

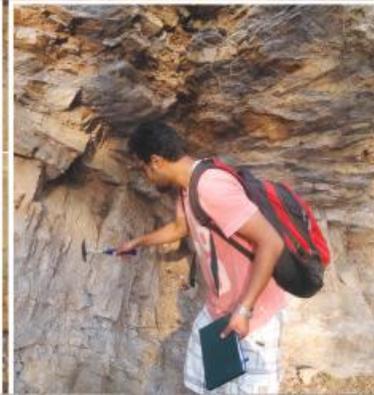


National Mission on Himalayan Studies (NMHS)

Proceedings of the 1st Himalayan Researchers Consortium

Volume I

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Proceedings of the 1st Himalayan Researchers Consortium Volume I

Broad Thematic Area
Biodiversity Conservation & Management

Editors
Puneet Sirari, Ravindra Kumar Verma & Kireet Kumar



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पर्यावरण, वन एवं जलवायु परिवर्तन मंत्रालय

GOVERNMENT OF INDIA
MINISTRY OF ENVIRONMENT, FOREST &
CLIMATE CHANGE

Foreword

Taking into consideration the significance of the Himalaya necessary for ensuring "Ecological Security of the Nation", rejuvenating the "Water Tower for much of Asia" and reinstating the one among unique "Global Biodiversity Hotspots", the National Mission on Himalayan Studies (NMHS) is an opportune initiative, launched by the Government of India in the year 2015–16, which envisages to reinstate the sustained development of its environment, natural resources and dependent communities across the nation. But due to its environmental fragility and geographic inaccessibility, the region is less explored and hence faces a critical gap in terms of authentic database and worth studies necessary to assist in its sustainable protection, conservation, development and prolonged management.

To bridge this crucial gap, the National Mission on Himalayan Studies (NMHS) recognizes the reputed Universities/Institutions/Organizations and provides a catalytic support with the Himalayan Research Projects and Fellowships Grants to start initiatives across all IHR States. Thus, these distinct NMHS Grants fill this critical gap by creating a cadre of trained Himalayan environmental researchers, ecologists, managers, etc. and thus help generating information on physical, biological, managerial and social aspects of the Himalayan environment and development. Subsequently, the research findings under these NMHS Grants will assist in not only addressing the applied and developmental issues across different ecological and geographic zones but also proactive decision- and policy-making at several levels.

In year 2018, the 1st Himalayan Researchers Consortium was organized with proactive participation of young researchers and institutions working in the Indian Himalaya, particularly on Biodiversity Conservation & Management theme. The key outcomes of the 1st Himalayan Researchers Consortium-2018 are documented in form of research articles submitted by these young Himalayan Researchers and Fellows after a brief review by NMHS Monitoring, Learning and Evaluation Panel Members and eminent subject experts.

*I am sure that all research findings and insights covered in the "**Proceedings of the 1st Himalayan Researchers Consortium**" will set this publication as valuable reference and ready-reckoner for future researchers working in the Indian Himalaya.*

Place: MoEF&CC, New Delhi

Dated: January 2019


(A.K. Jain)



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Preface

Taking ahead the journey of "learnings for and from the Himalaya as a Great Teacher to the Mankind" under the National Mission on Himalayan Studies (NMHS), the Himalayan Research Fellowship is part of a well-foresighted support system that brings "Young Researchers", "Institutional infrastructure", and "eminent experts/ mentors/ resource persons" together for achieving the vision envisaged for the Himalaya and its natural resources as well as dependent communities across the nation.

Observing the stark variations and vast range of the Himalayan ecosystems and its emanating services in unique biological systems under distinct climatic conditions, the Himalayan Studies demand genuine queries, inquisitive minds and innovative ways to attend, address and resolve the sustainable issues in the Indian Himalaya.

Under the National Mission on Himalayan Studies (NMHS), the Himalayan Research Fellowships have started emerging over the years as a bridge to fill the crucial gaps of datasets, information and thereupon decisions for the welfare of the communities dependent on the Himalaya and its natural resources.

Presented during the 1st Himalayan Researchers Consortium-2018, the research attempts and efforts of young Himalayan researchers are documented in form of the research articles after due review by the eminent experts, mentors and resource persons.

I am sure that the publication "Proceedings of the 1st Himalayan Researchers Consortium" will be another useful contribution towards facilitating prompt action research, innovative conservation strategies and demonstrative sustainable development with the committed participation of young Himalayan researchers under the National Mission on Himalayan Studies.

Wishing all contributors the best in their every endeavour to reinstate the resplendent Himalaya.

Place: GBPNIHESD, Almora, Uttarakhand

Dated: January 2019

Ranbeer S. Rawal





About the National Mission on Himalayan Studies (NMHS)

Observing the significance of the Himalaya important for ensuring “**Ecological Security of the Nation**” and reinstating the “**Water Tower of Asia**”, the **National Mission on Himalayan Studies (NMHS)** is a timely initiative, launched by the Government of India in year 2015–16 as a Central Sector (CS) Grant-in-Aid Scheme, which envisages to accomplish sustained development of both, i.e. the directly dependent upstream Himalayan population at the regional level and other indirectly dependent downstream population at the national level.

The NMHS targets to provide much needed focus, through holistic understanding of systems components and their linkages, in addressing the key issues relating to conservation, sustainable management and development of natural resources in Indian Himalayan Region (IHR). The mission is to launch and support innovative studies and related knowledge interventions to find scientifically sound solutions and best practices. The Mission strategy is to focus on enhancing livelihoods of local communities, in line with the **National Environment Policy, 2006 of the Government of India**.

Of global and national significance are the Himalayan Biodiversity Hotspots (HBH), crucial Protected Areas, life-sustaining Rivers, vital Watersheds, vast Forests cover, endemic Biodiversity and invaluable natural resources as boons of the Himalaya bestowed upon the whole nation, which also entail a responsibility entrusted upon the **Communities as Custodians of the Himalayan Resources and Environment**, directly and indirectly dependent on the Himalaya. Therefore, scientific, traditional and indigenous knowledge-based roles and responsibilities of communities, resource persons, young generation, and other stakeholders cannot be neglected towards ensuring the sustenance of the Indian Himalaya.

However, the constraints and challenges in terms of remoteness, difficult terrains, lack of resources and infrastructure have hampered and in fact deteriorated the extent and quality of research adversely for the IHR. Consequently, a continuous decline in the number of motivated and sincere researchers/ resource persons is observed as one among the grave concerns foreseen for the IHR. The National Mission on Himalayan Studies (NMHS) bridges this gap by provisioning the Himalayan Research Fellowships across 12 States of the Indian Himalayan Region (IHR).

About the NMHS Himalayan Research Fellowships

Under the National Mission on Himalayan Studies (NMHS), the Himalayan Fellowship Grant is aimed at facilitating knowledge building in IHR Institutions by way of (i) creating various fellowships and academic (national) exchange programmes; (ii) upgrading infrastructure of key academic and research institutions; and (iii) organizing regional, national and international conferences, workshops, events, etc. Initiated in year 2015, this is to attract the best talents from all across the nation to work on the specific research problems faced in Indian Himalayan Region (IHR) and find the scientifically sound solutions or best practices towards ensuring the sustainable development in the IHR.



To bridge this crucial gap, the National Mission on Himalayan Studies (NMHS) recognizes the reputed Universities/ Institutions/Organizations and grants the Himalayan Research Projects and Fellowships across all 12 IHR States. On one hand, these NMHS Grants seek to fill this critical gap, create a cadre of trained Himalayan environmental managers, ecologists and socio-economists and thus help generating information on physical, biological, managerial and social aspects of Himalayan environment and development in tandem. On the other hand, the research findings under these NMHS Grants will assist in not only addressing the applied and developmental issues across different ecological and geographic regions of the IHR States but also proactive decision-making at the local and regional levels.

Pre-Consortium Lectures by the Eminent Experts



Prof. V.K. Gaur interacted with the participants on 25th April 2018 and provided information on recent demand on biodiversity research on Himalaya. He appreciated the work of some of the Himalayan Fellows working in remote terrains in the Himalaya and advised following suggestions during an interactive session:

- Uniform methodologies needs to be followed for analyzing biodiversity elements across the Indian Himalayan Region.
- More focus on field studies are essential to understand the patterns of diversity in different ecosystem.
- Sharpening of objectives are essential to deliver better output.
- Essentiality of collaborative work among the Fellows towards achieving desirable Global Biodiversity Targets.

Prof. R.K. Kohli, Vice-Chancellor, Central University of Punjab, Bathinda, Punjab provided a brief lecture on "Methods of Biodiversity Data Analysis".

He appreciated the research approaches adopted by some of the Himalayan Fellows and stressed on the diversity analysis to be done in the field as well as available network in the world.

While deliberating a brief lecture on "Methods of Biodiversity Analysis", he shared best practices, methodologies and models and advised to establish linkages worldwide towards exchange of learnings and experiences in similar mountainous environmental conditions and regions.



About the 1st Himalayan Researchers Consortium-2018



Shri C.K. Mishra
Secretary, MoEF&CC

A two-day **1st Himalayan Researchers Consortium** was organized by the Project Management Unit (PMU) of the National Mission on Himalayan Studies (NMHS) at Vigyan Dham, Uttarakhand State Council for Science and Technology (UCOST), Dehradun, Uttarakhand on **26-27 April 2018**. The Consortium was **Chaired by Shri C.K. Mishra, Secretary, MoEF&CC, New Delhi**. The main aim was to review the progress of Himalayan Researcher Fellowships and induct an orientation with current needs of the mountainous region with the following objectives:

- To provide a platform for Himalayan Researchers to present and discuss their research findings for peer evaluation;
- To interact and gain knowledge/ guidance from the subject experts/ mentors;
- To enhance their capacity to exercise influence in scientific fraternity.

Eminent lectures by subject experts, one-to-one interaction, group discussion along with individual presentation by the Fellows were made during the consortium.



Session-wise Monitoring and Evaluation Panels

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- | | |
|--------------------------------------|--------------|
| 1. Prof. R.K. Kohli, PCU, Punjab | <i>Chair</i> |
| 2. Dr. G.S. Rawat, WII, Dehradun | Co-Chair |
| 3. Dr. S.S. Samant, GBPNIHESD, Kullu | Panelist |

Session II: Faunal and Microbial Diversity

- | | |
|---|--------------|
| 4. Shri Lalit Kapur, MoEF&CC, New Delhi | <i>Chair</i> |
| 5. Dr. Anita Pandey, GBPNIHESD, Almora | Co-chair |
| 6. Dr. Vikash Kumar, ZSI, Dehradun | Panelist |

Session III: Biodiversity Conservation and Management

- | | |
|---|--------------|
| 7. Dr. G.S. Rawat, WII, Dehradun | <i>Chair</i> |
| 8. Prof. Yashpal Sharma, YSPU, HP | Co-chair |
| 9. Dr. RS Rawal, GBPNIHESD, Almora | Panelist |
| 10. Dr. SK Uniyal, IHBT, Palampur | Panelist |
| 11. Dr Sunita Pradhan, ATREE, Bangalore | Panelist |



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SECTION I

FOREST ECOLOGY

The "Forest Ecology" section covers the research studies and demonstrative works presently progressing in the Indian Himalayan Region (IHR). Ecological monitoring, long-term monitoring plots, etc. are among the main initiatives taken by the young Himalayan Researchers under the National Himalayan Studies (NMHS).



Selection and Establishment of Long-Term Environmental and Ecological Monitoring Plots in Sikkim Himalaya

Rishi Kumar* and G.S. Rawat

Wildlife Institute of India, Dehradun, Uttarakhand

ABSTRACT: The Himalayan mountains, young and fragile, provide numerous ecosystem services to the people living within and in the downstream areas. These ecosystems are subject to changes brought by anthropogenic as well as climatic drivers. Most pronounced anthropogenic driver in the region is the change in land-use land cover (LULC) affecting biodiversity, ecosystem structure and functioning and the flow of ecosystem services. Cumulative effects of anthropogenic and climatic drivers have not been understood in the region. Therefore, collection of baseline data from multiple sites and long-term environmental monitoring are urgently needed in order to understand the critical linkages, ecosystem response and develop adaptation strategies. As part of the National Mission on Himalayan Studies (NMHS), we have initiated the establishment of long-term environmental and ecological research and monitoring (LTERM) sites in Sikkim Himalaya. These sites were selected on basis of altitudinal and anthropogenic pressure gradients. Logistics, accessibility, slope and forest types were other important criteria for selection of the 1-hectare monitoring plots in each of these sites. So far, a total of 24 sites have been identified in 13 Protected Areas (PAs) and Reserved Forests (RFs) and in each of these sites, single 1-hectare permanent plots have been established to monitor vegetation parameters. In the vicinity of such plots trails and transects have been established to record mammals and galliformes. Preliminary analysis of vegetation data reveals that tree species richness and abundances are slightly higher within PAs compared to RFs and higher diversity in the mid-altitudinal range (1500 – 2500 masl). Non-metric Multidimensional Scaling of tree species richness and abundances indicate the presence of 5 clusters in the 13 PAs and RFs. This paper deals with monitoring protocols and results of baseline surveys for vegetation.. ©2019

KEYWORDS: Long-Term Monitoring; flora; fauna; Sikkim; Teesta valley.

INTRODUCTION

All ecosystems continuously undergo autogenic or allogenic changes (Westman, 1968). Rapid allogenic changes in the environmental conditions due to increasing human populations and resultant over-exploitation of natural resources, habitat degradation and fragmentation, and rapid emission of greenhouse gases leading to global warming are of the immediate concern. Among the anthropogenic drivers leading to drastic changes in the land-use and land-cover (LULC), coupled with climate change are known to affect ecosystem services and human well-being. In order to evolve appropriate adaptation strategies and detect the changes in the environment, it is important to establish baseline data on biophysical as well as socio-economic parameters and establish a mechanism for long-term monitoring of such parameters. Similarly, monitoring of climatic parameters *vis-a-vis* ecosystem response is central to most assessment and adaptation strategies (Welch, 2006; Baron, 2009; Dudley *et al.*, 2010; Lemieux *et al.*, 2010). Environmental monitoring and

adaptation strategies are especially needed for the fragile and extremely vulnerable areas such as Himalaya which has been identified as a data-deficient region owing to scanty baseline data on biophysical, climatic and socio-economic parameters (IPCC, 2007). These data-gaps and uncertainties regarding ecosystem responses to climate change affect decision making. For the Himalayan region, baseline data are needed on several parameters including population status of various species, their habitats, human dependence on such species, status of cryosphere and hydrological regime, rate of glacial recession, invasive alien species, LULC, ecosystem health and disturbance patterns to name a few (Arrawartia & Tambe, 2012; Bolch *et al.*, 2012; Shrestha *et al.*, 2011; Sharma *et al.*, 2009; Xu *et al.*, 2009).

The need for long-term environmental and socio-ecological research in eastern Himalaya has long been felt by a number of authors (e.g., Tripathi, 2010; Rawat & Tambe, 2012; Chetry *et al.*, 2015; Gaira, 2015). The state of Sikkim and adjacent transboundary areas in Nepal and Bhutan, referred to as Khangchendzonga Landscape (KL) provides an excellent opportunity to initiate such monitoring effort as it represents one of the biodiversity rich landscapes with varied ecosystems and a rich array of socio-cultural groups.

Preliminary studies have indicated that distribution and abundance of several species of flora and fauna are likely to be affected due to climate change (Kholia, 2010; Acharya & Chettri, 2012). Human-driven LULC changes

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have also been observed particularly in low to mid-elevation regions of the state and along Highway 310 (Atreya, 2007). However, to track the effect of these drivers and to propose mitigation measure for not only biodiversity, ecosystems but also for human adaptations to these changes, more data are needed in all these aspects. In order to drive this initiative, long-term monitoring plots have been proposed in Teesta valley, Sikkim. This paper deals with basic approach and methodology of setting up long-term monitoring plots across the altitudinal range along Teesta Valley covering different vegetation types and gradients of anthropogenic pressures.

LITERATURE REVIEW

Global warming and the accompanying climate change, particularly in mountains have been severe with greater warming trends in last few decades. This has affected community composition, vegetation structure, and ecosystem services. One of the major changes induced by climate change is changes in the distribution of a number of species across the altitudinal gradient. This warming has resulted in the upward migration of species (e.g., Parolo & Rossi, 2008; Walther *et al.*, 2002) and increase in species richness at summits during the last century (Walther *et al.*, 2005). The upward shifting of snowline in the Himalayas over the years (Xu *et al.*, 2009) has created novel niches for birds in the alpine areas, causing upward extension in their altitudinal ranges. Consequently, such shifts have also resulted in the upward altitudinal shifting of bird species of low to mid-elevation areas. Advancement or delay of the breeding season is also observed in many species due to changes in the environmental cues with warming (Acharya & Chettri, 2012).

In addition to the vulnerability of wildlife, human societies depending on these are also highly vulnerable. Climate change effect on human social systems are basically in form of changes in agriculture systems as with increasing carbon-dioxide, high temperatures and erratic rainfall, results in crop failures, increased weeds, pathogens and pests (IPCC, 2007; Schmidhuber & Tubiello, 2007; Iglesias, 2011; Müllera *et al.*, 2011).

Several agencies and institutions around the globe have contributed to an in-depth understanding of the complex ecosystems and their responses to environmental changes through long-term ecological research, LTER (Vanderbilt & Gaiser, 2017). World over there are as many as 1197 LTER sites in 43 countries. In addition, there are over a dozen long-term studies on different environmental and ecological problems (Vihervaara *et al.*, 2013; Likens, 2013). Recently, need has been felt to include human social dimensions in long-term monitoring. Thus, the LTER concept has now

been upgraded to LTSER (Long Term Socio-Ecological Research) in many of these sites (Haberl *et al.*, 2006).

In India, the first long-term monitoring initiative was taken by the Indian Institute of Science, Bengaluru, with the establishment of a 50 ha permanent plot in Mudumalai Wildlife Sanctuary (Sukumar *et al.*, 1992). In recent years, long-term initiatives have been taken up by many other institutions. Permanent plots have been set up by the National Centre for Biological Sciences (Long-term Ecosystem Monitoring Network – India; LEMoN India 2011). The LEMoN network comprises seven 1-ha plots in collaborations with Ashoka Trust for Research in Ecology and Environment (ATREE), with Forest Department of Nagarjunasagar Srisailam Tiger Reserve (NSTR), Andhra Pradesh, with the Arunachal Pradesh Forest Department and with the Andaman and Nicobar Island's Environmental Team (ANET).

In 2016, the Ministry of Environment, Forest & Climate Change, Government of India announced setting up of eight – Long-Term Monitoring Ecological Observatories (I-LTEO) in different bio-geographic regions of the country. This programme visualizes interdisciplinary, multi-institutional collaboration throughout the country.

ENVIRONMENTAL CHANGES IN SIKKIM

In Sikkim, systematic meteorological data collection was initiated in 1955 with a single station in Gangtok (1812 m asl). Only in 1978, another meteorological station was set-up under the Agricultural Advisory Service (AAS). Analysis of the climatic data for 24 years (1987–2011) measured at Gangtok revealed that this city experienced a rise of about 1.08°C rise in the mean summer (minimum) temperature and about 2.08°C rise in the winter (minimum) temperature, indicating hotter summers and warmer winters. Sikkim has also been developing fast with industrialization and urban sprawl. In recent years, there have been large-scale changes in LULC in Sikkim, particularly along Teesta river and South and East Sikkim. In 2007, changes in Sikkim Registration of Companies Act (1961) ensured further industrialization by attracting more companies to the state (Atreya, 2007) and by the end of 2007 the state also signed contracts with private and public developers for the construction of over 30 dams on the rivers and by 2016 there was a spur of pharmaceutical plants and hydropower projects in the state, bringing in more roads, buildings, bridges and dams (Larkin, 2013).

Alpine meadows have shown to change its species

composition, diversity and structure, and altitudinal shifts with increasing temperature (Telwala *et al.*, 2013). The distribution of 38 species of *Rhododendron* in Sikkim shows a barrel shape distribution along the altitudinal gradient of 1500–6000 m (9 different altitudinal ranges; Pradhan & Lachungpa, 1990). The climatic modelling indicated that the bioclimatic envelope suitable for *rhododendron* has shrunk considerably for the envisaged climatic changes.

A preliminary climate change vulnerability assessment of rural Sikkim by Tambe *et al.* (2012a, b) identified the most vulnerable areas, concentrated in the subtropical zone of the South and West districts. However, areas which were not highly exposed (to pest, floods, draughts) were still highly vulnerable due to higher sensitivity (people growing rain-fed cropping) and lower adaptive capacity (due to illiteracy, poverty and lack of infrastructure), e.g. Karzi-Mangnam and Sakyong-Pentong villages.

Most of the studies on the effect of climate change on flora and fauna of Sikkim (Acharya & Chettri, 2012; Chettri & Bhupathy, 2007) report the opportunistic

presence of a low elevation species at a higher elevation or a few cases of breeding advancement or breeding failure. There is a lack of long-term study to establish changes in the altitudinal distribution of taxa, their response to climate change and also possible mitigation measures. Studies on Long-term sites have been very successful in establishing effects of various drivers like climate change, anthropogenic pressure or natural changes on species and ecosystems (Vihervaara *et al.*, 2013; Likens, 2013), and long-term monitoring of environmental quality is essential to robustly estimate sustainable resource and effect of management decisions (Wilby & Dessai, 2010). Long-term data are also essential to establish policy interventions in the management of wildlife, agriculture and human societies and the adaptive measures communities can take for climate change mitigation.

STUDY AREA AND METHODS

Study Area

Teesta river originates from “Tso Lhamo” lake at an altitude of 5330 m asl fed by the Kangtse glacier. It flows through the altitude gradient of the state finally reaching nearly 270 m asl

Table 1: Biophysical features of monitoring sites

Protected Areas/ Reserved Forests	No. of Sites	Elevation of sites (m)	Forest Type	Human Disturbances	Important Bird Areas
Kitam Bird Sanctuary	4	260-760	Tropical semi-deciduous and wet forest	None to High	IN-SK-07
Sombok RF	2	450 -700		Low	
Majhitar	2	330-700		High	
Namthang RF	1	500-800		High	
Tumberlong RF	1	350-600		Low	
Deling RF	1	350-600		High	
Singba Rhododendron Sanctuary	2	3000-3500	Temperate conifer and Sub-alpine vegetation	None to Low	IN-SK-11
Yumthang RF	2	3600-3850		Low to High	
FambongLho WLS	3	2050-2450	Temperate broad-leaved forest	None to Low	IN-SK-03
Dalley RF	1	2000-2300		High	IN-SK-08
Yangyang RF	1	1600-2100		Low to High	
Tendong RF	1	2000-2500		Low	
Maenam WLS	3	2250-3300		None to Low	
Kanchendzonga NP	(2)To be selected		Temperate broad-leaved forest, Temperate coniferous forestand Sub-alpine vegetation		IN-SK-04
Thangu – Lashar valley	(2)To be selected	3900 -4800	Sub-Alpine and Alpine	High to None	IN-SK-10
Total					
13+2 PA/RF	24 sites, 4 sites to be selected	260-4800m			

at the border with Darjeeling district of West Bengal. With its numerous tributaries, the Teesta basin covers the whole state. Sikkim as the mountainous state covers a natural elevation gradient from tropical to alpine zone over a short distance of 100 km and thus it also harbours a very high number of endemics (Pandit *et al.*, 2007, 2014).

Establishment of Monitoring Plots and Baseline Data Collection

Long-term ecological monitoring sites in the Teesta valley were identified covering major (six) vegetation types along the altitudinal gradient *viz.*, Tropical semi-deciduous and wet forest (300–900 m), Tropical broad-leaved forest (900–1800 m), Temperate broad-leaved forest (1800–2800 m), Temperate coniferous forest (2800–3200 m), Sub-alpine vegetation (3200–4000 m), Alpine vegetation (>4000 m). Protected Areas (PA) and Reserved Forests (RF) were selected for three gradient of disturbances or extraction pressure, *i.e.* high to nearly undisturbed. A total of 24 sites in 13 PAs/RFs were identified based on the altitudinal and anthropogenic pressure gradients available at these sites. In each of these PAs/RFs, along with different forest trails, anthropogenic disturbance data were collected, and permanent plots were placed within representative vegetation types. The vegetation parameters of each of these trails were analyzed along with the logistics, altitude, forest type and human disturbances to identify the sites within long-term monitoring plots. Sites in RFs close to village with extraction pressure were identified as high anthropogenic pressure areas, sites in RF with low human disturbances (lacking continued extraction pressures) were identified as low anthropogenic pressure zones and undisturbed sites were selected in PAs. In some PAs development activities have resulted in high disturbance areas (with low vegetation diversity), these areas were avoided during site selection for the undisturbed region.

We used accessibility, representativeness, distance from human habitation and varying gradient of elevation as main criteria for selection of long-term monitoring sites. The disturbance and vegetation data collected during trail walks and random vegetation plots were used to classify areas with low human disturbance to high human disturbance. In each of these identified sites, a single 1-ha plot was selected based on the key ecosystem and important sub-forest types. Key biophysical features of the identified sites are listed in Table 1. A list of these plots with background information and geo-tagged maps would be given to the forest department for providing protection to the sites in protected areas and for maintaining a status quo for the plots in RFs. The baseline data collection and monitoring methodologies used are as follows.

Long-Term Monitoring Plots in Sikkim

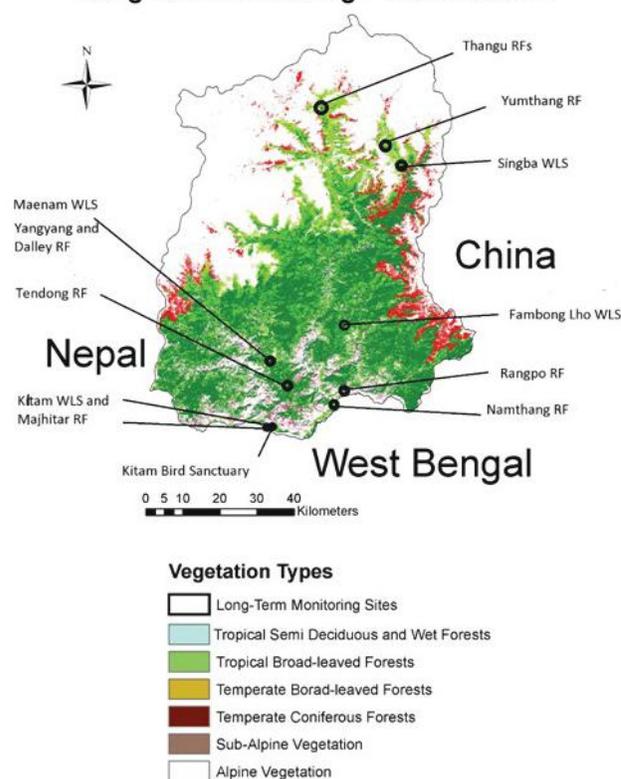


Figure 1: Map of the Study Area.

Flora

Flora in and around the plots is being studied based on already established protocols of Kailash Sacred Landscape Community Development Initiative (KSLCDI). In each location, sampling was done randomly on both sides of a 1–5 km trail transect. In the 1-ha plot, a more intensive vegetation sampling is being performed for creating baseline data.

In trails and in the 1-ha plots, multiple 20 m × 20 m quadrats were placed randomly on both sides of transect. In each of these quadrats trees, saplings, seedlings, shrubs and herbs were monitored. Girth at breast height (GBH at 1.37 m from the ground) and height of all trees in the plot were measured. Canopy cover of the trees within the plot, signs of lopping and cutting were also be recorded. The GBH and other parameters for classifying trees, saplings, seedlings and shrubs are listed in Table 2. Two nested plots for shrubs and saplings, *i.e.* 5 m × 5 m were placed in this quadrat, and similarly multiple plots of herbs and seedlings, 1 m × 1 m were nested inside the 20 m × 20 m plot.

Mammals

For assessing the mammalian fauna in and around the 1-ha plot, trail transects (Hiby & Krishna, 2001), sign surveys, scanning from vantage points surveys are

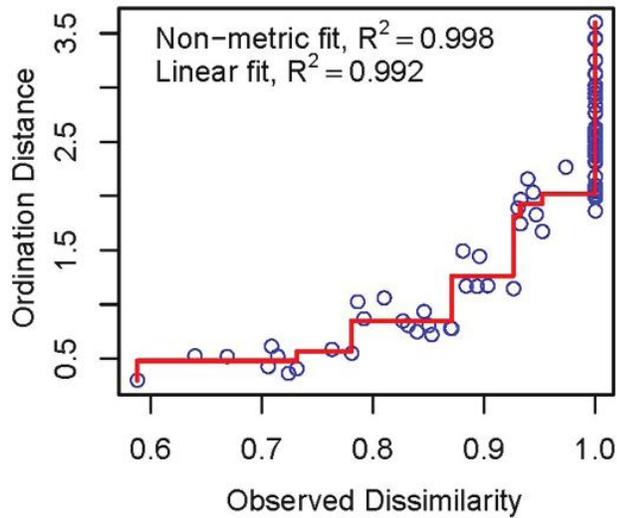


Figure 2: NDMS Shepard Plot.

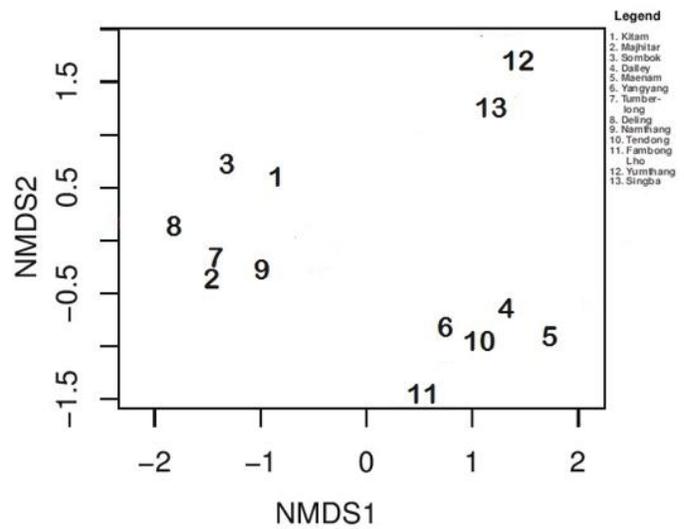


Figure 3: NMDS of PAs and RFs based on tree diversity and abundances.

being conducted (Wilson & Delahay, 2001). Monitoring frequency is planned to be seasonal to assess diversity, abundance and seasonal changes in species composition.

Multiple trails / transects of 1–5 km length were walked in morning and evening in the habitat. All species encountered, the number of each species encountered and distance from the trail was recorded and geo-referenced. In addition, all signs and tracks of mammals within 2 m to left and right of the transect were also recorded. For species identification, measurements of the track

and signs will also be taken. At some vantage points where a large area of the habitat can be seen clearly, a 20-minute scanning is conducted with binoculars for recording the presence of mammals. The distance of mammals seen and their number are also being recorded. Monitoring would be seasonal with same tracks would be walked in pre-monsoon and post-monsoon.

Aves

Aves are being assessed using variable radius point count for 10 minutes, vantage point scanning, and call counts (Bibby,

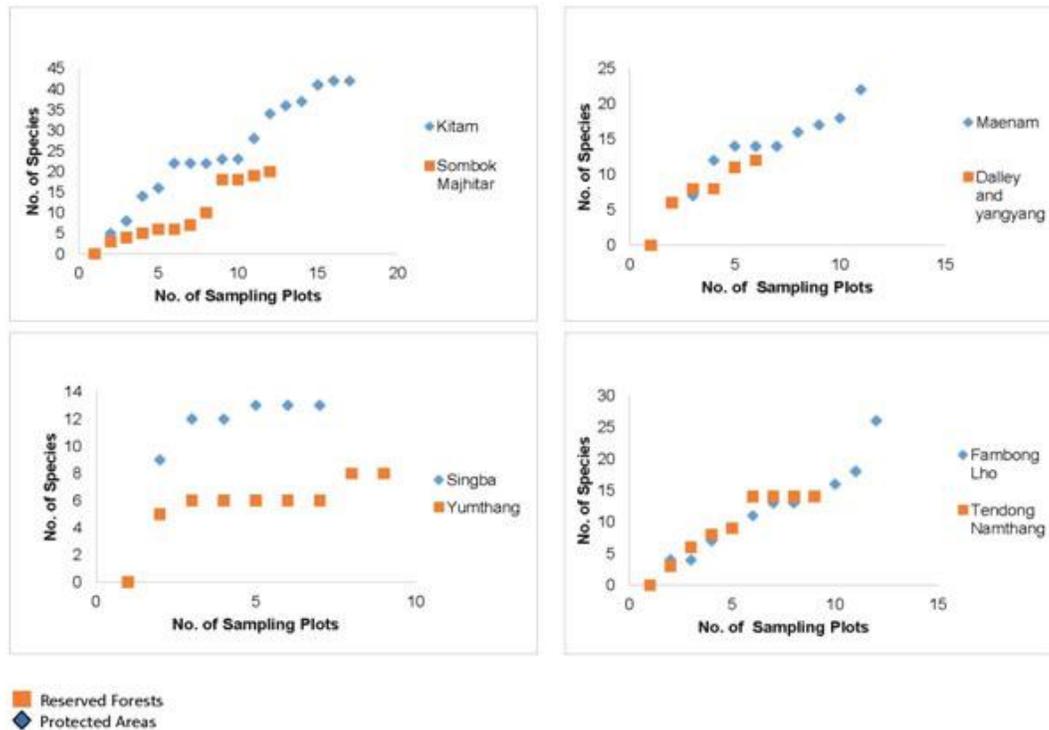


Figure 4: Species area curve of tree species in Protected Areas and adjoining Reserved Forests.

Table 2: Monitoring protocol for Flora and Fauna in the protected areas and reserved forests

Vegetation monitoring: The method to record saplings, seedlings, shrubs and herbs is as follows:

Type	Specifics	20 m × 20 m	5 m × 5 m (2 or more)	1 m × 1 m (2 or more)
Trees	All trees ≥ 9 cm dbh	Species; Tag ID; location; <i>dbh</i> ; height; canopy; tree condition	P or NP	No record
Saplings	2.5 cm ≥ <i>dbh</i> ≤ 9 cm dbh	No record	Species; <i>dbh</i> ; height; percent cover	No record
Seedlings	Trees < 2.5cm <i>dbh</i> and ≤ 15 cm tall	No record	No record	Count #
Shrubs	Shrubs ^a ; ferns	No record	Species; % Cover	P or NP
Herbs	Forbs; graminoids	No record	P	Species; % Cover
Non-vascular plants	Lichens and bryophytes ^b	No record	No record	P
Mean tree height (m)	5.02±0.00013			

Note: ^aDoes not include low growing woody species; ^bDoes not include species on logs that do not contact the ground.

2004; Gaston, 1980). For galliformes multiple trails of 1–5 km length are being walked in morning and evening within representative habitat. All species encountered, number of each species and the distance from the trail are being recorded and geo-referenced. Call counts of easily recognizable species are also being recorded at fixed intervals. At each 100 m distance on the transect, a bird count point is being created. At each of these points, the observer would stop for 10 minutes and record all the species encountered using a variable radius point count along with their distance from the observer. Monitoring along the pre-determined trails and bird count points would be seasonal in both pre-monsoon and post-monsoon.

For vegetation and faunal assessment we used GPS (Garmin Map 64s) to geo-locate the sites, a camera was used

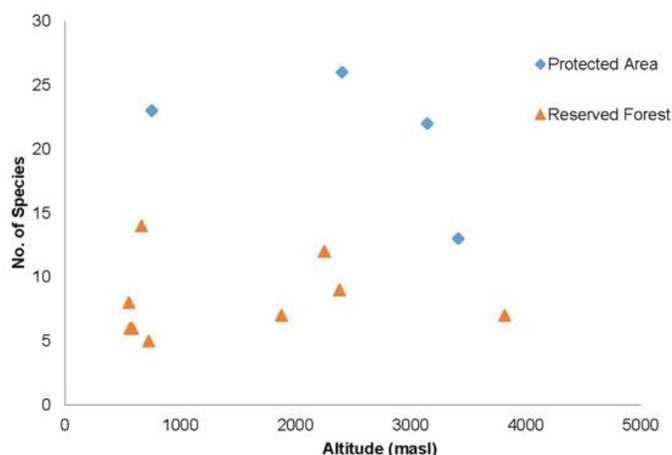


Figure 5: Species richness of sites with increasing altitude.

to photograph sites and species, binoculars were used for locating and identification of fauna on trail transect and birds invariable radius point count. Environmental monitoring is being conducted under project National Mission on Sustainable Himalayan Ecosystem (Wildlife Institute of India) by placing data loggers, this data would be shared with Forest Department of Sikkim and NMHS-PMU.

MONITORING STRATEGIES AND BASIC TRENDS

A total of 24 sites with a single 1-ha plot each were identified along 3 anthropogenic pressure gradient and 5 forest categories. These plots are spread across 13 habitats (13 sites in PAs and 11 in RFs) covering the altitudinal gradient of the state (Fig. 1).

A total of 77 vegetation plots have been surveyed at these sites. The data of 24 sites including the 1-ha plots and random vegetation plots in the same site was pooled for each RF or PA for analysis. The vegetation analysis of the 13 PA / RF locations reveals that the diversity of tree species are much better in protected areas compared to Reserved Forests with anthropogenic pressures.

To assess the similarities and dissimilarities in between these sites based on tree species presence and abundance Non-metric Multi-dimension Scaling (NMDS) analysis was performed on the tree species. The indirect gradient analysis of Non-metric multi-dimensional scaling (NMDS) produces ordination-based

distance or dissimilarity matrix. NMDS represent the pair-wise dissimilarity in a low-dimension space. Any dissimilarity coefficient or distance measure may be used to build the distance matrix used as input. The weights are given by the abundances of the species. The axes are arbitrary as is the orientation of the plot. A stress value near 0.05 or lower is considered a good fit.

Here the species abundances and presence was used to prepare the dissimilarity matrix for representing the PAs and RFs. NMDS analysis (tree species, Stress= 0.03902596, $k = 2$; Shepard Plot, Fig. 2) indicate these sites can be placed in 5 groups *viz.*, 1. Singba PA and Yumthang RF segregate very differently from all other PAs and RFs, 2. Sombok RF and Kitam PA form a separate complex close to the 3rd complex, 3. Majhitar RF near Kitam segregates with Tumberlong RF (Rangpo), Deling (Rangpo) and Namthang RF, 4. Maenam PA segregates with nearby RFs of Dalley, Yangyang and also Tendong RF and. 5. Fambong Lho PA segregates separately but closer to Maenam-Tendong complex (Fig. 3: Sites – indicated by numbers). Even though the RFs seems to have lost of many species due to degradation, the species composition is similar to the PAs near the RF. The altitudinal gradient and broad forest category within each of these five groups were similar.

The comparison of species-area-curve between PAs and nearby RFs shows the presence of a higher number of species only for Singba WLS in comparison to Yumthang RF. For other PAs and RFs, the number of species in RF may also be restricted by sampling effort (Fig. 4). In general, PAs show a slightly high number of species for similar sampling effort. PAs show a higher number of species with increasing altitude and this number decreased gradually, indicating a possible mid-domain effect (Fig. 5). Mid-domain species richness was also observed by Acharya *et al.* (2011a,b), who reports that species richness (tree species and birds) in Sikkim Himalaya follow a unimodal pattern. However, these are preliminary results and more sampling plots are required at each site to conclusively state any such effect.

CONCLUSION AND FUTURE STRATEGY

This paper summarizes the broad approach of monitoring and baseline data of tree diversity and richness. More vegetation plots and faunal surveys are being undertaken and more rigorous data can only give insights into the variation in species diversity across habitats, similarities and dissimilarities in vegetation across the sites and mid-domain effect in vegetation observed in these sites. More intensive vegetation and faunal sampling are being

carried out in and around the 1-ha plots in 24 sites covering sites in Thangu/Lashar valley and Khangchendzonga National Park for covering more vegetation types. Data on shrubs, herbs and fauna is yet to be analyzed. An institutional mechanism is being developed in consultation with various stakeholders to finally hand over the long-term monitoring to the local stakeholders with the baseline data collected during this project. As indicated earlier, full details and background information on these plots and sites would be handed over to the Forest Department of Sikkim for providing protection to sites as required and for the continuation of the long-term monitoring.

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Ecological Niche Models of Two Alpine Rhododendron Species in the Himalayas

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ABSTRACT: Mountain top plant species are vulnerable to rapid climate change and climate is considered to be a primary determinant in the distribution of plant species. Our study aimed to estimate the future and present distribution of two high altitude Rhododendron species. We projected them to current and future climate conditions for the year 2050 under the moderate (rcp 4.5) and extreme (2050 rcp 8.5) climate change scenario. We considered a widely distributed (*R. lepidotum*) and a narrowly distributed (*R. setosum*) species to quantify the effect of climate change. Our model suggests that the future climate change will alter the distribution of these species. The projected future model predicted a decrease in the habitat of *R. lepidotum* and an increase in *R. setosum* habitat. This study provides information for the conservation planners to adopt a strategy at a species level for conserving Rhododendrons in the face of climate change. ©2019

KEYWORDS: Climate change, Himalaya, Rhododendron, Ecological niche models, Species range shift.

INTRODUCTION

Climate change has affected many ecosystems and taxonomic groups worldwide (Parmesan & Yohe, 2003; Parmesan, 2006). The species range is one of the important ecological factors that are affected in response to climate change (Woodward, 1996; Cox & Moore, 2000). Mostly species with a restricted range distribution may be more vulnerable to the changes in climatic factors that determine the boundaries of their distributions (Thuiller *et al.*, 2005; Manish *et al.*, 2016). The rate of warming in the mountains across the world has been 0.13°C/decade in the past 50 years (Pepin & Seidel, 2005). It is further expected to increase to 0.25–0.48°C/decade by 2085 (Nogues Bravo *et al.*, 2007). In the Himalayan region, approximately three times higher rate of warming (0.06°C/year) than the global average range is predicted (Shrestha *et al.*, 2012). Therefore, understanding of the patterns of change is critical to developing appropriate conservation strategies.

Despite growing interest in studying impacts of climate change in the mountain region, there is still a shortage of scientific information available for the Himalaya (but *ref.* Shrestha *et al.*, 2012; Telwala *et al.*, 2013; Manish *et al.*, 2016). Especially, alpine plant species on mountain ranges have restricted habitat availability; an increase in

temperature will affect the existing communities and might even increase the risk of its extinction if they are unable to move to higher elevations (Dullinger *et al.*, 2012).

Rhododendron forms the dominant component of the high altitude plants distributed across the wide elevation range of 1800–5000 m, which makes them a model species for the climate change related study. The subalpine to alpine transition zone that includes timberline is the most fragile ecosystem in the Himalaya. Rhododendron is the only plant group that has a continuum in the ecotone as mentioned above. Therefore, it is imperative to understand the vulnerability of the keystone species such as Rhododendron (Singh *et al.*, 2009) in the Himalayas in response to climate change.

Against this backdrop, Ecological Niche Models (hereafter ENM) of a narrowly and widely distributed Rhododendron species we explore (1) the areas of climatically suitable conditions in the present scenario and (2) and the role of global climate change in determining the distribution of these species in Himalayas and adjacent areas by projecting it to two scenario for the year 2050s. Furthermore, we discuss future direction for research and conservation management in the wake of global change.

LITERATURE REVIEW

Rhododendrons are inhabitants of high altitude and cover a vast region of south-eastern Asia between the north-western Himalaya through Nepal, Sikkim, Eastern Tibet, Bhutan, Arunachal Pradesh, upper Burma and central China (Kumar P, 2012). Across the globe, Rhododendrons

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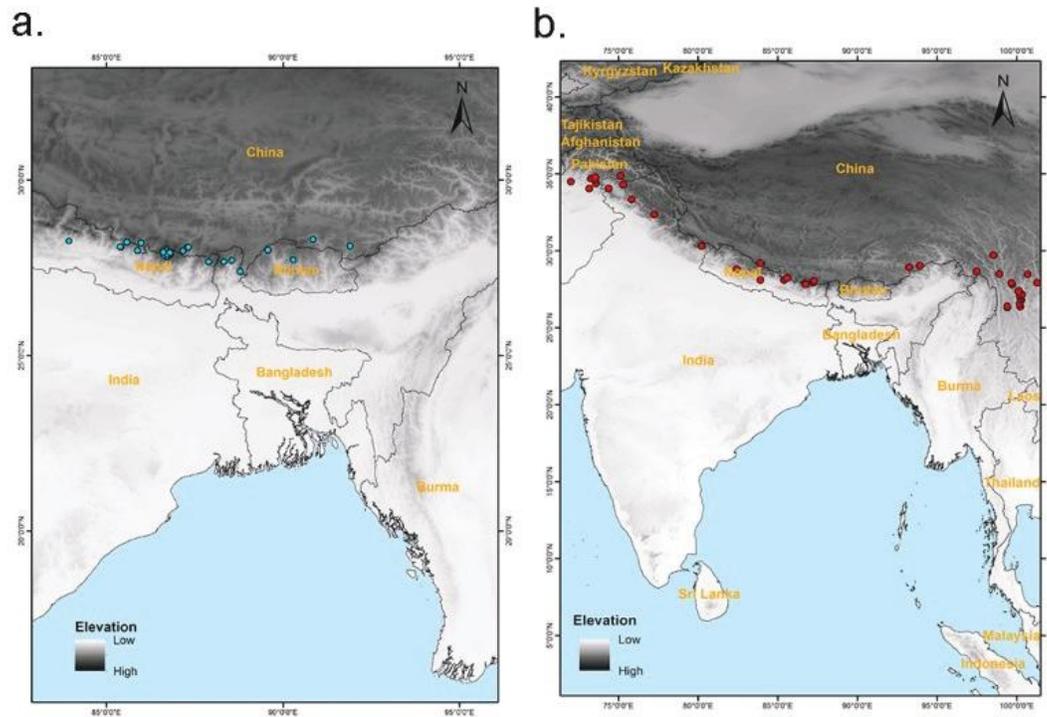


Figure 1: Map showing the distribution range of the two study species (a) *Rhododendron setosum* and (b) *Rhododendron lepidotum*.

are known for its high ornamental value. (Basnett & Devy, 2013). However, apart from having attractive flowers Rhododendron also have high ecological importance (Singh *et al.*, 2009). They form a dominant part of the important forests types as they are distributed all along the temperate, subalpine and alpine region supporting a wide range of animals and plants. They play a significant role in sustaining a wide range of insect and bird pollinator populations due to its profuse flowering and nectar provisioning from early spring to early summer (Ranjitkar *et al.*, 2013; Singh *et al.*, 2009). Especially in the Himalayan context, the major threats to Rhododendrons are deforestations and unsustainable extraction for firewood and incense by local communities. Due to the presence of polyphenols and flavonoids, Rhododendrons also make excellent fuel that burns even in the cold weather. Rhododendron also attracts lots of tourists during its flowering time. However, we still have very less information on its recreational services and neither the impact of high tourism on Rhododendrons habitat is evaluated.

Another important threat to Rhododendron is the climate change. The phenology of Rhododendron is highly sensitive to temperature (Rangitkar *et al.*, 2013), and any shifts in its phenology can have a detrimental effect on its reproductive success further affecting

the species functioning and its distribution. One way of understanding the effect of climate change is using ENMS. For example, Kumar P (2012) demonstrates the possible impact of climate change on Rhododendrons niches and its distributions using a Maximum entropy approach (known as MaxEnt, Phillips *et al.*, 2006) the study suggests that the suitable bioclimatic envelope for Rhododendrons will be shrunk considerably under the envisaged climate change scenario. Furthermore, Ranjitkar *et al.* (2014) used an ENM ensemble method to map the suitable climatic space for two tree Rhododendrons. The wide range of occurrence highlighted the adaptive capacity of these species and their distribution limits were associated with Isothermality, temperature ranges, the temperature of the wettest quarter, and precipitation of the warmest quarter of the year. The recent study on distributional overlaps of closely related Rhododendron sister species across the current and future climates highlights that precipitation variables are associated with *R. lowndesii* distribution, whereas temperature variables are associated with distributions of *R. lepidotum* and *R. cowanianum*. These research findings till present make it evident that it is difficult to generalize the effect of climatic factors across Rhododendron species.

STUDY AREA AND METHODS

This study was carried out within the distribution range of

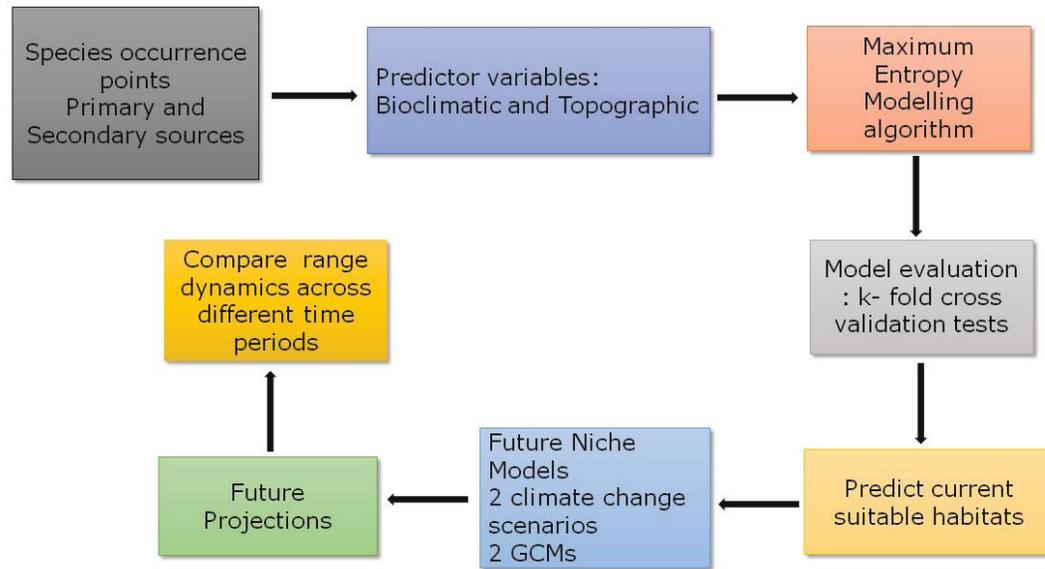


Figure 2: Flow diagram explains the steps taken for building the Ecological Niche Models.

R. lepidotum and *R. setosum* which covered the Himalayan belt (Fig. 1). *R. lepidotum* is widely distributed ranging from 72.0012°E to 103.8015°E and 25.4962°N to 34.8700°N whereas *R. setosum* is narrowly distributed and ranges from 83.9380°E to 91.9030°E and 27.3870°N to 28.3002°N. *R. lepidotum* covers an elevation gradient of 3000–4000 m and *R. setosum* of 3500–4800 m. These species occur as short shrub of about 1–2 feet height and are highly aromatic. We compiled occurrence data from field sampling and secondary sources which includes the Global Biodiversity Information Facility. We recorded 28 presence data for *R. setosum* and 80 for *R. lepidotum*. We filtered out the initial set of occurrence

points after checking for spatial autocorrelation using package spThin in R (Aiello-Lammens, 2014). 19 bioclim variables and one topographic variable-altitude were downloaded from global climate data (<http://www.worldclim.org>). We used Maximum Entropy (MaxEnt) approach estimate to develop the ecological niche models (Fig. 2). The maximum entropy (MaxEnt) approach estimates a species environmental niche by finding a probability distribution of a species occurrence that is based on a distribution of maximum entropy (Philips *et al.*, 2006). Jackknife test was performed to evaluate the variable importance and model performances were

Table 1: List of bioclimatic variables (✓) used for Maxent modeling

Bioclimatic variables	<i>R. lepidotum</i>	<i>R. setosum</i>
BIO1 = Annual Mean Temperature		
BIO2 = Mean Diurnal Range (Mean of monthly (max temp - min temp)		
BIO3 = Isothermality (BIO2/BIO7) (* 100)	✓	✓
BIO4 = Temperature Seasonality (standard deviation *100)	✓	✓
BIO5 = Max Temperature of Warmest Month		
BIO6 = Min Temperature of Coldest Month		
BIO7 = Temperature Annual Range (BIO5-BIO6)	✓	
BIO8 = Mean Temperature of Wettest Quarter		
BIO9 = Mean Temperature of Driest Quarter		
BIO10 = Mean Temperature of Warmest Quarter		
BIO11 = Mean Temperature of Coldest Quarter		
BIO12 = Annual Precipitation	✓	✓
BIO13 = Precipitation of Wettest Month		
BIO14 = Precipitation of Driest Month		
BIO15 = Precipitation Seasonality (Coefficient of Variation)		
BIO16 = Precipitation of Wettest Quarter	✓	✓
BIO17 = Precipitation of Driest Quarter		
BIO18 = Precipitation of Warmest Quarter		
BIO19 = Precipitation of Coldest Quarter		
Altitude	✓	✓

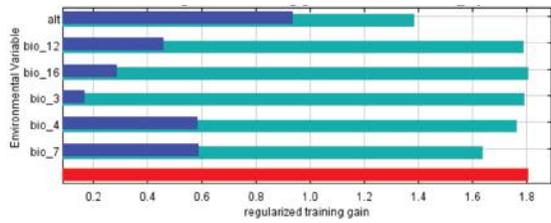


Figure 3a: Jackknife test of regularized training gain for *R. lepidotum*.

assessed using Area under the curve (AUC) statistic.

RESULTS

Model Performance and Importance of Predictors

The final set of the variable used for Maxent modeling after cross-correlation test consists of isothermality (BIO_3), temperature seasonality (BIO_4), annual precipitation (BIO_12), precipitation of the wettest quarter (BIO_16), altitude for both the species. For *R. lepidotum* in addition to these we considered annual temperature range as well (BIO_7) (Table 1). Jackknife tests illustrate that among the topographic variable altitude has a major contribution to the models of both the species (Figs. 3a,b and Table 2). The entire model performed better than random with AUC values >0.5 with *R. lepidotum* having an AUC_{cv}=0.92 and *R. setosum* AUC_{cv} = 0.95. The final models consist of the linear and hinge feature with beta multiplier 2. We performed ten-fold cross-validation tests by using the ten percentile minimum presence as the logistic threshold. The maximum iteration values were set to 5×10³.

The response curves (Fig. 4) illustrate the probability values of the change in occurrence of Rhododendron species concerning key climatic and topographic variables (Figs. 3a,b). The habitat suitability is highest between elevation gradient of 2200–4200 m for *R. lepidotum*, and we noticed a sharp increase in its habitat suitability up to ~6600 m for *R. setosum* (Figs. 4a,e). The habitat suitability was found higher towards lower values of annual temperature (Bio_7) (Fig. 4b) and lesser with the increase in temperature. Similarly, probability values for *R. setosum* also peaked around lower values of temperature seasonality (BIO_4) and the higher values of isothermality (BIO_3) (Figs. 4c,d).

Predicted Range of *R. lepidotum* and *R. setosum*

Under current climatic conditions, the model predicted a total suitable area of 9,01,176 km² for wide-ranging *R. lepidotum*, and we observed a decrease

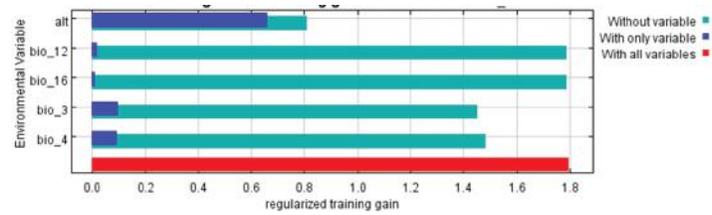


Figure 3b: Jackknife test of regularized training gain for *R. setosum*.

in its overall suitability area in both the future scenarios. Whereas in case of narrow ranging *R. setosum*, the present scenario model predicted a total area of 6,87,338 km² for *R. setosum* and we observed an increase in the overall suitability area in both the future scenarios for the year 2050. For *R. lepidotum*, the 2050 rcp (representative concentration pathway) 4.5 predicted 1,22,494 km² (reduction of 7,78,682 km² in the total area from the present) and 2050 rcp 8.5

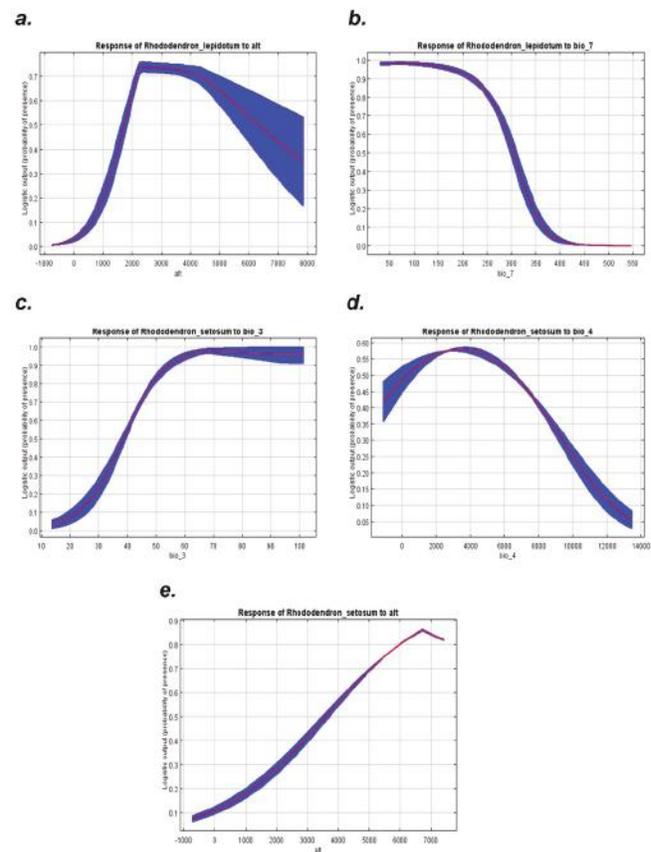


Figure 4: Marginal response curves illustrating the major environmental variables affect the suitability predictions for *R. lepidotum* and *R. setosum* (a) Response of Rhododendron lepidotum to a) altitude (b) temperature annual range (c) Response of Rhododendron setosum to isothermality (d) temperature seasonality (e) altitude.

Table 2a: Result of variables contribution for *R. setosum* distribution

Variable	Percent contribution	Permutation importance
Isothermality (BIO2/BIO7) (* 100)	37.8	8.4
Altitude	37	65.9
Temperature Seasonality (standard deviation *100)	24.5	23.9
Annual Precipitation	0.3	0.7
Precipitation of Wettest Quarter	0.3	1

Table 2b: Result of variables contributions for *R. lepidotum* distribution

Variable	Percent contribution	Permutation importance
Altitude	54.6	29.6
Temperature Annual Range (BIO5-BIO6)	20.7	33.6
Annual Precipitation	20.1	5.1
Temperature Seasonality (standard deviation *100)	3.6	29.7
Isothermality (BIO2/BIO7) (* 100)	0.8	1.8
Precipitation of Wettest Quarter	0.2	0.4

scenario predicted 1,27,652 km² (reduction of 7,73,524 km² in the total area from the present). In case of *R. setosum* 2050 rcp 4.5 predicted 9,07,011km² (an increase of 2,19,673 km² in the total area from the present) and 8,65,838 km² (increase in 1,78,500 km² in the total area from the present) (Fig. 5).

DISCUSSION

The ecological niche models developed in this study showed that *R. lepidotum* a wide-ranging species is likely to face a reduction in the highly suitable habitats whereas the narrowly distributed *R. setosum* may have an increase in its

suitability area for both future climate change scenarios for the year 2050. For both species altitudes was evident to be an important factor associated with its distribution. With an increase in altitude, other environmental factors change consecutively. For instance, with a lapse rate of 0.6°C / 100 m of elevation, a plant population spread over 500 m of elevation range will experience mean temperature differences of ca. 3°C (Ranjitkar *et al.*, 2013). This indicates that these species distributed across the elevation gradient are highly vulnerable to future warming. The temperature variables such as temperature

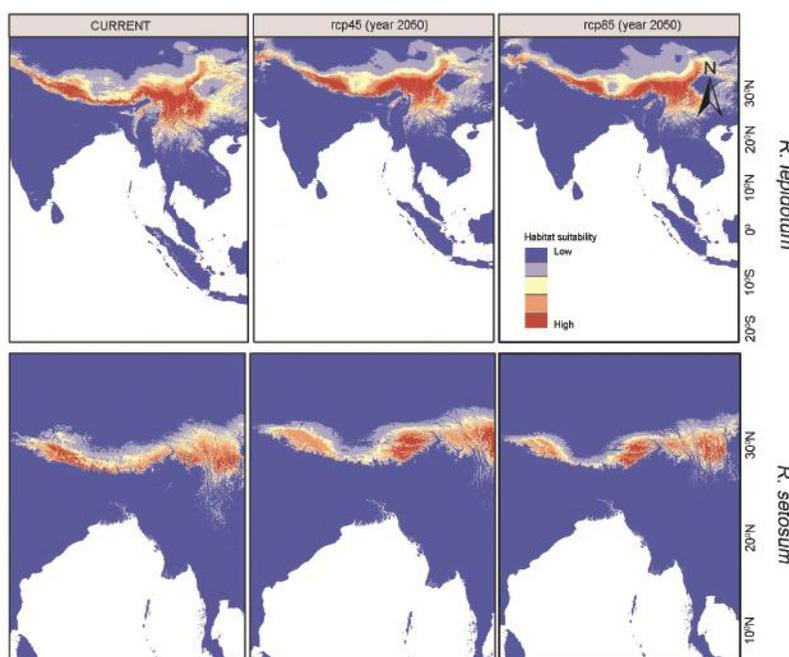


Figure 5: Habitat suitability maps of *R. setosum* and *R. lepidotum* in different climate change scenario for the time period 2050 under rcp 4.5 and 2050 rcp 8.5. The warmer color indicates areas with highly suitable areas.

annual range, temperature seasonality including isothermality are also predicted to be an important factor in determining the niche of *Rhododendron* species and hence changes in this in future can have an impact on their potential ecological niches. However, our models lack true absence records (Peterson *et al.*, 2007) which often raise the issue of over-fitting. The prediction accuracy and model performance do not only depend on the number of presences but are also affected by the number of pseudo-absences (VanDerWal *et al.*, 2009).

Apart from the environmental factors *Rhododendrons*, ecological niches might also be influenced by the biotic factors. The floral morphology denotes its attractiveness towards pollinators, and many studies have highlighted the dependence of *Rhododendrons* on pollinators (Huang *et al.*, 2017). Hence, understanding the pollinators influence on its ecological niches also becomes one of the crucial areas of investigation for its survival. Another important factor the land use change is one such aspect which can also induce niche shifts (Titeux N *et al.*, 2016). Even though MaxEnt is effective in predicting the niche of a species, but some caveats still exist when we consider presence only points (Baldwin, 2009).

