Integrating Biological Resources for Prosperity

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A Proceedings of the National Conference, Biratnagar
February 6-7, 2020

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Publishers

Botanical Society of Nepal, Kathmandu
Nepal Biological Society, Biratnagar
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Integrating Biological Resources for Prosperity

ISBN: 978-9937-0-9013-1

Date of Publication: April, 2021

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Publication Support: USAID's Hariyo Ban Program, WWF Nepal

Citation: Siwakoti, M., Mandal, T.N., Rai, S.K., Rai, S.K., Gautam, T.P., Aryal, H.P. & Limbu, K.P. (2021, eds.). *Integrating Biological Resources for Prosperity.* Botanical Society of Nepal, Nepal Biological Society and Department of Plant Resources.

Printing: M.S. Offset Press, Jyatha, Kathmandu, Tel: 5904388

Layout: Mahesh Maharjan

Prologue

Nepal represents a part of biodiversity hotspot due to its unique geographic position, wide altitudinal variations and diverse climatic conditions, where 35 forest types and 118 ecosystems exist. The country houses a high number of endemic and globally threatened species of flora and fauna along with rich agrobiodiversity. About 21% total land of the country has been used for agriculture, a prime source for livelihood. Sustainable management of the biological resources has been closely linked with livelihood and economic well-being of the people in all aspects of life for food, health, nutrition, culture, climate and aesthetic values. However, the precious resources are being threatened by poverty, ecological fragility, environmental instability, inappropriate farming practices and unsustainable management of the natural resources.

Nepal has signed several biodiversity related multilateral and bilateral treaties, agreements and conventions including Convention on Biological Diversity. The Government of Nepal has also formulated a number of national policies and acts for translating the obligations of the treaties, agreements and conventions. As a result of the commitments and management of the government, the protected areas in Nepal covers 23.39% and forest covers 44.74% of total area of Nepal. Currently, the conservation paradigm of the country has also been shifted from species conservation to landscape management. The landscape management of ecosystems are crucial for conserving biodiversity of the protected areas as well as beyond the protected areas at the ecosystem, species and genetic levels.

Constitution of Nepal (2015) has adopted the federal democratic system. The constitution has also addressed the biodiversity related issues to be managed by the federal government, provincial governments and local levels. The conservation of biological resources is a major concern to Nepal. Therefore, the sustainable use and management of biological resources is crucial to promote the prosperity of people in Nepal. The government is keen on research and development with the proper and wise use of its biological resources.

The Botanical Society of Nepal, the Nepal Biological Society, the Ministry of Forests and Environment, the Government of Nepal; and the Ministry of Industry, Tourism, Forest and Environment of Province No. 1 have jointly organized a two-day National Conference on "*Integrating Biological Resources for Prosperity*" in Biratnagar, Nepal on February 6-7, 2020. The conference theme was focused on the state of plant and animal diversity, wetlands and water, conservation and utilization of agriculture and forest resources, important medicinal and other non-timber forest products, biotechnology, climate change scenarios and its impacts, invasive species, environmental issues and so on. The conference was a big success with active participation of students, professors, free-lance scientists, biodiversity managers, government officials and policy makers. The conference was concluded with the announcement of "Biratnagar Declaration 2020" which is presented herewith in this book.

We are grateful to all participants, organizers, collaborators and supporting organizations of the conference for making a very smoothly. We express our gratitude to Mr. Sherdhan Rai, Chief Minister of Province No. 1 for inaugurating the National Conference and Mr. Jagdish

Prasad Kusiyait, Minister for Industry, Tourism, Forest and Environment, Province No. 1 for chairing the inaugural session. We are thankful to the Ministers of the Government of Province No. 1, parliament members of the Province No. 1, the vice president and members of the Province Planning Commission of Province No. 1. We acknowledge our sincere thanks to Dr. Bishwa Nath Oli, Secretary, the Ministry of Forests and Environment for his active support and participation. We are also grateful to Dr. Tirtha Bahadur Shrestha, Dr. Kamal Krishna Joshi (former Vice Chancellor of Tribhuvan University and former Chairperson of the University Grants Commission) and Prof. Dr. Sanu Devi Joshi for their educative presentation and to Professor Emeritus Dr. Pramod Kumar Jha and Professor Emeritus Dr. Ram Prasad Chaudhary for delivering the Keynote speech. We are thankful to the Executive Committee and Advisory Committee members of Botanical Society of Nepal and Nepal Biological Society for their continuous suggestion and cooperation for conference and the publication of the book.

This book is a proceeding of the conference which comprises of a wide range of biological science related original research articles including some informative and review articles. We are also thankful to all authors, reviewers and editors for their kind support and hard work to publish the proceedings in a timely manner. We acknowledge to Prof. Dr. Shiva Kumar Rai of Post Graduate Campus, Tribhuvan University, Biratnagar for his very appreciable coordination and painstaking effort needed while processing the articles for this book. We also express sincere acknowledgement to the USAID's Hariyo Ban Program, WWF Nepal for the generous support to publish this book. This publication is a joint venture of the Botanical Society of Nepal, the Nepal Biological Society, and the Department of Plant Resources of Ministry of Forests and Environment, Government of Nepal. We hope the continuation of harmonious collaboration of the organizations in future, too.

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Date: April 2021

Preface

Biodiversity plays an essential role to maintain life support system and quality of life. Conservation of plants and animals in their habitat is needed to ensure the supply of human needs for food, shelter, wellbeing including the ecosystem services. Fair and sustainable use of such biological resources should be only option so as to ensure their continuity for upcoming generations. Nepal is rich in biological resources, the conservation and sustainable utilization of resources is essential for the prosperity of the country. However, the biological resources are rapidly depleting due to habitat destruction, growing human population, overharvesting, invasion by alien species, unsustainable exploitation of resources, unmanaged tourism, pollution, infrastructure expansion, climate change, etc. To address the situation on biodiversity, the government of Nepal is implementing the obligations set by the conventions, formulating and implementing national and provincial legislation, awareness and readiness programmes and activities. The National Conference on *Integrating Biological Resources for Prosperity* organized at Biratnagar on February 6-7, 2020 is one of the campaigns to exchange scientific information and to promote awareness to the public, academic institutions and the government organizations.

The organizers got the support from distinguished personalities and many government, academic, non-government and private organizations. We acknowledge our sincere thanks to all of them, specially to the organizing and advisory committees and subcommittees of the conference as well as executive committee and advisory committee members of the Botanical Society of Nepal and the Nepal Biological Society. We extend our special acknowledgements to the following organizations for their generous support, namely, the Department of Plant Resources, Kathmandu / Plant Research Center, Ilam; the Department of Environment, Kathmandu; USAID's Hariyo Ban Program, WWF Nepal, Kathmandu; REDD Implementation Center, Kathmandu; Biratnagar Metropolis, Morang; Nepal Academy of Science and Technology (NAST), Lalitpur; IUCN Nepal, Kathmandu; CODEFUND, Kathmandu; Post Graduate Campus, Biratnagar; Mahendra Morang Adarsha Multiple Campus, Biratnagar; Central Campus of Technology, Dharan; Mechi Multiple Campus, Bhadrapur; Purbanchal University, Biratnagar; Chamber of Industries, Morang; Morang Merchants Association, Biratnagar; HISSAN, Morang; and PABSON, Nepal.

This book, an output of the National Conference includes informative and reviewed articles along with the original research articles presented during the conference. A wide diversity of articles have been incorporated in the book, ranging from the research of microorganisms and lower groups to higher groups of plants and animals, endemic plants, medicinal plants, invasive alien plants, environment, traditional knowledge and ethnobotany, biotechnology, botanical garden, food science, fish diversity, etc. We highly appreciate the intellectual contribution of all authors, without their support it was almost difficult to bring the publication in time. We also acknowledge our sincere thanks to all referees for reviewing the articles in a timely manner. We heartily acknowledge to the Department of Plant Resources and USAID's Hariyo Ban Program, WWF Nepal for the support to publish the Proceedings. We also acknowledge Mr. Mahesh Maharjan for layout and printing. Happy Nepali New Year 2078.

Editors

Date: April 2021

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Biological Resources and Prosperity in Nepal

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Abstract

Human beings have always made use of biological resources for livelihoods and prosperity. Despite being rich in biodiversity and biological resources, Nepal remains economically one of the poorest countries in the world. Once a food exporting country, Nepal is now spending every year large amounts in importing agricultural and livestock products. In the last few years, the country has also started to import timber wood. The export of bio-products is not up to the national potential. Biodiversity and the ecosystem services they provide are remarkable; however, these need to be translated as resources for prosperity. Nepal has comparative advantages in biological resources, including hydropower, tourism, and cheap labor, and these sectors should be wisely addressed for prosperity.

Key words: Agriculture, Biodiversity, Forest, Global rankings of Nepal, Natural capital, Plant genetic resources.

Introduction

There are nearly 200 countries in the world, all with diverse resources, culture, and prosperity. Based upon national gross domestic product, countries have generally been categorized as developed-developing and least developed. In 1990, the United Nations Development Programme (UNDP) started ranking countries based on their Human Development Index (HDI), which encompasses per capita gross national income, life expectancy at birth, and expected year of schooling. UNDP modified this indexing by adjusting the planetary pressure in the Human Development Index, now known as Planetary pressure adjusted Human Development Index (PHDI) (UNDP, 2020). An indexing (Legatum Prosperity Index) was also launched by the Legatum Institute of Prosperity in the United Kingdom in 2010. Legatum Prosperity Index includes 12 criteria basic for prosperity, which is useful in measuring transformation, and offers a unique insight into how prosperity is forming and changing across the world. The purpose of all these categorizations of countries is to reveal their status and performance in socio-economic and environmental sectors.

Nepal, a land-locked country with a great range of topography within 200 km south and north distance, is unique and holds the 91st global position in land area with 30 million people. The country has been recently promoted to medium human development country at 142nd HDI position in 2020 from the low human development country. The country is endowed greatly with natural resources and different climatic zones. Despite that, not only is the country lagging behind in the developmental indices, but it is gradually moving downward in several indices. Here, a brief description and assessment of resources and Nepal's potential for prosperity is presented.

Natural Capital

In the 20th century, the Himalayan kingdom was known for its natural beauty and cultural heritage. In the global context, Nepal holds the 50th position in population size, 4th in runoff basis water (42nd in inland freshwater), 50th in economically feasible hydropower, 25th in flowering plant biodiversity, 49th in overall biodiversity, and 29th in ecosystem services (Table 1). The Terai forest was regarded as the biggest and richest sanctuary of wildlife in South Asia. Nepal had a reputation as a food exporting country. International tourists used to visit the country to experience its nature and culture. After political change in 1950, Nepal started its journey towards modern development, and good progress was made. The first modern protected areas were established in 1973 as Sagarmatha National Park and Chitwan National Park. Both were declared natural world heritage sites by UNESCO in 1984. Ecosystem vitality and environmental performance were appreciable. One of the major components of natural capital on the earth is biological resources, in which Nepal has significant advantage.

Biological Resources

Biological resources refer to the living landscape –plants, animals, microbes – which are extremely important to people for the various services they provide. In the context of human beings, biological resources play major roles in the form of (i) genetic resources and scientific inputs, (ii) agriculture (cultivated plants, livestock, poultry, fishery, pests), and (iii) sensitive biological resources, natural goods and services (habitat, wildlife, pollinators, ornamentals, medicinal and industrial sources, scenic beauty, ecosystem services).

Nepal is rich in biological resources. The country has77.6% land under green cover (40% forest, 4.6% shrub land, 21% agricultural cultivated land, and12% rangeland). In the global context, it stands in the 110th position in the world. In agricultural land, the country holds the136thposition (Table 1).

Table 1. Global ranking of Nepal in ecological indicators.

Indicators	Global rank (remarks)
Land area	91
Population size	50 (0.36% of world)
Population density	54 (48 in 2000)
Population growth	70 (77 in 2000)
Total renewable inland freshwater^	42
Economically feasible hydropower^	50
Runoff basis water@	4 (1630 mm/yr)
Precipitation (mm/yr)@	57
Agriculture land (% of total land	126 (95 in 2000)
Food production	50 (112 in 2000)
Crop production index	55 (123 in 2000)
Cereal yield	86 (91 in 2000)
Global hunger index	73 (83 in 2000)
Threat to agroecosystem due to biological invasion*	3
Livestock production	28 (102 in 2000)
Forest area	110 (108 in 2000)
Floral biodiversity**	25

Overall biodiversity**	49
Ecosystem vitality	120 (40 in 2000)
Ecosystem services	29
Climate change vulnerability***	4
Vulnerability due to earthquake ***	11
Vulnerability due to flood risks ***	13
CO_2 emission	166
Environmental performance index #	145 (38 in 2010)

Sources: The Global Economy.com (https//www.theglobaleconomy.com/texts). * Paini et al. (2016), ** UNDP, 2020, *** Environmental Performance Index (epi.yale.edu/epi-results 2020/component/epi), ^ Thapa, 2013

Biodiversity

Biodiversity (biological diversity) in simple words is referred as the varieties of life, and it is the source of all the biological resources. It is measured at three levels: genetic, species, and ecosystem level. There are 1.9 million species known and named on the earth (Champion 2009). It is believed that a huge number of plants, animals and microbes are still unexplored and unidentified.

Nepal encompasses around 28,000 species (15,000 faunal and 13,000 floral species) with 1.138% of overall biodiversity in 49th rank and 2.8% of flowering plant diversity in the 25th rank in the world. Current estimates of number of plant species includes 1,001 species of Algae (Prasad, 2013), 2,182 species of Fungi (Kost & Adhikari, 2015), 850 species of Lichens (Sochting, 2015), 1,217 species of Bryophytes (Pradhan, 2016), 550 species of Pteridophytes (Fraser-Jenkins et al., 2015), and 22 species and seven varieties of Gymnosperms (Rajbhandari et al., 2020). There are diverse data on the number of species of Angiosperms in Nepal - ranging from 5,309 (Rajbhandari & Rai, 2017) to 6,653 (Kunwar et al., 2010) to 6,973 (Groombridge & Jenkins, 2002; MoFSC, 2014).

The faunal diversity recorded in Nepal includes 168 species of Platyhelminths (Gupta, 1997), 117 amphibians (ICIMOD & MoEST, 2007), 192 mollusks (Budha, 2015), 12,957 insects (Thapa, 2013), 230 fishes (Rajbanshi, 2012), 871 birds (Baral, 2013), 7 reptiles (Shah, 2013a), and 210 mammals (Shah, 2013b), totaling around 15,000 species.

Plant Genetic Resources

Nepal is rich in indigenous wild and landrace plant genetic resources of value for agronomic, horticultural, forestry, or medicinal uses. This diversity is largely a result of varied topography and climatic conditions. Unfortunately, a large number of local varieties and landraces of crops and livestock have been lost. The National Gene Bank has adopted four conservation strategies, viz., *ex-situ*, on-farm, *in-situ*, and breeding along with 60 different good practices. The National Gene Bank had more than 18,765 accessions of agricultural genetic resources in seed bank, tissue banks, and field (Joshi et al., 2020).

Jha et al. (1996) have listed 485 species (genera for ornamental plants) in 14 categories (aromatic plants, cash crops, cereals, fibers, fodder, fruits, legume grains, medicines, oils, ornamentals, spices, timber, vegetables, wild relatives of food plants), and compared these to

the germplasm collections in Nepal and USA to highlight collection and conservation needs. Many germplasms still need to be collected, characterized, and used for crop improvement; however, it will not be economically feasible to collect, increase, identify, maintain, characterize, and distribute all of the species in gene banks. Hence, habitat preservation is equally needed for protection of genetic resources.

Nepal's need for agricultural improvement has led to the development of a Plant Genetic Resources Program and many field stations of the National Agricultural Research Council, and the Department of Agriculture and Livestock has been established. These are located in the representative environments throughout the country and evaluate indigenous landraces and advanced cultivars. Unfortunately, little research has been done on the local agrobiodiversity.

Agriculture

Nepal is a predominantly agrarian country with about 66% of its population involved in agricultural occupation. The country has roughly 0.1 ha agriculture land per person, whereas global agricultural land availability is 0.21 ha per person. 28% of the biodiversity in Nepal are agricultural genetic resources (AGRs), generally termed as agrobiodiversity (Joshi et al., 2020).

The agriculture sector, including forest and fisheries, contributes to around one-fourth of Nepal's gross domestic product (GDP), and its share in national GDP is gradually decreasing. The contribution of agriculture and forestry sectors to GDP was estimated at 26.5% in the FY 2018/19 (MoF, 2019). Two decades back, the country used to export food to neighboring countries, but now its reputation as a food exporting country has vanished. The country's agricultural production heavily depends upon rainfall, which has become erratic due to climate change. Nepal produced 10.66 million tons of food grain in the year 2018-19 (380 kg per person per year (MoAL, 2020) and is 55th in the world in terms of crop production and 86th in cereal production. In the fiscal year 2019/20, Nepal spent Rs. 243 billion for importing food, which was an increase of Rs.19 billion compared to the previous fiscal year, and the spending on food imports has increased by around 62% in the last five years.

Nepal is rich and important in agrobiodiversity due to its climatic and topographical diversity. Crop and livestock species have adapted well and evolved in harsh environmental conditions, including in the high mountains. However, its agrobiodiversity resources are not well documented and evaluated. It is a general fear that many landraces of crop species may disappear without their documentation.

Although the Government of Nepal has given due importance to the agriculture sector by developing the 20-year Agricultural Development Strategy (2015-2035), its success depends upon various factors, including quality of governance. To be prosperous, Nepal must give serious attention to agriculture, not only for food sufficiency but for better livelihoods, employment opportunities, and earnings through export.

Livestock

The livestock sector contributes about 11.5% of the total GDP and 25.7% of the agricultural GDP (AGDP) (MoAL, 2020). In livestock production, Nepal holds the 28th position in the

world. In 2017/18, there were 7.37 million cattle and 5.22 million buffalo in the country, and their annual growth rate in the last decade was 0.38 and 1.28% per year (MoAL, 2020). Nepal has made remarkable growth in the last two decades. The contribution of livestock and poultry sectors is not only the food and nutrition through meat, milk, and eggs, but also provides raw materials for industries, manure and draught power.

Fisheries

The contribution of the fishery sector to GDP was estimated at 0.5% in FY 2018/19 (MoF, 2019). Nepal's fish production has increased from 24,295 tons in 2006/07 to 62,725 tons in 2018/19, i.e., 158%. In this 12-year period, fish yield increased 36% from 3,607 to 4,920 kg/ha. Subedi (2020) reported that 143,241 people are directly involved in aquaculture and 421,345 people are involved in capture fisheries in Nepal. Some ethnic groups such as Bhote, Darai, Majhi, Gurung, and Kunwar depend on fisheries for their livelihood.

Floriculture

Floriculture has emerged as a new business in Nepal. Cut flowers, bulbs, perennial flowers, ornamental plants, landscape and gardening, floral arrangements, and specific decorations are important business areas currently practiced under the floriculture sub-sector in the country. The country exported floricultural items Rs. 9.3 million worth, whereas imported NRs 418 million worth in 2018/19 (Malla, 2020). With floriculture emerging as an attractive commercial activity, Nepal has also entered in export of ornamentals in the international market though it is currently at a low scale.

Vegetation and Forest

The vegetation of Nepal exhibits a remarkable diversity as a result of great variation in elevation and climate. Hagen (1998) identified 12 major vegetation types, Stainton (1972) described 35 types of vegetation, and Dobremez (1976) with Nepalese colleagues initially identified 198 parcels of vegetation, then finalized all these in 118 categories, ultimately simplifying the vegetation to 36 types (TISC, 2002). Vegetation generally occurs up to 5000 m elevation; however, there are reports of species up to 6300 m in Himalaya.

The forest statistics of Nepal can be confusing. According to Government of Nepal, the forest cover is 5.962 million ha, i.e., 40.36% of the total land area, and other wooded land has 0.648 million ha, totaling 44.74% of the national land (DoFRS, 2015). 15% of the forest occurs in protected areas, 40% of forest area managed by communities, including 34% area as community forest (DoFSC, 2019), and the remainder is managed through provincial governments. The UNDP's publications show 25.4% forest area in the country in 2016, a 24.7% decrease between 1980 and 2016 (UNDP, 2020). Hence, the international ranking at 110th is based upon the data given by international organizations.

Forest is the major dominant ecosystem on earth, covering 30.8% of the total land areas, i.e., 0.6 ha per person and 422 trees per person. Nepal encompasses 0.2 ha of forest areas per person and 111 trees per person, which is lower than the global average (MoF, 2019). Nepal's forest on average has 430 trees/ha (DoFRS, 2015). Among south Asian countries, Bhutan has the

highest forest area with 72.5% and Pakistan has the lowest with 1.9% forest area (MoF, 2019).

Forests play an important role in the national economy of Nepal. FAO (2010) estimated that Nepal's forestry sector contributed 3.5% to the GDP of the country in 2000 and 4.4% from 1990 to 2000. There are varying reports on the forest and biodiversity in Nepal. Ecosystem services of forests and biodiversity are known to everyone, but its services have not been quantified in terms of monetary value.

Sensitive Biological Resources

Sensitive biological resources are those species and biological communities that receive special consideration by the government and communities through plans, policies, regulations and scientific importance. Endemic, threatened, invasive, pollinator, and priority species fall under the category of sensitive biological resources.

Endemic Plants and Animals: 312 species of endemic flowering plants (Rajbhandari et al., 2020), 8 species of pteridophytes, 31 species of bryophytes (Pradhan, 2020), 16 species of fungi, 3 species of algae, and 9 species of lichens have been reported from Nepal. There are 16 endemic fish species and 12 invasive alien fish species in Nepal (MoFSC, 2014). The list of the endemic animal species documented by MoFSC (2002) covers one species of mammal, two species of birds, 11 species of amphibians and reptiles, 30 species of butterflies and moths, and 108 species of spiders. This list needs to be updated.

Threatened Plants and Animals: There are nine plants, 55 mammals, 149 birds, 64 herpetofauna, and 21 fish included in the IUCN Red List, and15 species of plants, 52 mammals, 108 birds. 19 reptiles and three insects have been listed in the CITES Appendices. Similarly, the Nepal government has protected 41 faunal species (27 mammals, 8 birds, three reptiles and two amphibians), 18 floral species (14 angiosperms and four gymnosperms) and one plant group (lichens) (MoFSC, 2014). Realizing the endangerment of species due to trade, the country has imposed restrictions on the export of 17 plant species under the Forest Act 1993.

Medicinal and Aromatic Plants: There are varied estimates of medicinal plants in Nepal – from 700 species to 1,792 species. Bhattarai and Ghimire (2006) estimated that 49% of the traded medicinal plants are herbs, 29% trees, 14% shrubs, and 8% climbers. Ghimire et al. (2018) analyzed the trade of medicinal and aromatic plants (MAP) of Nepal, and concluded that the export value of MAP products increased from US $ 27.49 million in 2005 to $ 60.09 million in 2014. Pyakurel et al. (2019) recorded 300 species in trade of medicinal plants of Nepal, most of these occurring in subtropical and lower temperate regions, indicating an economic potential for increased cultivation and domestication at middle mountains.

There is a major trade of medicinal and aromatic plants in the world. Demand of herbal medicine is growing in developed countries. Himalayan herbs have a historical reputation and global recognition. Medicinal and aromatic plants (MAPs) of Nepal can offer good opportunities for sustainable economic growth. The country currently does not hold a significant share of global market for MAP, but has potential. Nepal's export performance of MAPs and essential oils has been erratic, with volatile year-on-year trade flows. The number of manufacturers along the MAPs value chain registered as members of the Nepal Herbs

and Herbal Products Association (NEHHPA) grew from 20 in 2012 to 85 in early 2018. Nepali firms have had some success in diversifying export markets in recent years, but India remains by far the most important trade partner and destination in the MAPs value chain (WB, 2018). Trade data indicate that Nepali products need consistency and quality control.

Pollinators: Around 350,000 animal species act as pollinators and 90% of the wild flowering plant species depend on animal pollinators (IPBES, 2016). Over 20,000 flowering plants depend on insect pollination and over 30,000 species of insects are effective pollinators including bees, beetles, moths, flies, wasps and butterflies (Thapa, 2013). About 100 crop species provide 90% of food, of which 71 species are bee pollinated and several others by many other insects (Thapa, 2013). In any ecosystem, insects are important elements as plant feeders, predators, decomposers, pollinators, vectors of diseases, forensic entomology and biocontrol agents. Insect pollination is worth mentioning of its ecosystem service.

Biocontrol Agents: There are a few alien insects and fungi acting as biocontrol agents on invasive plants (the Mexican beetle *Zygogramma bicolorata* and winter rust *Puccinia abrupta* var. *parthenicola* on *Parthenium hysterophorus*; stem gall fly *Procecidochaeres utilis* and a fungus *Passalora ageratinae* on *Ageratina adenophora*). Nepal Agricultural Research Council has imported the two weevils *Neochetina eichornae* and *N. bruchi* Hustache from the USA for biological control of water hyacinth in Phewa and Begnas lakes in Pokhara valley (Anonymous, 2015).

Biological Invasion

Biological invasion has emerged as one of the serious challenges for biological resources of Nepal. The recent pandemic of COVID 19 is an example of biological invasion disrupting the global economy and impacting billions of lives. There are at least a dozen agricultural pests and pathogens that are alien invasive to Nepal. Recent invasion of *Tuta absoluta* on tomato crop and fall army worm (*Spodoptera frugiperda*) on maize crop are some of the worst examples of invasion. Invasive plants and animals threaten natural vegetation, agroecosystems, and aquatic ecosystems seriously. Paini et al. (2016) have identified that Nepal's agriculture is the third most threatened country in the world due to biological invasion. The impacts of invasive alien plant species are on plant species composition, interference in tree seedling regeneration, habitat degradation, and health and livelihoods.

There are 179 species of naturalized alien plant species in Nepal (Shrestha, 2019), and among these, 27species are invasive (Adhikari et al., 2021). Among them, four species (*Mikania micrantha, Chromolaena odorata, Lantana camara* and *Eichhornia crassipes*) are in the list of 100 of the world's worst invasive alien plant species (Lowe et al., 2000).

Budha (2015) listed 64 species of alien fauna in Nepal, 7 species of mammals, 6 species of birds, 22 species of arthropods, 9 species of mollusks and one species of platyhelminths. Among these, 10 species are listed in 100 of the world's invasive alien faunal species.

Prosperity

Prosperity is generally defined as flourishing, thriving good fortune. Nepal is considered a poor country in the world, but an ecologically prosperous country, rich in natural capital.

Modern development started in the Himalayan kingdom after 1950, and the country started its journey towards economic growth. There is positive growth in education, health, infrastructure sectors and market access in the country; nonetheless, there are issues of environmental degradation and biodiversity loss. In the 1950s, Simon Kuznet advanced a hypothesis known as environmental kuznet curve, stating that economic development initially leads to a deterioration in the environment, but after a certain level of economic growth, a society begins to improve its relationship with the environment.

Three years ago, the Government of Nepal gave a slogan: "Prosperous Nepal-Happy Nepali." Nepal holds the 114th position in 2020, showing a jump in the last five years from 128th position, in the Legatum Prosperity Index (LPI). Data reveal improvement in LPI, but at the same time degradation in the natural environment and loss of natural capital are evident. The standard of living has increased and quality of living has been challenged. The Legatum Institute of Prosperity started the prosperity index in 2010, considering the following 12 criteria: (i) safety and security, (ii) personal freedom, (iii) governance, (iv) social capital, (v) investment environment, (vi) entrepreneur conditions, (vii) market access, (viii) economic quality, (ix) living conditions, (x) health, (xi) education, and (xii) natural environment (LPI, 2020).

Technologies, knowledge, and resources can help in achieving prosperity. Any country willing to be prosperous should analyze, plan, and harness its area of comparative advantages. Nepal has comparative advantages in biological resources, hydropower, tourism, and cheap labor. Resources itself are no guarantee of prosperity until and unless they are wisely used.

Table 2. Global ranking/perception of status/performance of socio-economic indicators of Nepal.

Indicators	**Global rank (remarks)**
Human development index	142 (140 in 2000)
Legatum prosperity index*	114
World happiness index	100 (128 in 2000)
Gross domestic products (GDP)	0.031% of the world
GDP per capita	164 (175 in 2000)
Economic growth	40
GDP share of agriculture	17 (9 in 2000)
Export (% of GDP)	154 (125 in 2000) (0.01% of the world)
Import (% of GDP)	68 (114 in 2000) (0.06% of the world)
International tourist arrival	104 (98 in 2000)
Revenue from international tourists	113
Foreign aid and development assistance	22 (44 in 2000)
Corruption perception index	113
National health condition	110
Innovation index	95 (135 in 2015)

Challenges

There are several challenges in the journey from poverty to prosperity. The most serious challenges for a developing democratic country like Nepal are ethical governance, proper implementation of plans and policies, and wise use of resources without compromising the environment. Climate change and increasing biological invasions threaten natural resources and slow down the growth. Strong political will is necessary to overcome these challenges. Indicators to achieve or improve are given in tables 1 and 2.

Conclusion

Prosperity comes from productivity, wise use of resources, application of science and technology, innovative ideas, investment in research, and good governance. Nepal has all the essential ingredients needed for prosperity. The agriculture sector has great potential for national development as it not only meets the basic needs, but also provides employment, income, and a balanced ecosystem. The country jumped from an agricultural to a service economy at lower level of per capita GDP, by passing growth in the industrial sector. The Human Development Report of Nepal 2020 emphasizes that the agriculture, manufacturing, energy, and tourism sectors are the most prominent sectors for economic development of Nepal (GoN, 2020). The forest sector has excellent potential of ecological as well as economic prosperity. Biologists should involve themselves in innovative research, leading to the sustainable use of biological resources and value addition to biological products. The government should have commitment and fixed targets to achieve. The ecotourism sector is the strength of the country but not well managed. A country cannot develop without availability of cheap energy. Despite having enormous economically feasible hydropower, the energy is expensive.

The most important biological resource is human resource. Nepal has achieved remarkable progress in human resources. There are experts in most of the fields related to modern development and prosperity. The country must properly exploit its precious biological resources, including the human resources, for optimized prosperity.

Acknowledgements

The author is thankful to Prof. Dr. Mohan Siwakoti, Dr. Bharat Babu Shrestha, and Ms Sara Hendery for suggestions.

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Biocultural Diversity

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Diversity in plants and that in animals
Is the wealth embedded in nature?
Save them and conserve them
For those that will come in future.

Abstract

Loss of culture and language diversity greatly affects negatively in biodiversity conservation. Importance of the knowledge of indigenous names has been well-recognized by ethnobiologists for the success of initiatives related to the recovery and restoration of endangered species. Evidently, studies have demonstrated that efforts to conserve biodiversity can greatly benefit from engaging with local communities, anthropologists and linguists. While local communities and anthropologists can share their unique traditional ecological knowledge, linguists can serve as bridges between traditional knowledge and eco-science. It is well considered that safeguarding of traditional knowledge and the indigenous languages are promising tools for the conservation and sustainable management of biodiversity. Conservation biologists have realized the importance of focused research exploring the relationship between biological, linguistic and cultural diversity for developing integrated strategies designed to conserve species, culture and languages.

Key words: Biodiversity, Conservation strategies, Culture and language diversity

Introduction

Climate change, deforestation and habitat loss, overexploitation of nature and natural resources, introduction of and invasion by alien species into ecosystem and increased pollution are considered widely as main threats to biodiversity. Lots of money has been spent and many efforts have been made to conserve biodiversity throughout the globe. Yet attempts to control the increased loss of biodiversity are still beyond success.

Studying on the linkage between biodiversity conservation and conservation of local languages Gorenflo et al. (2012) found that the less biologically diverse regions in the world have less linguistically and culturally diverse as well. Biologists estimate annual loss of species at 1,000 times or greater than historic rates, and linguists predict that 50-90% of the world's languages will disappear by the end of this century. Using greatly improved datasets to explore the co-occurrence of linguistic and biological diversity in regions containing many of the Earth's remaining species: biodiversity hotspots and high biodiversity wilderness areas, the authors indicate that these regions often contain considerable linguistic diversity, accounting for 70% of all languages on Earth. They observed that the languages involved are frequently unique (endemic) to particular regions, with many facing extinction. Linkage

of biological diversity with linguistic diversity is complex and found varying in different localities, in spite of the concordance of strong geographical, biological and linguistic diversity in many areas indicating the occurrence of some form of functional connection between them. They have also observed that languages in high biodiversity regions also often co-occur with one or more specific conservation priorities. They are defined as endangered species and protected areas, marking particular localities important for maintaining both forms of diversity.

UNESCO (2003) assessed language vitality endangerment and documented through its Intangible Cultural Heritage Unit's Ad Hoc Expert Group on Endangered Languages. There is a gradation of languages from safe to extinct as shown in the following table.

Table 1. Vitality of languages

Degree of Endangerment	Intergenerational Language Transmission
Safe	Language is spoken by all generations; intergenerational transmission in uninterrupted
Vulnerable	Most children speak the language, but it may be restricted to certain domains (e.g., home)
Severely endangered	Language is spoken by grandparents and older generations; while the parent generation may understand it, they do not speak it to children or among themselves
Critically endangered	The youngest speakers are grandparents and older, and they speak the language partially and infrequently
Extinct	There are no speakers left

High Biodiversity Wilderness Areas (HBWA) of the World

The High Biodiversity Wilderness Areas (HBWA) approach has been developed by Olson et al. (2001) for Conservation International (CI) and adopted in 2012 by WWF. Accordingly, HBWA consists of five of the 24 wilderness areas that hold globally significant levels of biodiversity, as identified by Mittermeier et al. (2002). The five HBWAs are:

- Amazonia
- The Congo forests of Central Africa
- New Guinea
- The Miombo-Mopane Woodlands of Southern Africa (including the Okavango Delta), and
- The North American desert complex of Northern Mexico and the Southwestern part of United States of America.

The intact portion of these areas covers 8,981,000 km^2 (76% of their original extent), and 6.1% of the planet's land area. The geographic boundaries of the HBWA's coincide with the boundaries of several amalgamated (WWF, 2012). Biodiversity hotspots, on the other hand, are biogeographic region that is both significant reservoir of biodiversity and is threatened with destruction. The term biodiversity hotspots specifically refer to 25 biologically rich areas around the world that have lost at least 70% of the original habitat.

Co-occurrence of linguistic and biological diversity in biodiversity hotspots (BH)

Gorenflo et al. (2012) reported the co-occurrence of linguistic and biological diversity in biodiversity hotspots (BH) and HBWA areas. A biodiversity hotspot is widely known as a biogeographic region that is considered as a significant reservoir of biodiversity and, however, is threatened of destruction. The term biodiversity hotspots specifically refer to 25 biologically rich areas around the world that have lost at least 70% of their habitat. The authors infer that as the world grows less biologically diverse, it is becoming less linguistically and culturally diverse. Referring to the biologists' estimate of annual loss of species, they noted that it was 1,000 times or greater than historic rates, and greater than linguists' prediction of disappearing 50-90% of the world languages by the end of century. Results of the studies on co- occurrence of linguistic and biological diversity in regions containing many of the Earth's remaining species: biodiversity hotspots and HBWA indicate that these regions often contain considerable linguistic diversity accounting for 70% of all languages on Earth. Reasons for co-occurrence of linguistic and biological diversity are complex and appear to vary among localities, although strong geographic concordance between biological and linguistic diversity in many areas argues for some form of functional connection.

Harmon (2014) gave reasons for the belief that our planet, the Earth is said to be "alive" as the existence of a profuse variety of organisms, divergent streams of human thought and behavior and geophysical features that provide a congenial setting for the workings of nature and culture. All three realms of difference have evolved so that they interact with and influence one another. Earth's interwoven variety - what is called biocultural diversity – is nothing less than the pre-eminent fact of existence.

Anonymous (2010) in a report of the conference on biological diversity for development states that from genes, species, ecosystems, landscapes and seascapes, to languages, practices, traditions, artistic expressions and belief, value and knowledge systems, these diversities are facing unprecedented changes, and most importantly loss. The impact of reduction in bio-cultural diversity on the resilience of the planetary systems is profound. In the current global change context, the loss of biological diversity, with the simultaneous loss of languages knowledge systems and specific ways of life, has resulted in new challenges for coupled social-ecological systems.

The report further states that in order to address those challenges mentioned above it is critical that the links between biological and cultural diversities - encompassing, inter alia, languages as repositories of knowledge and practices, tangible and intangible heritage related to nature, modes of subsistence, economic and social relations and belief systems – taken into consideration in policy development at all scales.

Global studies revealed that there was significant role of different cultures of the world such as Buddhism culture in China, Sacred grove culture in India on conservation and protection of biodiversity. Similar findings of the study were obtained from Samahni area, where Gujar and Jat tribes implemented their traditional cultural customs not only to their daily life but also on conservation of biodiversity in their vicinity region (Ishtiaq et al., 2012).

Kollmair and Turin (2007) urged that mountains particularly the Himalayas are not only centers of extraordinary biodiversity; they are also cultural and linguistic hotspots. Of the

approximately 600 languages found in the Himalayas, over 400 are spoken by groups of less than 100,000 people, and most of these are in danger of extinction. Analogous to the threat of species extinction, the extinction of languages should be regarded as an unrecoverable loss of diversity for all of humankind.

Inference

Shrestha (1999) presented a picture of biocultural diversity of Nepal describing the Himalayan country as one of the key hotspots of both biodiversity and cultural and linguistic diversities in South Asia. Nepal links six floristic provinces of Asia and two major world realms, the palearctic and the oriental. The country covers only 0.1% of the world's land but claims 8.5% of its bird species, 4.2% of butterflies, 2.2% of freshwater fishes, and houses 2.2% of the world's flowering plants. Nepal is equally rich in its cultural diversity as well with people in Mongolian characters in the Himalayan region, Mongolian and Aryan mixed in the mid hills and Aryan in lowlands, Tibetans dwelling in the highlands, Gorkhalis and wide range of other ethnic groups living in the mid hills, and tharus and others in the lowlands Most of these ethnic groups of people in Nepal have their own languages and cultures. Among all important communities of different parts of Nepal, Ayurvedic medicines, Trans-Himalayan Tibetan medicines practiced by local practitioners known as Amchis, and other ethno-medicines practiced by different local communities of Nepal are worth mentioning here. Knowledge of ethno-cultural aspect of the use of different plants and animals for different purposes and in different occasions and festivals by local communities along with information of their habitat is passed to the next generation through local languages and practices. Once the language is extinct inheritance of culture and knowledge becomes stranded. Once culture is forgotten, all those plants and animals which are used in different occasions and the knowledge of their existence in local vegetation including those of forests will also be forgotten. Recent studies made by environmentalists, biologists, anthropologists and related experts of the world suggest that without successful conservation of local languages and culture successful conservation of biodiversity will remain limited as a mere slogan.

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Integrating Biological Resources for Prosperity, 2021, 18-22

Plant Science Education and Society

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Abstract

With the inception of human civilization plants have being used by man for their basic needs. Recent urbanization and other development activities have been found to be responsible for careless and imprudent encroachment of natural habitat. Plant sciences today have to face challenges from global issues including climate change, global warming and increasing requirement of food, shelter, health, fodder, energy and so on for growing population. Education that cannot combat such challenges and solve the problems of society will certainly loose its values. People are now aware of responsibilities of educational institutions towards societies on which they subsist. In order to meet local and global needs of our education of plant sciences, it must be able to keep pace with social needs, global change and technology development. Time has come or rather late to look back on our curriculum of plant sciences in higher education and update it giving much attention to needs of the societies.

Key words: Plant science education, social needs, global change, curriculum.

Introduction

"Knowing is not enough, we must apply
Willing is not enough, we must do."
– Johann Wolfgang Von Goethe.

Technology is used or scientific knowledge is applied for specific goals or purposes in the society. Science, technology and society are interlinked with each other with their contributions and benefits. Science seeks to improve society and informs technology; while technology demands more science for its further development. Technology contributes to the society by making life of people in society easier. Society on the other hand, demands more and more from science and is benefited from technology.

American Society of Plant Biology (ASPB) put forward 12 principles of plant biology related to education transformation. The topic Transforming Education in Plant Biology is well studied to faculties who are planning to build courses or curricula in order to incorporate evidence-based methods of teaching and learning. Based on the principles courses are suggested for middle schools on 12 different topics. They are given in different titles *viz*., sugar makers (biological process and photosynthesis); biodiversity big and small (diversity and 350,000 species; feed your veggies (inorganic elements and biosphere); a rainbow of uses (fibers medicines and other important products); seeds of change (evolution, oxygen and ozone); mean and green (defense from pests, injury and illness); sex, bugs and pollen's role (flowers and seeds: asexual propagation); plant plumbing (water circulation, growth and structure); plants respire too (respiration and energy); which way to grow; green ecology

(hormones and external signals); and thanks for the support (cell walls and building materials). Lab works based on these 12 topics have also been well designed.

International Association of Universities also showed its deep concern on transformation education and organized an International Conference on Transforming Higher Education for the Future. The conference was held in Puebla, Mexico in 13-15 November, 2019. For further information concerning the conference may visit: www.iau-aiu.net or contact IAU Secretariat - UNESCO House – 1, rue Miollis, F-75732 Paris Cedex 15, France Tel: +33 (0)1 45 68 48 06.

Effective learning on any topics of sciences or all other disciplines may need some systematic method of learning and develop capacity of applying for the benefit of the society. The learner must also develop ability of creating new knowledge. Benjamin Bloom suggested a classification of educational objectives and developed a theory of mastery learning, today well known as Bloom's Taxonomy (Fig. 1).

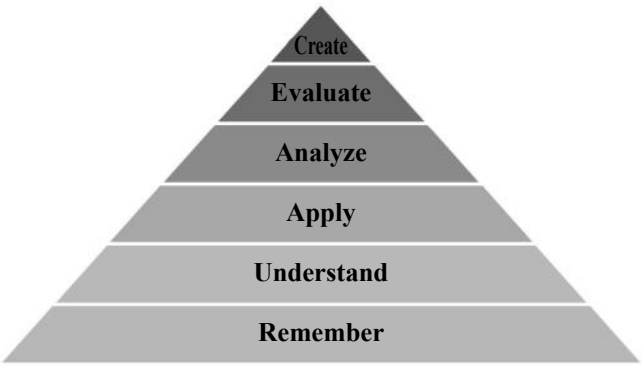

Figure 1. Bloom's Taxonomy (Remember- recall facts and basic concepts, Understand- explain ideas or concepts, Apply- use information in new situation, Analyze- draw connection among ideas, Evaluate- justify a stand or decision, Create- produce new or original works)

Dragomir and Panzaru (2015) highlighted the main connections between education and development entrepreneurship, understanding the significant aspects related to Romania and EU member states. Al-Zubeidi (2005) showed the importance of relationship between college educational background and all business successes in Texas. The author found that the often-argued relationship between college education in general, and education in business in specific, and small business success continues to be critical and evidently realized the importance of such relationship in the continuing need for learning to cope with rapid technological advances, competition, and the changing global economy.

Eilks (2014) discussed the insights into some theoretical foundations of the UN Decade of Education for Sustainable Development (ESD) by presenting different models of implementing ESD in teaching of science and technology and illustrating by various case studies. The paper suggests that thoroughly combining the ESD framework with science teaching that follows a socio-scientific issues-based approach to education has great potential for helping students develop many general educational skills. Lippman in Mallick (2017) states that one of the most severe limitations of nature is that it has not provided enough

genetic variation for breeders to work with, especially for the major yield traits that can involve dozens of genes. Mallick (2017) opined that the sense of security that farmers feel when they have advanced tools to protect and improve their harvests while protecting natural resources, benefits the whole family and ultimately the entire communities. To achieve sustainable development goals of UN to eradicate poverty, provide food security and improved nutrition, more such researches are needed to develop GM crops for disease resistance, drought resistance, and for other agronomic traits (Fig. 2).

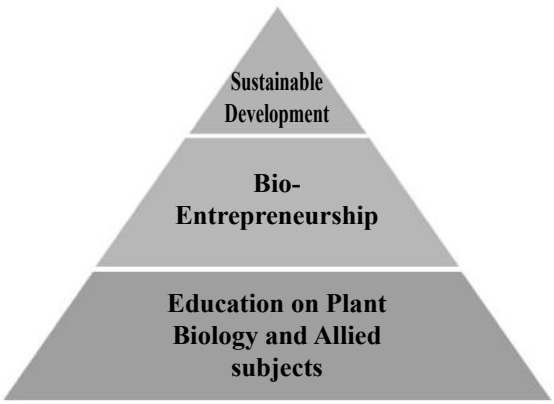

Figure 2. Relationship between education, entrepreneurship and sustainable development.

Most of the universities in our South Asian region do not revise and introduce new knowledge in their syllabi in time as per industries and other user' requirement, mostly because senior faculties are reluctant to update and enable themselves for teaching and undertaking research works in the fields they never learnt before. Grant is very right to say that the mark of higher education is not the knowledge we accumulate in our head. It is the skills we gain about how to learn.

Research, an Integral Part of Education for Development

Research produces knowledge. It also helps in confirming previous knowledge and finding truth in our beliefs. It is important in achieving success in competitive local and global markets. It also helps in combating existing and forthcoming health problems of all lives successfully and building society stronger, happier and compassionate. Research is considered crucial for quality higher education throughout the globe. Research receives great importance in universities and institutions delivering higher education both as degree-oriented *viz*., undergraduate, graduate, doctoral and post-doctoral programs and non-degree-oriented programs. For achieving a better position in world-class university ranking also research is considered one of the most important criteria both in its quality and quantity. In universities and higher education institutions of Nepal budget allocation for academic research is too poor. Yet, faculties by their own efforts are able to receive small or moderately bigger supports from national and foreign donors. More efforts are expected to increase research budgets of Central and State Governments for different universities, academic and research institutions.

Distribution of Human Resources in Botany

The history of science teaching is not very old in Nepal. It was introduced in Tri Chandra College in 1945. So far, the products of seven decades of Nepal universities and colleges absorbed in government organizations (viz., Department of Plant Resources, Nepal Agriculture Research Centre, Food Research Lab, Drug Research Lab and different ministries and departments, etc.), teaching institutions (viz., TU campuses, community campuses, private campuses, different community and private schools) and non-government organizations (viz., ICIMOD, IUCN, WWF, ANSAB, nurseries, herbal farms and herbal medical companies). Many graduates have left country and sought opportunities mostly in USA, Australia, New Zealand, UK, and different countries of Europe and Asia for further studies and jobs.

Promotion of Job Opportunities

Job opportunity for plant science graduates in Nepal is too limited. Self-employment among them is extremely low. It is partly because of their incapability in investing for small or medium entrepreneurial ventures and partly because of limitations in courses of studies. Revision of courses of studies is highly recommended here.

Curricula are to be revised giving emphasis on following requirements:

- keep pace with global trends
- fulfill social demands
- enable to develop entrepreneurship
- encourage consultancy services to relevant professionals
- enable graduates in solving related problems such as:
    - improvement of plants
    - prevention from and care of diseases and different stresses
    - increase in quality and productivity
    - imparting knowledge of modern technologies
    - introduction of internship at all levels of higher education
- involve job providers in curricula development and revision and
- interactions with all stakeholders for making curricula updated all the time.

Evidently, in recent years, students are attracted more in applied subjects of plant sciences. In Central Department of Botany of Tribhuvan University, special and applied subjects (in addition to general subjects) are offered to students. M.Sc. in Biodiversity and Environment Management is also a new addition as a separate self-sustaining subject. Students are found willing to pay the required amount for its cost recovery. For all other subjects' tuition fee is comparatively very low. Students' choice and job opportunities suggests faculties to think further on giving priority to subjects of students' interests linked mostly with more job opportunities.

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Endemic Flowering Plants of Nepal: Status and Distribution

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Abstract

This paper aims to present the status and distribution of Nepal's endemic flowering plants by revising their existing lists and updating them based on the published literature. The recently updated list shows that Nepal has 293 endemic flowering plant species to date. Among 289 species of endemic flowering plants analyzed, Central Nepal has 181 species, the highest number of species than in the west (60 species) and east (49 species) Nepal. Endemic flowering plants are found in 39 districts of Nepal. Mustang district (34 species) has the highest number of endemic flowering plants. Thirty-two herbaria in Nepal and abroad preserve the herbarium specimens of Nepalese endemic flowering plants. Among them, Natural History Museum (BM, London), Royal Botanic Garden Edinburgh (E, Edinburgh), University of Tokyo (TI, Tokyo) and National Herbarium of Nepal (KATH, Lalitpur) have more than 50 type specimens of the Nepalese endemic flowering plants. Several herbaria have one type of specimen only. The data can help to do further research, develop the planning/policy, and know the important plant areas (IPA) for the conservation of the endemic plants in Nepal. Districts with high diversity of endemic flowering plants such as Mustang, Dolpa, Rasuwa, Kaski, Gorkha, Sankhuwasabha, Jumla, Manang and Taplejung should be given importance for conservation and for further study to analyze them.

Key words: Flora of Nepal, Important Plant Areas, National Herbarium of Nepal, Plant Systematics, Vegetation zones

Introduction

Endemic flowering plants of Nepal are those plants whose distributions are confined to Nepal. If the plant is found outside Nepal, then it is not endemic to Nepal. In the broader sense, any endemic taxonomic unit's distribution is confined to a particular country or geographical region (Good, 1974). Isolation of geographical areas, such as high mountains and islands, plays a vital role in forming endemic plants. These areas have shown greater endemism in general. Importance of the endemic plants lies in the fact that if it is lost from a particular country or region, it will be lost from the world. Therefore, utmost importance has to be given to the conservation of these plants. Recently, some papers on the endemic flowering plants of Nepal have appeared with their lists. However, they have not been analyzed from the distribution point of view. With the help of the published literature, we revised the list of Nepal's endemic flowering plants, updating them wherever necessary and worked out their distribution in different climatic and vegetation zones of Nepal and different districts

of Nepal to see their diversity and distribution pattern. A single type specimen sometimes represents endemic flowering plant species of Nepal. Therefore, we also checked the national and international herbaria where these specimens are preserved to see in how many herbaria Nepalese endemic flowering plant specimens are available. The data can help in further research and develop the planning/policy of the conservation of Nepal's endemic plants.

The information on the flowering plants of Nepal was provided by Hara and Williams (1979) and Hara et al. (1978, 1982) in their books '*An enumeration of the flowering plants of Nepal volumes 1-3*' which later became a source for preparing the list of the endemic flowering plants of Nepal (Rajbhandari et al., 2016). Based on these books, Shrestha et al. (1986) reported 242 species of endemic flowering plants of Nepal. Joshi and Joshi (1991) provided a list of 283 endemic flowering plants of Nepal. Shrestha and Joshi (1996) reported 246 species of endemic flowering plants of Nepal. Chaudhary (1998) has given a list of endemic flowering plants of Nepal. Ghimire (2005) reported endemic flowering plants of Dolpo (northwest Nepal) area. Bhuju et al. (2007) in '*Nepal biodiversity resource book: Protected areas, Ramsar sites and world heritage sites*' have included as an Annex 1.5 a list of the endemic flowering plants and have recorded 316 species out of total 399 taxa (species, subspecies, variety). Rajbhandari and Adhikari (2009) and Rajbhandari and Dhungana (2010, 2011a) have given descriptions of 282 species of endemic flowering plants of Nepal. Rajbhandari and Dhungana (2011b) and Rajbhandari (2013) analysed the diversity of Nepalese endemic flowering plants and noted that the families having a large number of endemic species were *Asteraceae*, *Saxifragaceae*, *Papaveraceae* and *Ranunculaceae* and the genera having a large number of endemic plants were *Saxifraga*, *Pedicularis*, *Meconopsis* and *Impatiens*. Miehe et al. (2015) have followed Bhuju et al. (2007) and noted that 399 endemic flowering plants of Nepal are unevenly distributed. Rajbhandari et al. (2015) reported 81 type specimens preserved in Nepal's National Herbarium (KATH), which included 45 endemic flowering plants of Nepal. The previous lists of the endemic flowering plants were revised by Rajbhandari et al. and reported 324 species in 2016 and 312 in 2017. Tiwari et al. (2019) also gave a list of 312 species of endemic flowering plants of Nepal and analysed their phytogeographical implications. In the book '*Flowering plants discovered from Nepal*' published in 2019, Rajbhandari et al. compiled information about the new flowering plants discovered from Nepal, including endemic plants and clarifying their whereabouts type specimens. This paper aims to present the status and distribution of Nepal's endemic flowering plants by revising their existing lists and updating them based on the published literature and the examination of the type specimens in the National Herbarium of Nepal.

Results

Diversity of Endemic Flowering Plants of Nepal

The recent updated list showed that there are 293 endemic flowering plant species in Nepal belonging to 129 genera and 45 families (Tables 1, 11). This is about 5% of the total flowering plant species of Nepal. Previously in 2017, we had shown that there were 312 species of

endemic flowering plants in Nepal. The reduction from 312 to 293 is due to recent taxonomic studies by various experts. Some species have been reported from neighbouring countries, and some species have become synonyms of the species having distribution outside Nepal. A few new species have been recently added to the flora of Nepal thus adding to the endemic list of the flowering plants. Twelve families have ten or more than ten species of endemic flowering plants (Table 2). Among them, the families having a large number of endemic species are Apiaceae (29 species), Saxifragaceae (20 species), Fabaceae (19 species), Orchidaceae (19 species), Asteraceae (18 species) and Papaveraceae (17 species). Fourteen genera have five or more than five species of endemic flowering plant species (Table 3). Genera having a large number of endemic species are *Saxifraga* (20 species), *Pedicularis* (15 species) and *Corydalis* (9 species). *Aconitum*, *Acronema*, *Astragalus*, and *Papaver* each have 8 species. They are all much diversified high altitude plants (Rajbhandari et al., 2017).

Table 1. Diversity of endemic flowering plants of Nepal.

Family	No. of species	Family	No. of species
Acanthaceae	5	Hypericaceae	1
Amaryllidaceae	1	Iridaceae	1
Apiaceae	29	Juncaceae	1
Apocynaceae	3	Lamiaceae	10
Asparagaceae	1	Lauraceae	1
Asteraceae	18	Oleaceae	1
Balsaminaceae	6	Onagraceae	3
Begoniaceae	5	Orchidaceae	19
Berberidaceae	2	Orobanchaceae	16
Boraginaceae	4	Papaveraceae	17
Brassicaceae	10	Plantaginaceae	3
Campanulaceae	4	Poaceae	15
Caryophyllaceae	10	Polygonaceae	4
Crassulaceae	2	Primulaceae	4
Cucurbitaceae	1	Ranunculaceae	16
Cyperaceae	7	Rosaceae	7
Elaeagnaceae	1	Rubiaceae	3
Ericaceae	2	Salicaceae	2
Eriocaulaceae	3	Saxifragaceae	20
Euphorbiaceae	1	Scrophulariaceae	2
Fabaceae	19	Urticaceae	1
Gentianaceae	8	Zingiberaceae	3
Gesneriaceae	1		

Table 2. List of families having 10 or more than 10 species of endemic flowering plants of Nepal.

Family	No. of species	Family	No. of species	Family	No. of species
Apiaceae	29	Asteraceae	18	Poaceae	15
Saxifragaceae	20	Papaveraceae	17	Brassicaceae	10
Fabaceae	19	Orobanchaceae	16	Caryophyllaceae	10
Orchidaceae	19	Ranunculaceae	16	Lamiaceae	10

Table 3. List of genera having 5 or more than 5 species of endemic flowering plants of Nepal.

Genus	Family	No. of species
Saxifraga	Saxifragaceae	20
Pedicularis	Orobanchaceae	15
Corydalis	Papaveraceae	9
Aconitum	Ranunculaceae	8
Acronema	Apiaceae	8
Astragalus	Fabaceae	8
Papaver	Papaveraceae	8
Oxytropis	Fabaceae	7
Silene	Caryophyllaceae	7
Impatiens	Balsaminaceae	6
Begonia	Begoniaceae	5
Carex	Cyperaceae	5
Himalayacalamus	Poaceae	5
Saussurea	Asteraceae	5

Endemic flowering plants in different vegetation zones of Nepal

Among 289 endemic flowering plant species analyzed, Central Nepal has 180 species (highest number of endemic species) than in west (60 species) and east (49 species) Nepal (Table 4). In central Nepal, the alpine zone contains the largest number (76 species) of endemic flowering plants. Similarly, the subalpine zone of central Nepal has 50 species (the second largest number). The lowest number of endemic flowering plants (5 species) is found in east Nepal's temperate zone. *Strobilanthes nutans*, *Cyperus trisulcus*, *Cyperus wallichianus*, *Epilobium indicum* could not be included due to lack of altitudinal data. The highest altitude record of the endemic flowering plant in Nepal is *Lagotis nepalensis*, which was found in Bajhang district of west Nepal at 5700 m altitude. Similarly, the lowest altitude record of the endemic flowering plant in Nepal is *Begonia tribenensis*, which was found in Sunsari district of east Nepal at 130 m altitude.

Table 4. Number of endemic flowering plant species of Nepal in different vegetation zones.

Vegetation zone	West Nepal	Central Nepal	East Nepal	Total
Alpine (above 3800 m)	25	76	22	123
Subalpine (3001 m – 3800 m)	15	50	12	77
Temperate (2001 m – 3000 m)	13	30	5	48
Tropical and Subtropical (up to 2000 m)	7	24	10	41
Total	60	180	49	289

West Nepal: western border to 83°E longitude. Central Nepal: 83° to 86°30'E longitude. East Nepal: 86°30'E longitude to eastern border.

Endemic flowering plants in different districts of Nepal

Endemic flowering plants are found in 39 districts of Nepal. Mustang district (34 species) has the highest number of endemic flowering plants (Tables 6, 7, 8). Other districts having more than 10 endemic flowering plants of Nepal are Dolpa (26 species), Rasuwa (24 species), Kaski (21 species), Gorkha (18 species), Sankhuwasabha (17 species), Jumla (14 species),

Manang (13 species), and Taplejung (11 species). One species of endemic flowering plant is found in nine districts of Nepal. Gandaki Province (with 102 species) has the highest number of endemic flowering plants of Nepal. Similarly, Bagmati Province has 66 species, Karnali Province has 60 species, Province 1 (east Nepal) has 44 species, Lumbini Province has 9 species and Far-western Province has 8 species (Table 5). In Province 2 endemic flowering plant has not been recorded.

Table 5. Number of endemic flowering plant species in the Provinces of Nepal.

Province	No. of species	Percentage (%)
1	44	15.22
Bagmati (3)	66	22.84
Gandaki (4)	102	35.29
Lumbini	9	3.11
Karnali (6)	60	20.76
Far-western (7)	8	2.77

Table 6. Number of endemic flowering plants of Nepal in different districts.

No. of species	District (s)
34	Mustang
26	Dolpa
24	Rasuwa
21	Kaski
18	Gorkha
17	Sankhuwasabha
14	Jumla
13	Manang
11	Taplejung
9	Dhading
8	Mugu, Myagdi, Rukum East
7	Dolakha, Humla
6	Kathmandu, Baglung, Lalitpur, Ramechhap
5	Solukhumbu
4	Doti
3	Darchula, Dhankuta, Jajarkot, Jhapa
2	Ilam, Lamjung, Makwanpur, Sindhupalchok, Sunsari
1	Bajura, Bhaktapur, Chitwan, Kalikot, Khotang, Nuwakot, Palpa, Salyan, Sindhuli

Table 7. Districts with number of endemic flowering plants of Nepal.

Province	District	No. of species
Far Western Province (7)	Darchula	3
,,	Bajura	1
,,	Doti	4
Karnali Province (6)	Humla	7
,,	Jumla	14
,,	Dolpa	26
,,	Mugu	8
,,	Kalikot	1

,,	Jajarkot	3
,,	Salyan	1
Lumbini Province (5)	Rukum Purba (east)	8
,,	Palpa	1
Gandaki Province (4)	Baglung	6
,,	Gorkha	18
,,	Kaski	21
,,	Lamjung	2
,,	Manang	13
,,	Mustang	34
,,	Myagdi	8
Bagmati Province (3)	Bhaktapur	1
,,	Chitwan	1
,,	Dhading	9
,,	Dolakha	7
,,	Kathmandu	6
,,	Lalitpur	6
,,	Makwanpur	2
,,	Nuwakot	1
,,	Ramechhap	6
,,	Rasuwa	24
,,	Sindhuli	1
,,	Sindhupalchok	2
Province 1	Solukhumbu	5
,,	Khotang	1
,,	Sankhuwasabha	17
,,	Dhankuta	3
,,	Sunsari	2
,,	Taplejung	11
,,	Ilam	2
,,	Jhapa	3

Table 8. Districts of Nepal with list of endemic flowering plants.

Endemic flowering plants	**Locality**
Far Western Province	
Darchula district	
1. *Pimpinella kawalekhensis* Farille & Lachard (Apiaceae)	Kawa Lekh, 2950 m
2. *Scrophularia laportifolia* T. Yamaz. (Scrophulariaceae)	Tologaon (Marma), 2895.6 m
3. *Vicatia nepalensis* Kljuykov (Apiaceae)	Tologaon (Marma), 2590.8 m
Bajura district	
1. *Impatiens bajurensis* S. Akiyama & H. Ohba (Balsaminaceae)	Kaudegaon (Babali), 1520 m
Doti district	
1. *Calanthe himalaicum* Raskoti (Orchidaceae)	Wagalek, 2200 m
2. *Millettia nepalensis* R. N. Parker (Fabaceae)	Chusiakana
3. *Taraxacum nepalense* Soest (Asteraceae)	Khaptar forest
4. *Thunbergia nepalensis* Bh. Adhikari & J. R. I. Wood (Acanthaceae)	Doti, 2105 m

Endemic flowering plants	Locality
Karnali Province	
Humla district	
1. *Draba poluniniana* Al-Shehbaz (Brassicaceae)	Dozam Khola, near Simikot, 3810 m
2. *Galium saipalense* Ehrend. & Schoenb. -Tem. (Rubiaceae)	Saipal, 4700 m
3. *Lagotis nepalensis* T. Yamaz. (Plantaginaceae)	Urai Langna, 5791.2 m
4. *Noccaea nepalensis* Al-Shehbaz (Brassicaceae)	Dozam Khola, near Simikot, 3200.4 m
5. *Papaver simikotense* (Grey-Wilson) Christenh. & Byng (Papaveraceae) *Meconopsis simikotensis* Grey-Wilson	Dozam Khola, near Simikot, 3505.2 m
6. *Saussurea ramchaudharyi* S. K. Ghimire & H. K. Rana (Asteraceae)	Upper Chungsa valley, above Selimari lake, 4650 m
7. *Sisymbrium nepalense* Al-Shehbaz (Brassicaceae)	Kharpunath, 2200 m
Jumla district	
1. *Cirsium flavisquamatum* Kitam. (Asteraceae)	Ghurchi Lekh, Lumra – Churma, 3352.8 m
2. *Corydalis calycina* Liden (Papaveraceae)	Maharigaon, 3200 m
3. *Corydalis spicata* Liden (Papaveraceae)	Kapra, 2580 m
4. *Elymus nepalensis* (Melderis) Melderis (Poaceae) *Agropyron nepalense* Melderis	Lumsa, NW Jumla, 2895.6 m
5. *Festuca poluninii* E. B. Alexeev (Poaceae)	Maharigaon, 4114.8 m
6. *Impatiens williamsii* H. Hara (Balsaminaceae)	Jumla – Garjigoth, 3048 m
7. *Odontostemma paramelanandrum* (H. Hara) Rabeler & W. L. Wagner (Caryophyllaceae) *Arenaria paramelanandra* H. Hara	Chakhure lekh, S. of Jumla, 4267.2 m
8. *Oxytropis arenae-ripariae* Vass. (Fabaceae)	Sisne Himal, 4572 m
9. *Papaver chankheliense* (Grey-Wilson) Christenh. & Byng (Papaveraceae) *Meconopsis chankheliensis* Grey-Wilson	Maharigaon, 4572 m
10. *Pedicularis yamazakiana* R. R. Mill (Orobanchaceae)	Ratamata, Chakure Lekh, 3810 m
11. *Primula poluninii* Fletcher (Primulaceae)	Sisne Himal, 4876.8 m
12. *Ranunculus himalaicus* Tamura (Ranunculaceae)	Sisne Himal, 4572 m
13. *Roscoea nepalensis* Cowley (Zingiberaceae)	N. of Jumla, 2440-2740 m
14. *Saxifraga poluniniana* Harry Sm. (Saxifragaceae)	Kabhre, Padmara – Bumra, Padmara Lagna, 3505.2 m
Dolpa district	
1. *Allium hypsistum* Stearn (Amaryllidaceae)	About 4 miles S.W. of Saldanggaon, c. 5500 m
2. *Aphragmus nepalensis* (H. Hara) Al-Shehbaz (Brassicaceae) *Staintoniella nepalensis* H. Hara	Tarap, 5029.2 m
3. *Asparagus penicillatus* H. Hara (Asparagaceae)	Dunaihi, Bheri valley, 2438.4 m
4. *Astragalus chateri* Vassilcz. (Fabaceae)	Barbung Khola, Kakkotgaon, 3657.6 m
5. *Clematis phlebantha* L. H. J. Williams (Ranunculaceae)	Suli Gad, 2895.6 m
6. *Corydalis clavibracteata* Ludlow & Stearn (Papaveraceae)	Ringmigaon, Phoksumdo Tal, 4724.4 m
7. *Corydalis simplex* Liden (Papaveraceae)	Jangla Bhanjyang, 4300 m

Endemic flowering plants	Locality
8. *Corydalis uncinata* Liden (Papaveraceae)	Phoksumdo Tal, 3657.6 m
9. *Cyananthus hayanus* C. Marquand (Campanulaceae)	Panjen, 4572 m
10. *Festuca nepalica* E. B. Alexeev (Poaceae)	Tingjegaon, 5059.68 m
11. *Galium nepalense* Ehrend. & Schoenb. -Tem. (Rubiaceae)	Met Khola, Chharkabhot, 4724.4 m
12. *Malaxis dolpensis* M. R. Shrestha, L. R. Shakya & S. K. Ghimire (Orchidaceae)	Khorakchhang, 4200 m
13. *Nepeta staintonii* Hedge (Lamiaceae)	Barbung Khola, 3962.4 m
14. *Onosma bheriensis* H. Hara (Boraginaceae)	Narku – Ila, Bheri River, 1981.2 m
15. *Oxytropis fasciculiflorum* Vass. (Fabaceae)	Mukdem Khola, Chharkabhot, 5638.8 m
16. *Oxytropis torrentium* Vass. (Fabaceae)	Phuksumdo Khola, 4267.2 m
17. *Oxytropis williamsii* Vass. (Fabaceae)	Barbung Khola, near Bandar Phatka, 3505.2 m
18. *Pedicularis anserantha* T. Yamaz. (Orobanchaceae)	Central Nepal, 3780 m
19. *Pedicularis odontoloma* T. Yamaz. (Orobanchaceae)	Tsarka (Chharka), N. of Mukut Himal, 4700 m
20. *Primula ramzanae* W. W. Sm. & H. R. Fletcher (Primulaceae)	Nahure, 5181.6 m
21. *Saussurea platyphyllaria* Ludlow (Asteraceae)	Ringmigaon, Phoksumdo Tal, slopes beneath Kanjiroba, 4724.4 m
22. *Saxifraga mira* Harry Sm. (Saxifragaceae)	Barbung Khola, Kaya Khola, 4419.6 m
23. *Saxifraga rhodopetala* Harry Sm. (Saxifragaceae)	Phoksumdo Tal, 3962.4 m
24. *Scrophularia bheriensis* T. Yamaz. (Scrophulariaceae)	Ila, Bheri River, 1828.8 m
25. *Silene greywilsonii* Rajbh. & Mitsuo Suzuki (Caryophyllaceae)	Jengla, West of Namdo, c. 5300 m
26. *Solms-laubachia haranensis* (Al-Shehbaz) J. P. Yue, Al-Shehbaz & H. Sun (Brassicaceae) *Desideria haranensis* Al-Shehbaz *Ermaniopsis pumila* H. Hara	Sya Gompa, 5029.2 m
Mugu district	
1. *Aconitum poluninii* Lauener (Ranunculaceae)	Mugu – Purana Mugu, Mugu Khola, 3810 m
2. *Astragalus poluninii* Podlech (Fabaceae)	Gum, near Rara, 2134 m
3. *Eskemukerjea megacarpum* (H. Hara) H. Hara (Polygonaceae) *Fagopyrum megacarpum* H. Hara *Eskemukerjea nepalensis* Malick & Sengupta	Karnali valley, 2438.4 m
4. *Isodon namikawanus* Murata (Lamiaceae)	Mu, 3750 m
5. *Oreocome depauperata* Pimenov & Kljuykov (Apiaceae)	Daha Kharka, 3500 m
6. *Pedicularis muguensis* T. Yamaz. (Orobanchaceae)	Eding, 3600 m
7. *Silene davidlongii* Rajbh. & Mitsuo Suzuki (Caryophyllaceae)	Khaptang, Mugu Khola, 4572 m
8. *Veronica emodi* T. Yamaz. (Plantaginaceae)	Below Mugu, Mugu Khola, 3700 m
Kalikot district	
1. *Delphinium williamsii* Munz (Ranunculaceae)	Gilam, Tila valley, 1828.8 m
Jajarkot district	

Endemic flowering plants	Locality
1. *Ceropegia poluniniana* Bruyns (Apocynaceae)	Bheri River valley, 1500 m
2. *Discretitheca nepalensis* (Moldenke) P. D. Cantino (Lamiaceae) *Caryopteris nepalensis* Moldenke	Pokhra, 1066.8 m
3. *Prunus jajarkotensis* H. Hara (Rosaceae)	Jajarkot, 914.4 m
Salyan district	
1. *Rhynchosia nepalensis* H. Ohashi & Tateishi (Fabaceae)	Sitalpati, 1066.8 m
Lumbini Province	
Rukum Purba (east) district	
1. *Achnatherum staintonii* (Bor) M. Nobis & P. D. Gudkova (Poaceae) *Stipa staintonii* Bor *Stipella staintonii* (Bor) Roeser & Hamasha *Stipellula staintonii* (Bor) Roeser & Hamasha	Seng Khola, 3810 m
2. *Aconitum tabatae* Tamura (Ranunculaceae)	Purbang, 3850 m
3. *Ceropegia nepalensis* (Radcl. -Sm.) Bruyns (Apocynaceae) *Riocreuxia nepalensis* Radcl. -Sm. *Brachystelma nepalense* (Radcl. -Sm.) Bruyns	Ranmagaon, 3048 m
4. *Corydalis megacalyx* (Papaveraceae)	E. of Chalike Pahar, 4572 m
5. *Corydalis uncinatella* (Papaveraceae)	Toridwari Bhanjyang, 3800 m
6. *Oxytropis morenarum* (Fabaceae)	Chalike Pahar, 3962.4 m
7. *Saussurea chrysotricha* Ludlow (Asteraceae)	E. of Chalike Pahar, 4267.2 m
8. *Silene helleboriflora* Exell & Bocquet (Caryophyllaceae)	Mulmuley Khola, 4267.2 m
Palpa district	
1. *Habenaria palpensis* Raskoti (Orchidaceae)	Mujhung, 1500 m
Gandaki Province	
Baglung district	
1. *Aconitum amplexicaule* Lauener (Ranunculaceae)	Dogadi Khola, 3810 m
2. *Astragalus pseudorigidulus* Podlech (Fabaceae)	Near Dogado Khola, 4572 m
3. *Codonopsis reflexa* D. Y. Hong (Campanulaceae)	Near Dogadi Khola, 3960 m
4. *Neottia nepalensis* (N. P. Balakr.) Szlach. (Orchidaceae) *Listera nepalensis* N. P. Balakr.	Phagune Dhuri, 3352.8 m
5. *Pimpinella inundata* (Farille & S. B. Malla) P. K. Mukh. & Constance (Apiaceae) *Ligusticum inundatum* Farille & S. B. Malla	Dhorpatan, 2840 m
6. *Taraxacum amabile* Soest (Asteraceae)	Okhaldhungagaon, S. of Dhorpatan, 3048 m
Gorkha district	
1. *Aconitum dhwojii* Lauener (Ranunculaceae)	Khorlak, 4572-4876.8 m
2. *Arnebia nepalensis* (Kitam.) H. Hara (Boraginaceae) *Macrotomia nepalensis* Kitam.	Thaple Himal, 4100 m
3. *Astragalus nakaoi* Kitam. (Fabaceae)	Manaslu, 3800 m
4. *Clinopodium nepalense* (Kitam. & Murata) Braechler & Heubl (Lamiaceae) *Micromeria nepalensis* Kitam. & Murata	Bangu Khola, 3400 m
5. *Croton nepalensis* T. Kuros. (Euphorbiaceae)	Tatopani to Dovan, 970 m
6. *Delphinium unifolium* Tamura (Ranunculaceae)	Banga Khola, 3500 m

Endemic flowering plants	Locality
7. *Hedysarum manaslense* (Kitam.) H. Ohashi (Fabaceae) *Astragalus manaslensis* Kitam. *Hedysarum nepalense* H. Ohashi var. *subhirtellum* H. Ohashi	Gorkha District, Manaslu, 3800 m
8. *Leontopodium makianum* Kitam. (Asteraceae)	Kalun – Bajon, Thaple Himal, 4000 m
9. *Papaver manasluense* (P. A. Egan) Christenh. & Byng (Papaveraceae) *Meconopsis manasluensis* P. A. Egan	Manaslu Himal, east of Samdo, S. side of Sanam Khola, 4000 m
10. *Papaver regium* (G. Taylor) Christenh. & Byng (Papaveraceae) *Meconopsis regia* G. Taylor	Barpak, 3657.6-4572 m
11. *Prunus himalaica* Kitam. (Rosaceae)	Chum Gompa, 3900 m
12. *Roscoea ganeshensis* Cowley & W. J. Baker (Zingiberaceae)	Buri Gandaki valley, near Abuthum Lekh, 1900 m
13. *Roscoea tumjensis* Cowley (Zingiberaceae)	Shiar Khola river, above Tumje, 2740 m
14. *Saussurea dhwojii* Kitam. (Asteraceae)	Pongsing, 4572 m
15. *Saxifraga hypostoma* Harry Sm. (Saxifragaceae)	Jargeng Khola, 4876.8 m
16. *Sedum pseudo-multicaule* H. Ohba (Crassulaceae)	Bee – Namura, E
17. *Synclinostyles exadversum* Farille & Lachard (Apiaceae)	Lari, 4400 m
18. *Tetrataenium lallii* (C. Norman) Cauwet, Carb. & Farille (Apiaceae) *Heracleum lallii* C. Norman	Booshki (Pangsing), 3962.4-4419.6 m
Kaski district	
1. *Acronema refugicolum* Farille & Lachard (Apiaceae)	Annapurna Himal, Seti Khola, 4000 m
2. *Begonia taligera* S. Rajbh. (Begoniaceae)	Bharat Pokhari, near Pokhara, 700-740 m
3. *Conioselinum nepalense* Pimenov & Kljuykov (Apiaceae)	S of Annapurna mountain massif, valley of Modi Khola, 3100-3400 m
4. *Corydalis terracina* Liden (Papaveraceae)	N. of Pokhara, 2440 m
5. *Fallopia filipes* (H. Hara) Holub (Polygonaceae) *Bilderdykia filipes* H. Hara	Mardi Khola, 1981.2m
6. *Gastrochilus nepalensis* B. B. Raskoti (Orchidaceae)	Above Deurali on the way to Kande, ca. 2350 m
7. *Himalayacalamus asper* Stapleton (Poaceae)	Karuwa, Pipar
8. *Himalayacalamus cupreus* Stapleton (Poaceae)	Karuwa, Pipar
9. *Impatiens gorepaniensis* Grey-Wilson (Balsaminaceae)	Gorepani, N. W. of Pokhara, 2000 m
10. *Odontochilus nandae* Raskoti & H. Kurzweil (Orchidaceae)	Panchase forest, 2400 m
11. *Papaver taylorii* (L. H. J. Williams) Christenh. & Byng (Papaveraceae) *Meconopsis taylorii* L. H. J. Williams	Seti Khola, Annapurna Himal, 4572m
12. *Pedicularis annapurnensis* T. Yamaz. (Orobanchaceae)	Annapurna Himal, Seti Khola, 4267.2m
13. *Pedicularis chamissonoides* T. Yamaz. (Orobanchaceae)	Lamjung Himal, 3810m
14. *Pinalia annapurnensis* (L. R. Shakya & M. R. Shrestha)	Chhomrong, Annapurna

Endemic flowering plants	Locality
Schuit., Y. P. Ng & H. A. Pedersen (Orchidaceae) *Eria annapurnensis* L. R. Shakya & M. R. Shrestha	Conservation Area, north of Pokhara, 2000 m
15. *Pinalia pokharensis* (D. M. Bajracharya, A. Subedi & K. K. Shrestha) Schuit., Y. P. Ng & H. A. Pedersen (Orchidaceae) *Eria pokharensis* D. M. Bajracharya, A. Subedi & K. K. Shrestha	Lumle, Pokhara, 900-1000 m
16. *Saxifraga excellens* Harry Sm. (Saxifragaceae)	Mardi Khola, Annapurna Himal, 3810m
17. *Silene stellariifolia* Bocquet & Chater (Caryophyllaceae)	Kanra/Lumle, 1676.4m
18. *Sinocarum latifoliolatum* Pimenov & Kljuykov (Apiaceae)	South slopes of Annapurna Mts., valley of Modi Khola, right bank, between Himalaya Hotel and Deorali, 3100-3400 m
19. *Sinocarum meeboldioides* Pimenov & Kljuykov (Apiaceae)	South slopes of Annapurna Mts.,valley of Modi Khola, right bank, between Chhomrong and Dovan, 2000-2300 m
20. *Strobilanthes saccata* J. R. I. Wood (Acanthaceae)	Dhumpus/Lumle, 1930 m
21. *Thamnocalamus chigar* (Stapleton) Stapleton (Poaceae) *Borinda chigar* Stapleton	Karuwa – Pipar, 3000 m
Lamjung district	
1. *Acronema dyssimetriradiata* Farille & S. B. Malla (Apiaceae)	Annapurna, Lamjung Himal, north of Namun col, 4000-4200 m
2. *Lalldhwojia staintonii* Farille (Apiaceae)	Versant nord de Lamjung – Range (massif de l'Annapurna), 3800-4000 m
Manang district	
1. *Astragalus barclayanus* Podlech (Fabaceae)	Tilicho Pass path, 4820 m
2. *Astragalus lobbichleri* Podlech (Fabaceae)	Manangbhot, Sabzi Khola, 3700 m
3. *Codonopsis bragaensis* Grey-Wilson (Campanulaceae)	Marsyangdi valley, slopes of Annapurna III to the S. W. of Braga, c. 3800 m
4. *Crepis himalaica* Kitam. (Asteraceae)	Thumje, near Shiar Khola, 3300 m
5. *Draba macbeathiana* Al-Shehbaz (Brassicaceae)	Thorong La, Marsyandi valley, 5273.04m
6. *Gentianella lowndesii* Harry Sm. (Gentianaceae)	Central Nepal, Manang District, Bimtakothi, 3900 m
7. *Pedicularis breviscaposa* T. Yamaz. (Orobanchaceae)	Pisang – Tat Pani, 3100 m
8. *Rhododendron lowndesii* Davidian (Ericaceae)	Marsyangdi valley, 4114.8m
9. *Saxifraga alpigena* Harry Sm. (Saxifragaceae)	Marsyandi (Marsyangdi), 3505.2m
10. *Saxifraga cinerea* Harry Sm. (Saxifragaceae)	Marsiandi, 2743.2m
11. *Saxifraga lowndesii* Harry Sm. (Saxifragaceae)	Manang?, Sabze Khola, 4114.8m
12. *Silene hideakiohbae* Rajbh. & Mitsuo Suzuki (Caryophyllaceae)	Suggi Khola, 2520 m

Endemic flowering plants	Locality
13. *Synotis managensis* S. Joshi, Kanti Shrestha & D. Bajracharya (Asteraceae)	Manang district, 3432 m
Mustang district	
1. *Artemisia mustangensis* Yonek. (Asteraceae)	Marpha – Syang, 2550 m
2. *Artemisia nepalica* Yonek. (Asteraceae)	Tukuche – Yak Kharka, 3200 m
3. Astragalus nepalensis Podlech (Fabaceae)	Trail above Tukuche, Kali Gandaki, 2590.8m
4. *Astragalus notabilis* Podlech (Fabaceae)	Lower Puyun Khola, between Yara and Dhi, 3450 m
5. *Berberis mucrifolia* Ahrendt (Berberidaceae)	Tegar, N. of Mustang, 4419.6m
6. *Berberis pendryi* Bh. Adhikari (Berberidaceae)	Below Muktinath
7. *Carex gandakiensis* Katsuy. (Cyperaceae)	Chimgaon (N. of Tukuche), 3352.8m
8. *Carex mallae* (Rajbh. & H. Ohba) O. Yano (Cyperaceae) *Kobresia mallae* Rajbh. & H. Ohba	Nr. Pudamigaon, nr. Suli Gad, 3657.6m
9. *Ceropegia meleagris* H. Huber (Apocynaceae)	Ghasa (S. of Tukucha), Kali Gandaki, 2286m
10. *Cicerbita nepalensis* Kitam. (Asteraceae)	Tukucha, Kali Gandaki, 3000 m
11. *Clematis bracteolata* Tamura (Ranunculaceae)	Near Sangda, 3700 m
12. *Epilobium brevisquamatum* P. H. Raven (Onagraceae)	Tukucha, Kali Gandaki, 3200 m
13. *Epilobium staintonii* P. H. Raven (Onagraceae)	Pura, near Muktinath, 3650 m
14. *Eremogone mukerjeeana* (Majumdar) Rabeler & W. L. Wagner (Caryophyllaceae) *Stellaria mukerjeeana* Majumdar	Muktinath, 4260 m
15. *Gentiana tetramera* Miyam. (Gentianaceae)	Around Sangda La, 4660 m
16. *Gomphogyne nepalensis* W. J. de Wilde & Duyfjes (Cucurbitaceae)	Kalopani
17. *Juncus mustangensis* Miyam. & H. Ohba (Juncaceae)	Between Sangda Pass and Phalyak, 3870 m
18. *Justicia tukuchensis* V. A. W. Graham (Acanthaceae)	Ghasa, S. of Tukucha, Kali Gandaki, 2286m
19. *Keraymonia nipaulensis* Cauwet-Marc & Farille (Apiaceae)	Muktinath range, N. E. Annapurna, 4500 m
20. *Lepidostemon williamsii* (H. Hara) Al-Shehbaz (Brassicaceae) *Draba williamsii* H. Hara	Near Pudamigaon, Suli Gad, 4114.8m
21. *Microula mustangensis* Yonek. (Boraginaceae)	Lhetak Kharka – Ghumi, 4490 m
22. *Oxytropis graminetorum* Vass. (Fabaceae)	Taglung (S. of Tukucha), Kali Gandaki, 4267.2m
23. *Oxytropis nepalensis* Vass. (Fabaceae)	Tukucha, Kali Gandaki, 4114.8m
24. *Papaver staintonii* (Grey-Wilson) Christenh. & Byng (Papaveraceae) *Meconopsis staintonii* Grey-Wilson	Kali Gandaki valley, above Lete, 3657.6m
25. *Poa muktinathensis* Rajbh. (Poaceae)	Thorung La, above Muktinath, 5200 m
26. *Primula sharmae* H. R. Fletcher (Primulaceae)	Muktinah, 4114.8m
27. *Rhodiola nepalica* (H. Ohba) H. Ohba (Crassulaceae) *Sedum nepalicum* H. Ohba	Tegar, N. of Mustang, 4572m

Endemic flowering plants	Locality
28. *Salvia transhimalaica* Yonek. (Lamiaceae)	Valley of Ghemi Khola (N side), N of Ghemi, 3520 m
29. *Saussurea kanaii* K. Fujikawa & H. Ohba (Asteraceae)	Yak Kharka, 4420 m
30. *Saxifraga namdoensis* Harry Sm. (Saxifragaceae)	Namdo, N. Mustang, 4572m
31. *Saxifraga staintonii* Harry Sm. (Saxifragaceae)	Samargaon, N. Tukucha, 4876.8m
32. *Saxifraga williamsii* Harry Sm. (Saxifragaceae)	Muktinath, 4114.8m
33. *Silene vautierae* Bocquet (Caryophyllaceae)	Tukucha, Kali Gandaki, 3810m
34. *Swertia nepalensis* J. Shah (Gentianaceae)	Tukucha, Kali Gandaki Valley, 3810m
Myagdi district	
1. *Aconitum williamsii* Lauener (Ranunculaceae)	N. W. of Gurjakhani, 3275 m
2. *Cyananthus himalaicus* K. K. Shrestha (Campanulaceae)	S. of Gurjakhani, 3200.4m
3. *Draba staintonii* Jafri ex H. Hara (Brassicaceae)	Barse, 4572m
4. *Oreorchis porphyranthes* Tuyama (Orchidaceae)	S. of Gurjakhani, 3139.44m
5. *Saccharum williamsii* (Bor) Bor ex Cope (Poaceae) *Erianthus williamsii* Bor	Near Gurjakhani, 2895.6m
6. *Saxifraga micans* Harry Sm. (Saxifragaceae)	S. of Gurjakhani, 3810m
7. *Saxifraga roylei* Harry Sm. (Saxifragaceae)	S. of Gurjakhani, 3810m
8. *Sinocarum staintonianum* P. K. Mukh. ex Farille & Lachard (Apiaceae)	Above Sauwala Khola, 4500 m
Bagmati Province	
Bhaktapur district	
1. *Thunbergia kasajuana* Bh. Adhikari & J. R. I. Wood (Acanthaceae)	Nagarkot, 2020 m
Chitwan district	
1. *Eria nepalensis* Bajrach. & K. K. Shrestha (Orchidaceae)	Sauraha, Royal Chitwan National Park, 200 m
Dhading district	
1. *Acronema bryophilum* Farille & Lachard (Apiaceae)	Ganesh Himal, haute Mailung Khola, basin W, 4520 m
2. *Acronema phaeosciadeum* Farille & Lachard (Apiaceae)	Ganesh Himal, sur les cretes separant les vallees de Mailung Khola et de Manjor Khola, 4000 m
3. *Corydalis stipulata* Liden (Papaveraceae)	Mailung Khola, S. of Ganesh Himal, 3350 m
4. *Euphrasia nepalensis* Pugsley (Orobanchaceae)	Chisey, 4267.2m
5. *Iris staintonii* H. Hara (Iridaceae)	Abuthum Lekh, Ganesh Himal, 3505.2m
6. *Papaver ganeshense* (Grey-Wilson) Christenh. & Byng (Papaveraceae) *Meconopsis ganeshensis* Grey-Wilson	Ganesh Himal, Ankhu Khola, 4114.8m
7. *Pleione coronaria* P. J. Cribb & C. Z. Tang (Orchidaceae)	Ganesh Himal, 2850 m
8. *Poa hideaki-ohbae* Rajbh. (Poaceae)	Pati Kharka near Pabil Kharka, 3400 m
9. *Synclinostyles denisjordanii* Farille & Lachard (Apiaceae)	S. of Ganesh Himal, haute Mailung Khola, 4030 m

Endemic flowering plants	Locality
Dolakha district	
1. *Aconitum bhedingense* Lauener (Ranunculaceae)	Beding, 3657.6-3962.4m
2. *Acronema pneumatophobium* Farille & Lachard (Apiaceae)	Rolwaling Himal, entre Na et Tesi Lepcha, 4500 m
3. *Chamaesium shrestaeanum* Farille & S. B. Malla (Apiaceae)	Beding, Na, Rolwaling valley, 4200 m
4. *Pedicularis yalungensis* T. Yamaz. (Orobanchaceae)	Yalung Kharka – Yalung La – Pam Lhang, Rolwaling Himal, 4300–5300 m
5. *Saxifraga amabilis* H. Ohba & Wakab. (Saxifragaceae)	Rolwaling Khola, Na-Sangma-Khabun, 4300 m
6. *Saxifraga zimmermannii* Baehni (Saxifragaceae)	Beding, 4130 m
7. *Swertia acaulis* Harry Sm. (Gentianaceae)	Dolakha district, 3657.6-5486.4m (BM)
Kathmandu district	
1. *Bambusa nepalensis* Stapleton (Poaceae)	Bansbari
2. *Bulbophyllum nepalense* Raskoti & Ale (Orchidaceae)	Sivapuri National Park, 2300 m
3. *Herminium hongdeyuanii* B. B. Raskoti (Orchidaceae)	Chandragiri, 2200 m
4. *Himalayacalamus fimbriatus* Stapleton (Poaceae)	Kathmandu, 1200 m
5. *Hypericum cordifolium* Choisy (Hypericaceae) *Hypericum bracteatum* Buch.-Ham. ex D. Don	Tancote
6. *Machilus pubescens* Blume (Lauraceae)	Central Nepal
Lalitpur district	
1. *Carex rhombifructus* Ohwi (Cyperaceae)	Godawari – Phulchauki, 1600-2500 m
2. *Cirsium phulchokiense* Kitam. (Asteraceae)	Phulchoki, S. Kathmandu, 1500 m
3. *E1aeagnus tricbolepis* Momiyama (Elaeagnaceae)	Godawari, 1615.44m
4. *Isodon phulchokiensis* (Murata) H. Hara (Lamiaceae) *Rabdosia phulchokiensis* Murata	Phulchoki, S. of Kathmandu, 2400-2700 m
5. *Pinalia baniae* (Bajracharya, L. R. Shakya & Chettri) Schuit., Y. P. Ng & H. A. Pedersen (Orchidaceae) *Eria baniaii* Bajracharya, Shakya & Chettri	E. North facing slope Khani Gaon, Godavari, 1600 m
6. *Wulfeniopsis nepalensis* (T. Yamaz.) D. Y. Hong (Plantaginaceae) *Wulfenia nepalensis* T. Yamaz. *Wulfenia amherstiana* Benth. var. *nepalensis* (T. Yamaz.) T. Yamaz.	Godavari-Phulchoki, 2300 m
Makwanpur district	
1. *Bulbophyllum raskotii* J. J. Verm., Schuit. & de Vogel (Orchidaceae)	Makwanpur district, 2400 m
2. *Habenaria wallichii* (Kolan., Kras & Szlach.) J. M. H. Shaw (Orchidaceae) *Habenella wallichii* Kolan., Kras & Szlach.	Sahid Smarak, Hetauda
Nuwakot district	
1. *Begonia nuwakotensis* S. Rajbh. (Begoniaceae)	Kakani, Doman, 1700 m
Ramechhap district	
1. *Eriophyton staintonii* (Hedge) Ryding (Lamiaceae) *Lamium staintonii* Hedge	Panch Pokhari, Khimti Khola, 3810m

Endemic flowering plants	Locality
2. *Gentiana radicans* Harry Sm. (Gentianaceae)	Sermabee, 4876.8m
3. *Impatiens harae* H. Ohba & S. Akiyama (Balsaminaceae)	Neju – Choarma, 3651-2760 m
4. *Maharanga verruculosa* (I. M. Johnst.) I. M. Johnst. (Boraginaceae) *Onosma verruculosa* I. M. Johnst.	Tatey, 2743.2m
5. *Saxifraga harae* H. Ohba & Wakabayashi (Saxifragaceae)	Beni Kharka – Tschokarma, 4550 m
6. *Saxifraga mallae* H. Ohba & Wakab. (Saxifragaceae)	Neju – Luk Kharka (W. slope of Zurmoche Glacier), 4500 m
Rasuwa district	
1. *Acronema johrianum* Babu (Apiaceae)	Ghopte – Gosainthan, 4290 m
2. *Acronema mukherjeeanum* Farille & Lachard (Apiaceae)	Ganesh Himal, Paldol, 3450 m
3. *Arenaria globiflora* (Fenzl) Wall. ex Edgew. & Hook. f. (Caryophyllaceae) *Dolophragma globiflorum* Fenzl *Cherleria grandiflora* D. Don	Gossainthan, 1819m
4. *Begonia flagellaris* H. Hara (Begoniaceae)	Gul Bhanjang – Latsu, 2300-2400 m
5. *Crotalaria kanaii* H. Ohashi (Fabaceae)	Dhunche, Trishuli Khola – Singum Gompa, 2300 m
6. *Festuca eriobasis* H. Scholz (Poaceae)	Langschisa Karka, 4530 m
7. *Herminium fimbriatum* (Raskoti) X. H. Jin, Schuit., Raskoti & L. Q. Huang (Orchidaceae) *Bhutanthera fimbriata* Raskoti	Way to Gosainkunda, Langtang National Park, 3800 m
8. *Himalayacalamus* planatus Stapleton (Poaceae)	Syabru, ca. 2438.4m
9. *Himalayacalamus porcatus* Stapleton (Poaceae)	Syabru, 2286m
10. *Lalldhwojia pastinacifolia* Pimenov & Kljuykov (Apiaceae)	Langtang National Park, basin of Trisuli Khola, between Cholang Pati and Lauribinayak, 4000 m
11. *Leontopodium montisganeshii* S. Akiyama (Asteraceae)	Lipchet Kharka – Makgan Kharka, 3490 m
12. *Liparis langtangensis* B. B. Raskoti & Ale (Orchidaceae)	Kyangjin Kharka, Langtang National Park, 3700-3900 m
13. *Oreocome involucellata* Pimenov & Kljuykov (Apiaceae)	Langtang National Park, basin of the Trisuli Khola, between Sing Gompa and Shalang Pati, 3400 m
14. *Oxygraphis nepalensis* Tamura (Ranunculaceae)	Langtang, 3810-4114.8m
15. *Papaver autumnale* (P. A. Egan) Christenh. & Byng (Papaveraceae) *Meconopsis autumnalis* P. A. Egan	Ganesh Himal, Tulo Bhera Kharka – Jaisuli Kund, 4160 m
16. *Pedicularis pseudoregeliana* P. C. Tsoong (Orobanchaceae)	Dudhkund, E. Timure, 4725 m
17. *Pilea kanaii* H. Hara (Urticaceae)	Sim Chotala – Ramche, 1500 m
18. *Pimpinella acronemastrum* Farille & Lachard (Apiaceae)	Ganesh Himal, Pangjung, 2000 m
19. *Rhododendron cowanianum* Davidian (Ericaceae)	Langtang lateral valley, 3657.6m
20. *Rohmooa kirmzii* Farille & Lachard (Apiaceae)	Ganesh Himal, Paldol, rive

Endemic flowering plants	Locality
	gauche de Manjor Khola, 4270 m
21. *Saxifraga ganeshii* H. Ohba & S. Akiyama (Saxifragaceae)	Jaisuli Kund-Paldol Base Camp, 4250 m
22. *Silene fissicalyx* Bocquet & Chater (Caryophyllaceae)	Mailung Khola, 4267.2m
23. *Sinocarum normanianum* (Cauwet-Marc & Farille) Farille (Apiaceae) *Similisinocarum normanianum* Cauwet-Marc & Farille	North of Kathmandu, Pong-Sing (Pangsing?), 4572m
24. *Sorbus sharmae* M. F. Watson, V. Manandhar & Rushforth (Rosaceae)	Langtang, 3170 m
Sindhuli district	
1. *Impatiens kharensis* S. Akiyama, H. Ohba & Wakab. (Balsaminaceae)	Khare Khola, Bitta Kharka – Patale Pokhari, 3300-4100 m
Sindhupalchok district	
1. *Begonia leptoptera* H. Hara (Begoniaceae)	Kalingchok, Thala – Tale Bisauna, 2500 m
2. *Primula wigramiana* W. W. Sm. (Primulaceae)	Sherkhatan, 5181.6m
Province 1	
Solukhumbu district	
1. *Aphragmus hinkuensis* (Kats. Arai, H. Ohba & Al-Shehbaz) Al-Shehbaz & S. I. Warwick (Brassicaceae) *Lignariella hinkuensis* Kats. Arai, H. Ohba & Al-Shehbaz	Thasing Dingma – Chhatarwa, 3550 m
2. *Carex esbirajbhandarii* (Rajbh. & H. Ohba) O. Yano (Cyperaceae) *Kobresia esbirajbhandarii* Rajbh. & H.Ohba	Below Dudhkund – upper Dudhkund glacier, 4600 m
3. *Eriophyton nepalense* (Hedge) Ryding (Lamiaceae) *Lamium nepalense* Hedge	Beni Khola, 4114.8m
4. *Gentiana sagarmathae* Miyam. & H. Ohba (Gentianaceae)	Namche – Phurte, 3500 – 3600 m
5. *Sibbaldia emodi* H. Ikeda & H. Ohba (Rosaceae)	Tangna – Dik Kharka, 4015 m
Khotang district	
1. *Synotis panduriformis* (Kitam.) C. Jeffrey & Y. L. Chen (Asteraceae) *Senecio panduriformis* Kitam.	Aisyalu Kharka, 2100 m
Sankhuwasabha district	
1. *Aconitum angulatum* Tamura (Ranunculaceae)	Near Deoma, 3900 m
2. *Acronema cryptosciadeum* Farille & Lachard (Apiaceae)	Jaljale Himal, Khola Pokhari, 4070 m
3. *Anemone fuscopurpurea* H. Hara (Ranunculaceae)	Banduke Pokhari – Saju Pokhari, 4200-4000 m
4. *Bistorta diopetes* H. Ohba & S. Akiyama (Polygonaceae)	Banduke, Jaljale Himal, 4150 m
5. *Bistorta milletioides* H. Ohba & S. Akiyama (Polygonaceae)	Tinpokhari – Banduke, Jaljale Himal, 4150 m
6. *Carex himalaica* T. Koyama (Cyperaceae)	Arun valley, Kasuwa khola, N. of Num, 3657.6m
7. *Impatiens arunensis* Grey-Wilson (Balsaminaceae)	Arun valley, Upper Kashwa Khola, above Hedangna, 2800 m

Endemic flowering plants	Locality
8. *Kuepferia chateri* (T. N. Ho) Adr.Favre (Gentianaceae) *Gentiana chateri* T. N. Ho	Kasuwa Khola, 3962.4m
9. *Microtoena nepalensis* Stearn (Lamiaceae)	Tinjure Danda, 2286m
10. *Pedicularis gruiflora* T. Yamaz. (Orobanchaceae)	Around Cha Ding Kharka, 4500 m
11. *Pedicularis koshiensis* T. Yamaz. (Orobanchaceae)	Cha Ding Kharka, 4500 m
12. *Potentilla makaluensis* H. Ikeda & H. Ohba (Rosaceae)	Shipton Pass, 4120 m
13. *Ranunculus makaluensis* Kadota (Ranunculaceae)	Merek, 4340 m
14. *Salix staintoniana* Skvortsov (Salicaceae)	Arun valley, Barun Khola, N. of Num, 3657.6m
15. *Solms-laubachia nepalensis* (H. Hara) J. P. Yue, Al-Shehbaz & H. Sun (Brassicaceae) *Desideria nepalensis* H. Hara	Barun valley, 5394.96m
16. *Swertia barunensis* P. Chassot (Gentianaceae)	Makalu Barun National park, Shipton pass, c. 4200 m
17. *Synotis brunneo-villosa* (Kitam.) C. Jeffrey & Y. L. Chen (Asteraceae) *Senecio brunneo-villosus* Kitam.	Chyamtang, 2590.8m
Dhankhuta district	
1. *Didymocarpus nepalensis* Bh. Adhikari & Mich. Moeller (Gesneriaceae)	Pakhribas Municipality, Bokre, 1829 m
2. *Isodon dhankutanus* Murata (Lamiaceae)	Dhankuta, 1200 m
3. *Malaxis tamurensis* Tuyama (Orchidaceae)	Dhankuta, 1200 m
Sunsari district	
1. *Begonia tribenensis* C. R. Rao (Begoniaceae)	Barakshetra – Tribeni, ca. 130 m
2. *Jasminum amabile* H. Hara (Oleaceae)	Sanguri Danda, near Dharan, 762m
Taplejung district	
1. *Aconitum staintonii* Lauener (Ranunculaceae)	Walungchunggola, Tamur valley, 3505.2m
2. *Cortia staintoniana* Farille & S. B. Malla (Apiaceae)	Topke Gola, Arun-Tamur watershed, 3962.4m
3. *Cortiella lamondiana* Fullarton & M. F. Watson (Apiaceae)	Kambachen – Lhonak, 4200 m
4. *Eriocaulon trisectoides* Satake (Eriocaulaceae)	Mul Pokhari – Dumhan, 2100-700 m
5. *Pedicularis cornigera* T. Yamaz. (Orobanchaceae)	Lamni Nama, 4000 m
6. *Pedicularis oxyrhyncha* T. Yamaz. (Orobanchaceae)	Tasagon – Topke Gola, 4100 m
7. *Pedicularis tamurensis* T. Yamaz. (Orobanchaceae)	Mewa Khola, Tamur valley, 3352.8m
8. *Pedicularis terrenoflora* T. Yamaz. (Orobanchaceae)	Shewaden – Mewa Khola, 2200 m
9. *Prunus taplejungnica* H. Ohba & S. Akiyama (Rosaceae)	Chairam – Dorongden, 3520 m
10. *Prunus topkegolensis* H. Ohba & S. Akiyama (Rosaceae)	Topke Gola, 3700 m
11. *Saxifraga jaljalensis* H. Ohba & S. Akiyama (Saxifragaceae)	Shuwan Kharka – Topke Gola, Jaljale Himal, 4300 m
Ilam district	
1. *Habenaria sandiegoensis* Raskoti (Orchidaceae)	Kutidanda, 1600 m
2. *Ophiorrhiza nepalensis* Deb & Mondal (Rubiaceae)	Soktim Tea Estate, 450 m
Jhapa district	
1. *Eriocaulon exsertum* Satake (Eriocaulaceae)	Ghorwa – Sanichare, 300-200 m

Endemic flowering plants	Locality
2. *Eriocaulon obclavatum* Satake (Eriocaulaceae)	Ghorwa – Sanichare, 300-200 m
3. *Salix plectilis* Kimura (Salicaceae)	Mahara Bahara – Kathgara, 200 m

Endemic flowering plants of Nepal in different herbaria

32 herbaria in Nepal and abroad preserve the herbarium specimens of Nepalese endemic flowering plants (Table 9, 10). Among them herbaria having more than 50 type specimens of the Nepalese endemic flowering plants are Natural History Museum (BM) with 154 species, Royal Botanic Garden Edinburgh (E) with 85 species, University of Tokyo (TI) with 74 species and National Herbarium of Nepal (KATH) with 60 species. Several herbaria (BLAT, F, GB, HBG, JE, MAK, MSB, NEU, NY, PE, UPS) have one type specimen of endemic flowering plants of Nepal (Table 9, 10).

Table 9. Herbaria with number of endemic flowering plant species of Nepal.

Acronym of herbarium	Name of herbarium	Country	No. of species
A	Harvard University, Cambridge	U. S. A.	8
B	Botanischer Garten, Berlin	Germany	3
BLAT	St. Xavier's College, Mumbai	India	1
BM	Natural History Museum, London	U. K.	154
CAL	Botanical Survey of India, Howrah	India	15
E	Royal Botanic Garden Edinburgh, Scotland	U. K.	85
F	Field Museum of Natural History, Chicago	U. S. A.	1
G	Conservatoire et Jardin Botaniques, Geneve	Switzerland	12
GB	University of Gothenburg, Gogeborg	Sweden	1
GH	Harvard University, Cambridge	U. S. A.	2
HBG	University of Hamburg, Hamburg	Germany	1
JE	Friedrich-Schiller-Universitat, Jena	Germany	1
K	Royal Botanic Garden Kew, Kew	U. K.	27
KATH	National Herbarium and Plant Laboratories, Lalitpur	Nepal	60
KYO	Kyoto University, Kyoto	Japan	20
L	National Herbarium Nederland, Leiden	Netherland	2
LE	Komarov Botanical Institute, Saint Petersburg	Russia	2
M	Botanische Staatssammlung Munchen, Munchen	Germany	2
MAK	Tokyo Metropolitan University, Tokyo	Japan	1
MO	Missouri Botanical Garden, Missouri	U. S. A.	2
MSB	Ludwig-Maxmillians. Universitat, Munchen	Germany	1
MW	Moscow State University, Moscow	Russia	5
NEU	Universite de Neuchatel, Neuchatel	Switzerland	1
NY	New York Botanical Garden, Bronx	U. S. A.	1
P	Museum National d'Histoire Naturelle, Paris	France	12
PE	Chinese Academy of Sciences, Beijing	China	1
TI	University of Tokyo, Tokyo	Japan	74
TNS	Natural Museum of Nature and Science, Tsukuba	Japan	3
TUCH	Tribhuvan University, Kathmandu	Nepal	2
TUS	Tohoku University, Sendai	Japan	4
UPS	Museum of Evolution, Uppsala	Sweden	1
US	Smithsonian Institute, Washington	U. S. A.	2

Table 10. Endemic flowering plants of Nepal preserved in different herbaria (includes holotype, isotype, lectotype, syntype and paratype)

A (Harvard University, Cambridge, U.S.A)

BRASSICACEAE (CRUCIFERAE)
Draba poluniniana Al-Shehbaz (A, isotype)
Draba staintonii Jafri ex H. Hara (A, isotype)
Lepidostemon williamsii (H. Hara) Al-Shehbaz
Draba williamsii H. Hara (A, isotype)
Noccaea nepalensis Al-Shehbaz (A, isotype)
Solms-laubachia haranensis (Al-Shehbaz) J. P. Yue, Al-Shehbaz & H. Sun
Ermaniopsis pumila H. Hara (A, isotype)

RANUNCULACEAE
Clematis phlebantha L. H. J. Williams (A, isotype)

ROSACEAE
Potentilla makaluensis H. Ikeda & H. Ohba (A, isotype)

SAXIFRAGACEAE
Saxifraga ganeshii H. Ohba & S. Akiyama (A, isotype)

B (Botanischer Garten und Botanisches Museum Berlin-Dahlem, Zentraleinrichtung der Freien Universitaet Berlin, Berlin, Germany)

APIACEAE (UMBELLIFERAE)
Lalldhwojia pastinacifolia Pimenov & Kljuykov (B, isotype)

CAMPANULACEAE
Codonopsis reflexa D. Y. Hong (B, paratype)

POACEAE (GRAMINEAE)
Festuca eriobasis H. Scholz (B, holotype)

BLAT (St. Xavier's College, Mumbai, Maharashtra, India)

BEGONIACEAE
Begonia tribenensis C. R. Rao (BLAT, holotype)

BM (Natural History Museum, London, U.K.)

ACANTHACEAE
Justicia tukuchensisV. A. W. Graham (BM, holotype)
Strobilanthes saccata J. R. I. Wood (BM, holotype)

AMARYLLIDACEAE
Allium hypsistum Stearn (BM, holotype)

APIACEAE (UMBELLIFERAE)
Acronema dyssimetriradiata Farille (BM, isotype)
Acronema refugicolum Farille & Lachard (BM, holotype)
Cortia staintoniana Farille & S. B. Malla (BM, isotype)
Keraymonia nipaulensis (BM, paratype)
Lalldhwojia staintonii Farille (BM, isotype)
Pimpinella kawalekhensis Farille & Lachard (BM, isotype)
Sinocarum normanianum (Cauwet-Marc & Farille) Farille
Similisinocarum normanianum Cauwet-Marc & Farille (BM, holotype)
Sinocarum staintonianum P. K. Mukh. ex Farille & Lachard (BM, holotype)
Tetrataenium lallii (C. Norman) Cauwet-Marc, Carb. & M. Farille
Heracleum lallii C. Norman (BM, isotype)
Vicatia nepalensis (BM, holotype)

APOCYNACEAE
Ceropegia meleagris H. Huber (BM, holotype)
Ceropegia nepalensis (Radcl.-Sm.) Bruyns (BM, holotype)
Riocreuxia nepalensis Radcl.-Sm. (BM, holotype)

ASPARAGACEAE
Asparagus penicillatus H. Hara (BM**,** holotype)

ASTERACEAE (COMPOSITAE)
Cicerbita nepalensis Kitam. (BM**,** holotype)
Cirsium flavisquamatum Kitam. (BM**,** holotype)
Saussurea chrysotricha Ludlow (BM**,** holotype)
Saussurea dhwojii Kitam. (BM**,** holotype)
Saussurea platyphyllaria Ludlow (BM**,** holotype)
Synotis brunneovillosa (Kitam.) C. Jeffrey & Y. L. Chen
Senecio brunneo-villosus Kitam. (BM**,** holotype)
Synotis panduriformis (Kitam.) C. Jeffrey & Y. L. Chen
Senecio panduriformis Kitam. (BM**,** holotype)
Taraxacum amabile Soest (BM**,** holotype)
Taraxacum nepalense Soest (BM**,** holotype)

BALSAMINACEAE
Impatiens williamsii H. Hara (BM**,** holotype)

BERBERIDACEAE
Berberis mucrifolia Ahrendt (BM**,** holotype)

BORAGINACEAE
Onosma bheriense H. Hara (BM**,** holotype)

BRASSICACEAE (CRUCIFERAE)
Aphragmus nepalensis (H. Hara) Al-Shehbaz
Staintoniella nepalensis H. Hara (BM**,** holotype)
Draba poluniniana Al-Shehbaz (BM**,** isotype)

Draba staintonii Jafri ex H. Hara (BM**,** holotype)
Lepidostemon williamsii (H. Hara) Al-Shehbaz
Draba williamsii H. Hara (BM**,** holotype)
Noccaea nepalensis Al-Shehbaz (BM**,** holotype)
Solms-laubachia haranensis (Al-Shehbaz) J. P. Yue, Al-Shehbaz & H. Sun
Ermaniopsis pumila H. Hara (BM**,** holotype)
Solms-laubachia nepalensis (H. Hara) J. P. Yue, Al-Shehbaz & H. Sun
Desideria nepalensis H. Hara (BM**,** holotype)

CAMPANULACEAE
Codonopsis reflexa D. Y. Hong (BM**,** isotype)
Cyananthus hayanus C. Marquand (BM**,** holotype)
Cyananthus himalaicus K. K. Shrestha (BM**,** holotype)

CARYOPHYLLACEAE
Arenaria globiflora (Fenzl) Edgew. & Hook. f.
Cherleria grandiflora D. Don (BM**,** syntype)
Odontostemma paramelanandrum (H. Hara) Rabeler & W. L. Wagner
Arenaria paramelanandra H.Hara (BM**,** holotype)
Silene fissicalyx Bocquet & Chater (BM**,** holotype)
Silene helleboriflora Exell & Bocquet (BM**,** holotype)
Silene stellariifolia Bocquet & Chater (BM**,** holotype)
Silene vautierae Bocquet (BM**,** holotype)

CRASSULACEAE
Rhodiola nepalica (H. Ohba) H. Ohba
Sedum nepalicum H. Ohba (BM**,** holotype)

CYPERACEAE
Carex gandakiensis Katsuyama (BM**,** isotype)
Carex himalaica T. Koyama (BM**,** holotype)
Carex mallae (Rajbh. & H. Ohba) O. Yano (BM, paratype)
Cyperus trisulcus D. Don (BM**,** syntype)
Cyperus wallichianus Spreng.
Cyperus pulcher D. Don (BM**,** syntype)

ERICACEAE
Rhododendron cowanianum Davidian (BM**,** holotype)
Rhododendron lowndesii Davidian (BM**,** holotype)

FABACEAE (LEGUMINOSAE)
Astragalus chateri Vassilcz. (BM**,** holotype)
Astragalus pseudorigidulus Podlech (BM**,** isotype)
Hedysarum manaslense (Kitam.) H. Ohashi
Hedysarum nepalense H. Ohashi var. *subhirtellum* H. Ohashi (BM**,** holotype)
Oxytropis arenae-ripariae Vass. (BM**,** holotype)
Oxytropis fasciculiflorum Vass. (BM**,** holotype)
Oxytropis graminetorum Vass. (BM**,** holotype)

Oxytropis morenarum Vass. (BM**,** holotype)
Oxytropis nepalensis Vass. (BM**,** holotype)
Oxytropis torrentium Vass. (BM**,** holotype)
Oxytropis williamsii Vass. (BM**,** holotype)
Rhynchosia nepalensis H. Ohashi & Tateishi (BM**,** holotype)

GENTIANACEAE
Gentiana sagarmathae Miyam. & H. Ohba (BM**,** isotype)
Gentianella lowndesii Harry Sm. (BM**,** holotype)
Kuepferia chateri (T. N. Ho) Adr.Favre (BM**,** holotype)
Swertia acaulis Harry Sm. (BM**,** holotype)
Swertia nepalensis J. Shah (BM**,** isotype)

HYPERICACEAE
Hypericum cordifolium Choisy (BM**,** syntype)
Hypericum bracteatum Buch.-Ham. ex D. Don (BM**,** lectotype)

IRIDACEAE
Iris staintonii H. Hara (BM**,** holotype)

LAMIACEAE (LABIATAE)
Discretitheca nepalensis (Moldenke) P. D. Cantino
Caryopteris nepalensis Moldenke (BM**,** holotype)
Caryopteris nepalensis Moldenke var. *parvifolia* Moldenke (BM**,** holotype)
Eriophyton nepalense (Hedge) Ryding O
Lamium nepalense Hedge (BM**,** holotype)
Eriophyton staintonii (Hedge) Ryding O
Lamium staintonii Hedge (BM**,** holotype)
Isodon dhankutanus Murata (BM**,** isotype)
Microtoena nepalensis Stearn (BM**,** holotype)
Nepeta staintonii Hedge (BM**,** holotype)

OLEACEAE
Jasminum amabile H. Hara (BM**,** holotype)

ONAGRACEAE
Epilobium brevisquamatum Raven (BM**,** holotype)
Epilobium staintonii P. H. Raven (BM**,** holotype)

ORCHIDACEAE
Oreorchis porphyranthes Tuyama (BM**,** holotype)
Pleione coronaria P. J. Cribb & C. Z. Tang (BM**,** holotype)

OROBANCHACEAE
Euphrasia nepalensis Pugsley (BM, holotype)
Pedicularis annapurnensis T. Yamaz. (BM, holotype)
Pedicularis anserantha T. Yamaz.
Pedicularis ingentoides T. Yamaz. (BM, holotype)

Pedicularis chamissonoides T. Yamaz. (BM, holotype)
Pedicularis oxyrhyncha T. Yamaz. (BM, paratype)
Pedicularis pseudoregeliana P. C. Tsoong (BM, holotype)
Pedicularis tamurensis T. Yamaz. (BM, holotype)
Pedicularis terrenoflora T. Yamaz. (BM, Isotype)
Pedicularis yamazakiana R. R. Mill (BM, Isotype)

PAPAVERACEAE
Corydalis calycina Liden (BM, holotype)
Corydalis clavibracteata Ludlow (BM, holotype)
Corydalis megacalyx Ludlow (BM, holotype)
Corydalis simplex Liden (BM, holotype)
Corydalis stipulata Liden (BM, holotype)
Corydalis terracina Liden (BM, holotype)
Corydalis uncinata Liden (BM, holotype)
Corydalis uncinatella Liden (BM, holotype)
Papaver autumnale (P. A. Egan) Christenh. & Byng
Meconopsis autumnalis P. A. Egan (BM, paratype)
Papaver chankheliense (Grey-Wilson) Christenh. & Byng
Meconopsis chankheliensis Grey-Wilson (BM, holotype)
Papaver ganeshense (Grey-Wilson) Christenh. & Byng
Meconopsis ganeshensis Grey-Wilson (BM, holotype)
Papaver regium (G. Taylor) Christenh. & Byng
Meconopsis regia G. Taylor (BM, holotype)
Papaver simikotense (Grey-Wilson) Christenh. & Byng
Meconopsis simikotensis Grey-Wilson (BM, holotype)
Papaver staintonii (Grey-Wilson) Christenh. & Byng
Meconopsis staintonii Grey-Wilson (BM, holotype)
Papaver taylorii (L. H. J. Williams) Christenh. & Byng
Meconopsis taylorii L. H. J. Williams (BM**,** holotype)

PLANTAGINACEAE
Lagotis nepalensis T. Yamaz. (BM**,** holotype)
Veronica emodi T. Yamaz. (BM**,** holotype)

POACEAE (GRAMINEAE)
Achnatherum staintonii (Bor) M. Nobis & P. D. Gudkova
Stipa staintonii Bor (BM**,** isotype)
Elymus nepalensis (Melderis) Melderis
Agropyron nepalense Melderis (BM**,** holotype)
Festuca nepalica E. B. Alexeev (BM**,** isotype)
Festuca poluninii E. B. Alexeev (BM**,** holotype)
Saccharum williamsii (Bor) Bor ex Cope
Erianthus williamsii Bor (BM**,** holotype)

POLYGONACEAE
Eskemukerjea megacarpum H. Hara (BM**,** holotype)

Fallopia filipes (H. Hara) Holub
Bilderdykia filipes H. Hara (BM**,** holotype)

PRIMULACEAE
Primula poluninii Fletcher (BM**,** holotype)
Primula ramzanae Fletcher (BM**,** holotype)
Primula sharmae Fletcher (BM**,** isotype)
Primula wigramiana W. W. Sm. (BM**,** isotype)

RANUNCULACEAE
Aconitum amplexicaule Lauener (BM**,** holotype)
Aconitum bhedingense Lauener (BM**,** isotype)
Aconitum dhwojii Lauener (BM**,** holotype)
Aconitum poluninii Lauener (BM**,** holotype)
Aconitum staintonii Lauener (BM**,** holotype)
Aconitum williamsii Lauener (BM**,** holotype)
Clematis phlebantha L. H. J. Williams (BM**,** holotype)
Delphinium williamsii Munz (BM**,** holotype)
Oxygraphis nepalensis Tamura (BM**,** holotype)
Ranunculus himalaicus Tamura (BM**,** holotype)

ROSACEAE
Potentilla makaluensis H. Ikeda & H. Ohba (BM**,** isotype)
Prunus jajarkotensis H. Hara (BM**,** holotype)
Sibbaldia emodi H. Ikeda & H. Ohba (BM, isotype)

RUBIACEAE
Galium nepalense Ehrend. & Schoenb.-Tem. (BM**,** holotype)
Galium saipalense Ehrend. & Schoenb.-Tem. (BM**,** holotype)
Ophiorrhiza nepalensis Deb & Mondal (BM**,** holotype)

SALICACEAE
Salix staintoniana Skvortsov (BM**,** holotype)

SAXIFRAGACEAE
Saxifraga alpigena Harry Sm. (BM**,** holotype)
Saxifraga cinerea Harry Sm.(BM**,** holotype)
Saxifraga excellens Harry Sm. (BM**,** holotype)
Saxifraga hypostoma Harry Sm. (BM**,** holotype)
Saxifraga lowndesii Harry Sm. (BM**,** holotype)
Saxifraga micans Harry Sm. (BM**,** holotype)
Saxifraga mira Harry Sm. (BM**,** holotype)
Saxifraga namdoensis Harry Sm. (BM**,** holotype)
Saxifraga neopropagulifera H. Hara (BM, holotype)
Saxifraga poluniniana Harry Sm. (BM**,** holotype)
Saxifraga rhodopetala Harry Sm. (BM**,** holotype)
Saxifraga roylei Harry Sm. (BM**,** holotype)
Saxifraga staintonii Harry Sm. (BM**,** holotype)

Saxifraga williamsii Harry Sm. (BM**,** holotype)
Saxifraga zimmermannii Baehni (BM**,** isotype)

SCROPHULARIACEAE
Scrophularia bheriensis T. Yamaz. (BM**,** holotype)
Scrophularia laportiifolia T. Yamaz. (BM**,** holotype)

ZINGIBERACEAE
Roscoea nepalensis Cowley (BM**,** holotype)
Roscoea tumjensis Cowley (BM**,** holotype)

CAL (Botanical Survey of India, Howrah, West Bengal, India)

APIACEAE (UMBELLIFERAE)
Acronema bryophilum Farille & Lachard (CAL, isotype)
Acronema cryptosciadeum Farille & Lachard (CAL, isotype)
Acronema johrianum Babu (CAL, holotype)
Acronema mukherjeeanum Farille & Lachard (CAL, isotype)
Acronema phaeosciadeum Farille & Lachard (CAL, isotype)
Acronema pneumatophobium Farille & Lachard (CAL, isotype)

BRASSICACEAE (CRUCIFERAE)
Draba staintonii Jafri ex H. Hara (CAL, isotype)

CARYOPHYLLACEAE
Eremogone mukerjeeana (Majumdar) Rabeler & W. L. Wagner
Stellaria mukerjeeana Majumdar (CAL, holotype)

ORCHIDACEAE
Neottia nepalensis (N. P. Balakr.) Szlach.
Listera nepalensis N. P. Balakr. (CAL, holotype)

POACEAE (GRAMINEAE)
Saccharum williamsii (Bor) Bor ex Cope
Erianthus williamsii Bor (CAL, isotype)

POLYGONACEAE
Eskemukerjea megacarpum (H. Hara) H. Hara
Eskemukerjea nepalensis Malick & Sengupta (CAL, holotype)

SAXIFRAGACEAE
Saxifraga excellens Harry Sm. (CAL, isotype)
Saxifraga micans Harry Sm. (CAL, isotype)
Saxifraga poluniniana Harry Sm. (CAL, isotype)
Saxifraga williamsii Harry Sm. (CAL, isotype)

E (Royal Botanic Garden, London, U.K.)

ACANTHACEAE
Thunbergia kasajuana Bh. Adhikari & J. R. I. Wood (E, isotype).
Thunbergia nepalensis Bh. Adhikari & J. R. I. Wood (E, holotype)

APIACEAE (UMBELLIFERAE)
Acronema dyssimetriradiata Farille (E, isotype)
Chamaesium shrestaeanum Farille (E, isotype)
Cortia staintoniana Farille & S. B. Malla (E, holotype)
Cortiella lamondiana Fullarton & M. F. Watson(E, holotype)
Keraymonia nipaulensis Cauwet-Marc & Farille (E, isotype)
Lalldhwojia staintonii Farille (E, isotype)
Pimpinella inundata (Farille & S. B. Malla) P. K. Mukh. & Constance
Ligusticum inundatum Farille & S. B. Malla (E, isotype)
Sinocarum normanianum (Cauwet-Marc & Farille) Farille
Similisinocarum normanianum Cauwet-Marc & Farille (E, isotype)
Tetrataenium lallii (C. Norman) Cauwet-Marc, Carb. & Farille
Heracleum lallii C. Norman (E, holotype)

APOCYNACEAE
Ceropegia meleagris H. Huber (E, isotype)

ASTERACEAE (COMPOSITAE)
Saussurea chrysotricha Ludlow (E, isotype)
Saussurea kanaii K. Fujikawa & H. Ohba (E, isotype)
Saussurea platyphyllaria Ludlow (E, isotype)

BALSAMINACEAE
Impatiens arunensis Grey-Wilson (E, isotype)

BEGONIACEAE
Begonia nuwakotensis S. Rajbh. (E, holotype)
Begonia taligera S. Rajbh. (E, holotype)

BERBERIDACEAE
Berberis mucrifolia Ahrendt (E, isotype)
Berberis pendryi Bh. Adhikari (E, holotype)

BORAGINACEAE
Maharanga verruculosa (I. M. Johnst.) I. M. Johnst. (E, holotype)

BRASSICACEAE (CRUCIFERAE)
Aphragmus nepalensis (H. Hara) Al-Shehbaz
Staintoniella nepalensis H. Hara (E, isotype)
Draba macbeathiana Al-Shehbaz (E, holotype)
Draba staintonii Jafri ex H. Hara (E, isotype)
Lepidostemon williamsii (H. Hara) Al-Shehbaz
Draba williamsii H. Hara (E, isotype)
Noccaea nepalensis Al-Shehbaz (E, isotype)

Sisymbrium nepalense Al-Shehbaz (E, holotype)
Solms-laubachia haranensis (Al-Shehbaz) J. P. Yue, Al-Shehbaz & H. Sun
Ermaniopsis pumila H. Hara (E, isotype)

CAMPANULACEAE
Cyananthus hayanus C. Marquand (E, isotype)
Cyananthus himalaicus K. K. Shrestha (E, isotype)

CRASSULACEAE
Rhodiola nepalica (H. Ohba) H. Ohba
Sedum nepalicum H. Ohba (E, isotype)

CYPERACEAE
Carex himalaica T. Koyama (E, isotype)

ERICACEAE
Rhododendron cowanianum Davidian (E, isotype)
Rhododendron lowndesii Davidian (E, isotype)

FABACEAE (LEGUMINOSAE)
Astragalus poluninii Podlech (E, holotype)

GENTIANACEAE
Gentiana radicans Harry Sm. (E, holotype)
Swertia acaulis Harry Sm. (E, isotype)
Swertia nepalensis J. Shah (E, holotype)

GESNERIACEAE
Didymocarpus nepalensis Bh. Adhikari & Mich. Moeller (E, holotype).

LAMIACEAE (LABIATAE)
Discretitheca nepalensis (Moldenke) P. D. Cantino
*Caryopteris nepalensis*Moldenke (E, isotype)
Eriophyton nepalense (Hedge) Ryding O
Lamium nepalense Hedge (E, isotype)
Eriophyton staintonii (Hedge) Ryding O
Lamium staintonii Hedge (E, isotype)
Nepeta staintonii Hedge (E, isotype)

OROBANCHACEAE
Pedicularis annapurnensis T. Yamaz. (E, isotype)
Pedicularis yamazakiana R. R. Mill (E, holotype)

PAPAVERACEAE
Corydalis clavibracteata Ludlow (E, isotype)
Corydalis stipulata Liden (E, isotype)
Corydalis uncinatella Liden (E, isotype)
Papaver autumnale (P. A. Egan) Christenh. & Byng

Meconopsis autumnalis P. A. Egan (E, holotype)
Papaver ganeshense (Grey-Wilson) Christenh. & Byng
Meconopsis ganeshensis Grey-Wilson (E, paratype)
Papaver manasluensis (P. A. Egan)Christenh. & Byng
Meconopsis manasluensis P. A. Egan (E, holotype)
Papaver staintonii (Grey-Wilson) Christenh. & Byng
Meconopsis staintonii Grey-Wilson (E, holotype)

PLANTAGINACEAE
Veronica emodi T. Yamaz. (E, isotype)

POACEAE (GRAMINEAE)
Bambusa nepalensis Stapleton (E, holotype)
Festuca nepalica E. B. Alexeev (E, isotype)
Himalayacalamus asper Stapleton (E, holotype)
Himalayacalamus cupreus Stapleton (E, holotype)
Himalayacalamus fimbriatus Stapleton (E, holotype)
Himalayacalamus porcatus Stapleton (E, holotype)
Thamnocalamus chigar (Stapleton) Stapleton
Borinda chigar Stapleton (E, holotype)

PRIMULACEAE
Primula poluninii Fletcher (E, isotype)
Primula ramzanae Fletcher (E, isotype)
Primula sharmae Fletcher (E, holotype)
Primula wigramiana W. W. Sm. (E, holotype)

RANUNCULACEAE
Aconitum amplexicaule Lauener (E, isotype)
Aconitum bhedingense Lauener (E, holotype)
Aconitum dhwojii Launer(E, isotype)
Aconitum poluninii Lauener (E, isotype)
Aconitum staintonii Lauener (E, isotype)
Aconitum williamsii Lauener (E, isotype)
Anemone fuscopurpurea H. Hara (E, isotype)
Clematis phlebantha L. H. J. Williams (E, isotype)

ROSACEAE
Potentilla makaluensis H. Ikeda & H. Ohba (E, isotype)
Sibbaldia emodi H. Ikeda & H. Ohba (E, isotype)
Sorbus sharmae M. F. Watson, V. Manandhar & Rushforth (E, holotype)

RUBIACEAE
Galium nepalense Ehrend. & Schoenb.-Tem. (E, isotype)

SAXIFRAGACEAE
Saxifraga excellens Harry Sm. (E, syntype)
Saxifraga ganeshii H. Ohba & S. Akiyama (E, isotype)

Saxifraga micans Harry Sm. (E, isotype)
Saxifraga mira Harry Sm. (E, isotype)
Saxifraga poluniniana Harry Sm. (E, isotype)
Saxifraga rhodopetala Harry Sm. (E, isotype)
Saxifraga williamsii Harry Sm. (E, isotype)

ZINGIBERACEAE
Roscoea nepalensis Cowley (E, isotype)
Roscoea tumjensis Cowley (E, isotype)

F (Field Museum of Natural History, Chicago, Illinois, U.S.A.)

HYPERICACEAE
Hypericum cordifolium Choisy (F, syntype)

G (Conservatoire et Jardin botaniques de la Ville de Geneve, Geneve, Switzerland)

APIACEAE
Acronema bryophilum Farille & Lachard (G, isotype)
Acronema cryptosciadeum Farille & Lachard (G, isotype)
Acronema mukherjeeanum Farille & Lachard (G, isotype)
Acronema phaeosciadeum Farille &Lachard (G, isotype)
Acronema pneumatophobium Farille & Lachard (G, isotype)
Keraymonia nipaulensis Cauwet-Marc & Farille (G, isotype)
Sinocarum staintonianum P. K. Mukh. ex Farille & Lachard (G, isotype)
Synclinostyles denisjordanii Farille & Lachard (G, isotype)

BRASSICACEAE (CRUCIFERAE)
Draba staintonii Jafri ex H. Hara (G, isotype)
Lepidostemon williamsii (H. Hara) Al-Shehbaz
Draba williamsii H. Hara (G, isotype)

CAMPANULACEAE
Codonopsis reflexa D. Y. Hong (G, isotype)

SAXIGRAGACEAE
Saxifraga zimmermannii Baehni (G, holotype)

GB (University of Gothenburg, Goteborg, Sweden)

PAPAVERACEAE
Corydalis spicata Liden (GB, holotype)

GH (Harvard University, Cambridge, U.S.A.)

CAMPANULACEAE
Cyananthus himalaicus K. K. Shrestha (GH, isotype)

ROSACEAE
Sibbaldia emodi H. Ikeda & H. Ohba (GH, isotype)

HBG (University of Hamburg, Hamburg, Germany)

BEGONIACEAE
Begonia tribenensis C. R. Rao (HBG, isotype)

JE (Friedrich-Schiller-Universitat, Jena, Germany)

ONAGRACEAE
Epilobium indicum Hausskn. (JE, lectotype)

K (Royal Botanic Garden Kew, Kew, England, U.K.)

ACANTHACEAE
Strobilanthes nutans (Nees) T. Anders.
Ruellia strobilina Wall. (K, syntype)

APOCYNACEAE
Ceropegia poluniniana Bruyns (K, holotype)

BALSAMINACEAE
Impatiens arunensis Grey-Wilson (K, holotype)
Impatiens gorepaniensis Grey-Wilson (K, holotype)

BRASSICACEAE (CRUCIFERAE)
Solms-laubachia haranensis (Al-Shehbaz) J. P. Yue, Al-Shehbaz& H. Sun
Ermaniopsis pumila H. Hara (K, isotype)

CAMPANULACEAE
Codonopsis bragaensis Grey-Wilson (K, holotype)

CARYOPHYLLACEAE
Arenaria globiflora (Fenzl) Edgew. & Hook. f.
Dolophragma globiflorum Fenzl (K, syntype)
Silene greywilsonii Rajbh. & Mitsuo Suzuki (K, holotype)

CUCURBITACEAE
Gomphogyne nepalensis W. J. de Wilde & Duyfjes (K, holotype)

ELAEAGNACEAE
Elaeagnus tricholepis Momiyama (K, isotype)

FABACEAE (LEGUMINOSAE)
Astragalus barclayanus Podlech (K, holotype)
Millettia nepalensis R. N. Parker (K, holotype)

HYPERICACEAE
Hypericum cordifolium Choisy (K, syntype)

LAURACEAE
Machilus pubescens Blume (K, isotype)

ORCHIDACEAE
Habenaria wallichii (Kolan., Kras & Szlach.) J. M. H. Shaw
Habenella wallichii Kolan., Kras & Szlach. (K, holotype)

POACEAE (GRAMINEAE)
Achnatherum staintonii (Bor) M. Nobis & P. D. Gudkova
Stipa staintonii Bor (K, holotype)
Bambusa nepalensis (K, paratype)
Festuca nepalica E. B. Alexeev (K, holotype)
Festuca poluninii E. B. Alexeev (K, isotype)
Himalayacalamus asper (K, paratype)
Himalayacalamus fimbriatus (K, paratype)
Himalayacalamus planatus Stapleton (K, holotype)
Himalayacalamus porcatus (K, paratype)
Thamnocalamus chigar (K, paratype)

RANUNCULACEAE
Clematis phlebantha L. H. J. Williams (K, isotype)

ZINGIBERACEAE
Roscoea ganeshensis Cowley & W. J. Baker (K, holotype)
Roscoea nepalensis Cowley (K, isotype)

KATH (National herbarium and Plant Laboratories, Godavari, Lalitpur, Nepal)
(*holotype not found in KATH)

ACANTHACEAE
Justicia tukuchensis V. A. W. Graham (KATH, isotype)
Thunbergia kasajuana Bh. Adhikari & J. R. I. Wood (KATH, holotype)
Thunbergia nepalensis Bh. Adhikari & J. R. I. Wood (KATH, isotype)

APIACEAE
Acronema bryophilum Farille & Lachard (*KATH, holotype)
Acronema cryptosciadeum Farille & Lachard (*KATH, holotype)
Acronema dyssimetriradiata Farille (*KATH, holotype)
Acronema mukherjeeanum Farille & Lachard (*KATH, holotype)
Acronema phaeosciadeum Farille & Lachard (*KATH, holotype)
Acronema pneumatophobium Farille & Lachard (*KATH, holotype)
Chamaesium shrestaeanum Farille (*KATH, holotype)
Keraymonia nipaulensis Cauwet-Marc & Farille (*KATH, holotype)
Lalldhwojia pastinacifolia Pimenov & Kljuykov (KATH, isotype)
Lalldhwojia staintonii Farille (*KATH, holotype)

Oreocome depauperata Pimenov & Kljuykov (*KATH, holotype)
Oreocome involucellata Pimenov & Kljuykov (KATH, isotype)
Pimpinella acronemastrum Farille & Lachard (*KATH, holotype)
Pimpinella inundata (Farille & S. B. Malla) P. K. Mukh. & Constance
Ligusticum inundatum Farille & S. B. Malla (*KATH, holotype**)**
Rohmooa kirmzii Farille & Lachard (*KATH, holotype**)**
Sinocarum staintonianum P. K. Mukh. ex Farille & Lachard (KATH, isotype)
Synclinostyles exadversum Farille & Lachard (*KATH, holotype)
Vicatia nepalensis Kljuykov (KATH, isotype)

ASTERACEAE (COMPOSITAE)
Artemisia mustangensis Yonek. (KATH, isotype)
Artemisia nepalica Yonek. (KATH, paratype)
Saussurea ramchaudharyi S. K. Ghimire & H.K.Rana (KATH, isotype)
Synotis managensis S. Joshi, Kanti Shrestha & D. Bajracharya (KATH, holotype)

BEGONIACEAE
Begonia flagellaris H. Hara (KATH, isotype)

BERBERIDACEAE
Berberis pendryi Bh. Adhikari (KATH, isotype)

BRASSICACEAE (CRUCIFERAE)
Draba poluniniana Al-Shehbaz (KATH, isotype)
Solms-laubachia haranensis (Al-Shehbaz) J. P. Yue, Al-Shehbaz & H. Sun
Ermaniopsis pumila H. Hara (KATH, isotype)

CARYOPHYLLACEAE
Eremogone mukerjeeana (Majumdar) Rabeler & W. L. Wagner
Stellaria mukerjeeana Majumdar (KATH, isotype)
Silene davidlongii Rajbh. & Mitsuo Suzuki (KATH, isotype)
Silene hideaki-ohbae Rajbh. & Mitsuo Suzuki (KATH, isotype)

CYPERACEAE
Carex esbirajbhandarii (Rajbh. & H. Ohba) O. Yano
Kobresia esbirajbhandarii Rajbh. & H.Ohba (KATH, isotype)
Carex mallae (Rajbh. & H. Ohba) O. Yano
Kobresia mallae Rajbh. & H. Ohba (KATH, isotype)

GENTIANACEAE
Gentianella lowndesii Harry Sm. (KATH, isotype)

GESNERIACEAE
Didymocarpus nepalensis Bh. Adhikari & Mich. Moeller (KATH, isotype).

IRIDACEAE
Iris staintonii H. Hara (KATH, isotype**)**

LAMIACEAE (LABIATAE)
Isodon dhankutanus Murata (KATH, isotype**)**
Salvia transhimalaica Yonek. (paratype)

ORCHIDACEAE
Bulbophyllum nepalense Raskoti & Ale (KATH, holotype)
Calanthe himalaicum Raskoti (KATH, holotype)
Eria nepalensis D. M. Bajracharya & K. K. Shrestha (KATH, holotype)
Gastrochilus nepalensis B. B. Raskoti (KATH, holotype)
Habenaria palpensis Raskoti (KATH, holotype)
Habenaria sandiegoensis Raskoti (*KATH, holotype).
Herminium fimbriatum (Raskoti) X. H. Jin, Schuit., Raskoti & L. Q. Huang
Bhutanthera fimbriata Raskoti (KATH, holotype)
Herminium hongdeyuanii B. B. Raskoti (KATH, holotype)
Liparis langtangensis B. B. Raskoti & Ale (KATH, holotype)
Malaxis dolpensis M. R. Shrestha, L. R. Shakya et S. K. Ghimire (KATH, holotype)
Odontochilus nandae Raskoti & H. Kurzweil (KATH, holotype)
Pinalia annapurnensis (L. R. Shakya & M. R. Shrestha) Schuit., Y. P. Ng & H. A. Pedersen
Eria annapurnensis L. R. Shakya & M. R. Shrestha (KATH, holotype)
Pinalia baniae (Bajracharya, L. R. Shakya & Chettri) Schuit.
Eria baniaii Bajracharya, Shakya & Chettri (KATH, isotype)
Pinalia pokharensis (D. M. Bajracharya, A. Subedi & K. K. Shrestha) Schuit., Y. P. Ng & H. A. Pedersen
Eria pokharensis D. M. Bajracharya, A. Subedi & K. K. Shrestha (KATH, holotype)

OROBANCHACEAE
Pedicularis annapurnensis T. Yamaz. (KATH, isotype)
Pedicularis chamissonoides T. Yamaz. (KATH, isotype)
Pedicularis muguensis T. Yamaz. (KATH, paratype)
Pedicularis terrenoflora T. Yamaz. (KATH, isotype)

PAPAVERACEAE
Corydalis magacalyx Ludlow (KATH, isotype)
Corydalis spicata Liden (KATH, isotype)
Corydalis stipulata Liden (KATH, isotype)
Corydalis terracina Liden (KATH, isotype)
Papaver autumnale (P. A. Egan) Christenh. & Byng
Meconopsis autumnalis P. A. Egan (KATH, isotype)
Papaver chankheliense (Grey-Wilson) Christenh. & Byng
Meconopsischankheliensis Grey-Wilson (KATH, isotype)
Papaver manasluensis (P. A. Egan) Christenh. & Byng
Meconopsis manasluensis P. A. Egan (KATH, isotype)
Papaver staintonii (Grey-Wilson) Christenh. & Byng
Meconopsis staintonii Grey-Wilson (KATH, isotype)

POACEAE (GRAMINEAE)
Himalayacalamus porcatus Stapleton (KATH, isotype)
Poa hideaki-ohbae Rajbh. (KATH, holotype)

Poa muktinathensis Rajbh. (KATH, holotype)

POLYGONACEAE
Bistorta diopetes H. Ohba & S. Akiyama **(**KATH**,** isotype**)**
Bistorta milletioides H. Ohba & S. Akiyama **(**KATH, isotype**)**
Fallopia filipes (H. Hara) Holub
Bilderdykia filipes H. Hara (KATH, isotype)

RANUNCULACEAE
Aconitum tabatae Tamura (KATH, isotype)
Clematis phlebantha L. H. J. Williams (KATH, isotype)

ROSACEAE
Prunus taplejungnica H. Ohba & S. Akiyama (KATH, isotype)
Sorbus sharmae M. F. Watson, V. Manandhar & Rushforth (KATH, isotype)

KYO (Kyoto University, Kyoto, Japan)

ASTERACEAE (COMPOSITAE)
Artemisia nepalica (KYO, paratype)
Crepis himalaica Kitam. (KYO, holotype)
Leontopodium makianum Kitam. (KYO, holotype)

BORAGINACEAE
Arnebia nepalensis (Kitam.) H. Hara
Macrotomia nepalensis Kitam. (KYO, holotype)

CRASSULACEAE
Sedum pseudo-multicaule H. Ohba (KYO, holotype)

FABACEAE (LEGUMINOSAE)
Astragalus nakaoi Kitam. (KYO, holotype)
Hedysarum manaslense (Kitam.) H. Ohashi
Astragalus manaslensis Kitam. (KYO, holotype)

LAMIACEAE (LABIATAE)
Clinopodium nepalense (Kitam. & Murata) Braechler & Heubl
Micromeria nepalensis Kitam. & Murata (KYO, holotype)
Isodon dhankutanus Murata (KYO, isotype)
Isodon namikawanus Murata (KYO, holotype)
Isodon phulchokiensis H. W. Li
Rabdosia phulchokiensis Murata (KYO, isotype)

OROBANCHACEAE
Pedicularis anserantha T. Yamaz. (KYO, holotype**)**
Pedicularis breviscaposa T. Yamaz. (KYO, holotype)
Pedicularis muguensis T. Yamaz. (KYO, holotype)
Pedicularis odontoloma T. Yamaz. (KYO, holotype)

RANUNCULACEAE
Aconitum angulatum Tamura (KYO, holotype)
Aconitum tabatae Tamura (KYO, holotype)
Clematis bracteolata Tamura (KYO, holotype)
Delphinium unifolium Tamura (KYO, holotype)

ROSACEAE
Prunus himalaica Kitam. (KYO, holotype)

L (National Herbarium Nederland, Leiden Universiteit Branch, Leiden, Netherland)

HYPERICACEAE
Hypericum cordifolium Choisy (L, syntype)

LAURACEAE
Machilus pubescens Blume (L, holotype)

LE (Komarov Botanical Institute of RAS, Saint Petersburg, Russia)

APIACEAE (UMBELLIFERAE)
Sinocarum latifoliolatum Pimenov & Kljuykov (LE, isotype)
Sinocarum meeboldioides Pimenov & Kljuykov (LE, isotype)

M (Botanische Staatssammlung Munchen, Munchen, Germany)

FABACEAE (LEGUMINOSAE)
Astragalus lobbichleri Podlech (M, holotype)
Astragalus pseudorigidulus Podlech (M, holotype)

MAK (Tokyo Metropolitan University, Tokyo, Japan)

SAXIFRAGACEAE
Saxifraga amabilis H. Ohba & Wakab. (MAK, isotype)

MO (Missouri Botanical Garden, Missouri, U.S.A.)

BRASSICACEAE (CRUCIFERAE)
Aphragmus hinkuensis (Kats. Arai, H. Ohba & Al-Shehbaz) Al-Shehbaz & S. I. Warwick
Lignariella hinkuensis Kats. Arai, H. Ohba & Al-Shehbaz (MO, isotype)
Sisymbrium nepalense Al-Shehbaz (MO, isotype)

MSB (Ludwig-Maxmillians-Universitat, Munchen, Germany)

FABACEAE (LEGUMINOSAE)
Astragalus notabilis Podlech (MSB, holotype)

MW (Moscow State University, Moscow, Russia)

APIACEAE (UMBELLIFERAE)
Conioselinum nepalense Pimenov & Kljuykov (MW, holotype)
Lalldhwojia pastinacifolia Pimenov & Kljuykov (MW, holotype)
Oreocome involucellata Pimenov & Kljuykov (MW, holotype)
Sinocarum latifoliolatum Pimenov & Kljuykov (MW, holotype)
Sinocarum meeboldioides Pimenov & Kljuykov (MW, holotype)

NEU (Universite de Neuchatel, Neuchatel, Switzerland)

GENTIANACEAE
Swertia barunensis P. Chassot (NEU, holotype)

NY (The New York Botanical Garden, Bronx, New York, U.S.A.)

CYPERACEAE
Carex himalaica T. Koyama (NY, isotype)

P (Museum National d'Histoire Naturelle, Paris, France)

APIACEAE (UMBELLIFERAE)
Acronema bryophilum Farille & Lachard (P, isotype)
Acronema cryptosciadeum Farille & Lachard(P, isotype)
Acronema mukherjeeanum Farille & Lachard (P, isotype)
Acronema phaeosciadeum Farille & Lachard (P, isotype)
Acronema pneumatophobium Farille & Lachard (P, isotype)
Keraymonia nipaulensis Cauwet-Marc & Farille (P, isotype)
Pimpinella acronemastrum Farille & Lachard (P, isotype)
Pimpinella kawalekhensis Farille & Lachard (P, holotype)
Rohmooa kirmzii Farille & Lachard (P, isotype)
Synclinostyle sdenisjordanii Farille & Lachard (P, holotype)
Synclinostyles exadversum Farille & Lachard (P, isotype)

BRASSICACEAE (CRUCIFERAE)
Draba staintonii Jafri ex H. Hara (P, isotype)

PE (Institute of Botany, Chinese Academy of Sciences, Beijing, China)

CAMPANULACEAE
Codonopsis reflexa D. Y. Hong (PE, holotype)

TI (University of Tokyo, Tokyo, Japan)

ACANTHACEAE
Thunbergia nepalensis Bh. Adhikari & J. R. I. Wood (TI, isotype)

ASTERACEAE (COMPOSITAE)
Artemisia mustangensis Yonek. (TI, isotype)
Artemisia nepalica Yonek. (TI, holotype)

Cirsium phulchokiense Kitam. (TI, holotype)
Leontopodium montisganeshii S. Akiyama (TI, holotype)
Saussurea kanaii K. Fujikawa & H. Ohba (TI, holotype)

BALSAMINACEAE
Impatiens bajurensis S. Akiyama & H. Ohba (TI, holotype)
Impatiens harae H. Ohba & S. Akiyama (TI, holotype)
Impatiens kharensis S.Akiyama, H. Ohba & Wakabaya.(TI, holotype)

BEGONIACEAE
Begonia flagellaris H. Hara (TI, holotype)
Begonia leptoptera H. Hara (TI, holotype)

BORAGINACEAE
Microula mustangensis Yonek. (TI, holotype)

BRASSICACEAE (CRUCIFERAE)
Aphragmus hinkuensis (Kats. Arai, H. Ohba & Al-Shehbaz) Al-Shehbaz & S. I. Warwick
Lignariella hinkuensis Kats. Arai, H. Ohba & Al-Shehbaz (TI, holotype)
Aphragmus nepalensis (H. Hara) Al-Shehbaz
Staintoniella nepalensis H. Hara (TI, isotype)
Draba poluniniana Al-Shehbaz (TI, holotype)
Lepidostemon williamsii (H. Hara) Al-Shehbaz
Draba williamsii H. Hara (TI, paratype).

CARYOPHYLLACEAE
Odontostemma paramelanandrum (H. Hara) Rabeler & W. L. Wagner
Arenaria paramelanandra H. Hara (TI, isotype)
Silene davidlongii Rajbh. &Mitsuo Suzuki (TI, holotype)

CRASSULACEAE
Rhodiola nepalica (H. Ohba) H. Ohba
Sedum nepalicum H. Ohba (TI, isotype)

CYPERACEAE
Carex esbirajbhandarii (Rajbh. & H. Ohba) O. Yano
Kobresia esbirajbhandarii Rajbh. & H.Ohba (TI, holotype)
Carex gandakiensis Katsuyama (TI, holotype)
Carex himalaica T. Koyama (TI, isotype)
Carex mallae (Rajbh. & H. Ohba) O. Yano
Kobresia mallae Rajbh. & H. Ohba (TI, holotype)
Carex rhombifructa Ohwi (TI, holotype).

ELAEAGNACEAE
Elaeagnus tricholepis Momiyama (TI, holotype)

ERIOCAULACEAE
Eriocaulon exsertum Satake (TI, holotype)

Eriocaulon obclavatum Satake (TI, holotype)
Eriocaulon trisectoides Satake (TI, holotype)

EUPHORBIACEAE
Croton nepalensis T. Kuros. (TI, holotype)

FABACEAE (LEGUMINOSAE)
Astragalus chateri Vass. (TI, isotype)
Crotalaria kanaii H. Ohashi (TI, holotype)
Hedysarum manaslense (Kitam.) H. Ohashi
Hedysarum nepalense H. Ohashi var. *subhirtellum* (TI, isotype).

GENTIANACEAE
Gentiana sagarmathae Miyam. & H. Ohba (TI, holotype)
Gentiana tetramerus Miyam. (TI, holotype)

JUNCACEAE
Juncus mustangensis Miyamoto & H. Ohba (TI, holotype)

LAMIACEAE (LABIATAE)
Isodon dhankutanus Murata (TI, holotype)
Isodon phulchokiensis H. W. Li
Rabdosia phulchokiensis Murata (TI, holotype)
Salvia transhimalaica Yonek. (TI, holotype)

ORCHIDACEAE
Malaxis tamurensis Tuyama (TI, Holotype)

OROBANCHACEAE
Pedicularis annapurnensis T. Yamaz. (TI, isotype)
Pedicularis anserantha T. Yamaz.
Pedicularis ingentoides T. Yamaz. (TI, isotype)
Pedicularis breviscaposa T. Yamaz. (TI, isotype)
Pedicularis chamissonoides T. Yamaz. (TI, isotype)
Pedicularis cornigera T. Yamaz. (TI, holotype)
Pedicularis gruiflora T. Yamaz. (TI, holotype)
Pedicularis koshiensis T. Yamaz. (TI, holotype)
Pedicularis muguensis T. Yamaz. (TI, isotype)
Pedicularis oxyrhyncha T. Yamaz. (TI, holotype)
Pedicularis terrenoflora T. Yamaz. (TI, holotype)
Pedicularis yalungensis T. Yamaz. (TI, holotype)

PAPAVERACEAE
Papaver autumnale (P. Egan) Christenh. & Byng
Meconopsis autumnalis P. A. Egan (TI, isotype)
Papaver staintonii (Grey-Wilson) Christenh. & Byng
Meconopsis staintonii Grey-Wilson (TI, isotype)
Papaver taylorii (L.H.J. Williams) Christenh. & Byng

Meconopsis taylorii L. H. J. Williams (TI, isotype)

PLANTAGINACEAE
Lagotis nepalensis T. Yamaz. (TI, isotype)
Veronica emodi T. Yamaz. (TI, isotype)
Wulfeniopsis nepalensis (T. Yamaz.) D. Y. Hong
Wulfenia nepalensis T. Yamaz. (TI, holotype)

POLYGONACEAE
Bistorta diopetes H. Ohba & S. Akiyama (TI, holotype)
Bistorta milletioides H. Ohba & S. Akiyama (TI, holotype)

RANUNCULACEAE
Anemone fuscopurpurea H. Hara (TI, holotype)
Ranunculus makaluensis (TI, holotype)

ROSACEAE
Potentilla makaluensis H. Ikeda & H. Ohba (TI, holotype)
Prunus taplejungnica H. Ohba & S. Akiyama (TI, holotype)
Prunus topkegolensis H. Ohba & S. Akiyama (TI, holotype)
Sibbaldia emodi H. Ikeda & H. Ohba (TI, holotype)

SALICACEAE
Salix plectilis Kimura (TI, holotype)

SAXIFRAGACEAE
Saxifraga amabilis H. Ohba & Wakab. (TI, holotype)
Saxifraga ganeshii H. Ohba & S. Akiyama (TI, holotype)
Saxifraga harae H. Ohba & Wakabayashi (TI, holotype)
Saxifraga jaljalensis H. Ohba & S. Akiyama (TI, holotype)
Saxifraga mallae H. Ohba & Wakab. (TI, holotype)
Saxifraga neopropagulifera H. Hara (TI, isotype)
Saxifraga rhodopetala Harry Sm. (TI, paratype)

SCROPHULARIACEAE
Scrophularia bheriense T. Yamaz. (TI, paratype)

URTICACEAE
Pilea kanaii H. Hara (TI, holotype)

TNS (Natural Museum of Nature and Science, Tsukuba, Japan)

RANUNCULACEAE
Ranunculus makaluensis Kadota (TNS, isotype)

ROSACEAE
Prunus topkegolensis H. Ohba & S. Akiyama (TNS, isotype)

SAXIFRAGACEAE
Saxifraga ganeshii H. Ohba& S. Akiyama (TNS, isotype)

TUCH (Tribhuvan University, Kathmandu, Nepal)

ASTERACEAE (COMPOSITAE)
Saussurea ramchaudharyi S. K. Ghimire & H. K. Rana (TUCH, holotype)

ORCHIDACEAE
Pinalia baniae (Bajracharya, L. R. Shakya & Chettri) Schuit.
Eria baniaii Bajracharya, Shakya & Chettri (TUCH, holotype)

TUS (Tohuku University, Sendai, Miyagi, Japan)

ASTERACEAE (COMPOSITAE)
Artemisia mustangensis Yonek. (TUS, holotype)

CARYOPHYLLACEAE
Silene hideaki-ohbae Rajbh. & Mitsuo Suzuki (TUS, holotype)

EUPHORBIACEAE
Croton nepalensis T. Kuros. (TUS, paratype)

LAMIACEAE
Salvia transhimalaica Ynek. (TUS, paratype).

UPS (Museum of Evolution, Uppsala, Sweden)

BRASSICACEAE (CRUCIFERAE)
Draba staintonii Jafri ex H. Hara (UPS, isotype)

US (Smithsonian Institute, Washington, U.S.A.)

APIACEAE
Cortiella lamondiana Fullarton & M. F. Watson (US, paratype).

FABACEAE (LEGUMINOSAE)
Astragalus nepalensis Podlech (US, holotype)

Table 11. List of the endemic flowering plants of Nepal (only holotypes are used).
(West: West Nepal. Central: Central Nepal. East: East Nepal).

ACANTHACEAE
Justicia tukuchensis - Central: Mustang district, 2286m (BM).
Strobilanthes nutans - Central: Nepalia (K).
Strobilanthes saccata - Central: Kaski district, 1930 m (BM).

Thunbergia kasajuana – Central: Bhaktapur district, 2020 m (KATH).
Thunbergia nepalensis - West: Doti district, 2105 m (E).

AMARYLLIDACEAE
Allium hypsistum – Central: Dolpa district, 5,500 m (BM).

APIACEAE
Acronema bryophilum - Central: Rasuwa district, 4520 m (KATH).
Acronema cryptosciadeum - East: Sankhuwasabha district, 4070 m (KATH).
Acronema dyssimetriradiata – Central: Lamjung district, 4000-4200 m (KATH).
Acronema johrianum - Central, 4290 m (CAL).
Acronema mukherjeeanum - Central: Rasuwa district, 3450 m (KATH).
Acronema phaeosciadeum - Central: Rasuwa district, 4000 m (KATH).
Acronema pneumatophobium - Central: Dolakha district, 4500 m (KATH).
Acronema refugicolum - Central: Kaski district, 4000 m (BM).
Chamaesium shrestaeanum - Central: Dolakha district, 4200 m (KATH).
Conioselinum nepalense - Central: Kusma district, 3100-3400 m (MW).
Cortia staintoniana - East: Taplejung district, 3962.4m. (E).
Cortiella lamondiana - East: Taplejung district, 4200 m (E).
Keraymonia nipaulensis - Central: Mustang district, 4500 m (KATH).
Lalldhwojia pastinacifolia - Central Nepal: Rasuwa district, 4000 m (MW).
Lalldhwojia staintonii - Central Nepal: Lamjung district, 3800-4000 m (KATH).
Oreocome depauperata - West Nepal: Humla district, 3500 m (KATH).
Oreocome involucellata - Central Nepal: Rasuwa district, 3400 m (MW).
Pimpinella acronemastrum - Central Nepal: Rasuwa district, 2000 m (KATH).
Pimpinella inundata - Central Nepal: Baglung district, 2840 m (KATH).
Pimpinella kawalekhensis - West Nepal: Darchula district, 2950 m (P).
Rohmooa kirmzii - Central Nepal: Dhading district, 4270 m (KATH).
Sinocarum latifoliolatum – Central Nepal: Kaski district, 3100-3400 m (MW).
Sinocarum meeboldioides – Central Nepal: Kaski district, 2000-2300 m (MW).
Sinocarum normanianum - Central Nepal: Gorkha district, 4572m (BM).
Sinocarum staintonianum - Central Nepal: Myagdi district, 4500 m (BM).
Synclinostyles denisjordanii - Central Nepal: Rasuwa district, 4030 m (P).
Synclinostyles exadversum - Central Nepal: Gorkha district, 4400 m (KATH).
Tetrataenium lallii - Central Nepal: Gorkha district, 3962.4-4419.6m (E).
Vicatia nepalensis - West Nepal: Darchula district, 2590.8m (BM).

APOCYNACEAE
Ceropegia meleagris - Central Nepal: Mustang district, 2286m (BM).
Ceropegia nepalensis - West Nepal: Rukum district, 3048m (BM).
Ceropegia poluniniana - West Nepal: Jajarkot district, 1500 m (K).

ASPARAGACEAE
Asparagus penicillatus - West Nepal: Dolpa district, 2438.4m (BM).

ASTERACEAE
Artemisia mustangensis - Central Nepal: Mustang district, 2550 m (TUS).
Artemisia nepalica - Central Nepal: Mustang district, 3200 m (TI).

Cicerbita nepalensis - Central Nepal: Mustang district, 3000 m (BM).
Cirsium flavisquamatum - West Nepal: Mugu district, 3352.8m (BM).
Cirsium phulchokiense - Central Nepal: Lalitpur district, 1500 m (TI).
Crepis himalaica - Central Nepal: Manang district, 3300 m (KYO).
Leontopodium makianum - Central Nepal: Gorkha district, 4000 m (KYO).
Leontopodium montisganeshii - Central Nepal: Rasuwa district, 3490 m (TI).
Saussurea chrysotricha - Central Nepal: Rukum district, 4267.2m(BM).
Saussurea dhwojii - Central Nepal: Pongsing, 1524m (BM).
Saussurea kanaii - Central Nepal: Mustang district, 4420 m (TI).
Saussurea platyphyllaria - West Nepal: Dolpa district, 4724.4m (BM).
Saussurea ramchaudharyi – West Nepal: Humla district, 4650 m (TUCH).
Synotis brunneovillosa - East Nepal: Sankhuwasabha district, 2590.8m (BM).
Synotis managensis - Central Nepal: Manang district, 3432 m (KATH).
Synotis panduriformis - East Nepal: Solukhumbu district, 2100 m (BM).
Taraxacum amabile - Central Nepal: Baglung district, 3048m (BM).
Taraxacum nepalense - West Nepal: Doti district (BM).

BALSAMINACEAE
Impatiens arunensis - East Nepal: Sankhuwasabha district, (K).
Impatiens bajurensis - West Nepal: Bajura district, 1520 m (TI).
Impatiens gorepaniensis - Central Nepal: Kaski district, 2000 m (K).
Impatiens harae - East Nepal: Ramechhap district, 3651-2760 m (TI).
Impatiens kharensis - Central Nepal: Dolakha district, 3300-4100 m (TI).
Impatiens williamsii - West Nepal: Jumla district, 3048m (BM).

BEGONIACEAE
Begonia flagellaris - Central Nepal: Rasuwa district, 2300 – 2400 m (TI).
Begonia leptoptera - Central Nepal, Dolakha district, 2500 m (TI).
Begonia nuwakotensis - Central Nepal: Nuwakot district, 1700 m (E).
Begonia taligera - Central Nepal: Kaski district, 700-740 m (E).
Begonia tribenensis - East Nepal, Sunsari district, ca. 130 m (BLAT).

BERBERIDACEAE
Berberis mucrifolia - Central Nepal: Mustang district, 4419.6m (BM).
Berberis pendryi - Central Nepal: Mustang district (E).

BORAGINACEAE
Arnebia nepalensis - Central Nepal: Gorkha district, 4100 m (KYO).
Maharanga verruculosa - Central Nepal: Tatey, 2743.2m (E).
Microula mustangensis - Central Nepal: Mustang district, 4490 m (TI).
Onosma bheriense - West Nepal: Dolpa district, 1981.2m (BM).

BRASSICACEAE
Aphragmus hinkuensis - East Nepal: Solukhumbu district, 3550 m (TI).
Aphragmus nepalensis - Central Nepal: Dolpa district, 5029.2m (BM).
Draba macbeathiana - Central Nepal: Manang district, 5273.04m (E).
Draba polunininana - West Nepal: Humla district, 3810m (TI).
Draba staintonii - Central Nepal: Myagdi district, 4572m (BM).

Lepidostemon williamsii - Central Nepal: Mustang district, 3200.4m (BM).
Noccaea nepalensis - West Nepal: Humla district, 3200.4m (BM).
Sisymbrium nepalense – West Nepal: Humla district, 2200 m (E).
Solms-laubachia haranensis - Central Nepal: Dolpa district, 5943.6m (BM).
Solms-laubachia nepalensis - East Nepal: Sankhuwasabha district, 5394.96m (BM).

CAMPANULACEAE
Codonopsis bragaensis - Central Nepal: Manang district, ca. 3800 m (K).
Codonopsis reflexa - Central Nepal: Baglung district, 3960 m (PE).
Cyananthus hayanus - Central Nepal: Panjen, 4572m (BM).
Cyananthus himalaicus - Central Nepal: Myagdi District, 3200.4m (BM).

CARYOPHYLLACEAE
Arenaria globiflora – Central Nepal: Rasuwa district (K).
Eremogone mukerjeeana - Central Nepal: Mustang district, 4260 m (CAL).
Odontostemma paramelanandrum - West Nepal: Jumla district, 4267.2m(BM).
Silene davidlongii - West Nepal: Mugu district, 4572m (TI).
Silene fissicalyx - Central Nepal: Rasuwa district, 4267.2m (BM).
Silene greywilsonii - West Nepal: Dolpa district, c. 5300 m (K).
Silene helleboriflora - Central Nepal: Mulmuley Khola, 4267.2m (BM).
Silene hideakiohbae - Central Nepal: Manang district, 2520 m (TUS).
Silene stellariifolia - Central Nepal: Kaski district, 1676.4m (BM).
Silene vautierae - Central Nepal: Mustang district, 3810m (BM).

CRASSULACEAE
Rhodiola nepalica - Central Nepal: Mustang district, 4572m (BM).
Sedum pseudo-multicaule - Central Nepal: Gorkha district, (KYO).

CUCURBITACEAE
Gomphogyne nepalensis - Central Nepal: Mustang district (K).

CYPERACEAE
Carex esbirajbhandarii - East Nepal: Solukhumbu district, 4600 m (TI).
Carex gandakiensis - Central Nepal: Mustang district, 3352.8m (TI).
Carex himalaica - East Nepal: Sankhuwasabha district, 3657.6m (BM).
Carex mallae - Central Nepal: Mustang district, 4000 m (TI).
Carex rhombifructus - Central Nepal: Lalitpur district, 1600-2500 m (TI).
Cyperus trisulcus – Central Nepal (BM).
Cyperus wallichianus – Central Nepal (BM).

ELAEAGNACEAE
Elaeagnus tricholepis - Central Nepal: Lalitpur district, 1615.44m (TI).

ERICACEAE
Rhododendron cowanianum - Central Nepal: Rasuwa district, c. 3657.6m (BM).
Rhododendron lowndesii - Central Nepal: Manang district, 4114.8m (BM).

ERIOCAULACEAE
Eriocaulon exsertum - East Nepal: Jhapa district, 300-200 m (TI).
Eriocaulon obclavatum - East Nepal: Jhapa district, 300-200 m (TI).
Eriocaulon trisectoides - East Nepal: East Nepal, 2100-700 m (TI).

EUPHORBIACEAE
Croton nepalensis - Central Nepal: Gorkha district, 970 m (TI).

FABACEAE
Astragalus barclayanus - Central Nepal: Manang district, 4820 m (K).
Astragalus chateri - Central Nepal: Dolpa district, 3600 m (BM).
Astragalus lobbichleri - Central Nepal: Manang district, 3700 m (M).
Astragalus nakaoi - Central Nepal: Gorkha district, 3800 m (KYO).
Astragalus nepalensis - Central Nepal: Mustang district, 2590.8m (US).
Astragalus notabilis - Central Nepal: Mustang district, 3450 m (MSB).
Astragalus poluninii - West Nepal: Mugu district, 2134 m (E).
Astragalus pseudorigidulus - Central Nepal: Central Nepal, 4572m (M).
Crotalaria kanaii - Central Nepal: Rasuwa district, 2300 m (TI).
Hedysarum manaslense - Central Nepal: Gorkha district, 3800 m (KYO).
Millettia nepalensis - West Nepal: Doti district (K).
Oxytropis arenae-ripariae - West Nepal: Jumla district, 4572m (BM).
Oxytropis fasciculiflorum - Central Nepal: Dolpa district, 5638.8m (BM).
Oxytropis graminetorum - Central Nepal: Mustang district, 4267.2m (BM).
Oxytropis morenarum - Central Nepal: Rukum district, 3962.4m (BM).
Oxytropis nepalensis - Central Nepal: Mustang district, 4114.8m (BM).
Oxytropis torrentium - West Nepal: Dolpa district, 4267.2m (BM).
Oxytropis williamsii - Central Nepal: Dolpa district, 3505.2m (BM).
Rhynchosia nepalensis - West Nepal: Salyan district, 1066.8m (BM).

GENTIANACEAE
Gentiana radicans - Central Nepal: Sermabee, 4876.8m (E).
Gentiana sagarmathae - East Nepal: Solukhumbu district, 3500 – 3600 m (TI).
Gentiana tetramera - Central Nepal: Mustang district, 4660 m (TI).
Gentianella lowndesii - Central Nepal: Manang district, 3962.4m (BM).
Kuepferia chateri - East Nepal: Sankhuwasabha district, 3962.4m (BM).
Swertia acaulis - Central Nepal: Dolakha district, 3657.6-5486.4m (BM).
Swertia barunensis - East Nepal: Sankhuwasabha district, c. 4200 m (NEU).
Swertia nepalensis - Central Nepal: Mustang district, 3810m (E).

GESNERIACEAE
Didymocarpus nepalensis – East Nepal: Dhankuta district, 1829 m (E).

HYPERICACEAE
Hypericum cordifolium - Central Nepal: Kathmandu district (G).

IRIDACEAE
Iris staintonii - Central Nepal: Gorkha district, 3505.2m (BM).

JUNCACEAE
Juncus mustangensis - Central Nepal: Mustang district, 3870 m (TI).

LAMIACEAE
Clinopodium nepalense - Central Nepal: Gorkha district, 3400 m (KYO).
Discretitheca nepalensis - West Nepal: Jajarkot district, 1066.8m (BM).
Eriophyton nepalense - East Nepal: Solukhumbu district, 4114.8m (BM).
Eriophyton staintonii - Central Nepal: Ramechhap district, 3810m (BM).
Isodon dhankutanus - East Nepal: Dhankuta district, 1200 m (TI).
Isodon namikawanus - Central Nepal: Dolpa district, 3750 m (KYO).
Isodon phulchokiensis - Central Nepal: Lalitpur district, 2400-2700 m 17 (TI).
Microtoena nepalensis - East Nepal: Tinjure Danda, 2286m (BM).
Nepeta staintonii - Central Nepal: Barbung Khola, 3962.4m (BM).
Salvia transhimalaica - Central Nepal: Mustang district, 3520 m (TI).

LAURACEAE
Machilus pubescens - Central Nepal (L).

OLEACEAE
Jasminum amabile - East Nepal: Sunsari district, 762m (BM).

ONAGRACEAE
Epilobium brevisquamatum - Central Nepal: Mustang district, 3200 m (BM).
Epilobium indicum - Central Nepal: (JE).
Epilobium staintonii - Central Nepal: Mustang district, 3650 m (BM).

ORCHIDACEAE
Bulbophyllum nepalense - Central Nepal: Kathmandu district, 2300 m (KATH).
Calanthe himalaicum - West Nepal: Doti district, 2200 m (KATH).
Eria nepalensis - Central Nepal: Chitwan district, 200 m (KATH).
Gastrochilus nepalensis - Central Nepal: Kaski district, ca. 2350 m (KATH).
Habenaria palpensis - Central Nepal: Palpa district (KATH).
Habenaria sandiegoensis – East Nepal: Ilam district (KATH).
Habenaria wallichii - Central Nepal: Makwanpur District (K).
Herminium fimbriatum - Central Nepal: Rasuwa district, 3800 m (KATH).
Herminium hongdeyuanii - Central Nepal: Kathmandu district, 2200 m (KATH).
Liparis langtangensis - Central Nepal: Rasuwa district, 3700-3900 m (KATH).
Malaxis dolpensis - West Nepal: Dolpa district, 4200 m (KATH).
Malaxis tamurensis - East Nepal: Dhankuta district, 1200 m (TI).
Neottia nepalensis - Central Nepal: Baglung district, 3352.8m (CAL).
Odontochilus nandae - Central Nepal: Kaski district, 2400 m (KATH).
Oreorchis porphyranthes - Central Nepal: Myagdi District, 3139.44m (BM).
Pinalia annapurnensis - Central Nepal: Kaski district, 2000 m (KATH).
Pinalia baniaii - Central Nepal: Lalitpur district, Kathmandu valley, 1600 m (TUCH).
Pinalia pokharensis - Central Nepal: Kaski district, 900-1000 m (KATH).
Pleione coronaria - Central Nepal, 2850 m (BM).

OROBANCHACEAE
Euphrasia nepalensis - Central Nepal, 4267.2m (BM).
Pedicularis annapurnensis - Central Nepal: Kaski district, 4267.2m (BM).
Pedicularis anserantha - Central Nepal, 3780m (KYO).
Pedicularis breviscaposa - Central Nepal: Manang district, 3100m (KYO).
Pedicularis chamissonoides - Central Nepal: Lamjung district, 3810m (BM).
Pedicularis cornigera - East Nepal: Sankhuwasabha district, 4000 m (TI).
Pedicularis gruiflora - East Nepal: Sankhuwasabha district (TI).
Pedicularis koshiensis - East Nepal: Sankhuawasabha district (TI).
Pedicularis muguensis - West Nepal: Mugu district, 3600m (KYO).
Pedicularis odontoloma - Central Nepal: Dolpa district, 4700m (KYO).
Pedicularis oxyrhyncha - East Nepal: Taplejung district, 4100m (TI).
Pedicularis pseudoregeliana - Central Nepal: Rasuwa district, 4725m (BM).
Pedicularis tamurensis - East Nepal: Taplejung district, 3352.8m (BM).
Pedicularis terrenoflora - East Nepal: Taplejung district, 2200m (TI).
Pedicularis yalungensis - Central Nepal: Dolakha district, 4300–5300m (TI).
Pedicularis yamazakiana - West Nepal: Jumla district, 3810m (E).

PAPAVERACEAE
Corydalis calycina - West Nepal: Jumla district, 3200 m (BM).
Corydalis clavibracteata - West Nepal: Dolpa district, 4700 m (BM).
Corydalis megacalyx - Central Nepal: Rukum district, 4572m (BM).
Corydalis simplex - West Nepal: Dolpa district, 4300 m (BM).
Corydalis spicata - West Nepal: Jumla district, 2580 m (GB).
Corydalis stipulata - Central Nepal: Rasuwa district, 3352.8m (BM).
Corydalis terracina - Central Nepal: Kaski district, 2438.4m (BM).
Corydalis uncinata - West Nepal: Dolpa district, 3657.6m (BM).
Corydalis uncinatella - West Nepal: Rukum district, 3810m (BM).
Papaver autumnale - **Central Nepal: Rasuwa district, 4160 m (E).**
Papaver chankeliense - West Nepal: Jumla district, 4572m (BM).
Papaver ganeshense - Central Nepal: Ganesh Himal, 4114.8m (BM).
Papaver manasluense - **Central Nepal: Gorkha district, 4000 m (E).**
Papaver regium - Central Nepal: Gorkha district, 3657.6-4572m (BM).
Papaver simikotense - West Nepal: Humla district, 3505.2m (BM).
Papaver staintonii - Central Nepal: Mustang district, 3657.6m (BM).
Papaver taylorii - Central Nepal: Kaski district, 4572m (BM).

PLANTAGINACEAE
Lagotis nepalensis - West Nepal, Urai Langna, 5791.2m (BM).
Veronica emodi - West Nepal: Mugu district, 3700 m (TI).
Wulfeniopsis nepalensis - Central Nepal: Lalitpur district, 1600-2500 m (TI).

POACEAE
Achnatherum staintonii - West Nepal: Rukum district, 3810m (K).
Bambusa nepalensis - Central Nepal: Kathmandu district (E).
Elymus nepalensis - West Nepal: Jumla district, 2895.6m (BM).
Festuca eriobasis - Central Nepal: Rasuwa district, 4530 m (B).
Festuca nepalica - Central Nepal: Dolpa district, 5059.68m (K).

Festuca poluninii - West Nepal: Jumla district, 4114.8m (BM).
Himalayacalamus asper - Central Nepal: Kaski district, 2000 m (E).
Himalayacalamus cupreus - Central Nepal: Kaski district, 2500 m (E).
Himalayacalamus fimbriatus - Central Nepal: Kathmandu district, 1200 m (E).
Himalayacalamus planatus - Central Nepal: Rasuwa district, ca. 2438.4m (K).
Himalayacalamus porcatus - Central Nepal: Rasuwa district, 2286m (E).
Poa hideaki-ohbae - Central Nepal: Dhading district, 3400 m (KATH).
Poa muktinathensis - Central Nepal: Mustang district, 5200 m (KATH).
Saccharum williamsii - Central Nepal: Myagdi district, 2895.6m (BM).
Thamnocalamus chigar - Central Nepal: Kaski district, 3000 m (E).

POLYGONACEAE
Bistorta diopetes - East Nepal: Sankhuwasabha district, 4150 m (TI).
Bistorta milletioides - East Nepal: Sankhuwasabha district, 4150 m (TI).
Eskemukerjea megacarpum - West Nepal: Mugu district, 2438.4m (BM).
Fallopia filipes - Central Nepal: Kaski district, 1981.2m (BM).

PRIMULACEAE
Primula poluninii - West Nepal: Mugu district, 4876.8m (BM).
Primula ramzanae - Central Nepal: Dolpa district, 5181.6m (BM).
Primula sharmae - Central Nepal: Mustang district, 4114.8m (E).
Primula wigramiana - Central Nepal: Sindhupalchok district, 5181.6m (E)

RANUNCULACEAE
Aconitum amplexicaule - Central Nepal: Baglung district, 3810m (BM).
Aconitum angulatum - East Nepal, 3900 m (KYO).
Aconitum bhedingense - Central Nepal: Dolakha district, 3657.6-3962.4m (E).
Aconitum dhwojii - Central Nepal: Gorkha district, 4572-4876.8m (BM).
Aconitum poluninii - West Nepal: Mugu district, 3810m (BM).
Aconitum staintonii - East Nepal: Taplejung district, 3505.2m (BM).
Aconitum tabatae - West Nepal: Purbang, 3850 m (KYO).
Aconitum williamsii - Central Nepal: Myagdi district, 3276.6m (BM).
Anemone fuscopurpurea - East Nepal: Sankhuwasabha district, 4200 – 4000 m (TI).
Clematis bracteolata - Central Nepal: Mustang district, 3700 m (KYO).
Clematis phlebantha - West Nepal: Dolpa district, 2895.6m (BM).
Delphinium unifolium - Central Nepal, 3500 m (KYO).
Delphinium williamsii - West Nepal: Kalikot district, 1828.8m (BM).
Oxygraphis nepalensis - Central Nepal: Rasuwa district, 12500-4114.8m (BM).
Ranunculus makaluensis - East Nepal: Sankhuwasabha district, 4340 m (TI).

ROSACEAE
Potentilla makaluensis - East Nepal: Sankhuwasabha district, 4120 m (TI).
Prunus himalaica - Central Nepal: Gorkha district, 3900 m (KYO).
Prunus jajarkotensis - West Nepal: Jajarkot district, 914.4m (BM).
Prunus taplejungnica - East Nepal: Taplejung district, 3520 m (TI).
Prunus topkegolensis - East Nepal: Taplejung district, 3700 m (TI).
Sibbaldia emodi - East Nepal: Solukhumbu district, 4015 m (TI).
Sorbus sharmae - Central Nepal: Rasuwa district, 3170 m (E).

RUBIACEAE
Galium nepalense - Central Nepal: Dolpa district, 4724.4m (BM).
Galium saipalense - West Nepal: Humla district, 4724.4m (BM).
Ophiorrhiza nepalensis - East Nepal: Ilam district, 450 m (BM).

SALICACEAE
Salix plectilis - East Nepal: Jhapa district, 200 m (TI).
Salix staintoniana - East Nepal: Sankhuwasabha district, 3657.6m (BM).

SAXIFRAGACEAE
Saxifraga alpigena - Central Nepal: Manang district, 3505.2m (BM).
Saxifraga amabilis - Central Nepal: Dolakha district, 4300 m (TI).
Saxifraga cinerea - Central Nepal: Manang district, 2743.2m (BM).
Saxifraga excellens - Central Nepal: Kaski district, 3810m (BM).
Saxifraga ganeshii - Central Nepal: Rasuwa district, 4250 m (TI).
Saxifraga harae - East Nepal: Solukhumbu district, 4550 m (TI).
Saxifraga hypostoma - Central Nepal: Gorkha district, 4876.8m (BM).
Saxifraga jaljalensis - East Nepal: Sankhuwasabha district, 4300 m (TI).
Saxifraga lowndesii - Central Nepal: Manang district, 4114.8m (BM).
Saxifraga mallae - East Nepal: Ramechhap district, 4500 m (TI).
Saxifraga micans - Central Nepal: Myagdi district, 3810m (BM).
Saxifraga mira - Central Nepal: Dolpa district, 4419.6m (BM).
Saxifraga namdoensis - Central Nepal: Mustang district, 4572m (BM).
Saxifraga neopropagulifera - Central Nepal: Dolpa district 4500 m (BM).
Saxifraga poluninana - West Nepal: Jumla district, 3505.2m (BM).
Saxifraga rhodopetala - West Nepal: Dolpa district, 3962.4m (BM).
Saxifraga roylei - Central Nepal: Myagdi district, 3810m (BM).
Saxifraga staintonii - Central Nepal: Mustang district, 4876.8m (BM).
Saxifraga williamsii - Central Nepal: Mustang district, 4114.8m (BM).
Saxifraga zimmermannii - Central Nepal: Dolakha district, 4130 m (G).

SCROPHULARIACEAE
Scrophularia bheriensis - West Nepal: Dolpa district, 1828.8m (BM).
Scrophularia laportifolia - West Nepal: Darchula district, 2895.6m (BM).

URTICACEAE
Pilea kanaii - Central Nepal: Rasuwa district, 1500 m (TI).

ZINGIBERACEAE
Roscoea ganeshensis - Central Nepal: Gorkha district, 1900 m (K).
Roscoea nepalensis - West Nepal: Jumla district, 2438.4-2743.2m (BM).
Roscoea tumjensis - Central: Gorkha district, 2700 m (BM).

Conclusion

For the proper conservation measures to be undertaken for the endemic flowering plants of Nepal a systematic investigation of these plants should be carried out in order to understand their natural habitat, which will furnish the ecological requirements of different species.

Recent count shows that there are 125 species (including 60 type species) of herbarium specimens of endemic flowering plants of Nepal preserved in the National Herbarium of Nepal (KATH). Most of the type specimens of the endemic flowering plants are preserved in the herbaria outside Nepal. For the identification and the locality and habitat of these endemic plants, it would be convenient if the specimens of all the Nepalese endemic species are collected and represented in the Herbarium of Nepal. The data presented in this paper can help to do further research, develop the planning/policy, and know the important plant areas (IPA) for the conservation of the endemic plants in Nepal. Districts with high diversity of endemic flowering plants such as Mustang, Dolpa, Rasuwa, Kaski, Gorkha, Sankhuwasabha, Jumla, Manang and Taplejung should be given importance for conservation and for further study to analyze them.

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Role of Botanical Gardens of Nepal in Biodiversity Conservation, Education and Research

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Abstract

Botanical gardens are the living museum for plants conservation and open to the public for the purposes of education, research and recreation. The history of botanical gardens dates back to thousands of years and linked to the history of botany itself. There are over 2800 botanical gardens around the word. The botanical garden in Nepal started only after the establishment of the Department of Medicinal Plants in 1960. The first established Royal Botanical Garden in Nepal traces linkage with the British botanic gardens and institutions. The Department of Medicinal Plants was renamed as Department of Plant Resources in 1994. Similarly, the Royal Botanical Garden as the National Botanical Garden after the nation was transformed as the Federal Republic of Nepal. Currently, the department is managing 12 botanical gardens in different climatic zones of Nepal. Among these, five botanical gardens are situated in tropical region, four in subtropical and three in temperate region. The National Botanical Garden, Godawari, Lalitpur is the largest botanical garden of Nepal which conserves about 1000 species of plants. The conserved plants are also for public display. These botanical gardens are playing important role in *ex situ* and *in situ* conservation of rare, endangered and threatened plants. Botanical gardens and their living collections are of immense value which can support in scientific research and development. Further, they are considered as education and awareness center for communicating the importance of conserving plants and their sustainable utilization as well as recreational center to the people.

Key words: Department of Plant Resources, *Ex situ* and *in situ*, National Botanical Garden, Recreation

Introduction

Botanical gardens are the living museum of plants conserved *in situ* and *ex situ* conditions. Such gardens are meant for conservation, education, research on biodiversity and also places of recreation. Botanical Gardens Conservation Strategy (1989) has defined botanical garden as the scientifically ordered and maintained collection of living plants usually documented and labeled and open to the public for the purposes of recreation, education and research (Huxley, 1992).Similarly, Botanic Gardens Conservation International (BGCI) has defined the botanical garden as an institution holding documented collection of living plants for the purpose of scientific research, conservation, display and education (Wyse Jackson et al., 2000).

The value of the botanical gardens is not limited only to the professionals, but equally valuable to the botanists, horticulturists, foresters including the common public and school

children who get admiration on nature and learn the biodiversity conservation. The botanical gardens are usually run by the Universities or the research organizations and which have associated herbaria and the programmes in plant taxonomy or some other subjects of plant science.

The history of botanical gardens and the cultivation of plants in the world can be traced back to thousands of years. The former gardens were built around 3,000 years ago in ancient Egypt and Mesopotamia. However, the first botanic gardens with an underlying scientific foundation were the physic gardens of Italy created in the 16th and 17th centuries. The first of these physic gardens were the garden of the University of Pisa which was created by Lucsa Ghini in 1543 followed by the gardens of Padua (1545) and Florence (1550) of Italy (Borsch & Löhne, 2014). The botanical gardens have been significantly increased during the last decades, currently, there are over 2,800 botanical gardens in 2010 around the world (Sharrock et al., 2010, Borsch & Löhne, 2014). Among them the largest one is the Royal Botanic Garden, Kew which was founded in 1759. The green houses of the garden conserve over 13000 live plant specimens (https://www.kew.org). In addition, some others major botanical gardens of the world are, the Royal Botanic Garden Edinburgh (Scotland), UK, Missouri Botanic Garden, Missouri, USA, New York Botanic Garden, New York, USA, Calcutta Botanic Garden, Calcutta, India, etc.

The botanical gardens of the 16th and 17th centuries were medicinal gardens, but the idea of a botanical garden changed to display the plants which are beautiful, strange, new to the locality and sometimes economically important. Such plants were collected as trophies while the people returned from the European colonies and other distant lands (Hill, 2015). Seed-exchange program was established in 1682 and still continues (https://en.wikipedia.org/wiki/Botanical_garden#History_and_development).

Later, in the 18th century, botanical gardens became more educational in function, demonstrating the latest plant classification systems devised by botanists working in the associated herbaria as they tried to order these new treasures (Hill, 2015). The late 18th and early 19th centuries were marked by the establishment of tropical botanical gardens as a tool of colonial expansion mainly by the British and the Dutch people in India, South East Asia and the Caribbean. Then, in the 19th and 20th centuries, the trend was towards a combination of specialist and diverse collections demonstrating many aspects of both horticulture and botany. A large number of civic or municipal botanical gardens were founded in the 19th and 20th centuries (https://en.wikipedia.org/wiki/Botanical_garden#History_and_development/). The second half of the 20th century botanical gardens started to botanical exhibits on themes of evolution, ecology or taxonomy and established consistent methods for *ex situ* conservation in living collections and in seed banks as well as public education in raising awareness for sustainability (Borsch & Löhne, 2014). Botanical gardens rules were evolved in the twenty first century, then the contracting parties must follow the international rules and regulations of the Convention on Biological Diversity (1992), the Nagoya Protocol (2010) and the provision of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Krishnan & Novy, 2016). Further, BGCI (2018) updated the criteria and defined the botanic garden by giving greater emphasis on conserving rare and threatened plants compliance with international policies sustainability and livelihood.

Role of Botanical Gardens

Botanical gardens play a central role for scientific research for both *in situ* and *ex situ* conservation, plant resource utilization, public education and citizen science (Chen & Weibang, 2018). They are also important for aesthetic, cultural and scientific establishments that contribute to the well-being of our society. Currently, the botanic gardens are also playing a critical role for addressing global issues such as climate change, food security, biodiversity conservation, environmental education, sustainability and human wellbeing (Krishnan & Novy, 2016). They also provide many benefits to society including positive impacts on mental and physical health, particularly in urban settings.

In addition to various supplementary roles, the main roles of botanical gardens are:

1. Conservation: Botanical gardens are important for the *in situ* and *ex situ* conservation of plants around the world. Horticulture and cultivation skills allow botanical gardens to grow plants that might be lost in the wild. The living collections and seed banks safeguard the species and enable the restoration and rehabilitation of degraded habitats.
2. Research and development**:** Botanical gardens are also scientific research center as they play important role in plant taxonomy, genetics, phytochemistry, developing useful plant properties and informing selection of plants that can withstand degraded and changing environments.
3. Public education**:** Botanical gardens are considered as education center for informing the importance of conserving plants, reaching out to diverse audiences, and also to communicate how this may be achieved.
4. Linking plants with the wellbeing of people: They also help to conserve the indigenous and local knowledge, as well as encourage the sustainable use of plant resources for the benefit of people.

History and Development of Botanical Gardens in Nepal

The true botanical garden in Nepal started only after the establishment of the Department of Medicinal Plants in 1960. The Department of Medicinal Plants (DMP) was renamed as Department of Plant Resources in 1994. The history of developmental phase of the Botanical Gardens in Nepal can be divided into 3 phases.

i. Establishment Phase (1960-1990)

The history of botanical garden of Nepal is closely linked with British botanic gardens and institutions. The King Mahendra was inspired by the Royal Botanic Garden Edinburgh (RBGE), Scotland during his official visit to UK (1960) where he also planted a tree (*Betula utilis*). Knowing his interest, the Queen of Britain had deputed Dr. Geoffrey Herklots (a plant landscape designer and orchid specialist) to establish the botanical garden in Nepal. The DMP had selected a piece of land near the village of Godavari, Lalitpur about 16km south-east from Kathmandu with the assistance of Dr. Geoffrey Herklots to establish a Botanical Garden. Where then King Mahendra inaugurated the Royal Botanical Garden (RBG) on October 28, 1962. RBG was renamed as National Botanical Garden (NBG) on July 6, 2006 after the Government of Nepal abolished monarchy and turned into the Federal

Democratic Republic (Suwal, 2010; Shrestha, 2010). NBG is one of the central offices of the Department of Plant Resources (DPR). Its activities are mainly focused on *in situ* and *ex situ* conservation of plants, education, research and recreation. It is open for the public ever since its establishment (Hughes & Lamichhane, 2017).

DMP had also established nine herbal farms in different climatic zones of Nepal for introduction, domestication, promotion of agro-technology development, cultivation, production, processing and germ plasm conservation of commercially important Medicinal and Aromatic Plants (MAPs) (Bhattarai et al., 2005).

ii. Development Phase (1990-2000)

As the DMP is renamed as DPR in 1994 (Bhattarai, 2010), former herbal farms, botanical survey units and gardens were restructured as the seven District Plant Resources Offices (DPRO) with their own botanical gardens in 1998. The gardens are now managed by the Plant Research Centers (PRC) formerly known as DPROs. The main objectives of these botanical gardens are *in situ* and *ex situ* conservation, herbal research and providing quality planting materials to local farmers and community forest user groups for commercial cultivation. These botanical gardens are also contributing in the field of research and development of the MAPs. Following are the list of ten botanical gardens managed by seven PRCs (Table 1).

Table 1. Botanical Gardens under Plant Research Center (PRC)

S.N.	Botanical Gardens	Plant Research Center (PRC)	Establishment
1.	Maipokhari Botanical Garden, Maipokhari	Ilam	1992
2.	Dhanushadham Botanical Garden, Dhanushadham	Dhanusha	1998
3.	Brindaban Botanical Garden, Hetauda	Makawanpur	1962
4.	Mountain Botanical Garden, Daman	Makawanpur	1965
5.	Tistung Botanical Garden, Tistung	Makawanpur	1965
6.	Dhakeri Botanical Garden, Dhakeri	Banke	1990
7.	Mulpani Botanical Garden, Kapurkot	Salyan	1990
8.	Dhitachaur Botanical Garden, Dhitachaur	Jumla	1990
9.	Debariya Botanical Garden, Dhangadhi	Kailali	1998
10.	Godawari Botanical Garden, Godawari	Kailali	1998

iii. Research and Extension Phase (2000 onwards)

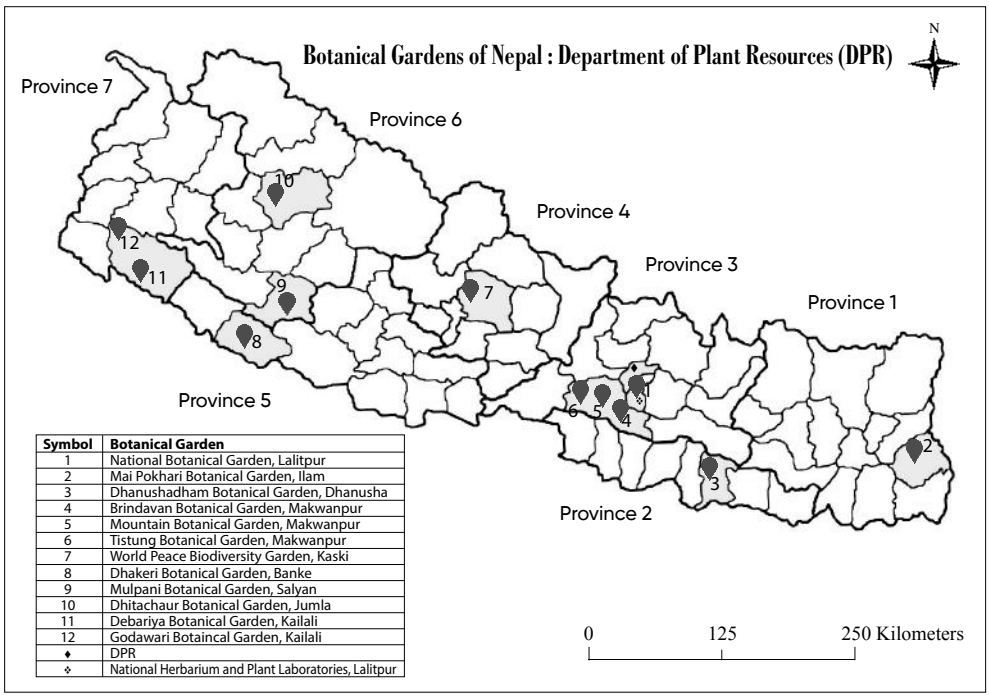
The Government of Nepal established the World Peace Biodiversity Garden (WPBG) in Pokhara on 2013. The garden is now managed by the Department of Plant Resources. It is in the designing and construction phase now. The beautiful forest south-east of the Fewa lake is a part of the garden. The place was formerly known as Raniban and was managed by the District Forest Office, Kaski as a protected forest (DPR, 2013).

Milestone of Forestry Sector Strategy (2016-2025) addresses the importance of botanical gardens in Nepal and considered there will be 20 botanical gardens by 2025 with better coverage in all physiographic regions (MoFSC, 2016). Under the new federal structure of Nepal, the federal government, province governments and local levels have the right to establish and manage the botanical gardens (MoFE, 2019). The District Plant Resources

Office (DPRO) has been renamed as the Plant Research Center (PRC) after restructuring of DPR in 2018. Increasing the roles and responsibilities, the PRCs are now focus their activities more in research and development of Medicinal and Aromatic Plants (MAP). Current activities include variety development, extension of cultivation of MAPs in pocket area of herbal zone in a large scale. They also provide grant to herbal farmers in cultivation and processing of MAPs. For research activities DPR also provide research fund to researchers (students). The ultimate goal of these activities is bio-prospecting of MAPs and release of their final products. Although the province and local government can establish and run the botanical gardens but all these botanical gardens of Nepal are under the jurisdictions of the Federal Government of Nepal, The Department of Plant Resources under the Ministry of Forests and Environment (MoFE) is the focal agency.

Botanical Gardens of Nepal

There are 12 botanical gardens (Fig. 1) under the premises of different PRCs including NBG and WPBG (DPR, 2021) in different ecological regions (Chaudhary et al., 2020), covering 794.7 ha of land. The Dhanushadham Botanical Garden, Brindaban Botanical Garden, Dhakeri Botanical Garden, and Debariya Botanical Garden and Godawari Botanical Garden are located in tropical region, Similarly, the National Botanical Garden, World Peace Biodiversity Garden, Tistung Botanical Garden, and Mulpani Botanical Garden are located in sub-tropical region, whereas, Maipokhari Botanical Garden, Dhitachaur Botanical Garden, and Dhitachaur Botanical Garden, located in temperate region (Lamichhane, 2018).



Symbol	Botanical Garden
1	National Botanical Garden, Lalitpur
2	Mai Pokhari Botanical Garden, Ilam
3	Dhanushadham Botanical Garden, Dhanusha
4	Brindavan Botanical Garden, Makwanpur
5	Mountain Botanical Garden, Makwanpur
6	Tistung Botanical Garden, Makwanpur
7	World Peace Biodiversity Garden, Kaski
8	Dhakeri Botanical Garden, Banke
9	Mulpani Botanical Garden, Salyan
10	Dhitachaur Botanical Garden, Jumla
11	Debariya Botanical Garden, Kailali
12	Godawari Botaincal Garden, Kailali
♦	DPR
❖	National Herbarium and Plant Laboratories, Lalitpur

Figure 1. Botanical gardens of Nepal.

Brief introduction of each botanical gardens are as follows:

1. National Botanical Garden (NBG) is situated in Godawari, Lalitpur district, Bagmati province. It is one of the internationally recognized botanical gardens and awarded the member of Botanical Garden Conservation International (BGCI) in 2015. NBG conserves the plant in both *in situ* and *ex situ* conditions, it extends over 82 ha of land at an altitudinal range of 1515-2000 m with natural forest of *Schima wallichii-Castanopsis indica* and *Alnus nepalensis*. The Garden offers fantastic view of the Phulchowki hill (2760 m), the highest peak of Kathmandu valley. Initial layout of this botanical garden was designed by two British scientists Geoffrey Herklots and Tony Schilling. Since then much of its activities have been focused on enriching the diverse collection of indigenous and exotic plant species by integrating to scientific research, conservation, education, demonstration and recreation. A prestigious VVIP (Very Very Important Persons) garden is designated in the botanical garden, where important species of plants have been planted by the Head of the states and Head of the Governments of different countries since 1974. Queen Elizabeth and Prince Philips of the United Kingdom also planted *Ilex excelsa* (Nepalese holy tree) in 1987 (Lamichhane et al., 2016). The garden conserves over 1000 species of rare endangered, endemic, medicinal and ornamental plants including wild orchids. An information and exhibition center at the entrance of the garden also serves the purpose of public information. The garden has also established a seed bank in 2019 which preserves 300 accessions of seeds. NBG has thematic gardens, some of them are physic garden, biodiversity education garden, terrace garden, special garden with water fountain and ornamental flower beds, orchid garden, rhododendron garden, Japanese style garden, fern garden, rock garden, ethno-botanical garden, VVIP plantation garden, rose garden, sand garden, coronation pond, taxonomic family garden, lily garden, rare and endangered plants garden. A collection of tree and shrub species are planted on the arboretum outside the main garden area. There is a short-graveled ring-road inside the garden which is being developed as an avenue of *Ginkgo biloba* in 2018. Similarly, there are glass houses for *ex situ* conservation of tropical plants, ferns, ornamental plants, cacti and medicinal plants. The ornamental plants are planted in the beds for public display. In addition, more than 80 species of butterflies and 200 species of birds are reported in the garden area. There is also shrine of *Masta* inside the garden where Chhetri people of *Bista* lineage worship during their *dewali* ritual. Mt. Manaslu can be seen from Manaslu house which also adds aesthetic value to the visitors. Visitors can buy ornamental plants from the sales house near the main gate. More than 450,000 visitors from Nepal, SAARC countries and other nationalities visit the garden in a year. Among them about twenty-five percent are students from many schools and colleges.

2. Mai Pokhari Botanical Garden is situated 15 km north of Ilam Bazaar, Province No. 1. at an altitude of 2121 m with an area of 200 ha. Small forest around an orbicular Maipokhari lake make this place majestic. This area is also a sacred place for the Hindu and Buddhist people. The forest has a mix of indigenous trees as well as an exotic tree *Cryptomeria japonica* which might have introduced from Darjeeling and adjoining area where massive plantation has been done. Mai Pokhari lake is listed as a Ramsar site in 2008. More than 20,000 domestic or foreign visitors enter here every year to enjoy the beauty of nature and to worship their sacred gods and goddesses. Some of the special features of the garden

are *in situ* and *ex situ* conservation of about 300 species of rare, endangered, endemic and native flora including wild orchids, different species of rhododendrons, medicinal plants, endemic bryophyte (*Sphagnum nepalense*) and endemic Salamander. The garden is divided into thematic gardens as an education garden, rock garden, orchid garden, fern garden, ornamental garden and rhododendron garden. There is also herbal nursery which produces seedlings of Himalayan yew (*Taxus* species).

3. Dhanushadham Botanical Garden is a tropical botanical garden situated about 20 km far from Janakpur airport of Dhanusha district, Province No. 2. The garden is nearby the Dhanushadham, a sacred place for the Hindus. According to legend, Lord Shiva's bow broke into pieces when it was arched by Lord Ram in a competition and a piece of the bow fell into this place. The garden extends over 107 ha of land at an altitude 276 m. Among the total area, 88.14 ha of land is covered by natural forest with tropical plant diversity. Some special features of the garden are *in situ* and *ex situ* conservation of about 103 species of rare, endangered and native plants. The garden has different thematic gardens such as education garden, rose garden, herbal garden, ornamental plants garden, ethno-botanical garden, religious garden and aquatic garden. The garden also conserves 30 plant species with religious value. The herbal nursery of the garden produces seedlings of *Cymbopogon citratus*, *Asparagus racemosus* and *Phyllanthus emblica*.

4. Brindavan Botanical Garden is situated 3 km south west from Hetauda, capital city of Bagmati Province. The covers 57 ha of land at the altitude of 405 m, where32 ha of land is covered with natural *Shorea robusta* forest and deciduous riverine forest. Visitors can enjoy the natural beauty of the garden along with the beautiful view of Rapti, Karra and Kukhreni River. Some of the features of the garden are *in situ* and *ex situ* conservation of over 315 species of rare, endangered, and native plants. The garden has different thematic gardens such as education garden, rock garden, herbal garden, sandalwood garden, rose garden, orchid garden, fern garden, taxonomic family garden, also exhibition sites for *Churia* plants and herbal nursery for producing seedlings of *Cinnamomum tamala, Rauvolfia serpentina, Aegle marmelos, Phyllanthus emblica, Asparagus racemosus* etc.

5. Mountain Botanical Garden is situated 80km south west of Kathmandu, Daman area, north side of Makawanpur district, Bagmati Province. The garden lies at an altitude of 2320 m and covers an area of 65 ha. It comprises natural forest of *Pinus wallichiana*, *Quercus semicarpifolia* and *Rhododendron arboreum*. Visitors can enjoy the garden with panoramic view of the Himalayan peaks and multilayered landscape of undulating hills and mountains. Special features of the garden are *in situ* and *ex situ* conservation of more than 220 species of rare, endangered, endemic and native plants especially of temperate and subalpine climatic zones. The garden has also thematic gardens such as ornamental plants garden, orchid garden, medicinal plant garden followed by domestication and germ-plasm conservation of 10 species of rhododendron and herbal nursery for producing seedlings of *Taxus* species.

6. Tistung Botanical Garden is situated 55 km far from Kathmandu, north side of Makawanpur district, Bagmati province. It lies at an altitude of 1900 m and spreads over 45 ha of land. The garden consists of natural forest of *Schima waliichii*, *Myrica esculenta and Pinus wallichiana*. The special features of the garden include *in situ* and *ex situ* conservation

of more than 300 species of rare, endangered and native plants followed by thematic gardens such as orchid garden, medicinal plant garden and ornamental plant garden including herbal nursery of *Zanthoxylum armatum* and demonstration plot of *Swertia chirayita.*

7. World Peace Biodiversity Garden is the first biodiversity garden situated at south west part of Fewa lake, Pokhara, Kaski district, Gandaki province. The garden lies at an altitudinal ranges of 775-1078 m and covers 164.76 ha of land. The garden comprises natural forest of *Schima wallichii* and *Castanopsis indica*. Special features of the garden are *in situ* and *ex situ* conservation of more than 300 Plants species including rare, endangered and native plants followed by conservation of tree fern (*Cyathea spinulosa*) in their natural habitat. The garden has also beautiful plant landscape and thematic garden (orchid garden, biodiversity education garden).

8. Dhakeri Botanical Garden is situated 11 km east from Nepalgunj, Banke district, Lumbini Province. The garden lies at an altitude of 170 m which covers 5.29 ha of land. It comprises natural forest of *Shorea robusta*. The special features of the garden are *in situ* and *ex situ* conservation of 400 plant species including rare, endangered, and native plants. There is also thematic garden such as orchid garden, ethno-botanical garden, fern garden, red sandalwood garden and aquatic garden along with herbal nursery of *Rauvolfia serpentina, Piper longum, Phyllanthus emblica* and domestication and germ plasm conservation plots of MAPs.

9. Mulpani Botanical Garden is situated 25 km north from the Dang Valley. Is lies in Salyan district, Karnali Province. It is situated at an altitude of 1420 m and covers 5.5 ha of land with a natural forest of *Pinus roxburghii*. The special features include plant landscape and thematic garden such as biodiversity education garden, fern garden, rock garden and orchid garden) along with herbal nursery of *Zanthoxylum armatum*, *Cinnamomum tamala* and *Rauvolfia serpentina* as well as domestication and germ plasm conservation plots of MAPs.

10. Dhitachaur Botanical Garden is situated at Dhitachaur, 3 km from Khalanga, Jumla district, Karnali Province. It lies at an altitude of 2500 m and covers 4.5 ha of land with natural forest of *Pinus wallichiana*. The special features of the garden are *in situ* and *ex situ* conservation of 47 species including rare, endangered and native plants. There is also thematic garden such as rose garden, lily garden, primula garden, orchid garden, fern garden. It also has herbal nursery of *Delphinium himalayii*, *Cedrus deodara, Juglans regia* and domestication and germ plasm conservation plots of MAPs.

11. Debariya Botanical Garden is situated 5 km from Dhangadi city in Kailali district, Sudurpaschim Province. It lies at an altitude of 170 m and covers 149.5 ha of land with natural forest of *Shorea robusta*. The garden is rich in wetlands and beautiful landscape with ornamental plants. The three natural lakes namely Jokhar, Murfutta and Murfutti are inside the garden. The garden is rich in wetland biodiversity, local and migratory birds. The special features of the garden are *in situ* and *ex situ* conservation of 500 species of rare, endangered and native plants. The garden has also Plant landscape and thematic garden sites such as education garden, taxonomical family garden, herbal Garden, ornamental flower garden, fern garden, ethno-botanical garden, aquatic plant garden, CITES garden, rock garden, Churia plant demo garden. It also has herbal nursery of *Asparagus racemosus* and domestication and germ plasm conservation plots of MAPs.

12. Godawari Botanical Garden is situated at 21 km north from Dhangadi city in Kailali district, Sudurpaschim Province. It lies at an altitude ranging from 185-1500 m covers 100 ha of land with the natural forest of *Shorea robusta, Holarrhena pubescens, Alstonia scholaris, Pterocarpus marsupium, Cassia fistula* and *Oroxylum indicum*. The special feature of the garden is *in situ* conservation of rare, endangered and native plants with unique landscape of Churiya hill.

Discussion

The botanical gardens of Nepal hold large collections of living plants and provide materials for teaching about the incredible diversity of the plants, the relationship among them, people and environment, importance of plants in our daily life with economically, culturally and aesthetically significance. They are playing significant role in *in situ* and *ex situ* conservation of rare, endangered, threatened, endemic and other useful plant species of Nepal. The *in situ* and *ex situ* conservation of plant species in botanical gardens serve the purpose of buffer against extinction of rare and endangered species and also promote in research, education and display (Chaudhary, 1998). These botanical gardens of Nepal are also involved in the implementation of species conservation plans (Rhododendron*, Nardostachys jatamansi, Dactylorhiza hatagirea, Pterocarpus marsupium,* etc.) by collecting and conserving their germplasm. These gardens are also conducting their activities for rescuing, collection, propagation and conservation of rare, endangered, threatened, endemic and other useful plant species. They also conserve the germ plasm by creating suitable condition inside the glass house and in seed bank (Lamichhane, 2018). The tropical house of NBG has been conserving more than 50 species of tropical plants. The cactus house conserves about 40 species of cacti and succulent plants. The seed bank of NBG has preserves germplasm (seeds) of 300 plant species (NBG, 2021). The botanical gardens work for the public education and awareness centre for local people, visitors and students. About 70,000 students out of 4,50,000 visitors from different schools and colleges visited the NBG every year for educational purpose (NBG, 2020). They are also raising awareness by sharing information about the plant diversity and their importance. Conservation education and interpretation in botanical gardens are going on mainly related with the horticultural and conservation techniques of plants and their sustainable use. Therefore, botanical gardens are important to increase conservation awareness. Similarly, the gardens also provide training programs to the local people for the conservation of MAPs, their cultivation technique and utilization. 'Friends of Botanical Gardens' program conducted by NBG is focused on school's children to enhance their knowledge on plants diversity.

Biodiversity Education Garden (BEG) of NBG was established in 2016 on the auspicious occasion of British-Nepal 200 years of diplomatic relationship. This is a collaborative project between Government of Nepal and British Embassy, Kathmandu as well as RBGE, Scotland, UK. BEG represents about 120 species of native plants of tropical, subtropical and temperate regions of the Nepal Himalaya. Main objective of this garden is to provide information about the plants and their uses to students and general public. Training program has been an important function of botanical gardens and many of the people now involved in cultivation of MAPs by receiving their training at botanical gardens. Botanical gardens

of Nepal are conducting conservation education and awareness programs to local people, visitors and school children. The aim is to increase conservation and awareness and to educate people about the urgent need to conserve plants and their sustainable utilization. The gardens also offer informal education to visitors, so that they can learn about botanical gardens and their role to save and conserve the flora by gaining the first-hand experience of plants and appreciate 'nature'. They will acquire practical skills and theoretical aspects of plant diversity conservation, propagation and landscaping.

The research on domestication and cultivation technology of commercially important MAPs (*Asparagus racemosus, Cymbopogon winterianus, Rauvolfia serpentina, Mentha spicata, Zanthoxylum armatum, Cinnamomum tamala*, etc.) had already been developed from different botanical gardens of Nepal. Research on variety development of Swertia, Mentha and Chamomile is going on. Study and research on ornamental plants like Chrysanthemum and other indigenous ornamental plants in NBG also helps to promote the development of floriculture in Nepal (Shrestha, 2010). These botanical gardens are also playing important role in the documentation of traditional knowledge (TK) of local people and local flora publication also. The botanical gardens of Nepal are closely linked with the livelihood support and sustainable utilization of MAPs by distributing quality seedlings of MAPs. These botanical gardens also provide technical support and facilitation in seedling production, cultivation, processing and marketing of MAPs (DPR, 2020).

The botanical gardens attract large number of visitors for observation of plant diversity as well as medicinal plants, rare and endangered plants, ornamental plants and other curious plants, so botanical gardens also provide public recreation and garden therapy too. Study shows that majority of the visitors visited this botanical garden for the purpose of recreation and education (Lamichhane, 2019).

Conclusion

Botanical gardens are playing important role in plant conservation in natural habitat specially to threatened species, in addition, to aesthetic and recreational value. Collection and propagation of native taxa, and maintenance of plant stocks for *ex situ* conservation and sustainable utilization, exchange of seeds among other botanical gardens for growing large collections of rare and endangered plants and holding them safely in cultivation or seed banks are crucial roles of botanical garden. Botanical gardens of Nepal are also conserving a large number of rare and threatened species of plants in different ecological regions. The Botanical gardens also serve as a research center for MAPs and other useful plants; also serving for conservation education and awareness programs to local people, visitors and school children to increase conservation and awareness. Plant diversity of Nepal is facing the threat of extinction due to unsustainable harvesting and over exploitation, destructive agricultural and forestry practices, urbanization, environmental pollution, land-use changes, exotic invasive species, global climate change, forest fire and other developmental activities. To cope with such risks, there is a need to increase the efforts to develop integrated conservation approaches for plant species conservations and to educate people for the conservation of plants and their sustainable utilization as a priority.

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Status of Fish Diversity and Production in Bangladesh

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Abstract

Data and information sources are used from the direct interview with the individual, publication of the Department of Fisheries (DoF) and related non-published grey literature. The country has an inland water area of about 45,000 km^2 and about 710 km long coastal belt. The fisheries sector contributes 3.50% to the national GDP, 25.71% to the agricultural GDP and more than 2.0% to the total export earnings. The total production was 4.384 m.mt fish in 2018-2019. In contrast, inland open water (capture) contributes 28.19%, and inland closed water (culture) contributes 56.76% to total production. This sector contributes significantly to food security by providing safe and quality animal protein; about 60% (62.58 g/day/capita) of animal protein in daily dietary requirement comes from fish. About 11% of the total population is engaged with this sector on a full and part-time basis for their livelihoods. Bangladesh is blessed with substantial open water resources with a wide range of enriched aquatic diversity, comprising almost 260 freshwater fish species and other aquatic lives. However, due to the decline and degradation of wetland resources, the stock of inland capture fisheries has been reduced remarkably. Bangladesh's fisheries sector has been facing challenges by natural and anthropogenic causes such as climate change, environmental pollution, industrialization, overexploitation, using destructive fishing gear, pesticide, and agrochemical. As a result, commercial important 03 species were extinct (E), 07 species critically endangered (CR), 30 endangered (EN), 27 vulnerable (VU), 29 not threatened (NT), 122 least concern(LC) and 42 data deficient (DD) position from the point of biodiversity. For the development of biodiversity, healthy ecosystem and safety food, hilsa fishery management technology, improved biological management technology of fish sanctuary, beel nursery, fingerlings stocking, fish habitat rehabilitation, breeding ground conservation, pen culture and fish regulation act-1950 is to be developed to restrict the declination of resources and enhance production and number of population.

Key words: Beel nursery, Biodiversity, Conservation, Critically endangered, Endangered, Extinct, Hilsa fishery management, Illegal fishing.

Introduction

Bangladesh is situated in the north eastern part of South Asia between 88º01' and 92º41' east longitude and 20º34' and 26º38' north latitude. India bounds the country on the west, north and northeast while Myanmar on the south-east and the Bay of Bengal on the south. The area of the country is 1,47,570 sq. km. The population of the Bangladesh is at 162.18 million in 2016 (Latest United nation estimate). The growth rate of population census is 1.3 per annum. The alluvial soil enriched by heavy silts deposited by rivers during the rainy season. There are six seasons in a year. Winter, summer and monsoon are prominent. Winter

begins in November and ends in February. In winter, the minimum temperature is recorded at 7ºC and the maximum temperature recorded in summer at 37ºC. This period accounts for 80% of the total rainfall. The average annual rainfall varies from 1429 to 4338 mm.

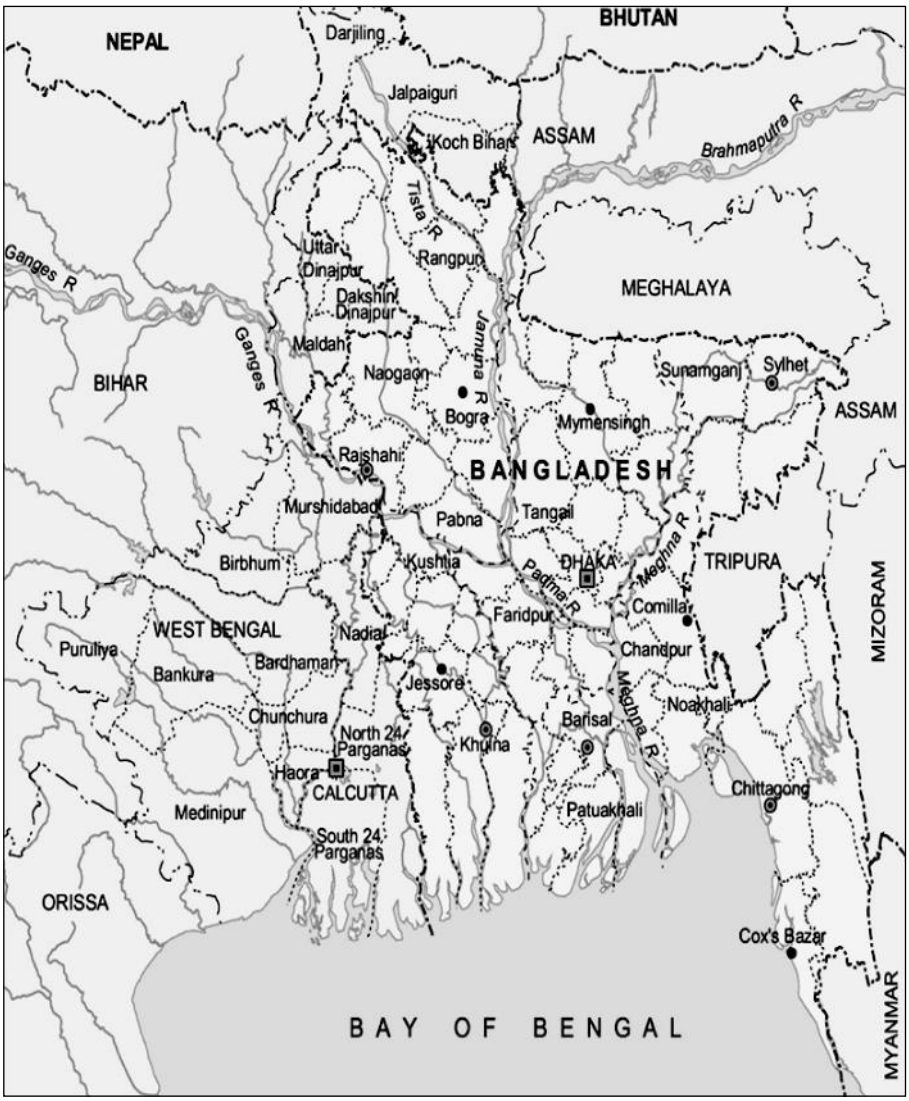

Figure 1. Map of Bangladesh showing essential rivers.

Fish habitats in Bangladesh are primarily a deltaic country in the Ganges, Brahmaputra and Meghna (GBM) drainage systems. Important Rivers the Padma, Jamuna, Teesta, Brahmaputra, Surma, Meghna and Karnaphuli exists in the country (Fig. 1). There are about 700 rivers, including 230 tributaries with a total length of about 24140 km. These form the main perennial water areas that provide both breeding and feeding habitats. The country is mainly an agrarian economy is naturally endowed with a vast fresh and coastal water resources and the world's longest continuous sea beach. The country is blessed with

huge open water resources with a wide range of enriched aquatic diversity, comprising almost 260 freshwater fish species (Fisheries Statistical Yearbook of Bangladesh, 2018). Of the reported species, 104 are considered riverine species, 36 migratory (travelling rivers and floodplains), and the rest 113 are floodplain resident species (FAP 17, 1994). Besides, a total of 20 species of prawns, 4 species of crabs and 26 species of molluscs are known to occur in Bangladesh's freshwaters (Siddiqui et al., 2008). However, due to mainly decline and degradation of wetland resources, the share of inland capture fisheries has been reduced remarkably during recent past decades.

The natural migration and recruitment of ûshes and other aquatic lives between rivers and ûoodplains have been blocked. As a result, many important ûsh, prawn species and other aquatic lives of rivers, ûoodplains and estuaries have become threatened and endangered (IUCN, 2000). The multipurpose Bridge of river, which was constructed recently, has caused severe impacts, particularly on the existing ecosystem, the open water (capture) production of ûsh spawn collections in Jamuna river both upstream and downstream of the bridge site and its adjacent rivers and ûoodplains downstream (Ali, 1997).

It has been estimated that Bangladesh has total open water bodies 3.917m. ha, inland closed (culture) water bodies 0. 80 m. ha and marine water bodies 710 km. These three areas were rich biodiversity of native wild fish species, prawn, snail, crabs, turtles and other aquatic life. Due to over-exploitation and various ecological changes, some important fish species have disappeared from the wetland. The aquatic fauna of Bangladesh is under severe threat due to over-exploitation and environmental degradation.

Materials and Methods

Study Area

The study was conducted in 64 districts of Bangladesh.

Data Collection

A sampling plan was run for a long time to get an accurate picture of the catch and catch composition. A semi-structured questionnaire was developed. Primary data was collected by focus group discussion (FGD), local ecological knowledge (LEK) and direct interviews with the individual respondent. Questions were asked systematically, with the framed questionnaire. Being a rapid survey, the study gives only a broad picture of a stock of fauna that was recorded through fish landing centers, Wholesaler, different market survey, directly from fishers', hatchery owner and fish farmer. Current data were collected through different stakeholder related to fisheries sectors.

Secondary data were collected mainly from the Department of Fisheries (DoF). Relevant literature was reviewed, and internet sites were explored to have relevant information. Fish harvester's, producers, transporters and packers were also met alongside to get more information about needs and gaps of the field.

Analysis of Experimental Data

The data were analyzed through one-way ANOVA using SPSS program to find out whether any significant difference existed among different data (Duncan, 1955; Zar, 1984). Standard deviation in each parameter was calculated and expressed as mean ±S.D.

Results

Fish Production

The fisheries sector contributes 3.57% to the national GDP, 25.30% to the agricultural GDP and more than 2.0% to the total export earnings (Fisheries Statistical Yearbook of Bangladesh, 19). Fish alone supply a per capita fish consumption of 62.58 g/day in our daily dietary requirement. Bangladesh is one of the world's leading fish producing countries with a total production of 4.384 mill. mt in 2018-19 (Fig. 2).

Contribution of inland capture fisheries

Inland open water area belongs to 3.917 (mill. ha) which is included rivers and estuaries, Sundarbans water resource in the forest, beels, Kaptai Lake and floodplain. In 1983-84, the contribution of inland capture fisheries fish production was 0.472 mill.mt and production percentage was 62.59% and in 2007-08, inland capture fisheries fish production was 1.027 mill.mt and decreased at 41.36%. Finally, in 2018-19, inland capture fisheries fish production was 1.236 mill.mt. and total fish production was decreased sharply with a value of 62.59% to 28.19% during last 36 years (Fig. 3). The regression type was Liner Trendline and the equation was y= - 17.20x +78.44 and where, R^2 is 0.982.

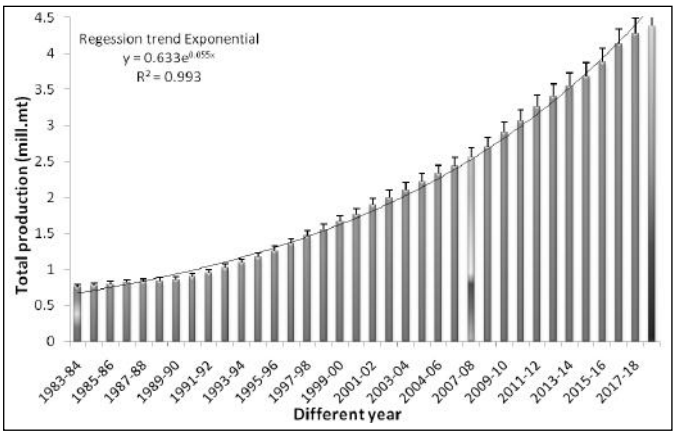

Figure 2. Last 36 years' production trends of fish in Bangladesh.

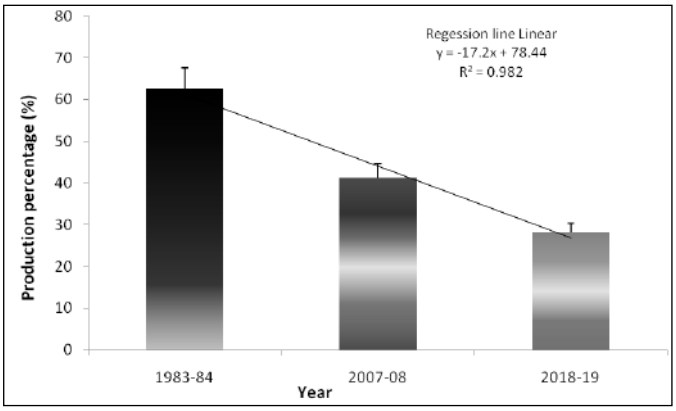

Figure 3. The fish production percentage (%) of open water (capture) last 36 years in Bangladesh.

Contribution of inland culture fisheries

Inland closed water area is belonged to 0.80 (mill.ha) which includes Pond, Seasonal cultured waterbody, Baor, Shrimp/Prawn

/crab Farm, pen and cage culture. In 1983-84, the contribution of inland closed water fish production was 0.117 mill.mt and production percentage was 15.53% and in 2007-08, inland capture fisheries fish production was 11.006 mill. mt and was increased at 39.23%. Finally, in 2018-19, contribution of inland culture fisheries fish production was 2.489 mill.mt. and total fish production was increased sharply with a value of 15.53% to 56.76% during last 36 years (Fig. 4). The regression type was Liner Trendline and the equation was y= 20.61x - 4.056 and where, R^2 is 0.992.

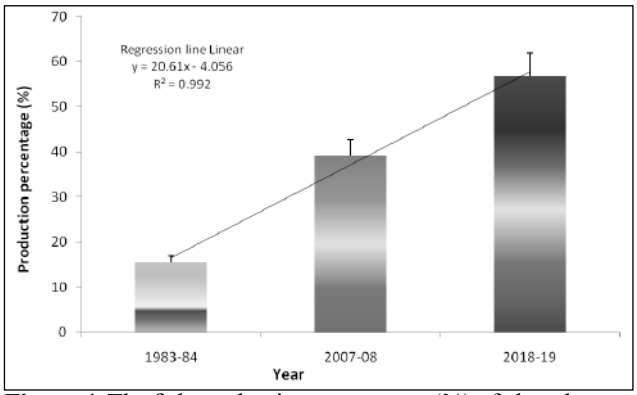

Figure 4. The fish production percentage (%) of closed water (culture) last 36 years in Bangladesh.

Contribution of marine fisheries

The area of marine fisheries is included Industrial (Trawler Fishing) and Artisanal. In 1983-84, the contribution of marine fish production was 0.165 mill. mt and production percentage was 21.38% and in 2007-08, marine fish production was 0.498 mill. mt and was increased at 19.41%. Finally, in 2018-19, contribution of marine fish production was 0.66 mill. mt. and total fish production was decreased sharply with a value of 21.38% to 15.05% during last 36 years (Fig. 5). The regression type was Liner trendline and the equation was y= - 3.415x + 11.95 and where, R^2 is 0.975.

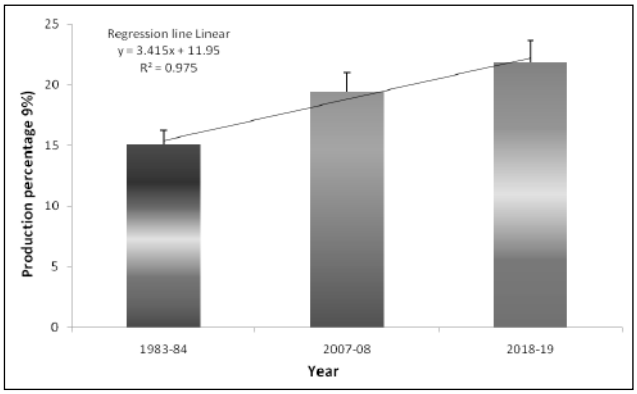

Figure 5. The fish production percentage (%) of marine water last 36 years in Bangladesh.

Carp and cat fish hatchling

Carp hatchling production was recorded in different river at 9.28 mt and about 926 government and private carp and cat fish hatcheries produced at 664.02 mt hatchling in the year 2018-19. Total production of hatchling was recorded at 666.52 mt in 2018-19 (Table 1).

Table 1. Annual production of carp and cat fish hatchling and PL

Source of production	No. of hatchery	Hatchling production (mt)
Natural	-	2.5
Artificial	1038	664.02
Total	1038	666.52

Post larvae (PL) production

The number of PL production in government and non-government Galda hatcheries was recorded at 1.58 crores and the number of PL production in government and non-government Bagda hatcheries was noted at 979.37 crores in the year 2018-19. Total production of PL was recorded at 980.95 crores in 2018-19 (Table 2).

Table 2. Annual post larvae (PL) production in 2019.

Source of Production	Galda Hatchery		Bagda Hatchery		Total	
	No. of Hatchery	PL Production (Crore)	No. of Hatchery	PL Production (Crore)	No. of Hatchery	PL Production (Crore)
Govt. Hatchery	27	0.4	0	0	27	0.4
Private Hatchery	8	1.18	42	979.37	50	980.55
Total	35	1.58	42	979.37	77	980.95

Current scenario of fisheries population

There are 162.18 million peoples live in Bangladesh. Near about 17.84 million (11%) people are somehow directly or indirectly related with different sectors of fisheries sector which is shown in the figure 6.

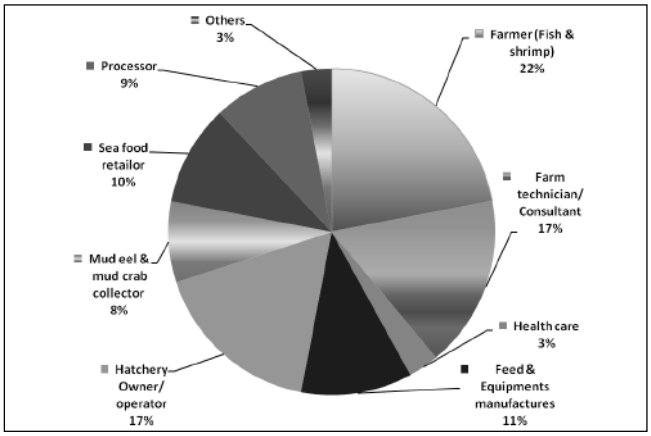

Figure 6. Current scenario of manpower of Fisheries sector due to COVID 19.

Enriched in fish biodiversity

Figure 7 showed that a well enrich fish biodiversity status is existing in Bangladesh. Fresh water fish species number is recorded 260, exotic fish species 12, marine fish species 486, fresh and marine shrimp species 48, tortoise 36 and crab 12, respectively (Fisheries Statistical Yearbook of Bangladesh, 2018-19). From biodiversity view point, figure shows important 02 fishes were Extinct (E), 07 Critically endangered (CR), 30 Endangered (E), 27 Vulnerable status (V), 29 Not Threatened (NT), 123 Least Concern (LC) and 42 Data Deficient (DD) position (IUCN, 2020).

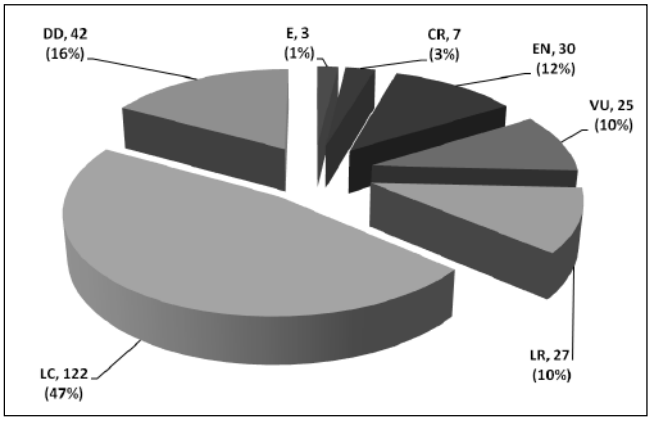

Figure 7. Status of fresh water fish biodiversity of Bangladesh.

Socio-eco-friendly Technology Applied to Enrich Production and Biodiversity

Hilsha (*Tenualosa ilisha*) conservation and management

National fish Hilsha contributed about 12.15% of total production comes from hilsa. Figure 8 is shown that hilsa production increased from 1.99-5.33 lakh mt in between 2003-04 to 2018-19 (Fisheries Statistical Yearbook of Bangladesh, 2019).

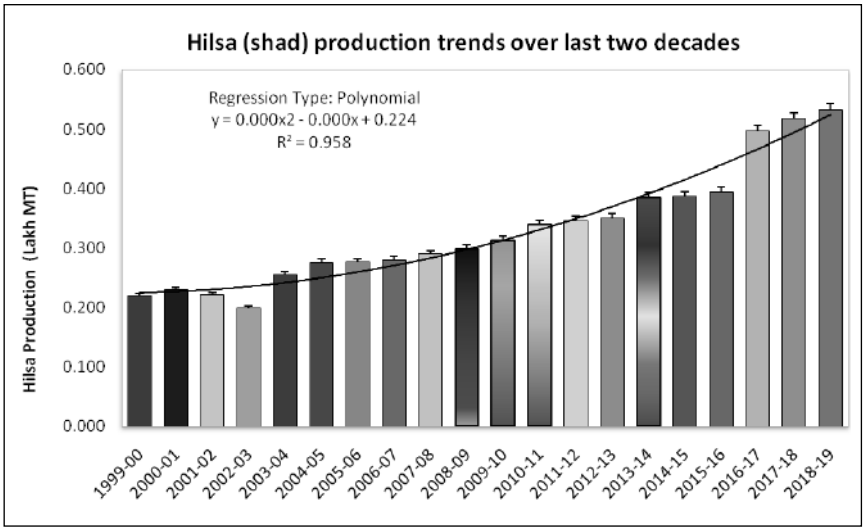

Figure 8. The production of Hilsha (*Tenualosa ilisha*) during 1999-10 to 2018-19 in Bangladesh.

The growth rate is 4.19%. Hilsha sector contribute on livelihood of coastal fisher's. Hilsa has been declared as Geographical Indicator (GI) of Bangladesh. Conservation of brood hilsha in peak spawning period (September-October), banning illegal catching of Jatka, provide food grain to fishermen during ban period of Jatka (fingerlings) catching. About six centaury zones of hilsa habitat in the Meghna and other rivers are declared for the better management of hilsa stock development. All kinds of fishing are prohibited in these zones between March and April. About 40 km stretch of the Andharmanik nursery ground in Patuakhali's Kalapara, fishing is banned here between November and January. Every year, jatka conservation week across the country aiming to mobilize people to conserve the Hilsa fry. In the same time inputs for alternative income generation (AIG) distributed to 32,509 fisher's families.

Hilsa production has doubled over the 12 years, by taking the government's efforts, including its ban on catching brood fish and fry, implementation of jatka conservation program, management of fish sanctuary and implementation of hilsa spawning protection activities.

Floodplain management

Stocking of fingerlings program:

Native carp and fishes were declined due to loss of habitat by siltation, pollution, over-exploitation of fish, collection of fish fry from natural resources, introduction of exotic species without proper research, indiscriminate use of pesticide for crop production etc. To improve

the productivity from open waters regular program of releasing fingerlings in floodplains was implemented in every year. Figure 9 is shown that in 2018-19, about 48994 mt fish fingerlings was released and extra fish production was 0.456 mt/ha annually and regression type was Polynomial Trendline and the equation was $y = - 18403x^2 + 24828x - 43561$ and where, R^2 is 0.981. At this period various regional endangered fish species was reappeared. b. Establishment of beel nursery Technology of beel nursery applied to increase the productivity floodplain (beel). Area of beel nurseries were 14770 ha (2018-19). Number of producing fingerlings was 120.30 million (Annual Report, 2018). Figure 10 is shown that total fish production in beel nurseries was16294 mt (1.103 mt/ha, 2018-19) and regression type was Polynomial Trendline and the equation was $y = - 1342.42x^2 + 19648x + 28674$ and where, R^2 is 0.969. As a result, extra fish production was increased and endangered fish species was reappeared.

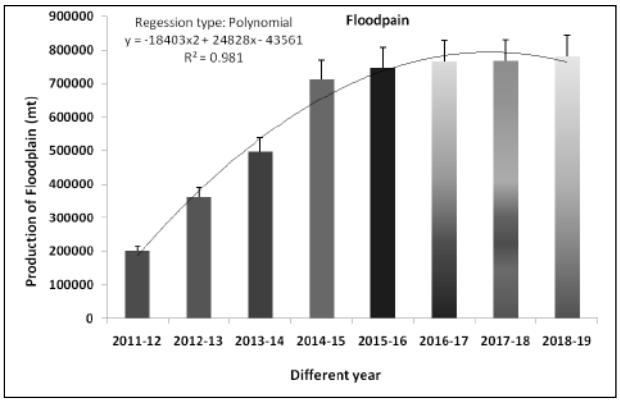

Figure 9. The fish production of floodplain during 1999-10 t0 2018-19 in Bangladesh.

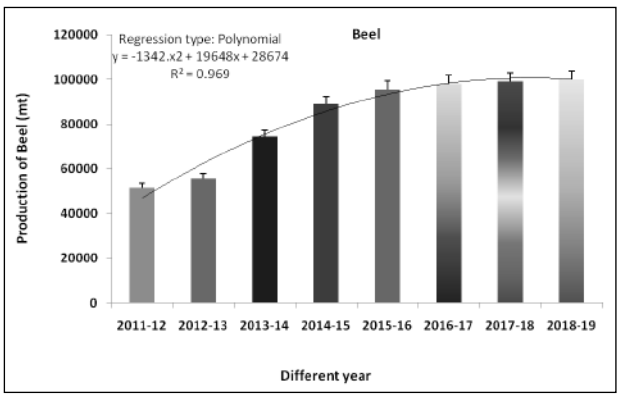

Figure 10. The fingerling production of beel nursery during 1999-10 t0 2018-19 in Bangladesh.

Establishment of fish sanctuary

Fish sanctuary is a scientific approach to conserve and propagate fish including endangered fish species (Fig. 11). A total number of 432 sanctuaries have established in different selective water bodies. Number of open water sanctuaries is 426 with an area 848.73 ha. Number of Hilsa centauries is 06 and its area is 432 km. Managed these fish

Figure 11. Figure shows the technology of a fish sanctuary in Bangladesh.

sanctuaries by the local beneficiaries of fisheries community. Protect and conserve fish species (fauna) from extinction and safe the status of aquatic biodiversity. At the same time recorded abundance of endangered species like as chital, Foli, Kalibaosh, Air, Tengra, Meni, Rani, Sarputi, Pabda, Kajoli, Gojar, Tara baim, etc.

Fish habitat rehabilitation

To restore those habitats government was taken various initiatives. During last 6 years 2100 hector water area were excavated/ re-excavated through 10 development projects. Besides in 2016-17, 280 hector water areas excavated/re-excavated (Fig. 12). About 3,000 mt additional fish/ yr. was produced by implementing these activities. Fish habitat was improved by applying Community based fisheries management policy. As a result, employment opportunities were created and increase income of local beneficiaries.

Figure 12. Figure shows how to habitat rehabilitation of a wetland in Bangladesh.

Natural breeding ground (Halda river) conservation

Department of fisheries has taken to conserve the natural breeding ground of major carps in the river, haor, and baor. The Halda River is only Indian major carp natural breeding ground in the country. Declared area is 40 km of Halda River as a fish sanctuary. Catching of fish is prohibited throughout the year in the Halda River. Six hatcheries are established at the bank of Halda River. Collecting eggs are hatched in hatcheries and supply throughout the country. During last 5 years (2011 to 2017) 6,042 kg hatchlings was recorded from the collected eggs. Hatchlings production

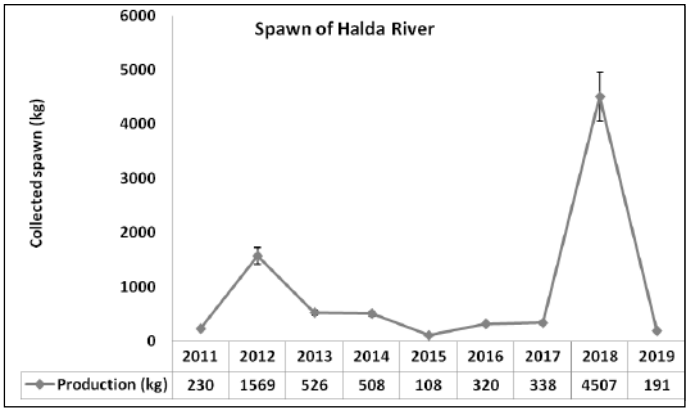

Figure 13. The production of spawn in the Halda River during 1999-10 to 2018-19 in Bangladesh.

was 191 kg (2019). Annual fry collection was about 609 kg. Before conservation collected only 832 kg in 2007 - 2010 and average production was only 208 kg (Fig. 13).

Expansion of pen farming in potential waterbodies

Pen farming is one of the technologies to increase the production of fish. It is one type of enclosure fish culture. The bottom of the enclosure is formed river, beel or any other water body bottom. Pens are constructed nylon or polyethylene mesh nets with traditional bamboo fences. Total fish production has been increased from 0.11 lakh MT in 2017-18 to 0.124 lakh MT in 2018-19 (Fig. 14).

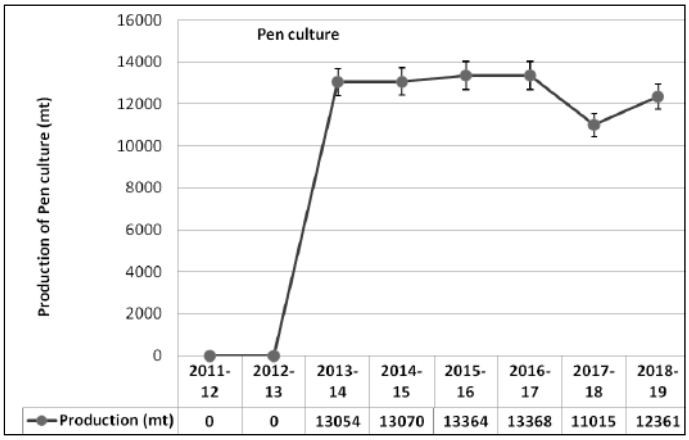

Figure 14. The production of fish in pen farming system between 2011-12 to 2018-19 in Bangladesh.

Expansion of cage culture in potential waterbodies

Cage culture is the best technology to increase the production in Bangladesh. Cage is blocked with nets, bamboo and floats in water. Cages are usually floated in rafts. A cage is totally enclosed on all, but the top side by mesh or netting. Fixed cages are used in shallow waters with appropriate muddy bottoms. Total fish production has been increased from 0.03523 MT in 2017-18 to 0.03802 lakh MT in 2018-19. Cage culture practice and production both are increasing day by day.

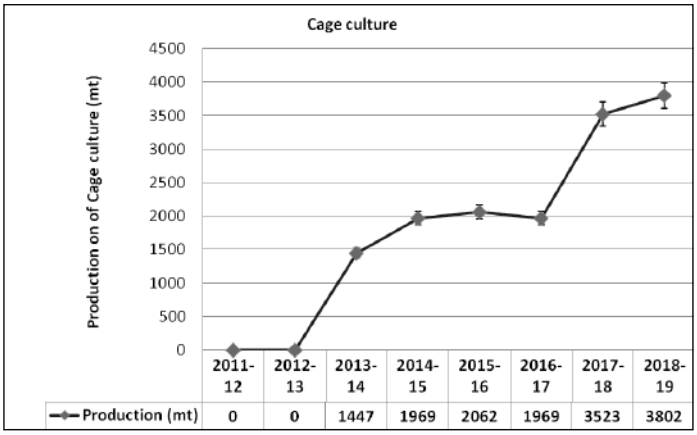

Figure 15. The production of fish in case culture system between 2011-12 to 2018-19 in Bangladesh.

Culture of Cuchia (Mud eel) in Bangladesh

Monopterus cuchia is a medicinery fish because of percentage of higher protein (18.7 gm protien, 0.8 gm fat, 2.4 gm carbohidrate and 185 mg calcium per 100 gm muscle (www.mcgill.ca). This species acts as a digestive trip, acts as a pain killer, Control high blood pressure and piles, Control asthma, acts as a remedy anemia and pox, per 100gm muscle contain > 1400 µgm of Retinol (Vitamin A_1), > 450 µgm of Dehydroretinol (Vitamin A_2) and > 3500 µgm of

Provitamin A[11] (Mishra et al., 1977). Amino acids (Alanine, Arginine, Glycine, Histidine, Leucine and Methionine) are available (www. genderaqua fish.files).

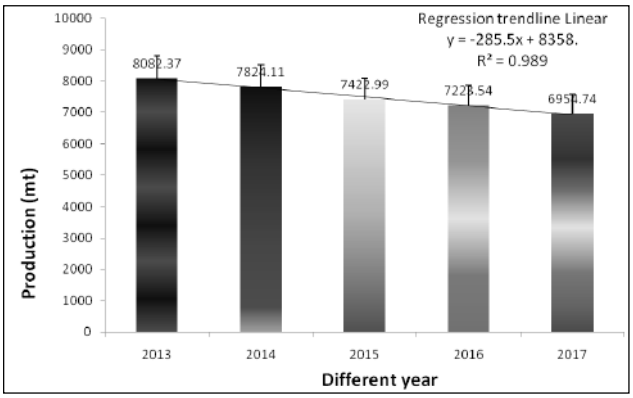

Figure 16. The status of mud eel (*Monopterus cuchia*) between 2013 to 2017 in Bangladesh.

A decreasing tendency of mud eel population was recorded in the 64 districts of Bangladesh between 2013 and 2017. In 2013 the recorded catch statistics was 8082.37 mt. But in 2017, the decreased catch statistic was recorded only 6954.74 mt (Fig. 16) and regression type was Liner Trendline and the equation was y= - 698.4 In(x) + 8170.20 and where, R^2 is 0.9563. About two culture technologies on eel culture developed. These are Aquaculture method and Natural resource management (NRM) System.

Mud crab status of Bangladesh

A decreasing tendency of mud crab population was recorded in the 64 districts of Bangladesh between2013 and 2017. In 2013 the recorded catch statistics was 25255.06 mt. But in 2017, the decreased catch statistic was recorded only 17406.64 mt (Fig. 17) and regression type was Liner Trend line and the equation was y= - 1953x + 26956 and where, R^2 is 0.995. About three culture technologies on mud crab culture was developed. These are Crab let culture Method, Pen culture Method and Cage Culture Method.

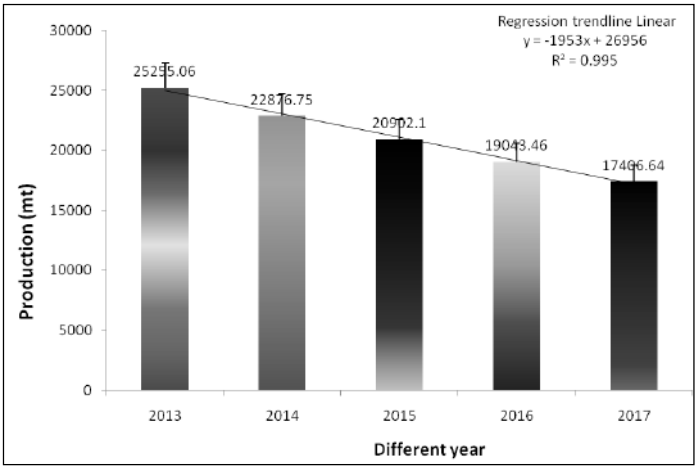

Figure 17. Decreasing tendency of mud crab population in the coastal area in between 2013 and 2018.

Major act and regulations

To accelerate the development activities of the fisheries sector, major acts and regulations are implemented in the fisheries sector. These are Tank Improvement Act, 1939; Fish Protection and Conservation Act, 1950 (amended in 1982, 1995, 2002); The Protection and Conservation of Fish Rules, 1985 (amended in 2008); The Fish and Fish products (Inspection and Quality Control), ordinance 1983; The Fish and Fish products (Inspection and Quality Control) Rules, 1997 (amended in 2008). The Marine Fisheries Ordinance, 1983. Fish Feed and Animal Feed

Act, 2010, Fish Hatchery Act, 2010. Implementation of Act, 2002, Fish Feed and Animal Feed Act, 2010 and Fish Hatchery Act, 2010 in the field level through mobile is resulted a wonderful production of fish in the country.

Climate change and fisheries sector

Climate change is a burning issue for the fisheries sector. It changes the genetics and breeding season of fishes. It increases harmful algal blooms and release toxins in water, decrease dissolved oxygen; increase incidents of disease, enhance breeding and growing season, changes ecosystems, alters the location and migratory rout of species, loss of areas for aquaculture and a considerable coastal area may go under salt water within 2030, loss of mangroves that act as nursery areas for fish species and decline river water level and low water flow and shrink water area in dry season. As a result, biodiversity of wetland is destructed and lost ecosystem health.

ID card for fishers

To ensure the rights of a fisher in the waterbody are provided ID card to every fisherman is to be established his right to the community and for management the waterbodies.

Discussion

In 2001-02, the total production was 1.89 mill.mt. But in 2018-19, the total production was increased gradually and reached at 43.84 mill.mt, whereas, open water contributes 28.19%, inland culture fisheries donate 56.76% and marine fisheries sector provided 15.05% (Fisheries Statistical Yearbook of Bangladesh, 2018-19). The catch statistics indicate that fishing pressure of the inland capture fisheries was increased sharply between 2001-02 and 2018-19. As a result, a decreasing trend in production percentage of the inland capture fisheries was clearly pronounced which is very similar to the report of Moyle and Leidy (1992). According to Moyle and Leidy (1992), worldwide 20% of all freshwater species are extinct, endangered or vulnerable. The total catch statistics of aquatic lives in the inland capture fisheries indicated that percentage of aquatic lives was sharply decreased which are very similar to the study of Chakraborty (2009, 2010).

The study clearly indicated that the ecosystem health is changing due to global affect, construction of flood control barrage, soil erosion, siltation and drainage structures and agro-chemicals. The aquatic lives are under severe threat due to over-exploitation and environmental degradation (Disaster, 1990). Stock of the wildlife brood fishes in their breeding ground was also suffered significant damages resulting in a reduction of biodiversity as noted by Allendroff (1988), Nishat (1993), Zaman (1993), and Chakraborty (2018).

National fish Hilsha contributed about 12.15% of total production comes from hilsa. Hilsa production increased from 1.99-5.33 lakh mt in between 2003-04 to 2018-19. The growth rate is 4.19%. Hilsha sector contribute on livelihood of coastal fisher's. Biological data indicated that hilsa goes through multiple reproductive cycles; therefore, a comprehensive understanding of reproductive biology, recruitment, stock abundance and habitats across the life cycle are necessary to accurately impose fishery regulatory measures, such as fishing ban in spawning season in Bangladesh (Hossain et al., 2019)

Native carp and fishes were declined due to loss of habitat by siltation, pollution, over-exploitation of fish, collection of fish fry from natural resources, introduction of exotic species without proper research, indiscriminate use of pesticide for crop production etc. To improve the productivity from open waters regular program of releasing fingerlings in floodplains was implemented in every year. Technology of beel nursery applied to increase the productivity floodplain (beel). Number of producing fingerlings was 120.30 million. Total fish production in beel nurseries was 1.103 mt/ha, 2018-19. As a result, extra fish production was increased and endangered fish species was reappeared which is agreed by Chakraborty (2011) and Chakraborty et al. (2013).

A community co-management committee was formed in every site of wetland to participate the activities of fingerling stocking, beel nursery, fish sanctuary and breeding ground. Participation of communities and their active involvement played an important role in overall management of open water body (Chakraborty, 2018).

Fish sanctuary is a scientific approach to conserve and propagate fish including endangered fish species.

A total number of 432 sanctuaries have established in different selective water bodies. Number of open water sanctuaries is 426 with an area 848.73 ha. Local beneficiaries of fisheries community managed these fish sanctuaries. According to Lakra (2010) and Lucia et al. (2008), conservation of fish diversity is essential to maintain ecological/nutritional and socio-economic equilibrium. A major portion (41.72%) of the total fish species recorded from the river was found threatened in Bangladesh (Galib et al., 2013). Technology, legislation, educational knowledge, action plan strategy, conservation practices are required to manage wetlands. So, it needs a comprehensive approach, strategy and integrated system combining political, economic, social, technological and institutional supports to address sustainable wetland conservation and the newly added crisis, climate change (Siddiquee & Hoque, 2011).

Halda River is the only pure Indian major carp breeding ground in Bangladesh. Fertilized eggs, spawn and fry were collected by local fisher's during March to June yearly. Spawn collection in the Halda river from the year 2011 to 2018 was 230, 1569, 526, 508, 107, 320 and 238 kg, respectively and this type of ups and downs indicated poor number of broods, poor management of breeding grounds and unhealthy ecosystem health of the Halda river (Chakraborty et al., 2019).

A decreasing tendency of mud eel population was recorded in the Bangladesh between 2013 and 2017. In 2013 the recorded catch statistics was 8082.37 mt. But in 2017, the decreased catch statistic was recorded only 6954.74 mt (Chakraborty, 2018). Decreasing tendency of mud crab population was recorded in the 64 districts of Bangladesh between 2013 and 2017. In 2013 the recorded catch statistics was 25255.06 mt and in 2017, the decreased catch statistic was recorded only 17406.64 mt (Chakraborty et al., 2018)

Climate Change increase harmful algal blooms, decrease dissolved oxygen; increase incidents of disease, enhance breeding and growing season, alter ecosystems, changes the location and migratory rout of species, loss of areas available for aquaculture and a considerable coastal area may go under salt water within 2030. Finally, affected biodiversity and lost ecosystem health of wetland, which is agreed by Brander, 2010.

Conclusions

World population will be grown from 6.9 billion to 9.0 billion and Global cereal demand to feed such population will grow from 2.1 billion tons to 3.0 billion tons in 2050 (FAO, 2009). The population of Bangladesh will be risen up and would be a great challenge to secure the future dietary demand of the increasing population. The sustainability in aquaculture in Bangladesh is under tremendous threat due to increasing population growth, changing climatic conditions, continuous decline in water area, vulnerable to the hazards of climate change-flood, drought, salinity; depleting water resources, declining production through indiscriminate use of agro-chemicals, insufficient scope and investment in research.

The action plan efforts for saving the biodiversity and stock of fishes to implement hilsa fishery management technology, enrich ecosystem of the river and wetland, declare the basins of the river as conservation including other's deeper wetland, upgrade management method for the conservation, enforce fishing rules, control over exploitation and illegal fishing, prohibition on harvesting of brood fishes and ensure sufficient water flow in the river.

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Invasive Alien Plants in the Protected Areas of Nepal: Diversity, Impacts and Management

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Abstract

Biological invasion is one of the threats to biodiversity conservation in the protected areas (PAs) globally. Although the problems of invasive alien plant species (IAPS) have been recognized in the PAs of Nepal, there is lack of comprehensive information to support management decisions. This paper provides an inventory of the IAPS in the PAs of Nepal based on a narrative review of the available literature and authors' personal experiences. Most of National Parks, Wildlife Reserves, Conservation Areas and Ramsar Sites located in Tarai, Siwalik and Middle Mountains are invaded by several IAPS, including globally worst species, whereas the PAs of the High Mountain and High Himalayan regions are relatively less invaded by the IAPS. Out of 27 IAPS present in Nepal, 23 species have been reported from the PAs of Nepal. Among them, the most frequently reported species in the PAs are *Ageratum conyzoides, Ageratina adenophora, Chromolaena odorata* and *Lantana camara*. Ramsar sites are invaded mainly by *Eichhornia crassipes, Ipomoea carnea* ssp. *fistulosa,* and *Leersia hexandra.* Degradation of wildlife habitat by *Mikania micrantha* in Chitwan National Park and reducing native plant species diversity by *Lantana camara* in Bardiya National Park have been reported. Management plans of most PAs have identified IAPS as one of the major management issues. Yet, control measures are largely ineffective and limited to small areas, thereby allowing unabated spread of the IAPS inside the PAs. Further research on aimed to identify drivers of the biological invasions in the PAs, and their dispersal pathways and ecological impacts, together with regular monitoring and survey of the IAPS in each PAs, can generate data and knowledge required for the management decision by the PAs authorities. The presence of a low number of IAPS or their absence in some mountain PAs provides the opportunity to prevent IAPS from invading these PAs. Prioritization of IAPS control in Nepal's PAs management will prevent further spread of the IAPS and help meet the long-term goal of biodiversity conservation inside the PAs.

Key words: Hunting Reserves, National Parks, Plant management, Ramsar sites, Wildlife Reserves

Introduction

Invasive alien species (IAS) affect the native biota and threaten biological diversity in virtually every ecosystem types of the world (Lowe et al., 2000; UNEP, 2001; Matthews & Brand, 2004; Matthews, 2004, 2005). However, the impacts of IAS are more severe in developing countries, mainly due to poor management responses, than in the developed countries (Pysek, 2008; Early et al., 2016). The IAS has been one of the main drivers of biodiversity

loss over the last 50 to 100 years (UNEP, 2005), whose invasion results in the loss of native species, biodiversity and ecosystem functioning, ecosystem services, and negatively affect livelihoods (Richardson et al., 2000; GISP, 2007; Blackburn et al., 2019). The Convention on Biological Diversity (CBD) recognizes the IAS problem as a global issue and calls on contracting parties to "prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats and species" (Article 8h). Similarly, resolution VIII/18 (Invasive Species and Wetlands) of the Ramsar Convention urges Ramsar Parties, among other, "to address the problems posed by invasive species in wetland ecosystems in a decisive and holistic manner" (GISP, 2007).

The IAS are expected to be less in protected areas (PAs) due to low human disturbances, high wilderness, and high biodiversity. However, the PAs could not remain pristine. Several IAS has invaded them due to tourism activities, construction works, and drainage system inside the area (Shackleton et al., 2020a). The IAS may be released, deliberately or accidentally, within a protected area, or may move into the PAs from surrounding areas (Mulongoy & Chape, 2004). Biodiversity inside many PAs is not fully explored yet, but they are already under threat by the IAS (Rico-Sanchez et al., 2020). The spread of IAS is a driver of change in the world's protected areas (Foxcroft, 2013; Shackleton et al., 2020b) and considered as a major problem for the managers of PAs (Foxcroft et al., 2017). Many PAs are also designated as World Heritage Sites, where the IAS has been reported as problematic (Shackleton et al., 2020a). The Convention on Biological Diversity (CBD) also recognized the threat of IAS in the PAs. Until 1980 biological invasion was considered to be confined in anthropogenically disturbed sites. But the problem of biological invasion is rampantly surging inside PAs. Researches have revealed that the IAS could have severe negative impacts on protected species and ecosystems (Foxcroft et al., 2017).

The PAs are designed to protect representative portions of natural landscapes, ecosystem processes, and biodiversity, contributing significantly to economic development (Margules & Pressey, 2000). In the studies of PAs of the world, problems like logging, land clearing, hunting, unplanned fires, overgrazing are mentioned but unfortunately impacts of IAS are seldom included. The PAs buffer biodiversity from many threatening processes. Specifically, park boundaries are thought to resist the spread of non-native plants into the PAs' core habitats. The probability of attaining PA goals increases by linking PAs through biological corridors. However, these biological corridors, together with rivers' drainage system, provide pathway for long-distance dispersal of non-native species (Foxcroft et al., 2011). Human settlement surrounding PAs is the source of propagule for the introduction of IAS, whereas roads significantly exacerbate IAS's dispersal in to the PAs (Foxcroft et al., 2011). The number and proportion of IAS often declines monotonically with increasing distance from park boundary to the core areas of the PA (Foxcroft et al., 2011).

In Nepal, biological invasion has been identified as an emerging threat to biodiversity and ecosystem services (Shrestha, 2019). At least 182 flowering plants are naturalized (i.e. alien species established in natural ecosystems) in Nepal (Shrestha & Shrestha, 2021). Among these naturalized plants, 27 species are considered as invasive alien plant species (IAPS) in Nepal. Most of the naturalized and invasive plants in Nepal are native to tropical and sub-tropical regions of the Americas (Tiwari et al., 2005; Bhattarai et al., 2014). Among them,

23 IAPS have been reported from the PAs and adjoining regions with negative impacts on agriculture, livelihood and ecosystems (Shrestha et al., 2019). Four of the IAPS in Nepal (*Chromoleana odorata, Eichhornia crassipes, Lantana camara* and *Mikania micrantha*) are also among the 100 of the world's worst invasive alien species (Lowe et al., 2000). Majority of the IAPS in Nepal are currently confined to low land below 2000 m asl (Tiwari et al., 2005). The diversity and abundance of the IAPS are high in lowlands and southern region of Nepal, including Tarai, Siwalik and Middle Mountains from east to west (Shrestha, 2016). However, climatically suitable areas of the most IAPS are likely to climb up and shift to higher elevation with future climate change scenarios (Shrestha & Shrestha, 2019). Mountain regions generally are considered to be at low risk from the IAS. However, recent studies show that mountain ecosystems are no longer resistant to biological invasions. Due to increasing tourists, climate warming and increasing human disturbance, future invasion risk is likely to increase (Kueffer et al., 2013). Changing climate will make mountain regions, including the mountain PAs, more favourable to lowland species than they are currently (Pauchard et al., 2009).

Protected Areas of Nepal

The protected areas (PAs) of Nepal cover 23.39% of the total land of Nepal. The Protected Areas system include 12 National Parks, 1 Wildlife Reserve, 1 Hunting Reserve, 6 Conservation Areas, and 13 Buffer Zones extending from lowland Tarai to high mountains (Fig. 1, Table 1; www.dnpwc.gov.np, accessed on January 2021). Out of the total area of PAs, 67.84% is in High Mountains and High Himalaya, 1.33% is in Middle Mountains and the remaining 30.83% in Tarai and Siwalik regions (Shrestha et al., 2010).

Rapid spread of the IAPS in PAs of Nepal has been identified as one of the threats to PAs management (Pandey et al., 2020). Nearly half (49%) of the previous studies on IAPS of Nepal have been conducted in PAs, and nearly two thirds (62%) of the studies in PAs have been conducted in the Chitwan National Park (Pandey et al., 2020). The PAs located in remote and mountains areas remain unexplored in the context of plant invasions. Such type of geographical biasness in biological invasion studies exists globally (Pysek et al., 2008). Since the status of IAPS in protected areas is not known adequately (Shrestha, 2019), an attempt has been made to synthesize the current state

Figure 1. Map of Nepal showing protected areas (Source: DNPWC)

Table 1. Brief information about protected areas of Nepal

	Name	Area (km^2) (Core area + Buffer zone)	Gazetted year	Location	Geographical region
National park	Banke NP	550+343	2010	Banke, Dang, Salyan	Tarai-Siwaliks
	Bardia NP	968+327	1976	Banke, Bardia, Surkhet	Tarai-Siwaliks
	Chitwan NP	952.63+729.37	1973	Chitwan, Makawanpur, Parsa, East Nawalparasi	Tarai-Siwaliks
	Khaptad NP	225+216	1984	Bajhang, Bajura, Doti, Achham	Middle Mountains
	Langtang NP	1710+420	1976	Nuwakot, Rasuwa, Sindhupalchok	High Mountains
	Makalu-Barun NP	1500+830	1992	Solukhumbu, Sankhuwasabha	High Mountains
	Parsa NP	627.39+285.30	1984	Chitwan, Makawanpur, Bara, Parsa	Tarai
	Rara NP	106+198	1976	Mugu, Jumla	High Mountains
	Sagarmatha NP	1148+275	1976	Solukhumbu	High Himal
	Shey-phoksundo NP	3555+1349	1984	Dolpa, Mugu	High Himal
	Shivpuri Nagarjun NP	159+118.61	2002	Kathmandu, Sindhupalchok, Nuwakot, Dhading	Middle Mountains
	Suklaphanta NP	305+243.50	1976	Kanchanpur	Tarai
Conservation area	Annapurna CA	7629	1992	Mustang, Pokhara	Lower Himal- High Himal
	Api-nampa CA	1903	2010	Darchula	High Himal
	Krishnasar CA	16.95	2009	Bardia	Tarai
	Kanchanjunga CA	2035	1998	Taplejung	High Himal
	Manaslu CA	1663	1998	Gorkha	Middle Mountains - High Himal
	Gaurishankar CA	2179	2010	Dolakha, Sindhupalchok, Ramechhap	High Himal
Wildlife reserve	Koshi Tappu WR	176+ 173	1976	Sunsari, Saptari, Udyapur	Tarai
Hunting reserve	Dhorpatan HR	1325	1983	Rukum, Myagdi, Baglung	High Mountains

Source: Compiled from management plans of the respective protected areas

of knowledge of IAPS in Nepal's PAs, which is mainly based on the published literature and authors' field observations. In addition to the PAs, this paper also provides a brief account of the IAPS problems in Nepal's Ramsar sites, many of which are located inside the PAs.

Diversity and Impacts of Invasive Alien Plant Species in the Protected Areas

In the following sections, we have described basic features of different PAs of Nepal with highlight of major IAPS that have invaded the PAs. List of IAPS reported in different PAs has been presented in table 2. Twenty-three out of 27 species reported from Nepal are known to present in the PAs of Nepal. Species like *Mimosa diplotricha* C. Wright, *Myriophyllum aquaticum* (Vellozo) Verdcourt, *Spergula arvensis* L. and *Spermacoce alata* Aubl. have not been reported yet from the PAs of Nepal.

1. National Parks

Chitwan National Park (CNP): It is the first national park declared in Nepal and is located in the subtropical Inner-Tarai lowlands and Siwaliks of south-central Nepal. UNESCO granted the status of the World Heritage Site in 1984 as having universal value and conservation importance (Shackleton et al., 2020b). Over 16 IAPS have been reported from the CNP (CNP, 2018). The most common among them are *Mikania micrantha, Chromolaena odorata, Lantana camara,* and *Parthenium hysterophorus*. *Mikania micrantha* has degraded the habitat of one-horned rhinoceros. A study has revealed that 44% of the rhinoceros habitats in Chitwan National Park were highly affected by *M. micrantha*, including the riverine/subtropical mixed hardwood forests, tall grass and wetland habitats, which were the prime habitats of rhinoceros and ungulates (Murphy et al., 2013). Invasion by *M. micrantha* has significantly reduced the native diversity and tree regeneration (Sapkota, 2007). It is one of the high risk-possessing IAPS in Nepal (Tiwari et al., 2005). Manual cleaning of *M. micrantha* is almost impossible in CNP as it has terrifically invaded the area (Khadka, 2017). The open places and forest edges are largely invaded by *Chromolaena odorata* and *Lantana camara.* In the upper Siwalik hills, *Ageratina adenophora* is also common. Wetland habitats inside the park and buffer zone are highly invaded by *Eichhornia crassipes* and *Pistia stratiotes.* The Besshazari tal (a Ramsar Site) of buffer zone is heavily invaded by *Eichhornia crassipes* and *Leersia hexandra* (Siwakoti & Karki, 2009).

Parsa National Park (PNP): It is located to the east of Chitwan National Park in south-central Nepal. Tarai and Siwalik regions are represented in the Park. Over 14 IAPS have been reported from this Park; among them, *Chromolaena odorata* is the most frequent and abundant species, followed by *Senna tora*, *Ageratum conyzoides, Mikania micrantha* (Chaudhary et al., 2020). The frequency and cover of the IAPS were found to be higher in the location closer to the settlement than in the core areas of the Park. High cover of the IAPS has negatively affected tree regeneration (Chaudhary et al., 2019).

Bardia National Park (BNP): Located in the western Nepal, this Park extends from Tarai in the south to the Siwalik Hills in north. A total of 12 IAPS has been reported from the

park; among them *Chromolaena odorata, Lantana camara, Ageratum houstonianum* are the most frequent and abundant species. *Lantana camara* had significantly reduced native plant species richness and diversity (Bhatta et al., 2019).

Banke National Park (BaNP): It is located to the east of Bardia National Park and connects the Suhelwa Wildlife Sanctuary of India towards south. The flood plains of Tarai and foothills of Siwaliks within the Park are highly invaded by IAPS. More than 13 IAPS have been reported from the Park (BaNP, 2018). *Lantana camara* and *Ipomoea carnea* ssp. *fistulosa* have invaded grasslands of the park, the prime habitats for royal Bengal tiger, Asiatic wild elephant and four-horned antelope (Napit, 2015).

Suklaphanta National Park (SuNP): Located in the Tarai region of far-west Nepal, the SNP has extensive floodplain grasslands, the habitat for Bengal tiger, rhinoceros and swamp deer, among others. These grasslands are invaded by 16 IAPS which has altered the assemblages of grass species with potential negative impacts on the grassland ecosystem (Bhattarai, 2012). *Lantana camara* is a major IAPS invading terrestrial ecosystem and *Eichhornia crassipes* is dominant in wetland ecosystem (Bhattarai, 2012).

Shivpuri Nagarjun National Park (SNNP): It is located in the Middle Mountain regions at the northern fringe of Kathmandu Valley. About a dozen of IAPS have been reported from the SNNP. Common IAPS includes *Ageratina adenophora, Ageratum conyzoides, Lantana camara, Parthenium hysterophorus* and *Bidens pilosa* (SNNP, 2017). Management plan has included IAPS as a problem but there is no scientific research carried out till now focusing IAPS in the SNNP.

Khaptad National Park (KNP): The KNP is located in the Middle Mountains of far-west Nepal and expands on Bajhang, Bajura, Doti and Achham districts. The Park is famous for medicinal herbs. Study about the IAPS in the KNP is lacking, however, *Lantana camara* and *Ageratum conyzoides* have been reported to occur in the lower belt of the Park (KNP, 2019).

Langtang National Park (LNP): It is the first Himalayan national park of Nepal. Vegetation ranges from subtropical to alpine scrub. *Ageratina adenophora* has been reported from the LNP and a large area within the Park has been predicted to be climatically suitable for the growth of *A. adenophora* (Poudel et al., 2021).

Makalu Barun, Rara, Sagarmatha and Shey-Phoksundo National Parks: These four national parks are located in the high mountain regions of Nepal and IAPS has not been reported from these Parks (Table 2).

Table 2. Distribution of invasive alien plant species in protected areas of Nepal.

Invasive species name	National park												Wildlife reserve	Conservation Area					
	BaNP	BNP	CNP	KNP	LNP	MbNP	PNP	RNP	SNP	SpNP	SNNP	SuNP	KTWR	ACA	ANCA	KrCA	GCA	KCA	MCA
Ageratina adenophora (Spreng.) R. King & H. Rob	+	+	+		+		+				+	+	+	+	+		+	+	+
Ageratum conyzoides L.	+	+	+	+			+				+	+	+	+	+	+	+	+	
Ageratum houstonianum Mill.	+	+	+				+					+	+	+	+				
Alternanthera philoxeroides (Mart.) Griseb.							+						+						
Amaranthus spinosus L.	+						+				+	+	+	+	+		+		
Argemone mexicana L.		+					+					+	+						
Bidens pilosa L.	+		+				+					+		+			+	+	
Chromolaena odorata (L.) R. King & H. Rob.		+	+				+						+	+			+		
Eichhornia crassipes (Mart.) Solms	+	+	+									+	+						
Erigeron karvinskianus DC.															+				
Galinsoga quadriradiata Ruiz & Pav.														+	+				
Ipomoea carnea subsp. *fistulosa*	+	+	+	+								+	+			+			
Lantana camara L.	+	+	+				+				+	+	+	+					
Leersia hexandra Sw.			+																
Mesosphaerum suaveolens (L.) Kuntze		+	+				+					+	+						
Mikania micrantha Kunth			+				+						+						
Mimosa pudica L.	+		+				+					+	+	+			+		
Oxalis latifolia Kunth													+	+	+				
Parthenium hysterophorus L.	+	+	+				+					+	+		+	+			
Pistia stratiotes L.			+									+							
Senna occidentalis (L.) Link	+	+	+				+				+	+	+	+					
Senna tora (L.) Roxb.	+	+	+				+				+	+	+	+		+	+		
Xanthium strumarium L.	+	+					+				+	+			+				
Total	**13**	**13**	**16**	**2**	**1**	**0**	**16**	**0**	**0**	**0**	**7**	**16**	**17**	**12**	**9**	**4**	**7**	**3**	**1**

BaNP= Banke National Park, BNP= Bardia National Park, CNP= Chitwan National Park, KNP= Khaptad National Park, LNP= Langtang National Park, MbNP= Makalu-barun National Park, PNP= Parsa National Park, RNP= Rara National Park, SNP= Sagarmatha National Park, SpNP= Shey-phoksundo National Park, SNNP= Shivapuri Nagarjun National Park, SuNP= Suklaphanta National Park

KTWR= Koshi-tappu Wildlife Reserve

ACA= Annapurna Conservation Area, ANCA= Api-nampa Conservation Area, BCA= Blackbuck Conservation Area, GCA= Gaurishankar Conservation Area, KCA= Kanchanjunga Conservation Area, MCA= Makalu-barun Conservation Area

2. Wildlife Reserve

Koshi Tappu Wildlife Reserve is only one wildlife reserves in Nepal, which is located in the Eastern Tarai along the floodplains of Sapta Koshi river. It provides habitat for the Nepal's last remaining population of the wild water buffalo (*Bubalus arnee)*. The Reserve is the first Ramsar Site of Nepal designated in 1987 which is also invaded by 17 IAPS. The grasslands of the Reserve have been invaded by *Mikania micrantha, Chromolaena odorata, Lantana camara, Mesosphaerum suaveolens, Ageratum conyzoides,* and *Parthenium hysterophorus,* resulting the degradation of the habitat of wild water buffalo. Similarly, the wetlands, key habitats for water birds and fish, have been degraded by the infestation of *Eichhornia crassipes, Ipomoea carnea* ssp. *fistulosa* and *Alternanthera philoxeroides* (Siwakoti, 2006).

3. Conservation Areas

Annapurna Conservation Area (ACA): It is the first and largest conservation area of Nepal, extending from 790-8091m in the Annapurna mountain range of the Himalayas. Over 8 species of IAPS have been reported from the ACA (Thapa & Maharjan, 2014). The most problematic species are *Ageratina adenophora, Chromolaena odorata, Ageratum houstonianum* (Thapa & Maharjan, 2014). Anthropogenic activities are considered responsible factor for the spreading of invasive species in the area. Climatically suitable area for *A. adenophora* is likely to increase in ACA under future climate change scenarios (Poudel et al., 2021).

Api-Nampa Conservation Area (ANCA): The ANCA is located in the Kailash Sacred Landscape, a transboundary landscape of Nepal, India and China. Over 10 IAPS including *Ageratina adenophora, Ageratum conyzoides, Erigeron karvinskianus, Lantana camara* and *Parthenium hysterophorus* have been recorded from the ANCA with potential threats to biodiversity, agriculture and livestock production of this region (Bisht et al., 2016; Shrestha et al., 2018; DNPWC, 2019).