CONCLUSIONS

In conclusive remarks, our model suggests that altitude is the primary factor that determines the ecological niches of these species. Even though our models indicate the putative role of climate change in *Rhododendron* species distributions, we recommend interpreting this with caution. For example, there could be several factors which we could not consider in the current modeling approach (land use and land cover changes, aspect, slope). We propose these models as a hypothesis which needs to be tested with several approaches to arrive at a strong conclusion. However, our model broadly describes the trajectory of change in *Rhododendron* species distribution in the phase of climate change.

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Vegetation Patterns of Treeline Ecotone in the Pangi Valley, Western Himalaya

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ABSTRACT: Treelines represent an ecotone from closed-canopy forests to open grasslands. They are highly sensitive to climate change and consequently studying treelines is a contemporary issue of global implication. The present study was, therefore, conducted across a treeline ecotone in the Pangi valley of Himachal Pradesh. A permanent site of 30 × 100 m was marked and a soil temperature data logger was installed. For population estimation, total count was carried out for trees in the marked site and their height and girth was recorded. Herbaceous flora was sampled by laying quadrats of 1 × 1 m ($n = 10$). A total of 34 flowering plant species were recorded in the marked treeline site. *Betula utilis* was the only tree species present at the treeline site and reported a density of 490 individuals/ha. With respect to herbs, *Cortusa* (6.6/m²) and *Picrorhiza kurroa* (4.9/m²) reported the highest density. Shrubs were not present. Higher species diversity ($H' = 2.62$) and richness (18) was recorded in the sampling plots above the treeline. Altogether, species were more evenly distributed above the treeline and their composition varied sharply across the current treeline ecotone. The annual mean soil temperature varied from 19.21 °C during the growing season to -2.82 °C during the winter season. It is expected that the baseline primary information thus generated will help in better understanding of the hitherto less explored treelines of Western Himalaya. ©2019

KEYWORDS: Himalaya; Treeline; Climate change; Vegetation; Diversity; Richness.

INTRODUCTION

Trees at their upper altitudinal range in the mountains are thermally limited for growth and development (Holtmeier, 2009; Butler et al., 2009). This thermally limited boundary of upright woody plants where individuals attain 3 m stem height is generally referred as treeline (Korner, 2012). Various theories have been put forwarded for explaining the altitudinal limits and prominence of treelines. Low temperature isotherm, carbon balance (growth limitation), short growing season, nutrient limitation and land use patterns are the prominent ones. Except for the low temperature limitation, others do not seem to be fundamental in explaining treeline positions globally (Korner, 2012). However, these factors are good proxies for defining treeline positions at regional scales. Treelines generally occur at higher altitudes in the tropical region and gradually drop down to sea level in northern boreal forest limit. In the arctic, tundra treelines occur close to sea levels (400–600 m). In Himalaya, treelines occur at an elevation of 3600±200 m asl and are used by the trans human communities for meeting their daily requirements (Shrestha & Vetaas, 2009; Schickhoff et al., 2015). They

occur at higher altitudes in the eastern Himalaya as compared to western Himalaya.

Contrary to as one would expect, treeline is not a smooth line but a transition zone that forms an ecotone between the open alpine grasslands at higher altitudes and closed canopy forests at lower elevations (Holtmeier & Broll, 2007) (Fig. 1).

The variability in climatic conditions across this zone determines the distinct vegetation composition of treelines (Korner, 2003; Camarero et al., 2006). However, human influences have also resulted in their modification (Aitor et al., 2010; Schickhoff et al., 2015). It is argued that in response to changing climatic conditions plant species will migrate to higher elevations, and thus treelines have been prioritized for monitoring impacts of climate change (Smith et al., 2009; Pauli et al., 2014). This migration



Figure 1: (a) Vegetation sampling and (b) Data logger installation at treeline site.

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of treeline position could be an outcome of enhanced tree growth and recruitment processes over a period of time (Hoch & Korner, 2005; Hofgaard *et al.*, 2009; Aakala *et al.*, 2014). These growth patterns are mediated by various biotic and abiotic factors. Evidences in support of this are already coming (Gamache & Payette, 2005; Batllori & Gutierrez, 2008; Harsch *et al.*, 2009).

The Himalaya has the highest and most diverse treelines in the world (Miehe *et al.*, 2007) and therefore imprints of climate change are expected to be more pronounced here. Unfortunately, this climate sensitive ecotone in the Himalayas has been much less studied than its European counterparts probably due to its rugged nature and remoteness (Schickhoff, 2005; Shi & Wu, 2013). Little is known about the spatial distribution of treelines and their characteristics in the Himalayan region (Zhang *et al.*, 2009; Burzle *et al.*, 2017). The dearth of information from Himalaya is reflected in the controversies that originate and surround it. Be it be treeline movement or the glaciers recession (IPCC, 2014; SAC, 2015; Schickhoff *et al.*, 2015).

It is with this background the present study was initiated. The objectives framed for the study include (i) vegetation documentation along treeline ecotone, and (ii) documentation of spatial patterns of tree species.

LITERATURE REVIEW

Thematic work on treelines extends back over the 200 years (Holtmeier, 2009). However, it was cursory and observational. It was in the early phase of the 20th century when treelines were studied from an ecological perspective. Earlier studies were more focused on the effect of temperature on tree growth across altitudinal limits. Tranquillini (1979) studied the Australian Alps and presented first general synthesis on experimental ecophysiological work. While Stevens and Fox (1991) discussed the possible causes of treelines. Later, several authors have put forward the possible mechanisms for treeline position (James *et al.*, 1994; Korner, 1998; Smith *et al.*, 2003; Hoch & Korner, 2003).

A significant rise in the global temperature is expected to impact the alpine vegetation more in near future (Korner, 2003). In this context, Gamache and Payette (2004) reported significant patterns of height and growth in black spruce of Canada. Similarly, Daniel and Velben (2004) assessed the influence of climate and the results endorsed the upslope migration of treeline. Spatio-temporal patterns of trees at the Spanish Mountain were studied by Camarero *et al.* (2006). Further work or studies (Harsch *et al.*, 2009; Basler & Korner, 2012; Aakala *et al.*, 2014) provided detailed ecological insights into treeline distribution in the Rocky

Mountains, Scandes and Australian Alps. Ameztegui *et al.* (2010) studied the forestline at Pyrenees and noted that changes in patterns of tree recruitment are directly governed by changes in land use characteristics. Due to climatic warming, Basler and Korner (2014) reported shift in the phenological traits of tree species. Treml *et al.* (2017) noticed treeline sensitivity to recent climatic change at Colorado range in USA.

In the Himalayan region, studies on general characteristics of timberlines in the Tibetan Plateau (Li, 1993) were amongst the pioneering ones. Shi *et al.* (2006) assessed the carbon reserve in woody taxa of treeline forest in Tibetan Plateau. Their findings revealed depletion of carbon reserve, which was also indicating the carbon sink limitation. Other workers like Shrestha and Vetaas (2009), Zhang *et al.* (2009) attempted to explore the spatial patterns of plant species at treeline ecotone in Nepal Himalaya and Tibetan Plateau, respectively. Liang *et al.* (2011) noticed very little change in the position of treeline over the past two hundred years in Tibetan Plateau. Similarly, Wang *et al.* (2012) have reported site-specific effects on treelines.

In recent times studies like Fang *et al.* (2009), Suwal *et al.* (2016), and Du *et al.* (2017) noticed significant uphill migration of treeline in the Tibetan Plateau and Nepal Himalaya. To address the response of treelines, Schickhoff *et al.* (2015) evaluated the sensitivity of treeline to climate change over the Himalayan range. Their findings reveal that recruitment under current scenario shows sensitivity to climatic change. Other studies from the Himalayan region indicate importance of soil properties such as temperature, moisture and nutrient deficiency to treeline altitudinal limit (Muller *et al.*, 2016; Drollinger *et al.*, 2017). With respect to Indian Himalaya, Sundriyal & Bisht (1988) have studied the regeneration status of forests in timberline in Garhwal Himalaya. They noticed that harsh winter conditions affect the survival of sprouts and seedlings. Similarly, Rawal *et al.* (1991) documented the phenology of tree species in Kumaun Himalaya and found that deciduous tree species were in higher constitution. Rawal and Dhar (1997) studied the floral diversity of timberline in Kumaun Himalaya and reported the high percentage (>64%) of native taxa at this zone. Based on results of remote sensing, Singh *et al.* (2011) noticed a significant shift of treeline vegetation in Indian Himalayan region. Similarly, Bharti *et al.* (2012) documented shift in the position of alpine treeline. Uncertainties on uphill movement of treelines in the Himalaya make this subject interesting (Panigrahy *et al.*, 2010; Singh *et al.*, 2012).

Table 1: Characteristics of treeline ecotone site at Pangli valley

Characteristics	Value
Longitude	76° 26' 34.957" N
Latitude	33° 04' 34.436" E
Treeline elevation (m asl)	3780/3800
Plot elevation limit (m asl)	3780–3900
Aspect	North East
Mean slope (°)	36.02
Mean tree height (m)	5.02±0.00013

MATERIALS AND METHODOLOGY

Study Area

The present study was carried out in the Pangli valley of Himachal Pradesh, western Himalaya that is inhabited by Pangwal’s-local people. The area receives heavy snowfall during winters and is drained by the river Chenab. It shares boundary with Lahaul & Spiti in the south-east and Jammu & Kashmir in the north-west, and has prominence of precipitous cliffs. At lower elevations, coniferous taxa such as *Cedrus deodara* and *Pinus wallichiana* are dominant while at higher altitude forests of *Betula utilis* are prevalent. Due to its ruggedness and steep terrain, the area is relatively less disturbed. Pristine environment of this valley provides an ideal environment for studying treeline dynamics. A *Betula utilis* treeline was identified in the region. The characteristics of which

are provided in Table 1. The site elevation and coordinates were recorded using a Trimble Juno GPS while slope and aspect were noted using a clinometer (Nikon) and compass (Brunton), respectively. *Betula utilis* is a broadleaf deciduous tree species that occurs at the upper elevational limit of forest vegetation (Fig. 1). Most of the treelines in Himachal Pradesh are formed by this broadleaf species.

Field Sampling

Following reconnaissance surveys, a site offering accessibility, repeatability, and relatively undisturbed status was selected. A permanent site of 30 × 100 m was marked across the treeline ecotone with its lower axis parallel to the slope. Total count of trees was done for population assessment and their X–Y coordinates were recorded to the nearest 0.1 m. The point marking (X–Y = 0, 0) was done from the lower left corner of the rectangular site. Tree height (*h*) and circumference at breast height (*cbh*) was recorded for individual tree stem (Fig. 1a). The height of trees was measured using a Nikon clinometer, except for the plants that were ≤2 m, while the circumference was measured using a meter tape.

To estimate the understory plant species 10 plots of 1 m² size were laid. These plots namely P1 to P10 were positioned systematically along the elevational gradient inside the marked site (30×100 m). Presence of herbaceous species and their population was recorded in these 1 m² quadrats. Plant samples were brought to the laboratory for taxonomic identification. Habitat characteristics such as aspect, slope, and terrain of site were also recorded.

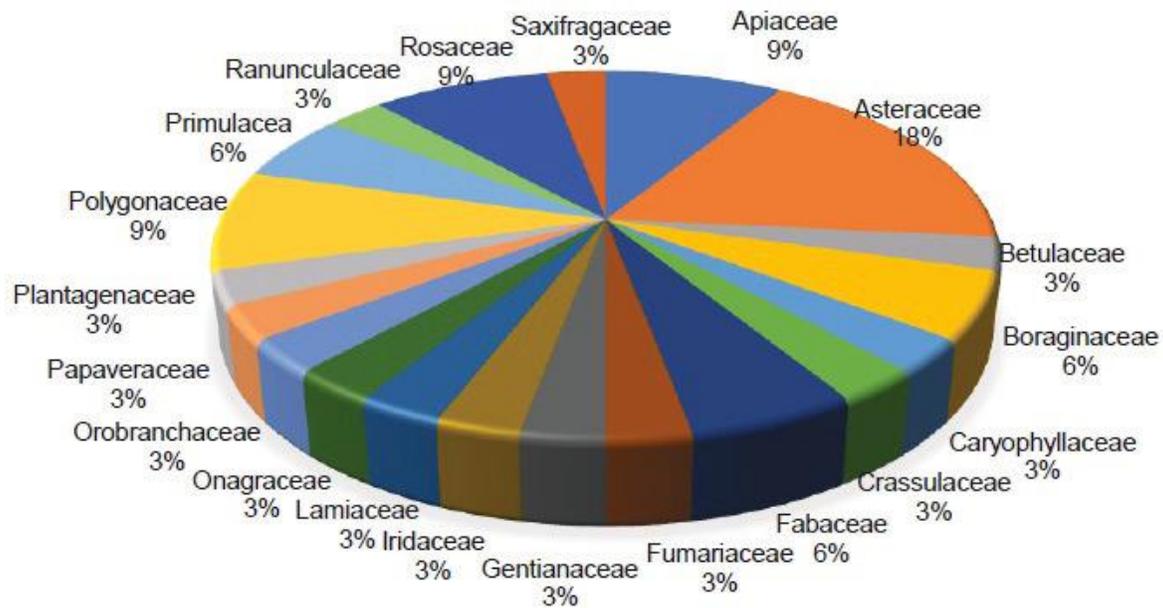


Figure 2: Family composition for quantified taxa at study site.

Table 2: Population status of plant species at the study site

Species	Family	Life Form	Density	Frequency (%)
<i>Achillea milifolium</i>	Asteraceae	Herb	1.5	30
<i>Anaphalis busua</i>	Asteraceae	Herb	1.7	40
<i>Anaphalis triplinervis</i>	Asteraceae	Herb	1	50
<i>Anemone tetrasepala</i>	Ranunculaceae	Herb	1	40
<i>Aconogonum molle</i>	Polygonaceae	Herb	0.3	30
<i>Astragalus rhizanthus</i>	Fabaceae	Herb	1.7	30
<i>Bergenia ligulata</i>	Saxifragaceae	Herb	0.8	30
<i>Bupleurum himalayense</i>	Apiaceae	Herb	1.5	50
<i>Chaerophyllum villosum</i>	Apiaceae	Herb	0.3	20
<i>Cortusa brotheri</i>	Primulaceae	Herb	6.6	60
<i>Corydalis govaniana</i>	Fumariaceae	Herb	1.2	40
<i>Cynoglossum wallichii</i>	Boraginaceae	Herb	0.6	30
<i>Epilobium sps.</i>	Onagraceae	Herb	1.7	40
<i>Gentiana argentea</i>	Gentianaceae	Herb	0.7	50
<i>Geum elatum</i>	Rosaceae	Herb	0.9	20
<i>Hedysarum sps.</i>	Fabaceae	Herb	1.1	30
<i>Iris kemaonensis</i>	Iridaceae	Herb	1.3	20
<i>Lappula sps.</i>	Boraginaceae	Herb	1	40
<i>Meconopsis aculeata</i>	Papaveraceae	Herb	0.3	20
<i>Pedicularis punctata</i>	Orobanchaceae	Herb	1.5	20
<i>Picrorhiza kurroa</i>	Plantagenaceae	Herb	4.9	70
<i>Polygonum affine</i>	Polygonaceae	Herb	1	60
<i>Potentilla argyrophylla</i>	Rosaceae	Herb	0.4	30
<i>Potentilla atosanguinea</i>	Rosaceae	Herb	0.7	20
<i>Primula denticulata</i>	Primulaceae	Herb	1.1	40
<i>Rhodiola himalensis</i>	Crassulaceae	Herb	1.7	30
<i>Rumex nepalensis</i>	Polygonaceae	Herb	0.7	30
<i>Saussurea taraxacifolia</i>	Asteraceae	Herb	0.5	20
<i>Selinum tenuifolium</i>	Apiaceae	Herb	4.1	60
<i>Silene aqualis</i>	Caryophyllaceae	Herb	1	50
<i>Tanacetum falconeri</i>	Asteraceae	Herb	1	30
<i>Taraxacum officinale</i>	Asteraceae	Herb	0.3	20
<i>Thymus linearis</i>	Lamiaceae	Herb	2.5	50
<i>Betula utilis</i>	Betulaceae	Tree	490	

Installation of Data Logger

For recording soil temperature data, a Geo precision data logger has been installed 10 cm below the soil in the center of the site. The logger has an accuracy of ± 0.10 C at 0 °C and resolution of 0.01 °C. The logger has been programmed to record temperature at a temporal resolution of 60 min (Fig. 1b).

Spatial Pattern and Diversity Analysis

The spatial distribution pattern of *Betula utilis* has been analyzed using the XLSTAT 2017 (version 2017.1). This requires one independent variable and one dependent variable. Here, X–Y coordinates are the independent variable while height (*m*) and girth (*cbh*) of individuals are dependent variables. The herbaceous layer was

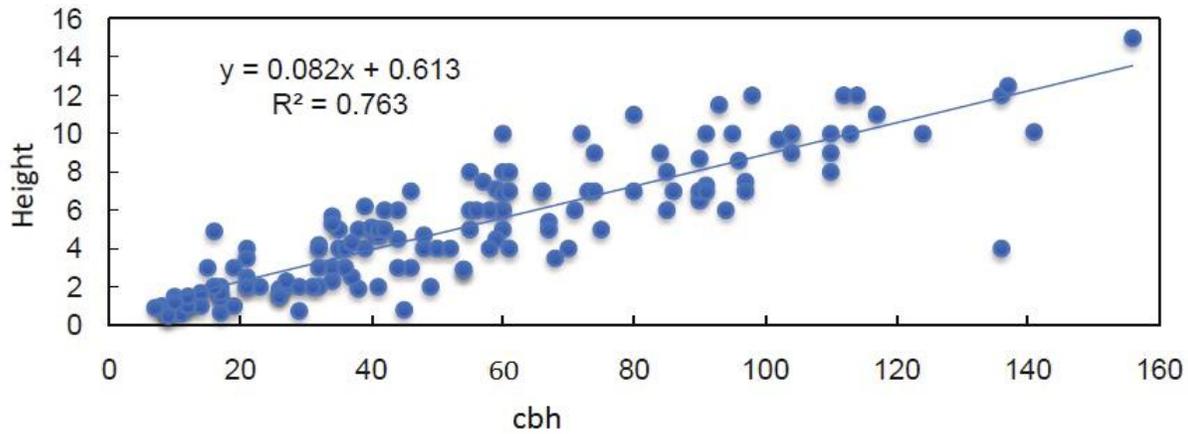


Figure 3: Scatter plot showing the relationship between the tree height (*h*) and *cbh*.

analyzed for its species composition. The Shannon–Weiner diversity index (*H'*) and evenness (*E*) index were calculated using the equations given below:

$$H' = \sum_{i=1}^s (pi) (\ln pi) \quad \text{Equation (1)}$$

where *S*: number of species; *pi*: proportion of individual or the abundance of the *i*th species; *ln*: log base_{*n*}.

$$E = \frac{H'}{H'_{max}} = - \frac{\sum_{i=1}^s (pi) (\ln pi)}{\ln s} \quad \text{Equation (2)}$$

where *S*: number of species; *pi*: proportion of individual or the abundance of the *i*th species; *ln*: log base_{*n*}.

RESULTS AND DISCUSSION

Floristic Composition at the Treeline Site

Altogether, 34 vascular plant species were recorded from the site. Of these, 33 are herbs and 1 is a tree species (Table 2). Shrubs were not observed at the site. These plant species belong to 20 families, of which Asteraceae (18%) was the dominant family (Fig. 2). Compared to the forested area, higher number of species were recorded in the open area above treeline.

Phytosociology

Betula utilis was the dominant and the only tree species present at the site. It's stand density was recorded to

be 490 individuals/ha (Table 2). The height of tree species ranged between 0.5 and 12.5 m while the girth ranged from 7 cm to 156 cm. The average tree height was 5.02±0.00013 m. This shows that most of the tree individuals were young and recruited in the recent past. It could be an early indicator of improved environmental conditions. Similar patterns of tree recruitment were also noticed by Camarero *et al.* (2000) in treeline ecotone of Spanish central Pyrenees. Several other workers have also noticed increased stem density in the transition ecotones (Camarero *et al.*, 2006; Burzle *et al.*, 2017). Shrubs were not found growing in the plot.

The density of the herbaceous species varied from 0.3 to 6.6 individuals/m² (Table 2). *Cortusa sp.* (6.6/m²) and *Picrorhiza kurroa* (4.9/m²) reported the highest density. It was noticed that these species had good populations in the upslope plots.

Diversity/ Dominance/ Evenness of Plant Species

The Shannon-Weiner diversity index, evenness and richness were calculated for the herbaceous species (Table 3). Diversity assessment reveals continuous changes in species composition along the vertical transect from the forest canopy to open landscape. The highest diversity value (*H'* = 2.62) was observed in the sampling plots above the treeline. On the other hand, lowest diversity (*H'* = 0.70) was recorded in the downslope plots under the tree canopy. In general, diversity increased from the forest canopy to open landscape. These findings were in line with work of Batllori *et al.* (2009). The value of evenness indicates that species

Table 3: Species diversity, evenness and richness of plant species at the study site

Density Indices/ Plots	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Diversity	1.59	0.70	1.39	1.95	1.89	2.57	2.63	2.48	2.61	2.53
Evenness	0.82	0.44	0.71	0.94	0.82	0.89	0.95	0.92	0.92	0.89
Richness	7	5	7	8	10	18	16	15	17	17

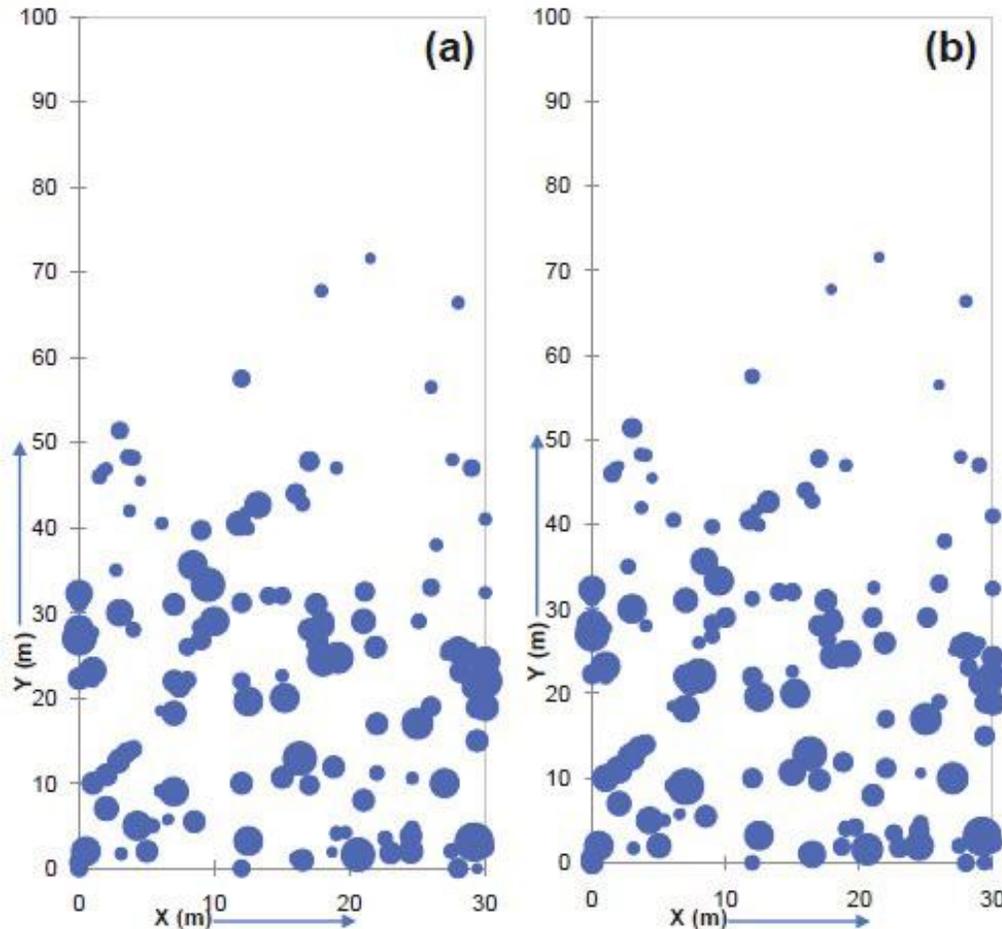


Figure 4: Spatial patterns of all the individual's height (h) and dbh along the treeline ecotone site at Pangri valley. (a) Spatial location of tree individual and height and (b) tree individuals and cbh .

are evenly distributed above the treeline. Highest evenness value ($E = 0.94$) was also recorded in the plot located in open areas. Similarly, richness of plant species also varied between the plots (Table 3). Highest species richness was found in P6 plot, where 18 plant species were recorded. A sharp change in species composition is one of the fascinating features of treeline ecotone (Young & Leon, 2007). The plant species diversity and richness at small scale is governed by multiple factors such as topography, light availability, moisture, temperature, nutrients, competition, herbivores, etc. However, large variations in number of species found above and below the treeline elevation could be explained on the basis of canopy cover. The open canopy in up slope may guide species richness along this transition zone. Low light availability and negative effect of dense canopy have already been documented by Camarero *et al.* (2006) and Shrestha & Vetaas (2009).

Spatial Distribution of Individual Trees

We analyzed the height and cbh of individual trees. There was a significant correlation between individual tree height

and cbh ($R^2 = 0.76$) (Fig. 3). The height and girth of the individuals decreased with altitude and along the treeline ecotone. Along the treeline ecotone, demographic traits like height and girth are highly governed by the altitude of the landscape and severity of climate (Fig. 4). Further, spatial pattern analysis revealed a large number of young individuals above the treeline (Fig. 4). It indicates recruitment of tree saplings during the recent times. This might be due to the improved complex microclimatic conditions or land use patterns along the treeline ecotone. The presence of saplings above the treeline also indicates reproductive potential of birch tree. The seeds of *Betula utilis* are light and mostly wind dispersed. Tree individuals were found to form clusters and the young individuals were found to be distributed in the vicinity of larger trees. This pattern is in agreement with tree recruitment patterns at treeline ecotone in Nepal Himalaya and Tibetan Plateau (Wang *et al.*, 2012; Gaire *et al.*, 2014). Herein nursing may be important (Lyu *et al.*, 2016). This would help young individuals to exploit the favorable conditions around the

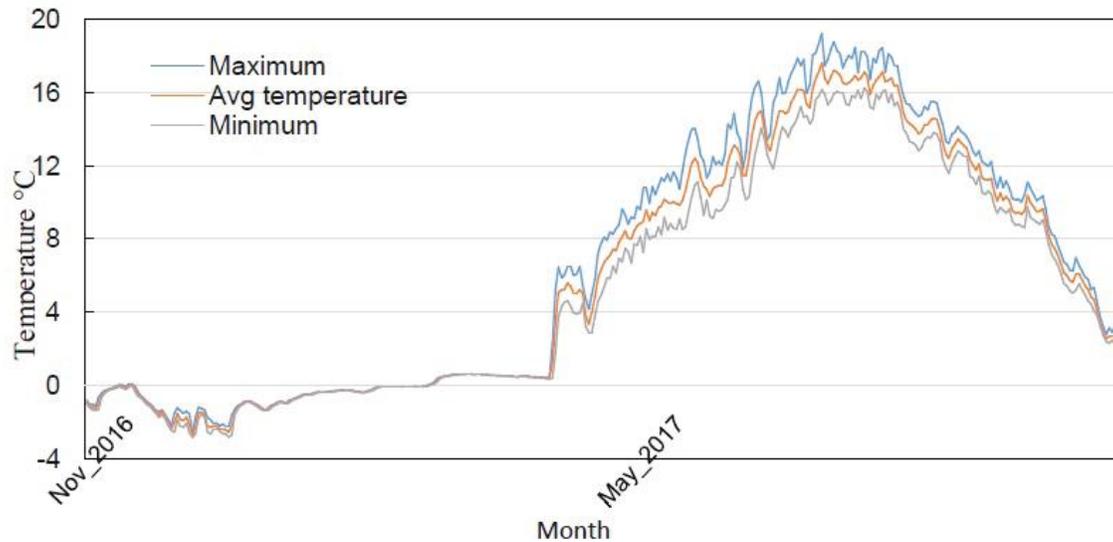


Figure 5: Soil temperature variation at treeline site.

mature trees. Uniform distribution of young individuals within the forest and also above the treeline indicate its potential for upslope migration in near future.

Soil Temperature

Soil temperature varied from -2.82 °C during the winter season to 19.21 °C in the month of growing season (Fig. 5). During the winters (November–March), average temperature was found to be -0.13 °C while during growing season (May–August) the average temperature was 11.28 °C. The maximum and minimum temperature recorded during this period was 17.61 °C and 1.94 °C, respectively. Altogether, soil temperature in the growing period was found to be above the hypothesized soil temperature (7 °C) in the treeline areas.

CONCLUSION

Betula utilis forms the tree line in the area. Patterns of species diversity and richness revealed the influence of forest canopy along the altitudinal gradient. Overall, it increased from closed canopy forests to open areas. Further, the growing season soil temperature was above the hypothesized soil temperature (7 °C) in the treeline areas.

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Identification of Sites for Long-Term Monitoring in Darjeeling

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ABSTRACT: Large scale transformation and a rapid loss of biodiversity have emphasized the need to focus efforts on long term monitoring. Long-Term Monitoring of ecosystems therefore becomes necessary in order to understand human induced changes on fragile ecosystems and also for better decision-making, planning and management. Here, we focus on a biodiversity hotspot, the Darjeeling Himalayas and develop a bottom up, participatory, interdisciplinary approach of identifying natural habitats under immense pressure, the threats involved and sites that should be under long-term monitoring efforts. Using a systematic sampling method in QGIS, we revealed 252 sites inside as well as outside of protection, should be placed immediately under long-term monitoring with support from the state. ©2019

KEYWORDS: Systematic sampling; Biodiversity loss; Long-term monitoring; Himalayas.

INTRODUCTION

Change has been an inevitable outcome of transformation. Since the Industrial Revolution, our planet has experienced large-scale changes in the form of land-use and land cover, pollution, movement of fauna and people, etc. (Turner *et al.*, 1990; Vitousek, 1994). There has been a shift in focus from studying pristine areas as they are to how human activities (Chin *et al.*, 2014) have influenced and brought about large-scale biodiversity changes through the practice of agricultural production, especially in areas that are considered fragile and ecologically sensitive. The Himalayas are one such area in being part of the world biodiversity hotspots (ENVIS Centre on Floral Diversity) which has undergone rapid change in the past twenty–thirty years (Pradhan, 2002). Darjeeling is a region that lies within this unique hotspot. It is home to the Red Panda and Asiatic Bear in the high altitudes, Leopards and Civets in the mid-lower altitudes to Pangolins. It is well known for its evergreen oak–rhododendron forests, the mixed moist evergreen and deciduous forests and low dry deciduous forests. It is where Hooker and Gamble first made their Himalayan journey. Although a region that was considered a goldmine for botanists and zoologists, there has been a lag in the effort to understand the impact of large-scale processes such as global warming and climate change on the natural habitats of the region. Given its unique nature, the Darjeeling region seems to be an ideal setting to study the effect of short- and long-term changes. However, there has been no such effort that addresses these concerns in the context of the Darjeeling Himalayas. In this study,

we describe an interdisciplinary, participatory approach of identifying areas that are under immense threat in the Darjeeling Himalayas and in dire need for long-term monitoring and research. We present results of an exercise carried out in the Darjeeling hills, where protected areas, reserved forests and production scape coalesce with one another and therefore are crucial for future conservation planning and action.

LITERATURE REVIEW

The accelerated loss of biodiversity has been a cause of concern which brought about the creation of the “Parties of Convention” who “committed” themselves “to achieve by 2010 a significant reduction of the current rate of biodiversity loss at the global, regional and national level as a contribution to poverty alleviation and to the benefit of all life on Earth” (CBD, 2002). This concern has led to a growing need to monitor data taken over a longer period of time (Butchart *et al.*, 2010). Perhaps the oldest and longest running example of long-term monitoring research is the Park Grass Experiment which was set up in 1856 to study how fertilizers affect hay meadows (Lawes & Gilbert, 1880; Silvertown *et al.*, 2006). This study systematically revealed two important outcomes that could have potential impact on plant biodiversity through change in soil pH over time and accumulation of soil nutrients (Silvertown, 1980; Crawley *et al.*, 2005). There have been other studies such as the Continuous Plankton Recorder studies which started in 1925 to study plankton communities in response to ocean warming (Kirby *et al.*, 2007). These long-term assessments formed the foundation of Long-Term Monitoring Research and highlighted how important they were to understand the impact of large scale, temporal changes on floral and faunal communities. Similarly, in the United States as part of their Long Term Monitoring Research, experimental plots were set up in Cedar Creek to

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assess whether observed variation in plant biomass over time, was dependent on the diversity of plant community that existed at the plot (Tilman *et al.*, 2006). In India, Long-Term Monitoring studies are comparatively rare; however, there is work from southern Western Ghats, another biodiversity hotspot that has focused on the long-term monitoring of structure and dynamics of

tropical deciduous forests (Sukumar *et al.*, 1992), modes of pollination of 86 tropical tree species and the fruiting phenology within the wet montane forest ecosystem of Kakachi (Devy & Davidar, 2003; Ganesh & Davidar, 2005).

Even though biodiversity loss has been cited as the primary reason for the need for long-term monitoring (Magurran *et al.*, 2010), it has also been emphasized that a good and effective design for environmental planning requires the information and understanding from long-term research (Lindenmeyer *et al.*, 2012). Additionally, it also helps us gain an understanding of how ecosystems are responding to changes such as disturbance events like hurricanes (Heartsill Scalley, 2010), addition of external inputs (Lawes & Gilbert, 1880), and environmental warming (Kirby *et al.*, 2007).

Table 1: Names and location of forest villages used in this study

Name of Forest Village	Location
Turzum	26.997765, 88.403849
Mangwa	27.059930, 88.410869
Peshok	27.044387, 88.340982
Deorali	26.906189, 88.301278
Poobong	27.005570, 88.211556
Gurdum	27.122499, 88.059021
Gurdum (Tonglu)	27.033522, 88.081394
Pulung Dong	26.983601, 88.178596
Group-Batasia	27.025715, 88.116539
Lopchu	27.059309, 88.370335
Barbatia	27.024499, 88.230156
Daragaon High School	27.141541, 88.081149
Namla	27.101060, 88.096415
Dhotray	27.049555, 88.110223
Lingsebung	27.093549, 88.087839
Simbung	27.023106, 88.130595
Khopi Dara	26.993045, 88.120404
Ghoom	27.000630, 88.242660
Majdhura	27.006797, 88.177157
Bich gaon (Majhia)	26.998685, 88.126041
10th Mile	27.027111, 88.118657
Sirikhola	27.130457, 88.060061
Rammam	27.154417, 88.078983
Samanden	27.182800, 88.070950
Gorkhey	27.187263, 88.072233
Rambi	26.984800, 88.305300
Rampuria	27.009275, 88.308457
Chatakpur	26.966793, 88.309916
Paschim Parmen	26.961817, 88.299633
Rangeroon	27.013894, 88.282609
Reshope	26.973999, 88.347270
Deorali Singhalila	27.041758, 88.097469

MATERIALS AND METHODS

Study Area

The site that was selected for this study included the Darjeeling Himalayas, a hill station in West Bengal, India. It is situated in the eastern part of the Himalayan range and is part of the world biodiversity hotspots. The Singalila National Park and Sinchal Wildlife Sanctuary along with the associated state-managed plantation forests and production scape were selected as an ideal settings for long-term monitoring.

Methods

Informal Interviews

Since there exists no baseline, we visited forest villages in the proximity to the two protected areas. Based on the Annual Report of the Forest Department (AAR, 2015–16), there are 41 forest villages under the Darjeeling Territorial and Wildlife Division. Out of the 41 forest villages, we gained access to only 30 due to various logistic constraints. We started by having conversations with different people from Darjeeling regarding the different land cover types and if they faced any kind of threats. Informal discussions with different interest groups such as forest department staff, community folk who lived close to the areas, were asked if they could list the perceived threats to natural habitats. The were also asked to identify locations where the perceived threats was the highest. A Participatory Rural Appraisal (Chambers, 1994) based method was followed during these interactions such as spider-web ranking and participatory listing.

Sampling Strategy

A systematic sampling method (Scott, 1998) was used to stratify the landscape and identify areas suitable for long-term monitoring. This procedure is based on methods



Figure 1(a): Google street satellite map with Darjeeling boundary. *Source:* Google map.

for assessing change in forest by setting up Continuous Forest Inventory or permanent plots (Scott, 1947; QGIS Development Team, 2016). Google maps were used to identify natural geographical boundaries of the area. These included roads that were considered boundary with Nepal on the North West, the river Mecheein the South West, the river Tista in the East, and North that separates Darjeeling from the state of Sikkim and the region of the Tarai and the Mohanodi in the South East. Google earth was used to delineate the boundaries and clip the region of interest while making sure it was correctly geo-referenced. The google street satellite map was accessed in the QGIS version 2.8.2 Wein and a boundary of Darjeeling District was added (Fig. 1a). Following this, the entire region was then stratified into 2×2 km grids (Fig. 1b), using a vector research function

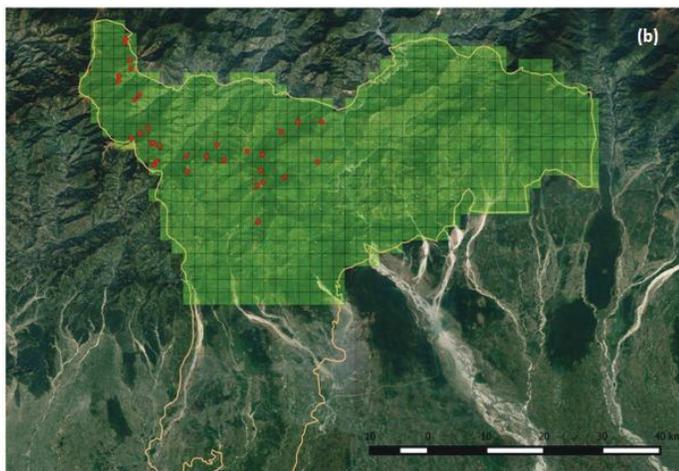


Figure 1(b): A $2 \text{ km} \times 2 \text{ km}$ grid layer to stratify the area into smaller independent units. The red dots represent locations of the forest villages under Darjeeling Territorial and Wildlife Division. *Source:* Google map.

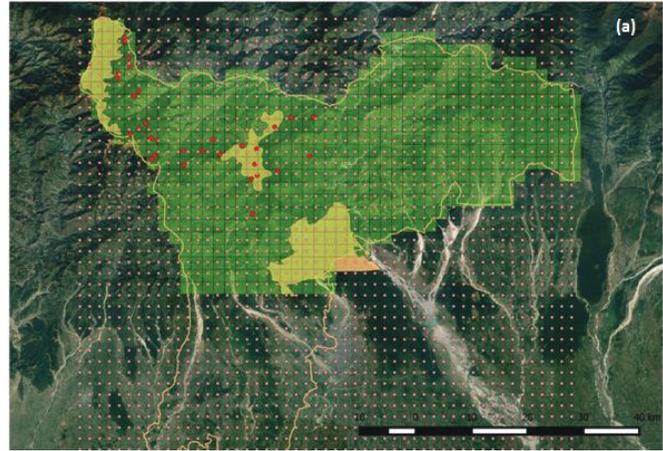


Figure 2(a): Systemtic sampling method used to generate regular sampling points along with replicates. *Source:* Google map.

“vector grid” in QGIS. Each grid had properties of a polygon and hence could be assigned extra attributes or be removed individually. Thereafter, GPS point of all forest villages (*ref.* Table 1) were converted into kml files (stackexchange) and converted to a shapefile with all villages using mmqgis plugin (QGIS Training Manual) in Google Earth Pro (Fig. 1b). Then Shapefiles of all protected areas in Darjeeling were imported into QGIS. A regular points vector function was used to generate points within each $2 \text{ km} \times 2 \text{ km}$ grid, where long-term monitoring plots could be set up (Fig. 2a). In order to prioritise areas, a buffer area was digitized in QGIS to generate a shapefile that included areas inside and outside of protection as well as the forest village locations. This shapefile was then used to clip the regular

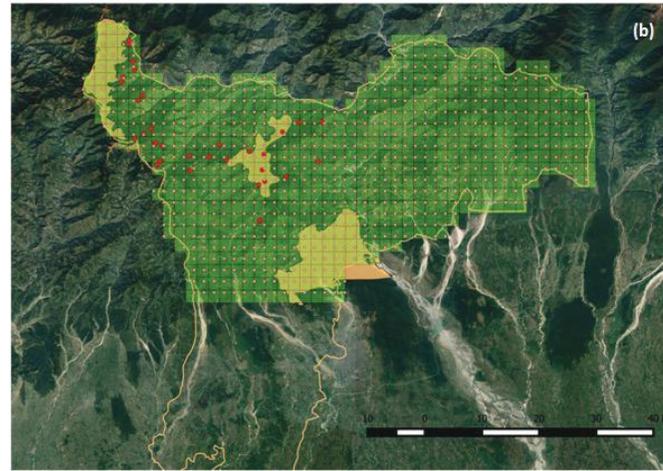


Figure 2(b): Sampling points clipped. *Source:* Google map.

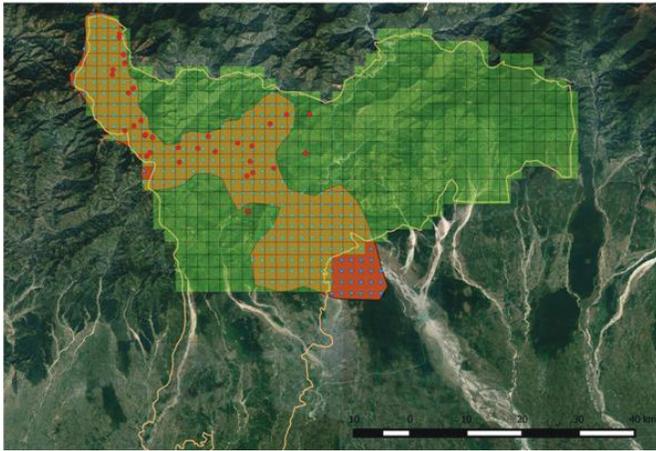


Figure 2(c): Digitised buffer area along with clipped sampling points for prioritisation. *Source:* Google map.

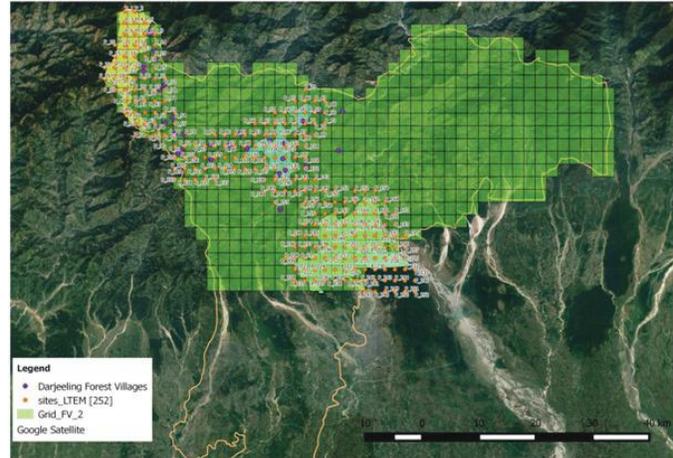


Figure 3: Generation of potential long-term monitoring sampling plot locations with ID. *Source:* Google map.

sampling points along with replicates that was generated earlier (Fig. 2b, QGIS Training Manual, 2016). Finally, the prioritized sites for long-term monitoring were given an ID using the attribute table (ref. Fig. 3).

RESULTS AND DISCUSSION

The aforementioned exercise resulted in the creation of a map with areas under perceived threat as well as generated using an established sampling scheme (Fig. 3). It revealed that not only areas inside protected areas but outside of protection require evaluation and monitoring.

Preliminary interactions in the study area with different key experts and community folk revealed that there are areas that are coming under massive stress due to increased anthropogenic pressures. People listed

that the primary threats to natural habitats are deforestation, landslides and large-scale developmental activities such as roads, unregulated tourism and waste disposal. When asked to rank the threats, people ranked the perceived threats as depicted in Figure 4.

When people were asked about identifying locations where the level of threats was the highest, people identified mostly areas under protection that were undergoing transformation due to these threats. They were of the opinion that the need to set up long-term monitoring plots was most necessary in areas where new roads were being developed, and selective removal of trees was occurring.

Based on our interactions with the people and visual assessment of the area, grids can be selected where there is an urgency of setting up long-term monitoring plots

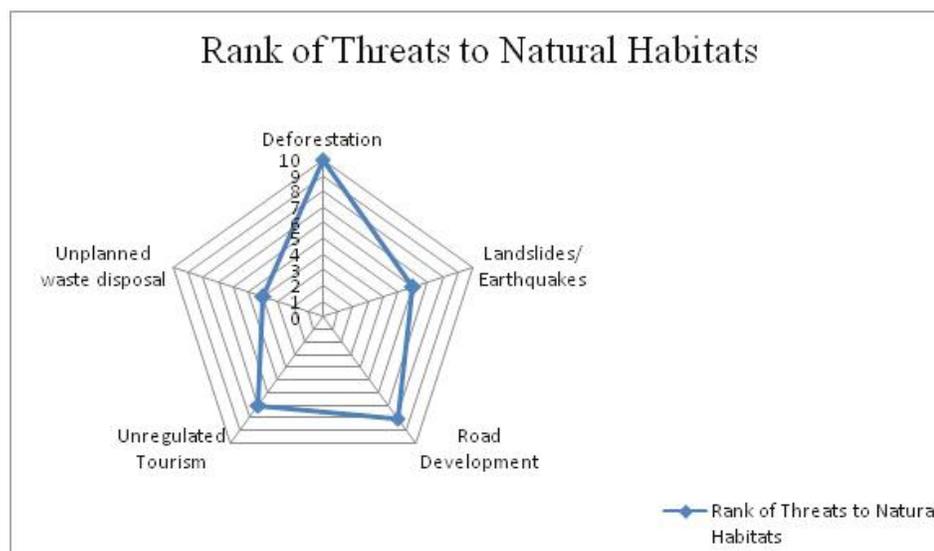


Figure 4: Rank of threats to natural habitats.

(Fig. 1c) in order to understand the impact of large-scale changes such as global warming and more local, place-based changes such as deforestation.

Based on this exercise, approximately 252 sites have been identified for long-term monitoring. However, depending on accessibility and logistic constraints, the number may decrease.

CONCLUSIONS & RECOMMENDATION

Based on the study, important sites were identified for long-term monitoring research plots. While some of the plots may lie inside protected areas, others may be present outside but inside the production scape. These plots could provide important insights into ecosystem dynamics in the face of change such as large-scale development and uncertainties and shocks such as environmental perturbations which include earthquakes and landslides that frequent this region. Such studies in the Darjeeling Himalayas are rare and therefore could be important source of data for future retrospective studies.

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Floristic Diversity of Kashmir Himalaya: An Update

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ABSTRACT: Kashmir Himalaya harbors rich floristic diversity which is of immense scientific interest and socio-economic importance. Therefore, the compilation of floristic database from Kashmir Himalaya is of immediate relevance not only for better understanding of floristic wealth but also for explicating the conservation strategies. In this study, floristic database of Kashmir Himalaya has been prepared based on information extracted from the available floristic literature dealing with the diversity of the region. This survey resulted in the compilation of 4143 species belonging to algae, fungi, bryophytes, pteridophytes, gymnosperms and angiosperms. Among angiosperms, dicotyledons contribute maximum number (2023) of plant species distributed in 657 genera and 121 families; whereas monocotyledons share 540 species grouped under 179 genera and 28 families. Gymnosperms are represented by 19 species belonging to 11 genera and 5 families. Pteridophytes comprised of 147 taxa belonging to 42 genera and 17 species. Among other cryptogams bryophytes contribute 273 species; algae hold 566 and fungi constitute 575 species. This study is expected to provide baseline scientific data for cutting-edge studies relating to long term ecological research, bioprospecting, possible impacts of changing climate and anthropogenic activities on vegetation and sustainable use of plants resources in this Himalayan region. ©2019

KEYWORDS: Kashmir Himalaya; Floristic database; Angiosperms; Pteridophytes.

INTRODUCTION

Owing to the vast variety of edapho-climatic and physiographic heterogeneity and diverse habitats including lakes, springs, swamps, marshes, rivers, cultivated fields, orchards, subalpine and alpine meadows, mountain slopes and terraces, permanent glaciers, etc., the Kashmir Himalaya harbours a rich diversity of almost all groups of organisms including, algae, fungi, lichens, bryophytes, pteridophytes, gymnosperms and angiosperms. Many species are distinct from those in the rest of the country and are endemic to this region. Although the first written record of floristic study from the region is of Moorcroft in 1822, a modern comprehensive flora of the region is still not available (Burkill, 1965; Dar *et al.*, 2002). No doubt during the past few decades, some significant contributions have been attempted but these remained largely confined either to a particular family or to a specific area. Therefore, current compilation of complete floral database of Kashmir Himalaya is taken into consideration. This database may help in understanding regional floristic wealth, the overall ecological conditions, and the presence of endemic and threatened plants. Moreover, it may alarm sustainable management and conservation of natural resources.

LITERATURE REVIEW

Kashmir Himalaya forms an important region of the

Northwest Himalayan biogeographic zone in India. Beset with considerable topographical, altitudinal and climatic variations, it depicts great habitat diversity and harbors a rich flora and fauna. It is a biological paradise with many of its plants and animal species being distinct from those in the rest of the country and endemic to this region. Being a mountain-girdled primarily agricultural province, the people living here have always remained in close association with and dependant on its biodiversity, so much so that it may be referred as a "biomass state". Kashmir has ever been famous for the various components of its biodiversity namely fruits, timber, furniture, wood carving, medicinal plants, scenic flowers, carpets, silk honey, wool, game animals, fishes, birds, etc.

During the last two centuries, its flora has attracted the attention of many foreign and local botanists. Many of its plants are cited in the illustrious works of Hooker's Flora of British India (1872–1897). After this monumental effort, several other authors also published their floristic works dealing with the region. Notable among them are Blatter (1928–29), Coventry (1923–1930), Lambert (1933), Lancaster (1980 a,b), Munshi & Javeid (1986), Javeid (1968, 1972), Kaul (1986), Naqshi & Javeid (1987), Persson (1938), Polunin & Stainton (1984), Singh & Kachroo (1976, 1983, 1994), Stewart (1972), Naqshi *et al.* (1988), Kaul *et al.* (1973), Dar & Kachroo (1982), Dhar & Kachroo (1983), Kak (1990), Sharma & Jamwal (1988, 1998), Ara *et al.* (1995), Vir Jee *et al.* (1989a,b), Navchoo & Kachroo (1995), Dar *et al.* (1995, 2002), Khuroo *et al.* (2004), etc. dealing with the seed plants.

Many workers have also contributed to other groups of plants of this Himalayan region. Among these groups

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Pteridophytes were studied by Stewart (1972), Wani *et al.* (2012), Mir *et al.* (2015); Bryophytes by Kaul & Dhar (1968), Vohra (1969), Srivastava (1979), Chopra & Kumar (1981), Qazi (1984), Townsend (1988), Banday *et al.* (1998), Banday (1997, 2012), Kour *et al.* (2015); Algae by Kaul *et al.* (1978), Khan & Zutshi (1980), Mam *et al.* (1987), Khan (2000), Rather (1994a–c), Sarwar (1987), Rather & Mir (1987, 1989), Sarwar & Zutshi (1987, 1988, 1989), Wanganeo & Wanganeo (1991), Ganai *et al.* (2010), Mir *et al.* (2010)); and Fungi (Sharma *et al.* (1969), Dar & Shah (1981), Dar *et al.* (2002), Saini & Atri (1984), Qasba & Shah (1991), Munshi *et al.* (1999), Walting & Abraham (1992), Rehman *et al.* (2006), Pala *et al.* (2012), Beig *et al.* (2008), Itoo *et al.* (2014), Dar *et al.* (2009, 2010).

Kashmir Himalaya also supports a rich and unique diversity of medicinal plants. The local persons having knowledge of Unani Medicine attribute some medicinal property to almost every plant species. Due to this reason, the Unani System of medicine has been a common and favourite system of treatment for the ailing humanity in Kashmir (Dar *et al.*, 2002). Various aspects of medicinal flora of Kashmir have been dealt in various publications, e.g. Chopra *et al.* (1956), Kapur & Sarin (1977), Kak (1983), Dar *et al.* (1984), Kaul *et al.* (1990), Navchoo & Buth (1994), Ara & Naqshi (1992), Naqshi *et al.* (1992), Khan *et al.*, (2004). Most of them dealt with the ethnobotany, agrotechniques, cultivation and phytochemistry of medicinal plants.

Despite this usefulness, the biodiversity of Kashmir valley has suffered greatly due to varied anthropogenic pressures, more so in the recent past. Our rich forests have been significantly destroyed or degraded, lakes and wetlands encroached upon, vast alpine meadows heavily grazed, wild flora and fauna overexploited, natural abodes of many endemic and other plant and animal species devastated, while genetic diversity of many economically important taxa has severely been eroded and so on. All these and other factors have together taken a heavy toll on our biocapital. According to rough estimate, about 40% of the endemic plant species in Kashmir Himalaya are currently threatened (Dar *et al.*, 2002). This calls for taking immediate conservation steps.

One of the major handicaps in evolving effective strategies for the wildlife conservation in Kashmir is the inadequate knowledge about its overall biodiversity. Though the angiosperm flora is now better known, much is to be desired in microorganisms' lower plants, fungi and animals. Even what is known so far is scattered and has not been consolidated effectively under one cover. Recently, Ahmedullah (1997) and Dar *et al.* (2002) have attempted a profile on the biodiversity of Jammu and Kashmir State.

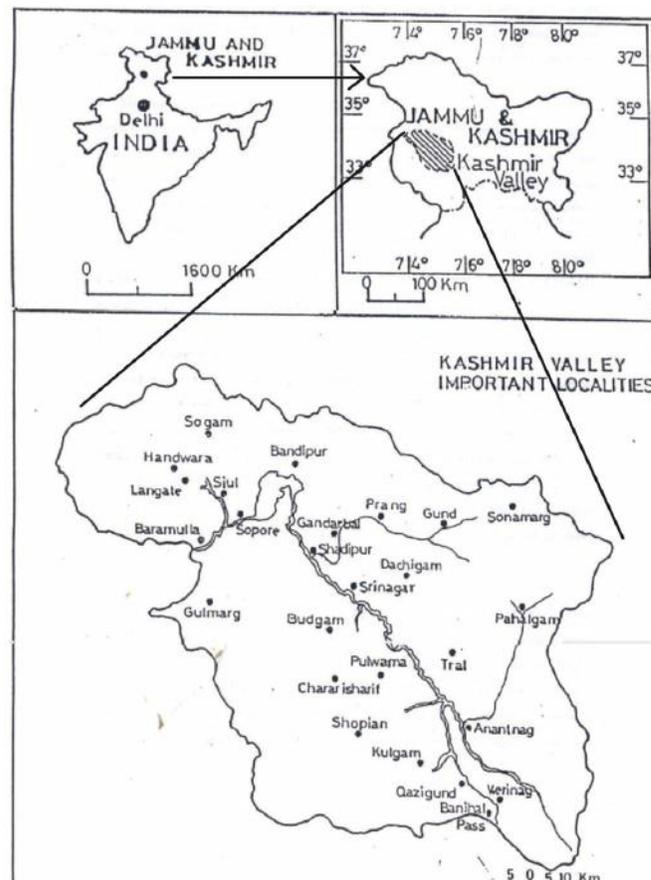


Figure 1: Map of Kashmir Himalaya showing various localities.

However, it is a fragmentary compilation, giving rather sketchy accounts of mainly angiosperms, invertebrates and vertebrates. Even information about these groups is far from complete, besides having unwarranted deficiencies in nomenclature. This database provides up-to-date information on the biodiversity of Kashmir Himalaya, focusing on the current status of species richness. It is the first comprehensive attempt in which scattered data available on the vast diversity of almost all the floristic groups including algae, fungi, bryophytes, pteridophytes, gymnosperms, and angiosperms in the Kashmir Himalaya have combined into a single synthesis.

STUDY AREA AND METHODOLOGY

Kashmir Himalaya, a picturesque south Asian region, is located in the northwestern extreme of the Himalayan biodiversity hotspot. The valley is a fertile basin, where soil is formed of deposits laid down on the floor of the lake that once covered it. Topographically, the region mainly comprises a deep elliptical bowl-shaped valley formed by a girdling chain of Himalayan ranges, namely the Zaskar Range of Greater Himalaya in southeast to

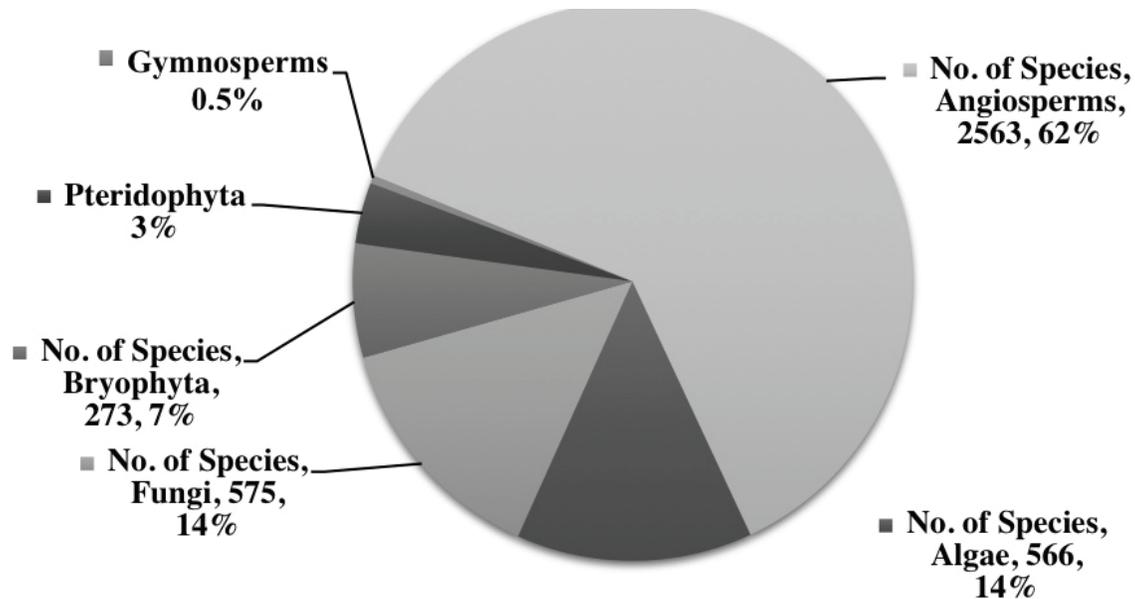


Figure 2: Floristic diversity of various taxonomic groups in Kashmir Himalaya.

northeast to west and the Pir Panjal range of the Lesser Himalaya in the south that separates the valley from the plains of the northern India. To the east of the Kashmir Himalaya lies Tibet, to the north lies China and some part of Afghanistan, to the west is Pakistan and to its south is the Jammu province and Himachal Pradesh. The valley has an area of about 15,948 km², with nearly 64% of the total area being mountainous and stands between coordinates of 33°20' and 34°54' North latitudes and 73°55' and 75°35' East longitude. The altitude of the valley plain at Srinagar is 1,600 m above mean sea level (amsl) and the highest peak among its surrounding mountains is that of the 'Kolahoi' with an altitude of 5,420 m amsl. Climate of the region is marked by well-defined seasonality that resembles with the mountainous and continental parts of the temperate latitudes. The temperature ranges from an average daily maximum of 31 °C and minimum of 15 °C during summer to an average daily maximum of 4 °C and minimum of -4 °C during winter. It receives annual precipitation of about 1,050 mm, mostly in the form of snow during the winter months. The valley has plentiful fresh water resources in the form of lakes, springs, streams, rivers and groundwater. Some of the important glaciers include Saichen, Kolahai, Nubra, Baltoro, Baifo, Hispur, etc. Springs in the valley are mostly concentrated in district Anantnag, SE Kashmir, along the foothills of Pir-Panjal range (Fig. 1). The study area of Kashmir Himalayas has been selected owing to its vast Biodiversity. The area is rich in flora and fauna, while there is lot of interference due to human activities resulting into loss of biodiversity.

For the compilation of floristic database of Kashmir Himalaya, all the written records of plant exploration (including journals, scientific reports, floras, and other published and unpublished records) dealing with the floristic diversity of Kashmir Himalaya were collected. The most important sources of information about the phanerogams of Kashmir Himalaya are *Flora of British India* (Hooker 1872–1897), *An annotated catalogue of the vascular plants of West Pakistan and Kashmir* (Stewart, 1972), *Forest flora of Srinagar and plants of neighbourhood* (Singh & Kachroo 1976), *Flora of Pulwama, Kashmir* (Navchoo & Kachroo 1993), *Medicinal plants of Kashmir and Ladakh: temperate and cold arid Himalaya* (Kaul 1997), *Indigenous and exotic trees and shrubs of Kashmir Valley* (Ara et al. 1995), *Flora of Upper Lidder Valley of Kashmir Himalaya - Vol. I & II* (Sharma and Jamwal 1988, 1998), *Biodiversity of the Kashmir Himalaya* (Dar et al., 2002), *Floristic diversity in the phanerogams of Langate* (Khuroo et al., 2004), *The alien flora of Kashmir Himalaya* (Khuroo et al., 2007), *The wealth of Kashmir Himalaya- Gymnosperms* (Dar & Dar, 2006), etc.