Krishnasaar (Blackbuck) Conservation Area (KrCA): It is located in the Bardiya district (mid-western Tarai) and was established to conserve the endangered antelope Blackbuck. The palatable grass species of Blackbuck habitat and grassland area have been declining due to increasing invasion by IAPS (KrCA, 2017; Gyawali et al., 2020). Common IAPS in KrCA are *Parthenium hysterophorus, Senna tora, Ipomoea carnea* ssp. *fistulosa, Ageratum conyzoides, Latana camara, Bidens pilosa* and *Eichhornia crassipes* (KrCA, 2017). The floods of 2015 replaced several species of palatable grasses and introduced many invasive species including *Ageratum conyzoides.*

Kanchenjunga Conservation Area (KCA): The KCA is situated in the eastern part of Nepal Himalaya. The area is popular for high diversity of rhododendron species. Invasion by *Ageratina adenophora, Ageratum conyzoides* and *Bidens pilosa* has been reported in the lower belts of the KCA (KCA, 2020).

Gaurishanker Conservation Area (GCA): The GCA is located between the Sagarmatha National Park and Langtang National Park, and is a prime habitat for the endangered Red Panda. The subtropical and lower temperature region of the conservation areas have been invaded by over seven species of IAPS. These includes *Ageratina adenophora, Ageratum*

conyzoides, Amaranthus spinosus, Bidens pilosa, Chromolaena odorata, Senna tora, and *Mimosa pudica* (NTNC, 2019).

Manaslu Conservation Area (MCA): It is located in the Gorkha district, and extends from the 600-8163m. Any study focusing IAPS is lacking in MCA, however, *Ageratina adenophora* is known from the lower elevation regions of the MCA (NTN, 2015) and the climatically suitable areas for this species has been predicted to increase in future in the MCA (Poudel et al., 2021)

4. Hunting Reserve

Dhorpatan Hunting Reserve, the only hunting reserve in Nepal, is one of the prime habitats for blue sheep, a highly prized trophy animal, which is the main target of hunters. There is lack of information regarding the occurrence of IAPS in this Reserve.

5. Ramsar Sites

Nepal has 10 Ramsar sites (Koshi Tappu, Beeshazar and associated lakes, Ghodaghodi Lake area, Jagadishpur Reservoir, Gokyo and associated lakes, Gosainkunda and associated lakes, Phoksundo Lake, Rara Lake, Mai Pokhari, Lake Cluster of Pokhara Valley). Among these, four sites, viz., Mai Pokhari of Ilam (eastern Middle Mountains), Lake Cluster of Pokhara Valley of Kaski district (central Middle Mountains), Jagdishpur Reservoir and the Ghodaghodi Lake area (western Nepal) are located outside the protected areas. Twenty-two IAPS have been reported from one or more Ramsar sites (Table 3). The Ramsar sites distributed in Tarai, Siwaliks and Middle Mountains are invaded by *Eichhornia crassipes, Alternanthera philoxerioides, Ipomoea carnea* ssp. *fistulosa, Leersia hexandra* and *Mikania micrantha*, (Siwakoti & Karki, 2009; Siwakoti, 2016; Pathak et al., 2021). There was no study on invasive species in the Ramsar sites located in the High Mountains (Gosainkunda, Gokyo, Rara and Phoksundo lakes). These wetlands of the High Mountains are currently free from the IAPS.

Efforts to Manage the Invasive Species

The recommended management strategies for the IAPS are prevention, eradication and control. Prevention program is intended to prevent the introduction of alien species, and eradication is recommended for an invasive species of limited distribution at the early stage of the establishment (Wilson et al., 2013). The eradication of IAPS is almost impossible once they are established in a large area. The PAs of Nepal are invaded by many IAPS (Table 2) and their eradication is impossible for most of the species. The abundance of the IAPS can be reduced and their further spread can be prevented by employing various control measures. Unfortunately, the implementation of control measures is very poor in most of the PAs. Available management plans of the PAs reviewed and components of IAS control strategies included in them have been presented in this section.

Chitwan National Park: The Management Plan of Chitwan National Park and its Buffer Zone (2018-2022) has identified *Mikania micrantha* as the most problematic IAPS in the

Table 3. List of invasive alien plant species reported from different Ramsar sites of Nepal.

Species name	Beeshazar	Ghodaghodi	Gokyo	Gosaikunda	Jagdishpur	Koshi tappu	Mai Pokhari	Phoksundo	Rara	Pokhara
Ageratina adenophora						+				+
Ageratum conyzoides	+	+			+	+				+
Ageratum houstonianum	+	+			+	+				+
Alternanthera philoxeroides	+	+			+	+				
Amaranthus spinosus	+	+				+				
Argemone mexicana						+				
Bidens pilosa					+					+
Chromolaena odorata					+	+				
Eichhornia crassipes	+	+			+	+	+			+
Ipomoea carnea subsp. *fistulosa*	+	+			+	+				+
Lantana camara						+				+
Leersia hexandra	+				+					
Mesosphaerum suaveolens					+	+				
Mikania micrantha						+				
Mimosa pudica		+			+	+				
Oxalis latifolia						+				
Parthenium hysterophorus					+	+				
Pistia stratiotes	+	+								
Senna occidentalis					+	+				
Senna tora		+			+	+				
Spermacoce alata										+
Xanthium strumarium					+					+
Total	**8**	**9**	**0**	**0**	**14**	**17**	**1**	**0**	**0**	**8**

grasslands of the Park and prioritized it for the control. Recommended interventions to control the rapid spread of this species is cutting and burning. The Plan has also recommended the control of *Chromolaena odorata* (CNP, 2018). The wetland habitats (lakes, floodplain) of the Park are invaded by *Eichhornia crassipes, Ipomoea carnea* ssp. *fistulosa* and *Leersia hexandra*. Physical removal of the IAPS biomass from wetlands has been also practiced. However, the control efforts have been ineffective due to improper linkage between research and management, and insufficient budget (CNP, 2018).

Parsa National Park: Wildlife habitat has been degraded by the spread of invasive species in the Park. The Management Plan of the Parsa National Park and its Buffer zone (2018-2022) has included the strategy to control IAS in the grassland of the Park and recommends for the IAPS related research programs. Some control activities such as burning, uprooting and removal of IAPS have implemented (PNP, 2018).

Bardia National Park: Degradation and shrinkage of rhinoceros habitat in the Bardia National Park is continued due to heavy infestation by the IAPS. The Park Management Plan of the Bardia National Park and its Buffer zone (2016-2020), highlights the problem of *Lantana camara* in the forest and grassland and *Eichhornia crassipes* in wetlands. The Plan recommends to control the invasive species. Some management practices such as burning and uprooting of species have been implemented (BNP, 2016).

Banke National Park: Most of the grassland and wetland habitats of the Park are degraded by IAPS. The Management Plan of the Banke National Park and its Buffer zone (2018-2022) has recommended to improve grassland habitats within 5 years by controlling the IAPS. The Plan also recommends for collaborative research to control IAPS (BaNP, 2018).

Khaptad National Park: The Management Plan of the Khaptad National Park and its Buffer zone (2019-2023) has identified the problem of IAPS in grasslands of the Park and recommended for their control without further detail (KNP, 2019).

Rara National Park: The Management Plan of the Rara National Park and its Buffer zone has identified IAPS as one of the problems in the rangeland of the Park (RNP, 2019).

Shivapuri Nagarjun National Park: The Management Plan of the Shivapuri Nagarjun National Park and its Buffer zone (2017-2021) has identified *Lantana camara* and *Ageratina adenophora* as the most problematic IAPS in the grasslands of the Park and has recommended activities such as uprooting, drying and burning for their control (SNNP, 2017).

Sagarmatha National Park: The problem of IAPS has not been reported from Sagarmatha National Park. Therefore, IAS control has not been included in the Management Plan of Sagarmatha National Park and it's Buffer zone (2016-2020) (SNP, 2016).

Koshi Tappu Wildlife Reserve: The Management Plan of Koshi Tappu Wildlife Reserve and its Buffer zone (2018-2022) has identified IAPS as one of the major problems of the Reserve and recommended for periodically uprooting, cutting and burning the terrestrial IAPS and removal of the wetland IAPS (KTWR, 2018).

Api Nampa Conservation Area: The problem of invasive species is not recognized in the Management Plan of Api Nampa Conservation Area (2015-2019) (DNPWC, 2017). However, the biodiversity profile of this Conservation area has identified the invasive species as one of the threats and provided a list of 10 IAPS (DNPWC, 2019).

Annapurna Conservation Area: The Management Plan of the Annapurna Conservation Area (2016-2020) has listed 8 IAPS and prioritized prevention as a key management strategy for the IAPS the Conservation Area (NTNC, 2016).

Kanchenjunga Conservation Area: A total of 3 IAPS (*Ageratum conyzoides, Bidens pilosa* and *Ageratina adenophora*) can be found in the list of flowering plants included in the Kanchenjunga Conservation Area Management Plan (2020-2024) but there is no further information included in the management plan to manage IAPS (KCA, 2020).

Krishnasar (Blackbuck) Conservation Area: The Management Plan of the Krishnasar Conservation Area has identified IAPS as one of the major threats for degradation of grassland habit and recommends some activities such as burning, manual uprooting and ploughing to control them (KrCA, 2017). The Plan has also recommended for the research related to the effect of IAPS on the Blackbuck habitat.

Manaslu Conservation Area: The Management Plan of Manaslu Conservation Area (2015-2018) has identified the problem of IAPS for the forest degradation in the Conservation Area (NTNC, 2015). But the Plan has not recommended any activity to control IAPS.

Gaurisankar Conservation Area: The Management Plan of the Gaurishankar Conservation Area (2019-2023) has identified the IAPS as a threat but the Plan has not included any strategy to control IAPS (NTNC, 2019).

Ramsar Sites: The problem of IAPS has been identified in most of the Ramsar Sites located in the Tarai, Siwaliks and Middle Mountains regions. The National Ramsar Strategy and Action Plan Nepal (2018-2024) has also identified IAPS as one of the threats to wetlands degradation in Ramsar sites and has recommended for the preparation of management plan of each Ramsar site (MoFE, 2018).

Conclusion

The protected areas (PAs) buffers biodiversity from many threatening processes and the boundaries of the PAs are believed to have a filtering effect against biological invasions. But anthropogenic disturbances provide windows of opportunities for the establishment and proliferation of invasive alien plant species (IAPS). Most of the PAs of Nepal, and all of the PAs located in Tarai, Siwalik and Middle Mountain regions, are invaded by one to several IAPS; some of these IAPS are globally worst species. Management plans of the majority of the PAs have identified IAPS as one of the major management issues, yet the control of IAPS, if any, has been limited to physical removal and burning in small areas. The management plans have not included survey and monitoring plans for the IAPS; species and habitat-specific control strategies have been missing. Therefore, the effectiveness of these control activities has remained very low, thereby allowing the unabated spread of the IAPS inside the PAs. Furthermore, some key information required for management decision is also

lacking. For example, there is a lack of comprehensive knowledge of biological invasions drivers to the PAs, dispersal pathways, and ecological impacts of the IAPS. The presence of a low number of IAPS or their absence in some mountain PAs provides the opportunity to prevent IAPS from invading these PAs. Further research on the above topics, together with regular monitoring and survey of the IAPS in each PAs, can generate data and knowledge required for the PAs authorities' management decision. Prioritization of IAPS control in the PAs management of Nepal will prevent further spread of the IAPS and help to meet the long-term goal of biodiversity conservation inside the PAs.

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Decomposition and Nitrogen Release in the Leaf Litter of Leguminous and Non-Leguminous Plant Species of Humse-Dumse Community Forest of Jhapa, Nepal

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Abstract

Leaf litter decomposition has a significant role in nutrient budget and biogeochemical cycle in the forest ecosystem. For the study of decomposition, twelve plant species were selected, out of which two species were leguminous (*Dalbergia* and *Cassia)*. The other ten species were non-leguminous (*Anthocephalus*, *Terminalia*, *Tectona*, *Careya*, *Dillenia*, *Shorea*, *Syzygium*, *Largerstroemia*, *Eichhornia*, and *Ipomoea*). The study was designed to estimate the rate of decomposition, nitrogen release, and carbon concentration variation in leaf litters of non-leguminous species combined with leguminous species. To assess the decomposition and nutrient dynamics, the litter-bag technique was used. Non-leguminous species, when combined with leguminous species, showed a faster rate of decomposition. The N concentration in fresh leaf litter was higher in *Eichhornia* (2.45%) and *Ipomoea* (2.17%), which decomposed singly. In combined form fresh litter, N concentration was low in *Dillenia+Cassia* (1.37%) and *Terminalia+Cassia* (1.58%). During the decomposition, the C: N ratio increased with time. According to weight loss and nitrogen release, the combined species like *Dalbergia+Anthocephalus* and *Dalbergia+Tectona* were considered fast decomposing species. Leaves of these plants may be used to synchronize the demand and supply of nitrogen in the cropping system. Plant species like *Eichhornia* and *Ipomoea* could be the ideal material for making pleasant composite organic manure. Some combinations showed a slow rate of weight loss and nitrogen release due to high carbon and low nitrogen content in non-leguminous plant species. These combinations of plant species may get a slow release of nitrogen for the long term in the sustainable agroecosystem.

Key words: Ecosystem, Litter bag, Nutrient budget, Organic manure, Plant residues

Introduction

Decomposition is the process through which dead material is broken down into simpler forms of mineral components. Litter decomposition is an essential process in the forest ecosystem. Leaf litter and non-leaf litter are dead plant material, such as leaves, bark, and twigs that have fallen to the ground. The decomposition of plant litter is crucial in the biogeochemical cycle (Decatanzaro & Kimmins, 1985). The litter decomposition process and its rate are vital in regulating the formation of soil organic matter and the release of nutrients for plants and microorganisms (Austin & Vivanco, 2006). Leaf-litter decomposition in terrestrial ecosystems has a significant role in recycling the nutrients to the soil. Nutrient dynamics is the way of the nutrients cycle in an ecosystem (Bhattarai & Bhatta, 2020). In the cycling of

such nutrients, decomposition processes have a crucial role by releasing complex organic compounds into the simple, usable form for proper growth and development of plants (Saha et al., 2016). Bacteria and fungi primarily carry out the process of decomposition; thus the rate of decomposition entirely depends on microbial activities, which in turn, get affected by soil character and climatic condition in an area (Berg & McClaugherty, 2014). Leaf decomposition by fungi and bacteria tends to be rapid at nutrient-enriched conditions (Krishna & Mohan, 2017).

Litter decomposition is regulated by two factors, the climatic factor and the chemistry of litter (Swift et al., 1979). Temperature and moisture together play a significant role in decomposition rates because they are not environmentally independent (Singh et al., 1992). Numerous other elements include secondary metabolites, soil traits, kinds of organisms in the detritus food chain, and herbivore- activity influence decomposition on a regional basis. Most of these factors are depended on water, temperature and the chemical nature of litter. Soil promotes the decomposition of litters by altering the microclimate surrounding leaf litter, enhancing suitable conditions for decomposer organisms, or acting as a vector for colonization by microbes, promoting the decomposition process (Hewins & Hyatt, 2009). A litter with a higher initial N concentration usually shows a higher mass loss; however, the importance of initial N concentration decreases with time (Ross et al., 2002). Slow decomposition rates result in the building up of organic matter and nutrient stocks in the soil; however, fast decomposition rates help to meet plant intake requirements (Isaac & Nair, 2005).

Variation in preliminary lignin content material or L: N ratio and in the C: N ratio seems to explain a large part of leaf litter of different species (Upadhyay & Singh, 1985). The relationship of these parameters with the decomposition rate is inverse. The rate of decomposition of leaves decreases from the floor layer to cover layer of the woodland probably because vegetation ought to produce greater mechanical tissue of their leaves to maintain them functioning in increasingly open surroundings. Leguminous plants used as green manure are a source of organic material with significant benefits for soil and crops due to the high N_2 fixation capacity, nutrient cycling and contribution to soil cover (Cobo et al., 2002). Leguminous species have a remarkable capacity to produce high amounts of biomass and accumulate high nutrient concentrations (Matos et al., 2008), which become available to crops after residue decomposition. Leaf litter of different tree species have a different decomposition rate. Leaf litters of leguminous trees have fast decomposition rate than non-leguminous leaf litters because of having a low C: N ratio (Upadhyay & Singh, 1989). If non-leguminous leaf litter is combined with leguminous leaf litter, then the decomposition rate becomes faster. The high nitrogen concentration of leguminous litter makes a closer C: N ratio required to enhance the rate of decomposition (Oli et al., 2018).

The information on decomposition and nitrogen release in leguminous species combined with non-leguminous plant species is limited. Therefore, the present study was undertaken to determine the patterns of nitrogen release, the variation of C: N ratio and effects of leguminous leaf litter on non-leguminous leaf litter during the decomposition process. Suitable combinations of these species would be useful for soil nutrients management.

Materials and Methods

Study Area

The leguminous and non-leguminous leaf litter samples were collected from the Humse Dumse Community Forest situated in Damak Municipality, Ward No.3, and 5 of Jhapa district. It is 4 km north-west of Damak Bazar. The area of the forest is 6.275 sq. km. The average altitude is of the forest is 135 m above msl. Maximum and minimum temperatures ranged between 10 and 35°C. Annual rainfall is 1800 mm per year. The forest is a tropical moist forest with *Shorea robusta* as dominant species and *Terminalia, Dillenia* and *Syzygium* as main associates.

Leaf Litter Decomposition

Decomposition of leaf litter was conducted on the following plant species present in the Sal bearing tropical moist forest of Humse-Dumse community forest (Table 1). As per the objective, non-leguminous leaf litters were combined with leguminous leaf litters, as mentioned in table 2.

The decomposition of leaf litter was determined using the litter bag technique (Hossain et al., 2011). The collected leaf litter samples were dried at 80°C to get the regular dry weight. Nylon litter bags were prepared (13cm×18cm size,1mm mesh), 5g of dried leaf litter was kept in each litter bag (2.5 g of leguminous species and 2.5 g of non-leguminous species), 20 bags were made for each combination. Litter bags were placed in rows on the soil surface, one row for each species where bags were arranged alternately. There were ten rows, eight rows for combined species and two rows for single species (Table 2). Rows were tagged for identity. Five random litter bags from each row were recovered at a 3-month interval from the soil surface. The recovered materials were separated from soil particles, dried at 80°C, weighed and ground in Willey Mill to pass a 1mm mesh screen.

Chemical Analysis of Litter

The fresh leaf litter and the decomposed sample of each species were dried in an oven at 80°C and ground in Willey Mill to obtain a powder sample.

Estimation of N was done by the micro- Kjeldahl method (Flindt & Lillebo, 2005). This method is based upon the determination of the amount of reduced nitrogen present. Estimation of N completes in 3 steps- Digestion, Distillation, and Titration.

For digestion of samples, 0.5 g of dried powder of plant samples were taken in the micro-Kjeldahl flask, 5g of catalyst mixture (potassium sulphate, copper sulphate and selenium powder in the ratio of 8:1:1) and 10 ml concentrated sulphuric acid were added. The flask was heated first at low a temperature, then at high temperature till the liquid became clear pale blue colour.

In a 100 ml conical flask, 5 ml of 2% boric acid solution with mixed indicator were taken. The distillation apparatus was connected with a conical flask dipping the delivery tube below the boric acid solution. A 30 ml digested solution was poured into the distillation apparatus

Table 1. Leaf litters of plant species used for decomposition.

SN	Plant species	Local Names	Family
1	*Dalbergia sissoo* Roxb	Sissoo	Fabaceae
2	*Tectona grandis* L.f.	Teak	Verbenaceae
3	*Anthocephalus chinensis* Walp	Kadam	Rubiaceae
4	*Syzygium cumini* (L) Skeels	Jamun	Myrtaceae
5	*Terminalia alata* Hyne ex. Roth Fam	Asna, Saaz	Combretaceae
6	*Dillenia pentagyna* Roxb.	Tantari	Dilleniaceae
7	*Shorea robusta* Gaertn.	Sal, Sakhuwa	Dipterocarpaceae
8	*Careya arborea* Roxb.	Kumbhi	Lecythidaceae
9	*Lagerstroemia parviflora* Roxb.	Bote Dhayaro	Lythraceae
10.	*Cassia siamena* Lam.	Kapur	Leguminosae
11	*Eichhornia crassipes* (Mart.) Solms	Water hyacinth	Pontederiaceae
12	*Ipomoea carnea* Jacq.	Ajamari	Convolvulaceae

Table 2. Combinations of leguminous and non-leguminous leaf litters.

S.N.	Combination of plant species
1	*Dalbergia* + *Shorea*
2	*Terminalia* + *Cassia*
3	*Dillenia* + *Cassia*
4	*Largerstroemia* + *Cassia*
5	*Careya* + *Cassia*
6	*Dalbergia* + *Tectona*
7	*Dalbergia* + *Anthocephalus*
8	*Dalbergia* + *Syzygium*
9	*Eichhornia crassipes* (Single)
10	*Ipomoea carnea* (Single)

through the side funnel, and 30 ml of 40% NaOH solution was also added through the side funnel. Distillation was done for 10 minutes to distil off the ammonia into the boric acid solution.

The distillate was titrated with 0.0775 N H_2SO_4 solution. For each sample, three replicates were titrated. One blank solution. was used as processed above to find out the presence of N as an error in the catalyst reagent. The blank titrated value was subtracted from each of the titrated value. The given formula determined % of N.

$$\% \text{ N} = 14/1000 \times \frac{\text{strength of } H_2SO_4 \times \text{titrated value} \times \text{dilution factor} \times 100}{\text{Weight of sample}}$$

Estimation of Carbon

For carbon estimation, the 5 g powder sample was taken in separate crucibles and burnt in Muffle Furnace at 600°C. Carbon concentration was assumed to be 50% of ash-free dry weight (McBrayer & Cromack, 1980). The following formula determined the % of carbon-

$$\text{Amount of carbon} = \frac{\text{Initial wt. of the sample- ash}}{2}$$

$$\% C = \frac{\text{Amount of C} \times 100}{\text{Initial wt. of sample}}$$

Data Analysis

The data analysis was done by using MS-Excel and SPSS.

Results

During decomposition, leaf litter weight loss with time showed considerable variation in different plant species. The percentage mass remaining during decomposition in different species is shown in figure 1. Based on mass remaining, *Dalbergia sissoo+Shorea robusta* and *Dalbergia sissoo+Sizygium cumini* showed slow decomposition. On the other hand, combinations of plant species like *Dalbergia+Tectona* and *Dalbergia+Anthocephalus* showed faster decomposition than other plant species (Table 3). Between two plant species decomposed singly, *Eichhornia crassipes* showed the fastest decomposition than *Ipomoea carnea.* In *Eichhornia* and *Ipomoea,* only 0.03 g and 0.11g mass remained respectively at the end of first three months. After 121 days, *Eichhornia* and *Ipomoea* showed 99.6% and 98.8% weight loss, respectively. Therefore decomposition experiment was terminated for these species. Among eight combinations, in *Dalbergia + Anthocephalus* and *Dalbergia+Tectona* only 5.0% and 8.2% mass remained respectively at the end of the experiment (Table 3). These showed 95.0% and 91.8% weight loss at the end of 365 days, which is greater than others. On the other hand, *Dalbergia+Shorea* and *Dalbergia+Syzygium* showed slower decomposition of 25.4% and 19.4% mass remained, respectively at the end of the experiment.

The nitrogen concentration (Table 4) in the fresh litter was maximum in *Dalbergia+Shorea (*2.41*%)* followed by the combination of *Dalbergia+Anthocephalus* (2.16%) and minimum in *Terminalia+Cassia* (1.58%) and *Dillenia+Cassia* (1.37%). Between *Eichhornia* and *Ipomoea,* nitrogen concentration in fresh leaf litter was maximum in *Echhornia* (2.45%). *Ipomoea* contained 2.17% of the nitrogen in fresh leaf litter. Nitrogen concentration in decomposed leaf litter samples was maximum in *Largerstroemia+Cassia* (0.98%) and minimum in *Dalbergia+Anthocephalus* (0.47%). In fresh leaf litter, there was variation in carbon concentration in different plant species (Table 4). *Dillenia+Cassia* (49.1%) and *Largerstroemia+Cassia*

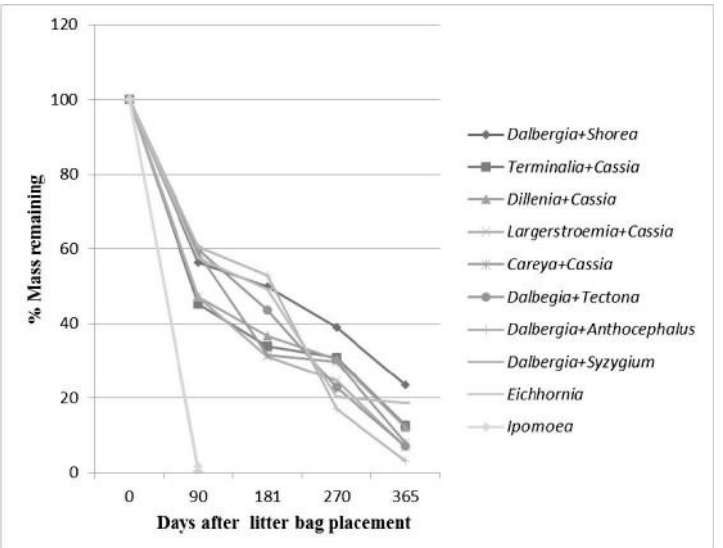

Figure 1. Relationship between per cent mass remaining (y-axis) with time in days (x-axis) in ten litter types.

(49%) showed a higher concentration of carbon. *Eichhornia* and *Ipomoea* showed a lower concentration of carbon i.e. 34.9% and 37.8%, respectively. Carbon concentration in decomposed leaf litter samples was higher in *Largerstroemia+Cassia* (33%) and *Dalbergia+Anthocephalus* (32.1%). *Dalbergia+Tectona* (29.9%) and *Dalbergia+Syzygium* (29%) showed comparatively lower concentration of carbon. The C: N ratio in fresh leaf litter was highest in *Dillenia+Cassia* (35.83%), whereas *Dalbergia+Tectona* showed a minimum (19.03%) among combining plant species. Between *Eichhornia* and *Ipomea*, *Eichhornia* showed a lower value of C: N ratio (14.2 4%) (Table 5).

Table 3. Amount of mass remaining (g) in the leaf litter samples during decomposition (Initial mass = 5 g).

Plant species	90 days	181 days	270 days	365 days
1. *Dalbergia + Shorea*	3.22 ± 0.4	2.69 ± 0.20	2.04 ± 0.09	1.27 ± 0.09
2. *Terminalia + Cassia*	2.5 ± 0.24	2.06 ± 0.33	1..75 ± 0.20	0.74 ± 0.11
3. *Dillenia + Cassia*	2.53 ± 0.18	2.01 ± 0.17	1.73 ± 0.21	0.72 ± 0.11
4. *Largerstroemia + Cassia*	2.57 ± 0.22	1.71 ± 0.15	1.29 ± 0.05	0.49 ± 0.15
5. *Careya + Cassia*	3.07 ± 0.12	1.84 ± 0.26	1.66 ± 0.17	0.53 ± 0.13
6. *Dalbergia + Tectona*	3.14 ± 0.15	2.45 ± 0.27	1.25 ± 0.11	0.41 ± 0.05
7. *Dalbergia + Anthocephalus*	3.18 ± 0.15	2.84 ± 0.19	0.94 ± 0.09	0.25 ± 0.09
8. *Dalbergia + Syzygium*	3.02 ± 0.14	2.57 ± 0.11	1.05 ± 0.04	0.97 ± 0.04
9. *Eichhornia crassipes*	0.03 ± 0.004	-	-	-
10. *Ipomoea carnea*	0.11 ± 0.02	-	-	-

Table 4. Concentration of C and N in fresh leaf litter and decomposed leaf litter of plant species.

Plant species	0 days		90 days		181 days		270 days		365 days	
	C %	N %	C %	N %	C %	N %	C %	N %	C %	N %
Dalbergia + Shorea	46.4	2.41	44.4	1.03	41.4	0.90	36.4	0.80	31.4	0.74
Terminalia + Cassia	44.9	1.58	42.0	1.07	40.0	0.84	35.0	0.76	31.9	0.96
Largerstroemia + Cassia	49.0	1.90	47.0	1.49	44.0	1.40	38.0	0.90	33	0.98
Dillenia + Cassia	49.1	1.37	44.0	1.09	39.1	0.97	34.1	0.88	30.1	0.8
Careya + Cassia	41.7	1.82	40.4	1.34	29.4	0.83	35.4	0.7	32	0.63
Dalbergia + Tectona	39.4	2.07	38.9	0.78	36.9	0.69	33.9	0.61	29.9	0.59
Dalbergia + Anthocephalus	41.95	2.16	40.1	0.86	38.1	0.68	35.1	0.64	32.1	0.47
Darbergia + Syzygium	40.75	2.07	40.0	0.87	37.0	0.76	32.0	0.66	29.0	0.64
Eichhornia crassipes	34.9	2.45	20.45	0.5	-	-	-	-	-	-
Ipomoea carnea	37.8	2.17	16.85	0.80	-	-	-	-	-	-

Table 5. C: N ratio in fresh and decomposed leaf litter of plant species.

Plant species	0 days	90 days	181 days	270 days	365 days
	C:N	C:N	C:N	C:N	C:N
Dalbergia + Shorea	19.25	43.1	46	45.5	42.43
Terminalia + Cassia	27.33	39.12	47.62	46.05	33.2
Largerstroemia + Cassia	25.79	31.54	31.43	42.22	33.67
Dillenia + Cassia	35.83	40.36	40.31	38.75	37.6
Careya + Cassia	22.91	30.14	47.46	50.57	50.7
Dalbergia + Tectona	19.03	49.87	53.47	55.57	50.6
Dalbergia + Anthocephalus	19.42	46.63	56.03	54.54	68.2
Darbergia + Syzygium	19.68	45.97	48.68	45.48	45.3
Eichhornia crassipes	14.24	40.9	-	-	-
Ipomoea carnea	17.42	21.06	-	-	-

Mass Loss and Nitrogen Release

The relationship between percentage litter mass loss and nitrogen release in the case of *Dalbergia* + *Anthocephalus* (Fig. 2) showed that in the initial 181 days of the decomposition experiment, nitrogen release was dominant over litter mass loss. On the other hand, at and after 270 days, litter weight loss became dominant over the nitrogen release. The same trend was also observed in the case of *Dalbergia* +*Tectona* (Fig. 3).

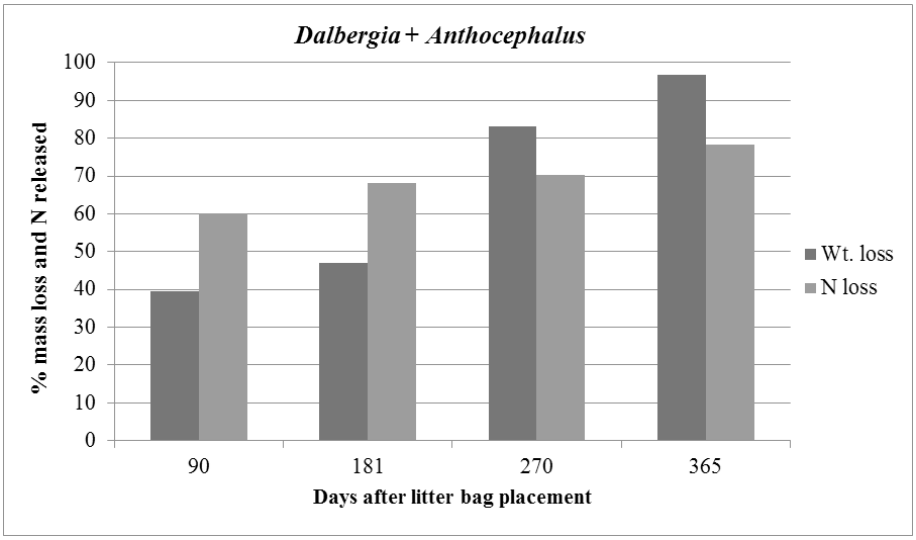

Figure 2. Relationship between percent mass loss and nitrogen release in Y-axis with time (days) in X-axis in litter type (*Dalbergia* + *Anthocephalus*).

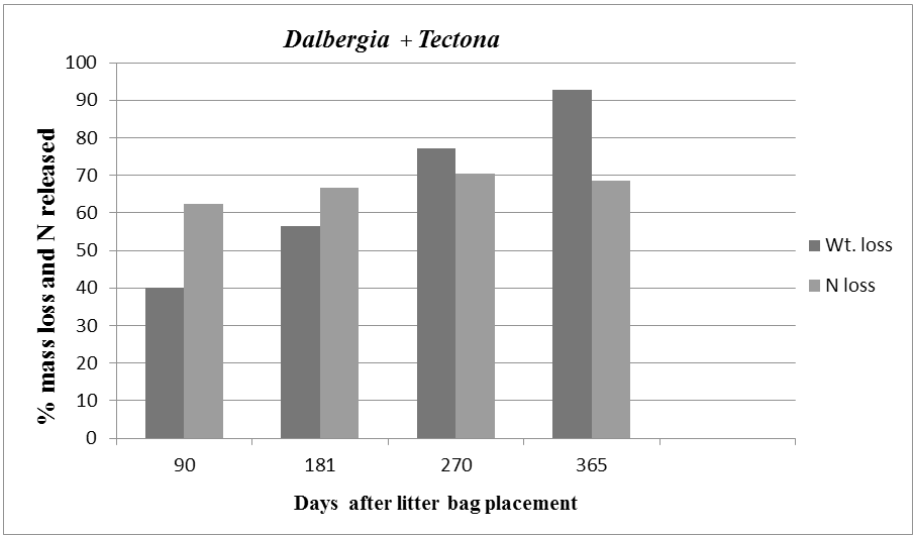

Figure 3. Relationship between per cent mass loss and nitrogen release in Y-axis with time (days) in X-axis in litter type (*Dalbergia* + *Tectona*).

Changes in absolute amount of N in littermass with time expressed as the percentage of initial N remaining is illustrated for *Dalbergia +Anthocephalus* and *Dalbergia +Tetona* in figure 4 and 5, respectively. The trend in the figure suggests that there is a sharp decrease in N remaining in the initial 90 days and then showed a slow process in the deconposition cycle. Percentage weight remaining in litter mass also showed the same trend but it is lesser than N remaining till initial 181 days.

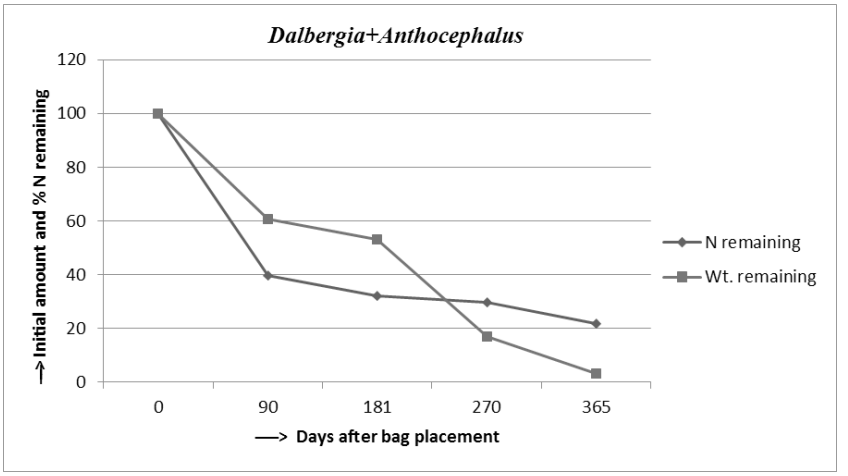

Figure 4. Changes in the absolute amount of N in litter mass (mg Ng^{-1}) with time expressed as the percentage of initial N remaining; the initial mass of N was 21.6 mg/g litter mass; also shown are changes (percentage weight remaining) in litter mass.

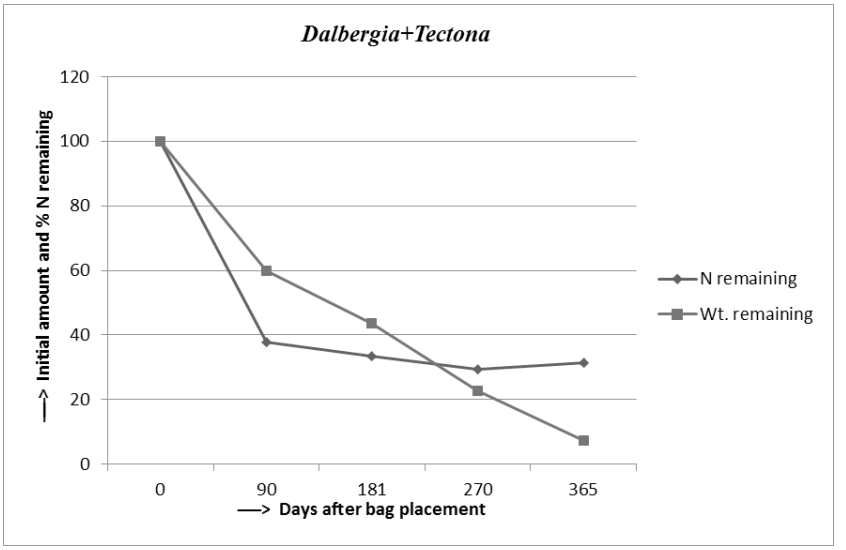

Figure 5. Changes in the absolute amount of N in litter mass (mg Ng^{-1}) with time expressed as the percentage of initial N remaining; the initial mass of N was 20.7 mg/g litter mass; also shown are changes (percent weight remaining) in litter mass.

Discussion

The decomposition of leaf litter is a significant source of nutrients in a forest ecosystem. The insects and microscopic decomposers break down the litter to release organically bound nutrients, becoming available for uptake by the plants. It is governed by the interplay of abiotic and substrate quality variables (Upadhyay & Singh, 1989). Under the influence of the prevailing climate, environment, different litter species have their specific decomposition rate. The nutrient dynamics of the decomposition of litter is complicated as the nutrients occur in various forms and are subject to various transformations. Differential immobilization and release patterns may be exhibited by various nutrients (Upadhyay et al., 1989). Therefore, the study on litter decomposition provides an opportunity to manipulate the timing of nutrients release, which is further useful in species selection for a sustainable agro-forestry system.

Litter decomposition plays a vital part in a forest ecosystem's nutrient budget, where the flora is influenced most significantly by nutrient recycling from plant litter (Vesterdal, 1999; Wedderburn & Carter, 1999). Leaf litter improves soil quality by adding organic matter and nutrient to the soil compared to other litter types. During decomposition, the nutrient may undergo sequential stages like leaching, immobilization, and the net mineralization stage in which an absolute decrease in nutrient concentration occurs in the residential litter mass (Berg & Staaf, 1981).

The Concentration of C and N

In the present study, the ranges of C and N concentration in different plant species were different shown in table 4. In fresh leaf litter the concentration of N was in the order of: *Eichhornia* > (*Dalbergia* + *Shorea*) > *Ipomoea* > (*Dalbergia* + *Anthocephalus*) > (*Dalbergia* + *Tectona*) > (*Dalbergia* + *Syzygium*) > (*Largerstroemia* + *Cassia*) > (*Careya* + *Cassia*) > (*Terminalia* + *Cassia*) > (*Dillenia* + *Cassia*).

Nutrient composition of litter is considered most influential in determining the rate of decomposition (Berg & Staaf, 1981). The initial leaf litter concentration of N was found to be maximum in *Eichhornia* and minimum in (*Dillenia*+*Cassia*). The species having the highest N percentage showed the fast decomposition rate and with lowest N content decomposed slowly. Higher initial N concentration causes the C: N ratio to approach the critical level more rapidly (Upadhyay & Singh, 1989). The C: N ratio decreases as decomposition proceeds to reach such a critical level (Singh, 1969). The rate of decomposition is high in species with extreme ash and nitrogen contents and the lowest C: N ratios and lignin contents (Krishna & Mohan, 2017).

The C: N ratio was found close in *Eichhornia* and *Ipomoea* while among combined species it was low in *Dalbergia*+*Tectona*. The low C: N ratio helps in fast decomposition. On the other hand, C: N ratio in non-leguminous plant species was wide, which showed the concentration of carbon is more significant than N. So, the non-leguminous plants like*, Careya, Tectona, Shorea,* and others showed slow decomposition even combined with leguminous species. During decomposition, N is released faster than C. Therefore, in all species the C: N ratio become wide with time and it was higher in the last decomposed samples. If C: N ratio is

higher, the decomposition rate becomes slow due to the higher content of lignified tissues, which result in high C: N ratio than the critical value. If L: N ratio is low, which is also reflected by C: N ratio, the decomposition becomes faster (Joshi et al., 2006).

Weight Loss and N Release

There was a rapid weight loss in the leaf litter of different plant species in the early month of litter incubation, which increased in the rainy season due to moisture and temperature conditions constituting a favourable condition for the activity of decomposers. In the present study, the higher weight loss in leaf litter was found during the initial stages. Then gradual slow trends were observed. This may be due to the high initial content of water-soluble material and simple substrates, breakdown of litter by decomposers, especially microorganisms and removal of leaf litter particles by soil animals (Devi & Yadava, 2007). Quick mass loss from leaves of *Dalbergia* and *Cassia* may be due to the leaching of water-soluble compounds like sugars, amino acids, and soluble phenolics. High soil moisture and optimum temperature during the rainy season could have facilitated more remarkable growth in microbial population that could induce faster decomposition. However, quick weight loss in *Eichhornia* and *Ipomoea* may be attributed to structurally softer leaves (thinner cuticle and less sclerenchyma). Quick weight loss may also be due to the incorporation of leaves into the soil. Wilson et al. (1986) found much more rapid decomposition and greater nitrogen recovery of *Dalbergia* when it was incorporated into the soil. Similar results have been obtained with many species with high and low-quality prunings (Mafongoya et al., 1998).

Conclusions

Based on weight loss and N release, the combined species like *Dalbergia+Anthocephalus* and *Dalbergia+Tectona* were considered as fast decomposing species. Leaves of these species could be used to synchronize the demand and supply of N in the cropping system. Moreover, species like *Eichhornia* and *Ipomoea* would be suitable materials for making good quality compost and organic manure.

On the other hand, some combinations of non-leguminous and leguminous plant species showed a slow rate of weight loss and nitrogen release. This may be due to high carbon and low nitrogen content in non-leguminous plant species resulting in high C: N ratio. These combinations of plant species can be used to get a slow release of nitrogen for a long time in sustainable agro-ecosystem.

Acknowledgements

We would like to thank the Head, Department of Botany, and the Campus Chief, Post Graduate Campus, Biratnagar and all the staffs of this campus for their co-operation and support.

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Himalayan Medicinal Plant Species Traded from Nepal to China

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Abstract

Despite an old millennial history of medicinal plants trade from Nepal to China, there has not been much comprehensive studies that overviews what species are traded from Nepal to China. This study attempts to generate a complete overview of Nepalese medicinal plant species traded with China and reveal their conservation status. We review the literature related to Nepal-China trade relations, including medicinal plants, track the government reports and data, and conduct interviews with forest officials and exporters trading to Tibet, China. The study documented 54 medicinal plant species of 48 genera under 41 families exported to China. Twenty-six species, including four orchids, fall in national or international conservation categories. With the industrialization of Tibetan medicine, access to modern transportation (roadways, seaways and airways) for supply of medicine ingredients and products, globalization of medicinal plant market, and high per capita income of Chinese people resulting change in consumer behaviour shifting to herbal medication have extended Nepalese medicinal plants trade beyond Tibet to mainland China. To mitigate the depletion of resources caused by high demand of medicinal plants, immediate action is required to address trade sustainability.

Key words: Comprehensive list, Conservation, Medicinal plant market, Tibetan Medicine Industry

Introduction

Nepalese medicinal plants have been exported for centuries (Dobremez, 1976), primarily to India and China (Kirkpatrick, 1811; Hamilton, 1819). With the industrialization of traditional medicine and globalization of medicinal plants trade, bulk of Nepalese medicinal plants are exported to India, China and beyond (Olsen, 2005; Ghimire et al., 2016; Pyakurel et al., 2018; Chapagain, 2020), where they are the subject of increasing interest in the herbal curative system (Singh et al., 1979). This high demand may be due to the significant efficiency (Phoboo et al., 2006) of bio-constituents in modern medicine, the proximity to centuries-old systems (Ayurveda, Tibetan Medicine and Traditional Chinese Medicine) (Jaiswal et al., 2016) or their use in the preparation of aromatic products, cosmetics, plant fibres, herbal dyes, food flavouring (NPC, 2011), and other uses like food supplements, herbal teas, massage oils, etc.

Out of 11,971 species of flowering and non-flowering plants, including fungi and lichens (MoFE, 2018), more than 2300 species have medicinal value (Rokaya et al., 2012), while 300 species are exported from Nepal (Pyakurel et al., 2019). About 39% of all traded medicinal plants are formally protected by the government of Nepal or included in national

and international conservation categories to control the over and pre-harvesting (Pyakurel et al., 2019). Pan-Himalayan people's tradition and culture are related to those plants for various purposes, including household activities (fence), religious beliefs (incense), medicinal purposes etc. In a recent study, the medicinal plants from Nepal are supplied to more than 50 countries worldwide (Ghimire et al*.,* 2016), out of which 13 countries continuously import every year (Chapagain, 2020). In addition, medicinal plant processing enterprises in Nepal are also experiencing continuous growth (Chapagain et al*.*, 2019).

The most recent and comprehensive account of traded medicinal plants is that by Pyakurel et al. (2019), although destination countries are not mentioned. Despite the historical and contemporary importance, there is no comprehensive overview of what species are traded from Nepal to China. Recent studies have estimated the number of traded species up to 17 (Saxer, 2009: 107; PSCN, 2014; Pyakurel & Panthi, 2015; He et al*.*, 2018). Here, the historical trade information is combined with the contemporary to create a comprehensive overview of species previously or currently trade (Pyakurel et al., 2019).

The present study aims create a comprehensive overview of species that have been or are in trade from Nepal to China and analyse conservation status of Nepalese medicinal plants traded with China.

Materials and Methods

A bibliography of 350 studies on medicinal plants, fungi, and lichens traded in and from Nepal compiled from 29 key resource institutions in Nepal (Smith-Hall et al., 2020) was reviewed to cover the period up to 2015. Existing literature and online journals relating to Nepal-China trade relations, including medicinal plants, were researched and reviewed to cover period from 2015 to 2020. Government reports and data from Customs and Tibetan border districts were also reviewed.

To support secondary information and capture contemporary traded species, field surveys were carried out in major medicinal plants trade hubs and routes to China in 2016 (Custom of Sindhupalchok; Tribhuvan International Airport; and exporters at Thamel, Kathmandu, number of species traded to China*, n=33*) and in 2020 (Kimathanka-Chentang border crossing, Sankhuwasabha, *n=8* and Divisional Forest Office, Kathmandu, *n=24*). Interviews were conducted on traded species (name, parts traded, uses etc.) with officials and Tibetan bordered exporters trading to China. The medicinal plants traded to China recorded from both primary and secondary sources were merged into a list, sorted, and any duplications removed. Finally, the listed species (*n=54*) were tabulated with families, recent scientific name with author citations; their English, Nepali, Tibetan and Chinese names; life form; parts traded; occurrence; distribution in Nepal with altitudinal variation; conservation status and source (Appendix I) following the CAMP (2001), Catalogue of Life (2018), Pyakurel et al. (2019), CITES (2020), Plants of the World online (2020), and IUCN (2020). The conservation status of investigated species was verified according to national (CAMP, 2001; GoN, 2001) and international (IUCN, 2020; CITES, 2020) conservation categories.

Results

A Comprehensive Overview of Species from Nepal to China

During the literature review, 13 studies mentioned the name and the final destination of species traded from Nepal to China. He et al. (2018) (reported number of species traded to China *n=17*); Pyakurel and Panthi (2015), and Saxer (2009) (each *n*=8); PSCN (2014) (*n= 6*); Acharya (2000) (*n= 3*); Sherpa (2001), and van Boeckel (2017) (each *n=2*); Amatya (2006); Cunningham et al. (2018); Devkota and Shrestha (2006); GoN (2011); IFA (2015), and Phoboo and Jha (2010) (each *n=1*).

A total of 54 species of medicinal plants of 48 genera under 41 families are recorded in a trade from Nepal to China from ancient to present. Orchidaceae is the largest family with four species, followed by Asparagaceae and Ranunculaceae (each three species) and Berberidaceae, Combretaceae, Elaeocarpaceae, Elaeagnaceae, Gentianaceae and Lauraceae (each two species). The remaining 32 families consist of one species each (Appendix I).

Most of the medicinal plants exported to China from Nepal are herbs (22 species contributing to 44.4%) (perennial herb-19, annual herb-1, herbaceous climber-2, epiphytic herb-1, saprophytic herb-1), 14 are tree (25.9%) (small tree-8, tree-1, large tree-5), 10 are shrubs (18.5%) (small shrub-2, shrub-8), 3 are Fungi (5.5%), 1 Lichen (1.8 %) and 2 are others (3.7%) (Pteridophyte-1, small tree/shrub-1) (Fig. 1).

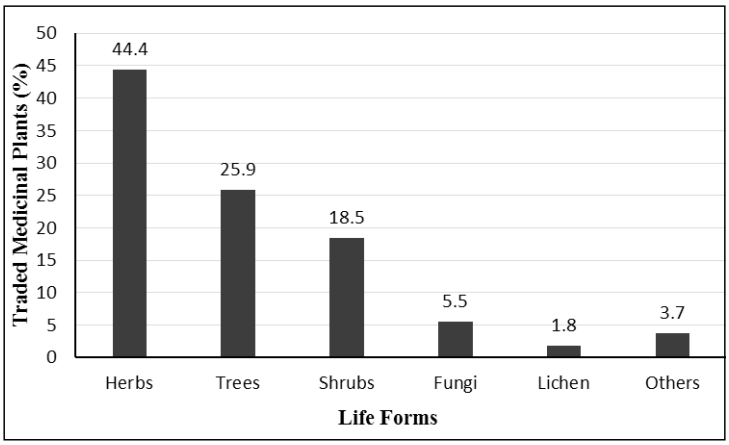

Figure 1. Life form of investigated medicinal plants traded from Nepal to China.

About 80% of the investigated medicinal plant species occur in native wild form and 15% in Native wild and cultivated form in Nepal. Two species are Exotic cultivated and one species in native cultivated (Appendix I).

Conservation Status of Investigated Species

About 59% of investigated medicinal plant species are harvested for whole plant, underground parts, bark, wood or stem, and 41% harvested for leaf, twig, fruit, seed, flower or resin (Appendix I).

Of the 54 investigated species traded from Nepal to China, 26 (48.15%) are included in one or more national (CAMP, *n=16* and GoN, *n=5*) or international (IUCN, *n=7* and CITES,

n=4) conservation lists (Appendix I). 4 species of orchids are traded, and all orchids are kept under threat category of CITES Appendix II. *Nardostachys jatamansi* (D.Don) DC. (jatamansi, bhutle / pangpö / spikenard) of family Valerianaceae is listed in all four categories. *Dactylorhiza hatagirea* (D. Don) Soó (panchaunle, hatajadi/ Salep, Marsh Orchid) of family Orchidaceace is listed in three of the conservation categories. Four species in two and remaining 20 species are listed in at least one of the conservation categories (Appendix I).

Discussion

The medicinal plant species exported from Nepal to China are in increasing order, almost twice the number of estimates by Saxer (2009:107) and thrice the number of estimates by Pyakurel and Panthi (2015) and He et al. (2018). The rise in per capita income for China from 1996 to 2018 increase by 13.8 folds (IndexMundi, 2020) has increased demand for consumer products containing medicinal plants, fungi, and lichen (Pyakurel et al., 2019). Some of the increase came from enumerating product groups at the species level, e.g., the two species of *Polygonatum* traded as Setakchini/Khiraula, similarly, the two species of *Berberis* traded as Chutro, and the two species of *Hippophae* traded as Seabuckthorn. The significant demand for new species such as the only recently traded *Ganoderma lucidum* (Curtis) P. Karst., and *Dendrobium nobile* Lindl. (He et al., 2018; Pyakurel et al., 2019) has also increased number of medicinal plants traded from Nepal to China.

Generally, underground parts (roots, rhizomes, and bulbs), bark, fruits, seeds, flowers, leaves, and twigs are the medicinal plants' traded parts. However, these are often harvested by uprooting the whole plant (Ghimire et al., 2008). The harvesting pattern of underground parts, the whole plant, or bark for collecting large quantities of the plant material is considered destructive (Cunningham, 1993) compared with the harvesting pattern of fruits, seeds, flowers, leaves and twigs (Gaoue & Ticktin, 2007).

About 59% of investigated Nepalese medicinal plant species exported to China are destructively harvested which is comparatively higher than the findings of Pyakurel et al. (2019) who reported 50% medicinal plant species exported from Nepal are harvested by destructive pattern. This indicates that China has a high demand in the underground parts, whole plant or bark of the commercial medicinal plants.

Conclusion

The medicinal plants from Nepal are traded towards Tibet for millennia with more medicinal and cultural functions. In contrast, present Nepalese medicinal plants trade to China is more economical due to the availability of high bioactive compounds, medicinal efficiency, ancient medication system (Tibetan and traditional Chinese Medicine), preparation of aromatic products, cosmetics, plant fibres, herbal dyes, food, flavours, gifts, etc.

Improving Nepal-China relations with Nepal's agreement on Chinese Belt and Road Initiative (BRI) and the cooperation of development of Traditional medicine during President Xi's state visit to Nepal in 2019 is expected to amplify the Nepalese medicinal plants trade, providing opportunity for nation's economic development.

Acknowledgements

The present research was conducted as a partial basis for the first author's Ph.D. dissertation at the Department of Public Policy, City University of Hong Kong. Research Activities Fund for the field work was obtained from Chow Yei Ching School of Graduate Studies, City University of Hong Kong (Scholarships and Financial Aid ID 000669). The researchers would like to thank the local officials of Divisional Forest Office of Kathmandu, Sindhupalchowk and Sankhuwasabha, members of the Herbal Entrepreneur Association Nepal (HEAN), the Nepal Herbs and Herbal Products Association (NEHHPA) and the Federation of Community Forestry User's Nepal (FECOFUN) and its district chapters, and the Transiting to Green Growth-Nepal (TGG-N) project at FECOFUN for providing information on medicinal plants trade and traded species in 2016. The researchers are grateful to Carsten Smith-Hall and Laxmi Raj Joshi for providing their valuable suggestions for framing the manuscript. The researchers are also thankful to the College Human Subjects Ethics Sub- Committee, City University of Hong Kong and the Department of National Parks and Wildlife Conservation, Government of Nepal for issuing research permission. The researchers would also like to thank the two anonymous reviewers for their comments and suggestions on an earlier version of the manuscript.

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Appendix I. Medicinal Plant species traded from Nepal to China.

S. N.	Family	Scientific Name	English Name	Nepali (N), Tibetan (T) and Chinese (C) Name	Life Form	Parts Traded	Occurrence Status	Distribution in Nepal (m)	Threat Status	Source
1	Anacardiaceae	*Pistacia chinensis* subsp. *integerrima* (J. L. Stewart) Rech. f.	Insect Gall in Pistacia	Kakadsinghi (N)	ST	Leaf, twig	Nat-W	W (600-2400)	-	Field Survey, 2016
2	Araliaceae	*Panax pseudoginseng* Wall.	Wild Potato	Saatgath / Ban Aalu (N)	PH	Underground Part	Nat-W	CE (2100-3800)	CAMP-V	DFO KTM, 2020
3	Asparagaceae	*Polygonatum cirrhifolium* (Wall.) Royle	-	Setakchini / Khiraulo (N)	PH	Underground part	Nat-W	WCE (1230-4600)	-	DFO KTM, 2020
4	Asparagaceae	*Polygonatum verticillatum* (L.) All.	-	Setakchini / Khiraulo (N)	PH	Underground part	Nat-W	WCE (2700-4700)	-	DFO KTM, 2020
5	Asparagaceae	*Asparagus racemosus* Willd.	-	Kurilo/Satawari (N)	SSH	Underground part	Nat-C&W	WCE (100-2200)	CAMP-V	DFO KTM, 2020
6	Asteraceae	*Artemisia indica* Willd.	-	Titepati (N)	SSH	Leaf, twig	Nat-W	(WCE 180-3100)	-	DFO KTM, 2020
7	Berberidaceae	*Berberis aristata* DC.	-	Chutro (N)	SH	Bark, Wood	Nat-W	WC (1150-4000)	-	DFO KTM, 2020
8	Berberidaceae	*Berberis asiatica* Roxb. ex DC.	-	Chutro (N)	SH	Bark, Wood	Nat-W	WCE (650-3150)	-	Field Survey, 2016
9	Bignoniaceae	*Oroxylum indicum* (L.) Kurz.	-	Tatelo (N)	ST	Bark, Seed	Nat-W	WCE (300-1400)	CAMP-EN	DFO KTM, 2020
10	Caprifoliaceae	*Nardostachys jatamansi* (D.Don) DC. *Syn:* Nardostachys grandiflora *Wall. ex DC.* *grandiflora Wall. ex DC.*	Spikenard	Jatamansi / Bhutle (N); Pangpö, spang spos (T); 甘松香(C)	ST	Und_prt, Rhizome	Nat-W	WCE (3200-5000)	GoN-RE, CAMP-V; IUCN-CR; CITES II	Acharya, 2000; Saxer, 2009; He et al., 2018
11	Combretaceae	*Terminalia bellirica* (Gaertn.) Roxb.	Belleric Myrobolan	Barro (N); Barura (T)	LT	Fruit, Seed, Bark	Nat-W	CE (100-1100)	-	Saxer, 2009; Field Survey, 2016
12	Combretaceae	*Terminalia chebula* Retz.	Chebulic Myrobolan	Harro (N); Arura (T)	LT	Fruit, Seed	Nat-W	CE (100-1500)	-	Saxer, 2009; Field Survey, 2016;
13	Compositae	*Saussurea gossipiphora* D.Don	-	Yazembawa/ Maikopila/ Kapse	PH	Fruit, Seed	Nat-W	CE (3300-5700)	-	Sherpa, 2001

			phool (N); bya-rgod-sug-po (T)						
14 Crassulaceae	*Rhodiola crenulata* (Hook.f. & Thomson) H. Ohba	-	Solomarpo (T)	PH	Underground part	Nat-W	E (4800-5300)	IUCN-EN	Saxer, 2009
15 Cucurbitaceae	*Herpetospermum pedunculosum* (Ser.) C. B. Clarke	-	Ban Karela (N)	HCL	Fruit, Seed	Nat-W	WCE (1500-3600)	-	DFO KTM, 2020
16 Cupressaceae	*Juniperus indica* Bertol.	-	Juniper / Dhupi (N); shug pa (T)	ST	Leaves; Essential Oil	Nat-W	WCE (2800-4800)	IUCN-LC	Field Survey, 2016; DFO KTM, 2020; Field Survey, 2020
17 Dipterocarpaceae	*Shorea robusta* Gaertn.	Resin of *Shorea robusta*	Saldhup (N)	LT	Resin	Nat-W	WCE (60-1500)	IUCN-LC; GoN-FTE	Field Survey, 2016
18 Elaeocarpaceae	*Elaeocarpus angustifolius* Bl.;Syn: *Elaeocarpus sphaericus* (Gaertn.) K. Schum	Utrasum Bead Tree	Rudrakshya (N)	T	Fruit ,Seed	Nat-C	CE (700-1700)	IUCN-LC	PSCN, 2014; Field Survey, 2016; Pyakurel, 2018; DFO KTM, 2020
19 Elaeocarpaceae	Elaeocarpus lanceifolius Roxb.	-	Bhadrakshya (N)	ST	Fruit, seed	Nat-C&W	E (1000-2010)	-	Field Survey 2020
20 Eleagnaceae	*Hippophae salicifolia* D. Don	Seabuckthorn	Dhale chuk (N); star bu (T)	SH	Fruit, Seed	Nat-W	WCE (2200-3900)	-	Field Survey, 2016
21 Eleagnaceae	*Hippophae tibetana* Schltdl.	-	Bhuin Chuk (N); star bu (T)	SH	Fruit, Seed	Nat-W	WCE (3300-4700)	-	Field Survey, 2016
22 Ephedraceae	*Ephedra gerardiana* Wall. ex Klotzsch & Garcke Garcke	-	Ephedra (N); Riwo Tsedum (T); 山▪麻黄 (C)	SH	Branches, Leaf, twig	Nat-W	WCE (1100-5400)	CAMP-EN	Field Survey, 2016; He et al., 2018
23 Ericaceae	*Rhododendron anthopogon* D. Don.	Rhododendron	Sunpati (N); da lis, ba-lu-dkar-po, dwa-li metog (T)	SH	Leaves, twig	Nat-W	WCE (2900-5500)	-	DFO KTM, 2020
24 Ganodermataceae	*Ganoderma lucidum* (Curtis) P. Karst.	Livlite mushroom	Rato Chhyau (N); Kosha (T); 灵芝(C)	Fungi	whole plant	Nat-W	WC (1600-3000)	-	Pyakurel and Panthi, 2015; He et al., 2018; DFO KTM, 2020
25 Gentianaceae	*Gentiana urnula* H. Smith	-	Gangachung (T)	PH	Whole plant	Nat-W	E (4700-6200)	IUCN-EN	Saxer, 2009
26 Gentianaceae	*Swertia chirayita* (Roxb.) Buch.-Ham.ex C.B. Clarke	Chireeta	Chirayita, Tite (N); Gyatig, Tigta,	AH	Whole plant	Nat-C&W	WCE (1200-3000)	CAMP-V	Saxer, 2009; Phoboo and Jha,

			Chaktig Nagpo (T)' 龙胆草(C)						2010; PSCN, 2014; Field Survey, 2016; Cunningham *et al*., 2018; He et al., 2018; Pyakurel, 2018; DFO KTM, 2020; Field Survey, 2020
27 Hypocreaceae	*Ophiocordyceps sinensis* (Berk.) G.H.Sung, J.M.Sung, Hywel-Jones & Spatafora; Syn: *Cordyceps sinensis* (Berk.) Sacc.	Caterpillar fungus	Yarshaguma (N); Yartsa Gumbu (T); 虫草(C)	Fungi	Whole part	Nat-W	CE (3500-5000)	IUCN-V	Amatya, 2006; Devkota, and Shrestha, 2006; Saxer, 2009; PSCN, 2014; Field Survey, 2016 (Thamel); van Boeckel, 2017; He et al., 2018; Pyakurel, 2018; DFO KTM, 2020; Field Survey, 2020
28 Hypoxidaceae	*Curculigo orchioides* Gaertn.	-	Black Musli (N); 地棕(C)	PH	Underground Part	Nat-W	CE (100-1700)	CAMP-V	Field Survey, 2016; He et al., 2018
29 Iridaceae	*Crocus sativus* L.	Saffron	Keshar (N); gur gum, Khache Shakam (T); 藏红花(C)	PH	Flower	Exo-C	(1500-2500)		Field Survey, 2016(Thamel), He et al., 2018
30 Lauraceae	*Cinnamomum tamala* (Buch.-Ham.) T.Nees	-	Dalchini / Tejpat (N); Shingtsa (T); 桂皮(C)	ST	Leaf and Bark	Nat-C&W	WCE (400-2300)	-	He et al., 2018
31 Lauraceae	*Machilus odoratissima* Nees; Syn: *Persea odoratissima* (Nees) Kosterm.	-	Kaulo (N)	LT	Bark of main stem	Nat-W	WCE (300-2200)	-	Field Survey, 2016
32 Leguminosae	*Butea monosperma* (Lam.) Taub.	Flame-of-the-forest, Bastard teak	Palas (N)	LT	Fruit, seed	Nat-W	WCE (150-1200)	CAMP-V	Field Survey, 2016
33 Liliaceae	*Fritillaria cirrhosa* D.Don	Snake's Head	Ban Lasun / Kakoli	PH	Underground	Nat-W	WCE	CAMP-V	PSCN, 2014; Field

		Fritillary	(N); Aikhashapa (T); 川贝母(C)		part		(3000-4765)		Survey, 2016(Thamel); He et al., 2018; Pyakurel, 2018; DFO KTM, 2020; Field Survey, 2020
34 Lycopodiaceae	*Lycopodium japonicum* Thunb. ex Murray; Syn: *Lycopodium clavatum* var. *wallichianum* Spring	Club Moss	Nagbeli (N)	Pteridophyta	Fruit, Seed, Spores	Nat-W	WCE (1600-3960)		Field Survey, 2016
35 Melanthiaceae	*Paris polyphylla* Sm.	Love Apple	Satuwa (N); 重楼(C)	PH	Underground part	Nat-C&W	WCE (1300-3560)	CAMP-V	PSCN, 2014; Field Survey, 2016; van Boeckel, 2017; He et al., 2018; Pyakurel, 2018; DFO KTM, 2020; Field Survey, 2020
36 Morchellaceae	*Morchella esculenta* (L.) Pers.; Synonyms: *Morchella conica* Pers.; *Morchella umbrina* Boud.; *Morchella vulgaris* (Pers.) Gray	-	Gucchi Chyau (N)	Fungi	Whole part	Nat-W	WC (2000-3500)	-	Field Survey, 2016 (Thamel); DFO KTM, 2020
37 Orchidaceae	*Brachycorythis obcordata* (Lindl.) Summerh.	-	Gamdol (N)	PH	Underground part	Nat-W	WC (580-2600)	CITES II	Field Survey, 2016
38 Orchidaceae	*Dactylorhiza hatagirea* (D. Don) Soó; Synonyms: *Orchis hatagirea* D.Don; *Orchis latifolia* var. *indica* Lindl.	Salep, Marsh Orchid	Panchaunle / Hatajadi (N); dbang-lag (T)	PH	Underground part	Nat-W	WCE (2800-4300)	CITES II; CAMP-EN; GoN Ban	Field Survey, 2016 (Thamel)
39 Orchidaceae	*Dendrobium nobile* Lindl.	Noble dendrobium	Sungava (N); Pushel Tse (T); 石斛(C)	Epi H	Stem	Nat-W	E (300-3400)	CITES II	He et al., 2018
40 Orchidaceae	*Gastrodia elata* Blume	Orchid	Sungava (N); Deng phung (T); 天麻(C)	Sp H	Stem	Nat-W	CE (400-3200)	CITES II; IUCN-VU	He et al., 2018
41 Parmeliaceae	*Everniastrum nepalense* (Taylor) Hale ex Sipman; Synonyms: *Hypotrachyna*	Lichens	Jhyau (N); rdo dreg (T)	Lichen	whole plant	Nat-W	WCE (1000-3000)	GoN-RE	Field Survey, 2016

	nepalensis (Taylor) Divakar; *Parmelia nepalensis* Taylor								
42 Phyllanthaceae	*Phyllanthus emblica* L.; Syn: *Emblica officinalis* Gaertn.	Emblic Myrobolan / Gooseberry	Amala (N); Kyarura / Kyaru (T)	ST	Fruit, Seed	Nat-C&W	WCE (100-1400)	-	Saxer, 2009; DFO KTM, 2020
43 Plantaginaceae	*Picrorhiza scrophulariiflora* Pennell; Syn: *Neopicrorhiza scrophulariiflora* (Pennell) D. Y. Hong	Gentian	Kutki (N); Honglen (T); 胡黄连(C)	PH	Underground part	Nat-W	WCE (3500-5300)	CAMP-V; GoN-Conditonal ban	Acharya, 2000; Sherpa, 2001; PSCN, 2014; Field Survey, 2016; He et al., 2018; Pyakurel, 2018; Field Survey, 2020
44 Polygonaceae	*Rheum australe* D. Don	Himalayan Rhubarb	Padamchaal (N); Chum Tsa (T); 藏边大黄(C)	PH	Underground part, Leaf, twig	Nat-W	WCE (2700-4400)	CAMP-V	Field Survey, 2016; He et al., 2018
45 Primulaceae	*Embelia ribes* Burm. F	-	Kalikath ko geda, Bayobiding, Kaladana (N)	SH	Fruit, Seed	Nat-W	WCE (400-1600)	-	DFO KTM, 2020
46 Ranunculaceae	*Delphinium himalayae* Munz; Syn: *Delphinium himalayense* Chowdhury ex Mukerjee	Aconite Root	Atis Root (N); Bongkar, bya rkang (T)	PH	Underground Part	Nat-W	WC (2000-4550)	CAMP-V; IUCN-R	Acharya, 2000; Field Survey, 2016; He et al., 2018; DFO KTM, 2020
47 Ranunculaceae	*Delphinium denudatum* Wall. ex. Hook. f. & Thomson	Larkspur	Nirmasi (N); bya rkang (T)	PH	Underground Part	Nat-W	WC (1500-3000)	-	Field Survey, 2016; DFO KTM, 2020
48 Ranunculaceae	*Aconitum palmatum* D. Don; Syn: *Aconitum bisma* (Buch.-Ham.) Rapaics	-	Bikhma (N)	PH	Underground Part	Nat-W	CE (3200-4500)	CAMP-DD	Field Survey, 2020
49 Rhamnaceae	*Ziziphus xiangchengensis* Y. L. Chen and P. K. Chou; Syn: *Ziziphus budhensis* K.R. Bhattarai and Pathak	-	Buddha Chitta, Bodhichitta (N)	ST/SH	Fruit, seed	Exo-C	(1200-2000)	-	IFA, 2015; DFO KTM, 2020
50 Rosaceae	*Argentina lineata* (Trevir.) Soják; Synonyms: *Potentilla fulgens* Wall. ex Hook.; *Potentilla lineata* Trevir	Cinquefoil, Silver Leaf	Bajradanti (N); Dumbu Rerel (T); 管仲 (C)	PH	Root	Nat-W	WCE (1700-4100)	-	Field Survey, 2016; He et al., 2018
51 Rubiaceae	*Rubia manjith* Roxb. ex	Madder	Majitho (N); Tsöpa	HCL	Root, Stem	Nat-W	WCE	CAMP-V	Field Survey, 2016;

	Fleming		(T); 茜草(C)				(1100-2900)		He et al., 2018; DFO KTM, 2020
52 Rutaceae	*Zanthoxylum armatum* DC.	Nepalese Pepper	Timur (N)	SH	Fruit, Seed	Nat-C & W	WCE (730-3100)	-	GoN, 2011; Pyakurel and Panthi, 2015; Field Survey, 2016; He et al., 2018
53 Sapindaceae	*Sapindus mukorossi* Gaertn.	Soapnut, Beads, Soapberry	Ritha (N)	ST	Fruit, Seed	Nat-C & W	W (900-1700)	-	Field Survey, 2016;
54 Saxifragaceae	*Bergenia ciliata* (Haw.) Sternb.	Rockfoil	Pakhanbed (N)	PH	Underground Part	Nat-W	WCE (900-2500)	-	Field Survey, 2016; DFO KTM, 2020

Notes on species list table

Species name with author citation following http://www.catalogueoflife.org/col/search/all

Catalogue of Life: 2018 Annual Checklist (http://www.catalogueoflife.org/annual-checklist/2018/) for nomenclature of angiosperms, gymnosperms and pteridophytes; and mycobank database (mycobank.org) for fungi and lichens.

Life form, Traded part, Occurrence, Global distribution (native range) http://www.plantsoftheworldonline.org/, Distribution in Nepal, Habitat and min-max altitude (m) following Pyakurel *et al*. 2019.

Life form: AH-Annual herb; Epi H-Epiphytic herb; HCL-Herbaceous climber; LT-Large tree; PH-Perennial herb; SH-Shrub; Sp H-Saprophytic herb; SSH-Small shrub; ST-Small tree; T-Tree

Occurrence status: Exo_C- Exotic cultivated; Exo_N- Exotic naturalized; Nat_C- native cultivated; Nat_C&W- native wild and cultivated; Nat_W- native wild

Conservation category: CAMP- Conservation Assessment Management Programme (CAMP, 2001); GoN- Government of Nepal (GoN, 2001); IUCN- International Union for Conservation of Nature (IUCN, 2020); DD- data deficient; LC- least concern; NT- near threatened; Vu- vulnerable; EN- endangered; CR- critically endangered https://www.iucnredlist.org/; Ban Government of Nepal (GoN, 2001) ban on collection, use, transport, trade and export; Ban RE- GoN ban on raw export; Ban FTE- GoN ban on felling, transportation and export; CITES-Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2020) http://checklist.cites.org/#/en accessed on 1June 2020.

Antioxidant Activity of Selected Fresh Green Leafy Vegetables Cultivated in Dharan Sub-Metropolitan City

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Abstract

Fresh green leafy vegetables; *Brassica juncea* (Broadleaf Mustard), *Chenopodium album* (Lamb's quarter), *Trigonella foenum graecum* (Fenugreek), *Anethum sowa* (Dill greens), and *Amaranthus tricolor* (Red Amaranth) were collected from Basantatar, Dharan, Sunsari district, Nepal and the antioxidant activity was determined. They were washed with distilled water and the phytochemicals were extracted in 99% methanol from fresh leaves to determine the three different parameters namely: Total antioxidant capacity, Reducing power assay, and 2,2-diphenylpicrylhydrazyl (*DPPH*) assay. The total antioxidant capacity (TAC) of *B. juncea, C. album, T. foenum graecum*, *A. sowa,* and *A. tricolor* was found to be 33.55±0.65, 38.78±0.35, 40.41±0.32, 50.87±0.28 and 36.53±0.73 mg AAE/100 g fresh weight, respectively. Similarly, the reducing power assay of *B. juncea, C. album, T. foenum graecum*, *A. sowa,* and *A. tricolor* was found to be 19.38±0.05, 18.28±0.08, 18.06±0.12, 41.02±0.65, and 19.06±0.13 mg AAE/100 g, respectively. Finally, the DPPH scavenging activity of *B. juncea, C. album, T. foenum graecum*, *A. sowa,* and *A. tricolor* was found to be 21.85±0.61%, 26.97±0.4%, 31.55±1.22%, 58.45±2.22%, and 41.38±1.12%, respectively. Overall, the study showed that the methanolic extract of fresh *A. sowa* possessed higher antioxidant activity in all three antioxidant assays among the other vegetables selected. Hence, it is recommended for improving defence systems in health and disease.

Key words: Antioxidant capacity, DPPH scavenging activity, Green leafy vegetables, Reducing power assay

Introduction

Green leafy vegetables are the most readily available sources of carbohydrates, fats, and essential protein (Bhat & Al-Daihan, 2014). They are a rich source of carotene, ascorbic acid, riboflavin, folic acids, and minerals like calcium, iron, and phosphorus (Fasuyi, 2006). Green leafy vegetables can be used fresh as a salad or can be cooked/processed as per the consumer's interest (Sharma & Rawal, 2013). These are becoming more popular for the masses day by day due to consumers' increased awareness about natural and organic foods. Bioactive substances have a wide range of biological functions, including antioxidant and antimicrobial activities (Burt, 2004). They can be helpful in the management of oxidative stress and age-related human aliments (Gacche et al., 2010). Being a photosynthetic tissue, leafy vegetables have higher vitamin K levels compared with other fruits and vegetables due to the direct involvement of vitamin K (phylloquinone) in the photosynthesis process. Vegetables as medicinal plants has none or less toxic effects (Souza et al., 2005), and can synthesize several secondary metabolites of relatively complex structures possessing antimicrobial activities (Dhiman et al., 2012).

Green leafy vegetables are also rich in antidiabetic, anti-histaminic, anti-carcinogenic, and hypolipidemic properties. They possess preventive or curative properties against cardiovascular disease, aging, obesity, hypertension, and insomnia. Leafy vegetables are a natural source of antioxidants and rich in phytochemicals (Bhat & Al-Daihan, 2014). Various research ûndings have demonstrated that changes in oxygen utilization in the body and increased formation of reactive oxygen species (ROS) contribute to many chronic diseases. Although an organism is naturally equipped with antioxidant protection systems to cope with ROS's harmful effects, the endogenous antioxidant defence system is not adequate to counteract the oxidative stress (Houston, 2010). Therefore, protection against oxidative stress depends partly on the adequacy of dietary antioxidants (Kaliora et al., 2006). Evidence suggests that phytochemicals from fruits and vegetables, including leafy vegetables, are capable of protecting against free radicals (Engwa, 2018). The burden over synthetic chemicals can be reduced by encouraging the use of green leafy vegetables in food and food products.

Green leafy vegetables represent an important proportion of foods with medicinal value (Sree et al., 2013). Limited information is available on the medicinal properties associated with leafy vegetable consumption in Nepal. During the fiscal year 2014/15, the production of vegetables was 3580085 metric tons (MT). The productivity of vegetables has increased from 9.5 to 12.2 MT/hector between 1998 and 2007. Due to health awareness and important nutrititional values of vegetables and fruits, the per capita consumption of vegetables in Nepal has increased from 49 kg/person/year to 60 kg/person/year but remains still below the human vegetable nutritional requirement, i.e., 104 kg/person/year (Shrestha & Rai, 2012). However, there is no detailed report on the evaluation of the antioxidant potential of green leaves which are the most consumed type of vegetables. Thus the present study takes into consideration the antioxidant activities of fresh green leafy vegetables which is considered to have plenty of antioxidant functions. Therefore, the extracts of green leafy vegetables were prepared, and their total antioxidant capacity, free radical scavenging activity, reducing power ability, were determined.

Materials and Methods

Raw Materials

The green leafy vegetable plants under the study were *Trigonella foenum graecum* (Fenugreek Leaves), *Anethum sowa* (Fresh Dill Greens), *Brassica juncea* (Broad Leaf Mustard), *Chenopodium album* (Lamb's Quarter), and *Amaranthus tricolor* (Red Amaranth) which were collected from Basantatar, Dharan, Sunsari, Nepal (26°48'51.7" N, 87°16.783' E). They were identified from the Department of Biology, Central Campus of Technology, Dharan.

Apparatus and Chemicals Required

The required apparatus and chemicals were obtained from the laboratory of Central Campus of Technology, Dharan.

List of equipments

- Centrifuge, 3000 rpm (Victolab, India)
- Electric balance (Phoenix instrument)
- Spectrophotometer (Labtronics, India)
- Soxhlet apparatus (Y.P. Scientific industries)
- Hot air oven (Victolab, India)
- Incubator (Victolab, India)
- Rotary Vacuum Evaporator (IKA RV 10)
- Refrigerator (Victolab, India)

List of chemicals

- Ammonium molybdate (Thermo Fisher Scientific India Pvt. Ltd.)
- Ascorbic acid (Thermo Fisher Scientific India Pvt. Ltd.)
- Disodium hydrogen orthophosphate (Thermo Fisher Scientific India Pvt. Ltd.)
- 2,2-diphenyl-1-picrylhydrazyl (DPPH, purity 95%) (Thermo Fisher Scientific India Pvt. Ltd.)
- Ferric chloride (Thermo Fisher Scientific India Pvt. Ltd.)
- Methanol (Merck (India) Ltd.)
- Sodium dihydrogen orthophosphate (Thermo Fisher Scientific India Pvt. Ltd.
- Sulphuric acid (Thermo Fisher Scientific India Pvt. Ltd)
- Trichloroacetic acid (Thermo Fisher Scientific India Pvt. Ltd.)
- Potassium ferricyanide (Thermo Fisher Scientific India Pvt. Ltd.)

Collection and Preparation of Sample

The plant specimens under study were collected in April 2018 from Basantatar, Dharan, Sunsari District of Nepal. The basic flow diagram of the methodology is made by modifications from the methodologies described by Jaradat et al. (2015) and is shown in figure 1.

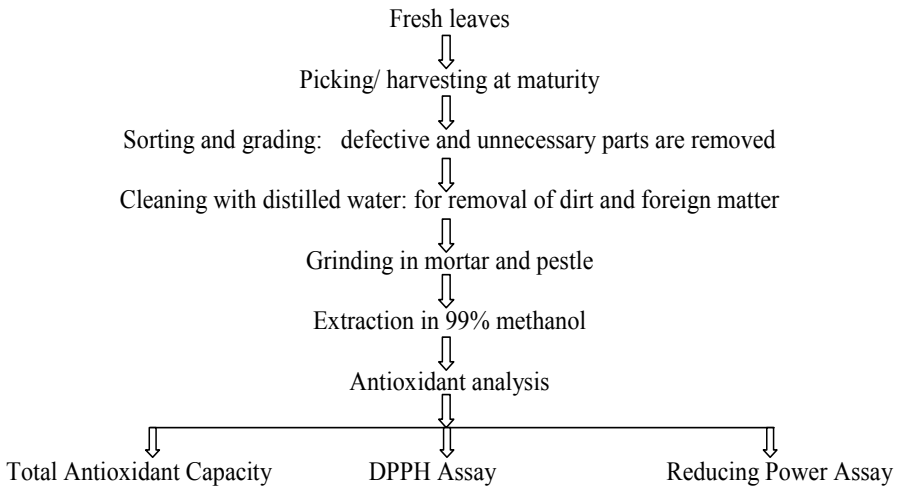

Figure 1. Flow diagram of the methodology

Preparation of Plant Extracts for Antioxidant Evaluation

About 10 g of the pulverized plant powder was soaked in 1 L of methanol (99%) and shaken by hand at 30 min interval for 8 h per day at room temperature for 3 days and stored in the refrigerator for 4 days. The extracts were then filtered using filter papers and concentrated under vacuum on a rotary evaporator. The crude extract was stored at 4ºC and the antioxidant test was done directly within 5 minutes (Jaradat et al., 2015).

Total antioxidant capacity

The total antioxidant capacity of leaf extracts was analyzed by the phosphomolybdenum reduction assay method according to the procedure described by Prieto et al. (1999). The assay is based on the reduction of Mo (VI) to Mo (V) by the methanol extract of different vegetables, and subsequent formation of green phosphate/Mo (V) complex at acid pH (Kumar et al., 2018). The tubes containing leaf extract (0.3 ml) and 3 ml reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) were incubated at 95ºC for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm spectrophotometrically against a blank. The antioxidant capacity was expressed as ascorbic acid equivalents (AAE) with the help of standard curve obtained from ascorbic acid.

The antioxidative potential of plant extracts can be measured using various *in vitro* assays and each assay is based on at least one feature of antioxidant activity. However, the total antioxidant properties of plants cannot be evaluated by any single method because of their complex nature of phytochemicals. Therefore, two or more methods should always be employed to evaluate the total antioxidative effects of plant extracts (Gunathilake & Ranaweera, 2016).

DPPH radical scavenging assay

The capacity of prepared extracts to scavenge the 'stable' free radical DPPH was monitored according to the method of Hatano et al. (1988) with slight modifications. Extracts (100 µl) were mixed in 3.9 ml freshly prepared methanolic solution of DPPH (1 mM). The mixture was vortexed at 3000 rpm in a centrifuge for 15 s and then left to stand at room temperature for 30 min in the dark. The absorbance of the resulting solution was read spectro-photometrically (UV/VIS spectrometer) at 517 nm. The percentage inhibition of the radicals due to the antioxidant activity of leaf extracts was calculated using the following formula.

$$\% \text{ inhibition} = (A_{control} - A_{sample})/A_{control} \times 100$$

$A_{control}$ is the absorbance of the DPPH solution with nothing added (control).

Lower absorbance of the reaction mixture indicates higher free radical scavenging activity (Zhao et al., 2008). Environmental temperature plays a significant role in antioxidant activity evaluation and it is more pronounced in cold weather (Iqbal & Bhanger, 2006).

Reducing power assay

The reducing power of the prepared extracts was determined according to the method of

Oyaizu (1986). Briefly, each extract (1 ml) was mixed with 2.5 ml of a 0.2M phosphate buffer (pH 6.6) and 2.5 ml of a 1% (w/v) solution of potassium ferricyanide. The mixture was incubated in a water bath at 50ºC for 20 min and then 2.5 ml of 10% (w/v) trichloroacetic acid solution was added and the mixture was then centrifuged for 10 min at 3000rpm. 2.5 ml aliquot of the upper layer was combined with 2.5 ml of distilled water and 0.5 ml of 0.1% (w/v) ferric chloride solution. The absorbance of the reaction mixture was read using a UV/VIS spectrometer (SP-3000) at 700 nm. Here, ascorbic acid was used as a reference standard, the reducing power of the samples was compared with the reference standard.

The various biological and environmental factors in which the plant grew also contribute to the plant's antioxidant power (Dimcheva & Karsheva, 2018). The reduction of Fe^{3+} has been described as an indicator of electron-donating activity which can demonstrate the antioxidant potential of the different phenolic compounds of Phyto origin (Gunathilake & Ranaweera, 2016). The reducing power is generally associated with the presence of reductones (Saritha et al., 2010), which has been shown to exhibit antioxidant potential by splitting the free radical chains by donating hydrogen atoms. Reductones can prevent peroxide formation by reacting with the precursors of peroxides.

Statistical Analysis

The analysis was carried out in triplicate. Statistical calculations were performed in Microsoft Office Excel 2010.

Results

Five different fresh green leafy vegetables were collected from Basantatar, Dharan, Sunsari district of Nepal, and bioactive components were extracted by soaking in 99% methanol for 72 h at room temperature and then concentrated in a rotary vacuum evaporator. After that each sample extracts were analyzed for TAC, reducing power assay, and DPPH scavenging assay.

Total Antioxidant Capacity (TAC)

In the study, TAC of 99% methanolic extract of the different vegetable extract is shown in figure 2.

TAC of 99% methanolic extract of *Brassica juncea*, *Chenopodium album*, *Trigonella foenum graecum*, *Anethum Sowa,* and *Amaranthus tricolor* leaves were found to be 33.55±0.65, 38.78±0.35, 40.42±0.32, 50.78±0.28, and 36.53±0.73 mg AAE/100 g fresh wt. (FW), respectively. *C. album* leaves TAC was found to be 38.78±0.35

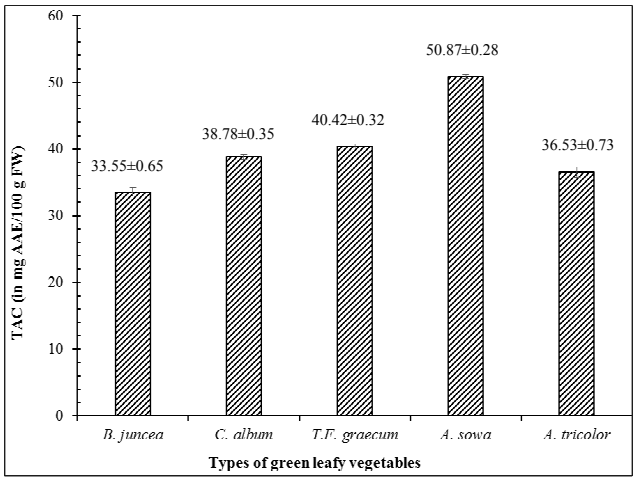

Figure 2. Total antioxidant capacity of green leafy vegetables. The values are expressed in mean ± standard deviation**.**

mg AAE/100 g while the TAC of *T. foenum graecum* and *A. sowa* was found to be 40.42±0.32 and 50.78±0.28 mg AAE/100 g, respectively.

Reducing Power Assay

The reducing power of 99% methanolic extract of fresh green leafy vegetables is shown in figure 3.

Reducing power of 99% methanolic extract of *B. juncea*, *C. album*, *T. foenum-graecum*, *A. Sowa,* and *A. tricolor* was found to be 19.38±0.04, 18.27±0.08, 18.05±0.12, 41.02±0.65, and 19.05±0.13 mg AAE/100 g fresh weight (FW), respectively.

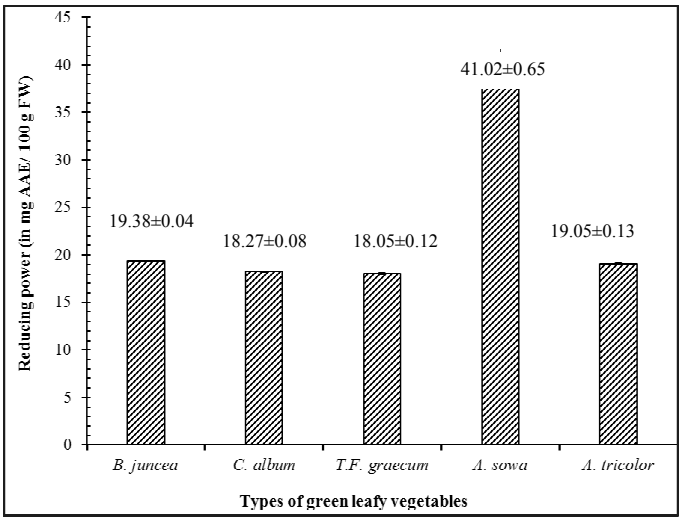

Figure 3. Reducing power of green leafy vegetables. The values are expressed in mean ± standard deviation.

DPPH Scavenging Assay

The DPPH scavenging activity of 99% methanolic extract of fresh green leafy vegetables is shown in figure 4.

The DPPH radical scavenging activity of 99% methanolic extract of *T. foenum graecum* and *A. sowa* were found to be 31.55±1.21% and 58.45±2.22%, respectively. Similarly, the scavenging activity of *B. juncea* and *A. tricolor* extract was observed to be 21.85±0.61% and 41.38±1.12%, respectively. The DPPH radical scavenging activity of 99% methanolic extract of *C. album* was found to be 26.97±0.4%.

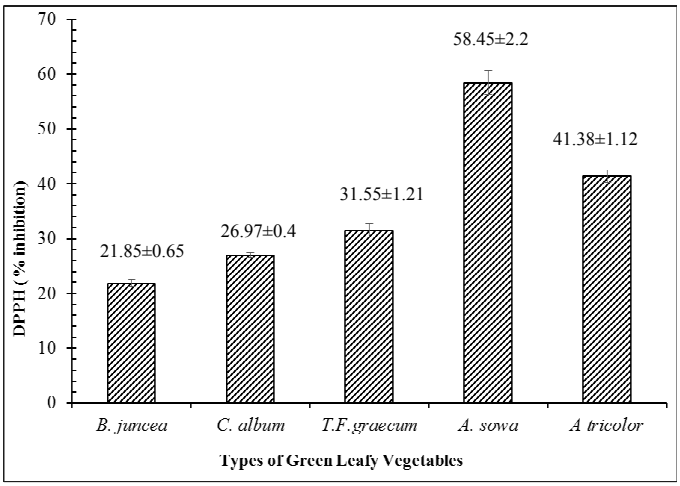

Figure 4. DPPH scavenging activity of green leafy vegetables. The values are expressed in mean ± standard deviation.

Discussion

The study was done by Kapoor et al. (2014) on *B. juncea* was found to be 46.2±3.25 mg AAE/100 g which is close to the current findings.. A study was done in India where the

TAC value of beetroot (which also belongs to the Chenopodiaceae family) was found to be 61.1±2.10 mg AAE/100 g fresh weight. The value is higher than the obtained value due to the polyphenol and flavonoid content of beetroot higher than that of *C. album* (Venkatachalam et al., 2014). The study done in Indian selected fruits and vegetables was in the range of 31.2 to 61.1 mg AAE/100 g. Hence, it can be concluded that the methanol extract of *T. foenum graecum* leaves has very strong antioxidant potential which might be associated with the high level of phenolic and flavonoids type compounds present in the extract (Bhanger et al., 2008). The value of TAC of *A. sowa* extract can be correlated with total phenolic content found in the essential oils of it (Gumus et al., 2016).

The study done by Akin-Idowu et al. (2017) in TAC of *A. caudatus* and *A. Cruentus* was found to be 14.02±4.92 and 14.5±8.40 mg AAE/100 g, respectively. This variation is due to the compositional variation of phytochemicals, methods of extraction, etc. The antioxidative potential of plant extracts can be measured using various *in vitro* assays and each assay is based on at least one feature of antioxidant activity. However, the total antioxidant properties of plants cannot be evaluated by any single method because of their complex nature of phytochemicals. Therefore, two or more methods should always be employed to evaluate the total antioxidative effects of plant extracts (Gunathilake & Ranaweera, 2016). Antioxidants prevent free radical-induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition (Young & Woodside, 2001).

A similar study was done on reducing the power of *B. juncea, C. album,* and *T. foenum graecum* leave extract by Meena et al. (2015) and was found to be 46.5±3.34, 55.4 ± 1.21, and 48.3±2.91 mg AAE/100 g fresh weight. This difference in value may be due to the different varieties used, stage of harvesting, environmental factors, etc.

The result obtained in reducing power assay is similar to the result obtained by Meena et al. (2015) where the reducing power of carrot leaves was 43.5 ± 3.18 mg AAE/100 g. *A. sowa* has been reported to contain flavonoids, phenolic, and essential oil. The excellent antioxidant activity of *A. sowa* is attributed not only to the presence of high content of polyphenols but also to that of volatile constituents that are present in dill (Ramadan et al., 2013). One research done by Akin-Idowu et al. (2017) showed that the reducing power of other variety of amaranth such as *A. caudatus* and *A. Cruentus* extract was found to be 16.8±0.16 and 19.2±0.12 mg AAE/100 g.

The various biological and environmental factors in which the plant grew also contribute to the plant's antioxidant power (Dimcheva & Karsheva, 2018). The reduction of Fe^{3+} has been described as an indicator of electron-donating activity which can demonstrate the antioxidant potential of the different phenolic compounds of Phyto origin (Gunathilake & Ranaweera, 2016; Juntachote & Berghofer, 2005). The reducing power is generally associated with the presence of reductones (Saritha et al., 2010), which has been shown to exhibit antioxidant potential by splitting the free radical chains by donating hydrogen atoms. Reductones can prevent peroxide formation by reacting with the precursors of peroxides.

According to one study, it was found that the scavenging activity of 50% ethanolic soxhlet extract of *T. foenum graecum* and *A. sowa* were 25.7±0.34% and 44.6±0.42% (Gacche et al., 2010). The higher activity of methanol extract of *A. sowa* can be related to its high phenolic

and flavonoid content (Kaur, 2018). However, the study done in Indian mustard ranged from 30.87% to 66.30% (Sarangthem et al., 2011). The research done in the *Chinese* variety of *A. tricolor* extract showed the result of 17.32±3.0% inhibition. The difference may be because the extract was prepared several times and was mixed up at last which decreased the concentration of antioxidants per volume. That's why the % inhibition was lower than the current value and the % inhibition increases with the increase in extract concentration per volume (Baang et al., 2015). According to Kwinana-Mandindi (2015), % inhibition of DPPH of *Chenopodium* extract was about 45%.

This variation in result from other workers might be due to different varieties which lead to genetic variability, time of harvest, stage of harvest, the analytical procedure applied, and climatic conditions (Kumar et al., 2018). It may be also due to the variation in the solvent used during extraction, extraction temperature, etc. On the other hand, an environmental condition such as temperature, light, water, or soil may concern the composition of compounds. Environmental temperature plays a significant role in antioxidant activity evaluation and it is more pronounced in cold weather (Iqbal & Bhanger, 2006).

Conclusion

The total antioxidant capacity (TAC) of 99% methanolic extract for *B. juncea*, *C. album*, *T. foenum graecum, A. sowa,* and *A. tricolor* were found to be 33.55±0.65, 38.78±0.35, 40.41±0.32, 50.87±0.28, and 36.53±0.73 mg AAE/100 g fresh weight, respectively. The reducing power of *B. juncea*, *C. album*, *T. foenum-graecum, A. sowa,* and *A. tricolor* was found to be 19.38±0.04, 18.28±0.08, 18.06±0.12, 41.02±0.65, and 19.06±0.13 mg AAE/100 g, respectively. The DPPH scavenging activity of *B. juncea*, *C. album*, *T. foenum-graecum, A. sowa,* and *A. tricolor* was found to be 21.85±0.61%, 26.97±0.4%, 31.55±1.22%, 58.45±2.22%, and 41.38±1.12%, respectively. From the above observation, it was clear that among the methanolic extracts of fresh green leafy vegetables, the extract from *Anethum sowa* had the highest antioxidant activity. Since *Anethum sowa* is a good source of antioxidants, its cultivation and utilization must be promoted at both local levels and the national level.

Acknowledgment

The authors appreciate all the faculties and staff of the Central Campus of Technology, Nepal for their support and technical assistance.

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Enumeration of Wall Flora of Pokhara Valley, Nepal

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Abstract

Certain plants design their life on wall in addition to their natural habitats. It is simply a matter of occupying space with limited nutrition and water. Present study enumerates the diversity of plants species growing naturally on four different types of wall (Stone-mud wall, Brick-mud wall, Stone-cement wall and Brick-cement wall) in Pokhara valley, Kaski District, Nepal. The wall flora of Pokhara had a total of 123 species of plants (40 families, 90 genera), where three species of bryophytes were the first colonizers and second were the pteridophytes containing nine species. Among them, 96 species (77.41%) were herbs, and 14 species (11.29%) each were shrubs and trees. Most of the herbs and shrubs species were found on the wall base and surrounding areas. Species diversity decrease while the percentage of typical rock xerophytes rise from the base to the top of the walls. This study might contribute to future urban ecology and restoration programs of National Heritage buildings for the Pokhara sub-metropolis.

Key words: Brick-cement wall, Bryophytes, Plant diversity, Pteridophytes, Xerophytes

Introduction

Ecological investigation of the artificial habitats within urban environment is generally neglected. The wall vegetation of Pokhara valley was studied to elucidate the ecology of this habitat. Pokhara is an ancient city having many old walls and buildings. Such walls and buildings provide new space for plant colonists. Certain plants design their life on walls in addition to their natural habitats. It is simply a matter of occupying space with limited nutrition and water by one plant on walls of building and fences without any special obligation. These plants have wide range of adaptations from mesic to xeric conditions.

Pokhara Sub-metropolitan city is the third largest city of Nepal. It is extended from 28º10'N to 28º16'N latitude and 83º58'30"E to 84º22'03"E longitude, covers an area of 201857 ha and 55.22 km in the western region of Nepal, which has well-maintained roads and extensive greenery with high annual precipitation coupled with congenial thermal and altitudinal variations.

Floristic variation in the vegetation of different walls was found to be associated with the nature of its substratum. Thus on the basis of material construction, walls are grouped into: (1) Brick and Cement wall (BC), (2) Stone and Cement wall (SC), (3) Brick and Mud wall (BM), (4) Stone and Mud wall (SM), and (5) Mud wall (MW). Since, Pokhara city is highly urbanized hence only brick-cement and stone-cement are more available than stone-mud and brick-mud walls.

The vegetation zones on the walls were characterized into (i) Horizontal top end of the wall (HT) (ii) Vertical face of the wall (V), and (iii) The base of the wall displaying variation in floristic composition (B).

In India, wall flora of Meghalaya (Chhetri, 1956), Nainital (Pangty & Rawat, 1987), Howrah, West Bengal (Ghosh & Pal, 1997), Midnapur, West Bengal (Gosh & Das, 1998) have been carried out. In Nepal, wall flora of Bhimkali Patan, Pokhara has been carried out (Thapa, 2015). It showed that, there is least studies on wall flora of Nepal.

Materials and Methods

Seasonal visits in winter, summer and rainy period of 2015, were made at regular monthly interval to different localities of Pokhara city for observation and collection of plant specimens from different types of wall habitats. The specimens were collected at the flowering and fruiting periods as far as possible. Field note for plant habits, habitats, and region of habitat they growing was maintained properly. Local names of the plants were collected as much as possible by interviewing the local peoples at collecting sites. The herbarium specimens were critically studied and identified following Hooker and Thomson (1855), Hooker et al. (1872-97), Hara et al. (1978-79, 1982), Rajbhandari (2002), Rajbhandari et al. (2011) and confirmed in the National Herbarium (KATH), Godawari, Kathmandu and Tribhuvan University Central Herbarium (TUCH), Kritipur. Voucher specimens were submitted at the Department of Botany, P.N. Campus, Pokhara.

Results

Present study enumerated a total 123 species of plants belonging to 90 genera and 40 families from different types of walls in Pokhara city. The family Poaceae was dominant with 19 species followed by asteraceae with 16 species and moraceae with 9 species. There were 86 species of dicotyledons, 25 species of monocotyledons, 9 species of pteridophytes, and three species of bryophytes under 61, 21, 5 and 3 genera, respectively (Tables 1, 2). Common tree species were *Bombax ceiba, Sapium insigne, Erythrina suberosa, Litsea monopetala, Toona ciliata, Morus indica, Ficus bengalensis, F. religiosa, F. benjamina, F. glaberrima, F. subincisa, F. lacor, F. Semicordata, F. hispida* and *Nyctanthes arbortristris*. Herb species were *Artemisia dubia, Cannabis sativa*, *Ricinus communis, Colebrookea, oppositifolia,* etc., they were very much stunted in growth perhaps due to deficiency of water and nutrient. But, the under shrubs like *Sida cardifolia, Solanum nigrum, Persicaria alatum* etc., and the herbaceous species such as *Oxalis corniculata*, *Ageratum conyzzoides*, *Centella asiatica, Amaranthus viridis*, *Sonchus asper, S. arvensis, Cardamine hirsuta, Commelia benghalensis,* etc. were found growing luxuriantly which attributes the ability of wall habitat to meet the optimum demand of water and nutrients of these species.

In table 1, families, genera and species are arranged in alphabetical order. The different seasons of collection, types of the wall habitat, region of habitats growing and local names are also mentioned to show the place and season of occurrence of different species. Numerous species of algae and lichens were also observed on such habitats but they are not considered in the present study.

Table 1. List of wall flora recorded from Pokhara valley.

Plant Species	Local Name	Habitats	Vegetation zone	W	S	R
A) BRYOPHYTA				+	-	+
Funariaceae						
1. *Funaria* sp.		BMW, SMW	BW	+	-	+
Marchantiaceae						
2. *Marchantia* sp.		"	"	+	-	+
Ricciaceae						
3. *Riccia* sp.		"	BW, VFW	+	-	+
B) PTERIDOPHYTA		"	" "	+	-	+
Davalliaceae						
4. *Nephrolepis auriculata* (L.) Trimen	Pani amala	"	" "	+	-	+
5. *N. cordifolia* (L.) Presl	Pani amala	"	" "	+	-	+
Equisetaceae						
6. *Equisetum debile* Roxb. ex Voucher	Kukur ghans	"	" "	+	+	+
7. *E. diffusum* D.Don	Ankhle jhar	"	BW	+	+	+
Pteridaceae						
8. *Adiantum* sp.	Kani unue	"	"	+	-	+
9. *Cheilanthes albomarginata* Clarke	Rani sinka-kani sinka	"	"	+	-	+
10. *C. dalhousiae* (Hook)	Rani sinka	"	"	+	-	+
Schizaeaceae						
11. *Lygodium flexuosum* (L.) Sw.	Janai lahara	"	" "	+	-	+
12. *L. japonicum* (Thunb.) Sw.	Janai lahara	"	BW, VFW	+	-	+
C) PHANEROGAMS						
Acanthaceae						
13. *Justicia japonica* Thunb.		"	" "	+	-	+
Amaranthaceae						
14. *Achyranthes* aspera L.	Datiwan	BMW, SMW	BW, VFW	+	+	+
15. *Alternanthera philoxeroides* (Mart.) Griseb.		"	" "	+	+	+
16. *A. sessilis* (L.) DC.	Bhiringi jhar	"	" "	+	+	+
17. *Amaranthus spinosus* L.	Lunre latte	"	" "	+	+	+
18. *A. viridis* L.	Latye sag	"	" "	+	+	+
Anacardiaceae						
19. *Rhus Javanica* L.	Bhaki amilo	BMW, SMW	BW, VFW	+	+	+
20. *R. parviflora* Roxb.	Satibayar	"	" "	+	+	+
21. *R. succedanea* L.	Bhalayo	"	" "	+	+	+
22. *R. wallichi* Hook. f.	Bhalayo	"	" "	+	+	+
Apiaceae						
23. *Centella asiatica* (L.) Urban	Ghod tapre	BMW, SMW	BW	+	+	+
24. *Hydrocotyle sibthorpioides* Lam.	Sano Ghodtapre	"	"	+	+	+
Araliaceae						
25. *Hedera nepalensis* K. Koch	Pipal Pate	BMW, SMW BCW, SCW	BW, VFW	+	+	+
Asteraceae						
26. *Ageratum conyzoides* L.	Gandejhar	BMW, SMW	BW, VFW	+	+	+
27. *A. houstonianum* Mill.	Nilo Gandhe	"	" "	+	+	+
28. *Anaphalis adnata* DC.	Seto eklay ghans	"	" "	-	+	+

Plant Species	Local Name	Habitats	Vegetation zone	W	S	R
29. *A. contorta* (D.Don) Hook. f.	Buki	"	" "	-	+	+
30. *Artemisia dubia* Wall ex. Bosser.	Titepati	"	" "	+	+	+
31. *Bidens pilosa* L.		"	" "	-	+	+
32. *Chromolaena odorata* (L.) R.M. King & H. Rob.	Banmara	"	" "	+	+	+
33. *Crassocephalum crepidioides* (Benth.) S. Moor	Anikale Jhar	"	" "	-	+	+
34. *Emilia sonchifolia* (L.) DC.		"	" "	-	+	+
35. *Erigeron* sp.		"	" "	-	+	+
36. *Galinsoga parviflora* Cav.	Chitlange	"	" "	-	+	+
37. *Gnaphalium affine* D.Don	Bokre phool	"	" "	+	+	+
38. *Parthenium sp.*				-	+	+
39. *Senecio cineraria* DC.	Marchajhar			-	+	+
40. *Sonchus arvensis* L.	Dudhe	"	" "	+	+	+
41. *S. asper* (L.) Hill		"	" "	-	+	+
Bombacaceae						
42. *Bombax ceiba* L.	Simal	BMW, SMW	BW, VFW	+	-	+
Brassicaceae						
43. *Cardamine hirsuta* L.	Bindrai	BMW, SMW	BW, VFW	-	+	+
44. *Nasturtium officinale* Br.	Betendra	"	" "	-	+	+
45. *Rorippa indica* (L.) Hiern		"	" "	-	+	+
Cannabinaceae						
46. *Cannabis sativa* L.	Ganja	BMW, SMW	BW, VFW	-	+	+
Caryophyllaceae						
47. *Drymaria cordata* (L) Roem ex Schult	Abijalo	BMW, SMW	BW, VFW	-	+	+
Chenopodiaceae						
48. *Chenopodium album* L.	Bethe	BMW, SMW	BW, VFW	-	+	+
Commelinaceae						
49. *Commelina benghalensis* L.	Kanejhar	BMW, SMW	BW, VFW	-	+	+
50. *C. paludosa* Blume	Sano kane	"	" "	-	+	+
51. *Murdania japonica* (Thunb.) Faden		"	" "	-	+	+
Convolvulaceae						
52. *Ipomoea purpurea* (L.) Roth	Jayanta	"	" "	-	+	+
53. *I. quamodit* L.		"	" "	-	+	+
Cyperaceae						
54. *Cyperus rotundus* L.	Mothe	"	" "	-	+	+
55. *Kyllinga brevifolia* Rottb.	Mothe	"	" "	-	+	+
56. *K. nemoralis* Rottb.	Mothe	"	" "	-	+	+
Euphorbiaceae						
57. *Euphorbia heterophylla* L.		"	" "	-	+	+
58. *E. hirta* L.	Dudhe jhar	BMW, SMW	BW, VFW	-	+	+
59. *Phyllanthus urinaria* L.	Bhui amala	"	" "	-	+	+
60. *Ricinus communis* L.	Ander	"	" "	-	+	+
61. *Sapium insigne* (Royle) Benth. ex Hook. f.	Khirro	"	" "	+	+	+
Fabaceae						
62. *Cassia occidentalis* L.	Panwar	BMW, SMW	BW, VFW	+	+	+
63. *C. tora* L.	Chinchin	"	" "	+	+	+

Plant Species	Local Name	Habitats	Vegetation zone	W	S	R
64. *Erythrina suberosa* Roxb.	Phadelo	"	" "	+	+	+
65. *Mimosa pudica* L.	Lazzawati	"	" "	+	+	+
Lamiaceae						
66. *Colebrookea oppositifolia* Sm.	Dhursuli			+	+	+
67. *Eisholtzia blanda* (Benth.) Benth.	Ban silam			-	+	+
68. *Justicia adhatoda* L.	Asuro			+	+	+
Lauraceae						
69. *Litsea monopetala* (Roxb.) Pers.	Kutimro			+	+	+
Lythraceae						
70. *Woodfordia fruticosa* (L.) Kurz.	Dhairo			+	+	+
Malvaceae						
71. *Sida cordifolia* L.	Balu	BMW, SMW	BW, VFW	+	+	+
Melastomataceae						
72. *Melastoma malabathricum* (L.) Sm.	Kali angeri			+	+	+
73. *Osbeckia capitata* Walp.	Angeri	BMW, SMW	BW, VFW	+	+	+
74. *O. stellata* Ker-gawl.	Rato Chulsi	"	" "	+	+	+
Meliaceae						
75. *Toona ciliata* Roem.	Tuni	"	" "	+	+	+
Moraceae						
76. *Ficus bengalensis* L.	Bara	"	" " "	+	+	+
77. *F. benjamina* L.	Swami	"	" " "	+	+	+
78. *F. glaberrima* Blume.	Pakhuri	"	" " "	+	+	+
79. *F. hispida* L	Khasreto	"	" " "	+	+	+
80. *F. lacor* Buch-Ham.	Kabhro	"	" " "	+	+	+
81. *F. religiosa* L.	Pipal	"	" " "	+	+	+
82. *F. semicordata* Buch-Ham. ex Sm.	Khnyo	"	" " "	+	+	+
83. *F. subincisa* Buch-Ham. ex Sm.	Bedulo	"	" " "	+	+	+
84. *Morus indica* L.	Kimbu	ALL	BW, HTW VFW	+	+	+
Nyctaginaceae						
85. *Mirabilis jalapa* L.	Lankasani (Malati)	BMW, SMW	BW, VFW	-	+	+
Oleaceae						
86. *Nyctanthes arbor-tristris* L.	Parijat	"	" "	-	+	+
Oxalidaceae						
87. *Oxalis corniculata* L.	Chari amilo	BMW, SMW	BW, VFW	+	+	+
88. *O. latifolia* Kunth	Thulo Chari amilo	"	" "	+	-	+
Plantaginaceae						
89. *Plantago erosa* Wall.	Isabgol	BMW, SMW	BW, VFW	-	+	+
Poaceae						
90. *Arunda donax* L.	Ekiro			+	+	+
91. *Arundinella nepalensis* Trin.	Musekharu			-	+	+
92. *Axonopus compressus* (Sw.) P. Beauv.	Chaure ghans	"	" "	-	+	+
93. *Chrysopogon aciculatus* (Retz.) Trin.	Kuro	"	" "	-	+	+
94. *Cynodon dactylon* Pers.	Dubo	BMW, SMW	BW, VFW	+	+	+
95. *Dactyloctenium aegypticum* (L.) Willd.		"	" "	+	+	+
96. *Desmostachya bipinnata* (L.) Stapf	Kush			-	+	+
97. *Digitaria setigera* Ruth. ex R & S		"	" "	+	+	+

Plant Species	Local Name	Habitats	Vegetation zone	W	S	R
98. *D. ciliaris* (Retz.) Koeler	chitre banso	"	" "	+	+	+
99. *Echinochloa colona* (L.) Link.	Sano Sama	"	" "	+	+	+
100. *Eleusine indica* (L.) Gaertn.	Kode jhar	"	" "	-	+	+
101. *Eragrostis tenella* (L.) P. Beauv. ex. R & S	Chiure Banso	"	" "	-	+	+
102. *Eulaliopsis binata* (Retz.) C.E. Hubbard	Babio	"	BW, VFW	-	+	+
103. *Imperata cylindrica* (L.) P. Beauv	Siru	"	" "	-	+	+
104. *Paspalum conjugatum* Bergium		"	" "	-	+	+
105. *P. disticum* L.			" "	-	+	+
106. *Pogonatherum crinitum* (Thunb.) Kunth	Kuro	"		-	+	+
107. *Saccharum spontaneum* L.	Kans	"	" "	-	+	+
108. *Setaria glauca* (L.) Beauv.		"	" "	-	+	+
Polygonaceae						
109. *Fagophyrum dibotrys* (D.Don) Hara	Ban Phaphar	BMW, SMW	BW, VFW	-	+	+
110. *Persicaria alatum* Ham.		"	" "	-	+	+
111. *P. capitatum* Hasssm.	Ratnaule jhar	"	" "	+	+	+
112. *P. perfoliatum* L.		"	" "	-	+	+
113. *Rumex nepalensis* Spreng		"	" "	+	+	+
Rosaceae						
114. *Rubus ellipticus* Smith	Ainselu	"	" "	+	+	+
115. *R. niveus* Wall.	Rato ainselu			-	+	+
Rubiaceae						
116. *Galium rotundifolium* L.		"	" "	+	+	+
117. *Hedyotis scandens* Roxb. ex D. Don		"	" "	+	+	+
Solanaceae						
118. *Datura stramonium* L.	Dhaturo	BMW, SMW	BW, VFW	-	+	+
119. *Nicandra physaloides* (L.) Gaertn.		"	" "	-	+	+
120. *Solanum nigrum* L.	Jangali bihin	"	" "	-	+	+
121. *S. torvum* Sw.	Thulo bihin	"	" "	-	+	+
122. *S. xanthocarpum* L.	Kantakari	"	" "	+	+	+
Verbenaceae						
123. *Lantana camara* L.	Masino Kanda	"	" "	+	+	+

BMW= Brick Mudwall, SMW= Stone Mud Wall, BCW = Brick Cement Wall, SCW = Stone Cement Wall, BW = Base of the Wall, VFW=Vertical Face of the Wall, HTW = Horizontal Top end of the Wall, W= Winter, S = Summer, R = Rainy.

Table 2. Statistical analysis of wall flora of Pokhara

Plant groups	Families	Genera	Species
Dicotyledons	30	61	86
Monocotyledons	3	21	25
Pteridophytes	4	5	9
Bryophytes	3	3	3
Total	40	90	123

Discussion

Plants seen growing on both horizontal top end and vertical face of the wall, often producing cracks and fissures on the walls. They were concentrated mostly at the cementing zones. Mud and cementing zones have been investigated as early successional areas unlike the cement mortaring zone which remains barren until the collection of thin humus layer through dry fallout of dust and other particles, that characterizes the higher density of plants on the mud, bricks and cemented walls than on cement mortared wall at the early stages (Mishra, 1948; Chhetry, 1956; Thapa, 2015). Species diversity has been observed highest on the bricks and mud wall, stone and mud wall and mud wall due to presence of large number of house made up of bricks, muds and cements in Pokhara.

Summer with mostly rainfall and rainy seasons seemed to be more favorable than winter with less frequent rainfall for growth and occurrence of different plants on walls because density and species diversity are observed increasing steadily from summer till the end of rainy season unlike winter with less species as well as density, which is in confirmation with the study of Pangtey and Rawat (1987). Similar work on wall flora on India (Gosh & Pal, 1997; Gosh & Das, 1998) is meager, although these plants occupy very important structural as well as factional positions in the trophic framework of the ecosystem.

The wall flora composes a very rich biodiversity and the walls act as a matrix for their conservation. Work of this kind can afford opportunity to formulate a way for conservation in more hospitable habitats of those threatened species seeking refuge on walls. Extensive studies are thus needed for a better understanding of the biology of the wall plants since they might have several benevolent implications still not known to us.

Acknowledgements

The first author is grateful to Mr. O.P. Dwa, Mr. N.D. Bastakoti, Mr. K. Panta for their continuous suggestion and encouragement throughout the research period.

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Ecology of *Nymphoides indica* (L.) Kuntze in Kashyap Lake, Kaski District, Nepal

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Abstract

Aquatic macrophyte, *Nymphoides indica* (L.) Kuntze and soil and water parameters of its habitat- Kashyap Lake, Kaskikot, Pokhara has been studied from July to January 2019. A total of 14 species of aquatic plants belonging to 11 genera and 8 families were recorded from Kashyap lake. Nitrogen, phosphorous and potassium in stem (0.64%, 0.40%, 5.7%) and leaf (1.96%, 1.37%, 11.6%) of *N. indica* was found highest in rainy season. Similarly, flower (2.10%, 0.06%, 0.08%) and seed (2.53%, 0.07%, 0.08%) were recorded in summer and winter season, respectively. The soil showed sandy clay texture, whereas water holding capacity (72.88%) and soil moisture (53.92%) was highest in winter. Soil nitrogen (0.18%) was highest in winter, but phosphorous (0.003) and potassium (0.015%) was highest in summer season. The pH of water remained almost acidic throughout the study period. Nitrite nitrogen and Nitrate nitrogen was found same in three seasons. Conductivity (58.67%) and total dissolved solid (31.4%) was maximum in summer season but turbidity (82.0%) was maximum in winter.

Key words: Aquatic plants, Banana plant, Hydrophytes, Kumudini, Macrophytes, Water analysis

Introduction

The floating-leaves banana plants, *Nymphoides indica* (L.) Kuntze of family Menyanthaceae, is known as "Kumudini" in Nepali. It grows in meso and eutropic lakes and ponds between altitudes 60 to 1800 m msl (Hara et al., 1979). The plant is a pretty, fast-growing, perennial water plant. New plants are formed all the time where the floating stolons form tufted plantlets along their length. The mother plant has a short, thick stem which is rooted in the mud at the bottom of the pond. Roots are props, long slender, vegetative stolons and possess runner stems. Leaves are large whorled, cauline and floating, ovate-orbicular, 5-10 cm, base deeply cordate with narrow sinuses, entire and fleshy, glossy green above, pale and gland-dotted beneath. Petioles are smooth, greenish-brown, length up to 2m long and are not strong enough. Secondary branches are sympodial, zig-zag, many-jointed, trailing on water surface; each joint uniphyllous. Flower are bisexual, solitary and distylous in umbellate clusters from the junction of the petiole and branch. Several flowers can arise from a single plant, clustered, white with a yellow center (2.5-3.5 cm wide). Flowers last for only one day. *N. indica* display a rare arrangement referred as "dimorphic heterostyly". Seeds are brown, spherical, smooth and less than 1.5 mm wide.

The excessive growth of aquatic plants can have negative effects on the lake ecosystem and water quality. Large aggregations of plants covering the water surface limit light penetration

and prevent water aeration. Dead plant parts deposited on the bottom of lake release nutrient into the lake water when they decay. Therefore, maintaining the aquatic vegetation density and biomass at a beneficial level becomes important for lake ecosystem management. Aquatic plant harvest has been widely considered as a plant management measure because it removes the targeted plant bodies quickly and efficiently. Furthermore, the nutrients contained in the plant tissues are also removed from the ecosystem, which reduced nutrient accumulation in the water column and sediments.

In Nepal although there are many reports on aquatic flora, community level studies are in infancy and are restricted to any lentic systems (Shrestha, 1997; Burlakoti & Karmacharya, 2004; Jha et al., 2005; Upadhyay et al., 2011; Niroula & Singh, 2012; Koirala, 2014). Mutreja et al. (2008) studied on effect of *Nelumbo nucifera* seeds on the reproductive organs of female rats and concluded that the ethanolic extract of *N. nucifera* seeds has anti-estrogenic effect in female rats. Pal and Dey (2013) carried out research on a review of Lotus (*N. nucifera*) seed and revealed that lotus seed are used as food and traditional medicinal purpose. Pollen viability, germination, and seed setting of *N. nucifera* have been studied by Khatfan et al. (2014) and found that pollen from un-bloom flower can be used for fertilization. The present paper elucidates the seasonal change in physico-chemical properties of soil and water and nutrients of *N. indica*.

Materials and Methods

Kashyap Lake is located at latitudes N28º15'59.5" and longitudes E83º54'31.7" at Kaskikot, Ward 24 of Pokhara Metropolitan city, Kaski District, northern-central Nepal (Fig. 1). Kaskikot shares its territory with Sarangkot, Chapakot, Dhikurpokhari and Hemja. The lake is oval shaped and the mean depth is approximately 20m. Kaskikot has alluvial soil and sub-tropical monsoonic climate with cool winter and maximum rainfall. The average annual

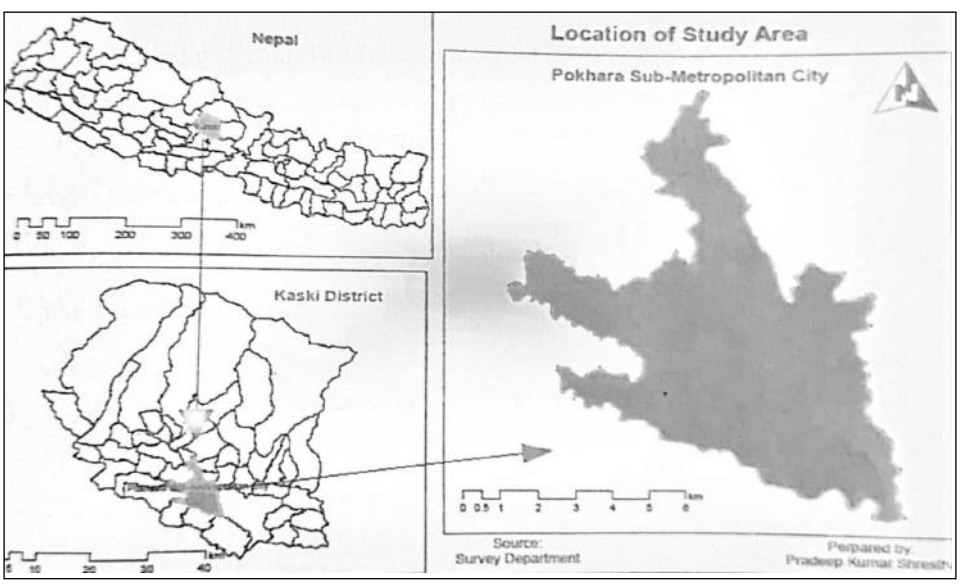

Figure 1. Map of Kashyap Lake, Kaskikot, Pokhara.

rainfall is 5869.4 mm, average monthly minimum and maximum temperature are16.6ºC and 29.1ºC, respectively (Hydrology and Metrology Office, Pokhara, 2014-2018).

The present study was carried out during July to January 2019 in Kaskikot, Pokhara of Kaski District. The aquatic macrophytes were sampled by laying ten quadrats of 30x30cm size randomly at monthly intervals. Collected sample were washed, brought to the laboratory and after proper sorting and processing, oven dried to a constant weight at 80ºC to determine plant nutrient contain in *N. indica*. The plants were identified following Siwakoti and Varma (1999) and confirmed from the Tribhuvan University Regional Herbarium, Department of Botany, Post Graduate Campus, Biratnagar. Physico-chemical properties of the water and soil were determined following APHA (1996).

Results

A total of 14 species of aquatic plants belonging to 11 genera and 8 families were recorded along with *Nymphoides indica* among which member of family cyperaceae was maximum followed by pteridaceae from Kashyap lake. They were hydrophytes-4, halophytes-4 and epiphytes-6 but all the species were categorized in hydrohytes.

Seasonal value of physio-chemical properties of soil is given in table 1. The soil showed sandy clay texture class. The average total value of sand, silt, water holding capacity and soil moisture as 61.73%, 13.0%, 25.1%, 58.77% and 36.43%, respectively. The pH value recorded (between 0.2 to 6.2) in the wetland habitat of Kashyap lake is slightly acidic in nature. However, soil organic carbon was maximum in rainy and minimum in summer season. Organic carbon showed positive relationship with total nitrogen which was higher in winter as the water remains stagnant. So, loss of carbon and nitrogen was maximum in the summer season. On the other hand, phosphorous and potassium were higher in winter and rainy season, respectively.

The physico-chemical properties of water of wetland is mentioned in table 2. The pH of water was acidic throughout the study period. Nitrite and nitrate nitrogen did not show seasonal

Table 1. Physico-chemical properties of soil of Kashyap Lake.

Soil properties	Seasons		
	Summer	Winter	Rainy
Texture			
Sand (%)	88.4±1.05	68.00±1.0	28.8±0.50
Silt (%)	7.2±1.1	27.75±0.65	4.18±1.07
Clay (%)	4.2±0.9	4.25±0.99	67.01±22.9
Texture class	Sandy	Sandy	Sandy
W.H.C (%)	46.28±1.02	72.88±7.68	57.17±3.07
Soil moisture (%)	23.26±4.99	53.92±3.04	32.11±8.25
pH	5.66±0.4	5.30±0.1	6.1±0.1
Organic Carbon (%)	0.69±0.23	2.95±0.15	3.59±0.46
Nitrogen (%)	0.07±0.01	0.18±0.01	0.11±0.01
Phosphorous (%)	0.003±0.01	0.0056±0.01	0.0035±0
Potassium (%)	0.013±0.04	0.012±2.12	0.0046±2.59

variation, while total nitrogen was maximum in rainy and winter season, i.e., 17.5%. Total dissolved solid content was higher in summer season, i.e., 31.4% which showed positive relationship with conductivity which was also higher in summer season, i.e., 58.67%. Turbidity was found highest in winter season, i.e., 82.0% and lowest in rainy season, i.e., 10.0%.

Table 2. Physico-chemical properties of water of Kashyap Lake.

Parameters	Summer	Rainy	Winter
pH	6.2	6.0	5.93
Conductivity (μS/cm)	58.67	20.0	36.00
Turbidity (NTU)	32.8	10.0	82.0
TDS (mg/L)	31.4	12.2	22.20
Ammonia (mg/L)	0.84	10.0	0.42
Nitrate Nitrogen (mg/L)	(<0.2) ND	(<0.02)	(<0.2)
Nitrite Nitrogen (mg/L)	0.08	0.08	<0.08
Total Nitrogen (mg/L)	6.37	17.5	17.5
Total Phosphorus (mg/L)	0.1	0.04	(<0.1)

Nutrient content in different parts of *N. indica* is mentioned in table 3. In the root of *N. indica*, nitrogen, phosphorous and potassium content were found as 1.44%, 0.20% and 2.23%, respectively in winter season. Similarly, in stem region nitrogen and potassium content was highest in rainy season, i.e., 6.4% and 5.7%, respectively and the lowest in winter season, i.e., 2.15% and 2.84%. Amount of phosphorous in stem region was found highest in winter season, i.e., 0.55%.

In leaf, nitrogen content was found highest in winter season, i.e., 4.83% and lowest in rainy season, i.e., 1.96%. Amount of phosphorous and potassium were found little higher in rainy season, i.e., 1.37% and 11.6%, respectively.

Flower of *N. indica* was recorded only in summer season. In flowers, nitrogen content was 2.10%, phosphorous content was 0.06%, and potassium content was 0.08%. Similarly, seed of *N. indica* was recorded only in winter season. In seeds, nitrogen content was 2.53%, phosphorous content was 0.07% and potassium content was 0.08%.

Table 3. Seasonal variation of nitrogen, phosphorous and potassium in different plant parts of *N. indica*

Plant parts	Winter (%)			Summer (%)			Rainy (%)		
	N	P	K	N	P	K	N	P	K
Stem	2.15	0.55	2.84	0.83	0.21	2.91	6.4	0.40	5.7
Root	1.44	0.20	2.23	0.57	0.10	2.23	1.32	2.08	31.3
Leaf	4.83	0.57	2.22	1.50	0.19	0.67	1.96	1.37	11.6
Flower	-	-	-	2.10	0.06	0.08	-	-	-
Seed	2.53	0.07	0.08	-	-	-	-	-	-

Discussion

The aquatic members of family cyperaceae was maximum in this lake which is followed by Pteridaceae. A similar finding was reported by Hickel (1973) in lakes of Pokhara valley, which may probably be Nepal's first report. In the winter season most of the cyperaceae

member disappeared which were once abundant in the rainy season. So, in the winter season dominance is shifted from cyperaceae to pteridaceae.

Soil texture can strongly influence the vegetation development and establishment of nutrient cycling (Robertson & Vitousek, 1981). The average value of pH was found that 5.66%, 5.30% and 6.1% during summer, winter and rainy seasons in the study site. This pH value is suitable for plant growth, as the optimum range for soil microorganisms' activity lies between pH 5.5 to 7.8. High organic matter content in the soil may lead to increase the water holding capacity, soil moisture and total nitrogen. Especially the average water holding capacity was recorded as 58.77%.

Physio-chemical properties of water affect the structure of macrophytes community and primary production of aquatic plants. The pH of water in wetland near Kashyap lake was 6.2%, 5.93%, 6.0% during summer, winter, and rainy seasons, respectively, which is slightly acidic. This results also reveals to Koirala (2014) that the water samples' pH ranged between 6.24% to 6.40% being slightly more acidic in rainy and winter seasons than the summer season.

Nymphoides indica is a freely rooted species in wetland habitat. In the stem and leaf of *N. indica*, N, P and K content were found more in winter season compared to rainy season. In the rainy season plant growth and nutrient demand are higher (Singh et al., 1989). So, the nutrient content (N, P, K) are higher in the leaf and stem during rainy season. If it is seen part-wise, then leaf showed maximum N, P, and K concentrations because of having high deposition. Moreover, as the root also functions as storage organ, N, P, and K's concentration were placed next to leaf, i.e., lower than leaf but higher than stem.

Among the flower and seed, the concentration of N, P and K were higher in seed. When flower is converted into fruit, the nutrients are migrating from flower to fruit and ultimately into the seed. Seed is the storage organ for nutrient. It conserves more nutrients for developing embryo.

Acknowledgements

We are thankful to the Department of Botany, Post Graduate Campus, T.U., Biratnagar, for providing laboratory facilities. The first author is thankful to Prof. Dr. Shiva Kumar Rai and Dr. Bhabindra Niroula, Department of Botany, Post Graduate Campus, T.U., Biratnagar for valuable suggestion, encouragement and identification of aquatic macrophytes.

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In-vitro Evaluation of *Rhododendron arboreum* Sm. for Potential Antibacterial Activity

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Abstract

In the present study, angiosperms medicinal plant *Rhododendron arboreum* Sm. with traditional folklore value were selected to assess their antimicrobial properties against pathogenic bacteria. Different parts of the plant studied showed many antibacterial properties against the bacteria (*Staphylococcus aureus, Escherichia coli, Vibrio cholerae, Shigella dysenteriae*, and *Shigella flexneri*) tested. *R. arboreum* possessed strong antibacterial potential. It is also evident that ethanol extract of leaf, stem and flower of *R. arboreum* were found to be potent in inactivating various Gram positive and Gram-negative bacteria. From this study the result obtained confirm the therapeutic potency of *R. arboreum* used in traditional medicine. In addition, these results form a reasonable basis for selection of the plant for the further phytochemical and pharmacological investigation. The present study results support the folkloric usage of the studied plant and suggest that the plant extract possess certain constituents with antibacterial property that can be used as antimicrobial agent in new drugs for the therapies of infectious disease caused by pathogen.

Key words: Antimicrobial property, *Escherichia coli,* Traditional medicine, *Vibrio cholerae*

Introduction

Plant is an integral part of human life to combat the sufferings from the down of civilization. Pathogenic microorganisms are always trying to develop resistance to various antimicrobial agents used for their control.

Resistances to antimicrobial agents are emerging in various organisms that pose a serious threat to infectious diseases (Tonin & Tomasz, 1986; Swiderski et al., 2004; Bhattacharyya, 2011). Therefore, the chemotherapy of infectious diseases has proved to be a continuous struggle. Scientists are always in search of new antimicrobial agents to control the ever-increasing menace of microbes. Thus, this is of paramount importance for the microbiologists to develop new antimicrobial agents effective against the resistant strain. Prolonged use of any kind out for the key demand of several infectious diseases also caused health hazard and different side effects. Hence, Researchers are very much interested to innovate new drugs that could not create only resistance mechanism in the microorganisms, against it and also unable to develop adverse side effects. The *Rhododendron arboreum* is an essential plant, which belongs to family Ericaceae. Moreover, abundance folklore knowledge exits according to plants produce many secondary metabolites and constitute an important source of pesticides, the use of plants in medicines. Herbal remedies are used extensively in many

parts of world particularly Asia and South Africa. The antimicrobial properties of certain Indian medicinal plants were based on folklore information (Nisar et al., 2011).

Plant extracts that inhibit pathogenic microorganisms without harming the host may have potential as therapeutic. The antimicrobial properties of certain Indian medicinal plants were based on folklore information. Plant extracts that inhibit pathogenic microorganism without harming the host may have potential as therapeutic. Therefore, preliminary screenings of this plant are undertaken for antibacterial activity against a variety of pathogenic bacteria.

Materials and Methods

The *R. arboreum* were collected from Bhimtal (elevation 448.05m), Uttarakhand, India, during the month of march, 2019. Fresh plants devoid of dead tissue with proper reproductivity organs were preferred. Sometimes serial plants had to be collected for scarcity of fertile specimens. The plants were freed from contaminants parts of the other plants, if present and carefully scooped out with certain amount of substratum, i.e., soil adhering to rhizoids of terrestrial spaces and a first issue to joining the growth of the epiphytic species to see whether those substrate they have any antimicrobial activity when was it if is also have found, specimen were discarded. The plants thus collected were kept in polythene bags which are subsequently sealed. In this condition, they could be prevented from dying on showing any signs of decay for several days. Since the same species in a different situation may not have the same antibiotic or antimicrobial activity information regarding time and place of collection, its state of maturity, habitat and altitude were recorded. The plant was identified and taxonomic authenticity was confirmed by Prof. G.G. Maity, Plant Taxonomist, Department of Botany, University of Kalyani, W.B, India.

The plant materials were freed of adhering soil particles washed in running tap water to blot. For aqueous extraction, tissues (either whole plant or different parts of plant) were grind with a small quantity of distilled water in a mortar with glass powder to yield a pulp. They were finally extracted with larger volume of water. Generally, 5 g of plant materials were extracted with 15 to 20 g of water, depending on the tissue's water content. For extraction at room temperature, the crushed tissues were covered with water and shaken in rotary shaker (200 rpm) for 14 h, filtered and filtrate passed through a sintered (G-5) glass filter. For Solvent extraction, the whole plant or different parts of the plant were shade dried and were blended ion a covering vendor or crushed with a mortar and pestle for extraction with organic solvents (ethanol, methanol and acetone). Crushed tissues were kept in contact with solvents for 24 h at room temperature followed by shaking for 14 h in a rotary shaker. Extracts were filtered through a bacteria concentrated to such a volume that 1 mL of extract would correspond to 5 g (gash weight) of tissue. Extract pH generally lied between 5.8 to7.2; values within this range had no inhibitory effect on test microorganism's growth.

However, when the pH was out of this range, it was adjusted to 7 before antibacterial activity assay. The bacterial strain onto which the antibacterial property of the solvent extracts of three different plants were tested including one Gram positive bacterium (*Staphylococcus aureus*) and four Gram negative bacteria (*Escherichia coli, Vibrio cholera, Shigella dysenteriae*, and *Shigella flexneri*). All the bacterial cultures were produced from Infectious Disease Hospital, Kolkata. These bacterial strains were maintained on nutrient agar slant at four degree

centigrade and were cultured before use. Antibacterial activity of extracts was assayed by the agar cup method. The antibacterial activity of different solvent extract was studied by agar well diffusion technique. Lawn of each organism was prepared on nutrient agar plate using overnight broth culture containing 10^6cfu/mL. 100 mL of solvent extract (containing approximately 5 mg of plant component/ml) was added to the well on the well on the agar plate. All the inoculated Petri dishes were incubated at 37°C for 24 h.

Results

In the present study of *Rhododendron arboretum* Sm. plants were selected which were used for the treatment of several disease in folk medicine since time immemorial. The plant details along with their medicinal values and active constituents present this plant depicted in table 1.

Table 1. Plant details selected for the present study.

Name of the plant	Brief description	Important chemical constituents	Medical properties
Rhododendron arboreum (Sm) Trautu (Ericaceae)	Is an evergreen tree to 35ft. tall in the temperate of Himalayas. Elevation of 8000-11000ft. large beautiful scarlet flower used for preparation of jam.	Quercitrin, Glycosides, Rhamose, Ursolic acid, Epifriedelanol, Fridelin, etc.	Headache, Cough, Diarrhoea, Menstrual disorder, etc.

The crude ethanol leaf extracts of *R. arboreum* Sm. exhibited maximum antimicrobial activity against *Shigella flexneri* and least activity against *Escherichia coli* (Table 2, Fig. 1). The highest antimicrobial activity against *Staphylococcus aureus* was recodes for the crude ethanolic flower extracts of *R. arboreum* Sm. and lowest activities *Shigella dysenteriae*. The ethanol stem extracts had considerable antimicrobial potential against Escherichia coli. However, *Shigella flexneri* was found to be insensitive to the ethanolic stem extracts of *R. arboreum* Sm. The present study indicated the considerable antimicrobial potential of crude ethanolic extract of *R. arboreum* Sm. against *Sheigella flexneri*. The crude methanol leaf extracts of *R. arboreum* Sm. showed highest antimicrobial activity against *Shigella dysenteriae* and *Vibrio cholera* and least antibacterial activity against *Escherichia coli* (Table 2, Fig. 2).

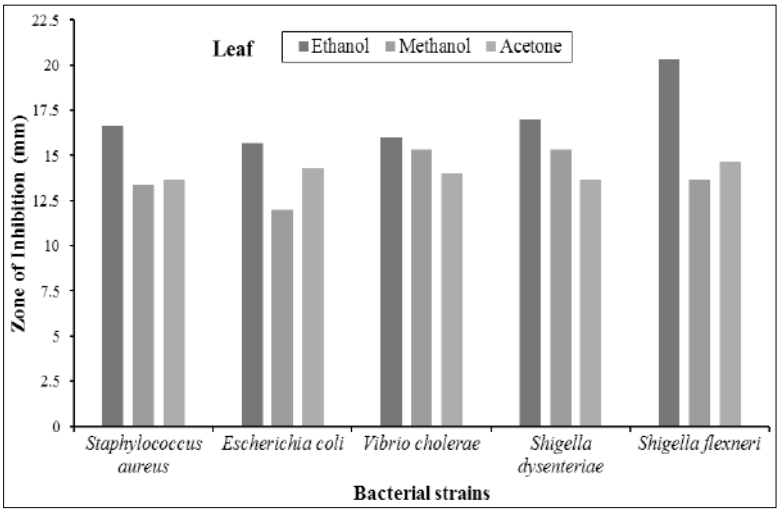

Figure 1. Graphical representation showing the zone of inhibition by leaf in different extract.

Table 2. Antibacterial activity of different parts of *Rhododendron arboreum* (Sm) Trautu.

Plant Parts	Solvents	Zone of Inhibition (mm)				
		SA	EC	VC	SD	SF
Leaf	Ethanol	16.67±3.21	15.67±0.57	16.00±4.14	17.00±2.00	20.33±1.52
	Methanol	13.33±1.15	12.00±1.00	15.33±4.16	15.33±3.51	13.67±2.08
	Acetone	13.67±2.51	14.33±1.15	14.00±2.00	13.67±0.57	14.67±0.57
Stem	Ethanol	15.00±1.00	16.00±1.41	15.00±1.00	15.50±2.12	ND
	Methanol	14.00±1.00	12.67±1.52	12.33±1.00	13.33±1.52	13.67±0.57
	Acetone	15.00±1.00	14.00±1.00	15.67±0.57	11.00±1.00	16.33±3.21
Flower	Ethanol	19.00±1.00	18.00±1.00	18.33±2.08	11.00±1.00	20.50±0.70
	Methanol	13.00±1.52	13.33±1.52	14.00±2.64	ND	18.00±2.64
	Acetone	NP	NP	NP	NP	NP

SA= *Staphylococcus aureus*, EC= *Escherichia coli*, VC= *Vibrio cholerae*, SD= *Shigella dysenteriae*, SF= *Shigella flexneri*, ± Standard deviation, ND= Not detected, NP= Not performed

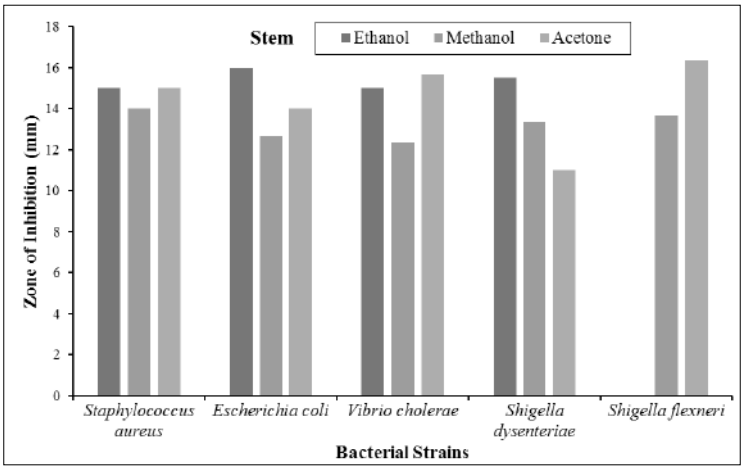

Figure 2. Graphical representation showing the zone of inhibition by stem in different extract.

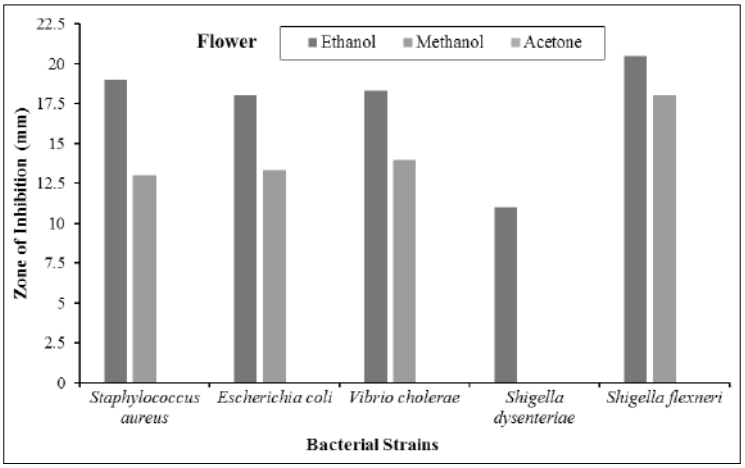

Figure 3. Graphical representation showing the zone of inhibition by flower in different extract.

Discussion

The crude methanol stem extract of this plant showed highest activity against *Staphylococcus aureus* while least antimicrobial activity was noted against *Vibrio cholera*. However, the crude methanol flower extract of *R. arboreum* Sm. showed its maximum antibacterial potential against *Shigella flexneri* with its maximum antibacterial potential against *Escherichia coli*. This study indicated potential of antimicrobial activity of crude methanol leaf extracts against *Shigella dysenteriae* and *Vibrio cholera.* The acetone extract of stem of *R. arboreum* Sm. showed maximum inhibited zone (14.67mm) against *Shigella flexneri* and minimum inhibited zone (13.67mm) against *Shigella dysenteriae* and *Staphylococcus aureus* (Table 2, Fig. 3) Similar highest antibacterial potential was recorded for acetone extract of stem of *R. arboreum* Sm. against *Shigella flexneri* while the minimum activity was recorded against *Shigella dysentriae*. However, no antibacterial activity was noted against any bacteria tested by acetone extract of the flower of *R. arboreum* (Sm) Trautu. From the least investigations, it was evident that ethanol extract of leaf, stem and flower of *R. arboreum* Sm. were found to be more potent in inactivating various Gram-positive bacteria followed by methanol and acetone extracts. All the plant parts showed significant antibacterial properties against both Gram positive and Gram-negative bacteria found to be more susceptible than Gram positive one.

These results contradict the previous reports that plant extracts are more active against Gram positive bacteria than Gram negative bacteria (Saklani & Chandra, 2015). It is therefore theorized that Gram positive bacteria are more susceptible than Gram negative organisms are considered to be more resistant due to their outer membrane acting as a barrier to many environmental substances including antibiotics (Bhandary & Kawabata, 2008; Anpin et al., 2010). However, the results revealed that crude ethanol extracts of *R. arboreum* Sm. contain certain bioactive components with significant antibacterial properties that enables the extract to overcome the barrier in Gram negative bacterial cell wall (Nisar et al., 2011; Verma et al., 2011). However, the leaf methanol extract of *Rhododendron smirnovii* Sm. was reported regarding its antibacterial activity against some Gram positive and Gram-negative bacteria (Tezgül Çakır et al., 2005). The most active extract can be subjected to isolation of the therapeutic antimicrobials and carry out further pharmacological evaluation.

Conclusion

In the present study, the angiosperm plant *R. arboreum* were selected to assess their antibacterial properties against pathogenic bacteria. The result of the present study supports the folkloric usage of the studied plant and suggests that the plant extract possess certain constituents with antibacterial property that can be used as antibacterial agent in new drugs for the therapies of infectious disease caused by pathogen.

Acknowledgements

I acknowledge CSIR, Pusa, New Delhi for funding agency and for providing Ph.D. fellowship, DST PURSE-II for fuding as well as University of Kalyani for providing laboratory.

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Study of Leaf Morphology and Anatomy of *Cinnamomum camphora* L. Plants Growing in Different Regions of Kathmandu, Nepal

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Abstract

Various studies indicate that *Cinnamomum camphora* L. possess intermediately air pollution tolerant index (APTI) value. These plants were planted on roadside areas previously as well as in newly constructed and expanded road side areas. So, the current study was done to examine morphological and anatomical parameters of leaf in *C. camphora* L. growing in Ringroad and Raniban forest areas of Kathmandu, Nepal. The Ringroad area was found disturbed with high vehicular emission and dust particles and Raniban forest area is the part of preserved Shivapuri Nagarjun National park area. Morphological characters like specific leaf dry mass content, specific leaf area, leaf length, leaf breadth and petiole length were examined. Similarly, anatomical characters like the stomatal frequency, thickness of the lower cuticle, thickness of the palisade tissue and thickness of the spongy parenchyma were examined. The specific leaf area, the specific leaf dry mass content and the length of leaf lamina were found more in the leaves of plant growing in Ringroad area of Kathmandu whereas, the leaf breadth and the petiole length were observed more in the leaves of plant growing in Raniban forest area. The stomatal frequency was found less in the leaves collected from Ringroad area than the leaves from Raniban forest area. The thickness of palisade tissue and the spongy parenchyma were found more in the leaves collected from Raniban forest area than the leaves from Ringroad area. The thickness of cuticle was measured equal in both study areas.

Key words: Cuticle, Leaf dry mass, Leaf lamina, National park, Stomata frequency

Introduction

Cinnamomum camphora L. is evergreen tree about 10 m height. Leaves stalked, alternate, 3.5-11.5 cm long, 1.5-5.5 cm wide, ovate to lanceolate, acuminate, glabrous, flowers yellowish in axillary and terminal panicles. The plants possess medicinal value. Leaves are boiled with water and the vapour is inhaled for cough and colds. The plant is source of camphor. Camphor oil is also used in perfumery (Manandhar, 2002). Kathmandu city resides in bowl-shaped valley, and it greatly enhances the likelihood of air pollution problems. The valley's unique shape prevents the escape of industrial and vehicular emissions. In the last one decade, the number of vehicles in the capital city has tripled. Two thirds of deadly pollutants are caused by vehicular emissions and dust according to Ministry of Health, Nepal Government (2017). Due to high vehicular emission and dust, Ringroad was considered as highly disturbed area whereas Raniban forest, a part of preserved Shivapuri Nagarjun National park, was selected as controlled site for the study.

Leaf is also called bioindicator of air pollution because it is the most sensitive due to its maximum exposure area to air and it is also house of many physiological reactions. The air pollutants emitted from automobiles are directly affecting leaf because they can penetrate into leaf and modify or destroy its cells and tissues, hence hampering its food producing capacity. Traffic pollution causes foliar injury, appearance of chlorotic spots on leaves, flower and fruits. There is twisting or curling and wilting of younger leaves, stunted growth due to shortening of internodes etc. as the effect of air pollutants. Leaves are usually the site of injury by pollutants that enter the leaves mostly through stomata. Deposition of pollutants on moist leaves results in the annular chlorosis or bleached patches on laminar surface (Chaphekar, 1982).

The increasing number of industries and automobiles vehicles is continuously adding toxic gases and other substances to the environment (Jahan & Iqbal, 1992). Such pollutants include sulphur and nitrogen oxides, carbon monoxides and soot particles as well as smaller quantities of toxic materials (Agbaire & Esiefarienrhe, 2009). Air pollution deteriorate ecological conditions of vegetation (Tripathi & Gautam, 2007). The vegetation plays an important role in atmospheric purification and air pollution reduction (Gheorghe & Ion, 2011). Urban trees play an important role in improving air quality of urban environment by absorbing gases and particulates (Woo & Je, 2006). The penetration of pollutants into plants is mainly through the leaves. Gaseous pollutants enter the plant through the stomata present on the surface of leaves like other atmospheric gases (www.encyclopedia-environment.org). Leaf is the most sensitive and exposed part to be affected by air pollutants than all other plant parts such as stems and roots (Leghari & Zaidi, 2013). Specific morpho-anatomical and physiological-biochemical characteristics are the results of plant adaptations on environmental conditions (Kovacic & Nikolic, 2005). The roadside vegetation of urban area acts as a sink for particulate matters and help for eco sustainable filter for pollution (Rai, 2016).

Incomplete combustion of fossil fuels leads to the formation of carbon monoxide in petrol powered motor vehicles. Road traffic is the main cause of air pollution in the metropolitan cities due to harmful emissions (Gidde & Sonawane, 2012). Kathmandu is Nepal's largest urban center with an annual population growth rate five percent. Rapid increase in vehicles number has seen in Kathmandu in last 15 years. According to DOTM Kathmandu, number of registered vehicles was 24,003 in 2000/1 and by 2015/16 it was increased up to 7, 79,822. Emissions from vehicles are particularly toxic as diesel powered vehicles which are considered deadly pollutant than the petrol powered vehicles. The low and middle country suffers more from transport generated pollution due to old and diesel vehicles (WHO, 2017). There are numerous factors to increase air pollution in Kathmandu city. The important factors are vehicles, haphazard digging of road, brick kilns, and unplanned expansions of roads, ill managed dumping of construction materials and old engine vehicles (Saud & Poudel, 2018). Air quality in Kathmandu is regularly surpassed very unhealthy levels and even reached hazardous in March and April 2016 (http//www.iied.org/clearing- air-Kathmandu). According to air quality index, the air quality level was found to be acceptable in Bhaktapur Sainik Awasiya Mahavidyalaya, Bhaisepati, Lalitpur and Dhulikhel areas whereas, it shows unhealthy air quality in Phora Darbur and Ratnapark areas of Kathmandu Valley (www.aqicn.org). A year continuous monitoring of ambient PM2.5, CO and NO_2 in

Kathmandu Valley showed that the Valley's ambient air (57.6% for PM2.5 and 56.4% for NO_2) has exceeded the daily National Ambient Air Quality Standards (NAAQS) for the majority of the days of monitoring, but in the case of CO, only a single day exceeded the National Standard (using 8 hour averages). Daily averages of PM2.5 are 3-5 times higher than the National Standard of 40µg/m^3. Moreover, concentrations of NO_2 in ambient air are also found to be high, with several very high spikes monitored above 1000 µg/m^3, which is around 12 times higher than 24-hour National Standard of 80µg/m^3. Station-wise results revealed that Kathmandu is more polluted with PM2.5 and CO throughout the year when compared to Lalitpur and Bhaktapur (Karki et al., 2016). Air pollution tolerant index (APTI) is an index that shows capability of a plant to tolerate air pollution (Hamal & Chettri, 2017). Biological monitoring and assessment studies due to urban road pollutants were carried out using air pollution tolerance index (APTI) of plants (Dhyani et al., 2019). *C. camphora* L. was found one of the important and dominant roadside tree species in Kathmandu Valley. APTI value of *C. camphora* was 28.16 which was found as intermediately tolerant species (Kanwar et al., 2016). *C. camphora* is the most air pollution tolerant plant among the four tree species, than *Jacaranda mimosifolia. Callistemon citrinus* and *Grevillea robusta* lie on the degree of tolerance. Therefore, *C. camphora* is recommended for plantation at the polluted areas like Kathmandu (Rawal et al., 2001). Hence, the present study is carried out on some morphological as well as anatomical characteristics of leaves of *C. camphora* tree growing in roadside of Kathmandu city, Nepal.

Materials and Methods

Leaf samples for the study were collected in the month of November, 2017 from the plant having similar diameter, uniform height and similar growth form. Leaf lengths, leaf breadth and petiole length were measured by scale in centimeter. Leaves were brought to laboratory in polyethene bags and some leaves were preserved in formalin for the anatomical study. Leaves area was determined by using graph paper. Study of stomata of lower surface of leaves was performed using calibrated optical microscope. Leaves were kept in hot air oven for two hours at 100°C for the calculation of the specific leaf dry mass content. Anatomical study was done with the help of fine anatomical section of preserved leaves under calibrated microscope. The thickness of the cuticle, palisade layer and the spongy parenchyma were measured under calibrated microscope using micrometer. Permanent slides were prepared after the completion of alcohol dehydration series. Leaves samples for all necessary data were taken in ten replicates. All the data were collected and analyzed with the help of Microsoft Excel 2013.

The present study has been carried out at ring road side of Kathmandu, Nepal as highly disturbed area and Raniban forest area of Kathmandu as controlled site. Ringroad is central part of Kathmandu with high vehicular emissions and heavy dust particles than the Raniban forest which is preserved area under the Shivapuri Nagarjun National park.

Results

The average dry mass content of leaves was observed 50.2% in Ringroad and 41.66% in Raniban area. The study revealed that the leaves area of *C. camphora* collected from Ringroad

area and Raniban forest area were found 9.4 sq cm and 15 sq cm, respectively. The average leaf length was found 6.42 cm in Ringroad and 7.9 cm in Raniban area. The average leaf breadth was measured 5.5 cm in Ringroad and 3.7 cm in Raniban area. The average petiole length was measured 1.07 cm in Ringroad and 1.68 cm in Raniban area. The stomata frequency in the leaves of Ringroad was found 6.27 per mm^2 whereas, in Raniban area, it was found 8.78 per mm^2. In case of thickness of lower cuticle, no difference was observed in the leaves in the study area. The average thickness of palisade layer was measured 68µ in Ringroad and 83µ in Raniban area. Similarly, the average thickness of spongy parenchyma layer was measured 140 µ in Ringroad and 168 µ in Raniban area. (Table 1, Figs. 1-5).

Table 1. Measurements of leaf parameters.

Leaf parameters	Ringroad area				Raniban forest area			
	Mean	SD.	Min.	Max.	Mean	SD.	Min.	Max.
Leaf dry mass content (%)	50.20	3.49	46.71	53.69	41.66	3.76	37.9	45.42
Specific leaf area (Sq. cm)	9.40	1.32	8.08	10.72	15.	1.84	13.16	16.84
Leaf length (cm)	6.42	0.71	5.71	7.13	7.9	0.86	7.04	8.76
Leaf breadth (cm)	5.50	0.56	4.94	6.06	3.7	0.36	3.34	4.06
Petiole length (cm)	1.07	0.22	0.85	1.29	1.68	0.22	1.46	1.9
Stomatal frequency	6.27	0.62	5.65	6.89	8.78	0.72	8.06	9.5
Thickness of lower cuticle (µm)	14.0	0.66	13.34	14.66	14	0.81	13.19	14.81
Thickness of lower palisade tissue (µm)	68.0	4.73	63.27	72.73	83	3.71	79.29	86.71
Thickness of spongy parenchyma (µm)	140.	4.58	135.42	144.58	168	2.91	165.09	170.91

Note: SD= Standard deviation, Min.= Minimum, Max.= Maximum

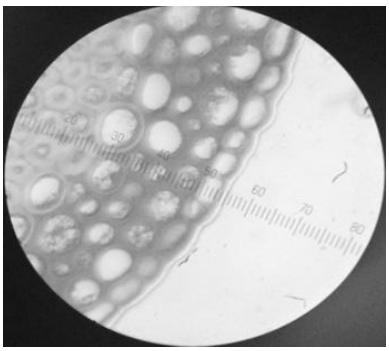

Figure 1. Leaf anatomy (Raniban area)

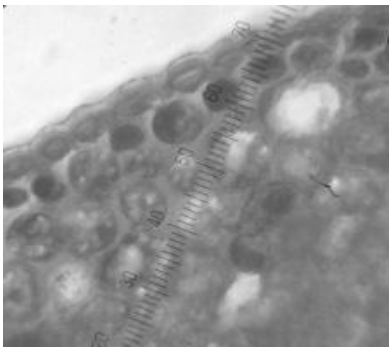

Figure 2. Leaf anatomy (Ringroad area)

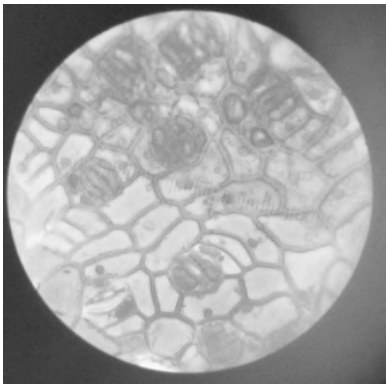

Figure 3. Epidermal peel (Raniban area)

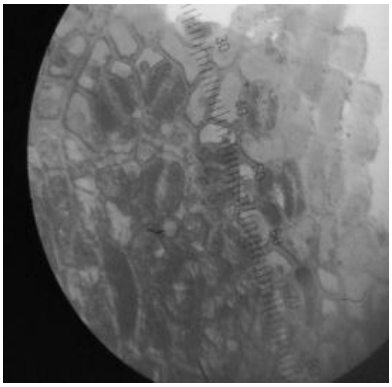

Figure 4. Epidermal peel (Ringroad area)

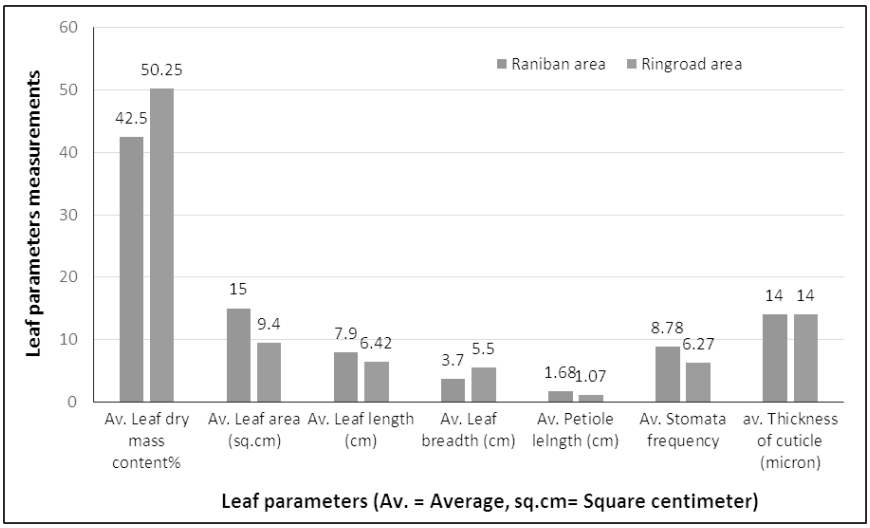

Figure 5. Measurements of leaf parameters in the study areas

Discussion

Air pollution is one of the severe problems in Kathmandu city. Air pollution stress leads to stomata closure which reduces carbon dioxide availability in leaves (Woo et al., 2007). Pollutants can cause leaf injury, stomata damage, premature senescence, decrease photosynthetic activity, disturb membrane permeability and reduce growth and yield in sensitive plant species (Tiwari et al., 2006). Reductions in leaf area and leaf number may be due to decreased leaf production rate and enhanced senescence. Plants subjected to air pollution showed reduction in leaf area and petiole length (Seyyednejad & Koochak, 2013). In the present study, leaf dry mass content of the leaves of *C. camphora* plant growing in Ringroad area of the Valley was found more than the Raniban area. Similar results were found in the leaves of *Ricinus communis* growing in the highly polluted areas of Kathmandu (Singh Suwal et al*.,* 2019). Bhatti and Iqbal (1988) reported that the leaf dry weight of *Guaiacum officinale, Ficus benghalensis* and *Eucalyptus* sp. was significantly reduced in the leaves of polluted sites of Karanchi as compared to the control site. The specific leaf area of studied plant was found reduced in Ringroad area than the Raniban forest area. The leaf length, petiole length and stomata frequency were found reduced in the plants growing in Ringroad as compared to the Raniban area. Similar results were observed by Jahan and Iqbal (1992), Munzuroglu et al. (2003), Dineva (2004), Tiwari et al. (2006), Sayyednejad et al. (2009), Assadi et al. (2011), Leghari and Zaidi (2013), Mahajan et al. (2015), EI-Khatib et al. (2016), and Sharma et al. (2017). These changes might be due to the cause of hidden injury or physiological disturbance occurring in morphological and anatomical characters of plants.

This study observed that there was no difference between the thicknesses of lower cuticle in the leaves from both study areas. Pourkhabbaz et al. (2010) observed thinner cuticle in *Platanus orientalis* L. growing in urban areas. The thickness of palisade layer and spongy parenchyma layer were found reduced in the plants growing in Ringroad area of Kathmandu

Valley. Similar results were observed by Jahan and Iqbal (1992) in the leaves of *Ficus bengalensis* L., *Guaiacum officinale* L., and *Eucalyptus* sp. growing in polluted area of Karachi city. Singh Suwal et al. (2019) have also observed the reduction in palisade layer and spongy parenchyma in the *Ricinus communis* L. leaves growing in the polluted areas.

Conclusion

C. camphora is the most important and dominant roadside tree in urban area of Kathmandu. The air pollution tolerance index value of this plant showed that it is intermediately tolerant species. The present study was mainly focused on the morphological and anatomical parameters of *C. camphora* growing in Ringroad and Raniban area of the Kathmandu Valley. The two study areas were found dominantly different in the contamination degree due to the motor vehicle exhaust. The leaves of *C. camphora* growing in Ringroad area possess reduced specific leaf area, leaf length, petiole length, stomata frequency, thickness of palisade layer and spongy parenchyma layer. The thickness of cuticle in the leaves of both study areas showed no difference. The leaf dry mass content and leaf breadth were found increased in the leaves of plant growing in Ringroad area. This study was found that the leaves of *C. camphora* adopted in Ringroad areas by decreasing most of the morphological and anatomical characters. In present day this plant is found dominantly planted in the urban areas. The present study aims to provide a good basis for further research on the effect of air pollution in the morphology and anatomy of plant leaf.

Acknowledgements

The authors are thankful to the University Grant Commission, Nepal for providing financial support and Patan Multiple Campus, Botany Department for providing laboratory facilities during this research work.

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Growth Potentials of the Greater Duckweed - *Spirodela polyrhiza* (L.) Schleiden

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Abstract

Among three roadside pools fed with rain water and domestic sewage, a pool at Katari fed with rain water and jute mill effluent, shore of the Singhia river receiving effluent from a soap factory, and a ditch filled with domestic sewage at Pichhara, Biratnagar, *Spirodela polyrhiza* had maximum frond number (57060/m^2), and fresh (1338 g/m^2) and dry biomass (76 g/m^2) in the Singhia river shore, whereas minimum fresh (448 g/m^2) and dry biomass (31 g/m^2) of the test species were recorded from Katari pool. Frond size of the test species was maximum (175 mm^2) in the roadside pool located adjacent to the Koshi project complex (habitat 2) and minimum (63.5 mm^2) in Pichhara ditch. Fresh biomass of *S. polyrhiza* ranged between 7.97 g/m^2 (February) and 669.02 g/m^2 (April) in habitat 1 (roadside pool located adjacent to the Bigyan Bhawan), between 21.76 g/m^2 (February) and 243.3 g/m^2 (May) in habitat 2, and between 95.19 g/m^2 (March) and 380.52 g/m^2 (January) in habitat 3 (roadside pool located adjacent to an orphanage north west to Bigyan Bhawan at 1 km distance). Sugar factory effluent at 5% dilution had stimulatory effect on biomass, whereas 6 mg/L glucose concentration increased root number, root length and biomass of the test species. The water pH for the optimum growth of the test species was 6. Anaerobically decomposed cow-dung for 21 days promoted the frond size and biomass of *S. polyrhiza* at 10 mg/L concentration.

Key words: Biomass, Cow-dung, *Ex-situ* and *in-situ* growth, Jute mill and soap factory effluents, Sugars

Introduction

Greater duckweed [*Spirodela polyrhiza* (L.) Schleiden, Family: Lemnaceae] is a free floating small aquatic plant lacking stem and leaves. The plant body consists of a few celled thick thalloid frond with adventitious roots but devoid of root hairs. The bulk of the frond is composed of chlorenchymatous cells separated by large intercellular spaces that are filled with air and provide buoyancy. Roots either stabilize the plant on the water surface or assist the plant to obtain nutrients (Leng, 1999). It grows in nutrient loaded water bodies and multiplies rapidly by vegetative means.

Greater duckweed is recognized as a promising new commercial crop suitable as high protein animal feed, organic fertilizer, and a source of bio-fuel ethanol. Further it has great applicability in wastewater purification [Haustein, 1992; Hasan & Chakrabarti, 2009; Jha & Pokharel (Bhattarai), 2015]. *Spirodela polyrhiza* is the most ubiquitous and robust duckweed found at Biratnagar, Nepal. Hence knowledge of growth conditions is essential for its utilization as biomass.

Materials and Methods

Study Site

Biratnagar (26°20'N and 87°16'E, 72 m msl) is characterized by ponds, pools, ditches along roadsides receiving municipal and industrial discharges and agricultural run off. It has alluvial soil, tropical moonson climate with three distinct seasons, viz., winter (November-February), summer (March-June) and rainy (July-October) in a year. The average annual rainfall is1312 mm and average annual minimum and maximum temperatures 14 and 30.6°C, respectively (Niroula & Singh, 2012).

In situ Growth

Three roadside pools receiving domestic sewage all year round were selected for the study of number (per unit area), morphology (frond size and root length), and biomass of *S. polyrhiza* between December 2017 and May 2018. Among the selected pools, first one was located near Bigyan Bhawan, Post Graduate Campus, Biratnagar (habitat 1), habitat 2 was situated adjacent to the Koshi Project complex along Biratnagar-Jogbani (Rani) road; and habitat 3 was a roadside pool located adjacent to an orphanage north west to Bigyan Bhawan at 1 km distance.

Other water bodies for the study were: Katari (Six km away from Biratnagar) pool fed with jute mill effluent; shore of the Singhia river receiving effluent from a soap factory (Nayabazar); and ditch filled with domestic sewage Pichhara north. Average water depth in the centre of all the selected pools and river was about 1 m in December and 0.3 in May.

Water and duckweed mixture were sampled in the first week of each month from all the selected pools using 10 cm ×10 cm quadrat (fitted with sieve and handle) in triplicate. The *S. polyrhiza* fronds were separated for determination of frond number and biomass (per unit area). Fifty fronds were selected randomly for the measurement of frond size (graphically) and root length.

Ex-situ Growth

Ten *S. polyrhiza* fronds were incubated in test solutions in the 500 ml plastic container and kept inside the laboratory of Department of Botany, Post Graduate Campus, T.U. Biratnagar, Nepal in triplicate. Number of fronds, root length, number, and fresh weight were recorded after 10 days of incubation.

Cow-dung

Cow-dung was anaerobically decomposed for 7 and 21 days by putting fresh dung in closed containers with borewell water. 2-30 g/L cow dung solution was prepared separately with dilution.

Glucose and sucrose

Glucose and sucrose solutions (3 to 50 mg/L) were prepared by dilution in bore-well water.

Industrial effluents

Effluents from the textile, sugar and soap factory were collected from their outlet. They were diluted in bore-well water to prepare 1 to 15 ml/L concentrations.

Water pH

Garden soil (2 kg/pot) was placed in 27 earthen pots (with sealed drainage) of 20 cm diameter and 15 cm depth, and all the pots were flooded with borewell water. They were arranged in 9 different groups (each group in triplicate) of water pH (2 to 10) either through adding hydrochloric acid or lime, and left overnight. *S. polyrhiza* fronds were collected from a eutrophic pool located near P.G. Campus, washed in running tap water. They were inoculated in each pot at the rate of 1 g fresh fronds in January. The pots were kept in open and watering was done regularly to compensate for evaporative loses. The fronds were harvested after 30 days of inoculation and fresh weight per pot was taken immediately after harvest.

Results

In-situ Growth

Growth of the test species in different water bodies during April is given in table 1. *Spirodela polyrhiza* had maximum frond number (57060/m^2), and fresh (1338 g/m^2) and dry biomass (76 g/m^2) in the Singhia river shore receiving soap factory effluent, although frond size was the biggest (175 mm^2) in habitat 2. The frond number (45040/m^2) and frond size (63.5 mm^2) of the test species were least in habitat 2 and Pichhara ditch, respectively. Similarly, minimum biomass of the test species (fresh 448 g/m^2, dry 31 g/m^2) occurred in Katari pond receiving jute mill effluent (Table 1).

Table 1. Growth of *Spirodela polyrhiza* in different water bodies during April (n=5, mean ± SD)

Habitats	Frond number / m^2	Frond size (mm^2)	Fresh weight/ m^2 (g)	Dry weight/ m^2 (g)
Pichhara North Ditch	49840±2125	63.5±23.6	553±29	37.2±14.9
Jute factory, Nayabajar	45100±894	68.1±18.1	448±86	31±3
Soap industry, Nayabajar	57060±3780	118.4±59.9	1338±83	76±2.9
Ditch highway (Brt-Jogbani)	45040±18249	175±57.2	1299.4±545	64.6±25.0

Water Temperature and pH in Selected Habitats

Range of temperature was 18.1 to 28.4°C in habitat 1, 17 to 27.5°C in habitat 2, and 16.5 to 27.7°C in habitat 3. The range of pH of water samples was 6.2 to 6.5 in habitat 1, 5.8 to 6.3 in habitat 2 and 5.8 to 6.3 in habitat 3 (Table 2).

Growth Variations

Monthly morphological growth variations (frond size, root length, frond number/m^2, fresh and dry weight) of *S. polyrhiza* in the selected habitats are given in table 3.

The fronds were largest (72.06 mm^2) in size in April and smallest (29.19 mm^2) in February in habitat 1; whereas largest (43.13 mm^2) and smallest (28.85 mm^2) fronds were recorded in

Table 2. Monthly variations in water temperature and pH of selected habitats of *Spirodela polyrhiza. (*mean ± SD; n = 3)

Month	Dec	Jan	Feb	Mar	Apr	May
Habitat 1						
Temperature (^oC)	19.2± 2.5	18.1 ± 2.4	18.5 ±	23 ± 2.5	26.7 ± 3.0	2 8.4 ± 3.1
pH	6.2	6.4	6.2	6.2	6.4	6.2
Habitat 2						
Temperature (^oC)	19.0 ± 2.5	17.0 ± 2.4	18.8 ± 2.5	22.9 ± 2.8	26.7 ± 3.0	27.5 ± 3.0
pH	6.3	5.8	6.0	6.3	6.2	6.3
Habitat 3						
Temperature (^oC)	17.4 ± 2.4	17.2 ± 2.4	16.5 ± 2.3	20.7 ± 2.6	27.0 ± 3.0	27.7 ± 3.0
pH	6.2	5.8	6.2	6.2	6.3	6.2

February (41.01 mm^2) and December (24.1 mm^2), respectively. Root length was minimum (0.58 cm) in May and maximum (1.17 cm) in March in habitat 1, between 0.68 cm (December) and 1.79 cm (February) in habitat 2, and between 0.78 cm (April) and 2.48 cm (December) in habitat 3. Number of fronds ranged between 3700 m^2 (February) and 50866/m^2 (January) in habitat 1, between 20300/m^2 (April) and 64067/m^2 (March) in habitat 2, and between 38676/m^2 (May) and 59100/m^2 (January) in habitat 3.

The fresh weight of *S. polyrhiza* fronds ranged between 7.97 (February) and 669.02 g/m^2 (April) in habitat 1, between 21.76 (February) and 243.3 g/m^2 (May) in habitat 2, and between 95.19 (March) and 380.52 g/m^2 (January) in habitat 3. The dry weight of fronds ranged between 0.35 (February) and 53 g/m^2 (April) in habitat 1, between 5.03 (February) and 21.06 g/m^2 (May) in habitat 2, and between 8.88 (March) and 38.22 g/m^2 (February) in habitat 3.

Table 3. Monthly variations in morphological attributes of *S. polyrhiza* in selected habitats (mean ± SD, n = 3)

Month	Dec	Jan	Feb	Mar	Apr	May
Habitat 1						
Frond size (mm^2)	42.8 ± 3.7	31.1 ± 3.2	29.1 ± 3.1	61.1 ± 4.5	72.06 ± 4.9	43.6 ± 3.8
Root length (cm)	0.80 ± 0.51	0.89 ± 0.54	0.75 ± 0.5	1.17 ± 0.62	1.09 ± 0.60	0.58 ± 0.43
Frond number/m^2	46200 ± 124	50866 ± 130	3700 ± 35	13533 ± 67	40533 ± 116	28940 ± 98
Fresh weight (g/m^2)	610.7 ± 14.26	425.5 ± 11.9	7.97 ± 1.62	101.8 ± 5.82	669.02 ± 14.9	427.3 ± 11.9
Dry weight (g/m^2)	22.56 ± 2.74	17.58 ± 2.42	0.35 ± 0.34	11.39 ± 1.94	53.0 ± 4.2	21.87 ± 2.7
Habitat 2						
Frond size (mm^2)	28.8 ± 3.1	30.6 ± 3.2	41.17 ± 3.7	27.3 ± 3.0	31.4 ± 3.2	28.4 ± 3.1
Root length (cm)	0.68 ± 0.47	1.27 ± 0.65	1.79 ± 0.77	1.5 ± 0.7	1.67 ± 0.74	1.5 ± 0.71
Frond number/m^2	21633 ± 84	39438 ± 114	15726 ± 72	64067 ± 146	20300 ± 82	36167 ± 109
Fresh weight (g/m^2)	87.32 ± 5.4	108.34 ± 6.0	21.76 ± 2.7	168.69 ± 7.5	107.1 ± 5.9	243.3 ± 9.0
Dry weight (g/m^2)	12.25 ± 2.0	12.96 ± 2.07	5.03 ± 1.3	9.35 ± 1.76	9.0 ± 1.73	21.06 ± 2.64
Habitat 3						
Frond size (mm^2)	24.10 ± 2.8	24.9 ± 2.9	43.17 ± 3.7	41.07 ± 3.7	34.4 ± 3.4	36.1 ± 3.4
Root length (cm)	2.4 ± 0.90	1.66 ± 0.74	1.87 ± 0.78	1.73 ± 0.75	0.78 ± 0.50	0.93 ± 0.55
Frond number/m^2	49700 ± 128	59100 ± 140	32800 ± 104	18400 ± 78	48700± 127	38767 ± 113
Fresh weight (g/m^2)	344.00 ± 10.70	380.52 ± 11.26	247.30 ± 9.07	95.19 ± 5.63	340.12± 10.6	269.11 ± 9.47
Dry weight (g/m^2)	36.45 ± 3.48	38.22 ± 3.56	14.39 ± 2.19	8.88 ± 1.72	16.04 ± 2.31	21.00 ± 2.64

Ex-situ Growth

Growth in water pH

pH tolerance range for the growth of *S. polyrhiza* was between 4 and 7. At 2, 3 and 8 pH, the plant did not survive. pH 6 was the optimum for growth of *S. polyrhiza* (Fig. 1).

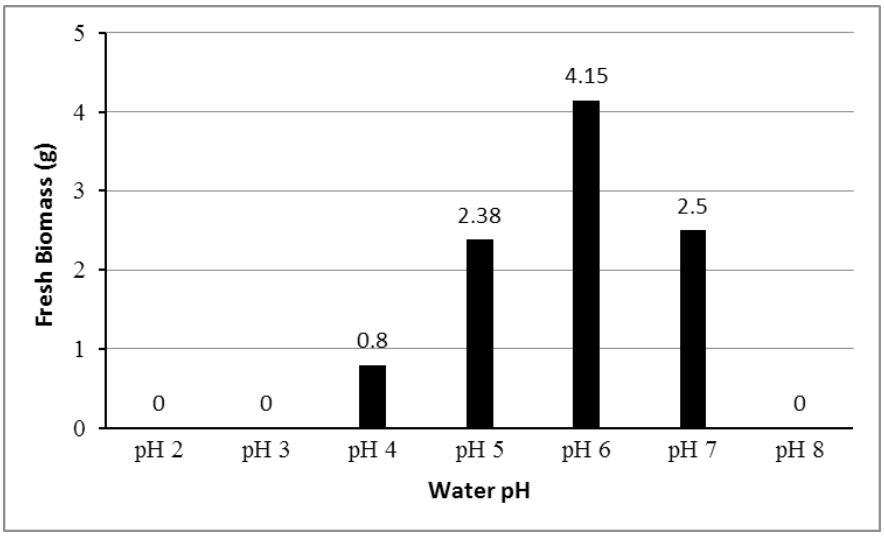

Figure 1. Increase or decrease in fresh biomass (g) of *Spirodela polyrhiza* at different water pH

Growth in cow-dung

Anaerobically decomposed cow-dung for 7 days stimulated growth of *S. polyrhiza*. 10 mg/L cow dung promoted frond size (48.3 mm^2), fresh biomass (3.6 g), and dry biomass (0.15 g) (Table 4). Similarly, 21 days anaerobically decomposed cow-dung stimulated the plant growth. It was effective at 10 mg/L; however, increasing concentration decreased the frond size, fresh weight, and dry weight (Table 5).

Table 4. Effects of anaerobically decomposed cow-dung for 7 days on *Spirodela polyrhiza* (1 g inoculum) growth after 15 days (initial frond size= 47.5 mm^2; n=3, mean ±SD)

Concentration	Frond size (mm^2)	Fresh wt (g)	Dry wt (g)
2 g/L	41.4±6.9	2.82±0.13	0.15±0.01
4 g/L	43.2±13.3	2.86±0.17	0.15±0.01
6 g/L	43.4±11.6	3.02±0.20	0.15±0.01
8 g/L	41.6±10.5	3.06±0.16	0.15±0.01
10 g/L	49.5±12.1	3.13±0.27	0.16±0.02
12 g/L	41.6±12.5	2.93±0.13	0.12±0.01
Control	41.4±6.9	2.3±0.17	0.11±0.09

Growth in glucose

All the tested glucose concentration was inhibitory on the growth performance parameters of *Spirodela polyrhiza* except 6 mg/L which increased the root number (7.3), root length (1.2 cm) and fresh weight (0.48 g), however, number of frond was inhibited (Table 6).

Table 5. Effects of anaerobically decomposed cow-dung for 21 days on *Spirodela polyrhiza* (1 g inoculum) growth after 15 days (initial frond size= 47.5 mm^2; n=3, mean ±SD)

Concentration	Frond size (mm^2)	Fresh wt (g)	Dry wt (g)
5 g/L	42.4±6.3	2.4±0.2	0.11±0.01
10 g/L	48.3±13.5	3.6±0.1	0.15±0.01
15 g/L	44.4±10.5	3.1±0.2	0.12±0.02
20 g/L	41.6±11.2	2.9±0.3	0.13±0.008
25 g/L	39.6±14.1	2.4±0.06	0.11±0.004
30 g/L	39.1±12.5	2.1±0.2	0.10±0.01
Control	35.8±6.7	1.9±0.2	0.08±0.01

Table 6. Effects of glucose concentration (mg/L) on the growth of *Spirodela polyrhiza* incubated for 10 days (December-January), n = 3, mean ± SD.

Treatment/ Concentration	Frond number	Root number	Root length (cm)	Fresh weight (g)
3 mg/L	25 ± 2.4	5.2 ± 2.1	0.7 ± 0.3	0.37 ± 0.05
6 mg/L	25 ± 0.8	7.3 ± 2.4	1.2 ± 0.3	0.48 ± 0.01
9 mg/L	25 ± 0.8	6.1 ± 2.3	0.9 ± 0.3	0.41 ±0.02
12 mg/L	24 ± 0.8	4.8 ± 2.2	0.7 ± 0.4	0.42 ± 0.o2
15 mg/L	24.6 ± 1.2	6.4 ± 2.8	0.8 ± 0.4	0.43 ± 0.01
Cotrol	27 ± 0.8	6.6 ± 1.6	1.0 ± 0.3	0.45 ± 0.04

Growth in sucrose

Number of fronds and roots, root length and fresh weight decreased with increasing concentration of sucrose from 10 to 50 mg/L. Frond number (27.6) and fresh weight (0.43 g) was least at 50 mg/L sucrose solution (Table 7).

Table 7. Effects of sucrose concentration (mg/L) on the growth of *Spirodela polyrhiza* incubated for 10 days (December-January), n=3, mean ± SD

Treatment/ Concentration	Frond number	Root number	Root length (cm)	Fresh weight (g)
10 mg/L	38.6±2.1	15.2 ± 6.7	1.41 ± 0.26	0.66 ± 0.01
20 mg/L	37.3±1.1	12.8± 4.8	1.1± 0.31	0.65 ± 0.01
30 mg/L	36.0±0.0	10.2 ± 4.1	0.91 ± 0.30	0.56 ±0.02
40 mg/L	33.6±3.8	6.6±3.4	0.7 ± 0.33	0.47 ± 0.04
50 mg/L	27.6±3.2	7.1± 4.1	0.8 ± 0.43	0.43 ± 0.05
Control	38±1.0	15.3± 5.4	1.6± 0.30	0.67 ± 0.03

Growth in industrial effluents

Sugar factory effluent was stimulatory on fresh biomass of *S. polyrhiza*, at 5% and 10% concentrations. Textile industry at 10% stimulated the fresh biomass (0.28 g) and frond number (30). Soap factory effluent was inhibitory to fresh biomass. However, root length and number was inhibited by all the test effluents (Table 8).

Table 8. Effect of industrial effluents on root number and length (cm), frond number and fresh weight (g) of *Spirodela polyrhiza* (n= 3, mean ± SD) (initial 10 frond/500ml container)

Industrial effluent/control	Concentration (%)	Frond number	Root number	Root length (cm)	Fresh weight (g)
Textile industry	5%	24.6±2.06	5.0±2.04	1.2±0.4	0.21±0.02
(Shah udyog)	10%	30.0±2.8	5.3±1.6	0.8±0.4	0.28±0.04
	15%	23.6±1.2	4.9±2.1	0.9±03	0.19±0.02
Sugar factory	5%	22.3±1.7	8.5±1.8	1.1±0.3	0.30±0.02
	10%	23.0±1.4	8.3±2.1	1.2±0.3	0.27±0.01
	15%	20.6±1.2	8.0±2.1	1.1±0.3	0.24±0.02
Soap factory	5%	26.6±3.6	8.0±2.1	0.7±0.3	0.21±0.02
	10%	28.0±2.4	5.6±1.4	0.6±0.2	0.24±0.04
	15%	22.6±2.1	7.4±2.1	0.5±0.2	0.21±0.04
Control	Tap water	27.6±0.5	10.6±3.6	1.2±0.3	0.25±0.01

Discussion

Growth Variations

The frond size ranged between 29.19 (February) and 72.06 mm^2 (April) in habitat 1, between 27.37 (March) and 43.13 mm^2 in habitat 2, and between 24.1 (December) and 41.07 mm^2 (February) in habitat 3. Ashbey and Wangermann (1949) and Wangermann and Ashbey (1950) have reported similar cycles of senescence and rejuvenation in the vegetative growth of *Lemna minor* associated with periodic reduction/increase in size and number of the frond cells.

The optimum range of some important physic-chemical factors of water for the growth of duckweeds is as follows: temperature 15-30°C, pH 6.5-8, nitrogen 7-12 mg/L, and phosphorus 4-8 mg/L (Hasan & Chakrabarti, 2009). Thus, except temperature, range of pH, nitrogen and phosphorus in the selected pools were inadequate for the optimum growth of *Spirodela*. Nevertheless, concentrations of nitrogen and phosphorus ranged between 3.37-5.25 mg/L (0.98-2.46 mg/L in habitat 3) and 0.05-0.19 mg/L, respectively, in the selected pools. Edwards et al. (1992) observed that pond water with less than 3 mg/L N and 0.3 mg/L P did not support normal growth of *Lemna perpusilla* and *Spirodela polyrhiza*, whereas Luond (1980) reported growth reduction in duckweeds only when P concentration in water dropped below 0.017 mg/L.

Maximum frond biomass per unit area was 53 g/m^2 in April (water temperature 26°C) in habitat 1, 21.06 g/m^2 (water temperature 27°C) in habitat 2, and 41.07 g/m^2 in February (water temperature 27°C) in habitat 3 in the present study. Khondker et al. (1993) reported that water temperature above 25°C is detrimental to the growth of *S. polyrhiza*, whereas Pokharel (Bhattarai) and Jha (2016) have observed healthy growth of the test species even at 31.2°C water temperature in a nutrient poor roadside pool at Biratnagar, Nepal.

Root length of *Spirodela* ranged between 0.58 (May) and 1.17 cm (March) in habitat 1, between 0.61 (December) and 1.79 cm (February) in habitat 2, and between 0.78 (April) and 2.48 cm (December) in habitat 3. Leng (1999) opined that roots either stabilize the frond on the water surface or assist the frond to obtain nutrients where these are in dilute

concentrations. They tend to lengthen as mineral nutrients in water are exhausted.

Growth on Different Water pH and Nutrient Media

Spirodela polyrhiza survived in the pH range of 4 to 7 and displayed optimum increase in fresh weight at pH 6. According to Tossavainen et al. (2018) mixed cultures can better tolerate potentially stressful changes in growth conditions in comparison to monoculture.

In the present study, appropriate concentration of glucose in water for the frond growth was 6 g/L. Number of fronds and roots, root length and fresh weight decreased with increasing concentration of sucrose. Leng (1999) stated that like other photosynthesizing organisms duckweeds grow with only requirements for minerals, utilizing solar energy to synthesize biomass; however, they have the capacity to utilize pre-formed organic materials particularly sugars and can grow without sunlight when provided with such energy substrates. In practice the ability to use sugars in the medium as energy source is irrelevant, as in most aquatic systems sugars do not exist. However, they could be of some importance where industrial effluents need to be purified and duckweed is considered for this process (e.g., wastewater from sugar industry, waste water from starch processing etc.).

Acknowledgements

We are thankful to University Grants Commission (UGC), Nepal for providing research grant. Thanks are also due to Head Prof. Dr. Umesh Koirala, Department of Botany Post Graduate Campus, T.U. Biratnagar for providing laboratory facilities.

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Integrating Biological Resources for Prosperity, 2021, 189-195

Ethno-medicinal Plants of Dhankuta District, Province 1, Nepal

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Abstract

The study of ethnomedicinal plants of Dhankuta district of province 1 was conducted from 2015 to 2016. Information was taken from 75 randomly selected individuals of three Municipalities and four Rural Municipalities of Dhankuta district using questionnaires. A total of 51 plant species of ethnomedicinal values belonging to 25 families under 35 genera were recorded during the survey. These plants have been used to cure 20 different ailments, such as diabetes, blood pressure, tonsillitis, dry cough, fever, dysentery, common cold, bone fracture, burns, etc. Traditional knowledge of plant utilization has been declined due to lack of continuous flow of indigenous knowledge and documentation to the young generation. The conservation and management of local ethnomedicinal plants with the involvement of ethnic people of Dhankuta must be encouraged by the joint venture of local and provincial governments.

Key words: Conservation, Indigenous knowledge, Joint venture, *Oroxylum*, Tonsillitis

Introduction

Nepal is a multilingual, multicultural and multiethnic country. There are more than 140 ethnic groups and they use about 123 different languages (CBS, 2012). Medicinal plants are very important and useful in the context of Nepal because of less side effect, scarcity of modern health services and the rural nature of the country (Bhattarai et al., 2009). Most of the people of Nepal are literate and in poverty. They are not aware of the modern health care and modern medicines. The ancient culture and train to use plants as traditional medicine continues to this day in our country.

Dhankuta is one of the hilly districts of the eastern region of Nepal. The total population of Dhankuta is 1,63,412 (CBS, 2012), and it has 891 km^2 in area. Rai and Limbu dominate the study area in association with Magar, Brahman and Chhetri. Indigenous people and ethnic groups used the traditional medicinal plants of the Dhankuta district. However, the present trend of urbanization and globalization leads to decreased cultural and traditional knowledge from the indigenous group. Deforestation and burning of forest cause continuous loss of essential and useful plants annually. The young generation wants to be modern. They do not like to take information about cultural and traditional knowledge about the importance and uses of local medicinal plants. There was a vast generation gap between the young generation and the adult one.

The proper documentation of medicinal plants began with the work of Banerji (1955) in Nepal who was followed by Manandhar (1971), Dobremez (1976), Gautam (2011), Acharya (1996),

Dangol and Gurung (1999), Basnet et al. (2001), Bhattarai (2002), Rai (2003), Chapagain et al. (2004), Siwakoti et al. (2005), and Malla and Chhetri (2009). However, the surveys of the ethnomedicinal plant in Dhankuta have been done by Dahal (2000) and Subba et al. (2016).

Materials and Methods

The study was done in three Municipalities (Mahalaxmi, Pakhribas, Dhankuta) and four Rural Municipalities (Chaubise, Sanguri Gadi, Khalsa Chhintang Sahid Vumi, and Chhathar Jorpati) (Fig. 1). Chaubise and Chhathar Jorpati were Limbu dominated Rural Municipalities, while Khalsa Chhintang Sahid Vumi Rural Municipalities were Rai dominated areas. Chhetri domination was seen in Mahalaxmi, Pakhribas, and Dhankuta Municipalities. Field trips were made to collect information about the ethnomedicinal plants in Dhankuta from 2015 to 2016. Information was taken by direct field visit and interview taken with knowledgeable villagers using ethnomedicinal plants in real life.

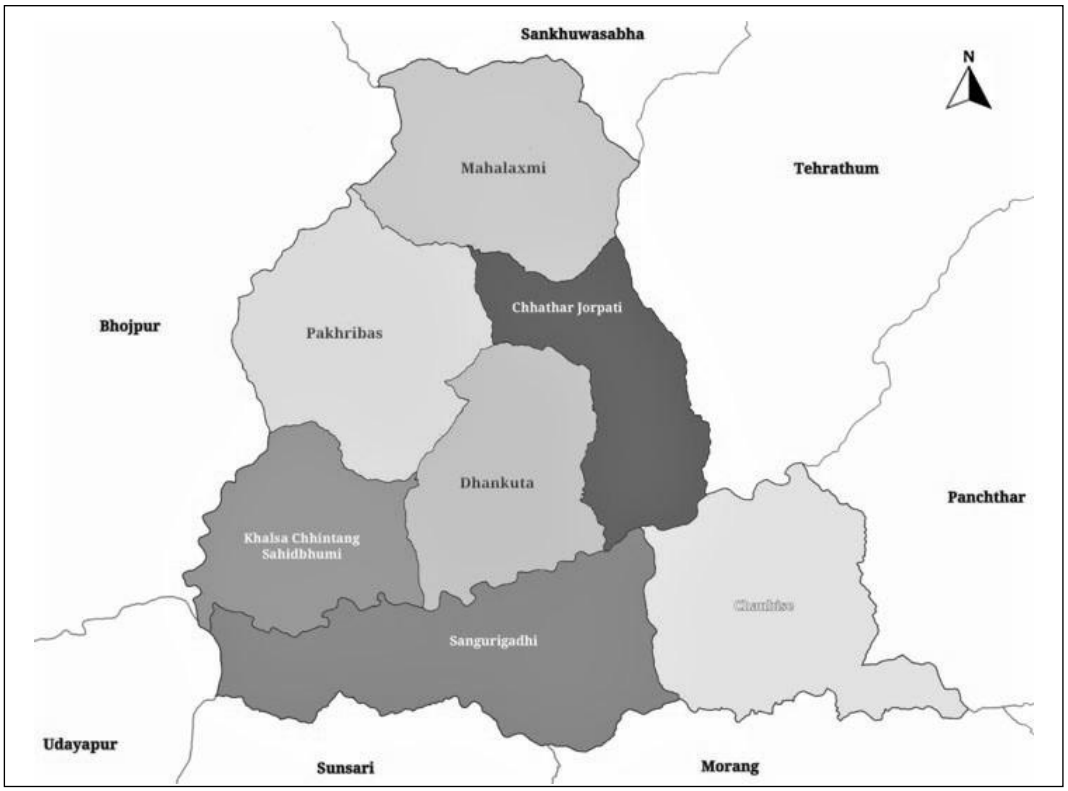

Figure 1. Map of Dhankuta District showing the study area (Source: google.com)

The specimen display method and the standard literature method were followed while taking information about the ethnomedicinal plants found in Dhankuta. Collected plants were shown to the local people and knowledgeable people to confirm the results. Plants were brought to the Department of Botany, Post Graduate Campus, Biratnagar and consulted the herbaria for identification. Specimens identification was confirmed by following Polunin and Stainton (1997), and Shrestha (1998).

Results

The scientific name, related family, local name and medicinal uses of the medicinal plants were listed in alphabetical order in table 1. Fifty-one plant species belonging to 26 families and 35 genera were recorded. The largest family was Rutaceae which included six medicinal plants, followed by families Asteraceae and Zingiberaceae, and each included 4 species (Table 2). Families, viz., Brassicaceae, Cucurbitaceae, Lamiaceae, and Oleaceae, consist of three medicinal plans. Similarly, families, viz., Asparagaceae, Combretaceae, Fabaceae, Gentianaceae, Musaceae, and Rosaceae, consist of two medicinal plants. Rest, 13 families consist of single medicinal plants. Among the 51 medicinal plants, 22 were herbs, 14 were shrubs, 5 were climbers, and 10 were trees (Table 2).

Most of the plants were used in throat pain, tonsillitis, gastric, jaundice, cuts, diarrhoea, dysentery and coughing. Seven plants were used in throat pain, and five plants were used in tonsillitis, five plants used in blood pressure, four plants in cough and the other four plants were used in cuts (Table 3). They mostly used externally in the form of paste as an ointment and used internally and in the form of juice or solution. *Oroxylum indicum* (L.) Kurz was used for the treatment of jaundice, *Tinospora cordifolia* (Wild.) Miers for the treatment of fever, uric acid as well as jaundice, followed by *Cissus quadrangularis* L. in bone fracture, *Musa superba* Roxb. in diarrhoea, *Centella asiatica* (L.) Urban and *Curcuma longa* L. for tonsillitis.

For the treatment of ailments, the maximum parts used was a leaf, followed by fruit, stem, seed, rhizome, root bark, etc. (Table 4).

Table 1. List of ethnomedicinal plants recorded in Dhankuta District.

SN	Plant name	Family	Local name	Habit	Use	Part	Method
1	*Acorus calamus* L.	Acoraceae	Bojho	H	Cough	Rh	Juice
2	*Aegle marmelos* (L.) Corr.	Rutaceae	Bel	T	Paralysis prevention	Fb	Paste
3	*Ageratina adenophora* (Spreng.) R.M. King & H. Rob.	Asteraceae	Kalijhar	T	Cut, Wound	L	Paste
4	*Allium sativum* L.	Amaryllidaceae	Lasun	H	Gastric	St	Juice
5	*Aloe vera* (L.) Burn.f.	Asphodelaceae	Ghiu kumari	H	Wound, Burn	L	Paste
6	*Amomum aromaticum* Roxb.	Zingiberaceae	Alaichi	H	Cough, Throat pain	Sd	Chew
7	*Artemisia indica* Willd.	Asteraceae	Titepati	S	Vomiting, High blood pressure	L	Chew
8	*Asparagus filicinus* Buch-Ham. ex D.Don	Asparagaceae	Ban kurilo	H	Low blood pressure	St	Vegetable
9	*Asparagus officinalis* L. var. altilis L.	Asparagaceae	Kurilo	H	Low blood pressure	St	Vegetable
10	*Berginia ciliata* (Haw.) Stemb.	Saxifragaceae	Pakhan ved	H	Body pain	Rh	Juice
11	*Brassica campestris* L. var. *sarson* Prain.	Brassicaceae	Sarsu	H	Common cold	Sd	Oil paste
12	*Brassica nigra* (L.) Koch	Brassicaceae	Rayo	H	Common cold	Sd	Oil paste
13	*Brassica rapa* L.	Brassicaceae	Tori	H	Common cold	Sd	Oil paste
14	*Calotropis gigantea* (L.) Dryand.	Apocynaceae	Ank	S	Cuts, Joint pain	L	Paste, Covering

SN	Plant name	Family	Local name	Habit	Use	Part	Method
15	*Centella asiatica* (L.) Urban.	Apiaceae	Ghodtapre	H	Throat pain	L	Eat
16	*Chromolaena odorata* (L.) R.M. King & H. Rob.	Asteraceae	Banmara	S	Skin errose	L	Paste
17	*Cissus quadrangularis* L.	Vitaceae	Hadjod	C	Bone fracture	St	Paste
18	*Citrus aurantifolia* (Christ.) Swingle	Rutaceae	Kagati	S	Oral wound	F	Eat
19	*Citrus reticulata* Blanco.	Rutaceae	Suntola	T	Jaundice	Fb	Juice
20	*Curcuma angustifolia* Roxb.	Zingiberaceae	Hardi	H	Cuts, Wound	Rh	Paste
21	*Curcuma longa* Linn.	Zingiberaceae	Besar	H	Tonsillitis	Rh	Powder
22	*Drymaria villosa* Cham & Schltdl.	Caryophyllaceae	Abilalo	H	Tongue wound	L , St	
23	*Eupatorium triplinerve* M.Vahl.	Asteraceae	Bhumiraj	H	Cut, Wound	L	Paste
24	*Jasminum humile* L.	Oleaceae	Jaiphul	S	Tonsillitis	L	Chew
25	*Jasminum nepalenses* Spreng.	Oleaceae	Ban Jaiphul	S	Tonsillitis	L	Chew
26	*Jasminum officinale* L.	Oleaceae	LahareJaiphul	S	Tonsillitis	L	Chew
27	*Mentha arvensis* L.	Lamiaceae	Pudina	H	Gastric	L	Prickle
28	*Mimosa pudica* L.	Fabaceae	Lajjawati	H	Tonsillitis	R	Chew
29	*Mormordica balsamina* L.	Cucurbitaceae	Barela	C	Gastric	F	Vegetable
30	*Momordica charantia* L.	Cucurbitaceae	Tite karela	C	High blood pressure, Gastric	F	Vegetable
31	*Momordica dioica* Roxb.ex Wild.	Cucurbitaceae	Ban karela	C	Gastric, Jaundice	F	Eat
32	*Musa paradisiacal* L.	Musaceae	Kera	T	Diarrhoea	F	Eat
33	*Musa superba* Roxb.	Musaceae	Bankera	T	Diarrhoea	F	Eat
34	*Ocimum basilicum* L.	Lamiaceae	Babari	H	Skin allergy	L	Paste
35	*Ocimum tenuiflorum* L.	Lamiaceae	Tulasi	H	Throat pain	L	Juice
36	*Oroxylum indicum* (L.) Kurz	Bignoniaceae	Totela	T	Jaundice	Sb	Juice
37	*Phylanthus emblica* L.	Phyllanthaceae	Amala	T	Oral wound	F	Eat
38	*Rubus ellipticus* Smith	Rosaceae	Ainselu	S	Throat pain	St	Chew
39	*Rubus niveus* Thunb.	Rosaceae	Rato ainselu	S	Throat pain	St	Chew
40	*Swertia angustifolia* Buch.-Ham. ex D.Don	Gentianaceae	Chiraito	H	Throat pain, Fever	Sl	Juice
41	*Swertia chirayita* (Roxb.ex Fleming) Karsten	Gentianaceae	Chiraita	H	Throat pain, Fever	Sl	Juice
42	*Tamarindus indica* L.	Fabaceae	Titri	T	Diarrhoea, Fever	F	Eat
43	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	Barro	T	Coughing	F	Eat
44	*Terminalia chebula* Retz.	Combretaceae	Harro	T	Coughing	F	Eat
45	*Tinospora cordifolia* (Wild.) Miers.	Menispermaceae	Gurjo	C	Fever, Uric acid, Jaundice	Sb	Juice
46	*Urtica dioica* L.	Utricaceae	Sisnu	S	Sugar, Blood pressure	L	Vegetable
47	*Zanthoxylum acanthopodium* DC.	Rutaceae	Boke timur	S	Gastric	Sd	Juice
48	*Zanthoxylum armatum* DC.	Rutaceae	Timur	S	Teeth pain	Rb	Juice
49	*Zanthoxylum oxyphyllum* Edgew.	Rutaceae	Siltimur	S	Gastric	Sd	Juice
50	*Zingiber officinale* Rosc.	Zingiberaceae	Aduwa	H	Tonsillitis	Rh	Powder
51	*Zizyphus mauritiana* Lam.	Rhamnaceae	Bayar	T	Teeth pain	Rb	Chew

T = Tree, H = Herb, S = Shrub, C= Climber, L = Leaf, St = Stem, Sb = Stem bark, R = Root, Rb = Root bark, F = Fruit, Fb = Fruit bark, Sd = Seed, Rh = Rhizome, Sl = Stem leaf

Table 2. Families and habits of medicinal plants.

S.N.	Family	Herb	Shrub	Climber	Tree	Total
1	Asparagaceae	2				2
2	Asteraceae	1	2	-	1	4
3	Brassicaceae	3	-	-	-	3
4	Combretaceae	-	-	-	2	2
5	Cucurbitaceae	-	-	3		3
6	Fabaceae	1	-	-	1	2
7	Gentianaceae	2	-	-	-	2
8	Lamiaceae	3	-	-	-	3
9	Musaceae	-	-	-	2	2
10	Oleaceae	-	3	-	-	3
11	Rosaceae	-	2	-	-	2
12	Rutaceae	-	4	-	2	6
13	Zingiberaceae	4	-	-	-	4
14	Others (13)	6	3	2	2	13
	Total	22	14	5	10	51

Table 3. Plants belonging to different habits used in various diseases.

S.N.	Disease	Herb	Shrub	Climber	Tree	Total
1	Blood pressure	2	1	-	2	5
2	Body pain	2	-	-	-	2
3	Common cold	3	-	-	-	3
4	Cough	2	-	-	2	4
5	Cuts	2	2	-	-	4
6	Diarrhoea	-	-	-	2	2
7	Fever	2	-	-	-	2
8	Gastric	1	1	1	-	3
9	Jaundice	-	-	1	1	2
10	Oral wound	-	1	-	1	2
11	Teeth pain	-	1	-	1	2
12	Throat pain	3	4	-	-	7
13	Tonsillitis	3	2	-	-	5
14	Others	2	2	1	3	8
	Total	22	14	5	13	51

Table 4. Plant parts belonging to different habits used as medicine.

Habit	Root	Root bark	Stem	Stem bark	Stem Leaf	Leaf	Fruit	Fruit bark	Seed	Rhizome	Total
Herbs	-	-	5	-	2	6	-	-	4	5	22
Shrubs	-	1	2	-	-	8	1	-	2	-	14
Climbers	-	-	1	1	-	-	3	-	-	-	5
Trees	-	1	-	1	-	-	6	2	-	-	10
Total	0	3	8	2	2	14	10	2	6	4	51

Discussion

Plant species listed were found to have been used for the treatment of different ailments. Leaf and stem parts were mostly used to prepare the medicine. The liquid extracted from different

parts of the plant was used as an ointment. The leaf of 14 plant species, fruits of 10 species and stem of eight plant species were used as medicine. The species of 22 herbs, 14 shrubs, five climbers and ten trees were used mostly as medicine. Six species of Rutaceae family, four species of Asteraceae and Zingiberaceae, three Brassicaceae species, Cucurbitaceae, Lamiaceae, and Oleaceae, two species of each six families, and single species of remaining every thirteen families were used as medicine. To treat throat pain, seven plant species, in blood pressure and tonsillitis five plant species, cough and cuts four plant species were found to have been used as medicine.

Most of people believe and depend on medicinal plants for the treatment of ordinary disease. Because of cultural and traditional belief, many people depend on local traditional healers. The present findings indicate that indigenous people maintain their health by the immediate use of local medicinal plants. Due to indigenous people's low economic status and rich traditional knowledge, people living in the remote areas of Dhankuta still depend on the traditional healing system. The reverse was found in new generation who do not appreciate to conserve traditional knowledge. Ethnomedicinal plants should be conserved locally, and knowledge of ethnomedicinal plant of the ethnic group must be transferred to the young generation.

Conclusion

Ethnomedicinal plants are the country's property, and indigenous knowledge is the identity of the ethnic group. So indigenous knowledge about the use of medicinal plant should be conserved by the government. Knowledge should be transformed to the young generation by conducting an awareness programme. The local people in a personal garden should cultivate medicinal plants as a new development of culture. Cultivation of medicinal plant must be encouraged by providing subsidy. The proper conservation and management of the ethnomedicinal plant of Dhankuta must be encouraged by joint venture of the local government and the province of the province.

Acknowledgements

I want to thank the local people and teachers of Dhankuta who provided me with essential information about ethnomedicinal plants. I am grateful to Dr. Bharat Raj Subba, and Dr. Bhabindra Niroula, Department of Botany, Post Graduate Campus, T.U., Biratnagar, Nepal for the support and encouragement.

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Status of Arsenic and Fluoride Pollutants in Groundwater of Biratnagar of Morang District, Nepal

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Abstract

Evaluation of arsenic and fluoride pollution status of groundwater in Biratnagar, Nepal, has been carried out to assess the water quality during the study period. The value of arsenic (As) ranged from nil (ward no 3) to 0.17±0.21 mg/L (ward no. 1), from 0.01±0.00 mg/L (ward no. 13) to 0.23±0.25 mg/L (ward no. 1), from nil (ward no. 3) to 0.17±0.21 mg /L (ward no. 1), from 0.01±0.01 mg /L (ward no. 13) to 0.37±0.00 mg/L (ward no. 6) during the period of post-monsoon 2015, pre-monsoon 2016, post-monsoon 2016, and pre-monsoon 2017, respectively. Out of 110 analyzed samples, the arsenic concentrations in 42 water samples (38.18%) during post-monsoon 2015, in 58 water samples (52.72%) during pre-monsoon 2016 (N = 110), in 37 water samples (33.64%) during post-monsoon 2016 and 59 water samples (53.64%) during pre-monsoon 2017 period exceeded the WHO permissible limit of 0.01 mg/L. As per BIS and NDWQS-Nepal guideline values for As in drinking water, only ten water samples (9.09%) during post-monsoon 2015, 22 water samples (20%) during pre-monsoon 2016, seven water samples (6.36%) during post-monsoon 2016, and 24 water samples (21.89%) during pre-monsoon 2017 (N=110) exceeded the prescribed limit. The concentrations of arsenic were relatively higher during the pre-monsoon period than the post-post-monsoon period throughout the study. In the present study, the fluoride level in all the groundwater samples analyzed had below the detection level.

Key words: Arsenic, Contamination, Fluoride, Groundwater, Tube wells

Introduction

Arsenic is a heavy metal with a name derived from the Greek word *arsenikon*, meaning potent. The elements occur in the environment in different oxidation states and form various species, e.g., As (V), As (III), As (0) and As (-III). In an oxidizing environment, As appears mostly as oxyanions (Cutter, 1992). Inorganic arsenic generally exists in two predominant oxidation states, arsenite ($NaAsO_2$) and arsenate (Na_2HAsO_4), both of which are toxic to man and plants. Inorganic arsenic is always considered a potent human carcinogen, associated with increased risk for cancer of the skin, lungs, urinary bladder, liver and kidney (National Research Council, Report, 1999). The toxicity of arsenic to human health ranges from skin lesions to cancer of the brain, liver, kidney, and stomach (Smith et al., 1992). Clinical symptoms of acute intoxication include abdominal pain, vomiting, diarrhoea, muscular pain, and weakness, with the skin's flushing (Asklund & Eldvall, 2005).

Arsenic contamination of drinking water is a global problem due to its detrimental effects on health. Presence of arsenic in groundwater in the Terai districts was known for the first time in 1999 from WHO's research work. Since then, there has been a growing concern among the Nepalese scholars towards understanding more on arsenic and its human health impacts. These effects range from skin ailments to severe diseases such as Cancer and death. Arsenic contamination of ground water has been reported from many countries, including Bangladesh, Vietnam, Argentina, China and parts of USA and now in India. The provisional limit of Arsenic in drinking water as recommended by WHO is 10 mg/L.

Fluoride is a naturally occurring chemical substance found in water, soil, foods and several other compounds in trace quantities (Harrison, 2005). Small amounts in ingested water are usually considered good to have a beneficial effect on the rate of dental caries, particularly among children. On the other hand, due to its strong electronegativity, fluoride is attracted by positively charged calcium ions in teeth and bones. Excessive intake results in pathological changes in teeth and bones, such as mottling of teeth or dental fluorosis followed by skeletal fluorosis (Saralakumari & Ramakrishna, 1993). Excessive fluoride intake over a long period may result in a serious public health problem called fluorosis, which is characterized by dental mottling and skeletal manifestations such as crippling deformities, osteoporosis, and osteosclerosis. Endemic fluorosis is now known to be global in scope, occurring on all continents and affecting many people (WHO, 2006).

The natural contamination of arsenic in groundwater has been reported worldwide, and most of these belong to South Asian and South American regions (Bundschuh et al., 2012; Hashim et al., 2019). The severely affected countries include Bangladesh (Yang et al., 2014), India (Chakraborti et al., 2018; Bindal & Singh, 2019; Dhillon, 2020), China (Guo et al., 2014), Nepal (Pokhrel et al., 2009; Das & Choudhary, 2018). WHO has reported worldwide 748 million people are exposed to contaminated groundwater resources crisis for drinking purpose in 2012, 200 million human population in 27 nations worldwide facing critical issue of fluoride contamination while 66.64 million people in India (Mumtaz et al., 2015). Similarly, the fluoridation of public drinking water supplies in the U.S. has proceeded at a steady rate, reaching 62% in 1992, with the goal of 75% by the year 2000 (Yiming et al., 2001). In this study, the contamination of arsenic and fluoride has been examined to evaluate the quality of drinking water in Biratnagar city of Nepal.

Materials and Methods

Study Area

Biratnagar city lies in the plain area of Morag district of eastern Nepal (Fig. 1). The city is situated in the south-west corner of Morang district at 26°232 N - 26°302 N latitudes and 87°142 - 87°182 E longitudes. Kesalia River borders the west, Tankisinwari Village Development Committee (VDC) in the north, Singhia River in the east and Jogbani, (Araria district of Bihar, India) in the south. Biratnagar is an industrial powerhouse of Nepal with many industries located in and around its suburbs. Biratnagar has traditionally been an agricultural hub and is home to many industries based on agriculture. Biratnagar city comprises 22 wards, and most of the people living in these wards depend on groundwater

sources, i.e., shallow and deep tube wells for domestic purposes, including drinking. According to 2011 census, the population of Biratnagar was 2,04,949.

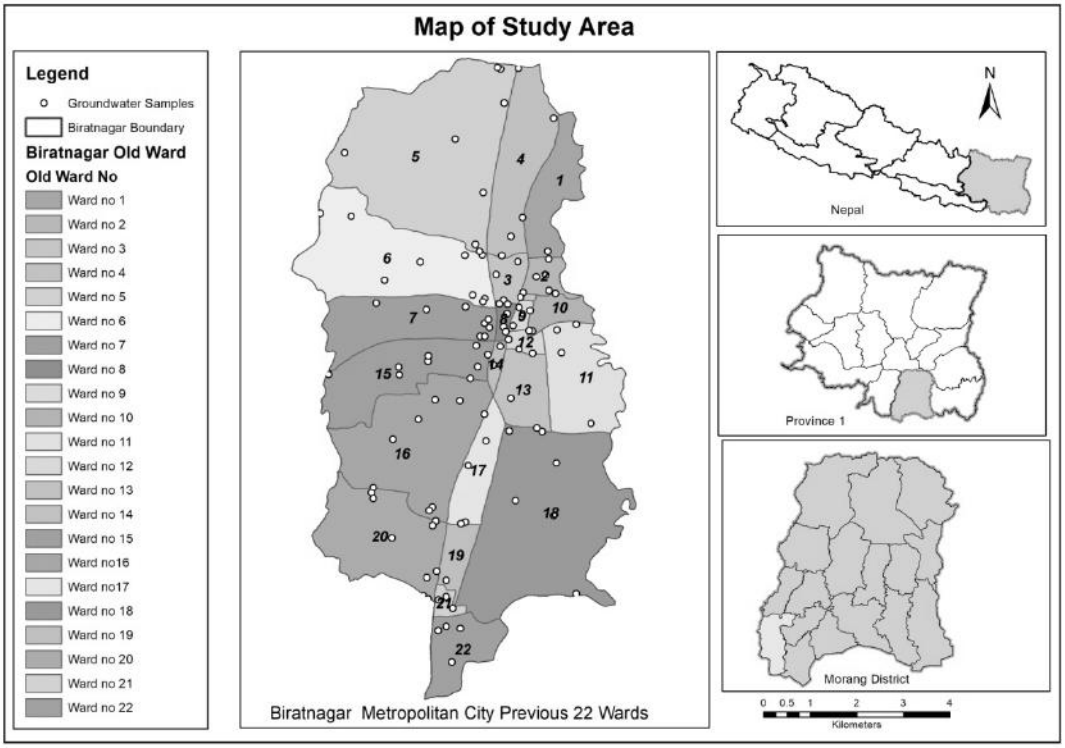

Figure 1. The map of study area (Biratnagar, Nepal) showing sampling sites.

Water Collection

Groundwater samples were collected from 110 representative tube wells (five tube wells from each ward) from the investigated area (22 Wards in Biratnagar city) for 2 years during post-monsoon-2015, pre-monsoon 2016, post-monsoon 2016 and pre-monsoon 2017 (4 seasons). Sample locations were recorded using a Global Positioning System (GPS).

Arsenic Test

The arsenic concentrations were determined on the spot using the HACH Chart Comparator. The reaction vessel was filled with sample to the 50 ml mark. The contents of one of the reagent number 1 and reagent number 2 powder pillows were added to the reaction vessel. Cap was immediately attached with the test strip inserted to the reaction vessel and swirled to mix. The mixture was allowed to react for 20 minutes, and the reaction vessel swirled twice during the reaction period. The test strip was removed, and immediately the developed colour compared with the chart on the strip bottle.

Fluoride Test

Fluoride was determined using the Water Testing Kit (Nice Chemical Pvt. Ltd. Cochin-682024; C.No.W25535). The test tube was filled with 5 ml of water sample. To it, 5 drops of Fluoride

reagent-1(1, 8-Dihydroxy-2-(4 sulfophenylazo) naphthalene-3,6- disulfonic acid) was added and shaken well. The colour that formed was compared with the Fluoride Colour Chart given in the test kit. The value was expressed in mg/L.

The overall analyses were done following Standard Methods (Trivedy & Goel, 1986; APHA, 2005). The results were tabulated and compared to the guide-lines for drinking water give by WHO, BIS (Bureau of Indian Standards) and NDWQS (National Drinking Water Quality Standards- Nepal) limits for drinking water. The depth of the groundwater resources (tube wells) in the present study ranged from 20 to 200 feet.

Results

The samples were regularly analyzed during post-monsoon 2015, pre-monsoon 2016, post-monsoon 2016 and pre-monsoon 2017. All the results are enumerated in table 1 and figure 2.

The value of Arsenic (As) ranged from nil (ward no 3) to 0.17±0.21 mg/L (ward no. 1), from 0.01±0.00 mg/L (ward no. 13) to 0.23±0.25 mg/L (ward no. 1), from nil (ward no. 3) to 0.17±0.21 mg /L (ward no. 1), from 0.01±0.01 mg /L (ward no. 13) to 0.37±0.00 mg/L (ward no. 6) during the period of post-monsoon 2015, pre-monsoon 2016, post-monsoon

Table 1. Post and pre-monsoon variations in Arsenic concentrations and depth of Tube-well water samples at Biratnagar, Nepal (2015-2017).

Ward No.	Arsenic Concentrations (mg/L)				Depth
	Post-monsoon 1015	Pre-monsoon 2016	Post-monsoon 2016	Pre-monsoon 2017	
1	0.17	0.16	0.35	0.23	63
2	0.12	0.17	0.35	0.11	57.8
3	0.00	0.17	0.35	0.02	63.4
4	0.02	0.18	0.36	0.04	50.8
5	0.02	0.18	0.36	0.03	56.2
6	0.04	0.17	0.37	0.04	54
7	0.02	0.18	0.36	0.03	56.6
8	0.03	0.16	0.34	0.08	65.2
9	0.14	0.16	0.36	0.02	69.6
10	0.02	0.17	0.36	0.03	83.4
11	0.02	0.16	0.36	0.02	84.2
12	0.00	0.16	0.36	0.11	54.8
13	0.01	0.16	0.36	0.01	59
14	0.01	0.16	0.38	0.01	56.2
15	0.01	0.16	0.35	0.02	53.6
16	0.01	0.16	0.38	0.02	56.6
17	0.00	0.16	0.36	0.023	61.8
18	0.01	0.17	0.37	0.02	66.6
19	0.02	0.16	0.36	0.02	46.6
20	0.01	0.17	0.36	0.02	59
21	0.01	0.16	0.36	0.01	50.4
22	0.02	0.16	0.36	0.02	53.2

2016 and pre-monsoon 2017, respectively. It showed positive correlation with iron. In the present study, all the groundwater samples analyzed had fluoride below the detection level. So, it has not been described in detailed here.

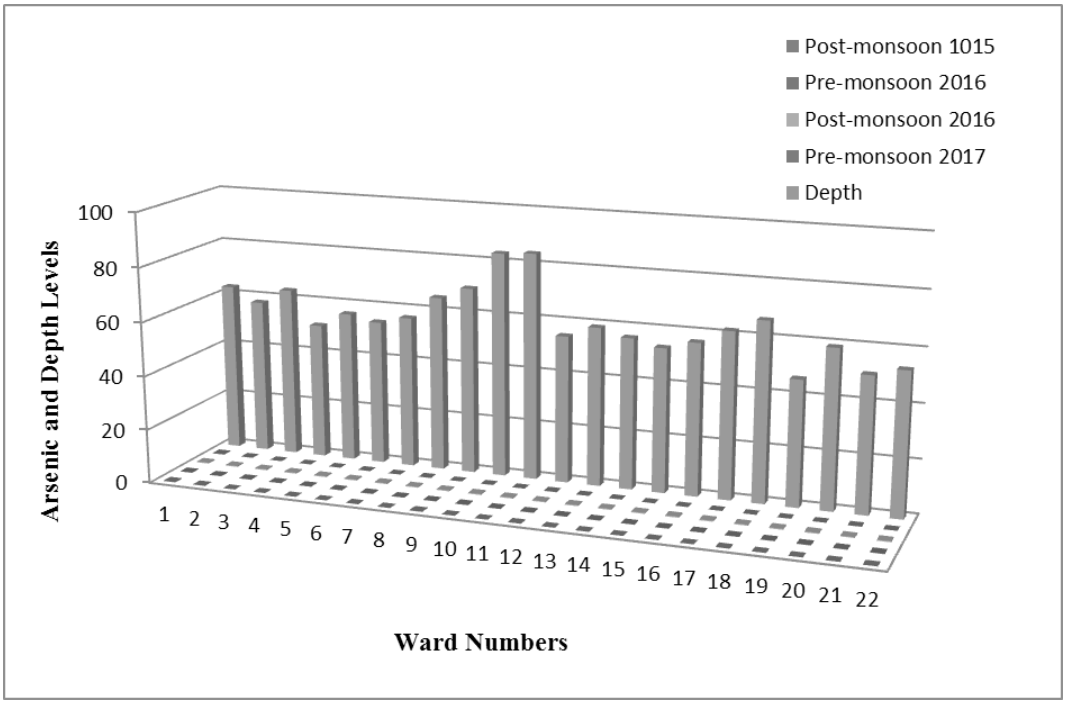

Figure 2. Post and pre-monsoon variations in Arsenic concentrations and Depth of Tube-well water samples at Biratnagar, Nepal (2015-2017)

Discussion

The concentrations of arsenic in groundwater in most of the wards were found very low (below detection limit) in both the pre-monsoon and post-monsoon period. However, arsenic concentrations in 42 water samples (38.18%) during post-monsoon 2015, in 58 water samples (52.72%) during pre-monsoon 2016 (N = 110), in 37 water samples (33.64%) during post-monsoon 2016 and in 59 water samples (53.64%) during pre-monsoon 2017 period exceeded the WHO permissible limit of 0.01 mg/L. As per BIS and NDWQS-Nepal guideline values for As in drinking water, only 10 water samples (9.09%) during post-monsoon 2015, 22 water samples (20%) during pre-monsoon 2016, seven water samples (6.36%) during post-monsoon 2016 and 24 water samples (21.89%) during pre-monsoon 2017 (N = 110) exceeded the prescribed limit. The highest level of arsenic was reported from ward number 3 whereas the least level of arsenic was recorded from Ward Number 3.

The depth of tube-wells in the study area varies from 20 ft to 200 ft. It does not show any relation with the arsenic concentration. Since same amount of arsenic (0.50 mg/L) content has been obtained from the depth of 20 feet as well as 200 feet and also BDL was noticed from the depth at 70 ft. Therefore, study showed insignificant positive correlations between arsenic concentration and depth of groundwater.

Source of arsenic for Chandigarh, West Bangladesh and Terai area of Nepal is Himalayas (Foster et al., 2000) and for Bihar (India) the source should also be the Himalayas (Chakraborti et al., 2003). After randomly taking interviews from some families in representatives' villagers during the field studies, no arsenical skin lesions have been noticed in the study area.

Conclusion

This paper describes the status of arsenic and fluoride pollutants in the study area. During the study higher values of arsenic were recorded from 58 water samples (52.72%) during pre-monsoon and 42 water samples (38.18%) during post-monsoon. It was observed that concentration of arsenic were relatively higher during pre-monsoon period than post-post-monsoon period. It might be due to lesser recharge rate of water table during pre-monsoon period. Based on the results obtained on groundwater (mainly shallow tube wells), it has been concluded that the groundwater sources with elevated levels of arsenic are unsuitable for drinking and other domestic purposes before proper treatment.

The status of fluoride was below the detectable level in all the analyzed groundwater samples. Water containing the F concentration up to 1.0 mg/L is safe. Whereas the F levels in between 1.1 and 2.5 mg/L are marginally contaminated. However, above 2.6 mg/L F level is determined as highly contaminated (Susheela, 1999). It was found that the level of F in groundwater is higher than the surface water as the F percolates from the soil to groundwater through the leaching process. Several factors are responsible for the presence of F in natural groundwater from the soil. Among them, geological factors, consistency of the soil, nature of rocks, pH and temperature of the soil, chelating action of other elements, depth of wells, leakage of shallow groundwater, and chemical and physical characteristics of water (Li et al., 2014).

Acknowledgements

The authors are grateful to the University Department of Botany, T.M. Bhagalpur University, Bhagalpur, Bihar (India), for providing a field kit for arsenic and fluoride examination. The authors are also thankful to the people of Biratnagar for their cheerful cooperation during the research. We are thankful to Mr. Madan Kumar Khadka (Senior Scientific Officer/Under Secretary (Technical), Plant Research Centre, Ilam, Department of Plant Resources, Ministry of Forests and Environment) for generating GIS map of study area.

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Public Perception on Impact of Climate Change on Agrobiodiversity and Livelihood of Rural Communities in Lamjung, Nepal

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Abstract

The study was conducted on Bajhakhet and Simpani VDC of Lamjung district from July to September 2016 to assess the impact of climate change on agro-biodiversity and livelihood. Interview survey, key informant interview, and focus group discussion were conducted in conjunction with long-term meteorological data. One hundred twenty households were sampled out of 1538 households (i.e., 60 from each VDC) with random sampling for the interviews survey. The result showed that Bahun/Chettri dominates the Simpani VDC with 820 households whereas the Bajhakhet VDC with 718 households is dominated by Gurung. Temperature and precipitation data analysis between 1987 and 2015 showed some changes. The maximum mean temperature was increasing at 0.057°C/year, and the minimum mean temperature was increasing at 0.0075°C/decade. Total rainfall was decreasing by 25.22 mm/year. Fifty percent of respondents believed that rainfall amount was decreasing in recent past with direct impacts on agricultural production and water resources. Changing climate of the area is showing effect on the flowering time of different species, including wheat, rice, maize, etc. and so were the germination, harvesting and maturing times of different crops had changed. All these changes in crop and livestock had influenced the income level of the people in study area. The income from agriculture and livestock had decreased, so people are separating from their traditional occupation, way of life, and seeking alternative professions.

Key word: Agriculture, Crops, Livestock, Rainfall, Temperature

Introduction

The term climate change is often used interchangeably with the term global warming. However, according to the National Academy of Sciences, the phrase 'climate change' is growing in preferred use to 'global warming' because it helps convey the meaning of other terms related to climate change and rising temperatures. The sudden and unexpected change in weather or season is known as climate change (Sapkota, 2008).

According to MoPE (2016), Nepal, the total GHGs emission share is negligible compared to the global community. It is only 0.027% of total global emission irrespective of the population size, however, Nepal is highly vulnerable to climate change impacts. Climate change is a global problem that affects all of us. Nepal's average temperature is rising at the rate of 0.03°C-0.06°C/annum between 1977 and 1994 with a higher rate in the mountains than in low lands (Gurung & Bhandari, 2009).

Nepal is significantly affected by the south-easterly monsoon, which provides most precipitation during the rainy summer months (June to September) (Shrestha & Aryal, 2011). Monsoonal precipitation is the most important climatic element for agriculture and water resources of the country (Malla, 2009).

However, due to Nepal's geological solid diversity, the levels of climate change impacts vary across the country. For example, temperature levels in western and central Nepal are expected to increase faster than in eastern Nepal (Timsina, 2011). Specifically, the observed impacts of climate change in Nepal include temperature increase, erratic rainfall, unpredictable monsoon seasons, increased storms, landslides, and droughts (Gentle & Maraseni, 2012; Devkota et al., 2013; Khanal, 2014). Furthermore, there is an increasing concern about Himalayan glacier melt. It has resulted in glacial lake outburst floods (GLOF), which have proven deadly (Mool et al., 2001; Bajracharya et al., 2007; Shrestha & Aryal, 2011). Warming was much in the Himalayan regions of Nepal, such as the Middle Mountain and high Himalaya. Nepal's temperature is rising by about 0.41°C/decade (SAGUN, 2009).

The concentration of GHGs in the atmosphere determines the temperature on earth. If the concentration of GHGs becomes the low temperature of the earth become less and if increased, it results in raising temperature on earth. GHGs forms layer on the atmosphere which allows entering sunlight and heat on earth through the atmosphere. The earth absorbs some of the sun's heat and reflects some fragment of heat on the atmosphere. If the GHGs layer becomes thin, the heat passes from the atmosphere and the temperature on earth becomes low, otherwise, the GHGs layer obstruct the passing process of heat and again reflect the heat to the earth, which causes increasing temperature on earth. GHGs comprise Carbon dioxide (CO_2), Methane (CH_4), Nitrous Oxide (NO_2), Hydrofloro Carbon (HFCs), Perfloro carbon (PFCs), Sulphur Hexafloride (SF6), these gases are emitting naturally, but human induced activities are accelerating the rate of emission of these gases from different activities (Dahal, 2009).

Anthropogenic greenhouse gas emissions have increased since the pre-industrial era, driven mainly by economic and population growth, and are now higher than ever. This has led to atmospheric concentrations of carbon dioxide, methane and nitrous oxide that are unprecedented in at least the last 800,000 years. Together with those of other anthropogenic drivers, their effects have been detected throughout the climate system and are *extremely likely* to have been the dominant cause of the observed warming since the mid 20th century (IPCC, 2014).

According to the study carried out by Metrological Department of Nepal, there is an increasing phenomenon of melting of glacier and Glacier Lake may cause outburst and increases flooding. According to the report, temperature is increasing by 0.12°C in Himalaya, 0.03°C in Hill, and 0.06°C in Terai annually (Sapkota, 2008).

In parts of Africa, Asia and Central America, yields of wheat and maize could decline by around 20 to 40% as temperature rises by 3 to 4°C, even assuming from level adjustment to higher temperature. With full CO_2 fertilization, the losses would be about half as large. Rice yields would also decline, though less than wheat and maize yields (WDR, 2008).

According to IPCC (2007a), the following are some important factors directly connected to climate change and agricultural productivity:

Average Temperature Increase

An increase in average temperature can 1) lengthen the growing season in regions with a relatively cool spring and fall, 2) adversely affect crops in regions where summer heat already limits production, 3) increase soil evaporation rates, and 4) increase the chances of severe droughts.

Change in Rainfall Amount and Patterns

Changes in rainfall can affect soil erosion rates and soil moisture, both of which are important for crop yields. The IPCC predicts that precipitation will increase in high latitudes and decrease in most subtropical land regions by about 20%. While regional precipitation will vary the number of extreme precipitation events is predicted to increase (IPCC, 2007b).

Rising Atmospheric Concentrations of CO_2

Increasing atmospheric CO_2 levels, driven by emissions from human activities, can act as a fertilizer and enhance the growth of some crops such as wheat, rice and soybeans. CO_2 can be one of a number of limiting factors that, when increased, can enhance crop growth. Other limiting factors include water and nutrient availability. While it is expected that CO_2 fertilization will have a positive impact on some crops, other aspects of climate change (e.g., temperature and precipitation changes) may temper any beneficial CO_2 fertilization effect (IPCC, 2007b).

The research's main aim is to assess the impact of climate change on livelihood of rural communities of Lamjung district, Nepal.

Materials and Methods

The research employed both qualitative and quantitative approach and was based on empirical data, literature and documents. Data was collected through survey and field observation.

Study Area

The research was conducted in Nepal's western hill, which was selected as a study site because inhabitants of this district show great variation in rainfall pattern. Bajakhet and Simpani VDCs of Lamjung district was purposively selected for the study (Fig. 1).

Lamjung district lies in the Gandaki Zone of Western Development Region of Nepal with geological extension from middle hill to northern Himalaya. Lying near the middle of Nepal, Lamjung district, with its 1692 km^2, is one of the agro-biodiversity-rich fragile hill region of Nepal. Lamjung district lies on 28°03'-28°30'. North latitude and the lowest area 385 m and highest 8162 m msl (CBS, 2006).

This study was carried out based on exploratory research design because it focused on investigating the impact of climate change on local communities.

Besides, the study attempted to describe the effects of climate change on agriculture, livestock based on local people's perception and explored findings is described. Thus, this is both descriptive and exploratory.

Data Collection and Analysis

Farmer groups of the selected VDCs were the target population for the study. Most of the farmer of these VDCs involved in the farmer groups was taken in the sampling frame for our study. The list of farmer groups in the district was recorded formally from DADO and also informal list of farmer groups was taken in consideration with the help of local key informants. Altogether 60 respondents from each VDC were selected by applying simple random sampling method. Therefore 120 households were randomly selected for this study.

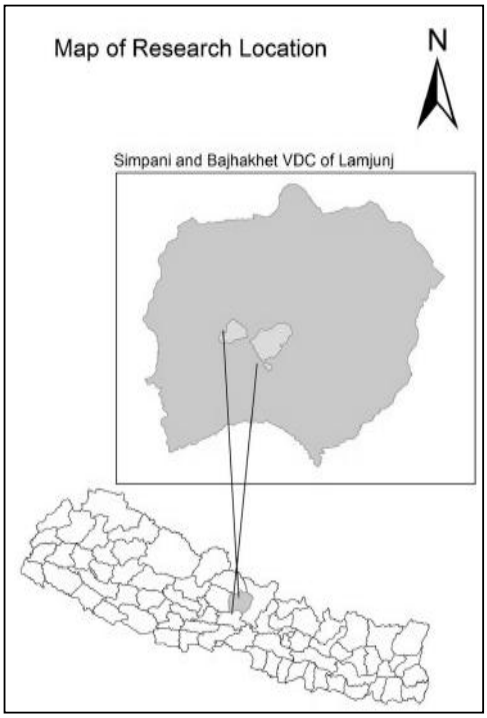

Figure 1. Study area showing Bajakhet and Simpani VDCs of Lamjung district.

Primary information was taken through the household survey, focus group discussion and key informant survey. Secondary data was collected from different reports like Climate Change Project Implementation in Lamjung: A case of Hariyo Ban Project (Sharma, 2015), published and unpublished documents, presentations from individuals, experts and organizations related to environment, and related websites. The study was conducted by taking 30 years' data on crop yield, precipitation, and temperature data.

An interview schedule was designed for primary data collection. Two set of data collection instruments was prepared for the collection of primary data. The first set has included interview schedule, which was prepared to collect information from farmer groups and second set was used to collect the information from subject matter specialists working in the district agriculture development office, Lamjung.

The interview schedule and checklists was pre-tested prior to administering to the actual respondents for checking reliability and validity of the interview schedule. The pre-testing was done by selecting 5 respondents near to the study area. The suggestion given during the pre-testing was incorporated in the final interview schedule.

The field survey was conducted in the month of July 15 to September 15, 2016. The interview time was fixed as per the farmer's convenience. Regular checking and validation was done immediately after filling the interview schedule.

The information collected was coded and sincerely entered in excel. SPSS version 20 was

used in the analysis of the data obtained. After secondary data collection of climate data, analysis was done by using correlation and regression. Regression analysis is widely used for prediction and forecasting. It is also used to understand among the independent variables are related to the dependent variable, and to explore the forms of these relationships. Monthly temperature and monthly rainfall data of nearby station for the periods of 30 years for the district was collected from the Department of Hydrology and Meteorology was taken as independent variables.

Trend analysis explains average annual production, average annual maximum temperature, average annual minimum temperature and average annual rainfall. The temporal variations of temperature and precipitation were analyzed using secular trend of time series analysis with simple linear regression. The least square curve fitting technique was used to fit a linear trend in the data. The linear trend between the time series of temperature or precipitation data (y) and time (t) is given by the equation as $Y = \alpha + \beta t + \varepsilon i$. Where, Y = Temperature or precipitation data at time t (year), α (intercept), i.e., amount of temperature or precipitation data at time zero, β (regression coefficient), i.e., the rate of change of temperature and precipitation over the time t, and εi is a random error.

Results

Trend Analysis of Temperature and Rainfall

The Khudi station data showed that both maximum and minimum temperatures were increasing sharply, with a maximum temperature increase of 0.23°C/decade and mean temperature increase of 0.18°C/decade. Therefore, the Khudi area is warming each year and expected to warm in years to come.

On the trend analysis of the maximum temperature, the maximum temperature increased at the rate of 0.057°C/year (Fig. 2). While looking to the minimum temperature trend analysis, minimum temperature increased at the rate of 0.0075°C/year (Fig. 3).

The 30 years' temperature

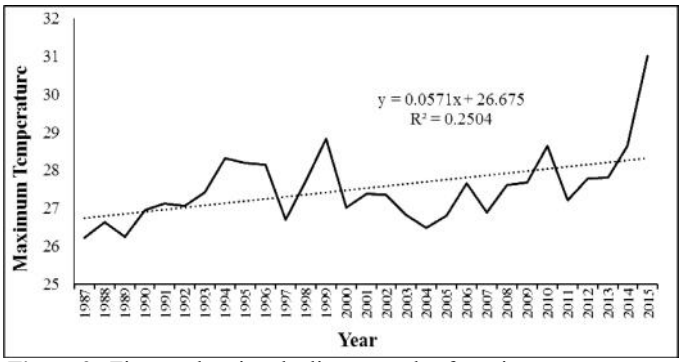

Figure 2. Figures showing the linear trends of maximum temperature trend in the Khudi station. The dotted line is the trend line, while the equation shows the direction and magnitude of the trend.

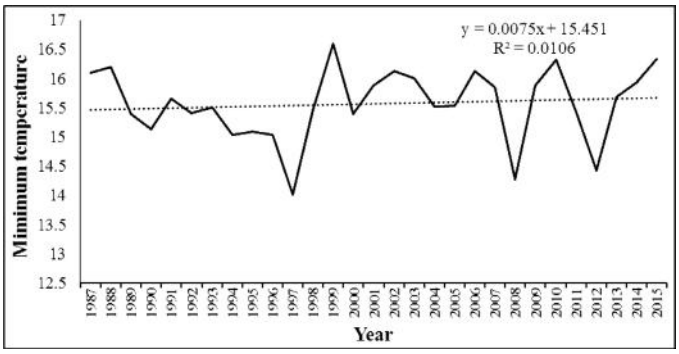

Figure 3. Figures showing the linear trends of minimum temperature trend in the Khudi station. The dotted line is the trend line, while the equation shows the direction and magnitude of the trend.

analysis showed that temperature in the area is in increasing trend. The average maximum temperature was increasing at 0.26°C/decade and minimum temperature increase was 0.81°C/decade, which is greater than national average increase of 0.4°C/decade. Winter temperature increase is alarming, the maximum temperature increases at 0.76°C/decade and minimum temperature increases at rate of 1.17°C/decade which indicates that the western mountain hill of Nepal is warming rapidly. The snow cover area of upper Khudi is decreasing each year, as winter temperature is increasing more, it's sure to increase the rate of melting in coming years Alarming increase in both maximum and minimum temperature both in average and season, especially in winter has great influence on the community's livelihood as it hampers cropping pattern and yield, water availability in the driest pre-monsoon months, chances of spread of diseases etc.

Figures 4 and 5 showed the trend of annual total rainfall for period of 1985 to 2015. Total rainfall decreases at the rate 2.2008 mm/year. There is insignificant increasing trend (10 mm year-1). Higher rate of increasing air temperature and lower rainfall results in greater evapotranspiration, therefore less water available for ground recharge, springs, and rivers. The high fluctuation in the rainfall patterns showed an increasing uncertainty in rainfall. It has great influence on agricultural practice and total production yield which directly influences community's socio-economic state.

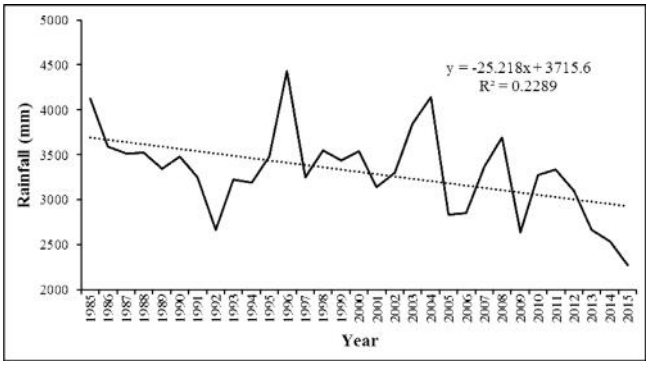

Figure 4. Total rainfall of study area.

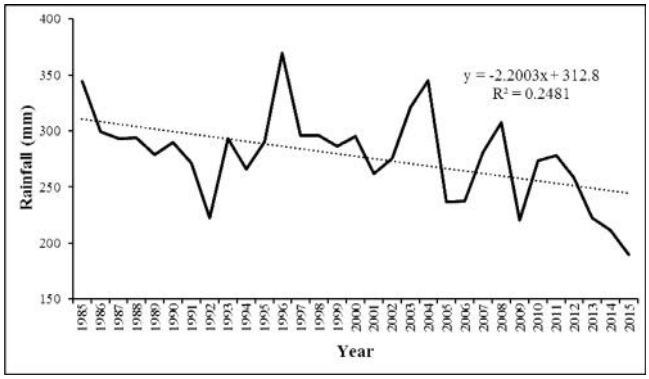

Figure 5. Average rainfall of study area.

People's Perception

About 96.7% respondent living in Bajhakhet felt that temperature has been increased while 90% respondent of Simpani accept the same but 10% said it has not changed (Fig. 6). When respondents were asked about the change pattern of the precipitation in their region, 83.3% of Bajhakhet and 73.3% of Simpani residents said that it is in decreasing trend these

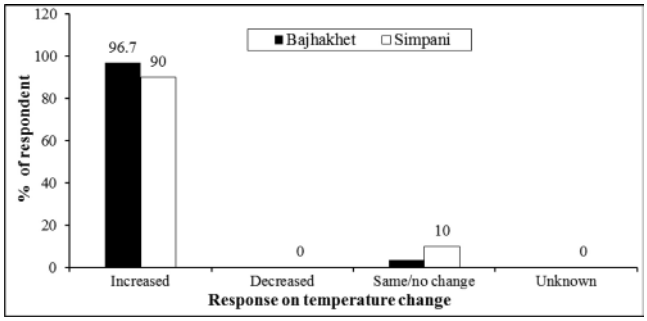

Figure 6. Peoples perception on temperature change

days. Those respondents who are directly related with agricultural practice have noticed rainfall change pattern more in depth and said it is decreasing (Fig. 7). About 85% respondent of Bajhakht and 78% respondent of Simpani felt that snowfall is decreasing while few respondents said it's also increasing (Fig. 8). This type of perception may be due to their short and long term experience in the snowfall pattern because there is very rare snowfall event in their area and they have to see it in upper belt.

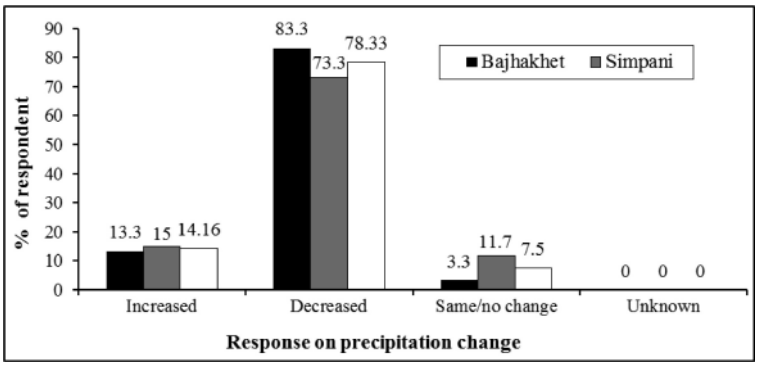

Figure 7. People's perception on precipitation change pattern.

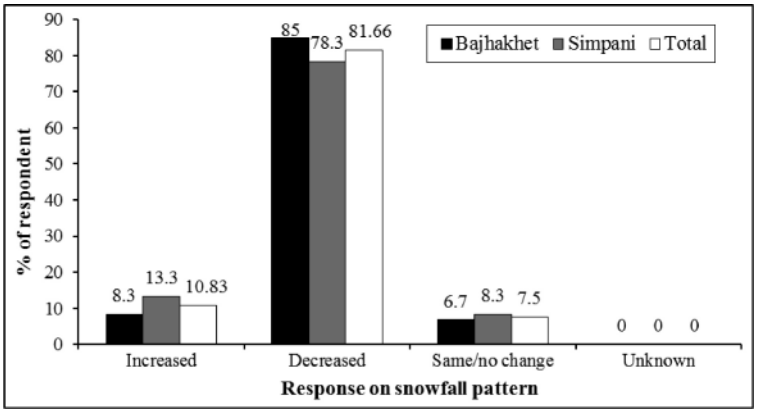

Figure 8. People's perception on snowfall pattern.

Impact of Climate Change on Agriculture

Water resources are decreasing and wetlands are also slowly disappearing. Among the respondents responded, 20.83% says water resources are drying out, 5% says wetlands were disappearing and level of water on ponds and river are decreasing (Table 1). Many of the respondents noticed less rainfall as the main cause of decreasing flow of water in river. Wetland has been lowered resulting to water scarcity for household use and irrigation area turning to arid region.

Table 1. Impact of less rainfall on water resources

S.N.	Effect on water resources	Respondents	
		Number	%
1	Drying water resources	25	20.83
2	Reduction in water level in rivers	20	16.66
3	Loss of wetland	4	3.333
4	Drying water resources, reduction in water level in rivers	40	33.33
5	reduction in water level in rivers, loss of wet lands	6	5
6	Drying water resources, loss of wet land	10	8.33
7	N.A. (Not answered)	15	12.5
	Total	120	100

Source: Field study (2016)

The pattern of production and productivity of crops has been changing day by day. 26.66% of respondents believe on seasonal rainfall causing decrease in production of crops, while 13.33% replied that difficult to cultivate (Table 2).

Table 2. Effects of rainfall on agriculture system.

S.N.	Effect of less rainfall	Respondents	
		Number	%
1	Rise in production	0	0
2	Decrease in production of crops	32	26.66
3	No change on production	8	6.66
4	Difficult to cultivate	16	13.33
5	Other	5	4.16
6	Decrease in production of crops, difficult to cultivate	42	35
7	Decrease in production of crops, difficult to cultivate and others	3	2.5
8	Difficult to cultivate, others	5	4.16
9	Rise in Production, others	2	1.66
10	N.A.	7	5.83
	Total	120	100

Source: Field study (2016)

Note: Others include, less production, threatening food security and causing famine, xerophytes species (species available in arid places) is being cultivated, no irrigation facilities)

Effect of Unseasonal Rainfall on Agriculture

Irregular and unseasonal rainfall has been increasing from last few years, which is affecting cultivation of crops. Among total respondents 7% replied they are facing problem on cultivation of crops while 41% of respondents replied irregularities on production of crops (Table 3).

Table 3. Effect of irregular rainfall on agriculture

S.N.	Effects of irregular rainfall	Respondents	
		Number	%
1	Effect on cultivation	7	5.83
2	Drawn of crops	0	0
3	Flooding	27	22.5
4	Irregularities on production of crops	41	34.16
5	Others	4	3.33
6	Effect on cultivation, irregularities on production of crops	19	15.83
7	Effect on cultivation, flooding and irregularities on production of crops	12	10
8	N/A	10	8.33
	Total	120	100

Source: Field study (2016)

Note: Other includes, decreasing production due to unseasonal rainfall, growth of crops is limited, Cultivation of crops is being impossible of irregular rainfall, more input low output

Effect of Rise of Temperature on Agriculture

Table 4 showed slightly more than 21.66% of the respondents of the study sites indicated wide spread diseases/insects due to increase in temperature and less rainfall, while the other 24.16% felt decreased the maturation period of crops and 3.33% of the respondents felt shortening the time of germinating seed.

Table 4. Effect of increasing temperature on crops

S.N.	Effect of increasing temperature on crops	Respondents	
		Number	%
1	Decreasing the maturation period of crops (A)	29	24.16
2	Decreasing the time period of germinating seed (B)	4	3.33
3	Wide spread of insect/diseases (C)	26	21.66
4	Others (D)	3	2.5
5	Decreasing the maturation period of crops, decreasing the time period of germinating seed	9	7.5
6	Decreasing the maturation period of crops, wide spread of insect/diseases	11	9.16
7	Decreasing the time period of germinating seed, wide spread of insect/diseases	7	5.83
8	Wide spread of insect/diseases, Others	4	3.33
9	Decreasing the maturation period of crops, decreasing the time period of germinating seed and wide spread of insect/ diseases	19	15.83333
10	N.A.	8	6.66
	Total	120	100

Source: Field study (2016)

Note: Others includes, irregularities in flowering of plants species, shedding time of plant is changed, change in harvesting time of crops and Increasing different pests and insects.

Impact of Climate Change on Agro-biodiversity

The key informants reported that forests, lakes, grass land, wetland and agricultural ecosystems are continuously degrading. Grasslands have been converted to barren wasteland due to less rainfall and drought. Many key crops species were lost in recent years. Many species of paddy like, *Achhame, Ghaiya Aanga, Khumal-4, Chhate, Kathe* and other crops like millet, bean species have disappeared. Likewise, local varieties of vegetable crops like *Local-Rayo*, *Cucumber, pumpkins, chilies,* etc., are not found these days because their seeds are replaced by improved and exotic varieties.

Spread of Invasive Species

Change in temperature, and rainfall pattern are creating favorable environment for pests, diseases and invasive species to emerge, spread and encroach in agriculture and forest lands. Respondents have already experienced the emergence of species that they have never seen in their field area. Invasive species like Banmara (*Lantana species*), Gande Jhar (*Ageratum conyzoides*), Aaloo Jhar (*Galinsoga parviflora*), Badame Jhar (*Parochetus communis*), Kande Jhar (*Amaranthus spinosus)*, and Titepati (*Artemisia vulgaris*) are evident in the study area.

Impact of Climate Change on Livestock

The scarcity of fodder and space for livestock rearing community is forcing the farmer to change their livestock pattern. Most of the people who have changed their livestock which needed less fodder, grass the study shows that majority of the people (40%) have changed their livestock, where and 35% said they haven't changed their livestock pattern (Fig. 9).

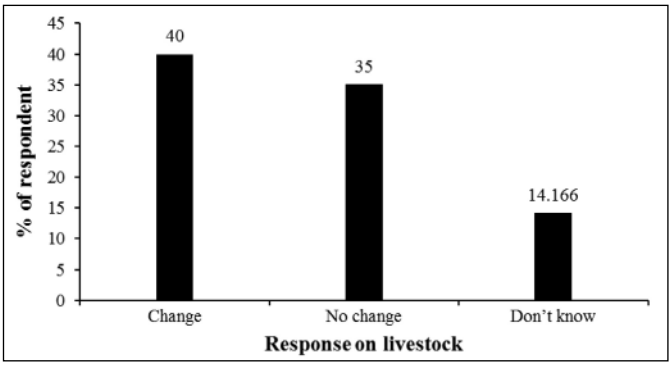

Figure 9. Change in livestock pattern (Source: Field study, 2016)

Due to scarcity of resources for livestock, change in livestock pattern has been observed. According to respondents' dairy products have decreased significantly, while meat product is increased due to the poultry farming (Fig. 10).

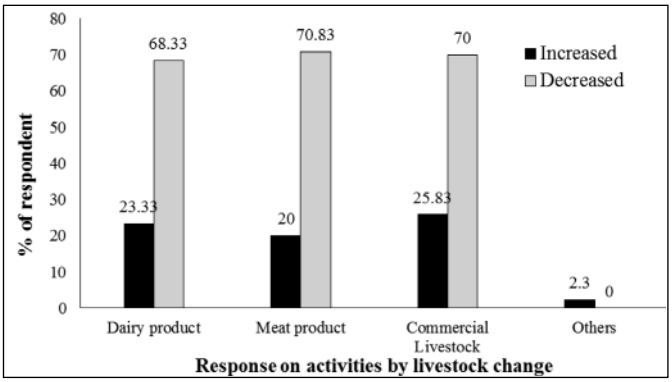

Figure 10. Effects on different activities by change in livestock pattern. (Source: Field study, 2016)

Impact of Climate Change on Overall Economy

Figure 11 showed that many respondents (49%) have said their income level are decreased, another 28% of the respondents said their income is increasing and other 21% of the respondents replied that their income is constant.

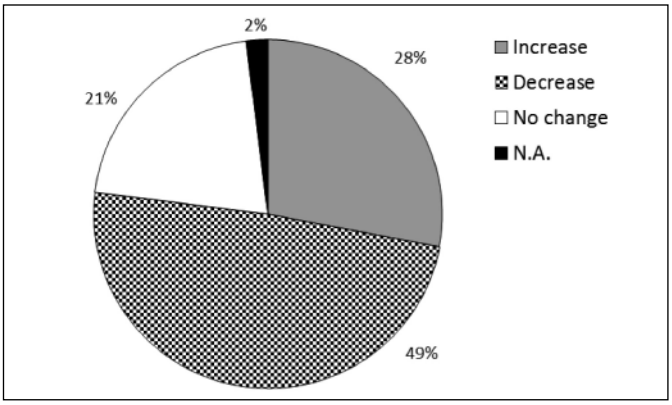

Figure 11. Overall income pattern. (Source: Field study, 2016)

Discussion

The analysis indicates continuously rising temperate, low volume of rainfall, and inconsistency in the local people's livelihood. Rainfall in this area shown by empirical data matches the farmers' perception from the lowland Rupandehi district (Manandhar et al., 2011). They have also interpreted these events as a result of climate change. In recent year rainfall pattern is recorded fluctuated, altered,

delayed monsoon, erratic and abnormal duration of rainfall etc. Winter season rainfall is also decreased and many activities of this season became uncertain. On the other hand, natural calamities like hailstone are occurring unpredictably and seem to have increased in recent days. Temperature rising pattern is also an unpredictable and strange matter. The plants' lifecycle behavior regarding flowering, shedding and germination of seed are shortening, maturation time of fruits and crops also indicates the pattern of increasing temperature. The production and productivity of agricultural product have been decreasing because of less rainfall. This is similar to the experiences that the Tharu indigenous community in Western Terai region has also experience especially in regards to agriculture loss (Manandhar et al., 2011). Scarcity of water for irrigation has been started so the communities are being unable to cultivate in time. Damp and Swampy areas and farm land are disappearing day by day. The availability of grass on forest and field is also in decreasing pattern, scarcity of grass and fodder is increasing, the pasture land has been converted to arid desert and due to the scarcity of water and fodder, it is resulting in decreased number of livestock. Because of the lack of adequate fodder and difficulty to manage it, livestock pattern has started to change. Ultimately, our results coincide and support other studies from Nepal. Chalise et al. (2015) showed in a survey of peasant farmers throughout the Central Region that the majority perceived a change in temperature and rainfall and had adapted by changing farming practices, cultural practices, or occupation.

Conclusion

Rural communities are experiencing increased temperature and changing pattern of rainfall. Rural communities are experiencing changing time of cultivation of crops and changing behavior of animal. The production and productivity of the food grain as well as cash crops has been decreasing every year. Due to the low productivity threat to food security is rising as a big issue. Income level is decreasing because of reduction on production of cash crops. Cultivation of crops is being difficult because of scarcity of water for irrigation. Area for livestock rearing, getting grass and fodder is reduced because of drought. Due to less availability of fodder livestock pattern has been changed, resulting reduction on milk and milk product. Agro-biodiversity also has been affected from the changing climate. Different crop species have disappeared and lost behavior of plant and animal is changing. Germination, flowering, maturation and shedding time of plants species have been changing. Different species of herbs and weeds (Aaloo Jhar) have been spreading on farm land and forest. Different species of beans, rice, millet and barley have disappeared already.

Acknowledgements

I would like to thanks the community of Bajhakhet and Simpani VDCs of Lamjung district for the willingness to share their information and incredible hospitality. A special thank goes to the women's group who provided me valuable insight and perspective. Without them, this work would not have been possible. Additionally, I would like to acknowledge Prof. Dr. Kalyani Mishra Tripathi, Prof. Dr. Indira Bhattarai, Mr. Shiva Chandra Dhakal, and Mr. Ananta Prasad Subedi for their continuous support, guidance, and feedback throughout this research. At last, I would like to thank to my family who is the source of inspiration during this study.

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Optimization of Cultural Media for Mycelia Growth of *Termitomyces robustus* (Beeli) Heim

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Abstract

The study was carried out for *in vitro* cultural characteristics, to determine the effects of various source of carbon, nitrogen, amino acid, vitamins and carbon/nitrogen ratios on mycelial growth to *Termitomyces robustus*. They were inoculated in culture tube from Pathari Forest, Eastern Nepal for the process using semi solid and liquid media. Data were used to showing the significant test. One-way ANOVA followed by Tukey HSD test were performed to compare the result of different treatments. Out of six carbon sources, the best growth was observed in maltose with somewhat compact mycelia density and lactose showed the least ($p<0.05$). While fresh and dry weight in liquid medium did not show significant. The best growth in both medium was seen in organic nitrogen as yeast extract with compact mycelia density. Serine provides to be the best amino acid having somewhat thick mycelia density and Aspartic acid medium showed least with thin mycelia density. Out of five vitamins tested, thiamine showed the best and in the folic acid, the least growth having somewhat thin mycelia density, in both semisolid and submerse medium. The combination of C: N ratio, the best growth was in 5:1 and least in 1:1, while in liquid medium, maximum fresh weight was observed in 5:1 and least in 1:1 where as in dry weight, maximum growth was observed in 4:1 and least in 1:1.

Key words: Biological efficiency, *In vitro* and *in vivo* culture, Lignocellulosic substrate

Introduction

In vitro studies on fungi have helped indirectly a great deal in understanding various phenomenon of Molecular biology (Heitefuss, 1966). Physiological genetics (Esser & Kuenen, 1967) morphogenesis (Barnett, 1968) and biophysics (Ingold, 1971). Termitophilous fungi are a monophyletic group of tropical gilled mushrooms with a single genus *Termitomyces.* They are unique, obligatory symbionts, grows in close and intimate association with termites. The contain higher dry matter, protein and fiber, but contain lower amount of fat and carbohydrates. They are rich source of important minerals, like phosphorus, potassium, calcium, copper, manganese, zinc, magnesium, sodium and iron. Besides nutrition, the species possess high medicinal value (Aryal, 2015). It is a good source of bioactive compounds (Aryal & Budhathoki, 2013). Due to presence of high concentration of diverse phytochemicals it is used in drug development (Aryal & Budhathoki, 2014).

Growth of fungi is measured in terms of changes in number of cells, in linear dimensions, in cell mass, in cell volume or in amount of some cellular component (Bilgrami & Verma, 1981). Various environmental and biochemical factors, affects growth; among them nutrients have significant influence on mycelial growth (Hawker, 1957; Bilgrami & Verma, 1981;

Kaul, 1999). Fungi received their food either parasitically or saprophytically from the host substrate. Nutritional requirement for growth and reproduction are carbon, nitrogen, sulphur, phosphorus, vitamin and certain metallic or trace elements sources (Kaul, 1999).

The mycelium accumulates food materials and synthesizes complex substances of proteins, polysaccharides, fats and enzymes (Hawker, 1957). Thus, to achieve maximum yield, optimum culture medium containing the nutrients in suitable amounts and combination should be formulated. Cultural characteristics optimization procedure aims at developing such a medium.

Nutritional components of the medium can be varied and the impact of these changes assessed in terms of fungal growth. All of these factors must be considered during optimization. In the production medium, the nutritional components of the defined medium are replaced with low-cost, complex substrates at less time. Use of this in the production media for specific nutritional information will be useful in developing production media for commercial mycelial production. They can be used as a source of nutrition and valuable products (Jackson, 1997). Thus, an optimization condition for mycelial growth of *Termitomyces robustus* was investigated.

Materials and Methods

Study Area

The survey of various localities of Pathari Forest, Morang, East Nepal was conducted for the collection of materials June-November, 2019, in order to explore the domestication aspect and was inoculated in culture plates and brought to the laboratory, Central Department of Botany, T.U. for the process. The coordinate range between 26°35'08"N-26°73'32"N and 87°30'02"E-87°59'27"E and attitude 190-220 m msl (Fig. 1).

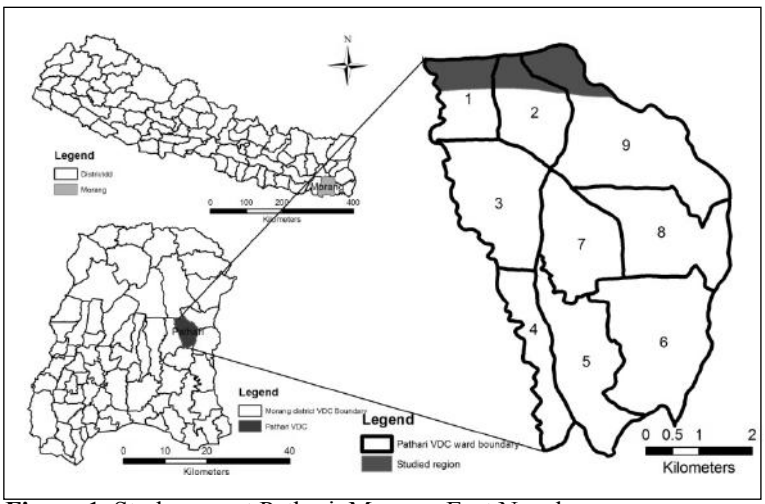

Figure 1. Study area at Pathari, Morang, East Nepal.

Laboratory Studies

The specimens were subjected to culture process using semi solid medium and liquid media (Shim et al*.,* 2005a), to observe the effects of various carbon, nitrogen, vitamins, amino acids and its Carbon/Nitrogen ratio sources on mycelial growth, under the pH 6 and the temperature 25±2°C. Measurements of linear growths were done by means of a standard scale. Weighing of mycelial mass growth was done by a sophisticated digital chemical balance.

Sterilization of instruments

Glass wares were wrapped in aluminum foil after cleaning with water and sterilized in the hot air oven at 160-170°C for 2 h. The media, cotton were sterilized in an autoclave, at 121°C and 15 psi pressure for about 30 min.

Isolation on PDA media

Fresh and healthy sample was surface sterilized by submerging in 0.4% sodium hypochlorite for 1 min and was washed with sterilized water to remove residual NaOCl. Using a sterilized blade, its pileus and stipe were separated. The stipe was split longitudinally into two equal halves and approximately 3×6 mm pieces of tissue were taken from depth of 1/4th thickness of the upper end of the stipe so that they contained neither outermost portion nor the central tissues of the stipe. That tissue pieces were inoculated in the PDA plates. The plates were sealed with parafilm tape and were covered with the help of aluminum foil.

The inoculated petriplates were incubated at 25±2°C in inverted position for seven days (Shim et al*.,* 2005a,b). Mycelial growth, diameter and texture of the colony were noted down. Then mycelium was observed to grow out of the inoculated tissue. Sub-cultures of the mycelium were done to obtain pure cultures. Then it was transferred to PDA/PD slants for further process.

Media preparation

Vegetative growths of mycelium of the species were done experimentally by adopting the standard procedures (Chandra & Purkayastha, 1977; Fasidi & Olorunmaiye, 1994; Shim et al*.,* 2005a) by which media optimized. Cultural characteristics was investigated, where composed of four important sources viz. carbon (Dextrose, Lactose, Sucrose, Fructose, Mannitol, Maltose and Control), nitrogen (Ammonium Nitrate, Peptone, Urea, Sodium Nitrate, Calcium Nitrate, Yeast extract and Control), vitamins (Ascorbic acid, Nicotinic acid, Folic Acid, Thiamine, D-priotine and Control), amino acids (Serine, Leusine, Valine, Glutamic acid, Arginine, Aspartic acid and Control), including C:N ratios and applied under five treatments in each experiment, with their five replication.

Statistical Analysis

Standard error (SE) of means significance test was used SPSS 20.0 version. One Way Analysis of Variance (ANOVA) followed by multiple range test, post-hoc and Tukey HSD Test were performed to compare the results of different treatments.

Results

The different nutrient sources were used and their effects on growth are listed below:

Effects of Carbon Sources

Linear mycelial growth varied significantly with change in sources at 5% level of significance; it showed differential preferences for carbon sources for its metabolism ($df = 6,28$; $F = 21.86$; $P=<0.001$). However, utilization varied from one carbon source to another. The best

linear growth was observed in maltose with somewhat compact mycelial density while the least growth was observed on lactose with compact mycelial density ($p<0.05$). However, in control medium, mycelial growth was better than in lactose. Dextrose, fructose and sucrose also stimulated good growth with somewhat compact mycelial density and better growth than control (Fig. 2). While both fresh weight ($df=6,28$; $F=1.77$; $P=0.14$) and dry weight ($df=6,28$; $F=0.655$; $P=0.68$) of mycelium in submerged did not vary significantly with change in carbon sources at 5% level of significance. It might be due to the agitation, lack of orbital incubation or continuation back up.

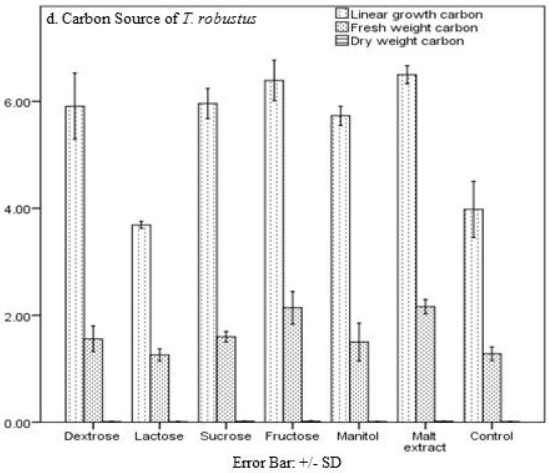

Figure 2. Mycelial growth on different carbon sources; One-way ANOVA, Tukey HSD Test ($p<0.05$), multiple range and mean test (indicate the different carbon source in the graph).

Effects of Nitrogen Sources

Linear growth varied significantly with change in sources at 5% level of significance ($df=6,28$; $F=116.78$; $P<0.001$). The best linear growth was seen in yeast extract with compact mycelium density ($p<0.05$) while, in urea minimum growth with compact mycelial density (Fig. 3) was observed. Similarly, both fresh weight ($df=6, 28$; $F=1.77$; $P=0.14$) and dry weight ($df=6, 28$; $F=0.655$; $P=0.68$) of mycelium in submerged did not vary significantly with change in nitrogen sources at 5% level of significance. The maximum fresh weight and dry weight was observed in yeast extract while minimum in urea. However, in control of mycelia had better growth than urea also showed optimum growth with somewhat compact mycelial density.

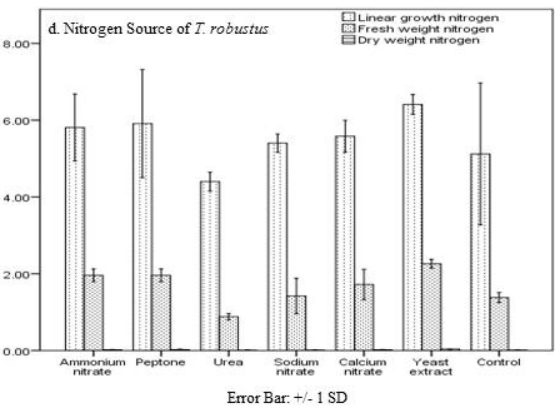

Figure 3. Mycelial growths on different nitrogen sources; One-way ANOVA, Tukey HSD Test ($p<0.05$), multiple range and mean test (indicate the different nitrogen source in the graph).

Effects of Vitamins

Linear growth was varied significantly with change in sources at 5% level of significance ($df=5, 24$; $F=27.34$; $P<0.001$). Out of five vitamins tested, liner growth was found in thiamine and least in folic acid ($p<0.05$). Control with somewhat compact mycelial density, ascorbic acid and D-priotine with somewhat thin mycelial density showed the optimum growth

(Fig. 4). However, fresh weight (df=5, 24; F=14.22; P<0.001) of mycelium in liquid medium also varied significantly with change in vitamin sources at 5% level of significance. Maximum weight was observed in D-priotine while minimum of ascorbic acid and dry weight (df=5, 24; F=2.56; P=0.054) of mycelium in submerged almost significant with change in vitamin sources at 5% level of significance.

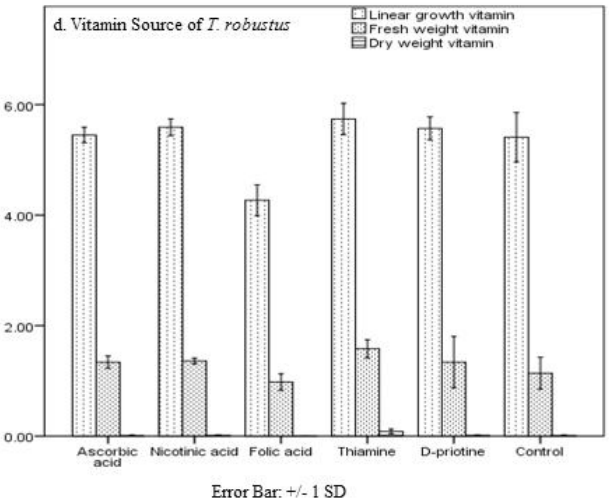

Figure 4. Mycelial growths on different vitamins sources; One-way ANOVA, Tukey HSD Test (p<0.05), multiple range and mean test (indicate the different vitamin source in the graph).

Effects of Amino Acids

Linear growth was varied significantly with change in sources at 5% level of significance (df=6, 28; F=49.33; P<0.001). Among them, glutamic acid proved to stimulate the maximum linear growth with somewhat thin mycelial density while control showed the least growth with thin mycelial density (p<0.05) (Fig. 5). The control treatment showed intermediate growth with somewhat thick mycelial density. Leusine, valine, aspartic acid and serine was also observed with somewhat thin mycelial density showed the medium growth. However, fresh weight (df=6, 28; F=1.36; P= 0.26) and dry weight (df=6, 28; F=1.45; P=0.23) of mycelium in submerged did not vary significantly with change in amino acid sources at 5% level of significance.

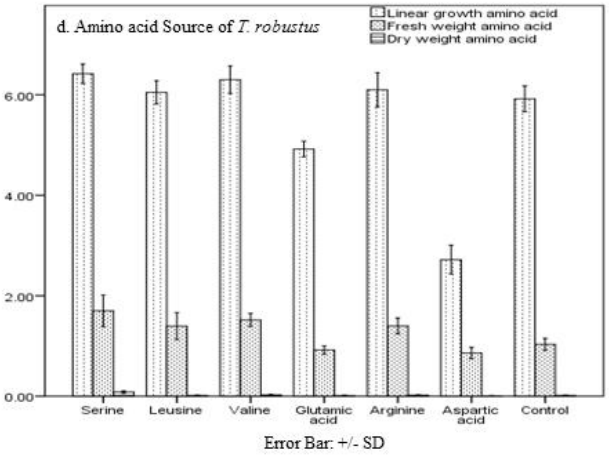

Figure 5. Mycelial growths on different amino acid sources; One-way ANOVA, Tukey HSD Test (p<0.05), multiple range and mean test (indicate the different carbon source in the graph).

Effects of (C: N) Ratios

Linear growths, did not vary significantly with change in sources at 5% level of significance (df=9, 40; F=30.99; P<0.001). The combination of carbon and nitrogen in various proportions showed that the growth rate increases with the proportional increase in carbon up to C: N ratio of 5:1 (p<0.05). Control and C: N ratio 1:1 showed the least growth with somewhat compact and thin mycelial density respectively. In the same manner, when the ratio of

nitrogen was increased, growth rate also increased gradually up to the C: N ratio of 1:4. So, the best growth observed was when the C: N was 1:4 and 1:5 respectively (Fig. 6). However, better growth was observed in control than in 1:1. However, both fresh weight (df=9, 40; F=2.40; P=0.028) and dry weights (df=9, 40; F=15.13; P<0.001) of mycelium in submerged were varied significantly with change in nitrogen sources at 5% level of significance. Maximum fresh weight was observed in C_5: N_1 while minimum in C_1: N_1. However, of control was observed better than in 1:1, 1:2 and 1:5 while equal with 2;1 and 3:1. Similarly maximum dry weight was observed in C_4: N_1 and minimum in C_1: N_1. However, of control was observed better than in 1:1, 2:1, 3:1, 1:2 and 1: 5 while equal with 5:1, 1:3 and 1:4.

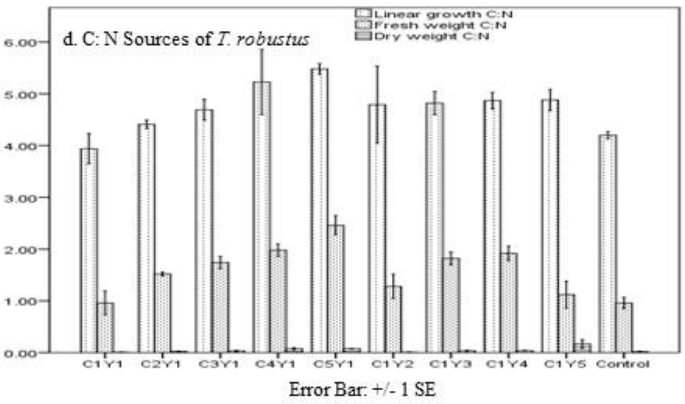

Figure 6. Mycelial growths on different C: N ratio sources; One-way ANOVA, Tukey HSD Test ($p<0.05$), multiple range and mean test (indicate the different carbon to nitrogen ratio source in the graph)

Discussion

Study on the Mycelial Growth

To assess the effect of different nutritional factors Subba (1975) obtained the optimal mycelial growth at 26°C and in the pH 6.5. According to him among the carbon sources, sucrose was the best and lactose supported poor growth. Similarly, among the nitrogen, ammonium nitrogen and aspartic acid were found to be the best and nitrate nitrogen were found to stimulate moderate growth, on *Choanephora infundibulifera* (Curry) D.D. Cunn. For the growth and reproduction of most fungi, the culture media must contain sources for carbon, nitrogen, sulphur, phosphorus, vitamins and certain trace or metallic elements (Kaul, 1999). Termitophilous mushrooms possess capability to produce lignocellulosic enzymes with potential to be efficient degrader of agro-wastes. Taprab et al. (2005) postulated that symbiotic fungi, viz., *Termitomyces* strain produces laccases which are potentially involved in fungus combs and facilitate mushroom growth. Hence, various nutrients were compared to identify their efficiency as the source of these elements for the mycelial growth of *T. robustus*. Poudel (2012) reported that *Volvariella taylorii* (Berk. & Broome) Singer, an edible mushroom of Nepal, was best at 25±2°C in maltose and sucrose, of carbon, yeast extract and peptone, of nitrogen, serine of vitamin, nicotinic acid of amino acid and C_5:N_1, C_1:N_4, and C_1:N_5 of carbon to nitrogen ratio were the best growth. Similarly, Acharya (2012) in his experiment "A study on mycelial growth pattern of *Amanita chepangiana*, a wild edible Mushroom, on Different Nutrient Sources" reported that malt extract of carbon supported the optimum growth at 22±2°C. Similarly, in nitrogen, sodium nitrate and in vitamins

ascorbic acid; in amino acid, arginine and in carbon to nitrogen ratio C_4:N_1 were the optimum growth.