The literature concerned with other groups of plants such as Pteridophyta (Stewart, 1972; Wani et al., 2012; Mir et al., 2015), Bryophyta (Kaul & Dhar, 1968; Vohra, 1969; Banday et al., 1998; Banday, 1997, 2012; Kour et al., 2015), Algae (Kaul et al., 1978; Khan & Zutshi, 1980; Rather, 1994a–c; Sarwar, 1987; Ganai et al., 2010; Mir et al., 2010), and Fungi (Sharma et al., 1969; Dar & Shah, 1981; Dar et al., 2002; Saini & Atri, 1984; Qasba & Shah, 1991; Itoo et al., 2012; Dar et al., 2009, 2010) was also consulted to prepare an exhaustive list of flora of Kashmir Himalaya. While

Table 1: Numerical distribution of diversity in various taxonomic groups in Kashmir Himalaya

Taxonomic Group	No. of Species	No. of Genera	No. of Families
Algae	566	137	7
Fungi	575	256	118
Bryophyta	273	102	42
Pteridophyta	147	42	17
Gymnosperms	19	11	5
Angiosperms	2563	764	149

preparing the present catalogue, original publications have been used in order to authenticate the identity of the species. During the present study, it was ensured that all the spelling mistakes of scientific names published in various floras were corrected. The nomenclature was validated from the following online floral indices: <http://www.theplantlist.org/> <http://www.arsgrin.gov/cgi-bin/npgs/html/index.pl>, <http://www.ipni.org/>, <http://www.algaebase.org/>, <http://www.indexfungorum.org/> <http://www.tropicos.org/> <http://www.stridvall.se/la/index.php> and <http://www.mycobank.org/>

RESULTS AND DISCUSSION

At the current stage of compilation, total 4143 species belonging to ‘plant group’ – algae, fungi, bryophytes, pteridophytes, gymnosperms and angiosperms have been recorded from Kashmir Himalaya. In all, these species are grouped under 1312 genera and 338 families. Among angiosperms, dicotyledons contribute maximum number (2023) of species distributed in 657 genera and 121 families; whereas monocotyledons share 540 species grouped

Table 2: First ten (10) dominant families in the flora of Kashmir

S. No.	Name of Family	Group	No. of Genera	No. of Species
1.	Asteraceae	Dicot	97	310
2.	Poaceae	Monocot	84	231
3.	Rosaceae	Dicot	26	131
4.	Leguminosae	Dicot	45	130
5.	Cyperaceae	Monocot	15	123
6.	Brassicaceae	Dicot	48	122
7.	Ranunculaceae	Dicot	20	105
8.	Lamiaceae	Dicot	35	99
9.	Apiaceae	Dicot	41	73
10.	Polygonaceae	Dicot	9	64

under 179 genera and 28 families. Gymnosperms are represented by 19 plant species belonging to 11 genera and 5 families. Pteridophytes comprise 147 taxa belonging to 42 genera and 17 species. Among other cryptogams bryophytes contribute 273 species; algae hold 566 and fungi constitute 575 species (Fig. 2).

The angiosperms are by far the largest and most well known groups of plants of Kashmir valley. Compilation of the list of plant species occurring in Kashmir Himalaya shows a total of 2563 species of angiosperms, belonging to 764 genera and 149 families. Of these, dicotyledons include 2023 (78.93%) species grouped under 657 genera and 121 families (Table 1), while the monocotyledons include 540 species (21.06%), 179 genera and 28 families (Table 2). The largest families of dicotyledons are Asteraceae holding 97 genera and 310 species, followed by Rosaceae (26 genera and 131 species); Leguminosae (45 genera and 130 species), Brassicaceae (48 genera and 122 species), Lamiaceae (35 genera and 99 species) and Ranunculaceae (10 genera and 105 species). Out of the 149 families, within which 2563 plant species are distributed, just ten families (Table 2) have a major share of 54.15% (1388) of the species in the total flora (Table 2), while as the remaining 139 families contribute 45.85% (1175) of the total flora. This indicates that in spite of a large number of families reported in the flora of Kashmir, only few families tend to dominate the botanical diversity. In addition, there are 37 families which are represented by a single species in the angiosperm flora of Kashmir Himalaya. Similarly, the largest genera of dicotyledons are Taraxacum (36 species), Ranunculus (31), Saussurea (28) and Polygonum (24 species). Of the 540 species of monocots listed from Kashmir Himalaya, 354 (65.55%) are alone shared by Poaceae (231 species) and Cyperaceae (123 species), the remaining 186 (34.45%) species are distributed among other 26 families. Of all monocot genera, the largest Carex and Poa shared 58 and 21 species, respectively (Fig. 3).

The gymnosperm species that dominate the forests of Kashmir Himalaya are of immense socio-economic

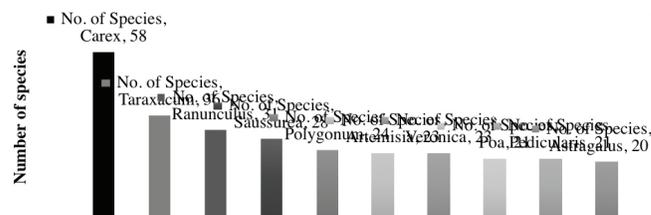


Figure 3: First ten (10) dominant genera in the flora of Kashmir.

genera with respect to number of species are Puccinia, Russula, Septoria and Aminata, respectively sharing 45, 29, 21 and 14 species.

CONCLUSION

The present compilation of floristic database growing in Kashmir valley will be of great utility for the scientific interest and socio-economic development of the state. During the present survey, base-line information on floral diversity existing in Kashmir valley has been generated. About 4143 species belonging to both cryptogams and phanerogams have been documented. The information gathered is of greater significance for exploitation of these resources for the welfare of human beings. The study further emphasizes on the need for thorough exploration of Kashmir Himalaya, especially for vascular and non-vascular cryptogams, at variable altitudes and under varied environments, which is still in infancy. With reference to spatial spread of the documented flora across the valley, only few areas are well explored namely Lidder Valley Sonamarg, Gulmarg and district Srinagar, the rest areas and difficult terrains in the hinterland are still either least- or totally-unexplored. It is also essential to develop on-line database for the description of plants.

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Patterns of Lichen Species Richness along the Altitudinal Gradient in Bhagirathi Valley, Western Himalaya: Preliminary Findings

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ABSTRACT: Lichens are considered as important indicators of the environmental changes. The aim of the present study is to investigate the patterns of the lichen species richness along various environmental gradients and to assess the influence of microclimatic conditions on lichen communities in the Himalayan region. Bhagirathi basin in the state of Uttarakhand was selected for the intensive study. This valley has undergone several changes in recent decades in terms of land use and land cover and retreat of glaciers at the higher altitude. As a precursor to assessment of lichen species richness and community composition, initial surveys were conducted along an environmental gradient and lichen specimens (Corticolous, Saxicolous, and Terricolous) were collected from a wide range of habitats. To assess impacts of changes in microclimatic condition simulated warming experiments based on Open Top Chambers (OTC) were also initiated in the alpine–subalpine zone of the area, equipped with climatic data loggers. Lichen species were marked inside and outside the OTCs for change detection in growth and secondary metabolites in simulated warming conditions. A total of 105 lichen species were recorded during the study. Parmeliaceae was the largest family with 33 species recorded in the study area and Cladonia as largest genus with 9 species showing rich soil lichen biota. Maximum species richness of lichens was recorded in 2500–3500 m amsl elevation range. Simulated warming experiment created the temperature elevated by 1–2 °C which caused the early and rapid growth of the vegetation. Impact on lichen communities composition, growth, and changes in biochemical composition in experimental warming and glacier retreat will be addressed in the future. ©2019

KEYWORDS: Bhagirathi Basin; Environmental Gradient; Indicators; Lichens; Western Himalaya.

INTRODUCTION

Lichens are the most fascinating and widely distributed organisms on earth. They are the pioneers of primary succession colonizing the inorganic substrate and yet considered more sensitive to changes in environmental conditions compared to all other plant forms (Rai *et al.*, 2010). Lichens lack vascular systems, hence completely depend on the surrounding environment for water and mineral nutrients. This dependency makes them vulnerable to changes in their habitat condition and an ideal indicator to track changes in the environmental conditions. Though lichens have great ability to survive in a harsh environment but are most sensitive to microclimatic changes, thus they act as a natural sensor and successfully utilized as a biological indicator of air quality and climate change studies. For a long time, lichens have been known as valuable plant resource and are still used as medicine, food, fodder, perfumes, and dyes and for miscellaneous purposes in different parts of the world. Besides these multipurpose uses, lichens play an important role in geochemical emission studies, in dating rock surfaces (Lichenometry), in plant succession and soil development, and because of their high sensitivity, lichens have been used as indicators of

air pollution also. Terricolous lichens (grows on the soil) are suitable indicators of various environmental disturbances of alpine and subalpine regions, because of their direct contact with the soil, their competition with other ground vegetation and their sensitivity to anthropogenic influences (Bilovitz *et al.*, 2014). The epiphytic lichens are among the most sensitive for microclimatic changes and are used in biomonitoring studies of an area. Due to their intimate dependency on the surrounding atmosphere for water, they are a most preferred indicator of changes in the environmental condition such as moisture, temperature, light and atmospheric pollutants.

The Indian Himalayan Region (IHR) is highly sensitive to the impact of global climate change, information about the effect of such alteration on plant distribution, especially on the lichens, are very scanty and rarely collected and analyzed in a systematic manner. According to IPCC (Intergovernmental Panel on Climate Change), projection Himalaya may suffer drastic climate changes. The rapid temperature increase and changes in precipitation, in combination with the importance of Himalayan snowpack and glaciers, make the region one of the most threatened nonpolar areas of the world (Xu *et al.*, 2009; Stocker *et al.*, 2013). In the IHR regions, studies in the glacier retreat regions are lacking and quite a few studies are done mainly focused on the dynamics of the vascular plant region indicated a directional pattern of increasing species richness along primary succession that is usually associated with compositional shifts but surprisingly a knowledge gap still exists about soil-dwelling lichens. Colonization of

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lichens in the ice cap retreat regions of polar regions and other mountainous region indicates the need of thorough study on the response of these pioneer colonizer organisms in the IHR also focussing on their community structure and functional roles in the region.

LITERATURE REVIEW

India has a rich diversity of lichens represented by more than 2,000 species (Awasthi, 2000), which is about 10% of the total species (20,000) known from the world. The lichens are common to abundant in temperate and alpine regions of the Himalaya and hilly regions of Peninsular India. Though the maximum diversity of lichens in the country is recorded for South India and Eastern Himalaya region, but they are common in occurrence in Western Himalaya (Awasthi, 2000).

Lichens are excellent bioindicators of changes in air quality due to their differential sensitivity to various air pollutants (Nash & Egan, 1988). Lichens have been used as bioindicators of environmental stress in a number of studies, using different techniques including single species distribution maps, analysis of the whole flora (Wetmore, 1983), transplant experiments, analysis of morphological injury (Brodo, 1961) and Indices of Atmospheric Purity (IAP) (LeBlanc & De Sloover, 1970). IAP, a quantitative approach, employing a mathematical calculation is the best-known method to evaluate the level of pollution-affecting epiphytic flora (Svoboda, 2007). IAP is frequently used for summarizing information about pollution tolerance of species and their spatial variation of abundance (LeBlanc & De Sloover, 1970) and reflects a gradation of lichen species richness from “good” (high diversity) to “worst” (low diversity) (Kricke & Loppi, 2002) and sums up the effect of long-term environmental conditions.

Glacial retreat is accelerating in most mountain regions of the world, and consequently, newly exposed land surfaces are available for biotic colonization (Nascimbene *et al.*, 2017). Several studies across the world observed a rapid colonization of the exposed surfaces in the forefields after glacier retreat during the last decades and several studies have used lichens as a bioindicator for changes in the environmental condition towards pollution and climate. Colonization of lichen in the Polar Regions have been reported in the recent decades (Valladares & Sancho, 1995; Nascimbene *et al.*, 2017) and fluctuation in the climate especially warming and cooling phases in the climate in this region and their impact on the lichen community diversity and abundance was highly noticeable (Sancho *et al.*, 2016).

The Indian Himalayan region (IHR) exhibits abundant

variety and luxuriant growth of subtropical-temperate-alpine lichens (Upreti, 1998). The lichens form dominant terrestrial vegetation in higher alpine habitats in the Himalaya. The studies focused on the lichen flora of IHR are floristic surveys, taxonomy, including different monographic and revisionary studies. The lichens are common to abundant in temperate and alpine regions of the Himalaya and hilly regions of peninsular India. The present numbers of lichens in the country appears a lower estimate as many areas especially mountains and the forest canopies are yet to explore (Negi & Gadgil, 1996, 2002). Awasthi and Singh (1978) reported 74 macrolichens lichen taxa from this Gangotri region. Upreti *et al.* (2004) reported 149 lichens species belonging to 50 genera and 21 families from this area. The area is dominated by macrolichens (foliose–fruticose form) represented by 120 species whereas microlichens (crustose–squamulose) are represented by 29 species. The member of lichens families Parmeliaceae and Physciaceae dominate the area as they are represented by 59 species (20 genera) and 28 species (8 genera) respectively. Among the microlichens families, Lecanoraceae and Rhizocarpaceae are represented by 6 species of Lecanora and 5 species of Rhizocarpon, respectively. Recently, Bajpai *et al.* (2016a) analyzed the change in lichen community composition and impacts of atmospheric deposition of pollutants on lichen diversity based on the comparative study of old herbarium and recent collections from eastern Himalaya. Lichenometry based study on the rate of glacier retreat in the Himalayan region revealed the importance of this tool to study climate change and shift of vegetation in the future scenario (Bajpai *et al.*, 2016b).

Therefore, the present study was carried out with the objectives: to understand how microclimatic variables impact the lichens communities in the Indian Himalayan region and to assess the response of lichens communities in terms of diversity patterns and changes in biochemical features towards retreating glaciers and experimental warming.

STUDY AREA AND METHODS

Bhagirathi basin in the state of Uttarakhand was selected as intensive study site (Fig. 1). The study area covers various habitat types along wide elevation gradient from 500 to 5000 m covering the subtropical, montane, subalpine, moist-alpine and dry alpine trans-Himalayan ecosystems. The vegetation of the study area falls in subtropical to subalpine forests. The sub-tropical forest is mainly dominated by deciduous tree species such as *Terminalia* spp., *Anogeissus latifolia*, *Bombax ceiba*,

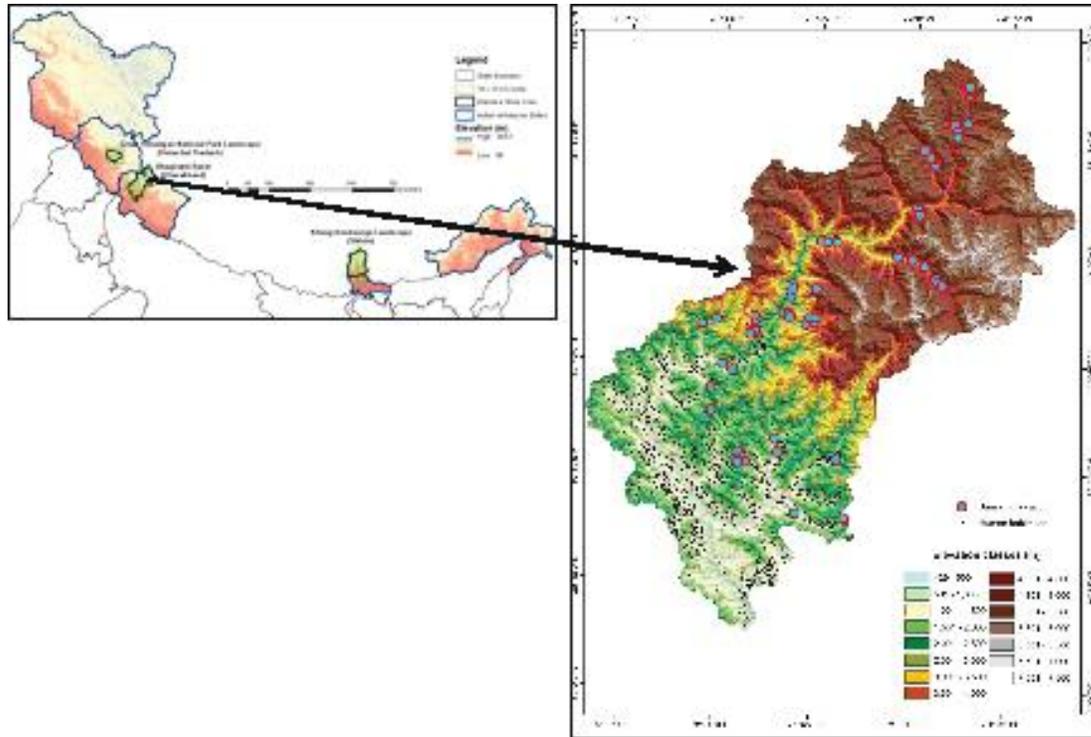


Figure 1: Location map of study area showing the Bhagirathi basin and the sampling locations.

Adina cordifolia and *Acacia catechu* in lower areas whereas Chir pine (*Pinus roxburghii*) forms a prominent vegetation in the higher areas. The montane zone lies between the altitudes of 1500 and 3000 m amsl, where one or more species of oaks are dominant and forms evergreen forests. The area is represented by 4 species of oak. The montane zone also has a dominant proportion of mixed deciduous forests with the prevalence of lauraceous forests. Subalpine forests are dominant above 3000 m amsl elevation zone with Birch (*Betula utilis*), Fir (*Abies* spp.) and *Q. semecarpifolia* as dominant forest forming species intermixed with Rhododendron, Viburnum and Sorbus species. The alpine zone was dominated by moist herbaceous and grassy meadows in the greater Himalayan region while dry scrub is dominant formations in the trans-Himalayan region of Nelang valley.

In the Bhagirathi basin, the Gangotri glacier has been under a state of continuous recession and the pattern appears to be continuing even at present. It is one of the largest glaciers in the Himalaya and the most studied areas in the Himalayan region in terms of glacial fluctuations and well as documentation of various ecological aspects. Its recession history in terms of the position of the snout is well defined and has been identified and marked on the boulders in the field. All these conditions and existing studies make this area an ideal site for the set objective.

Lichens samples were collected from different vegetation and micro-habitat types in the valleys and patterns of species assemblages were analyzed. Once collected, specimens were dried, mounted on herbarium sheets and identified with the help of Lichenology Laboratory, NBRI, Lucknow after proper examinations. The specimens were housed at the herbarium of Wildlife Institute of India (WII). To record climatic data, automatic data loggers (HOBO®) is installed at an elevation of 4000 m amsl, and data was obtained for air temperature and relative humidity after the regular interval (Fig. 2). More climatic data loggers will be installed in future to understand the patterns in the entire basin. The lichenological investigations was also initiated in the open-top chamber (OTC) to study long-term climate change effect on the lichens (Fig. 2).

Two crustose lichen species *Rhizocarpon geographicum* and *Xanthoria elegans* were identified as focal species for Lichenometry studies and thalli were marked for the future observations.

RESULTS AND DISCUSSION

Species Richness

A total of 105 lichens species were identified including micro and macrolichens from various habitat types with respect to different elevation zones and vegetation types. These 105 species of lichens belong to 42 genera and 23 families occur

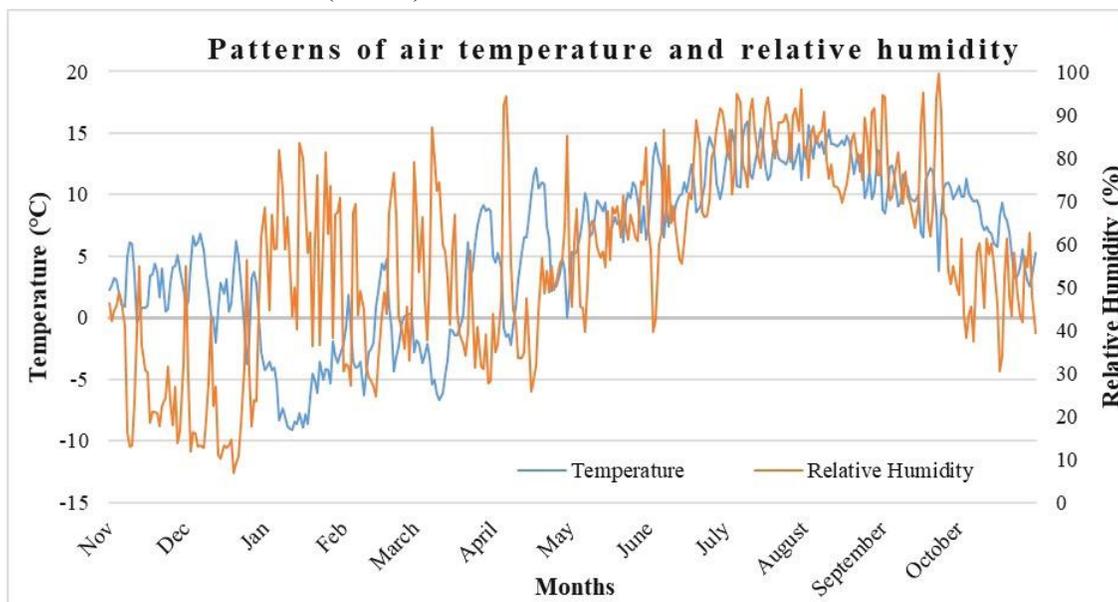


Figure 2: Patterns of air temperature and relative humidity at 4000 m amsl elevation in the study area.

on various substrata in subtropical, montane, subalpine and alpine regions of the basin. Parmeliaceae was the largest family with 33 species recorded in the study area followed by Physciaceae (11), Lecanoraceae (10) and Cladoniaceae (9) (Fig. 3).

The majority of Parmeliaceae species have a foliose, fruticose, or subfruticose growth form and grows abundantly in the soil, rocks or as epiphytes. Due to diverse nature of adaptability and growth in different substrates, the family was dominant in the region. Maximum diversity of the species was recorded in the montane and subalpine forests. Some interesting species such as, *Menegazzia terebrata*, which is the only known species under the genus *Menegazzia* in Northern hemisphere was also recorded during the study. Representation of soil-dwelling lichens like genus *Cladonia* also reflects good habitat conditions in the study area. Some of the ecologically important species, *Usnea longissima*, *U.*

orientalis which are important fodder for the Himalayan musk deer during the lean season were also recorded from the area. Many species are still to be identified, which may lead to increase the tally of the recorded species during the study.

Patterns of species Richness along elevation gradient

The species richness was maximum in elevation zones between 2000 and 3000 m amsl (Fig. 4). This zone has some of the undisturbed and dominant oak forests in the study area especially on way to Dayara from Raithal and Barsu and in the Bhilangana valley which supports the luxurious growth of the lichen with high species richness. Other studies also suggested a peak of high species richness between 2000 and 3000 m amsl elevation zone or major families of lichen in the Himalayan region

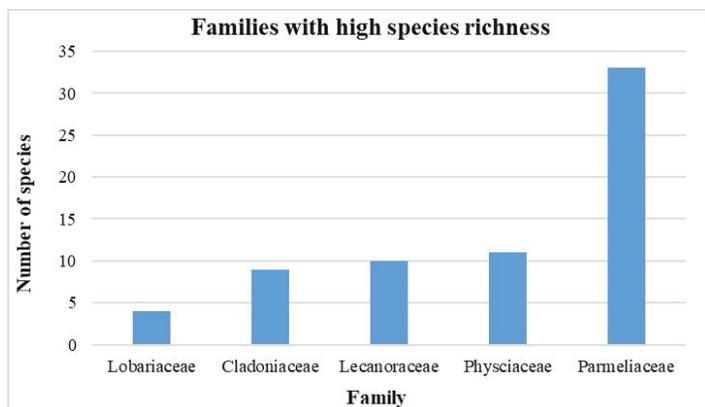


Figure 3: Dominant Lichen families recorded in the study area.

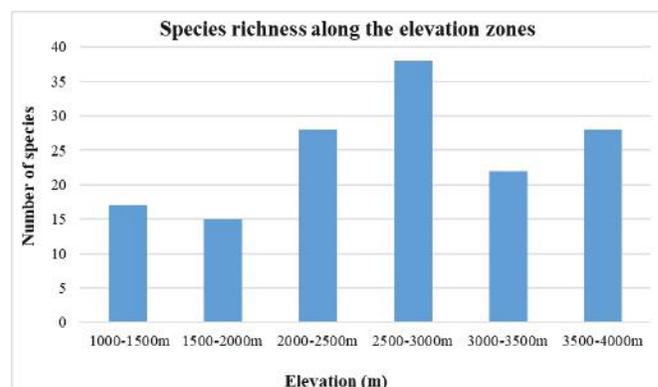


Figure 4: Dominant Lichen families recorded in the study area.

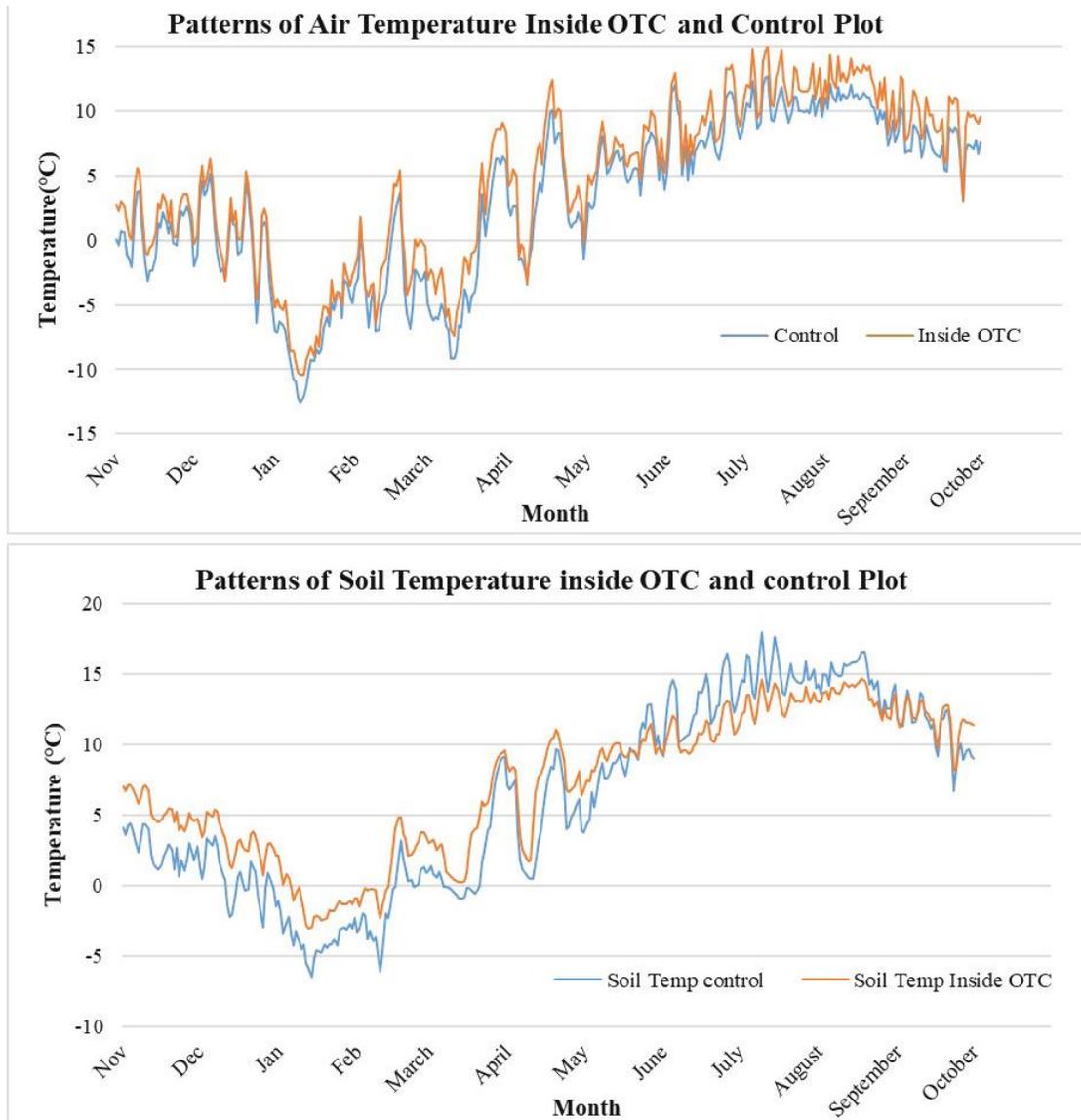


Figure 5: Patterns of air temperature inside OTC and control plot (Above) and Soil temperature inside OTC and control plot (Below) at 4000 m amsl in Gangotri NP.

(Baniya *et al.*, 2014). Areas below 2000 m amsl have low species richness due to comparatively low canopy cover and anthropogenic pressure of lopping and grazing. Elevation zone of 1500–2000 m asml in the valley has most of the hill settlements as this zone supports a large number of agricultural practices by local people. This condition leads to more pressure on the forest resources. As compared to this, high elevation zones have more protected area (PA) status and have low grazing and resource collections which might be suitable for the high lichen growth and richness.

Microclimatic Trends of Experimental Warming

The simulated warming under experimental setup lead to elevate the average annual mean temperature of the

air inside OTC up to 1.68 °C higher as compared to the controlled plots (natural conditions) whereas soil T was 0.98 °C higher inside the OTC (Fig. 5). These trends indicated a suitable condition for the monitoring change in community composition and function under experimental warming conditions which falls under various future climate change scenarios. Changes in the secondary metabolite composition due to altered climatic conditions will be evaluated and correlated with the temperature and humidity patterns in the future course of the study.

CONCLUSION

Lichens generally occupy pristine and least disturbed habitats and therefore serve as important indicators for monitoring habitat conditions. They can also be used as indicators to

monitor air quality and climate change. This study presents baseline data on the diversity of lichens along the altitudinal gradient in Bhagirathi Valley. The altitudinal range between 2500–3500 m amsl was found richest in lichen species. High diversity of terricolous lichens in the alpine areas reflect a natural conditions suitable for long term monitoring in the changes in lichen communities with shrinking snow and glacier covers in the area. Presence of soil-dwelling lichens along the glacial fore-fields indicates the suitability of these organisms towards climate change studies.

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SECTION 2

Traditional and Indigenous Knowledge System

The "Traditional and Indigenous Knowledge System" section covers the research studies and demonstrative works presently progressing in the Indian Himalayan Region (IHR). Ethnobotanical survey, conservation practices of medicinal plants, indigenous practices, etc. are among the main initiatives assessed by the young Himalayan Researchers under the National Himalayan Studies (NMHS).



Ethnobotanical Survey of Medicinal Plants for Assessment of Their Conservation Status in Chenab Valley, J&K

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ABSTRACT: The study presents results of ethnobotanical surveys and participatory rural appraisal (PRA) meetings with the local communities to document the traditional knowledge, usage and conservation status of medicinal plants in Chenab valley, Jammu and Kashmir. A total of 97 species are used heavily in the local folk medicine to cure 25 ailments using 88 formulations. These include 68 herbs, 16 shrubs, 11 trees and 2 climbers. From majority of the species, roots are extracted both for trade and usage in folk medicine which is a harmful practice since it involves uprooting of the whole plants resulting in loss of regeneration in the species. Among the 47 species traded for their use in traditional medicine systems 36 species are threatened under various categories as per CAMP (2010). The PRA meeting with indigenous ethnic community indicates a lack of awareness among a large proportion of population about the declining population trends of medicinal plants in the wild. About 59% respondents opined that there has been a decline in the availability of wild medicinal plants which is largely ascribed to increasing trade. Further, local communities believe in retaining their traditional rights for utilization of these plants both for trade as well as for traditional healthcare. ©2019

KEYWORDS: *Chenab valley; Ethnobotany; Medicinal plants; Participatory rural appraisal.*

INTRODUCTION

All cultures across globe use plants as a source of medicines since traditional therapy involves the use of plant extracts or their active principles. Mountain communities are more dependent on medicinal plants largely due to lack of modern healthcare facilities sometimes resulting in over exploitation and adverse impact on their natural populations. According to FAO “*Medicinal plants may be defined as those plants that are commonly used in treating and preventing specific ailments and diseases and are generally considered to have a beneficial role in healthcare*”. The collection and identification of medicinally important plants have led to the development of modern pharmaceutical products. Many drugs that are used in modern medicine have come from folk-use and use of plants by indigenous cultures (Batugal *et al.*, 2004). Demand for medicinal plant is increasing due to growing recognition of natural products, as they are non-narcotic, having no side-effects, easily available at affordable prices and sometime the only source of health care available to the poor (Nishteswar, 2014).

Medicinal plant sector has traditionally occupied an important position in the socio- cultural, spiritual and medicinal arena of rural and tribal lives of India. In India, 9500 registered herbal industries and a multitude of unregistered cottage-level herbal units using more than 6000 species of plants, depend upon the continuous supply of medicinal plants for manufacture of herbal medical formulations based

on Indian Systems of Medicine (Ved & Goraya, 2008). About 2000 tons of herbs are used annually in India by over 1.5 million practitioners of the Indian system with estimated 25000 effective plant based formulations in about 7800 drug manufacturing units (Ramakrishnappa, 2002). Most of the traded medicinal plants in Indian medicine system are collected from wild resulting in an unprecedented pressure on the population status of many species. Himalayan region harbors about 2500 species of medicinal value and many of these are highly traded and falls under the threatened category (Ved & Goraya, 2007). In Jammu and Kashmir 62 species of medicinal plants falls under threatened category (CAMP, 2010) in addition to many species used in traditional health care or traded for the pharmaceutical industry.

Chenab valley being a remote region in Himalaya, the aboriginal communities in many villages which are still devoid of modern amenities like roads, electricity and healthcare facilities. Thus they are largely dependent on the local plant resources like fuel wood, fodder, timber and handicraft for meeting their daily needs as well as on local plants of medicinal values to fulfill their healthcare requirements. Medicinal plants in Chenab valley are used heavily by local communities for traditional healthcare as well as trading. Further, intensive grazing by a large livestock adds additional pressure on the medicinal plants in different regions. In the present study we used the tools of ethnobotanical surveys and PRA meeting to document the traditional knowledge and wisdom of these aboriginal communities for assessment of conservation status of medicinal plants in Chenab valley, J&K.

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In addition, seasonal migration of nomads and transhumans pastoral communities occupies most of the pristine forests and alpine meadows during summers resulting in high level of impact on the local biodiversity including medicinal plants. Despite a high pressure on the medicinal plants due to trade, anthropogenic disturbance and local use, very few studies have been conducted and that only on documentation of medicinal plants. This study is designed to assess the population status of high value medicinal plants in wild and wisdom of local community for sustainable usage and conservation of medicinal plants.

LITERATURE REVIEW

The treasury of diverse medicinal plants available in the Himalaya has been recorded in ancient Indian scripts dated as far back as 1000 BC. In the Indian Himalayan Region (IHR), there are over 1748 plant species of medicinal plants including 1020 herbs, 335 shrubs and 330 trees of medicinal value (Samant *et al.*, 1998). Medicinal plants are an integral part in the lifestyle of Himalayan inhabitants and are highly valued both in folk medicine and in codified traditional medical systems, however there is a lack of management for Himalayan medicinal plants with reference to knowledge and practices of local people (Lama *et al.*, 2001).

A large number of studies on medicinal plants have been carried out in different parts of Indian Himalayan Region. The North-Western Himalaya embracing the states of Jammu and Kashmir, Himachal Pradesh and Uttarakhand is especially rich in diversity of wild medicinal plants. Some of the studies carried out on the ethnomedicobotany and status of wild medicinal plants in the North-Western Himalaya include: Ballabh and

Chaurasia (2009); Kaul (2010); Ballabh & Chaurasia (2011); Kaul (1997); Khan *et al.* (2004); Kumar *et al.* (2009); Kumar & Hamal (2011); Malik *et al.* (2011) and Pant & Pant (2011); Samant & Dhar (1997); Samant *et al.* (1998); Dhar *et al.* (2000) and Kala (2005a). In the state of Himachal Pradesh, key publications on the subject are Samant *et al.* (2007); Singh *et al.* (2009); Rana & Samant (2011); Singh & Rawat (2011). Major contributors in this field in the state of Uttarakhand works include Kala (2005b); Gangwar *et al.* (2010) and Kumar *et al.* (2011). From Jammu and Kashmir state, a total of 937 species belonging to 129 families and 509 genera have been reported from Jammu, Kashmir and Ladakh regions to have a traditional medicinal use by indigenous communities (Gairola *et al.*, 2014).

In Chenab valley of Jammu and Kashmir, previously four studies have been published on the diversity and uses of medicinal plants (Kumar *et al.*, 2009; Kumar & Hamal, 2011; Gupta *et al.*, 2013; Kumar & Sharma, 2013). To summarize these studies, more than 300 species of medicinal plants are being used by regional folk medicine system. Among these species, 284 species were found to be used in folk medicine system in different parts of Chenab valley, 90 species being used in Ayurvedic medicine system, 58 species in Unani medicine system, 50 species in Siddha medicine system, 37 species in Homeopathy medicine system, 23 species in Tibetan medicine system, 22 species in modern medicine system (Kumar & Sharma 2013).

STUDY AREA AND METHODS

Chenab valley in Jammu and Kashmir is located in the Greater Himalayan range, characterized by very steep, rugged and tough terrain. The human habitations are present even in the remote valleys areas ranging 1000–3000 m in elevation (Fig. 1).

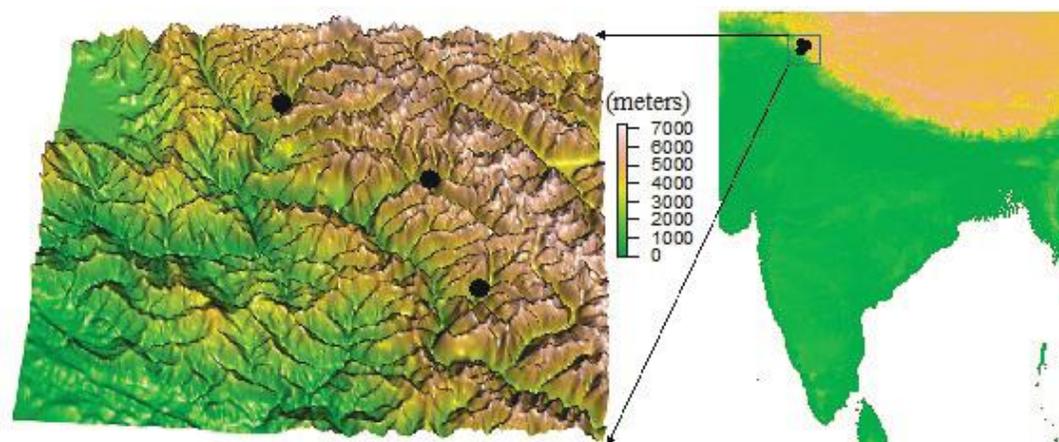


Figure 1: Digital elevation model of study area with GPS points in Paddar, Bhadarwah and Dachan catchments of Chenab Valley in Jammu and Kashmir.

A detailed literature review was conducted to assess diversity and uses of medicinal plants in Chenab valley. A total of 25 (10 men, 15 women) herbal healers from Paddar and Bhadarwah catchment were interviewed for documentation of traditional uses of medicinal plants in local folk medicine. A Prior Informed Consent (PIC) was taken from the respondents for the sharing of their traditional knowledge for scientific purpose. Since the herbal healers are familiar with local names of the plants only, if available plant specimen in their possession or our own photographs of the plants were used to ascertain the botanical name of each species. In addition, Participatory Rural Appraisal (PRA) method was applied to document the wisdom and malpractices impacting medicinal plants both in positive and negative ways. PRA meetings in a total of 16 villages (Paddar; 9, Bhadarwah; 7) were conducted involving participation of a total of 177 respondents in the groups of 9 to 17 during the meetings in different villages. Semi-structured questionnaires were used to document the responses of informants in each village community. Questions were asked on their opinions and evidence based information related to the status of medicinal plants in wild and prevalent threats responsible for decline of medicinal plants in Chenab valley (ref. Table 1).

RESULTS AND DISCUSSION

Ethnobotanical surveys and PRA interviews of local community shows that a total of 97 species of plants are used intensively for medicinal purposes in the local folk medicine. The life forms of these species include 69 herbs, 16 shrubs, 11 trees and 2 climbers. Among the species used for folk medicine, 47 species are also traded outside Chenab valley for economic gains. These include five (5) additional species which are not recorded in the list of species under trade in India by Ved and Goraya (2008). Majority of intensively used species in folk medicine by local herbal healers are also traded to meet the supply of various traditional medicine systems like Ayurveda, Unani, Siddha, Homeopathy. Notably,

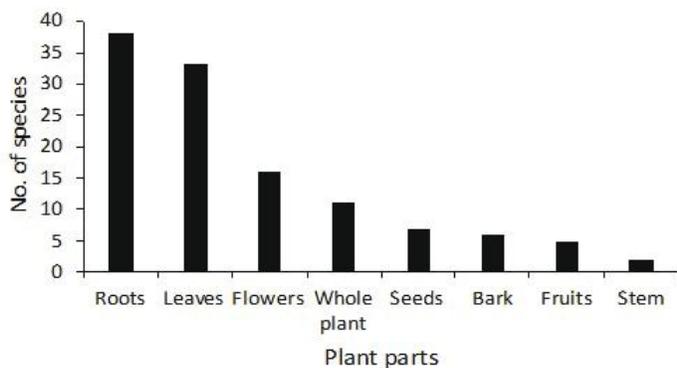


Figure 2: Number of species used for their parts in the folk medicine system in Chenab valley, J&K.

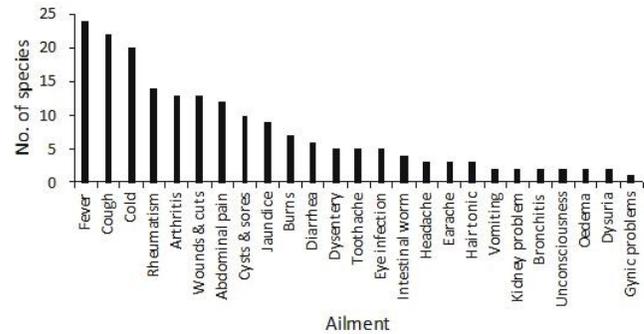


Figure 3: Number of species used by local herbal healers in various formulations for curing 25 ailments in Chenab valley, J&K.

all the 34 species present in the region and categorized as threatened by CAMP (2010), are also under trade for traditional medicine except two species viz., *Betula utilis* and *Rhododendron campanulatum*. Among these, five intensively traded species viz., *Saussurea costus*, *Dioscorea deltoidea*, *Picrorhiza kurrooa*, *Podophyllum hexandrum* and *Taxus wallichiana* are listed in CITES-I and II categories. Literature survey of the medicinal plants in Chenab valley (Kumar *et al.*, 2009; Kumar & Hamal, 2011; Gupta *et al.*, 2013; Kumar & Sharma, 2013) shows that more than 300 species are used in the valley. Among these, 284 species are used in folk medicine, 90 species in Ayurveda, 58 species in Unani, 50 species in Siddha, 37 species in Homeopathy, 23 species in Tibetan and 22 species in modern medicine. A total of 75 species growing in Chenab valley are recorded under trade in India for use in pharmaceutical industry (Ved & Goraya, 2008).

Ninety-seven species of medicinal plant are used by local herbal healers to cure 25 common ailments/diseases using 88 formulations. Roots are the most preferred parts of the medicinal plants both for folk medicine as well as for trade followed by leaves, flowers and whole plant (Fig. 2). Extraction of roots is the most harmful practice since it involves uprooting of the whole plants resulting in loss of regeneration of the species (Nishteswar 2014). For example, all critically endangered and endangered species (CAMP 2010) present in Chenab valley are harvested for their roots and are used both for trade as well as local folk medicine. Highest number of species are used for the cure of fever followed by cough, cold, rheumatism, arthritis etc. (Fig. 3). The usage pattern of medicinal plants shows a trend that higher number of species are used for the most common ailments prevalent in the temperate regions of Chenab valley. This signifies that the aboriginal communities benefit maximum possible usage of their surrounding plant resources using

Table 1: Medicinal plants used to cure various ailments in Chenab valley, J&K

Botanical name	Local Name	Family	In Trade	LF	Parts Used	Ailments
<i>Abies pindrow</i>	Reel	Pinaceae	No	T	Bk	Arthritis, rheumatism
<i>Aconitum heterophyllum</i> *	Atesh	Ranunculaceae	Yes	H	Rt	Cough, cold, fever, dysentery, diarrhea, jaundice, headache, abdominal pain
<i>Aconitum rotundifolium</i> *	Patish	Ranunculaceae	Yes	H	Rt	Fever
<i>Aesculus indica</i>	Guug	Sapindaceae	Yes	T	Sd	Arthritis, rheumatism
<i>Agrimonia pilosa</i>	Pili buti	Rosaceae	Yes	H	Fl	Cough, cold, fever
<i>Ajuga bracteosa</i>	Pitkaur	Lamiaceae	No	H	Lf, Rt	Cough, cold, fever, jaundice
<i>Allium roylei</i>	Kesher	Amoryllidaceae	No	H	WP	Abdominal pain
<i>Allium semenovii</i>	Dhar pyaz	Amoryllidaceae	Yes	H	WP	Abdominal pain
<i>Anemone obtusiloba</i>	Daler	Ranunculaceae	No	H	Rt	Wounds and cuts
<i>Anemone rivularis</i>	Daler	Ranunculaceae	No	H	Lf, Fr	Earache
<i>Angelica glauca</i> *	Choura	Apiaceae	Yes	H	Rt	Cough, cold, fever, vomiting
<i>Aquilegia fragrans</i>	Nepur	Ranunculaceae	No	H	Lf, Fr	Earache
<i>Arctium lappa</i>	Karpach	Compositae	No	H	Rt	Wounds and cuts
<i>Arnebia benthamii</i> *	Kahzaban	Boraginaceae	Yes	H	Fl	Jaundice, hair tonic
<i>Artemisia maritima</i> *	Sasem	Compositae	Yes	S	Lf	Cough, cold, fever, intestinal worm, abdominal pain
<i>Atropa acuminata</i> *	Beladona	Solanaceae	Yes	H	Rt	Fever, cough
<i>Berberis aristata</i>	Kemal	Berberidaceae	Yes	S	Rt	Cysts, sores
<i>Berberis lycium</i>	Kemal	Berberidaceae	Yes	S	Rt	Eye infection
<i>Bergenia ciliata</i>	Shupater	Saxifragaceae	Yes	H	Rt	Kidney, dysuria
<i>Bergenia stracheyi</i> *	Shupater	Saxifragaceae	Yes	H	Rt	Toothache, burns
<i>Betula utilis</i> *	Bhuj	Betulaceae	No	T	Lf	Cysts, sores
<i>Bunium persicum</i> *	Zeera	Apiaceae	Yes	H	Sd	Cough, cold, fever, vomiting
<i>Cassiope fastigiata</i>	Murki	Eriaceae	No	H	Lf	Burns, arthritis, rheumatism
<i>Cedrus deodara</i>	Harr	Pinaceae	No	T	Bk	Skin infection
<i>Chaerophyllum reflexum</i>	Ban zera	Apiaceae	No	H	Sd, Lf	Wounds and cuts
<i>Cirsium arvense</i>	Kanda	Compositae	No	H	Rt	Kidney stones, dysuria
<i>Colchicum luteum</i> *		Colchicaceae	Yes	H	Rt	Cold, cough
<i>Corydalis govaniiana</i>	Bhutkeshi	Papaveraceae	No	H	Fl, Fr	Abdominal pain
<i>Cremanthodium ellisii</i>		Compositae	Yes	H	Rt	Fever
<i>Cuscuta reflexa</i>	Bael	Convolvulaceae	No	C	WP	Jaundice
<i>Datisca cannabina</i> *	Kuur	Datisceae	Yes	S	Rt	Cough, cold, fever, jaundice
<i>Delphinium brunonianum</i>	Mundal	Ranunculaceae	No	H	Lf	Hair tonic
<i>Dioscorea deltoidea</i> *	Khed	Dioscoreaceae	Yes	C	Rt, Lf	Cough, cold, fever, bronchitis
<i>Ephedra gerardiana</i> *	Dhar ber	Ephedraceae	Yes	S	St	Toothache
<i>Equisetum arvense</i>	Chau	Equisetaceae	No	H	St	Toothache
<i>Ferula jaeschkeana</i> *		Apiaceae	Yes	H	Rt	bronchitis

*Threatened medicinal plants.

Abbreviations: H-herb, S-shrub, T-tree, C-climber, Lf-leaf, Rt-root, Fl-flower, Bk-bark, Fr-fruit, Sd-seed, St-stem, WP-whole plant.

Botanical name	Local Name	Family	In Trade	LF	Parts Used	Ailments
<i>Fragaria nubicola</i>	Bhaunch	Rosaceae	No	H	WP	Cough, cold, fever
<i>Fritillaria roylei</i> *	Salmdana	Liliaceae	Yes	H	Rt	Abdominal pain
<i>Fumaria indica</i>	Pitpapra	Papaveraceae	No	H	Lf, Sd	Cough, cold, fever, jaundice
<i>Galium aparine</i>		Rubiaceae	No	H	Lf	Wounds and cuts
<i>Heracleum candicans</i> *		Apiaceae	Yes	H	Rt	Fever
<i>Hypericum perforatum</i> *	Phul jhari	Hypericaceae	Yes	H	Rt	Fever
<i>Indigofera heterantha</i>	Shagal	Leguminosae	Yes	S	Lf	Unconsciousness
<i>Inula grandiflora</i>		Compositae	No	H	Fl	Wounds and cuts
<i>Inula royleana</i>		Compositae	Yes	H	Fl	Wounds and cuts
<i>Juglans regia</i>	Thanr	Juglandaceae	No	T	Bk	Cysts, sores, tuberculosis
<i>Juniperus recurva</i>	Bather	Cupressaceae	No	S	Lf	Arthritis, rheumatism
<i>Jurinea dolomiaea</i> *		Compositae	Yes	H	Rt	Gynic problems
<i>Lilium polyphyllum</i> *		Liliaceae	Yes	H	Rt	Cough
<i>Meconopsis aculata</i> *	Kunt	Papaveraceae	Yes	H	Rt, Lf	Cysts, sores
<i>Mentha longifolia</i>	Jangli pudina	Lamiaceae	Yes	H	Lf	Abdominal pain, diarrhea, stomachache, dysentery
<i>Origanum vulgare</i>	Muur	Lamiaceae	Yes	H	Fl, Lf	Cough, cold, fever, eye infection, abdominal pain
<i>Oxyria digyna</i>	Amlu	Polygonaceae	No	H	WP	Eye infection
<i>Pedicularis bicornuta</i>	Hurang	Orobanchaceae	No	H	Lf, Fl	Burns
<i>Pedicularis cheilanthis- folia</i>	Hurang	Orobanchaceae	No	H	Lf, Fl	Burns
<i>Physochlaina praealta</i> *		Solanaceae	Yes	H	Sd	Intestinal worms
<i>Picrorhiza kurrooa</i> *	Kaur	Scrophulariaceae	Yes	H	Rt	Cough, cold, fever, arthritis, rheumatism, dysentery, diarrhea, jaundice
<i>Pinus wallichiana</i>	Chei	Pinaceae	No	T	Lf	Unconsciousness
<i>Plantago lanceolata</i>		Plantaginaceae	No	H	WP	Wounds and cuts
<i>Podophyllum hexan- dram</i> *	Ban kakri	Berberidaceae	Yes	H	Rt, Fr	Abdominal pain
<i>Polygonum alpinum</i>	Panchalar	Polygonaceae	No	H	Rt	Arthritis, rheumatism
<i>Prunus armeniaca</i>	Cher	Rosaceae	No	T	Sd	Arthritis, rheumatism, body pain oedema
<i>Prunus persica</i>	Chana	Rosaceae	No	T	Sd	Arthritis, rheumatism, jaundice
<i>Prunus spinosa</i>	Auur	Rosaceae	No	T	Lf	Body pain, Oedema
<i>Quercus baloot</i>	Ere	Fagaceae	No	T	Bk	Abdominal pain
<i>Rabdosia rugosa</i>	Thapar	Lamiaceae	No	S	Lf	Cough, cold, fever, Wounds and cuts
<i>Rheum spiciforme</i> *		Polygonaceae	Yes	H	Rt	Rheumatism
<i>Rheum webbianum</i> *		Polygonaceae	Yes	H	Rt, WP	Rheumatism
<i>Rhodiola heterodonta</i> *	Dharber	Crassulaceae	Yes	H	Rt	Toothache
<i>Rhodiola himalensis</i>	Dharber	Crassulaceae	Yes	H	Rt	Toothache

*Threatened medicinal plants.

Abbreviations: H-herb, S-shrub, T-tree, C-climber, Lf-leaf, Rt-root, Fl-flower, Bk-bark, Fr-fruit, Sd-seed, St-stem, WP-whole plant.

Botanical name	Local Name	Family	In Trade	LF	Parts Used	Ailments
<i>Rhododendron anthopogon</i> *		Eriaceae	Yes	S	Rt, WP	Arthritis
<i>Rhododendron campanulatum</i> *	Shangur	Eriaceae	No	S	Lf	Arthritis, rheumatism
<i>Rosa brunonii</i>	Ghyal	Rosaceae	No	S	Lf	Cysts, sores
<i>Rosa webbiana</i>	Ghyal	Rosaceae	No	S	Lf, Fl	Cysts, sores, stomach pain
<i>Rumex nepalensis</i>	Ajobal	Polygonaceae	No	H	Lf	Cysts, sores
<i>Salix alba</i>	Beed	Salicaceae	No	S	Fl	Arthritis, rheumatism
<i>Saussurea costus</i> *	Kuth	Compositae	Yes	H	Rt	Arthritis, rheumatism, dysentery, diarrhea
<i>Saussurea gnaphaloides</i>	–	Compositae	Yes	H	WP	Burns
<i>Saussurea gossypiphora</i> *	Ghugi ban	Compositae	Yes	H	WP	Burns
<i>Saxifraga flagellaris</i>	Sitabal	Saxifragaceae	No	H	Lf, Fl	Hair tonic, abdominal pain
<i>Sedum ewersii</i>		Crassulaceae	No	H	Fl	Cysts, sores
<i>Skimmia anquetilia</i>	Shangel	Rutaceae	Yes	S	Lf	Cough, cold, fever
<i>Solanum nigrum</i>	Kagdash	Solanaceae	No	H	Fr	Earache, eye infection, skin infection
<i>Tanacetum dolichophyllum</i>	–	Compositae	No	H	Fl	Cough, cold, fever
<i>Tanacetum tibetica</i>		Compositae	No	H	Fl	Cough, cold, fever
<i>Taraxacum officinale</i>	Chupar	Compositae	No	H	WP	Wounds and cuts, cysts, sores
<i>Taxus wallichiana</i> *	Chuga	Taxaceae	Yes	T	Bk	Burns, cysts
<i>Thalictrum foliolosum</i> *	Phuljari	Ranunculaceae	Yes	H	Rt, Fl	Eye infection
<i>Trillium govaniatum</i>	–	Melanthiaceae	Yes	H	Rt	Cough, cold, fever
<i>Thymus linearis</i> *	Jash	Lamiaceae	Yes	H	Fl, Lf	Cough, cold, fever, abdominal pain, stomach pain
<i>Urtica dioica</i>	Aarn	Urticaceae	No	H	Rt, Lf	Arthritis, rheumatism, allergy
<i>Valeriana jatamansi</i> *	Jatmansi	Caprifoliaceae	Yes	H	Rt	Dysentery, diarrhea
<i>Verbascum thapsus</i>	Bantamuk	Scrophulariaceae	No	H	Lf	Cough, cold, fever, Wounds and cuts
<i>Viburnum cotinifolium</i>	Tung	Adoxaceae	No	S	Bk	Jaundice
<i>Viola betonicifolia</i>	Banafsa	Violaceae	No	H	Rt, Lf	Wounds and cuts, skin infections
<i>Viola canescens</i>	Banafsa	Violaceae	No	H	Rt, Lf	Wounds and cuts, skin infections
<i>Viscum album</i>	Mashur	Santalaceae	No	S	Lf	Skin infection

*Threatened medicinal plants.

Abbreviations: H-herb, S-shrub, T-tree, C-climber, Lf-leaf, Rt-root, Fl-flower, Bk-bark, Fr-fruit, Sd-seed, St-stem, WP-whole plant.

the traditional ethnobotanical knowledge for meeting their healthcare needs.

Responses of participants in PRA meetings shows a lack of awareness in a major proportion about the declining population trends and conservation importance of medicinal plants. For example, 41% respondents believed that medicinal plants are not facing any

problem of population decline in wild. However, 21% participants believe that medicinal plants are under the threat of population decline in Chenab valley whereas 38% participants believe that only some of the highly used species are under the threat of population decline and other species have sufficient population size in wild. Indigenous ethnic groups who do not believe in any population decline argued

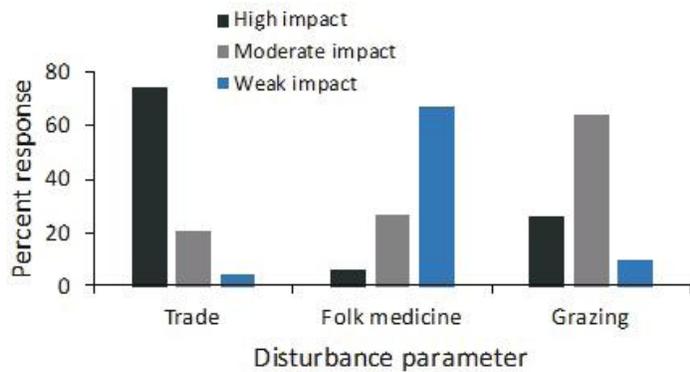


Figure 4: Level of threat on the wild population of medicinal plants based on the responses of local community during PRA meetings.

that adequate regeneration happens in nature which sustains the populations of medicinal plants in wild. As many as 59% respondents felt that there has been a population decline of medicinal plants in Chenab valley. Major reasons for the decline, according to the respondents were increasing commercial trade (major factor), habitat degradation due to excessive livestock grazing (second important factor) and use in folk medicine (not an important) factor responsible for decline of medicinal plants in wild (Fig. 4). Local inhabitants also believed that the traditional rights for the local use and trade of wild medicinal plants and their area should be retained which has been prevalent since several generations.

From Jammu, Kashmir and Ladakh regions of J&K state 937 species of plants are used for various purposes which is about 26% of the total floras of the state (Gairola *et al.*, 2014). In the Indian Himalayan region, there are over 1748 plant species of medicinal value (Samant *et al.*, 1998), 675 species of wild edible plants (Samant & Dhar, 1997), 279 species of fodder yielding plants, 118 species of oil yielding plants and 155 species of sacred plants (Samant & Pant, 2006) among which 121 species are rare and endangered plants (Nayar & Sastry, 1990). Medicinal plants are thus an integral part in the lifestyle of Himalayan inhabitants and are highly valued both in folk medicine and in codified traditional medical systems, however there is a lack of management of Himalayan medicinal plants with reference to knowledge and practices of local people (Lama *et al.*, 2001; Kanwar *et al.*, 2006). For example, a ten-year long monitoring of 60 threatened plants species in Himalaya shows that 22% of these species were critically endangered, 16% were endangered, and 27% were vulnerable (Kala, 2005b). The high demand of raw plant material for the traditional medicine systems has posed a serious threat on the population in wild, however assessment of such impact is very rare since most of the trade is unmonitored and illegal. Thus there is a dire need to assess the population status of medicinal plants in wild which

are under heavy trade. Participation of the aboriginal communities for the documentation of their traditional knowledge and wisdom can help in both conservation and management of the medicinal plants in Himalaya.

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Ethnomedicinal Use of Plants by Bhutia Tribe in Sikkim Himalaya

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ABSTRACT: Medicinal Plants (MPs) have been recognized as an important bioresource, are embodied with the tribal communities worldwide. A thorough understanding of the traditional use practices and appropriate field-based documentation requires conserving their bio-cultural values. Amongst three major tribes in Sikkim the Lepcha, Bhutia and Limboo, the Bhutias have hardly been attempted for such investigations. Therefore, we studied the utilization pattern of MPs among the Bhutia tribes of Sikkim. Our study explored 35 MPs belonging to 30 families, which are used by the Bhutia tribes for curing 31 different ailments and diseases. Among these MPs, Asteraceae emerged to be the dominant family. On the basis of plant part used for medicinal purpose, leaves of 12 species were extracted for treatment of various ailments and diseases followed by whole plant of 11 species, fruits of 6 species, roots of 5 species, tuber of 3 species, bark and stem of each 2 species and flower, seed, spikes, bulbs and rhizome of each 1 species. The highest 16 species are used for cough and fever followed by 8 species for diarrhoea and dyspepsia, and 6 species for stomachache. In addition, the maximum numbers of plants (18 species) used for the medicinal purpose are herbs followed by shrub (9 species) and tree (6 species). Our study suggests a strong need for conserving bio-cultural based traditional knowledge of Bhutia tribes in Sikkim Himalaya. ©2019

KEYWORDS: Bio-cultural, Bhutia tribe, Ethnomedicine, Traditional knowledge, Medicinal plants, Sikkim Himalaya.

INTRODUCTION

Sikkim is the second smallest state of India, which encompasses a diverse range of ecological zones, viz., subtropical, temperate, sub-alpine and alpine. Owing to high vertical variations, the area is rich in biodiversity. Over 490 medicinal plant species have been listed out for Sikkim and Darjeeling in some studies (Sharma & Sharma, 2010), whereas, the literature indicates that Sikkim-Darjeeling hills represent over 707 species of medicinal value (Badola & Aitken, 2003). The Sikkim has recently been assessed as a hotspot of ethnobiological plants, with over 995 species of ethnobotanical value (O'Neill *et al.*, 2017). The locals who mostly live in poverty, gather the medicinal plants and trade in the local market or work for collectors to supply plants to a larger market. Due to this, the threat of extinction of some important species in the wild was noted (Rai *et al.*, 2000); however, since the year 2000 Sikkim Government has made a ban on the *in-situ* harvesting of medicinal plants.

In Sikkim, 25 ethnic communities identified during its first ethnographic survey between 1988 and 1990 by Anthropological Survey of India (Chettri *et al.*, 1992). Recently, O'Neill *et al.* (2017) recognized 32 ethnic communities in Sikkim. Of which, Bhutias, Lepchas,

Limboos, Nepalese and Tibetans are prominent. However, the Lepchas are the oldest indigenous tribe of Sikkim (Pradhana & Badola, 2008). Bhutia tribe possesses Tibetan cultural life including the religion, language, and economic system, traditionally; this is a combination of pastoralism and semi-settled agriculture. Buddhism is the religion followed by the tribe and has been widely spreading in Sikkim with uprising monasteries and followers. Traditionally, the Bhutias dwell in clusters and at present settled in all the four districts of Sikkim.