Carbon sources

Carbon compounds serve two essential functions in fungal metabolism. The first function is to supply the carbon needed for the synthesis of compounds which comprise the living cell. Proteins, nucleic acids, reserve foods, etc., Second, the oxidation of carbon compounds produces appreciable amounts of energy. Fungi can utilize a wide range of carbon sources. *T. robustus* showed different preference for its metabolism. However, utilization varied from one carbon sources to another. Maltose and manitol were observed to be the most effective while lactose the least for growth (Fig. 2). According to Cochrane (1958) the ability of an organism to utilize carbohydrate depends on type of enzymes produced by the organism. This result showed that, this species produces enzymes that utilize maltose and fructose better than any other carbon sources. Shim et al. (2005b) who reported this result is in line with the work of that maltose was screened suitable for mycelial growth while lactose was the least for *Cystoderma amianthinum* var. *ruigosoreticulatum* (F. Lorinser) Bon. According to Manjunathan and Kaviyarasan (2011), for *Lentinus tuber-regium* (Fr.) Fr. dextrose was most effective while lactose was the least. Ayodele (2008) found that, in *Psathyrella atroumbonata* Pegler; glucose was the best which was followed by maltose, starch and mannitol in decreasing order. The least growth was observed in lactose and control. Chandra and Purkayastha (1977) reported that most of the tropical edible macrofungi were in favor of utilizing glucose than other carbon sources. Jayasinghe et al*.,* (2008) found that dextrose was the best on *Ganoderma lucidum* (Curtis) P. Karst. This was closely followed by galactose and fructose which were not considerably different from each other. Jonathan et al. (2006) found that in *Pleurotus florida* Singer, the most supportive sugars were among the monosaccharides. Aldohexose (glucose) stimulated greater biomass yield than ketohexose (fructose) under the same conditions. Generally, complex sugar and sugar alcohols produced little biomass with the exception of dextrin and mannitol. He attributed the little amount of mycelial production with polysaccharides and sugar alcohols to their complex nature. Since hydrolytic enzymes would be required to convert polysaccharides and sugar alcohols to simple sugar before they will enter respiratory pathways. Mannitol (a sugar alcohol) also supported good biomass yield of *P. florida.* The best carbon source suitable for promoting mycelial growth of *Lignosus rhinoceros* (Cooke) Ryvarden was glucose but with somewhat compact mycelial density according to Lai et al. (2011). Additionally, fructose and mannose also recorded a high radial mycelial growth rate. The combination of these three carbon sources indicated that *L. rhinoceros* preferred monosaccharaides. Imtiaj et al. (2008) found that, in *Schizophyllum commune* Fr., the suitable mycelial growth was found in dextrin and fructose; however, the lowest growth of mycelium was obtained in lactose, mannose and sorbitol.

Nitrogen sources

Nitrogen occurs in cells as a constituent of proteins which occur as enzymes as well as structural polymers, and of the purines and pyrimidines which are constituents of nucleic

acids and some growth factors. Within the cell, the basic units of nitrogen metabolism are amino acids, which may be obtained from the environment as such or formed from inorganic nitrogen. Fungi exhibit considerable versatility with regard to utilization of nitrogen sources. Among the organic and inorganic nitrogen sources, this species utilized organic nitrogen better than inorganic nitrogen. The best growth was found in yeast extract (Fig. 3). This finding was similar to that of Ayodele (2008), who reported that *Psathyrella atroumbonata* Pegler showed preference for organic nitrogen than inorganic nitrogen and the best yield was in yeast extract while the least was found in sodium nitrate and ammonium nitrate. Manjunathan and Kaviyarasan (2011) also found that *Lentinus tuber-regium* (Fr.) Fr. utilized organic nitrogen better than inorganic nitrogen, yeast extract being the best for its mycelial growth in the same line. They suggested that the stimulatory effect of yeast extract in their study may have been due to its amino acids, protein and vitamins. According to Adebayo et al. (2011), comparatively organic nitrogen supported optimum production of mycelial by *Pleurotus ostreatus* (Jacq.) P. Kumm. when inorganic nitrogen was used poor biomass growth was reported. Urea had been recorded as the best nitrogen source for biomass production by *P. ostreatus*. Jayasinghe et al. (2008) reported that *Ganoderma lucidum* (Curtis) P. Karst. showed optimum mycelial growth on ammonium acetate, glycine, arginine and calcium nitrate. Jayasinghe et al. (2008) observed that inorganic nitrogen sources also enhanced the mycelial growth of *G. lucidium*. Jonathan et al. (2006) found that in *Pleurotus florida* (Jacq.) Singer, inorganic compounds supported moderate biomass production. The best biomass yield was found with ammonium nitrate closely followed by potassium nitrate. Among the complex nitrogen compounds, yeast extract and casein hydrolysate supported significant biomass. Lai et al. (2011) observed that the best nitrogen source was potassium nitrate for promoting mycelial growth of *Lignosus rhinoceros* (Cooke) Ryvarden. Imtiaj et al. (2008) found that the most suitable nitrogen source were calcium nitrate, potassium nitrate, and alanine, and the most unsuitable were ammonium phosphate, histidine, urea and arginine for growth of *Schizophyllum commune* Fr., Shim et al. (2005a) found that the best nitrogen source was glycine for *Macrolepiota procera* (Scop.) Singer. However, in this study, urea, despite being an organic nitrogen source, showed the least support for the mycelial growth of the mushroom under study.

Vitamins

All living organisms require vitamins and other growth factors though in minute quantity. Some fungi are able to synthesize most of the vitamins required by them. Thiamine was the best, among the different vitamin sources used. Control treatment with somewhat compact, ascorbic acid with somewhat thin mycelial density and folic acid with somewhat thin mycelial density showed the least growth (Fig. 4). This finding was in line with the report of Ayodele (2008), who proved that thiamine was the best vitamin, followed by nicotinic acid and riboflavin and the least growth was observed in folic acid for *Psathyrella atroumbonata* Pegler. This observation was also in line with the report of Landers (1964) who found that thiamine stimulated mycelial growth of *Cercospora arachidicola* Hori in liquid culture. Manjunathan and Kaviyarasan (2011) also proved that thiamine was the best among the vitamins followed by biotin and tocopherol for *Lentinus tuber-regium* (Fr.) Fr. Adebayo et al.

(2007) found that riboflavin and pyridoxine promoted the mycelial growth for *Schizophyllum commune* Fr. Adebayo et al. (2011) observed that among the vitamins tested, ascorbic acid and folic acid had the highest stimulatory effect on mycelia of *Pleurotus ostreatus* (Jacq.) P. Kumm. Pokhrel et al. (2009a) reported that riboflavin and thiamine were the most stimulatory vitamins for mycelial growth of *Lyophyllum decastes* (Fr.) Singer.

Amino acids

Amino acids constitute one of the largest and most diverse groups of substances. Some fungi can use amino acids as the sole source of nitrogen and carbon although they are able to grow very poorly in most cases. Their utilization depends upon a number of factors such as permeability, enzymatic capacity of the organism and to some extent pH of the medium. Out of six amino acids tested, serine proved to be the best, while aspartic acid showed the least for mycelial growth. Arginine, Leucine and glutamic acid showed similar growths as the control with arginine showing thin mycelial density, and lucine and glutamic acid showing somewhat thin mycelial density (Fig. 5). According to Ayodele (2008), Asparagines proved to be the best amino acid followed by aspartic acid for *Psathyrella atroumbonata* Pegler. According to Chandra and Purkayastha (1977), amides such as asparagines and aspartic acid have been employed in increasing mycelial growth and fruiting body production in *Agaricus bisporus* (Lange) Imbach. Hayes and Hand (1981) observed that higher and lower concentrations of amino acids are found to be either ineffective or inhibitory for the growth of mycelia. According to Manjunathan and Kaviyarasan (2011) glycine proved to be the best amino acid followed by L-ornithine monohydrochloride for the mycelial growth of *Lentinus tuber-regium* (Fr.) Fr. Hayes and Hand (1981) reported that higher and lower concentrations of these amino acids were found to be either ineffective or inhibitory for growth. Pokhrel et al. (2009b) investigated the effect of light, moisture, amino acids, vitamins and mineral nutrients on mycelial growth of *Lyophyllum decastes* (Fr.) Singer on solid media. Glutamic acid was the best amino acid among the eight amino acids tested. Riboflavin was the best among the vitamins tested. Adebayo et al. (2011) observed that some of the amino acids had a stimulatory effect on mycelial yield of *Pleurotus ostreatus* (Jacq.) P. Kumm. Optimum mycelial growth was recorded when glycine was used. Jonathan et al. (2006) found that in *Pleurotus florida* Singer, although all the twelve amino acids used in his study enhanced biomass production, the most stimulatory amino acid was tryptophan. Pokhrel et al. (2009a) found that proline and glutamic acid showed significant growth than other amino acids tested in *Lyophyllum decastes* (Fr.) Singer. Gottlieb and Cieferri (1956), while working on *Streptomyces venezuelae*, suggested that the difference in the growth of fungi on various amino acids may be due to different degrees of availability of carbon skeleton of amino acids for the synthesis of carbohydrates, proteins and fats. Jennison et al. (1955) reported that there was correlation between the amount of the growth of certain Basidiomycetes and the type or structure of amino acids, although this was not consistently true for the individual organism. They suggested that both environmental factors and intrinsic differences in molecular structure may be involved in the differences noticed in the utilization of various amino acids.

C: N ratios

Growth of fungi is essentially limited to the terminal portion of the hyphae. Change must occur in the cell walls fairly soon after they are laid down which prevent elongation or expansion. This phenomenon of apical growth was studied carefully by Smith (1923) in *Botrytis* and also in *Fusarium, Phytophthora, Aspergillus, Penicillium, Rhizoctonia, Pyronema,* and *Rhizopus*. It was observed that the mycelial growth of the fungi under study increased with the proportional increase of carbon in comparison to nitrogen up to C: N ratio of C_5:N_1. Control treatment and C: N ratio C_1:N_1 showed the least growth with somewhat compact and thin mycelial density respectively. When the ratio of nitrogen was increased, mycelial growth was also found to increase gradually in the same manner, up to C: N ratio C_1:N_4. So, the best growth observed was when the C: N ratio was C_5:N_1 (Fig. 6). Lai et al. (2011) reported that C: N ratio of C_{10}:N_1 was favorable for mycelial growth of *Lignosus rhinoceros* (Cooke) Ryvarden. Notably, no growth occurred at C_{40}:N_1 of C: N ratio. Shim et al. (2003) observed that the optimum C: N ratio suitable for favorable growth of *Paecilomyces fumosoroseus* (Wize.) Brown and Smith were on culture media which were adjusted to C: N ratio of C_{40}:N_1. Shim et al. (2005b) found the C: N ratio of C_{30}:N_1 was the best for the mycelial growth of *Cystoderma amianthinum* var. ruigosoreticulatum (F. Lorinser) Bon. Chandra and Purkayastha (1977) observed C: N ratio of C_3:N_1, C_5:N_1 and C_1:N_3 were favorable for the growth of *Lentinus subnudus* Berk., *Volvariella volvacea* (Bull.) Singer and *Termitomyces eurrhizus* (Berk.) R. Heim, respectively. According to Manjunathan and Kaviyarasan (2011), carbon to nitrogen ratio of C_1:N_3 and C_1:N_5 were the best for the growth of *Lentinus tuber-regium* (Fr.) Fr. Ayodele (2008) found that the C: N ratio of C_4:N_1 and C_1:N_4 supported best growth of *Psathyrella atroumbonata* Pegler.

Conclusion

The growth processes of the filamentous fungi are still complex than those of yeast, because of greater differentiation in structure. Especially aerial myceliums are nourished through the mycelium in contact with the medium. This involves translocation of nutrients over considerable distances. In view of the popularity with rural masses and capability to utilize lignocellulosic substrates there is need to pursue further work for the cultivation of the present investigated mushrooms, so as to find out their biological efficiency of converting agro-forest by-product and other such substrate available in plenty in different parts of the country. Through domestication and bulk production, they can be made easily available to the consumers and this can revenue to the country and at the same time this will result in reduced pressure for their bulk hunting from nature. This is how the dual purpose of meeting the human demand and conservation aspects can be targeted simultaneously which is so vital for conservation of the natural ecosystem and sustainability. That's why there is need to domesticate this mushroom, for their mycelium and to assay for capability to degrade the lignocellulosic substrate. Hence it is time to conserve the fungus *in situ* condition to prevent it from going to extinct. In this regards the optimal medium for the growth of this mushroom in pure culture in the lab may maltose of carbon, yeast extract of nitrogen, Thiamine of vitamins, serine of amino acid and C_5:N_1 of C: N sources.

Growth in the fungi, as in other organisms, follows a definite pattern. The way this development takes place depends upon the species and the environmental and nutritional conditions. In batch culture, growth proceeds through a series of arbitrarily defined phases. The mycological resources are far from investigated. In view of their composition, it needs to be used directly in diet so as to promote health, taking advantage of the additive and synergistic effects of all the bioactive compounds present in them. Therefore, this species needs to be domesticated for their large scale production and subsequent use as natural nutrition sources. For this, developing a specific strategies to preserve small pocket rich area in *Termitomyces* on cultivated lands (e.g., Tea, Coffee, Areca, Millet and Maize farms).

Acknowledgement

I am thankful to Prof. Dr. M. Siwakoti, CDB, TU; Mr. P. B. Lakhey, DPR, GoN; Prof. S.C. Singh, Former Director of RECAST,TU; Prof. Dr. U. Budhathoki, CDB,TU; Dr. G.P. Rao, Pusa Campus, New Delhi, India; Prof. Dr. M.V. Rao, Bharathidasan University, Triuchirappalli, Tamil Nadu, India; for their encouragement and support. I am also thankful to Central Department of Botany, TU and Local informent during field for their kind help.

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Ex-situ Conservation of *Zanthoxylum armatum* DC. in Khokana Village, Lalitpur District, Nepal

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Abstract

Zanthoxylum armatum DC., a native medicinal and aromatic plant of Nepal, distributed in the mid hills of Nepal. The species is listed in prioritized high valued and market demand medicinal plants by the Department of Plant Resources, Nepal. The plant is disappearing in the natural habitat because of deforestation and infrastructure development. Successful propagation and *ex-situ* conservation of this species was needed for improving ecological balance and livelihood improvement. We conducted experiments to study the propagation requirements of this native tree species in Khokana village, which lies at an altitude 1300 m. Seeds were propagated in 2016 at a medicinal plant nursery in Khokana village. Seed germination and seedling growth of this species in nursery environmental condition were observed. The germination percentage of seeds ranged from 50-70% and the length of germination time ranged from six to eight weeks. Nursery grown seedlings were distributed to educational institutions, local people, and Non-government organizations for educational purposes, one house one tree concept and obviously for *ex-situ* conservation. Based on observation in 2018, about 99% of seedlings were successfully conserved and the plants produced fruits after three year of seed germination. This is one of the pioneer projects in Khokana, supported by the Department of Plant Resources, Kathmandu. Based on this experiment, we recommend that this type of research is necessary for the development of the medicinal plants area in Nepal. This is one of the suitable species for propagation and *ex-situ* conservation.

Key words: Germination, Medicinal and aromatic plants, Nursery, Seed propagation

Introduction

Zanthoxylum armatum DC. (Family- Rutaceae) is commonly known as Nepalese pepper, *Timur* in Nepali. It is a native medicinal and aromatic plant, a globally valuable natural resource and economic plant distributed in the mid-hills of Nepal. It is semi-deciduous tree of 2-4 m tall with spiny branches, dark green, aromatic leaves; white small flowers; and dark red aromatic fruits (Fig. 1).

Figure 1. *Zanthoxylum armatum* DC. plant

Timur plant has multipurpose uses. Fruits are used as cough and cold, gastric problems, toothache (Joshi et al., 2020) and consumed as spices. Stem is used as tooth brush and mouth purifier. Leaves and fruits are chewed in teeth enamel disease (Joshi & Joshi, 2001). Pounded leaves, stem, bark and fruits are

given by the Limbus of Morang District in Stomach pain. Paste of immature fruit is applied on to treat wounds at Sankhuwasabha District (Rajbhandary, 2001). Leaves are used externally in ring worm. Plant decoction is used in acute bronchitis (Baral & Kurmi, 2006). Similarly, root decoction is taken for curing as an anthelmintic. Brushing teeth with the branches stem is beneficial for toothache. Leave paste is applied for leukoderma. A paste of immature fruits is also used for cuts and wounds. This paste is also taken in cases of cough. Two or three seeds and a small clove of garlic are chewed for indigestion (Manandhar, 2002). The species is listed in prioritized high valued and market demand medicinal plants by the Department of Plant Resources, Nepal's Government (DPR, 2006, 2007). Decoction of the fruits is used to treat gastritis in the Rasuwa district (Shrestha & Shrestha, 2008; Shrestha et al., 2002). Fruits are pickled and uses as spices (Shrestha and Shrestha, 2004). One teaspoonful of root powder is taken twice a day for one week used for gastritis in the Lamjung district. Fruits are also used as gastritis disease (Shrestha et al., 2001-2002). Root powdered is taken for the treatment of gastritis in Makawanpur district (Shrestha, 2017a). Fresh and dry fruits are pickled and used as spices (Shrestha, 2017b). It is avoided to use during the fever and blood pressure in Makawanpur district.

The plant is considered for conservation aspect due to its natural habitat destruction (Manandhar, 2002). It is losing in the natural habitat because of deforestation, forest degradation and infrastructure development, e*x situ* conservation is one of the good approaches for making survival of the species. Propagation and cultivation of medicinal plants in farmlands by farmers' participation would be more fruitful for effective conservation of high valued medicinal plants (Mukherjee et al., 2009).

Successful propagation and *ex situ* conservation of this species were needed for improving ecological balance and livelihood. Thus, this study's main objective is about initiation of *ex situ* conservation of *Z. armatum* in Khokana Village of Lalitpur district.

Materials and Methods

Study Site

Field propagation experiment was conducted at Khokana Village. There is an interesting legend about the Khokana Village. According to the legend, the village was established by a couple of priest at Pachali Bhairab in Kathmandu. When the priest was dead, the funeral procession was brought to Pachali ghat with his wife for sati. Fortunately, there was a heavy storm and ran away all people came into the funeral procession. The priest got conscious, who was about to set fire for cremation, and asked his wife all about what had happened. And, they escaped from the ghat along the bank of Bagmati River fearing that people will kill him again when they come back. Ultimately, when they arrived at Sikali Chaur, they decided to live there forever. Later, they set a new settlement area near by the Sikali Chaur and called it as Khokhana. The meaning of Khokahana in Newar language is weeping. Later, Khokhana is called as Khokana.

It is a pure medieval newar settlement of the Kathmandu Valley. It is situated at the top and surrounded by terraced green fields. It is about seven kilometers distant south from Patan, the

district headquarter of Lalitpur district. The study site is located at Lat. 27°28' - 27°38'30"N and Long. 85°17'20" - 85°17'60"E (Fig. 2). The altitude of study site ranges from 1200-1400 m above sea level. The climate of the Khokana is subtropical type as of the Kathmandu Valley, spring, summer, monsoon, autumn and winter (Tuladhar, 2019). However, monsoon season plays pivotal roles in the livelihood as almost all residents are farmers. In Khokana, 4927 people reside in 1056 households and their primary language is Nepal Bhasha (Newar) (CBS, 2011). The main occupation of inhabitant is agriculture. The place is also popular for mustard oil production since ancient time. Recently, it is designated as a ward no. 21 of Lalitpur Metropolitan City.

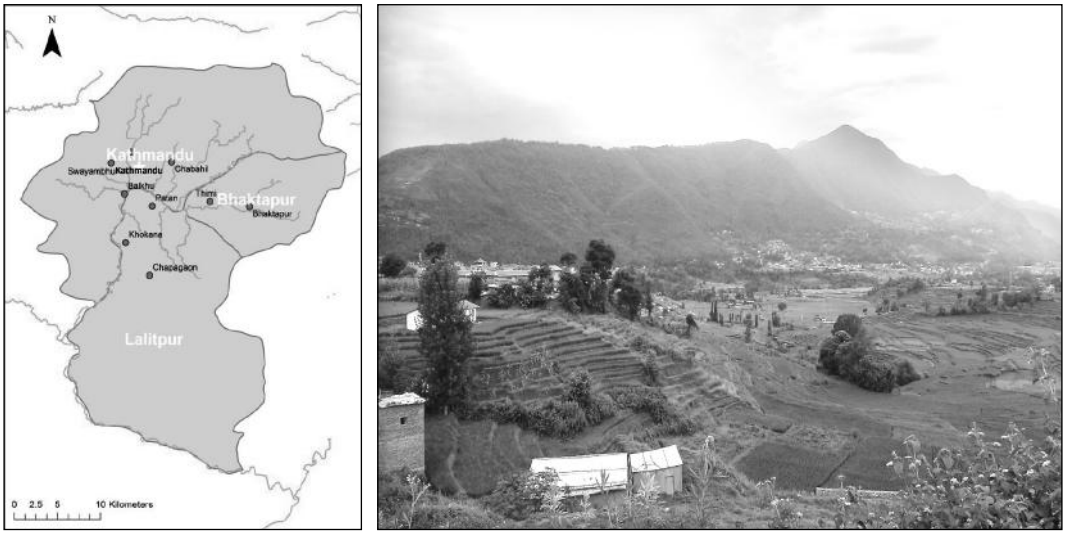

Figure 2. Map of study site.

The Newar community of this area was rich in traditional knowledge and culture. The place was almost independent on food security except salt and some spices. Traditional irrigation system was still in practice for arable lands. However, the modern development has been impacted slowly in the system. Traditional knowledge and information have been losing gradually among community as the education system undermined the traditional values. Similarly, *Z. armatum* was also disappearing in the locality, however, its utilization was still in practice.

Preparation of Nursery Bed and Soil

The nursery beds were prepared by digging about 20-25 cm dip properly and removed all unwanted materials found in the soil such as shrubs, weeds, gravel stone, etc. Margins of the bed size 1 m x 5 m were fixed with using bricks and wooden flat plank. Soil was crushed as much possible as fine and soft using traditional field tools. The top layer of the soil bed was covered at the time of seed sowing with newly prepared soil mixture of soil, sand and manure (1:1:1) in equal ratio.

Seed Collection, Grading, Treating and Sowing

Seeds were available from the Department of Plant Resources, Thapathali which were collected from Salyan District. The seeds were manually graded by removing unwanted materials and damaged seeds to get a high percentage of germination in the nursery. The seeds were treated for soaking into water for 12 h before sowing in nursery bed (Fig. 3). The floated seeds were removed considering that they were non-viable seeds for germination. The soaked seeds were desiccated using newspapers. The treated seeds were sown individually 3 cm deep and 4 cm apart each other in the bed (Fig. 4).

Figure 3. Seed treatment

Figure 4. Seed sowing

Mulching and Watering

The seed beds after sown were covered with straw completely in order to prevent the top soil and seeds during watering and cold winter time (Fig. 5). Watering was done twice a day (morning and evening time) regularly (Fig. 6). When germination of targeted plant species were ready in the bed, the mulch was removed carefully without damage seedlings.

Figure 5. Mulching

Figure 6. Watering

Seed Germination

Seed germination was observed every 2/3 days after one month and calculated the germination percentage based on number of seedlings grown and seed sown.

Seedling Growth and Survival

Seedling growth and survival was done by field observation in site. The survival was based on healthy and morphological study of the tree. Meantime, farmers were also asked about its fruiting, challenges to make survival of the tree. Fifty planted trees of *Z. armatum* were observed in Khokana.

Shading, Weeding and Transplantation

When germination of the plants was well developed, straw shading for bed was prepared to prevent form wilting and dying (Fig. 7). Furthermore, clearing of weeds were carried out regularly. It was done manually by bare hands without using any tools and weedicides. The seedlings were transplanted into polybags when they were about 4-5 cm tall with 4-5 leaves (Fig. 8). During transplantation, the seedlings were taken out very carefully from seedbed without disturbing root systems. The transplanted poly-bags were kept into shade house to prevent from wilting and dying. Watering and manuring was done regularly for keeping the plant healthy.

Figure 7. Shading

Figure 8. Seedling transplanting

Survival of the Species

The survival rate of *Z. armatum*, which were planted for conservation and educational purposes, were monitored by field visits. During the visits, physical condition of the species was observed and discussed about its fruiting, difficulties and implication for growing (Figs. 9, 10).

Figure 9. Seedling survive in the field

Figure10. Fruiting after 3 years in *ex-setu* conservation.

Distribution and Plantation

About 25-30 cm tall saplings were distributed for plantation to different educational institutions, community forest user groups, local people, and social organization with *ex situ* conservation, educational purposes and for its utility.

Results

Seed Germination Performance

The total number of seed sown of *Z. armatum* was 2000 and only 1100 seeds (55%) were germinated.

Seedling Distribution

Seedlings were distributed to local people of Khokana, community forest user group of Attarkhel, Sindhupalchowk, and other nearby villagers. Similarly, seedlings were provided to non-government organizations community service organization such as Society for Community Development Professionals (SOCODEP), Inner Wheels of Rotary Club of Balaju, and Om Bridashram, Bhaktapur. Seedling were also provided to some educational institution such as Natural History Museum, Tri-Chandra Campus, Amrit Science Campus, Patan Multiple Campus, Tribhuvan University, and Zing Boarding School of Khokana.

Seedling Growth and Survival Performance

Out of 1100 germinated seeds, only 1090 were grown as seedlings. Similarly, out of 50 plants transplanted into the soil, only 49 plants were survived.

Discussions

Seed Germination

Seed germination probability of *Z. armatum* is found 60-80% and it may also depend upon seed quality (DPR, 2011). One thousand and one hundred seedlings were grown from 2000 seeds. So, the germination percentage is 55% and the length of germination period took nearly two months. In this experiment seed germination percentage was found low. This might have happened due to seed quality and seed collection period. Usually, seeds are sown in bed within a week of harvesting time to get higher percentage of germination (DPR, 2011). For this experiment, seed were provided by Department of Plant Resources collecting from field offices.

Seedling Growth

Seedling growth rate in this experiment was about 99% which is very high. There should be very careful during seedling transplantation to get higher performance. If the root system of the seedlings is disturbed at the time of transplantation, it may be die. There should be regular watering and take caring activities.

Seedling Survival

On the basis of field observation in 2018, about 99% of seedlings were successfully conserved in Khokana and the plants produced fruits after three years from seed germination.

Conclusion

The distribution of seedling in local community and educational areas are the crucial for *ex situ* conservation. On the basis of this experiment, we recommend that this type of research is necessary for the development of the medicinal plants in local areas. This is one of the suitable species for propagation and *ex situ* conservation.

Acknowledgements

We would like to thank the Department of Plant Resources (DPR), Thapathali, Kathmandu for providing financial as well technical support for nursery development of Timur. We acknowledge to the local Newar community of Khokana for their valuable participation in nursery development, seedling production, and conservation activities. We are grateful to the Society for Community Development Professionals (SOCODEP) for necessary administration supports and thankful to Devaki Shrestha, Prof. Dr. Narendra Nath Tiwari, and Ms. Rajani Shrestha for their valuable help during the program implementation. Thanks also due to local inhabitants and institutes for growing and management of Timur in their fields.

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Occurrence of *Plasmodiophora* sp. on the Root of *Cascabela thevetia* (Apocynaceae)

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Abstract

Cascabela thevetia was planted in a pot in the year 1991 at the Department of Botany, M.M.A.M., Campus, T.U., Biratnagar. The plant with yellow flowers grown continuously and dried in February 2016 except main stem which was covered with lichens. The microscopic observation in the cyst of root-hairs found zoospores in inner portion of root system. The pathogen was identified as *Plasmodiophora* sp.

Key words: Cyst, Hypertrophy, Pot, Soil, Temperature

Introduction

The parasitic fungus *Plasmodiophora* sp. attacks on the root hair, secondary root, primary root and other allied portion of root of host plant and forms the cysts like structure. *Cascabela thevetia* was planted at the premises of the Botanical garden of M.M.A.M. Campus, Biratnagar, Nepal in 1991 and died in 2016 due to attack of *Plasmodiophora* sp. Similar type of infection was studied in other plants by the following workers. Dube (1983) has described *Plasmodiophora* in detail especially *P. brassicae*. Ingram and Tommerup (1972) mentioned the life history of *P. brassicae* in details, whereas Karling (1968) described the order "Plasmodiophorales". Kole and Gielink (1963) have mentioned about the significance of zoosporangial stage in the life cycle of Plasmodiophorales. Rangaswami (1994) wrote the text book "Diseases of crop-plants in India", as the same Singh (1980) wrote one book "Introduction to principles of plant pathology" and another text book "Plant diseases" in 1968. He has also described *P. brassicae* favoured the acidic soil and moderate temperature in nature. Waterhouse (1973) described the class "Plasmodiophoromycetes". Williams and Yakuwa (1967) mentioned about the ultra-structural studies on the host-parasite relation of *P. brassicae.*

Materials and Methods

C. thevetia was planted in the pot at Biratnagar Ward No. 15 in the year 1991. The plant used to give yellow flowers continuously and became dried without any branches except main stem in February 2016. The main stem also bearing the different scales of lichens, i.e., fully covered morphologically. After uprooted the whole plant from the pot, hypertrophy in root system was noticed. Root hair contained cyst and zoospores in the inner portion of root system was observed during microscopic study on 12/09/2016 at the Department of Botany, Biratnagar.

Results

The root hairs of host plant *C. thevetia* were found cyst like structure due to the growth of some pathogen caused hypertrophy in the root system. The microscopic study of cyst, identified the pathogen as *Plasmidiophora* sp.

Discussion

Host

Cascabela thevetia [Syn. *Thevetia peruviana* (L.) Juss. ex Endl] (Apocynaceae).

An evergreen poisonous shrub. Leaves linear lanceolate covering with wax coating. Flower's funnel shaped with yellow colours. The Tropical American origin plant is commonly cultivated in gardens, it has also medicinal value.

Pathogen

C. thevetia (Yellow oleander 'Pilakaner') was selected as a host plant to the present study. The plant saplings were planted in two pots in 1991, it was well grown and died in February 2016. The main stem of the plant was covered with lichens. Both plants were removed carefully along with root system on 09/09/2016 at 3 pm for the study.

The root system of both plants was observed as followings:

A. Morphological structure – On 12/9/2016 at 2-2.5 pm.
   First plant$_{1:}$
   (a) Colour - Greyish to black
   (b) Main branch above root zone, i.e., Stem length – 61 cm
   (c) Root bearing part - 6.2 cm
   (d) The longest tap root system - 37 cm
   (e) The smallest root hairs and rootlets - 3.1 cm
   (f) Total no. of roots - 51
   Second plant$_{2}$:
   (a) Colour - Greyish to black
   (b) Stem length - 31 cm
   (c) The longest tap root system - 37 cm
   (d) The smallest root hairs and rootlets - 1.8 cm
   (e) Total no. of roots - 43
B. Microscopic observation of the root hairs of host plant- On 12/9/2016 at 12-12.20 pm.
   (a) Cyst or nodule like structure of the root examined under 40x.
   (b) Several rows of zoospores present inside the root hair or root lets.
   (c) Some germinating spores freely moved in 100x magnification.
   (d) Due to zoospores aggregated give the symptom of hyper trophied and club-shaped.
   (e) According to Ainsworth (1966, 1973) it must have included in as a class of Myxomycotina.
   (f) Resting spores rupturing rest system come out in the soil of pot and again attack on the rest system.

(g) Therefore, the root system become weaker continuously, from 1991 to Feb. 2016.
(h) As a result, the shoot system become weak and due to weak root system internally and growth of lichen externally.
(i) Finally, it was identified the pathogen as *Plasmodiophore* sp. which infects the members of Apocynaceae including *C. thevetia.*

Conclusion

Acidic soil favours the growth of pathogen *Plasmodiophora* sp. on host plant. The growth of host plant (*C. thevetia*) reduced and finally died in 2016. The formation of cysts and hypertrophy on root system externally and internally zoospores due to infection of *Plasmodiophora* sp.

Acknowledgements

I am thankful to former Head of the Department, Prof. Dr. P.K. Jha, Central Department of Botany, Tribhuvan University for his guidance.

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Assessment of Heavy Metal Concentrations in Water of Koshi at Kursela, Katihar, Bihar

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Abstract

The study was undertaken to assess heavy metal concentration in water of Koshi at Kursela, Katihar, Bihar. The Koshi river at Kursela, Katariya, Koshikipur, Bintoli Tinghatiya and Simara of Katihar, Bihar was selected randomly by considering the population, location and source of pollutions to study heavy metal concentration in water. Periodic samplings in September (wet season) and January (dry season) were carried out in two consecutive years 2016-2017 and 2017-2018. River water samples were collected at depths varying from 15cm to 30cm and the water was then transferred into pre-cleaned polypropylene bottles. These samples were used to determine heavy metal concentrations by Atomic Absorption Spectrophotometer (Perkin-Elmer model 800, USA), the standard methods recommended by American Public Health Association. Metals in the water exhibited a significant seasonal and regional variation. The minimum concentration of Co, Cu, Cr, Ni, Cd, Zn and Pb was found 0.0, 0.016, 0.001, 0.003, 0.009, 0.017, 0.007 mg/L and maximum concentration was found 0.036, 0.037, 0.008, 0.018, 0.038, 0.012, 0.027 mg/L respectively in the various sites. Most of the above values were found either below or closed the permissible limit set by World Health Organization and United State Public Health Services.

Key words: Cadmium, Koshi river, Lead, Pollution, Zinc.

Introduction

"Clean water is a key factor for economic growth. Deteriorating water quality is stalling economic growth, worsening health conditions, reducing food production, and exacerbating poverty in many countries" said David Malpass, World Bank Group President (Malpass, 2019). Water pollution due to heavy metals is a significant environmental problem and has a negative impact on human health and agriculture. Assessment of water resource quality from any region is an important aspect for the region's development activities because the rivers, lakes, and manmade reservoirs are used for water supply to domestic, industrial, agriculture and fish culture use (Sarwade & Kamble, 2014). A previous study indicated that potential sources of elevated levels of heavy metals were sewage wastes, wastes from metal processing industries and other household refuse (Lokhande et al., 2011). Very little assessment in the related areas has been made in a developing country like Nepal (Napit et al., 2020). Heavy metals concentrations in aquatic ecosystem can reflect the present pollution status of these areas.

Heavy metal is a naturally occurring element with a high atomic weight and high density five times greater than that of water. Among all the pollutants, heavy metals have received a

paramount attention to the environmentalists due to their toxic nature. Heavy metals become toxic when the body does not metabolize them and accumulate in the soft tissues (Hujare, 2008). They may enter the human body through food, water, air or absorption through the skin when they come in contact with humans in agriculture, manufacturing, pharmaceutical, industrial or residential settings. Water pollution due to heavy metals is a worldwide problem because the metals are in destructive and most of them have toxic effects on living organisms when metals exceed a certain concentration (MacFarlane & Burchette, 2000; Pekey, 2006; Kayastha, 2015).

Heavy metals are usually present in trace amounts in natural waters but many of them are toxic even at very low concentrations. Metals such as arsenic, lead, cadmium, nickel, mercury, chromium, cobalt, zinc and selenium are highly toxic even in minor quantity (Singh et al., 2013). Increasing the quantity of heavy metals in our resources is currently an area of greater concern for people's prosperity, especially since a large number of industries are discharging their metal containing effluents into fresh water without any adequate treatment.

Surface water quality of river is greatly affected by various heavy metals (Lokhande et al., 2011). These are a special group of trace elements which have been shown to create definite health hazards when taken up by aquatic biota. These include Cr, Cd, Ni, Zn, Cu, Pb and Fe. These are called heavy metals and their densities are greater than 4g/cc. The Koshi river basin is one of the river system's major river tributaries in the Himalayan region. In Nepal, more than five million people are living in the Koshi river basin (WECS, 1994). Glaciers dominate the basin in the upstream areas and snowmelt contributes to stream flow (Nepal, 2012).

Heavy metals released from tanneries are kept under environment pollutant category due to their toxic effects on plants, animals and human beings. They interfere with plants' physiological activities such as photosynthesis, gaseous exchange and nutrient absorption and cause reduction in plant growth, dry matter accumulation and yield (Sharma & Agrawal, 2005). They cause direct toxicity, both to human and other living beings due to their presence beyond specified limits. The metals present in the soil can enter in the aquatic system by weathering, percolation, and surface runoff from agricultural land (Pinay et al., 1992). Soil can also be polluted by waste water irrigation. These contaminated soils may have an impact on water quality.

Water quality monitoring and evaluation is the foundation of water quality management; thus, there has been an increasing demand for monitoring water quality of many rivers by regular measurements of various water quality variables (Bartram & Balance, 1996). Assessment of seasonal changes in surface water quality is an important aspect for evaluating temporal variations of river pollution due to natural or anthropogenic inputs of point and nonpoint sources (Ouyang et al., 2006). The present paper describes the heavy metals concentration in the water of the river Koshi. It provides a scientific basis for pollution control and its monitoring. The obtained data provide essential information for the preventive measures and/or remedial actions to be taken to overcome the risk and impact increasing population in the river basin area.

Materials and Methods

The quality of water resources is usually described according to its physical, chemical and biological characteristics. For confirming the good quality of water resources, large numbers of physicochemical and biological parameters are to be studied in detail and must be found in normal range.

Five different experimental sites (Fig. 1) were selected to study heavy metal concentration in water of Koshi. The study sites were: Site-1 (Kursela), Site-2 (Katariya), Site-3 (Koshikipur), Site-4 (Bintoli Tinghatiya), and site-5 (Simara) at Katihar district, Bihar, India. It lies between 26º31'35"N and 25º27'02"N latitude and 87º14'46"E and 86º35'22"E longitudes. The study area was periodically visited in September (wet season) and January (dry season) and the surface water samples were collected by using samplers, from five sites of the river. Water samples were collected from these five sampling sites on seasonal basis from 2016-17 to 2017-18.

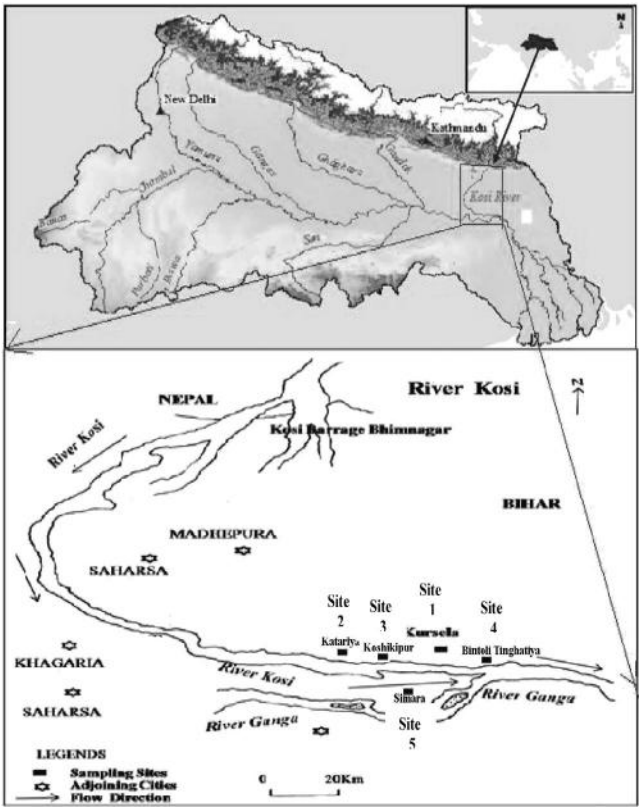

Figure 1. The study area showing sampling sites: Site 1 Kursela, Site 2 Kataria, Site 3 Koshikipur, Site 4 Bintoli Tinghatiya, and Site 5 Simara

Water Sampling

The sampling site was selected randomly by considering the population, location and source of pollutions. Water samples were collected at depths varying from 15 to 30 cm with the

help of a water sampler which consisted of a glass bottle and a cord tied to a lid. The whole assembly was lowered into water to the desired depths and the cord of the lid was pulled and released only when displaced air bubble ceased to come to the surface. The whole assembly was withdrawn and the water was then transferred into pre-cleaned polypropylene bottles. All the containers which used in sampling purposes were thoroughly washed and rinsed with 10% HNO_3 following by double distilled water. The bottles were filled leaving no air space, and then the bottle was sealed to prevent any leakage. Each container was clearly marked with the name and address of the sampling station, sample description, and sampling date. All the procedures were adopted according to the standard methods recommended by APHA-AWWA-WPCF (1985).

Preparation of Water Sample for the Analysis of Heavy Metals

For determination of heavy metals in water, water samples (50 ml) were digested with 10 ml of conc. HNO_3 at 80ºC until the solution became transparent (APHA, 2005). The solution was filtered through Whatman No. 42 filter paper and diluted to 50 ml with double distilled water. These samples were used to determine heavy metal concentrations by Atomic Absorption Spectrophotometer (Perkin-Elmer model 800, USA). For evaluating precision and accuracy of the analytical procedure used in the above experiment, duplicates and analytical blanks were prepared and analyzed using the same procedures and reagents. Care was taken during sampling, handling and analysis to prevent the samples coming in to the contact with dust and metals.

Heavy metals were determined by Atomic Absorption Spectrophotometer (AAS) which is a spectroanalytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state. AAS is based on absorption of light by free metallic ions. In AAS, the sample is first converted at a selected wavelength, which is characteristic of each individual element. The same experimental condition was also applied for the determination of the reference samples of known composition.

Results

The heavy metals concentration recorded in the water of Koshi River are described below.

Cobalt (Co^{++})

It was found that the concentration of Co shows different level of variability at different sites in different seasons. The maximum concentration of Co was measured 0.026 mg/L in dry

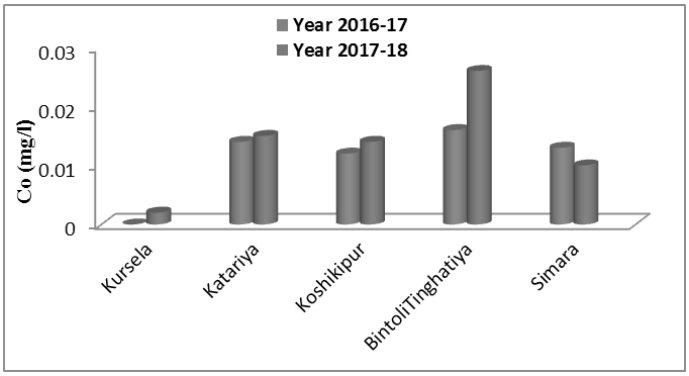

Figure 2. Concentration of Co^{++} at different sites of Koshi River in 2016-17 and 2017-18.

season at Bintoli Tinghatiya in the year 2017-18 followed by 0.015 mg/L at Katariya, 0.014 mg/L at Koshikipur, 0.013 mg/L at Simara. The minimum value was recorded as 0.0 mg/L in wet season at Kursela in the year 2016-17 (Fig. 2).

Copper (Cu^{++})

The concentration of Cu was maximum 0.026 mg/L in dry season at Bintoli Tinghatiya in the year 2017-18 followed by 0.02 mg/L at Katariya and Koshikipur, 0.018 mg/L at Simara, 0.017 mg/L at Kursela. The minimum value was recorded as 0.016 mg/L in wet season at Simara in the year 2017-18 (Fig. 3).

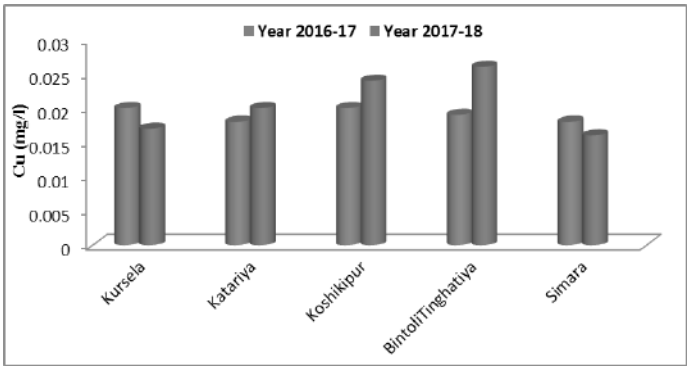

Figure 3. Concentration of Cu^{++} at different sites of Koshi River in 2016-17 and 2017-18.

Chromium (Cr^{++})

The concentration of Cr was maximum 0.006 mg/L in dry season at Simara in the year 2017-18 followed by 0.005 mg/L at Bintoli Tinghatiya, 0.004 at Kursela. The minimum value was recorded as 0.001 mg/L in wet season at Koshikipur in the year 2016-17 and Katariya in 2017-18 (Fig. 4).

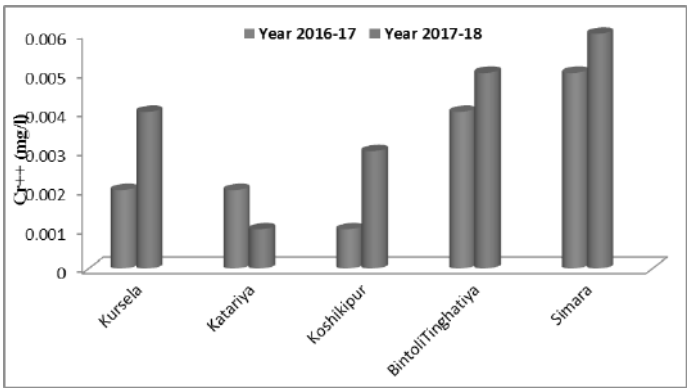

Figure 4. Concentration of Cr^{++} at different sites of Koshi River in 2016-17 and 2017-18.

Nickel (Ni^{++})

The concentration of Ni was maximum 0.018 mg/L in dry season at Koshikipur in the year 2017-18 followed by 0.017 mg/L at Bintoli Tinghatiya, 0.012 at Kursela and Simara. The minimum value was recorded as 0.003 mg/L in wet season at Koshikipur in the year 20216-17 and Katariya in the year 2017-18 (Fig. 5).

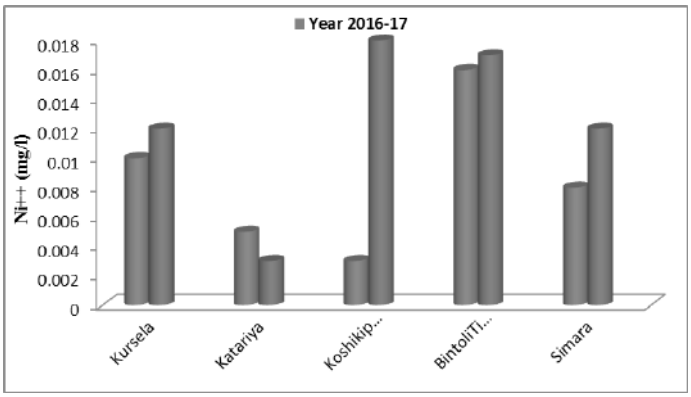

Figure 5. Concentration of Ni^{++} at different sites of Koshi River in 2016-17 and 2017-18.

Cadmium (Cd^{++})

Concentration of Cd was maximum 0.026 mg/L in dry season at Bintoli Tinghatiya in the year 2016-17 followed by 0.024 mg/L at Koshikipur, 0.02 mg/Lat Katariya, 0.016 at Kursela. The minimum value was recorded as 0.009 mg/L in wet season in the year 2017/18 at Simara (Fig. 6).

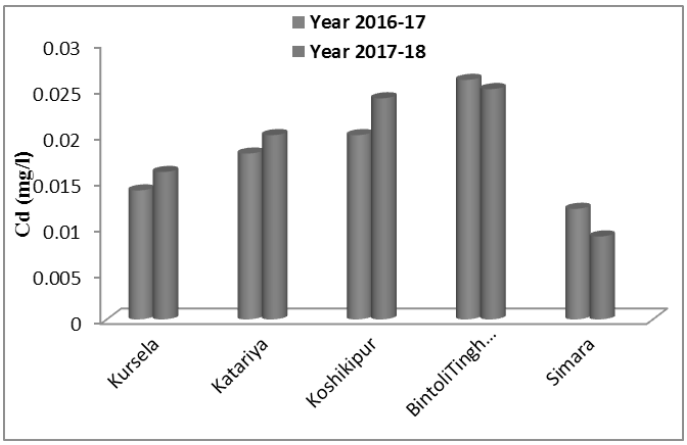

Figure 6. Concentration of Cd^{++} at different sites of Koshi River in 2016-17 and 2017-18.

Zinc (Zn^{++})

In the river water of Koshi the concentration of Zn was maximum 0.124 mg/L in dry season at Bintoli Tinghatiya in the year 2017-18 followed by 0.092 mg/L at Katariya, 0.09 mg/L at Koshikipur, 0.021 mg/L at Simara. The minimum value was recorded as 0.017 mg/L in wet season at Kursela in the year 2017-18 (Fig. 7).

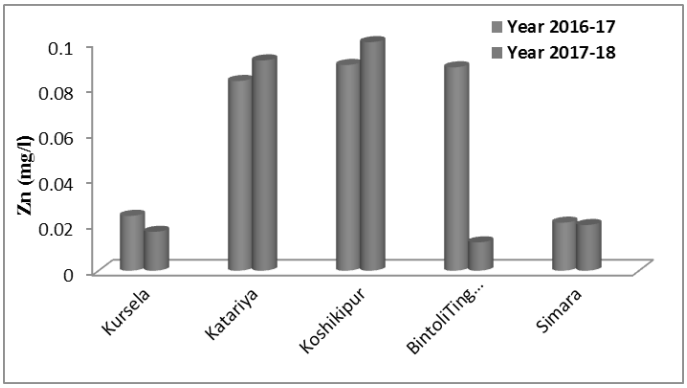

Figure 7. Concentration of Zn^{++} at different sites of Koshi River in 2016-17 and 2017-18.

Lead (Pb^{++})

The concentration of Pb was maximum 0.023 mg/L in dry season at Bintoli Tinghatiya in the year 2017-18 followed by 0.015 mg/L at Koshikipur, 0.02 mg/L at Simara, 0.009mg/L at Kursela. The minimum value was recorded as 0.004 mg/L in wet season at Kataria in the year 2016-17 (Fig. 8).

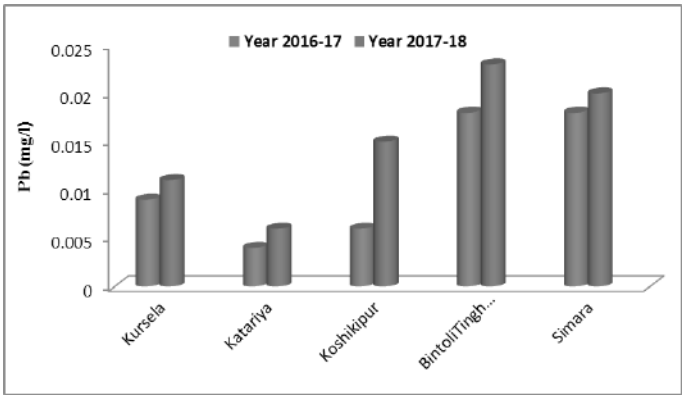

Figure 8. Concentration of Pb^{++} at different sites of Koshi River in 2016-17 and 2017-18.

Table 1. Heavy metals concentration in water of the Koshi River at different sites and periods.

Heavy metals	Kursela		Katariya		Koshikipur		BintoliTinghatiya		Simara	
	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18
Co (mg/L)	0.0± 0.002	0.007± 0.002	0.014± 0.009	0.015± 0.006	0.012± 0.005	0.014± 0.008	0.016± 0.011	0.026± 0.010	0.013± 0.004	0.010± 0.005
Cu (mg/L)	0.020± 0.005	0.017± 0.006	0.018± 0.009	0.020± 0.006	0.020± 0.004	0.024± 0.007	0.019± 0.007	0.026± 0.011	0.018± 0.009	0.016± 0.014
Cr (mg/L)	0.002± 0.001	0.004± 0.001	0.002± 0.00	0.001± 0.000	0.001± 0.002	0.003± 0.002	0.004± 0.001	0.005± 0.002	0.005± 0.002	0.006± 0.002
Ni (mg/L)	0.01± 0.006	0.012± 0.007	0.005± 0.002	0.003± 0.001	0.003± 0.005	0.018± 0.009	0.016± 0.008	0.017± 0.011	0.008± 0.002	0.012± 0.004
Cd (mg/L)	0.014± 0.009	0.016± 0.011	0.018± 0.009	0.020± 0.009	0.020± 0.001	0.024± 0.011	0.026± 0.012	0.025± 0.008	0.012± 0.007	0.009± 0.004
Zn (mg/L)	0.024± 0.012	0.017± 0.010	0.083± 0.043	0.092± 0.035	0.090± 0.053	0.1± 0.035	0.086± 0.062	0.124± 0.015	0.021± 0.004	0.020± 0.007
Pb (mg/L)	0.009± 0.003	0.011± 0.002	0.004± 0.003	0.006± 0.003	0.006± 0.010	0.015± 0.012	0.018± 0.008	0.023± 0.011	0.018± 0.008	0.020± 0.007

Discussion

The concentrations of heavy metals like Co, Cu, Ni, Zn and Pb in water of the river Koshi were recorded below the permissible limit at most of the selected sites, whereas the level of Cd in water exceeded the permissible limit of 0.005 mg/L set by WHO (Table 2). Cd is contributed to the surface waters through paints, pigments, glass enamel, deterioration of the galvanized pipes etc. This indicates the natural input of the Cd into the river.

Table 2. Comparative description of ranges between concentrations of heavy metals of the River Koshi with standard permissible limits.

Heavy Metals	Concentration (mg/L)		WHO Permissible Limit (mg/L)
	Min	Max	
Co	0.005	0.016	
Cu	0.014	0.026	1.0
Cr	0.001	0.006	0.05
Ni	0.003	0.018	0.21
Cd	0.009	0.026	0.005
Zn	0.017	0.124	5.000
Pb	0.004	0.023	0.05

A low value of metals in wet season can also be attributed to water levels rising in the rivers due to rainfall. The large stretch of the river passes through the agricultural field which may add some inorganic elements in to the river.

Lead is one of the oldest metals known to man and is discharged in the surface water through paints, solders, pipes, building material, gasoline etc. Lead is a well known metal toxicant and it is gradually being phased out of the materials that human beings regularly use. Zinc is one of the important trace elements that play a vital role in the physiological and metabolic process of many organisms. Nevertheless, higher concentrations of zinc can be toxic to the organism (Rajkovic et al., 2008). It plays an important role in protein synthesis and is a metal

which shows fairly low concentration in surface water due to its restricted mobility from the place of rock weathering or from the natural sources (Rajappa et al., 2010).

There are many anthropogenic sources of Copper. Industrial uses include alloys, paints, pigments, electroplating, batteries, automotive parts, coatings and electrical wiring. Copper is also used as insecticides or fungicides. It is present in industrial wastes and sewage sludges. It has limited mobility in soil, and anthropogenic impacts to water are small.

Conclusion

The minimum concentration of Co, Cu, Cr, Ni, Cd, Zn, and Pb was recorded as 0.005, 0.014, 0.0.001, 0.003, 0.009, 0.017 and 0.004 mg/L respectively in wet season whereas the maximum value was recorded 0.016, 0.026, 0.006, 0.018, 0.026, 0.124 and 0.023 mg/L respectively in dry season at different sites in surface water of the river Koshi. Most of the above values were found either below or closed the permissible limit set by World Health Organization (WHO, 2011). The present experimental data indicates that the pollution level due to heavy metal concentration along the river Koshi is not very high but the increasing population load in the basin may cause irreparable ecological harm in the long-term.

The quality of water resources is usually described according to its physical, chemical and biological characteristics. For confirming the good quality of water resources, large number of physicochemical and biological parameters is to be studied in detail and must be found in normal range. In any rational formulation and deciding quality of water resources an adequate knowledge of existing nature of physico-chemical parameters, magnitude and source of any pollution load must be known, for which monitoring of physico-chemical parameters and pollutants is essential (Agarwal & Rozgar, 2010).

The data generated may provide useful information to Governmental agencies to control the heavy metal pollution of the river at these urban centers which may even be worst in future scenario. The water resource is being used for various purposes such as domestic use, agriculture and fish culture etc. by local community. In future there is threat of contamination of water from the surface runoff of used fertilizers and pesticides. It also suggests a need for consistent, internationally recognized data-driven strategy to assess the quality of waste water effluent and the generation of international standards for evaluating contamination levels. Although extensive works on the physico-chemical parameters have already been carried out, there is no sufficient baseline data about heavy metal concentrations of Koshi river water; hence the present work has been undertaken for monitoring the water characteristics.

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Garden Flowers in Tansen Municipality, Palpa

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Abstract

Flowers are the most commonly used for decoration purposes and necessary to all communities and religious groups to celebrate various ceremonies. The demand for floriculture products is increasing day by day in the national and international markets. This study was carried out to identify the significant garden flowers in Tansen Municipality, Palpa. A total of 70 ornamental garden flowers belonging to 60 genera and 35 families were recorded from the study area. Among these, herbaceous species were the most dominant, comprising 58% of species. In comparison, shrubs comprise 30% and both the trees and climber consist of 6% each. The perennial flowers cultivated in gardens comprise 73% of total species reported. The number of annual flowers was the highest, i.e., 24% and the biennial flowers were the least consisting of only 3%. The highest number of species included in the family Asteraceae (11 species) followed by five Malvaceae species and four species of Solanaceae. The genus *Hibiscus* and *Petunia* comprise three species each. The genus *Chrysanthemum*, *Dracaena*, *Euphorbia*, *Pelargonium*, *Sedum,* and *Tagetes* include two species in each. The results of this study showed that diverse types of garden flowers are grown in Tansen Municipality.

Key words: Asteraceae, Floriculture, Herbaceous, Ornamental, Perennial

Introduction

A close relationship between plants and people has occurred since time immemorial. The plants provide food and fiber, wood, medicine, store carbon. Plants also have inspiration, recreation and aesthetic value.

Flowers are a symbol of grace and elegance. Flowers are necessary in most of the religious activities of various communities and ethnic groups. These are also used in both formal and informal programs to welcome, as a gift in ceremonies, to visit sick people in hospitals (bouquet) and used in social gatherings and funerals. Flowers add beauty and are used for ornamental purposes. Flowers are also essential and raw material to extract essential oils and prepare perfumes and scents. Cut flowers are most widely used for indoor decoration. Cut flowers and cut foliage are used for vase decoration and indoor decoration.

Garden of Dreams was built in 1895 at Kaiser Mahal, Kathmandu and many exotic species of garden plants were introduced in Nepal during the period of Ranas (Rai et al., 2010). Later on, several parks were developed in Kathmandu valley like Ratna Park, National Botanical Garden at the Godavari, Coronation Garden at Tribhuvan University, Balaju Park, Tribhuvan Park, etc. Birendra Fulbari, Amar Narayan Fulbari and Lakhan Park are some of the parks in Tansen Municipality.

Floriculture Association Nepal (FAN) was established in 1992 to organize and promote the floriculture business in Nepal. The floriculture field creates employment opportunities. There are currently 697 plant nurseries and about 43,500 people are directly or indirectly involved in Nepal's floriculture sector (Pun, 2019).

A total of 190 indoor and outdoor garden flowers of Nepal belonging to 61 families were enumerated by Bajracharya et al. (1997) with their coloured photographs. They provided a scientific name, common name, local name, description, flowering season, and propagation method. Sharma (2003) illustrated 122 species of ornamental plants in the National Botanical Garden, Lalitpur. Similarly, 54 common ornamental garden flowers with their botanical name, description, flowering season, gardening notes, and photographs were published by Rai et al. (2010). Batth (2014) reported 73 Indian garden flowers in his book, where he categorized 58 flowers as winter-blooming, nine as summer blooming, and six as rainy season blooming. Another study was carried out in the Singh Durbar area of Kathmandu valley, and including 212 angiosperms and 17 gymnosperms species, a total of 229 plant species were reported belonging to 176 genera and 88 families (Bhattarai, 2019). Present research work is carried out to identify the varieties of garden flowers that are grown in Tansen Municipality

Materials and Methods

This study was carried out in the Tansen Municipality of Palpa district, ranging from altitude 700 m to 1500 m asl. Among the total 14 wards, only 7 wards (1-7) were covered in this study. A total of 120 households in the study area were visited to collect information about garden flowers. Photographs of garden flowers were taken, and specimens were collected for herbarium preparation. The taxonomic characters and other necessary information about plants were also noted. The plant species were identified following previously published literature (Bajracharya et al., 1997; Shrestha, 1998; Rai et al., 2010; Batth, 2014). The herbarium specimens were deposited at the Department of Botany, Tribhuvan Multiple Campus, Tansen Palpa.

Results

In this study, 70 species of garden flowers belonging to 60 genera and 35 families were identified (Table 1). The most abundant genera were *Hibiscus* and *Petunia,* with three species each followed by *Chrysanthemum*, *Dracaena*, *Euphorbia*, *Pelargonium*, *Sedum* and *Tagetes* with two species each. The garden flowers were grouped into four life forms, among which herbs were found most dominant comprising 41 species which was followed by shrubs (21), climbers (4), and trees (4). Perennial flowers were highest in distribution and grown in the study area which includes 51 species. In comparison, biennial flowers were least distributed having only two species. The annual flowers bear 17 garden flowers out of total plants.

Among the 35 families, Asteraceae was the dominant family with 11 species of garden flowers, followed by Malvaceae (5), Solanaceae (4), Amaranthaceae (3), Apocynaceae (3), Araceae (3), Crassulaceae (3), Lamiaceae (3), Acanthaceae (2), Amyrillidaceae (2), Asparagaceae (2), Euphorbiaceae (2), Geraniaceae (2), Liliaceae (2), Nyctaginaceae (2) and Oleaceae (2). Rest all families have only one species each.

The plants in gardens have ornamental value, but they also have medicinal and food values. 57.14% of plants have medicinal value, and 7.14% of plants have food value.

Table 1. Garden flowers in Tansen Municipality

S.N.	Scientific Name	Family	Local Name	English Name	Remarks*
1	*Albizia julibrissin* Durazz.	Fabaceae	Rato siris	Silk tree	T, P
2	*Aloe vera* (L.) Burm. f.	Asphodelaceae	Ghiu Kumari	Barbados aloe	H, P, M
3	*Amaranthus caudatus* L.	Amaranthaceae		Love lies bleeding	H, A
4	*Asclepias curassavica* L.	Asclepiadaceae	Khursani kose phool	Blood flower	S, P, M
5	*Bougainvillea glabra* Choisy	Nyctaginaceae	Kagaje phool	Paper flower	C, P, M, F
6	*Brassica oleracea* var. *acephala* L.	Brassicaceae	Gobi phool	Kale, Ornamental cabbage	H, B, M
7	*Calendula officinalis*L.	Asteraceae	Asharfi	Pot marigold	H, A, M
8	*Callistemon lanceolatus* DC.	Myrtaceae	Kalki phool	Bottle brush	T, P
9	*Callistephus chinensis* (L.) Nees	Asteraceae	Gyanthunge	China aster	H, A
10	*Canna hybrida* Hort.	Cannaceae		Canna	H, P
11	*Catharanthus roseus* (L.) G. Don	Apocynaceae	Barhamase phool	Periwinkle	S, P, M
12	*Centaurea cyanus* L.	Asteraceae	Makai phool, Naurangi, Pancharangi	Corn flower	H, A, F
13	*Cestrum nocturnum* L.	Solanaceae	Raatki rani	Night blooming jasmine	S, P, M
14	*Chrysanthemum coccineum* Willd.	Asteraceae		Common pyrethrum	H, P
15	*Chrysanthemum morifolium* Ramat.	Asteraceae	Godavari phool	Florist's Chrysanthemum	H, P, M
16	*Clerodendrum thomsoniae* Balf.	Lamiaceae		Bleeding heart vine	S, P
17	*Coleus blumei* Benth.	Lamiaceae	Sindure	Beauty of lyon	H, P, M
18	*Coreopsis grandiflora* Hogg ex Sweet	Asteraceae		Tickseed	H, P
19	*Crassula ovata* (Mill.) Druce	Crassulaceae		Jade plant	H, P, M
20	*Dahlia pinnata* Cav.	Asteraceae	Lahure phool	Garden dahlia	H, A, M
21	*Delphinium elatum* L.	Ranunculaceae		Delphinium	H, P
22	*Dianthus barbatus* L.	Caryophyllaceae		Sweet William	H, B
23	*Dracaena goldieana* Bullen ex Mast. & T.Moore	Asparagaceae		Dracaena	H, P
24	*Dracaena sanderiana* Mast.	Asparagaceae		Song of India	T, P
25	*Duranta repens* L.	Verbanaceae	Nil kanda	Golden dewdrop	S, P
26	*Epipremnum aureum* (Linden & Andre) G.S. Bunting	Araceae		Money plant	C, P
27	*Euphorbia milii* Des Moul.	Euphorbiaceae	Kande phool	Crown of thorns	S, P, M
28	*Euphorbia pulcherima* Willd. ex Klotzsch	Euphorbiaceae	Lalupate	Christmas flower	S, P, M
29	*Fuchsia hybrida* Voss	Onagraceae	Krishnakali	Fuchsia	S, P, F
30	*Gomphrena globosa* L.	Amaranthaceae	Supari phool, Makhamali	Globe-amaranth	H, A, M

S.N.	Scientific Name	Family	Local Name	English Name	Remarks*
31	*Helianthus annus* L.	Asteraceae	Suryamukhi	Sunflower	H, A, M, F
32	*Hibiscus mutabilis* L.	Malvaceae	Naalu phool	Cotton-rose	S, P, M
33	*Hibiscus rosa-sinensis* L.	Malvaceae	Barhamase phool	China rose	S, P, M
34	*Hibiscus syriacus* L.	Malvaceae	Datiwane phool	Rose of sharon	S, P, M, F
35	*Hippeastrum vittatum* Herb.	Amaryllidaceae	Dhwang phool	Amaryllis	H, P, M
36	*Hydrangea macrophylla* (Thunb.) Ser.	Hydrangeaceae	Hansaraj	Common hydrangea	S, P, M
37	*Hypoestes phyllostachya* Baker	Acanthaceae		Polka dot plant	H, P
38	*Impatiens balsamina* L.	Balsaminaceae	Tiuri	Garden Balsam	H, A, M
39	*Ipomoea hederacea* Jacq.	Convolvulaceae	Bhurungko lahara	Calico flower	C, P, M
40	*Iresine herbstii* Hook . f.	Amaranthaceae	Ganesh phool	Chicken gizzard, Blood leaf	H, P, M
41	*Jacobinia carnea* (Lindl.) G. Nicholson	Acanthaceae		Shrimp plant, Water willow	S, P
42	*Jasminum gracile* Andr.	Oleaceae	Chameli	Jasmine	S, P, M
43	*Kochia scoparia* (L.) Schrad.	Chenopodiaceae		Kochia, Kokia	S, A, M
44	*Lilium wallichianum* J.A. & J.H. Schult	Liliaceae	Lily	Yellow diamond lily	H, P, M
45	*Liriope muscari* (Decne.) L.H. Bailey	Liliaceae	Seto dubo	Big-blue-lily-turf	H, P
46	*Malva rotundifolia* L.	Malvaceae		Common mallow	H, P
47	*Malvaviscus arboreus* Cav.	Malvaceae	Ghanti phool	Ladies tear drops	S, P, M
48	*Mirabilis jalapa* L.	Nyctaginaceae	Malati phool	Four O'clock	S, P, M
49	*Nerium oleander* L.	Apocynaceae	Karbir	Rose bay	S, P
50	*Nyctanthes arbor-tristis* L.	Oleaceae	Parijat	Tree of sadness, Tree of sorrow	S, P, M
51	*Pelargonium hortorum* Ait.	Geraniaceae		Garden geranium, Zonal geranium	H, P
52	*Pelargonium zonale* Ait.	Geraniaceae		Horseshoe geranium	H, P, M
53	*Petunia axillaris* (Lam.) Britton, Sterns & Poggenb.	Solanaceae		White moon petunia, Wild white petunia	H, A
54	*Petunia hybrida* Vilm.	Solanaceae		Petunia	H, A
55	*Petunia integrifolia* (Hook.) Schinz & Thell.	Solanaceae		Violet petunia	H, A
56	*Philodendron erubescens* K.Koch & Augustin	Araceae		Blushing philodendron	C, P
57	*Plumeria rubra* L.	Apocynaceae	Chuwa	Frangipani	T, P, M
58	*Rosa alba* L.	Rosaceae	Gulaf	Rose	S, P
59	*Russelia equisetiformis* Schltdl. & Cham.	Plantaginaceae	Lwang phool	Coral blow	S, P, M
60	*Salvia splendens* Sello. ex J.A. Schultes	Lamiaceae	Thulo tulasi	Scarlet sage	H, P, M
61	*Sedum rubrotinctum* R.T.Clausen	Crassulaceae		Francesco Baldi	H, P
62	*Sedum morganianum* E.Walter	Crassulaceae		Donkey tail, Burrow tail	H, P
63	*Spathiphyllum floribundum*	Araceae		Peace lily	H, P

S.N.	Scientific Name	Family	Local Name	English Name	Remarks*
	(Linden & Andre) N.E.Br.				
64	*Tagetes erecta* L.	Asteraceae	Sayapatri, Thunge phool	Africal marigold	H, A, M
65	*Tagetes patula* L.	Asteraceae	Sayapatri	French marigold	H, A, M
66	*Tropaeolum majus* L.	Tropaeolaceae		Garden nasturtium, Indian cress	H, A, M
67	*Viola tricolor* L.	Violaceae		Pansy, Heart sease	H, A, M
68	*Zebrina pendula* Schnitzl.	Commelinaceae		Wandering jew	H, P, M
69	*Zephyranthes candida* Herb.	Amaryllidaceae		Zephyr lily	H, P, M
70	*Zinnia elegans* Jacq.	Asteraceae		Pumila liliput	H, A, M

*A = Annual, B = Biennial, C = Climber, F = Food, H = Herb, M = Medicinal, P = Perennial, S = Shrub, T = Tree

Discussion

A total of 70 garden flowers grown in the gardens of Tansen Municipality were studied and listed in this study. The number of perennial herbaceous garden flowers was high in the study area. The perennial plants are popular because these plants always remain green and provide beauty. The herbaceous plants are often grown because such species need small spaces to grow and can be placed in indoor and outdoor spaces. On comparing the total number of garden flowers in this study with previous studies (Bajracharya et al., 1997; Sharma, 2003; Rai et al., 2010; Batth, 2014), the number seemed few. This might be because the study covered only seven wards of Tansen Municipality. If more wards were explored, there would be more specific. So far, the garden flowers of Tansen Municipality were studied for the first time; more extensive study is necessary to have complete information about the garden flowers grown in the study area.

Conclusion

Since ancient times, people are growing garden ornamental plants around their homes and offices to decorate and for a pleasant environment. This study lists 70 various species grown as garden plants in Tansen Municipality. Perennial herbaceous garden flowers are primarily grown in gardens. Some of the ornamental plants also have medicinal and food value. Further research is necessary to list more garden flowers in this municipality.

Acknowledgements

We are thankful to Mr. Chandra Bahadur Thapa, Chairman of the Botany Department, Tribhuvan Multiple Campus for his kind support and suggestions during this research work. It is our privilege to thank former Assistant Campus Chief, Mr. Atiullah Khan for his kind help. We would also like to thank Mr. Toyanath Belbase, Mr. Bal Bahadur Birkatta, Mrs. Meena Devi Lamsal (Sharma), and Mr. Keshav Karki for their continuous support. We are also thankful to local people for their valuable information without whom this work would not be completed.

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Ethnomedicinal Uses of Plants among Newah Community of Chitlang, Makawanpur District, Central Nepal

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Abstract

Diverse ethnic groups of Nepal rely on plants based traditional medicine for relief from different ailments. The research was carried out during 2009 to 2011 in the Chitlang area of Makawanpur district where Newah (Newa, Newar) community is dominantly inhabited. Ethnomedicinal uses of wild and cultivated plants were documented through open interview with local knowledgeable respondents. Altogether 95 medicinal plant species belonging to 92 genera and 54 families were recorded. Out of total species, 51 species were in wild habitat, 35 species cultivated in farmers' home garden and nine species were cultivated and wild habitats. The 95 medicinal plant species were used to treat 49 types of ailments including cough and cold, diabetes, skin diseases, cuts and wounds, blood pressure, burns, itching, gastric, diarrhoea and dysentery, bone fracture, jaundice, etc. The common mode of application of drug was decoction and paste. The commonly used plant parts were leaves, bark, fruit, root, tuber, rhizome and bulb. The traditional knowledge related to health care by using medicinal plants help to aware the people and concerned institutions for conservation of such important plants before they are lost forever.

Key words: Conservation, Illnesses, Medicinal plants, Traditional knowledge, Utilization

Introduction

Wild plants collection for medicine is an ancient tradition in Newah (Newar) communities. The Newah communities live in rural and peri-urban regions of Makawanpur district and have a rich indigenous knowledge for utilization of traditional medicine. Utilization of medicinal plants for health care is demanding in all over the world (WHO, 2013). However, uses of traditional medicinal knowledge to young generation are losing. In Nepal common diseases in rural areas are fever, diarrhoea, dysentery, cough, cold, skin diseases, itching, blood pressure, diabetes, gastric, rheumatism, typhoid, heart diseases (NHRC, 2019). About 80-85% of the population depends upon use of medicinal plants for their healthcare (Manandhar, 2002). Local knowledge for medicinal use is a social product that has been part of the specific cultural system (Antweiler, 1998). Nepal has 125 ethnic groups (NPHC, 2012), each ethnic group has a rich traditional knowledge for utilization of plants for medicines and other uses. Various ethnobotanical studies on medicinal plants have encompassed in Chepang (Manandhar, 1989), Tamang (Manandhar, 1991; Shrestha & Siwakoti, 2009), Tharu (Dangol & Gurung, 1991), Limbu (Siwakoti & Siwakoti, 1998), Satar (Siwakoti & Siwakoti, 2000), Gurung (Shrestha et al., 2001), Meche (Rai, 2004), Newah (Balami, 2004;

Joshi & Siwakoti, 2020, Joshi et al., 2020), and Bankaria (Uptrety et al., 2008).The present study documented the indigenous knowledge on medicinal plants that are used by the Newah community of Chitlang village.

Materials and Methods

Study Sites

Chitlang village is located in Makawanpur district, Narayani zone, Bagmati Province, Nepal (Fig. 1). This village is bounded by Phakhel to the east, Markhu to the south, Bajrabarahi to the west and Kathmandu to the North. Historically, Chitlang is connected with the Emperor Ashok who had visited Nepal during 273-232 BC or 2265 years ago and built an Ashok pillar. This Ashok pillar was called Chaitya. Chitlang village was formerly well known village as Chilanche (ci hai-gu lang in Newar) or Chitrapur due to presence of Chaitya. The total area of this village covers 33 sq. km. This place is an ancient Newah ethnic settlement, located between 1700 m to 1800 m altitude above sea level. It is situated in between latitude 27º38'55.06"N and longitude 85º10'42.16"E. The total population of this village is 9138, of which 4549 are females and 4589 males in 1366 households (CVDC, 2006). Among 1366 households, Newar ethnic group holds 841 households (61%), i.e., about 6000 Newar population lives in Chitlang village, including Balami (3000), Gopali (1600), Gamal (1000) and Dyola (400) are most dominated (CVDC, 2006). The ethnic composition of Chitlang covers Newar (61%), Tamang (13%), Kshetri (11%), Brahmin (8%), Magar (4%), and others (3%). The occupation of most of the ethnic community is agriculture and trades.

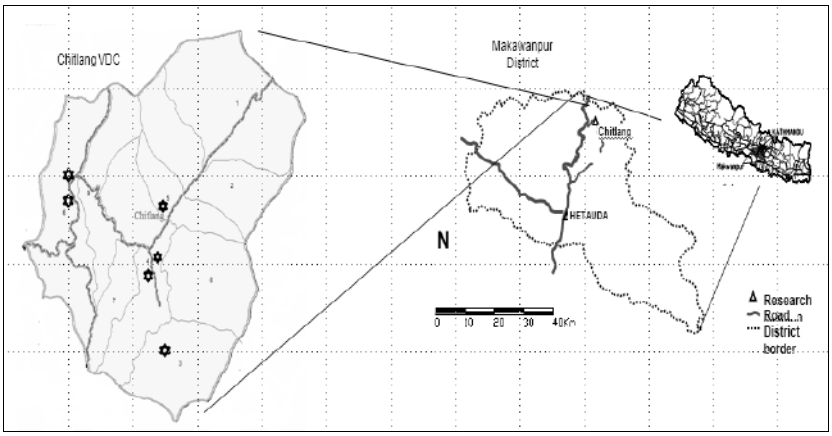

Figure 1. Map showing study site of Chitlang

Data Collection

Field visits were carried out in Chitlang village between 2009 and 2011. A focus group discussion was organized with local people including village head, key informants, healers to explain the aim of research and to receive the consent from the respondents. Ethnomedicinal knowledge of plants was collected through forest walks with knowledgeable people and open interviews were conducted with 34 respondents of both male and female. The age group of

respondents was ranged from 28 to 77 years, whereas, the number of female respondents was 20 (59%) and male 14 (41%). The respondents of the Newar community were selected from then Chitlang VDC of Kapugaon Ward No. 3, Narayanahiti, Kuchabu, Podegaon, Tupikhel Ward No. 4, Majhgaon Ward No. 5, Taukhel, Nhulgaon Ward No. 6, Tallo Bishinkhel Ward No. 9. (Fig. 1).

Medicinal plants were collected from natural habitat and cultivated field. Local names of medicinal plants, plant part used, kind of illness treated were recorded. During the interviews, herbarium specimens, plant photographs were also shown to respondents for the correct identification of plant names. Medicinal plant species were identified in the field with the help of respondents' given local names, and identification was done by personal expertise. The identified scientific name and families of medicinal plants were checked with www.catalogueoflife.org. The status of plant species was collected during field visit observation.

The use categories of illness were recorded from International Classification of Primary Care (ICPC) and listed by the WONCA (World Organization of Family Doctors) WONCA International Classification Committee (2005).

All the medicinal plant species are arranged in alphabetical order by scientific names, followed by family, habit, local name (Newar name), status, plant part, illness, no. of citations and collection number. The collection number is given for wild species.

Results

Enumeration of Medicinal Plants

Altogether 95 species of plants belonging to 54 families and 92 genera were recorded (Table 1). Asteraceae was the largest family with eight medicinal plant species followed by Fabaceae with five species, and Apiaceae, Cucurbitaceae, Lauraceae with four each. Out of 95 species, 49 species were herbs followed by 25 species of trees, 13 shrubs and eight climbers. Regarding the plant part used to cure diseases, leaves were the mostly used (27 species), followed by fruit (18 species), seed (15 species), root/rhizome/bulb (14 species), bark (7 species), stem and tender shoot (7 species). A few species were cited for flower (3 species), entire plant (3 species), and wood (1 species). Most of medicinal plants were collected from wild (51 species) and 35 species from cultivated land or local market, while nine species were found both in wild as well as cultivation. These wild and cultivated medicinal species were *Cinnamomum tamala, Prunus cerasoides, Terminalia chebula, Phyllanthus emblica, Choerospondias axillaris, Piper longum, Mentha spicata, Zanthoxulum armatum* etc. Some medicinal plants *w*ere also planted in their home gardens, mainly for vegetable (*Bauhinia variegata*), fodder and vegetable (*Ficus concinna*), pickles and medicine (*Mentha spicata*), fuel and timber (*Prunus cerasoides*).

Illness Treated and Use Categories

A total of 95 medicinal plant species were used for curing about 49 types of illnesses according to the 34 respondents (Table 2). These 49 illnesses were classified into 11 use categories out of the 17 categories according to the International Classification of Primary Care (ICPC)

Table 1. Enumeration of 95 medicinal plants used by Newar ethnic of Chitlang, including scientific name, family, habit, Newar name, status, plant part, illness, No. of citation.

Scientific name	Family	Habit	Newar name	Status	Plant part	Illness	No. of citation	Collection no.
Acorus calamus L.	Acoraceae	H	Bisaha	C/W	Rhizome	Cough, Throatsore	2	0931156 NJ
Ageratum conyzoides L.	Asteraceae	H	Ramune	W	Leaves	Cut & wound	8	0911303 61NJ
Allium cepa L.	Amaryllidaceae	H	Pyaj	C	Bulb	Blood pressure	7	x
Allium sativum L.	Amaryllidaceae	H	Laba	C	Bulb	Blood pressure	1	x
Alnus nepalensis D. Don	Betulaceae	T	Gwayachhasi	W	Bark	Burns, Cut & wound	3	0112567 2NJ
Aloe vera (L.) Burm. f.	Asphodelaceae	H	Kunhu	C	Leaf pulp	Blood pressure, Headache, Burn, Diabetes	4	x
Amaranthus caudatus L.	Amaranthaceae	H	Bakacha	W	Root	Fever, Diarrhoea & dysentery	1	0113157 1NJ
Amaranthus spinosus L.	Amaranthaceae	H	Kabanka	W	Leaves	Weakness, Urine problem	1	0113177 36NJ
Anaphalis busua (Buch.-Ham.) Hand.-Mazz.	Asteraceae	H	Buswan/ Bhorighyan	W	Leaves	Cut & wound	2	0101044 69NJ
Artemisia indica Willd.	Asteraceae	H	Khafya/ Dhuswa	W	Leaves	Ringworm/Cut & wound	1	0112464 2NJ
Asparagus filicinus Buch.-Ham. ex D. Don	Asparagaceae	H	Kurilo	W	Tender shoot	Weakness, Fever	2	0112462 6NJ
Astilbe rivularis Buch.-Ham. ex D. Don	Saxifragaceae	H	Fakuwasa	W	Rhizome	Delivery Stomachache	9	0101010 586NJ
Azadirachta indica A. Juss.	Meliaceae	T	Niha	C	Leaves, Stem	Blood pressure, Toothache	1	x
Baccharoides anthelmintica (L.) Moench	Asteraceae	H	Haji	C	Seeds	Diabetes, Worm parasites	1	x
Bauhinia variegata L.	Fabaceae	T	Vabro/Varabo	C	Flower/ Flower bud	Blood pressure, Diarrhoea & dysentery	5	0951151 NJ
Begonia picta Sm.	Begoniaceae	H	Makarche	W	Entire plant	Leech bite, Itching, Wound in between toes	4	0982324 5NJ
Berberis aristata DC.	Berberidaceae	S	Chhurasi	W	Root	Eye problem	1	0951130 NJ
Bergenia ciliata (Haw.) Sternb.	Saxifragaceae	H	Quatiwasa	W	Rhizome	Post-delivery stomachache	9	0981521 3NJ
Blumea lacera (Burm. fil.) DC.	Asteraceae	H	Khichabhwath	W	Leaves	Post-delivery stomachache, Post-delivery ovarian disease, Loss of lactation	5	0931160 NJ

Scientific name	Family	Habit	Newar name	Status	Plant part	Illness	No. of citation	Collection no.
Bombax ceiba L.	Malvaceae	T	Simaha	W	Dried flower calyces	Dysentery, Sotmachache	1	092936 NJ
Brassica juncea (L.) Czern.	Brassicaceae	H	Tu chika	C	Seed oil	Bone weakness, Cold	5	x
Cannabis sativa L.	Cannabaceae	H	Lupu	W	Seed	Diarrhoea & dysentery	5	0115156 NJ
Carica papaya L.	Caricaceae	T	Mewa	C	Unripe fruit	Diarrhoea & dysentery, Jaundice	5	x
Castanopsis indica (Roxb.ex Lindl.) A. DC.	Fagaceae	T	Syanguli	W	Fruits	Weakness	5	0101085 08NJ
Centella asiatica (L.) Urb.	Apiaceae	H	Hiloghayn	W	Leaves	Appetite loss, Jaundice, Ring worm, Skin inflammation	9	0101010 571NJ
Chenopodium album L.	Amaranthaceae	H	Ika	W	Leaves	Eye sight	1	0931164 NJ
Choerospondias axillaris (Roxb.) B. L. Burtt & A. W. Hill	Anacardiaceae	T	Amali	C	Seed	Burns, Cough & cold	4	0992428 0NJ
Cinnamomum camphora (L.) J. Presl	Lauraceae	T	Kapu	C	Leaf, seed	Cough & old	1	x
Cinnamomum tamala (Buch.-Ham.) T. Nees & Eberm.	Lauraceae	T	Tejpat	C/W	Bark	Blood pressure, Heart disease, Cough & cold	1	091252 NJ
Cirsium wallichii DC.	Asteraceae	H	Chwanka	W	Stem pith	Urine problem	2	0981524 1NJ
Citrus x aurantifolia (Christm.) Swingle	Rutaceae	T	Jhamsi	C	Fruit	Cold	1	x
Coriaria nepalensis Wall.	Coriariaceae	S		W	Fruit	Weakness	1	092839 NJ
Cucumis sativus L.	Cucurbitaceae	C	Tusi	C	Seed	Mouth ulcer	1	x
Cuminum cyminum L.	Apiaceae	H	Jee	C	Seeds	Cough & cold	2	x
Curcuma longa L.	Zingiberaceae	H	Halu	C	Rhizome	Asthma, Throatsore, Burn, Cough & cold	15	x
Cynodon dactylon (L.) Pers.	Poaceae	H	Situ	W	Leaves	Cut & wound, Sinusitis	1	0992436 3NJ
Cynoglossum zeylanicum (Vahl) Thunb. ex Lehm.	Boraginaceae	H	Wachuwasa	W	Leaves	Cut & wound	2	0112462 8NJ
Datura stramonium L.	Solanaceae	H	Dhatuma	W	Leaves	Cut & wound	1	0982391 2NJ
Drymaria cordata (L.) Willd. ex Roem & Schult.	Caryophyllaceae	H	Kholchaghye	W	Leaves	Gastric, Sinusitis, Skin inflammation	3	0951132 NJ

Scientific name	Family	Habit	Newar name	Status	Plant part	Illness	No. of citation	Collection no.
Eclipta prostrata (L.) L.	Asteraceae	H	Atalicha	W	Tender shoot	Eye sight	2	09823266NJ
Equisetum ramosissimum Desf.	Equisetaceae	H		W	Entire plant	Toothache, Boils	1	01131774 5NJ
Euphorbia hirta L.	Euphorbiaceae	H		W	Leaves	Wound	1	0951140 NJ
Ficus concinna (Miq.) Miq.	Moraceae	T	Kavro	C	Latex	Boils	1	09320611NJ
Foeniculum vulgare Mill.	Apiaceae	H	Tagajee	C	Seeds	Diarrhoea & dysentery	7	x
Gaultheria fragrantissima Wall.	Ericaceae	S	Charasin	W	Fruit	Worms & parasites	2	0101010 585NJ
Hedera nepalensis K.Koch	Araliaceae	C	Kaikal	W	Leaves/Frui t	Scabies/Skin disease	1	09103308NJ
Helianthus annuus L.	Asteraceae	H	Suryamuki Swan	C	Seeds	Mouth ulcer	1	**x**
Juglans regia L.	Juglandaceae	T	Khosi	C/W	Fruit shell/ Leaves	Toothache	1	0932687 NJ
Justicia adhatoda L.	Acanthaceae	S	Aleha	W	Leaves	Itching, Bone fracture	8	01124644NJ
Lindera neesiana (Wall. ex Nees) Kurz	Lauraceae	T	Katabasi	W	Fruit	Stomachache	1	09711183NJ
Linum usitatissimum L.	Linaceae	H	Tisi	C	Seeds	Cholesterol, Constipation	2	x
Lobelia nicotianifolia Roth	Campanulaceae	H	Eklebir	W	Leaves	Fever	1	09424103NJ
Lycopodium japonicum Thunb.	Lycopodiaceae	C	Banmala	W	Spores	Cracks of feet	2	01125668NJ
Lyonia ovalifolia (Wall.) Drude	Ericaceae	T		W	Leaves	Skin diseases	1	01124627NJ
Machilus duthiei King ex Hook. fil.	Lauraceae	T	Fawyanakal	W	Wood/Bark	Skin diseases	3	09823526NJ
Macrotyloma uniflorum (Lam.) Verdc.	Fabaceae	C	Kola	C	Seeds	Kidney stone	3	x
Mahonia napaulensis DC.	Berberidaceae	S	Mitasi	W	Bark	Eye inflammation	1	0116199 04NJ
Mangifera indica L.	Anacardiaceae	T	Aa	C	Endocarp	Diarrhoea & dysentery	1	x
Meizotropis buteiformis Voigt	Fabaceae	S	Palavi	W	Fruit	Worms & parasites	6	0116199 04NJ
Melia azedarach L.	Meliaceae	T	Khaeebasi	C/W	Fruit	Worms & parasites	1	x
Mentha spicata L.	Lamiaceae	H	Haswanghya	C	Leaves	Cough & cold, Gastric, Skin inflammation, indigestion	3	09823249NJ
Momordica charantia L.	Cucurbitaceae	C	Khaeekakacha	C	Fruit	Diabetes	1	**x**
Morella esculenta	Myricaceae	T	Kawasi/	W	Bark	Toothache	3	0112462

Scientific name	Family	Habit	Newar name	Status	Plant part	Illness	No. of citation	Collection no.
*(*Buch.-Ham. ex D. Don) I.M.Turner			Kapase					3NJ
Musa paradisiaca L.	Musaceae	S	Kera	C	Unripe fruit	Diarrhoea & dysentery	1	x
Myristica fragrans Houtt.	Myristicaceae	T	Jifo	C	Seeds	Weakness, Stomachache	1	x
Nicotiana tabacum L.	Solanaceae	H	Surti	C	Leaves, oil	Itching, Skin diseases	1	x
Ocimum tenuiflorum L.	Lamiaceae	H	Tulsi	C	Leaves	Cold and cough	4	x
Osbeckia chinensis L.	Melastomaceae	H	Jogiamkhara	W	Leaves	Burns	1	0112566 7NJ
Oxalis corniculata L.	Oxalidaceae	H	Paulaghyan	W	Leaves	Ringworm	2	092953 NJ
Phyllanthus emblica L.	Phyllanthacae	T	Ambali	C/W	Fruit	Appetite loss, Constipation, Vitamin deficiency,	3	0101044 54NJ
Phyllanthus parvifolius Buch.-Ham. ex D. Don	Phyllanthacae	S	Chhusyaguli	W	Leaves/Frui t	Bone fracture	1	0112462 2NJ
Pieris formosa (Wall.) D. Don	Ericaceae	S	Pote	W	Young leaves	Scabies	2	0910331 0NJ
Piper longum L.	Piperaceae	C	Pipi	C	Fruits	Cough & cold	1	0101085 36NJ
Potentilla indica (Andr.) Wolf	Rosaceae	H	Dyapasi, Bwakapasi, Byakapasi	W	Fruits	Mouth ulcer	1	0924117 NJ
Prunus cerasoides D. Don	Rosaceae	T	Fasipa	C/W	Stem bark	Burns	4	0112464 1NJ
Psidum guajava L.	Myrtaceae	T	Amasi	C	Leaves	Toothache	2	x
Punica granatum L.	Lythraceae	S	Dhale	C	Fruit shell	Diarrhoea & dysentery	1	x
Ricinus communis L.	Euphorbiaceae	S	Alama	W	Seeds oil	Heel cracks	1	x
Rubus ellipticus Sm.	Rosaceae	S	Airsi/Yansi,	W	Fruit/root	Cough & cold, Diarrhoea	3	0112569 3NJ
Saccharum officinarum L.	Poaceae	H	Tu	C	Stem	Jaundice	1	x
Schima wallichii (DC.) Korth.	Theaceae	T	Gwayachhasi	W	Wood	Heal cracks	2	092943 NJ
Senegalia catechu (L.f.) Hurter & Mabb.	Fabaceae	T	Hikhyo	C	Wood	Toothache	3	0113202 2NJ
Solanum lycopersicon L.	Solanaceae	H	Golveda	C	Fruit	Burns	1	x
Solena amplexicaulis (Lam.) Gandhi ex Saldanha & Nicolson	Cucurbitaceae	C	Talansi/ Kothuse. Gutusi	W	Root/seeds	Fever, Urinary inlfammation/Post-delivery stomachache	6	0101044 77NJ
Sonchus arvensis L.	Asteraceae	H	Khaeke	W	Leaves	Fever, Diabetes	1	0912780 NJ
Swertia chirayita	Gentianaceae	H	Khalu	C/W	Entire plant	Fever, diabetes, blood	9	0101010

Scientific name	Family	Habit	Newar name	Status	Plant part	Illness	No. of citation	Collection no.
(Roxb.) H. Karst.						pressure		587NJ
Terminalia chebula Retz.	Combretaceae	T	Hala	C/W	Fruit	Cough	1	010104463NJ
Thalictrum foliolosum DC.	Ranunculaceae	H	Ganuwasa	W	Root	Gastric	1	09815209NJ
Trachyspermum ammi (L.) Sprague	Apiaceae	H	Imu	C	Seeds	Cough & cold	14	x
Trichosanthes wallichiana (Ser.) Wight	Cucurbitaceae	C	Kokochasin	W	seed	Fever	1	010108520NJ
Trigonella foenum-graecum L.	Fabaceae	H	Mee	C	Seeds	Diabetes, Cholesterol, Blood pressure	1	x
Urtica dioica L.	Urticaceae	H	Nhake	W	Leaves	Blood pressure, Kidney stone, Diabetes, Vitamin deficiency	14	092930 NJ
Zanthoxylum armatum DC.	Rutaceae	S	Tepura/ Tebasi	C/W	Fruit, stem	Toothache, Gastric	7	0942494 NJ
Zephyranthes carinata Herb.	Amaryllidaceae	H	Mahariswaya	W	Bulb	Wound	1	0992431 1NJ
Zingiber officinale Roscoe	Zingiberaceae	H	Palu	C	Rhizome	Appetite loss, Cough & cold	8	x

Life forms: C = Climber, H = Herb, S = Shrub, T = Tree; Status: C = Cultivated, W = Wild, C/W = Cultivated/Wild

and listed by the WONCA (World Organization of Family Doctors) (WONCA International Classification Committee (2005).

The largest numbers of plant species (13 species) were used for the treatment of cough and cold, followed by eight species each to treat for blood pressure and diarrhoea and dysentery, respectively, seven species each for fever and toothache, respectively whereas five species each for using burns and diabetes, respectively (Table 1). The most common medicinal plant species mentioned by total 34 respondents were from wild species, i.e., *Urtica dioica* (14 respondents), *Swertia chirayita* (14 respondents) and cultivated species, i.e., *Curcuma longa* (15 respondents) and *Trachyospemum ammi* (14 respondents) (Table 1).

Discussion

The utilization of medicinal plants for various illnesses in Newar community in this area have indigenous knowledge for health care. The present study identified 95 medicinal plant species from Newar ethnic of Chitlang, Makawanpur District. When comparing the species names of the present study with earlier published studies from Newar community from Kathmandu District (Balami, 2004) and Makawanpur District (Joshi et al., 2020), this study documented 26 and 52 similar species that were utilized in different diseases, respectively.

Higher number of useful medicinal plant species belonging to the family Asteraceae and Fabaceae are species rich families in Nepal and elsewhere as well as most commonly

Table 2. Eleven different use categories and the 49 illnesses treated following the classification of WONCA (World Organization of Family Doctors) WONCA International Classification Committee (2005) according to 34 respondents in Chitlang, Makawanpur district, Nepal.

S.N.	Use category	Illness	Total plant species
1	Cardiovascular	Blood pressure, Heart disease, Cholesterol	9
2	Digestive	Jaundice, Mouth ulcer, Stomachache, Diarrhoea and dysentery, Gastric, Indigestion, Toothache, Worms and parasites, Constipation	30
3	Endocrine/Metabolic and Nutrition	Appetite loss, Diabetes, Vitamin deficiency	9
4	Eye	Low eye sight, Eye problem, Eye inflammation	4
5	General and unspecified	Fever, Weakness	12
6	Musculoskeletal	Bone weakness, Bone fracture	2
7	Neurological	Headache	1
8	Pregnancy, Child bearing, Family planning	Post-delivery stomach ache, Post-delivery ovarian disease, Loss of lactation	4
9	Respiratory	Cough and cold, Throat sore, Cold, Asthma, Tonsillitis, Cough, Sinusitis	13
10	Skin	Boils, Burns, Feet crack, Hair loss, Heel crack, Itching, Leech bite, Pimples, Ringworm, Scabies, Wound in between toes, Cut and wounds, Skin diseases, Wound in between toes, Skin inflammation	31
11	Urological	Kidney stone, Urine inflammation, Urine problem,	5

Note: Six use categories of WONCA were not covered in the present study.

found in the present study sites as well. Many previous studies carried out in Central Nepal (Uprety et al., 2010; Shrestha et al., 2014), have also recorded high number of medicinal plant species belonging to the family Asteraceae, Rutaceae, Gentianaceae, Polygonaceae and Saxifragaceae. Regarding plant parts used, out of 95 species, 27 species of leaves were used for various medicinal purposes. Similar study of utilization of leaf parts was reported in Makawanpur district by (Joshi et al., (2020). With regard to locations for collections of medicinal plants, 51 species were collected from wild habitat such as forest, fallow land, whereas 35 species were from cultivated land (home garden and farmer field) and nine species were from both wild and cultivated lands. Collection of medicinal plants from wild habitat is also reported in previous ethnomedicinal studies done in Rasuwa district (Uprety et al., 2010) and Makawanpur district (Joshi et al., 2020). Due to unsustainable collection and overexploitation of plants from natural habitat, these days more valuable medicinal plants is being threaten in the study area according to respondents. However, *Acorus calamus*, *Bauhinia variegata, Choerospondias axillaris, Cinnamomum tamala*, *Juglans regia*, *Justicia adhatoda*, *Mentha spicata, Prunus cerasoides, Swertia chirayita* and *Zanthoxylum armatum* being cultivated in home gardens show highly valuable species to the local communities.

Cough and cold diseases were found the most common diseases in community and *Acorus calamus, Choerospondias axillaris, Cinnamomum tamala, Mentha spicata, Piper longum, Rubus ellipticus* were used to relief cough. During utilization of medicinal plants collection, single plant species *Centella asiatica* was used for many diseases such as appetite loss,

jaundice, ringworm, skin inflammation. Similarly, *Urica dioica* was used for blood pressure, kidney stone, diabetes, vitamin deficiency and *Mentha spicata* in cough and cold, gastric, indigestion.

Conclusion

Indigenous knowledge on medicinal plants for treating various diseases are still used in the Newah community of Chitlang. They also plant some wild medicinal plants in their home gardens for various uses. It indicated that domestication and cultivation of most important species should be emphasized to ensure continuous supply of the raw materials and conservation of the genetic resources. More research is needed to perform studies on the pharmacological evaluation of the most important medicinal plant species, such information is largely missing. This could also help to aware the community for conservation of such important plants before they are lost forever.

Acknowledgements

The authors are grateful to the respondents of Newah ethnic communities of Chitlang village for sharing their valuable indigenous knowledge of medicinal plant use and for their assistance during field work. We are also thankful to the Chitlang Village Development Committee, for providing necessary documents. The Nepal Academy of Science and Technology (NAST) is highly acknowledged for awarding a fellowship to the first author to conduct the research.

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Ethnobotanical Study of Medicinal Plants of Banskharka Community Forest, Kabhrepalanchowk District, Nepal

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Abstract

This study was conducted to identify medicinal plants of Banskharka Community Forest user group (CFUG), Mandandeupur Municipality, ward no.12, Kabhrepalanchwok district of Central Nepal. The main characteristic features of the study area were the endowment of medicinal resources. The data to require the objective of the study consists of resource data and socio-economic data. Both primary and secondary information sources were used. Primary data were collected from the study area through participatory resource mapping, questionnaire survey, informal meeting along with local healers, village persons, experienced knowledgeable persons, key informant survey and observation, household survey as well as personal contact with local ethnic groups, Vaidhyas, Herbalist, and medicinal plant collectors and traders. Secondary data were collected from published materials regarding medicinal plants. Altogether 46 species were identified and listed and consist of different life forms such as herbs, shrubs, trees and climbers. Generally, the local people use these species to cure and treat common human disorders like diabetes, fever, headache, cold and cough, dysentery, stomach pain, urinary disorders, injuries and wounds. It was found that Brahmins and Chhetry's were more than Tamang's and Rai's in the study area. The most preferred species by the local people in their CF in descending order are namely Alaichi (*Amomum subulatum*), Pakhanbed (*Bergenia ciliata*), Jatamasi nakkali (*Valeriana hardwickii*), Chutro (*Berberis aristata*), Dhasingre (*Gaultheria fragrantissima*), Timur (*Zanthoxylum armatum*), Thulo Okhati (*Astilbe rivularis*), Utis (*Alnus nepalensis*), Aiselu (*Rubus ellipticus*), Siltimur (*Litsea cubeba*).

Key words: Brahmins, Chhetry's, Ethnobotany, Rai's, Tamang's, Vaidyas.

Introduction

Nepal is a small hilly country with its land area of 1, 47,181 sq. km. It lies between the latitudes of 26°22' to 30°27'N and longitudes of 80°04' to 88°12'E occupying the central of the massive Himalayan chain 2500 km long. It has a length of about 885 km and average width of 193 km. the area covers 0.03% of land area of the earth (Rijal, 2016). There is remarkable altitudinal variation with this small area ranging from about 57 m above mean sea level in Terai to 8848 m in the high mountain. It presents several habitats on the slopes of the Himalayan Mountains that contributed to the richness of biodiversity. Its' physiographic diversity under five broad categories, viz., Terai, Siwalik, Midhill, High Mountain and High Himalaya, accommodates six different bio-climatic zones. As a result, it presents the dense tropical forest of the Terai in the South that gradually changes over to sub-tropical and lower temperate, upper temperate broad-leaved and coniferous forest in the middle region sub-alpine and alpine vegetation in the Himalayas.

The term NTFPs (Non-Timber Forest Products) encompasses all biological materials other than timber, fuelwood, and fodder which are extracted from the natural forest for human use. These include foods, medicines, ornamental and aromatic plants, gums and resins, dyes and tannins and raw materials, notably rattan, bamboo, small wood and fibres. Man's dependence on plants for his existence dates back to the beginning of the human race. NTFPs play a crucial role in supporting the livelihood of rural people. The rural people of Nepal depend on plant resources to sustain their life. They derive food, fodder, timber, building material and medicine from plants. The country comprises about 7000 plant species. More than 800 species are reported to be medicinal value, about 100 species are fodder, 70 for fibre and 450 species for food have been utilized by the rural people (Manandhar, 1995). About 70-80% of the rural population in the mountainous region have since time immemorial been involved in collecting NTFPs for sale and household use because there are no medicinal facilities in most of Nepal's remote hilly areas.

In 1895, the American botanist John W. Harshberger first applied the term ethnobotany to studying plants used by primitive and aboriginal people. Later ethnobotany was described as "the study of direct interaction between human and plant population through its culture. Each human population classifies plants develop attitudes and beliefs, and learns the use of plants. In contrast, human behaviour has a direct impact on plant communities with their interaction. The plant themselves also impose a limitation on humans; these mixture interactions are the focus of ethnobotany" (Ford, 1978).

Today ethnobotany is widely accepted as a science that investigates human interaction with plants and their ecosystem. The most recent development in ethnobotany in some Hindu Kush Himalaya (HKH) region (China, India, Nepal and Pakistan) has been towards traditional herbal medicine indigenously managed plant resources, traditional agroecosystem and cultural interpretation of the plant world. There is a focus on minorities and the values of the ethnobotany in rural development and biodiversity conservation. These new concerns are allied with a strong applied approach.

More than 60 ethnic groups reside in different geographic belts in Nepal, speaking about 75 languages and are rich in indigenous knowledge about plant in medicine (Manandhar, 1998). Most of the tribal people are very poor economically. For their health care, they depend on local plant-based therapy, which is cheap and readily available. Moreover, they believe that these traditional remedies are effective; Sherpa, Tamang, Rai and Tharu are some of the oldest ethnic groups of Nepal. Ethnobotany of various ethnic groups of Nepal has been studies, viz., Sherpa (Sacherer, 1979; Bhattarai, 1989), Tharu (Manandhar, 1985; Dangol & Gurung, 1991; Acharya & Acharya, 2009; Chaudhary & Rai, 2017; Singh, 2017), Mooshar (Manandhar, 1986), Chepang (Manandhar, 1989; Basnet et al., 1998; Khan, 1998), Danuwar (Manandhar, 1990), Tamang (Manandhar, 1991; Tamang, 2003), Satar (Siwakoti et al., 1997), Gurung (Shrestha, 1998; Shah et al., 2019), Limbu (Siwakoti & Siwakoti, 1998; Limbu & Rai, 2013), Rajbansi (Karki, 1998), Raute (Manandhar, 1998), Aathpaharia Rai (Dahal, 1999), Darai (Dangol & Gurung, 1999), Meche (Rai, 2004), Bantar (Acharya & Pokhrel, 2006), Magar (Ale et al., 2009; Singh et al., 2018), Raji (Thapa et al., 2013), Lapcha (Tamang & Singh, 2014), Rai (Rai & Singh, 2015), Sardar and Malaha (Joshi &

Baidar, 2018), Thami (Bhattarai, 2018). In the present study, we have tried to determine the medicinal values of different locally available plants in Banskharka Community forest of Kabhrepalanchwok District.

Materials and Methods

The present study aims to review the indigenous knowledge and use of plant resources and sketch the interrelationship between humans, plants, and their ecosystem. The indigenous knowledge perceived from human societies on plants use was collected by interviewing local people. The study area was visited frequently from November 2018 to February 2019 in Banskharkha CF of Kabhrepalanchowk. Different ethnic people, mainly Brahmin, Chhetri's, Rai's, and Tamang, were taken as resource persons in this research. The different plant specimens collected from the study area were identified by their local name, parts use, and purpose. For the scientific approach, collected plant specimens were compared with the herbarium of Tri-Chandra Campus, Botany Department, to confirm the identification. The data collection was carried out based on primary data collection and secondary data collection.

Banskharka Community Forest is located in Mandandeupur Municipality, Ward No. 12 of Kavrepalanchowk District (Fig. 1). The altitude variation of the district is 275-3018m. The Community Forest occupies an area of 192ha. The average rainfall is 1581mm with a temperature range 10-31°C. The vegetation is tropical and subtropical type.

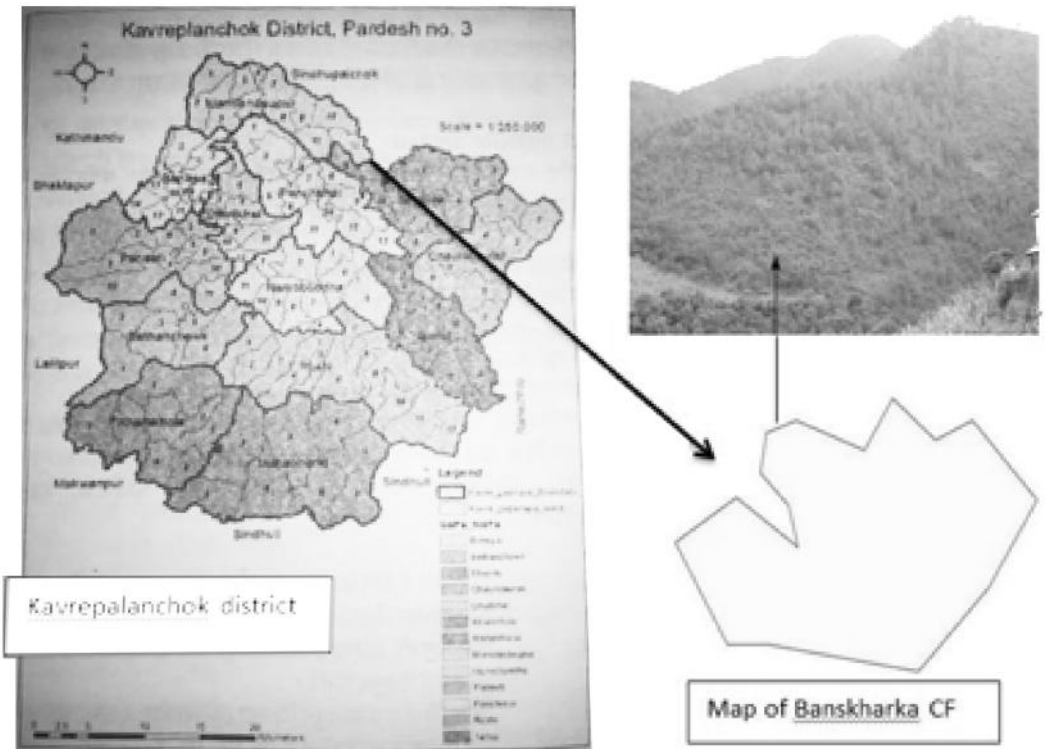

Figure 1. Map of study area.

Primary Data Collection

Key informants survey

Local healer, Vaidhya, local elites, older people, local leaders, Lama etc. were taken as key informants for getting different kinds of information. At first, the objectives of research were convinced to key informants before data collection. This was done to ensure informants co-operation and make them comfortable to provide reliable information.

Participatory resources mapping

Participatory resources map of the community forest was drawn to identify the medicinal plant available areas inside the community forest. The committee members of CF and key informants were asked to prepare the medicinal plants' participatory resources map.

Group discussion

Group discussion was conducted to obtain information about the dependency of people and the abundance of medicinal plants. It also provided information about people's knowledge to present trading chain, use, price, etc. Importance in health care, access to medicinal plants, plants' availability in their CF, storage of plant for future use, daily household use of plants, and market demand of particular medicinal plants were also discussed.

Interview

It included formal and informal interviews with government staff (DFO, Ranger, and forest guard), key informants, collectors, and road head traders. Altogether 45 informants were interviewed of age group 20-60 from CF. The questionnaire comprising local name, parts use, resource conditions and local uses were administered during interview and group discussions (Martin, 1995).

Direct field observation

Since the research work focused on ethnobotany, field visits were made targeting indigenous inhabitants' areas. The study area was observed to know the actual way of using plants and their importance in their daily lives. Based on the need, one of the local inhabitants was taken for the survey to the field. The collected plant species were consulted with local healers to confirm their medicinal uses. The information collected from the group discussion, participatory mapping, and key informant interview were verified through actual observation.

Matrix ranking

The potential medicinal plants were listed after the detail participatory inventory. Then the criteria and indicators for the preferred species were identified through group discussion. As it is used to compare services available, few informants were selected and discussed the plants on selected criteria to elicit information. The ranks were given accordingly by scores (1-3), the lowest score being 1 and the highest score of 3 on each criterion. Finally, matrix ranking was conducted to identify up to the top ten preferred species.

Secondary Data Collection

Secondary data regarding the research was collected from different published and unpublished literature in DFO Office and TU Library. Review of different literature was carried out with the help of books, journals, articles and from internet resources. Operational plan of community forest was also reviewed.

Results

Altogether 46 plant species were identified and listed as medicinal plants from the study area, i.e., Banskharka CF and its adjoining areas. Out of the listed forms are trees-13, climbers-2, shrubs-14, herbs-15, and grasses-2 (Table 1). Most of the medicinal plant species available in the study area were used as traditional medicine. Traditional medical practitioners in the area were engaged in folklore medicines, and few Lama's, Vaidya's were practising local medicinal treatment in this area. Most of the people believed in such treatment. Present practitioners hesitate to give all information regarding the knowledge and practice they use. They think if they transfer all knowledge without reservation, their popularity in society will be lost. It indicates that local medical practitioners' indigenous knowledge and used practice was not properly transferred to the new generation. They were very conservative. This was a substantial drawback of the knowledge transfer in society. Some of the essential medicinal plants commonly used in the study area were Chutro (*Berberis aristata*), Bojho (*Acorus calamus*), Pakhanbed (*Berginia ciliata*), Titepati (*Artimisia vulgaris*), Ghodtapre (*Centella asiatica*), Ghiukumari (*Aloe vera*), Thulo okhati (*Astilbe rivularis*) etc.

Medicinal plants were applied to recover from different types of diseases like sinus (pinas), jaundice, abdominal pain, eye and skin diseases, urinary problems, cold and cough, fever, etc. Along with medicinal properties, medicinal plants were also used for various religious purposes, construction works, food and vegetables, fibres, oils and other purposes. Different parts of the plant, including fruit, seed leaf, root, stem, bark, bud, rhizome, flower, and sap, were traditionally used (Table 1).

Table 1. Ethnobotanical uses of identified medicinal plants

S.N.	Nepali Name	Botanical name/Family	Habit	Parts used	Ethnobotanical use
1	Abijalo	*Drymaria diandra* Blume. (Caryophyllaceae)	Herb	Whole plant	Used in Pinas (Nepali term for sinus)
2	Aiselu	*Rubus ellipticus* Sm. (Rosaceae)	Shrub	Flower, roots, fruits	fruit edible, whole plant used in abdominal pain
3	Akasbeli	*Cuscuta reflexa* Roxb. (Cuscutaceae)	Climber	Leaves	Juice used in Jaundice treatment
4	Alaichi	*Amomum subulatum* Roxb. (Zingiberaceae)	Herb	Fruit	Spices and medicinal use
5	Amliso	*Thysanolaena maxima* Roxb. (Poaceae)	Herb	Inflorescence and roots	Inflorescence are used to prepare broom; grass; erosion control; root is used as a paste for measles and juice in jaundice
6	Angeri	*Lyonia ovalifolia* (Wall.) Drude. (Ericaceae)	Medium sized tree	Leaves	Used as fuel, insecticides and to treat skin diseases of parasitic origin
7	Asuro	*Justicia adhatoda* L.	Shrub	Whole plant	Used in treatment of gastric; good for

S.N.	Nepali Name	Botanical name/Family	Habit	Parts used	Ethnobotanical use
		(Acanthaceae)			compost; biological control of insect/pests
8	Bakaino	*Melia azedarach* L. (Meliaceae)	Tree	Root, leaves, fruit, bark	Leaf juice is used as pesticide and insecticide
9	Banmara	*Ageratina adenophora (*Spreng.) (Asteraceae)	Undershrub	Leaf	Leaf juice is used in cut wounds of human body as a homeostatic and antiseptic and leaves used for mulching and green manure
10	Bans	*Dendrocalamus sp.*(Poaceae)	Grass	leaf, culm	Culms are used as food in the young stage; used to make handicrafts; sticks; fencing purpose; different agricultural instruments; having religious values
11	Batule Pat	*Stephania glandulifera* Miers. (Menispermaceae)	Climber	Leaf	1-2 teaspoonful of the root juice is diluted in water and drunk twice in a day as a vital tonic
12	Bhalayo	*Rhus wallichii* Hook. f. (Anacardiaceae)	Tree	Fruit, root, bark	Fruit is used in diarrhoea; gum used as preservative and root paste is applied on wound
13	Bhang/Ganza	*Cannabis sativa* L. (Cannabaceae)	Shrub	Fruits, flower	Used in abdominal pain, gastritis, seeds are pickled and leaf juice is given to cattle suffering from diarrhoea and cold
14	Bhojo	*Acorus calamus* L. (Acoraceae)	Perennial herb	Roots	Used as throat problem, common cold and teeth problem, scalp problem to small dog. It is kept with stored grains to protect from insect and pest.
15	Bhuikafal	*Fragaria indica* Andr. (Rosaceae)	Herb	Fruit	Used as antiseptic
16	Chanp	*Magnolia champaca* L. (Magnoliaceae)	Tree	Flower	Flowers used in different cultural ceremonies
17	Chari amilo	*Oxalis corniculata* L. (Oxalidaceae)	Herb	Whole plant	Used as antiseptic
18	Chilaune	*Schima wallichii* (DC.) Korth. (Theaceae)	Tree	Seed	Seed is used for cure scalp problem
19	Chutro	*Berberis aristata* DC. (Berberidaceae)	Shrub	Fruits, bark, root	Fruits edible, bark and roots are used in medicine
20	Dhasingre	*Gaultheria fragrantissima* Wall. (Ericaceae)	Shrub	Leaf, fruits	Ripened fruits are eaten, oil from leaves is rubbed on body for rheumatic pains
21	Dubo	*Cynodon dactylon* (L.) Pers. (Poaceae)	Grass	Whole plant	Used for decorations, bioengineering and religious ceremony. Exhibits carminative action
22	Ghiukumari	*Aloe vera* (L.) Burm. f. (Asphodelaceae)	Perennial Herb	Leaf	Used in piles, constipation, and menstrual suppression, useful in eye disease, tumors, liver complaints, skin diseases, ulcer, veterinary use, leaf gum used to cure fire burnt

S.N.	Nepali Name	Botanical name/Family	Habit	Parts used	Ethnobotanical use
23	Ghodtapre	*Centella asiatica* (L.) Urb. (Apiaceae)	Herb	Whole plant	Juices used in tonsil, fever and urinary problem
24	Gogan	*Saurauia nepaulensis* DC. (Actinidiaceae)	Tree	Leaf	Used to cure cattle dysentery and gastric, young shoots are used as vegetable
25	Halhale	*Elephantopus scaber* L. (Asteraceae)	Herb	Leaf, young shoots, roots	Antipoison , jaundice and young shoot used as vegetable,cure for skin diseases(Nepali name Dubi)
26	Jamuno	*Eugenia jambolana* Lam. (Myrtaceae)	Tree	Fruits	Decoction of fruit and seed powder is to cure diabetes
27	Jatamasi Nakkali	*Valeriana hardwickii* Wall. (Caprifoliaceae)	Perennial herb	Rhizomes	Bitter tonic, stimulant, antispasmodic, and to treat hysteria and epilepsy
28	Kafal	*Myrica esculanta* Buch.-Ham. Ex D. Don. (Myricaceae)	Tree	Fruits, bark, root	Edible fruits and used in cough, diarrhoea
29	Khareto	*Phyllanthus parvifolius* Buch.-Ham. Ex D. Don. (Phyllanthaceae)	Herb	Foliage	Used as broom
30	Kukurdaino	*Smilax aspera* L. (Smilacaceae)	Climber	Flowers, young shoots	Stem used as stick, young shoot used as vegetable and seed used to prepare pickle.
31	Kurilo	*Asparagus racemosus* (Willd.) (Asparagaceae)	Perennial herb	Roots, whole plant	Root powder is used diarrhoea, to minimize skin burn problem and whole plant feed to cow and buffalo as medicine to cure milk problem.
32	Kuro	*Bidens pilosa* L. (Asteraceae)	Herb	Roots	Roots paste is used in hand and legs fracture problem
33	Laligurans	*Rhododendron* sp. (Ericaceae)	Tree	Flower	Decorative, religious, medicine to take off fishbone from throat and fruits, flowers are used in dysentery
34	Nagbeli	*Lycopodium clavatum* L. (Lycopodiaceae)	Perennial herb	Whole plant	Spores medicine to impair memory and gunpowder and plant for decoration in different ceremonies
35	Neem	*Azadirachta indica* A. Juss. (Meliaceae)	Tree	Bark , leaf	Used in fever, bronchitis, scabies
36	Pakhanbedh	*Bergenia ciliata* (Haw.) Sternb. (Saxifragaceae)	Shrub	Whole plants	Dry plant dust used to feed ladies after childbirth to recover body and root used in fever, flowers pickled
37	Pani Amala	*Nephrolepis auriculata* (L.) Trimen. (Nephrolepidaceae)	Perennial Herb	Fruits	Fruit is used to recover dehydration in human body
38	Sano Jhingane	*Eurya acuminate* DC. (Theaceae)	Tree	Leaves	Leaves can be used for treatment of stomach diseases and used to adulterate tea.
39	Siltimur	*Litsea cubeba* (Lour.) Pers. (Lauraceae)	Tree	Fruits	Used for teeth pain and as spices
40	Sisno	*Urtica dioica* L. (Urticaceae)	Shrub	Whole plants	Young shoots used as vegetable food by human, fed to pigs, root powder for

S.N.	Nepali Name	Botanical name/Family	Habit	Parts used	Ethnobotanical use
					abdominal colic, root juices for dog-bite and leaf paste for burnt
41	Thakal	*Argemone mexicana* L. (Papaveraceae)	Shrub	Stem	The white pith portion and the ripe fruits are eaten
42	Thulo Okhati	*Astilbe rivularis* Buch.-Ham. Ex D. Don. (Saxifragaceae)	Herb	Roots	Used in curing pain in the body and dry root dust to feed to ladies after childbirth to maintain her body
43	Timur	*Zanthoxylum armatum* DC. (Rutaceae)	Shrub	Bark, fruit, seeds	Used in rheumatism and skin diseases, blood pressure, fever
44	Tite Pati	*Artemisia vulgaris* L. (Asteraceae)	Herb	Whole plant	Used in abdominal pain, headache and religious importance, leaf juice used for fish poison and insecticides
45	Tulsi	*Ocimum tenuiflorum* L. (Lamiaceae)	Herb	Fruits, leaves and inflorescence	Used in cold, cough and fever.
46	Uttis	*Alnus nepalensis* D. Don. (Betulaceae)	Tree	Bark	Religious purpose, bark can be used in dyes

Matrix Ranking

Preference ranking of 10 medicinal plants was reported based on health care, access, availability, profitability, storage, daily use and market demand. The findings showed that Alaichi (*Amomom subulatum*) scored highest mark and ranked first indicating the most effective and preferred medicinal plant, while Siltimur (*Litsea cubeba*) least preferred (Table 2).

Table 2. Matrix preference ranking of selected ten most important medicinal plants.

Species / Criteria	Alaichi	Chutro	Aiselu	Pakhanbed	Timur	Siltimur	Dhasingre	ThuloOkhati	Jatamasi Nakkali	Utis
Health care	3	2	2	2	1	1	2	2	2	1
Access	3	2	2	2	1	1	2	1	3	2
Availability	3	2	1	2	1	2	1	2	2	3
Storage	3	2	1	3	3	2	2	2	2	1
Daily use	2	1	1	2	2	1	1	1	1	1
Profitability	3	2	1	3	2	1	2	2	3	1
Market demand	3	3	1	3	2	0	3	1	3	1
Total	20	14	9	17	12	8	13	11	15	10
Rank	I	IV	IX	II	VI	X	V	VII	III	VIII

Discussion

Traditionally, herbal medicines were used by the Nepalese community from pre-historic times. Although allopathic medicines' availability somewhat diminished the importance of traditional medicinal systems, these medicines are still in use basically in rural communities.

Hanif et al. (2014) suggested that *Lycopodium* may be used as a drug of choice in a condition of memory impairment due to its beneficial effect on Cerebral Blood Flow (CBF). Ruffo et al. (2002) also stated the wide use of *Bidens pilosa* to treat a wide range of digestive complaints, cough, diabetes, muscular pain and malaria. Lozano (2001) stated *Cannabis* was known to hold curative power and used as a diuretic, anti-epileptic, anti-inflammatory and painkilling virtues. Giri and Paudyal (2017) reported using *Ageratina adenophora* as antiseptic and biofuel, *Drymaria diandra* in eye diseases, *Ocimum tenuiflorum* in medicinal and religious purposes and *Artemisia vulgaris* to treat fever and as insect repellant. Likewise, *Rubus ellipticus* is used for dysentery (Prasai, 2007); *Amomum subulatum* in indigestion, *Zanthoxylum armatum* in gastric, *Astilbe rivularis* used for uterine contraction during birth, *Berberis aristata* in Jaundice, *Litsea cubeba* in headache, *Gaultheria fragrantissima* in swellings and pain *Oxalis corniculata* as antiseptic and astringent, and *Aloe vera* in burns (Rai, 2003). Other than the human ailments, medicinal plants like *Alnus nepalensis* is used for construction purposes (Thapa, 2000) and *Eurya acuminata* is used in reforestation projects in Thailand (Pakkad et al., 2002). Prakash and Gupta (2005) reported that *Ocimum sanctum* is used for the treatment of bronchitis, bronchial asthma chronic fever, insect bites, etc. Kanwal, et al. (2015) showed the effect of different parts of *Zanthoxylum armatum* used for the ailment of chest infection, gas problems, indigestion problems in addition to fever, rheumatism and skin diseases. Plants are used for various other purposes beside medicinal values such as religious purpose, construction, fibres, oils, etc. as reported by various workers worldwide.

Conclusion

The tribal livings in the community forest were highly dependent upon the traditional folklore medicines due to lack of allopathy, and deep faith on old tradition and treaties. Most medicinal plants were also used as food, vegetables, dye and tan, poisons, pesticides, veterinary, religious, and handicrafts. Local healers are Lamas, Vaidhyas having good knowledge of medicinal plants for different ailments should be encouraged to transfer knowledge to young generations. Considering the importance of medicinal plants in today's world in the health care system, these should be cultivated and propagated. However, lack of technical knowledge inhibit the local people from cultivating the MP's in their private land. So the concerned agencies should provide the technical knowledge and skill for cultivation. Further research should be carried out on the regeneration, biomass production capacity and sustainable management of the MPs species in this Community Forest.

Acknowledgements

We are very much thankful to the local people of Mandhandeupur Municipality, Ward No.12, who helped a lot to carry out this work. Department of Botany, Trichandra College, Ghantaghar, Kathmandu is highly acknowledged for providing us necessary support in the successful completion of the study.

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Effect of Alcoholic Fermentation on FOS Levels and Radical Scavenging Activity of Yacon (*Smallanthus sonchifolius*) Root Slices

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Abstract

The effects of alcoholic fermentation on the radical scavenging activities (RSA) and fructooligosaccharides (FOS) of yacon (*Smallanthus sonchifolius*) roots were investigated. The experiment was carried out using mature yacon roots locally grown in Pakhribas, Dhankuta, Nepal. The said effects were investigated by fermenting thin slices (3 mm) of yacon in 10% glucose solution, using commercial wine yeast (*Saccharomyces cerevisiae* (ex) *bayanus*, Lalvin EC1118, Canada), at ~ 30°C and following the course of changes in FOS, RSA (% DPPH inhibition) and half maximal inhibitory concentration (IC_{50}) for a period of up to 240 h. Yacon slices exhibited a camel-hump (rise-and-fall) pattern in RSA during alcoholic fermentation while having no significant effect on FOS content. The maximum values of RSA (91.57% DPPH inhibition, IC_{50} = 6.48 µg of dry sample) were obtained at 144 h fermentation. The finding implies that fermentation is beneficial but should be carried out only for a limited time period (144 h in the present case) to obtain the benefit of improved level of RSA. Also, since wine yeasts cannot ferment FOS, it is possible to produce fermented yacon slices (and possibly yacon wine) that is rich in nutraceuticals (prebiotic in particular).

Key words: Fructooligosaccharides (FOS), Half maximal inhibitory concentration (IC_{50}), Nutraceuticals, Radical scavenging activity (RSA)

Introduction

Yacon [*Smallanthus sonchifolius* (Poepp. & Endl.) H. Robinson], syn. *Polymnia sonchifolia*, is one of the 23 species of the genus *Smallanthus* (Vitali et al., 2015). It is related to the sunflower but produces large tuberous roots (Lachman et al., 2003) that are sweet and crunchy (Grau & Rea, 1997).

Yacon is a multifunctional food (Delgado et al., 2013; de Almeida Paula et al., 2015): it contains several bioactive compounds, including phytoalexins which have antimicrobial activity (Inoue et al*.,* 1995), phenolic compounds that exert antioxidant activity, and fructans (inulin and fructooligosaccharides) that have prebiotic properties (Geyer et al., 2008). Yacon has been found to be one of the richest plant sources of fructooligosaccharides (FOS), which are claimed to have numerous health benefits (Caetano et al., 2016). Besides being prebiotics, FOS are non-cariogenic, impart moderate sweetness and provide negligible calorie. FOS are recognized as a soluble fiber which causes several favorable effects during digestion (Manrique et al., 2005).

Review of literature shows that FOS, because of its health-promoting properties, has remained the focus of most researches on yacon. Shrestha (2015) and Pokhrel (2018) have optimized methods for making yacon wine but they have delimited their study to aspects of value-addition and sensorial optimization. Chemical properties as affected by aging of fermented yacon root have been described by Brandao et al. (2014) but there is no indication as to what type of fermentation was used.

It is still unclear whether or not the FOS is utilized by the yeast to convert it into ethanol. If it indeed does, the very purpose of value-addition (by way of supplying FOS through wine) will be marred. The few research papers available to date on this aspect also have contradictory reports. The fate of FOS, antioxidant activity during alcoholic fermentation is therefore still far from clear, hence the need for quantifying changes in functional compounds/activities in yacon during alcoholic fermentation.

The antioxidant property of yacon has been studied by many workers (Yan et al., 1999; Pereira et al., 2016), mostly in terms of radical scavenging activity by DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay. One parameter that has been introduced recently by Brand-Williams et al. (1995) for the interpretation of the results from the DPPH method is the half-maximal effective concentration EC_{50} (otherwise called the half-maximal inhibitory concentration, IC_{50}). This is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color) (Molyneux, 2004). Some of the standard methods (including suitable modifications thereof) along with references are given in table 1.

Changes in content and/or properties of phytochemicals may occur during processing. For example, Adetuyi et al. (2014) and Hur et al. (2014) have discussed changes in polyphenols that occur during fermentation. Of the very number of studies carried out on relationship between fermentation process and antioxidant activities is that of Chu and Chen (2006), who studied kombucha (fungus tea) fermentation and its relation to antioxidant activities.

Table 1. Standard methods for antioxidant capacity and FOS determination (including suitable modifications thereof) along with references.

Analysis	Principle	Reference(s)
Antioxidant capacity	DPPH assay	Khajehei et al. (2018)
Fructooligosaccharides (FOS)	Resorcinol reaction	Roe et al. (1949) Steele (1969) Pencheva et al. (2012) Petkova et al. (2013) Somogyi (1930)

Materials and Methods

Materials

Yacon roots locally grown in Pakhribas (27°3′5.93″N, 87°17′7.01″E, Elevation 1752 m), Dhankuta district, were collected on the 2nd week of March, 2018 (one week after harvest). Wine yeast (*Saccharomyces cerevisiae* [ex *bayanus*], Lalvin EC-118) was used for the fermentation. All the reagents (DPPH, methanol, fructose and ascorbic acid) used for the

analyses were purchased from Sigma-Adrich Chemicals, India. Microprocessor U-VIS Spectrophotometer – 2371, India, was used for spectrophotometric measurements.

Experimental Design and Data Analysis

The outline of experimental design for the present work is given in figure 1, followed by necessary explanations (in the text).

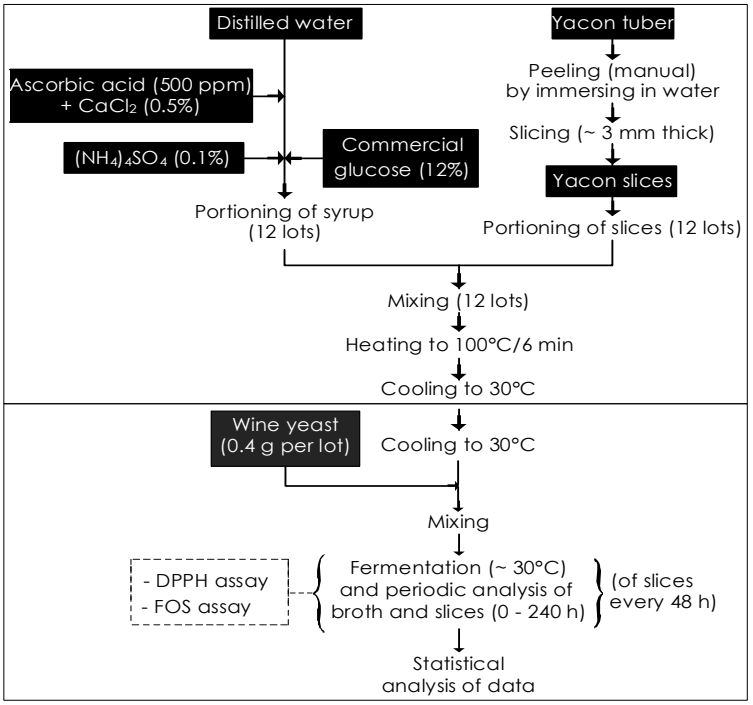

Figure 1. Outline of experiment

Data analysis was done using Genstat 12th edition (Payne et al., 2009) for ANOVA. Microsoft Excel 2019 Add-in was used for correlation analysis and graphs. The necessary explanations for Figure 1 are as follows:

- *Peeling by immersing in water:* To prevent browning.
- *Heating of slices:* To (i) destroy browning enzymes, (ii) destroy pectinases and pectin esterases, and (iii) improve transport of soluble components to and from the slices.
- *Addition of $(NH_4)_2SO_4$:* Yeast food.
- *Glucose supplementation:* Glucose was used instead of sucrose because it is free from fructose, which interferes with the fructose present the yacon. A concentration of ~ 10% glucose in the syrup (which is lower than that for any wine fermentation) was used to achieve two purposes, viz.: (i) avoid catabolite repression (prevent the yeast from depending solely on the supplied glucose), and (ii) faster depletion of glucose (so that the yeast may begin using its cellular mechanisms to utilize fructose present in yacon FOS).
- *Use of slices instead of juice or pulp:* Processing (cutting, juice extraction, etc.) incurs significant loss of FOS (Duar et al., 2015)

Physicochemical Analysis

For the analysis of changes in RSA, FOS during the alcoholic fermentation of yacon slices, extracts were prepared from dried samples (stock powder). The data for drying and particle size were taken from L'homme et al. (2003).

Extraction of DPPH Reductants for Analysis

Following the method described by Khajehei et al. (2018), extraction of phytochemicals from the dried sample (stock powder) was performed in a 15 mL test tube by adding ~ 5 mL of methanol to 0.25 g of dried powder (< 200 μm size) of yacon slices and vigorously agitating in a vortex mixer for 20 min at room temperature (~ 30°C).

Afterwards, the mixture was centrifuged at 3000 rpm for 10 min to separate the supernatant from the solid residuals. Two more washings were done by resuspending the residue in 5 mL methanol and centrifuging for 3 min (for each additional washing). The methanol extracts were pooled and filtered through Buchner funnel with fritted glass (16-40 μm pore size), and volume made up in a 25 mL graduated measuring cylinder with methanol. The extracts were used for the determination of DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals scavenging activity (DPPH-RSA).

Preparation of Reagents

- **Preparation of DPPH solution:** 4 mg of DPPH in 100 mL methanol to give a solution of 100 μM. The DPPH solution is then kept in dark for 2 h (covered with aluminum foil) to allow formation of a stable purple color.
- **Preparation of ascorbic acid for "positive" control:** Ascorbic acid solution (positive control) is prepared by dissolving 8.8 mg (in powdered form) in 100 mL distilled water to give 500 μM stock solution and then diluted 10 times (10 mL stock + 90 mL distilled water) to get a working solution of 50 μM.
- **Preparation of dilution series for IC_{50}:** For IC_{50} (or EC_{50}) calculation, a series of concentrations (of the sample/reductant) is required. Since 25 μM is the end-point concentration (the highest concentration to be used), a series of 5, 10, 15, 20, and 25 μM ascorbic acid means, respectively, use of 0.4, 0.8, 1.2, 1.6 and 2.0 mL of the ascorbic acid standard, the remaining volume (to make 2.0 mL) being made up with either distilled water or methanol.
- **Preparation of "negative" control:** Negative control implies mixture of 2.0 mL DPPH solution and 2.0 mL methanol (solvent).