The indigenous knowledge on plants and its usage in local healthcare is massively declining in the Himalayan region; it is, therefore, important to document this traditional wisdom before it completely disappears forever (Pradhan & Badola, 2008). The field-based documentation will strengthen the human-plant relationship and brace preservation approach of useful species. Except for some fragmentary knowledge there is hardly any specific work on ethnomedicinal plants is available in literature from Sikkim. The present paper aimed to study the medicinal and religious plants used by Bhutia tribe inhabiting the five villages in East Sikkim; study focused on their use practices in curing different diseases and ailments and for various religious beliefs.

LITERATURE REVIEW

World Health Organization estimated that about 80% of the world's population depends on traditional medicine for their primary health care needs (Azaizeh *et al.*

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2003). In many rural communities, traditional medicine is still recognized as the primary health care system (Bannerman *et al.*, 1983). Such type of knowledge survives because it is transferred from one generation to another (Manandhar, 1989). Over the past several decades, the humans have been rapidly and over using the existing natural resources, which has questioned the very future of biological resources for coming generations (Chan *et al.*, 2016; Badola, 2017). The biocultural significance is not limited to the use of plants for food, clothing, and shelter, but it also includes their use in religious ceremonies, ornamentation and healthcare (Schultes, 1992; Badola & Aitken, 2010). Lately later on, the international conservation agencies have been increasingly acknowledging the importance of biocultural knowledge vital to biodiversity conservation (Maffi, 2014; Badola, 2017). The prolonged utilization has widened the knowledge of plants passing the understanding to generations which either has been limited within the tribe or transferred to neighboring tribes. In India, many rural communities are still dependent on medicinal plants for health care and 7500 plant species are used by tribal people (Badola & Aitken, 2003). However, due to the insufficient written records and with the impact of urbanization and ongoing globalization process, the knowledge on medicinal and

religious plants is declining very prominently (Pirker *et al.*, 2012).

MATERIALS AND METHODS

Study Area

The five villages selected for the study purpose in East Sikkim are Linkey, Parkha, Machong, Menchu, and Barapathing. These villages are located along 1200 m to 1600 m asl extending from 88°39'09.5" E to 88°41'33.8" longitude and 27°13'30.2" N to 27°14'40.3" N latitude (Fig. 1). The vegetation type of these villages is subtropical to temperate-broad-leaved. The other communities residing in these areas are Gurung, Chettri, Brahmins, Rai, *etc.*

Household Survey

Available literature was thoroughly reviewed with a view to getting baseline information especially for the Sikkim context, and accordingly, the field survey design prepared. Questionnaires based survey was done in the five villages of East Sikkim. The present study was focused on the ethnomedicinal knowledge of Bhutia tribe on medicinal plants used in curing different ailments and diseases along with its used parts and procedures. Different aged group of locals was approached and requested to share their knowledge on the use of different plants for the medicinal and religious purpose. In each of the 05 villages, 20 households were randomly surveyed. Youngsters disagreed to get questioned as they lacked knowledge of the plants. This clearly indicated the fading knowledge on plants in the upcoming generations. As the locals were more comfortable in communicating in their own language, the mode of communication was largely done in Bhutia language. The Bhutia names, Nepali name of the local plants were collected and plants were identified by asking local people, monks and by using local regional flora, e-flora, photographs and relevant literatures.

Of total 101 respondents interacted, 54 were males and 47 females. The respondents belonged to an age group between 18 and 85. The elderly people were the most preferred respondents. The male were mostly farmers, retired employee and few monks. The female were mostly housewives who visited nearby forest for fodder, fuelwood, wild edibles and other resources. Youngsters were mostly educated and lived out of the village pursuing studies or working and rarely visited home.

RESULTS

The study explored 35 ethnomedicinal plants (MPs) belonging to 30 families and 35 genera used by the Bhutia tribe of Sikkim (Table 1). Among 35 MPs, 18 represented

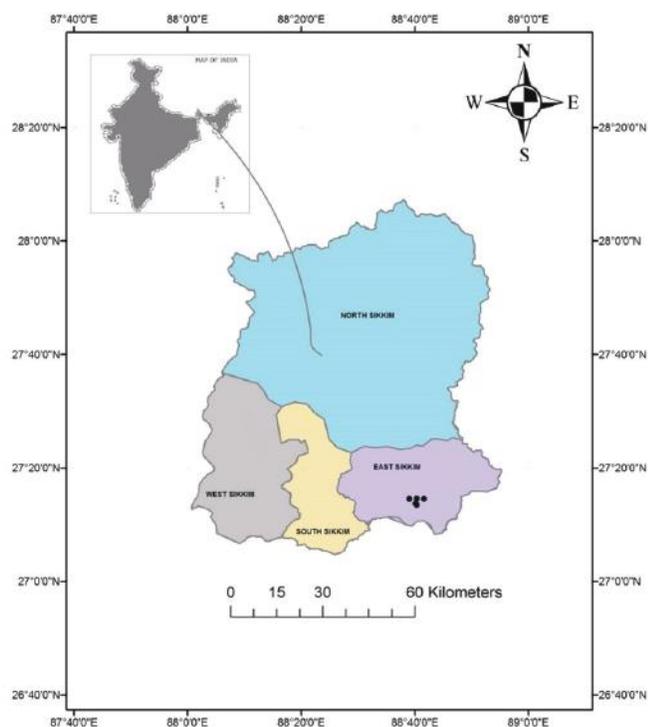


Figure 1: Location map of the study sites in the Sikkim Himalaya.

Table 1: Ethno-medicinal plants used by the Bhutia tribe in Sikkim

S. No.	Scientific Name	Local Name	Family	Habit	Parts Used	Uses
1.	<i>Aconitum ferox</i> Wall. ex Ser.	Bikh	Ranunculaceae	Herb	Tubers	Tuber piece or paste is taken against acute dysentery, fever, body ache, piles, hysteria, cough, throat infection, stomach disorder, and vomiting
2.	<i>Acorus calamus</i> L.	Syotakko	Acoraceae	Herb	Rhizome and leaves	Rhizome is used as a tonic against fever, diarrhoea, and dyspepsia.
3.	<i>Aeschynanthus parviflorus</i> (D. Don) Spreng.	Baklay Patay	Gesneriaceae	Shrub	Whole plant	Decoction of root is taken against fever, fresh flower is taken to cure throat pain and the plant paste is applied on bone fracture.
4.	<i>Ageratum conyzoides</i> (L.) L.	Yung-kokham	Asphodelaceae	Herb	Whole plant	Leaf juice is applied on cuts to stop bleeding and wounds and allergy.
5.	<i>Allium sativum</i> L.	Ghokoo	Amaryllidaceae	Herb	Bulbs and leaves	Bulbs are taken during whooping cough, cold and fever.
6.	<i>Aloe vera</i> (L.) Burm.f.	Khado minto	Asphodelaceae	Shrub	Leaves	Fresh levees are usually used in skin problem and the juice is taken against diabetes.
7.	<i>Artemisia vulgaris</i> L.	Khempaa	Asphodelaceae	Shrub	Whole plant	Fresh leaves are crushed and inserted in nostrils as a plug to stop bleeding and the leaf juice is applied to cuts and wound to stop bleeding and infection.
8.	<i>Azadirachta indica</i> A. Juss.	Nimpata	Meliaceae	Tree	Whole plant	Leaves are basically used in vomiting, fatigue, fever.
9.	<i>Bergenia ciliata</i> (Haw.) Sternb.	Bhya mentho	Saxifragaceae	Herb	Whole plant	Fresh rhizome is used for curing diarrhoea, vomiting, fever, cough, and boils.
10.	<i>Solanum torvum</i> Sw.	Jangali Bee	Solanaceae	Shrub	Leaves	Leaves are used to control microbial activities.
11.	<i>Chenopodium album</i> L.	Shambin	Amaranthaceae	Herb	Whole plant	Plant juice is taken against ulcer, stomachache, joint pain, and piles.
12.	<i>Costus speciosus</i> (Koen. ex. Retz.) Sm.	Bet laure	Costaceae	Shrub	Tubers and stem	Tuber and stem juice extract are taken orally in empty stomach to cure urinary tract infections.
13.	<i>Brugmansia suaveolens</i> (Humb. & Bonpl. ex Willd.) Bercht. & J. Presl	Radhong minto	Solanaceae	Shrub	Leaves	Leaves are used in rheumatic swelling, decoction of seeds are applied in dog bites.
14.	<i>Drymaria cordata</i> (L.) Willd. ex Schult.	Byapin	Caryophyllaceae	Herb	Whole plant	Leaves and stem rolled in a bigger leaf and kept in flames should not burn the whole lot, while hot then transferred to a thin cotton cloth and the emanating vapor is smelled immediately to cure sinus.
15.	<i>Eupatorium adenophorum</i> Spreng.	Banmara	Asphodelaceae	Herb	Roots and leaves	Fresh tender leaves and stems are mashed and the juice is applied to cut and bruises it will help in stopping bleeding and infection.
16.	<i>Heracleum wallichii</i> DC.	Chimphing	Apiaceae	Shrub	Roots and fruits	Dried fruits are taken against stomach disorder and chewed to treat sinusitis.

S. No.	Scientific Name	Local Name	Family	Habit	Parts Used	Uses
17.	<i>Centella asiatica (L.) Urb.</i>	Golpatta	Apiaceae	Herb	Leaves	Fresh leaves and stem are crushed and taken orally in empty stomach to relieve blood pressure, stomach disorders and as a brain tonic.
18.	<i>Kaempferia rotunda L.</i>	Bhui champa	Zingiberaceae	Herb	Tubers	Paste of the tuber is applied immediately on the bone fracture to avoid swelling.
19.	<i>Lindera neesiana (Wall. ex Nees) Kurz</i>	Narik	Lauraceae	Tree	Fruits	Fruits are taken during stomachache
20.	<i>Mentha spicata L.</i>	Shyamen	Lamiaceae	Herb	Roots and leaves	Roots, leaves are used in fever and bronchitis, oil is used for rheumatism.
21.	<i>Oroxylum indicum (L.) Kurz</i>	Paksang mentho	Bignoniaceae	Tree	Bark, fruits and seeds	Dry seeds are taken orally to prevent throat complications and hypertension.
22.	<i>Phytolacca acinosa Roxb.</i>	Khim	Phytolaccaceae	Herb	Leaves	Mashed leaves are applied in cuts and wounds to stop bleeding and infection.
23.	<i>Picrorhiza kurrooa Royle</i>	Kutki	Scrophulariaceae	Herb	Whole plant	The dried roots are orally used in malaria, asthma and chronic fever.
24.	<i>Polygonum molle D. Don</i>	Thomcha	Polygonaceae	Shrub	Leaves	Leaf juice is taken against diarrhoea and dysentery.
25.	<i>Pteris baurita L.</i>	Kyeem	Pteridaceae	Fern	Stem and leaves	Mashed stem applied on cuts to stop bleeding and infection and wounds
26.	<i>Rhododendron arboreum Sm.</i>	Altho minto	Ericaceae	Shrub	Bark and Flowers	Flower is consumed in diarrhea and dysentery.
27.	<i>Smilax aspera L.</i>	Kukur dainey	Smilacaceae	Climber	Whole plant	Used in urinary problem and dysentery, roots are taken as a tonic
28.	<i>Swertia chirayita (Roxb.) Buch.-Ham. ex C.B. Clarke</i>	Tiktha	Gentianeae	Herb	Leaves	Dried leaves and stem are used during fever.
29.	<i>Terminalia chebula Retz.</i>	Arru	Combretaceae	Tree	Fruits	Fruits are taken to cure cough, bronchitis, jaundice, pneumonia, and piles.
30.	<i>Tetradium fraxinifolium (Hook. f.) T.G. Hartley</i>	Khuno	Rutaceae	Tree	Fruits	Fruits powder along with water is given to cure dysentery, gastritis, cough, and cold.
31.	<i>Thysanolaena latifolia (Roxb. ex Hornem.) Honda</i>	Pasham	Poaceae	Herb	Roots	It can be used as dried as well as fresh. Paste is applied to check boils and roots extract is used as a mouthwash.
32.	<i>Tupistra nutans Wall. ex Lindl.</i>	Barma	Asparagaceae	Herb	Roots and spikes	Decoction of root is taken against urinary problems, insomnia, constipation and rheumatic pain. Roots are used during food poisoning.
33.	<i>Urtica dioica L.</i>	Shocha	Urticaceae	Herb	Whole plant	Paste of the plant is applied on the minor fracture. Leaves and stem are used as vegetables it will help to control high blood pressure, cough, cold, stone problem.
34.	<i>Viscum articulatum Burm. f.</i>	Harchur	Santalaceae	Herb	Whole plant	Decoction of the herb is used for muscular pain, injuries and bone fracture.
35.	<i>Zanthoxylum acanthopodium DC.</i>	Narik	Rutaceae	Tree	Fruits	Fruits are taken during stomachache, gastritis, toothache, and cold.

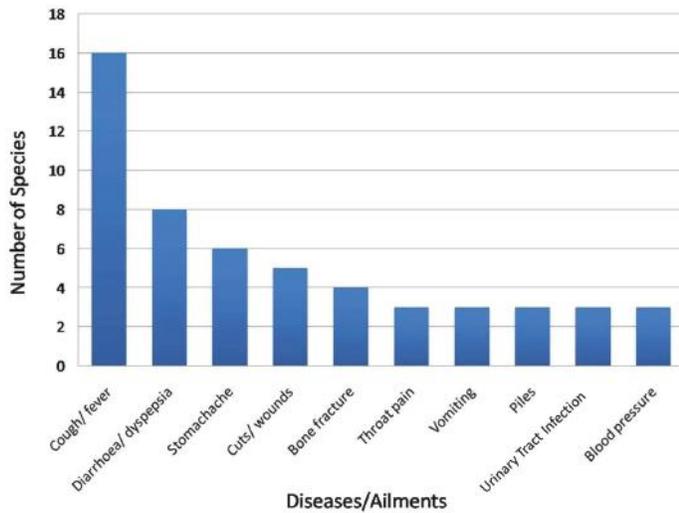


Figure 2: Different groups of ailments and diseases cured by using plant species in East Sikkim.

herbaceous group followed by shrubs (9 MPs), trees (6 MPs) and one MP each of fern and climber. Asteraceae emerged as the most dominating family (3 species), followed by Apiaceae, Rutaceae and Solanaceae (2 species each), while 26 families are monospecific.

The 35 MPs recorded from East Sikkim are used for curing 31 different ailments and diseases. Villagers use 16 species in curing cough and fever, 8 species in diarrhoea and dyspepsia, 6 species are orally taken during stomachache, 5 species they apply on cuts and wounds, 4 species of plants used during bone fracture, 3 species in throat pain, vomiting, piles, urinary tract infection and blood pressure. These plants are used either externally or internally. As per our study, the preparation of plant for treating different ailments and diseases includes single plant species and in few cases, villagers use more than one species (Fig. 2).

The twelve medicinal plant species were reported to be used to treat different ailments by using leaves. The whole plant of 11 plant species is used for other problems. The fruits of 6 species; roots of 5 species; tuber of 3 species; bark and stem of 2 species each; seed, flower, spikes, bulbs, and rhizome of 1 species each are used to cure several ailments and diseases (Fig. 3).

The age group of respondents was selected on a random basis (Fig. 4). Age group 18–30 had less knowledge on the use of medicinal plant in the area and they also disagreed on being questioned. Age group 30–50 had basic knowledge on the use of some of the plants. But the age groups 50–85 were the most resourceful respondents in all the targeted villages, as they had immense knowledge on the previous and prevailing usage of medicinal plants in the place.

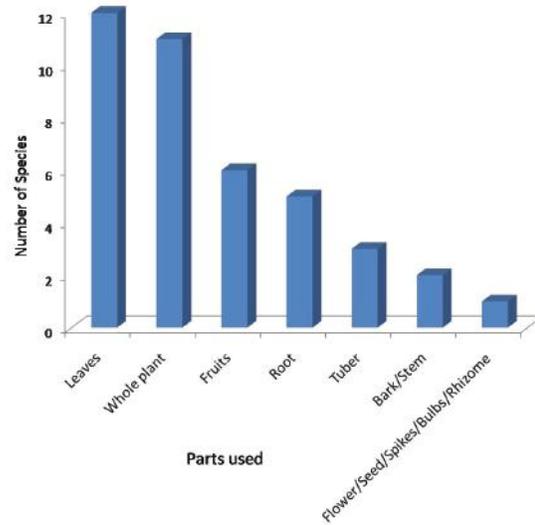


Figure 3: Different plant parts used for treating different ailments and diseases in East Sikkim.

DISCUSSION

Indigenous people play a vital role in environmental management and development and the bio-cultural approaches may offer valuable clues and tools (Badola, 2017) helping conservation and sustainable utilization of bio-resources especially in the Himalaya (Dhar *et al.*, 2002; Badola, 2017). Several indigenous communities’ posses incredible knowledge of ethnomedicinal practices for the well-being of human for long-time. As the time plunging by, such knowledgebase is gradually declining and suggesting urgent need to document these practices for their long-term conservation (Pradhan & Badola, 2008). We surveyed the Bhutia dominated villages in the eastern part of Sikkim and explored 35 medicinal

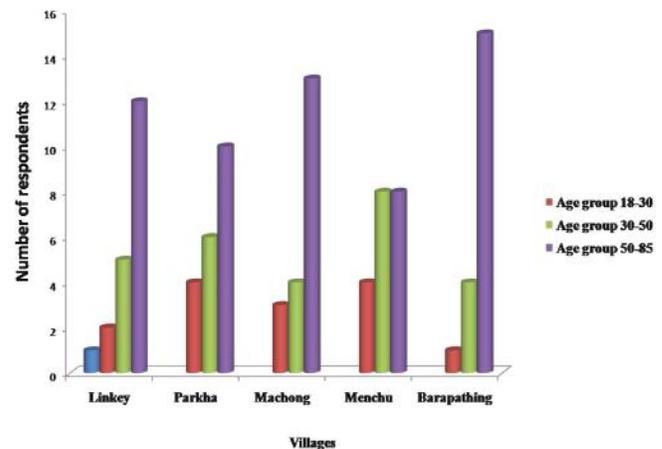


Figure 4: Graphical representation of different age groups in five villages of East Sikkim.

plants belonging to 30 families and 35 genera. Our study focusing the current status of the ethnobiological interaction of Bhutia tribe is the first of its kind in Sikkim. Our assessment followed more or less similar approaches that investigated earlier in Sikkim, for example, ethnomedicinal plant use by Lepcha tribe (Pradhan & Badola, 2008) and by Limboo tribe (Badola & Pradhan, 2013). In addition, recently O’Neill *et al.* (2017) highlighted the integration of ethnobiological knowledge into biodiversity conservation in Sikkim Himalaya and they traced the history and trends of ethnobiological documentation, identifying priority species and habitat types and analyzed within and among community differences pertaining to species use and management. Sikkim Himalaya is a bio-cultural hotspot where six ethnic communities, like Bhutia, Lepcha, Limboo, Nepali, Sherpa, and Tibetan communities, and 1128 species engage in bio-cultural associations (O’Neill *et al.*, 2017). However, an earlier review mentioned the highest number of NTFPs from India (377 species), followed by Nepal (363) and Bhutan (245) in Kangchenjunga landscape (Uprety *et al.*, 2016). These initiatives suggest further necessity field-based documentation of ethnomedicinal plants.

We realized through field investigations that the Bhutia tribe is an important community connected with the biocultural relationship. Ironically, in our study, over 90% respondents from the five villages of east Sikkim (Bhutia-dominated villages) shared that the use of plant species for the treatment of certain ailments and diseases has drastically declined with the development of allopathic treatment facilities provided in primary health centres and sub-centre in the area.

Our results further showed that the ethnomedicinal knowledge on plants was more preserved in the age group 50–85. Villagers also relied on the ritual practices performed by Monks (lama) at monasteries and by folk healers in the villages. They mentioned the total dependency of people during older times on the medicinal plants in healing which has been replaced by the invasion of advanced medication. This aged group people still do not prefer visiting doctor, instead, they rely on medicinal plants. Age group 30–50, representing government employees, housewives, and farmers, is the generation that swings between the old practices and allopathic mode of treatment; they find the latter is the beneficial and prominent mode of medication. For having weak knowledge in the application of the medicinal plants, they have stopped using the old traditional methods or

even rituals. However, species like *Aloe vera*, *Artemisia vulgaris*, and *Eupatorium cannabinum* are commonly used for minor cuts or injuries.

Age group 18–30 disagreed on getting interviewed due to their lack of knowledge, and they too rely on allopathy. Such generation gap does not help to transfer valuable ethnomedicinal knowledge from old generation to younger generation and indicates its weak future. In comparison, the youths of Limboo community in Khangchendzonga Biosphere Reserve (west Sikkim) have better preserved such ethnomedicinal knowledge (Badola & Pradhan, 2013).

In comparison to other studies in Sikkim (124 plants by Limboo tribe: Badola & Pradhan, 2013; 118 species by Lepcha tribe: Pradhan & Badola, 2013), our exploration (35 species) shows limited knowledge on the ethnomedicinal use of plants by Bhutia tribe (Fig. 5).

In our study area, Bhutia people commonly use *Eupatorium cannabinum* and *Artemisia vulgaris* during cuts and bleedings. *Aloe vera* is potted in almost every household that was surveyed. *Ageratum conyzoides* is an invasive plant which is widely spread in the east Sikkim having medicinal property is also applied to cuts to stop bleeding and wounds. Some medicinal plants that were listed from these villages are not found growing in these areas; rather they are found in the higher altitude and are collected sometimes and kept for medication purpose, especially *Cordyceps sinensis*. The plants taken orally to cure are consumed either with warm water, skimmed milk, honey, and sugar or raw. In west Sikkim, local people in general use 36 medicinal plants as anti-diabetic (Gurung *et al.*, 2014). The fresh leaves and stem of *Centella asiatica*, commonly known as Golpatta is taken to relieve blood pressure, against stomach disorder

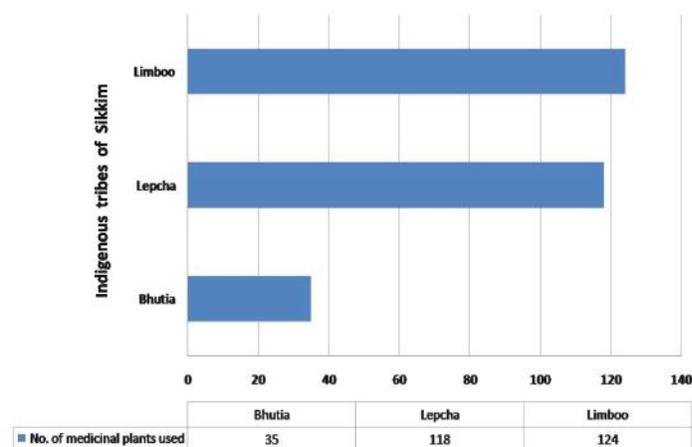


Figure 5: Comparison of the number of medicinal plants used by major tribes of Sikkim (Based on Pradhan & Badola, 2008; Badola & Pradhan, 2013).

and is one of the most commonly used medicinal plants by different tribal communities of North East India (Bhuyan, 2015).

The Limboos and Lepchas of Sikkim mostly use the underground parts like rhizome, root, and bulbs as medicines. They believe that the underground parts contain more healing properties in comparison to the other parts (Badola & Pradhan, 2013). But, the present study revealed the more use of leaves and whole plant parts by the Bhutia tribe in east Sikkim. Like Limboo tribe from west Sikkim and Lepcha tribe from Dzongu, north Sikkim (Badola & Pradhan, 2013; Pradhan & Badola, 2008), our study revealed the high use of herbs as medicine.

CONCLUSION AND RECOMMENDATION

Our present study was the first attempt to record the use of medicinal plants by Bhutia tribe in east Sikkim. Traditional knowledge of MPs acquired in Sikkim is continuously diminishing, with its composition influenced by urbanisation and ongoing globalisation processes and challenged by shifts from traditional healing practices to modern healthcare facilities. Our field-based ethnomedicinal plants' documentation would be of enormous use to researchers, pharmaceuticals, and society. In essence, there is an urgent need to initiate conservation of ethnomedicinal knowledge of Bhutia tribe. In addition to conservation awareness; scientific-based ex-situ conservation would help to provide incentives to villagers for their livelihoods.

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SECTION 3

Management of Invasive Species



Inventory of the Wooden Alien Flora of Uttarakhand Himalayas: A Review

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ABSTRACT: The woody plants were initially introduced for ornamental and economic purposes around the globe, however a number of trees and shrubs now have occupied vast areas thus becoming invasive. Uttarakhand being a Himalayan state, encompasses a rich biodiversity both in terms of flora and fauna including invasive species. The present study is an attempt to inventories alien woody flora of Uttarakhand based on the existing literature. A total of 125 alien woody flora belonging to 48 families and 81 genera including 72 angiosperms and 53 gymnosperms have been identified in this study. In terms of life form these species represented 91 trees and 34 shrubs. Of the 48 families, Cupressaceae was the most dominant (19 species) followed by Pinaceae (15), Solanaceae (7 species) and Mimosaceae (6 species). As per the nativity the majority of the woody alien flora was from Tropical America (15) followed by China (14) and Australia (13). This inventory will help in assessing the current status of woody alien flora in the state which will further help in managing the invasive species effectively. ©2019

KEYWORDS: *Invasive; Biodiversity; Woody alien flora; Himalaya.*

INTRODUCTION

Uttarakhand, a state lying in the lap of Himalaya is endowed with a rich diversity of flora and fauna throughout its geographical extent. The state encompasses an area of 53,483 sq km (Uniyal *et al.*, 2007) with altitude ranging from 300 m asl to 7,817 m asl. This variation has supported the successful establishment of alien woody flora (trees and shrubs) in the state. With increasing globalization and trade connections around the world, various alien species have been introduced in the state (Sekar, 2011) intentionally or unintentionally and their number is expected to increase in the near future.

Globally, exotic woody plants were primarily introduced for ornamental and commercial purposes due to their adaptability, rapid growth and absence of native pests (Nair, 2001). Though not widely considered as invasive, some of the woody aliens have subsequently become noxious invaders, which might have significant detrimental impacts on biodiversity across the ecosystems. These impacts include biodiversity loss, habitat degradation and alteration of various edaphic parameter including the nutrient dynamics (Dogra *et al.*, 2010). As per the International Union on Conservation of Nature and Natural Resources (IUCN) alien invasion is the second only cause of species endangerment and extinction after habitat loss (Lowe, 2000). Despite of their destructive impacts, these alien species have significantly contributed in the overall rural and national economy throughout the world (Pant & Sharma, 2010).

The present study is an attempt to bring together the existing information on woody alien plant species found in Uttarakhand with the aim to prospect their appropriate management strategy.

LITERATURE REVIEW

The expansion of the alien woody flora in different biogeographic zones across the world has raised enormous concern for the biodiversity conservation and management. In a study on vascular plant diversity in a modified habitat of the Shivalik foot hills with special reference to the invasion by the alien species. Singh *et al.* (2017) found that 63.35% species were non-native to the region. Similar study was carried out in the sub-tropical Kandi Siwaliks of Jammu province by Sharma and Kant (2014) who studied the species diversity of woody plant communities and recorded a total of 112 species.

Many of the exotic tree species that were planted in new environments have naturalized thus impacting the ecosystems (Zalba & Villamil, 2002). Thus, in order to manage the invasive woody alien species, the first step is to document these species. Efforts were made by Sankaran and Suresh (2013) who documented the invasive alien plant species of the Asia-Pacific region. In context of the Indian Himalayan region Sekar *et al.* (2012) has documented a comprehensive list of invasive alien species and also specifically with respect to Uttarakhand Himalayas (2012). Another study in the Uttarakhand Himalayan region by Pant and Sharma (2010) documents 18 exotic tree species of Doon valley along with their ethnobotanical uses. Tewari *et al.* (2010) also studied the diversity of gymnosperms in Uttarakhand and reported a total of 63 species both wild and exotic.

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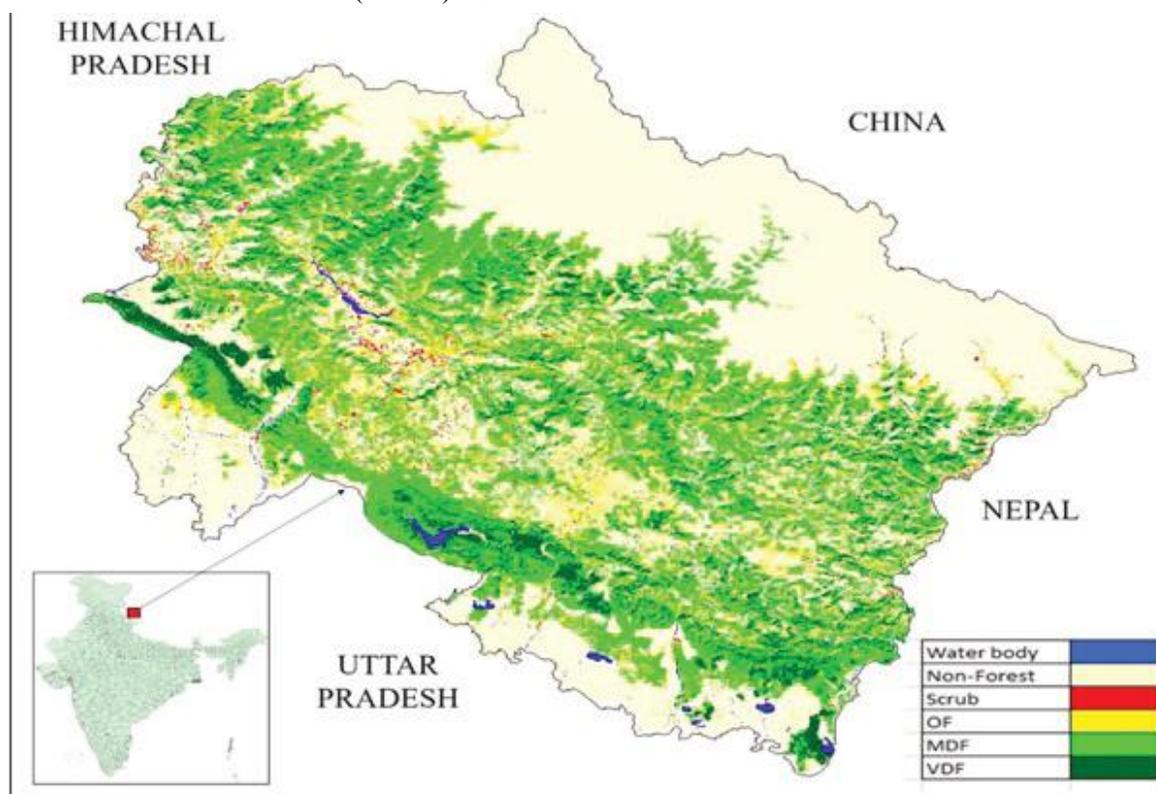


Figure 1: Location of study area.

Along with documentation, it is also very important to devise effective management plans that can help in controlling the spread of such species. Reshi and Khuroo (2012) proposed an integrated research and policy framework for the management of alien plant invasions in India. They further constructed an index of invasion severity which will help in prioritization of species along with their management.

STUDY AREA AND METHODS

Uttarakhand state encompasses an area of 53,483 sq km. Located at the foothills of the Himalayan mountain ranges, it is largely a hilly State, having international boundaries with China (Tibet) in the north and Nepal in the east. On its north-west lies Himachal Pradesh, while on the south is Uttar Pradesh (Fig. 1) (uk.gov.in-2018). Extensive literature review was conducted to inventories the wooden alien flora of Uttarakhand. The information available in the research articles, books, technical reports, and herbaria in the Botanical Survey of India and Forest Research Institute, Dehradun were incorporated. The present study is thus based on the information compiled from available inventories (Table 1) of alien species in Uttarakhand, to develop an exhaustive list of woody alien plants.

The nativity of these plants was established using the published information (Table 1) and online resources (www.theplantlist.org; www.efloras.org). Plants enumerated in the list were then categorized on the basis of their life form (trees, shrubs), family, and altitude. A detailed database (Table 2) has been prepared on woody alien plant species of Uttarakhand Himalaya on the basis of available information. In order to avoid taxonomic inflation, the synonyms of the species were removed (Khuroo *et al.*, 2012).

RESULT AND DISCUSSION

The study documents a total of 125 woody alien plant species, belonging to 81 genera and 48 families in Uttarakhand. Out of these species, 72 were angiosperms and 53 gymnosperms. The life form analysis showed that 91 species were trees and 34 shrubs, found in different habitat types of the state ranging from forest in Shivalik foothills to high altitude scrubs. Among the recorded 48 families, Cupressaceae was the most dominant family which includes 19 species which was followed by Pinaceae (15 species), Solanaceae (7 species) and Mimosaceae (6 species). As per the nativity the majority of the woody alien flora was from Tropical America (15) followed by China (14) and Australia (13). A majority of the woody alien species of Uttarakhand Himalayas originate from America which has been documented in other studies as

Table 1: Literature available on alien plant species of Uttarakhand

S. No.	Reference (Author, Year and Title)	Details
1.	Sekar <i>et al.</i> (2012), <i>Invasive Alien Plants of Uttarakhand Himalaya</i>	A total of 163 plant species were recorded as alien invasive in Uttarakhand Himalayas based on literature review, field observations and herbarium records
2.	Tewari <i>et al.</i> (2010), <i>Wild and Exotic Gymnosperms of Uttarakhand, Central Himalaya, India</i>	The paper deals with the gymnospermic diversity, both wild and exotic species present in Uttarakhand.
3.	Pant and Sharma (2010), <i>Inventory of some exotic cultivated tree species of Doon valley and their ethnobotanical uses</i>	The study reports 18 exotic tree species of Doon valley which are mostly cultivated for food, fodder, timber and medicinal purposes.
4.	Singh <i>et al.</i> (2017), <i>Vascular plant diversity with special reference to invasion of alien species on the Doon University Campus, Dehradun, India</i>	The study documents a total of 191 plant species in the Doon University campus situated in the foot hills of Shivalik mountains.
5.	Sankaran and Suresh (2013), <i>Invasive alien plants in the forests of Asia and the Pacific</i>	The book list the invasive alien plants of the Asia-Pacific region that would help in recognizing and managing these species.
6.	BP Uniyal (2007), <i>Flowering plants of Uttarakhand – A Checklist</i>	The book enumerates more than 4700 flowering plants that occur in the state of Uttarakhand. Prior to this checklist the state has no published flora.

well (Weber *et al.*, 2008., Srivastava *et al.*, 2014). Majority of the woody species were introduced for ornamental purposes, however, as per the global database many of them such as *Lantana camara*, *Arundo donax*, *Eichhornia crassipes*, *Imperata cylindrica*, *Leucaena leucocephala*, *Opuntia stricta* and *Pueraria montana* have become invasive (Lowe *et al.*, 2000).

The management of many alien species reported in the state has been an issue of concern, because of their unknown or poorly known resource value. Substantial amount of money is being spent every year by the government and private agencies for clearing invasive alien species and the cost is expected to increase in the future. Thus, utilizing their

potential as a commercial resource, would be crucial for both the economic development and their management (Table 2).

CONCLUSIONS AND RECOMMENDATION

Uttarakhand Himalaya, being a part of the global biodiversity hotspot, the alien invasive species contribute significantly to the floral diversity of the state. In addition, many of these species are important in farming and forestry systems of the state and support the local livelihood and consequently the national economy. A detailed information on their current status is crucial to understand their positive and negative impacts which

Table 2: Ethno-medicinal plants used by the Bhutia tribe in Sikkim

Scientific Name	Div.	Family	Nativity	Life Form	Altitude (m asl)	Reference
<i>Abies spectabilis</i> (D. Don) Spach	G	Pinaceae	Asia Temperate	T	3000–3600	Tiwari <i>et al.</i> , 2010
<i>Acacia dealbata</i> Link	A	Mimosaceae	Australia	T	450–1,400	Sekar <i>et al.</i> , 2012
<i>Acacia farnesiana</i> (L.) Willd.	A	Mimosaceae	Trop. South America	T	Up to 1,000	Sekar <i>et al.</i> , 2012
<i>Agathis robusta</i> (C. Moore ex F. Muell.) F.M. Bailey	G	Araucariaceae	Australia	T	400–1900	Singh <i>et al.</i> , 2017; Uniyal, 2007
<i>Agave vivipara</i> L.	A	Asparagaceae	North America	S	200–1000	Singh <i>et al.</i> , 2017
<i>Araucaria angustifolia</i> Bertal	G	Araucariaceae	Brazil	T	600–2000	Tiwari <i>et al.</i> , 2010
<i>Araucaria bidwillii</i> Hook.	G	Araucariaceae	Australia	T	1500–2000	Tiwari <i>et al.</i> , 2010

Scientific Name	Div.	Family	Nativity	Life Form	Altitude (m asl)	Reference
<i>Araucaria columnaris</i> (Forst.) Hook.	G	Araucariaceae	New Caledonia	T	1000–2000	Tiwari <i>et al.</i> , 2010
<i>Araucaria cunninghamii</i> Sweet	G	Araucariaceae	Australia	T	1000–2000	Tiwari <i>et al.</i> , 2010
<i>Bauhinia variegata</i>	A	Caesalpina- ceae	Southeastern Asia	T	Up to 1800	Pant & Sharma, 2010
<i>Bombax malabaricum</i>	A	Malvaceae	Asia, Australia and Malaysia	T	200–1400	Pant & Sharma., 2010
<i>Borassus flabellifer</i> L.	A	Arecaceae	Trop. Africa	T	Up to 800	Sekar <i>et al.</i> , 2012
<i>Bougainvillea spectabilis</i> Willd.	A	Nyctaginaceae	South America	S	150–2500	Singh <i>et al.</i> , 2017
<i>Broussonetia papyrifera</i>	A	Urticaceae	Burma	T	250–1000	Pant & Sharma, 2010
<i>Buddlejadavidii</i> Franch.	A	Buddlejaceae	China	S	–	Sankaran & Suresh, 2013
<i>Butea monosperma</i>	A	Papilionaceae	Southern Asia	T	–	Pant & Sharma, 2010
<i>Calliandra haematocephala</i> Hassk.	A	Mimosaceae	Trop. America	T	250–1100	Singh <i>et al.</i> , 2017
<i>Callistemon citrinus</i>	A	Myrtaceae	Australia	T	700–900	Pant & Sharma., 2010
<i>Callitrisco lumellaris</i> F. Muell.	G	Cupressaceae	Australia	T	1200–2000	Tiwari <i>et al.</i> , 2010
<i>Calotropis gigantea</i> (L.) R. Br.	A	Asclepiada- ceae	Trop. Africa	S	Up to 600	Sekar <i>et al.</i> , 2012
<i>Calotropis procera</i> (Ait.) R. Br.	A	Asclepiada- ceae	Trop. Africa	S	Up to 600	Sekar et al. , 2012
<i>Cassia javanica</i> L.	A	Caesalpina- ceae	Indonesia	T	–	Pant & Sharma, 2010, Uniyal, 2007
<i>Casuarina equisetifolia</i> L.	A	Casuarinaceae	Australia, south Pacific Islands, Southeast Asia	T	0–1400	Sankaran & Suresh, 2013; Uniyal, 2007
<i>Ceiba speciosa</i> (A.St.-Hil.) Ravenna	A	Bombaceae	North America	T	400–1600	Singh <i>et al.</i> , 2017
<i>Cephalotaxusbarringtonia</i> Koch	G	Cephalotaxa- ceae	Japan	T	2000–3700	Tiwari <i>et al.</i> , 2010
<i>Cephalo taxus griffithii</i> Hook.f.	G	Cephalotaxa- ceae	Japan	T	2000–3700	Tiwari <i>et al.</i> , 2010
<i>Cestrum parqui</i> L'. Herit.	A	Solanaceae	Central and South America	S	–	Sankaran & Suresh., 2013; Uniyal, 2007
<i>Cinnamomumcamphora</i>	A	Lauraceae	China	T	1350–1800	Pant & Sharma, 2010
<i>Cocculuslaurifolius</i>	A	Menisperma- ceae	Japan and China	T	Up to 1500	Pant & Sharma, 2010
<i>Cryptomeria japonica</i> (Linn. f.) D.Don	G	Taxodiaceae	Japan, China	S	1100–2500	Tiwari <i>et al.</i> , 2010
<i>Cunninghamialanceolata</i> (Lambert) Hook	G	Taxodiaceae	China	T	600–2200	Tiwari <i>et al.</i> , 2010
<i>Cuphea hissisifolia</i> Kunth	A	Lythraceae	Trop. America	S	300–2000	Singh <i>et al.</i> , 2017
<i>Cupressus arizonica</i> Greene	G	Cupressaceae	Mexico	T	1760–2400	Tiwari <i>et al.</i> , 2010
<i>Cupressus cashmeriana</i> Royle ex Carrie	G	Cupressaceae	China (Tibet)	T	1760–2400	Tiwari <i>et al.</i> , 2010
<i>Cupressus funebris</i> Endl.	G	Cupressaceae	China	T	1760–2400	Tiwari <i>et al.</i> , 2010
<i>Cupressus goveniana</i> Gord	G	Cupressaceae	California	T	1760–2400	Tiwari <i>et al.</i> ,2010
<i>Cupressus lusitanica</i> Mill.	G	Cupressaceae	Mexico	T	1760–2400	Tiwari <i>et al.</i> , 2010

Scientific Name	Div.	Family	Nativity	Life Form	Altitude (m asl)	Reference
<i>Cupressus sempervirens</i> L	G	Cupressaceae	Cyprus	T	1760–2400	Tiwari <i>et al.</i> , 2010
<i>Cupressus torulosa</i> D. Don	G	Cupressaceae	Bhutan	T	1500–2500	Tiwari <i>et al.</i> , 2010
<i>Cycas revoluta</i> Thunb.	G	Cycadaceae	South Japan	S	600–2000	Tiwari <i>et al.</i> , 2010
<i>Datura fastuosa</i> L.	A	Solanaceae	South America, Mexico	S	Up to 1,200	Sekar <i>et al.</i> , 2012
<i>Datura innoxia</i> Mill.	A	Solanaceae	Trop. America	S	Up to 1,200	Sekar <i>et al.</i> , 2012
<i>Datura metel</i> L.	A	Solanaceae	Trop. America	S	Up to 1,200	Sekar <i>et al.</i> , 2012
<i>Datura stramonium</i> L.	A	Solanaceae	Trop. America	S	Up to 1,500	Sekar <i>et al.</i> , 2012
<i>Delonix regia</i>	A	Caesalpiniaceae	Madagascar	T	450–1400	Pant & Sharma, 2010; Singh <i>et al.</i> , 2017
<i>Dracaena reflexa</i> Lam.	A	Asparagaceae	Madagascar	T	100–1500	Singh <i>et al.</i> , 2017
<i>Duranta erecta</i> L.	A	Verbenaceae	North America	S	200–1600	Singh <i>et al.</i> , 2017
<i>Elaeagnus umbellata</i> Thunb.	A	Elaeagnaceae	China, Korea and Japan	S	–	Sankaran & Suresh, 2013; Uniyal, 2007
<i>Ephedra gerardiana</i> Wall. ex Stapf.	G	Ephedraceae	Europe, Asia	S	2000–4500	Tiwari <i>et al.</i> , 2010
<i>Eriobotrya japonica</i>	A	Rosaceae	Japan and China	T	Up to 700	Pant & Sharma, 2010
<i>Eucalyptus tereticornis</i>	A	Myrtaceae	Australia	T	0–1000	Pant & Sharma, 2010
<i>Euphorbia milii</i> Des Moul.	A	Euphorbiaceae	Madagascar	S	200–1800	Singh <i>et al.</i> , 2017
<i>Ficus benjamina</i> L.	A	Moraceae	North East Asia	T	250–1400	Singh <i>et al.</i> , 2017
<i>Ginkgo biloba</i> L.	G	Ginkgoaceae	China	T	1800–2400	Tiwari <i>et al.</i> , 2010
<i>Grevillea robusta</i>	A	Proteaceae	Australia	T	760–2000	Pant & Sharma, 2010
<i>Hibiscus mutabilis</i> L.	A	Malvaceae	North East Asia	S	200–1200	Singh <i>et al.</i> , 2017
<i>Hyophorbelagenicaulis</i> (L. H Bailey) H.E. Moore	A	Araceae	Mascarene Islands	T	50–800	Singh <i>et al.</i> , 2017
<i>Indigoferatritra</i> L.f.	A	Fabaceae	Trop. Africa	S	Up to 1,400	Sekar <i>et al.</i> , 2012
<i>Ipomoea carnea</i> Jacq. subsp. <i>fistulosa</i> (Mart. ex Choisy) Austin	A	Convolvulaceae	Trop. America	S	Up to 900	Sekar <i>et al.</i> , 2012
<i>Jacaranda mimosifolia</i> D. Don	A	Bignoniaceae	South America	T	400–1200	Singh <i>et al.</i> , 2017
<i>Juniperus bermudiana</i> L.	G	Cupressaceae	Bermunda	T	1700–3400	Tiwari <i>et al.</i> , 2010
<i>Juniperus chinensis</i> L.	G	Cupressaceae	China	T	1700–3400	Tiwari <i>et al.</i> , 2010
<i>Juniperus communis</i> L.	G	Cupressaceae	Northern hemisphere	T	2500–4500	Tiwari <i>et al.</i> , 2010
<i>Juniperus deppeana</i> Steud.	G	Cupressaceae	Mexico	T	1700–3400	Tiwari <i>et al.</i> , 2010
<i>Juniperus oxycedrus</i> L.	G	Cupressaceae	Syria	T	1700–3400	Tiwari <i>et al.</i> , 2010
<i>Juniperus phoenicea</i> L.	G	Cupressaceae	Algeria	T	1700–3400	Tiwari <i>et al.</i> , 2010
<i>Juniperus procera</i> Hochst.	G	Cupressaceae	Kenya	T	1700–3400	Tiwari <i>et al.</i> , 2010
<i>Juniperus scopulorum</i> Sarg.	G	Cupressaceae	United States of America	T	1700–3400	Tiwari <i>et al.</i> , 2010
<i>Juniperus semiglobosa</i> Regel	G	Cupressaceae	Central Asia	T	1700–3400	Tiwari <i>et al.</i> , 2010
<i>Lantana camara</i> L. a	A	Verbenaceae	Trop. America	S	Up to 2,000	Sekar <i>et al.</i> , 2012
<i>Lagerstromia speciosa</i>	A	Lythraceae	North America	T	-	Pant & Sharma, 2010
<i>Leucaena latisiliqua</i> (L.) Gilli.	A	Mimosaceae	Trop. America	T	Up to 1,800	Sekar <i>et al.</i> , 2012
<i>Leucaena leucocephala</i> (Lam.) De Wit	A	Mimosaceae	Trop. America	T	Up to 2,000	Sekar <i>et al.</i> , 2012

Scientific Name	Div.	Family	Nativity	Life Form	Altitude (m asl)	Reference
<i>Ligustrum lucidum</i> W.T. Aiton	A	Oleaceae	China	S	Up to 2000	Sankaran & Suresh, 2013; Uniyal, 2007
<i>Litchi chinensis</i>	A	Sapindaceae	China	T	0–1000	Pant & Sharma, 2010
<i>Magnolia grandiflora</i> L.	A	Magnoliaceae	North America	T	60–700	Singh <i>et al.</i> , 2017
<i>Melaleuca bracteata</i> F. Muell.	A	Myrtaceae	Australia	T	300–750	Singh <i>et al.</i> , 2017
<i>Melia azedarach</i>	A	Meliaceae	Iran	T	–	Pant & Sharma, 2010
<i>Opuntia elatior</i> Miller	A	Cactaceae	South America	S	Up to 1,600	Sekar <i>et al.</i> , 2012
<i>Opuntia stricta</i> Haw. var. <i>dillenii</i> (Ker Gawl.) Benson	A	Cactaceae	Trop. America	S	Up to 900	Sekar <i>et al.</i> , 2012
<i>Opuntia vulgaris</i> Miller	A	Cactaceae	South America	S	Up to 1,800	Sekar <i>et al.</i> , 2012
<i>Picea smithiana</i> (Wall.) Boiss	G	Pinaceae	Southern Asia	T	2000–3300	Tiwari <i>et al.</i> , 2010
<i>Pinus canariensis</i> Sm.	G	Pinaceae	Canary Island	T	1800–2100	Tiwari <i>et al.</i> , 2010
<i>Pinus caribea</i> Mor.	G	Pinaceae	Cuba	T	1800–2100	Tiwari <i>et al.</i> , 2010
<i>Pinus densiflora</i> Sieb. & Zucc.	G	Pinaceae	Japan	T	1800–2100	Tiwari <i>et al.</i> , 2010
<i>Pinus echinata</i> Mill.	G	Pinaceae	Mexico	T	1800–2100	Tiwari <i>et al.</i> , 2010
<i>Pinus elliotti</i> Engler	G	Pinaceae	United States of America	T	1800–2100	Tiwari <i>et al.</i> , 2010
<i>Pinus halepensis</i> Mill.	G	Pinaceae	Cyprus	T	1800–2100	Tiwari <i>et al.</i> , 2010
<i>Pinus hartwegii</i> Lindl.	G	Pinaceae	Mediterranean	T	1800–2100	Tiwari <i>et al.</i> , 2010
<i>Pinus kesiya</i> Role	G	Pinaceae	Myanmar	T	1800–2100	Tiwari <i>et al.</i> , 2010
<i>Pinus merkusii</i> Jungh & de Vries	G	Pinaceae	Myanmar	T	1800–2100	Tiwari <i>et al.</i> , 2010
<i>Pinus oocarpa</i> Schiede	G	Pinaceae	Mexico	T	1800–2100	Tiwari <i>et al.</i> , 2010
<i>Pinus patula</i> (Shide) Deppe	G	Pinaceae	Mexico	T	1800–2100	Tiwari <i>et al.</i> , 2010
<i>Pinus radiata</i> D. Don	G	Pinaceae	United States of America	T	1800–2100	Tiwari <i>et al.</i> , 2010
<i>Pittosporum tobira</i> (Thunb.) W.T. Aiton	A	Pittosporaceae	North East Asia	S	–	Singh <i>et al.</i> , 2017
<i>Plumeria alba</i> L.	A	Apocynaceae	Trop. America	T	400–1400	Singh <i>et al.</i> , 2017
<i>Plumeria obtusa</i> L.	A	Apocynaceae	Trop. America	T	400–1400	Singh <i>et al.</i> , 2017
<i>Podocarpus gracilior</i> Pilger	G	Podocarpaceae	Kenya	T	1500–3000	Tiwari <i>et al.</i> , 2010
<i>Podocarpus latifolius</i> (Thunb.) R.Br. ex Mirb.	G	Podocarpaceae	Kenya	T	600–1550	Tiwari <i>et al.</i> , 2010
<i>Podocarpus macrophylla</i> (Thunb.) D. Don	G	Podocarpaceae	Chian, Japan	T	1000	Tiwari <i>et al.</i> , 2010
<i>Podocarpus neriifolius</i> D. Don	G	Podocarpaceae	China	T	300–1500	Tiwari <i>et al.</i> , 2010
<i>Pongamia pinnata</i>	A	Fabaceae	Asia	T	Up to 1000	Singh <i>et al.</i> , 2017
<i>Prosopis juliflora</i> (Sw.) DC. a	A	Mimosaceae	Mexico	S	Up to 700	Sekar <i>et al.</i> , 2012
<i>Prunus persica</i>	A	Rosaceae	China	T	500–2100	Pant & Sharma, 2010
<i>Psidium guajava</i> L.	A	Myrtaceae	Central, South America	T	400–1200	Sankaran & Suresh, 2013; Uniyal, 2007
<i>Rhapis excelsa</i> (Thunb.) Henry	A	Araceae	North East Asia	S	500–2500m	Singh <i>et al.</i> , 2017
<i>Ricinus communis</i> L.	A	Euphorbiaceae	Trop. Africa	S	300–2500	Singh <i>et al.</i> , 2017
<i>Robinia pseudoacacia</i> L.	A	Fabaceae	United States of America	T	1000–3000	Sankaran & Suresh, 2013; Uniyal, 2007

Scientific Name	Div.	Family	Nativity	Life Form	Altitude (m asl)	Reference
<i>Sesbaniabi spinosa</i> (Jacq.) W.F. Wight	A	Fabaceae	Trop. America	S	Up to 1,500	Sekar <i>et al.</i> , 2012
<i>Shorearobusta</i>	A	Dipterocarpaceae	Nepal	T	100–1700	Pant & Sharma, 2010
<i>Solanum hispidum</i> Persoon	A	Solanaceae	Peru	S	Up to 1,500	Sekar <i>et al.</i> , 2012
<i>Solanum torvum</i> Sw.	A	Solanaceae	West Indies	S	Up to 1,000	Sekar <i>et al.</i> , 2012
<i>Tabebuia rosea</i> (Bertol.) Bertero ex DC.	A	Bignoniaceae	North America	T	100–1200	Singh <i>et al.</i> , 2017
<i>Taxodium distichum</i> (L.) Rich.	G	Taxodiaceae	Florida	T	600–2000	Tiwari <i>et al.</i> , 2010
<i>Taxodium mucronata</i> Tenore	G	Taxodiaceae	Mexico	T	600–2000	Tiwari <i>et al.</i> , 2010
<i>Taxus baccata</i> L.	G	Taxaceae	Western Europe, Asia, Afghanistan	T	2000–3400	Tiwari <i>et al.</i> , 2010
<i>Tecomaca stanifolia</i> (D. Don) Melch	A	Bignoniaceae	South America	T	100–2200	Singh <i>et al.</i> , 2017
<i>Thujaocci dentalis</i> Bailey	G	Cupressaceae	Canada/ United States of America	T	600–2400	Tiwari <i>et al.</i> , 2010
<i>Thujaorientalis</i> Linn	G	Cupressaceae	China	T	600–2600	Tiwari <i>et al.</i> , 2010
<i>Toonaciliata</i> M. Roem.	A	Meliaceae	Australia	T	300–1000	Singh <i>et al.</i> , 2017
<i>Tsuga dumosa</i> (D. Don) Eichler	G	Pinaceae	Asia	T	2400–3000	Tiwari <i>et al.</i> , 2010
<i>Urenalobata</i> L.	A	Malvaceae	Trop. Africa	S	Up to 1,500	Sekar <i>et al.</i> , 2012
<i>Vachelliani lotica</i> (L.) P.J.H. Hurter & Mabb.	A	Fabaceae	Australia	T	100–2000	Singh <i>et al.</i> , 2017
<i>Xanthium indicum</i> Koenig	A	Asteraceae	Trop. America	S	Up to 1,800	Sekar <i>et al.</i> , 2012
<i>Zamia furfuracea</i> L.F. in Aiton	A	Zamiaceae	N. America	T	Upto 1500	Singh <i>et al.</i> , 2017

Note: G, Gymnosperm; A, Angiosperm; T, Tree; S, Shrub.

would contribute in the effective management of these alien species. Thus, following recommendations are needed to be undertaken:

- Involvement of the stakeholders;
- Understanding the short and long-term impacts of these alien species;
- Detailed investigation is needed to recognize the un-utilized potential of invasive alien species in term of ecological and economic aspects;
- Careful management plus a proper marketing channel can form a basis for the social upliftment and environmental management.

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Probability Distribution Modeling of *Ageratina adenophora* and *Lantana camara* in Kailash Sacred Landscape, Uttarakhand

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ABSTRACT: Biological invasion has become one of the major causes of economic and environmental damage. The Himalayan region, one of the biodiversity hotspots is rapidly getting invaded by alien invasive species this study aims to understand the pattern of invasion by *Ageratina adenophora* (Kala Bansa) and *Lantana camara*. Intensive field surveys were conducted to record the invasion by major invasive plants across agricultural lands, fallow lands, forests and grasslands of Kailash sacred landscape–India situated in district Pithoragarh of Eastern Uttarakhand in Western Himalaya. Site selection was carried out on the basis of intensive reconnaissance surveys at different altitudinal gradients. MaxEnt modeling was done to predict potential distribution of these two alien invasive plants. The models were calibrated using data from observations and geographic distribution records and then a combined model was prepared to get the approximate area under invasion in the landscape. The average model was chosen among 5 replicates on the bases of AUC value (94 ± 0.01 for *Eupatorium* and 94 ± 0.006 for *Lantana*). Among the 19 bio-climatic variables selected precipitation of wettest month was found to be the most important variable for the distribution of *Ageratina adenophora*, followed by annual mean temperature and elevation, however, elevation was the most important factor followed by precipitation seasonality and aspect in the distribution of *Lantana*. ©2019

KEYWORDS: *Invasive species; Kailash Sacred Landscape; Ageratina adenophora; Principal component analysis; MaxEnt.*

INTRODUCTION

The plant diversity around the world is facing various threats and is reducing very rapidly (Dogra *et al.*, 2009). Invasive species are a major threat to the Earth's biodiversity because they often dramatically affect the structure and functioning of ecosystems. Altering Climate and anthropogenic factors accelerates the growth of Alien invasive plants as they favor the area where they are not limited by climatic constrains. Change in climatic conditions may increase the opportunities for their introduction and spread (Mooney, 2000). Due to which they have spread their horizons from lower to higher altitudes in Himalaya. In areas where they spread, invasive can destroy natural pasture, displace native trees, and reduce grazing potential of rangelands (Admasu, 2008). The invasion of alien plant species has become the second highest threat to plant diversity in the new regimes after the habitat loss (Hobbs & Humphries, 1995). It may be due to invasive species has faster rates of growth and biomass production compared to native species, higher competitive ability, high reproductive efficiency including production of a large number of seeds, efficient dispersal, vegetative reproduction, rapid establishment, other traits that help them adapt to new habitats (Simberloff *et al.*, 2005; Sharma *et al.*, 2005) and broader range of tolerance (Walther *et al.*, 2009; IUCN Report, 2013). Some of the widely spread and

documented invasive plant species all over the world are *Ageratina adenophora*, *Lantana camara*, *Parthenium adenophorum* and *Bidens pilosa*. It is widely accepted fact that preventing biological invasions or tackling them at a very early stage is the most efficient and cost-effective approach (Brunel *et al.*, 2011). This demand awareness of the threats they pose, preventive measures to stop new invasions and control of those species that have already invaded the ecosystems. Distribution maps can be useful tool for community awareness and formulation of appropriate long-term management strategies for AIP species invasion from hotspots and areas which are at risk of becoming climatically suitable for invasion under the future climatic scenarios.

LITERATURE REVIEW

Himalaya being the youngest mountains on earth is very well known for their vast biodiversity due to which they become one of the best sites for study of climate change on the earth. It has been observed that species of higher elevations are projected to shift higher, due to which few invasive alien plant species which were earlier limited to the lower areas are now shifting towards the higher altitudinal areas of Himalaya (Telwala *et al.*, 2013). About 50% of the alien species are deliberately introduced in the Himalaya and others came through trade and grain imports (Sekar *et al.*, 2012). There are still many regions in the world where basic information on naturalized plant taxa and plant invasions is only superficial or completely lacking like Asia and neighboring regions (Corlett, 1988;

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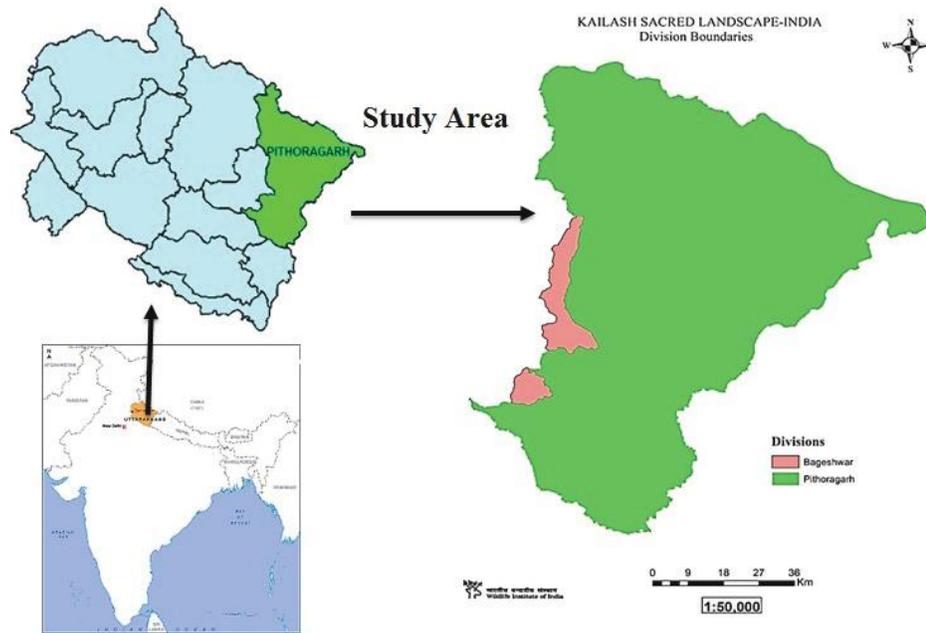


Figure 1: Map showing the study area.

Enomotto, 1999). Therefore, an urgent need was felt to study implications of climate and environmental change on distribution and abundance of major invasive alien species in the Himalayan region along with the impact assessment and management of such species.

MaxEnt (Phillips *et al.*, 2006) has been used to model the potential distribution of invasive species (Pawar *et al.*, 2007; Thomas *et al.*, 2010; Roger *et al.*, 2015; Adhikari *et al.*, 2015; Choudhury *et al.*, 2016; Qin *et al.*, 2016; Mungi *et al.*, 2018) and is being used in the current study.

MATERIALS AND METHODS

Study Area

The present investigation will be conducted mainly for two major invasive alien species *viz.*, *Ageratina adenophora* and *Lantana camara* in Pithoragarh district of Kumaon region, Uttarakhand which falls under Kailash Sacred Landscape (KSL). This landscape is mountainous, remote with steep topography, high spatial heterogeneity, and difficult to access. Of the 31,000 km² KSL area, nearly 7114 km² falls within the Indian territory, that too mainly in Pithoragarh district (96%) followed by Bageshwar (4%). The Indian part of KSL (*ref.* Fig. 1) is culturally rich, ecologically diverse and geographically fragile but with high rates of poverty and facing enormous environmental challenges including fragmentation of natural habitats and degradation (Zomer & Oli, 2011). The KSL-India predominates in diverse

forest (from moist subtropical broadleaf to temperate oak forests, sub-alpine conifers, high altitude birch forests, while extensive alpine pastures in area >3000–3500 m asl). These forests have been intersected by numerous rivers, tributaries and springs. Human habitation includes district headquarter (Pithoragarh), towns, and villages along with their agro-ecosystems covered 1252.73 km² area and represented 17.6% area of the landscape.