Calculation of DPPH Radical Scavenging Capacity

The DPPH-RSA is calculated using the equation (KamLeshiya et al., 2012):

$$\text{RSA (\%)} = \frac{A_c - (A_s - A_1)}{A_c} \times 100$$

Where, A_c is the absorbance of the negative control, A_s is the absorbance of the test sample,

and A_1 is the absorbance of the blank.

In case the instrument has been set to zero with blank, the equation for scavenging capacity simplifies to:

$$\text{RSA (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

For IC_{50} calculation, a series of dilutions of extract was used and absorbance recorded. The IC_{50} of ascorbic acid is similarly determined and "ascorbic acid equivalent antioxidant capacity" (AEAC) of the extract was calculated (Van De Velde et al., 2013). The calculation was done in a computer (e.g., using Microsoft Excel®) by plotting inhibition ratios (y) against the sample concentrations (x) and drawing a regression line ($y = mx + c$), from which concentration needed for 50% inhibition was calculated.

DPPH (2,2-diphenyl-1-picrylhydrazyl) Radicals Scavenging Capacity

The DPPH radical scavenging capacity was measured employing a slight modification of the method described by Khajehei et al. (2018). A generalized method synthesized after thorough review of work by several authors (Molyneux, 2004; Sharma & Bhatt, 2009; Xie & Schaich, 2014) was used as the working outline.

Briefly, 2 mL of the methanolic extract was mixed with 2 mL of DPPH (0.004% in methanol, corresponding to 100 µM) in 4.5 mL plastic cuvette (Molyneux, 2004) and incubated at 37°C in dark (wrapped with aluminum foil) for 20 min (for completion of reaction) before spectrophotometric analysis. Absorbance was measured at 517 nm for sample as well as the standard after setting the absorbance to zero for the blank. Ascorbic acid (50 µM stock) was used as the standard, the absorbance data of which were used for drawing the reference curve for calculating the IC_{50} and RSA. The RSA was expressed as mg ascorbic acid equivalent per 100 g dry weight (mg AAE/100 g, dw). Details of the amounts of reagents/standard/extract used before incubation are given in tables 2 and 3.

Table 2. Amounts of reagents/extract used for the DPPH assay (for extract analysis)

Reagent	Distribution in cuvettes						
	(-) ve control	Blank 1	Extract				
DPPH, mL	2.0	0	2.0	2.0	2.0	2.0	2.0
Extract, mL	0	2.0	0.4	0.8	1.2	1.6	2.0
Methanol, mL	2.0	2.0	1.6	1.2	0.8	0.4	0
AA, mL	0	0	0	0	0	0	0
Total, mL	4.0	4.0	4.0	4.0	4.0	4.0	4.0

Table 3. Amounts of reagents/ascorbic acid used for the DPPH assay (for standard curve)

Reagent	Distribution in cuvettes				
	Blank 2	(+) ve control / standard			
DPPH, mL	0	2.0	2.0	2.0	2.0
Extract, mL	0	0	0	0	0
Methanol, mL	2.0	1.5	1.0	0.5	0
AA, mL	2.0	0.5	1.0	1.5	2.0
Total, mL	4.0	4.0	4.0	4.0	4.0

The (-) ve control contains DPPH and solvent only, the (+) ve control contains both ascorbic acid (in graded concentrations) and DPPH, Blank 1 and Blank 2 imply blanks for extract and ascorbic acid standard (respectively).

FOS Determination

Preparation of extract for FOS determination

In this work, FOS determination methods described by Roe et al. (1949), Steele (1969), Pencheva et al. (2012) and Petkova et al. (2013) were adapted. Briefly, the method involved following steps: Yacon powder was taken for the analysis. 0.25 g of stock powder was weighed into 50 mL beaker and 15 mL distilled boiling water was added to the sample. It was placed on hot plate of the magnetic stirrer. The extraction process was carried out at 80°C for 10 min under the constant stirring. Thereafter, the sample was allowed to cool down to room temperature and Somogyi deproteinizing reagents (Somogyi, 1930) were added to it (5 mL of 10% (w/v) $ZnSO_4.7H_2O$ and 5 mL of 0.5N NaOH). The sample was mixed, transferred to 25 mL measuring cylinder, volume made up with distilled water (if needed), rested for 15 min, and filtered through fritted glass Buchner funnel (125 mL capacity, 16-40 µ pore size). The filtrate was used for the analysis of FOS after appropriate dilution.

The dilution to be carried out was determined separately by comparing the color developed (after carrying out test as in table 3) in two test tubes, viz., one containing the fructose standard with the highest fructose content and another containing 1 mL of extract. In case the sample extract showed color intensity higher than that of the standard (having the highest fructose content) approximate dilution was made with distilled water so that the color intensity was less than that of the standard. The trial data on dilution was used for diluting the sample extract before carrying out the analysis as shown in table 4.

Preparation of reagents

Reagents were prepared according to Steele (1969) and Pencheva et al. (2012).

- *Resorcinol-thiourea:* 0.1 g resorcinol and 0.25 g thiourea was dissolved to a total volume of 50 mL in glacial acetic acid with gentle heating. The reagent was stored in amber bottle.
- *30% hydrochloric acid:* To 15 mL of distilled water 75 mL of 36% conc. HCl was added.
- *Standard fructose solution:* 100 mg of fructose was dissolved in distilled water to make 100 mL in a volumetric flask. 1 mL of this solution was again diluted to 100 mL in distilled water to give a standard solution containing 10 µg fructose/mL.

Spectrophotometric analysis

Spectrophotometric methods for FOS determination are based on the well-known Seliwanoff's reaction. The amounts of reagents, sample, and standard fructose solution used for the spectrophotometric analysis were as in table 5. The mixtures were prepared in 20 mL glass test tubes.

The mixture tubes thus prepared were kept in water bath at 80°C for exactly 10 min. To avoid loss due to evaporation, each tube was lidded with a glass marble of matching size (Fig. 2). After the heating period, tubes were immediately cooled in water bath for 5 min.

Absorbances were read in a spectrophotometer (Microprocessor UV-Vis-2371, India) at 520 nm for standards and sample after adjusting the reading to 0 (i.e., 100% transmittance) for blank. The calculation was done on dry basis, as mg fructose content/kg dry matter.

Table 4. Arrangement of reagents/samples for spectrophotometric analysis of FOS

Reagents/Sample	Analyte					
	Standard		Sample		Blank	
Fructose std (10 µg/mL), mL	0.5	1.0	1.5	2.0	0.0	0.0
Thiourea-resorcinol, mL	1.0	1.0	1.0	1.0	1.0	1.0
Conc. HCl (30%), mL	7.0	7.0	7.0	7.0	7.0	7.0
Distilled water, mL	1.5	1.0	0.5	0.0	1.0	2.0
Aliquot, mL	0.0	0.0	0.0	0.0	1.0	0.0
Total, mL	10.0	10.0	10.0	10.0	10.0	10.0

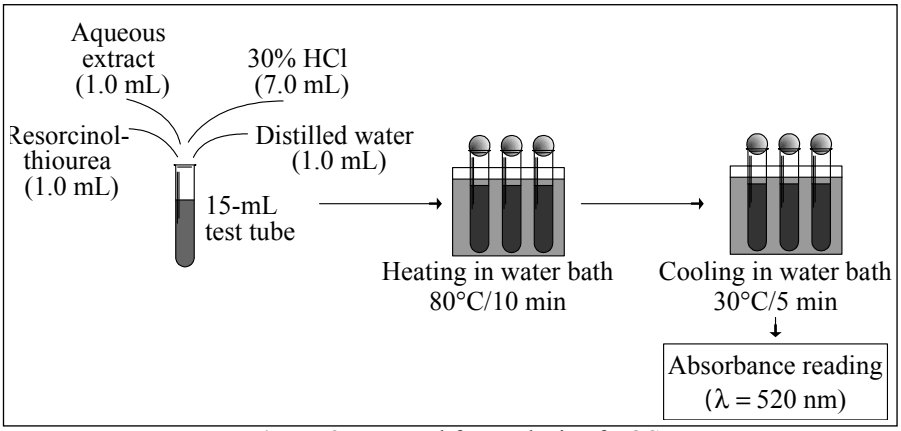

Figure 2. Protocol for analysis of FOS

Results

Effect of Fermentation Time on FOS

ANOVA of mean values (Table 5) showed no significant change ($p < 0.05$) in FOS content with the fermentation time (Fig. 3).

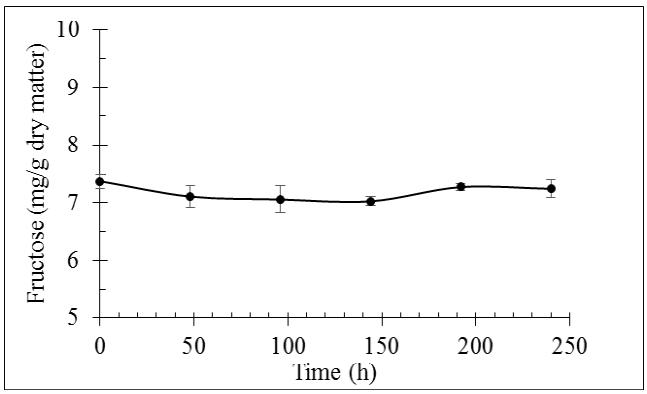

Figure 3. Effect of fermentation time on FOS. (Vertical error bars represent standard deviation of triplicate data)

Effect of Fermentation Time on DPPH Radical Scavenging Capacity

The change in radical scavenging activity (RSA, expressed as % DPPH inhibition) of the yacon root slices as affected by alcoholic fermentation is shown in figure 4. The RSA increased steadily from 78.31% (0 h) to 91.57% (144 h) and then decreased to 81.93 (240 h).

As expected, significant ($p < 0.05$) change in IC_{50} value was observed as the fermentation progressed (Table 5) the lowest (6.48 µg), corresponding to 91.57% inhibition, for the 144 h fermented sample. The IC_{50} at 144 h was better than (in terms of RSA) even the unfermented (0-h) sample. Since the IC_{50} value is more meaningful when expressed in terms of standard antioxidant (e.g., ascorbic acid), the result recalculated in terms of ascorbic acid equivalent, AAE (using standard curve, Fig. 5) is given in table 5.

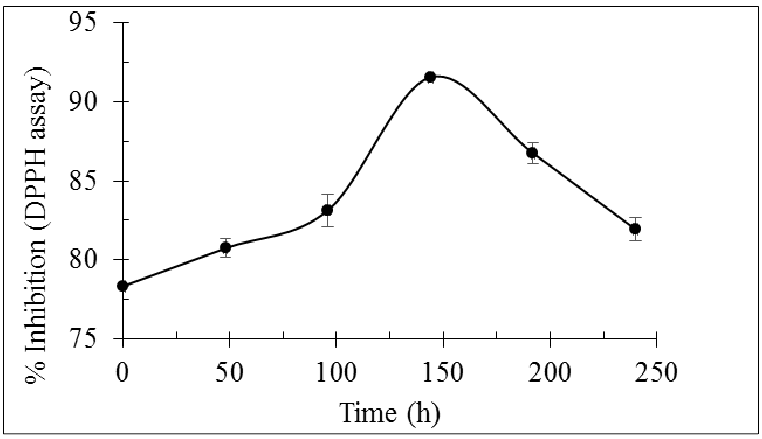

Figure 4. Effect of fermentation time on RSA

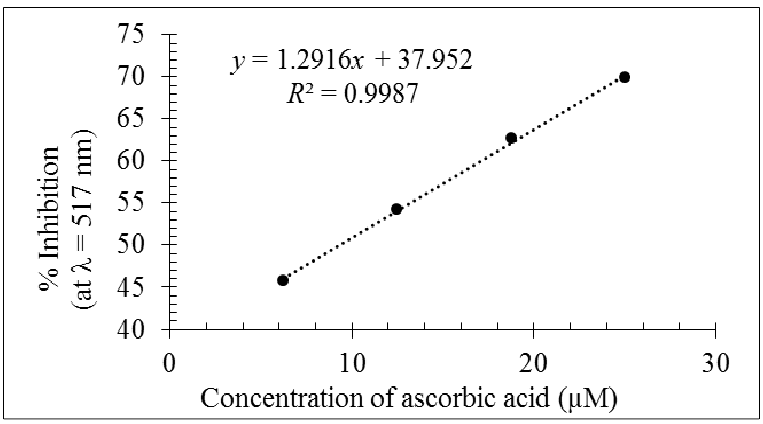

Figure 5. Standard curve of % DPPH inhibition by ascorbic acid

Values are means of triplicates, with standard deviations in the parentheses. Values in the columns bearing the same superscripts are not significantly different at 5% level of significance. LSD (5%) denotes least significant difference at 5% level of significance.

Table 5. Summary of ANOVA: Changes in FOS, RSA and IC_{50} during fermentation of yacon root slices

Fermentation time (h)	FOS (mg/g, dm)	RSA (% DPPH inhibition)	RSA (µmol AAE/g sample, dm)	IC_{50} (µg)
0	7.37 (0.13)	78.31 (0.03)[a]	1233 (4.88)[a]	7.57 (0.03)[f]
48	7.11 (0.19)	80.72 (0.58)[b]	1264 (1.71)[b]	7.38 (0.01)[e]
96	7.06 (0.23)	83.13 (1.03)[d]	1312 (1.85)[d]	7.11 (0.01)[c]
144	7.03 (0.08)	91.57 (0.03)[f]	1440 (2.22)[f]	6.48 (0.01)[a]
192	7.27 (0.06)	86.75 (0.65)[e]	1376 (2.02)[e]	6.78 (0.01)[b]
240	7.24 (0.15)	81.93 (0.73)[c]	1296 (1.79)[c]	7.2 (0.01)[d]
LSD (5%)	1.790	1.101	4.733	0. 02739

FOS = Fructooligosaccharide, RSA = Radical scavenging activity, AAE = Ascorbic acid equivalent.

Discussion

Effect of Fermentation Time on FOS

FOS typically represents 32-70% of the yacon root carbohydrate (Manrique et al., 2005). The average value of FOS (7.18 ± 0.12 mg/g, dm) found in this study works out to be 77.08% of carbohydrate, which is slightly higher than the reported values.

The result on FOS in the present study appears to run contrary to report by de Almeida Paula et al. (2015). This could either be due to some (unknown) protective mechanism involved, or systemic error(s) in the analysis.

Nevertheless, the result of this study unequivocally establishes that wine yeast does not ferment yacon FOS, indicating that yacon roots can be advantageously utilized for the preparation of prebiotic wine. However, this result runs contrary to the findings of Brandao et al. (2014), who reported that yeasts are able to ferment yacon FOS. Since the authors did not disclose the fermentation- and aging protocols of their experiment, the results leave room for speculations.

Since FOS was not significantly affected by alcoholic fermentation in the present study, the wine yeast (*Saccharomyces cerevisiae* (ex) *bayanus*) used was probably devoid of FOS utilizing capability.

Earlier works (dissertations in particular) (Shrestha, 2015; Pokhrel, 2018) dealt only with preparation of yacon wine, without even alluding to the potential therapeutic property. It is indubitably apparent from the present work that wine from yacon root has both prebiotic and enhanced therapeutic properties.

Effect of Fermentation Time on DPPH Radical Scavenging Capacity

The present work shows that fermentation significantly increases the antioxidant activity but only up to some point. Further fermentation may not be desirable in terms antioxidant activity.

DPPH radical scavenging activity of yacon has been studied by various authors, including Yan et al. (1999), Pereira et al. (2016), and Khajehei et al. (2018). Yan et al. (1999) found that DPPH radical scavenging activity of yacon ranged from 13.8 ± 4.7 to 93.2 ± 0.6%. The difference in antioxidant can be due to variability of genotypes, agronomic characteristics, soil types, climatic conditions, and use of fertilizers, as well as post-harvest time (Pereira et al., 2016).

Effect of fermentation on antioxidant activity had been studied by various authors. Chu and Chen (2006), Adetuyi and Ibrahim (2014) and Hur et al. (2014) found out that fermentation increased the DPPH radical-scavenging activity. Increase in the antioxidative activity of plant-based foods by fermentation is influenced by various factors, including the microorganism species, pH, temperature, solvent, water content, fermentation time, kind of food and aerobic conditions. Fermentation improves antioxidative activity by increasing the release of flavonoids and total phenolic content. Phenolic compounds can act as reducing agents, hydrogen donors and singlet oxygen quenchers (Hur et al., 2014).

Adetuyi and Ibrahim (2014) reported that during fermentation of okra seeds release of phenolic isoflavoneaglycones by the catalytic action of β-glucosidase and the formation of reductones contributes to increase in antioxidant activities. Synergistic effect of the phenolic content of the fermented okra seeds with other components present in the aqueous extract could also cause an increase in the antioxidant activities. The author maintains that the total antioxidant activity of the aqueous extract cannot be predicted based on its total phenolic content alone but a synergism of soluble polyphenolic compounds, with one another, and/or other components present in the extracts, may contribute to the overall observed antioxidant activity.

In the same study (Adetuyi & Ibrahim, 2014), the antioxidant activity decreased as fermentation progressed. They attributed the improvement in antioxidant activity during the fermentation to breaking of the linkages of phenolic- and flavonoid compounds by microorganisms, thereby freeing the compounds to actively play the role of antioxidants. Increasing the fermentation time might allow the microorganisms to use those available compounds as substrates for their growth. Fermentation period is therefore critical to ensure optimal breaking down of the compound linkages but not to allow compounds to be substrates for microbial growth.

Chu and Chen (2006), who studied kombucha (fungus tea) fermentation, also observed significant increase in DPPH-RSA in 8 kombucha samples during fermentation for 15 days.

Conclusion

Alcoholic fermentation significantly affects radical scavenging activity (RSA) of yacon slices but has no effect on Fructooligosaccharides (FOS). The changes in RSA during fermentation exhibit a camel-hump (rise-and-fall) pattern. This implies that fermentation is beneficial but should be carried out only for a limited time period (144 h in the present case) to obtain the benefit of improved levels of RSA. Since wine yeasts cannot ferment FOS, it is possible to produce fermented yacon slices (and possibly yacon wine) that is rich in nutraceuticals (prebiotic in particular).

Acknowledgements

First of all, we would like to express our deep sense of gratitude to Prof. Pashupati Mishra, HOD of Food Science and Technology, Dharan Multiple Campus for his kind support and constant encouragement. We acknowledge Dharan Multiple Campus for providing necessary facilities during the work and entire library and laboratory staffs of Dharan Multiple Campus for their cooperation and support during the work.

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Algal Diversity and Water Quality of Tamor River at Mulghat, Dhankuta District

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Abstract

Algal diversity and water quality of Tamur River at Mulghat, Dhankuta District have been studied during autumn (September 2018), winter (December 2018), and spring (March 2019) seasons. The river is one of the tributaries of Saptakoshi River originates at the Himalaya region of Kanchanjunga and joins Sunkoshi and Arun River at Tribeni Ghaat. Water temperature and pH were measured in the field. Simultaneously, dissolve oxygen and conductivity of water and algae diversity were studied in the laboratory. Temperature, pH, DO, and water conductivity were 18.16°C, 8.03, 6.53 mg/l, and 74.1 ìS/cm, respectively. About 92% algae reported were belonged to class bacillariophyceae and the most prominent genus was *Gomphonema* (17%). The maximum number of algae genera was reported during the autumn season (85.18%), while the least number was reported during the winter season (51.85%). The richest site was site-1. *Gomphonema*, *Navicula*, and *Ulnaria* were the dominant genera; *Cymbella*, *Encyonema*, and *Hannae* were common; and the least occurred genera were *Achnanthes, Amphora*, *Cosmarium*, *Oscillatoria*, *Placoneis* etc.

Key words: Conductivity, Diatoms, Epilithic algae, Phytoplankton, Sapta Koshi River

Introduction

Algae are the lower group of an organism having the photosynthetic and thalloid body and grow in aquatic or wet habitats. Diatoms (Bacillariophyceae) are a group of algae, primarily microscopic and have a unique cell wall made up of silica. They are an essential source of energy-rich molecules that are food for the entire food web, from zooplanktons to aquatic insects to fishes (Sharma, 1992). Diatoms can be used as a biological indicator for water quality and determine former water quality and trends over the years (Jüttner et al., 1996; Lobo et al., 2016).

Nepal is rich in lotic water system and there are more than 6,000 rivers with their total cumulative length of 45,000 km (MoUD, 2005). However, rivers in Nepal were little studied for algae. Only the rivers like Narayani (Upadhyaya, 1979), Punyamati (Aryal & Lacoul, 1996), Mahakali (Habib & Chaturvedi, 1997), Koshi (Jha & Kargupta, 2012), Bagmati (Rai & Khadka, 2017) etc, were studied. More algal works carried on rivers belonged to diatoms, a fascinating group of algae. Various workers have also studied the diatoms of lentic water bodies in Nepal. Rai and Rai (2005) reported 12 diatoms from Biratnagar. Similarly, Rai (2005) described ten diatoms from Maipokhari Lake of Ilam district. From Itahari and its adjoining area, seven diatoms have been recorded (Shrestha et al., 2013). Dahal and Jüttner (2004) studied the water quality of middle hill springs using diatoms. Simkhada (2006)

studied the diatoms as indicator of environmental change in lakes and ponds of low land, middle hills and high Himalaya of Nepal and concluded that the species richness was highest in Kathmandu (i.e., 213 sp.) in contrast to Koshi Tappu (i.e., 119 sp.) and high altitude lakes (i.e., 77 sp.). Similarly, Simkhada and Jüttner (2006) studied the diatoms of 12 ponds and four Lakes of Kathmandu valley and Koshi Tappu, including their relationship with hydro-chemicals and habitat characters and reported 213 diatoms. However, the algae of Tamor River has not been studied yet. Thus, this study is the preliminary work for this river.

Materials and Methods

Study Area

Tamor River is one of the major tributary of Saptakoshi River which originates from Eastern Himalayan region near Kanchanjunga, flows through Taplejung, Panchthar, Terhathum and Dhankuta Districts and ends at Tribeni Ghat where it joins with Sunkoshi and Arun river. Its elevation ranges from 358 to 8387 m asl, and mean water flow from 1976-2010 is 226.20 m^3/s (Khadka et al., 2015). It covers about 190 km in length with a catchment area of about 5817 km^2 (Shrestha et al., 2009). The river flow gradient varied considerably, resulting in a series of rapids, runs, riffles, and pools along the river (Shrestha et al., 2009).

The study sites in Tamor River are located at Mulghat and its surrounding areas, Dhankuta District. Here, the river flows more or less from east to west direction with a discharge rate ranges from 13 m^3/s to 5120 m^3/s (Shahi et al., 2013). Site 2 lies near to the bridge at N 26°55'47.54", E 87°19'02.28", alt. 262.4 m; site 1 lies about 1 km downstream from the bridge at N 26°55'38.58", E 87°18'25.73", alt. 258.47 m; and site 3 lies about 1 km upstream from the bridge at N 26°55'44.92", E 87°19'41.67", alt. 263.3 m (Fig. 1).

The climate of Dhankuta district is warm and temperate type. The annual rainfall and average temperature of this district are 1002 mm and 20.6°C, respectively.

Algae Collection and Identification

A total of 27 algal samples were collected from three different sites along the river at an average distance of 1 km each, in September 2018 (Autumn), December 2018 (Winter), and March 2019 (Spring) at an interval of 3 months. Algae were collected by brushing the slippery surface of submerged stones from the edge of the river and using a Plankton net (mesh size 10 μm). They were tagged and labelled correctly, preserved in 4% formaldehyde solution and then brought to the laboratory for further study. Water temperature and pH were also measured with a digital thermometer and Hanna portable pH meter, respectively. Water samples were also taken in separate bottles to measure dissolved oxygen and conductivity in the lab.

Samples were screened under light microscopes and selected samples were performed for frustules cleaning of diatoms using the nitric acid treatment. Photomicrography of diatoms was taken under 40X and 100X objectives using Olympus Chi20 microscope. Dimensions of frustules were measured with the help of ocular and stage micrometres. Algae were

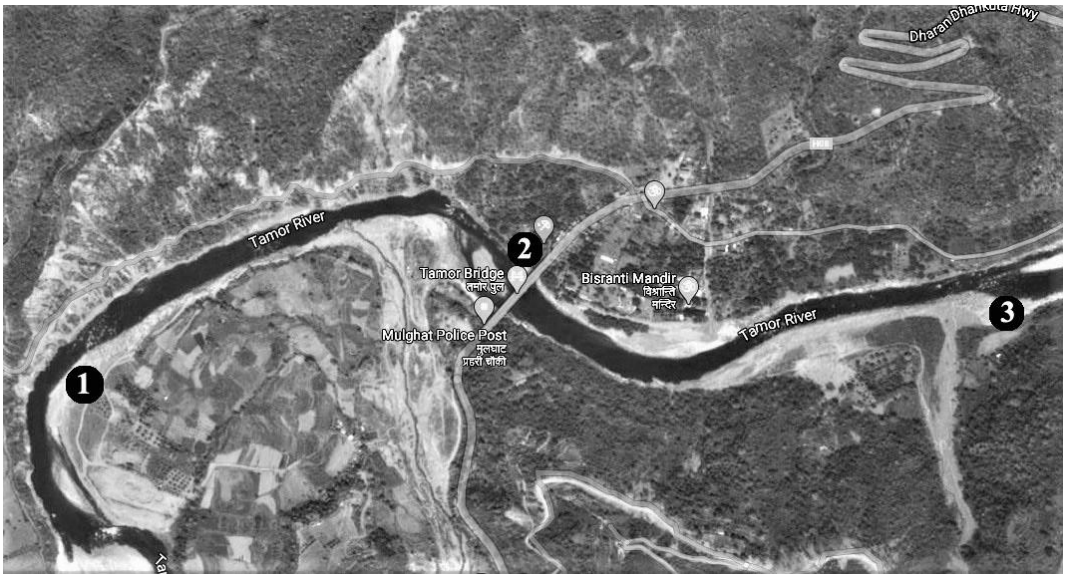

Figure 1. Map of Tamor river showing three algae sampling sites 1, 2 and 3 (Source: google.com/maps/place/Dhankuta/@...)

counted using Sedgwick Rafter Cells. Identification was done with the help of monographs and literature, viz., Tiffany and Britton (1952), Prasad and Srivastava (1992), Krammer (1997), Lange-Bertalot (2001), Rai (2006), Agata (2009), and Karthick et al. (2013). All the collected materials and slides were deposited in the Phycology Research Lab, Department of Botany, Post Graduate Campus, Biratnagar. Water analysis of Tamor river was done in the SEAM-N MMA Environmental Laboratory, Biratnagar.

Results

In the present study, only four algae classes were reported from Tamor river, viz., bacillariophyceae, chlorophyceae, cyanophyceae, and zygnematophyceae. The river was dominated by class bacillariophyceae (diatoms). A total 44 algal species (91.66%) out of total 48 were diatoms. Other classes of algae were least enumerated, i.e., chlorophyceae 2 species (4.16%), cyanophyceae 1 species (2.08%) and zygnematophyceae 1 species (2.08%).

Family-wise representation of algae showed that among the 21 families, Gomphonemataceae was found maximum (27.08%) followed by Naviculaceae (14.58%), Cymbellaceae and Ulnariaceae (6.25% each), and so on (Fig. 2).

Among the identified 27 genera, *Gomphonema* had maximum species represented by eight species (17%). They were *G. insularum*, *G. lacusrankaloides*, *G. lagenula*, *G. minutum*, *G. olivaceum*, *G*. cf. *parvulum*, *G*. cf. *pumilum*, and *G*. cf. *sarcophagus*. Similarly, genera *Navicula* was represented by 7 species (15%), viz., *N*. cf. *angusta*, *N. cryptocephala*, *N. cryptotenella*, *N. margalithii*, *N. mongolreinhardtii*, *N. radiosa*, and *N. rostellata* which is followed by *Encyonema* with 3 species (6%), viz., *E. brevicapitatum*, *E. neogracile*, and *E. silesiacum*. Genera represented by 2 species (4%) were *Cymbella*, *Nitzschia, Surirella*,

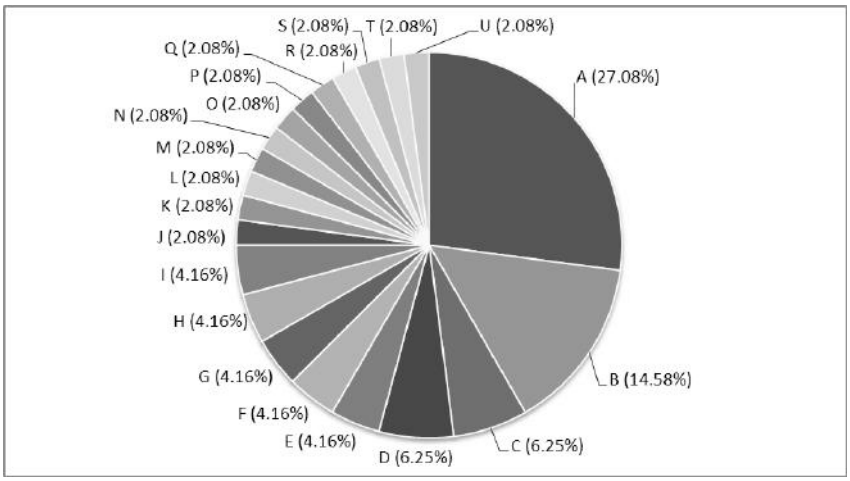

Figure 2. Family-wise representation of total algal taxa reported from Tamor River at Mulghat, Dhankuta. (A= Gomphonemataceae, B =Naviculaceae, C= Cymbellaceae, D= Ulnariaceae, E= Achnanthidiaceae, F= Bacillariaceae, G= Fragilariaceae, H= Oedogoniaceae, I= Surirellaceae, J= Achnanthaceae, K= Amphipleuraceae, L= Catenulaceae, M= Cocconeidaceae, N= Desmidiaceae, O= Eunotiaceae, P= Gesneriaceae, Q= Oscillatoriaceae, R= Rhopalodiaceae, S= Sellaphoraceae, T= Stauroneidaceae, U= Tabellariaceae

Fragillara, and *Hannaea*, and represented by single species (2%) were *Achnanthes, Achnanthidium, Planothidium, Cocconeis, Eunotia, Sellaphora, Frustulia, Craticula, Cymbopleura, Rimeria, Placoneis, Amphora, Rhopalodia, Ulneria, Diatoma, Epithema, Oscillatoria* and *Cosmarium* (Fig. 3).

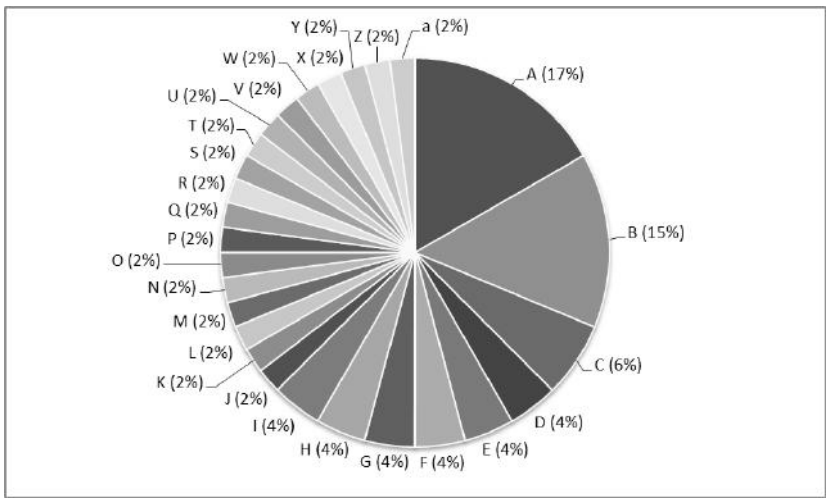

Figure 3. Total algae belonging to various genera reported from Tamor River at Mulghat, Dhankuta. (A= *Gomphonema*, B= *Navicula*, C= *Encyonema*, D= *Cymbella*, E= *Fragilaria*, F= *Hannaea*, G= *Nitzschia*, H= *Oedogonium*, I= *Surirella*, J= *Achnanthes*, K= *Achnanthidium*, L= *Amphora*, M= *Cocconeis*, N= *Cosmarium*, O= *Craticula*, P= *Cymbopleura*, Q= *Diatoma*, R= *Epithemia*, S= *Eunotia*, T= *Frustulia*, U= *Oscillatoria*, V= *Placoneis*, W= *Planothidium*, X= *Rhopalodia*, Y= *Reimeria*, Z= *Sellaphora*, a= *Ulnaria*

Seasonal occurrence showed that maximum algal genera (85.18%) were reported during September (autumn season) and minimum (51.85%) in December (winter season)

(Fig. 4, Table 1). On the basis of collection sites, in general, site 3 harbor maximum numbers of algal genera (77.77%) and site 1 with least number of genera (55.55%) among the three collection sites (Fig. 5, Table 1). The highest number of algal genera (66.66%) was found in site 3 during autumn season and lowest number of algal genera (22.22%) was found in site 1 during winter (Fig. 6).

The genus *Gomphonema* was reported in all sites in all three seasons (Table 1). *Ulnaria* was found in all sites in all three seasons except at site 2 in winter and *Encyonema* in all sites in all three seasons except site 1 in autumn. *Cymbella* was reported in all three sites but only in autumn and spring seasons.

The dominant genera of Tamor river were *Gomphonema*, *Navicula,* and *Ulnaria*. *Cymbella*, *Encyonema*, and *Hannaea* were common genera. The least reported genera were *Achnanthidum, Cosmarium, Craticula, Cymbopleura, Epithemia, Frustulia, Oscillatoria*, and *Placoneis*.

Water temperature of Tamor river was recorded highest (18.33°C) in autumn and lowest (18.10°C) in winter seasons. Water pH was recorded highest (8.03) in spring and lowest (7.6) in autumn seasons. Dissolved oxygen and conductivity of water recorded in spring were 6.53 mg/l and 74.1 µS/cm, respectively (Table 2).

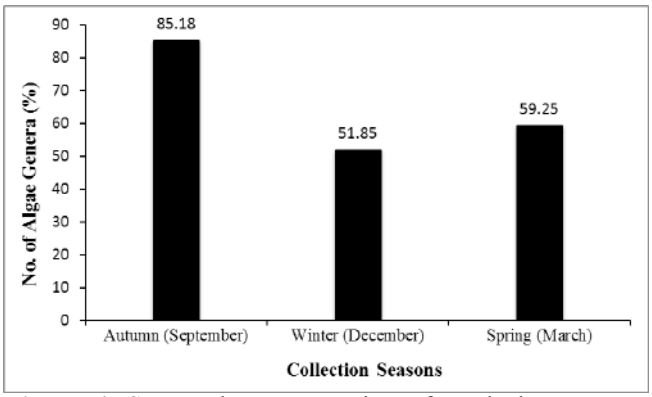

Figure 4. Seasonal representation of total algae genera reported from Tamor River at Mulghat, Dhankuta.

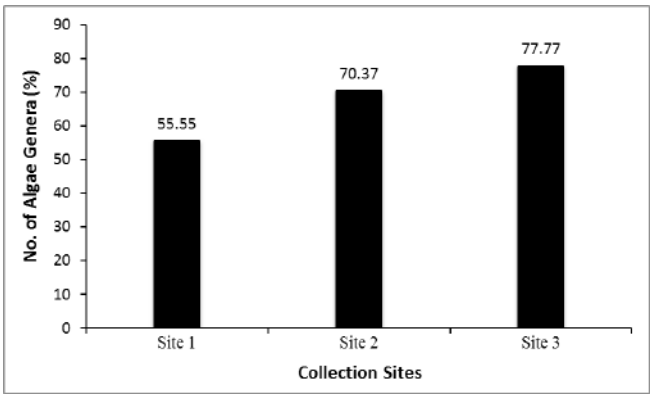

Figure 5. Collection sites-wise representation of total algae genera reported from Tamor River at Mulghat, Dhankuta.

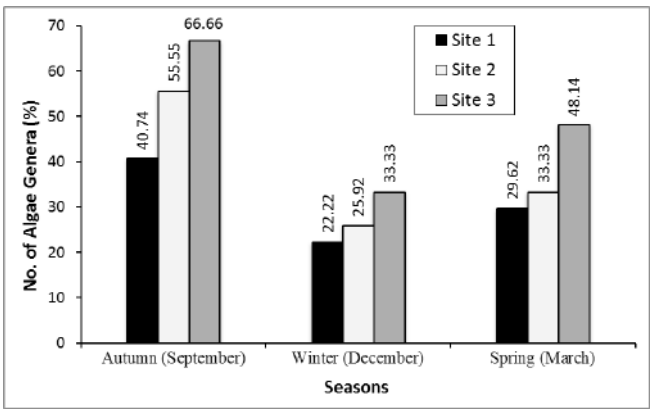

Figure 6. Total algae genera reported in different seasons from different sites of Tamor River at Mulghat, Dhankuta.

Table 1. Occurrence of algae genera in different collection sites in different period of collection at Tamor River, Mulghat, Dhankuta.

S.N.	Genera	Occurrence of algae genera in different sites in different seasons											
		Autumn				Winter				Spring			
		Site 1	Site 2	Site 3	Total sites	Site 1	Site 2	Site 3	Total sites	Site 1	Site 2	Site 3	Total sites
1	*Achnanthes*	+	-	+	2	-	+	+	2	-	+	-	1
2	*Achnanthidium*	-	-	-	-	-	+	-	1	-	-	-	-
3	*Amphora*	-	-	+	1	-	+	+	2	-	-	+	1
4	*Cocconeis*	+	-	+	2	-	+	++	2	-	+	+	2
5	*Cosmarium*	-	-	-	-	-	-	-	-	+	-	-	1
6	*Craticula*	-	-	-	-	-	-	-	-	+	-	-	1
7	*Cymbella*	+	++	++	3	-	-	-	-	++	++	++	3
8	*Cymbopleura*	-	+	-	1	-	-	-	-	-	-	-	-
9	*Diatoma*	-	+	+	2	-	+	-	1	-	-	+	1
10	*Encyonema*	-	++	+	2	+	++	+	3	+	+	++	3
11	*Epithemia*	-	+	-	1	-	-	-	-	-	-	-	-
12	*Eunotia*	+	-	+	2	-	-	-	-	-	-	-	-
13	*Fragilaria*	+	+	+	3	-	-	-	-	-	-	-	-
14	*Frustulia*	-	+	-	1	-	-	-	-	-	-	-	-
15	*Gomphonema*	+++	+	+++	3	++	++	+++	3	+++	+++	+++	3
16	*Hannaea*	+	+	++	3	-	-	+	1	-	++	++	2
17	*Navicula*	+++	++	+++	3	+	-	-	1	++	+++	+++	3
18	*Nitzschia*	-	+	+	2	-	-	-	-	-	++	+	2
19	*Oedogonium*	+	-	-	1	-	-	+	1	-	-	-	-
20	*Oscillatoria*	-	-	-	-	-	-	+	1	-	-	-	-
21	*Placoneis*	-	-	+	1	-	-	-	-	-	-	-	-
22	*Planothidium*	-	-	+	1	+	-	-	1	-	-	+	1
23	*Reimeria*	-	-	+	1	-	-	-	-	+	-	+	2
24	*Rhopalodia*	-	+	-	1	-	-	-	-	-	-	+	1
25	*Sellaphora*	-	+	++	2	-	-	-	-	-	-	-	-
26	*Surirella*	+	+	++	3	++	-	-	1	-	-	-	-
27	*Ulnaria*	++	+	+++	3	+	-	++	2	+++	++	+++	3
	Total genera	**11**	**15**	**18**	**23**	**6**	**7**	**9**	**14**	**8**	**9**	**13**	**16**

Dominant (+++), Common (++), Scarce (+), Absent (-)

Table 2. Water analysis of Tamor River in three different seasons.

S.N.	Water Parameters	Seasons		
		Autumn	Winter	Spring
1	Temperature (°C)	18.33	18.1	18.16
2	pH	7.6	7.97	8.03
3	Dissolved Oxygen (mg/l)	-	-	6.53
4	Conductivity (µS/cm)	-	-	74.1

Discussion

In hilly rivers, diatoms are frequently attached on the submerged pebbles and stones making the substrates brown and slippery coating. Due to water current, rivers are less suitable habitats for desmids and some other planktonic algae (Kiran, 2016). In Tamor river also,

diatoms specially of epilithic form were the major constituent in comparison to the other groups of algae.

The maximum number of species of *Gomphonema* and *Navicula* found in Tamor river is also supported by the diatoms recorded from Bagmati river (Rai & Khadka, 2018). The common diatoms found in both Tamor and Bagmati rivers were *Achnanthes crenulata*, *Cymbella tumida*, *Diatoma hiemale* var. *mesodon*, *Epithemia adnata*, *Fragilaria voucheriae*, *Frustulia vulgaris*, *Navicula radiosa*, *N. rostellata*, *Nitzschia linearis*, *N. palea*, *Planothidium lanceolatum*, and *Ulnaria ulna*. In Bagmati River, the poor representation throughout the study period were of following genera, viz., *Amphora, Aulacoseira, Cocconeis, Cyclotella, Diatoma, Diploneis, Fragilaria, Meridion, Neidium, Planothidium, Sellaphora* and *Ulnaria*. These were presented by single taxa whereas in Tamor River genera, viz., *Placoneis, Planothidium, oscillatoria, Craticula, Amphora, Eunotia, Cosmarium, Sellaphora, Rimeria, Frustulia, Cocconeis, Achnanthes* were found scarcely.

In general, dominant diatom taxa of Bagmati river were *Achnanthes crenulata, Gomphonema pseudoaugur, Nitzschia linearis, N. palea, Pinnularia* cf. *divergens* and *Surirella linearis* and rarely present species were *Amphora ovalis, Aulacosiera granulata, Cymbella aspera, C. tumida, Diploneis ovalis, Encyonema ventricosum, Epithemia adnata, E. sorex, Eunotia bidens, E. botuliformis,* and *E. minor* (Rai & Khadka, 2018). Similarly, dominant taxa of Tamor river were *Ulneria ulna, Navicula rostellata, N. radiosa, Gomphonema lacusrankaloides, G. minutum, G. insularum, G. lagenula, Cymbella tropica,* and *C. tumida.*

In autumn season, warm temperature prefers the rapid growth and reproduction of algae (Singh & Singh, 2015). Maximum number of algal flora was available in this season. It may be due to availability of light, nutrition and favourable temperature. Where as in winter season (December), least number of algae was available and it may be due to lack of proper sunlight to conduct photosynthesis, suitable pH and temperature. However, during spring (March) number of algae was greater in comparison to winter.

Diatom flora during summer, winter and rainy seasons observed at Sundarijal site of Bagmati river showed that *Achnanthes crenulata, Nitzschia linearis* and *Surirella linearis* were dominant taxa throughout all three seasons. Maximum number of taxa were reported during summer followed by rainy and least during winter (Rai & Khadka, 2018), whereas diatom flora during autumn, winter and spring observed at Tamor River showed that *Achnanthes*, *Amphora*, *Cocconeis*, *Diatoma*, *Encyonema*, *Gomphonema*, *Hannaea*, *Navicula*, *Planothidium*, and *Ulnaria* were available throughout all seasons. Maximum number of algae was reported during autumn followed by spring and least during winter.

Conclusion

The epilithic form of algae in Tamor River was dominated by diatoms. Gomphonematacea was the richest family with 13 species and *Gomphonema* was the richest genera with eight species. Maximum number of algae was recorded during autumn season whereas minimum number was recorded during winter. The dominant genera during the study period were *Gomphonema*, *Navicula,* and *Ulnaria* whereas least reported genera were *Achnanthidum, Cosmarium, Craticula, Cymbopleura, Epithemia, Frustulia, Oscillatoria*, and *Placoneis*.

Among the three seasons, highest water temperature was recorded in autumn season and lowest in winter. Similarly, highest water pH was recorded in spring season and lowest in autumn. Dissolved oxygen and conductivity of water in spring were 6.53 mg/l and 74.1 µS/cm, respectively. Algal flora specially the diatoms of Tamor River is rich and diverse. It needs further detail studies to update documentation and conservation of algae.

Acknowledgements

We are thankful to the Chairman, Department of Botany, Post Graduate Campus, T.U., Biratnagar for laboratory facilities. The first author is grateful to the Department of Plant Resources, Thapathali, Kathmandu, Nepal for financial support to conduct this work under Global Taxonomic Initiative Program.

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Formulation of Amylolytic Starter using Yeasts and Molds Screened from Traditional *Murcha*

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Abstract

Amylolytic starter (*murcha*) was formulated in the laboratory using different ratios of yeasts and molds screened from traditional *murcha. Murcha* samples (collected from Saangu, Udayapur, Kerabari, Dhankuta, Laxmimarga, Belbari, Dandaghopa, Bishnupaduka, Panmara, Letang) were screened for amylolytic molds and fermentative yeasts, characterized and preserved for the study. Mold was used in the form of wheat bran *koji* (fungal culture) and yeast in the form of actively growing suspension. Experimental design was generated by Design Expert® to formulate *murcha* using *koji* (1, 2, and 3 g) and yeast (0.25, 0.50, and 0.75 mL) in 50 g of rice flour (moisture ~ 30% and particle size < 280m). Physicochemical analysis, performance test, sensory analysis and statistical analysis were carried out to select the best formulation.

Mold (identified as *Amylomyces rouxii* Calmette) and yeast (identified as *Saccharomyces cerevisiae* Meyen ex Hansen) from Udayapur *murcha* were found to be the most suitable. The *murcha* formulation with 2 g *koji* (~ $2´10^8$ spores) and 0.5 mL yeast suspension (~ 510^5 live yeast cells) per 50 g of rice flour was significantly superior to other *murcha* formulations in terms of physicochemical- and sensory quality. In conclusion, the ratio of mold to yeast in *murcha* formulation is critical in the preparation of *murcha* of very good quality. The method developed in this work is sustainable (does not depend on harvesting of exotic plants), reliable (in terms of culture purity), consistent (in terms of quality), and applicable even in the rural setting.

Key words: Amylolytic starter, *Amylomyces rouxii*, *Jand*, *Murcha*, *Saccharomyces cerevisiae*

Introduction

Marcha or *murcha* is a mixed dough inoculum used as an amylolytic starter to produce ethnic alcoholic beverages such as *jand* (undistilled) and *raksi* (distilled) in the Himalayan regions of India, Nepal, Bhutan, and Tibet (Tamang, 2010). The traditional method of *murcha* preparation entails use of around 38 identified wild plants, hereafter called *murcha* plants. The most prized *murcha* plant is *Polygala arillata*, the semi-dried root barks of which reportedly sell at NRs 4,000/kg. Other *murcha* plants of importance are *Polygala abyssynica* R. Br. ex Fresen*, Vernonia cinerea* (L.) Less, and *Inula* spp. These plants harbor a complex association of essential microorganisms, a feature that a *murcha* maker exploits for the amylolytic fermentation (KC et al., 1999).

Amylolytic starters similar to *murcha* are used in many different countries of the world for cereal-based fermentations. However, the most dominant production and use of such

starters occur in the Asian countries. As a result of serious researches, many of them (for instance, Chinese *Chu*, Korean *nuruk*, Malaysian *ragi*, and Japanese *koji*) have now evolved as commercial commodities (Lee, 1999).

Microbiologically, *murcha* is a mixed culture containing saccharifying molds, fermenting yeasts, and acidifying bacteria (KC et al., 2001). Several researchers, including Rai (2006) and Rai (2015) have successfully prepared *murcha* in the laboratory using isolated molds and yeasts from local *murcha*. Their findings indicate that it is possible to produce good-quality *murcha* using defined cultures.

Review of literature reveals that researches on *murcha* so far are limited to survey, *murcha* preparation, screening, and characterization of *murcha* flora. Quantitative aspects of the inocula, which is very important in the preparation of *murcha* of consistent quality, appears to have been ignored. Likewise, suitable choice of raw-materials for substrate, particle size of substrate, drying condition, shelf-life of *murcha*, cake/ball size have not been studied yet. Moreover, the sanitary and phytosanitary (SPS) aspect of the starter has not been studied.

It is expected that this finding will aid researchers in identifying areas and scope for future research. One of the aims of this study was to devise a simplified protocol for the improvement and preparation of *murcha* in the laboratory and at the same time offering practicability in the tribal setting so that ultimate beneficiary of this work will be the tribes who have depended on this traditional art for so long. This work is also a small attempt to upgrade quality of *murcha* such that it can meet the stringent criteria of food safety/SPS measures laid down by developed countries in terms of trading of foods. Lastly, the developed method will have sustainability implications whereby the producers will not have to depend on exotic (and sometimes already rare) plants/herbs.

Materials and Methods

Sample Collection

Murcha samples (prepared by the natives) were collected from 10 sites (Saangu, Udayapur, Kerabari, Dhankuta, Belbari, Laxmimarga, Dandaghopa, Bishnupaduka, Panmara and Letang) to represent 5 districts (Morang, Sunsari, Dhankuta, Udayapur, and Taplejung).

Rapid Screening of *Murcha* Sample

Amylolytic molds and fermentative yeasts are the essential microorganisms in *murcha*. Given the diverse materials and methods used by the natives in a tribal setting, the quality of *murcha* is subject to significant variation. Method described by Rai and Subba (2016) was used to reduce the number of *murcha* samples by rejecting the poor ones. For this, ~ 100 g of cooked rice was inoculated with 1 g of *murcha* sample and left for fermentation left (in small plastic jars) for 15 days. *Jand* produced in this preliminary step were subjected to general analysis (liquefaction, taste, smell, sourness and overall) as the important criteria for selection.

Screening of Fermentative Yeasts from Screened *Murcha*

The screened *murcha* samples were used for screening of fermentative yeasts by the technique described by KC et al. (1999). Accordingly, 10 g of *murcha* powder was suspended in 100 mL of sterile enrichment broth (15% molasses broth, pH 4.5 with H_2SO_4, boiled and cooled) and incubated at 30°C for a week. Microscopic examination of the fermenting broth was carried out by negative staining. The yeast-positive broths were spread-plated on a series of 5-6 molasses agar plates and incubated again at 30°C for 2-3 days. The plates that bore the well-isolated colonies were selected and subcultured in molasses agar for stock culture (Rai & Subba, 2016).

Testing of Yeast Performance

About 4 loopfuls of yeast culture from the preserved slant were pitched in 750 mL of sterile high-test cane molasses broth (5°brix and pH 4.5). Fermentation was carried out in cotton plugged 1 L PET bottle at 30°C until the TSS ceased to decrease further. The characteristics of the isolates and the beer itself were noted to get an idea regarding their suitability in starter preparation. The beer was analyzed for sensory attributes using 9 point hedonic rating (Rai & Subba, 2016) and data were analyzed to best isolate for further study.

Identification and Preservation of Fermentative Yeasts

The selected yeasts were subjected to microscopic examination by negative staining with nigrosine (Aneja, 2003). The colony characteristics of isolated yeasts were studied and noted following method in Barnett et al. (1990). The isolates were further characterized by modified auxanography (sugar assimilation test) (Rai & Subba, 2004) and sugar fermentation test (Suh et al., 2008). The isolated yeasts were preserved in normal slants (Kirsop, 1987).

Screening of Molds from *Murcha*

The screening of the most desirable mold was done using method given by Rai and Subba (2016). Small pieces (~ 3 mm × 3 mm) of the screened *murcha* were embedded at 2-3 places with the help of tweezers in molasses agar, keeping at least 1.5 cm space between the pieces. The plates were incubated at 27-28°C for 2-3 days and observed daily for cottony growth. The distinctly different colonies were subcultured on a fresh molasses agar plate by spot-culturing with the help of inoculating needle. The most desirable type of mold was selected first by testing for performance. Briefly, the performance test involved inoculating cooked rice (~ 100 g) in transparent, wide-mouthed PET jars with mold isolates from the plate, incubating the jars for 10 days at 28-30°C, and subjecting the product to sensory- and liquefaction tests.

Identification and Preservation of Amylolytic Molds

The selected mold was grown in molasses agar plates and flat bottles until luxuriant growth and then preserved at refrigeration temperature. Characterization of the mold was done by tape culture (Rai & Subba, 2016) and dichotomous keys (Harrigan & McCance, 1976; Malloch, 1997).

Rice Flour Preparation for *Murcha* Making

Non-sticky variety of rice (2 kg) was washed and steeped for 3 h in warm water (40°C) that was acidified to pH 2.5 with 2% aqueous citric acid. The steeped rice was drained and ground in a mortar-and-pestle followed by an electric grinder to give a moist rice flour containing ~ 30% moisture. The flour used for *murcha* preparation was subjected to sieve analysis to get an idea about the distribution of particle size. Only the flour fraction that was < 280 μ was used for *murcha* making.

Mold Mropagation (Koji Making)

The selected mold was propagated aseptically in sterile wheat bran to produce *koji* (a term for fungal culture). The process involved profuse sporulation of mold on molasses agar (at 28-30°C, for 4-5 days) before propagation. Propagation was done in sterilized, moist wheat bran in a 1 L conical flask for 5-6 days at 28-30°C and ~ 95% RH. The *koji* was taken out, mycelial network broken, dried in an electronic dryer at 50±1°C until around 7% moisture, packed in a reclosable polyethylene bag, and stored at ~ 5°C until needed (Rai & Subba, 2016). In the meantime, mold count was performed in the *koji* by plating on Potato Dextrose Agar (PDA) amended with 35 ppm of Rose Bengal (Rai, 2012).

Yeast Propagation

A thick yeast suspension was prepared by growing the yeast culture in 10% molasses broth in a conical flask. In the meantime, yeast count was done by direct microscopic count (Rai & Subba, 2016).

Starter (*Murcha*) Preparation and Experimental Design

Wheat bran *koji* was mixed with yeast isolates and propagated in moist rice flour as a carrier-cum-medium. For every 50 g of moist rice flour, calculated amount of dry *koji* (~ 7% moisture content) and thick yeast suspension (obtained by propagating in the laboratory in 10% molasses broth in conical flask) were added.

The experimental design (Central Composite, Face-centered, 2 Factors, 3 Levels, and 13 Runs, Table 1) was done with Design Expert V7.25. For every 50 g of rice flour three levels of *koji* (1, 2 and 3 g) and 3 levels of yeast suspension (0.25, 0.5 and 0.75 mL) were used. The details of the runs are given in the experimental plan (Table 2).

Table 1. Experimental design for the formulation of amylolytic starter using yeast and mold isolates

Mold (g)[a]	2	2	1	2	3	1	3	1	2	3	2	2	2
Yeast (mL)[b]	0.5	0.5	0.5	0.5	0.75	0.75	0.50	0.25	0.50	0.25	0.50	0.25	0.75

(g)[a] = g of *koji* (1 g *koji* =10^8 *Rhizopus oryzae* spores): (mL)[b] = mL of yeast suspension (*Saccharomyces cerevisiae*), where 1 mL ~ 10^6 cells

Preparation of the cakes was done according to Rai and Subba (2016) (Fig. 1). Briefly, the admixture was formed into a stiff dough (~ 43% moisture, adjusted by adding calculated amount of distilled water). The dough was then molded into circular cakes by placing over muslin-lined regular Petri plate. The plate was inverted on a muslin-lined wire-mesh to get

Table 2. Experimental plan for response surface analysis

Standard	10	11	8	3	1	5	7	2	12	13	9	6	4
Run	1	2	3	4	5	6	7	8	9	10	11	12	13
Factor A: Mold (g)[a]	2	2	2	1	1	1	2	3	2	2	2	3	3
Factor 2: Yeast (mL)[b]	0.5	0.5	0.75	0.75	0.25	0.5	0.25	0.25	0.5	0.5	0.5	0.5	0.75
Response 1: Alcohol (% abv)													
Response 2: Amylase (Unit)[c]													

(% abv) = % alcohol by volume; (Unit)[c] = amount of enzyme (*murcha*) needed to liberate 1 µM of maltose equivalent in 1 min at 40°C

circular cakes. The molded cakes were finally covered with sterile wet muslin cloth and allowed to ferment for 2 days at 28-30°C.

The swelling of cake, appearance of profuse mycelia, and prevalence of sweet alcoholic smell were taken as indicator for the completion of fermentation. The cakes were dried at 50±1°C in a cabinet drier (having provision for hot air circulation) for 6 h to reduce moisture content to ~ 9%. The cakes were finally packed in polyethylene bag, sealed and stored at refrigeration temperature (~ 4°C).

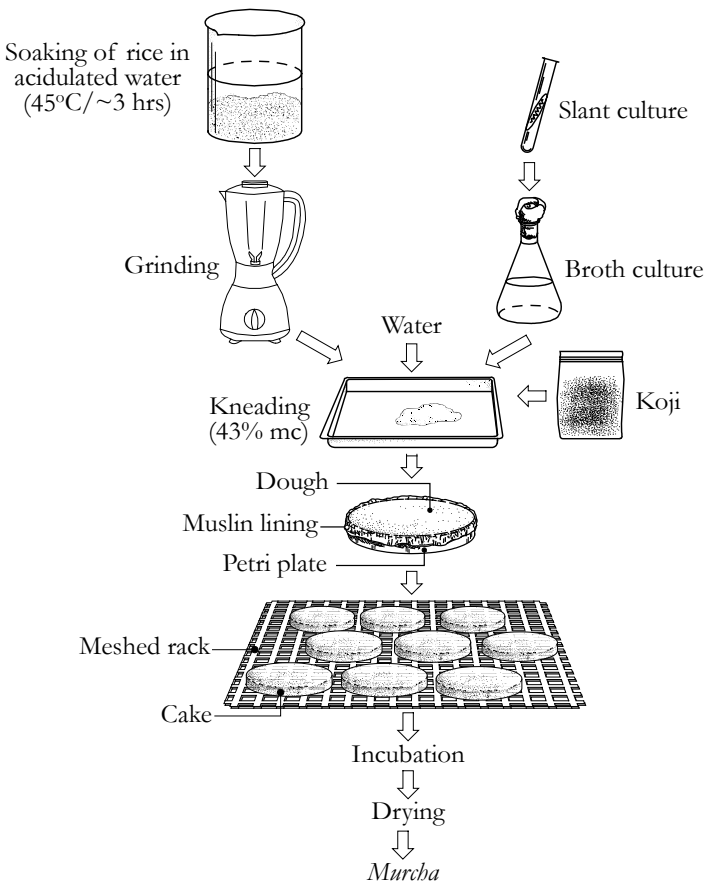

Figure 1. A recapitulative diagram for murcha preparation

Testing of Performance of Formulated Starter

Performance of the formulated starter was tested by trial fermentation (15 days at 28-30°C) on cooked rice (100 g cooked rice inoculated with 1% prepared *murcha*), followed by sensory and chemical analysis of the product. The best formulation was selected on the basis of sensory (taste, acidity balance, liquefaction of fermented mash), diastatic activity (amylase activity), and alcohol content.

Physicochemical Analysis of Formulated Starter

Amylase activity was carried out using modified method given by Rai and Subba (2016), a working outline of which is given in figure 2. The amylase activity was calculated as amount of enzyme that liberates 1 µM of maltose equivalent in 1 min at 40°C (Mulimani & Lalitha, 1996). Alcohol content was determined by pycnometric method (Rai & Subba, 2016).

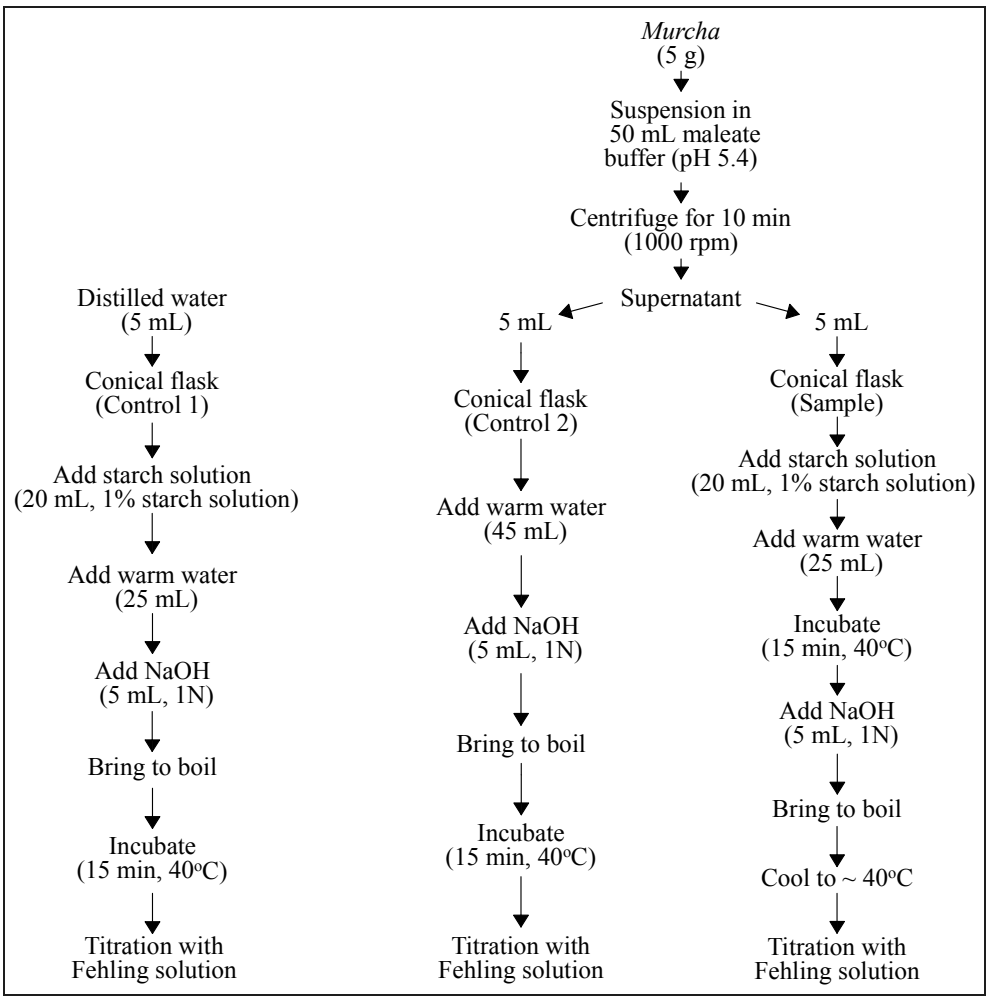

Figure 2. Schematic outline of amylase activity determination

Data Analysis

Data on sensory quality of *jand* from different *murcha* were statistically processed by Genstat Release v12 (Payne et al., 2009) for Two Way Analysis of Variance (ANOVA). Means of the data were compared using LSD (least significance difference) method at 5% level of significance. Data on alcohol content was processed using Design Expert® for response surface curves, contour plots, and model equations.

Results

Prelimary *Murcha* Screeing

Jands produced in this preliminary step were subjected to liquefaction and sensory tests (taste, smell, sourness and overall) as the criteria for selection. ANOVA of samples for liquefaction, taste, smell, sourness and overall showed significant difference ($p < 0.5$) between *murcha* samples whereas there was no difference among the panelists. A summary of difference in liquefaction properties and sensory attributes between the *murcha* samples is given in Table 3. Based on the statistical evidence, two *murcha* samples, viz., *murcha* from Dhankuta and Letang were rejected while the remaining 8 were retained for subsequent tests.

Table 3. Summary of difference between the liquefaction properties of murcha samples and sensory attributes of jand prepared using the murcha samples

***Murcha* sample**	**Liquefaction#**	**Taste**	**Smell**	**Sourness**	**Overall**
Belbari	3.8^{a} (0.45)	3.8^{c} (0.45)	3.8^{cd} (0.45)	4.2^{b} (0.45)	3.8^{b} (0.45)
Dhankuta	3.8^{a} (0)	2.2^{b} (0.45)	1.2^{a} (0.45)	1.2^{a} (0.45)	1.2^{a} (0.45)
Panmara	3.8^{a} (0.45)	3.8^{c} (0.45)	3.8^{cd} (0.45)	3.8^{b} (0.45)	3.8^{b} (0.45)
Saangu	3.8^{a} (0.45)	4.8^{d} (0.45)	3.6^{c} (0.55)	3.8^{b} (0.45)	4.2^{bc} (0.45)
Udayapur	3.8^{a} (0.45)	5.0^{d} (0)	4.8^{e} (0.45)	4.8^{c} (0.45)	4.4^{cd} (0.55)
Laxmimarga	4.0^{a} (0.45)	4.2^{c} (0.45	4.2^{d} (0.45)	4.8^{c} (0.45)	4.2^{bc} (0.45)
Letang	4.0^{a} (0)	1.2^{a} (0.45)	1.2^{a} (0.45)	1.4^{a} (0.55)	1.2^{a} (0.45)
Bishnupaduka	4.2^{a} (0.45)	4.2^{c} (0.45)	3.0^{b} (0)	4.8^{c} (0.45)	4.0^{bc} (0)
Dandaghopa	4.8^{b} (0.45)	4.8^{d} (0.45)	4.0^{cd} (0)	4.8^{c} (0.45)	4.8^{d} (0.45)
Kerabari	4.8^{b} (0.45)	3.8^{c} (0.45)	3.8^{cd} (0.45)	3.8^{b} (0.45)	3.8^{b} (0.45)

Under conditions described in the methodology, all of those mold isolates were found to saccharify cooked rice and sensory analysis of the saccharified rice was carried out. The statistical part of the test data is given in table 4.

Table 4. Summary of difference between the liquefaction properties of mold isolates and sensory attributes of saccharified rice prepared using the isolates

Mold isolates	**Liquefaction**	**Sweetness**	**Smell**	**Sourness**	**Overall**
Belbari	4.2^{b} (0.45)	3.2^{a} (0.45)	4.2^{c} (0.45)	3.2^{cd} (0.45)	3.8^{c} (0.45)
Laxmimarga	2.8^{a} (0.45)	4.0^{c} (0)	3.0^{b} (0)	2.0^{b} (0)	3.0^{b} (0)
Panmara	3.8^{b} (0.45)	4.0^{c} (0)	2.8^{b} (0.45)	2.8^{c} (0.45)	2.8^{b} (0.45)
Udayapur	5.0^{c} (0)	4.0^{c} (0)	4.0^{c} (0)	3.8^{e} (0.45)	5.0^{d} (0)
Kerabari	4.8^{c} (0.45)	4.0^{c} (0)	1.2^{a} (0.45)	1.0^{a} (0)	1.2^{a} (0.45)
Dandaghopa	5.0^{c} (0)	3.6^{b} (0.55)	3.8^{c} (0.45)	3.4^{de} (0.55)	4.2^{c} (0.45)
Bishnupaduka	3.8^{b} (0.45)	4.0^{c} (0)	3.2^{b} (0.45)	1.0^{a} (0)	2.8^{b} (0.45)
Saangu	5.0^{c} (0)	4.0^{c} (0)	4.0^{c} (0)	3.2^{cd} (0.45)	4.0^{c} (0)

It is evident from table 4 that only the Udaypur *murcha* had the significantly superior ($p < 0.05$) overall property and hence was the only *murcha* retained for further study. Other *murcha* samples failed to meet the criteria set up for smell and sourness attributes of *jand*.

Screening of Mold

The isolated mold was identified to be *Rhizopus oryzae* (*Amylomyces rouxii*). The KEYS referred for the identification of the mold genera are given by Malloch (1997).

Screening of Yeast

The best yeast isolate was selected based on fermentation vigor which was determined by trial fermentation test. The fermented molasses broth was subjected to sensory analysis (9 point hedonic rating) and the statistical part for which is given in table 5. Based on the test data, yeast isolates from Udayapur and Laxmimarga *murcha* were the best but only Udayapur yeast was selected (excelled in few counts). They both were identified to be strains of *Saccharomyces cerevisiae*.

Table 5. Summary of difference between sensory attributes of fermented molasses broth prepared using the yeast isolates

Yeast isolates	Alcohol	Clarity	Color	Smell	Sourness
Bishnupaduka	1.20^{a} (0.45)	5.20^{a} (0.45)	6.20^{a} (0.45)	4.80^{b} (0.45)	5.60^{c} (0.55)
Kerabari	1.20^{a} (0.45)	5.00^{a} (0.71)	6.00^{a} (0.71)	3.60^{a} (0.55)	5.60^{c} (0.55)
Panmara	1.20^{a} (0.45)	4.80^{a} (0.45)	5.60^{a} (0.55)	5.60^{c} (0.55)	2.40^{a} (0.55)
Belbari	1.40^{a} (0.54)	5.40^{a} (0.55)	7.00^{b} (0.71)	6.40^{d} (0.55)	4.80^{b} (0.45)
Dandaghopa	2.80^{b} (0.45)	5.40^{a} (0.55)	6.00^{a} (0.71)	3.80^{a} (0.45)	5.80^{c} (0.45)
Saangu	2.80^{b} (0.45)	5.40^{a} (0.55)	5.60^{a} (0.55)	5.80^{cd} (0.45)	5.80^{c}(0.45)
Laxmimarga	8.20^{c} (0.45)	7.40^{b} (0.55)	8.40^{c} (0.55)	8.40^{e} (0.55)	7.40^{d} (0.55)
Udayapur	8.20^{c} (0.45)	8.40^{c} (0.55)	8.20^{c} (0.45)	8.60^{e} (0.55)	7.40^{d} (0.55)

Formulation and Testing of *Murcha*

Using the selected mold (*Amylomycess rouxii*) *koji* and yeast (*Saccharomyces cerevisiae*), both screened from Udayapur *murcha*, starters were formulated in terms of their proportion. The formulated starters were prepared using calculated amounts of yeast and mold isolates derived from experimental design (Table 2).

The best formulation was found to be 2.00 g *koji* and 0.50 mL yeast suspension. The analyzed statistical data for the same is shown in tables 6 and 7. The contour plot and Response Surface are given in figures 3 and 4, respectively.

Alcohol Content

The alcohol content in cooked rice after fermenting for 15 days at 30°C using 1% starter in 100 g of cooked rice ranged from 6.35±2.81 to 10.3±3.15% abv (Table 6). A summary of statistical test for alcohol production by varying yeast levels is given in table 7. The same by varying *koji* levels (Table 7) was 5.57±1.42 to 10.57±1.66% abv.

Table 6. Summary of statistical test for alcohol production by varying yeast levels

Source of variation	Amount of yeast (mL)	Alcohol (% abv)	LSD (5%)
vYeast	0.25	6.82 (2.25)[a]	2.58
	0.5	6.34 (2.81)[ab]	
	0.75	10.3 (3.15)[b]	

Table 7. Summary of statistical test for alcohol production by varying koji levels

Source of variation	Amount of *koji* (g)	Alcohol (% abv)	LSD (5%)
Mold	1	5.57 (1.42)[a]	2.58
	2	9.32 (2.64)[b]	
	3	10.57 (1.66)[b]	

The analyzed data show that *koji* content at concentrations of 2-3 g and yeast concentrations at 0.5-0.75 mL give significantly higher amounts of alcohol. Both lower levels of *koji* and yeast gave significantly poorer results.

A contour plot or iso-response curve (Fig. 3) and 3D response curve (Fig. 4) relating amounts of mold and yeast levels in starter vis-à-vis alcohol content (the response) showed that some relations exist between the amounts of mold and yeast in the starter for alcohol production.

The final equation of the quadratic model in terms of actual factors is:

$$\text{Alcohol (\% abv)} = -12.47 + 8.52 \times \text{Mold} + 50.59 \times \text{Yeast} + 1.94 \times \text{Mold} \times \text{Yeast} - 1.75 \times \text{Mold}^2 - 51.42 \times \text{Yeast}^2$$

Tables 6 and 7 showed that the alcohol content is influenced by both mold and yeast concentration in *murcha*. Thus, there exists a range of mold-yeast ratios where alcoholic fermentation is most efficient.

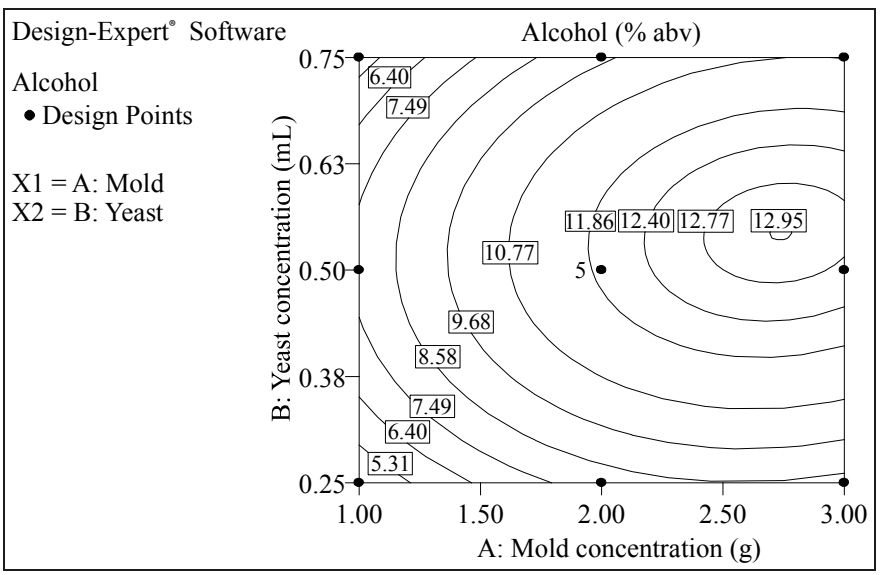

Figure 3. Contour plot showing relation of amounts of mold and yeast in starter for the response alcohol content (% by volume)

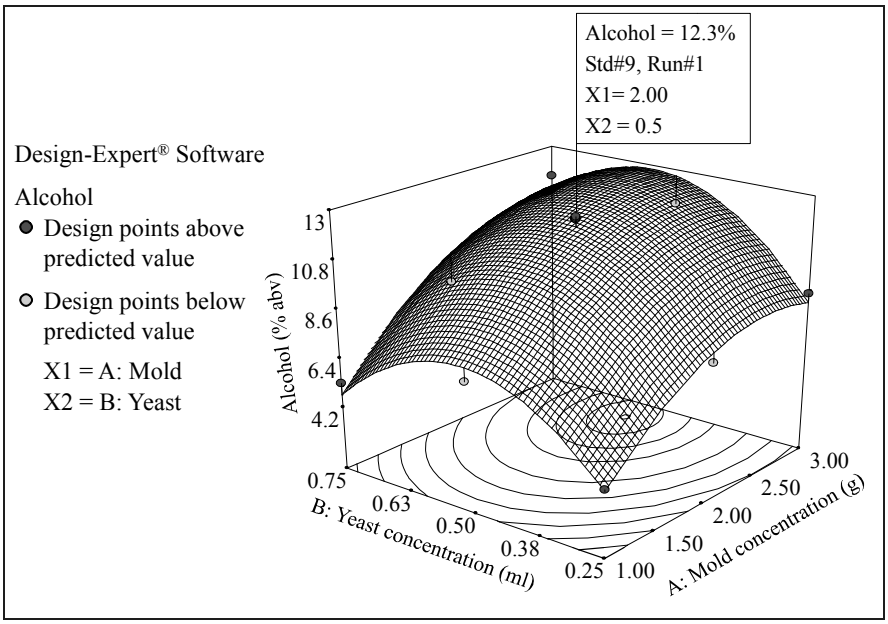

Figure 4. Contour plot showing relation of amounts of mold and yeast in starter for the response alcohol content (% by volume)

Amylase Activity

The highest and the lowest amylase activity in the laboratory *murcha* were 18.69 Unit (at mold level = 1 g, yeast level 0.5 mL) and 6.23 Unit (at mold level = 2 g, yeast level = 0.75 mL). However, the amylase content of *murcha* was not found to significantly change ($p < 0.05$) with the change in mold-to-yeast ratio in the formulation (Table 8).

Table 8. ANOVA table of amylase activity

Source of variation	df	SS	MS	VR	F pr.
Mold	2	49.109	24.554	2.68	0.183
Yeast	2	15.08	7.54	0.82	0.502
Residual	4	36.691	9.173		
Total	8	100.88			

Discussion

Preliminary Screening of *Murcha*

Murcha from Dhankuta was rejected because of the strange flavor and a "strong" acetone taste. *Murcha* from Letang was rejected because of the "very" sour taste. Both acetone flavor and excessive sourness are not desirable. The reason behind this property, however, is still not clear. The acidity and strange flavors may well have been due to dominance of undesirable bacteria in *murcha*. The sensory overall score therefore heavily depends on smell and sourness of the product.

Selection of Mold

Mold isolates from Panmara, Bishnupaduka, Kerabari and Laxmimarga *murcha* samples were rejected by the panelists as these mold isolates failed to meet the criteria set up for smell and sourness attributes of *jand*.

Alcohol Yield

Under the short duration of fermentation carried out in the experiment, the plot shows that 12.95% abv is the maximum that can be achieved. The corresponding mold and yeast contents are 3 g and 0.55 mL. In the semi-commercial fermentation, however fermentation time is usually more than 15 days, and often several months (Rai, 1991). The alcohol content may then slowly reach up to 18% (because of parallel fermentation, with no role of mold in the later phases).

Amylase Activity

Explanation similar to that given for alcohol concentration can be offered here also. It is obvious that the parallel and solid-state natures of fermentation are the main sources of complication (unpredictability). The highly variable micro-environment existing in the *murcha* cake during incubation may be another reason for variations in amylase activity. As such, the amylase level in *murcha* is of minor significance (Rai & Subba, 2016).

Unlike *koji* used in *sake* (Japanese wine) fermentation, the purpose of *murcha* is to serve as an inoculum rather than as an enzyme source. In *jand* production, both the growth of mold and the concomitant production of amylase are thus obligatory (Rai & Subba, 2016).

In the present study, the interaction between the amounts of molds and yeasts in the starter, as far as alcohol yield is concerned, was not found to be significant. Again, this result can be partly attributed to the complex "parallel fermentation" pattern that occurs in cereal "beer" fermentation. Simple sugars are utilized for growth and deriving energy by both yeasts and molds. This apart, yeasts also utilize the sugars to produce ethanol, the primary metabolite. The synthesis of alcohol occurs only under "Crabtree effect", which implies that the reducing sugar level must always be more than 5% in the fermenting mash (Rai, 2012). Considering the varying fates and rates of utilization of simple sugars, it is presumably difficult to predict the nature of interaction(s) between the quantities of amylolytic molds and fermentative yeasts in cereal fermentation that is predominantly of solid-substrate nature. Thus, although the interaction between molds and yeasts in cereal fermentation is clear, the effect(s) of their quantities in the starter is still elusive.

Conclusion

Based on the study, it can be concluded that a good quality *murcha* necessarily contain strains of *Amylomyces rouxii* (*Rhizopus oryzae*) as the amylolytic mold and *Saccharomyces cerevisiae* as the fermentative yeast. *Murcha* of very consistent and good quality can be easily prepared using the method developed in this work. This entails use of pure cultures of mold (in the form of koji) and yeast suspension in a carefully worked out proportion. Following

the method developed in this work, *murcha* makers will not have to depend on herbs/plants (which are sometimes rare and thus pose threat to floral diversity).

Acknowledgements

We owe a debt of gratitude to Dharan Multiple Campus for providing the research facilities. The first author would like to thanks friend and classmate Ms. Jeny Subba for her kind help and cooperation throughout the research period.

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Integrating Biological Resources for Prosperity, 2021, 313-320

Ferns and Fern-allies of Dharan, Eastern Nepal

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Abstract

The pteridophytes covering ferns and fern-allies constitute a significant part of Nepalese flora. This research was conducted from September 2017 to April 2018 to provide an overview of fern and fern-allies of Dharan. A total of twenty-seven species belonging to twenty genera and eleven families were recorded. Out of these, the dominant families are Pteridaceae, Thelypteridaceae, Dryopteridaceae and Polypodiaceae. The dominant genera are *Pteris* and *Thelypteris*. Among them, four species have food value, three have medicinal value, and five have ornamental value.

Key words: Frond, *Nephrolepis cordifolia*, Pteridophytes, Rhizome

Introduction

Pteridophytes are seedless, flowerless, vascular plants found chiefly in humid areas. They require water to grow, survive and at least during sexual reproduction for male gamete to swim to the non-motile female gamete (Sporne, 1975). Broadly, the pteridophytes are divided into ferns and fern-allies. The ferns bear rhizome, scale, fronds, similar spores, therefore called homosporous ferns. The fern-allies are distinct in having rhizomatous roots, reduced scaly leaves and microspore, and megaspore, thus called heterosporous ferns.

There are about 13600 species of fern and fern-allies distributed worldwide (Moran, 2006). There exist some 580 species and subspecies of pteridophytes in Nepal (Fraser-Jenkins et al., 2015). Different tribal communities utilize pteridophytes for food, medicine, agriculture and horticulture. Majority of them are edible. The pioneer plant-exploration and taxonomic study on Nepalese pteridophytes and other groups of plants started with the work of British botanists. The first collections of pteridophytes from Nepal came about at the beginning of the 19th century when the famous botanist Francis Buchanan (Later Francis Hamilton) made collection in 1802-1803 Kathmandu Valley. Although the collection of Nepalese pteridophytes started earlier, the taxonomic and biogeographic study started since David Don when he published a book *Prodromus Florae Nepalensis* in 1825, consisting of some 87 pteridophytes mainly based on collection made by Hamilton and Wallich, being the first ever treatment on Himalayan pteridophytes (Don, 1825). Japanese botanists also played an important role in the enumeration and other research in ferns and fern-allies. The University of Tokyo organized many expeditions to the Eastern Himalaya between 1960 and 1972. The results were published in the three volumes of the *Flora of Eastern Himalaya* (Hara, 1966; Hara, 1971; Ohashi, 1975). Christopher Fraser-Jenkins has made a major contribution on Nepalese Pteridophytes (Fraser-Jenkins, 1997; Fraser-Jenkins, 2011). B.D. Pandey, formerly head of Central Department of Botany, T.U. collected pteridophytes from Kathmandu valley

(1948-1949) and published a list of 65 species (Pandey, 1962). Dr. Vidya Laxmi Gurung published accounts of pteridophyte ecology (Gurung, 1982; Gurung 1984; Gurung, 1985; Gurung, 1992; Gurung, 1994; Gurung, 1995; Gurung, 1997), ethnobotany (Gurung, 1979; Gurung, 1988b) and threatened forms conservation (Gurung, 1988a). One of the important book published by Gurung (1991) is a book on *Ferns the Beauties of Nepalese Flora,* consisting of 93 species under 51 genera and 13 families (Gurung, 1991). Siwakoti and Sharma (1998) recorded 95 species of ferns that belong to 50 genera and 32 families of eastern Nepal, Koshi zone (Shiwakoti & Sharma, 1998). Similarly, Jha (2000) also described 61 species of pteridophytes belonging to 44 genera and 23 families from Morang district (Jha, 2000). Thapa (2000) reported 79 species of ferns from Jaljale-milke area of east Nepal (Thapa, 2000). Pathak et al. (2012) recorded 133 species ferns and fern-allies belong to 59 genera and 26 families from Sankhuwa-sabha district, eastern Nepal with notes on medicinal values. (Pathak et al., 2012). Rajbhandary (2013) collected pteridophytes from Daman V.D.C. Makawanpur district with the application of GIS (Rajbhandary, 2013).

Although several works on ferns and fern-allies in eastern Nepal were carried out but particularly at Dharan, no specific work is done regarding the ferns and fern-allies except for food value of some edible ferns from Dharan (Subba et al., 2001). In this scenario, the present study was carried out to fill the gap in the knowledge of pteridophytic flora of the Dharan.

Materials and Methods

Study Area

Dharan is one of the three major urban centers of Sunsari District of Eastern Development Region. It is sub-metropolitan city, situated on the foothills of the Mahabharat range in the north with its southern tip touching the edge of the Terai region. It is located at Latitude 26°42'41" - 26°52'42" and Longitude 87°12'04" - 87°21'23" and at an elevation of 119m to 1778m above mean sea level (Fig. 1). It covers 192.32 km^2 area. The first locality was Bishnu Paduka, second was Bhedetar, third was Panchakanya, fourth was Hattisar and the last was Panbari. The climate of Dharan is tropical to sub-tropical. The temperature varies from 5°C to 37°C. The average rainfall is 2626mm (Dharan Sub-Metropolitan City, 2021). Southwardly it is extended to the edge of the Tarai in Charkoshe forest, in east to the Morang district, north Dhankuta district and west Baraha Municipality. Major vegetation constituting the dense forest of Sal (*Shorea robusta*), Bot Dhainyaro (*Lagerstromea parviflora*), Karma (*Adina cordifolia*), Asari (*Mussaenda frondosa*), Chilaune (*Schima wallichii*), Sirish (*Albizia saman*), Saj (*Terminalia alata*) and others.

Fern Collection and Identification

In September to October, the field was visited once in post-monsoon to cover both significant growing and flourishing seasons in 2017. Mature and healthy plants were selected for herbarium. The specimens were collected from all four sampling stations with rhizome, hairs and/or scales and fertile portion as far as possible. Before collecting the plant, all the information like color of fertile parts and their natural habitat arrangement was noted down according to Bridson and Forman (1998). The specimens were collected in polythene bags

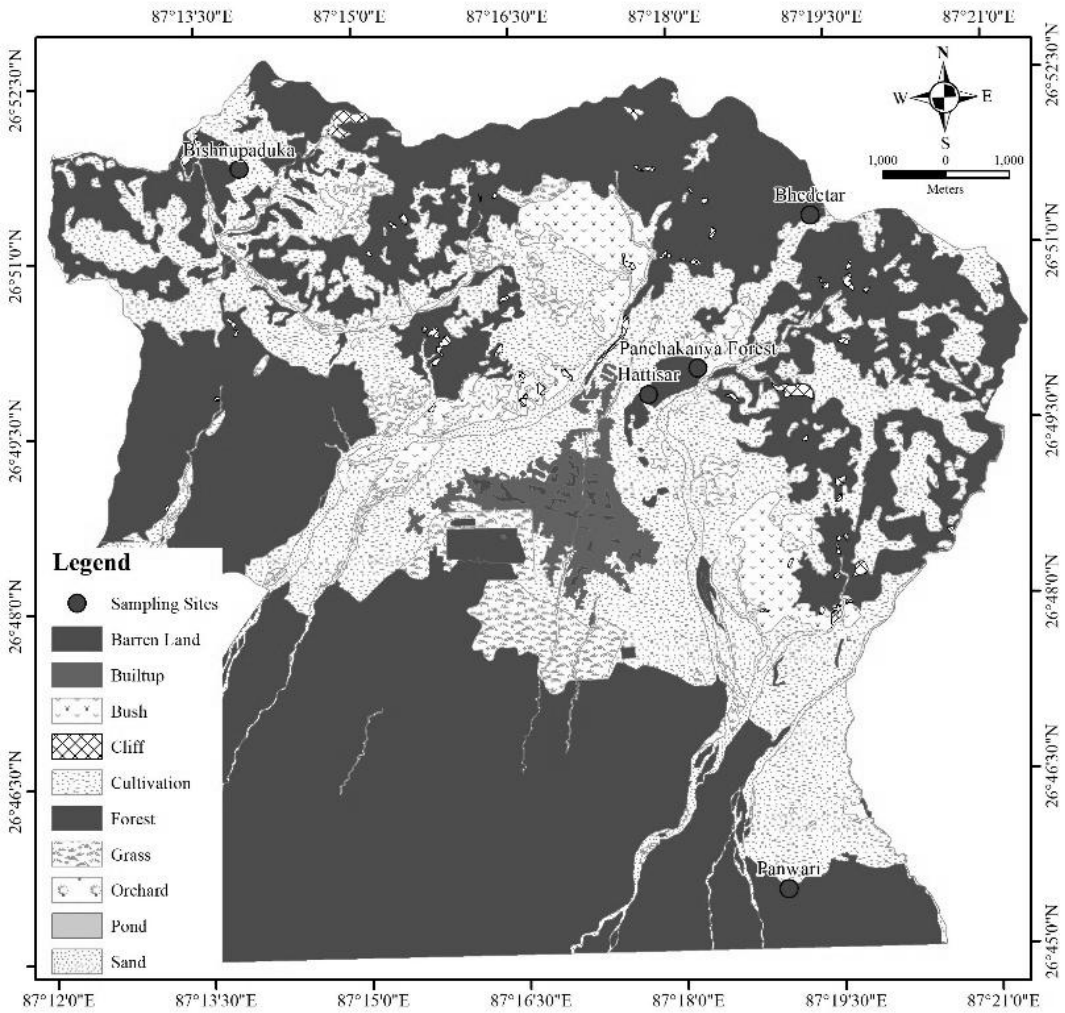

Figure 1. Dharan Sub-metropolitan city showing sampling sites.

and pressed in herbarium press as soon as possible. Photographs of the specimens were taken in their natural habitat and immediately after collecting from the habitat to examine the sori and scales. Photographs were very much helpful in delimiting the taxa, especially at species level. The plant specimens were dried from the same day of preparation. For pteridophyte specimens, simple drying was enough just by properly pressing, regularly checking the specimens during drying and changing paper to obtain neat and clean herbarium. The dried specimens were then mounted on the herbarium sheet. Specimens collected from the field were identified at National Herbarium and Plant Laboratories (KATH), Godawari.

Results

A total 27 species of ferns and fern-allies belonging to 20 genera and 11 families have been found in the study area (Table 1). The dominant genera were *Pteris* (3 sp.) and *Thelypteri*s

(3 sp.). Out of these, the dominant families were Pteridaceae (8 sp.), Thelypteridaceae (3 sp.), Dryopteridaceae (4 sp.) and Polypodiaceae (4 sp.) (Fig. 2). One family Woodsiaceae, was represented by 2 species. Six families, viz., Selaginellaceae, Equisetaceae, Lygodiaceae, Dennstaedtiaceae, Lindsaeaceae, and Nephrolepidaceae were represented by single species. A number of ferns and fern-allies have food, medicinal and ornamental valuees. Ferns used as green vegetables were *Thelypteris prolifera, Diplazium esculentum, Dryopteris cochleate* and *Tectaria coadunate* Similarly, three fern species with medicinal values recorded were *Adiantum incisum, Adiantum philippense*, and *Aleuritopteris bicolor*. Ferns and fern-allies with ornamental values were *Onychium siliculosum, Polystichum lentum, Nephrolepis cordifolia, Phymatosorus cuspidatus*, and *Pyrrosia costata.* Some ferns reported from Dharan are given in figures 3 to 12.

Table 1. Ferns and fern-allies of Dharan

Family	Scientific name
Pteridaceae	1. *Adiantum incisum* Forssk.
Pteridaceae	2. *Adiantum philippense* L.
Pteridaceae	3. *Aleuritopteris bicolor* (Roxb. in Griff.) Khulia & Punetha (Rani Sinka)
Woodsiaceae	4. *Athyrium pectinatum* (Wall. ex Mett.) T. Moore
Woodsiaceae	5. *Diplazium esculentum* Sw.
Polypodiaceae	6. *Drynaria quercifolia* (L.) J. Sm.
Dryopteridaceae	7. *Dryopteris cochleata* (Ham. ex D.Don) C. Chr. (Gheu Niguro)
Equisetaceae	8. *Equisetum arvense* L. subsp. *diffusum* (D.Don) Fraser-Jenk.
Dennstaedtiaceae	9. *Hypolepis polypodiods* (Bl.) Hook.
Lygodiaceae	10. *Lygodium flexuosum* (L.) Sw.
Nephrolepidaceae	11. *Nephrolepis cordifolia* (L.) C. Presl (Pani Amala)
Lindsaeaceae	12. *Odontosoria chinensis* (L.) J. Smith
Pteridaceae	13. *Onychium siliculosum* (Deav.) C. Chr.
Polypodiaceae	14. *Phymatosorus cuspidatus* (D.Don) Pich. Serm.
Pteridaceae	15. *Pityrogramma calomelanos* (L.) Link.
Dryopteridaceae	16. *Polystichum lentum* (D.Don) T. Moore
Pteridaceae	17. *Pteris biaurita* L.
Pteridaceae	18. *Pteris venusta* Kunze subsp. *matsudae* (Masam.) Fraser-Jenk. & Kandel
Pteridaceae	19. *Pteris vittata* L.
Polypodiaceae	20. *Pyrrosia costata* (C. Presl ex Bedd.) Tagawa & K. Iwats.
Polypodiaceae	21. *Pyrrosia porosa* (C. Presl) Hoven. Kamp
Selaginellaceae	22. *Selaginella subdiaphana* (Wall. ex Hook. & Grev.) Spring
Dryopteridaceae	23. *Tectaria coadunata* (Wall. ex J. Sm.) C. Chr. (Kali Niguro)
Dryopteridaceae	24. *Tectaria polymorpha* (Wall. ex Hook.) Copel.
Thelypteridaceae	25. *Thelypteris dentata* (Forssk.) E. St. John
Thelypteridaceae	26. *Thelypteris ornata* (Wall. ex Bedd.) Ching
Thelypteridaceae	27. *Thelypteris prolifera* C. Reed

Discussion

Bhagat and Shrestha (2010) reported 35 species of fern and fern-allies belonging to 28 genera and 23 families from eastern Tarai. The present study recorded 27 species of fern and fern-allies belonging to 20 genera and 11 families from Dharan. Bhagat and Shrestha (2010) reported Polypodiaceae family as dominant family having 15 species and *Adiantum*

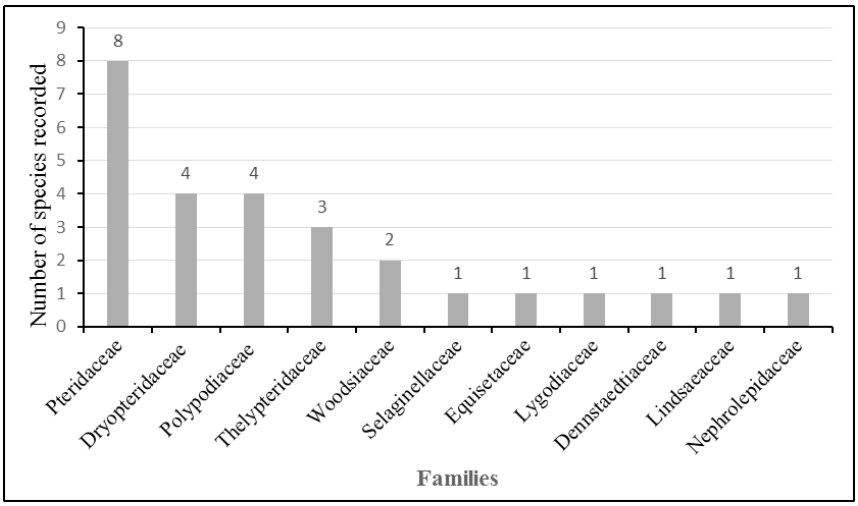

Figure 2. Familywise composition of ferns and fern-allies.

Figure 3. *Adiantum incisum*

Figure 4. *Adiantum philippense*

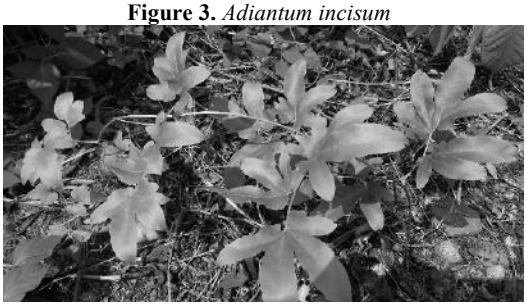

Figure 5. *Lygodium flexuosum*

Figure 6. *Phymatosorus cuspidatus*

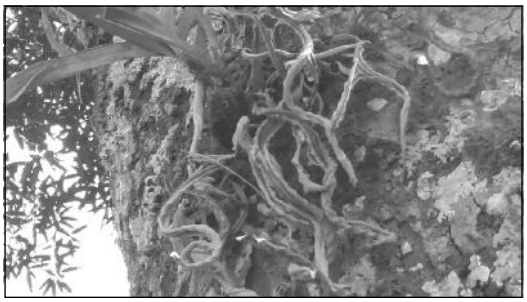

Figure 7. *Pyrossia costata*

Figure 8. *Thelypteris dentata*

Figure 9. *Diplazium esculentum*

Figure 10. *Onychium siliculosum*

Figure 11. *Pteris biaurita*

Figure 12. *Pteris vittata*

genus with four species. In this study, I reported Pteridaceae family having 8 and *Pteris* and *Thelypteris* gerera with three species for each as dominant species. It may be due to small coverage study area of the present research. Bhagat and Shrestha (2010) reported four edible ferns and fern-allies, viz., *Diplazium esculentum*, *Dryopteris cochleata*, *Tectaria coadunate* and *Ophioglossum* sp. which matches the first three species in the present study. In addition, I have also reported *Thelypteris prolifera* as an edible fern. Similarly, Bhagat and Shrestha (2010) reported four species of medicinal ferns and fern-allies, viz., *Lygodium flexuosum*,

Adiantum incisum, *Adiantum philippense* and *Adiantum capillus-veneris* and four species of ornamental ferns and fern-allies, viz., *Adiantum capillus-veneris*, *Cyathea spinulosa*, *Phymatosorus cuspidatus* and *Nephrolepis cordifolia*. First three of medicinal ferns and fern-allies matches with the findings of present study. Whereas only two species of ornamental ferns and fern-allies, viz., *Phymatosorus cuspidatus* and *Nephrolepis cordifolia* matches with the findings of present study.

Conclusion

The present study reported a total of 27 species of ferns and fern-allies belonging to 20 genera and 11 families from Dharan Sub-metropolitan city. Out of these, the dominant families are Pteridaceae, Thelypteridaceae, Dryopteridaceae and Polypodiaceae. The dominant genera are *Pteris* and *Thelypteris*. Among them, four species have food value, three have medicinal value and five have ornamental value.

Acknowledgements

I would like to thanks the Dean Office, Institute of Science and Technology, Tribhuvan University, Kirtipur for providing financial support. I acknowledge Dr. Bimala Subba, Mr. Kamal Maden and Dr. Bhabindra Niroula for their valuable feedback and suggestions to complete this research work.

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Pollution Load and the Diversity of Phytoplankton in the River Mahananda, Malda, West Bengal, India

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Abstract

The present study enlightens us on the pollution and pollution impact on the phytoplankton in Mahananda river, Malda district, West Bengal, India. The water quality was monitored for a period of six months from January to June 2019. The surface water samples were collected month-wise from the three sites of the Mahananda river, namely one from the non-polluted area and two from sewage-mixing areas in the river's north bank. The physico-chemical parameters measured were pH, temperature, acidity, alkalinity, hardness of calcium and magnesium, total hardness, salinity, chloride, sulfate, nitrate, DO, BOD and COD, and their correlations were also assessed. The physico-chemical parameters exhibited clear-cut spatial and seasonal fluctuations. Total 57 species of phytoplankton were identified during the study period which consisted of 29 genera belonging to four taxonomic classes namely Cyanophyta, Chlorophyta, Bacillariophyceae and Euglenophyta. The class Bacillariophyceae was the dominant group of algae in all the three sites of the Mahananda river throughout the study period. The present study revealed that eleven numbers of pollution-tolerant algal genera namely *Oscillatoria, Lyngbya, Nitzschia, Navicula, Cocconeis, Cymbella, Cyclotella, Melosira, Synedra, Stauroneis,* and *Euglena* were found in the river which indicated that the quality of the river was deteriorated at all the selected three sites in the Mahananda river and were polluted due to human interventions. The analysis of physico-chemical parameters also indicated deterioration of water quality of the Mahananda river at all the study sites during the period of study.

Key words: Algae, Diatoms, Dissolved oxygen, Physico-chemical parameters, Water quality

Introduction

Over the past several decades, rivers worldwide have become increasingly polluted due to increasing pressure from human activities, affecting their ecological equilibrium, inevitably affecting flora and fauna of water bodies. The algae are used for assessing the degree of pollution and as a water pollution indicator (Palmer, 1969). The phytoplankton indicates the impact of pollutants on the aquatic environment. Any effect on the lower level of the food chain has consequences with higher-level organisms.

Pollution of India's major rivers through the discharge of industrial effluents and domestic sewage is the major threat in recent times (Singh et al., 2007). The biological communities change with the change in the environment in which they occur. The relative proportion of abundance of the species in a community often provides a good indication of pollution (APHA, 1998).

The river Mahananda acts as a water resource for the Malda town, Malda District. The river receives untreated sewage and domestic wastes from different areas of the Malda town in both banks, thus continuously polluting the river. The river water resources in West Bengal state are subjected to substantial stress due to riverine ecology changes. River Mahananda near Malda town is of no exception. With this view in mind, the present work was carried out with the following objectives: to collect water samples from selected sites of the river, analyze physico-chemical parameters of the water, and enumerate phytoplankton.

Materials and Methods

The present study was conducted in Mahananda River at three sites, namely (S1) (24.993º N/88.151º E), (S2) (24.989º N/88.145º E), and (S3) (24.978º N/88.153º E) for a period of six months (January 2019 to June 2019). The surface water samples were collected monthly for above-mentioned period (Fig. 1).

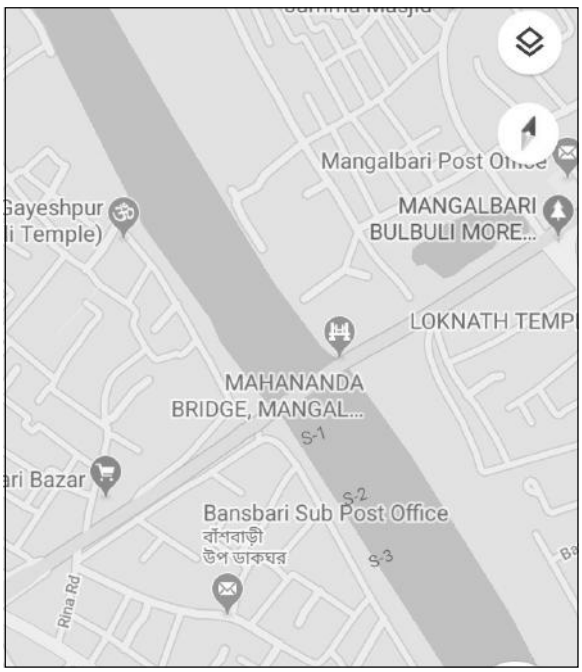

Figure 1. Course of Mahananda river showing different sampling sites

Determination of Physico-chemical Parameters

The temperature and pH of water samples were noted by standard methods on the spot at the time of collection. The samples for the determination of dissolved oxygen (DO) were collected and fixed on the spot. The physico-chemical parameters namely acidity, alkalinity, calcium hardness, magnesium hardness, total hardness, chloride, salinity, nitrate, sulphate, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were analyzed in the laboratory by standard methods (APHA, 1998).

Analysis of Phytoplankton

The plankton nets were used to collect samples for the plankton's qualitative and quantitative estimation by filtering a known volume of water (100 L) through the net with 60µ mesh size. The sample was allowed to settle for 24-48 h and was further concentrated to approximately 50 mL by decanting. The numbers of phytoplankton per liter was counted. Sedgwick-Rafter (S-R) cell method was used for counting the phytoplankton (APHA, 1998). The filtrate was immediately preserved in 4% formaldehyde for the identification of phytoplankton upto genera according to identification keys given by Edmondson (1959), APHA (1998) and Roy and Datta Munshi (2010). Correlation studies were performed by the methods of Das and Das (1998).

Results

The study revealed that the water quality parameters fluctuated with sites and seasons (Figs. 2-4). The growth and reproduction of phytoplankton are influenced by the physico-chemical characters of water (APHA, 1998). The analysis of the physico-chemical parameters of water indicated that the Mahananda River's water was deteriorated and showed monthly fluctuations. The pollution was severe during pre-monsoon season (February to May). The pollution was minimized in June due to the rainfall.

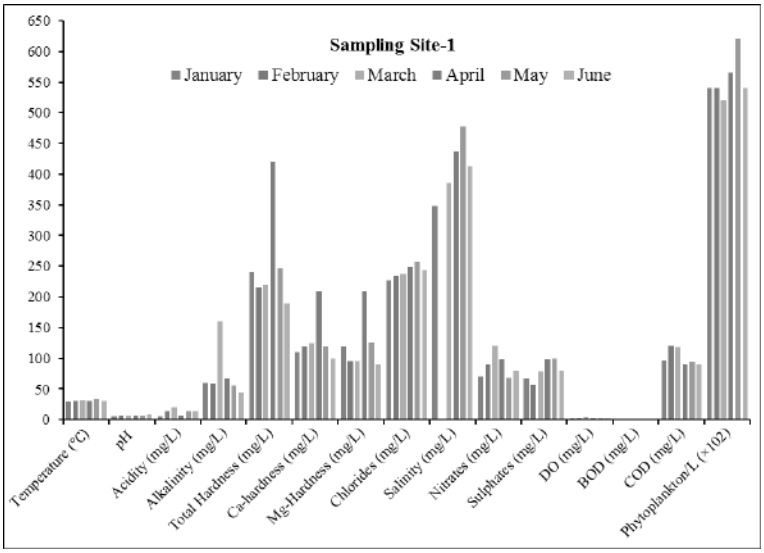

Figure 2. Physico-chemical parameters and phytoplankton in sampling site-1 of Mahananda river.

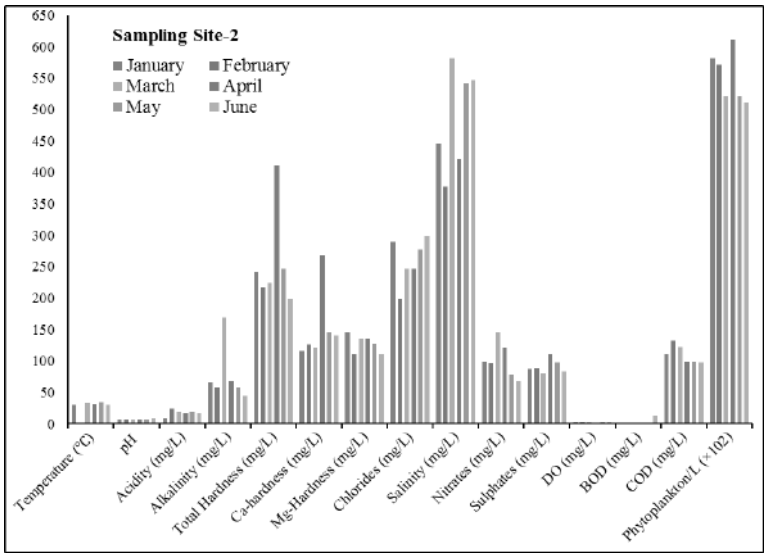

Figure 3. Physico-chemical parameters and phytoplankton in sampling site-2 of Mahananda river.

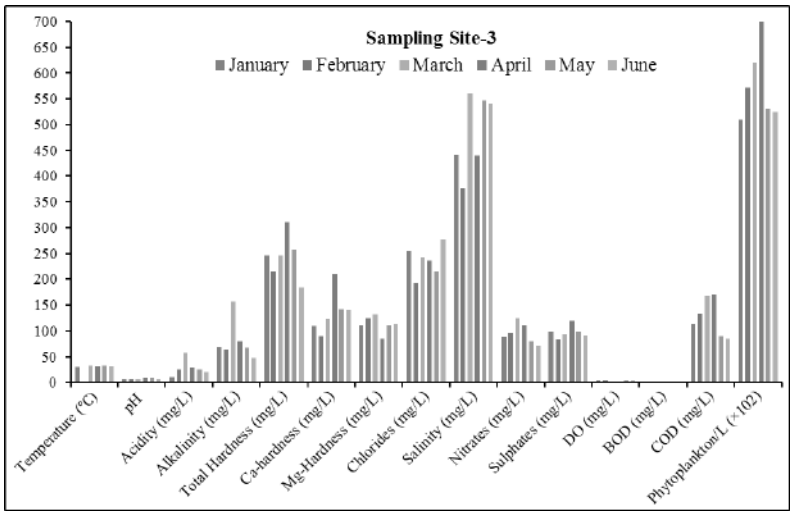

Figure 4. Physico-chemical parameters and phytoplankton in sampling site-3 of Mahananda river.

The range of temperature observed in the present study was from 29.2°C (January) to 34.4°C (May). The range of variation of sulfate was from 57.6 (February) to 119.95 (April). Maximum values were recorded during April (119.97 mg/L) and minimum values during February (57.6 mg/L). The fluctuations of pH in the Mahananda river's water ranged between 6.4 (February) and 7.9 (June). The dissolved oxygen content of the surface water of the river varied greatly. A high oxygen concentration was typical of the late winter (March). The minimum oxygen concentration was recorded in summer (April). Dissolved oxygen of the surface water of the river varied from 2.0 to 3.8 mg/L. The river was moderately higher in total alkalinity, which varied from 44.0 (June) to 168.98 (March).

Chloride of water was maximum (298.5 mg/L) at site 3 in June and minimum (192.55 mg/L) at the same site during February (Fig. 4). Salinity was found to be ranged between 348.78 mg/L and 560.24 mg/L. The maximum value was found at S3 in March, and minimum value was found at S1 in January.

The hardness of water was comparatively high and was mainly due to calcium and magnesium salts. According to BIS (2012), the desirable limit of hardness for drinking water was 200 mg/L and the permissible limit of hardness was 600 mg/L. The present study revealed that the total hardness of the water in three sites exceeded the desirable limit except June. The value of nitrate ranged from 67.8 to 145.52 mg/L. The present study showed that the COD values ranged from 85.4 to 169.6 mg/L.

The correlations between the different water parameters for the three sites were presented. The total hardness showed significant positive correlation with calcium, magnesium and nitrate in all the three sites studied. The total hardness also showed significant positive correlation with chloride, salinity and acidity at site 3. The sulphate showed significant negative correlation with pH at site 3. The salinity showed significant positive correlation with total hardness and calcium at site 3 and with magnesium at site 1 and site 3.

The COD is positively and significantly correlated with alkalinity at site 1, with calcium and nitrate at site 2, with acidity at site 3 and with total hardness at site 2 and 3. Magnesium showed significant positive correlation with COD in all the three sites studied.

The chloride at site 1 and site 3 and acidity at site 2 and 3 indicated significant positive correlation with Magnesium. The magnesium showed significant positive correlation with alkalinity at site 1, with calcium at site 2 and with nitrate at site 3. The calcium exhibited significant positive correlation with acidity and chloride at site 3. The nitrate showed significant positive correlation with calcium and magnesium at site 2 and with acidity and calcium at site 3.

During the present study, 57 species of phytoplankton belonging to 29 genera falling under four taxonomic divisions namely Cyanophyta, Chlorophyta, Bacillariophyta and Euglenophyta were identified from the Mahananda River, Malda district, West Bengal. Out of the 57 algal taxa recorded, 39 belong to Bacillariophyceae, 14 to Cyanophyceae and 2 each to Chlorophyceae and Euglenophyceae (Table 1). Bacillariophyceae (diatoms) was the major group consisted of 39 taxa (68.3%) belonging to 20 genera followed by Cyanophyceae (blue-green algae) represented by 14 taxa (24.5%) belonging to 7 genera. Chlorophyceae and Euglenophyceae were represented by 2 taxa (3.6%) belonging to 1 genus each (Table 1).

Table 1. Phytoplankton diversity of Mahananda river.

Classes of algae	Number of genera	Number of species	Percent
Bacillariophyceae	20	39	68.3
Cyanophyceae	7	14	24.5
Chlorophyceae	1	2	3.6
Euglenophyceae	1	2	3.6

Members of Bacillariophyceae (Diatoms) showed the dominant group of algae in the Mahananda River during the period of study (Table 1). The diatoms are ecologically resistant and are highly adapted to the riverine environment. The phytoplankton showed fluctuations due to monsoon and more numbers of phytoplankton were found during pre-monsoon season (Figs. 2-4). The *Euglena* was recorded at site 1 during pre-monsoon and onset of monsoon and it is pollution indicating organism (Palmer, 1969). Comparatively more numbers of Chlorophycean members (17.9%) were recorded on the onset of monsoon at site 2. Phytoplankton strongly influences certain non-biological aspects of water quality and they are a part of water quality (APHA, 1998). The phytoplankton is responsible for the process of primary production in water bodies and forms a vital energy source. They serve as a tool for assessing the health of the aquatic ecosystem. The high concentration of nutrients in water resources increases the growth of algae and triggers eutrophication.

Discussion

The study area at all sites is slightly acidic to alkaline in nature. The alkaline nature of water indicates that the pollutants and decomposition of organic matter received by the river are not suppressed. All chemical and biological reactions are depending upon the hydrogen ion concentration. The higher pH value can attribute to increased production in the aquatic ecosystem (Zaffer, 1966).

The temperature is one of the vital factors that control the abundance of phytoplankton. The increment of water temperature leads to the acceleration of chemical reactions in water, reduces the solubility of gases and amplifies the taste and odour. Temperature is also an essential factor in determining various other parameters such as pH, conductivity and alkalinity (Trivedi et al., 1998). The wastewater discharge from industries, settlements and microbial decomposition of organic matter present in the surface water bodies are the alkalinity inducing components. High alkalinity in river water indicates a high pollution load (Koshy & Nayer, 2001). Increasing temperature reduced the rate of oxygen but increases the rate of oxidation of organic matter and, hence, the rate of oxygen consumption (Zindge et al., 1981).

Dissolved oxygen was found higher at site 1. This may be due to the presence of aquatic vegetation and resulted in rapid photosynthetic activity producing more oxygen (Jhingran, 1982). Dissolved oxygen was less at site 2. This may be due to presence of domestic sewage and industrial waste. High temperature also affects the decreasing oxygen. Hardness of water is caused due to the presence of sulfate and chlorides of calcium and magnesium. Chloride content of water was observed high in site 2 because of high mixing of detergents in water.

Nitrate was more at site 2 may be due to the contribution from sewage water, surface runoff of the water and nitrification of ammonia. Nitrate was less found at site 1, may be due to absorption utilization by the phytoplankton growth (Gonzalves & Joshi, 1946; Singh, 1965; Sahai & Singda, 1969).

Eleven pollution tolerant algal genera namely *Cocconeis, Cyclotella, Cymbella, Euglena, Lyngbya, Melosira, Navicula, Nitzschia, Oscillatoria, Stauroneis* and *Synedra* were identified from the study area during the present investigation on the basis of Palmer (1969). The phytoplankton responds quickly to environmental changes and hence the species composition of phytoplankton indicates the water quality (APHA, 1998). These algal species might be used as indicators of pollution in the Mahananda river. The present water quality studies of the river also revealed that the quality of the river water deteriorated during the period of study.

The most dominant phytoplankton genera in Indian River system are *Oscillatoria, Nitzschia, Navicula, Synedra* and *Melosira*. The Ithikkara River in Kerala showed the predominance of diatoms as reported by Sheeba and Ramanujan (2005). The *Nitzschia, Navicula* and *Cymbella* were the dominant diatom taxa in Periyar River (Joy et al., 1990). During the period of the present investigation, Bacillariophyceae members were dominant in all the sites followed by Cyanophyceae except site 2 during June.

Algae are found frequently in polluted and non-polluted water. Because of this behaviour, they are generally considered beneficial to determine the quality of water. Phytoplankton was employed to assess the degree of water pollution or as the indicator of water pollution of different aquatic ecosystems (Trivedy, 1986; Sudhaker et al., 1994). *Synedra, Melosira*, and *Nitzschia* were the diatoms highly resistant to pollution and they dominate depending upon the severity of the pollution level. These diatom genera were reported from the study area.

Conclusion

The present study revealed that the selected three sites in the Mahananda River were polluted due to human interventions. The pollution was severe during pre-monsoon and on the onset

of post-monsoon seasons. In the present investigation, pH, total hardness, calcium hardness, magnesium hardness, chloride, salinity, sulphate, DO, COD and BOD were not within the permissible limit and desirable limit in all the three sites studied except nitrate and BOD. The salinity content in water was high in all three sites, contaminants came mainly from the nearby fish market and municipal sewage. The investigation concluded that all the three sites studied were polluted and the physico-chemical parameters have a profound influence on the abundance of phytoplankton in the Mahananda river.

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Distribution of Invasive Plant Species along Elevation Gradient in Eastern Nepal

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Abstract

Invasion is considered as the second most significant threat to biodiversity after habitat destruction. This study was done to find the distribution of invasive plant species along the elevation gradient in Eastern Nepal. We used stratified random sampling methods for this study. We laid a total of 24 quadrates of size 10 m x 10 m randomly in different sites (agricultural land, roadside, fallow land) and elevation bands (60-120 m, 580-640 m, and 900-960 m). A total of 13 invasive species belonging to eight families were recorded. In the elevation range of 60-120 m, five IAS types were found in fallow land to be higher and *Ageratum conyzoides* highly dominating. In the elevation range of 580-640 m, *A. conyzoides* was found to be higher and *Hyptis suaveolens* species found to be lower. Similarly, in the elevation range of 900-960 m, *Lantana camara* species was found to be higher and *Mikania micrantha* species found to be lower. The invasive species richness was found significantly different from the elevation band. The invasive richness and number were not significant.

Key words: *Ageratum conyzoides*, Elevation band, Invasive species, Invasion.

Introduction

Biological invasions are one of the major drivers for human made environmental changes. It poses greatest threat and it's increasing (Shackleton et al., 2020). Invasive species threaten ecosystem services and biodiversity (Shah et al., 2020). It threats biodiversity by causing disease, acting as predators or parasites, acting as competitors, altering habitat or hybridizing with local species. Invasive alien species have caused land and sea use changes, exploitation in the organism, climate change, and pollution (IPBES, 2019). Invasions have complex and often immense long-term direct and indirect impacts. Invasive alien species break down biogeographic realms, affect native species richness and abundance, increase the risk of native species extinction, affect the genetic composition of native populations, change native animal behavior, alter phylogenetic diversity across communities, and modify trophic networks (Hulme et al., 2020).

Elevation gradient is one of the significant factors that govern species distribution and richness (Sanders & Rahbek, 2012). Nepal has an altitudinal range from 63 m asl to 8848 m asl (i.e., Tarai, which is the plain region to the world highest peak). Nepal's topography shows extreme altitudinal variation where climatic variation makes the availability of invasive alien species different. There is no actual invasion data, but it has started with the

introduction of plant species by gardeners, horticulturists, foresters, British Gorkha soldiers, and traders in Nepal (Kunwar, 2003).

In Nepal at least 219 alien species of flowering plants are naturalized in which 26 species of which have been reported to be invasive (Shrestha, 2016). Among 26 invasive species, four of them [*Lantana camara* L., *Mikania micrantha* Kunth, *Chromolaena odorata* (L.) King & Rob., and *Eichhornia crassipes* (Mart.) Solms] are listed on 100 of the world's worst invasive alien species in ecosystem and range lands. Bhattarai et al. (2014) reported a higher number of naturalized and invasive species from the eastern and central parts than western Nepal. Invasive alien species amount is higher in southern lowlands, i.e., Tarai and Siwalik (Tiwari et al., 2005). In developing countries like Nepal, the spread of invasive species is a serious worsen livelihood (Sapkota, 2007). It has severe threats in biodiversity and the biggest threat in agriculture in Nepal, resulting in crop loss (Shah et al., 2020). Invasion change ecosystem functioning and the distribution of ecosystem services, thereby severely impacting human livelihoods (Kunwar, 2003). This study will provide insight into IAS distribution pattern of alien invasive species along the altitudinal gradient.

Materials and Methods

Study Area

The field survey was conducted from January to March 2018. The study was done from Lat. 26°24'56"N to 26°49'54"N and Long. 88°01'14"E to 88°06'64"E (Fig. 1). Similarly, elevation point ranges from 58 to 500 m. The annual rainfall is about 2000 m, and temperature varies with altitude in the studied area. The climate of the region varies from tropical to temperate. The soil of the studied area contains alluvial, clay fertile soil and sandy soil. At the same time, it contains rocks like shale, slate, sandstone, graphitic schist, quartzite, phyllite and amphibolite.

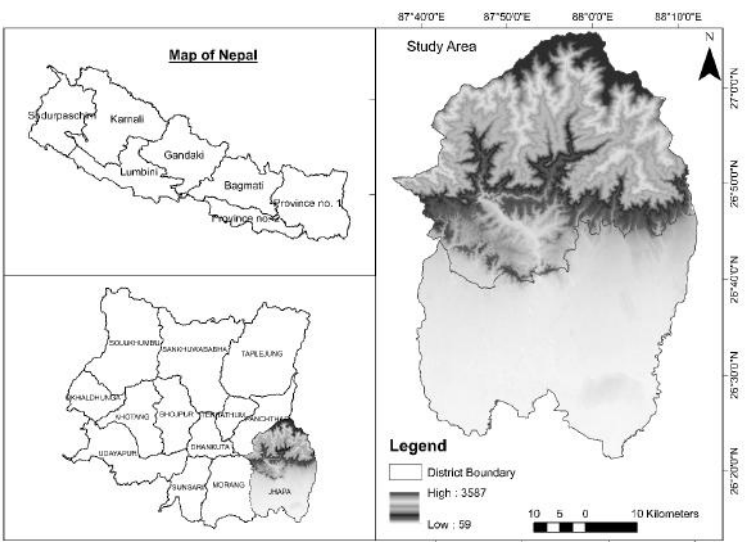

Figure 1. Map showing the Jhapa and Illam districts with elevation gradients

Methods

Stratified random sampling techniques methods used in this study. A total of 24 quadrates of size 10 m x 10 m were laid randomly in different sites (agricultural land, roadside, fallow land) and elevation bands (60-120 m, 580-640 m, and 900-960 m). Each quadrate was divided

into four parts (5 m x 5 m). After that, two subplots were used to record vegetation data (herbs, shrubs and climbers). Identification of invasive species was carried out by following (Shrestha, 2019) and also by comparing specimens deposited at National Herbarium and Plant Laboratories (KATH, Godawari, Lalitpur, Nepal) and referring 'Plant Diversity of Eastern Nepal: Flora of Plains of Eastern Nepal' (Siwakoti et al., 1999).

Data Analysis

The density of each invasive species was calculated. Invasive species were listed following Shrestha (2019). Data analysis was done using R programming 2016. Kruskal Wallis test was used to find the relationship between invasive species richness and the number of species with different elevation bands and numbers.

Results

Invasive Species

A total of thirteen invasive species belonging to eight families (Asteraceae, Amaranthaceae, Verbenaceae, Mimosaceae, Oxalidaceae, Pontederiaceae, Lamiaceae, Caesalpiniaceae) were recorded (Table 1). IAS was recorded from three sites (Agricultural land, roadside and fallow land). Species richness was more in fallow land followed by the road and agricultural land (nine, eight and six, respectively). In agricultural land and roadside, *M. micrantha* has higher density; *Galinsoga parviflora* has lower density in agriculture and *Hyptis suaveolens* lower density in roadside (Table 1)

Table 1. List of Invasive species recorded.

SN	Scientific Name	Family	Growth Forms	Agricultural Land (/ ha.)	Road Side (/ ha.)	Fallow Land (/ha.)
1.	*Ageratum conyzoides* L.	Asteraceae	AH	2111	2978	3678
2.	*Alternanthera sessilis* (L.) R. Br. ex DC.	Amaranthaceae	AH	2667	911	611
3.	*Amaranthus leucocarpus* S.Wats.	Amaranthaceae	AH	3022	0	0
4.	*Ageratina adenophora* (Spreng.) R.M. King & H. Rob.	Asteraceae	AH	811	844	756
5.	*Galinsoga parviflora* Cav.	Asteraceae	AH	56	700	1056
6.	*Lantana camara* L.	Verbenaceae	S	1811	2822	744
7.	*Mikania micrantha* Kunth	Asteraceae	CH	3456	4556	1978
8.	*Mimosa pudica* L.	Mimosaceae	AH	200	278	1544
9.	*Oxalis corniculata* L.	Oxalidaceae	AH	1256	1189	1578
10.	*Eichhornia crassipes* (Mart.) Solms	Pontederiaceae	S	0	189	0
11.	*Hyptis suaveolens* (L.) Poit.	Lamiaceae	AH	0	133	511
12.	*Ageratum houstonianum* Mill.	Asteraceae	AH	0	0	1867
13.	*Senna tora* (L.) Roxb.	Caesalpiniaceae	AH	0	0	167

AG= Annual grass, AH= Annual herb, C= Shrubby climber, CH= Climber herb, PG= Perennial herb, S= Shrub, SH= Succulent herb

Invasive Species and Elevations

In the elevation range of 60 to120 m, five types of IAS were found in fallow land to be higher and *A. conyzoides* highly dominating. In elevation range of 580 to 640 m, *A. conyzoides* was found to be higher, and *H. suaveolens* species were lower. Similarly, in the elevation range of 900 to 960 m, *L. camara* species was found to be higher and *M. micrantha* species found to be lower.

The invasive species richness was found significantly different (χ^2= 10.937, df = 2, p-value = 0.004) with the elevation band. The invasive species were recorded in the entire three elevation band which richness varies from 0 to 6. More invasive species were in elevation band of 580-640 m followed by 60-120 m (Fig. 2).

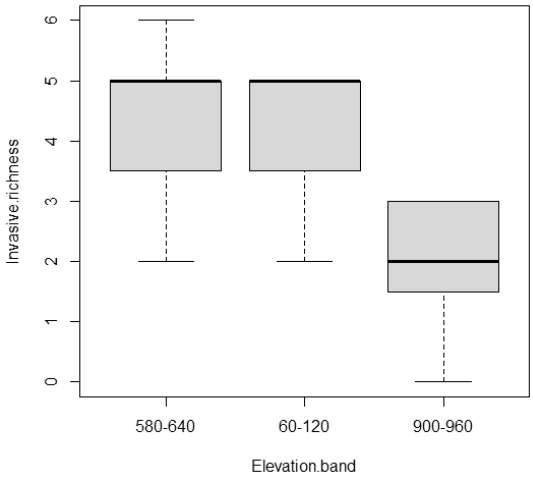

Figure 2. Invasive species richness in different elevation band

The number of invasive species were significantly different (χ^2= 11.34, df = 2, p-value = 0.003) with the elevation band. The numbers of invasive species were more in lower elevation band (60-120 m) followed by middle elevation band (580-640 m) and the range of species number were 0-499 (Fig. 3).

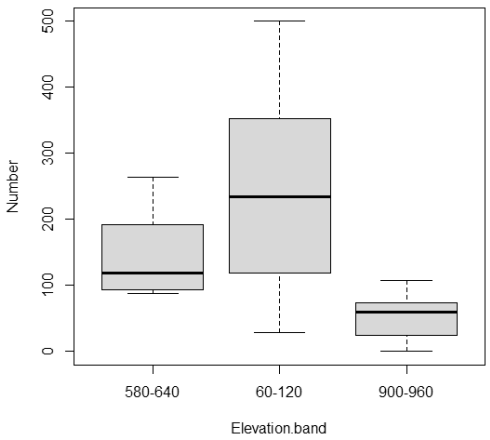

Figure 3. Invasive species number in different elevation band.

Discussion

Out of the 13 species recorded in the study area, fallow land constituted nine types of invasive species, followed by roadside land with eight types and then agriculture land with six types. This result was similar to the study by Bacaro et al. (2015). Additionally, fallow lands have higher species richness which can be directed towards their rich in nutrients and thus are more susceptible to invasion (Pathak et al., 2019). The roadside regions are dissimilar to other kinds of disturbances. These regions are linear structures acting as biological corridors (Christen & Matlack, 2009). However, these regions are also under constant usage, thus giving ample sunlight and water through drainage, allowing for their proper growth (Hastings et al., 2005). Due to frequent anthropogenic involvement in agriculture land, the region is more susceptible to introducing invasive species (Manley et al., 2015). Even though, agriculture land is under the constant threat of biological invasion, when proper care is taken through invasion control, the invasive species richness can be fairly reduced (Naylor, 1996).

This study is similar to a research by Rejmanek et al. (2016) and Pauchard and Alaback (2004). Higher elevation can result in lower invasive species count (Dai et al., 2020). Invasive species are widespread in lower elevations mostly because these regions have higher anthropogenic disturbances such as grazing or agriculture. Additionally, higher elevation in the study had lower species number, which accounts for less space availability, resulting in constraints in the growth of invasive species (Pathak et al., 2019). The elevation is considered a significant contributor that stresses plant invasion (Wilson et al., 1992). In Nepal, *A. conyzoides* are highly prevalent on the lower elevation around the altitude of 75 to 2000 m (Shrestha, 2016), which is also apparent from this study. The majorities of Invasive plant species to Nepal are of neo-tropical origin (South America). They have been introduced to Nepal via India (Tiwari et al., 2005), related to the plant species available in the eastern part of Nepal. Higher native species are invaded by the higher number of invasive plant species. Human disturbance and environmental stress affect invasions by changing resource availability. However, it is also important to note that the richness of invasive species in a geographical region is also dependent on resource availability and human activities (Spear et al., 2013).

Conclusion

A total of thirteen invasive species belonging to eight families was recorded. In the elevation range of 60-120 m, five types of IAS were found in fallow land to be higher and *A. conyzoides* highly dominating. In elevation range of 580-640 m also, *A. conyzoides* species was found to be higher and *H. suaveolens* species found to be lower. Similarly, in the elevation range of 900-960 m, *L. camara* species was found to be higher and *M. micrantha* species found to be lower. The invasive species richness and number were found significantly different from the elevation band.

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Integrating Biological Resources for Prosperity, 2021, 335-341

Plants and Culture of Santal and Meche Ethnic Groups of Nepal

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Abstract

Santals and Meches are highly marginalized and endangered ethnic groups in Nepal due to deprive economic, social and political rights, opportunities and benefits. Traditionally, they practiced nomadic lifestyle and followed agriculture later on. The Santals live in Jhapa and Morang districts of Province Number 1, Eastern Nepal with a total population of 51735 and commonly called Satars in Nepal. Whereas, Meches live only in Jhapa district with a total population of 4867 and considered as endangered ethnic group. The Meche and Bodo tribes of Assam in India are considered as of the same ethnic group. Both Santal and Meche communities worship the nature and believe the universe is inhabited by numerous invisible supernatural beings and powers which always influence their daily activities and always try to appease them through religio-cultural activities. An attempt was made to understand the plants and its relations with their culture. Information was collected from both primary and secondary sources. It found that the festivals, religious rituals and life cycle rites of both ethnic groups were closely associated with plants. The plants like Sal, Kadam, mango were closer to Santals culture, and Seudi, Dubo, Tulsi, bamboo were closer to Meche's culture.

Key words: East Nepal, Ethnic groups, Ethnoculture, Plants, Traditional knowledge

Introduction

Nepal is a multiethnic, multi-lingual, multi-religious and multi-cultural country which houses 26,494,504 population of 126 caste/ethnic groups and 123 languages (CBS, 2012). Among them, 59 ethnic groups are listed as indigenous peoples or indigenous nationalities by the National Foundation for Development of Indigenous Nationalities (NFDIN) Act 2002, based on their mother language and traditional customs (HMG/N, 2002). The National Foundation for Development of Indigenous Nationalities (NFDIN) formed in 2006, defined the ethnic groups as those having their own mother tongue and traditional culture and yet do not fall under the conventional fourfold Varna of Hindu. NFDIN also classified the ethnic groups into five categories, i.e, endangered, highly marginalized, marginalized, disadvantaged and advantaged groups (Gellner, 2007). The Santals and Meches are classified as highly marginalized and endangered groups, respectively due to deprive of economic, social and political rights, opportunities and benefits. Both ethnic groups are considered as Tarai Adivasi.

Santals are commonly called "Satars" in Nepal and are considered as highly marginalized ethnic group. The total population of Santals in Nepal is 51,735 and live in the Jhapa and Morang districts, Province Number 1, Eastern Tarai (CBS, 2012). In addition, a large population of the Santals is found in India, Bangladesh, Bhutan and Myanmar border areas.

It is considered that the Santals migrated to Nepal from the central and eastern part of India (Dumka district of the Santhal Paraganas of Jharkhand state and the Malda district of West Bengal), probably during the 19th century, when the Tarai region of Nepal was thinly populated and densely covered by Sal forest. They involved in clearing the forests and made a cultivable land. They were employed by landowners to sow seeds, transplant, and cut or thresh the rice paddy during agricultural season (Siwakoti Olee, 2015). Whereas, Meches live mostly in Jhapa district with the total population 4867. They are considered as endangered groups of *Aadivasi Janjati* due to low population number. In addition to Nepal, they also live in India, mainly in the Dooars region of West Bengal, Dimapur, Nagaland and Assam, where they are commonly known as *Bodo* tribe (Meche, 2012; Siwakoti Olee, 2016a). Both Santal and Meche communities believe, the universe is inhabited by numerous invisible supernatural beings and powers which always influence their daily activities. They worship house, celestial bodies (sky), forest trees, rivers, lands, birds and animals considering as abodes of supernatural beings and powers. In both communities the celebrations of festivals, social rituals including communal disputes are discussed and settled in their traditional social organizations with a common consent.

Traditionally, the preparation of homemade liquor (alcohol) was common in both communities for their rituals and own consumption. Usually, the Meches sell the liquor at local markets. Both ethnic groups are traditionally associated with plants for material and cultural requirements. The previous studies showed, the Santals used 52 species of wild plants for food (Siwakoti et al., 1997) and more than 110 species for traditional medicines (Siwakoti & Siwakoti, 2000; Siwakoti Olee 2011a, 2013), whereas, 46 species of wild plants were used by Meche community for traditional medicines (Rai, 2004). In addition, they have a close relation with plants for various cultural rituals.

Materials and Methods

Information about the plants and their relations with the culture of Santal and Meche people were taken from both primary and secondary sources. Haldibari and Jalthal areas of Jhapa district and Sijuwa village of Morang district were selected as study sites for Santal community. Whereas, Dhaijan and Jalthal villages of Jhapa district were selected for Meches community. Any knowledgeable persons of Santal and Meche communities (elder persons, members of their traditional and contemporary social organizations, ethnic activists, etc.) were considered as respondents. Most of the data were collected by the village headman (*Majhi Hadam*) and associates (*Paranik, Jogmanjhi, Jogparanik, Naike, Kudum Naike, Godet*) of traditional political organization of Santals. Similarly, in case of Meches' village headman (*Makhal*), priest (*Roja*) and *Phanthol,* school teachers were used as primary source of information. The published materials (books, articles, reports, web sites, etc.) relevant to the present study were used as secondary source. The author mostly used qualitative data collection technique range from interviews, observational techniques such as participant and non-participant observations, focus group discussion, documents and researcher's impressions and reactions, for analysis and discussion. The scientific names of the plants were confirmed consulting with Plant Taxonomist.

Results

In both Santal and Meche communities, many cultural rituals revolved around the plants by considering the abode of benevolent spirits. They have strong faith and belief on the deities/ spirits residing in the nature and always try to appease them. Some of the following important plant species related to culture of Santals and Meches are described below:

Sal tree (*Shorea robusta*): Sal tree is very important in the life of Santals. Traditionally, Santals live in Sal forest and their traditional religion, named as *Sarna* religion is considered derived from Sal tree. According to the mythology of the Santal community, the Santals that had gone to the forest for hunting started the discussion about their Creator and Savior when they were taking rest under a tree. They questioned themselves, who is their God? Whether the sun, the wind or the cloud? Finally, they came to conclusion that they would throw an arrow in the sky and wherever the arrow would target that will be the God's home. They left an arrow in the sky; which fell down under a Sal tree (*Sarjom tree* in Santali). Then they started worshipping the Sal tree and named their religion as *Sarna*, because it was derived from a Sal tree (Siwakoti Olee, 2013). Every Santal village had sacred grove inside Sal forest considered as abode of supernatural spirits where worshipping is done. The sacred *Manjhithan* and the *Jaherthan* of Santals are traditionally related to Sal culture. The *Manjhithan* (a temple like structure) was one of the traditional institutions of the Santal village which was built at the centre of village or near the village head's (*Manjhi HaŢam)* house. It is believed that the *Manjhi Bonga* (the spirit of their ancestral Manjhi *Haṛam*) resides in the *Manjhithan* and is represented by the stone or the central wooden post. The sacred place is used to settle the community disputes by the traditional village council. Traditionally it was built by Sal wood. The *Jaherthan* was built at the outskirt of the village, usually at the Sal forest, where Santals gather in various religio-cultural festivals (*Sorhae, Baha*, etc.) to worship the deities (*Bongas*). For *Jaherthan,* three stones marked at the base of the three Sal trees were considered as abodes of *Jaher Bongas* (the village deities) (Siwakoti Olee, 2015).