Site selection was conducted based on the intensive reconnaissance survey of the KSL-India depending on the availability of the alien plant species, *i.e.* *Ageratina adenophora* (*Eupatorium adenophorum*) and *Lantana camara*. Intensive field surveys were conducted to record the distribution, Geographical coordinates and patch size of these two invasive species using GPS to study the patterns and extent of invasion, presence/absence, abundance and general cover in four major eco-systems *viz.*, forest, fallow land, agricultural and grassland. Colonies with different population intensity of selected species such as dense, moderate and sparse were identified and each was sampled.

The bio-climatic variables and DEM (slope aspect and elevation) of the study area were directly downloaded from the WorldClim and MODIS websites, respectively. We used Maximum Entropy (MaxEnt) distribution modelling, for analysis of data obtained. It is a habitat distribution/probability distribution model which uses presence only data to predict potential distribution of a particular species. MaxEnt model was calibrated using data from observations and geographic distribution records to get the approx area under invasion in Kailash Sacred Landscape.

RESULTS

A potential distribution model was developed with MaxEnt. 25 runs were performed for model, building replicates were taken, and the average model was chosen on the basis of AUC value (for *Ageratina adenophora*: 94 ± 0.01 and *Lantana camara*: 94 ± 0.006). A total of 19 bio-climatic variables were considered including the topographic variables (slope aspect and elevation). The maximum probability of occurrence of *Ageratina adenophora* and *Lantana camara* is shown with red colour in Figure 2, and the least probability of occurrence in green colour in KSL-India landscape.

Different variables were found to be responsible for distribution of the two alien plant species. The most important bio-climatic variable which was responsible for the distribution of *Ageratina adenophora* was found to be precipitation of wettest month (bio_13), followed by annual mean temperature (bio_1) and elevation (DEM; Table 1), whereas in case of *Lantana camara*, the elevation (DEM) was found to be the most important factor responsible for the distribution of the species, followed by precipitation seasonality (bio_15) and aspect, respectively. DEM (elevation) was found to be the most important factor for invasion of *Lantana camara* (35.5%), whereas it contributed only 12% in case of *Ageratina adenophora*. Similarly,

Table 1: Percentage contribution of *Ageratina adenophora* and *Lantana camara* with respect to different variables

<i>Ageratina adenophora</i>		<i>Lantana camara</i>	
Variables	Percentage Contribution	Variables	Percentage Contribution
bio_13	21.4	DEM	35.5
bio_1	12.6	bio_15	31.2
DEM	12	aspect	5.1
bio_8	11.5	bio_2	4.8
bio_6	10	bio_13	3.9
bio_19	7.5	bio_8	3.9
bio_17	5.9	bio_14	3.6
Aspect	3.1	Slope	3.5
bio_12	2.4	bio_18	2.7
bio_14	2.2	bio_19	1.8
bio_2	2	bio_17	1
bio_4	1.7	–	–
bio_11	1.6	–	–
bio_5	1.3	–	–
bio_10	1	–	–
bio_16	1	–	–

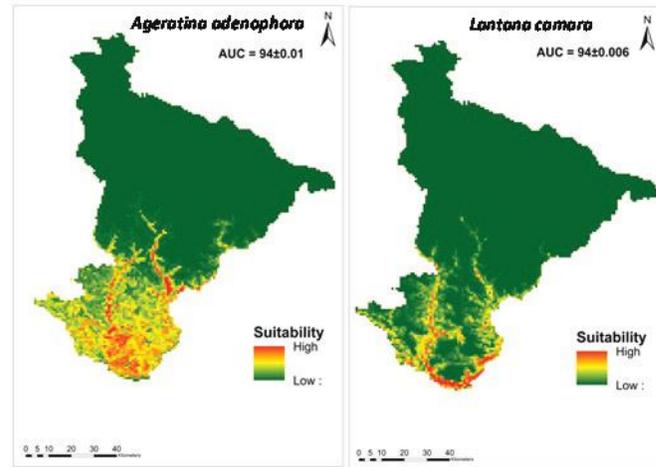


Figure 2: Distribution map of *Ageratina adenophora* *Lantana camara* through MaxEnt modeling in KSL-India.

bio_13 (precipitation of wettest month) was found to be the most affecting environmental variable for distribution of *Ageratina adenophora* (21.4%), whereas it was found to be contributing only 3.9% in case of *Lantana camara*. Thus, it is evident from our results that the degree of impact of eco-climatic variables varies with species, indicating both the species have different habitat and microclimatic preferences. Our results also suggest that the 80.9% of invasion was dependent on seven variables for *Ageratina adenophora*, whereas only five factors were mainly contributing (80.5%) towards the distribution of *Lantana camara*. When compared with the forest density map, open forests were more vulnerable to invasion followed by moderate and dense forests of the landscape.

DISCUSSION

Our results indicate that open areas adjoining forest fringes are more vulnerable to the growth of both the alien invasive plant species and their distribution is influenced by many eco-climatic factors such as elevation, temperature, precipitation and water bodies along with the anthropogenic disturbance such as roads, settlements and degraded lands which greatly facilitates the invasion of invasive species. Elevation, aspect, and slope are the three main topographic factors that control the distribution and patterns of vegetation in mountain areas (Titshall *et al.*, 2000). The dominating variables which were responsible for the distribution of *Ageratina adenophora* were precipitation, followed by annual mean temperature and elevation. The invasion of *Ageratina adenophora* requires low temperature and high precipitation (Sun *et al.*, 2004; Tereraia & Wood, 2014),

and it has also been reported that invasive plants have a major impact on catchment hydrology (Geldenhuys, 1986); we also recorded the maximum presence (82%) of *Ageratina adenophora* near water bodies (secondary and tertiary tributaries). The annual mean temperature is also important bio-climatic variable as the suitability of habitat of *Ageratina adenophora* increases with increase in annual mean temperature.

Elevation was found to be the most dominant factor followed by precipitation seasonality and aspect for the distribution of *Lantana camara*. The species preferred disturbed micro-habitats either naturally (land-slides and flood sites) or anthropogenic disturbed sites (Mungi *et al.*, 2018) and was found that the intensity of invasion was directly proportional to increase in temperature, whereas inversely proportional to the elevation. Among this elevation was found to be the more dominant limiting factor as the distribution was recorded between 600 and 1700 m asl only. Our results suggest that *Ageratina adenophora* and *Lantana camara* compete with the native species and are capable of excluding native species from any micro-habitat. The invasion of exotic plant species in any ecosystem reduces the biodiversity and alters important ecosystem functions and services (Houlihan & Findlay, 2004). It was observed during the study that the exotic species had a significant negative effect on the native plant community. One reason could be the poor competitive ability of the native species (Gaston, 1994).

CONCLUSION

Plant invasions in the new areas cause huge economic and ecological imbalance by altering native community composition, depletion in species diversity, thus affecting ecosystem process. The increased incidence of invasion in high altitudinal ecosystems possesses a major threat to indigenous biological diversity of the region. Alien invasive species prefers and occupies such habitats which are degraded, disturbed and the habitats where the anthropogenic pressure is much higher than other habitat types in any ecosystem. Some serious mitigation techniques and measures are required to check the further distribution of this alien species in the western Himalayan region. The further distribution of this alien species may cause a serious threat to the local biodiversity and a change in ecosystem process of the area. The results of the present study show the present distribution scenario of two major alien invasive plants in Kailash Sacred Landscape. Red colour shows the area where there is maximum probability of occurrence of *Ageratina adenophora* and *Lantana camara* in KSL-

India landscape. It was observed that the highest invasion of *Ageratina adenophora* was in between 1700 and 1800 m asl elevation gradient and the lowest presence detected between 700 and 800 m asl. *Ageratina adenophora* was recorded highest at Northern aspect which gives the species more sheltered conditions whereas in case of *Lantana camara* its distribution increased with increase in temperature and decrease in elevation. The average model performance may not lose any place where there is high probability of invasion, therefore, the distribution map of alien invasive plants can be used by risk managers for monitoring the spread of these invasive plant and eco-restoration of degraded ecosystems.

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Invasion Management through Lessons Learned from Phylogenetic Clustering

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ABSTRACT: Biological invasions involve enormous biodiversity losses and huge economic impacts that call for considerable management efforts. However, the funds and capacity available to control these invasions are limited, particularly in the Indian Himalayan region and there is a need to identify priority invasive taxa. Solving this prioritization problem is a major concern for policy makers, conservationists, and land managers. Here we compared the alien plant families present at different stages of naturalization–invasion continuum, distributed along an altitudinal range of 1600–3200 masl, within Kashmir Himalaya, for phylogenetic analysis. The results reveal that co-occurrence of invasive species is mostly structured by dispersal and stochastic forces (neutral theory) at naturalization stage, while species functional traits (niche theory) influence species co-occurrence at the final stage of invasion. Such an understanding of the effect of coexistence mechanisms on invasive species richness may further be employed to predict the areas that can be at highest risk of invasion allowing us to design more targeted management and prevention measures. ©2019

KEYWORDS: *Invasive species; Naturalization; Functional traits; Phylogenetics.*

INTRODUCTION

Human-assisted intentional and unintentional introductions of alien species to regions outside of their native range has increased manifold due to unprecedented upsurge in trade and travel across continents and countries (Hulme, 2009). In fact, human population size and various socio-economic indicators that serve as surrogates of increased propagule pressure and anthropogenic disturbance of natural habitats have shown positive correlation with numbers of alien plant and animal species in different regions and countries (Essl et al., 2011). Studies have revealed significant negative impact of invasive alien species on the structure, function and productivity of natural ecosystems (Panetta and Gooden, 2017), reduction in diversity and fitness of resident species (Ehrenfeld, 2010; Vilà et al. 2011; Pyšek et al. 2012), alterations in nutrient or water cycles leading to changes of whole ecosystem properties (Blackburn et al. 2014; Cameron et al. 2016), adverse effect on ecosystem services, regional economies and public health (Ricciardi et al. 2017).

Economic damages associated with non-indigenous species invasions are also huge (Paini et al. 2016) and economic costs have been estimated to be approximately \$120 billion/year for US (Pimentel et al. 2005) and 12.5 billion EUR/year in Europe (Kettunen et al. 2009). Recently, Hoffmann and Braodhurst (2016) estimated that the annual cost of invasive species in agricultural and natural ecosystems in Australia is about \$ 13.6 billion. Likewise, Bradshaw et

al. (2016) reported a minimum cost of US\$ 70.0 billion/year globally due to invasive insects, in addition to health costs that exceed US\$6.9 billion/year.

In view of above mentioned ecological and economic impacts, Convention on Biological Diversity and its Strategic Plan for Biodiversity 2011-2020 recognizes the issue and impacts of invasive alien species. Specifically the Aichi Target 9 states that “By 2020, invasive alien species and pathways are identified and prioritized, priority species are controlled or eradicated and measures are in place to manage pathways to prevent their introduction and establishment”. This recognition has provided necessary impetus for exploring management strategies for alien invasive species so as to minimize their adverse ecological and economic impact (Latombe et al. 2017). Management strategies available to the decision-makers include prevention, early detection, rapid response and eradication, control/mitigation and adaptation/restoration.

Understanding the mechanisms by which alien species establish and become invasive, and finding strategies to anticipate new invasions, is a key step for mitigating their impacts. Several hypotheses postulate that species invasion is affected by interplay between the phylogenetic position of the invading species and the phylogenetic structure of the invaded community type (Keddy, 1992; Procheş *et al.*, 2008). Some of them suggest that phylogenetic relatedness of invaders to native species promotes naturalization, because phylogenetically related alien species tend to have similar environmental adaptations as native species (Kempel et al. 2013; Ordonez 2014). Others predict that

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phylogenetic relatedness hampers naturalization because of stronger competition of aliens with native species and shared enemies (Colautti et al. 2004; Hierro et al. 2005; Blumenthal 2006). Here we ask how phylogenetic diversity of invasive families affects invasions across community types.

LITERATURE REVIEW

At least 4% of all currently known vascular plant species on Earth have become naturalized outside their natural ranges because of humans (van Kleunen et al. 2015). In Europe alone number of alien plants and animals is more than 2,000 species (Vilà et al. 2010). In India, Khuroo et al (2012) reported 1599 alien vascular plants. All these introduced alien species progress through different stages of invasion (Theoharides and Dukes, 2007) and after overcoming abiotic and biotic filters, some alien species become invasive in the non-native regions (Lodge et al. 2016). Three main assembly processes (or filters, according to the community ecology filtering metaphor, Keddy 1992) are thought to influence the success of alien species outside their native range which include:(1) environmental, (2) biotic and (3) dispersal filtering (Theoharides and Dukes, 2007, Richardson and Pyšek, 2012). The interplay of these three simple processes determines which species become invasive and which communities are invaded over large biogeographical scales (Richardson and Pyšek, 2012). While there is broad consensus on the importance of these processes in structuring invasion patterns, studying them at the large spatial scales relevant for invasion management and prevention has proven extremely challenging. However, knowledge about the relationship between the phylogenetic structure of plant community types and their level of invasion (i.e. the actual number or proportion of alien species) may provide insights for understanding and predicting biological invasions at a larger scale (e.g. Lambdon & Hulme, 2006; Strauss et

al., 2006; Diez et al., 2008; Cadotte et al., 2010; Gerhold et al., 2011). Several hypotheses converge in suggesting that invasion is driven by interplay between the phylogenetic position of the alien species and the phylogenetic structure of the invaded communities (Ma et al., 2017), with phylogeny often being considered as an indirect proxy for missing functional trait information.

Both pre-introduction (aimed at preventing introduction of potentially invasive species) and as well as post-introduction risk assessment protocols (aimed at prescribing appropriate management strategies ranging from rapid response to effective control and containment through physical, cultural, mechanical, biological and chemical methods for alien species at different stages of invasion) are necessary for management of invasive alien species.

Preventing introduction of alien species is considered as the most effective management option (Hussner et al., 2017; Novoa et al., 2018) and if it fails, early detection and rapid response are the available options besides control and eradication options which are suitable for already established invasive species. There are many pre-introduction risk assessment predictive schemes (Pheloung et al., 1999; Magarey et al., 2018)that help in prediction of potentially invasive species and prevent their introduction into nonnative regions. For prioritizing the alien species that require immediate attention, several post-introduction risk assessment protocols are also available (Randall et al., 2008; McGeoch et al., 2016). In addition to conventional physical, mechanical, chemical and biological control methods, emerging technologies such as CISPER-Cas9 gene drives may also aid in control and eradication of invasive species at a larger scale (Ricciardi et al., 2017; Kopf et al., 2017).

STUDY AREA AND METHODS

Study Area

Kashmir Himalaya represents a unique biospheric unit in the northwestern Himalayas (Rodgers & Panwar, 1988). The

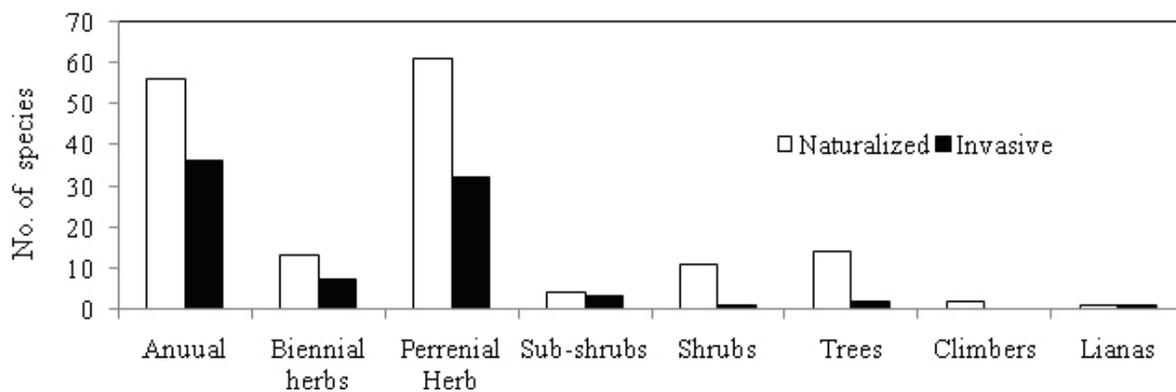


Figure 1: Relative contribution of growth form to naturalized and invasive alien flora.

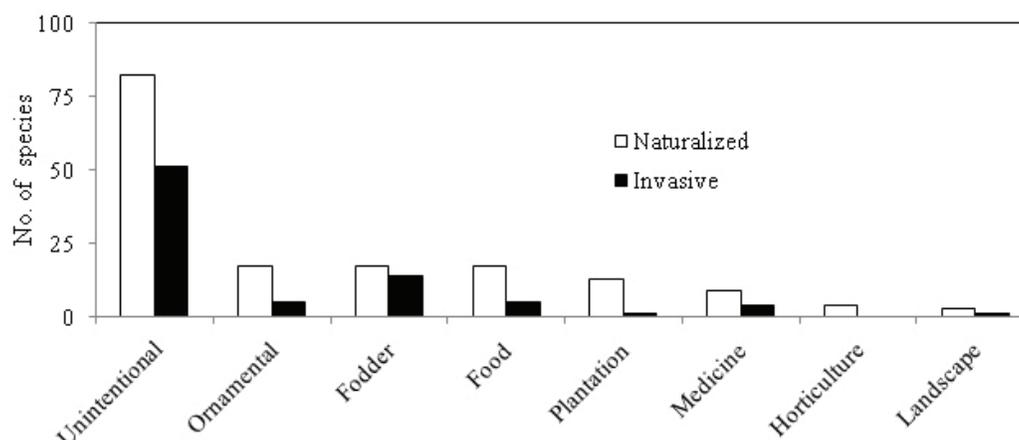


Figure 2: Relative contribution of introduction purpose to naturalized and invasive alien flora.

region has an area of about 15,948 km², with nearly 64% of the total area being mountainous. The region lies between coordinates of 32°20' to 34°50' North latitude and 73°55' to 75°35' East longitude (Husain 2002). Topographically, the region mainly comprises of a deep elliptical bowl-shaped valley bounded by the Pir Panjal range of Lesser Himalaya in the south and south-west, and the Zaskar range of the Greater Himalaya in the north and north-east. The altitude of the valley plain at Srinagar is 1,600 m above mean sea level (amsl) and the highest peak among its surrounding mountains is that of the 'Kolahoi' with an altitude of 5,420 m (amsl). Climate of the region, marked by well-defined seasonality,

resembles that of mountainous and continental parts of the temperate latitudes. The temperature ranges from an average daily maximum of 31°C and minimum of 15°C during summer to an average daily maximum of 4°C and minimum of -4°C during winter. It receives annual precipitation of about 1,050 mm, mostly in the form of snow during the winter months.

Methods

The present study is based on the database compiled by the authors after perusal of the available floristic literature dealing with the vascular flora of Kashmir

Table 1: List of Invasive plants

Family	Name of Plant Species	Origin	Altitudinal Range (m asl)
Amaranthaceae	<i>Amaranthus caudatus</i> L.	South America	1600–2500
	<i>Amaranthus caudatus</i> L.	North America; South America	1600–2800
	<i>Amaranthus caudatus</i> L.	South America	1600–2800
Amaryllidaceae	<i>Narcissus pseudonarcissus</i> L.	Europe	1600–2200
	<i>Narcissus tazetta</i> L.	Europe	1600–2500
Apiaceae	<i>Daucus carota</i> L.	Africa; Europe	1600–3100
	<i>Eryngium billardieri</i> Del.	Africa; Europe	1700–2700
Araliaceae	<i>Hedera helix</i> L.	Europe	1600–2600
Asteraceae	<i>Achillea millefolium</i> L.	Europe	1600–3100
	<i>Ageratum conyzoides</i> L.	South America	1600–2400
	<i>Anthemis cotula</i> L.	Europe	1600–2800
	<i>Arctium lappa</i> L.	Europe	1600–3200
	<i>Artemisia absinthium</i> L.	Europe	1800–3000
	<i>Carduus edelbergii</i> Rech. f.	Europe	1700–3000
	<i>Centaurea iberica</i> Trev. ex Spreng.	Asia; Europe	1700–2600
	<i>Cichorium intybus</i> L.	Europe	1600–2500

Family	Name of Plant Species	Origin	Altitudinal Range (m asl)
	<i>Cirsium arvense</i> Scop.	Asia	1600–2700
	<i>Conyza canadensis</i> Cronquist	North America	1600–2500
	<i>Crepis sancta</i> Bab.	Asia	1600–2300
	<i>Galinsoga parviflora</i> Cav.	South America	1600–2200
	<i>Siegesbeckia orientalis</i> L.	Africa	1600–1900
	<i>Sonchus arvensis</i> L.	Asia; Europe	1600–2600
	<i>Taraxacum officinale</i> Weber	Europe	1600–3100
	<i>Xanthium spinosum</i> L.	South America	1600–2200
	<i>Xanthium strumarium</i> L.	Africa	1600–2100
Boraginaceae	<i>Lithospermum arvense</i> L.	Asia; Europe	1600–2800
Brassicaceae	<i>Capsella bursa-pastoris</i> Medic.	Europe	1600–3000
	<i>Sisymbrium loesellii</i> L.	Africa; Europe	1600–2800
Butomaceae	<i>Butomus umbellatus</i> L.	Asia; Europe	1600–2000
Caprifoliaceae	<i>Sambucus wightiana</i> Wall. ex Wt. and Arn.	Asia; Africa	2200–3000
	<i>Arenaria serpyllifolia</i> L.	Asia; Europe	1600–2400
	<i>Lychnis coronaria</i> Desr.	Europe	1600–2700
Caryophyllaceae	<i>Sagina saginoides</i> Karst.	Europe	2100–3000
	<i>Stellaria media</i> Cyr.	Europe	1600–2900
	<i>Ceratophyllum demersum</i> L.	North America	1600–2100
	<i>Chenopodium album</i> L.	Europe	1600–2900
Chenopodiaceae	<i>Chenopodium foliosum</i> Aschers.	Asia; Europe	1600–2800
	<i>Chenopodium hybridum</i> L.	Asia; Europe	1700–3000
Convolvulaceae	<i>Convolvulus arvensis</i> L.	Europe	1600–3100
Cyperaceae	<i>Carex notha</i> Kunth.	Asia	1600–3000
	<i>Cyperus difformis</i> L.	Africa; Europe	1600–3100
	<i>Cyperus globosus</i> All.	Africa; Europe	1700–2500
	<i>Cyperus rotundus</i> L.	Europe	1600–2400
Euphorbiaceae	<i>Euphorbia helioscopia</i> L.	Asia; Europe	1600–2500
Fabaceae	<i>Medicago polymorpha</i> L.	Africa; Europe	1600–2500
	<i>Robinia pseudoacacia</i> L.	North America	1600–2200
	<i>Trifolium pratense</i> L.	Europe	1600–3000
	<i>Trifolium repens</i> L.	Europe	1600–3400
Iridaceae	<i>Iris ensata</i> Thunb.	Asia	1600–2200
	<i>Juncus articulatus</i> L.	Asia; Europe	1700–2800
	<i>Marrubium vulgare</i> L.	Asia; Europe	1700–2500
	<i>Mentha longifolia</i> L.	Africa; Europe	1800–3700
	<i>Thymus serpyllum</i> L.	Europe	1600–3400
Lamiaceae	<i>Lemna minor</i> L.	Asia; Africa	1600–2500
	<i>Spirodela polyrhiza</i> Schleid.	Asia; Africa	1600–2600

Family	Name of Plant Species	Origin	Altitudinal Range (m asl)
	<i>Marsilea quadrifolia</i> L.	Europe	1600–2200
	<i>Nymphoides peltatum</i> Kuntze	Asia; Europe	1600–1800
Onagraceae	<i>Epilobium hirsutum</i> L.	Africa; Europe	1600–2600
	<i>Oenothera rosea</i> Ait.	South America	1600–2900
Plantaginaceae	<i>Plantago lanceolata</i> L.	Africa; Europe	1600–3000
	<i>Plantago major</i> L.	Europe	1600–2800
Poaceae	<i>Aegilops tauschii</i> Cosson	Africa	1700–2500
	<i>Agrostis stolonifera</i> L.	North America	1700–2800
	<i>Bothriochloa ischaemum</i> Keng	Africa	1600–3000
	<i>Bromus inermis</i> Leys.	Europe	1600–2800
	<i>Dactylis glomerata</i> L.	Asia; Europe	1600–3200
	<i>Eragrostis pilosa</i> P. Beauv.	Africa	1600–2600
	<i>Lolium temulentum</i> L.	Europe	1600–2700
	<i>Phragmites australis</i> Trin.	South America	1600–2600
	<i>Poa annua</i> L.	Europe	1600–3200
	<i>Setaria viridis</i> P. Beauv.	Asia; Africa	1600–2900
	<i>Sorghum halepense</i> Pers.	Europe	1600–2700
	<i>Themeda anathera</i> Hack.	Asia	1800–2900
	<i>Vulpia myuros</i> Gmel.	Europe	1800–3200
Polygonaceae	<i>Polygonum aviculare</i> L.	Europe	1600–2500
	<i>Polygonum hydropiper</i> L.	Europe	1600–3000
	<i>Rumex hastatus</i> D. Don	Asia	1700–2600
Potamogetonaceae	<i>Potamogeton crispus</i> L. var. <i>serrulatus</i> Reichb.	Europe; South America	1600–2400
Primulaceae	<i>Anagalis arvensis</i> L.	Europe	1600–2800
Ranunculaceae	<i>Ranunculus arvensis</i> L.	Africa; Europe	1600–3200
	<i>Ranunculus laetus</i> Wall. ex Hk. f. and T.	Europe	1600–2800
	<i>Ranunculus muricatus</i> L.	Africa; Europe	1600–2700
Rosaceae	<i>Rubus ulmifolius</i> Schott.	Europe	1600–2800
	<i>Salvinia natans</i> All.	Africa; Europe	1600–2900
Scrophulariaceae	<i>Verbascum thapsus</i> L.	Europe	1600–3500
	<i>Veronica persica</i> Poir.	Asia	1600–3100
Simaroubaceae	<i>Ailanthus altissima</i> Sw.	Asia	1600–3000
Solanaceae	<i>Datura stramonium</i> L.	North America	1600–2700
	<i>Solanum pseudo-capsicum</i> L.	Asia; Africa	1600–2400
	<i>Sparganium ramosum</i> Huds.	Europe	1600–2600
	<i>Trapa natans</i> L.	Europe	1600–2500
	<i>Typha angustifolia</i> L.	Europe; North America	1600–2600
Urticaceae	<i>Urtica dioica</i> L.	Africa; Europe	1600–3000

Himalaya and the information from the field surveys conducted during 2017. To enumerate and record the plant species, eighty sites were randomly selected along the whole Himalayan region so as to have holistic representation of the study site. In order to capture the vegetation turnover during the year, and to ensure that no species is missed while recording the species growing in different seasons, field surveys/collection trips were conducted in spring (April and May), summer (June and July) and autumn season (September and October) during the year. Small herbaceous plants were collected as a whole, while as for shrubs, climbers and trees, the flowering twigs and branches were collected. The plant species were recorded and were taxonomically determined up to species level using regional floras, field manuals, monographs, e-floras viz., Calflora (<http://www.calflora.org/>), eFloras (<http://www.efloras.org/>), Flowers of India (<http://www.flowersofindia.net/>), Jepson eFlora (<http://ucjeps.berkeley.edu/eflora/>) and expert taxonomic scrutiny. Family arrangement of taxa was based on the Angiosperm Phylogeny Group-III Classification of 2009 (Angiosperm Phylogeny Group III 2009). The Plant List (www.plantlist.org) was used for assigning accepted names to identified plants. The compiled database includes the minimum and maximum elevations for each species, and the life form (tree, shrub, or herb), and origin of each species. Species were categorized on the basis their extent of spread along the altitudinal gradient of 1600 to 3200 masl. The higher altitudes were usually devoid of naturalized and invasive species, although some alien casuals were present. The species were

categorized as per families and PCA was done to delimit the distribution of these families based on species richness.

RESULTS

Results reveal that Kashmir Himalaya is dominated by 81 invasive and 160 naturalized species belonging to 28 and 48 families, respectively (Tables 1 and 2). Largest number of alien plant species belongs to families of Poaceae and Asteraceae in both naturalized as well as invasive categories. While estimating the contribution of source floras, present study revealed that annual and perennial herbs predominate the alien flora and most of the species have been introduced unintentionally (Figs. 1 and 2).

Total 95% of the naturalized species were represented by 48 families with restricted distribution along the altitudinal gradient (Figs. 3 and 4). At this stage the three largest families outside range were represented by Brassicaceae, Asteraceae and Poaceae, with Poaceae being the most dominant family. The members of Asteraceae and Poaceae were represented in almost same altitudinal range when they were at the naturalization phase (Fig. 3); however the families separated into different altitudes when the species of the two families crossed the naturalization barrier.

DISCUSSION

Results reveal that the herbs dominate (annual, biennial and perennial) both naturalized and invasive alien flora of the Kashmir Himalaya. This is a trend observed in other regional alien floras as well (see for example Pysek et al. 2002; Weber

Table 2: List of naturalized plants

Family	Name of Plant Species	Origin	Altitudinal Range (m asl)
Amaranthaceae	<i>Achyranthes bidentata</i> Blume.	Asia	1700–1900
	<i>Alternanthera sessilis</i> DC.	South America	1600–2300
	<i>Amaranthus graecizans</i> L.	Europe	1700–1900
Apiaceae	<i>Anethum graveolens</i> L.	Europe	1700–2400
	<i>Berula erecta</i> Cov.	Europe	1800–2600
	<i>Conium maculatum</i> L.	Europe	1900–2400
	<i>Sanicula elata</i> Buch.-Ham.	Africa; Europe	1700–2600
	<i>Scandix pecten-veneris</i> L.	Europe	1600–3100
	<i>Torilis japonica</i> DC.	Asia	1800–2800
Apocyanaceae	<i>Vinca major</i> L.	Europe	1600–1700
Asteraceae	<i>Artemisia scoparia</i> Waldst. and Kit.	Asia; Europe	2200–2900

Family	Name of Plant Species	Origin	Altitudinal Range (m asl)
	<i>Artemisia tournefortiana</i> Reichb.	Asia	1700–2100
	<i>Bellis perennis</i> L.	Europe	1600–2100
	<i>Bidens cernua</i> L.	Europe; North America	1600–3000
	<i>Carpesium abrotanoides</i> L.	Asia; Europe	1700–2300
	<i>Chrysanthemum cinerariifolium</i> Vis.	Europe	1600–1800
	<i>Cirsium wallichii</i> DC.	Asia	1600–1900
	<i>Filago arvensis</i> L.	Africa; Europe	1700–2200
	<i>Filago pyramidata</i> L.	Asia; Europe	2300–2900
	<i>Gaillardia aristata</i> Pursh	North America	1600–2000
	<i>Gnaphalium affine</i> D. Don	Africa; Europe	1700–2500
	<i>Hieracium crocatum</i> Fries	Europe	1800–2400
	<i>Lactuca dissecta</i> D. Don	Europe	1700–2800
	<i>Onopordum acanthium</i> L.	Europe	1800–2300
	<i>Senecio vulgaris</i> L.	Europe	1600–2000
	<i>Tagetes minuta</i> L.	South America	2000–2800
Boraginaceae	<i>Myosotis caespitosa</i> Schultz	North America	1600–2000
Brassicaceae	<i>Arabidopsis thaliana</i> Heynh.	Africa; Europe	1600–2600
	<i>Barbarea intermedia</i> Boreau.	Asia; Europe	2200–2900
	<i>Cardamine hirsuta</i> L.	Europe	1600–2600
	<i>Coronopus didymus</i> Sm.	South America	1700–2200
	<i>Descurainia sophia</i> Webb.	Africa	1700–2900
	<i>Diplotaxis muralis</i> DC.	Europe	2200–2900
	<i>Erophila verna</i> Besser	Africa	1600–2300
	<i>Eruca sativa</i> Miller	Europe	1700–2000
	<i>Lepidium sativum</i> L.	Asia	1700–2600
	<i>Nasturtium officinale</i> R. Br.	Europe	1600–2100
	<i>Turritis glabra</i> L.	Europe	1700–2500
Buddlejaceae	<i>Buddleja lindleyana</i> Fortune	Asia	1700–1900
Buxaceae	<i>Buxus sempervirens</i> L.	Asia; Africa; Europe	1600–1700
Caprifoliaceae	<i>Lonicera quinquelocularis</i> Hardw.	Asia	1800–2400
Caryophyllaceae	<i>Cerastium glomeratum</i> Thuill.	Europe	1600–2600
	<i>Dianthus barbatus</i> L.	Asia	1600–2100
	<i>Gypsophila muralis</i> L.	Europe	1800–2800
	<i>Silene conoidea</i> L.	Asia	1800–3100
Chenopodiaceae	<i>Chenopodium ambrosioides</i> L.	South America	1700–2100
	<i>Chenopodium botrys</i> L.	Africa; Europe	1600–3700
	<i>Chenopodium murale</i> L.	Africa; Europe	1700–2000
Convolvulaceae	<i>Ipomoea purpurea</i> Roth.	South America	1700–2100
Cyperaceae	<i>Carex diluta</i> M. Bieb.	Australia	1600–2500

Family	Name of Plant Species	Origin	Altitudinal Range (m asl)
Dioscoreaceae	<i>Dioscorea bulbifera</i> L.	Asia	1600–1800
Ebenaceae	<i>Diospyros kaki</i> L.	Asia	1800–2500
Elaeagnaceae	<i>Elaeagnus umbellata</i> Thunb.	Asia	1700–2200
Euphorbiaceae	<i>Euphorbia hispida</i> Boiss.	Asia	1800–2000
Fabaceae; Fagaceae	<i>Caragana versicolor</i> Benth.	Asia	2500–2800
	<i>Lathyrus aphaca</i> L.	Africa; Europe	1600–1900
	<i>Lotus corniculatus</i> L.	Asia; Europe	1600–2500
	<i>Medicago lupulina</i> L.	Africa; Europe	1600–2300
	<i>Medicago sativa</i> L.	Africa; Europe	1600–1900
	<i>Trifolium fragiferum</i> L.	Europe	1600–1700
	<i>Vicia faba</i> L.	Asia; Africa	1600–2100
	<i>Quercus baloot</i> Griffith	Europe	1600–1700
Geraniaceae	<i>Erodium cicutarium</i> L'Herit. ex Ait.	Africa; Europe	1700–2200
	<i>Geranium rotundifolium</i> L.	Asia; Europe	1600–2100
Grossulariaceae	<i>Ribes alpestre</i> Dcne	Africa; Europe	2300–3400
Hippocastanaceae	<i>Aesculus indica</i> Hook. f.	Not available	1600–2600
Hypericaceae	<i>Hypericum perforatum</i> L.	Europe	1600–2900
Iridaceae	<i>Iris germanica</i> L.	Europe	1600–2400
	<i>Iris reticulata</i> M. Bieb.	Europe	1600–1900
Juncaceae	<i>Juncus bufonius</i> L.	Asia; Europe	1800–2400
Lamiaceae	<i>Clinopodium umbrosum</i> C. Koch	Asia; Europe	1800–3100
	<i>Clinopodium vulgare</i> L.	Asia; Europe	1700–2900
	<i>Lycopus europaeus</i> L.	Europe	2000–2800
	<i>Mentha arvensis</i> L.	Africa; Europe	1600–2600
	<i>Nepeta cataria</i> L.	Europe	1700–2800
	<i>Origanum vulgare</i> L.	Europe	2000–2800
	<i>Prunella vulgaris</i> L.	Africa; Europe	1600–2900
	<i>Thymus linearis</i> Benth.	Asia	1600–2000
Liliaceae	<i>Asparagus officinalis</i> L.	Asia; Europe	1800–2600
	<i>Hemerocallis fulva</i> L.	Europe	1600–2200
	<i>Tulipa lanata</i> E. Regel	Asia; Europe	1600–2700
Lythraceae	<i>Ammania auriculata</i> Willd.	Asia; Australia	1600–2600
	<i>Ammania baccifera</i> L.	Asia; Australia	1700–2700
	<i>Rotala densiflora</i> Koehne	Asia; Australia	1600–2200
Malvaceae	<i>Hibiscus rosa-sinensis</i> L.	Asia	1600–2600
	<i>Hibiscus trionum</i> L.	Africa	1600–2000
	<i>Malva neglecta</i> Wall.	Asia; Africa	1600–2400
Moraceae	<i>Ficus carica</i> L.	Europe	1600–1900
	<i>Ficus religiosa</i> L.	Europe	1600–1700

Family	Name of Plant Species	Origin	Altitudinal Range (m asl)
	<i>Morus alba L.</i>	Asia	1600–2500
	<i>Morus nigra L.</i>	Asia	1600–2200
Oleaceae	<i>Jasminum humile L.</i>	Asia	1800–2300
	<i>Olea europaea L.</i>	Europe	1600–1800
	<i>Syringa emodi Don.</i>	Asia	2100–2900
Onagraceae	<i>Epilobium cylindricum D. Don</i>	Asia	1700–1800
	<i>Epilobium parviflorum Schreb.</i>	Africa; Europe	1700–2500
	<i>Oenothera biennis L.</i>	North America	1600–2400
	<i>Oenothera glazioviana Micheli</i>	North America	1600–2300
Oxalidaceae	<i>Oxalis corniculata L.</i>	Asia; Europe	1600–2500
Papaveraceae	<i>Papaver hybridum L.</i>	Asia; Europe	1600–1800
Plantaginaceae	<i>Plantanus orientalis L.</i>	Asia; Europe	1600–2200
Poaceae	<i>Agrostis canina L.</i>	Europe	2500–3000
	<i>Agrostis palustris Huds.</i>	Europe	1700–2100
	<i>Agrostis tenuis Sibth.</i>	Europe	1900–2500
	<i>Alopecurus aequalis Sobol.</i>	North America	1700–2000
	<i>Alopecurus arundinaceus Poir.</i>	Europe	1800–2600
	<i>Avena fatua L.</i>	Europe	1600–2000
	<i>Bothriochloa pertusa A. Camus</i>	Asia; Africa	1800–2000
	<i>Bromus japonicus Thunb.</i>	Europe	1600–2600
	<i>Capillipedium parviflorum Stapf</i>	Asia; Australia	1700–1900
	<i>Digitaria longiflora Pers.</i>	Africa	1900–3100
	<i>Eragrostis poaeoides P. Beauv.</i>	Asia; Africa	1700–2000
	<i>Helictotrichon pratense Pilger</i>	Europe	2400–3200
	<i>Imperata cylindrica P. Beauv.</i>	Asia; Europe	1800–2000
	<i>Koeleria macrantha Schult.</i>	Asia; Europe	2400–2900
	<i>Lolium perenne L.</i>	Asia; Europe	1600–2300
	<i>Paspalum paspaloides Scribner</i>	North America	1600–1800
	<i>Pennisetum orientale Rich.</i>	Asia; Africa	1800–2500
	<i>Phalaris arundinacea L.</i>	Europe; North America	1800–2200
	<i>Phleum pratense L.</i>	Europe	2400–3000
	<i>Poa angustifolia L.</i>	Europe	1600–2200
	<i>Poa palustris L.</i>	North America; South America	1900–2400
	<i>Poa pratensis L.</i>	Europe; North America	1800–2200
	<i>Polypogon fugax Nees ex Steud.</i>	Asia	1700–2400
	<i>Sorghum nitidum Pers.</i>	Asia; Australia	1700–1900
	<i>Sorghum vulgare Pers.</i>	Africa	1600–2500
Polemoniaceae	<i>Polemonium coeruleum L.</i>	Europe	2400–2900
Polygonaceae	<i>Rumex crispus L.</i>	Asia; Europe	1700–2000

Family	Name of Plant Species	Origin	Altitudinal Range (m asl)
	<i>Rumex dentatus L.</i>	Africa; Europe	1800–2200
	<i>Rumex nepalensis Spreng.</i>	Africa; Europe	1800–2600
	<i>Rumex palustris Smith</i>	Europe	2200–2800
Portulacaceae	<i>Portulaca oleracea L.</i>	Africa; South America	1600–1900
Ranunculaceae	<i>Adonis aestivalis L.</i>	Europe	1900–2400
	<i>Anemone biflora DC.</i>	Africa; Europe	1900–2700
	<i>Ceratocephalus falcatus Pers.</i>	Europe	1800–2200
	<i>Ranunculus sceleratus L.</i>	Europe	1600–2300
	<i>Thalictrum minus L.</i>	Europe	1700–2400
Rosaceae	<i>Fragaria nubicola Lindel. ex. Lacaita</i>	Europe	1600–3100
	<i>Potentilla reptans L.</i>	Asia; Europe	1700–2600
	<i>Potentilla supina L.</i>	Africa; Europe	1800–3000
	<i>Prunus tomentosa Thumb</i>	Asia	1800–2500
	<i>Rosa brunonii Lindl.</i>	Africa; Europe	1700–2400
Rubiaceae	<i>Galium asperifolium Wall.</i>	Europe	1600–3000
	<i>Galium elegans Wall.</i>	Africa; Europe	1800–2700
	<i>Galium palustre L.</i>	Africa; Europe	1700–2600
	<i>Galium tenuissimum Bieb.</i>	Asia	1900–2700
	<i>Rubia cordifolia L.</i>	Asia; Africa	1800–2600
Salicaceae	<i>Populus alba L.</i>	Europe	1600–2100
	<i>Populus caspica Bornm.</i>	Europe	1800–2600
	<i>Salix babylonica L.</i>	Asia	1600–2300
Scrophulariaceae	<i>Digitalis lanata Ehrh.</i>	Europe	1900–2300
	<i>Digitalis purpurea L.</i>	Europe	2000–2500
	<i>Mazus japonicus Kuntze</i>	Asia	1600–3000
	<i>Veronica biloba L.</i>	Asia	1900–3100
Solanaceae	<i>Hyoscyamus niger L.</i>	Africa; Europe	1800–2900
Valerianaceae	<i>Valeriana officinalis L.</i>	Asia; Europe	1800–2500
Violaceae	<i>Viola sylvestris Lam</i>	Europe	1900–2700
Vitaceae	<i>Vitis vinifera L.</i>	Asia; Europe	1600–1900
Zygophyllaceae	<i>Tribulus terrestris L.</i>	Asia; Africa	1900–3000

et al. 2008) or even broader regions (see Lambdon et al. 2008). The perennial life cycle, which implies vegetative propagation, a trait correlated with alien abundance (Hulme et al. 2008; Milbau and Stout 2008), was equally frequent with the annual one. The ability to predict which alien plants will transition from naturalized to invasive is a key goal for conservation and has the potential to increase the efficacy of a priori weed risk assessments, and the generalization about the growth form gives us an

idea about the same. However, our study clearly shows that most of the introductions are unintentional, thus hampering our ability to stop the alien species at the introduction phase of the invasion process. This necessitates the development of broad generalizations about invasion process. Here we show that alien species not only invade the community types with phylogenetically clustered species pool at disproportionately high numbers, but also increase the degree of clustering of these species pools during naturalization stage of the

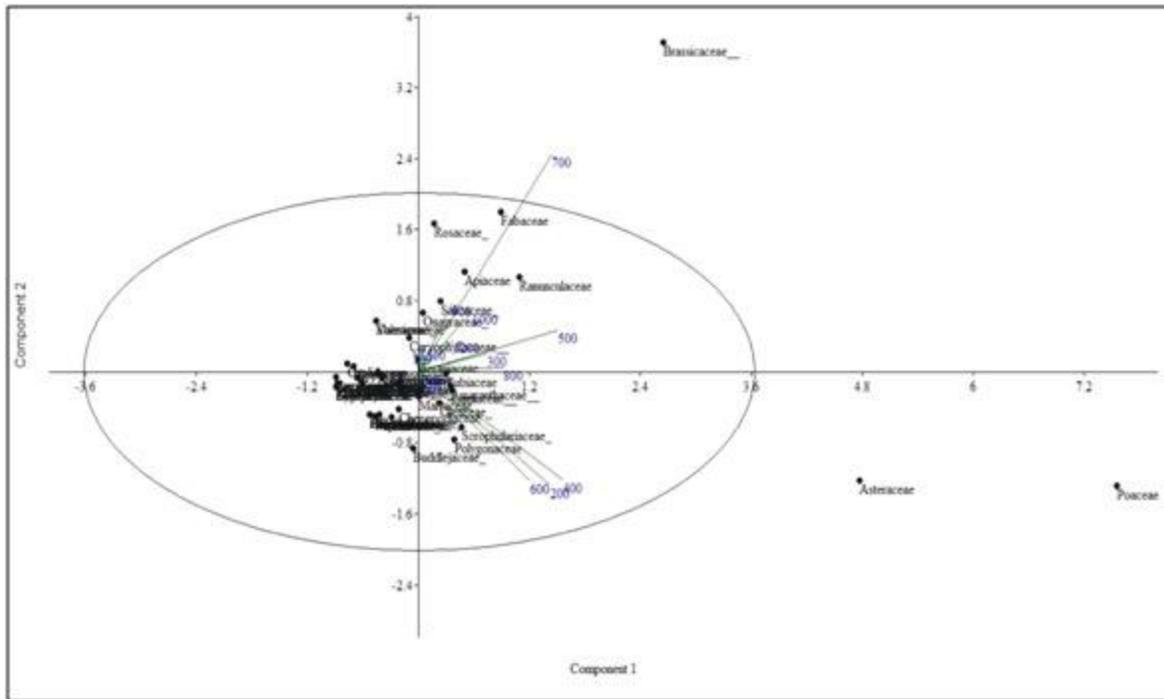


Figure 3: PCA of the naturalized alien species with species richness and extent of spread as the predictors.

invasion process. According to Darwin’s naturalization hypothesis (Daehler, 2001) alien species closely related to native species may be favoured during invasion, as closely related species tend to share similar traits that allow them to adapt to the same environments (the pre-adaptation hypothesis, PAH (Ricciardi and Mottiar, 2006)). Empirical tests of Darwin’s hypotheses have been quickly accumulating

in recent years (summarized by Proches et al. (2008), Thuiller et al. (2010) and Jones et al. (2013)), coinciding with the emergence of phylogenetic community ecology that links species evolutionary relationships with species interactions (Webb et al., 2002).

The clustering that was detected in our study

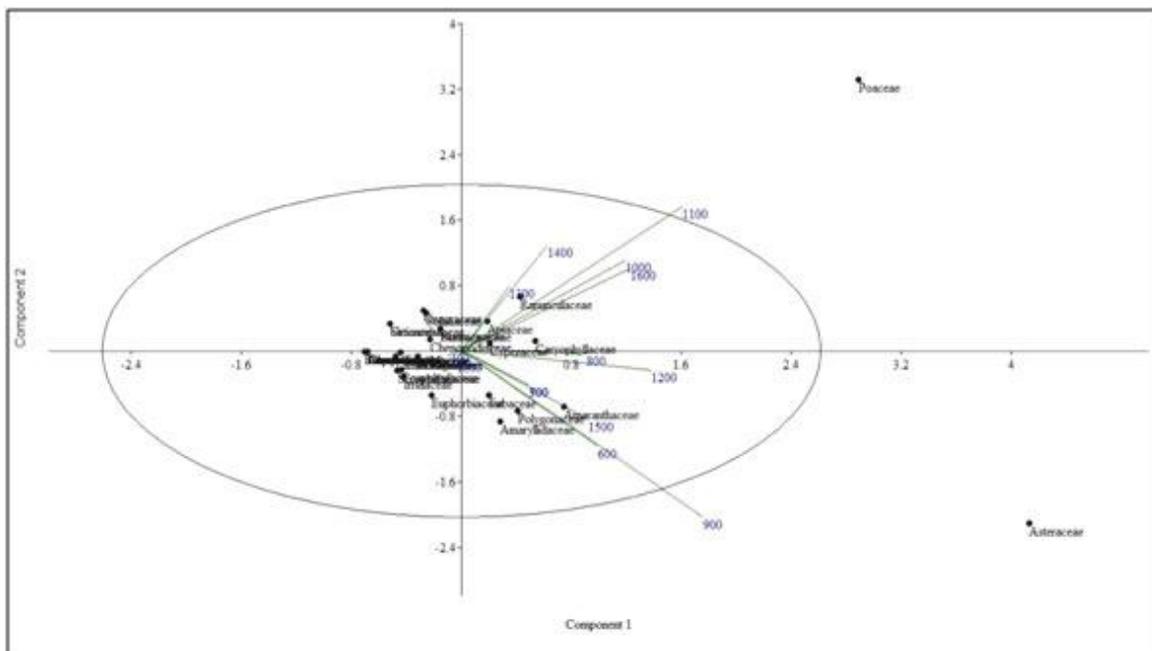


Figure 4: PCA of the invasive alien species with species richness and extent of spread as the predictors.

should indeed provide a first robust indication that alien species which share ecological adaptations with other alien species are mainly successful invaders in phylogenetically clustered plant community types at naturalization stage. In contrast to community types with phylogenetically clustered species pools, the smallest numbers of alien species were detected for community types with phylogenetically diverse species pools. Although the pattern for phylogenetically diverse species pool remained more or less same for the less rich invasive families, the families with larger species richness specialized when they reached the ‘final stage of invasion’. Darwin (Darwin 1859) postulated that alien species more closely related to native communities are less likely to invade, based on the premise that native species more closely related to alien invaders tend to share more similar niches (i.e. phylogenetic niche conservatism (Harrison et al. 2010)) with them, and thus offer stronger biotic resistance (Darwin’s naturalization hypothesis, (Daehler 2001)). Our study clearly shows that the species pool specializes as per their niche requirements to avoid the competition and occupy different niches to become successful invaders in the region.

CONCLUSION

Our findings support the hypothesis that relatedness of invaders to other invasive species promotes invasion because of their shared adaptations to the same environments during naturalization. However, the communities specialize during further course of invasion process and the phylogenetic traits determine the distribution of species to avoid competition at later stages of invasion.

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SECTION 4

Eco-Restoration and Conservation Strategies



Pattern of Plant Functional Traits (PFTs) Variation between Two Populations of *Morina longifolia* Wall. at Western Himalaya

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ABSTRACT: Plant Functional Traits (PFTs) are critical life-history traits that influence success, distribution, and adaptation of species. We, therefore, analyzed variations in PFTs between low (2500–2900 m) and high altitude (3000–3500 m) populations of *Morina longifolia*. The study was conducted along a 2500–3500 m altitudinal transect in Dhauladhar mountain range of western Himalaya. Nine permanent nine plots of 20 × 20 m in dimension were established at every 100 m rise in altitude. Mature and healthy *M. longifolia* samples were collected from these plots and analyzed for vegetative, belowground and reproductive traits. Correlation between altitude and traits was assessed using the Pearson's parametric product moment (*r*) test. One-way linear ANOVA was used to test the effect of elevation on PFTs. Flower display area was calculated using the equation: $A = 2\pi \times \{(d_1/2) + (d_2/2)\} / 2 \times h + \pi \times (d_1/2) \times (d_2/2)$, where d_1 is largest, d_2 represent the diameter and h is height of inflorescence. All measurements were done using a digital caliper. Across altitude, significant variation in the flower area $F(1,8) = 4.26$ ($P = 0.04$) and flower size $F(1,8) = 4.82$ ($P = 0.03$) were recorded among populations. Flower size, flower area, and seed mass reported higher values in the high-altitude populations while flower count and seed count decreased with increasing altitude. Similarly, most of the vegetative and belowground traits also decreased from low to high altitude. Trait variations may help the species initiate ecological performance. ©2019

KEYWORDS: Altitude; PFTs; Adaptation; *Morina longifolia*.

INTRODUCTION

Mountain ecosystem is characterized by rapid environmental changes across altitudes that promote diversification of plant species and plant functional traits (PFTs) (Ackerly, 2009). Altitude plays an important role in shaping mountain ecosystems as it governs the availability of nutrients, pollination, and diversity of pollinators (Körner 2007; Pescador *et al.*, 2015). Therefore, elevation gradient is of utmost importance for studying PFTs variations in plant species (Körner, 2007).

Alpine plant species are distinctly small in size and allocate more resources to flower biomass compared to lowland plants (Körner & Renhardt, 1987; Kieltyk, 2017). Plants growing at higher altitudes have shorter stem, lower number of flower, seed and flower head, but larger flowers as compared to their lowland counterparts (Schemske & Bradshaw, 1999; Hattori *et al.*, 2014). The flower size is relatively more important at high altitudes than other parts of the plant (Fabbro & Körner, 2004). Alpine plants with greater pollen quantity, large flowers, and of different colors accounts for higher visitation by pollinators, which ultimately guides reproductive fitness and success (Childs *et al.*, 2004; Fabbro & Körner, 2004). PFTs are governing factors of biological diversity, ecological functioning with significant influence on establishment, survival, and fitness of plant species

(Körner, 2003; Trunschke & Stöcklin, 2017). Identifying PFTs of newly appearing or disappearing species helps us to understand these selection processes which are important for predicting the future of plant species population with respect to climate change (Körner, 2003; Hattori *et al.*, 2014). Himalaya being the youngest and highest mountain range in the world with high degree of endemism is perfectly suited for trait based studies along altitudes (Körner, 2007).

We therefore addressed (1) how PFTs of *M. longifolia* vary between the low and high altitude population and (2) how resource allocations differ between them. We hypothesize that PFTs does not vary between the two populations of *M. longifolia*. To test this hypothesis, we measured reproductive, vegetative and belowground traits of both the selected populations.

LITERATURE REVIEW

Darwin, Wallace, and von-Humboldt were amongst the pioneer workers who observed, how natural world changes with changing elevation (Lomolino 2001). Gradient studies in mountains formed the basis of research in ecology, adaptation, and evolution including niche theory, life zones, community assembly, environmental pressures, and adaptation of PFTs (McCain & Grytnes 2010). Cochrane *et al.* (2016) studied that selection pressures along altitudinal gradients gave rise to variation in PFTs, with strong evidence to show that many plant traits are functionally linked to spatial scales.

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PFTs studies were initially used as precursors of organism performance. Variations in PFTs with respect to biotic as well as abiotic interactions such as pollination, climate change, soil nutrients, and light intensity were studied by Junker and Parachnowitsch (2011); Cadotte *et al.* (2009); Fabbro and Körner (2004); Körner *et al.* (1989); Flynn *et al.* (2011). Recent studies on the subject are focusing on flower colour (Shrestha *et al.*, 2014), flower display area (Fabbro & Körner, 2004), flower duration (Lessard-Therrien *et al.*, 2014a), flower longevity (Trunschke & Stöcklin, 2017), specific flower area (SFA), corolla allocation (Herrera, 2009) and seed characteristics (Wang *et al.*, 2014).

Most of the earlier studies have also documented the phenology and phylogeny of PFTs between different populations and communities (Lessard-Therrien *et al.*, 2014b). In the context of Himalaya as an ecological unit, flower color patterns have been studied by Shrestha *et al.* (2014) while seed traits of forty-two (42) Rhododendron species have been analyzed by Wang *et al.* (2014). The pattern of carbon, nitrogen and phosphorus ratio in leaf (Zhao *et al.*, 2014), leaf stomata traits (Wang *et al.*, 2014) and seedling traits have been recorded (Li *et al.*, 2004). Xu *et al.* (2009) and Shrestha *et al.* (2014) analyzed functional traits in the Himalayan Mountains. The literature perused revealed that though such studies are much desired, they are presently lacking from the Indian Himalayan region. This formed the basis for the present study.

MATERIALS AND METHODOLOGY

Study Area

The study was conducted in the Dhauladhar mountain range of Western Himalaya. We established five 20 × 20 m² permanent plots between 2500 and 2900 m as land four 20 × 20 m² permanent plots between 3000 and 3500 m. The area is characterized by typical high altitude vegetation comprising of lush temperate oak and conifer forests, and herbaceous dominated alpine meadows. Snowfall is common in the region with temperatures plunging to sub-zero. The annual rainfall in the area is reported to be close to 2500 mm (Gupta, 2014).

Morina longifolia Wall. ex DC. is an important aromatic plant that is referred to as the Himalayan whorl flower. It is a member of Caprifoliaceae family and occurs between 2400 and 4000 m in the Himalaya (Singh & Singh, 1987). It is an evergreen plant with spiny, fragrant, linear, and dark-green basal leaves. The leaves are spiny-margined and the flowers are initially white and later turn pink. The flowering period of the species spans from June to September. Flower, root, and leaf of the plant are highly aromatic and have a citrus-like

scent (Chauhan *et al.*, 2012). The plant attains a height of about 1 m.

PFTs Methods

For trait analyses, 10–20 individuals were collected from each permanent plot, and three replicates of soil samples were also collected. The plant samples were selected with an aim to compare growth stages, habitat and flowering. All the samples were collected at maturation and in full bloom stage for flower analyses. For seed trait analyses, seeds were collected when mature but prior to dehiscence.

The leaves were scanned on the scanner (HP Scan jet 7400C) at a resolution of 300 dpi such that their abaxial side faced the glass with a scale used to standardize size. The images were saved in “jpeg” format for the purpose of measuring leaf area by Image J Software (Pérez-Harguindeguy *et al.*, 2013). All plant parts were carefully separated and wrapped in paper. The stem height, root length, and blossom length were measured using scale. All plant parts were separately dried in a hot oven at 60 to 70°C for 48 h (Fabbro & Körner, 2004) and the dry materials were weighed by the electronic balance (Mettler Toledo-ME104, 0.0001g). Specific leaf area (SLA) was obtained from scanned leaf. The one-sided area of the fresh leaf was divided by its oven-dry mass (Pérez-Harguindeguy *et al.*, 2013).

The plant height and root length (root tuber length) was measured using a measuring tape. Plant stem and root diameter (root tuber breath) were measured with the help of digital Vernier caliper (LCD 150 mm Electronic Digital Vernier Caliper). Flowers/ inflorescences were counted on individual plants. The display area (A) of flowers was calculated following Fabbro and Körner (2004); Herrera (2009) and Bernhardt (2016).

$$A = \frac{2\pi \left\{ \left(\frac{d_1}{2} \right) + \left(\frac{d_2}{2} \right) \right\}}{2} * h + \pi \left(\frac{d_1}{2} \right) \left(\frac{d_2}{2} \right)$$

where d_2 is largest diameter and d_1 is the smallest diameter of the blossoms and h is the height of inflorescence. Each representative flower was oven dried at 60–70°C, and the dry inflorescence was weighed on electronic balance. The specific flower area (SFA) was calculated just as specific leaf area (Cheng *et al.*, 2016). SFA refers to one-sided surface area of fresh flower, divided by its own dry flower weight (Pérez-Harguindeguy *et al.*, 2013). The seeds were collected separately from 10–20 individual

plants from each altitude. The seed number was counted on graph paper (by the grid) (Peart *et al.*, 1989) and weighed on electronic balance for seed mass.

The soil samples were analyzed for physico-chemical properties. Organic matter was estimated following Hach (1988), Phosphorus and Potassium using Mehlich (1984) and Nitrogen by using the Kjeldahl process (Hach, 1988; Ahmad *et al.*, 2014). The soil pH was detected by pH digital meter.

Statistical Analyses

Samples from 2500 to 2900 m were treated as low altitude population while the ones from 3000 to 3500 m as high altitude population for statistical analysis. The relationship between population and traits was determined using the Pearson's parametric product moment (*r*) test at 95% level of confidence. The linear model of ANOVA analysis was used to test the effect of population on plant traits (Wang *et al.*, 2014). In the model, population and plant traits were used as fixed factor and response variables, respectively. Plant traits were clustered into three qualitative groups in case of multivariate analyses *viz.*, (1) reproductive traits (Flower area, SFA, flower count, flower mass, seed count, and seed mass), (2) vegetative traits (leaf area, SLA, leaf mass, stem mass, stem diameter, and stem height), and (3) below ground

traits (root length, root diameter, and root mass). Significant effects were evaluated with the Tukey's honest significance test for multiple comparisons of group means. All statistical analyses were performed in statistical stats-package and graphical representation has been done in ggplot2-package of R 3.4.1 (Team, 2016). All values in text and tables are presented as mean \pm SE and both the sample population have been used for making graphical visualizations. Normality of the data was confirmed by normality distribution test and all differences were tested for significance at $P < 0.05$ level.

RESULTS

PFTs were found to be significantly correlated between population of *M. longifolia* except for SLA and SFA along the elevation. Most of the reproductive traits (FSA, blossom mass and seed weight) were significantly positively correlated between populations. While for the flower count, SFA and seed count; correlation was significantly negative. In the context of vegetative traits, the correlation was significantly negative, except for SLA (R^2 0.20, $P = 0.06$) (Table 1).

The maximum flower area ($318111 \text{ mm}^2 \pm 1142.50 \text{ mm}^2$) was found at high altitude and the minimum ($35474.04 \text{ mm}^2 \pm 13.75.51 \text{ mm}^2$) was observed in low altitude population (Table 2). Most of the vegetative traits such as leaf area ($3.06 \text{ mm}^2 \pm 0.23 \text{ mm}^2$ low altitude and $1.69 \text{ mm}^2 \pm 0.15 \text{ mm}^2$ high altitude), leaf mass ($7.16 \pm 0.47 \text{ gm}$ low altitude and $2.82 \pm 0.17 \text{ gm}$ high altitude), stem diameter ($5.65 \pm 0.31 \text{ mm}$ low altitude and $2.75 \pm 0.16 \text{ mm}$ high altitude), and stem length ($20.11 \pm 0.50 \text{ cm}$ low altitude and $14.42 \pm 0.48 \text{ cm}$ at high altitude) recorded higher values for low altitude population. Reproductive traits had higher values for high altitude population. Thus, higher resources were channelized towards reproductive traits in the high altitude population.

The studied PFTs of *M. longifolia* significantly varied between the two populations. However, traits like SFA ($F(1,8) = 0.03$, $P = 0.86$), and SLA ($F(1,8) = 3.68$, $P = 0.06$) did not report significant differences. Among all the measured PFTs, only SFA, and SLA showed a non-plastic response along the elevation gradients (Table 2). Rest of measured traits (i.e. FSA, flower mass, seed weight, seed count, leaf area, leaf mass, stem mass, stem diameter, stem length, root mass, root diameter and root length) showed plastic responses at 0.05% significant level (Table 2).

Multivariate analysis (MANOVA) and Wilk's lambda (λ) test was used to test the significance of quantitative grouped traits. The estimates of multivariate partial eta squared indicated that reproductive (43%), vegetative (37%) and below ground traits (52%) were associated with population (Table 3).

Table 1: PFTs correlation between populations of *M. longifolia* SFA: Specific Flower Area, SLA: Specific Leaf Area

Plant Functional Traits	R^2	P Value
Flower area (mm ²)	0.22	0.041
Flower mass (gm)	0.23	0.03
SFA (mm ² /gm)	-0.02	0.86
Flower count	-0.68	<0.001
Seed weight (gm)	0.21	0.05
Seed count	-0.60	<0.001
Leaf area (mm ²)	-0.45	<0.001
SLA (mm ² /gm)	0.20	0.058
Leaf mass (gm)	-0.69	<0.001
Stem diameter (mm)	-0.63	<0.001
Stem length (cm)	-0.65	<0.001
Stem mass (gm)	-0.52	<0.001
Root diameter (mm)	-0.55	<0.001
Root length (cm)	-0.42	<0.001
Root mass (gm)	-0.51	<0.001

Table 2: Results of Linear Model (ANOVA) to test for differentiation among sites SFA: Specific Flower Area, SLA: Specific Leaf Area

Plant Functional Traits	Low Altitude (mean ± SE)	(n)	High Altitude (mean ± SE)	(n)	d.f.	F	P Value
Flower area (mm ²)	31811.31 ± 1142.50	(50)	35474.04 ± 1375.51	(40)	1,8	4.26	0.04
Flower mass (gm)	2.118 ± 0.07	(50)	2.43 ± 0.12	(40)	1,8	4.82	0.03
SFA (mm ² /gm)	16108.06 ± 884.93	(50)	15881.57 ± 913.55	(40)	1,8	0.03	0.86
Flower count	126.3 ± 5.32	(50)	67.52 ± 3.75	(40)	1,8	73.75	<0.001
Seed weight (gm)	117.59 ± 4.37	(50)	131.96 ± 5.94	(40)	1,8	3.95	0.04
Seed count	63.28 ± 2.48	(50)	42.00 ± 1.52	(40)	1,8	48.81	<0.001
Leaf area (mm ²)	3.06 ± 0.23	(50)	1.69 ± 0.15	(40)	1,8	21.92	<0.001
SLA (mm ² /gm)	0.64 ± 0.05	(50)	0.872 ± 0.11	(40)	1,8	3.68	0.059
Leaf mass (gm)	7.16 ± 0.47	(50)	2.82 ± 0.17	(40)	1,8	78.49	<0.001
Stem diameter (mm)	5.65 ± 0.31	(50)	2.75 ± 0.16	(40)	1,8	57.16	<0.001
Stem length (cm)	20.11 ± 0.50	(50)	14.42 ± 0.48	(40)	1,8	63.97	<0.001
Stem mass (gm)	4.31 ± 0.26	(50)	2.44 ± 0.15	(40)	1,8	32.26	<0.001
Root diameter (mm)	13.46 ± 0.47	(50)	9.61 ± 0.37	(40)	1,8	38.05	<0.001
Root length (cm)	11.31 ± 0.43	(50)	8.69 ± 0.39	(40)	1,8	18.97	<0.001
Root mass (gm)	3.59 ± 0.23	(50)	1.94 ± 0.15	(40)	1,8	31.02	<0.001

Note: Significant level *0.01 when $P < 0.05$, **0.001 when $P < 0.01$.

Soil Characteristics

The value of pH in soil samples ranged from 4.36 to 5.28. The maximum pH was recorded at 3100 m altitude followed by 2500, 2600, 2700, 3000, 3200, 2900, 3300, 3400 and 3500 m (Table 4). Soil Organic ranged between 4.15% and 4.56%. The maximum organic matter 4.56% was found at 2700 m, followed by 3200 (4.48%) and 3300 (4.48%) m. Total nitrogen ranged between 0.41 (%) and 0.82 (%) at 2500 and 3400 m, respectively. Total phosphorus varied from 0.07% to 0.18% at 3500 altitudes and 3400 m. Total potassium ranged between 0.94% and 1.70% (Table 4).

Table 3: MANOVA used for multivariate tests to study effect of the sites on three grouped traits (reproductive, vegetative and below ground traits)

Functional Groups	d.f.	Wilk's Lambda	F	error d.f.	P Value
Reproductive trait	1,88	0.433	18.043	83	<0.001
Vegetative trait	1,88	0.373	23.19	83	<0.001
Below ground trait	1,88	0.525	25.914	86	<0.001

Note: Significant level *0.01 when $P < 0.05$, **0.001 when $P < 0.01$.

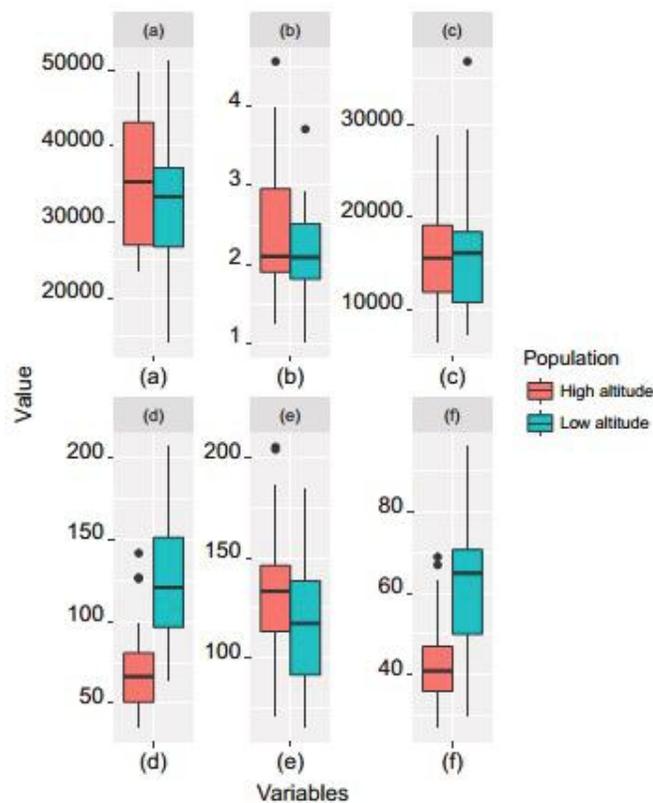


Figure 1: Reproductive traits variation of *Morinalongifolia* (a) Flower area, (b) Flower mass, (c) SFA, (d) Flower count, (e) Seed weight and (f) Seed count.

Table 4: Soil chemical parameter from selected sites

Altitude	pH	Organic matter %	Total Potassium %	Total Nitrogen %	Total Phosphorus %
2500	4.73±0.01	4.37	1.01±0.01	0.41±0.00	0.08±0.00
2600	4.72±0.00	4.23	1.70±0.05	0.59±0.04	0.10±0.00
2700	4.68±0.00	4.56	1.13±0.07	0.54±0.06	0.11±0.00
2800	4.44±0.00	4.46	1.36±0.02	0.57±0.06	0.10±0.00
2900	4.55±0.00	4.38	1.39±0.13	0.66±0.06	0.13±0.00
3000	4.62±0.01	4.23	1.10±0.03	0.58±0.03	0.09±0.00
3100	5.28±0.00	4.15	1.60±0.06	0.63±0.03	0.13±0.00
3200	4.57±0.00	4.48	1.68±0.01	0.63±0.03	0.11±0.00
3300	4.52±0.01	4.48	0.97±0.01	0.60±0.03	0.15±0.01
3400	4.49±0.00	4.02	1.02±0.02	0.82±0.05	0.18±0.00
3500	4.36±0.00	4.52	0.94±0.01	0.68±0.05	0.07±0.00

DISCUSSION

The plant functional traits (PFTs) of *Morinalongifolia* populations varied along altitudinal gradient (Tables 1, 2 and 3). We found that reproductive traits such as flower area, blossom mass, and seed size increased with altitude, while flower count and seed count decreased with altitude (Fig. 1).

Altitude is an important gradient in mountains that governs patterns of plant reproductive, vegetative and belowground traits which have direct effect on plant fitness and species composition (Körner, 2003; Herrera, 2009). Elevational variations play an important role in shaping plant morphology and phenology whereas pollinators result in adaptation of plants and evolution of flower traits (Trunschke

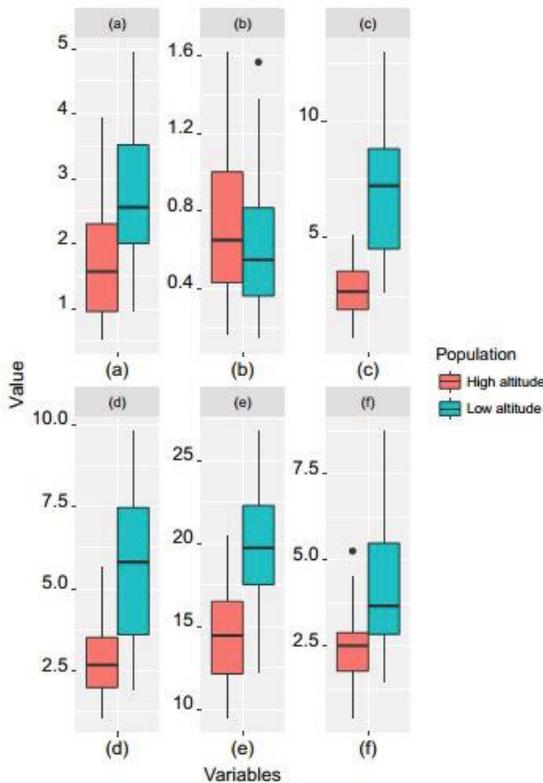


Figure 2: Vegetative traits variation of *Morina longifolia*. (a) Leaf area, (b) SLA, (c) Leaf weight, (d) Stem diameter, (e) Stem length, and (f) Stem mass.

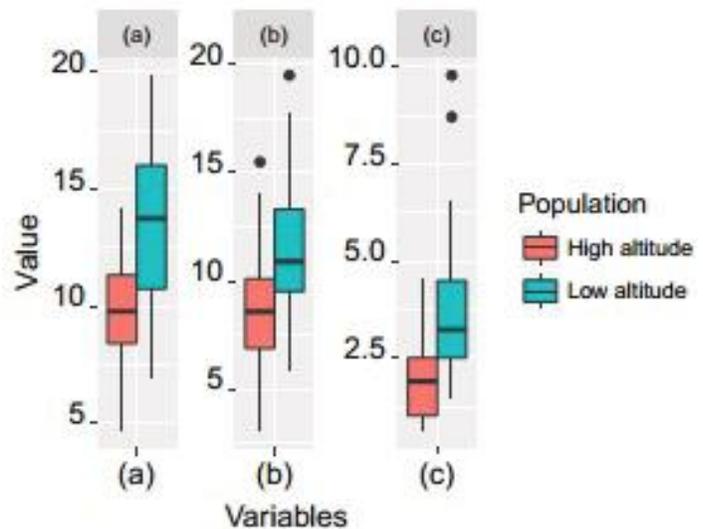


Figure 3: Below ground traits variation of *Morina longifolia* (a) Root diameter, (b) Root length and (c) Root mass.

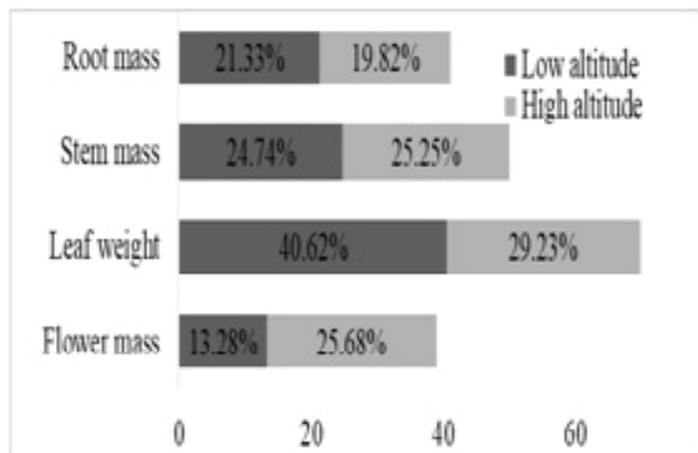


Figure 4: Below ground and above ground shoot mass fractions of *Morina longifolia*.

& Stöcklin, 2017). This shows that investment for progeny and ensuring their survival is high in high altitude populations as has also observed in the present study. For evolutionary concepts, it acts as divergent adaptive response to the changing environment and thereby influences reproductive evolution and adaptation of population along altitudes. At the same time, reproduction success is also governed by flower size/inflorescence which attracts pollinators (Körner, 2003; Fabbro *et al.*, 2004; Herrera, 2009; Zhao *et al.*, 2016). Enhanced flower size influences visits by pollinators which in turn governs reproductive success (Körner, 2003; Herrera, 2009; Zhao *et al.*, 2016).

In the study we observed that seed mass was high at high altitude population. Fewer seeds but of larger size in the high altitude population ensure seedling establishment in harsh environments (Herrera, 2009; Junker & Parachnowitsch, 2011; Cadotte *et al.*, 2009). They act as reservoirs of foods (McWilliams *et al.*, 1968; Keeley, 1991; Milberg *et al.*, 1998) that are advantageous for early seedling establishment (Rodríguez-Pérez & Traveset, 2007), and in enabling seedlings to succeed in resource limited environments (Leishman *et al.*, 2000).

In case of vegetative traits, the resource investment was low along altitude. In our study leaf area, leaf mass, stem length, stem diameter, and size decreased with increasing altitude (Figs. 2 and 3). The reduction in the plant height, leaf size, and vegetative resource allocation may also have positive effects on seed set and attractiveness of pollinators for helping the reproductive fitness of alpine plants (Zhao *et al.*, 2016; Trunschke & Stöcklin, 2017). As altitude increases, the growing season for plant decreases and thus limited time is available to plants for completing their life cycle. This may account for lesser resource allocation to

vegetative parts in high altitude populations (Fabbro & Körner, 2004). Below ground trait also revealed a similar trend, we found root length, root mass, and diameter to decrease along altitude (Figure 3). This has also been reported from other mountain ranges (Yaqoob *et al.*, 2015). Interestingly, fine root length has been reported to increase along altitude so as to maximize water and nutrients absorption in ecosystems are limited in nutrients (Fabbro & Körner, 2004; Wu *et al.*, 2013).

The maximum difference was found in leaf weight which was (21.23) time more at low altitude when compared to high altitude population. This was followed by root mass fraction (1.51). The flower mass fraction of high altitude population was 12.40 more time as compared to the low altitude population (Fig. 4).

An increased reproductive and decreased vegetative resource investment across altitude was in favor of reproductive fitness of plants species at high altitude environment. Whereas larger seed size in populations of high altitude species provides them a chance to survive in harsh environment and increased flower visibility seems to ensure pollination at high altitude. Thus, PFT variations along altitude seem advantageous for ensuring plant survival.

CONCLUSIONS

Traits in *M. longifolia* differed between high and low altitude populations. Higher reproductive investment was observed in high altitude population while higher vegetative allocation was noted in low altitude populations. These seem to ensure survival of the species.

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Conservation Status of Indigenous Crops and Traditional Agriculture in Pithoragarh District, Uttarakhand

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ABSTRACT: The agrarian societies in the Indian Himalayan Region (IHR) have inherited a rich tradition of farming with multiple native crops that are suitable for the marginal lands and highly seasonal environment. Many of the traditional farming practices and landraces/local varieties are currently threatened due to a preference for high yielding varieties (HYV), loss of traditional knowledge pertaining to farming, climate change, and outmigration of rural communities to urban areas. This study aims to investigate the influence of aforementioned factors on the native crop varieties and traditional agriculture practices in Pithoragarh district of Uttarakhand. We conducted questionnaire surveys across 50 villages in six Administrative Blocks covering 201 households. The study reveals that a total of 25 crop species are cultivated in the surveyed villages. Paddy has the highest number of landraces (local varieties) in the study area (n=69), followed by wheat (n=21). We report abandoning of 32 crop species by the farmers at various intensities. Increase in the intensity of cash cropping (potato and French beans) have negatively influenced the crop species diversity. The land under cultivation, especially at higher altitudes, has declined in the study area by 23% over the last 10 years. Traditional knowledge about the farming practices is vital to enhance resilience and adaptive capacity of the farmers to the complex issues that contribute to the decline in agriculture and landraces. In addition, developing a modeling framework that considers multiple parameters are also important to sustain the agriculture in IHR. ©2019

KEYWORDS: *Agriculture vulnerability; Crop diversity loss; Landraces; Traditional agriculture; Western Himalaya.*

INTRODUCTION

Humans have domesticated, improved and conserved numerous crop species and varieties over thousands of years. With the increasing demand for food, the agriculture sector has seen an enormous technological advancement such as the use of sophisticated machinery, development of high yielding crop varieties (HYV), promotion of crop monocultures, use of fertilizers and pesticides during past few decades. However, these advancements fail to make up for the age-old holistic farming practices inherited by the majority of the farmers in the developing countries (Shiva and Bhatt, 2009). The indigenous ethnic communities in various parts of the world have conserved numerous varieties of native crops and landraces (Jarvis et al. 2008). These landraces are often well adapted to the local conditions tested and selected through several generations as opposed to recently introduced high yielding modern varieties (Emperaire and Peroni, 2007). Perusal of literature on the status and trends of diversity of crop landraces around the globe reveals that most of traditional crops are on the decline (Bisht et al. 2007; Maikhuri et al. 1997). Several factors are responsible for the decline in traditional crop varieties. Some of them include (i) erosion of traditional knowledge, (ii) outmigration of rural population to the urban areas, (iii) low economic returns

from the traditional crops when compared to modern high yielding varieties, and (iv) climate change. Most of these factors are interlinked and have a cumulative effect on the agricultural production (Morton, 2007).

Infusion of modern technology in agriculture to increase the yield has had both positive and negative outcomes. Though the yield has increased multi-fold, the mono-cropping system and preference for high yielding varieties has drastically reduced the cultivation of landraces that were meticulously developed over many generations through the traditional knowledge (Shiva 2016; Verma et al. 2010). These crops often have high tolerance to extreme environmental conditions such as short growing period, drought and pest and require less or minimal capital investment compared to the modern high yielding varieties. The sustainability of high yielding varieties under the recent trends in unpredictable climatic conditions pose major challenges for the agriculture sector (Maikhuri et al. 2001).

The Indian Himalayan Region (IHR) is among the places with the highest diversity of native crop varieties (Pande et al. 2016). However, the preference for high yielding varieties and other factors have drastically reduced the cultivation of landraces that were meticulously developed considering the local environmental conditions in the IHR. Documenting and conserving these landraces are important for the sustainable livelihood of the mountain human communities.

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LITERATURE REVIEW

The Indian Himalayan Region (IHR) is among the centre of origin for numerous cultivated plants and supports multitudes of farming communities and agricultural production systems (Vavilov and Dorofeev 1992; Maikhuri et al. 1997). The high diversity of crop varieties in IHR includes native pulses, buckwheat, barley, and millets (Pande et al. 2016). The local communities here have inherited a rich tradition of farming that is intimately associated with the cultural milieu. Most of the traditional crops are well suited for the marginal lands and harsh climatic conditions. These landraces often have special nutritive qualities that are superior to their counterparts in the plains (Shiva and Bhatt 2009). Agricultural systems in IHR are backed up by an extremely rich traditional knowledge accumulated over several generations on weather, soil, crop characteristics, selecting superior quality seeds, and pest management (Pande *et al.*, 2016).

As in other parts of the globe, the local communities in the IHR have been strongly influenced by globalization, socio-economic transformation and climatic uncertainties (Zomer and Oli 2011). In many areas, traditional farming is no longer economical and sufficient to meet the aspirations of the younger generations. Many remote areas have undergone a drastic transformation in their social structure especially due to outmigration for education and alternative livelihood opportunities. The outmigration, coupled with rapid changes in climatic conditions, have negatively influenced the traditional farming practices (Shiva and Bhatt 2009). According to Ford et al. (2006), the increased alienation of youth from older generations in terms of farming practices, and the degradation of social networks have led to the decline in native crop varieties. If relationships between generations continue to degrade 'the younger generations would have difficulty making sense of their observations because it is the elders that help frame knowledge and lead the discourse through which observations are translated into new knowledge (Berkes 2009). The outmigration from rural to urban areas across the developing countries has also resulted in the reduction of human resource required for the traditional farming practices.

Climate change is predicted as a major threat to the agriculture systems in the IHR where majority of the farming is based on rain-fed agriculture (Partap and Partap 2009). In some cases, the increasing temperatures may have a positive impact on agriculture in the mountain areas, for instance through shortening of growing period for the winter season crops. The shortening of the growing season length due to rising temperature could be beneficial in the mountain areas as it would help the winter crops in timely maturity and as

such would allow the crop to mature in the optimal period of time, with beneficial effects on crop area and yields (Hussain and Mudasser, 2004). However, unseasonal precipitation and prolonged drought will negatively impact agriculture in the mountains, as these systems are predominantly based on rain-fed agriculture.

Earlier studies in IHR have largely documented the crop diversity and cropping patterns (Agnihotri and Palni 2007; Pande et al. 2016). An extensive information on the landraces of paddy and its straw quality as a fodder are available for the Kumaun region (Agnihotri 2002; Agnihotri et al. 2003). Recently, Pandey et al. (2016) have documented 161 crops grown in the Kumaun Himalaya. Similarly, Atkinson (1882), Bhatt and Chauhan (1999), and Pandey and Pandey (2004) have also documented the landraces/ local varieties of different crops in Kumaun region. However, information on the exact location and intensity of use of the landraces are yet unavailable. Besides, recent changes in cropping patterns owing to cumulative effects of socio-economic transformations and increased intensities of factors influencing agriculture have not been documented adequately. Kaushalya et al. (2014), based on a satellite remote sensing study, have estimated that 20 – 33 % of agriculture land in India, especially in the mountain regions, is vulnerable to climate change. However, analysis based on primary data and site-specific cropping patterns that are currently unavailable are vital to understand the intensity of climate change related vulnerability. Therefore, our main objective for the current study is to understand how different factors are interacting with the traditional agriculture and contributing to the vulnerability of agro-system in a mountain region. The specific research questions of this study include (i) what is the pattern of diversity among traditional crops in the district? (ii) How the cash crops and village amenities influence crop diversity and cropping area? (iii) What is the current rate of change in cropping area?, and (iv) How farmers perceive the contribution of identified threats to the decline in agriculture?

STUDY AREA AND METHODS

Pithoragarh district, situated in the state of Uttarakhand, is bordered by Nepal in the east, by Tibetan Autonomous Region of China in the north (Fig 1). The district forms part of the Kailash sacred landscape and is known for its rich diversity of traditional crop varieties that are suited for the extreme weather condition in the Himalayan region. The altitude in Pithoragarh varies between 500 m

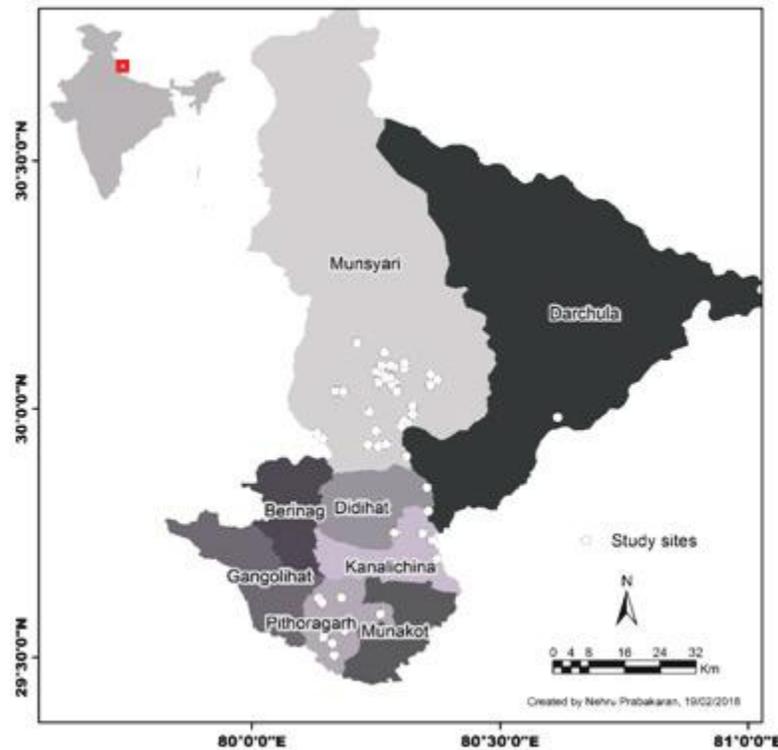


Figure 1: Map showing the Pithoragarh district and the sampling locations.

ASL to 7000 m asl, where farming is practiced generally until 2700 m ASL. The high variation in the altitude and weather condition allows the farmers to grow multiple crop varieties suited for the particular environment. The erratic rainfall coupled with other factors have resulted in the decline of land under agriculture in Pithoragarh. Therefore, it provides a suitable conditions to study the above-mentioned objective.

The diversity of crops, landraces, intensity of use, common trends in agriculture and socio-economy of the farming communities were quantified using both structured and non-structured interviews. The study villages were selected along an altitudinal gradient of 700 to 2500 m asl to assess if any change in the agricultural system with increasing elevation. During the survey, we documented all the major crops and their varieties cultivated during the past one year with the extent of land under each crops. We considered crops that are cultivated ≥ 1 naali of land as major crops (the measure of agricultural land throughout the district is ‘naali’; 1 ha = 50 naali). In addition, we also recorded various ancillary parameters such as the presence of basic amenities in the villages, viz., school, road, electricity, public transportation, distance from town, and healthcare facility; each village infrastructure was quantitatively evaluated based on the presence/absence of these amenities. Each amenity had

a specific score and the cumulative score for a village varies between 1 and 13, where 1 indicates least infrastructure and 13 indicates highest infrastructure. These scores are then correlated with the agriculture land use change over the past decade to assess the influence of village infrastructure on the agriculture land use. influence of cash crop cultivation on the overall crop diversity was assessed based on the landholding of each farmer and the proportion of land under cash crop cultivation. The data was normalized by deriving relative percentage for land under cash crops with individual formers. We used Pearson’s correlation to assess the relationship between land under cash crops and number of crops grown by individual farmers; similarly, relationship between land under cash crops and number of crops abandoned by each farmer was also assessed to check the influence of cash crop intensity on abandoning of traditional crops. The data were analysed in the statistical program R (ver. 3.4.3).

The interview data were also used to assess the changes in the land holdings of individual farmers over the last 10 years. Additionally, Landsat images of 30 m resolution that are freely available at the Global Land Cover Facility (<http://www.glcg.org/>), were used to assess the patterns of land use changes in the agricultural landscapes in the study area between 1995–2005 and 2005–2016. We used ArcGIS (ver. 10.4) for land use change analysis. The respondents were asked to rank the five most commonly perceived threat to

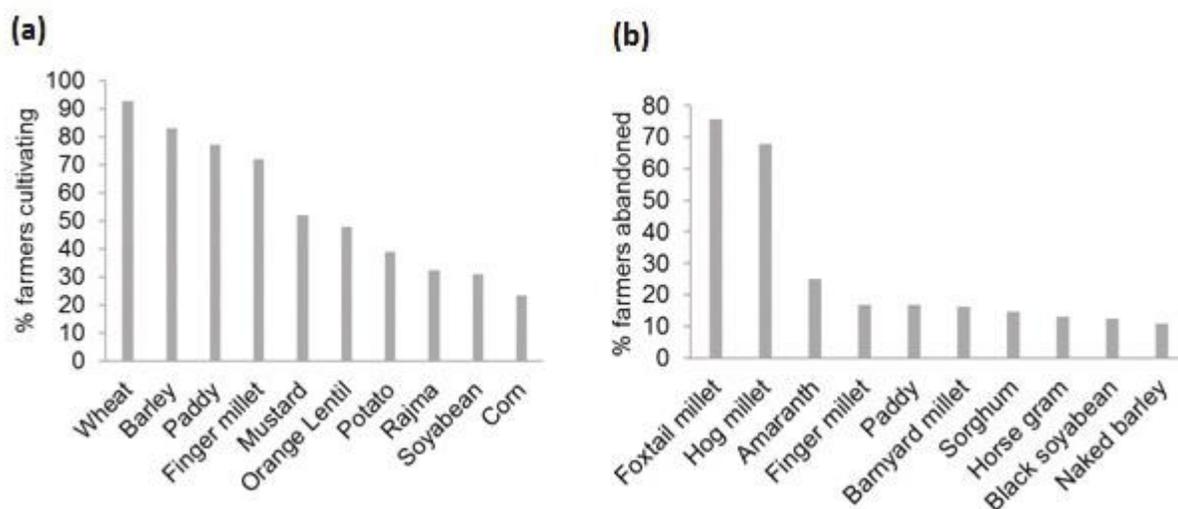


Figure 2: Details of major crops cultivated (a) and abandoned (b) by farmers in the study villages.

agriculture in the study area viz. climate change, wildlife damage, insect pest, human out-migration, and market forces that contribute to the reduced interest for agriculture among the farmers (Rank 1-5, where Rank 1 indicates high influence and Rank 5 indicates least influence)

We report the results of our study based on the interviews with 201 farmers from 50 villages across five administrative blocks (viz., Bin, Munsyari, Kanalichina, Didihat, Dharchula, and Monakot) of Pithoragarh district. The elevation of the study villages ranged from 600 m to 2400 m ASL. Age of the respondents ranged between 29 – 90 (55.3 ± 14.28) and the gender participation was 32% women and 68% men. It may be a meagre percentage of adult population in the study district, but as the samples are distributed across the district, the results below are representative of the major population.

RESULTS

A total of 25 crop species were identified as major crops that are presently cultivated in the study area. Wheat, barley, and paddy are cultivated by over 80% of the farmers (Fig. 2a). We recorded abandoning of 32 crop species by the interviewed farmers at different intensities; for example, Foxtail millet, vern Koni (*Setaria italica*) was abandoned by 75% of farmers, likewise Hog millet, vern Cheena (*Panicum miliaceum*), Amaranth, vern Chuwa (*Amaranthus hypochondriacus*) and Finger millet, vern Maduwa (*Eleusine coracona*) are abandoned by 67%, 25% and 17% farmers respectively (Fig. 2b). Crop variety was highest in paddy (80 varieties – 69 indigenous, 11 HYV) and wheat (24 varieties – 21 indigenous, 3 HYV).

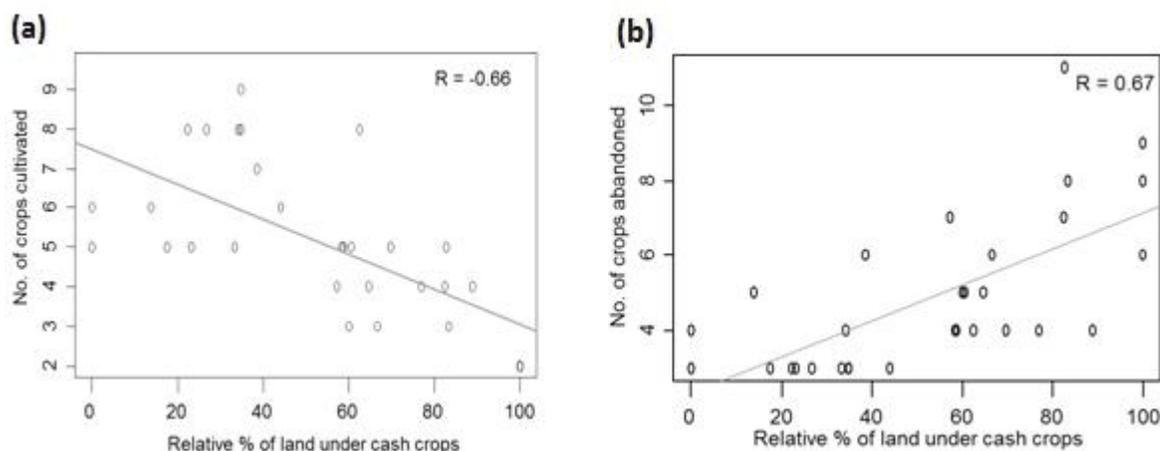


Figure 3: Influence of cash crop cultivation on traditional crop diversity (a) and abandoning of traditional crop cultivation (b).

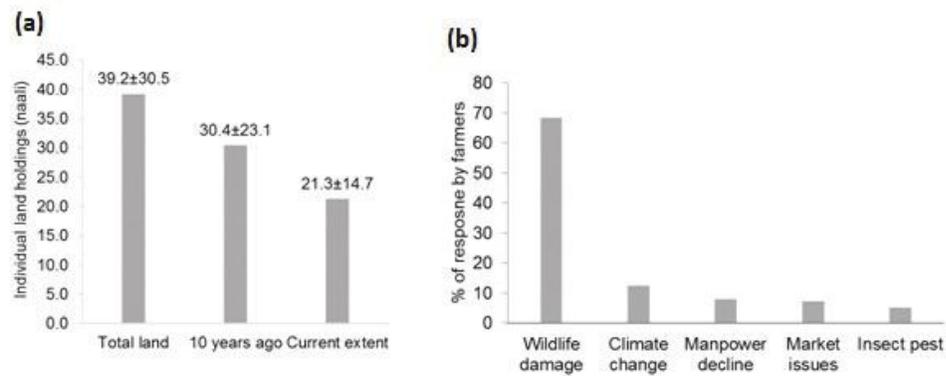


Figure 4: (a) Graph showing the decline in land under cultivation and (b) major threat to the agriculture perceived by the farmers.

We observed that increase in cultivation of cash crops viz., potato and French bean had negatively influenced the diversity of native crops at the higher altitudes (>1700 m). The diversity of agriculture crops cultivated during the study period was negatively correlated with the relative percentage of land under cash crops (Pearson’s $r = -0.66$; Fig. 3a). Meanwhile, the number of traditional crops abandoned was positively correlated with the relative percentage of land under cash crop cultivation (Pearson’s $r = 0.67$; Fig. 3b).

The landholdings of individual farmers have declined by 23% (Fig. 4a) over the last 10 years (Paired sample t -test: $p < 0.001$) and the land under cultivation is accounted for 57% of total land available for agriculture. The land use change analysis based on satellite data also showed that 18% of decline in agricultural land during 1995–2016. A minor decline in agricultural area (3.5 ± 5.4) was observed in villages with least basic amenities. Interestingly the villages with more basic amenities showed less decline (19.3 ± 13.5) than the villages with medium basic amenities (36.2 ± 18.8). Wildlife damage was perceived as the major threat to farming followed by climate change and manpower decline due to out-migration (Fig. 4b).

DISCUSSION

Our study has documented the rich diversity of traditional crop varieties, particularly for paddy and wheat, in Pithoragarh, Uttarakhand. The recorded 80 varieties of paddy, almost close to the 85 varieties recorded for the entire Kumaun region by Pande and Pande (2004), indicate rich landrace diversity in the study region. In general, diversity of crops and landraces are high in the lower and middle elevation 600–1700 m asl compared to the higher altitudes above 1700 m asl. The lower

elevations are known to provide suitable climatic condition for many crops; whereas in the higher altitudes, the growth and cultivation of certain crops are limited by the cold climatic condition (Bhatt & Singh, 2009). For example, we observed that the villages above 2200 m asl does not cultivate paddy. The high diversity of landraces also provides multiple options to the local farming communities as each landrace has a special property in terms of yield, nutritive value, taste, adaptation to local environment, pest tolerance, and sustaining the soil fertility. For example, a paddy variety called Thapchini provides better yield, and the grains have a mild scent with a good taste, therefore is widely preferred by farmers. Additionally, landraces also have other ancillary utilities such as fodder, mulching, and household uses (Agnihotri, 2002; Agnihotri *et al.*, 2003).

Decline in Crop Varieties

Our study shows that there is a sharp decline in crop diversity across the sampled villages. It is noteworthy that the most frequently abandoned crops in the study area belong to millet group (e.g. Foxtail millet and Hog millet) that often provide relatively less yield than the two most commonly cultivated staple food crops namely, paddy and wheat. The same trend is also observed in other parts of the Himalaya (Shiva & Bhatt, 2009). The common reason for abandoning these crops is often the change in food habit among the local community during the past few decades, where rice and wheat had replaced the millets and became the staple food of majority of the human population. In addition, cultivation of rice and wheat were also highly promoted by the green revolution and other food security policies (Shiva, 2016). Meanwhile, other factors such as crop depredation by wildlife and the extensive cultivation of crops that provide monetary benefits (e.g. potato and French bean) had further marginalized the cultivation of many minor crops. Particularly, it may be

inferred that the economic benefit from the cash crops drives the farmers to cultivate more of the cash crops, which in fact reduces the land available for the cultivation of economically less benefiting crops – usually the traditional crops with less market hope.

Major Threats to Mountain Agriculture

The drastic decline (23% in 10 years) in land under cultivation in the study area may be due to the cumulative impact of the human out migration, climate change and crop depredation by wildlife. Particularly the human out migration can have cascading impacts on the farming system, such as, (i) erosion of tradition knowledge about weather and agriculture, (ii) as the traditional rules and regulation degrade, the cooperation among the farmers may decline in group activities such as patrolling the farm land to reduce the crop damage caused by wildlife, (iii) less reliability on agriculture as the economic income from non-farm activities will increase the buying potential of the farming communities. Studies have suggested that the economy of the sustenance farming communities across the developing countries are already contributed 30 – 50 % by the non-farming activities (Morton 2007). This may also be true with the farming communities in the study area where most of the young men between 20 – 40 years of age are often living in the urban areas to generate an income through non-farm activities, which support their families living in the rural areas.

Crop damage by climatic uncertainties, such as, hailstorm, unseasonal precipitation and drought are also among the major constrain in the study area. A recent study has predicted that the decreasing agriculture crop yield in India is highly linked to the increasing temperatures (Carleton 2017). According to Morton (2007), the sustenance farmers in the developing countries – such as the farmers in the study area – are more vulnerable to climate change. A recent study on the climate change vulnerability of Indian districts has suggested that the study area is one among the seven district in Uttarakhand state that are highly vulnerable to climate change. Most importantly, the adaptive capacity (i.e. climate change resilience) in the study area is much less than the national average (Rao et al. 2017). Therefore, it is highly critical to improve the adaptive capacity of the farmers in order to sustain the agriculture systems in the study area and similar sites across the Himalaya. We observed two practices that increases the adaptive capacity of the farmers in the study area (i) advancing the winter cropping season by two to three weeks to minimize damages caused by drier weather conditions towards the end of growing season, and (ii) choosing crop varieties that are less preferred by wildlife. For example, the Rhesus monkeys less damage a rice variety

locally known as ‘Sirmari’, which possesses long awns (hair) on the grain. However, more such practices need to be developed and adapted to reduce the agriculture vulnerability.

CONCLUSIONS

This study provides information on the diversity of traditional crop varieties and the impact of various factors on the agricultural system in the Pithoragarh district of Uttarakhand. The study reveals that there has been nearly 18 % reduction in the area under cultivation in the district since last 20 years and in most of the study villages, the farmers now prefer to grow only cash crops. Low inputs to agriculture due to outmigration of younger generation and climatic uncertainties are reported to be the major drivers of change in farming system. More efficient weather prediction and cooperative farming would be needed to conserve the large pool of landraces to increase the adaptive capacity of farmers to mitigate complex issues around the mountain agriculture. In addition, predictive models that use updated information on weather, traditional crop diversity, and socio-economy of farmers are important to map the agriculture vulnerability and formulate mitigation strategies in the IHR.

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Indian Himalayan Biodiversity and Climate Change: Leads and Gaps

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ABSTRACT: The fifth assessment report of Intergovernmental Panel on Climate Change (IPCC) clearly mentioned the threats to the Himalayan region. The concern is mainly on the Himalayan Biodiversity, which has its own significance for the survival of all life forms. Many studies have already been done on the effect of changing climate on biodiversity of IHR. Which mainly emphasised on forest, vegetation, phenology and agridiversity. Assessment of the impact of climate change on biodiversity have also been done using climate models (Joshi et al., 2012; Kumar et al., 2016). Moreover, few studies have been conducted in recent years on long term ecological monitoring (LTEM) with climate change in IHR (Sekar et al., 2017; Tiwari et al., 2017). The significant lacking of data has been discussed in the fourth assessment report of IPCC as there is no comprehensive data of climate change effects for most of the biodiversity components. this limitation of knowledge is due to rapid changes in climatic scenario, gaps between the studies, irrelevant data sets and technological insufficiency. There is a considerable need for generating systematic data sets on Himalayan biodiversity and climate change for long term monitoring. Also, to increase the knowledge sharing and networking among the multi-stakeholders of IHR, Himalayan Biodiversity and Climate Change Knowledge Network (HBCC-KN) has been introduced. ©2019

KEYWORDS: Indian Himalayan region, IHR, Climate change, Himalayan biodiversity.

INTRODUCTION

Indian Himalaya region (IHR) is a long chain of mountains covering a total area of 5.3 lakh km² and one of the 35 global biodiversity hotspots. The region is a rich repository of plant and animal wealth distributed in diverse ecological zones. The biodiversity hotspot of IHR includes 10,000 plant species, 300 mammals, 977 birds, 176 reptiles, 105 amphibians and 269 freshwater species. The region also covers 3.8% of total human population of the country and exhibits diversity of ethnic groups (171 in numbers) (<http://www.biodiversityhotspots.org>).

The Himalaya is prone to climate change (Xu *et al.*, 2009), clearly depicted by the scientists in the *Fifth assessment report of Intergovernmental Panel on Climate Change* (IPCC). However, few studies have been conducted on the effect of climate change on Himalayan Biodiversity.

Biodiversity is important for the survival of Human being as well as for functioning of the ecosystem (Kumar & Chopra, 2009). The mountain ecosystem has been recognised as a fragile ecosystem and is vulnerable to the climate change. Moreover, these mountains are having high biodiversity and endemism which makes them a conservation priority area (Dhar *et al.*, 2000; Xu *et al.*, 2009). However, the Himalaya has been considered as data deficient on

Himalayan Biodiversity and Climate change (Negi *et al.*, 2012; Singh & Thadani, 2015).

Climate change is considered as one of the major threat to the biodiversity loss which increases pressure on its various functions (Schulze and Mooney, 1994). Moreover, with increasing temperature and precipitation, Himalaya will likely to experience some of the most drastic climate change (Beaumont *et al.*, 2011), thus need utmost concern.

Taking in consideration the aforementioned concerns, the objectives of this review paper is to compile studies related to effect of climate change on biodiversity in IHR and to give major knowledge gaps for better understanding of present and future research.

METHODS

For systematic literature search, we followed a systematic approach for synthesizing information through a pragmatic step-wise process for selecting available peer-reviewed literature sources. We searched using Google scholar with the keywords related to 'Himalayan Biodiversity', 'Indian Himalayan Biodiversity', 'Forest', 'Agriculture', 'Flora', 'Fauna', etc. along with 'Climate Change'. After removing gray literature (reports, conference proceedings, and notes) from the lists of searches, we selected articles based on the scope of the study (*ref.* Table 1).

RESULTS

Forest and Vegetation

A study on the effect of geometric constrains and

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environmental factor in shaping forest structure and composition (Sinha et al., 2018 revealed that the actual and potential evapotranspiration along with moisture are the major drivers which shape the composition and structure of the forest. Also these environmental variables have significant positive correlation with the species richness and negative correlation with altitude (Sinha et al., 2018).

In another study the assessment of vulnerability of Himalayan forest, meadows and mountain ecosystem due to climate change have been reviewed (Maikhuri et al., 2003). The increase in temperature induces stimulation of growth with increase in elevation. For instance, *Q. leucotrichophora* populations at low altitude showed lower production of acorn in comparison to their high altitude counterpart. The leaf life span was getting reduced with increasing temperature in north-eastern Himalaya, however the trend was opposite in western Himalaya (Maikhuri et al., 2003). Similarly, a study has also been done to determine the effect of climate change on forest cover shift of western Himalayan (700–4500 m) (Joshi et al., 2012). The study was conducted on the effect of different modelling scenario, i.e., current, changing precipitation, temperature and both temperature and precipitation on forest cover type. Study revealed that with increase in precipitation (8% increase), the model showed downward shift in temperate evergreen and tropical semi-evergreen forest, however sub-tropical conifer and tropical moist-deciduous forest will likely to move upward with no change in temperate conifer forest. Moreover, changing temperature (1.07°C increase) have more profound upward shift in all forest type except sub-tropical conifer forest. Similarly, when changing both precipitation and temperature a slight upward shift was expected in all forest type except sub-tropical conifer. The projected climate change on the forest ecosystems will give a better understanding and thus corresponds better management and conservation strategies (Joshi et al., 2012).

The effect of changing climate on species shift, physiological function, distribution pattern were determined. As such, it was found that the Oak species and some of the fodder tree species (e.g., *Grewia*, *Celtis*, *Boehmeria* spp.) of western Himalaya have higher photosynthesis rate with increasing CO₂ level, however some species like *Betula utilis* showed completely reverse trend (Joshi et al., 2007). Also with increasing temperature, the shift of Himalayan Pine as 1.9m/year on south and 1.4m/year north slope was found (Dubey et al., 2003). It was also found that the distribution pattern

of *Quercus* spp. and *Pine* spp. was completely changed with changing temperature and precipitation (Saran et al., 2010; Chakraborty et al. 2016). Moreover it was found that changing climate is support to some of the species. For instance, Himalayan birch (*Betula utilis*) species was having potential to grow in sub-alpine region (Singh et al., 2013), while the treeline in Himalaya was recognised as most vulnerable region which have higher sensitivity to climate change (Telwala et al., 2013).

Studies have been also conducted on projected climate change effect on forest and vegetation. In one of the study, the upper Himalayan region forest are found to be most vulnerable to projected impact of climate change by 2085, while northeast forests are more resilient (Chaturvedi, 2011). Moreover, under climate changing scenario (temperature and precipitation), the net primary productivity (NPP) will increase for the Himalayan dry temperate forest and sub-alpine and alpine forests. Also climate change effect will likely to be on Himalaya moist temperate forest which has much smaller increase in soil organic carbon (SOC) by 2085. Percentage change in NPP by 2085 would be much more in Himalayan dry temperate forest as compared to Himalayan moist temperate forest. Similar was found for SOC. It was also estimated that climate change is predicted to be higher for regions that have high elevation. Also the percentage of vegetation changes by 2085 is much more in Himalayan dry temperate forest as compared to Himalayan moist temperate forest (Chaturvedi, 2011).

In another climate change projection study, the distribution pattern of major plant community in Sikkim Himalaya was studied using maximum entropy model (Kumar et al., 2016). Species distribution models (SDMs) has been used for current climate (average climate for 1950–2000), 2050 (average climate for 2041–2060), and 2070 (average climate for 2061–2080). Result revealed that by 2050 about 16% of angiosperm endemic species loose habitat, while the percentage increases up-to 18 % by 2070. Similarly, the meadows will have lower 1% of the current distribution habitat by 2050 and 3% by 2070 (Kumar et al., 2016). In another study a transboundary approach on projection of climate changes effect on spatial distribution of bioclimatic strata and ecoregions were assessed (Zomer et al., 2014). Under transboundary Kailash Sacred Landscape (KSL) of China, India and Nepal study area, 12 ecoregion and 7 bioclimatic zones were developed based on vegetation distribution and function. The climatic change model was used to predict the changes by 2050. Result showed that by 2050, there is substantial shift in climatic condition with 76% shift to a different stratum, 55% to different bioclimatic zone and 36.6% to different ecoregion (Zomer et al., 2014).

Table 1: Studies conducted on effect of climate change on Himalayan biodiversity in IHR

Study Type	Study	Climate Change Effect	References
Forest and Vegetation	Effect of geometric constrains and environmental factor in shaping forest structure and composition in Eastern Himalaya	<ul style="list-style-type: none"> Actual and potential evapotranspiration along with moisture are the major drivers 	Sinha et al., 2018
	Assessment of vulnerability of Himalayan forest, meadows and mountain ecosystem due to climate change	<ul style="list-style-type: none"> Increase in temperature induce stimulation of growth with increase in elevation. Leaf life span getting reduced with increasing temperature in north-eastern Himalaya, however the trends is opposite in western Himalaya. 	Maikhuri et al., 2003
	Shifts in forest cover types of western Himalayan eco-region (700–4500 m) with changing climate	<p><i>Increase in precipitation (8% increase)</i></p> <ul style="list-style-type: none"> The model showed downward shift in temperate evergreen and tropical semi-evergreen forest. Sub-tropical conifer and tropical moist-deciduous forest will likely to move upward with no change in temperate conifer forest. Increase in temperature (1.07increase) Upward shift in all forest type except sub-tropical conifer forest. <p><i>Increasing both precipitation and temperature</i></p> <ul style="list-style-type: none"> Slight upward shift was expected in all forest type except sub-tropical conifer 	Joshi et al., 2012
	Effect of changing climate on species shift, physiological function, distribution pattern	<ul style="list-style-type: none"> Oak species and some of the fodder tree species (e.g., Grewia, Celtis, Boehmeria spp.) of western Himalaya have higher photosynthesis rate with increasing CO2 level. Species like Betula utilis showed completely reverse trend. With increasing temperature, the shift of Himalayan Pine as 1.9m/year on south and 1.4m/year north slope. Distribution pattern of Quercus spp. and Pine spp. is completely changed with changing temperature and precipitation. Himalayan birch (Betula utilis) species was having potential to grow in sub-alpine region. 	Joshi et al., 2007; Dubey et al., 2003; Saran et al., 2010; Chakraborty et al. 2016; Singh et al., 2013
	Projected climate change effect on forest and vegetation	<ul style="list-style-type: none"> Upper Himalayan region forest are found to be most vulnerable to projected impact of climate change by 2085, while northeast forests are more resilient. Net primary productivity (NPP) will increase for the Himalayan dry temperate forest and sub-alpine and alpine forests. Climate change effect will likely to be on Himalaya moist temperate forest by 2085. Percentage change in NPP and SOC by 2085 would be much more in Himalayan dry temperate forest as compared to Himalayan moist temperate forest. Percentage of vegetation changes by 2085 is much more in Himalayan dry temperate forest as compared to Himalayan moist temperate forest. 	Chaturvedi, 2011

Study Type	Study	Climate Change Effect	References
	Distribution pattern of major plant community in Sikkim Himalaya using maximum entropy model	<ul style="list-style-type: none"> • By 2050 about 16% of angiosperm endemic species loose habitat, while the percentage increases up-to 18 % by 2070. • Meadows will have lower 1% of the current distribution habitat by 2050 and 3% by 2070. 	Kumar et al., 2016
	Projection of climate changes effect on spatial distribution of bioclimatic strata and ecoregions in transboundary Kailash Sacred Landscape (KSL) region	<ul style="list-style-type: none"> • By 2050, there is substantial shift in climatic condition with 76% shift to a different stratum, 55% to different bioclimatic zone and 36.6% to different ecoregion. 	Zomer et al., 2014
	Current and future habitat of five invasive species of the Himalayan region using MaxEnt	<ul style="list-style-type: none"> • Two invasive species, <i>Ageratum conyzoides</i> and <i>Parthenium hysterophorus</i>, will lose overall suitable area by 2070. • <i>Ageratina adenophora</i>, <i>Chromolaena odorata</i> and <i>Lantana camara</i> will gain suitable areas and all of them will retain most of the current habitat as stable by 2070. • The southern Himalayan foothills will mostly conserve species ecological niches. 	Lamsal et al., 2018
	Historical (1849–50) and the recent (2007– 2010) data on temperature and endemic species elevation ranges in two alpine valleys of Sikkim Himalaya	<ul style="list-style-type: none"> • Around 87% of endemic plant species were found to be shifted in a temperature driven shift, which results in increase in the species richness in upper alpine zone. • A shift of 23 to 998m in the species upper elevation and a mean upward displacement of 27.53 m/decade were recorded. 	Telwala et al., 2013
	Spatiotemporal changes from 1969 to 2008 in the land cover land use of the Hokersar wetland	<ul style="list-style-type: none"> • 18.75 km² wetland area in 1969 was decreased to 13 km² in 2008. • Reduction in the marshy land habitat of the migratory birds from 16.3 km² in 1969 to 5.62 km² in 2008. • Reduction in the area of forest cover, settlement and water bodies were recorded from 1969 to 2008. 	Ramshoo & Rashid, 2014
Agriculture	Effect of climate change over agriculture system of IHR	<ul style="list-style-type: none"> • Rise in minimum temperature leads to decline in apple yield in Himachal Pradesh. • Apple production has significantly decreased during 1981–2000. • Shift in apple and kiwi cultivation to higher elevation. • Net cereal production in the region is projected to decline by 4 to 10% by the end of this century. • Grassland productivity in the region is also expected to reduce as much as 40 to 90%, with increase in 2 to 3°C temperature combined with reduced precipitation. • North-eastern Himalaya it is projected that by 2030 the production of rainfed rice increased by 5%, however the total rainfed rice area decreased by 10%. • The potato crop production increased by 5%. • Wheat and maize production will be decreased by 20% and 40%, respectively by 2030. 	Sharma and Chauhan, 2011; Vishvakarma et al. 2003; Vedwan and Rhoades 2001; Gautam et al., 2014; Lal, 2005; Smith et al., 1996; Ramay, 2011

Study Type	Study	Climate Change Effect	References
Phenology	Climate change effect on land use change and crop production were estimated in middle to lower altitude area of IHR	<ul style="list-style-type: none"> Rainfall intensity, temperature, runoff and urbanization in the mid-Himalayan region increased which changes the crop pattern, productivity and land use changes. 	
	Climate change effect on traditional crops in IHR	<ul style="list-style-type: none"> More than 60% decline was recorded in last three decades in IHR. Glycine spp., <i>Hibiscus sabdariffa</i>, <i>Panicum miliaceum</i>, <i>Perilla frutescens</i>, <i>Setaria italica</i>, Vigna spp., to name a few were about to extinct. With increasing CO₂ and temperature, <i>Hordeum himalayans</i> crop production and photosynthesis rate was decreased. 	Maikhuri et al. 2001; Negi & Joshi 2002; Joshi and Palni, 2005
	Determining the effect of climate change on phenological pattern using herbarium collection	<ul style="list-style-type: none"> Significant early flowering during the last 100 years in <i>Aconitum heterophyllum</i> Maximum and minimum temperature have been increased resulting in early flowering. Endemic herb species will likely shifted upward by 55.2 m/decade with impulsive variation in chemical constituents. Stomatal density decreases from the past years with increasing temperature. The early flowering in some of the species such as <i>Pyrus</i> spp., <i>Prunus</i> spp. and <i>Rhododendron</i> spp. 	Gaira et al., 2011; Agnihotri et al., 2017
	Forest tree phenology under climate change	<ul style="list-style-type: none"> Bud breaking, flowering, fruiting and leaf drop, was found to be influenced by temperature and rainfall variations. Change in flowering time of <i>Rhododendron</i> from Sikkim Himalaya. 	Negi, 1989; Gaira et al., 2014
Long-Term Ecological Monitoring (LTEM)	Distribution of <i>Rhododendron</i> species in Sikkim Himalaya w.r.t. climate change using Maxent modelling algorithm	<ul style="list-style-type: none"> Probability distribution and future distribution of <i>Rhododendron</i> in Sikkim Himalaya under climate change. 	Kumar, 2012
	Phenological changes in alpine pasture from 1988 to 1998	<ul style="list-style-type: none"> Phenology of different species were affected by soil moisture, humidity, temperature and photoperiod, and indicate a hemicytopytic and geophytic plant climate life form. 	Nautiyal et al., 2001
	Phenological changes from 1982 to 2006 w.r.t climate change using NDVI (Normalized Difference Vegetation Index) values from remotely sensed imagery	<ul style="list-style-type: none"> Annual mean temperature and precipitation during the 25 year of time has increased, which affected the start time and length of the growing season. Growing season started earlier by 4.7 days without any change in the end time. 	Shrestha et al., 2012
	Permanent plots established in Himachal Pradesh at above 3500 m	<ul style="list-style-type: none"> The study is under process. 	Chawla et al., 2012
	Global Observation Research Initiative (GLORIA) sites in alpine environments established in Chaudash Valley, Pithoragarh district, Uttarakhand	<ul style="list-style-type: none"> The study is under process. 	Sekar et al., 2017

Climate change will impact the dynamics of invasive alien plant species. However, it is lesser known about the effect of climate change on alien species in IHR. In one of the study, the current and future habitat of five invasive species of the Himalayan region using MaxEnt was estimated (Lamsal et al., 2018). The result revealed that two invasive species, *Ageratum conyzoides* and *Parthenium hysterophorus*, will lose overall suitable area by 2070, while *Ageratina adenophora*, *Chromolaena odorata* and *Lantana camara* will gain suitable areas and all of them will retain most of the current habitat as stable. The southern Himalayan foothills will mostly conserve species ecological niches, while suitability of all the five species will decrease with increasing elevation. Such invasion dynamics in the Himalayan region could have impacts on numerous ecosystems and their biota, ecosystem services and human well-being, thus transboundary approaches should be a suitable strategy to study and applying managing plans (Lamsal et al., 2018).

In another study the historical (1849–50) and the recent (2007– 2010) data on temperature and endemic species elevation ranges in two alpine valleys of Sikkim have been performed (Telwala et al., 2013). The results showed that the winter temperature increased in the region as compared to the last two centuries. Around 87% of endemic plant species were found to be shifted in a temperature driven shift, which results in increase in the species richness in upper alpine zone. A shift of 23 to 998m in the species upper elevation and a mean upward displacement of 27.53 m/decade were recorded. It was concluded that the upper shift of species will likely to happen and species extinction particularly at the mountain tops (Telwala et al., 2013).

Wetland is considered to be an important ecosystem and likely be affected by climate change. In this regard, a study has been conducted to determine the spatiotemporal changes from 1969 to 2008 in the land cover land use of the Hokersar wetland (Ramshoo and Rashid, 2014). The catchment area were also examined using satellite, historical and field data. It is reported that the 18.75km² wetland area in 1969 was decreased to 13km² in 2008, with reduction in water depth. Moreover, significant reduction in the marshy land habitat of the migratory birds from 16.3km² in 1969 to 5.62km² in 2008 was reported. Also reduction in the area of forest cover, settlement and water bodies were recorded from 1969 to 2008. The possible explanation for such change could be urbanization, deforestation and climate change (Ramshoo and Rashid, 2014).

Agriculture

Climate change is not only threatening the species but also produce threats on livelihood and food security of people across IHR. Some studies have been conducted on determining the effect of climate change over agriculture system of IHR. It was found that the rise in minimum temperature leads to decline in apple yield in Himachal Pradesh (Sharma and Chauhan, 2011) and also shift in apple and kiwi cultivation to higher elevation (Gautam et al., 2014). Also, the net cereal production in the region is projected to decline by 4 to 10% by the end of this century (Lal, 2005). Moreover the grassland productivity in the region is also expected to reduce as much as 40 to 90%, with increase in 2 to 3°C temperature combined with reduced precipitation (Smith et al., 1996). It was estimated that the eastern Himalayan region is likely to face the highest reduction in agriculture potential due to climate change.

Climate change will affect differently for different crops. The effect on agriculture particularly rainfed agriculture will likely to be affected by climate change (Ramay, 2011). In north-eastern Himalaya it is projected that by 2030 the production of rainfed rice increased by 5%, however the total rainfed rice area decreased by 10%. Similarly, the potato crop production increased by 5%. By 2030, it was reported that wheat and maize production will be decreased by 20% and 40%, respectively. In another study the climate change effect on land use change and crop production were estimated in middle to lower altitude area of IHR (Isaac and Isaac, 2017). From 1951 to 2013 data were collected in climate change and crop production in different locations. It was found that the rainfall intensity, temperature, runoff and urbanization in the mid-Himalayan region increased which changes the crop pattern, productivity and land use changes (Isaac and Isaac, 2017).

In Himachal Pradesh, it has been reported that the apple production has significantly decreased during 1981-2000 (Vishvakarma et al. 2003; Vedwan and Rhoades 2001). It has also been reported that during 1990s the minimum and maximum temperature is increased by 0.25 to 1°C with decrease in rainfall (Vishvakarma et al. 2003). Considering traditional crops, more than 60% decline was recorded in last three decades in IHR and some crops such as *Glycine* spp., *Hibiscus sabdariffa*, *Panicum miliaceum*, *Perilla frutescens*, *Setaria italica*, *Vigna* spp., to name a few were about to extinct (Maikhuri et al. 2001; Negi & Joshi 2002). It was also reported that with increasing CO₂ and temperature, *Hordeum himalayans* crop production and photosynthesis rate was reduced (Joshi and Palni, 2005).

Phenology

Phenology is one of the most sensitive indicators to climate change. In IHR studies have been conducted on finding the effect of climate change on phenology. As such, some studies have been conducted on determining the effect of climate change on phenological pattern using herbarium collection. For instance, in a study, *Aconitum heterophyllum* herbarium was used to determine the phenological pattern of alpine/sub-alpine area (Gaira et al., 2011). The result showed significant early flowering during the last 100 years. The maximum and minimum temperature have been increased resulting in early flowering (Gaira et al., 2011). Similar studies using herbarium collection was conducted on endemic herb species of Himalaya (Agnihotri et al., 2017). It was reported that the species will likely shifted upward by 55.2m/decade with impulsive variation in chemical constituents. Also the stomatal density decreases from the past years. It was recorded that the mean maximum and minimum temperature increased by 0.31°C and 0.79°C, respectively (Agnihotri et al., 2017). With increasing temperature, the early flowering in some of the species such as, *Pyrus* spp., *Prunus* spp. and *Rhododendron* spp. were revealed. In Kumaun Himalaya, it was reported that the forest tree phenology changes w.r.t bud breaking, flowering, fruiting and leaf drop, which was found to be influenced by temperature and rainfall variations (Negi, 1989). Moreover, the change in flowering time of *Rhododendron* from Sikkim Himalaya was recorded with early flowering (Gaira et al., 2014).

In another study on the distribution of *Rhododendron* species in Sikkim Himalaya w.r.t. climate change, Maxent modelling algorithm was used (Kumar, 2012). The modelling estimated the target probability distribution and future distribution of *Rhododendron* in Sikkim Himalaya under climate change which need to adopt suitable conservation strategy (Kumar, 2012).

The western Himalaya (Garhwal region) were studied for phenological changes in alpine pasture from 1988 to 1998 (Nautiyal et al., 2001). It was found that the phenology of different species were affected by soil moisture, humidity, temperature and photoperiod, and indicate a hemicryptophytic and geophytic plant climate life form (Nautiyal et al., 2001). Similarly, another study was conducted to collect data from 1982 to 2006 on temperature, rainfall and vegetation phenology. The data were analysed by NDVI (Normalized Difference Vegetation Index) values from remotely sensed imagery (Shrestha et al., 2012). It was found that the annual mean temperature and precipitation during the 25 year of time has increased, which affected the start time and length of the growing season. Also, the

growing season started earlier by 4.7 days without any change in the end time (Shrestha et al., 2012).