Baha festival is one of the largest festivals of Santals, celebrated during the Sal blossom period for welcoming the spring season. The festival is celebrated in the month of *Falgun* (February-March) for 3 days when Sal tree start to blossom (*Baha* means flower in a Santali word). The Santals don't collect any wild flower, and don't construct a new house or thatch the houses until the *Baha* festival is over. The *Naike* (Santal priest) and other villagers offer Sal flowers to every family of the village. A Santal woman of each house receives flowers in the folds of her sari from the priest after she has ceremonially applied oil on the feet of priest

Sal tree is also used to join and separate the relationship between Santals couple. During marriage ceremony, a Santal carries a Sal branch with leaves at nearby market, the Santals know the date of marriage' by counting the number of leaves on the branch. The marriage alter (*Mandwa*) is constructed by using the branches of Sal tree, Sal leaves are used for worshipping the deities. During divorce, the couple holds three Sal leaves in their hands and tears the Sal leaves for the separation of marital union and upsets a pot full of water by taking the name of the *Sin Bonga* for the separation of marital union. If leaves are not completely torn out and water pot is not completely empty, they believe there is a possibility of reunion. The Sal branches with leaves are also used for inviting the Santal people for sanctioning a

social punishment (*Bitlaha*). *Bitlaha* means out casting the Santal from the society as a social punishment for violating the norms of exogamy and endogamy. They also burn a Sal resin for worshipping the various deities at the cremation spot. Culturally, the use of Sal plant was less in Meche community, they mainly used the leaves for various religious activities.

Seudi/Sijo (*Euphorbia royleana*): This plant is closely related to the *Bathouism*, a religion of Meche community. Traditionally, the *Sijo* plant represents as a symbol of *Bathou* (God Shiva-the supreme god). Every Meche household constructs a *Bathou* (a worshipping place) where a *Seudi/ Sijo* plant is planted. According to Meche/ Bodo language, *Ba* means five an *thou* means deep, they believe five mighty elements of God are land, water, air, fire and ether, the five number has significant values in Meche culture (https://en.wikipedia.org/wiki/Boro_people). The cultural value of the *Seudi* plant in Santals community was not found.

Bamboo (*Bambusa* and *Dendrocalamus* spp.): Bamboos have significant cultural and socio-economic values in both ethnic groups, the plant is one of the essential materials to perform various rituals in birth, marriage and death. Both communities use bamboo plants for construction of house, wall, pole, ladder and roof of their house including fencing, handicrafts, and baskets. They also make hunting and fishing materials, musical and domestic instruments from the bamboos. Young bamboo shoots are used for vegetable and prickle. Santals welcome the new baby by beating the house roof with a bamboo pole, and also keep in a bamboo cot (*Parkam*). Santals also erect the bamboo made arrow at the corner of cot during the time of labour pain to save her from evil spirits. Similarly, in Santals, if child is born in inauspicious day, the bamboo winning fan and broom are used to save baby from evil spirits. Santals make a dancing horse of bamboo (*Sin sadan*-celestial horse) in marriage processions which symbolize the creation. The bride of Santal is taken in bamboo basket for various marriage rituals including *Sindurdhan*. The Santal corpse is also carried on bamboo cot for cremation. Santals practice to plant bamboo on Tuesday, they considered Saturday and Sunday inauspicious for bamboo planting and cutting. In poor Santal family, the bride enters the groom's house by carrying a small bamboo basket (*Tunki*), with some clothes over the head (*Tunki Dipil Bapla*), *Bapla* means marriage in Santals.

The Meches also use bamboo materials during marriage negotiation ceremony and other rituals. The groom party keeps turmeric colored betel nut, betel leaf, rice and coin in banana leaves and puts inside bamboo basket by tying with 5 bamboo strips. Traditionally, after the birth of a child, the umbilical cord is cut down by using five sharp bamboo blades for male child and seven for female child. Meches make *Jholungo* (baby cot) with bamboo strips for keeping baby. Feeding ceremony of Meche baby takes place at *Bathau than* (a sacred hut for Lord Shiva) with Seudi/ Sijo (*Euphorbia* sp.) which is fenced by bamboo in five layers. Meches use bamboo to carry dead body, if dead person is male, they use five sticks of bamboo and in case of female, they use seven sticks. Meches make bamboo tripod to offer the food for dead body. When Meche boy/ girl indulge in physical relationship within the same clan or with a person of taboo, then they are punished by keeping them inside bamboo made pig stay and also given pig food.

Neem tree (*Melia azadirachta*): Neem plant is important for Santal culture, during the new born of child, the leaves of the plant are used for the purification of mother and name giving

ceremony (*Nawaran*) of child. A special type of food, called as *Neem Dak'Mandi* is prepared by the midwife (*Dargin Buŗi)* by mixing rice and leaves of Neem tree with sufficient amount of water. The food is offered to the *Maran Buru* (the ancestor deity), then served to all the relatives and invited men, women (including mother) and children by putting in a Sal leaf plate. The usual day for *Neem Dak'Mandi* is fifth day for a male and third day for a female child. Some families observe this ceremony on the same number of days of birth for both boy and girl without making any difference.

Mahuwa plant (*Madhuca longifolia*): It is an important plant for Santal culture, used in various religious ceremonies. The flowers are important food item. The Santals ferment the flowers to produce the alcoholic drink *mahua*. They enjoy it during festival cerebration. However, they informed that this plant is not seen nowadays. Both ethnic people during ceremonies, use *tadi* sap of toddy palm (*Borassus flabellifer*), if available.

Kadam tree (*Neolamarckia cadamba*): Santals consider Kadam tree as a sacred plant for marriage and other ritual performance. Traditionally, they use to marry under the Kadam tree called as *Kadam Bapla*. If Kadam plant grows at burial ground, they consider the plant as a sacred abode and worship it. When a Santal boy and girl fall in love, they go towards the forest and exchange the garland by plucking the wild flowers of forest (*Baha Dor Bapla*). Santal has a third type of kinship, where a bond of friendship is seen that is fictive kinship (*Sange, Mit system),* they use branches of this plant during the ritual along with exchange of flower garland.

Besar/Haldi (*Curcuma longa*): In addition to spices, turmeric is used for various rituals including marriage ceremony. Both ethnic groups use turmeric powder/ paste, obtained from the rhizome of this plant for rubbing the bodies of bridal couple, they believe the sacred plant protect them from malignant spirits. The turmeric powder is also used to soak the wedding dress of bride and groom in water to clean it and purify it ritually. Santals believe that turmeric dyed clothes of bride and groom brings a sign of their oneness.

Khursani (*Capsicum annuum*): In addition to kitchen spices, the red chillies are also used in cultural ritual. Sometimes, a Santal girl likes a Santal boy if he can't accept her proposal then she forcibly enters his house by carrying a small pot of *handi* (local liquor). The boy's family tries to expel her by producing smoke of red chilies, if she remains in the house for a whole day, they need to accept her.

Jute plant (*Corchorus* sp.): Santals also hang knotted jute fiber or rope at the main door of house, the number of knots indicate the day for marriage or other important ceremonies and one knot is untied every day, the last knot is the day of marriage or ceremony. Both ethnic groups use the blast fiber of jute plant for making ropes in domestic purposes, jute stalks for fuel and fencing. In addition, Dubo (*Cynodon dactylon*), Tulsi (*Ocimum sanctum*)*,* Pan (Betel leaves), Supari (*Areca nut*) are used by both ethnic groups for various religious and life cycle rituals.

Discussion

Originally, both Santal and Meche ethnic groups practiced the nomadic pattern of lifestyle and their way of life revolved around the forest-based resources. When forest resources and

area become scarce and limited, they started shifting to settled agriculture. From the very beginning, the Santals were hunter-gatherers, they hunt the wild animals and gather the wild plants (fruits, rhizomes, etc.) for subsistence, the agrarian way of living was brought by the Aryans who came to their homeland (Chhotanagpur area of India) at about the 1500 B.C. (Archer,1974; Siwakoti Olee, 2013). Similarly, Meches used to practice the slash and burn cultivation before settled agriculture (HMG/N, 1974). Most of the festivals of both communities are revolved around the agricultural calendar, they also first started to cultivate the wet rice in their respective homelands.

Plants serve an intimate role in human culture since times immemorial which were used as symbols in many festivals and rituals including birth, marriage and death. The plants insights in the tribal culture during religion, festivals and rituals of life cycle ceremony. The Birth, marriage and death rites of both ethnic groups cannot be fulfilled without paying homage to the sal, mango, banyan plants. In addition to cultural value, they use bamboo for making several kinds of hunting and fishing instruments, musical instruments and domestic instruments (Siwakoti Olee, 2011b, 2016b, 2017). As both communities are poor and live-in rural areas where the modern facilities of hospitals are limited, they need to depend on the plant resources for treating the various aliments (Rai, 2004; Siwakoti Olee, 2011a). Many species of plants also used for their domestic purposes in addition to cultural utility. Even today they are far away from the modern technology and rely on plant resources for their subsistence livelihood.

Conclusion

Both Santals and Meches communities are highly marginalized and endangered ethnic groups of Nepal. They depend on plants for their subsistence livelihoods and various socio-cultural rituals. They have strong faith on their culture and tradition which revolve around the forests, agriculture and plant resources. Santals and Meches still proud on their forest-based traditions of and celebrate many traditional religio-festivals. Some of the forest trees particularly, Sal, Kadam, Mahuwa plants are very close to Santals culture, whereas, Seudi, Bamboo, dubo plants are closer to Meches culture. It indicates, the ethnic groups are not only associated with plants for shelter, clothes, foods and traditional medicines but also culturally connected. The cultural value of plants also supports to conserve the plants. We need to taught from them how to survive with nature without damaging them.

Acknowledgements

I am grateful to the Santals and Meches respondents of the study areas for their support to provide the information. I am also thankful to Prof. Dr. Mohan Siwakoti for encouragement and confirming the scientific names of plants.

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Taxonomy and Distribution of Himalayan Plant *Senecio* L. in Nepal

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Abstract

Nepal houses 14 species of *Senecio* L. from family asteraceae. These species exhibit a remarkable amount of morphological variations and occur in extremely diversified habitats from tropical to alpine zones of different phytogeographical regions of Nepal. Among the 14 species, 11 species, viz., *Senecio albopurpureus* Kitam., *S. candolleanus* Wall. ex DC., *S. echaetus* Y.L. Chen & K.Y. Pan, *S. graciliflorus* Wall. ex DC., *S. kumaonensis* Duthie ex C. Jeffrey & Y.L. Chen, *S. nudicaulis* Buch.-Ham. ex D. Don., *S. panduliformis* Kitam., *S. ramosus* Wall. ex DC., *S. raphanifolius* Wall. ex DC., *S. royleanus* DC., and *S. topkegolensis* Kitam. were endemic to the Himalayas and within the Himalayan endemic, two species, viz., *S. panduliformis*, and *S. topkegolensis* were endemic to Nepal Himalayas. These species showed huge gross and micromorphological variations. Based on the morphological characters, an identification key was prepared for the species. The Global and national conservation status of these species was unknown, but ten species out of a total of 14 species were reported from the different protected areas of Nepal. However, there was no report of the occurrence of four species, viz., *S. candolleanus*, *S. panduliformis*, *S. ramosus*, and *S. topkogolensis* Kitam. from any of the protected areas of Nepal. The species, *S. panduliformis*, endemic to Nepal, had been reported only as the type specimen in 1969 from Aisyalu Kharka, Khotang, East Nepal.

Key words: Asteraceae, Endemic, Gross morphology, Micromorphology, Habitat.

Introduction

Senecio L. is the largest genus of the subtribe Senecioninae of tribe Senecioneae of family Asteraceae. The genus was described by Linnaeus (1753) in A. Paris's (1749) *Florae Parsiensis prodromus*. Being a huge genus, it had been divided into subgroups by different taxonomists (De Candolle, 1838; Clarke, 1876; Hooker, 1882; Koyama, 1969; Jeffrey & Chen, 1984). The genus traditionally comprised of ca. 3000 species (Jeffrey et al., 1977) and this notion was perceived as highly artificial (Bremer, 1994; Vincent, 1996) because of the fact that many species assigned to the genus *Senecio sensu lato* (*Senecio s.l.*) were found to be closely related to the species of other genera than *Senecio* in the strict sense (*s.s*), the type species of which is the *S. vulgaris* L. This enormous size genus with a remarkable amount of morphological variations between and within the species has been the subject of diverse taxonomic interpretation (Ediger, 1970; Barkley, 1978; Vincent, 1996) for a long time and till now as well. A bulk of literature had been published to make the genus more homogenous, and based on morphological, karyological and later on a molecular basis, many species had been transferred to other existing or new or resurrected genera to make the genus more homogenous, and the genus is becoming narrower and homogenous.

Jeffrey and Chen (1984) had erected the subgenus *Synotis* (C.B. Clarke) with distinctly caudate anther base and a clearly defined geographical area to the rank of genus *Synotis* (C.B. Clarke) C. Jeffrey & Y.L. Chen. Similarly, on the basis of distinct characters such as the absence of bracts, having the corolla tube 3-4 longer than lamina, the Sect. Nemosenecio Kitam. was erected to rank of genus *Nemosenecio* (Kitam.) B. Nord., The sect. Tephroseris Rchb., with anther collar having the same sized cells, corolla tube 1-1.5 as long as the other parts had been erected to a rank of genus *Tephroseris* (Rchb.) Rchb. Similarly, many other infragenus had been erected to the status of the genus. Besides erecting the infragenus to the rank status of the genus, a number of anomalous species from *Senecio s.l* had been transferred to other genera as *comb nov*., viz., *Senecio buimalia* Buch.-Ham. ex D.Don. to *Cissampelopsis* (DC.) Miq. as *Cissampelopsis buimalia* (Buch.-Ham. ex D. Don) C. Jeffrey & Y.L. Chen; *Senecio chola* W.W. Smith to *Parasenecio* W.W. Sm. & J. Small.; *Senecio lancifera* J.R. Drummond to *Ligularia* Cass. Moreover, Jeffrey (1992), based on morphological characters, had transferred a number of *Senecio* species in other genera like *Iranecio* B. Nord., *Packera* A. Love & D. Love., *Emilia* (Cass.) Cass., *Humbertacalia* C. Jeffrey, *Hubertia* Bory., *Faujasia* Cass., *Monticalia* C. Jeffrey, *Dendrophorbium* (Cuatrec.) C. Jeffrey, *Pentacalia* Cass., *Dresslerrothamnus* H. Robins, *Jacmaia* B. Nord., *Psacaliopsis* H. Robins & Brettell, *Roldana* La Llave, *Telanthophora* H. Robins & Brettell, *Nelsonianthus* H. Robins & Brettell, etc. The *Senecio* species, viz., *Senecio cariensis* Boiss., *S. davisii* Matthews, *S. eriospermus* DC., *S. farfarifolius* Boiss & Kotschy, *S. hypochionaeus* Boiss., *S. jurineifolius* Boiss., *S. kubensis* Grossh., *S. lazicus* Boiss & Bal., *S. lipskyi* Lomak., *S. lorentii* Hochst, *S. massagetovii* Schischk., and *S. pandulifolius* C. Koch. had also been transferred in the genus *Iranecio* B. Nord., the genus characterised by the 4-lobed corolla. Based on molecular studies, Pelser et al. (2006) had also proposed to transfer 27 species of *Senecio* assigned to *Senecio* sect. Jacobaea from *Senecio* to genus *Jacobaea* Mill. Riva et al. (2009), based on the micromorphological studies of style branches and anther, also proposed to remove *Senecio* sect. Senecio ser. Otopteri from genus *Senecio,* to make the genus *Senecio,* monophyletic. While solving the taxonomic problems in the genus, both gross and micromorphological characters should be considered. One of the eminent plant anatomists, Metcalfe and Chalk (1979), also stressed the supplementary addition of anatomy and chemistry to overcome taxonomic problems that remain unsolved.

Previously 26 species and infraspecies of *Senecio* had been reported from Nepal (Hara et al., 1982; Press et al., 2000). However, according to present generic delimitation, those 26 species of *Senecio* fall in 5 well-established genera, viz., *Cissampelopsis*, *Ligularia*, *Parasenecio*, *Senecio,* and *Synotis*. Presently, in Nepal Himalaya, there are 14 species of *Senecio,* viz., *Senecio albopurpureus* Kitam., *S. biligulatus* W.W. Sm., *S. candolleanus* Wall. ex DC., *S. echaetus* Y.L. Chen & K.Y. Pan, *S. graciliflorus* Wall. ex. DC., *S. kumaonensi*s Duthie ex C. Jeffrey & Y.L. Chen, *S. laetus* Edgew., *S. nudicaulis* Buch.-Ham. ex D. Don, *S. panduliformis* Kitam., *S. ramosus* Wall. ex DC., *S. raphanifolius* Wall. ex DC., *S. royleanus* DC., *S. scandens* Buch.-Ham. ex D. Don., and *S. topkegolensis* Kitam. The genus with about 1200 species is worldwide in distribution except in Antartica (Chen et al., 2011). However, the generic limits in some areas are still uncertain (Chen et al., 2011). The *Senecio* species exhibit huge morphological variations with diverse habitats. Study of morphological variations plays the huge role in finding the interrelations among the species and also is of paramount importance

for identification and to remove the anomalous species from the genus. Most of the species of *Senecio* are chiefly distributed in temperate and subalpine regions of the world. However, some species are also reported from tropical region and alpine climates as well.

Materials and Methods

Study Area

The present research covered the study of genus *Senecio* L. from different localities from Western to Eastern regions along the different bio-climatic vertical gradient of Nepal Himalaya. Among the 14 species of *Senecio*, in the present study, the morphological variations of only 12 species of *Senecio*, viz., *S. albopurpureus*, *S. biligulatus*, *S. echaetus*, *S. graciliflorus*, *S. kumaonensi*s, *S. laetus*, *S. nudicaulis*, *S. ramosus*, *S. raphanifolius*, *S. royleanus*, *S. scandens*, and *S. topkegolensis* were carried out. The morphological variations of two species, viz., *S. candolleanus*, and *S. panduliformis* had been excluded from the present study because of unavailability of voucher specimens and live collection. However, their conservation status was studied from the literature.

Methodology

The study was primarily based on the voucher specimens deposited at different herbaria like KATH, TUCH, BM, CAL and G-DC (Holmgren et al., 1990) and the live specimens collected from different localities of Nepal Himalaya from the year 2010 to 2013. Both the gross and micromorphological variations were studied. All vegetative parts were measured in a dry state except for the capitulum and its components. To study the characters of capitulum and its components, the dried capitula were taken out and wet on the soft detergent for few minutes, washed with pure water and dissected.

For the study of micromorphological characters such as trichomes and stomata of leaves, the method described by Carpenter (2005; with some modification) was followed. A mature leaf was selected from the plant specimen. The selected leaf was taken out and immersed in 5% potassium hydroxide solution overnight, rinsed with deionised water and again immersed in a fresh solution of potassium hydroxide for a few hours. The leaf was then washed in water and treated with glacial acetic acid for a few minutes (2-5 min). The epidermal layers (both adaxial and abaxial) of the leaf were peeled and then treated with 4% Sodium Hypochlorite until the tissue got discoloured. It was then washed in water several times. The material was stained in 1% aqueous safranine, and the excess stain was washed by water; and the material was then mounted on glycerin and observed under the light microscope.

The habitats of species from voucher specimens and live collections were recorded in the data matrix and analyzed whether the species are located inside the protected area or not and conservation status evaluated.

Identification and Authentication of Specimens

The specimens were identified and authenticated by comparing the character states with the protologue texts and valid literature (Linnaeus, 1753; Don, 1825; De Candollea, 1838;

Edgeworth, 1846; Clarke, 1876; Hooker, 1882; Koyama, 1969; Kitamura, 1979; Kitamura, 1981; Chen et al., 1981; Jeffrey & Chen, 1984; Hajra et al., 1995; Srivastava & Jeffrey, 1996; Grierson & Long, 2001). Moreover the voucher specimens were also compared with the type specimens deposited at different herbaria acquired from a different website (www.jstorplant.; www.kew.org; www.cvh.ac.cn) and deposited in different herbaria (CAL, TUCH, KATH).

Results

Keys to the identification of species

1a. Capitula discoid, presence of white tomentose hairs on abaxial surface of leaves .....***S. kumaonensis***
1b. Capitula radiate, absence of white tomentose hairs on abaxial surface of leaves..... 2
2a. Phyllaries in capitula upto 8 or less 3
2b. Phyllaries in capitula more than 8 6
3a. Auricles present, involucre narrowly campanulate, disc florets 7 – 10.....***S. royleanus***
3b. Auricles absent, involucre tubular, disc floret upto 3 4
4a. Capitula in terminal and axillary compound corymb, presence of tricytic and tetracytic stomata .....***S. graciliflorus***
4b. Capitula in terminal compound corymb, absence of tricytic and tetracytic stomata ..5
5a. Ligule minute, more or less equal to disc floret, nerves absent .....***S. topkegolensis***
5b. Ligule large, larger than disc floret, nerves present .....***S. biligulatus***
6a. Ray florets more or less equal to disc florets, presence of anisocytic stomata ...***S. ramosus***
6b. Ray florets larger than disc florets, absence of anisocytic stomata 7
7a. Plant up to 26 cm, capitula solitary or few, presence of biseriate trichomes ...***S. albopurpureus***
7b. Plant more than 26 cm, capitula many, absence of biseriate trichomes 8
8a. Ovaries of ray and disc florets densely pubescent.....***S. nudicaulis***
8b. Ovaries of ray and disc florets smooth 9
9a. Plant scandent, leaf surface hypostomatic.....***S. scandens***
9b. Plant erect, leaf surface amphistomatic 10
10a. Phyllaries more than 16, ray florets more than 16, pappus deciduous.....***S. echaetus***
10b. Phyllaries less than 16, ray florets less than 16, pappus persistent 11
11a. Ray florets 10-13, ray floret with copious pappus, pappus white or light yellow ..***S. laetus***
11b. Ray florets 13 -15, ray florets with scanty pappus or lacking, pappus dark yellow or reddish brown .....***S. raphanifolius***

Based on the gross and micromorphological characters, the above identification keys for the species are prepared.

Taxonomy of *Senecio*

Species of *Senecio* showed huge gross and micromorphological variations. There was a great variation in habit, the height of plant, rhizome, and type of branching and surface of stem (Table 1). Similarly, the variations were also found in the structure and texture of leaves as well as in capitulescence and capitula (Tables 2, 3). The variations were also found in the type of trichome and stomata in different species (Tables 4, 5).

Table 1. Distribution range and habit types of *Senecio*

SN	Name of Species	Habit	Height of plant (cm)	Rhizome	Surface of stem
1.	*S. albopurpureus*	Perennial erect dwarf herb	5-26	Present	Striated; upper arachnoid, lower smooth
2.	*S. biligulatus*	Perennial erect herb	57-98	Present	Striated; upper pubescent
3.	*S. echaetus*	Perennial erect herb	27-57	Present	Striated; upper arachnoid
4.	*S. graciliflorus*	Perennial erect herb	100-150	Present	Striated; glabrous
5.	*S. kumaonensis*	Perennial erect herb, woody	40-150	Present	Ribbed; arachnoid
6.	*S .laetus*	Perennial erect herb	50-125	Present	Striated; arachnoid
7.	*S .nudicaulis*	Perennial erect herb	75	Present	Striated; arachnoid
8.	*S. raphanifolius*	Perennial erect herb	50-150	Present	Striated; arachnoid
9.	*S. ramosus*	Annual erect herb	6-22	Absent	Ribbed
10.	*S. royleanus*	Perennial erect	100-200	Present	Striated; smooth
11.	*S. scandens*	Perennial scandent herb	100-600	Present	Striated; smooth
12.	*S. topkegolensis*	Perennial erect herb	56-99	Present	Ribbed; smooth

Distribution of Species

Among the 14 species, 11 species, viz., *S. albopurpureus*, *S. candolleanus, S. echaetus*, *S. graciliflorus*, *S. kumaonensis*, *S. nudicaulis*, *S. panduliformis*, *S. ramosus*, *S. raphanifolius*, *S. royleanus*, and *S. topkegolensis* were endemic to Himalayas and with the Himalayan endemics, 2 species, viz., *S. panduliformis* and *S. topkegolensis* were endemic to Nepal. Among the 14 species, three species *S. biligulatus*, *S. laetus*, and *S. scandens* are not endemic to Himalayan range and also found in other places. The data analysis of the habitat of voucher and live collections revealed that among 14 species of *Senecio*, ten species, viz., *S. albopurpureus, S. biligulatus, S. echaetus, S. graciliflorus, S. kumaonensis*, *S. laetus, S. nudicaulis, S. raphanifolius, S. royleanus*, and *S. scandens* were reported from protected areas and four species, viz., *S. candolleanus*, *S. panduliformis*, *S. ramosus*, and *S. topkegolensis* were reported outside the protected areas only (Table 6). Among the species outside the protected areas, *S. panduliformis* was reported hitherto from the type locality only (Stainton, 6615; Holotype in BM) which was collected by Stainton in 1969 at 2100 m from Aisyalu Khorka, Khotang, East Nepal. Similarly, *S. ramosus* was collected from Bangmati river, Makawanpur district in 1969 (Kanai & Bista, 11848; KATH).

Discussion

The species of *Senecio* have the diverse habitats from tropical to alpine region. Different gross morphological characters such as texture of leaf surface, base of leaf, type of capitulescence, size and type of capitulum, number of phyllaries, shape of involucres, number of ray and disc florets in a capitulum were found to be significant in delimiting the taxa. In the present study, among the species studied, *S. kumaonensis* is the only taxa with discoid capitulum. Similarly persistence, abundance and colour of pappus were found to be taxonomically significant and *S. echaetus* was the only species in which the pappus is deciduous and almost absent in mature achenes. However, in *S. raphanifolius*, the pappus is dark yellow or reddish brown in colour and in other remaining species, the pappus are either stramineous or white

Table 2. Variations in leaf structure.

SN	Species	Shape	Petiole (Stem leaf)	Distinct auricles	Radical leaf at anthesis	Base of stem leaf	Leaf lamina (stem leaf)	Margin of lamina	Abaxial surface
1.	*S.albopurpureus*	Elliptical /obovate	Present	Absent	Present	Semi-amplexicaul	Unipinnate	Sublobulate	Purplish tomentose
2.	*S. biligulatus*	Triangular	Present (winged)	Absent	Absent	Semiamplexicaul	Not pinnate	Dentate	Not tomentose
3.	*S. echaetus*	Oblong/ lanceolate	Sessile	Absent	Absent	Amplexicaul	Unipinnate	Dentate	Not tomentose
4.	*S. graciliflorus*	Ovate/ Oblong	Present	Absent	Absent	Simple	Unipinnate	Dentate	Not tomentose
5.	*S. kumaonensis*	Ovate oblong	Present	Absent	Absent	Simple	Not pinnate	Dentate	White tomentose
6.	*S. laetus*	Ovate/elliptical	Sessile	Absent	Absent	Amplexicaul	Unipinnate	Dentate	Not tomentose
7.	*S. nudicaulis*	Oblong	Sessile	Absent	Present	Semiamplexicaul	Not pinnate	Crenate lobulate	Not tomentose
8.	*S. raphanifolius*	Oblong	Sessile	Absent	Absent	Semiamplexicaul	Uni or subpinnate	Dentate	Not tomentose
9.	*S. ramosus*	Linear /oblong	Sessile	Absent	Absent	Semiamplxicaul	Uni or bipinnate	Dentate	Not tomentose
10.	*S. royleanus*	Ovate/ Oblong	Present	Present	Absent	Simple	Unipinnate	Dentate	Not tomentose
11.	*S. scandens*	Ovate/ triangular	Present	Absent	Absent	Simple	Not pinnate	Dentate	Not tomentose
12.	*S. topkegolensis*	Oblong/ triangular	Present (winged)	Absent	Absent	Semiamplexicaul	Not pinnate	Dentate	Not tomentose

Table 3. Capitulescence and capitula characters.

SN	Species	Capitulescence type	No of capitula in capitulescence	Type of capitula	Shape of involucres	No. of phyllaries	No. of ray florets	No. of disc florets	Pappus colour
1.	*S.albopurpureus*	Solitary or terminal corymb	Ca. 5	Radiate	Campanulate	15	7-11	∞ (ca.38)	White/Stramineous
2.	*S.biligulatus*	Terminal compound corymb	Numerous	Radiate	Tubular	5	2-3	2-3	White
3.	*S.echaetus*	Terminal & upper axillary corymb	Lax(9/10)	Radiate	Campanulate	19-21	18-21	∞ (Ca.80)	White/Stramineous
4.	*S.graciliflorus*	Terminal & axillary compound corymb	Numerous	Radiate	Tubular	5	2	3	White
5.	*S.kumaonensis*	Terminal & upper axillary panicle	Numerous	Discoid	Tubular	5-6	-	5-7	Stramineous
6.	*S.laetus*	Terminal & axillary corymb	Numerous	Radiate	Campanulate	10-13	10-13	∞ (Ca. 50)	White/Stramineous
7.	*S.nudicaulis*	Terminal compound corymb	Numerous	Radiate	Campanulate	13-15	13	∞ (Ca. 70)	White
8.	*S. raphanifolius*	Terminal simple or compound corymb	Numerous	Radiate	Campanulate	13-15	13-15	∞ (Ca. 80)	Dark yellow or reddish brown
9.	*S.ramosus*	Terminal corymb	Lax (Ca. 5/6)	Radiate	Hemispherical	14-21	8-11	∞ (Ca. 48)	White
10.	*S.royleanus*	Terminal & upper axillary compound corymb	Numerous	Radiate	Narrowly campanulate	8	3-5	7-10	White
11.	*S.scandens*	Terminal & axillary loose divaricately branched thyrses	Numerous (upto ca. 20)	Radiate	Campanulate	10-13	8-10	∞ (Ca. 30-50)	White
12.	*S.topkegolensis*	Terminal compound corymb	Numerous	Rradiate	Tubular	5	2	3	White

Table 4. Trichome variations in *Senecio* species.

SN	Species	Stem	Leaf trichome	Phyllaries	Ray floret trichome	Ovary trichome
1.	*S. albopurpureus*	Sparsely arachnoid	Underside sparsely purplish	Uni and biseriatetrichomes, biseriate with 2 row all along	Absent	Absent
2.	*S.biligulatus*	Smooth	Uniseriate with terminal cell shriveled, cells not exceeding, 4	Absent	Absent	Absent
3.	*S. echaetus*	Sparsely arachnoid	Uniseriate usually without shriveled cells	Absent	Uniseriatetrichomes, upper cell long hair like or shriveled	Absent
4.	*S.kumaaonensis*	Sparsely tomentose	Underside white tomentose	Uni and biseriatetrichomes, 2 rows cells below &1 row above	-	Absent
5.	*S. graciliflorus*	Smoth	Uniseriate with shriveled cells	Uniseriate with shriveled cells	Absent	Absent
6.	*S. laetus*	Sparsely arachnoid	Abent	Absent	Uniseriate multicellular	Smooth/sparsely pubescent
7.	*S.nudicaulis*	Sparsely arachnoid	Uniseriatewith usually terminal cell shriveled	Absent	Absent	Densely pubescent
8.	*S. ramosus*	Smooth	Absent	Uniseriate, sparse	Absent	Densely pubescent
9.	*S. raphanifolius*	Sparsely arachnoid	Uniseriate with or without shriveled cells	Absent	Uniseriate&biseriate	Absent
10.	*S. royleanus*	Smooth	Uniseriatewith upper cell shriveled	Uniseriatewith shrivelled cells	Uniseriatewith shrivelled cells	Smooth
11.	*S. scandens*	Smooth	Uniseriate without shriveled cell	Absent	Uniseriate with shrivelled cells	Absent
12.	*S. topkegolensis*	Smooth	Uniseriate with shrivelled cells,3-4	Absent	Absent	Absent

Table 5. Qualitativeand quantitative characteristics of stomata and epidermal cell of leaf of *Senecio*

SN	Species	LS	Type of stomata					Epidermal cells	
			Ano	Tri	Ani	Tet	Con	Cont. cells	Shape
1.	*S. albopurpureus*	Amphi	+	-	-	+	+	4-6	Irr-Poly
2.	*S. biligulatus*	Hypo	+	-	-	-	+	5-6	Irr-poly
3.	*S. echaetus*	Amphi	+	-	-	+	+	4-6	Irr-Poly
4.	*S. kumaonensis*	Hypo	+	-	-	-	+	5-7	Irr
5.	*S.graciliflorus*	Hypo	+	+	-	+	+	3-7	Irr
6.	*S. laetus*	Amphi	+	-	-	+	+	4-6	Irr-Poly
7.	*S. nudicaulis*	Amphi	+	+	-	+	+	3-5	Poly-Irr
8.	*S. ramosus*	Amphi	+	+	+	+	+	3-5	Irr-ploy-ract
9.	*S. raphanifolius*	Amphi	+	-	-	+	+	4-5	Poly-rect
10.	*S. royleanus*	Hypo	+	+	-	+	+	3-7	Irr
11.	*S. scandens*	Hypo	+	+	-	+	+	3-5	Irr
12.	*S. topkegolensis*	Hypo	+	-	-	-	+	5-6	Irr

LS = Leaf surface, Hypo = Hypostomatic, Amphi = Amphistomatic, Ano = Anomocytic stomata, Tri = Tricytic Stomata, Ani = Anisocytic stomata, Tet = Tetracytic Stomata, Con. = Contiguous stomata, Cont. = Contact, Irr = Irregular

Table 6. Species reported from Protected Areas of Nepal.

SN	Species	State of species	Reports from protected area
1.	*S.albopurpureus*	Endemic to Himalayas	LNP, KCA
2.	*S.biligulatus*	-	LNP, GCA
3.	*S.candolleanus*	Endemic to Himalayas	-
4.	*S.echaetus*	Endemic to Himalayas	LNP
5.	*S.graciliflorus*	Endemic to Himalayas	KNP, ACA, LNP, MBNP
6.	*S.kumaonensis*	Endemic to Himalayas	LNP, GCA
7.	*S.laetus*	-	ACA, LNP, GCA, MBNP
8.	*S.nudicaulis*	Endemic to Himalayas	ShNP, SNNP
9.	*S.panduliformis*	Endemic to Nepal Himalayas	-
10.	*S.ramosus*	Endemic to Himalayas	-
11.	*S.raphanifolius*	Endemic to Himalayas	ACA, LNP, SNP, MBNP
12.	*S.royleanus*	Endemic to Himalayas	MCA, LNP
13.	*S. scandens*	-	ACA, LNP
14.	*S.topkegolensis*	Endemic to Nepal Himalayas	-

LNP = Langtang National Park, KCA = Kanchanjanga Conservation Area, GCA = Gaurishankar Conservation Area, KNP = Khaptad NP, ACA = Annapurna CA, MBNP = Makalu Barun NationalPark, ShNP = Sukhlaphanta National Park, SNNP = ShivapuriNagarjun, MCA = Manaslu CA.

in colour. Besides the gross morphological characters, the micromorphological characters like trichomes and stomata were revealed as the good parameters to solve the taxonomic problems. In leaves, only the uniseriate trichomes are present in all species. However, some biseriate trichomes are also present in phyllaries of *S. albopurpureus* and *S. kumaonensis*, and ligules of ray florets in *S. raphanifolius*. The species, *S. biligulatus, S. kumaonensis, S. graciliflorus, S. royleanus, S. scandens*, and *S. topkegolensis* have hypostomatic leaf surface while *S. albopurpureus, S. echaetus, S. laetus, S. nudicaulis, S. ramosus*, and *S. raphanifolius* have amphistomatic leaf surface. The species, *S. ramosus* is characterized by the presence of anisocytic stomata as well. The contiguous stomata were of common occurrence in all species. The occurrence of contiguous stomata was reported as the feature associated with polyploidy (Ikechukwu & Bosa, 2006).

The micromorphological characters thus seem a good parameter in solving the taxonomic problems in *Senecio s.s.* and can be extrapolated in solving the taxonomic problems in *Senecio* species of the world to make the genus more homogenous and remove the anomalous species presently under the genus (Joshi & Bajracharya, 2015). The species like *S. ramosus* was found in tropical region, while *S. biligulatus* and *S. topkegolensis* were distributed in subalpine and alpine regions of Nepal Himalaya. Three species, viz., *S. biligulatus*, *S. laetus*, and *S. scandens* were not endemic to Himalayas and distributed in other habitat as well. The species *S. biligulatus* is distributed in Bhutan, China, India (Sikkim), and Myanmar; *S. laetus* is distributed in Bhutan, India (NE, NW), China, and Pakistan (NW), and *S. scandens* in Bhutan, China, India, Japan, Laos, Myanmar, Nepal, Philippines, Sri Lanka, Thailand, and Vietnam (Chen et al., 2011).

Conclusion

The morphological study revealed that the characters like stalk of leaf, dissection of leaves lamina, type of capitulescence, type of capitula, number of phyllaries, nature and number of

ray and disc florets, relative length of ovary of ray and disc florets and colour and abundance of pappus were taxonomically significant in delimiting the species of *Senecio.* Besides that, micromorphological characters like type of trichome, distribution of stomata on leaf surface like amphistomatic and hypostomatic leaf surface, and type of stomata like tricytic, anisocytic, tetracytic stomata and epidermal cell type were found to be taxonomically significant in the genus. Many species are Himalayan endemic and distributed in the protected areas and outside the protected areas of Nepal. However, the present trends of habitat destruction due to natural processes and anthropogenic activities have indicated the situation for need of conservation of species outside the protected areas as well.

Acknowledgements

The author is grateful to the Central Department of Botany, Tribhuvan University; Department of Botany, Amrit Science Campus and Patan Multiple Campus; National Herbarium and Plant Laboratories (KATH); and Natural History Museum (BM).

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Foliicolous Black Mildews on Fagaceae at Shivapuri and Phulchoki Hill Forest, Nepal

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Abstract

The present investigation involves the general survey and taxonomic characterization of black mildew found in Fagaceae at different altitudinal ranges of Shivapuri and Phulchoki hills of Kathmandu valley. A total of 11 species, viz., *Appendiculella* sp., *Asterina* sp., *Asterotexis* sp., *Fulviomyces* sp., *Meliola* sp., *Sarcinella* sp., *Stenella* sp., *Paraphialocephala* sp., *Verrucispora* sp., *Tretospora* sp., and *Triposporium* species were identified and studied in detail in two different sites on different host plants and belonged to the class Ascomycetes and Deuteromycetes. All the reported fungi were recorded on the seven host species (*Castanopsis indica, C. tribuloides, Lithocarpus spicata, Quercus glauca, Q. lanuginosa, Q. lineata, Q. semicarpofolia*) 900m-2700m at Phulchocki and Shivapuri hills. The single genus *Meliola* species was found in *Lithocarpus spicata*. The highest degree of infestation was observed between 1900m-2300m altitude while less degree of infestation was below 900m and above 2300m comparatively. The present investigation was carried out to enumerate the foliicolous black mildews and altitude's effect on their distribution.

Key words: *Appendiculella,* Fungi, Host, Infestation, Pathogens, Symptoms

Introduction

Knowledge about the time of origin and evolution of fungi has been lost in antiquity. However, their interaction with human beings can be traced back only to civilization. A detailed study about fungi could have been started only after microscopy discovery during the 17th century. Among the living organisms, the fungi are unique in having the adaptability to adverse environmental conditions. They are eukaryotic spore bearing, achlorophillous organism occurring as saprophytes, parasites and symbionts. Fungi are helpful in many ways such as in making antibiotics, alcohol industries, baking industries, preparation of various types of enzymes and acids. They destroy crops, vegetables, fruits and cash crops.

In occurrence, fungi inhabit all the components of the global environment, the lithosphere, the atmosphere and the hydrosphere, and almost all the living and dead plants and animals. Nutritionally they are entirely heterotrophic but of different categories like saprophytes and parasites. Fungi are pathogenic and cause root, stem or leaf diseases which are technically referred to as foliicolous. The foliicolous form cause different types of symptoms like leaf spot, necrosis, sooty moulds, tar spots, downy and powdery mildew and black mildew. Black mildews are most common in tropical and subtropical areas and reduce biomass production in economically important plants (Alexopolous & Mims, 1966). They destroy the

photosynthetic pigment (Verma, 1997) and produce some toxic substances (Bessey, 1968). The black mildew fungi develop black, thick and velvety covering and are ectoparasites of vascular plants (Stevens & Dowell, 1928).

Black mildews develop remarkable interwoven hyphae, hypopodia, setae, ascocarps etc. The systemic studies of black mildews were begun with the investigation done by Berkeley (1856) with the records of *Asterina cineta.* At the end of 19th century, Gaillard (1892) described several species of *Meliola*. The most significant contributions in taxonomy and biology of some black mildew, with particular reference to *Asterina*, *Meliola* and *Sarcinella* were made by Patial and Thita (1977). Alexopolous and Mims (1966) reported 149 and 103 genera of class Ascomycetes and Deuteromycetes, respectively. The present investigation was carried out to enumerate the foliicolous black mildews and altitude's effect on their distribution.

Materials and Methods

The present survey was carried out in two different sites, i.e., site 1 (Shivapuri) and site 2 (Phulchoki) hills of Kathmandu valley situated at 1336 m above the sea level. Site 1 is located in the northern part and site 2 is situated in the south-east corner of Kathmandu valley (Fig. 1). The average temperature varies from 22.5-26°C, and average annual rainfall was 1881.7-2910.6 mm during the study periods (DHM, 2000). The climate was subtropical to temperate type and characterized by the dominance of *Quercus* spp. and *Castanopsis* spp. The associated species were *Acer* spp., *Rhododendron* spp., *Schima* spp., *Lithocarpus* spp. and so on.

The survey was carried out during 1998 to 2000 by systematic visits of the different study sites, i.e., Site 1 and Site 2 for noting black mildew. During the field visits, the natural habit, growth form, phenology of the sample species was determined by visual observation. Quadrats of 20×20 m size were kept randomly in the study sites with three replicate nearly 30m inside the tracking route and calculate the density as per Misra (1968). Periodic visits were made to collect the foliicolous black mildews which occur on members of Fagaceae. The infected leaves were collected in separate polythene bags. The collected specimens were labeled as per locality, host, and altitude and identified by consulting different literatures

Site 1. Shivapuri Site 2. Phulchoki

Figure1. Photographs of the study area

and herbariums. The herbarium preparation was done as per Jain and Rao (1977). The detailed analysis was done with staining and section cutting under compound microscope. The sketches and necessary measurement of the section was done by Camera Lucida and micrometer.

Results

A total of 11 species of black mildews fungi was identified and studied in detail in two different sites on different host species of Fagaceae like *Appendiculella* sp., *Asterina* sp., *Asterotexis* sp., *Fulviomyces* sp., *Meliola* sp., *Sarcinella* sp., *Stenella* sp., *Paraphialocephala* sp., *Verrucispora* sp., *Tretospora* sp., and *Triposporium* sp. (Table 1).

Although vegetation pattern of site 1 and site 2 have been described in study area. However, efforts have been put to show the comparative study of host plants with their altitudinal distribution in both the sites. From the study, it is clear that the altitudinal range of same genus of host is different in different areas, i.e., *Castanopsis indica* was found from 1800m-1900m at site 2 only where as the other host species were found common with both sites with different altitude like *Castanopsis tribuloids* was found from 1600m-2300m at site 1 and 1900m-2300 at site 2. Similarly, *Quercus glauca* was found from 1600m-2100m and 1800m-2300m at site 1 and site 2, respectively. *Quercus lanuginosa* was found from 1600m-2300m at site 1 and 1900m-2300m at site 2. *Quercus lineata* 2000m-2500m and *Q. semicarpofolia* 2000m-2700m were found in some altitude in both sites. *Lithocarpus spicata* was found from 2000m-2200m and 1800m-2300m at site 1 and site 2, respectively (Table 2).

In the present survey seven species of *Quercus* and *Castanopsis* belonging to family Fagaceae with different symptoms, i.e., leaf spots, necrosis, powdery mildews, sooty moulds, tar spots and black mildews were collected during the study periods. A total of eleven black mildews were identified in seven different hosts. In majority of cases, it was found that more than two genus of fungi occurred in same host. In *Castanopsis indica; Meliola, Paraphialocephala, Sarcinella, Stenella, Verrucispora* species and in *Castanopsis tribuloides*; *Asterina*, *Fulviomyces*, and *Meliola* species were found from an altitude of 1800m-1900m at Phulchoki hills only. Similarly, in *Quercus glauca; Asterina, Asterotexis, Meliola, Stenella* and *Triposporium* species were found from an altitude of 1800-2300m and 1600m-2100m at Phulchocki and Shivapuri hills, respectively. The single genus *Meliola* species was found in *Lithocarpus spicata*. In the present study genus *Meliola* was frequently found from an altitude of 1800m to 2700m at Phulchoki and 1600m to 2700m at Shivapuri in different host species of family Fagaceae.

In the laboratory conditions, the identification key of individual species such as type of mycelium, spore type, spore bearing structure and size of spore were analyzed critically. Of the total species the highest size of appendage was 286.38-643.4 µm and lowest size was 6.66-19.98 µm in *Paraphialocephala* sp. and *Fulviomyces* sp. species, respectively. Similarly, the highest size of spore was 59.94×13.32 µm and lowest was 6.66×3.23 µm in *Meliola* sp. and *Paraphialocephala* species, respectively. All the identified black mildews are tabulated on the basis of host plants (Table 1). All the investigated genera of foliicolous fungi are represented as in figures 2-12.

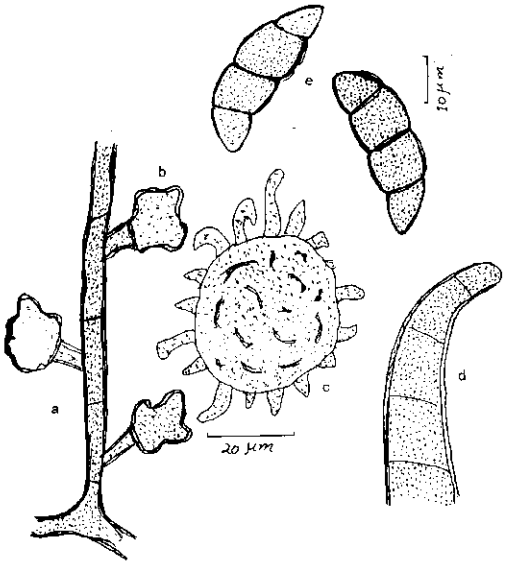

Figure 2. *Appendiculella* sp.: a & b. Hyphae with hyphopodia, b & c. Ascocarp with appendages, c. Ascospores

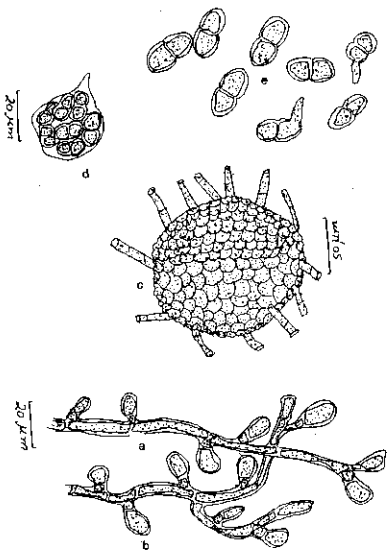

Figure 3. *Asterina* sp.: a-b. Hyphae with hyphopodia, c & d. thyriothecium and asci, c. Ascospores

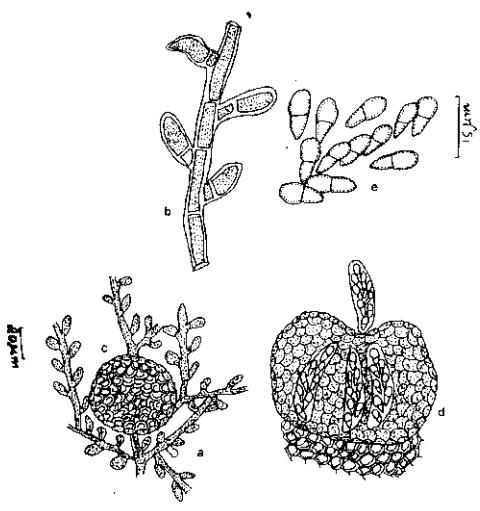

Figure 4. *Asterotexis* sp.: a & b. Hyphopodiate mycelium, c & d. Hyphae and ascometa, e. Ascospores

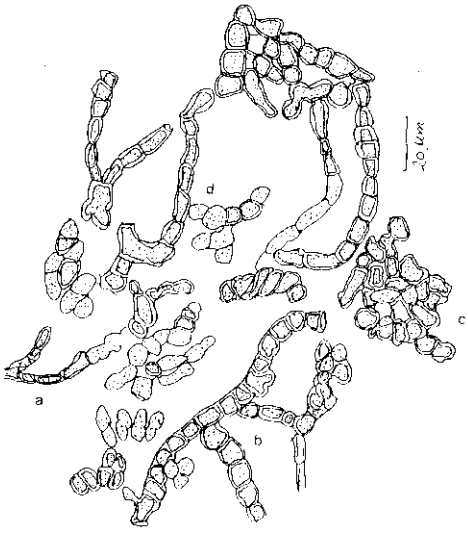

Figure 5. *Fulviomyces* sp.: a & b. Hyphae and conidiophores, c. Rosette of cells, d. Conidia

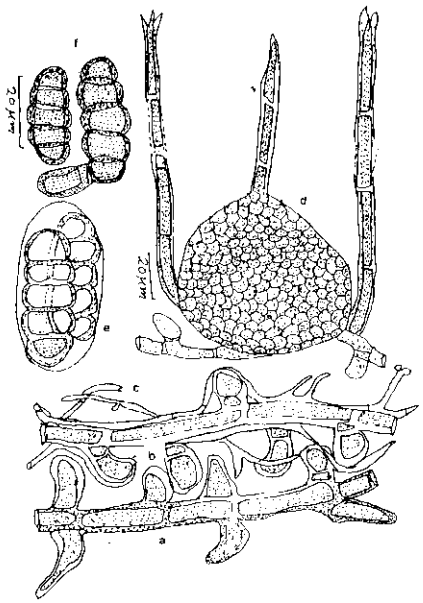

Figure 6. *Meliola* sp.: a & b. Hyphae and hyphopodia, c & d. Hyphae and asci

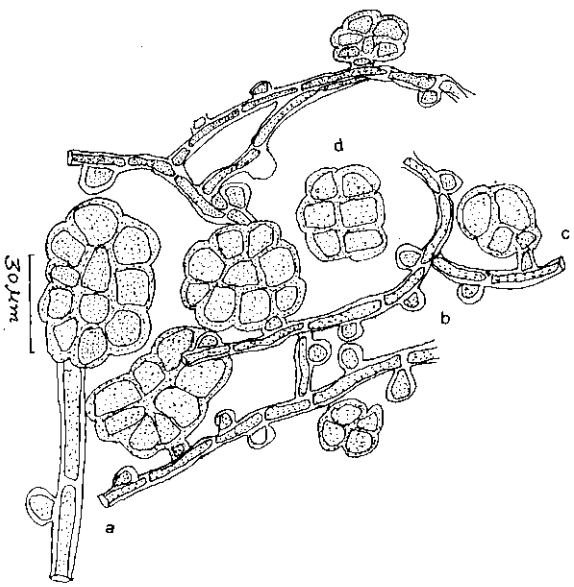

Figure 7. *Sarcinella* sp.: a & b. Hyphae and hyphopodia, c. Conidium with conidiogenous cell

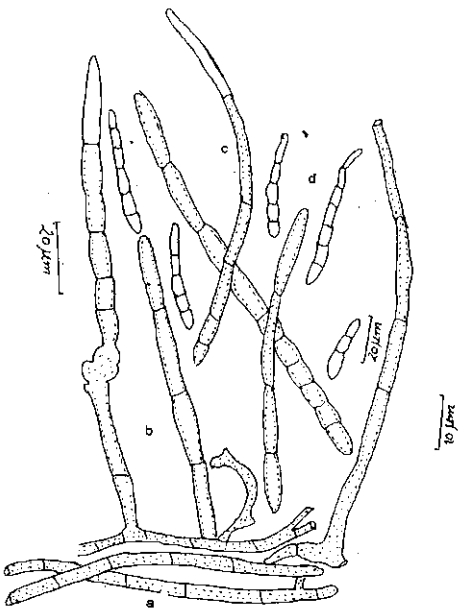

Figure 8. *Stenella* sp.: a. Vegetative hyphae, b. Conidiophore, c. Conidia

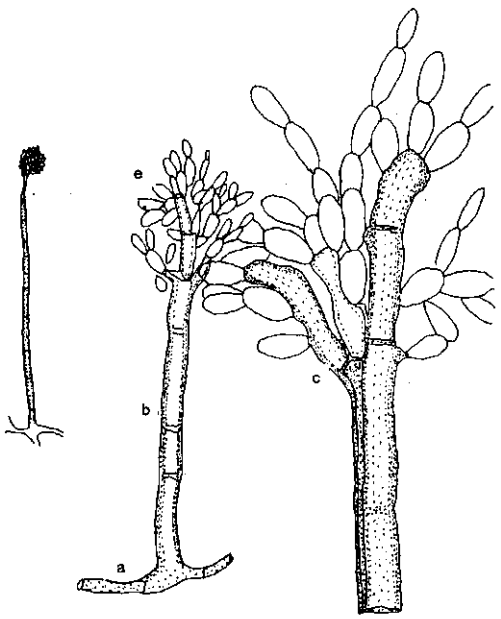

Figure 9. *Paraphialocephala* sp.: a. Vegetative hyphae with conidiophores, b. Conidiophore, c & d. Stipe and Conidia in chain

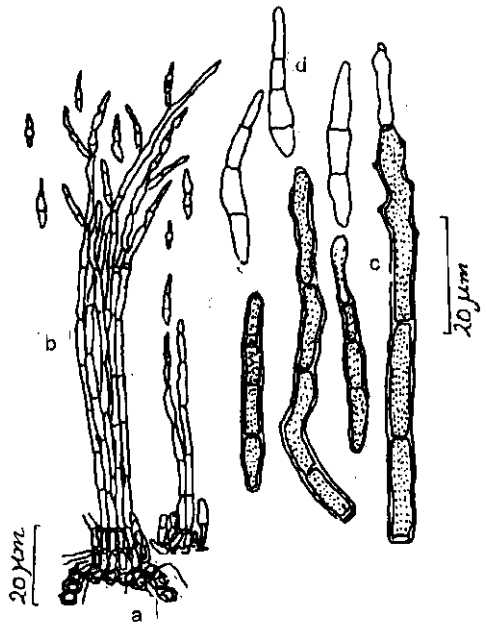

Figure 10. *Verrucispora* sp.: a. Stroma and conidiophores, a. Stroma and conidiophores, c & d. Conidia

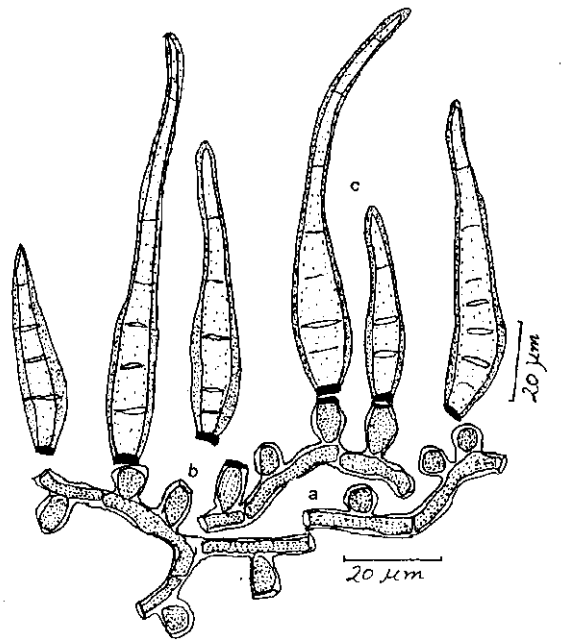

Figure 11. *Tretospora* sp.: a. Hyphae, b. Hphopodia, c. Conidigenous cells, d. Conidia

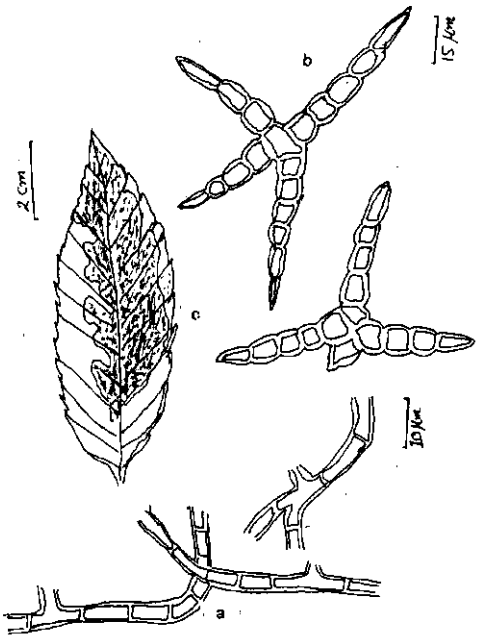

Figure 12. *Triposporium* sp.: a. Septate branched hyphae, b. Conidia, c. Conidium

The hyphae are of different shape and size depending upon the species, generally branched, septate, simple or interwoven with smooth and rough surface. The size of hyphae ranged from 3.32 µm in *Meliola* sp. to 37.29 µm in *Verrucispora* sp. The hyphopodia was measured 3.93 µm in *Sarcinella* sp. to 22 µm in *Astertaxis* sp. All the black mildews like *Stenella*, *Tretospora*, *Verrucispora* and *Paraphialocephala* do not possess ascus and ascospores but give rise to conidiophores conidia. The dominance of host plants and black mildews fungi are tabulated on the basis of host plants (Table 1).

Table1. Host plant species and total number of pathogens found in site 1 and site 2 with their classes.

Host plants	Pathogens (Genera)	Total No.	Class
	Meliola sp.		Ascomycetes
	Paraphialocephala sp.		Deuteromycetes
Castanopsis indica	*Sarcinella* sp.	5	Deuteromycetes
	Stenella sp.		Deuteromycetes
	Verrucispora sp.		Deuteromycetes
	Asterina sp.		Ascomycetes
Castanopsis tribuloides	*Fulviomyces* sp.	3	Deuteromycetes
	Meliola sp.		Ascomycetes
Lithocarpus spicata	*Meliola* sp.	1	Ascomycetes
	Asterina sp.	5	Ascomycetes
	Asterotexis sp.		Ascomycetes
Quercus glauca	*Meliola* sp.		Ascomycetes
	Stenella sp.		Deuteromycetes
	Triposporium sp.		Deuteromycetes
	Appendicullela sp.		Ascomycetes
Quercus lanuginosea	*Asterina* sp.	3	Ascomycetes
	Meliola sp.		Ascomycetes
	Meliola sp.		Ascomycetes
Quercus lineate	*Sarcinella* sp.	3	Deuteromycetes
	Tretospora sp.		Deuteromycetes
	Asterina sp.		Ascomycetes
Quercus semicarpofolia	*Meliola* sp.	3	Ascomycetes
	Sarcinella sp.		Deuteromycetes

Discussion

Nepal is a mountainous country with diverse mycoflora due to sudden change in climate and topography. The form causing leaf disease is called foliicolous. The Himalayan region of the country is represented by a turnover of rich pathogenic vegetation all over the year around sub-tropical climatic conditions and provide suitable substratum for the occurrence of foliicolous fungi. The black mildews are found most common in humid tropical and sub-tropical areas with different symptoms.

The detailed study was carried out in two different sites, i.e., site 1 and site 2 from where the species belonging to Fagaceae with different symptoms such as leaf spots, necrosis, powdery mildews, sooty moulds, tar spots. From the collected specimens, the most frequently occurring species were *Meliola* sp. which was seven in number followed by *Asterina* sp. four in number, three species of *Sarcinella*, two species of *Stenella* and one species of

Table 2. List showing host plants with their sites, density, frequency (%) and altitude (m).

Name of host species	Family	Altitude m	1600m		1800m		2000m		2200m		2400m		2600m	
			D	F	D	F	D	F	D	F	D	F	D	F
Site 1														
Castanopsis indica	Fagaceae	1800-1900	-	-	106.2	100	-	-	-	-	-	-	-	-
Castanopsis tribuloides	Fagaceae	1800-2200	-	-	200	100	162.5	100	31.2	50	-	-	-	-
Lithocarpus spicata	Fagaceae	1800-2300	-	-	50	75	12.5	25	12.5	25	-	-	-	-
Quercus glauca	Fagaceae	1800-2300	-	-	68.7	100	81.2	100	62.5	75	-	-	-	-
Quercus lanuginosea	Fagaceae	1900-2300	-	-	25	25	137.5	75	81.2	50	-	-	-	-
Quercus lineatea	Fagaceae	2000-2500	-	-	-	-	56.2	100	62.5	75	137.5	100		
Quercus semicarpofolia	Fagaceae	2000-2700	-	-	-	-	12.5	25	137.5	75	187.5	100	731.2	100
Site 2														
Castanopsis indica	Fagaceae	1800-1900	-	-	-	-	-	-	-	-	-	-	-	-
Castanopsis tribuloides	Fagaceae	1600-2300	25	75	256.2	75	312.5	100	62.5	75	-	-	-	-
Lithocarpus spicata	Fagaceae	2000-2200	-	-	-	-	43.7	25	31.5	100	-	-	-	-
Quercus glauca	Fagaceae	1600-2100	25	50	62.5	25	137.5	100	-	-	-	-	-	-
Quercus lanuginosea	Fagaceae	1600-2300	43.7	75	62.5	75	25	50	50	75	-	-	-	-
Quercus lineatea	Fagaceae	2000-2500	-	-	-	-	56.2	25	12.5	50	50	100	-	-
Quercus semicarpofolia	Fagaceae	2000-2700	-	-	-	-	12.5	25	87.5	100	387.5	100	512.5	100

Appendicullela, *Asterotexis*, *Triposporoum*, *Fulviomyces*, *Treataspora*, *Paraphialocephala*, and *Verrucispora*.

The detailed studies on black mildews have been very few reported from Nepal. The previous records of *Meliola* species from Nepal were study on *Citrus aurantum* from Kathmandu by Khadka and Shah (1967), *M. Sacchari* on *Sorghum* (Singh & Kamal, 1978), *Hedera nepalensis* from Godawari (Adhkari et al., 1987). Budhathoki (1988) described *Meliola* species on the host *Jasmine humile*, *Quercus glauca*, *Hedra nepalensis*, *Desmodium heterocarpum*, *Castanopsis indica,* etc. The present study is more or less similar with the findings of Budhathoki (1988). *Sarcinella* sp. was previously reported by Budhathoki (1988) on *Lyonia ovalifolia*, *Prunus rufa*, *Oryris* species and *Celastrus* species, whereas in the present research it was found on *Castanopsis indica*, *Quercus lineate* and *Q. semicarpolifolia*. Present study finding of *Q. linuginosa* agree with the findings of Adhikari and Manabdhar (1984) and Budathoki (1988).

Asterotexis quercina and *Fulviomyces* sp. was previously reported by Budhathoki (1988) on leaves of *Quercus glauca* and *Eurya acuminate*, respectively. In the present study it was found on same host from site 2 and *Castanopsis indica* at site 1. *Tretospora* sp. and *Stenella* sp. previously has been reported by Kamal et al. (1986) and Singh and Kamal (1978) on living leaves of *Xeromphis* sp. and leaves of *Rhododendron* sp., respectively and in the present study it was found on *Q. lineata* on both sites. *Paraphialociphala* sp. and *Triposporium* sp. were reported by Budhathoki and Singh (1994) on leaves of *Castanopsis tribuloides* and *Q. glauca* from Kathmandu and in the present study it was observed in living leaves of *Castanopsis indica*. Similarly, *Verrucispora* sp. was also reported on the leaves of *Luculia gratissima* but the present study observed in living leaves of *Castanopsis indica*.

The ascocarp is globose or flask shaped in *Meliola*. Thyriothecia is the characteristic features of *Asterina* sp. which size ranged from 46.62-193.42 µm. In *Asterina* sp., ascus

is the sac like structure with 2-8 ascospores. The results of present study are similar with the findings of Sahni (1964). The smallest and largest size of ascus was recorded as 39.62 µm and 61.27×43.29 µm in *Asterina* sp. and *Appendicullela* sp., respectively. The sizes of conidiophores were found in between 7.3×6.98 to 286.38µm. The smallest size of conidia was 6.66×3.23 µm in *Paraphialocephala* sp. and 120×18.5 µm in *Tretopspora* sp. The variations in shape and size of these species from the previously recorded are due to the eco-climatic variation (Kamal et al., 1986). The abundance of infection and pathogen was found in between the altitude of 1900m-2300m whereas less was found below 1900m and above 2300m. Such variations in the distribution of black mildew fungi were due to moderate humidity, temperature or eco-climatic variation (Stevens & Dowell, 1928).

The biodiversity of foliicolous black mildew fungi within the member of Fagaceae is well established in the present study sites. Altitude plays an important role in the distribution of black mildew fungi. Among the studied fungi *Meliola* species are very common and frequently occurred in all the members of Fagaceae. The present investigation shows that the maximum number of black mildews is found in *Castanopsis indica* and *Castanopsis tribuloides* while less number in *Lithocarpus spicata*. The advancement of the knowledge on mycoflora will certainly be possible by the taxonomic studies on different mycotaxa.

Acknowledgements

The authors are thankful to the Head of Central Department of Botany, Kritipur and Prof. Usha Budathoki for their support and facilities provided throughout the study period.

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Birds of Dharan Sub-Metropolitan City, Eastern Nepal: Status and Threats

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Abstract

Proper understanding regarding the status and threats to avian diversity is a key to formulating an effective conservation-dependent species management strategy. This study aims to assess the bird species status, distribution, and associated threats in East Nepal's Dharan sub-metropolitan city. Based on habitat suitability, vegetation, altitude, and anthropogenic disturbances, 12 different sites were selected within the study area. Altogether 181 bird species belonging to 13 orders and 53 families were recorded during the field survey. Passeriformes records were the highest, i.e., 62.2% (113 species) of total data. Most of the bird species (72.3%) were found to be residential. In total, 84% of the total bird species were recorded from the tropical region. Apart from this, forest area habitats were mostly preferred by the recorded bird species. The increasing anthropogenic pressures, including haphazard developmental works, expanding settlements and agricultural conversions, have affected the distribution and abundance of birds in the study area. This study will provide critical baseline information about the distribution, related threats and overall avian status of the city, including some parts of IBAs . Further, this research opens up avenues to conduct rigorous studies and devise conservation action plans hereafter.

Key words: Avian diversity, Conservation status, Migratory birds, Passeriformes

Introduction

Eight hundred eighty-six bird species have been reported in Nepal, representing around 9% of the world's total bird species (DNPWC & BCN, 2018). Of these, 42 species are globally threatened and 35 are globally near endangered. Despite its small size, Nepal is recognized to have vibrant avian diversity. Birds are exemplary bioindicators of ecosystem health, environmental/ecological change and biodiversity status overall (Mekonen, 2017; Kandel et al., 2018). Among vertebrates, birds are the best-researched class that possesses a broad array of ecological functions; from producing soil to shaping primates' behaviour (Sekercioglu, 2006). Avian ecological diversity is an essential descriptor to assess natural and human- dominated landscapes (Sandströem et al., 2006). Ornithological researchers select birds' ecological groups as a surrogate method to study biodiversity status in urban environments (Sandströem et al., 2006). For the reason that birds are mobile organisms and relatively conspicuous, many researches on urban landscapes have been conducted using avifauna as indicators of habitat quality (Mortberg & Wallentinus, 2000; Bolger et al., 2001; Fernandez-Juricic, 2004).

Habitat types used by birds play an indispensable role in their survival; nevertheless, fragmentation and loss eventually have colossal effects on their population. The 2010 study on "The state of Nepal birds" narrated that the most significant overall threat to a total of 128 nationally threatened bird species is due to the increasing human pressures. Important Bird & Biodiversity Areas (IBAs) are sites of international importance for the sustainable conservation of birds and other wildlife in the world (BirdLife International, 2014). There are 27 (IBAs) that cover 2,651,592 ha of the total land of Nepal (Bird Life International, 2021a). These areas have been classified under standardized global criteria.

The recent IBAs monitoring and assessment data have attributed increasing human-induced disturbances as a major threat for overall avian survival (BirdLife International, 2021b). At one-third of the Nepal IBAs, anthropogenic disruptions have already affected theprovision of ecosystem services. These facts were supported by other past periodical studies (Basnet, 2009; Baral et al., 2012). Besides, over-exploitation of wildlife either by hunting and capturing is regarded as another gravechallenge at over 20% (IUCN, 2013). IBAs play a major role in bird conservation however, adequate and reliable information about such areas including Dharan IBAs is still lacking (Baral & Inskipp, 2005; Baral et al., 2012).

It is estimated that around 300 bird species occur in the Dharan forest, however, anofficial comprehensive inventory is not available yet (BirdLife International, 2021a). Some of the past studies have focused on Dharan IBAs (Basnet, 2009). So far, Subba (1995) conducted research on birds of Dharan Sub-metropolitan city; although robust datasets are scarce. Strenuous land topography and inaccessible remote area is among a major hindrance for carrying out ornithological researches. This study mainly focused on assessing the bird species status, distribution, and threats in northern part and urban areas of Dharan while also attempted integrating the past corresponding researches.

Materials and Methods

Study Area

The study was conducted in the Dharan Sub-metropolitan city in eastern Nepal, province no.1. The city is situated within the Chure/Siwalik hills (26°49'00"N, 87°17'0"E) and has an area of 192.32 km^2 (Fig. 1). The elevation of this study arearanges from 119 to 1778 m msl. The study mainly focused on the northern andurban areas and covered some regions of Dharan IBAs. The study site has a tropical and subtropical ecosystem with high dominance of evergreen tree species like *Shorea robusta* (Bird Life International, 2021a).

Data Collection and Processing

The entire study area was divided into 12 different sites. They were selected on the basis of potential habitat, vegetation, altitude, and human-induced disturbances. We collected data from Feb 2017-Dec 2019 from each of the 12 selected sites. Linetransect sampling method was followed with multiple replications. We recorded the name of the species and their location using a handheld Garmin GPS Etrex 10. Subsequently, we took notes regarding the key anthropogenic activities such as land conversions, deforestation, fires and livestock grazing. We used Nikon Monarch binoculars, PORRO PRISM spotting scope, and Canon 7D DSLR

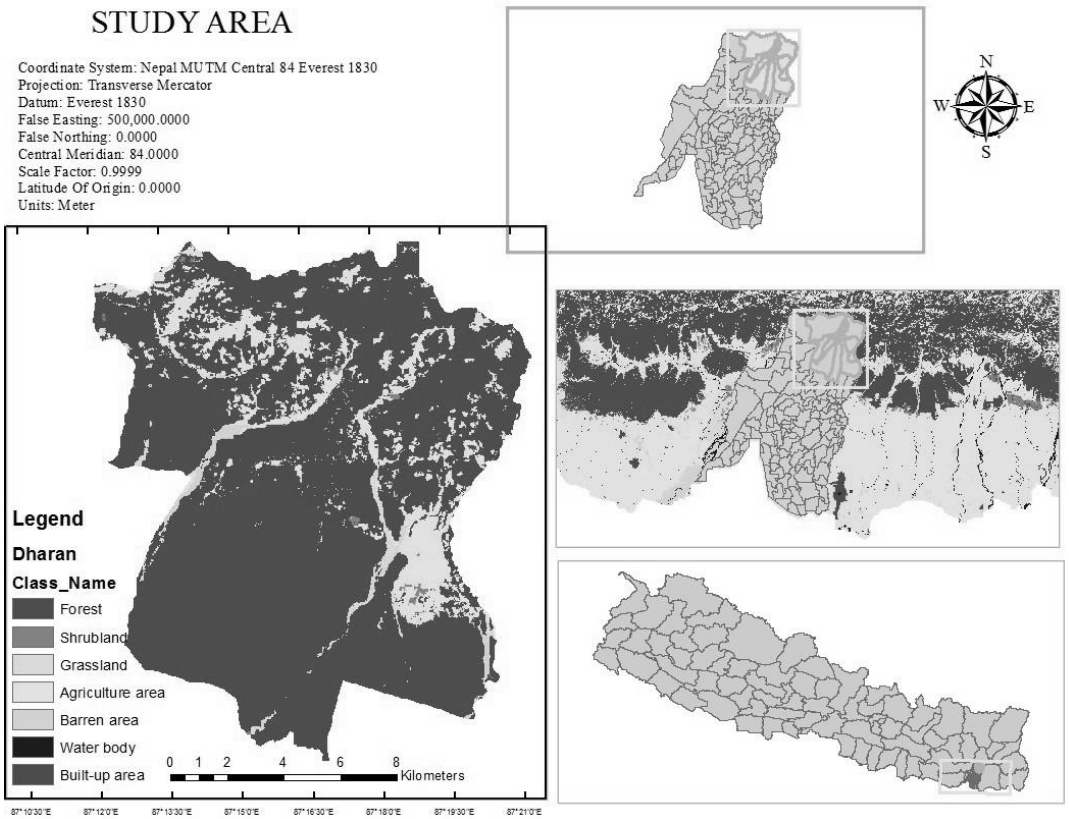

Figure 1. Map locating study area

with 180-600mm Tamron lens to identify and record the species. Most of the bird's species were identified through direct sighting method. However, in case of shy and skulking species, we recorded their sound and identified their taxonomy via online database of Xeno-canto website. Helm Field Guides and photographic field guide books were also used to identify the bird's species. Later, we tabulated and analyzed the data using Microsoft Excel 2016.

Results

Taxonomic Coverage

We collected data of 181 bird species belonging to 13 orders and 53 families (Table 1). Passeriformes (largest order of birds) records were the highest, i.e., 62.2% (113sp.) of total data. Pelecaniformes is the second largest order in case of the family but Acciptriformes is the second largest in terms of species number. Among families, Muscapidae (16 sp.) is the most diversified family, followed by Accipitridae (12 sp.), Cuculidae (9 sp.), Pylloscopida and Columbidae.

Table 1. Checklist of Dharan Birds (Baral et al., 2012; DNPWC & BCN, 2018)

S.N.	Order/Family/English name	Scientific name	Status
		GALLIFORMES	
Phasainaidae			
1	Red Junglefowl	*Gallus gallus*	
		COLUMBIFORMES	
Columbidae			
2	Eurasian Collard-dove	*Streptopelia decaota*	
3	Oriental Turtle-dove	*Streptopelia orientalis*	
4	Red Turtle-dove	*Streptopelia tranquebarica*	
5	Western Spotted Dove	*Spilopelia suratensis*	
6	Ashy-headed Green-pigeon	*Treron phayrei*	
7	Yellow-footed Green-pigeon	*Treron phoenicopterus*	
8	Wedge-tailed Green-pigeon	*Treron sphenurus*	
9	Emerald Dove	*Chalcophaps indica*	
10	Thick-billed Green-pigeon	*Treron curvirostra*	
		CAPRIMULGIFORMES	
Caprimulgidae			
11	Grey Nightjar	*Caprimulgus jotaka*	
Apodidae			
12	White-rumped Spinetail	*Zoonavena sylvatica*	
13	House Swift	*Apus nipalensis*	
		CUCULIFORMES	
Cuculidae			
14	Greater Coucal	*Centropus sinensis*	
15	Lesser Coucal	*Centropus bengalensis*	
16	Green-billed Malkoha	*Phaenicophaeus tristis*	
17	Jacobin Cuckoo	*Clamator jacobinus*	
18	Common Hawk-cuckoo	*Hierococcyx varius*	
19	Common Cuckoo\ Eurasian	*Cuculus canorus*	
20	Western Koel	*Eudynamys scolopaceus*	
21	Lesser Cuckoo	*Cuculus poliocephalus*	
22	Grey-bellied Cuckoo	*Cacomantis passerinus*	
		PELECANIFORMES	
Ardeidae			
23	Indian Pond-heron	*Ardeola grayii*	
24	Cattle Egret	*Bubulcus ibis*	
		SULIFORMES	
25	Little Cormorant	*Microcarbo niger*	
Charadriidae			
26	Red-wattled Lapwing	*Vanellus indicus*	
Scolopacidae			
27	Common Snipe	*Gallinago gallinago*	
		STRIGIFORMES	
Tytonidae			
28	Common Barn-owl	*Tyto alba*	II, VU
Strigidae			
29	Asian Barred Owlet	*Glaucidium cuculoides*	
30	Mountain Scops-owl	*Otus spilocephalus*	
31	Spotted Owlet	*Athena brama*	

S.N.	Order/Family/English name	Scientific name	Status
32	Little Owlet	*Athena noctua*	
33	Collard Owlet	*Glauicidium brodiei*	
	ACCIPITRIFORMES		
Accipitridae			
34	Black-winged Kite	*Elanus caeruleus*	II
35	Crested Serpent-eagle	*Spilornis cheela*	II
36	Black Baza	*Aviceda leuphotes*	II
37	Oriental Honey-buzzard	*Pernis ptilorhynchus*	II
38	White-rumped Vulture	*Gyps bengalensis*	CR,II,CR
39	Himalayan Griffon	*Gyps himalayensis*	II, VU
40	Changeable Hawk-eagle	*Nisaetus cirrhatus*	II
41	Hen Harrier	*Circus cyaneus*	II,VU
42	Crested Goshawk	*Accipter trivirgates*	II
43	Shikra	*Accipter badius*	II
44	Besra	*Accipter virgatus*	II
45	Mountain Hawk-eagle	*Nisaetus nipalensis*	II
	BUCEROTIFORMES		
Bucerotidae			
46	Indian Grey Hornbill	*Ocyceros birostris*	
47	Oriental Pied Hornbill	*Anthracoceros albirostris*	II
Upipidae			
48	Common Hoopoe	*Upupa epops*	
	CORACIIFORMES		
Meropidae			
49	Asian Green Bee-eater	*Merops orientalis*	
50	Chestnut-headed Bee-eater	*Merops leschenaultia*	
51	Blue-bearded Bee-eater	*Nyctyornis athertoni*	
52	Blue-tailed Bee-eater	*Merops philippinus*	
53	Oriental Dollarbird	*Eurystomus orientalis*	
54	Indochinese Roller	*Coracias affinis*	
55	Indian Roller	*Coracias benghalensis*	
Alcedinidae			
56	White-breasted Kingfisher	*Halcyon smyrnensis*	
57	Common Kingfisher	*Alcedo atthis*	
	PICIFORMES		
Megalaimidae			
58	Coppersmith Barbet	*Psilopogon haemacephalus*	
59	Great Barbet	*Psilopogon virens*	
60	Lineated Barbet	*Psilopogon lineatus*	
61	Blue-throated Barbet	*Psilopogon asiaticus*	
Picidae			
62	Lesser Yellownape	*Picus chlorolophus*	
63	Greater Yellownape	*Picus flavinucha*	
64	Grey-capped Pygmy Woodpecker	*Dendrocopos canicapillus*	
65	Himalayan Flameback	*Dinopium shorii*	
66	Greater Flameback	*Chrysocolaptes guttacristatus*	
	CARIAMIFORMES		
Falconidae			
67	Collared Falconet	*Microhierax caerulescens*	II

S.N.	Order/Family/English name	Scientific name	Status
68	Common Kestrel	*Falco tinnunculus*	II
	PSITTACIFORMES		
Psittacidae			
69	Plum-headed Parakeet	*Psittacula cyanocephala*	II
70	Alexandrine Parakeet	*Psittacula eupatria*	II
71	Rose-ringed Parakeet	*Psittacula krameri*	
	PASSERIFORMES		
Eurylaimidae			
72	Long-tailed Broadbill	*Psarisomus dalhousiae*	
Oriolidae			
73	Black-hooded Oriole	*Oriolus xanthornus*	
74	Indian Golden Oriole	*Oriolus kundoo*	
75	Slender-billed Oriole	*Oriolus tenuirostris*	
Campephagidae			
76	Long-tailed Minivet	*Pericrocotus ethologus*	
77	Scarlet Minivet	*Pericrocotus flammeus*	
78	Indian Cuckooshrike	*Coracina macei*	
79	Black-winged Cuckooshrike	*Lalage melaschistos*	
Vangidae			
80	Large Woodshrike	*Tephrodornis virgatus*	
81	Common Woodshrike	*Tephrodornis pondicerianus*	
Artamidae			
82	Ashy Woodswallow	*Artamus fuscus*	
Aegithinidae			
83	Common Iora	*Aegithina tiphia*	
Rhipiduridae			
84	White-throated Fantail	*Rhipidura albicollis*	
Dicruridae			
85	Black Drongo	*Dicrurus macrocercus*	
86	Crow-billed Drongo	*Dicrurus annectens*	
87	Ashy Drongo	*Dicrurus leucophaeus*	
88	Bronzed Drongo	*Dicrurus aeneus*	
89	Spangled\Hair-crested Drongo	*Dicrurus hottentottus*	
90	Lesser Racquet-tailed Drongo	*Dicrurus remifer*	
91	Greater Racquet-tailed Drongo	*Dicrurus paradiseus*	
Monarchidae			
92	Black-naped monarch	*Hypothymis azurea*	
93	Indian Paradise-Flycatcher	*Terpsiphone paradisi*	
Laniidae			
94	Brown Shrike	*Lanius cristatus*	
95	Isabelline Shrike	*Lanius isabellinus*	
96	Grey-backed Shrike	*Lanius tephronotus*	
97	Great Grey Shrike	*Lanius exactitor*	CR
Corvidae			
98	Grey Treepie	*Dendrocitta formosae*	
99	Rufous Treepie	*Dendrocitta vagabunda*	
100	Red-billed Blue Magpie	*Urocissa graculus*	
101	Common Green Magpie	*Cissa chinensis*	
102	House Crow	*Corvus splendens*	

S.N.	Order/Family/English name	Scientific name	Status
103	Large-billed Crow	*Corvus macrorhynchous*	
Steenostridae			
104	Grey-headed Canary Flycatcher	*Culicica paceylonensis*	
Paridae			
105	Sultan Tit	*Melanochlora sultanea*	EN
106	Green-backed Tit	*Parus monticolus*	
107	Great Tit	*Parus major*	
108	Black-lored Tit	*Machlolophus xanthogenys*	
Cisticolidae			
109	Zitting Cisticola	*Cisticola juncidis*	
110	Plain Prinia	*Prinia inornata*	
111	Grey-breasted Prinia	*Prinia hodgsonii*	
112	Striated Prinia	*Prinai crinigera*	
Hirundinidae			
113	Asian Plain Martin	*Riparia riparia*	
114	Red-rumped Swallow	*Ceropis daurica*	
115	Asian House Martin	*Delichon dasypus*	
116	Nepal House Martin	*Delichon nipalense*	
Pycnonotidae			
117	Red-whiskered Bulbul	*Pycnonotus jocosus*	
118	Himalayan Bulbul	*Pycnonotus leucogenys*	
119	Red-vented Bulbul	*Pycnonotus cafer*	
120	Black-Crested Bulbul	*Pycnonotus flaviventris*	
121	Black Bulbul	*Hypsipetes leucocephalus*	
122	Ashy Bulbul	*Hemixos flavala*	
Phylloscopidae			
123	Hume's Leaf-warbler	*Phylloscopus humei*	
124	Lemon-rumped Leaf-warbler	*Phylloscopus jocosus*	
125	Buff-barred Warbler	*Phylloscopus pulcher*	
126	Dusky Warbler	*Phyllosscopus fuscatus*	
127	Large-billed Leaf-warbler	*Phylloscopus mangnirostris*	
128	Blyth's Leaf-warbler	*Phylloscopus reguloides*	
129	Greenish Warbler	*Phylloscopus trochiiloides*	
130	Grey-bellied Tesia	*Tesia cyaniventer*	
Sylviidae			
131	Lesser Whitethroat	*Sylvia curruca*	
Zosteropidae			
132	Oriental White-eye	*Zosterops palpebrosus*	
Timaliidae			
133	Slender-billed Scimitar-babbler	*Pomatorhincus superciliaris*	VU
Pelorneidae			
134	Puff-throated Babbler	*Pellorneum ruficeps*	
Leiotrichidae			
135	Common Babbler	*Argya caudata*	VU
136	Jungle Babbler	*Turdoides striata*	
137	White-crested Laughingthrush	*Garrulax leucolophus*	
138	Rufous Sibia	*Heterophasia capistrata*	
Strurnidae			
139	Chestnut-tailed Starling	*Sturnia malabarica*	

S.N.	Order/Family/English name	Scientific name	Status
140	Brahminey Starling	*Sturnia pagodarum*	
141	Common Myna	*Acridotheres tristis*	
142	Common Hill Myna	*Gracula religiosa*	II
143	Jungle Myna	*Acridotheres grandis*	
Sittidae			
144	Chestnut-bellied Nuthatch	*Sitta cinnamoventris*	
144	Velvet-fronted Nuthatch	*Sitta frontalis*	
145	Wallcreeper	*Tichodroma muraria*	
Turdidae			
146	Orange-headed Thrush	*Geokichla citrina*	
147	Scaly Thrush	*Zoothera dauma*	
148	Grey-winged Blackbird	*Turdoides boulboul*	
Muscicapidae			
149	Oriental Magpie-robin	*Copsychus saularis*	
150	Indian Robin	*Saxicoloides fulicatus*	
151	White-rumped Shama	*Kittacincla malabarica*	
152	Verditer Flycatcher	*Eumyias thalassinus*	
153	Pale-chinned Flycatcher	*Cyornis poliogenys*	
154	Siberian Rubythroat	*Calliope calliope*	
155	Indian Blue Robin	*Larvivora brunnea*	
156	Black-backed Forktail	*Enicurus immaculatus*	
157	Blue Whistling-thrush	*Myophonus caeruleus*	
158	Ultramarine Flycatcher	*Ficedula superciliaris*	
159	Blue-fronted Redstart	*Phoenicurus frontalis*	
160	White-capped Water-redstart	*Phoenicurus leucocephalus*	
161	Plumbeous Water-redstart	*Phoenicurus fuliginosus*	
162	White-throated Bushchat	*Saxicola insignis*	VU, EN
163	Pied Bushchat	*Saxicola caprata*	
164	Common Stonechat	*Saxicola torquatus*	
Regulidae			
165	Goldcrest	*Regulus regulus*	
Chlorospeidae			
166	Golden-fronted Leafbird	*Chloropsis aurifrons*	
167	Orange-bellied Leafbird	*Chloropsis hardwickii*	
Nectariniidae			
168	Purple Sunbird	*Cinnyris asiaticus*	
169	Crimson Sunbird	*Aethopyga siparaja*	
170	Fire-tailed Sunbird	*Aethopyga ignicauda*	
Estrildidae			
171	White-rumped Munia	*Lonchura striata*	
172	Scaly-breasted Munia	*Lonchura punctulata*	
Passeridae			
173	House Sparrow	*Passer domesticus*	
174	Russet Sparrow	*Passer cinnamomeus*	
175	Eurasian Tree Sparrow	*Passer montanus*	
Motacillidae			
176	Tree Pipit	*Anthus trivialis*	
177	Olive-backed Pipit	*Anthus cervinus*	
178	Paddy Field Pipit	*Anthus rufulus*	

S.N.	Order/Family/English name	Scientific name	Status
179	Grey Wagtail	*Motacilla cinerea*	
180	Citrine Wagtail	*Motacilla citreola*	
181	White-browed Wagtail	*Motacilla maderaspatensis*	

The dataset includes 17 families that have only one genus and species (Figs. 2, 3).

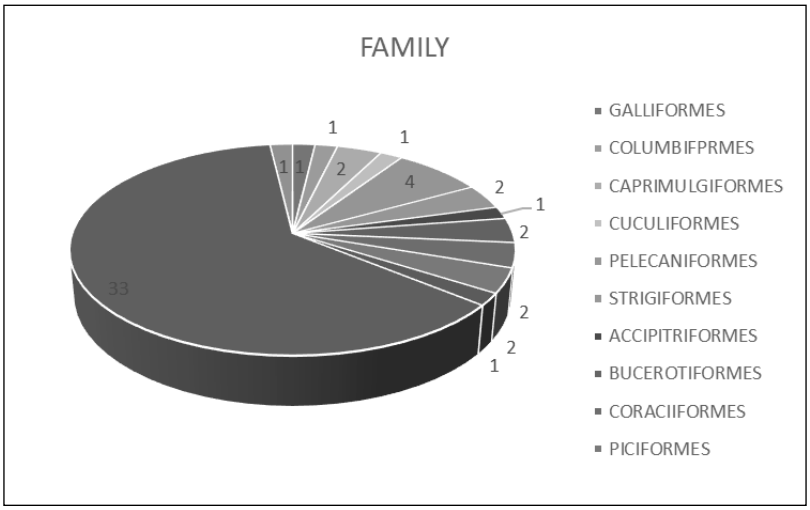

Figure 2. Taxonomic representation by order

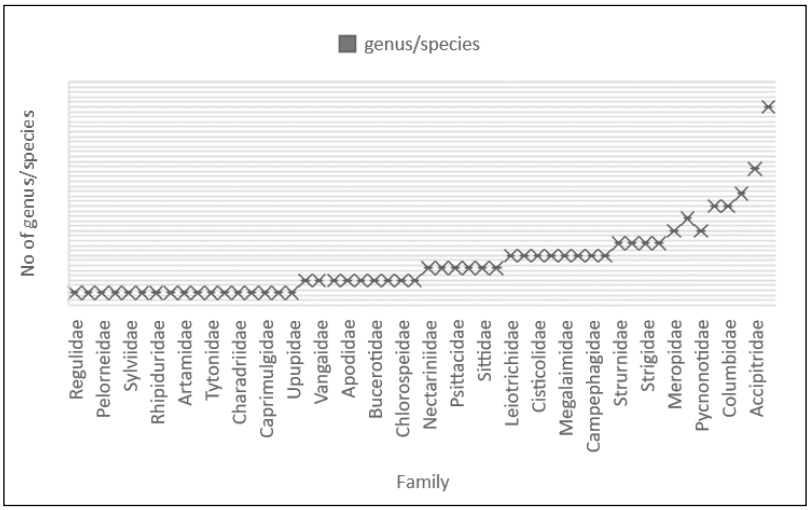

Figure 3. Taxonomic representation by family

Conservation Status

Out of 181 species, two species (1.1%), viz., Great Grey Shrike and White-rumped Vulture are critically endangered as per the IUCN Red Data book. Dharan Sub- metropolitan city harbors 11 species (6%) that are globally and nationally threatened. Similarly, 19 species

are listed in Appendix II of CITES. Two species are listed endangered and have nationally threatened status: Sultan Tit (*Melanochlora sultanea*) and White-throated Bushchat (*Saxicola insignis*). Similarly, 6 species (3.3%) are categorized as "vulnerable" whereas remaining (94.42%) species falls under least concern conservation status (Table 2).

Table 2. Conservation status of recorded birds

Status	Number	Percentage (%)
Critically Endangered	2	1.1
Endangered	2	1.1
Vulnerable	6	3.3
Least Concern	171	94.42

Note: There are 19 species of Birds which are listed in CITES (Appendix II).

Distribution Pattern and Threats

Distribution along season

Of all the birds documented in the study area, 72.3% were residential and 23% (42 sp.) were winter migratory (Fig. 4). Besides, 3.3% and 1.1% were summers migratory and passage migrants. Most of the summer migratory birds are from Cuculidae family. Every year, particularly during the winter season, critically endangered *Gyps bengalensis* and vulnerable *Gyps himalayensis* are recorded in Dharan.

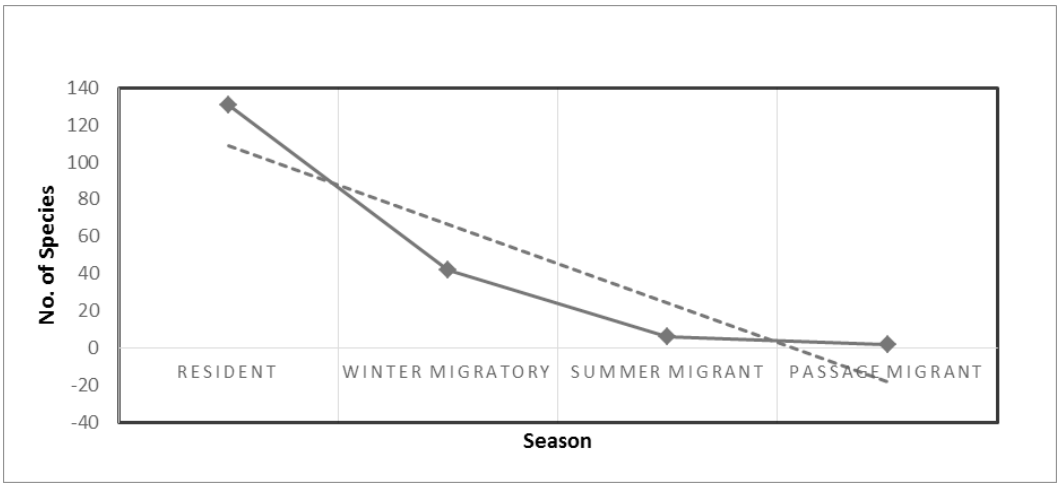

Figure 4. Distribution along the season

Distribution along elevation

Most of the bird species (84%) were documented below 1000 m (Fig. 5). This record shows higher abundance of birds mainly in the tropical region. The most preferable sites included Dharan IBAs, Pindeswori areas, Panchakanya forest, Bishnu Paduka areas, Patnali forest, and riverine areas of Seuti and Sardu river. The elevation range between 1200-1500 m also show greater species richness, i.e., 98 species.

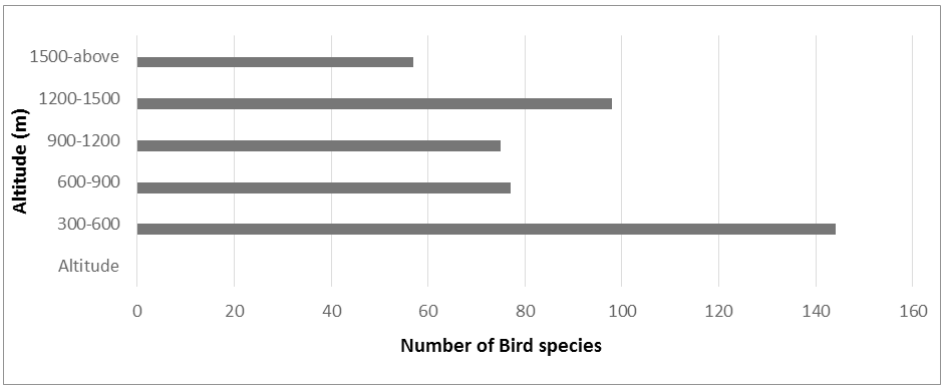

Figure 5. Distribution of birds along elevation

Distribution along with habitat types

In total 70 recorded birds (38%) favors Dharan's forest area while 17.67% of the total bird species were documented from agricultural land. Approximately 16.1% and 15.46% of the total records were from riverine and scrubland, respectively (Fig. 6). Peak cliff also harbors critically endangered *Gyps bengalensis* and vulnerable *Gyps himalayensis*. Common Barn-owl (*Tyto alba*) showed greater resilience in human settlement areas.

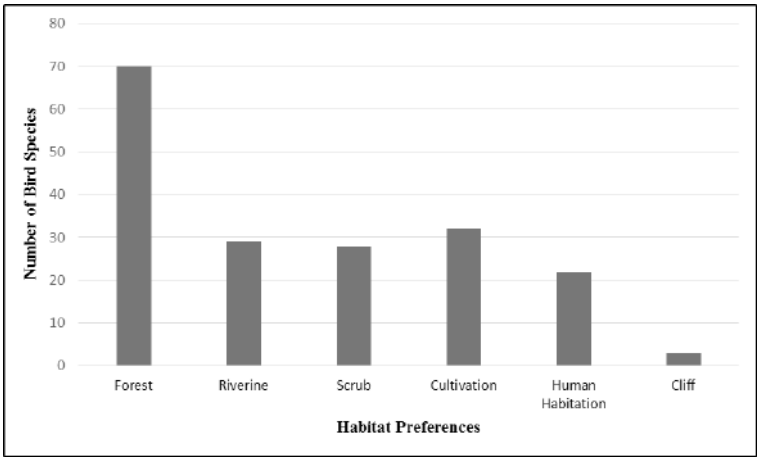

Figure 6. Distribution along with habitat types

Discussion

This study is a comprehensive documentation with regards to avian taxonomy, conservation status, habitat preferences, threats and seasonal and elevational distribution. We were able to record a total of 181 species belonging to 13 orders and 53 families in 12 different surveyed areas. Samples collected were based on the habitat suitability, vegetation, and elevation as well as prevailing anthropogenic impacts. In accordance with the official checklist of birds of Nepal by Baral et al. (2012) and DNPWC and BCN (2018), about 57% (13 order) and 65% (33 families) were found to be inhabitants of Dharan Sub-metropolitan city: this covers 20.40%

of the total recorded birds in the study. Ornithological survey of Dharan IBAs in the year 2007-2008 documented a totalof 197 bird species of which 143 species were residential, 45 species were winter migratory and 9 species were summer migratory (Basnet & Sapkota, 2008). Though our study comprised only the southern portion of Dharan IBAs, we recorded 144 similar species even in such a confined area. Forest areas below 1000 m elevation have greater species abundance; less species diversity as we move higher (Kattan & Franco, 2004; Acharya et al., 2011; Kandel et al., 2018). As most of our selected study sites fall in the tropical region, we could record a higher number of birds' species in such alimited portion of Dharan IBAs. Apart from this, an elevation range of 1200-1500 m also demonstrated larger species abundance (Fig. 5): this might be attributable to the junction point between tropical and higher altitude birds. The southern faced jungle harbors species such as Red jungle fowl (*Gallus gallus*), Thick-billed Green-Pigeon (*Treron curvirostra*), Great Barbet (*Psilopogon virens*), Changeable Hawk-eagle (*Nisaetus cirrhatus*), Black Bulbul (*Hypsipetes leucocephalus*), Ashy Bulbul (*Hemixos flavala*), White-crested Laughing thrush (*Garrulax leucolophus*), Slender-billed Schmitar-babbler (*Pomatorhincus superciliaris*) and Rufous Sibia (*Heterophasia capistrata*). Dharan's forest areas hold greater species abundance in comparison with other habitats. Food availability and prey might have influenced their presence (Holmes & Schultz, 1988) or they might have received greater infer constrainton forest movements (Bélisle et al., 2001). Human dominated areas also showed the occurrence of some vulnerable species such as Common Barn-Owl (*Tyto alba*).Nepal's geography also supports summer migratory birds (Acharya et al., 2011; Paudel et al., 2012), and most of the summer migratory are from Cuculidae family.

A total of 11 species were identified as nationally and globally threatened according to the IUCN Red Data book. A group of five critically endangered *Gyps bengalensis* was recorded during the winter season along with 25 individuals of vulnerable *Gyps himalayensis* in the Bishnu Paduka area and southern side of Vedetar area. More importantly, the result shows that Dharan is an important area for bird diversity. Despite its inherent rarity and representative qualities, the avian diversity is under immense threat from anthropogenic factors like deforestation, fragmentation, and unplanned urbanization. Over two years, large portions of scrub and agricultural landof Pindeswori, Bijayapur, and Bishnu Paduka area were converted into plotting areas.These developmental projects have impacted the endangered and critically endangered species like White-throated Bushchat (*Saxicola insignis*), and Great Grey Shrike (*Lanius exactitor*) and distinctively to overall biological diversity. Gidde Vir, a possible breeding site of vulnerable *Gyps himalayensis* was damaged by forest fire. During our survey, we noticed both active and passive fires in the study region.

Conclusion

Based on our findings, we can say that Dharan is rich in avian diversity simultaneously threatened by pervasive and increasing human activities. It is also a major migratory junction for both winter and summer birds, however, supporting research index is limited. Locals are ignorant regarding the distribution status, population trend, conservation importance and threatsto birds and their habitats. The increasing anthropogenic factors might be the reason for the extinction of threatened species from our study area. For the sustainable biodiversity

management and conservation, Environmental Impact Assessment (EIA) should be conducted before running any developmental work. Specifically, conservation awareness programs should be organized for local people and concerned authorities in order to draw their attention towards the detrimental impacts of habitat fragmentation/loss on avian population and overall biological diversity. Moreover, local people, youths, and college students should be sensitized and mobilized to restore the habitat for these threatened species. This study provides further avenues to conduct in-depth research hereafter as well as urges to formulate conservation action plans in the long run.

Acknowledgments

We would like to acknowledge our sincere thanks to Department of Zoology, Central Campus of Technology, Dharan for providing the necessary field gears. We are grateful to our team members Mr. Nabin Basnet, Mr. Narayan Rai, Ms. Pranita Rai, and Ms. Sumnima Tumba who supported us during the field study.

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Faunal Diversity of Tapli Rural Municipality, Udayapur, Province 1, Nepal

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Abstract

The study of faunal diversity is an important attraction for biologists and conservationists around the globe. As habitat change issues caused by anthropogenic and climate change are active in imposing threats on fauna and flora, many species of animals have already been either uncommon or endangered. By the time rest are on the same race. Considering the study of faunal diversity as a vague topic, because of its coverage, for the present study only selected animals, viz., mammals, birds, reptiles, amphibians, fish and butterflies were chosen. The Tapli Rural Municipality lies in the rain shadow area of Udayapur District, within the lowest and highest elevations 307 msl and 2000 msl, respectively. The total area covered is 119.11 km^2, in which five wards of the Municipality lie. On the basis of altitudinal gradient, transect lines were drawn and points were fixed at each ward to be visited on foot. Altogether 17 species mammal belonging to 13 families, 74 bird species of 39 families, 10 species of reptiles under 6 families, 3 amphibian species pertaining to 3 families, 22 fish species spread over 8 families and 34 species of butterfly under 8 families were recorded. Because of physiographical condition of the place and climate change, only one species of slug representing phylum Mollusca was recorded.

Key words: Altitudinal gradient, Anthropogenic impacts, Climate change, Rain shadow area

Introduction

More slogans and more researches on biodiversity are emerging globally; it might be due to the remarkable impacts on living organisms throughout the world. Contribution to Nepal's faunal diversity has been made covering across the country; however, many more uncovered places are there in need of scientists' vision mainly for the protection of the biodiversity. A check list of herpetofauna of Nepal (Leviton, 1962) appeared in California's proceedings. Academy of Science, Sanfrancisco, USA. Red Data Book of Fauna of Nepal (BPP, 1995) has shared information about faunal diversity of Nepal. Regarding insect, contribution to Nepal's butterflies (Smith, 1977) and marvelous moths of Nepal (Allen, 1993) deserve special mention about butterflies and moths of Nepal. About the fish diversity of Nepal, contributions (Shrestha, 2008; Shrestha et al., 2009) are remarkable in the subject concerned. Similar works on birds, molluscs and butterflies have been carried out by Subba (1984, 1995 a b c, 1996, 1997, 2001, 2002a, 2002b, 2003, 2005), Subba and Pandey (2005), Surana et al. (2007), Subba and Ghosh (2008), Subba et al. (2017), have been informative fragmentary reports for faunal diversity of different places of Nepal. Likewise, works on herpetofauna of Nepal (Swan & Leviton, 1962; Schleich & Kastle, 2002; Shah & Tiwari, 2004) have been

remarkable contribution to amphibians and snakes from all ecological reasons of Nepal. Avifaunal diversity of Nepal has been worked out (Inskipp & Inskipp, 1991; Grimmett et al., 2000) thoroughly. Survey of mammals in Nepal has been carried out since long back and several reports have been published. However, a compilation of fragmentary reports on mammals (Jnawali et al., 2011) has shared the information in details about Nepal's diversity of mammals. A check list of mammals of Nepal prepared by Thapa (2014) emerged out with current data of mammals. Fish fauna of Deumai and Ratuwa rivers (Limbu et al., 2016; Limbu et al., 2019 a b) and a review of eastern Nepal's fish diversity (Limbu, 2019b) are important contributions. As this was the first study done on faunal diversity assessment of the study area, literature pertaining to adjoining areas were lacking. The physiography and climatic condition of Tapli Rural Municilpality differ from other adjoining areas it being a rain shadow area. The study of faunal diversity of this area was more interesting and important, as most animal species and their population were fewer because of insufficient multiple food resources scarcity except few.

Climate

Generally, the climate of the Rural municipality is the sub-tropical type but it ranges to warm temperate in the higher elevation. There is diversity in precipitation within the rural municipality. The north-facing slope of the Mahabharat range of the TRM is characterized by the rain shadow features, which receive less amount of rainfall. These rain shadow areas in TRM include the most parts of ward 4 (Thanagaun), ward 5 (Rupatar) and many parts of ward 1 (Okhle); whereas ward 2 (Lekhgaun) and ward 3 (Iname) recieves comparatively higher rainfall. There is no weather station in TRM, so weather pattern analysis has been done based on the data from the nearby stations in Udaypurgadhi and Kuruleghat by the Department of Hydrology and Meteorology (DHM). The data from Udaypurgadhi has information on both the temperature and rainfall; whereas Kuruleghat records only the precipitation.

Temperture

In general, January is the cold month with minimum temperature of 10.3ºC whereas May remains the hottest month with maximum temperature of more than 33ºC. From December to February it is the cold period and April to September the warm period exceeding the 30ºC. The average temperature ranges from 15.9ºC in January to 28.5ºC in June. The maximum and minimum annual average temperature is 28.6ºC and 19.1ºC, respectively. Average annual temperature is almost warm in Tapli RM, i.e., 23.9ºC, and a little lower than physiological temperature.

Precepitation

The analysis of precipitation data obtained from nearby weather stations Kuruleghat, Khotang and Udayapurgadhi indicates that Tapli has an average anual rainfall of 2090.60 mm which gives an impression of Tapli RM, the dry area with an average monthly rainfall of 174.22 mm. Further, there is a large variation in terms of precipitation within the rural municipality. For example, northward flow of rain bearing clouds in the parts of Thanagaun (ward 4) and Rupatar (ward 5) is obstructed by the Mahabharat range, which sheds rain to these areas. In

contrast, Iname and Lekhgaun receive higher rainfall, so are comparatively better for rain fed upland agriculture

Materials and Methods

Study area was Tapli Rual Municipality (TRM) of Province 1 (Fig. 1) which comprises five wards namely Lekhgaun (N27°02'41.7", E086°30'09.4"), Okhale (N27°01'47.4", E086°33'22.3"), Iname (N27°02'37.3", E086°33'52.8"), Thanagaun (N27°05'24.7", E86°34'37.1"), and Rupatar (N27°05'54.7", E086°32'22.0"). It is a north facing vertical rugged and rocky landscape, sparsely covered with thorny vegetation reflecting nature of rain shadow area, covers 119.11 km^2. It looks like a small valley from its outlook due to varied ecological gradients. The altitude of TRM ranged from 307 msl (Sunkoshi river bank) to 2000m of Tapli hillock. Geographical positions of each spot of study area were recorded with help of GPS.

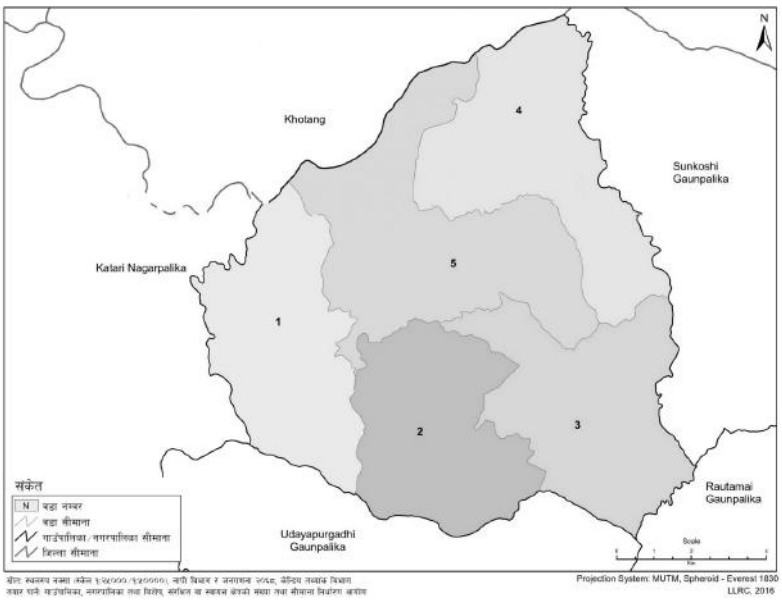

Figure 1. Map of the Tapli Rural Muncipality Nepal

A rapid assessment of faunal diversity was carried out in all five wards in September, 2018. Following scientific standard methods, the research was accomplished. Mostly point count method was followed because of nature of landscape and limitation of time, other methods were not used. However, maximum coverage of diversity rich areas in each ward was made and data obtained from both direct observations and contacts made with local people. Photography and collection of available specimens such as that of butterfly, other arthropods, slug, birds, reptiles, and amphibians were done.

Observed wild animals such as mammals, birds, herpetofauna, butterflies, were identified with the help of books: Wild mammals of Nepal (Baral & Shah, 2008), Birds of Nepal (Grimmett et al., 2016), Field guide to amphibians and reptiles of Nepal (Kastle et al., 2013), Illustrated checklist of Nepalese Butterflies (Smith, 1993), respectively. Unseen animals but their presence ascertained by local people's observations were verified by interviewing local people of each ward to reach the conclusion (Tables 1-5). Recording sounds of some birds that could not be observed but their sound could be heard was done and identified later on. The presence of some animals was confirmed with the help of some of the marks left by them and their observed behaviours, which indicated pangolin in TRM area. Similarly,

spines of porcupines at several places and availability of porcupine could be ascertained, but species were also confirmed.

Results

Assessment of Wildlife was conducted during the preparation of the profile report of Tapli Rural Municipality. A record of 160 species of wildlife which comprised 34 butterfly species, avian diversity was the highest number of species securing 76 species, (> 47% of total wildlife diversity) followed by Pisces (nearly 17%), butterfly 21.5%, Mammal (>11%), Reptile (6.9%), and Amphibian (2.5%) (Fig. 2). Two species of fish have been designated as vulnerable and endangered, and one spcecies each from reptiles and mammals were reported endangered. Further, the wildlife diversity at different levels is in (Fig. 3).

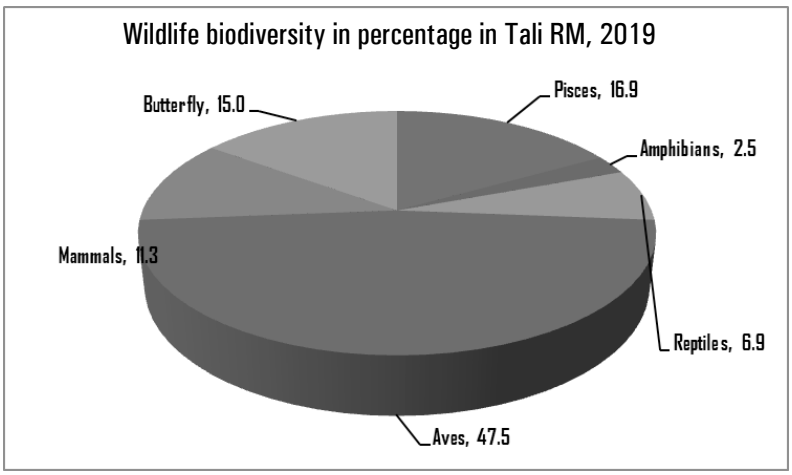

Figure 2. Wildlife biodiversity in percentage in Tapli RM, 2018.

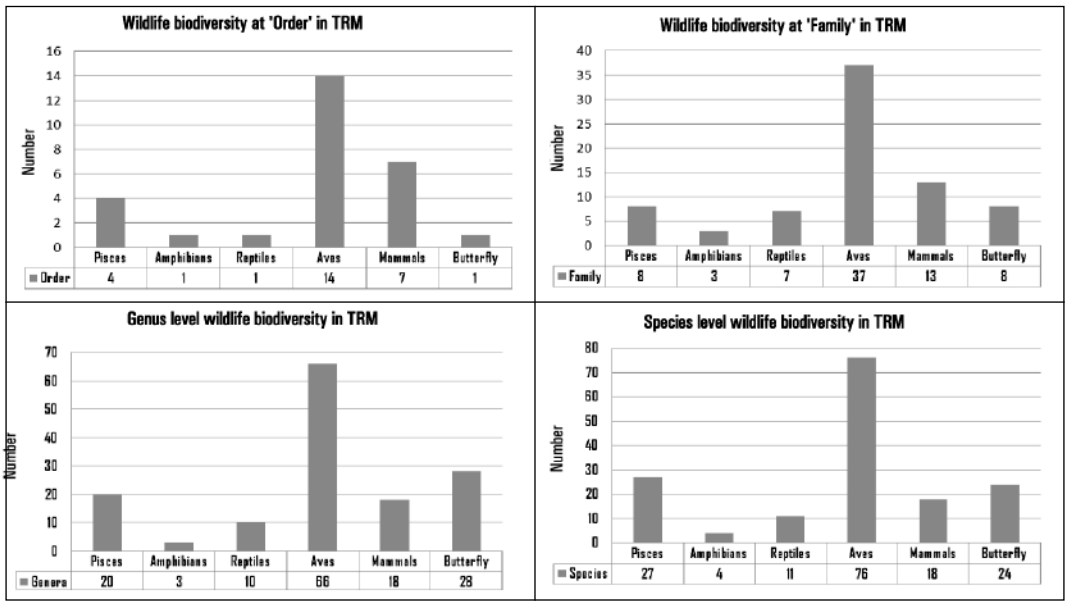

Figure 3. Wildlife biodiversity at different levels including genera and species levels in Tapli RM, 2018.

Fish

A total of 27 species of fish categorized under 4 orders, 8 families, and 19 genera were known to occur in the Sunkoshi River. Among orders Cypriniformes comprises four families, and families Cyprinidae and Sisoridae dominate by 56% and 19%, respectively. Among the total fish diversity, *Anguilla bengalensis bengalensis* (VU) and *Tor tor* (EN), two fish species belong to vulnerable and endangered groups (Table 1).

Table 1. Diversities of fish in the Tapli RM

Order	Family	Genus	Species	Percent
Anguilliformes	Anguillidae	1	1	3.7
Cypriniformes	Balitoridae	1	1	3.7
	Cabitidae	1	2	7.4
	Cyprinidae	9	15	55.6
	Psilorhynchidae	1	1	3.7
Perciformes	Channidae	1	1	3.7
Siluriformes	Schilbeidae	1	1	3.7
	Sisoridae	4	5	18.5
Total: 4	8	19	27	100

Amphibian

Four species of amphian were found in the study area of TRM which comperises three genera and two families such as Bufonidae and Ranidae.

Reptile

Tapli RM houses 11 species of reptile comprised under 10 genera and 7 families (Table 2). The dominating families were Colubridae and Varanidae. Many species were common but *Varanus flavescens* (Sungohoro) was endangered species reported from ward 1 (Okhale).

Table 2. Diversities of Reptiles in the Tapli RM.

Order	Family	Genus	Species	Percentage
Sauria	Viperidae	3	1	9.1
	Elapidae	1	1	9.1
	Colubridae	1	4	36.4
	Angamidae	1	1	9.1
	Scincidae	1	1	9.1
	Varanidae	1	2	18.2
	Gekkonidae	1	1	9.1
Total: 1	7	10	11	100

Avi fauna

A total of 76 species of birds under 14, 37 families and 66 genera have been reported from the municipality (Table 3). Among birds, family Muscicapidae was more diverse by nearly 8 % of total bird species followed by two families, Phasianidae and Cuculidae. Most of the species were common, and some occasional visitors.

Table 3. Diversities of Bird species in the Tapli RM.

Order	Family	Genus	Species	%	Order	Family	Genus	Species	%
Accipitriformes	Accipitridae	3	4	5.3	Galiformes	Hirundinidae	2	2	2.6
Apodiformes	Apodidae	1	1	1.3		Laniidae	1	1	1.3
Bucerotiformes	Upupidae	1	1	1.3		Leiothrichidae	1	1	1.3
Charadriformes	Charadridae	1	1	1.3		Motacillidae	2	2	2.6
Ciconiformes	Ciconiidae	1	1	1.3		Muscicapidae	6	6	7.9
Columbiformes	Columbidae	3	3	3.9		Nectariniidae	1	1	1.3
Coraciformes	Alcelinidae	1	1	1.3		Paridae	1	1	1.3
	Meropidae	1	1	1.3		Passeridae	3	3	3.9
Cuculiformes	Cuculidae	4	5	6.6		Phylloscopidae	1	3	3.9
Galiformes	Phasianidae	5	5	6.6		Pycnonotidae	3	3	3.9
	Aegithinidae	1	1	1.3		Rhipiduridae	1	1	1.3
	Alaudidae	1	1	1.3		Strunidae	2	2	2.6
	Campephagidae	2	2	2.6		Timaliidae	2	2	2.6
	Cettidae	1	1	1.3	Pelecaniformes	Ardeidae	1	1	1.3
	Cisticolidae	1	1	1.3	Piciformes	Picidae	2	2	2.6
	Corvidae	3	6	7.9		Ramphastidae	1	2	2.6
	Dicruridae	1	2	2.6	Psittaciformes	Psittacidae	1	1	1.3
	Estrildidae	1	1	1.3	Strigiformes	Strigidae	2	3	3.9
	Fringillidae	1	1	3.9					
Total		33	39	51.3			33	37	48.7

Mammal

Tapli RM harbors 18 species of mammals comprising 18 genera and 13 families. Family Muridae of the oder RodentiaOrder Rodentia of the Muridae family was found to have occupied higher diversity percentage, i.e., >22% followed by Family Mustelidae of the oder Carnivora Order Carnivora of the family Mustelidae (>11%) (Table 4). Some remarkable mammals include Nirbiralo (*Viverricula indica),* Ban Biralo (*Felis chaus*), Banel (*Sus scrofa*), Ratte Mirgha (*Muntiacus muntjak*), Salak (*Manis pentadactyla*), and Ban Musa (*Nesokia indica*). The present finding claims to have had biodiversity significant species

Table 4. Diversities of Mammals in the Tapli RM.

Order	Family	Genus	Species	Percentage
Artiodactyla	Bovidae	1	1	5.6
	Carividae	1	1	5.6
	Suidae	1	1	5.6
Carnivora	Canidae	1	1	5.6
	Felidae	1	1	5.6
	Herpestidae	1	1	5.6
	Mustelidae	2	2	11.1
	Viverridae	1	1	5.6
Insectivora	Sorcidae	1	1	5.6
Lagomorpha	Leporidae	1	1	5.6
Pholidata	Manidae	1	1	5.6
Primates	Carcopithecidae	2	2	11.1
Rodentia	Muridae	4	4	22.2
Total: 7	13	18	18	100

Municipality reports to hold biodiversity significant species such as Common Otter (*Lutra lutra*) in ward 5 (Rupatar).

Butterfly

A total of 34 species of butterfly belonging to 1 order Lepidoptera 8 families and 28 genera. Of this diversity, family Nymphalidae holds over 35% of species diversity of butterfly followed by Danaidae and Lycaenidae (Table 5, Fig. 3).

Table 5. Diversities of Butterfly in the Tapli RM

Order	Family	Genus	Species	%
Lepidoptera	Amathusiidae	3	3	8.8
	Danaidae	4	6	17.6
	Lycaenidae	6	6	17.6
	Nemeobiidae	1	1	2.9
	Nymphalidae	10	12	35.3
	Papilionidae	1	1	2.9
	Peiridae	2	4	11.8
	Satyridae	1	1	2.9
Total: 1	8	28	34	100

Discussion

As people didn't have permission to keep the guns for their personal safety, hunting was almost nil, so population of mammalian species such as barking deer, porcupine, wild boar, monkey, squirrels, wild cat, mongoose was noted. However, trappings cases of wild mammals and birds were heard to have been common in some areas. By nature, some of the species which create unwanted activities in the farm lands; they had been taken as unwanted guests by farmers. Severe poaching of Pangolin (*Manis pantadactyla*), this pholidot was rare there. Monkey's population was found in the top list and has been most troublesome to farmers. The case was similar throughout TRM. Population of *Arborophil rufogualis* was found to have increased fewer. Because of climate change, streams were found having less volume of water which has direct relation with aquatic as well as terrestrial faunal diversity that was also observed there. The climate was found not suitable for several plant species so did the faunal species. Except one species of slug, rest mollusca representatives were lacking there due to insufficient moisture that mollusks need. Towards higher altitudes beautiful moths and butterflies were observed but whatever numbers of species were sighted could not be collected because of range of their staying position. To get an opportunity to watch and record only 160 species of wildlife while covering 119.11 km^2 of different altitudinal gradients clearly indicated that in rain shadow areas wildlife species are fewer.

Protection and conservation of biodiversity of any place is paramount important in order to keep ecosystem in balanced state, which, in turn, will pay back humans heavily. Public awareness is the only effective measure for making stakeholders conscious about wildlife so it is recommended.

Conclusion

A rapid assessment of faunal diversity of Tapli Rural Municipality belonging to Province1 was carried out in all wards for one month in 2018 to enumerate fauna. The study recorded a total of 160 species of wildlife, they were mammal (11.30), bird (47.5), reptile (6.9), fish 16.9 amphibian (2.5), butterfly (21.25) in percentage of the total.

Acknowledgements

Tapli Rural Municipality is thankfully acknowledged for the financial support. A long list of names of persons who could be of immense help differently during the survey was impossible here to include, so we would like pay our sincere gratitude to them all in common. Last not the least, we would like to cordially acknowledge Mr. Ganga Shrestha for his sincere and most energetic performance during the study period.

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Bacteria Isolated from the Fishes Naturally Infected with Epizootic Ulcerative Syndrome (EUS) in Eastern Nepal

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Abstract

The study deals with isolation, characterization and identification of bacteria from EUS infected fishes and pathogenicity of isolated bacteria tested in *Heteropneustes fossilis*. EUS outbreaks were recorded in three fish farms from 2009-2015 in the months of December to April. Altogether 445 naturally EUS affected fishes were collected and examined. The order of susceptibility was about 60% in *Cirrhinus mrigala*, 30% in *Labeo bata / L. rohita* and 10% in *Catla catla*, *Channa striatus,* and *Puntius* sp. among affected fishes. Bacteria were isolated from the ulcers of naturally infected *Cirrhinus mrigala*, *Catla catla, Channa striatus, Puntius* sp.*, Mystus tengara* and *Labeo bata.* Among 23 isolates, two belong to *Pseudomonas* sp. from Lb_1 and Cc_4, two belong to *Micrococcus* sp. from Cm_2 and P_4, 14 bacteria were of *Aeromonas hydrophila* isolated from Cm_1, Cm_3, Cs_1, P_1, P_3, Lb_2, Lb_3, Cc_1, Cc_2, Cc_3, Mt_1, Mt_2, Mt_3 and Mt_4 one belongs to *Moraxella* sp. from P_2, one belongs to *Aeromonas veroni biovar sobria* from Lb_4 and three belong to *Aeromonas caviae* from Cm_4, Cs_2 and Cs_3. Altogether 20 bacterial isolates were pathogenic and three were non- pathogenic. EUS is the major microbial fish disease in eastern Nepal.

Key words: Babiya Birta, Fish disease, Fish farm, Pathogenicity, Tarahara

Introduction

EUS is a disease affecting wild and farmed fish first appeared in farmed Ayu (*Plecoglossus altivelis*) in Japan in 1971 (Egusa & Masuda, 1971) and named as mycotic granulomatosis (Miyazaki & Egusa, 1972). EUS is basically a disease of complex nature involving certain fungal and bacterial elements in its later stages, and probably one or more viruses (Chinabut, 1995).

Bacterial diseases such as streptococciosis, bacterial gill disease (Lumsden et al., 1994) have stunned both wild and cultured waters throughout the world. Some fish pathogenic bacteria like *Streptococcus fecalis, Micrococcus* sp., *Pseudomonas* sp., *Aerococcus* sp., and *Flavibacterium* sp. were reported from Nepal (Rayamajhi & Bajracharya, 2005). Among fish diseases, epizootic ulcerative syndrome (EUS) caused a loss of about 15-20% of total fish production during its initial outbreak in the Eastern Terai of Nepal (Phillips, 1989). Lilley et al. (2002) mentioned that among 37% of fish farms, 95% cases were of EUS affected and it was considered as the most destructive fish disease in Nepal (Phillips, 1989; ADB/ NACA 1995). People are unwilling to start aquaculture activities due to the perceived high risk of diseases mainly EUS outbreaks and lack of knowledge of how to deal with the fish disease (Callinan et al., 1999).

Although Mohan et al. (1999) suggested that an invasive fungus *A. invadans* was the primary pathogen of EUS, several other bacterial pathogens were also involved in EUS of fish. Ulcer disease due to *Aeromonas hydrophila* has been reported in *Catla catla* (Karunasagar et al., 1986).

Llobrera and Gacutan (1987) reported the association of *Aeromonas hydrophila* with necrotic ulcers and lesions in snakehead (*Ophiocephalus striatus*), Thai catfish (*Clarias batrachus*), crucian carp (*Carassius carassius*) and goby (*Glossogobius giuris*) in Laguna de Bay, Philippines from December, 1985 to February 1986. Boonyaratpalin (1989) reported that the EUS involving both wild and cultured fish in Burma, Indonesia, Lao PDR, Malaysia, Singapore and Thailand was associated with bacterial pathogens. *A. hydrophila* was also reported to be associated with EUS affected fishes in Sri Lanka (Subasinghe et al., 1990). Jhingran and Das (1990) induced the haemorrhagic ulcers inoculating pure bacteria isolated in healthy murrels.

Two fluorescent Pseudomonads, one Aeromonad and one *Micrococcus* sp. were isolated from skin lesions of air breathing fishes by Pal and Pradhan (1990). Several researchers in India and abroad reported associations of bacterial pathogens with EUS (Mukherjee et al., 1991; Torres et al., 1993; Qureshi et al., 1995; Lio-Po et al., 1998; Kar, 2000; Saha & Pal, 2000, 2002; Das et al., 2007, 2009). More epidemiological studies are required to get an insight into the role of various environmental risk factors responsible for EUS (Pradhan et al., 2014).Only sparse works on the fish diseases have been carried out in Eastern Nepal. So it is considered worthwhile to study the fish diseases prevalent and to identify the bacterial fish diseases especially associated with EUS in the study areas.

Materials and Methods

Study Sites

Baidya Fish Farm, Site 1 (S_1), latitude 26°31'11.12"N and longitude 87°16'25.64"E Tankisinwari, Morang; Babiya Birta Fish Farm, Site 2 (S_2), latitude 26°30'23.85"N and longitude 87°26'09.01"E, Morang; and Tarahara Fish Farm, Site 3 (S_3), latitude 26°42'05.77"N and longitude 87°16'38.50"E, Sunsari were selected in the disease prone areas of Eastern Nepal (Fig. 1). Diseased fishes were collected from these sites during 2009-2015.

Collection of Diseased Fish

Naturally infected fish *Catla catla*, *Cirrhinus mrigala*, *Channa striata* and *Puntius* sp., *Labeo rohita* and *Labeo bata* showing ulcerative lesions were collected during winter months of the year 2009-2015 from three different affected ponds of the Sunsari and Morang districts of eastern Nepal and were used for the isolation of bacteria.

Collection and Maintenance of Healthy Fish for Experimental Works

Healthy air breathing fish (*Heteropneustes fossilis*) collected from nearby fish farm with no history of EUS infections were used for experimental work. Fish were maintained in the laboratory in glass aquaria measuring 90×35×35cm^3 in which the depth of the static water

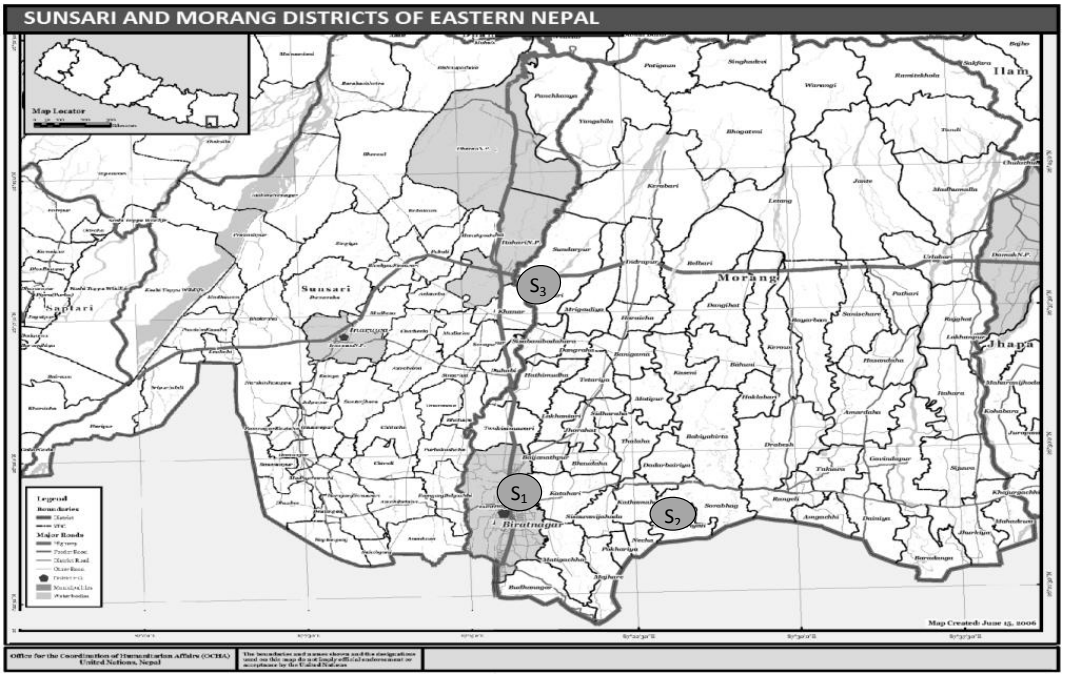

Figure 1. Map of Sunsari District showing sampling sites (1-3) (Source: OCHA, UN, Nepal, modified)

was 20 cm. Water temperature was maintained at 28-30°C. The fish were fed with chopped earthworms and acclimatized under laboratory conditions for 15 days before using them for experimental work.

Isolation of Bacteria from the Ulcers of Diseased Fishes and Culture

The ulcerated area of the diseased fish was dissected aseptically following Pal and Pradhan (1990) and placed in a conical flask containing 15 mL of nutrient broth (Hi Media) supplemented with glucose. The flask was incubated at 30°C for 72 hrs. Then 1 mL of each bacterial culture grown on nutrient broth was inoculated in a conical flask containing 20 mL of molten *Aeromonas* isolation medium supplemented with *Aeromonas* selective supplement (Hi Media) and mixed thoroughly. The mixture was then poured on sterile petridish and allowed to solidify for overnight at 30°C. Some colonies grown on the agar plates were selected and then streaked on to nutrient agar slants to incubate at 30°C for 24 hrs. Bacterial cultures grown on nutrient broth were also streaked on nutrient agar slants to incubate at 30°C for 24 hours. Each isolate was given a particular code name (Cm_1, Cm_2, Cm_3,C m_4 ,Cc_1, Cc_2, Cc_3, Cc_4, Cs_1, Cs_2, Cs_3, Mt_1,Mt_2,Mt_3 Mt_4, P_1, P_2 P_3, P_4, Lb_1, Lb_2,Lb_3 and Lb_4) and stored at 4°C. For routine experimental works, the isolates were sub cultured by growing in nutrient broth for 24 hrs at 30°C.Several media were used during the study for isolation and maintenance of isolates and for biochemical tests.

Characterization of the Isolated Bacteria

To identify the bacteria, a number of physiological and biochemical tests (Barrow & Feltham,

1993) were conducted following the identification scheme described by Popoff (1984), Carnanhan et al. (1991), and Abbott et al. (1992).

Pathogenicity Test of the Isolated Bacteria

All the isolates were tested for their ability to induce ulcers in healthy *Heteropneustes fossilis* fish weighing of 50-60 g by intramuscular application of 0.5 mL of bacterial cell suspension (1×10^7 c.f.u./mL) per 100 g of body weight in 0.85% NaCl. Each isolate was injected into a set of five fish. The control set of fish received 0.05 mL sterile saline. Fish were observed for changes in their behavioral patterns as well as development of hemorrhagic ulcers and tissue necrosis. Intramuscular injection was given at the trunk region on the right/left side of the fish from behind to the front at an angle of 20° to the body axis.

Results

A total 445 naturally infected fishes showing lesions on the body; 60% *Cirrhinus mrigala,* 30% *Labeo rohita* and *Labeo bata*, 10% *Catla catla, Channa* sp., *Puntius* sp. (Figs. 2-7) were collected during winter months of the year 2009-2015 from different affected ponds in various locations of the Sunsari and Morang districts of eastern Nepal .The infected fish were brought to the laboratory alive for further detailed observations.

In the early stage of lesion the fish showed single or multiple red spots on the body surface. Some fishes showed moderate type of ulcer with erosion of the epidermis (Figs. 3, 6, 7). In the advanced stage ulcer became deep and necrotic with occasional haemorrhages (Figs. 2, 5).

Figure 2. Naturally EUS infected *Cirrhinus mrigala*

Figure 3. Naturally EUS infected *Catla catla*

Figure 4. Naturally EUS infected *Labeo bata*

Figure 5. Naturally EUS infected *Channa striata*

Figure 6. Naturally EUS infected *Puntius* sp.

Figure 7. Naturally EUS infected *Mystus tengara*

Isolation of Bacteria and their Characterization

Four types of bacteria were isolated from ulcers of *Cirrhinus mrigala* (Table 1), *Catla catla* (Table 2). *Puntius* sp. (Table 4), *Mystus tengara* (Table 5), and *Labeo bata* (Table 6). Three types of bacteria were isolated from ulcers of *Channa striatus* (Table 3). Results of the morphological observations and biochemical test of the bacterial isolates from ulcers of different fishes are given in tables 2-7.

Altogether twenty-three bacteria were isolated from the ulcers of six infected fishes, out of which fourteen were *Aeromonas hydrophila*, three were *A. caviae*, one was *A. veroni biovar sobria*, two were *Pseudomonas* sp., two were *Micrococcus* sp. and one was *Moraxella* sp.

Out of fourteen *A. hydrophila*, two (Cm_1 and Cm_3) from *Cirrhinus mrigala,* three (Cc_1, Cc_2 and Cc_3) from *Catla catla*, one Cs_1 from *Channa striata*, two (P_1 and P_3) from *Puntius* sp., four (Mt_1, Mt_2, Mt_3 and Mt_4) from *Mystus tengara* and two (Lb_2 and Lb_3) from *Labeo bata* were isolated. Out of three *Aeromonas caviae*, one (Cm_4) from *C. mrigala* and two (Cs_2 and Cs_3) from *C. striata* were isolated. *A. veroni biovar sobria*, was isolated only from *Labeo bata*. Two *Pseudomonas* sp. (Cc_4 and Lb_1) were isolated one each from *Catla catla* and *Labeo bata*. Two *Micrococcus* sp. were isolated one each from *Cirrhinus mrigala* (Cm_2) and *Puntius* sp. (P_4). One *Moraxella* sp. was isolated from *Puntius* sp. (P_2) (Table 7).

Pathogenicity Test of the Isolated Bacteria

Among 23 bacterial isolates (Table 7), 20 were found to be pathogenic (86.95%) after intramuscular administration of these isolates to the healthy *Heteropneustes fossilis* fish. Two *Micrococcus* sp. (P_4 and Cm_2) and one *Moraxella* sp. (P_2) could not induce any ulcer at the site of injection in healthy fish.

Moderate to severe ulcers were found at the injection site. Initially red patches appeared at the site of injection, it swelled gradually and after 72 hrs, the skin and underlying muscle layer eroded and it developed into ulcer. In control set, the fish received only saline suspension. No disease sign was noticed. All fish, in which ulcers developed, however did not die. The moderate ulcers were healed in some fish. No notable change of the swimming behavior was also observed.