Research has also been conducted on people perception on changing climate and effect on phenological characteristics. Chaudhary and Bawa (2013), determine that majority of the people from IHR perceive early budburst and flowering. For instance, species such as magnolia (*Magnolia* sp.), rhododendrons (*Rhododendron* spp.), chrysanthemum (*Chrysanthemum indicum*), marigold (*Tagetes* spp.), peach (*Prunus persica*), plum (*Prunus cerasoides*) and some orchids showed early flowering. Also some of the species shifted upward with changing climate. Among these, *Castanopsis hystrix*, *Schima wallichii*, *Eurya acuminata*, *Ficus roxburghii*, *Alnus nepalensis*, *Saurauia nepaulensis* and *Albizia lebbek* showed upward shift. Considering the upward shift of species some crops and vegetables have been inhabited to high elevation. Such cultivated species include chilli, tomatoes, ginger, winter potato, onion, radish, carrot, cauliflower, cabbage, beat, millet, wheat and cardamom. The changing climate has also found to be favourable to new pests and weeds in IHR (Chaudhary and Bawa, 2011).

Long Term Ecological Monitoring (LTEM)

The IPCC in its fourth assessment report (AR4) describe the Himalayan region as data deficient in term of climate change impact on ecosystem and biodiversity (Bernstein et al., 2008). In order to meet this gap various approaches were already introduced. For instance, under LTEM, permanent plots have been established in Himachal Pradesh at above 3500m in the year 2012 (Chawla et al., 2012). Similarly, Global Observation Research Initiative (GLORIA) sites in alpine environments have been established by G.B. Pant National Institute of Himalayan Environment and Sustainable Development in Chaudash Valley, Pithoragarh district, Uttarakhand, India in the year 2014. Till now 4 summits have been establish under GLORIA protocol and 121 species were recorded, which will be further monitored w.r.t. climate change over the years (Sekar et al., 2017). These LTEM studies were used to determine the long term ecological changes w.r.t climate change in higher altitudinal zones of IHR. The high altitudinal zones mostly covered with snow for longer period of time played an important role in Himalayan ecology and biodiversity (Tiwari et al., 2017).

CONCLUSIONS

This present study provides a comprehensive review

on studies conducted so far on climate change effect over biodiversity components in IHR. Majority of the studies have been performed over climate change effect on phenology, forest composition, agriculture, species distribution and climate projection.

There exists a clear gap in data on effect of climate change on various biodiversity components of IHR. For instance, data on the effect of climate change over microbial community, its function and distribution, fauna species, secondary metabolite production, RET species, etc. w.r.t. climate change need to be considered for future studies. Moreover, technological advances need to be considered for long term monitoring of climate and its effect over biodiversity. For instance, use of high-resolution remote-sensing technology need to be implemented for generating high accuracy data for monitoring and understanding ecosystem, vegetation and biodiversity pattern along with climate change.

Approaches need to be performed on modelling climate factors, which will help in developing long-term strategies to monitor climate change impacts and propose policy briefs/management strategies to manage biodiversity for mitigation of climate change in IHR. For ensuring the sustainable utilization of Indian Himalayan Biodiversity, there is a need to integrate climate change with biodiversity components. The IHR has been identified as data deficient by the IPCC, indicating the need to develop regional database and sharing mechanisms. Under this, an initiative has been taken on generating Himalayan Biodiversity and Climate Change Knowledge Network (HBCC-KN) which will provide a platform to share knowledge and build network among different stakeholders of IHR. Also, to reduce the scientific uncertainty, a more collective inter-institutional approach need to be followed to address emerging challenges of climate change on IHR biodiversity.

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Perception of Local Communities towards Ecosystem Services from a Community Managed Forest in a Part of Dhauladhar Range, North West Himalaya

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ABSTRACT: Ecosystem services (ESS) from forests play a significant role in sustaining livelihood in the Himalaya. Traditionally, lifestyle of villagers has helped in their sustained flow. However, habitat degradation, over-exploitation, land use and climate change have a negative effect on them. On one hand the forests are undergoing rapid degradation; on the other hand we still lack adequate knowledge regarding ESS flowing from them. Therefore present study was initiated to identify key ESS flowing from a community managed forest in the Dhauladhar range and document the perceptions of villagers on its status and flow of ESS. Focus group discussions, Participatory rural appraisal and semi-structured interviews were conducted to collect data. ESS have been classified following Common International Classification of Ecosystem Services. Twenty five ESS identified by the villagers include provisioning (energy, food and water), regulating (spring recharge) and cultural (temple of a local deity) services. Provisioning (100% respondents) and cultural (18.18% respondents) services from the ecosystems have high significance for the rural communities while recharge of spring and enhanced biodiversity (46.97% respondents) are important for downstream communities. Villagers use 98 plant species for various purposes. The forest has ability to provide basic ESS for the well being of over 300 human beings (upstream) and > 10,000 in the downstream area. Villagers (>80%) reported increased flow of ESS from the forest after community based management, however, spread of invasive species and crop raiding by wild animals (48.48% respondents) and lack of ecological monitoring has serious conservation implications for its long term sustenance. ©2019

KEYWORDS: Community; Ecosystem services; Forest; Himalaya; Management; Water.

INTRODUCTION

Ecosystem services are the benefits that people obtain from ecosystems and have been classified as provisioning services such as food, water, timber, and fiber; regulating services that affect climate, floods, disease, wastes, and water quality; cultural services that provide recreational, aesthetic, and spiritual benefits; and supporting services such as soil formation, photosynthesis, and nutrient cycling (MA 2005). Himalaya, with its unique ecological and landscape diversity helps in providing multiple supporting, provisioning, regulating and cultural services spanning across spatial and temporal scales (Macchi 2010). At local level forests support the agro-ecosystem of mountain communities and fulfill their resource needs for survival. At regional level it maintains the precipitation patterns, water supply and soil fertility. Globally, Himalayan ecosystems help in reducing the impacts of global warming and carbon storage. Its recognition as one of the 34 biodiversity hotspots of the world highlights its ecological importance (Myers 2000). Most of the Himalayan ecosystems are intrinsically interwoven; sustainability of one ecosystem defines the sustenance of other (Singh and

Singh 1992). Traditional ways of resource management and sustainable use of its resources in the past helped in its present day sustenance (Uniyal and Rawat in press). However, in the recent decades unsustainable land use changes, socio-economic transformations and impact of globalization among local communities has led to the rapid degradation of these ecosystems affecting the lives and livelihood of millions (Tiwari 2008). This has raised concerns globally among ecologists as well as economists (Naeem et al. 1994; Perrings et al. 1995; Costanza et al. 1997). It has been realized that replacement of natural resources lost is either not possible or economically very demanding. Himalayan ecosystems are undergoing rapid degradation and fragmentation leading to reduced flow of goods and services (Messerli 2009) and we have still limited knowledge about these ecosystems (Kremen 2005; Singh 2007). This has serious repercussions for the livelihoods of local communities. In order to promote the enduring and sustainable development of mountain areas, it is essential to identify and assess the ecosystem services they offer and develop local and regional level policies (Zisenis 2015). Recognizing this, the present study has been initiated as a part of National Mission on Himalayan studies (NMHS) to identify and assess the status of ESS flowing from a community managed forest in the Dhauladhar range (North west Himalaya).

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LITERATURE REVIEW

Interaction between biotic and abiotic components of ecosystems results in many ecosystem processes, which sustain and fulfill human life. They are called ESS. They always flow but are treated as services when human beings use them in various ways (Daily 1997). The flow of ESS is dependent on the health of the ecosystem. It is the structure and functioning of ecosystem that regulate the quality and quantity of ESS flowing from it (Norberg 1999) which is a key to human wellbeing and sustainable development (Adams 2014). The nature's services to humans came into limelight after their values were estimated in economic terms (De Groot, 1992; Costanza et al., 1997). It is recognized that if ESS are accounted for, the cost of most of the goods would increase manifold. World over, there has been an increasing realization regarding the need for assessment of ecosystem services and their governance (CBD, UNEP-WCMC 2012; Birch et al., 2014; Badola et al., 2015; Zisenis 2015). Efforts have been undertaken to develop efficient methods for identification, mapping, classification and valuation of ESS (MA 2005; TEEB 2008; Fisher and Turner 2008; Young and Potschin 2011, Rasul et al., 2011; Young & Potschin 2017). Value of an ESS depends on its reach and use by human beings both inside and outside the ecosystem. Therefore, to maintain a sustained flow of goods and services from ecosystems, their conservation and participatory management at the local level, valuation of the ESS and payment for ecosystem services by the beneficiaries to the suppliers of services is being recognized world over (Clements 2010). The concept of ESS has been more talked, however, progress in its practical application in land use planning and decision making has been slow (Naidoo et al., 2008). Only few places across the world, PES has been successfully implemented (Corbera et al., 2007; Asquith et al., 2008). In the Himalayan countries such as Bhutan and Nepal, the concept of ESS has taken a shape and has been widely implemented (Kubiszewski et al., 2013; Bhatta et al., 2015; www.iucn.org 2015). Studies dealing with the mapping and documentation of ESS in parts of Indian Himalayan Region (IHR) have also been accentuated (Semwal et al. 2007; Singh & Sundriyal, 2009; Joshi & Negi, 2011; Kubiszewski et al., 2013; Birch et al., 2014; Sharma et al., 2015, Vogt et al., 2016, Murali et al., 2017)). Economic value of ESS has been well understood by the Indian government hence 12th Finance Commission granted PES of Rs. 1000 crores to the state of Uttarakhand for the maintenance of the forest ecosystems (Singh 2007). Another example

of PES is from Palampur (a small town in district Kangra), where an agreement was signed between Palampur Municipal Council (PMC) and village forest development society (VFDS) in October 2010 for 20 years to protect the catchment of Bohl spring (Anonymous, 2013). According to which PMC (beneficiary of the spring water) pays a sum of Rs. 10,000 annually to the VFDS for the conservation of the spring recharge zone (GIZ, 2011). Currently, PES in this area only considers drinking water service; however, the area supports many other services. Bundling other ecosystem services, such as provisioning, recreation, biodiversity conservation, carbon sequestration with drinking water services, may further enhance economic returns to the local community. Recognizing this, the present study has been initiated as a part of National Mission on Himalayan Studies (NMHS) with an aim to assess the status of ESS in Bohl spring shed. The main objective of the study is to document the ESS flowing from the forest through social preferences, document the perception of local people towards the status of forest and flow of ESS after the implementation of management initiatives, to assess the role of these ESS in their livelihood and document the socio-ecological threats for the long term sustainability of forest management. The outcome of this study aims to help policy makers to fill in the gaps identified in the current management plan and develop strategies for its improvement so that the long-term flow of ESS is maintained.

STUDY AREA AND METHODS

Study Area

The study was conducted in Bohl Spring Shed (BSS; ca. 286 ha; 31°13'51.1" to 32°08' 31.8" N & 77° 55' 33.2" to 76° 32' 59.4" E) in the Dhauladhar Range, North west Himalaya. The Spring shed lies in district Kangra, Himachal Pradesh. The altitude of the intensive study site ranges 900–2100 masl. Bohl spring provides drinking water to the downstream Palampur town. The Palampur Municipal council (PMC) purchased rights to use this water from the villagers in the year 1952. There are three hamlets (Bohl, Mandai and Odi) of Bandla Panchayat inhabited by transhumant *Gaddi* community in its lower recharge zone (Fig. 1). The local communities depend heavily on the biomass resources of the forest. However, these forests are designated as 'Protected Forest' and are formally under the State Forest Department (Banihal, 2010). Forest (mainly comprising of oak-rhododendron) occupies 55.97% of the total catchment area. Excessive lopping for fuel wood, fodder and overgrazing in early 1990's resulted in the degradation of forest. This led to scarcity of resources for local people and reduction in the discharge of spring, which caused scarcity of drinking water for the population

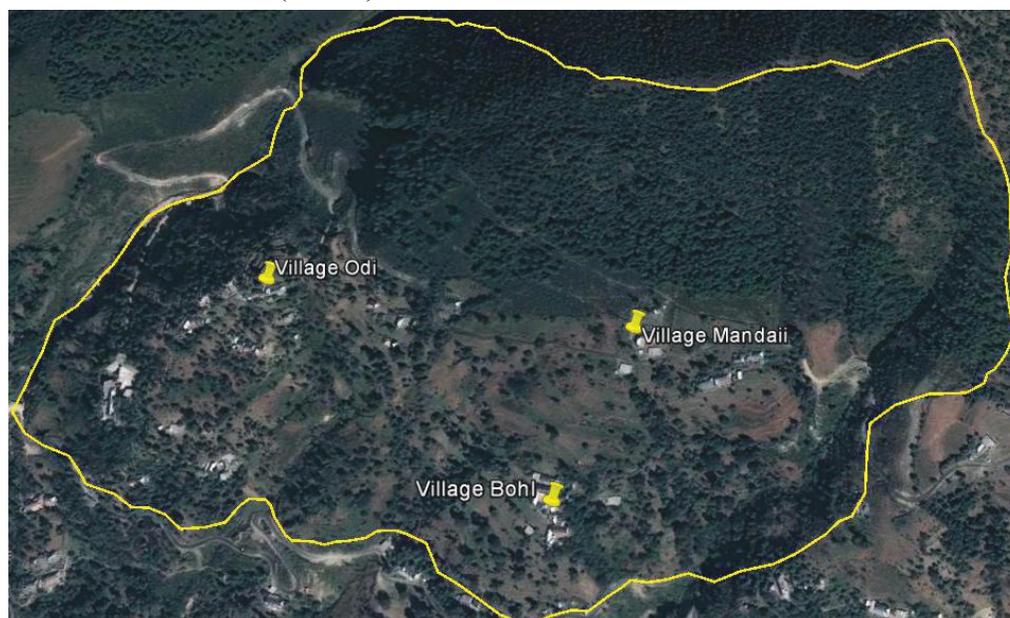


Figure 1: Map of the study area depicting location of study villages.

downstream. To overcome this, community forest protection started here in the year 1999 with the formation of village Mahila Mandal's (women self-help groups). Recently, community forest protection strengthened with the formation of Village Forest Development Society (VFDS). The forests are currently managed by the VFDS following the guidelines mentioned in the 20 years forest management plan. Of the sixty six families residing here, sixty are the members of VFDS and participate in the forest conservation activities. Ward Panch, PMC and Himachal Pradesh Forest Department are the other stakeholders of forest ecosystem.

Methods

Field surveys were carried out during August 2016 to August 2017. Prior to all group discussions and participatory rural appraisal, the concept of ESS (the benefits that the



Figure 2: Focus group discussions with the villagers.

ecosystems provide for human well-being) was explained to the local people who were already aware of nature's contributions to human well-being. Participatory rural appraisal including Focused Group Discussions (FGD, $n=5$) (Fig. 2), participant field observations and semi-structured interviews were conducted (Martin 1995) with the villagers to build confidence with the village communities and involve them in identifying different ESS, document their perception towards their flow and forest management activities. Group discussions were held in local dialect, facilitated by 2–3 local field assistant. People (mean number of people in each FGD = 30.5 ± 7.5) from all the three villages participated in these discussions. Household surveys ($n=66$ households, 100%) were carried out in the three hamlets using semi-structured questionnaires to get an unbiased respondent analyses. The interviews were restricted to individuals over 18 years of age as they are the one who participate in the forest related activities in the study area. Information on the socio-economic status and resource use patterns of the individual families, the perception of families towards pros and cons of forest conservation was collected. The data gathered through interviews was corroborated with the quantitative assessments of the resources collected by the people from forest following standard ecological methods (Ravindranath & Premnath 1997). Value estimate of the resources collected was based on the price of the resources in the local market. Preferences / perceptions of different respondents were measured on Conceptual Content Cognitive Mapping (3CM technique-a score based scale) following Kearney et al.

(1999) and Zahvoyska and Bas (2013). For classifying ESS, Common International Classification of Ecosystem Services (CICES) (Haines-Young & Potschin, 2011) has been followed. Descriptive statistics in MS Excel was used to analyze the data.

RESULTS

Key Ecosystem Services from the Forest

Key ecosystem services from the forest along with their perceived values given by the respondents on a 0–10 scale are given in Table 1. Though respondents identified a range of services, all of them recognized importance of provisioning services. Only 46% respondents identified

the regulating and 18 % recognized the cultural services of the forests. Of the provisioning services fuel wood was given highest score (9.82) followed by green fodder and lowest score was given to material for brooms and ropes (0.47). Other NTFPs such as timber, subsidiary food, medicinal and aromatic plants were given less scores as compared to fuel wood and fodder. A total of nine regulating services were identified by the villagers. Of these, spring recharge was given the highest mean score (9.19) followed by air purification (8.94) and regulation of soil moisture (5.98). Spring recharge has a regional reach as water from the spring is supplied to approximately 5000 residents and over 10,000 floating population of the downstream Palampur town. Most of the respondents (8.35) recognized that forests

Table 1: Key ecosystem services from the community forest as identified and perceived by the primary stakeholders

ESS	% Response (n=66)	Types	Sub-Types	Score ±SE	
Provisioning	100	Food	Green Fodder	9.36 ± 0.20	
			Dry Fodder	8.33 ± 0.29	
			Water	5.27 ± 0.44	
			Wild food	1.55 ± 0.21	
			Aromatic herbs	0.79 ± 0.09	
			Medicinal Plants	1.59 ± 0.22	
		Material	Bamboo for baskets/mats	3.76 ± 0.35	
			Wood for agricultural implements	1.35 ± 0.19	
			Timber for houses	0.76 ± 0.07	
			Poles for fencing	3.45 ± 0.36	
			Grass for broom/ropes	0.47 ± 0.07	
			Energy	Fuel wood	9.82 ± 0.04
				Flow Regulation	46.97
Regulating	46.97	Flow Regulation	Spring recharge	9.19 ± 0.15	
			Regulate soil erosion	7.49 ± 0.35	
			Purify air	8.94 ± 0.14	
			Purify water	7.21 ± 0.34	
			Soil moisture	5.98 ± 0.35	
			Soil fertility	6.8 ± 0.31	
			Regulation of physical environment	8.35 ± 0.21	
			Regulation of biotic environment	8.89 ± 0.15	
			Regeneration of native species/nursery function	8.42 ± 0.18	
			Cultural	18.18	Sacred
Aesthetic/experiential	Satisfaction for pure environment	6.76 ± 0.32			
Education	National/international students visit to study community management	5.076 ± 0.35			
Tourism	Nature lover	6.53 ± 0.36			

Table 2: Socio-economic profile of the three (3) study villages

Parameters	Bohl	Mandai	Odi
Location	32° 8' 16.778" N 76° 32' 59.853" E	32° 08' 21.038" N 76° 33' 06.866" E	32° 08' 31.8" N 76° 32' 59.4" E
Altitude	1598 m	1697 m	1672 m
Distance from road head	0–0.5 km	1.0 km	0–1.0 km
Distance from forest	0.5–1.0 km	0.1 km	0.1–1.2 km
Households	27	10	29
Total Population	133	42	138
Total Livestock Units	145.75 LU	15.85 LU	154.05 LU
VFDS members	25	10	25
Major Occupation	Agro pastoral	Other (driver/maid/worker)	Agro pastoral

have important role in regulating local rain and snowfall. They also reported that forests serve as natural habitat for wild animals such as goral, barking deer, sambar, and wild pig. Cultural services of the forest were given high scores (6.76%). A temple of local deity “Bheerni mata” is located close to the forest and highly revered and visited by the local people. Moreover, this springshed is frequently visited by a large number of researchers and students from far and wide to understand the mechanism of PES. Of the four cultural services identified, presently only one (educational) has a wide (national and international) reach while other services (sacred, experiential and tourism) have only local reach. The regulating and cultural services have indirect use values for the downstream stakeholders. However, the provisioning services have direct use values for the villagers.

Socio-Economic Structure of the Villages and their Resource Dependency

A socioeconomic profile (Table 2) of the three villages (Bohl, Mandai & Odi) reveals that there are a total of sixty six families residing here. Odi is the biggest village with 29 households and Mandai smallest with 10 households. Bohl is a medium size village with a population of 133. Bohl-Mandai- Odi has a geographical area of ca. 43 hectares and a total population of 313. Of these, 48% are males and 52% are females. The inhabitants primarily belong to trans-humant Gaddi community. Their main occupation has been animal husbandry since generations. Livestock composition of the village shows the dominance of sheep and goats i.e. 212.85 livestock unit (LU, 1LU is equivalent to 1cattle), followed by cattle (94.80 LU) remaining are the mules/horses. Land holdings are marginal and with limited production that meets only 30% of food grain requirement of the household per year. Most (65%) of the arable land is maintained as grazing lands and only 35% of this is under rain fed agriculture.

Main crops grown are barley, maize, wheat, and some vegetables such as potato, radish and beans.

All the households depend heavily on surrounding forests for fuelwood, fodder and NTFPs. Though, more than 80% houses have cooking gas supplied by the government at subsidized rate (Rs. 484 per 14.2 kg LPG cylinder), all the households prefer to store fuel wood as it is freely available and it helps them to heat the rooms during winter season. Per capita consumption of fuel wood is 3.15 kg/day during summers and 9.5 kg/day in winters. In terms of monetary value this amounts to approximately Rs. 10,000 per capita per annum. Twenty one species of woody plants, e.g., white oak (*Quercus leucotrichophora*) and rhododendron (*R. arboreum*) are collected as fuel wood. Only dead and fallen wood is collected, as cutting of trees is strictly banned in the community managed forest. A transformation in the livestock composition of Gaddis has been observed with more of bovine replacing sheep/goats. With reduced area under crop cultivation, availability of agricultural by-products as fodder has also reduced. This has significantly increased their dependency on the surrounding forest for green fodder and dry hay. The collection of leaf and grassfodder from the community managed forest is allowed only for 15–20 days in winter months. Of the total sixty six families, fifty eight families collect both green (45.27 kg/day/ head) and grass fodder or hay (22.88kg/ head/day) from the forest edges. Fodder (green and dry fodder both) worth Rs. 26,000 per livestock unit is collected annually from the forest. Dry fodder collected fulfills the demand for three months and for the remaining months villagers have to buy hay at a rate of Rs. 7/ 1.5kg. Of the 61 species collected in total for fodder purpose, 21 are collected as green (leaf) fodder and 34 as hay. Of the total 98 species collected

Table 3: Magnitude of key provisioning services derived by the villagers from the community managed forest

Provisioning Service	Number of Species	Quantity consumed kg/capita/day	Yearly consumption (kg/capita)	Market value (Rs./kg)	Total value (Rs.) capita/year
Fuel wood	21	6.33	2310.45	4.54	10,489.44
Green Fodder	27	15.09	2746.38	5.00	13, 731.9
Dry Fodder	34	7.62	2781.3	4.6	12, 793.98
Timber	3	Occasional	NE	NE	NE
NTFP	44	Occasional	NE	NE	NE

by villagers for various purpose, maximum (44) are used as NTFP’s that are collected occasionally and only three species are used as timber wood (Table 3).

The forest is rich in socially and ecologically important species such as Banoak (*Quercus leucotrichophora*), Moru oak (*Quercus floribunda*), Kaith (*Pyrus pashia*), Pajja or Padam (*Prunus cerasoides*), Burans (*Rhododendron arboreum*), and Deodar (*Cedrus deodara*) among others. Analyses of villager’s perception towards status of forest revealed an increase in the flow of services (86%) such as spring discharge, quality and quantity of fuelwood/ fodder, litter deposition, regeneration of native species

after community forest management. It was also revealed in the sampling of vegetation (Uniyal, unpublished data). Plots completely under protection had high species density (40.1 ± 13.43 trees/10m²) and richness compared to forest edges (3.2 ± 5.21 trees/10m²) where high infestation by invasive species has not only hampered the regeneration of native species but also degraded the quality of soil. Around 49% respondents reported that spread of invasive plant species and crop raiding by wild animals is emerging as a major ecological problem that may pose a threat for forest conservation and management in the near future (Table 4). Besides, lack of capacity building and awareness programs

Table 4: Response of villagers towards forest management

ESS	% Response (n=66)	Indicators	Score ±SE
Current status of forest		Forest is dense	9.18 ± 0.05
Very Good	81.81	Wild animals increased	9.08 ± 0.17
Good	18.18	Regeneration of oak	7.96 ± 0.18
Degraded	0	Leaf litter is rich	5.17 ± 0.33
Flow of Services		Quality & quantity of fuel wood and fodder enhanced	9.74 ± 0.06
Increased	86.36	Spring recharge enhanced	9.18 ± 0.11
		Mud erosion is less	6.86 ± 0.33
Maintained	13.64	Bamboo availability increased	6.59 ± 0.33
		Travel time for collection reduced	8.31 ± 0.17
Emerging Problems			
Invasive species	10.61	<i>Eupatorium adenophorum</i> & <i>Lantana camara</i> are spreading fast	9.09 ± 0.18
Wild animals	28.78	Crop raiding by wild pig and ungulates	7.24 ± 0.35
Both	48.48	Few cases of leopard attack on livestock	0.92 ± 0.14
Economic burden	7.58	Monthly charge for forest guard’s (Rakha) salary from each family	7.36 ± 0.36
		Fine from members not following rules	1.33 ± 0.33
Participation of other stakeholders		Invasive species eradication program and check dams done by FD	8.27 ± 0.16
		Plantation and Hedging of forest not yet planned	0.61 ± 0.14
Yes	10.61	Monitoring of biodiversity and wild life by FD not done	0.56 ± 0.11
No	89.39	Monitoring Spring discharge not done	0.16 ± 0.05
		Awareness & training program for local people not conducted by PMC	0.06 ± 0.03

involving other stakeholders (89% responses), economic burden on socio-economically weak families due to monthly contribution towards forest guard salary and inclination of young generation towards market oriented occupation are some other factors waning interest for forest management activities.

DISCUSSION

Present study revealed that forests delivered a total of twenty five goods and services in the Bohl springshed that have local as well as regional significance. Involvement of community participation in the forest management in the recent years has helped in the rejuvenation of forest. Similar findings have also been reported by Paudyal et al. (2015). They revealed that community managed forestry has substantially helped in the restoration of degraded lands which has a positive impact on the flow of ESS in Nepal. In the present area also, a small patch of intact forest currently supports energy, water and food requirements of over 300 villagers and >10,000 people in the downstream area (Uniyal & Rawat, in press). Income per family in the three villages is about Rs.99,000/annum, of which income based on livestock contributes significantly (30%). Hence the role of forest in sustaining their livelihood becomes even more important. Because fodder worth Rs 26,000/LU and fuel wood worth Rs. 10,000/capita is collected annually from the forest. These estimates are comparable with the studies done in other parts of the Himalaya (Joshi & Negi 2011, Dhyani & Dhyani, 2016). If these provisioning services are accounted for, the economic burden on villagers would increase substantially. Thus for the livelihood of villagers continued flow of these ESS play an important role in the present area as in other parts of the Himalaya (Sharma *et al.*, 2015). With the restoration of degraded forest in the Bohl spring shed the current vegetation and soil parameters reveal a healthy sign (Uniyal, unpublished data) that may be one of the reason for enhanced flow of ESS (Table 3). Regeneration of oak, rhododendron and deodar is very good in the protected forest as compared to the adjacent unprotected area (Uniyal, unpublished data). The area is also a good habitat for many medicinally important under storey species such as *Zanthoxylum armatum*, *Berberis asitatica*, *Valeriana wallichii*, and *Prinsepia utilis*. Respondents revealed that forest conservation has not only improved the status of provisioning services but also helped in spring discharge, increased soil moisture, regulating local rain and snow pattern. Discharge of spring water which was decreasing few years back due to forest degradation has substantially improved after community protection (Agarwal et al. 2007). The mud slide and erosion has also been regulated after the conservation of forest, however, no

empirical data is yet available to relate it ecologically. On one hand ecological measurements of water discharge, soil moisture and valuation of its services has been emphasized for the well being of humans (Garrick et al. 2017), on the other hand such studies are still lacking in the IHR. In the present area spring discharge is the reason for PES, however; none of the stakeholder is monitoring its flow till date (personal communication). Carbon sequestration by forest is another important service that has global implications (Yu and Han 2016). Forest carbon pool in Indian Himalaya is reported to be 5.4 billion tons. It is known that one hectare of forest helps in 3.7 tons carbon sequestration in a year (Singh 2007). So the role of ca. 160 hectares of conserved forest in the Bohl spring shed in sequestering carbon is quite perceptible. It is reported that trading of carbon credits with the involvement of local people has not only improved economic returns but also the livelihood options of the villagers (Banskota et al. 2007; Singh 2007). In the present area where there is a vast scope of carbon trading, such initiatives are still lacking in the management plan. Involvement of other stakeholders in capacity building and awareness programs is also weak, causing resentment among villagers. Conflict among people of different socio-economic backgrounds over the amount fixed as PES, fine charged and monthly contribution towards salary of forest guard has also been reported (Kovacs et al. 2016) and it was also evident during village meetings in the present study. Another emerging ecological problem in the area is the spread of invasive species such as *Eupatorium adenophorum* (Fig. 3) and *Lantana camara*. Approximately twenty hectares of land is degraded due spread of these species. This has severely hampered the regeneration of native



Figure 3: Spread of *Eupatorium adenophorum* in the forest ecosystem.

species in the forest edges and affected the soil quality (Uniyal, unpublished data). This is a cause of concern for the sustained flow of ecosystem services in the near future. It has been observed that the interplay of invasive species often results in diminishing other services of the nature (Wilcove et al. 1998). Degradation of ESS is well known to increase economic costs putting burden on economically backward societies. Hence, programs for scientifically eradicating invasive species and awareness generation for the local communities must be conducted on a priority basis. Besides, restoration of the cleared sites by planting multipurpose grass, fuel wood and fodder species should also be taken up simultaneously to strengthen the continuous flow of ESS from the forest.

CONCLUSIONS

The community managed forest in the present study area support numerous ESS which have local, regional as well as global significance, however, the health of ecosystem is threatened due to spread of invasive species and lack of awareness programs which may affect the flow of ESS from this forest in the near future.

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SECTION 5

Himalayan Mammals and Avian Diversity



Feeding Ecology and Methods to Study the Diet of the Red Fox (*Vulpes vulpes*)

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ABSTRACT: This review paper pertains to the feeding habits of the Red Fox *Vulpes vulpes* and various methods used for the study. It is essential to know about the diet of a species across its distributional range as it answers several questions like the distribution and abundance, solitary and group living behaviour, mating strategies as well as adaptability to the dynamic environment. The methodology of studies reviewed in this study deals with either scat analysis or the analysis of food content from stomach. While the former is non-invasive, replicable and permits a greater sample size, the latter can be done only after the death of the animal. For this purpose 145 scientific papers were reviewed, which were searched following a combinations of keywords: 'Vulpes vulpes' or 'red fox', 'diet' and 'feeding' or 'foraging'. Diet composition of a generalist predator, the red fox consists of a huge variety of food articles. The data from various papers confirms the opportunistic foraging habits of the Red Fox *Vulpes vulpes*. A total of 105 food items have been reported to be consumed by the red fox of which 12 food items were exclusively reported only from the stomach content analysis. The wide spectrum of food depends on the local food supply throughout its worldwide habitats. ©2019

KEYWORDS: Red Fox; Diet; Feeding; Carnivore; Scat analysis.

INTRODUCTION

Canids are a diverse group of carnivores with a wide range of body weights (1–80 kg), dietary habits (omnivory to strict carnivory), and habitat preferences from extreme deserts to ice fields to rainforests (Geffen et al. 1996). The 35 species of canids in the world represent a family of predators, many of which hunt on the run. Small sized canids or the foxes are the most adaptable and opportunistic feeders. The grey wolf was originally the most widely distributed terrestrial mammal; its successor to the throne is now another successful canid, the red fox. Red fox is the largest fox in the genus *Vulpes*. Distributed across the entire northern hemisphere from the Arctic Circle to North Africa, Central America, and the Asiatic steppes, the red fox has the widest geographical range of any member of the order Carnivora covering an area of nearly 70 million km² (Claudio Sillero-Zubiri et al., 2004) (Fig. 1) and is one of the most extensively studied carnivores (Nowak, 1999).

However in India where the mountain red fox is found in the Himalayan region, there is a dearth of knowledge pertaining to this animal. Despite living in human-dominated landscapes, red fox is rarely sighted owing to their highly elusive and secretive behavior.

Feeding ecology is one of the most studied features of carnivore ecology. It is interesting to understand the feeding ecology of this animal as it answers several questions. The

reason why the foxes live alone than in groups was given by Macdonald, 1983 emphasizing that foxes kill their prey that is the rodents by ambushing and stealth which can be better done when alone. In resource scarce environment, the territories of red foxes were larger and group smaller whereas when red foxes are exposed to rich food supply, they lived in territorial groups composed of one male and several females (Macdonald 1983). Foraging is mainly nocturnal and crepuscular, although more diurnal where they are undisturbed. Food availability is the primary factor for limiting fox numbers (Lindstorm 1989). In a notable study, (Zabel and Taggart 1989) it was observed that there was a shift in the mating system from polygamy to monogamy associated with scarcity in the food resources. Dispersal in the red foxes also depends on food resource (Errington 1935). The diet of red foxes has been studied in rural areas (Sequeira 1980) and towns (Harris 1981). The seasonal trend in the diet of foxes living in the rural areas was more pronounced as compared to foxes living in London (Harris 1981). The proportion of scavenged food items was found to be the highest in the diet of red fox by Doncaster (1990). In many habitats, foxes appear to be closely associated with man, even thriving in intensive agricultural areas (Claudio Sillero-Zubiri et al. 2004). Reshamwala et al. (2018) also reported the role of human culture and religion in the Trans-Himalayan region affecting the foxes diet and occurrence. Optimal foraging theory emphasises that animals promote their fitness by foraging in ways that maximise their net rate of energy gain (MacArthur and Pianka 1966). It is probably the red fox's generalist conformation and lack of specialist adaptations that makes it the widely successful species.

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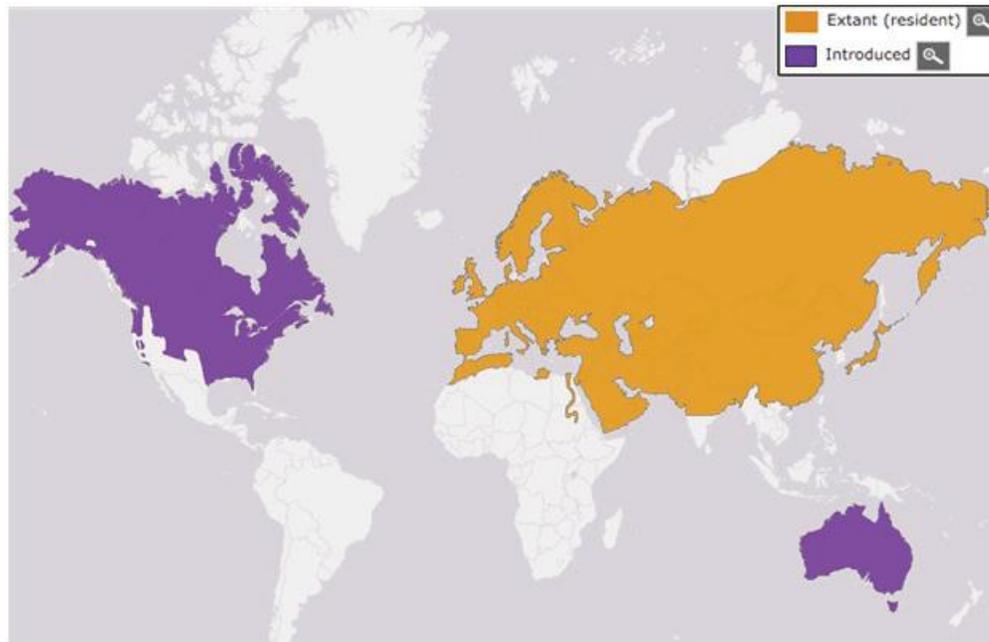


Figure 1: Distribution of the Red Fox across the Globe (IUCN, Hoffmann & Sillero-Zubiri, 2016).

METHODOLOGY

An exhaustive review of 145 scientific papers (Appendix I) from various sources of information was used to compile and comprehend the available literature. Web of Science and Google Scholar search engines were used to obtain the relevant scientific studies containing information about the trophic feeding ecology of the red fox worldwide. Search was carried out for the terms that were identified using the following combinations of keywords: ‘*Vulpes vulpes*’ or ‘red fox’, ‘diet’ and ‘feeding’ or ‘foraging’. The compiled total of 145 published studies concerning the diet of the red fox spanned over a period from 1932 to 2018. Most authors reported data seasonally, however, a few reported data pooled annually and several provided both annual and seasonal data and by classifying seasons into spring, summer, autumn and winter.

RESULTS

To study the foraging ecology of red fox most of the studies deal with either scat analysis or the analysis of food content from the stomach. Each of the ways has its own advantages.

Scat Analyses

In the reviewed papers the sample size of number of scats ranged from 58 (Dell’Arte et al. 2007), to as high as 2242 (Nentvichova et al. 2009). As a standard procedure in scat analysis the scats labelled with their GPS, date, and other information collected in zip lock plastic bags are first dried at 100°C. These are then washed in running water with the

help of sieve of 1mm mesh or smaller and the undigested remains are exploited to understand the diet. In an eminent study the diet of red fox was studied in the Sumava Mountains on the basis of scat analysis (2242 specimens) for over ten years (1998 to 2008) by Nentvichova et al 2009. The faecal samples were collected at about one month intervals. The samples were consequently divided into those collected during the growing season (April 16 to October 15) and the non-growing season (October 16 to April 15). Standard methods were used for samples analyses (Kozena 1988; Reynolds and Aebischer 1991). The mammal species were determined by teeth, remainders of skeleton and hairs. Mammal hairs and bird feathers were identified using a binocular magnifying glasses and field guides (Day 1966; Dziurdzik 1973; Teerink 2004) and with the help of macroscopic and microscopic comparative material obtained before the samples of faeces were analysed. The comparative material was obtained from carcasses of animals killed by a predator in the area of the study plots and from museum collections. Unidentifiable objects and remnants of other vertebrate species were consulted with specialists. Specimens of the family Lumbricidae were identified through the use of their bristles. The identification of beetles (Coleoptera) was based on elytrae, antennae, legs or parts of the head capsule with mouth parts. The Orthoptera species were determined by the remnants of their limbs. The categorization of the food items varies greatly based on the area and the objective of study, however, broadly they are classified as follows: 1) birds

2) rodents 3) insectivores 4) lagomorphs 5) wild carrion, 6) livestock carrion, 7) plants 8) Human-derived material (HDM) and 9) others.

Stomach Content Analysis

A variety of methods are employed to obtain the specimens including organized drives, trapping, fumigation of dens, and night shooting with the aid of spotlights. Most of the foxes are usually captured in steel traps by commercial fur trappers and the animals are killed shortly after capture and a scientist accompanying the trappers would immediately remove the stomach and preserve its contents in 10% formalin for subsequent laboratory analysis. In certain cases stomachs are removed and stored frozen within 12 hours of killing the animal until further analysis. Sometimes the stomach contents are

also supplied by hunters and local game wardens. Foxes are usually trapped during autumn, winter, spring and summer for the sake of completing a full annual cycle. Date, time, area of capture, and size of the animal are also recorded. At the laboratory, the stomach contents are weighed, examined and categorized into main items. The process of identification of food contents varies from place to place as the diet changes to a great extent. In an eminent study where 70 stomach contents were analyzed by Mohammad Basuony in Egypt, 2005 the following methodology was used plant content was identified by first collecting them from the different study sites and then with the help of epidermal remains found in the stomach. Storr's (1961) method was used to prepare the reference slides. On the basis of scale morphology and toe structures, reptiles were identified whereas birds with the help of skull morphology and feathers. In case of small

Table 1: List of all food items (species) found in the diet of the Red Fox

Species	Species	Species	Species	Species
List of food items found from scat analysis				
<i>Erinaceuseuropaeus</i>	<i>Rodentia sp.</i>	<i>Carabidae sp.</i>	<i>Tetraourogallus</i>	<i>Egg shell</i>
<i>Talpaeuropaea</i>	<i>Mustela sp.</i>	<i>Pyrus sp.</i>	<i>Cantharidae sp. larvae</i>	<i>Scarabaeidae sp. larvae</i>
<i>Sorexaraneus</i>	<i>Martes sp.</i>	<i>Cervidae sp.</i>	<i>Rubusidaeus</i>	<i>needles of conifers</i>
<i>S. minutus</i>	<i>Melesmeles</i>	<i>Carabidae sp. larvae</i>	<i>G. gallus</i>	<i>Zootoca vivipara</i>
<i>S.alpinus</i>	<i>Amphibia sp.</i>	<i>Malus sp.</i>	<i>F. domesticus</i>	<i>Coleoptera sp.</i>
<i>Crocidurasuaveolens</i>	<i>Hymenoptera sp.</i>	<i>Ovisammon</i>	<i>Geotrupidae sp.</i>	<i>pebbles and soil</i>
<i>Soricidae sp.</i>	<i>Felissilvestris</i>	<i>F. aries</i>	<i>Sorbusaucuparia</i>	<i>Lacertidae sp.</i>
<i>Lepuseuropaeus</i>	<i>F. catus</i>	<i>Byrthidae sp.</i>	<i>Galliformes sp.</i>	<i>Formicidae sp.</i>
<i>Oryctolagusuniculus</i>	<i>Cottusgobio</i>	<i>Vacciniummyrtillus</i>	<i>Staphylinidae sp.</i>	<i>Plastics</i>
<i>F. domesticus</i>	<i>Orthoptera sp.</i>	<i>Capra aegagrus</i>	<i>Avena sativa – grain</i>	<i>Ranatemporaria</i>
<i>Sciurus vulgaris</i>	<i>Carnivora sp.</i>	<i>F. hircus</i>	<i>Anasplatyrhynchos</i>	<i>Myrmica sp.</i>
<i>Muscardinusavellanarius</i>	<i>Cypriniformes sp.</i>	<i>Cuculionidae sp.</i>	<i>Chrysomelidae sp.</i>	<i>indigestible items</i>
<i>Arvicolaterrestris</i>	<i>Prunusdomestica</i>	<i>V. vitisidaea</i>	<i>Triticum sp. grain</i>	<i>Celtisaustralis</i>
<i>Microtusarvalis</i>	<i>Susscrofa</i>	<i>Bosprimigenius</i>	<i>Anseriformes sp.</i>	
<i>M. agrestis</i>	<i>Lumbricidae sp.</i>	<i>F. taurus</i>	<i>Cerambycidae sp.</i>	
<i>M. subterraneus</i>	<i>Prunus sp.</i>	<i>Silphidae sp.</i>	<i>Zea mays – grain</i>	
<i>Microtus sp.</i>	<i>Cervuselaphus</i>	<i>V. uliginosum</i>	<i>Passeriformes</i>	
<i>Clethrionomysglareolus</i>	<i>Helicidae sp.</i>	<i>Bonasabonasia</i>	<i>Elateridae sp.</i>	
<i>Rattusnorvegicus</i>	<i>Cerasusavium</i>	<i>Cantharidae sp.</i>	<i>Poaceae sp.</i>	
<i>Apodemus sp.</i>	<i>Capreoluscapreolus</i>	<i>Vaccinium sp.</i>	<i>Elateridae sp. larvae</i>	
List of food items found exclusively only from stomach content analysis				
<i>Ficuscarica</i>	<i>Salix safsaf</i>	<i>Phoenix sp.</i>	<i>Stenodactylus sp.</i>	<i>Chalcedisocellatus</i>
<i>Tomato sp.</i>	<i>Acacia sp.</i>	<i>Arundo sp.</i>	<i>Trapelusmutabilis</i>	<i>Psammophisshokari</i>
<i>Phragmites sp.</i>	<i>Capsicum annum</i>	<i>Scorpions</i>	<i>Laudakiastellio</i>	

mammals, hair morphology, teeth and whole or tail when available helped in the identification process. Insects could be identified upto the order level and the frequency of occurrence of animal and plant food items. Insects could be identified to the order level.

In another notable study where 509 stomach contents were analyzed by Saunders et al., 2004 in Australia the following methodology was adopted: –The stomach contents were washed in running water and the indigestible matter was separated using a sieve. Whole rabbit pieces such as ears and feet helped in the identification. The diet was subsequently analyzed with the help of microscopic examination of hair (Brunner and Coman 1975) and estimating the volume of each food item ingested.

Analysis for the Diet

The red fox diet is analysed using the frequency of occurrence (FO), defined as the proportion of scats containing a given food item with respect to the total sample size (Leckie et al. 1998). Even though this is a common way to present results of diet analyses, and it is useful in order to test how often a given food item was ingested, this method is not a good estimator of the nutritional importance of the different prey categories. All methods for extrapolating from occurrence in faeces to biomass or energy consumed are fraught with a range of errors and assumptions (Reynolds and Aebischer 1991). In order to obtain an estimate of the contribution in terms of the volume of each prey species, the method of estimated ingested volume (EIV) is used which is described by Kruuk and Parish (1981). This method has been widely used in meso-predator diet studies (Lucherini and Crema 1994). However, in order to compare the relative contribution of different prey categories to the estimated total volume ingested, the EIV have been converted into whole scat equivalents (WSEs) described by Angerbjorn et al. (1999). The WSE is a modified FO calculated with the estimated volume (EIV) of each prey category. This method has an advantage of maintaining the same unaltered sample size and, thus, allows for a direct comparison of the different proportions ingested. In order to combine FO with volume and, thus, provide a better visualization of the composition of a typical red fox meal, the average volume (AV) of each food item in a scat, defined as the total volume of a given food item in all scats divided by the number of scats containing that food item was estimated (Calisti et al. 1990).

Formulas

1. Frequency of occurrence (FO) = Of each food item in the faeces is calculated for each species using two methods:

$$(a) \text{ FO} = 100 \times \frac{\text{the proportion of faeces containing a certain food item}}{100}$$

$$(b) \text{ FO} = \left(\frac{\text{The frequency of each food item}}{\text{The total no. of occurrences of all food items}} \right) \times 100$$

$$2. \text{ Percentage of Volume} = \left(\frac{\text{Volume of a food item}}{\text{Total value of all food items}} \right) \times 100$$

$$3. \text{ Biomass Consumed} = \left(\frac{\text{Dry weight of remains of prey item} \times \text{Digestibility coefficient}}{\text{Total biomass}} \right) \times 100$$

Diet of Red Fox

A significant difference in the diet composition of red foxes between winter and summer seasons has been reported in most of the cases. In majority of the studies including both scat and stomach content analysis the food ingested was classified into birds, mammals, reptiles, invertebrates and plant material. Mammals are sometimes further classified. A large number of food articles were reported in a detailed study by Nentrichova 2009 through scat analysis (2242 samples). The study describes 88 taxa in the red foxes diet that were classified into 17 categories namely- Insectivora, Rodentia, Carnivora, Lagomorpha, Artiodactyla, Galliformes, Aves, Reptilia, Amphibia, Osteichthyes, Lumbricidae, Mollusca, Coleoptera, Other Insects, Fruits, Plant material and indigestible items. This list is comprehensive and covers all of the species reported from other studies as well (Table 1). The differences among seasons exactly reflected the availability of particular food items. As expected, there was a higher food diversity in the growing season due to additional food components such as fruit and insect as compared with the non-growing season. On the other hand, they become scarce during winter and are found minimally in the scat samples. Insects and other invertebrate species and fruit are well accessible only during a certain period of the year and were more prominent in studies which analysed the stomach content. For example, in the study by Basuony et al., 2005 in Egypt which followed the stomach content analysis the following plant materials were found- tomato, Ficus carica, Capsicum annum, Arundasp, Phoenix sp, Zea mays, Acacia sp, Salix safsaf, Phragmites sp. (Table 1).

DISCUSSION

Scat versus Stomach Content Analyses

Scat analysis is proved to be cost-effective, non-destructive

and most efficient by obtaining large number of samples. Concomitantly, Webbon et al. (2004) also suggested that faecal counts could be utilized to apprehend the relative fox density, especially in areas with low rainfall where the rate of decomposition of scats is low. Analysis of stomach content on the other hand, can be fruitful as it would give a better insight on the ingested food, as completely digested food is often missing in the scat. For example quantifying plant contents from scat and its actual proportion ingested is very difficult to determine. This was well reported by Basuony et al. (2005) in Egypt who found potato, tomato, dates, corn, capsicum etc. which are not reported in any of the studies which used scat analysis. For further analysis, percentage of occurrence often overestimates food items such as insects and fruits because of the lower digestibility of insect chitin and fruit seeds and their distinctive presence in the scats. Although food items like insect and fruit may be frequently present, lower biomass of such items may have been consumed in actual. Bigger prey items such as livestock carrion may also be underestimated due to their higher digestibility and lower hair representation in the scats as compared to rodents. Hence examining both the percentages of occurrence and biomass consumed gives a better understanding of the actual diet of red fox.

Diet

Based on published literature, the Red Fox is essentially an omnivore. There is a great diversity of food items like invertebrates such as crickets, beetles, earthworms, crabs, small mammals, birds, fishes, carrion and fruits (Flower 1932; Macdonald 1983). Rodents were found to be the main prey of the red fox. To maximize the net energy gain foxes also tend to hunt easy prey such as domestic farm animals like chicken, rabbit, pigeon and goat. Feeding on carrion would be cost effective. Anthropogenic food resources, including agricultural products, unmanaged waste and kitchen offal, are also getting increasing recognition in the diet of animal populations (Oro et al. 2013). Increased amount of indigestible items signifies the human presence. The incidence of big predators for example the lynx, wolf etc. causes that red fox to feed more on ungulate remnants coming from carcasses. The consumption of alternative prey is independent of its availability, and increases only when relative benefits of harvesting alternative food (e.g. fruits) increases, which is consistent with optimal foraging theory (Martinoli et al. 2001).

CONCLUSION

Scat analysis is the most widely used method for the red fox diet studies. Being non-invasive and economical, this method also offers a greater number of samples. It can also be used to cover larger landscapes and can be replicated easily temporally and spatially. The advantage of understanding the feeding habits of the red fox through the stomach contents is that the chances of missing anything that is ingested and might be completely digested and absent in the scats is out ruled. However, in studies pertaining to analysis of stomach contents the greatest disadvantage is perhaps the killing of the red foxes. The diet results confirm the generalist and opportunistic behavior of the red fox. It will exploit all sorts of food resources based on availability and season. One could also predict that items rich in sugar such as dates will be favored as immediate sources of energy when caloric requirements are greatest. Anthropogenic food subsidies are also playing an important role in determining the red foxes diet and its occurrence (Reshamwala et al. 2018). The trade-off between costs and benefits that gives maximum net benefit is what determines a predator's choice of prey species according to the optimal foraging theory (Krebs and Davis 1993). The dietary choices of small carnivores such as the Red Fox will depend primarily on the temporal variation of foraging costs (Zielinski 1988), which are mainly affected by availability of the primary prey species. Understanding these patterns in the feeding ecology of the red fox, the most abundant carnivore in the world (Aubry et al. 2011) will facilitate the understanding of the geographical variations in its abundance and behaviour, and improve the management and conservation of this species.

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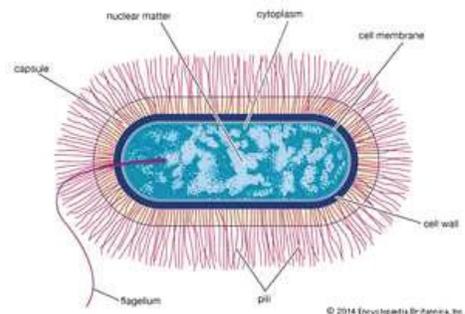
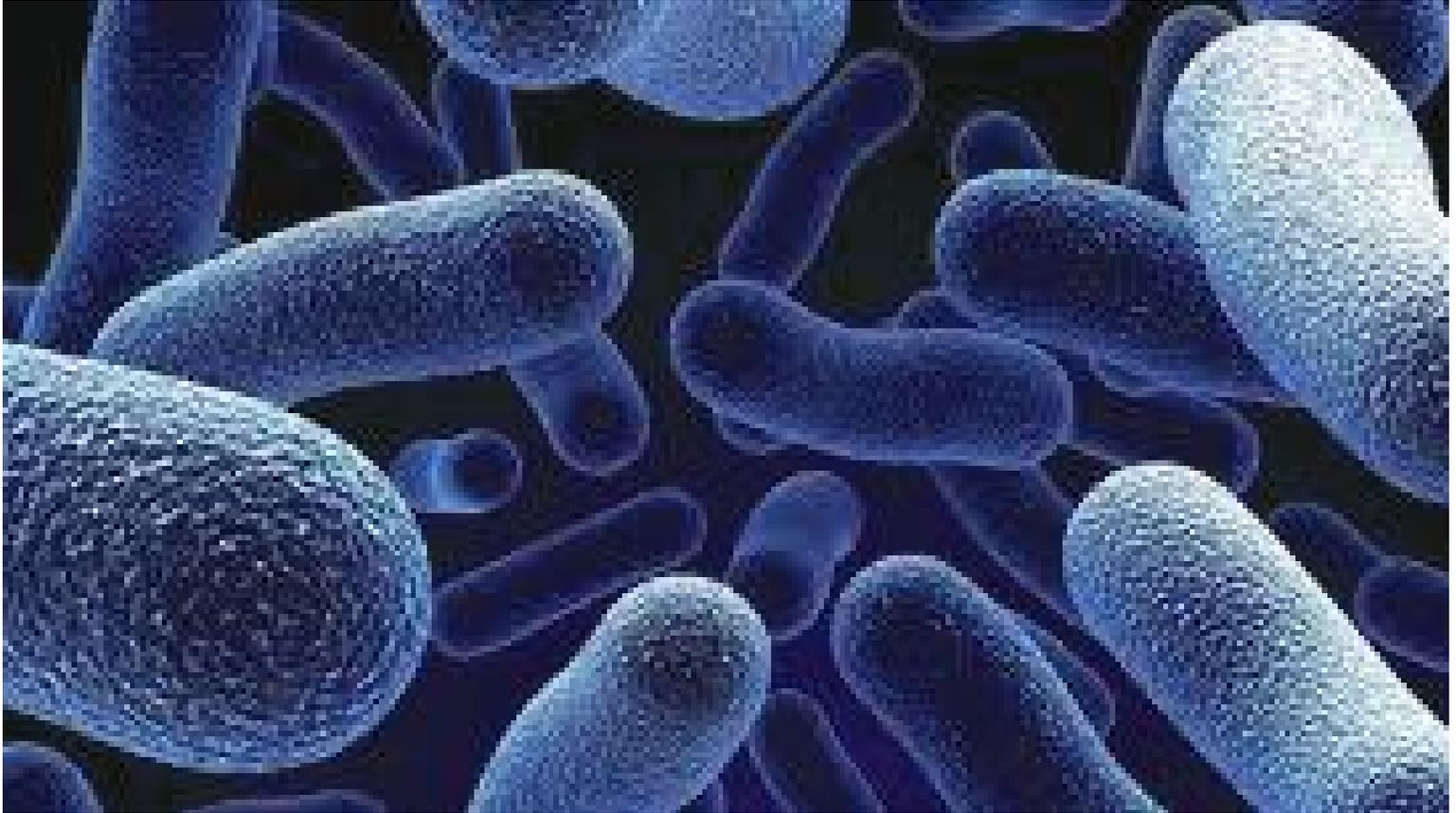
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SECTION 6

Medicinal & Antimicrobial Activity



Diversity of Endophytic Fungi Associated with Himalayan Yew (*Taxus wallichiana* Zucc.) Roots

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ABSTRACT: Microbial endophytes are being recognized for their contribution in adaptation and survival of the host plants under stressed environments. Present study is based on the diversity of the fungal endophytes, including arbuscular, dark septate and culturable fungi, associated with the root microbiome of *Taxus wallichiana* Zucc. Microscopic observations revealed heavy colonization of *T. wallichiana* roots by a variety of endophytic fungi, fungal mycelium being higher in comparison to the dark septate endophytes. Simple dip treatment in distilled water, up to 2–3 weeks, helped in cleaning the melanin like substances from *T. wallichiana* roots and providing clarity to the size and shape of the fungal structures. The culturable endophytic fungi, isolated from *T. wallichiana* roots and identified using phenotypic and molecular characters, belonged to the species of *Penicillium* and *Aspergillus*. In bioassays, the fungi showed tolerance to wide range of temperature (5–25°C), pH (0.5–12.0) and salt concentration (12–14%). These fungi also showed preferences in utilization of carbon and nitrogen sources. Colonization of these fungi in roots of *T. wallichiana* is likely to have implications in establishment of mutualistic plant–microbe association. The culturable fungi (*Penicillium* and *Aspergillus*) are well known for their role in plant growth and biocontrol. ©2019

KEYWORDS: Himalayan Yew, *Taxus wallichiana*; Plant–microbe association; Fungal endophytes; *Penicillium*; *Aspergillus*.

INTRODUCTION

Taxus wallichiana Zucc. (English name: Himalayan Yew; Hindi name: Thuner; family Taxaceae) is recognized as a medicinally important evergreen tree that grows under temperate locations of Indian Himalaya. It is a medium sized, slow growing and dioecious gymnosperm that can reach up to 20 m in height. In the Indian subcontinent, the species grows in the northern hemisphere with its distribution in the hills of Jammu & Kashmir, Himachal Pradesh and Uttarakhand and the states in northeast namely Meghalaya, Nagaland and Manipur, at an altitude range of 1800–3300 m asl. It is found in shady places, either in patches or associated with oak, silver fir, spruce and deodar forest. Regeneration of *T. wallichiana*, in nature, is through seed germination only which is reportedly very low (Anonymous, 1976; Pandey *et al.*, 2002). In medicine, it is well recognized as a source of anticancerous drug ‘taxol’ (Witherup *et al.*, 1990); antimicrobial and antioxidant activities are also being recognized (Juyal *et al.*, 2014; Adhikari & Pandey,

2017). The species has received considerable attention on account of its existing exploitation for the extraction of the drug (taxol) and also the removal of old forests (Rikhari *et al.*, 1998; Nadeem *et al.*, 2002).

Beneficial associations between endophytic fungi and medicinal plants are emerging as a frontline topic in plant–microbe interaction research (Jia *et al.*, 2016; Bakker *et al.*, 2018). Endophytic fungi live in symbiotic association with plants and play important role in plant growth. Colonization of the host plant by endophytic fungi while contributes in plant growth promotion, also provides resistance to the host against a range of biotic and abiotic stresses. Endophytic microorganisms have been recovered from almost every studied plant species, ranging from herbaceous plants, ferns, mosses and lichens to woody trees. A single plant species may harbour a diverse array of endophytic microorganisms. Classical methods to study endophytes involve isolation of endophytes from various plant parts following surface sterilization (Jain & Pandey, 2016), followed by their phenotypic and genotypic characterization. Most studies performed on *Taxus* spp. associated endophytes have been related to their taxol producing ability (Xiong *et al.*, 2013; Somjipeng *et al.*, 2015; Fatima *et al.*, 2016; Li *et al.*, 2017).

T. wallichiana Zucc. is placed in the IUCN Red List of Threatened Species (Thomas & Farjon, 2011) and, therefore, is important for its propagation and conservation

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in view of its enormous benefits. The species is being studied for its regeneration and plant growth related aspects (Kishor *et al.*, 2015; Aslam *et al.*, 2017; Das & Jha, 2018). In this background, understanding of its rhizosphere microbiome, endophytes in particular, will be a prerequisite. With this view, the present study focuses on the colonization of *T. wallichiana* roots by a variety of endophytic fungi including the culturable ones.

MATERIALS AND METHODS

Study Site and Plant Sample Collection

T. wallichiana root samples were collected from Jageshwar area (29°35′–29°39′N and 79°59′–79°53′E), District Almora in Uttarakhand, India. The total area of its habitat is approximately 120 ha where *T. wallichiana* grows between 1790–1950 m amsl (Nadeem *et al.*, 2002).

Microscopic Observations on Root Colonization by Endophytic Fungi

Total fungal colonization in roots was analysed by the trypan blue staining method (Phillips & Hayman, 1970). The roots were washed gently in the running tap water, and the fine roots were cut into small pieces (~1 cm). The root pieces were then treated with 10% KOH (1–2 h), bleached with alkaline H₂O₂ (15 min), acidified with 1% HCl (10 min) and then stained with 0.1% trypan blue in lactophenol (30 min). Total percent root colonization was measured by taking a minimum of 100 root segments/sample. The roots were kept in distilled water for 2–3 weeks before staining (an additional step in the prescribed procedure) with a view to remove the melanin-like substances and other debris from the roots and to provide clarity to the fungal structures.

Microscopic observations were recorded on the root segments with respect to fungal colonization, including vesicular arbuscular mycorrhizae, fungal mycelium and dark septate endophytes, using Nikon-Eclipse 50i, Japan. VAM colonization was identified on the basis of vesicle and arbuscular structures present in the root segments. The mycelial threads, spanning through the roots, are referred as fungal mycelium, and the dark septate endophytes are represented by the mycelium that did not take the trypan blue stain and appeared brown (Jain & Pandey, 2016).

Isolation of Culturable Fungal Endophytes

The lateral roots thoroughly washed in running water, treated with Tween-20 for 1 h, sterilized with HgCl₂ (0.01%) for 2–3 min, and finally washed with sterilized distilled water were

used for isolation of endophytic fungi. Sterilization was confirmed by plating the roots on Potato Dextrose agar (PDA). The surface sterilized roots were crushed in an autoclaved mortar and pestle. The paste was serially diluted and plated on PDA. The inoculated plates were incubated at 25 °C for 120 h. The discrete colonies obtained were subcultured on the fresh PDA plates. Five endophytic fungi, observed with distinct morphology, were preserved at 4 °C in a refrigerator. Each culture was designated with a code number. Fresh culture was raised for further experimental work.

Growth Requirements of the Endophytic Fungal Isolates

The fungal isolates were tested for their temperature and pH requirements using one week old cultures grown on PDA. For temperature, the inoculated plates were incubated at different temperatures ranging from 5 to 45 °C. For pH requirement, fungal cultures were inoculated into the medium, set between 1 and 14 (with an interval of 0.5), following incubation at 25 °C. In both cases, the plates were incubated for 7 days, and observations were recorded for optimum, minimum and maximum fungal growth. For determination of salt tolerance, the fungal isolates were inoculated on PDA supplemented with NaCl up to 15% concentration, following incubation at 25 °C for 7 days. Fungal isolates were also tested for their preference to carbon (glucose, fructose, maltose, sucrose, cellulose and starch) and nitrogen sources (ammonium ferrous sulphate, ammonium nitrate, ferrous nitrate and urea). Glucose and ammonium nitrate were taken as control.