Table 1. Morphological and biochemical characteristics of bacteria isolated from the ulcers of *Cirrhinus mrigala.*

	Bacteria Isolates			
	Cm_1	**Cm_2**	**Cm_3**	**Cm_4**
Shape	Rod	Sphere	Rod	Rod
Occurance	Single Pairs	Single Pairs Tetrads	Single	Single
Size	2.8-3.2x0.75-0.8 µm	1.2-1.6µm diameter	2.8-3.2x0.75-0.8 µm	2.8-3.2x0.75-0.8µm
Spores	–	–	–	–
Agar Colonies	Circular Smooth Convex	Circular Smooth Convex	Circular Smooth Convex	Circular Smooth Convex
Gram reaction	–	+	-	-
Motility	+	-	+	+
Growth at: 25°C	g	m	g	g
30°C	g	g	g	g
37°C	m	g	m	m
42°C	n	n	n	n
Growth at 6% NaCl	-	+	-	-
Indole Production	+	-	+	+
Resistance to Ch	-	-	-	+
VP	+	-	+	-
Nitrate	+	W	+	+
Gas from glucose	+	-	+	-
Oxidase	+	+	+	+
Catalase	+	+	+	+
O-F test	F	0	F	F
Acid from: Glucose	+	+	+	-
L-arabinose	+	-	+	+
Sucrose	+	+	+	+
Mannitol	+	+	+	+
Esculin hydrolysis	+	+	+	+
LDC	+	-	+	-
ODC	-	-	-	-
ADH	+	-	+	+
Pigment production	-	Bright yellow	-	-

+ positive, - negative, 0 = neutral, g = good growth, m = moderate growth, n = no growth, Ch = cephalothin, VP = Voges-Proskauer reaction, O-F = Oxidation – Fermentation, LDC = lysine decarboxylase, ODC = ornithine decarboxylase, ADH = arginine dihydrolase, w = weak

Table 2. Morphological and biochemical characteristics of bacteria isolated from the ulcers of *Catla catla.*

	Bacterial isolates			
	Cc_1	**Cc_2**	**Cc_3**	**Cc_4**
Shape	Rod	Rod	rod	rod
Occurrence	Single Chains	Single Chains	single chains	single pairs or chains
Size	2.8-3.2x0.75-0.8 µm	2.8-3.2x0.75-0.8 µm	2.8-3.2x0.75-0.8 µm	2.2-0.3x0.7-0.8 µm
Spores	-	-	-	-
Agar Colonies	Circular Smooth Convex	Circular Smooth Convex	circular smooth convex	circular smooth slightly convex /flat
Gram reaction	-	-	-	-
Motility	+	+	+	+
Growth at:				
25°C	g	g	g	m
30°C	g	g	g	g
37°C	m	m	m	g
42°C	n	n	n	n
Growth at 6% NaCl	-	-	-	-
Indole Production	+	+	+	-
Resistance to Ch	-	-	-	-
VP	+	+	+	-
Itrate	+	+	+	+
Gas from glucose	+	+	+	-
Oxidase	+	+	+	+
Catalase	+	+	+	+
O-F test	F	F	F	0
Acid from:				
Glucose	+	+	+	+
L-arabinose	+	+	+	+
Sucrose	+	+	+	+
Mannitol	+	+	+	+
Esculin hydrolysis	+	+	+	-
LDC	+	+	+	-
ODC	-	-	-	-
ADH	+	+	+	+
Pigment production	-	-	-	Yellowish green in King's B medium

Table 3. Morphological and biochemical characteristics of bacteria isolated from the ulcers of *Channa striata.*

	Bacterial isolates		
	Cs_1	**Cs_2**	**Cs_3**
Shape	rod	rod	rod
Occurance	single	single	single
Size	2.8-3.3x0.7-0.75µm	2.8-3.2x0.75-0.8µm	2.8-3.2x0.75-0.8µm
Spores	-	-	-
Agar Colonies	circular smooth convex	circular smooth convex	circular smooth convex
Gram reaction	-	-	-
Motility	+	+	+
growth at:			
25°C	g	g	g
30°C	g	g	g
37°C	m	m	m
42°C	n	n	n
Growth at 6% NaCl	-	-	-
Indole Production	+	+	+
Resistance to Ch	-	+	+
VP	+	-	-
Nitrate	+	+	+
Gas from glucose	+	-	-
Oxidase	+	+	+
Catalase	+	+	+
O-F test	+	+	+
Acid from:			
Glucose	+	+	+
L-arabinose	+	+	+
Sucrose	+	+	+
Mannitol	+	+	+
Esculin hydrolysis	+	+	+
LDC	+	-	-
ODC	-	-	-
ADH	+	+	+
Pigment production	-	-	-

Table 4. Morphological and biochemical characteristics of bacteria isolated from the ulcers of *Puntius* sp.

	Bacterial isolates			
	P_1	**P_2**	**P_3**	**P_4**
Shape	Rod	Rod	rod	Sphere
Occurance	Single Pairs Chains	Single	single pairs chains	Single Pairs tetrads or irregular clusters
Size	2.8-3.2x0.75-0.8µm	1.5-1.7x0.9-1.9 µm	2.8-3.2x0.75-0.8µm	1.2-1.6 µm diameter
Spores	-	-	-	-
Agar Colonies	Circular Smooth Convex	Circular Smooth Convex	circular smooth convex	Circular Smooth Convex
Gram reaction	-	-	-	+
Motility	+	+	+	-
Growth at:				
25°C	g	m	g	m
30°C	g	g	g	g
37°C	m	g	m	g
42°C	n	n	n	n
Growth at 6% NaCl	-	-	-	-
Indole Production	+	-	+	-
Resistance to Ch	-	-	-	-
VP	+	-	+	-
Nitrate	+	-	+	W
Gas from glucose	+	-	+	-
Oxidase	+	+	+	+
Catalase	+	+	+	+
O-F test	F	0	F	0
Acid from:				
Glucose	+	-	+	+
L-arabinose	+	-	+	-
Sucrose	+	-	+	+
Mannitol	+	-	+	+
Esculin hydrolysis	+	+	+	+
LDC	+	-	+	-
ODC	-	-	-	-
ADH	+	-	+	-
Pigment production	-	-	-	Bright yellow colonies

Table 5. Morphological and biochemical characteristics of bacteria isolated from the ulcers of *Mystus tengara.*

	Bacterial isolates			
	Mt_1	**Mt_2**	**Mt_3**	**Mt_4**
Shape	Rod	Rod	rod	rod
Occurance	Single Pairs Chains	Single Pairs	single pairs chains	single pairs chains
Size	2.8-3.2x0.75-0.8µm	2.8-3.2x0.75-0.8µm	2.8-3.2x0.75-0.8µm	2.8-3.2x0.75-0.8µm
Spores	-	-	-	-
Agar Colonies	Circular Smooth Convex	Circular Smooth Convex	circular smooth convex	circular smooth convex
Gram reaction	-	-	-	-
Motility	+	+	+	+
Growth at:				
25°C	g	g	g	g
30°C	g	g	g	g
37°C	m	m	m	m
42°C	n	n	n	n
Growth at 6% NaCl	-	-	-	-
Indole Production	+	+	+	+
Resistance to Ch	-	-	-	-
VP	+	+	+	+
Nitrate	+	+	+	+
Gas from glucose	+	+	+	+
Oxidase	+	+	+	+
Catalase	+	+	+	+
O-F test	F	F	F	F
Acid from:				
Glucose	+	+	+	+
L-arabinose	+	+	+	+
Sucrose	+	+	+	+
Mannitol	+	+	+	+
Esculin hydrolysis	+	+	+	+
LDC	+	+	+	+
ODC	-	-	-	-
ADH	+	+	+	+
Pigment production	-	-	-	-

Table 6. Morphological and biochemical characteristics of bacteria isolated from the ulcers of *Labeo bata.*

	Bacterial isolates			
	Lb_1	**Lb_2**	**Lb_3**	**Lb_4**
Shape	Rod	Rod	rod	Rod
Occurance	Single Pairs Chains	Single	single pairs chains	Single Pairs Chains
Size	2.2-0.3x0.7-0.8 µm	2.8-3.2x0.75-0.8µm	2.8-3.2x0.75-0.8µm	2.5-3.0x0.7-0.8µm
Spores	-	-	-	-
Agar Colonies	Circular Smooth Convex	Circular Smooth Convex	circular smooth convex	Circular Smooth Convex
Gram reaction	-	-	-	-
Motility	+	+	+	+
Growth at : 25°C	m	g	g	g
30°C	g	g	g	g
37°C	g	m	m	m
42°C	n	n	n	n
Growth at 6% NaCl	-	-	-	
Indole Production	-	+	+	+
Resistance to Ch	-	-	-	+
VP	-	+	+	+
Nitrate	+	+	+	+
Gas from glucose	-	+	+	+
Oxidase	+	+	+	+
Catalase	+	+	+	+
O-F test	0	F	F	F
Acid from:				
Glucose	+	+	+	+
L-arabinose	+	+	+	+
Sucrose	+	+	+	+
Mannitol	+	+	+	+
Esculin hydrolysis	+	+	+	-
LDC	-	+	+	+
ODC	-	-	-	-
ADH	+	+	+	+
Pigment production	Yellowish green in King's B medium	-	-	-

Table 7. Pathogenic and non-pathogenic bacteria isolated from EUS affected fish.

Bacteria	No. of isolates	Pathogenic	Non-Pathogenic
Aeromonas hydrophila (Cm_1,Cm_3,Cc_1,Cc_2,Cc_3,Cs_1,P_1,P_3,Mt_1,Mt_2,Mt_3, Mt_4, Lb_2 and Lb_3)	14	14	0
Aeromonas caviae (Cm_4, Cs_2, Cs_3)	3	3	0
A. veronii biovar sobria (Lb_4)	1	1	0
Pseudomonas sp. (Cc_4, Lb_1)	2	2	0
Micrococcus sp. (Cm_2, P_4)	2	0	2
Moraxella sp. (P_2)	1	0	1
Total	**23**	**20**	**3**

Discussion

In the present study, twenty-three bacterial isolates were collected from the ulcers of six different EUS affected fish. The morphological features and biochemical profiles of eighteen isolates suggested that they were motile, non-spore forming, glucose fermenting, gram-negative bacilli. They were straight rods, grown in agar with 0% but not in 6% NaCl and reduced nitrate to nitrite (Tables 1-6). Thus, they all belonged to the genus *Aeromonas* (Popoff, 1984). Among these, fourteen (Cm_1, Cm_3, Cs_1, P_1, P_3, Lb_2, Lb_3, Cc_1, Cc_2, Cc_3, Mt_1, Mt_2, Mt_3 and Mt_4) of them were positive to esculin hydrolysis test, sensitive to antibiotic cephalothin, produced gas from glucose and acid from arabinose (Carnanhan et al., 1991). These bacteria were positive to lysine decarboxylase and arginine dehydrolase test but negative to ornithine decarboxylase test, produced acid from mannitol and sucrose and gave a positive to VP test (Abbott et al., 1992). Thus, these bacteria were regarded as *Aeromonas hydrophila*. The isolate Lb_4 was negative to esculin hydrolysis test, positive to indole test, produced acid from sucrose and gave a positive VP test (Carnanhan et al., 1991). It was tested positive to arginine dihydrolase, lysine decarboxylase and negative to ornithine decarboxylase, produced acid from arabinose and mannitol (Abbott et al., 1992). Therefore, it belonged to *Aeromonas veronii biovar sobria.* The isolate Cm_4 and Cs_2 and Cs_3 (Tables 1, 3) were tested positive to esculin hydrolysis but could not produce gas from glucose (Carnanhan et al., 1991). It was tested positive to arginine dihydrolase, negative to lysine decarboxylase and ornithine decarboxylase, gave a negative VP test and produced acid from arabinose, mannitol and sucrose (Abbott et al., 1992). Thus these were identified as *Aeromonas caviae*.

The morphological features and bio-chemical profiles of the isolated pathogenic bacteria, Cc_4 and Lb_1 (Tables 2, 6, 7) revealed that these bacteria were gram negative, motile, catalase positive, utilized glucose oxidatively and produced yellowish green pigment in King's B medium. Thus they belonged to the genus Pseudomonas (Palleroni, 1984).

The biochemical profile of Cm_2 and P_4 (Tables 1, 4, 7) showed that these bacterial isolates belonged to the genus Micrococcus. These differed from the genus *Staphylococcus* and *Streptococcus* with respect to the breakdown of glucose. As these isolates utilized glucose oxidatively and produced a yellowish pigment, Cm_2 and P_4 resembled Micrococcus. The

sphere-shaped bacteria were gram positive, non-motile, non- spore forming, catalase positive, indole negative, oxidase negative and oxidative, occurring singly, in pairs, in tetrad, in short chain or in irregular cluster. Colonies were yellow and small, smooth, convex. It satisfied the characteristics of the species *Micrococcus varians* (Kocur, 1986), e.g., oxidase negative, oxidative in metabolism, reduction of nitrate and nitrite, good growth between 25-37ºC and non-pathogenic. From the morphological and biochemical profile, P_2 isolate (Tables 4, 7) resembled Moraxella.

Fourteen *Aeromonas hydrophila* (Table 7) were isolated from infected fishes, e.g., *C. mrigala, C. catla, C. striata, Puntius* sp., *Mystus tengara* and *Labeo bata* and all are pathogenic. Three *Aeromonas caviae* were isolated from ulcers of *Cirrhinus mrigala* and *Channa striata* and one *A. veronii biovar sobria* was isolated from ulcer of *Labeo bata* are also pathogenic. Two *Micrococcus* sp. were isolated from ulcer of *Cirrhinus mrigala* and *Puntius* sp., one *Moraxella* sp. was isolated from ulcer of *Puntius* sp. Two *Micrococus* sp. and one *Moraxella* sp. were non-pathogenic.

Aeromonas sp. is one of the most common bacteria associated with fish diseases. Jo and Onishi (1980) isolated *A. hydrophila* from all skin ulcer of diseased cultured ayu, *Plecoglossus altivelis*. Rahim et al. (1985) isolated *A. hydrophila* from the wounds of five species of fishes in Bangladesh. Esteve et al. (1993) isolated *A. hydrophila* and *A. jandaei* from diseased farmed European eel in Spain. Two pathogenic *Pseudomonas* sp. were isolated from ulcer of *Catla catla* and *Labeo bata*. *P. anguilliseptica* was identified as the causative agent of red spot disease in Japan (Nakai et al., 1985). Boonyaratpalin (1989) found primarily *A. hydrophila* and occasionally *Pseudomonas* sp. associated with the outbreak of EUS. Association of *A. hydrophila* with EUS affected fish in Sri Lanka was also reported (Subasinghe et al., 1990). Karunasagar et al. (1989) and Mc Garey et al. (1991) isolated *A. hydrophila* and *A. sobria*. Two virulent strains of *Pseudomonas* sp. and one virulent Aeromonad, *A. caviae* were isolated from ulcerative air breathing fish from North Bengal in 1988 and reported to be pathogenic to *A. testudineus* (Pal & Pradhan, 1990). *Micrococcus* sp. and some other bacteria were also found to be associated with EUS, (Jhingran & Das, 1990). Mc Garey et al. (1991), Torres et al. (1993), Cartwright et al. (1994), and Lio-Po et al. (1998) also reported the association of mainly *Aeromonas* sp. and *Pseudomonas* sp. with EUS. Ali and Tamuli (1991) isolated three types of bacteria from ulcers from four species of affected fish and reinfection studies showed that *Aeromonas* sp. produced only mild infection. *Micrococcus* sp. failed to induce any sign.

Mukherjee et al. (1991) isolated five distinct strains of *A. hydrophila* from EUS affected fish. Torres et al. (1993) isolated 54 strains of *Aeromonas* sp. from EUS affected fish. Karunasagar et al. (1995) isolated *A. sobria* and *A. hydrophila* from EUS affected fish of Karnataka, India. Aeromonads and Pseudomonads isolated from EUS affected fish were found to induce EUS like lesion when injected intramuscularly to healthy snakehead (*O. striatus*) and walking catfish (*C. batrachus*) (Leano et al., 1995). Prasad et al. (1995) observed that *C. mrigala* injected with virulent *A. hydrophila* strain isolated from EUS affected *M. armatus* was found to be highly pathogenic. Lio-Po et al. (1998) isolated four types of bacteria associated with EUS, namely *A. hydrophila*, *Aquaspirillum* sp., *Pseudomonas* sp. and *Streptococcus* sp. Out of these bacteria, *A. hydrophila* was highly pathogenic. Saha and Pal (2000) isolated

16 strains of bacteria from *C. punctatus, Puntius* sp. and *Mystus* sp. belonging to the genus *Pseudomonas, Aeromonas, Micrococcus, Bacillus, Vibrio* and *Moraxella*. Among these bacteria, only 6 strains of Aeromonads and Pseudomonads were pathogenic. Pal and Pradhan (1990) isolated *A. caviae*; McGarey et al. (1991) isolated *A. hydrophila* and *A. sobria.* Lio-Po et al. (1998) isolated *A. hydrophila* along with other bacteria. In the present studies *A. hydrophila, A. sobria* and *A. caviae* were isolated from different infected fish which produced ulcer when injected intramuscularly to healthy fish. EUS affected fishes often die due to bacterial septicemia caused by pathogenic aeromonads (Pal & Pradhan, 1990; Rahman et al*.,* 2002). Mastan and Qureshi (2001) reported that 17 species of common bacteria were isolated from the investigated water bodies and EUS affected fishes. Das et al. (2009) found that all the 15 strains *Aeromonas* isolated from the ulcers of EUS affected fishes *Catla catla, Cirrhinus mrigala* and *Puntius* sp.

From the present experimental work, it was found that out of 23 bacterial strains isolated from infected fish in which 14 *Aeromonas hydrophila,* three *Aeromonas caviae*, one *A. veronii biovar sobria* and two *Pseudomonas* sp. were pathogenic. Two *Micrococcus* sp. and one *Moraxella* sp. were non-pathogenic.

Conclusion

A*eromonas hydrophila, A. caviae*, *A.veroni biovar sobria* and *Pseudomonas* sp. of bacteria also involve as pathogens to cause EUS in fish. Among them *Aeromonas hydrophila* was found to be more pathogenic and *Cirrhinus mrigala* fish was more infected.

Acknowledgements

The authors are very thankful to UGC, Nepal to provide partial grants and authorities of R and D laboratory, Department of Microbiology, Central Campus of Technology, Dharan and Zoology research laboratory NBU, West Bengal to carryout isolation, characterization and identification of bacteria.

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Pattern of β-Thalassemia and Other Haemoglobinopathies: A Cross-sectional Study in the Ethnic Groups of Eastern Terai Nepal

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Abstract

Thalassemia and structural haemoglobinopathies the emerging global health problems are the major erythrocyte formation disorders prevalent in certain parts of the world including Nepal. Any population based data on the prevalence of β-thalassemia and haemoglobinopathies is lacking in Nepal. The disease requires lifelong blood transfusion accompanied with iron chelation therapy. Therefore, prevention of births of homozygotes constitutes a major armament in the management of thalassemia. The study was aimed to find out the prevalence of haemoglobinopathies by screening large population in three districts Jhapa, Morang and Sunsari of Eastern Terai, Nepal. Study Population comprised of Musahar Dalit, Koch Rajbanshi, Kochila Tharu, Muslim and Santhal ethnic groups. In this cross-sectional study, an automated electronic cell counter estimated haematological parameters of 1500 blood samples of all age groups. Peripheral Blood Smear was observed microscopically for red cell morphology. The cases with Hb, MCV and MCH less than 15 gm/dL, 80 femtolitre, and 27 picograms were analysed for Haemoglobin pattern and quantification. The Haemoglobin quantification was done by capillary electrophoresis (Sebia minicap flex printing). Of the 1500 samples, the cases with haematological parameters below the cut-off level were considered positive for β-thalassemia. Peripheral blood smear was microscopically examined for target cells. The cases with typical target cells were subjected for haemoglobin quantification by capillary electrophoresis test. Out of the 1500 cases 39 (2.6%) HbE homozgygotes, 48 (3.2%) HbE heterozygotes and 97 (6.46%) β-thalassemia heterozygotes were diagnosed based on HbA2 quantification. The haemoglobin disorders diagnosed were â-thalassemia heterozygote, HbE homozgygote and HbE heterozygote. This study showed that haemoglobin disorders are prevalent in Nepal. The prevalence of HbeE gene (3.8%) in the Koch Rajbanshi and β-thalassemia heterozygote (3.4%) in the Kochila Tharu, Musahar and Santhal ethnic groups is significant. Since cost of lifelong treatment is beyond the reach of the common people. The control and management programmes for birth of homozygotes and β-thalassemia/ HbE heterozygote must be promptly implemented.

Key words: Haemoglobin electrophoresis, HbE heterozygote, HbE homozygote

Introduction

Thalassemia and structural haemoglobinopathies, the emerging global health problems are the major erythrocyte formation disorders prevalent in certain parts of the world including Nepal (Thein & Rees, 2015). About 4.5% of the world population is affected by haemoglobinopathies. An estimated 300,000-400,000 children are born every year with

severe haemoglobin disorders worldwide, 80% of these births are in developing countries (WHO, 2007). The disorder arises from a mutation or deletion in one or more globin gene(s) that leads to a reduction or absence in haemoglobin production (Galanello & Origa, 2010). In general thalassemia is of two types: α-thalassemia and β-thalassemia. α-thalassemia is characterized by impaired synthesis of α-globin chain, and β-thalassemia is characterized by impaired synthesis of β-globin chain. β-thalassemia is the most common type of thalassemia (Safizadeh et al., 2012).

β-thalassemia presents in three forms namely heterozygous state (β-thalassemia Trait/ minor/carrier), intermediate state and homozygous state (β-thalassemia major/disease). Heterozygous β-thalassemia is asymptomatic and does not require blood transfusion. β-thalassemia intermedia is mild and may require occasional blood transfusion. Homozygous β-thalassemia also known as β-thalassemia major is a severe blood transfusion dependent lifelong disease. Heterozygous β-thalassemia is also known as β-thalassemia Minor/ trait or carrier state, the affected person carries one normal and one mutated thalassemia β-globin chain. β-thalassemia minor may be present in person without knowing that they are carriers. There are 25% chances of producing homozygous β-thalassemic child for a β-thalassemia heterozygous/carrier couple (Thein & Rees, 2015).

Mutation of the β-globin gene causes β-thalassemia and haemoglobinopathies of which HbS, HbE, HbD and HbC are the commonest genetic disorders (Weatherall, 2010). HbE is caused by a mutation of the 26th amino acid of a normal β-chain leading to the replacement of glutamine by lysine (Tangvarasittichai, 2011). This mutation produces a structurally abnormal haemoglobin as well as activates a cryptic splice site, resulting in abnormal messenger RNA (mRNA) processing. The β-chain of HbE (β^E) is synthesized at reduced rate than normal adult haemoglobin (HbA). The HbE may be present in three states. The homozygous state (genotype EE or Hemoglobin E disease), heterozygous state (genotype AE or HbE carrier/trait) and a variety of compound heterozygous states such as HbE/βthal (E/β-thal) and sickle cell haemoglobin E (SE genotype). Hemoglobin E maybe regarded as β-thalassemia hemoglobinopathy. (Bachir & Galacteros, 2004; Olivieri et al., 2011).

HbE/β-thalassemia the most frequent β-thalassemia with abnormal Hb or structural Hb variant with thalassemic characteristics. Worldwide 50 per cent of severe/major β-thalassaemia patients were reported to be Hb E/β-thalassaemia (Sandhya Rani et al., 2013). The prevalence of HbE/β-thalassemia stretches across Northern India, Bangladesh, Laos, Combodia, Thailand, Vietnam, Malaysia, the Philippines and Indonesia. In Thailand the carrier frequency of HbE/β-thalassemia is around 50%. Thalassemia is cost effective. Rakholia et al have estimated IRS 1, 25, 000/annum as the ideal treatment cost of one thalassemic child in India.

It is very important to have a reliable detection and identification methods for thalassemia heterozygous/minor/traits and Hb variants, because this can lead to the prevention of more severe disorders like thalassemia homozygous/major and HbE/β-thalassemia in infants. Many researchers have screened thalassemia minor across the world. The screening can be done in four different ways: prenatal, new born, premarital and random total population screening. Prevention of thalassemia is not a single procedure but a conjoint collaboration of the psychiatrist, physician, counsellors and the government.

The objective of the present study was to determine the pattern and prevalence of haemoglobinopathies in some districts of eastern Nepal.

Materials and Methods

This random cross-sectional study included 1500 samples collected from Jhapa, Morang and Sunsari districts of east Nepal. The study population comprised of five ethnic groups namely Muslims Koch Rajbanshi, Kochila Tharu, Santhal and Musahar. 3 mL intravenous blood samples were collected in EDTA-containing vacutainer blood collection tubes. The samples were subjected to testing within 2 hours of sampling using a fully automated blood cell counter (Sysmex XN1000). Peripheral Blood Smear was observed microscopically for red cell morphology. The cases with Hb (<15 gm/dL), MCV (<80 femtolitre) and MCH (<27 picogram) respectively were analysed for Haemoglobin pattern and quantification. The Haemoglobin quantification was done by capillary electrophoresis (Sebia minicap flex printing). The HbA2 value >3.5% was considered as a cut-off point for heterozygous beta-thalassemia.

For Statistical Analysis the raw data were managed on Microsoft ® Excel sheet then exported to R3.0.3. Software (R Core Team, 2014) for further analysis. The significance was calculated by Chi-square Test. P-values less tha 0.05 were considered significant

Results

Among the total sampled cases (1500) the haematological parameters revealed Microcytic Hypochromia in 285 cases. The Chi-square test of the entire study area showed the prevalence of Microcytic Hypochromia to be significant in the Ethnic groups. Therefore, anaemia was a serious problem among the ethnic groups of Eastern Nepal (Table 1).

In the microscopic examination of red cell morphology, the significance of the target cells was found in the Ethnic groups (Table 1). This was indication of the prevalence of genetic issue among the ethnic groups.

Haemoglobin quantification by capillary electrophoresis confirmed the prevalence of Beta thalassemia heterozygote, Haemoglobin E homozygote and Haemoglobin E heterozygote. The significance of beta thalassemia was higher in Jhapa (1.03e-08) compared to Morang (0.004) district but did not show any significance in Sunsari district. Haemoglobin E disease was significantly high in Jhapa (2.2e-16) followed by Sunsari (0.002) and was not significant in Morang. Whereas Haemoglobin E heterozygote was significant in all three districts, (745e-07) Jhapa, (5.50e-05) Morang and (0.0008) Sunsari (Table 2).

Table 1. Significance of MH and TC in the study area

Districts	MH		Remarks	TC		Remarks
	Chi-squared	p-value		Chi-squared	p-value	
Jhapa	60.786	1.983e-12	Significant	29.559	6.017e-16	Significant
Morang	21.41	0.0003	Significant	29.1	7.60E-06	Significant
Sunsari	46.3	2.087-09	Significant	17.2	0.002	Significant

MH = Microcytic Hypochromia, TC = Target cells

Table 2. Significance of Haemoglobinopathies in the study area

Districts	BTM		Remarks	HbEE		Remarks	HbEA		Remarks
	Chi-squared	p-value		Chi-squared	p-value		Chi-squared	p-value	
Jhapa	43.011	1.03e-08	Significant	96.436	<2.2e-16	Significant	33.999	7.45e-07	Significant
Morang	15	0.004	Significant	5.7	0.22	Non-significant	24.8	5.50e-05	Significant
Sunsari	6.07	0.19	Non-significant	17.3	0.002	Significant	18.96	0.0008	Significant

BTM = β-thalassemia heterozygous/carrier, HbEE = Hemoglobin E homozygous/disease, HbEA = haemoglobin E heterozygous/carrier

A total of 1500 cases were screened during the study period. 39 (2.6%) HbE homozgygotes, 48 (3.2%) HbE heterozygotes and 97 (6.46%) β-thalassemia heterozygotes were diagnosed based on HbA2 quantification. The haemoglobin disorders diagnosed were β-thalassemia heterozygote, HbE homozgygote and HbE heterozygote.

Discussion

Thalassemias and haemoglobinopathies, were mainly confined to certain areas, religions, castes and tribes, particularly with endogamous norms of marriages. These disorders are now widely prevalent all over the world due to global migration of various races over the ages (Patne & Shukla, 2009). In Southeast Asia α-thalassemia, β- thalassemia, Hb E and Hb CS (Constant Spring) are prevalent. HbE is the hallmark of Southeast Asia with a frequency of 50-60% at the junction of Thailand, Laos and Combodia (Shrestha & Karki, 2013).

In this population based random cross-sectional study, the prevalence of Hb disorders was found to be 12.26%, which was lesser than another hospital based study conducted by Jha (2015) in the haematology section of Department of Pathology of Tribhuvan University Teaching Hospital on cases sent for electrophoresis. Jha reported thalassemia trait as the most common (26.8%) followed by sickle cell disease (21.6%), out of 11 alpha thalassemia, 9 HbH and 2 HbJ, and 9.3% of the abnormal hemoglobins comprised of HbE beta thalassemia (Jha, 2015). In the present study the haemoglobin disorders diagnosed were β-thalassemia heterozygote (6.46%), HbE haemoglobin (5.8%) comprising of HbE homozgygote (2.6 %) and HbE heterozygote (3.2%), was the most common haemoglobin variant diagnosed. The importance of this study was the finding of HbE in the Koch Rajbhanshi of East Nepal.

With respect to haemoglobin E, the lethal effect to human health is not clear. In Northeast India many researchers have found that HbE homozygotes live a normal life (Singh et al., 2010). According to the findings of De et al. (1997) the HbE homozygotes in Tripura were not anaemic compared to the normal genotypes of the same. But later Sharma et al. (2013) reported a significant anaemic status with respect to presence of abnormal HbE either in homozygous or heterozygous condition in college going girls in Assam. A high frequency of anaemia among children with HbE either homozygote, heterozygote or compound heterozygote condition was detected by Pathak et al. (2014). Sikdar (2016) found a twelve years old tribal child with homozygous HbE received regular blood transfusion in Assam.

In Southeast Asia compound heterozygosity of HbE with thalassemia was a frequently observed condition. HbE/ beta thalassemia results from the co-inheritance of a β-thalassemia allele from one parent and the structurally variant HbE from the other parent. Worldwide approximately 50% of severe beta-thalassemia patients are of HbE beta-thalassemia (Olivieri et al., 2011). Patients with combined HbE/beta-thalassemia present variable clinical symptoms varying from a mild form of thalassemia to a severe transfusion dependent condition indistinguishable from thalassemia major. Data collected over recent years indicate that HbE-beta thalassemia is emerging as a serious public health problem throughout the India and parts of South East Asia.

Beta thalassemia is a hereditary disease allowing for a preventive treatment by carrier screening and prenatal diagnosis. It can be prevented if one parent is normal, giving rise to screenings that empower carriers to select partners with normal hemoglobin. Many countries which adopted thalassemia programs that distribute information about reproductive risks associated with carriers of haemoglobinopathies have definitely reduced the incidence of thalassemia. In Italy prevalence of thalassemia reduced from 1:250 to 1:4000 with a 95% decrease.

Conclusion

The significant prevalence of β-thalassemia and haemoglobinopathies indicate the emergence of HbE-β-thalassemia as a serious public health problem in the ethnic groups of Eastern Terai Nepal in the near future. Since cost of lifelong treatment is beyond the reach of the common people. The control and management programmes for birth of homozygotes and compound heterozygote HbE-β-thalassemia must be promptly implemented.

Acknowledgements

We are highly obliged to the local people and health assistants for their voluntary support during sample collection. We also indebted to all participants who willingly allowed me to collect their blood samples for testing.

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Status and Identification of Fishes in the Markets of Saptari District, Eastern Nepal

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Abstract

Freshwater resources in Nepal are the sole of fish diversity and aquaculture of the nation. Regular visits to the different markets of Rajbiraj, Rupani, Kanchanpur, Phattepur, Bhardaha, Hardiya and Belahi were made once each month from April 2018 to March 2019. The collected fishes were preserved in 5% to 10% formaldehyde. A total of 66 fish species belonging to 44 genera, 22 families and 7 orders were identified from the fish markets. The largest order was Cypriniformes (35, 53%) then Siluriformes and Perciformes (12, 18.2% for each), Symbranchiformes (4, 6%) and only one species (1.5%) for each Osteoglossiformes, Anguiliformes and Beloniformes. About eight exotic fishes (*Cyprinus carpio*, *Hypophthalmichthys nobilis*, *H. molitrix*, *Ctenopharyngodon idella*, *Pangasius pangasius, Clarias gariepinus*, *Oreochormis nilotica*, and *O. mossambica*) were recorded in the selected markets. *Labeo rohita*, *Cirrhinus mrigala* and *Catla catla* were indigenous fishes coming from captive fisheries. Large sized indigenous fishes obtained from capture fisheries were *Wallago attu*, *Channa marulius* and *Aorichthys aor*. Others small fishes carried directly from the natural resource were *Channa striatus*, *C. orientalis*, *C. punctatus*, *Mystus tengera*, *M. cavasius*, *M. bleeveri*, *M. vittatus*, *Mastacembalus armatus*, *M. puncalus*, *Macrognathus aral*, *Anabus testudineus*, *Puntius* species, etc. Enhancement of domestic captive fisheries and strictly banned illegal fishing are necessary for the conservation of indigenous fishes.

Key words: Cypriniformes, Fish market, Indigenous fishes, Province 2.

Introduction

Nepal, a landlocked country, is rich in freshwater. The major rivers are Gandaki, Koshi, Karnali, Narayani, Bheri, Trisuli, Kamala, Bagmati, Rapti, Seti, etc. These rivers and their tributaries, lakes and wetlands are suitable for freshwater fishes (Limbu & Gupta, 2019), inhabiting 250 fish species (Shrestha, 2019). These freshwater occupy about 745,000 ha and 5% of the total area (Sharma, 2008). Out of 12500 ha of the suitable area available, only 1225 ha (approx.) was being recently used for aquaculture (Budhathoki & Sapkota, 2018). The country has 29,270 fish ponds, of which 95% are in terai alone (DOFD, 2017). Province No. 2 has the highest number of ponds, i.e., 9397 and the surface area, i.e., 4055 ha. (Budhathoki & Sapkota, 2018).

Since ancient time, some tribes like Majhi, Tharu, Kumal, Kewat, Mushar, Bote, etc., has been practised capture fishery for their livelihood through out the country (Bhattarai, 2012). The national fish production in fiscal year 2001-02 was 33,270 mt (Sharma, 2008) and 37427 mt with fish consumption per capita was 2.1 kg in the year 2013-14 (Budhakoti & Sapkota, 2018) and 65,770 mt in the year 2013-2014 (DOFD, 2017). Thirty-five percent of the national production is from eastern Nepal (Shrestha & Mishra, 2014).

Only a few works have been done on the ichthyofauna of eastern Nepal by some researchers like Shrestha et al. (2009), Pinky (2016), Shrestha (2016), Subba et al. (2017), Yadav (2017), Limbu et al. (2018), and Shrestha and Yadav (2019). They made notable contributions on this field. So, an attempt was made to document the fishes found in different markets of Saptari district, eastern Nepal.

Materials and Methods

Study Area

Seven study sites were Rajbiraj (26°32.3602 N and 86°45.1852 E, alt. 99 msl), Rupani (26°622 N and 86°702 E, alt. 100 msl) Kanchanpur (26°38.4362 N and 86°54.5742 E, alt. 97 msl) Fattepur (26°442 N and 86°562 E, alt.78 msl), Bhardaha (26°33.4412 N and 86°53.0402 E, alt. 71 msl), Hardiya (26°452 N and 86°322 E, alt. 126) and Belahi (26°322 N and 86°282 E, alt. 81 msl) were in the Saptari district (Fig. 1).

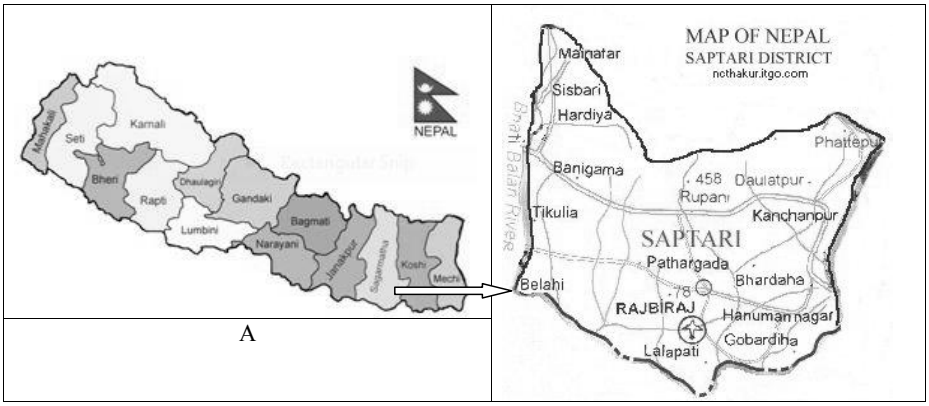


Figure 1. Maps of Nepal 'A' and Saptari district showing fish collection sites (Google map).

Fish Collection and Identification

Regular visits to the study sites (Rajbiraj, Rupani, Kanchanpur, Fattepur, Bhardaha, Hardiya, and Belahi) were made once a month from April 2018 to March 2019. Snaps and systematic collections of fishes were done from every study site in each visit for taxonomic identification based on Shrestha (2008). The collected fishes were preserved in 40% formaldehyde solution for 6 to 8 hours and then in 5% to 10% formaldehyde depending on size of fishes facing their head downward for further identification. A longitudinal incisor was made in large-sized fishes to protect the gut contents.

Results

Fish Diversity in the Markets of the District

In the study period, fishes were composed of 66 species belong to 44 genera, 22 families, and 7 orders presented in table 1. About eight exotic fishes and 58 indigenous species were recorded in the different market of study areas.

Table 1. Check list of fishes found in markets of Saptari district, Province-2, Nepal.

S.N.	Fish species in the markets	Common name	Conservation status
	Family: Notopteridae		
1	*Notopterus notopterus* (Pallas) 1767	Grey Featherback	LC
	Family: Cyprinidae		
2	*Cirrhinus reba* (Hamilton-Buchanan) 1822	Reba Carp	LC
3	*Cirrhinus mrigala* (Hamilton-Buchanan) 1822	Mrigal	LC
4	*Labeo rohita* (Hamilton-Buchanan) 1822	Rohu	LC
5	*Labeo caeruleus* Day 1877	Sind Labeo	NE
6	*Labeo dero* (Hamilton-Buchanan) 1822	Kalabans	LC
7	*Catla catla* (Hamilton-Buchanan) 1822	Catla	LC
8	*Cyprinus carpio* Linnaeus 1758	Common Carp	VU
9	*Puntius sarana* (Hamilton-Buchanan) 1822	Olive Barb	LC
10	*Puntius sophore* (Hamilton-Buchanan) 1822	Spotfin Swamp Barb	LC
11	*Puntius ticto* (Hamilton-Buchanan) 1822	Ticto Barb	LC
12	*Puntius conchonius* (Hamilton-Buchanan) 1822	Rosy Barb	LC
13	*Chagunius chagunio* (Hamilton-Buchanan) 1822	Chaguni	LC
14	*Osteobrama cotio cotio*(Hamilton-Buchanan) 1822	Cotio	DD
15	*Ctenopharyngodon idella* Valenciennes 1844	Grass Carp	NE
16	*Danio dangila* (Hamilton-Buchanan) 1822	Dangila Danio	LC
17	*Danio devario* (Hamilton-Buchanan) 1822	Devario Danio	LC
18	*Aspidoparia jaya* (Hamilton-Buchanan) 1822	Jaya	LC
19	*Esomus danricus* (Hamilton-Buchanan) 1822	Flying Barb	LC
20	*Barilius barna* (Hamilton-Buchanan) 1822	Barna Baril	LC
21	*Barilius bendelisis* (Hamilton-Buchanan) 1822	Hamilton's Barila	LC
22	*Barilius vagra* (Hamilton-Buchanan) 1822	Vagra Baril	LC
23	*Barilius shacra* (Hamilton-Buchanan) 1822	Shacra Baril	LC
24	*Parluciosoma daniconius* (Hamilton-Buchanan) 1822	Blackline Rasbora	LC
25	*Hypophthalmichthys molitrix* Valenciennes 1844	Silver Carp	NT
26	*Hypophthalmichthys nobilis* Richardson 1845	Bighead Carp	DD
27	*Chela labuca* (Hamilton-Buchanan) 1822	Glass Barb	LC
28	*Salmostoma bacaila* (Hamilton-Buchanan) 1822	Large Razorbelly Minnow	LC
29	*Garra annandalei* Hora 1921	Annandale Garra	LC
30	*Garra gotyla gotyla* Gray 1830	Gotyla	LC
	Family: Balitoridae		
31	*Acanthocobotis botia* (Hamilton-Buchanan) 1822	Pate Gadela	LC
	Family: Cobitidae		
32	*Botia lohachatta* Chaudhari 1912	Y- Loach	NE
33	*Lepidocephalus guntea* (Hamilton-Buchanan) 1822	Guntea Loach	LC
34	*Somileptes gongota* (Hamilton-Buchanan) 1822	Spindle Loach	LC
	Family: Psilorhynchiidae		
35	*Psilorhynchus sucatio* (Hamilton-Buchanan) 1822	Sucatio Minnow	LC
36	*Psilorhyncus pseudecheneis* (Menon &Datta) 1964	Nepalese Minnow	LC
	Family: Bagridae		
37	*Aorichthys aor* (Hamilton-Buchanan) 1822	Long-Whiskered Catfish	LC
38	*Mystus bleekeri* (Day) 1878	Day's Mystus	LC
39	*Mystus cavasius* (Hamilton-Buchanan) 1822	Gangetic Mystus	LC

S.N.	Fish species in the markets	Common name	Conservation status
40	*Mystus tengara* (Hamilton-Buchanan) 1822	Tengara Mystus	LC
41	*Mystus vittatus* (Bloch) 1797	Striped Dwarf Catfish	LC
	Family: Clariidae		
42	*Clarias batracus* Linnaeus 1758	Magur	LC
43	*Clarias gariepinus* Burehell 1822	African Catfish	LC
	Family: Heteropneustidae		
44	*Heteropneustes fossilis* (Bloch)1794	Stinging Catfish	LC
	Family: Siluridae		
45	*Ompok bimaculatus* (Bloch) 1794	Butter Catfish	NT
46	*Wallogo attu* Bloch &Schneider 1801	Boal /Buhari	NT
	Family: Chacidae		
47	*Chaca chaca* (Hamilton-Buchanan) 1822	Squarehead Catfish	LC
	Family: Pangasidae		
48	*Pangasius pangasius* (Hamilton-Buchanan) 1822	Pungas	LC
	Family: Anguillidae		
49	*Anguilla bengalensis* Gray 1831	Long fin Freshwater Eel	LC
	Family: Belonidae		
50	*Xenantodon cancila* (Hamilton-Buchanan) 1822	Freshwater Garfish	LC
	Family: Gobidae		
51	*Glossogobius giuris* (Hamilton-Buchanan) 1822	Tank Goby	LC
	Family: Channidae		
52	*Channa marulius* (Hamilton-Buchanan) 1822	Giant Snakehead	EN
53	*Channa orientalis* (Bloch and Schneider) 1801	Asiatic Snakehead	NE
54	*Channa punctatus* (Bloch) 1793	Spotted Snakehead	LC
55	*Channa striatus* (Bloch) 1793	Striped Snakehead	LC
	Family:Belontiidae		
56	*Colisa faciatus* (Bloch and Schneider) 1801	Striped Gourami	LC
	Family:Nandidae		
57	*Badis badis* (Hamilton-Buchanan) 1822	Badis	LC
	Family: Cichlidae		
58	*Oreochromis nilotica* (Linnaeus) 1758	Nile Tilapia	LC
59	*Oreochromis mossambica* (Peters) 1852	Mozambique Cichlid	NT
	Family: Anabantidae		
60	*Anabus testudineus* (Bloch) 1792	Climbing Perch	DD
	Family: Chandidae		
61	*Chanda nama* (Hamilton-Buchanan) 1822	Elongate Glass-perchlet	LC
62	*Pseudambassis ranga* (Hamilton-Buchanan) 1822	Glassy Fish	LC
	Family:Symbranchiidae		
63	*Monopterus cuchia* (Hamilton-Buchanan) 1822	Chuchia	LC
	Family: Mastacembellidae		
64	*Mastacembellus armatus* (Lacepede) 1800	Tire-Track	LC
65	*Macrognathus aral* (Bloch and Schneider) 1801	Gainchi	LC
66	*Macrognathus pancalus* (Hamilton-Buchanan) 1822	Kathgaichi	LC

The fishes' threat status in the river is 53 least concern, four not evaluated, one vulnerable, three data deficient, four near threatened and one endangered (Fig. 2).

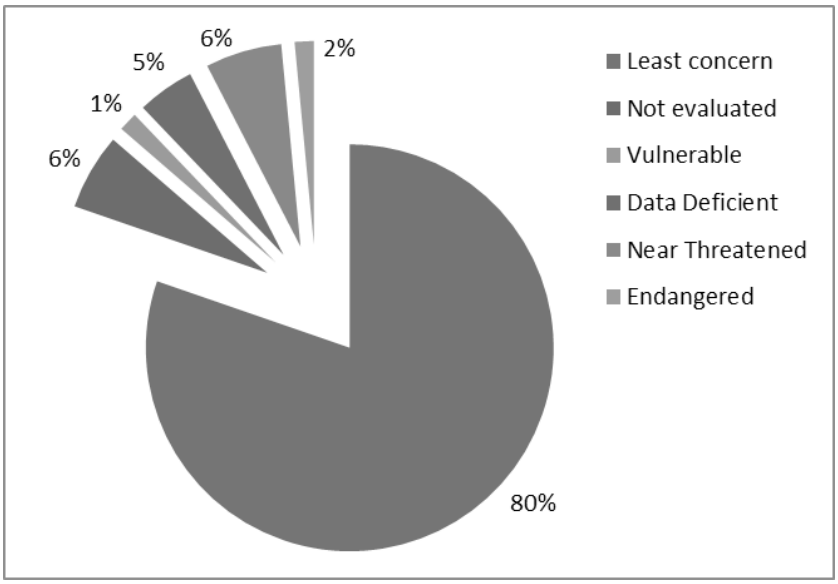


Figure 2. Percentage of the conservation status of fishes present in the markets.

There were 29 Cyprinidae, five Bagridae, four Channidae, three for each Cobitidae and Mastacembellidae, two for each Psilorhynchiidae, Clariidae, Chandidae, Siluridae and Cichlidae, one for each Heteropneustidae, Chacidae, Pangasidae, Anguillidae, Belonidae, Gobidae, Belontiidae, Nandidae, Anabantidae, Symbranchiidae, Notopteridae, and Balitoridae. Similarly, the largest order was Cypriniformes (35, 53%) then Siluriformes and Perciformes (12, 18.2% for each), Symbranchiformes (4, 6%) and only one species (1.5%) for each Osteoglossiformes, Anguilliformes and Beloniformes (Fig. 3).

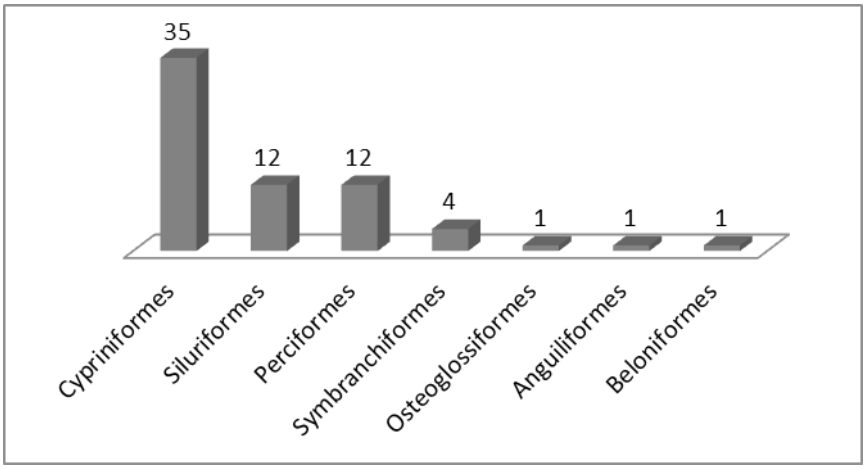
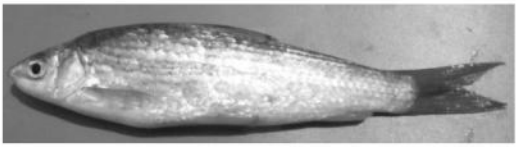


Figure 3. Order wise fish diversity of the markets of Saptari district.

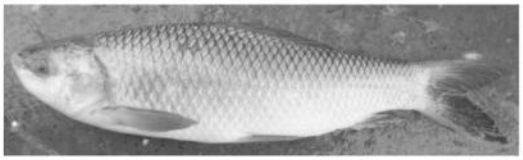
Photographs of some of the fishes found in the markets of Saptari District were given in figure 4.

Notopterus notopterus



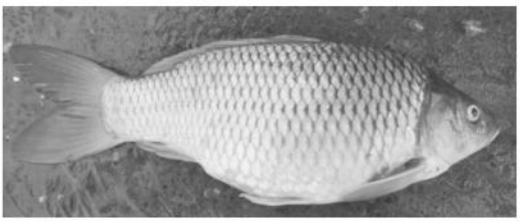
Cirrhinus reba

Cirrhinus mrigala

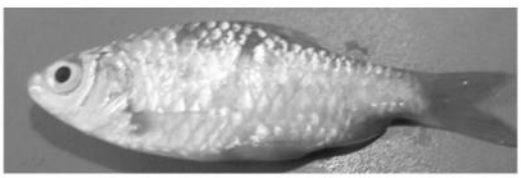


Labeo rohita

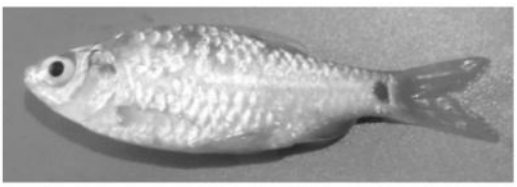
Catla catla



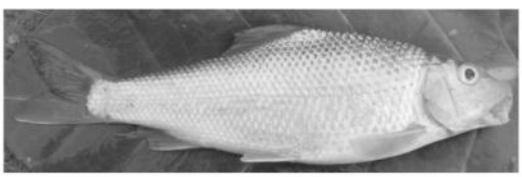
Cyprinus carpio



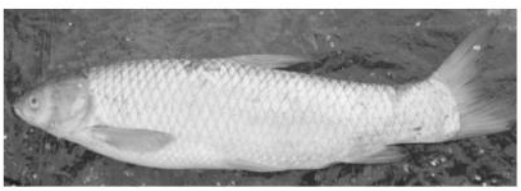
Puntius sarana



Puntius sophore



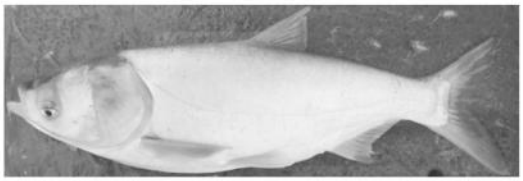
Chagunius chagunio



Ctenopharyngodon idella

Aspidoparia jaya

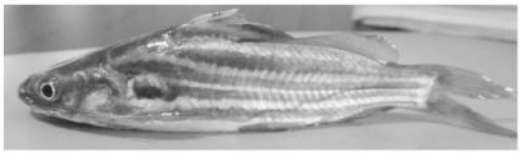
Barilius barna



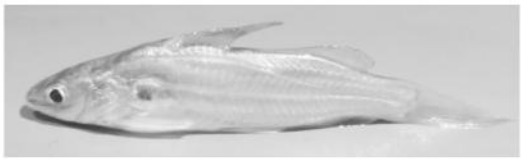
Hypopthalmichthys molitrix

Hypopthalmichthys nobilis

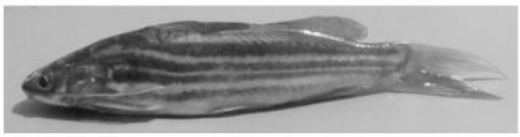
Lepidocephalichthys guntea



Mystus bleekeri



Mystus cavasius



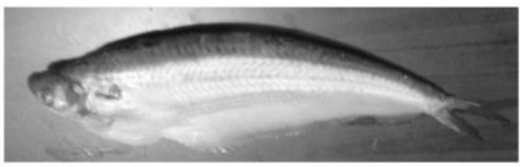
Mystus tengera

Mystus vittatus

Clarias batracus

Clarias gariepinus

Heteropneustes fossilis



Ompok bimaculatus

Wallago attu

Pangasius pangasius

Xenantodon cancila

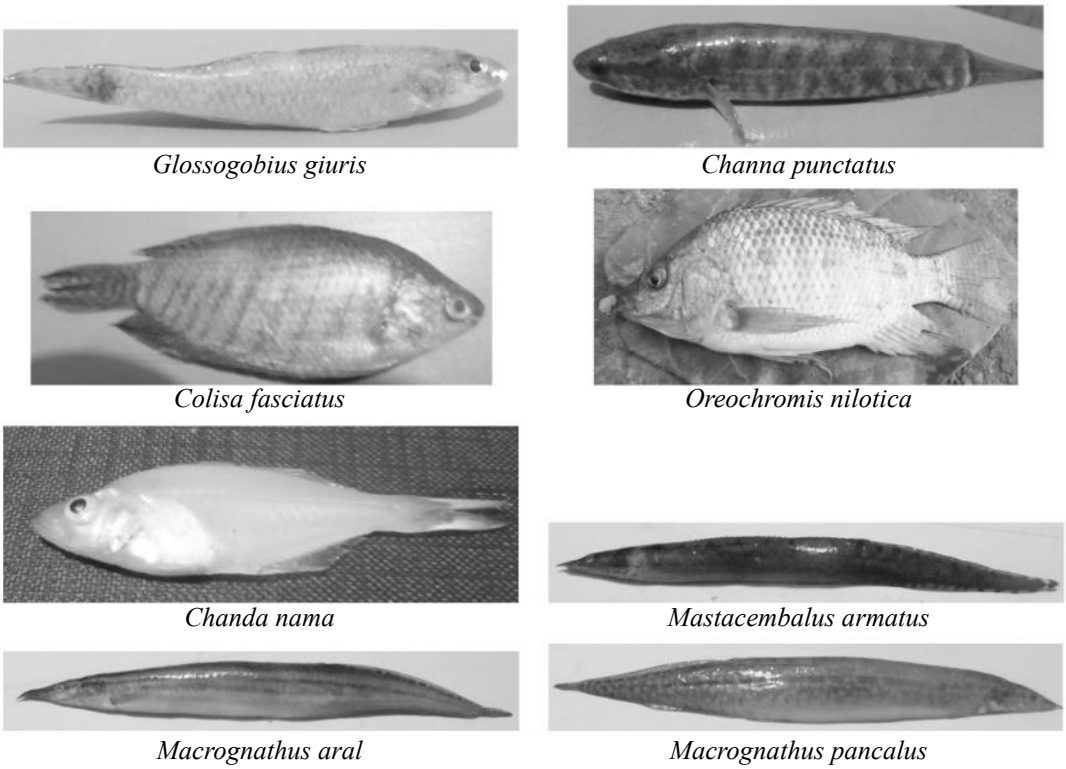


Figure 4. Recorded available fish species in the markets of Saptari District.

Most of the fishes in markets of the district were cultivated fishes supplied by captive fisheries. They were iced fish. The ratio of fish and ice used in iced fish were 1:1. *Labeo rohita*, *Pangasius pangasius*, *Cirrhinus mrigala*, *Cyprinus carpio* and *Catla catla* were commonly found.

Other cultivated fishes found in the markets were *Hypopthalmichthys nobilis*, *Ctenopharyngodon idella*, *Clarias gariepinus*, *Oreochormis nilotica* and *O. mossambica*. *Oreochormis nilotica* and *O. mossambica* were found seasonally in the markets.

Discussion

The indigenous fishes came from capture fisheries, mainly from rivers. The presence of Cyprinidae in the markets as dominant species (29) advocates the result of Nepal (Shrestha, 2008; Shrestha & Yadav, 2019). They reported 86 Cyprinidae (Shrestha, 2008) among 232 fish species and 20 Cyprinidae (Shrestha & Yadav, 2019) among 55 fish species. The cultivated fishes like *L. rohita*, *C. catla*, *C. mrigala* and *P. pangasius* were also found in the rivers of Nepal but others like *C. idella*, *C. carpio*, *H. nobilis*, *H. molitrix*, *C. gariepinus,* and tilapia species were not reported from the rivers of Nepal.

Fishes like *Anguilla bengalensis*, *Xenentodon cancila*, *C. catla*, *C. chagunio*, *C. mrigala*, *C. reba*, *L. dero*, *L. rohita*, *O. cotio cotio*, *Puntius conchonius*, *P. sarana*, *P. sophore*, *P. ticto*,

Salmostoma bacaila, *Barilius barna*, *B. bendelisis*, *B. vagra*, *Danio devario*, *E. danricus*, *Parluciosoma daniconius*, *Garra annandalei*, *G. gotyla*, *Lepidocephalus guntea*, *Semileptus gongota*, *Psilorhynchus pseudecheneis*, *Acanthocobitis botia*, *Chanda nama*, *Parambassis ranga*, *Badis badis*, *Anabus testudineus*, *Botia lohachata*, *N. notopterus*, *Aorichthys aor*, *Mystus bleekeri*, *M. cavasius*, *M. tengara*, *M. vittatus*, *Glossogobius giuris*, *Colisa fasciatus*, *Channa marulius*, *C. orientalis*, *C. punctatus*, *C. striatus*, *Monopterus cuchia*, *Ompok bimaculatus*, *Wallago attu*, *P. pangasius*, *Clarias batrachus*, *Heteropneustes fossilis*, *Chaca chaca*, *Macrognathus aral*, *Macrognathus pancalus*, and *Mastacembelus armatus* were also reported in Rapti and Narayani river but some indigenous fishes like *Labeo caeruleus*, *Danio devario*, *Aspidoparia jaya*, *B. vagra*, *B. shacra*, *Chela labuca*, *Psilorhynchus sucatio* and exotic cultivated fishes such as *O. nilotica*, *O. mossambica*, *C. idella*, *C. carpio*, *H. molitrix*, *H. nobilis* and *C. gariepinus* were not reported in the river (Jha & Bhujel, 2014).

Some indigenous fishes such as *Labeo caeruleus*, *L. dero*, *P. ticto*, *P. conchonius*, *O. cotio cotio*, *D. dangila*, *E. danricus*, *B. barna*, *B. shacra*, *P. daniconius*, *C. labuca*, *S. bacaila*, *G. annandalei*, *G. gotyla gotyla*, *P. sucatio*, *P. pseudecheneis*, *M. vittatus* and *A. testudineus* were fishes of the markets but not recorded from Koshi river (Rijal et al., 2014). All fishes reported from the markets were also reported from Koshi except some exotic fishes like *C. idella*, *C. carpio*, *H. molitrix*, *H. nobilis*, *C. gariepinus*, *O. nilotica* and *O. mossambica* (Rajbabshi, 2012).

N. notopterus, *C. chagunio*, *B. shacra*, *C. cachius*, *S. bacaila*, *G. annandalei*,*B. badis*, *M. cuchia* and *M. pancalus* were reported in the study but these species were not found from Koshi river (Limbu & Subba, 2011).

B. lohachata, and *A. aor* from Karnali river; *C. cachius*, *P. sophore*, *B. lohachata*, *M. bleekeri* and *A. aor* from Mahakali river were not recorded (Rajbanshi, 2012). But these species were found in present study from the markets of Saptari district. Fishes like *Barilius guttatus, Tor putitora, Pseudeutropius atherinoids* and *Olyra longicaudata* were not found in the markets. These fishes were reported in Triyuga river (Shrestha, 2016). Shrestha and yadav (2019) reported *Aspidiparia morar*, *Amblypharyngodon mola*, *Salmostoma acinaces*, *Clupisoma garua*, *Garra rupecula*, *Bagarius bagarius*, *Gagata cenia*, *Glyptothorax pectinopterus*, *Hara hara*, *Nangra nangra*, *N. viridescens*, *Sisor rhabdophorus* and *Tetradon cutcutia* from Keshaliya river, Morang. These fishes were not found in the markets of Saptari district.

Captive breeding is being practised in some district fishes like *C. batracus* (Fisheries Research Center, Pokhara; Regional Agricultural Research Station, Tarahara), *B. lohachata*, *G. annandalei* and *C. chagunio* (Kaligandaki Fish Hatchery, Syanja).

Moreover, the poor arrangement of fish markets like lack of infrastructures, lack of storage facilities, lack of electricity, financial crisis, etc., are also the cause of local production supply to other countries.

Conclusion

A large amount of fishes in the markets of Saptari district has to be imported from India. So, the enhancement of domestic captive fisheries is necessary to discourage the imported

Indian fishes. Illegal fishing of indigenous fishes from the rivers should be strictly banned for conserving the fishes in their natural habitats.

Acknowledgements

The authors like to thank fish sellers of different markets of Saptari district for their valuable contribution to collect primary data. We would also give a special thank to Nisha Acharya, M. Sc. Students (Zoology) to help in a survey on the fields to collect the data.

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Integrating Biological Resources for Prosperity, 2021, 419-423

Traditional Uses of Animals as Medicine Practiced by Dhimal Tribe at Damak, Jhapa District, Nepal

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Abstract

Nepal is a multiethnic and multilingual country. There are more than 50 ethnic groups in Nepal. Dhimal is also an ethnic group with its tradition, culture, language, and indigenous skills. They are found to have settled mainly in two districts of Nepal - Jhapa and Morang. The study was carried out from May 2004 to April 2005. The primary purpose of the study was to document the animals used by Dhimal people for medicinal purpose. Direct involvement, interview and questionnaires methods were applied during the research work. Dhimal has preserved their traditional culture and indigenous knowledge to some extent. Overall 24 species were used in medicine. Dhimal uses wild animals more than domesticated animals. They have sound indigenous knowledge about traditional medicine against various diseases.

Key words: Conservation, Ethnozoology, Indigenous, Jaundice, *Varanus flavescens*

Introduction

Nepal is one of the wealthiest countries in the world for its ethnic diversity and cultural heritage. The prefix "ethno" is generally used to refer to various types of indigenous knowledge widely prevalent among the different ethnic groups, e.g., ethnozoology means the study of animals and animal products by people of different cultures. The term indigenous knowledge is defined as the system generated by internal initiative within a local community. Dhimal is moderately rich in ethnozoology. They utilize various animals for different purposes like medicine, food, agriculture, ritual value, recreation etc. They are related with different mammals, birds, fishes, reptiles, amphibians and invertebrates. An ethnic group is a social group of the population in a large society set apart and bound together by common ties of race, language, culture and nationality. In other words, an ethnic group is an aggregate of distinct people in religion, language, culture, or nationality from other members of the society where they live (Rai & Dhungana, 2002). The different geoclimatic regions of Nepal are inhabited by various ethnic groups that have developed their own culture based on available national resources regarding biological diversity. Nepal has an extreme topographical difference and comprises almost all climatic zones of the world. More than 53 different tribes speaking about 75 different languages dwell in Nepal (Singh, 1995). Dhimal belongs to the ancient Nepalese ethnic group. The rural areas of Damak municipality are mostly dominated by Dhimal community. So they are the major tribe of the place. Dhimal is distributed only in two districts of Nepal-Jhapa and Morang. Recently, very little number of Dhimal has settled

in Sunsari as well. The origin place of Dhimal in Nepal is believed as Letang, Morang. The study site lies on Terai plain and bottom of Churiya mountains. The climate of Damak is tropical type. Due to urbanization and modern technology, many tribes have been forced to change their lifestyle, rituals and indigenous knowledge. It is, in fact, a very serious matter. Their traditional lifestyle should be protected (Rai, 2003).

In Ethiopia, the honey of the bee *Apis mellifera* was traditionally used for relieving various ailments with highest fidelity value while the upper coats of snake *Naja naja* and the teeth of crocodiles *Crocodylus* sp. had the lowest fidelity value (Kendie et al., 2018). In Cuicatec culture and subsistence, animals are essential elements for complementing their diet and medicine. In our country, Lapcha people from Ilam used various animal and plant species to treat different diseases and ailments such as respiratory tract infections, skeletomuscular problems, gastrointestinal disorders and dermatological infections (Tamang & Singh, 2014). Similarly, Rai community is rich in traditional medicinal knowledge. It has been using 27 different animal species belonging to 23 families in healing 28 ailments in their day to day life (Rai & Singh, 2015). The ethnic groups like Pahari and Danuwar from the central mountainous region of the country followed zoo-therapeutic practices. Mass production of such animals could help provide not only protein supplement but also animal-based medicines to the people (Lohani, 2012). The forests should be conserved to ensure the maintenance of animals which are a valuable part of nature, culture and the beauty of their territory (Solis & Casas, 2019).

Materials and Methods

Damak is one of the oldest municipalities situated in Jhapa District in Province No. 1 of Nepal (Fig. 1). It is surrounded by the Ratuwa River from the east and the Maawa River in the west. There is Sivalik Hills in its north and the intersection of Ratuwa River and Maawa River in the south. The population status of Dhimal in Damak is 6.5%. Damak Municipality has announced Dhimal people as the first citizen of the municipality.

The research procedure incorporates different ways of getting information about the ehnozoological data from the Dhimal people. The different materials needed to complete the work were ancient scripts, books, research papers, newspapers, journals, internet, camera, polythene bag, formalin, microscope, etc. Various methods were applied to get the information of medicinal value of various animals. Monthly field visits were made to get the detailed information. At every visit, the information about their indigenous knowledge was collected from the focused group like old persons, Vaidhya (Ayurvedic physician), wizard (Dhami, Ojha), knowledgeable persons, local leaders, teachers and ordinary people as well. The unknown animals collected during the visit were preserved in formalin and later classified with specialists' help. Interviews were conducted with local Dhimal to know their ethnicity, tradition, and animal uses in different purposes, especially medication. Questionnaires mostly containing objective and short questions were prepared and asked to the respondent. PRA method was applied to collect various ethnozoological data by active participation in their festivals, ceremonies, and rituals.

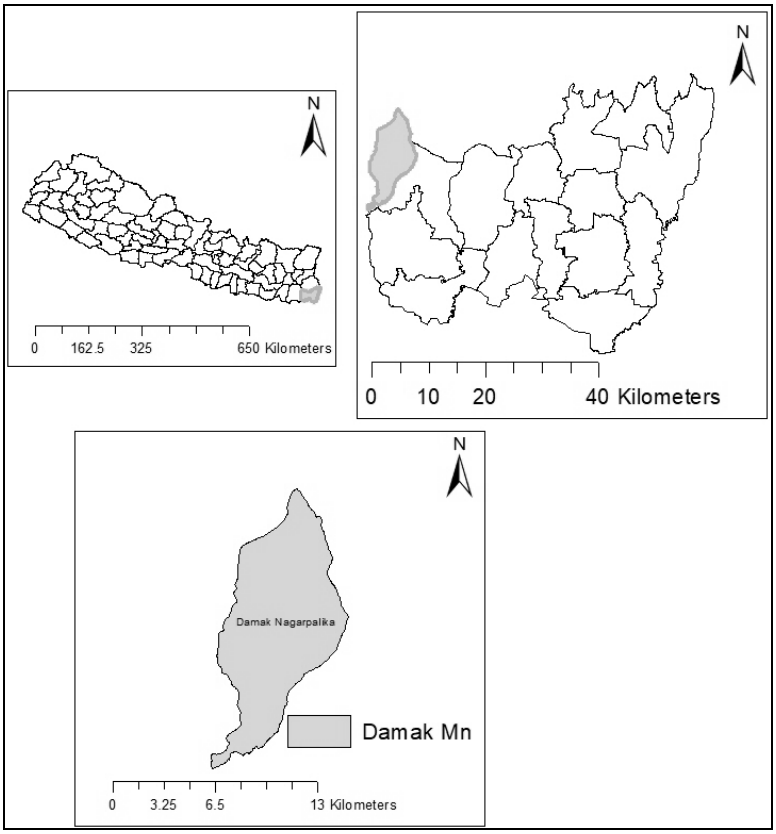

Figure 1. Map of study area.

Results

The indigenous knowledge of Dhimal in various aspects was fishing, farming, exorcism, hunting, liquor making, food varieties, medicinal skills, knitting, etc. This knowledge is passed from one generation to another. Dhimal still believes the sorcerers (Dhami, Ojha) to diagnose and cure the diseases. They called Dhamis on delivery case and other rites. Some of Ojhas really had a bit of knowledge about herbs and local medicines for common diseases like headache, fever, stomach pain, cuts, wounds, etc. Dhimal has knowledge on traditional medicine. Such medicines have been used since ancient time. Dhimal utilized altogether 24 species for medical purposes as given below.

Wild Fauna

Mammals: The meat of jackal (*Canis aureus*) has medicinal value for patients of tuberculosis. The wine extracted from its flesh is beneficial for Arthritis. The dried faeces of porcupine (*Hystrix brachyura*) are useful in abdomen pain and continuous cough. The meat of bat (*Rhinolophus* sp) is helpful in relieving the patient from asthma and night blindness. The fat of wild boar (*Sus scrofa*) is used to cure measles. The shell parts of pangolin (*Manis* sp) are used in necklace especially wreathed to the children. It is believed that a necklace

gives energy to children. The gall bladder of the bear (*Melursus ursinus*) is beneficial to the patient of body pain and Arthritis.

Aves: The blood of the crow (*Corvus splendens*) is externally applied on the ankle and cracks of sole of foot. The meat of common Myna (*Acridotheres tristis*) is consumed by the people suffering from jaundice. Similarly the meat or eggs of sandpiper (*Vanellus indicus*) are eaten by the patients of rheumatism for remedy.

Reptiles: The meat of Sungohoro (*Varanus flavescens*) is consumed in physical weakness, laziness and fever. Dhimals believe that they do not suffer from jaundice due to their habit of eating its meat. Its fat is applied on swelling and itching surface of the skin. The meat of Kalogohoro (*Varanus bengalensis*) is beneficial to piles and asthma. Its gall bladder is useful to malaria. The shell of turtle (*Lissemys* sp) is used to treat several infectious diseases to children. A paste is prepared by rubbing the shell on a stone with water and applied externally to treat measles and tonsillitis. The paste is also applied on the open wounds. The gall bladder of python (*Python molurus*) is used to cure wounds and cuts.

Amphibia: The meat of frog (*Hoplobatrachus tigrinus*) is used to cure malnutrition among children.

Pisces: Magur (*Clarias batrachus*) is very nutritious and beneficial to those suffering from physical weakness. The meat of Bam (*Amphipnous cuchia*) is consumed to get energy and to cure piles.

Invertebrates: The burning incense or Dhoop of scorpion (*Palamnaeus bengalensis*) is used to warm the newly born baby to prevent from allergy in future. The young earthworms (*Pheretima posthuma*) are used to cure the infected wounds and pneumonia. The shells of freshwater mussels (Unio sp.) are traditionally used to cut the cord (Sal) of newly born child to protect the child from tetanus. Their fried dishes are beneficial for the patient of diarrhoea. The flesh of Freshwater snails (*Bellamya bengalensis*) enrich the eye sight.

Domestic Fauna

Mammals: The fat of pig (*Sus domestica*) is used to cure pimples and smallpox. The brain of horse (*Equus* sp.) is used in typhoid and pneumonia.

Aves: The fat of local hen (*Gallus gallus domesticus*) is applied to the skin's burning part to reduce pain. The hot soup of pigeon (*Columba livia*) is consumed during common cold or flue.

Discussion

The Dhimal tribe is very close to nature. They worship nature such as land, river and forest. Most rural folk in Nepal depend on traditional medicine since most remote areas do not have access to modern medical services (Shaha & Tiwari, 2004). The indigenous knowledge of ethnic people should be protected. However, sometimes their activities affect biodiversity and natural resources in various ways. So conservation education is a must. The problem exists by killing the protected animal species like monitor lizards, pangolin, turtle, bat, etc. The leather of different animals is used to make musical instruments (Schleich & Kastle, 2002). The majority of Dhimal is unknown to the conservation act. It is a severe problem. Since

Dhimal is fond of meat, they kill or hunt animals. They kill some animals for medicine too. Anyway, wildlife and their habitat destruction cause negative impacts on the environment and natural balance. Firstly, ethnic people should be well educated to understand the value of animals and animal products. Awareness programmes should be carried out in the local community. They should be encouraged to use alternative animals instead of endangered species. Besides, the concept of wildlife farming may be developed. The conservation process may be practised in a better way by local people's direct participation.

Acknowledgments

I am grateful to Prof. Dr. Kalu Ram Rai, Mechi Multiple Campus, Bhadrapur for his continuous guidance and valuable suggestions to complete my research work. My sincere gratitude goes to Mr. Ganesh Tamang, Lecturer, Central Campus of Technologys, Hattisar, Dharan, for providing essential study materials. I acknowledge Mr. Ram Bahadur Dhimal, Mr. Bhumi Nanda Dhimal, and the whole Dhimal community for their kind co-operation throughout my field visit.

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Identification of Gastro-intestinal Parasites from Faecal Matters of Rhesus Monkeys (*Macaca mulatta* Zimmermann, 1780) of Dharan, Nepal

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Abstract

The study was conducted to identify the gastrointestinal parasites in rhesus monkeys of Dharan. Sampling was done in the morning (6-10 AM) twice a week for five months. Macroscopic and microscopic observations were done for the identification of parasites. Among 124 examined samples, 54.03% (n=67) samples were found to be positive with at least one parasites, 31.19% (n=39) samples were negative for the parasites and 14.51% (n=18) of them were unidentified. Out of 67 positive samples, 40 % (n=27) samples were found to be positive with protozoans, whereas 60% (n=40) samples were found to be positive with helminths. A total of 68% (n=45) of the samples showed single parasitic infestation, 24% (n=16) of the samples showed double and 8% (n=6) of the samples showed multiple parasitic infestations. The parasites identified were four species of protozoa and six species of helminths. The protozoans include *Entamoeba coli, E. histolytica, Balantidium coli* and *Eimeria* sp. The helminths include *Ascaris lumbricoides, Strongyloides stecoralis, Taenia* sp.*, Enterobius vermicularis, Trichuris trichuria* and *Ancyclostoma duodenale*. The study shows that the *Ascaris lumbricoides* has the highest prevalence (34.32%, n =23) in the rhesus monkeys of Dharan.

Key words: *Ascaris lumbricoides*, Dharan, *Entamoeba coli*, Faecal matters, Gastro-intestinal parasites, Rhesus monkey

Introduction

Intestinal parasitic infection includes both the protozoans and helminths, which are the most common infections worldwide. Faeces are the most frequent specimen collected and examined for gastrointestinal parasites' presence (Khanna et al., 2014). Macaques are primates having very high adapting characteristics and are distributed more widely than any other non-primate genus. They are found in tropical forests, dry savannas, mountains, villages, temples and even in large cities (Van Hoff, 1990). Three species of non-human primates, viz., Hanuman langur, Rhesus and Assamese monkeys, have been recorded from Nepal (Chalise, 1999). Trees are the primary sources of food for all the macaques. They depend upon various plant parts such as fruits, leaves, flowers, barks, etc., as well as vines and epiphytes. Rhesus macaques living in urban areas are dependent on human cooked foods such as chapattis, bread, roasted grains, groundnuts, splashed items, and even junk foods (Fooden, 1980). Rhesus macaques (*Macaca mulatta*) are adapted well and co-existing with the human in both the urban and agricultural areas (Cawthon, 2005). Jha et al. (2011) stated, 'parasites play a central part in

the ecosystem and affect the ecology and species interactions (Esch & Fernandez, 1993), host population and regulation (Hudson et al., 1998; Hochachka & Dhont, 2000) and community diversity (Hudson et al., 2002). Since the monkeys live near human residents, they share not only human foods but also parasites. Their close phylogenetic relationship with humans often results in a high potential of pathogen exchange (Cheng, 1986). Thus, the rhesus monkey population of Dharan may provide a current status of intestinal parasites, both of zoonotic and anthropologic importance. Rhesus monkey and humans are very close in terms of physiologic and genetic characters thus, they share infectious agents like intestinal parasites along with the foods. Many shreds of evidence show that many emerging parasites in humans have originated from the primates (zoonosis) and in the same way, there is a great risk of transmission of human diseases to the primates (Jones-Engel et al., 2006). Monkeys tend to remain around the periphery of the temples; thus Dharan consists of many monkeys. This research also can be helpful for those who are interested in similar fields. Rhesus macaque is one of the species of old world monkey that reside near the human residents and often feed on plants, insects, and human leftovers and refusals. Due to their unhygienic feeding habits, they could be infested with intestinal parasites that deteriorate their health conditions. As these are non-human primates living together with humans, there is a high chance of transferring zoonotic diseases. Humans are always prone to be infected by most of the rhesus' parasites; thus, they are zoonotic importance. The study's primary purpose is to identify the intestinal parasites from the faecal matter of the rhesus monkeys of Dharan and its significance in the transfer of the various zoonotic diseases.

Materials and Methods

Study Area

Dharan is a sub-metropolitan city located in Province 1 of Nepal in the Sunsari district. It is situated at the foothills of Mahabharat range in the north while it joins the Terai at the south's tip. It lies at an altitude of 349 m from the sea level. It has the coordinates of 26°49'0"N, 87°17'0"E. Dharan is a religious place having different temples such as Budhasubba, Dantakali, Panchakanya, etc., study area divided into major blocks: A (Vijaypur forest area) and block-B (Dharan town area). Block-A was sub-divided into five sampling stations, viz., Panchkanya temple area, Dantakali temple area, Pindeshowri temple area, Hattisar Campus area, and Budhasubba temple area. Similarly, block-B was also sub-divided into five sampling stations, viz., Bhanu Chowk area, Chhata Chowk area, Krishi Amarhat, Sabji Mandi and Khatri Dhara area (Fig. 1).

Sampling

The collection of faecal matters was done randomly in the morning time between 06.00 to 10.00 a.m., twice a week, from ten different sites of Dharan from February to August 2019. In every attempt, about 5 g of fresh faecal samples were collected with a single-use wooden spoon and kept in sterile vials containing 2.5% potassium dichromate preservative solution 6 samples were discarded after found to be contaminated. Thus, 124 samples were taken for laboratory examinations. Macroscopic and microscopic observations were done in the laboratory of Biology department at Central Campus of Technology, Dharan.

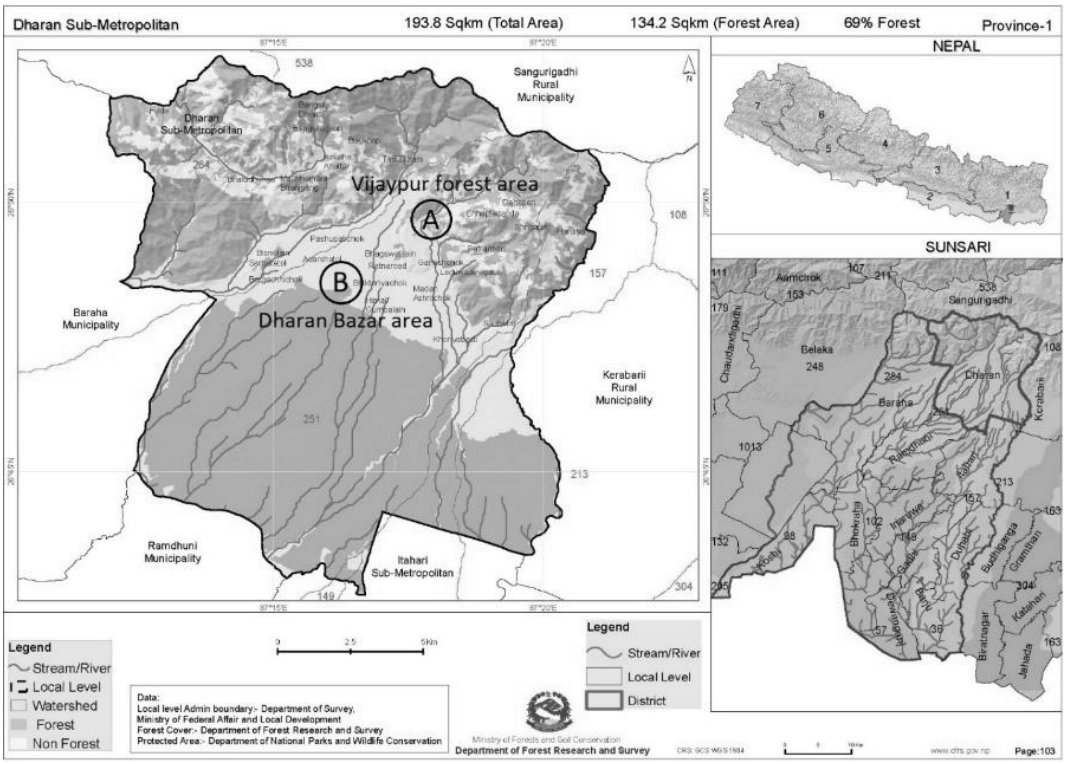

Figure 1. Sampling sites (block- A and block- B)

Observations

Temporary slides of each sample were examined under light microscopy at 10X and 40X magnification. One hundred and thirty samples were collected using a random sampling method from ten sampling sites. Staining of slides was done using a wet mount method using Lugol's iodine, concentration techniques like faecal floatation and sedimentation. Microscopic examinations were done to detect and identify the cysts, oocytes and trophozoites of protozoan parasites, eggs and larvae of helminthic parasites. Identification of parasites was made consulting literature of Arora and Arora (2007), Dubey and Maheshwari (2013), Parija (2013) and Chatarjee (2017). The photographs of parasites were taken using a Canon digital camera mounted on the top of the microscope.

Results

The laboratory examination of the total 124 samples of the faecal matters of the rhesus monkeys showed 54.03% (n= 67) samples to be positive for the presence of at least one type of parasites, 31.45% (n=39) of the samples were found to be negative for the parasites whereas 14.52% (n=18) of the samples contained possibly unidentified parasitic species (Fig. 2). Similarly, 60% (n=40) of total positive samples were infested with helminths and 40% (n=27) with protozoan parasites (Fig. 3).

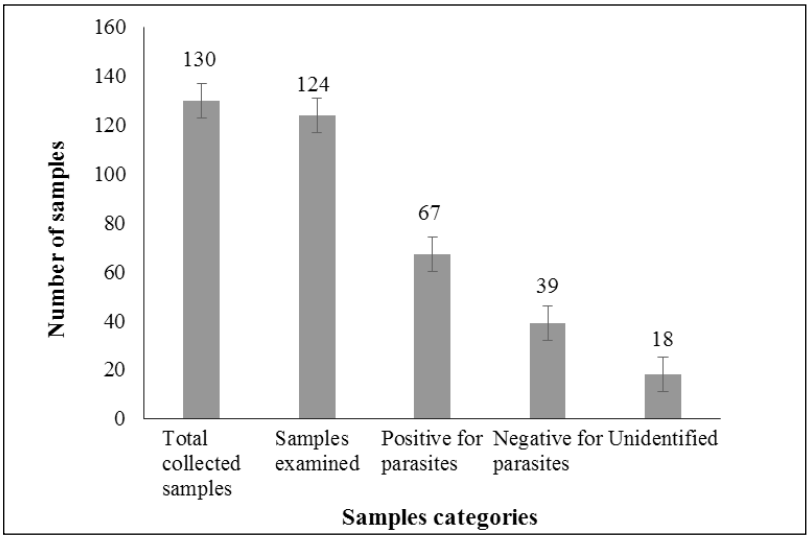

Figure 2. Consideration of collected samples

The study showed the presence of four species of protozoa which include *Entamoeba coli* 25.37% (n=17), *E. histolytica* 7.46% (n=5), *Balantidium coli* 5.97% (n=4), and *Eimeria* sp 1.49% (*n*=1) (Table 1). The helminths include six different species which are *Ascaris lumbricoids* 34.32% (n=23), *Ancyclostoma duodenale* 17.91% (n=11), *Enterobius vermicularis* 4.47% (n=3), *Trichuris trichuria* 1.49% (n=1), *Strongyloids* sp. 1.49% (n=1), *Taenia* sp. 1.49% (n=1) (Fig. 4). Out of 67 positive stool samples, 68% (n=45) of the samples were found to have a single parasitic infestation, 24% (n=16) of them had double parasitic infestations whereas 8% (n=6) of them had multiple parasitic infestations (Fig. 5).

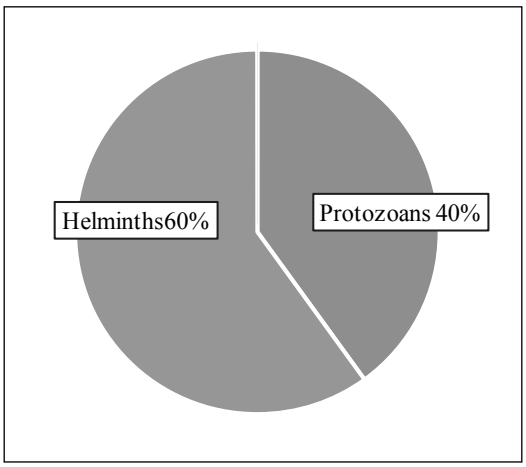

Figure 3. Prevalence of parasitic infestations in positive samples**.**

Table 1. Number of parasites infested samples.

Protozoan parasites	**No. of positive samples**
Entamoeba coli	17 (15C, 2T)
E. histolytica	5 (C)
Balantidium coli	4 (2C, 2T)
Eimeria sp.	1 (C)
Total no. of protozoans infected samples	27
Helminths parasites	
Ascaris lumbriciodes	23 (14A, 9E)
Ancylostoma duodenale	11 (E)
Taenia sp.	1 (P)
Enterobius vermicularis	3 (A)
Trichuris trichuria	1 (E)
Strongylois stecoralis	1 (L)
Total no. of helminths infested samples	40
Total no. of parasites infested samples	67

A= Adult, C = Cyst, E = Egg, O = Ova, T = Trophozoite, P= Proglottids, L=Larva

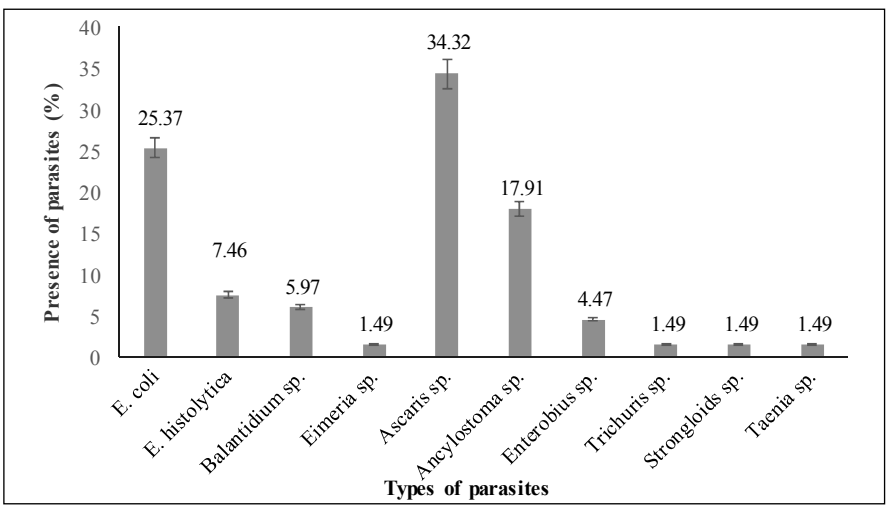

Figure 4. Prevalence of intestinal parasites in collected samples.

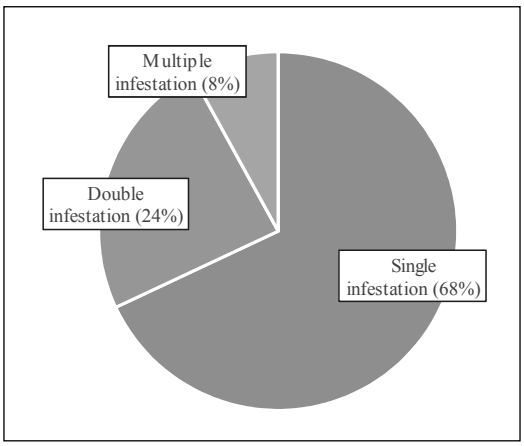

Figure 5. The intensity of parasitic infestations (based on the type of parasites)

Discussion

All the parasites identified in this study are in support of Soulsby (1982). He has listed *Entamoeba, Balantidium, Strongyloides, Oesophagostomum, Trichostrongylus* and *Trichuris* all as infecting non-human primates. Thus, these findings support the result of the present study. According to this study, the prevalence of helminth infection (47.05%) was higher than protozoal infection (21. 17%). The result coincided with Jha et al. (2011), who also reported similar results, viz., 59.5% and 53.72% for helminth and protozoal infection, respectively. The present study shows conflict with Hilser et al. (2011), who recorded that 62% of monkeys were positive for helminth infection, and 82% were protozoans. These differences may be due to geographic conditions, source of feeds and feeding behaviour of monkeys.

According to the present research, the prevalence of *Ascaris lumbricoides was* the highest (34.32%) among all the parasites reported. The result was dissimilar to that of a study carried out in Devghat, Chitwan by Adhikari and Dhakal (2018). They found a comparatively lower (11.82%) prevalence of *Ascaris lumbricoides*. This was also confirmed from Red langur (Hilser et al., 2011), Hanuman langur and Rhesus macaque (Parmar et al., 2012) and Assamese Macaque (Pokhrel & Maharjan, 2015). However, Arunachalam et al. (2015) reported it to be 5%. The widespread infection of *Strongyloides* sp. was 1.49%. Adhikari and Dhakal (2018) reported to be 10.75% did not support it. The result was also conflicted with the research done by Hilser et al. (2011) from Red langur and by Pokhrel and Maharjan (2015) from Assamese macaque. Hookworm was found to be 17.91% as found in the research. The result was found to be much higher than that of the report given by Adhikari and Dhakal (2018) which was 3.22%. It is dissimilar to the reports of Pokhrel and Maharjan (2015) with 4.7% and contrary to the result of Hilser et al. (2011) and Mutani et al. (2003) with 8% and 34%, respectively.

Regarding the intensity of infection, the study shows that 68% of the monkeys have single infection, 24% of them have double infections whereas 8% of them had multiple infections. The result shows conflict with the result as given by Jha et al. (2011) where 27.96% of monkeys had a single infection, 39.78% had double and 32.26% had multiple infections. In this study, 54.03% (n=67) samples were found to be positive for the presence of at least one type of parasite. The result differs from the study done by Adhikari and Dhakal (2018) where 74.20% (n=69) samples were found positive for at least one type of parasites in the faecal matters of Rhesus Macaque and Hanuman langur. Jha et al. (2011) revealed 72.94% and 76.86% positive cases from Assamese macaque and Rhesus macaque, respectively. This was almost similar to the findings of Adhikari and Dhakal (2018). In captive monkeys, the prevalence rate was lower (13.63%) in Assam State Zoo (Nath et al., 2012). This could be due to regular screening of faecal samples and periodical anti-helminthic treatment in most of the zoos, as per the protocol of zoo authority.

Conclusion

The monkeys of Dharan were found infected with various protozoans and helminthic gastro-

intestinal parasites. Among the protozoal infections, *Entamoeba coli* was the highest prevalent (25.37%) as compared to other protozoa whereas among helminths *Ascaris lumbricoides* (34.32%) showed the highest prevalence. Since, 54.03% of the monkeys showed parasitic infestations, they are at higher risks to critical conditions of gastro-intestinal parasites. Thus, it makes it clear that 32% of monkeys harboured more than one type of parasite. Multiple infections show impacts on the growth pattern, reproduction, fecundity, and establishment and the death of the monkeys. The high transmission rate might be possible due to high population density or favourable environmental conditions for parasites. It would be rational to consider these monkey populations as the reservoir hosts of humans' intestinal parasites.

Thus, it is recommended that local people be aware of the transmission of various diseases by contaminating the monkeys' faecal matters. The concerned authorities should treat the monkeys by giving anti protozoan and anti-helminths drugs to reduce loads of the parasites in their bodies and to prevent the transmission of the zoonotic diseases to the local people. 31.45% (n= 39) of the samples were found to be free from parasites. The reason may be due to the less burden of parasitic infections or they are parasites free. The reason is not apparent as the experiment was solely based on the monkeys' faecal matters.

Acknowledgements

We want to acknowledge the Department of Biology, Central Campus of Technology (CCT), Dharan for providing laboratory facilities to conduct this study. We want to extend our thanks to the library staff of CCT for providing concerning literature.

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Summary of the National Conference

The National Conference on *Integrating Biological Resources for Prosperity* was organized on February 6-7, 2020 (Magh, 23-24, 2076 B.S.) at Hotel Eastern Star, Biratnagar in collaboration of the Botanical Society of Nepal, Kathmandu; the Nepal Biological Society, Biratnagar; the Government of Nepal, the Ministry of Forests and Environment (MoFE), Kathmandu; and the Ministry of Industry, Tourism, Forest and Environment (MoITFE), Province No. 1, Biratnagar.

In the two-day program, the first day sessions were divided into (a) Inaugural session, (b) Plenary session, (c) Special symposium, and (d) Technical (oral and posters) session, and in second day it was conducted as (a) Technical (oral and posters) session, (b) Special symposium, and (c) Resolution and valedictory session. The overview of program schedule was as follows.

10.00-06.00PM	**Registration (5 February 2020)**				
	DAY 1 Thursday (6 February 2020 / 23 Magh 2076)				
07.30-09.00AM	**Registration/Breakfast**				
09.00-11.00AM	**Hall A (Sagarmatha): Inaugural session**				
11.00-12.30PM	**Hall A (Sagarmatha) Plenary session**		**Hall C (Kanchenjunga) Special Symposium: Department of Environment (DoE) and Department of Plant Resources (DPR)**		
12.30-01.30PM	**Lunch**				
Location	**Hall A (Sagarmatha)**	**Hall B (Lhotse)**	**Hall C (Kanchenjunga)**	**Hall D (Makalu)**	**Poster (Outside)**
01.30-03.45PM	**General**	**Biotechnology and Biochemistry**	**Ecology and Environment**	**Higher Plant Diver. & Systematics**	**Faunal Diversity and Ichthyology**
03.45-04.00PM	**Tea Break**				
04.00-05.30PM	**Ichthyology**	**Lower Plant Diversity: Algae, Bryophyte and Pteridophyte**	**Ecology and Environment**	**Biological Invasion**	**Biotechnology, Biochemistry, Fungi and Plant Pathology**
05.30-05.45PM	**Tea Break**				
06.30-07.00PM	**Hall A (Sagarmatha): Visit Nepal 2020 Documentary**				
07.00-08.00PM	**Welcome Dinner**				
	DAY 2 Friday (7 February 2020 / 24 Magh 2076)				
07.30-08.00AM	**Breakfast**				
08.00-10.15AM	**Faunal diversity**	**Medicinal Plants and Ethnobiology**	**Ecology and Environment**	**Biological Invasion**	**Lower Plant Diversity: Algae, Bryophyte and Pteridophyte**
10.15-10.30AM	**Tea Break**				
10.30-12.30PM	**Wildlife Conservatn**	**Medicinal Plants and Ethnobiology**	**Ecology and Environment**	**Fungi and Plant Pathology**	**Ecology and Environment**
12.30-01.30PM	**Lunch**				
01.30-03.15PM	**Wildlife Disease**	**Special Symposium: REDD Implementation Centre, DoE and DPR**	**Ecology and Environment**	**Fungi and Plant Pathology**	**Medicinal Plants and Ethnobiology**
03.15-03.30PM	**Tea**				
03.30-05.00PM	**Hall A (Sagarmatha): Resolution and Valedictory session**				
07.00-08.00PM	**Farewell Dinner**				

Inaugural Session

The inaugural session of the conference was chaired by Mr. Jagadish Prasad Kusiyait, Honorable Minister for Industry, Tourism, Forest and Environment, Province No. 1. The conference was

inaugurated by Honourable Chief Minister, Mr. Sherdhan Rai of Province No. 1. It was attended by the Cabinet Ministers of the Province, Vice-Chairman and Member of the Provincial Planning Commission, Chairperson and Members of Province Public Service Commission, Province No. 1 as the guests.

The secretary of the Ministry of Forests and Environment, Kathmandu; the Secretaries of the Government of Province No. 1; the dignitaries of Tribhuvan University and Purbanchal University; Mayors; Professors of Tribhuvan University and Purbanchal University; the C.D.O. of Morang; Campus Chiefs of Mahendra Morang Adarsha Multiple Campus; Post Graduate Campus, Biratnagar; Central Campus of Technology, Dharan; Mechi Multiple Campus, Bhadrapur; Chairperson and dignities of valued partner organizations including CODEFUND Kathmandu; Chamber of Industries, Province No. 1; Morang Merchants Association, Biratnagar; Chief of REDD Implementation Center; Director Generals of the Department of Environment and the Department of Plant Resources, Plant Resource Center, Ilam; Nepal Academy of Science and Technology (NAST); HISSAN, Morang; PABSON, Nepal; media persons were also attended the inaugural session.

Prof. Dr. Mohan Siwakoti, the Chairman of Conference Organizing Committee and President of Botanical Society of Nepal delivered the welcome address and highlighted the profile of the conference. During the ceremony, two keynote addresses were delivered by Professor Emeritus Dr. Pramod Kumar Jha on "*Importance of biological resources for prosperity in Nepal*" and by Professor Emeritus Dr. Ram Prasad Chaudhary on "*Importance of biodiversity conservation for human well-being*". Dr. Bishwa Nath Oli, Secretary of the Ministry of Forests and Environment, Government of Nepal delivered his remark on state of biodiversity in Nepal.

Highlighting the importance of the conference, the inaugural address was delivered by Honorable Chief Minister as a Chief Guest Mr. Sherdhan Rai assuring to implement the resolution of the conference. On the occasion, "Plant Diversity in Nepal" a book published by the Botanical Society of Nepal has launched for the public by the Honorable Chief Minister. Dr. Kamal Krishna Joshi, former Chairman of University Grants Commission, Nepal and former Vice Chancellor of Tribhuvan University reviewed the book which covers the diversity of the plants ranging from algae to higher plants. Prof. Dr. Sasinath Jha, the president of Nepal Biological Society, Biratnagar delivered vote of thanks to the Honorable Guests, dignitaries, distinguished participants and all the supporters of the conference. Concluding remark in the inaugural session highlighting the scope and conservation of biodiversity and development of touristic places in the Province No. 1 was forwarded by the Chair, Mr. Jagadish Prasad Kusiyait, Honorable Minister for Industry, Tourism, Forest and Environment.

Plenary Session

During the plenary session, five thematic papers were presented by senior biologists. Dr. Tirtha Bahadur Shrestha and Prof. Dr. Sanu Devi Joshi pointed out the importance of plant science education for the society. Dr. Kamal Krishna Joshi argued the importance of biocultural diversity. Similarly, Dr. Keshab Raj Rajbhandari delivered the status and distribution of endemic flowering plants of Nepal and Dr. Binay Kumar Chakraborty reviewed the status of aquatic biodiversity of Bangladesh.

Technical Session

During the two-day conference, altogether 27 technical sessions (20 oral and 7 poster sessions) were conducted. Four parallel oral sessions were conducted at the same time in four different spacious and interactive halls. Each session was chaired by senior professors and eminent personalities of concerning fields and was accompanied by rapporteurs to note the comments upon.

In technical session following thematic papers were presented (figures inside the brackets indicated number of papers) as Biological invasion (13), Biotechnology and biochemistry (9), Ecology and environment (42), Faunal diversity (9), Fungi and plant pathology (13), General fields (7), Higher plants and systematics (9), Ichthyology (7), Lower plant diversity (algae, bryophytes and pteridophytes) (7), Medicinal plants and ethnobiology (16), Wildlife conservation (7), and Wildlife diseases (6).

Altogether, 153 oral presentations were delivered accompanied by 62 poster presentations in the technical sessions. The total number of presentations were 215 including two keynotes and five plenary presentations. The number of the papers was maximum from ecology and environment (59) followed by medicinal plants and ethnobiology (26) and biological invasion (13) combining both oral and poster presentations. Altogether about 350 distinguished participants and dignitaries from Nepal, India and Bangladesh attended the conference to update and share their knowledge and experiences.

Two special symposia were conducted by the Department of Environment (DOE); the Department of Plant Resources (DPR), Kathmandu / Plant Resources Center, Ilam; and the REDD Implementation Center, Kathmandu. Those symposia were focused on the current trends and issues of the environments, REED implementation, and medicinal plants with the interactions to targeted people. The chief guest of second special symposium was Honorable Ms. Sita Thebe Jabegu, the President, Industry, Tourism and Environment Parliamentary Committee, Province No. 1, and chaired by Dr. Bishwa Nath Oli, Secretary (MoFE). The first special symposium was chaired by Ms. Abha Shrestha, Director General of the Department of Environment, Kathmandu.

Resolution and Valedictory Session

A feedback form was distributed to each participant to collect their opinion about the expectation, experience, and output of the conference. On the basis of participants' view and experts' comments, a resolution was drafted and discussed in the resolution session. A resolution drafting committee was formed including Prof. Dr. Ram Prasad Chaudhary, Prof. Dr. Mohan Siwakoti, Prof. Dr. Min Raj Dhakal, Prof. Dr. Sasinath Jha, Prof. Dr. Tej Narayan Mandal, Prof. Dr. Sangeeta Rajbhandary, and Dr. Bharat Babu Shrestha for the preparation of Biratnagar Declaration 2020. The resolution was endorsed in valedictory session.

Valedictory Session

The valedictory session was chaired by Prof. Dr. Mohan Siwakoti, Chairman of the conference organizing committee. The chief guest for the closing session was Honorable Mr. Narayan Bahadur Burja Magar, Member of the Parliamentary Committee for Industry, Tourism and Environment, Province No. 1. Prof. Dr. Shiva Kumar Rai, Co-Convener of the conference summarized the conference activities. Prof. Dr. Ram Prasad Chaudhary presented the resolution of the conference (Biratnagar Declaration) for endorsement. Dr. Bishwa Nath Oli, the guest of honour, the Secretary of MoFE mentioned the conference as a successful conference and thanked to all participants and supporting institutions. Senior botanist Dr. Tirtha Bahadur Shrestha also congratulated to the organizing committee and all the participants to make the conference a grand success. Ms. Tilmaya Dhakal of Siddhanath Science Campus, Kanchanpur and Ms. Gita Shrestha of Mechi Multiple Campus, Jhapa presented their remarks on the behalf of participants. Prof. Dr. Mohan Siwakoti concluded the conference with vote of thanks to all participants and supporting organizations and individuals. The program was conducted by Mr. Sanjeev Kumar Rai, Convener of the conference and the Secretary of MoITFE, Province No. 1.

Conference Committees

For conducting the historic and well-managed conference, an advisory committee, organizing committee, and technical committee were formed in the beginning as follows.

Advisory Committee

1. Hon. Mr. Jagadish Prasad Kusiyait - Minister; Industry, Tourism, Forest and Environment, Province No.1
2. Hon. Mr. Subodh Raj Pyakurel - Vice Chairman, Province Planning Commission, Province No. 1
3. Mr. Bhim Parajuli - Mayor, Biratnagar Metropolitan City, Biratnagar, Morang
4. Dr. Bishwa Nath Oli - Secretary, Ministry of Forests and Environment, GoN
5. Prof. Dr. Sanu Devi Joshi - Academician NAST
6. Prof. Dr. Pramod Kumar Jha - Professor Emeritus, CDB, TU; Academician NAST
7. Dr. Baburam Timalsena - Campus Chief, Mahendra Morang A. M. Campus, TU, Biratnagar
8. Mr. Mahesh Kumar Khatri - Campus Chief, Post Graduate Campus, TU, Biratnagar
9. Prof. Dr. Dhan Bahadur Karki - Campus Chief, Central Campus of Technology, TU, Dharan
10. Prof. Dr. Ram Kailash Prasad Yadav - Head, Central Department of Botany, TU, Kathmandu
11. Prof. Dr. Tej Bahadur Thapa - Head, Central Department of Zoology, TU, Kathmandu
12. Prof. Dr. Sasinath Jha - President, Nepal Biological Society, Biratnagar
13. Prof. Dr. Ram Bahadur Thapa - Post Graduate Campus, Tribhuvan University, Biratnagar,
14. Prof. Dr. Min Raj Dhakal - Director, Research Division, Purbanchal University, Biratnagar
15. Dr. Bharat Raj Subba - Post Graduate Campus, TU, Biratnagar
16. Prof. Dr. Kalu Ram Khambu - Mechi Multiple Campus, Bhadrapur, Jhapa
17. Prof. Dr. Damodar Thapa Chhetry - Head, Department of Zoology, Post Graduate Campus, TU, Biratnagar
18. Mr. Dhananjaya Poudel - DG, Department of Plant Resources, Thapathali, Kathmandu
19. Dr. Buddi Sagar Poudel - Chief, REDD Implementation Centre, Kathmandu
20. Mr. Sushil Kumar Jha - Post Graduate Campus, TU, Biratnagar
21. Dr. Ganesh Bahadur Thapa - Chief, Natural History Museum, TU, Kathmandu
22. Mr. Shailendra Kumar Pokharel - Founder President/ED, Conservation Development Foundation, Kathmandu

Organizing Committee

Chairman	:	Prof. Dr. Mohan Siwakoti (BSON)
Convener	:	Mr. Sanjeev Kumar Rai (BSON/Secretary, MOITFE, Province No. 1)
Co-Convener	:	Prof. Dr. Shiva Kumar Rai (BSON/NBS)
Secretary	:	Dr. Tilak Prasad Gautam (NBS)
Treasurer	:	Prof. Dr. Umesh Koirala (NBS)
Members	:	Dr. Hari Prasad Aryal (BSON/NBS)
	:	Dr. Dil Kumar Limbu (NBS)
	:	Dr. Bhabindra Niroula (NBS)
	:	Dr. Narayan Prasad Ghimire (BSON)
	:	Mr. Dipak Lamichhane (BSON)
	:	Mr. Kul Prasad Limbu (NBS)
	:	Mrs. Radha Adhikari (BSON/NBS)
	:	Mr. Ram Chandra Adhikari (NBS)

Technical Committee

Co-Ordinator	:	Prof. Dr. Tej Narayan Mandal
Members	:	Dr. Tilak Prasad Gautam
	:	Mr. Kul Prasad Limbu

The contact person of the conference was Co-Convener Prof. Dr. Shiva Kumar Rai, who has communicated and updated the information/notices to all the participants and corresponding authors through email and phone and received abstracts. To conduct the conference timely and smoothly six sub-committees and a team of volunteers were also formed as follows.

Sub-Committees

Finance:	Prof. Dr. Umesh Koirala Mr. Sanjeev Kumar Rai	Prof. Dr. Mohan Siwakoti Dr. Prakash Kumar Yadav
Abstract Publication:	Prof. Dr. Tej Narayan Mandal Dr. Tilak Prasad Gautam	Prof. Dr. Shiva Kumar Rai Mr. Kul Prasad Limbu
Accommodation/Transport/ Food Management:	Ms. Radha Adhikari Mr. Lalit Narayan Mandar	Mr. Anil Kumar Das Dr. Narayan Prasad Ghimire
Venue Management/ Publicity, Press and Media:	Dr. Bhabindra Niroula Mr. Ram Chandra Adhikari	Mr. Shaligram Adhikaree Dr. Krishna Prasad Bhattarai
Programme Management:	Prof. Dr. Shiva Kumar Rai Mr. Kul Prasad Limbu	Dr. Hari Prasad Aryal Mr. Rajesh Tamang
Registration:	Mr. Jay Narayan Shrestha Ms. Bindu Pokharel (Bhattarai)	Mr. Bishnu Dev Das Ms. Sabitri Shrestha

Volunteers

To assist the conference, total 34 students of Post Graduate Campus, Biratnagar and Mahendra Morang A.M. Campus, Biratnagar were nominated as volunteers with following responsibilities.

Accommodation	: Om Prakash Aryal, Mohan Sharma, Radha Shrestha, Reedima Rai
Transport	: Bheshraj Gautam, Sanjeev Majhi, Nabaraj Ghimire, Upendra Sahni
Food management	: Asbina Sharma Mainali, Roma Shris Thapa, Mamata Kumari Mandal
Decoration	: Bidhya Regmi, Karishma Dhakal, Radha shrestha, Raju Chaudhary
Registration and enquiry desk	: Pushpa Poudel, Rashmi Shrestha, Rubina Rai, Julee Meheta
Book display desk	: Bheshraj Gautam, Susmita Parajuli, Nirmal Mandal
Health desk	: Yasir Jamal, Nidhi Saha
Technical session (Poster)	: Rijan Ojha, Kalpana Chemjong
Technical session (Oral):	
Sagarmatha Hall	: Birendra Bista, Sabina Dahal, Ram Chandra Wagle, Reedima Rai
Kanchanjangha Hall	: Raju Chaudhary, Mohan Sharma, Radha Shrestha, Mamata Kumari Mandal
Makalu Hall	: Sanjeev Majhi, Bheshraj Gautam, Pushpa Poudel, Rashmi Shrestha
Lhotse Hall	: Anup Khatiwada, Om Prakash Aryal, Biddhya Regmi, Karishma Dhakal

All Rounder (to link and communicate four halls): Rijan Ojha, Biddhya Regmi

Accommodation, Transport and First Aid

To provide a congenial stay and better hospitality, all the delegates, dignitaries, distinguished guests and participants outside from Biratnagar were accommodated free of charge at Hotel Harrison Palace, Hotel Eastern Star, Hotel Asiatique, Hotel Swagatam, Hotel Shree Krishna, Hotel Vintuna, Marwari Sewa Sadan, and Balaji Atithi Sadan. Similarly, two-way travel fare by airplane for some distinguished guests and keynote speakers from Kathmandu were managed by organizing committee. The organizers had also managed two deluxe buses for two-ways for 74 participants from Kathmandu. The health desk from Koshi Zonal Hospital was also conducted throughout two days during the conference.

Conference Supporting Organizations

The conference was supported financially and morally by the following institutions and organizations.

- USAID's Hariyo Ban Program, WWF Nepal
- Department of Environment, Ministry of Forests and Environment, Kathmandu
- Department of Plant Resources, Kathmandu / Plant Research Center, Ilam
- REDD Implementation Centre, Kathmandu
- Biratnagar Metropolitan, Morang
- CODEFUND, Conservation Development Foundation, Kathmandu
- Nepal Academy of Science and Technology (NAST), Khumaltar, Kathmandu
- IUCN Nepal, Kathmandu
- Mahendra Morang Adarsha Multiple Campus, Tribhuvan University, Biratnagar
- Central Campus of Technology, Tribhuvan University, Dharan
- Post Graduate Campus, Tribhuvan University, Biratnagar
- Purbanchal University, Biratnagar
- HISSAN, Morang
- Sundar Haraicha Municipality, Morang
- Mechi Multiple Campus, Tribhuvan University, Bhadrapur, Jhapa
- Chamber of Industries, Province 1
- Morang Merchants Association, Biratnagar

Biratnagar Declaration 2020

- *Noting* that Nepal being a part of Himalayan biodiversity hotspot occupying about 0.1% of the global land area, is proportionately rich in biodiversity, and that local communities play a key role in the conservation and sustainable use of biological resources;
- *Acknowledging* that majority of people in the country are dependent upon biological resources and ecosystem services, including glaciers and river systems for drinking water and irrigation;
- *Acknowledging* that the high cultural diversity in the country possessed by indigenous peoples and local communities (IPLCs) that are reflected through rich indigenous knowledge and traditional practices are pertinent to conservation and management of biological resources for their livelihoods, and country's prosperity;
- *Recognizing* however, that the country faces a variety of anthropogenic and natural threats including climate change to biodiversity and ecosystem services that have serious negative impacts on the livelihoods of the population and environmental sustainability;
- *Further recognizing* that there are important gaps in knowledge and information of biological diversity, and ecosystem functions across the country including Province No. 1;
- *Further noting* that inadequate funds and weak management of biological resources constitute additional challenges to attain the goals of biodiversity conservation and development;
- *Recognizing further* that national assessment of the economic, ecological and cultural values of biodiversity are needed for its management as the public good, and the values be integrated into public and private decision making through the participation of local and indigenous communities and national stakeholders;
- *Noting with satisfaction* that Nepal, and its provinces are committed to address the existing and future issues related to biodiversity conservation and management through socio-ecological approach;
- *Aware* that Nepal is party to the Convention on Biological Diversity (CBD), the United Nations Framework Convention on Climate Change (UNFCCC), the Sustainable Development Agenda 2030, and related multilateral / bilateral international treaties / agreements, and conventions; and
- *Expressing gratitude* to the Honourable Chief Minister of the Province No. 1; the Province No. 1 Government; the organizers and co-organizers of the Conference, as well as the people for hosting the Conference and offering their warm hospitality.

*Therefore, the Resolut*ion *of the Conference* has been passed, and it is here followed by Activities to Accomplish the Recommendations and Background of the Conference.

The National Conference on Integrating Biological Resources for Prosperity (NCIBRP), 6-7 February 2020 / 23-24 Magh 2076, Biratnagar, Nepal:

1. *Recommends strongly* that Ministry of Forests and Environment (MoFE), Ministry of Industry, Tourism, Forests and Environment (MoITFE), Province No. 1 develop respectively national and provincial targets beyond 2020 for conservation and sustainable use of biodiversity in accordance with the national and its provincial priorities, and develop related indicators for assessing progress; the indicators as far as possible being measurable, time-bound and achievable, and building on the progress achieved towards the 2010-2020 Aichi Biodiversity Targets.
2. *Strongly recommends* MoFE and MoITFE to integrate biodiversity and related plans with national development plans of Government of Nepal.
3. *Recommends* the government, academia and society to develop strategies for sustainable use of biodiversity by integrating the knowledge of natural and social sciences including indigenous ecological knowledge (traditional knowledge) for informed policy, decision making and practices.

4. *Highly encourages* to link conservation plans with entrepreneurship including ecotourism, sustainable trade of medicinal and aromatic plant species (MAPs) and make integrated plans for sustainable utilization of biological resources.
5. *Recommends* scientific and research organizations to endeavor to develop and carry out research that addresses the needs of governments and peoples under the federal structure of the country, and as identified in National Biodiversity Strategies and Action Plans (NBSAPs).
6. *Urges* scientific and research organizations to collaborate with federal, provincial and local governments in assessing the present and future impacts of socio-ecological changes on biodiversity, ecosystem services and good quality of life in the country.

Calls upon Governments and organizations at all levels of governance to work in synergy to reduce the impacts of global and local drivers of changes on biodiversity, ecosystem services and human wellbeing through wider public awareness and participation. *Urges* to widely disseminate the outputs of the conference through effective means including in Nepali language.

Activities to Accomplish the Recommendations

A. *Regarding the Strategic Plan* (Resolutions 1-4)

- *Integrate* biodiversity values into national and provincial accounting systems to ensure that all sectors of governments and society take into account biodiversity values in the short and long term plans for national, provincial and local development including the Sustainable Development Goals.
- *Enhance* the effectiveness and sustainability in the management of ecosystems of the country, provinces and local levels. Specific efforts should be devoted to reduce the rate of loss of biodiversity and habitat as well as IPLC's indigenous ecological knowledge.
- *Enhance* management effectiveness of *in-situ* conservation of biodiversity at the landscape level through participatory approach; and *ex-situ* conservation of biodiversity at federal, province and local levels.
- *Encourage and develop* biodiversity based enterprises and bioprospecting, and share equitably the benefits arising from the use of biological resources among stakeholders including IPLCs.

B. *Regarding knowledge management* (Resolutions 5-7)

- *Put in place and/or strengthen* mechanisms that will facilitate and ensure implementation of measures taken in the federal, provincial and local levels strategies and action plans, in particular mobilization of financial resources and participation of all stakeholders including local communities.
- *Undertake participatory approaches* to manage the Protected Areas, Ramsar sites, and Landscape system to maintain and restore biodiversity, and increase the adaptive capacity of ecosystems, species and the resilience of human populations including IPLCs, in the face of global and regional environmental changes.
- *Strengthening capacity* of government, community based, educational and private institutions for effective implementation and monitoring of biological resources and fair and equitable sharing of benefits arising from their utilization in accordance with the existing national laws and international agreements.

C. *Regarding dissemination of the outcome* (Resolution 8)

- The Conference presentations will be published in a peer-reviewed volume for wider dissemination. The resolutions will be translated in Local Vernacular languages and widely disseminated.

Botanical Society of Nepal, Kathmandu

Background

Botanical Society of Nepal (BSON) is an autonomous, non-governmental, non-profitable and nonpolitical professional organization of Nepali botanists, dedicated for conservation and sustainable utilization of plant resources as well as strengthening the scientific capacities of Nepali botanists. The society primarily focuses on promotion of knowledge, skills, technology and innovation related to plant science through academic discourses and social activities dedicated to the prosperity and healthy environment of Nepal. BSON serves as a platform for organizing workshop, seminar, conference, training, field research/excursion, exhibition, publication and meeting for exchanging of ideas / knowledge among the botanists, policy makers and common people.

The history of BSON links with Nepal Botanical Society, founded in 1981 and registered in 2006. However, the society was inactive since a long time and many botanists worked in different institutions were interested for an active common forum. Considering it, a meeting was held at Central Department of Botany, Tribhuvan University on August 31, 2017 (Bhadra 15, 2074) in presence of former executive committee members of Nepal Botanical Society. An *ad hoc* committee was formed under the coordination of Prof. Mohan Siwakoti (then Head of Department) to renew the society but due to technical difficulty and lack of document of previous society, the committee was agreed to registered a new society rather than renew the previous one. To take the responsibility for preparing a constitution, registration of society and election of the new executive committee of society, a seven membered *ad hoc* committee, consisting of Prof. Dr. Mohan Siwakoti (President), Mr. Sanjeev Kumar Rai (Vice President), Dr. Hari Prasad Aryal (Secretary), Dr. Narayan Prasad Ghimire (Treasurer), Dr. Bhuvan Keshar Sharma (Member), Dr. Anjana Giri (Member) and Mr. Dipak Lamichhane (Member), was formed on May 23, 2018 (Jestha 9, 2075 BS). The Botanical Society of Nepal (BSON) was registered on August 18, 2018 (Shrawan 12, 2075 BS) at Kathmandu District Office as per the law of the Government of Nepal. An introductory program was organized on September 26, 2018 (Asoj 12, 2075 BS) at the Department of Plant Resources to exchange the festival greetings and share the information to botanists about the registration of the Botanical Society of Nepal.

Executive Committee

The first annual general meeting (AGM) of the society was held on November, 29, 2018 (Mangsir 13, 2075 BS) at Central Department of Botany, Kirtipur where an 11 membered full-fledged executive committee of the society was unanimously elected by the election committee for the society. The election committee was constituted by Professor Emeritus Dr. Pramod Kumar Jha (coordinator), Dr. Keshab Raj Rajbhandari and Prof. Dr. Mohan Prasad Panthi. The second Annual General Meeting (AGM) of BSON was held at Biratnagar in the last day of the national conference, February 7, 2020. The third general meeting (AGM) and second term election was held on December 4, 2020 (Mangsir 19, 2077), the AGM unanimously recommended to the Election Committee for repeating the previous Executive Committee for second term (2021-2022). The election committee was constituted by Dr. Tirtha Bahadur Shrestha (coordinator), Dr. Kamal Krishna Joshi and Prof. Dr. Mukesh Kumar Chhetri.

Following persons elected for the first Executive Committee of the society (2018 – 2020) and re-elected for the second term (2021-2022).

President:	Prof. Dr. Mohan Siwakoti	Members:	Dr. Man Dev Bhatt
Vice President:	Mr. Sanjeev Kumar Rai		Dr. Bhuvan Keshar Sharma
Secretary:	Dr. Hari Prasad Aryal		Dr. Anjana Giri
Joint Secretary:	Mr. Jaya Prakash Hamal		Mr. Dipak Lamichhane
Treasurer:	Dr. Narayan Prasad Ghimire		Ms. Jwala Shrestha
			Ms. Chandrakala Thakur

Advisory Committee

BSON has a 14 membered Advisory Committee for providing the technical advice for the progress of the society, namely: Prof. Dr. Sanu Devi Joshi (Kathmandu), Professor Emeritus Dr. Pramod Kumar Jha (Kathmandu), Professor Emeritus Dr. Ram Prasad Chaudhary (Kathmandu), Dr. Keshab Raj Rajbhandari (Kathmandu), Prof. Dr. Sangeeta Rajbhandary (Kathmandu), Prof. Dr. Chandra Bahadur Thapa (Pokhara), Prof. Dr. Ram Kailash Prasad Yadav (Kathmandu), Prof. Dr. Shiva Kumar Rai (Biratnagar), Mr. Mohan Dev Joshi (Kathmandu), Dr. Umesh Prasad Shrivastava (Birgunj), Mr. Vishnu Prasad Gautam (Chitwan), Dr. Anant Gopal Singh (Butwal), Ms. Tilmaya Dhakal Kharel (Mahendranagar), and Mr. Shibaraj Ghimire (Surkhet).

Major activities of the society

A book entitled "Plant Diversity in Nepal" was published with updated information on plant resources in Nepal. The book was launched on the Inaugural session of National Conference 2020 at Biratnagar by Mr. Sherdhan Rai, the Chief Minister of Province No. 1 and reviewed by Dr. Kamal Krishna Joshi, Former Vice Chancellor of Tribhuvan University and Former Chairman of the University Grants Commission.

A National Conference on "Integrating Biological Resources for Prosperity" was organized on February 6-7, 2020 by the Botanical Society of Nepal in collaboration with Nepal Biological Society, Biratnagar and the Government of Nepal, the Ministry of Forests and Environment, Kathmandu, and the Ministry of Industry, Tourism, Forest and Environment, Province No. 1, Biratnagar.

A Directory of Nepali Botanists was published by the Society in collaboration with the Department of Plant Resources. The book was launched on the third AGM of society (December 4, 2020) by Dr. Kamal Krishna Joshi and reviewed by Professor Emeritus Dr. Pramod Kumar Jha.

A talk program on "Botany and Budha: The Plants in Lumbini" and "Important Plants in Lumbini area" was delivered by Dr. Mark F. Watson and Prof. Mohan Siwakoti, respectively on March 1, 2019 at Thapathali. During the program, the Honourary membership of the Botanical Society of Nepal was awarded to Dr. Mark. F. Watson, Chief Editor of Flora of Nepal, the Royal Botanical Garden Edinburgh, UK.

A talk program on "Gurjo Plant for present Pandemic: Health Benefits and Safety Concern" was delivered by Dr. Rajendra Gywali, Associated Professor of Kathmandu University on October 22, 2020.

Membership

Till now, the Botanical society of Nepal awarded life membership for 122 botanists and honorary membership for one botanist and ordinary membership for 2020/2021 for 5 botanists. More memberships are in process. Further information with detail list of life membership is available in website of BSON (www.bson.org.np).

Nepal Biological Society, Biratnagar

Background

Nepal Biological Society (NBS) is a non-governmental and non-profitable, professional organization established in 2002 A.D. at Biratnagar, Nepal. The objectives of the organization are (1) to conduct and coordinate research activities pertaining to all facets of the biosciences and natural resources, (2) to publish bulletins, journals and books pertaining to biosciences and natural resources, (3) to organize seminars, conferences etc. related with biosciences and natural resources, (4) to collaborate with various governmental and non-governmental organization, and (5) to establish a documentation center cum library for dissemination of knowledge and information pertaining to biosciences and natural resources.

Executive Committee

The current Executive Committee of NBS is as follows:

President	: Prof. Dr. Sasinath Jha
Vice-President	: Prof. Dr. Damodar Thapa Chhetry
General Secretary	: Prof. Dr. Shiva Kumar Rai
Secretary	: Mr. Shaligram Adhikaree
Treasurer	: Dr. Prakash Kumar
Members	: Prof. Dr. Umesh Koirala
	: Dr. Bhabindra Niroula
	: Ms. Radha Adhikari
	: Mr. Ram Chandra Adhikari
	: Mr. Mohan Shrestha
	: Ms. Indira Pokhrel

Activities

NBS has been working actively on different aspects of biological and environmental researches and developmental activities. The NBS successfully organized "National Seminar on Natural Resource Management" on February 13-14, 2004 at Biratnagar in collaboration with Ecological Society (ECOS), Kathmandu and P.G. Campus, T.U., Biratnagar and published a proceeding book entitled "Natural Resource Management, ISBN: 99946-982-4-9" in 2006 which includes 73 articles in 474 pages. Another seminar on "Modern Trends in Science and Technology" was organized in December 28-29, 2012 at Biratnagar in the collaboration with Nepal Physical Society (NPS), Eastern Chapter, and Research Council of Science and Technology (RCOST), and published a proceeding book entitled "Modern Trends in Science and Technology, ISBN: 978-9937-2-6401-3" in 2013. Last year, NBS in collaboration of Botanical Society of Nepal (BSON), Kathmandu; the Government of Nepal, the Ministry of Forests and Environment (MoFE), Kathmandu; and the Ministry of Industry, Tourism, Forest and Environment (MoITFE), Province No. 1, Biratnagar, organized a grand successful National Conference on "Integrating Biological Resources for Prosperity" on February 6-7, 2020 at Biratnagar. The society has been organized many workshops, trainings, seminars, and talk programs at regional and local level too. The peer-reviewed official journal of Nepal Biological Society entitled "Nepalese Journal of Biosciences", is being published annually since 2011.

Address
Nepal Biological Society (NBS)
Science Block, Post Graduate Campus
Tribhuvan University, Biratnagar, Nepal
Contact: 977-021-435275
E-mail: nbs.brt.np@gmail.com

Department of Plant Resources, Kathmandu

The Department of Plant Resources (DPR) is one of the government institutions under the Ministry of Forests and Environment which was established in 1960 A.D. This organization is conducting researches and providing services on the plant resources in Nepal. It is a multidisciplinary organization comprising scientists mainly the botanists, the chemists and the pharmacists.

Objectives and activities:

- Resource survey and collection of plant materials and preservation of the specimens in the National Herbarium and Plant Laboratories (KATH).
- Identification and certification of plant and its products (KATH).
- Establishment and maintenance of Botanical Gardens in different physiographic regions of the country for *ex-situ* and *in-situ* conservation of rare, endangered, threatened and medicinal plants.
- Technical support for sustainable utilization of plant resources through cultivation and processing; floriculture and landscaping.
- Chemical and Biological researches for the utilization of medicinal, aromatic and other valuable plants.
- Biotechnology research, improvement and propagation of plants of economic value.
- Agro-technology development of important and medicinal plants to provide services to the farmers on techniques of commercial cultivation.
- Conduction of trainings on plant resources conservation, management and provide garden services.
- Information dissemination through publications on various aspects of Nepalese plant resources.
- Bio-prospecting of plants of economic value.
- Library service for public, researcher, policy maker and all concerned.

DPR also functions as the following

- National Scientific Authority of plant resources for the implementation of the Convention on International Trade in Endangered Species of Wild Fauna & Flora (CITES).
- National focal point of Global Taxonomic Initiative (GTI)
- National focal point of Bio-safety

The Department has two divisions, three central level offices, seven district level plant research centers and twelve botanical gardens.

1. **Research and Planning Division:** This division is mainly responsible to formulate research and management plan for research and development of diverse plant resources of the country. Under this division there are Planning and Monitoring section, Herbs promotion and development section, Biotechnology section, Biodiversity and CITES section and Urban garden development section.
2. **Management and Development Division:** This division is responsible for application of research for sustainable utilization, herbs promotion and development, development of plant resources of the country. Under this division there are Instrument section, Quality determination section, Biological section, Herbs utilization and pilot section and Documentation section.

Ongoing Programs

- Plant Research, Conservation & Garden Development Programme
- Medicinal Plants Development Programme

Central Level Offices

Natural Products Research Laboratory, Thapathali
This laboratory provides services to the public for industrial product development of plant origin and certification. Currently its main focus is on plant chemistry aiming for R&D activities. This lab also certifies plant products and essential oil as per ISO 17025/2017.
Natural Products Research Laboratory
Thapathali, Kathmandu, PO Box 2270
Tel: 977-01-4268247, 4266856
E-mail: nprl@dpr.gov.np

National Herbarium and Plant Laboratories
Established in 1961, the National Herbarium (KATH) aims to support the nation building through R&D on the diverse plant resources of the country. This is the only institution having the mandate of conducting country-wise plant exploration, collection, identification, preservation and housing of dried plant specimens called as Herbarium.
National Herbarium and Plant Laboratories
Godawari-5, Lalitpur, PO Box No. 3708
Tel: 977-1-5174277
E-mail: nhpl@dpr.gov.np, Website: www.kath.gov.np

National Botanical Garden (NBG)
Established in 1962, for the purpose of Collection, Conservation, Education and Scientific Research of living plants. This botanical garden is located at the foothill of Phulchoki hill, spreads over an area of 82 ha. More than 1000 different species of plants are conserved here. There are different landscapes and thematic gardens like Physic garden, Biodiversity Education Garden (BEG), Rock garden, Fern garden, Orchid house, Japanese style garden, Lily garden, Tropical garden, Taxonomic family garden, VVIP plantation area, Ethno-botanical garden, etc.
National Botanical Garden
Godawari, Lalitpur, PO Box No.3708
Tel: 977-01-5174279, 5174246, Fax: 977-01-5174279
E-mail: nbg@dpr.gov.np

Plant Research Center

There are seven plant research center (PRC) under DPR.
1. Plant Research Center, Ilam
2. Plant Research Center, Dhanusa
3. Plant Research Center, Makawanpur
4. Plant Research Center, Banke
5. Plant Research Center, Salyan
6. Plant Research Center, Jumla
7. Plant Research Center, Kailali

Botanical Gardens

In addition to the National Botanical Garden, DPR has been managed following 11 botanical gardens in the country directly (NBG, WPBG) or through respective PRCs:
1. Maipokhari Botanical Garden, Maipokhari, Ilam, 2100 m (estd. 1992)
2. Dhanushadham Botanical Garden, Dhanushadham, Dhanusha, 106.6 m (estd. 1998)
3. Brindaban Botanical Garden, Hetauda, Makwanpur, 500 m (estd. 1962)

4. Mountain Botanical Garden, Daman, Makwanpur, 2320 m (estd. 1965)
5. Tistung Botanical Garden, Tistung, Makwanpur, 1900 m (estd. 1965)
6. Dhakeri Botanical Garden, Dhakeri, Banke, 160 m (estd. 1990)
7. Mulpani Botanical Garden, Kapurkot, Salyan, 2000 m (estd. 1990)
8. Dhitachaur Botanical Garden, Ditachaur, Jumla, 2498 m (estd. 1990)
9. Debariya Botanical Garden, Dhangadhi, Kailali, 170 m (estd. 1998)
10. Godawari Botanical Garden, Godawari, Kailai, 185 m (estd. 1998)
11. World Peace Biodiversity Garden (WPBG), Raniban, Pokhara, Kaski. Established in 2013 as per Government of Nepal (Secretary Level) decision dated 2070/8/18 B.S.

Major Programs and Activities

Major programs and activities implemented by Government of Nepal in these botanical gardens are Medicinal plants (Jadibuti) Development Program and Research, Conservation and Garden Development.

The major activities under these programs are:

- Production of quality planting material of medicinal plants and distribution to farmers for commercial farming
- In-situ and ex-situ conservation of threatened and endemic plant species
- Research and study for agro-technology development of medicinal plants
- Documentation of indigenous knowledge related to plant resources
- Domestication and germ-plasm conservation of Medicinal and Aromatic Plants (MAPs)
- Variety development program of *Mentha* and *Chamomile*
- Friends of Botanical Garden (FoBG)
- Beautification of plant landscapes and thematic gardens
- Production of seasonal and perennial ornamental plants
- Research on indigenous ornamental plants
- Awareness program

Conference Photo Gallery

Conference inaugurated lighting the Panas by Hon. Chief Minister Mr. Sherdhan Rai

Book (Plant Diversity in Nepal) launched by Hon. Chief Minister Mr. Sherdhan Rai

Inaugural session

Conference participants at inaugural session

Photography in inaugural session with organizing committee

Inaugural address by Hon. Chief Minister Mr. Sherdhan Rai

Chair remarks at inaugural session by Hon. Minister Mr. Jagadish Prasad Kusiyait

Dr. Bishwa Nath Oli, Secretory MoFE

Dr. Kamal Krishna Joshi, Former VC, TU

Keynote speech by Prof. Dr. Ram Prasad Chaudhary

Keynote speech by Prof. Dr. Pramod Kumar Jha

Prof. Dr. Mohan Siwakoti, Chairman, Organizing Committee

Dr. Tirtha Bahadur Shrestha

Prof. Dr. Sanu Devi Joshi, Academician NAST

Prof. Dr. Sasinath Jha, President NBS

Dr. Keshab Raj Rajbhandari

Dr. Binay Kumar Chakraborty, Bangladesh

Prof. Dr. Sangeeta Rajbhandari, CDB, TU

Prof. Dr. Shiva Kumar Rai, Co-convener

Dr. Ganesh Bahadur Thapa, Chief, Nat.Hist. Mus., Kathmandu

Mr. Sanjeev Kumar Rai, Secretary, MoITFE

Dr. Bharat Raj Subba

Prof. Dr. Dilip Kumar Jha

Dr. Buddi Sagar Poudel, Chief, REDD Implementation Center

Mr. Mohan Dev Joshi, DDG, DPR

Mr. Dol Raj Luitel, DoE

Mr. Jeeban Pandey, DPR, Ilam

Mr. Shailendra Kumar Pokharel, Founder President, CODEFUND

Technical session (Oral presentation)

Chaired by Prof. Dr. Sanu Devi Joshi

Chaired by Prof. Dr. S.N. Sinha, India

Chaired by Prof. Dr. T.N. Mandal

Chaired by Prof. Dr. Mohan Panthi

Chaired by Prof. Dr. Sangeeta Rajbhandari

Chaired by Prof. Dr. Kanta Rijal Poudyal

Chaired by Prof. Dr. Hari Datta Bhattarai

Special Symposium

Participants

Participants

Group photo at Valedictory Session

Guests on Special Symposium (Legislators of Provincial Assembly)

Senior Botanists

Technical session (Poster presentation)

Book Journal Display Desk

Botanical Society of Nepal (Executive and Advisory members)

Meeting of Conference Organizing Committee

Health Desk

About Editors

Dr. Mohan Siwakoti: Professor in Tribhuvan University. His major fields of interest are systematics and ethnobotany, wetland biodiversity and alien invasive plant species. He was a Darwin scholar in 2003-2006 at Nepal and U.K. Prof. Siwakoti has supervised three Ph.D. and one more Ph.D. student submitted thesis. He has conducted more than 18 research projects, authored one book, co-authored two books, edited five books, and published about seven dozen papers in national and international peer reviewed journals. E-mail: mohansiwakoti@live.com

Dr. Tej Narayan Mandal: Professor in Tribhuvan University. His research areas are forest ecology and restoration ecology. He has supervised two Ph.D. and supervising three more Ph.D. Prof. Mandal has authored two books and published 61 research papers in reputed national and international journals. He has conducted eight research projects as principal investigator, also edited some books and journals. E-mail: tnmandal@gmail.com

Dr. Shiva Kumar Rai: Professor in Tribhuvan University. His major field of research is freshwater algae. Dr. Rai was awarded by the Young Science and Technology Award 2064/2065 from the NAST, Kathmandu. He has conducted about a dozen of research projects, authored one book, edited two books, and published about six dozen of scientific papers in reputed journals. Prof. Rai is an executive editor of *Our Nature* and *Nepalese Journal of Biosciences*. E-mail: shiva.rai@pgc.tu.edu.np

Mr. Sanjeev Kumar Rai: Director General of the DPR and former Secretary of the MoITFE, Province No. 1. Mr. Rai has long experiences for the promotion of medicinal and aromatic plants of Nepal. He has a good knowledge in statistical and geo-information analyses and various computing packages. He is also experienced photographer specialized in plants, butterflies, insects and landscapes. Mr. Rai has authored one book, co-authored more than six books, edited books and journals, and published many articles in peer reviewed journals. E-mail: sanjeevkrai4@gmail.com

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ISBN 993709013- X