Identification of the Endophytic Fungi

The identification of the fungal isolates was done following polyphasic approach, including phenotypic (colony morphology and microscopic features) and molecular characters.

Phenotypic Characters

Colony morphology of the fungal isolates was determined on PDA, following incubation of the inoculated plates at 25 °C for 7 days. The observations were recorded mainly for their size (diameter), colour (obverse and reverse), and exudation. Microscopy of the fungal isolates was performed following lacto-phenol cotton blue staining (magnification 1000X, Nikon Image analyzer). The observations were recorded on number, size and shape of conidia and sterigmata and branching pattern of the

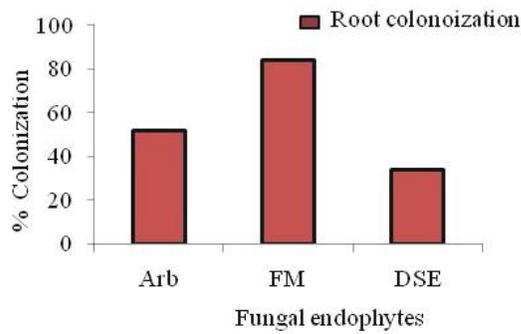


Figure 1: Fungal colonization in *T. wallichiana* roots. Arb= Arbuscules; FM= Fungal mycelium; DSE= Dark septate endophytes.

conidiophores.

Molecular Characters

Species level identification was performed by amplifying ITS region (ITS1-5.8S-ITS2) using PCR and sequencing of the amplified product (Courtesy: National Centre for Cell Science, Pune, India). The phylogenetic tree was inferred using the Neighbor Joining method, Tamura-Nei model (Kumar *et al.*, 2008).

RESULTS

Root Colonization

The roots of *T. wallichiana* were found to be colonized with diverse endophytic microorganisms, dominated by fungal mycelium, vesicular arbuscular fungi and dark septate endophytes. Maximum colonization (84%) was recorded for fungal mycelium; it was followed by vesicular arbuscular mycorrhizae (52%) and then dark septate endophytes (34%). Per cent colonization in *T. wallichiana* roots by various types of endophytic fungi is shown in Figure 1. The presence of

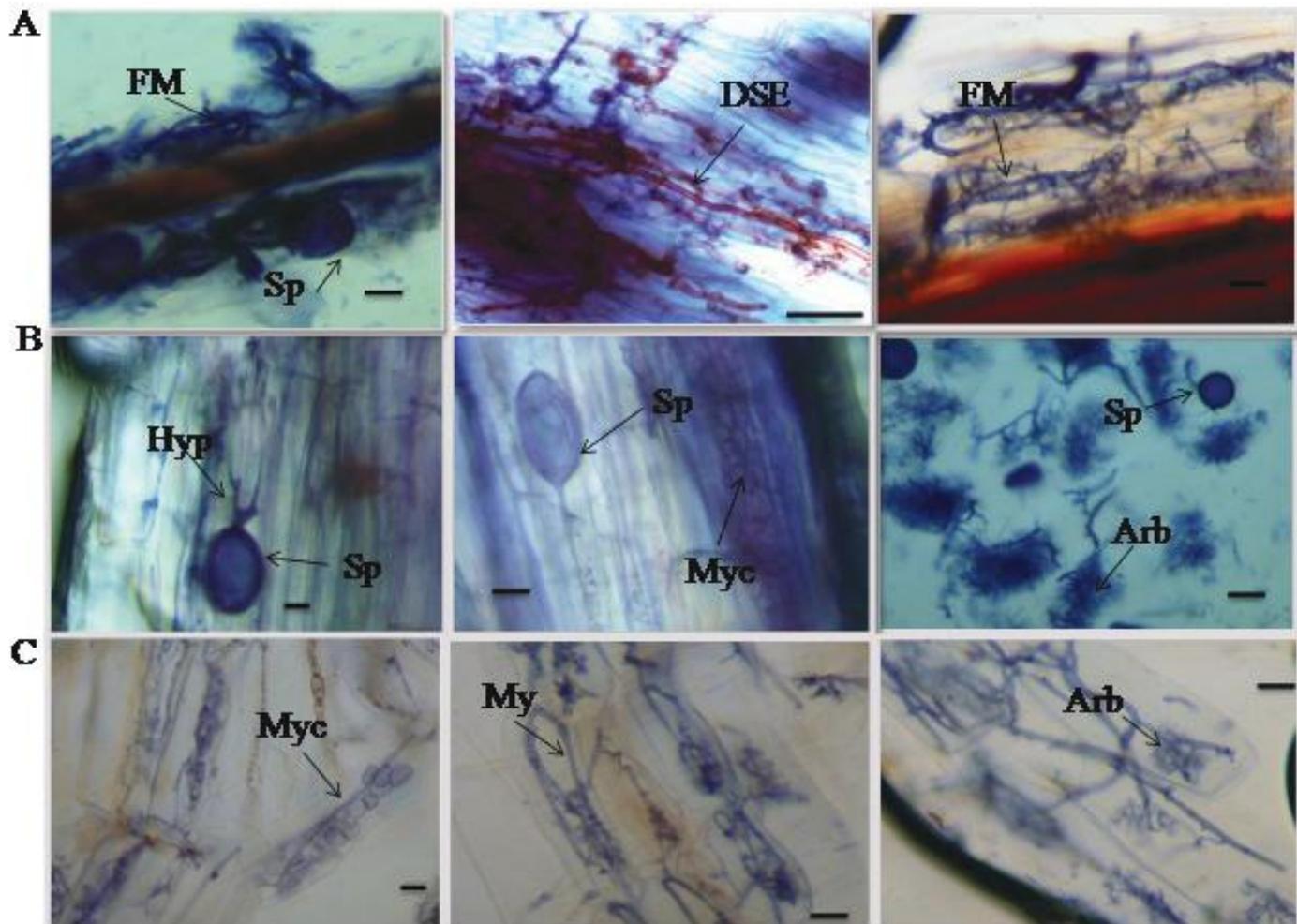


Figure 2: Fungal colonization of *T. wallichiana* roots by endophytes following distilled water dip treatment on Day 1 (A), Day 12 (B), and Day 24 (C). DSE= dark septate endophytes, Sp= spore; Hyp= hypha, Myc= mycosclerosia, FM= fungal mycelium, Arb= arbuscules, My= mycelium. Bar = 2 μ m.

Table 1: Growth (Temperature and pH) requirements and salt tolerance of the fungal isolates

S. No.	Fungal Isolate Code	Temperature (°C)	pH Requirement	Salt Tolerance (%)
1.	GBPITWR_F1	5–35, opt 25	0.5–12, opt 9	12
2.	GBPITWR_F2	5–35, opt 25	0.5–12, opt 9	12
3.	GBPITWR_F3	5–35, opt 25	0.5–12, opt 6	12
4.	GBPITWR_F4	5–35, opt 25	2.5–12, opt 6	14
5.	GBPITWR_F5	5–35, opt 25	2.5–12, opt 7	12

Opt = Optimum.

melanin content and extra debris in roots created hindrance while taking the observations on the endophytic structures. This was overcome by leaving the washed roots in distilled water up to 2–3 weeks. This resulted in giving clarity to the fungal structures (shape and size) when observed under microscope. Microscopic observations on diverse colonization by fungal endophytes in *T. wallichiana* roots, observed on Day 1, 12 and 24, are presented in Figure 2.

Culturable Fungal Endophytes

Five endophytic fungi were obtained on the prescribed media. These fungi were given the code nos. as GBPITWR_F1, GBPITWR_F2, GBPITWR_F3, GBPITWR_F4 and GBPITWR_F5 (Table 1). Their colony morphology and microscopic features are presented in

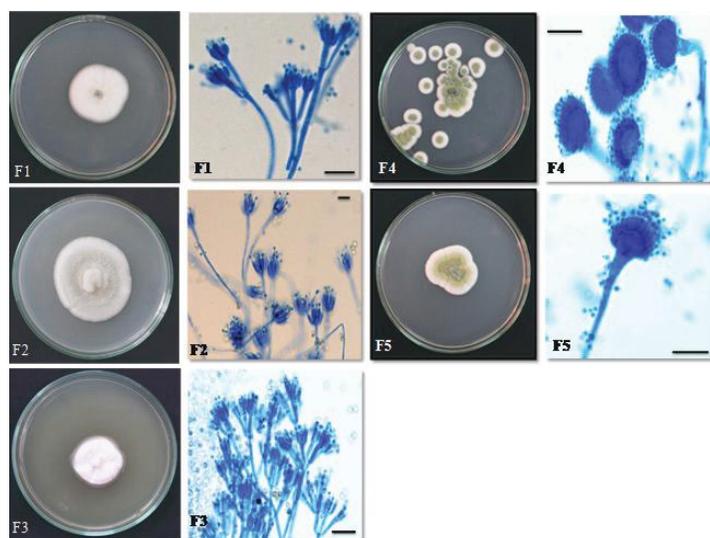


Figure 3: Colony morphology and microscopic features of the culturable endophytic fungi isolated from *T. wallichiana* roots. Bar=2µm. F1=GBPITWR_F1, F2=GBPITWR_F1, F3=GBPITWR_F3, F4=GBPITWR_F4, F5=GBPITWR_F5.

Figure 3.

Growth Requirements

The results on temperature and pH requirements and the salt tolerance of five fungal endophytes are presented in Table 1. The fungal isolates showed wide range of tolerance to temperature and pH as well. While the fungi could grow from 5 to 35 °C, the optimum temperature requirement for their growth was 25 °C. The range of pH tolerance of these fungi was between 0.5 and 12, optimum varying from 6 to 9. The fungal isolate GBPITWR_F1 grew optimally at pH 9, GBPITWR_F2, GBPITWR_F3 and GBPITWR_F4 at pH 6 and GBPITWR_F5 grew

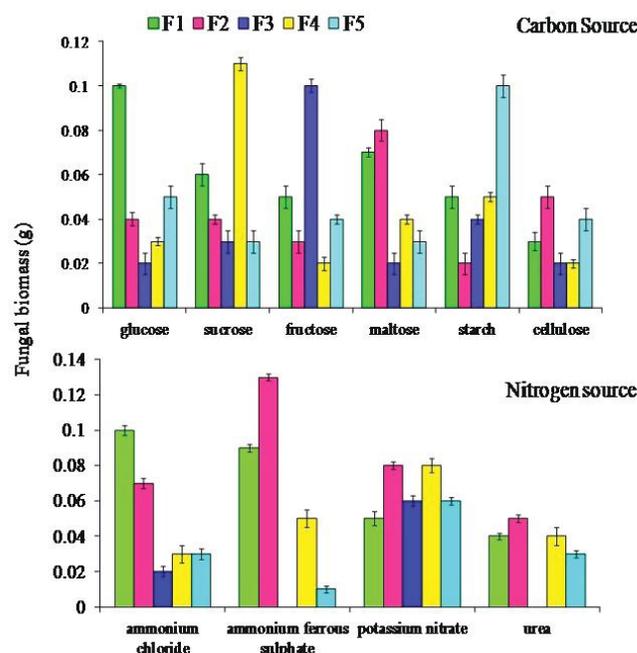


Figure 4: Utilization of carbon and nitrogen source by endophytic fungi. F1= GBPITWR_F1, F2= GBPITWR_F1, F3= GBPITWR_F3, F4= GBPITWR_F4, F5= GBPITWR_F5.

Table 2: Growth (temperature and pH) requirements and salt tolerance of the fungal isolates

S. No.	Fungal Isolate Code	Morphology	
		Colony	Microscopy
1.	GBPITWR_F1	Colony dia 4.5 cm with scanty sporulation, off white with grey margin, reverse plate brown	Sterigmata (size 4.5±0.30 µm, No. 4–6), metula (size 8.1±0.10 µm, No. 3), conidia size 1.30±0.10 µm, No. 1–2, hyphae width 1.30±0.10 µm
2.	GBPITWR_F2	Colony dia 4.0 cm with scanty sporulation, grey with cream margin, reverse plate brown	Sterigmata (size 6.8±0.10 µm, No. 4–6), metula (size 7.6±0.20 µm, No. 3), conidia (size 1.50±0.07 µm, No. 1–2), hyphae width 1.90±0.09 µm
3.	GBPITWR_F3	Colony dia 5.0 cm with scanty sporulation, off white with cream margin, reverse plate brown	Sterigmata (size 8.0±0.20 µm, No. 4–6), metula (size 8.3±0.10 µm, No. 4), conidia (size 1.30±0.08 µm, No. 1–2), hyphae width 1.9±0.10 µm
4.	GBPITWR_F4	Colony dia 5.0 cm with even distribution of spores, green with white margin, reverse plate yellow	Vesicles dia 10 µm, texture of conidia rough and spiny, conidia dia 0.3 µm, phialides biseriate
5.	GBPITWR_F5	Colony dia 5.0 cm with even distribution of spores, green with white margin, reverse plate cream	Vesicles dia 9 µm, texture of conidia rough and spiny, conidian dia 0.3 µm, phialides both uniseriate and biseriate

optimally at pH 7. The fungal isolates tolerated high salt concentration up to 12–14% (Table 1).

The fungal endophytes were found to possess the ability to utilize glucose, sucrose, fructose, maltose, starch and cellulose as carbon sources. However, the optimum growth of these fungi varied with the test carbohydrates. Similarly, the fungi could also utilize various test nitrogen sources namely ammonium chloride, ammonium ferrous sulphate, potassium nitrate and urea with variation in their optimum requirements. Figure 4 shows preference of the fungal isolates towards utilization of carbon and nitrogen sources.

Fungal colonies were obtained on PDA plates in 5–7 days of incubation at 25 °C. PDA supported both mycelial growth as well as the sporulation in all the fungal isolates. In microscopy, the fungi varied with respect to the number, size and shape of conidia and sterigmata, and branching pattern of the conidiophores. On the basis of colony morphology and microscopic features, the fungi GBPITWR_F1, GBPITWR_F2 and GBPITWR_F3 were designated to the genus *Penicillium*, while the fungal isolates GBPITWR_F4 and GBPITWR_F5 were designated to the genus *Aspergillus* (Table 2).

Morphological Identification

Molecular Identification and Phylogenetic Analysis

Table 3: Similarity of the fungal isolates with respect to their corresponding reference strain and the Accession Numbers given by NCBI

S. No.	Fungal Isolate Code	Reference strain	Similarity (%)	Nucleotide Accession No.
1.	GBPITWR_F1	<i>Penicillium</i> sp. KP747701.1	99	MH166337
2.	GBPITWR_F2	<i>Penicillium daleae</i> KF313087.1	99	MH191158
3.	GBPITWR_F3	<i>Penicillium</i> sp. MF588872.1	99	MH191159
4.	GBPITWR_F4	<i>Aspergillus versicolor</i> LN898740.1	100	MH191160
5.	GBPITWR_F5	<i>Aspergillus</i> sp. MG022169.1	100	MH191161

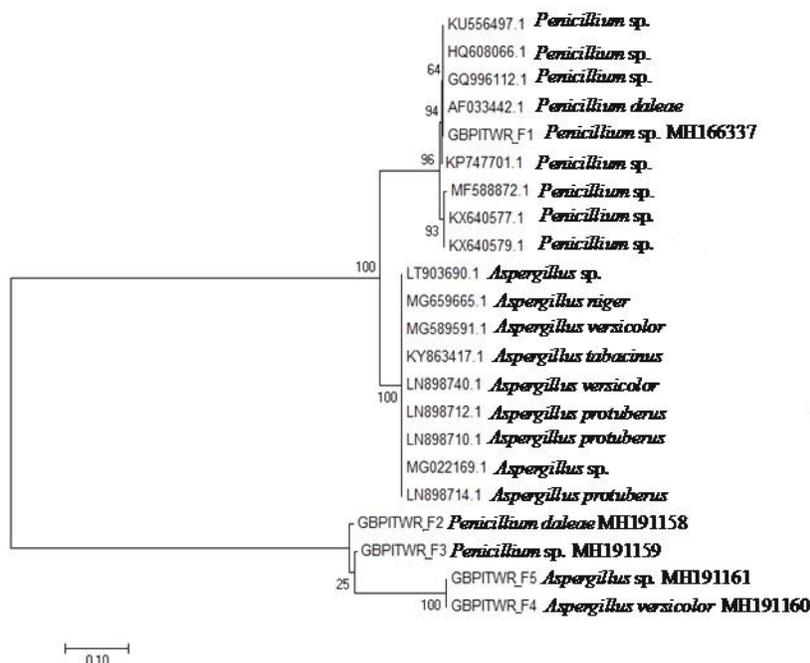


Figure 5: Neighbour joining tree of endophytic fungi isolated from *T. wallichiana* roots with bootstrap value of 1000.

The molecular identification while confirmed the genus level identification, also showed their closest references available in NCBI (National Center for Biotechnology Information, Bethesda, MD, USA 20894) database. The fungal isolates GBPITWR_F1, GBPITWR_F2 and GBPITWR_F3 showed 99% similarity to *Penicillium* sp. KP747701.1, *Penicillium daleae* KF313087.1 and *Penicillium* sp. MF588872.1, respectively. The fungal isolates GBPITWR_F4 and GBPITWR_F5 were close to *Aspergillus versicolor* LN898740.1 and *Aspergillus* sp. MG022169.1, respectively, with 100% similarity. The accession nos. designated by NCBI to the nucleotide sequences of these fungi are given in Table 3. The phylogenetic tree constructed on the basis of BLAST search analysis is shown in Figure 5.

DISCUSSION

Predominant colonization of roots by a variety of endophytic fungi, as observed in the present study, is likely to have ecological implications for growth of *T. wallichiana* under low temperature environment that often experiences heavy rainfall and snowfall. Most of the endophytic fungal structures, observed under microscope, appeared to be unculturable which can be attributed to the obligate nature of these fungi or limitation of the given growth conditions during their isolation under laboratory conditions. One of the most practical findings, in this study, was the use of simple dip treatment in distilled water up to 2–3 weeks that helped in removal of the melanin-like substances and other debris from

T. wallichiana roots. This simple treatment enhanced the clarity of the fungal structures (shape and size), mainly with respect to the dark septate endophytes. Occurrence of dark septate and other endophytes in crop as well as tree species has been highlighted for their ability to cope up with the biotic as well as abiotic stresses under low temperature environments in Himalayan mountain ecosystem (Chaurasia *et al.*, 2004; Kumar *et al.*, 2009; Jain & Pandey, 2016).

On the basis of microscopic observations, the endophytic fungi associated with *T. wallichiana* roots in the present study were recorded in three forms: arbuscules (Arb), Fungal mycelium (FM) and Dark septate endophytes (DSE). In records, the prolific growth of mycelium was referred as the vegetative fungal growth, and the vesicular arbuscular fungi were the branched structures residing in the cortical root cells. The dark septate endophytes were observed as distinct mycelial threads that appeared brown due to the presence of melanin-like substances in higher amount and not taking the colour of trypan blue. Endophytes residing in the tissues between living plant cells form a mutually beneficial relationship with the host plant that is most likely mutualistic or symbiotic. The fungal endophytes associated with the healthy tissues of all the plant taxa studied to date is mainly due to their chemical diversity instead of biological (Bakker *et al.*, 2018).

Natural habitat of the plant host is likely to provide the clues about the microbial endophytes and the

associated activities. Fungal endophytes are classified into four classes on the basis of the hosts they colonize. Class 1 consists of clavicipitaceous species that occur in cool and warm season grasses and exhibit fastidious growth in culture media; Class 2 endophytes comprise a small group with members restricted to the Dikarya (Ascomycota or Basidiomycota) and are known to provide tolerance toward various environmental stresses to their hosts; Class 3 endophytes exhibit great diversity within individual host, belonging to vascular to non-vascular plants and woody to herbaceous angiosperms that inhabit different climatic conditions; and Class 4 endophytes colonize the plant roots and are distinguished by the presence of dark melanized septa. Most members of Class 4 endophytes belong to Ascomycetes that form melanized structures within plant roots in form of inter and intracellular hyphae and microsclerotia (Singh & Pandey, 2017).

Plant associated endophytes may be culturable, and therefore, can be studied for their structural as well as functional aspects under laboratory conditions. *Taxus* roots have been studied for the colonization of a range of microorganisms including arbuscular mycorrhizae (Chaurasia *et al.*, 2004) and other root-associated microbes following both culture-dependent as well as culture-independent methods (Zhang *et al.*, 2015; Hao *et al.*, 2016). Isolation of culturable fungi, belonging to *Penicillium* and *Aspergillus* indicate the predominance of beneficial plant–microbe interactions in *T. wallichiana* roots. Cold adapted ascomycetes fungi, mainly the species of *Aspergillus*, *Paecilomyces*, *Penicillium* and *Trichoderma* have been reported to colonize the colder regions in Himalaya along with their plant growth related benefits (Pandey *et al.*, 2008; Ghildiyal & Pandey, 2008; Rinu & Pandey, 2010, 2011; Rinu *et al.*, 2012; Dhakar *et al.*, 2014). Based on the temperature tolerance, *T. wallichiana* associated species of *Aspergillus* and *Penicillium* can be referred as psychrotolerants. Besides, in view of their tolerance to wide range of pH and high salt concentration, they can also be referred as polyextremophilic fungi (Dhakar & Pandey, 2016; Pandey *et al.*, 2018) that are capable of establishing endophytic symbiosis with *T. wallichiana*. Species of both *Penicillium* and *Aspergillus* have been reported for inhabiting various plant species (Ali *et al.*, 2017; Pang *et al.*, 2017).

CONCLUSIONS

Taxus wallichiana Zucc. is a highly valuable medicinal

plant of the Indian Himalayan region due to its well-known anticancerous properties. In view of the critical status of the species, insight on its rhizosphere microbiome with respect to its propagation and conservation will be essential. The present study indicates the colonization of *T. wallichiana* roots by a variety of endophytic fungi. Fungal endophytes that comprise a diverse group of species and vary in symbiotic and ecological functions can influence the survival and fitness of plants in all the natural ecosystems. Isolation of the culturable fungi, species of *Penicillium* and *Aspergillus*, will be helpful in studying their contribution in the growth of *T. wallichiana* under low temperature environment. Optimization of media and growth conditions for isolation of more endophytic microbes from the host should be emphasized in future studies. Establishment of a propagation package, including agro-climatic conditions and microbial associates, will certainly help in propagation and conservation of this precious Himalayan species.

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Exploring Antimicrobial Activity of Selected Medicinal Plants from Remote Areas of Shimla District in Himachal Pradesh

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ABSTRACT: Ecosystem Himachal Pradesh, having varied climatic conditions is a rich repository of medicinal and aromatic plants. In a field survey of Shimla district, it was found that even in present age, people have great faith in effectiveness of herbal/traditional medicines in treatment of various human diseases. These medicinal herbs are being used in various forms, i.e. whole plant extract, decoction, juice, paste and powder. The increasing evolution of multi drug resistant bacteria has led to emergence of serious bacterial diseases which has resulted in growing interest in safe herbal alternatives to tackle human pathogens. The present study has been aimed to investigate the antimicrobial potential of locally available plant resources from Shimla district in Himachal Pradesh which have been traditionally used to cure several ailments. Few important plants from the study area, i.e. *Rhododendron campanulatum*, *Bryophyllum pinnatum*, *Allium ursinum*, *Mentha arvensis* and *Roylea cinerea* were subjected for evaluation of their antibacterial activity against certified pathogenic bacterial strains using agar well diffusion method. Acetone and methanolic extracts of *R. campanulatum* performed better against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Methanolic extract of *Bryophyllum pinnatum* showed activity against *S. aureus* and *P. aeruginosa*, methanol extract of *Allium ursinum* against *E. coli*, chloroform extract of *Mentha arvensis* against *E. coli* and *S. typhi*, acetone extract of *R. cinerea* against *E. coli* and methanol extract of *R. cinerea* showed good activity against *E. coli* and *Shigella sp.* Highest antimicrobial activity was recorded in case of *Roylea cinerea* and *Mentha arvensis*. In view of the significant antimicrobial activity exhibited in the extracts of selected medicinal plants support their traditional use as herbal medicines and also their potential utility in developing herbal drugs for curing various diseases after further R&D and clinical trials. ©2019

KEYWORDS: Antimicrobial activity; Medicinal plants; Herbal extracts; Traditional medicines.

INTRODUCTION

Traditional medicines encompass diverse health practices incorporating plant, animal and mineral based medicines, psychological treatment, spiritual therapies and manual techniques applied to maintain wellbeing, as well as to diagnose, treat and prevent illness. Himachal Pradesh, having varied climatic conditions due to variations in altitudes ranging from 450 meters to 6500 meters above mean sea level from west to east and from south to north, is a rich repository of various important medicinal and aromatic plants (Chauhan, 1999; Atkinson, 1882). People in the remote areas of Shimla district have great faith in effectiveness of these herbs in treatment of numerous ailments. These herbs play very important role in their primary healthcare system. The Shimla hills, located at 31.61 °N and 77.10 °E, lie in the south-western ranges of the Himalayas. The mid hill region of Himachal comprises regions between the elevation range of 1500 m to 3500 m above mean sea level and includes Shimla district along with other districts. This district has a rich biodiversity with most useful herbs. For a

long period of time, plants have been a valuable source of natural products for maintaining human health. Plants are good source of important compounds such as nitrogen containing compounds, phenolic compounds, vitamins and minerals which have anti-oxidant, anti-tumour, anti-mutagenic, anti-carcinogenic and diuretic activities. Microbial infections pose serious health problems all over the world, and various important plants have been found as a potential source of antimicrobial agents (Burapadaja & Bunchoo, 1995). Presently, research is going on worldwide to evaluate the effectiveness of herbal medicines for chronic diseases like cancer, heart diseases, tuberculosis, bronchitis, dengue, autoimmune disorders, rheumatism, AIDS etc.

Allium ursinum L. is widely used as a traditional medicine for the treatment of high blood pressure, high cholesterol, stomach upset, and other chronic diseases. *A. ursinum* extract can be used as a natural ingredient in food and/or pharmaceutical industries (Mihaylova et al., 2014). *Mentha arvensis* traditionally used for the treatment of digestive problems, gall bladder problems, coughs, stomach aches, rheumatism, gas pains and many other ailments. *M. arvensis* has potential to provide new weapon against bacterial resistance to antibiotics (Coutinho et al., 2008). *Roylea cinerea* is

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used for the treatment of fever, pimples, and tonsils and also used as blood purifier. The leaf extract *Roylaelegans* showed slight analgesic activity (Kumar et al., 1981). This study is an attempt to determine the antimicrobial activity of petroleum ether, chloroform, acetone, methanol and aqueous extracts of *Rhododendron campanulatum*, *Bryophyllumpinnatum*, *Allium ursinum*, *Menthaarvensis* and *Royleacinerea*, on selected pathogenic microbes such as *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Shigella* sp. and *Pseudomonas aeruginosa*. Attempts have been made to demonstrate the efficacy and effectiveness of some important herbal medicines using modern scientific investigations.

LITERATURE REVIEW

Traditional healing practices focus on different aspects of healthcare system such as psychological, social, spiritual and physical health. Although traditional healing practices for the treatment of diseases have been used from ancient times (Schulz et al., 2001), there is a requirement to use natural herbs in our primary healthcare system due to their therapeutic efficacy. In India, medicinal plants are widely used by all sections of people both directly as traditional medicines in different indigenous systems of medicine like Siddha, Ayurveda and Unani and indirectly in the pharmaceutical preparations (Srinivasan et al., 2001). Himachal Pradesh, having varied climatic conditions is a rich repository of medicinal and aromatic plants. Singh and Thakur (2014) enlisted 63 plant species in a field survey of medicinal plants in the Shimla Hills. These plants reported to be used in 126 medical conditions in human beings. In traditional systems of medicine, the Indian medicinal plants have been used in successful management of various disease conditions like bronchial asthma, chronic fever, cold, cough, malaria, dysentery, convulsions, diabetes, diarrhoea, arthritis, emetic syndrome, skin diseases, insect bite, etc. and in treatment of gastric, hepatic, cardiovascular & immunological disorders (Chopra et al., 1993). The Indian Himalayan Region (IHR) is a mega hot spot of biological diversity (Myers, 2000). The regions inhabit around 1748 species of medicinal plants with various traditional and modern therapeutic uses, 675 species of wild edible plants (Samant & Dhar, 1997) and 118 species of medicinal plants yielding essential oils. Use of medicinal plants around the world has significantly supported primary healthcare system (Maciel et al., 2002).

The increasing evolution of multi drug resistant bacteria leads to the emergence of untreatable bacterial

diseases. There is a growing interest to explore the alternative drugs from different plant species that have antimicrobial properties and can be used as antibiotic resources (Westh et al., 2004). Search for new antibacterial agents should be continued by screening many plant families. Recent work revealed the potential of several herbs as sources of drugs (Iwu & Wootton, 2002). The use of plant extracts and phytochemicals, both with known antimicrobial properties, are of great significance to therapeutic treatments. As a consequence, the pharmacological activity of many medicinal plants has been studied with increasing interest in most of the countries (Gottlieb et al., 2002). The effectiveness of plant extracts against various pathogenic microbes can be enhanced through the green synthesis of silver nanoparticles using biotechnological interventions (Thakur et al., 2017). The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003). Numerous studies have identified compounds within herbal plants that are effective antibiotics (Basile et al., 2000). Traditional healing systems around the world that utilize herbal remedies are important source for the discovery of new antibiotics (Okpekonet et al., 2004); some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria (Kone et al., 2004). This indicates the need for further research into traditional healthcare systems with constant explorations of medicinal plants (Romero et al., 2005). It also facilitates pharmacological studies leading to synthesis of a more potent drug with reduced toxicity (Manna & Abalaka, 2000). Extensive validations of the pharmacological actions are required for the reported uses of different medicinal plants (Narod et al., 2004).

MATERIALS AND METHODS

Collection of Plant Material

Five medicinal plants were selected from remote areas of Shimla district for the purpose of antimicrobial screening experiment. Fresh samples of selected plants viz., *Rhododendron campanulatum* (leaves), *Bryophyllumpinnatum* (leaves), *Allium ursinum* (bulbs), *Menthaarvensis* (leaves) and *Royleacinerea* (mixture of leaves and roots) free from diseases were collected from remote areas of Shimla district. The leaves were washed thoroughly 2–3 times with running water and once with sterile distilled water. These samples were then air-dried on sterile blotter under shade, and grinded into powdered form and stored in air tight container until further use (Fig. 1e).



Figure 1: Collection of samples from Kala Pani forest of Dodra village (31.20 °N and 78.01 °E) in Shimla district (a, b). *Bryophyllum pinnatum*, local Name: Patherchatt (c) and *Rhododendron campanulatum*, local name: Shimroo (d). Dried samples of *Rhododendron campanulatum*, *Bryophyllum pinnatum*, *Allium ursinum*, *Menthaarvensis* and *Royleacinerea* (e).

Field Surveys

Open informal interviews were performed to collect information regarding the use of medicinal and aromatic plants in primary healthcare system especially from herbal practitioners of Shimla district in Himachal Pradesh. Ethnobotanical information was collected through several visits, questionnaires and group discussions with local people. During field survey, the village heads, herbal practitioners as well as elderly men and women of the different villages were interviewed.

Growth and Maintenance of Test Microorganism for Antimicrobial Studies

The clinical isolates *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Shigella spp.* and *Pseudomonas*

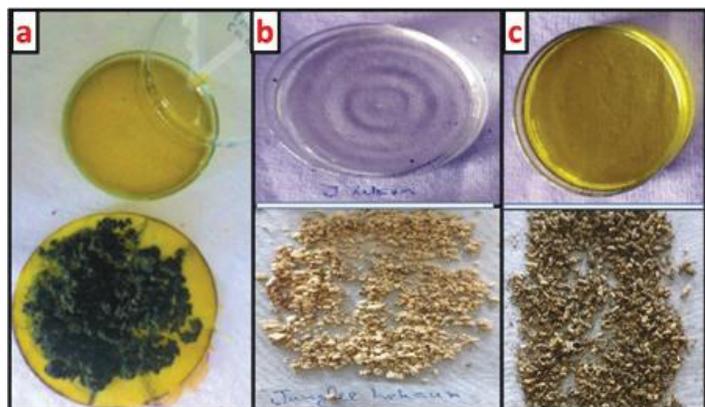


Figure 3: (a) Methanol extract of *Menthaarvensis*, (b) Petroleum ether extract of *Allium ursinum*, and (c) Petroleum ether extract of *Royleacinerea*.



Figure 2: Petroleum ether extracts of (a) *Rhododendron campanulatum* and (b) *Bryophyllum pinnatum*.

aeruginosa were obtained from Department of Microbiology, Indira Gandhi Medical College & Hospital (IGMC), Shimla, Himachal Pradesh and were used for antimicrobial test organisms. The bacteria were maintained on nutrient broth (NB) at 37 °C.

Preparation of Inoculum

The gram positive (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*) were pre-cultured in nutrient broth overnight in a rotary shaker at 37 °C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A610 nm).



Figure 4: Few prominent herbal practitioners/ vaidyas from the study area (a) Vaidya Sahajanand, (b) Vaidya Jai Ram, (c) Vaidya Shiv Kumar, (d) Vaidya Badri Prasad) of Dodra and Kawar (remotest villages of Shimla district), (e–f) Open interview to collect ethnobotanical information from local herbal practitioners of Dodra and Kawar.

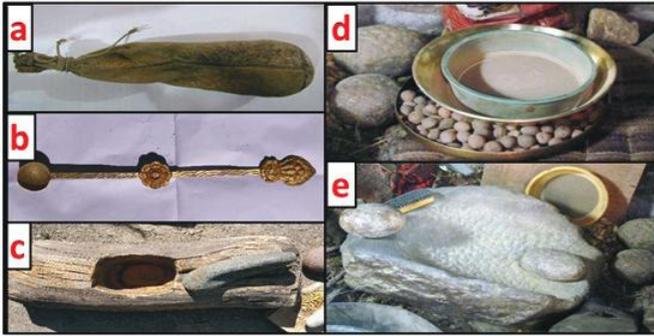


Figure 5: Conventional tools used by herbal practitioners of Dodra and Kawar villages for preparation of traditional medicines (a) Sample bag, (b) Spatula, (c) Wooden pestle mortar, (d) Sample sieve, and (e) Stonepestle mortar).

Preparation of Extracts

Cold percolation method of extraction was used for the preparation of plant extract. In this method powdered plant material was used for extraction using different solvents, viz. Petroleum ether, chloroform, acetone, methanol and water by cold percolation method as per the method detailed by Rosenthaler in 1930 (Figs. 2 and 3). For solvent extraction, 10 g of air dried powder was taken in 100 ml of organic solvent in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 160 rpm for 48 h. After 48 hours the filtrate was collected and the solvent was evaporated and stored at 4°C in airtight Eppendorf tubes.

Stock Solution

The stock solution of different plant extracts were prepared in such a way that the final concentration comes to be 100 mg/ml using 10% Dimethyl sulfoxide, the universal solvent. The extracts were mixed thoroughly and used for further experimentation.

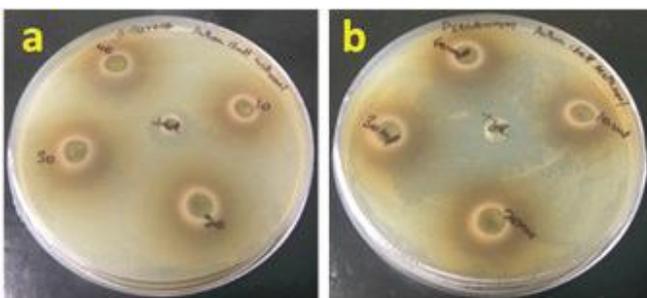


Figure 7: Inhibition zones of methanol extract of *Bryophyllum pinnatum* (a, b) against *Staphylococcus aureus* (a) and *Pseudomonas aeruginosa* (b).

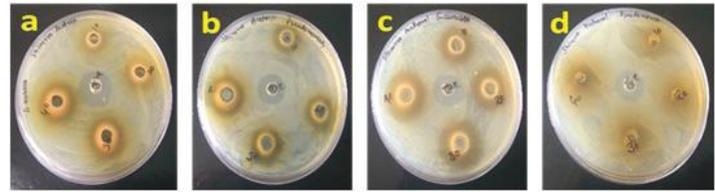


Figure 6: Inhibition zones of acetone (a, b) and methanol (c, d) extracts of *Rhododendron campanulatum* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Antimicrobial Agents

The antimicrobial activity of the plant extracts on human pathogenic bacteria was compared with the commercially available antibiotics as positive control and DMSO as a negative control. *Gentamycin* was used as positive control against *Staphylococcus aureus* and *Pseudomonas aeruginosa* whereas tetracycline, chloramphenicol and ciprofloxacin were used as positive control against *Escherichia coli*, *Salmonella typhi* and *Shigella sp.* respectively.

Determination of Antimicrobial Activity

The antimicrobial assay was performed by using agar well diffusion method (Perez et al., 1990) for solvent extracts. The molten Mueller Hinton Agar (HiMedia) was inoculated with the 30 µl of the inoculum (1 x 10⁸Cfu) and poured into the sterile Petri plates (Hi-media). For agar well diffusion method, wells were prepared in the plates with the help of a cork-borer. 30 µl of the test compound was introduced into the well. Different volumes of each plant extract i.e. 10µl, 20µl, 30µl and 40µl were added to respective wells. The plates were incubated overnight at 37 °C. The antimicrobial compounds present in the plant extracts were allowed to diffuse out into the medium and interact in a plate freshly seeded with the test microorganisms. The resulting zone of inhibition was observed as uniformly circular areas due



Figure 8: Inhibition zones of methanol extract of *Allium ursinum* against *Escherichia coli*.

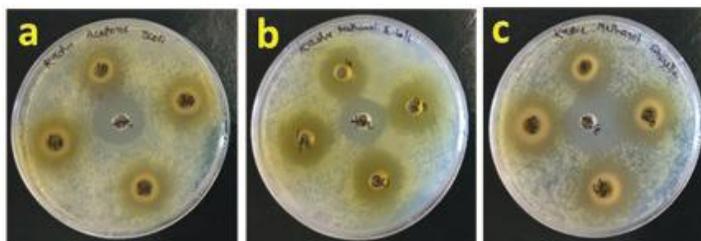


Figure 9: Inhibition zones of chloroform extract of *Mentha arvensis* against *Salmonella typhi* (a) and *E. coli* (b).

to the confluent lawn of growth. The diameter of zone of inhibition was measured in mm.

RESULTS AND DISCUSSION

Intensive ethnobotanical exploration were undertaken in different areas of Shimla district to find out various medicinal plants used for different ailments. Field surveys on the use of traditional medicines were conducted in different areas of Shimla district such as Rohru, Chirgaon, Theog, Narkanda, Kumharsain, Rampur, Kotgarh, Gopalpur, Jhakhari, Jeuri, Sarahan, Dobi, Manjhgaoon, etc. Field surveys were explored through several visits, questionnaires and group discussions with local people and provided valuable information regarding conventional remedies practiced against different

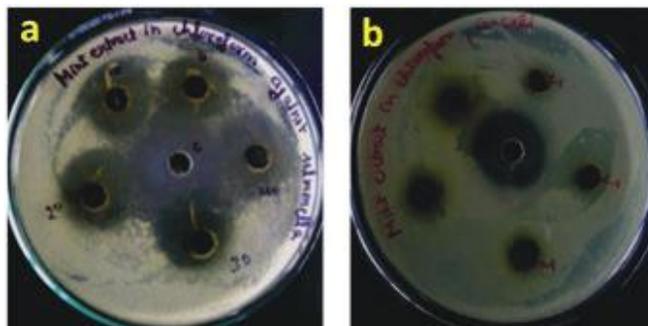


Figure 10: Inhibition zones of acetone extract of *Royleacinerea* against *Escherichia coli* (a) and methanol extract of *R. cinerea* against *Escherichia coli* (b) and *Shigella sp.* (c).

diseases by the herbal practitioners in the studied area. It was also found that remotest areas of the Shimla district in Himachal Pradesh i.e. Dodra(31.19 °N and 78.05 °E) and Kawar(31.21 °N and 78.09 °E) (Fig. 4) have a rich repository of traditionally important medicinal plants. Demographic features of informants and ethno medicinal uses of important medicinal plants were documented. More than 25 plant species covering the different pharmaceutical properties have been reported. 26 herbal practitioners shared information regarding

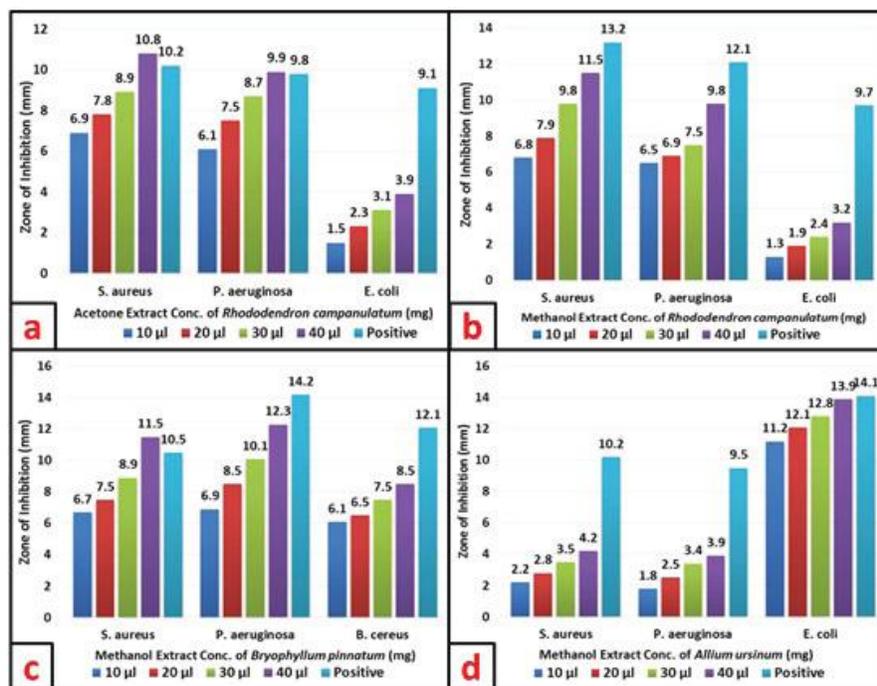


Figure 11: (a) Antimicrobial activity of acetone extract of *Rhododendron campanulatum*, (b) Methanol extract of *Rhododendron campanulatum*, (c) Methanol extract of *Bryophyllum pinnatum*, and (d) Methanol extract of *Allium ursinum* against different pathogenic bacterial strains.

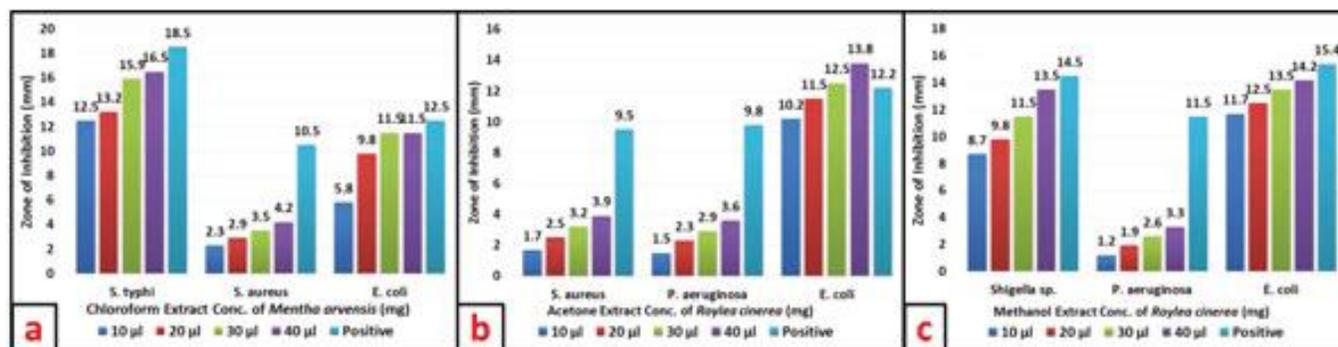


Figure 12: (a) Antimicrobial activity of chloroform extract of *Mentha arvensis*, (b) Acetone extract of *Roylea cinerea*, and (c) Methanol extract of *Roylea cinerea* against different pathogenic bacterial strains.

use of medicinal plants in their primary health care system. They were found to use conventional tools for preparation of traditional medicines (Fig. 5). It was found that information regarding the use of medicinal plants now seems to be restrained to elder people (within the age group of 40-95 years). Leaves were the most frequently used parts in preparing herbal remedies. Decoctions and oral route of administration were commonly used method of herbal medicine preparation and administration respectively. Hence, it was observed that a traditional health practitioner possesses an extensive knowledge of curative herbs, natural treatments and specializes in the use of herbal and other medicinal preparations for treating different diseases. Previously, a field survey of medicinal plants in the Shimla Hills was also conducted by Singh and Thakur in 2014 and enlisted 63 plant species. However, the scientific logic behind the treatment is not known. The vaidyas also do not easily share the knowledge with anyone including the team surveying the area and the younger generation either hesitate to go for the traditional knowledge or go away to cities in search of job which has resulted in decline of their very important treasure of traditional medication. The results obtained during the current study seem quite promising not only for promoting herbal medicines but also to preserve the traditional knowledge, conserve the important bioresource and improve the healthcare with blend of traditional knowledge and modern scientific interventions in the larger interest of mankind.

Medicinal plants have been explored by different researchers to use as herbal alternate to tackle human pathogens because they are rich source of novel drugs and their bioactive principles form the basis in medicine besides other advantages (Ncube et al., 2008). Most of the medicinal plants have been found as a good source of antibacterial substances (Srinivasan, 2001). Plants contain phytoconstituents like alkaloids, flavonoids,

tannins, essential oil and other aromatic compounds which have been reported to act as good antimicrobial agent and have been used for treating various ailments (Lawrence et al., 2015). The solvent extracts of selected plants was prepared by using five solvents i.e. petroleum ether, chloroform, acetone, methanol, and aqueous by the cold percolation method (Rosenthaler, 1930) and stock solutions was prepared by using universal solvent DMSO (100 mg plant extract/ml of DMSO). It was found that the antimicrobial activity was high in solvent extract compared to aqueous extract. Then these plant extracts were tested against pathogenic strains like *Escherichia coli*, *Bacillus cereus*, *Salmonella typhi*, *Staphylococcus aureus*, *Shigella* spp. and *Pseudomonas aeruginosa* by well diffusion method (Perez et al., 1990). Different concentrations of plant extract i.e. 10 µl to 40 µl were used. The experiment was recorded after 24 hours while incubating the culture at 37°C and effect of plant extract was recorded by visualizing the zones of inhibition in each plate.

Acetone and methanol extract of *Rhododendron campanulatum* showed activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Highest activity has been observed against *Staphylococcus aureus*, i.e. 10.8 mm in acetone extract and 11.5 mm in methanol extract at 40 µl conc. (Figs. 2a, 6 and 11a,b). Previously, the diethyl ether and methanol extracts exhibited dose-dependent antimicrobial activity against *Staphylococcus aureus* and *Streptococcus faecalis* (Paudelet et al., 2011). Methanol extract of *Bryophyllum pinnatum* showed activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Highest activity has been observed against *Pseudomonas aeruginosa*, i.e. 12.3 mm at 40 µl conc. (Figs. 2, 7b and 11c). Akinsulire et al. (2007) also recorded high antimicrobial activity in the methanol extract of *Bryophyllum pinnatum*. Methanol extract of *Allium ursinum* showed activity against *Escherichia coli*. Highest activity has been observed, i.e. 13.9 mm at 40 µl conc. (Figs. 3b, 8, and 11d). Previously, pressurised-liquid extraction of *Allium ursinum* was used to

determine antimicrobial activity (Mihaylova et al., 2014). Chloroform extract of *Mentha arvensis* showed high level of activity against *Escherichia coli* and *Salmonella typhi*. Highest activity has been observed against *Salmonella typhi*, i.e. 16.5 mm at 40 µl conc. (Figs. 3a, 9, 12a). *M. arvensis* extract was classified as very active against *S. aureus*, active against *E. coli*, *K. pneumoniae* and *S. flexneri* and partially active against *Pseudomonas aeruginosa*. *Mentha arvensis* could serve as a source of plant-derived natural products with antibiotic resistance-modifying activity to be used against multi resistant and *Staphylococcus aureus* (Rios et al., 1988; Alves et al., 2000). Coutinho et al. (2008) used ethanol extract of *M. arvensis* L. and recorded low level of antimicrobial activity against *E. coli*. Acetone extract of *Royleacinerea* showed activity against *Escherichia coli* and methanol extract of *Royleacinerea* showed activity against *Escherichia coli* and *Shigella* sp. Highest activity has been observed in acetone and methanol extract of *Royleacinerea* against *Escherichia coli*, i.e. 13.8 mm and 14.2 respectively at 40 µl conc. (Figs. 3, 10, 12b, c). Analgesic activity was recorded in the leaf extract of *Royleaelegans* (Kumar et al., 1981). The antimicrobial agents are particularly useful in situations in which the normal host defence cannot be relied on to remove or destroy pathogens. A given antimicrobial can be bactericidal in one situation, yet bacteria static in another, depending on the concentration of the drug and the growth stage of the microorganism (Nester et al., 2004).

CONCLUSIONS & RECOMMENDATIONS

The present study shows that remotest area of the Shimla district, i.e. Dodra and Kawar have a rich repository of traditionally important medicinal plants and local people are enriched with knowledge of its uses in their healthcare system. They are extensively using the traditional medicines to cure various ailments. Medicinal plant species with high medicinal values have been subjected to pharmacological investigation. The results indicated the presence of potent antibacterial compounds in leaves, roots and bulbs extracts of selected plants using different solvent system. These can serve a source of new therapeutic agents to control the various pathogenic bacteria. The demonstration of activity against gram positive and gram negative bacteria is an indication of broad spectrum activity of these plants thus can be used as a source of antibiotic substances in drug development for control of various bacterial infections. Further work on identification and purification of chemical constituents and toxicological investigations of plant extracts are required to be carried out with a view to develop novel drugs for human healthcare.

This ethnobotanical and traditional knowledge contributes to the conservation of biodiversity and provides resource of economic and ecological interest. An attempt has been made in the direction to document valuable information regarding the use of traditional medicines in the present study areas. A complete database of information regarding traditional healthcare practices are required to develop, that should be further utilized in identification of medicinal plant conservation areas. There is a constant need to develop location and region specific management plan for conservation of rare medicinal and aromatic plants. There is great need of promotion in implementation of medicinal plants farming especially in remote areas of Himachal Pradesh. This will pave way for development of economic status of local community and conservation of important medicinal plants in the state.

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Increased Production of Extracellular Cellulase from Bacterial Isolate KCPS-76, Isolated from the Kinnaur District of Himachal Pradesh

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ABSTRACT: Waste management is one of the major challenges to overcome, nowadays and turning waste into energy can be a beneficial option to deal with. Keeping in mind, the study was carried out to utilize agricultural and industrial waste to useful by-products like ethanol and nutraceuticals etc. Agricultural waste contains the lignocellulosic biomass which is nature's richest energy source, i.e. sugars. Cellulase, is multi-component enzyme system which hydrolyze cellulose into soluble sugars. Hence in the present investigation 80 cellulase producing microbes were isolated from various sites of Lahaul Spiti and Kinnaur. Among them 48 isolates were positive but isolate showing the maximum cellulase activity was KCPS-76. The optimisation of culture and reaction conditions of CPS 76 resulted into 13.9 fold increase in the overall enzyme activity (36.2 U/ml) over the control (2.6 U/ml). Maximum cellulase activity was recorded with 7% inoculum (24 hr old culture) using 1% CMC (w/v) as sole carbon source. Among the various nitrogen sources tested, 0.6% yeast extract (w/v) was found as the best nitrogen source for cellulase production. The relative production of enzyme at varied pH (4-11) and temperature ranges (20-55 °C) revealed that this enzyme was equally effective over a wide pH range (6 to 9.5) with maximum activity at pH 9 and temperature of 35 °C. The properties of hyper cellulase producing KCPS-76 will further be utilised for the saccharification of various lignocellulosic wastes. For the fermentation process ethanol fermenting microorganisms were also isolated from local brewery of Kinnaur. Among the 7 isolates 4 isolates were positive. Maximum ethanol fermenting isolate was EFS-3. Hence, Utilization of KCPS-76 and ethanol fermenting organisms can be used for development of consortia to carry out simultaneous saccharification and fermentation so as to convert lignocellulosic waste into the useful products. ©2019

KEYWORDS: Carboxymethyl cellulose; Cellulase activity; Fermentation.

INTRODUCTION

Millions of tons of waste are produced as a result of various agricultural and industrial activities around the globe every year, and its proper disposal can resolve many health and environmental issues. Agricultural waste produces a major part of cellulosic biomass which is the most abundant and renewable biomass waste in the biosphere. It has also been recognized as a potential low-cost source of mixed sugars for fermentation to fuel ethanol and some other useful products. Lignocellulosic materials are mainly composed of lignin, cellulose, and hemicellulose. Among these cellulose is the most abundant carbohydrate source and has significant potential for conversion into liquid and gaseous biofuels. Cellulose and hemicellulose typically comprise up to two-thirds of the lignocellulosic biomass and are the main sources of sugars for second generation biofuel production (Hamelinck et al., 2005). To access these cellulosic and hemicellulosic fractions, pre-treatments are used prior to enzymatic hydrolysis to open the

lignin sheath. After pre-treatment, cellulose deconstructing enzymes, cellulases and hemicellulases, are used to release fermentable sugars.

Since the microorganisms are characterized by their rapid growth, therefore, bioconversion by cellulolytic microorganisms which is controlled and processed by the enzymes cellulases seems to be an economical and ecofriendly option. The enzymatic degradation of cellulosic biomass to corresponding sugars has received high attention because it will not generate an environmental load. Full enzymatic hydrolysis of cellulose requires at least three major cellulase components including β -1,4-endoglucanases (EG, EC3.2.1.4), β -1,4-cellobiohydrolases (CBH, EC3.2.1.91) and β -glucosidase (BGL, EC3.2.1.21), working together in a synergistic style (Yamada et al. 2011). The expression ratios and synergistic effects of these enzymes both significantly influence the extent and specific rate of substrate degradation (Kotaka et al. 2008).

However, saccharification and by-product manufacturing from cellulosic biomass are complex and lengthy processes. The current schemes for the biotechnological conversion of plant cell wall polysaccharides rely on first reducing biomass recalcitrance through a pretreatment step, and afterward, enzymatic cocktails are needed to breakdown biomass into more simple, fermentable saccharides, which could be fed

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into several bioprocesses, such as bioethanol production (Berlin et al. 2005).

Despite the advantages of enzyme-catalyzed processes, i.e., speed, specificity and mildness, the high cost of enzyme production and low catalytic efficiency are still major hindrances for cellulosic bioethanol. As microflora from the Himalayan region is still unexplored, it is of prime interest to explore the potential microbes for the utilisation of industrial and other wastes for the production of viable products. Thus, relevant biotechnological challenges in this field include the improvement of the catalytic efficiency of enzymes like cellulases, xylanases and lignin peroxidase for the economic benefit and the synergy between the type of pre-treatment and enzymatic load, and reduction of the cost of enzyme production.

LITERATURE REVIEW

The rise of crude oil prices and the high emissions of greenhouse gasses have resulted in growing interest in finding alternate source of energy. Furthermore as the transportation sector accounts for about 16% participation in global greenhouse gases (Fuglestvedt, 2008), hence one of our greatest challenges is to reduce our Nation's dependence on petroleum and diesel. According to the International Energy Agency's report "World Energy Outlook 2007", the worldwide energy demand would be 50 % higher in 2030 than that of today's (Hasan and Kalam 2013). In 2003, the Ministry of Petroleum and Natural Gas (India) has launched the first phase of the Ethanol Blended Petrol (EBP) Program that mandated the blending of 5% ethanol in gasoline for nine states (out of a total of 29) and four union territories (out of a total of 6) (Singh, 2017). Although food crops are at the leading front of the biofuel industry (Somerville et al. 2010), but as these are the food stocks, it is really not possible to rely on food crops for fuel in developing countries. Fortunately, there is an alternate -the cellulosic and hemi-cellulosic material. For example, forests comprise about 80% of the world's biomass. Being abundant and outside the human food chain makes cellulosic materials relatively inexpensive feed stocks for ethanol production (Hamelinck, 2005).

According to the today's scenario second-generation (2G) ethanol produced from lignocellulosic biomass has been considered to be the biofuel with the greatest potential to replace oil-based fuels (Seabra et al. 2010; Macrelli et al. 2012). Hence, fuels derived from cellulosic biomass—the fibrous, woody, and generally inedible portions of plant matter—offer one such alternative to conventional energy sources that can dramatically impact National economic growth, National energy security, and environmental goals (Bhatt et al., 2018).

Bioconversion of cellulose, nature's most abundant polysaccharide is accomplished by the enzyme 'cellulases'. Cellulases, a complex of sequentially acting component enzymes (endoglucanase, exoglucanase, cellobiase as) are found to be synthesized by a variety of microbes (Karmakar & Ray, 2011).

Both bacteria and fungi can produce cellulases for the hydrolysis of lignocellulosic materials. These microorganisms can be aerobic or anaerobic, mesophilic or thermophilic. Bacteria, belonging to *Clostridium*, *Cellulomonas*, *Bacillus*, *Thermomonospora*, *Ruminococcus*, *Bacteriodes*, *Erwinia*, *Acetovibrio*, *Microbispora*, and *Streptomyces* can produce cellulases (Maki et al., 2012; Coughlan, 1985). *Cellulomonas fimi* and *Thermomonospora fusca* have been extensively studied for cellulase production. Many of other hyper-producing cellulolytic microbes have been isolated by researchers like *Anoxybacillus flavithermus*, *Bacillus amyloliquefaciens* and *Bacillus megaterium*, (Ibrahim & EI-diwany, 2007). Behera et al. (2018) isolated around 15 cellulase producing microbes. The aim of Onsoni et al. (2005) was to identify an *Aspergillus* sp. with over production of endo-1,4-glucanase. Properties of endo-1,4-glucanase/ carboxymethyl cellulase (CMCase) from a culture filtrate of the *Aspergillus* sp. were also studied.

Saccharification of pre-treated biomass by cellulase from *Aspergillus niger*, *Trichoderma viridae*, *Aspergillus ellipticus* and *A. fumigates* was also shown by Bhatt et al. (1992); Sharma et al. (1996); Ingale et al. (2005). Sherief et al. (2010) utilised the cellulase for saccharification of wheat bran and rice straw. Zhu et al. (2006) studied the aptness of production of ethanol from physical and chemical treatment in comparison to treatment by cellulase from *Trichoderma reesei* and fermentation by *Saccharomyces cerevisiae* YC-097 cells.

Improvement of the process of cellulase production and development of more efficient lignocellulose-degrading enzymes are necessary in order to reduce the cost of enzymes required in the biomass-to-bioethanol process (Elliston et al., 2015). Keeping in view the work was to isolate a potential microbe for cellulase production, also to isolate some fermentative microbes.

MATERIALS AND METHODS

Sample Collection

For the isolation of cellulolytic microorganisms soil, water, cowdung and horse dung samples were collected from different areas of Kullu (i.e. Rohtang, Manikaran and Parvati river), Lahaul-Spiti (i.e. Keylong, Biling,

Jispa, Darcha and Gramphu lake) and Kinnaur (i.e. Tapri, Sangla, Chitkul, ReckongPeo, Rarang, Nako, Pooh). The samples were collected in sterile polythene bags/plastic bottles and were sealed and brought to the lab carefully for further processing.

Isolation and Primary Screening of Cellulolytic Microorganisms

Soil samples were serially diluted to 10⁴-10⁶ times with physiological saline and 0.1 ml of diluted inoculum was spread on petri plates containing CMC medium. Plates were incubated at 30 °C for 48h. Pure line cultures were established by repeatedly streaking a single bacterial colony on the above medium. In order to visualize the hydrolysis zone, the plates (isolates on CMC agar plates) were flooded with 0.1% Congo red solution and washed with 1 M NaCl. The formation of a clear zone of hydrolysis indicated cellulose degradation (Ingale et al. 2014). The ratios of the clear zones were compared for screening the highest cellulase activity producer. The largest ratio was assumed to contain the highest activity.

Secondary Screening by Cellulase Assay

Newly isolated strains were screened for cellulase enzyme production in CMC media [10g CMC, 1g meat extract, 2.5g (NH₄)₂SO₄, 0.25g K₂HPO₄·3H₂O, 0.1g NaCl, 0.125g MgSO₄, 0.0025 FeSO₄, 0.025 MnSO₄ in 1000 ml Distilled water at pH-7]. The 100ml production medium was inoculated with 1 ml of selected bacterial isolate in a 250ml conical flask, incubated in a shaker at 30 °C for 24 h at 150 rpm. After 24 h broth was centrifuged at 10000 × g for 10 min at 4 °C to obtain the supernatant that served as a source of crude enzyme. Cellulase activity was quantitatively assayed by determining the reducing sugars produced as a result of enzymatic hydrolysis of CMC following the method developed by Miller (1959). The assay system contained 4.5 ml having 1% carboxymethyl cellulose (CMC) in 0.055 M citrate buffer (pH-5.0) and 0.5 ml of enzyme preparation. Reaction mixture was incubated at 70 °C for 30 min and the reaction was stopped by adding DNS reagent. Absorption of reaction mixture was recorded at 540 nm. Concentration of reducing sugars was calculated using a standard curve of glucose (20-200 µg/ml). One unit of CMCase is defined as the amount of enzyme required to produce one µmole of D-Glucose from substrate per min under the assay conditions. The isolate with the maximum activity (KCPS-76) was further utilised for optimisation of production and reaction conditions.

Optimization of Culture Conditions for Cellulase Production from KCPS-76

In order to achieve maximum production of cellulase from selected bacterial isolate, attempts were made to optimize various production parameters viz. inoculum size, inoculum age nitrogen sources, pH, incubation temperature etc. using the selected bacterial isolate KCPS-76.

Effect of Inoculum Age

To study the effect of age of inoculum on cellulase production, KCPS-76 grown in the medium was withdrawn after 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 h and used for production of cellulase for 48 h at 30 °C and enzyme activity was measured in each case.

Effect of Inoculum Size

Different volumes of inoculum (1-10% v/v) were added to the production medium separately and the culture contents were incubated at 30 °C for 24 h and cellulase activity was assayed to see the effect of inoculum size on subsequent enzyme production.

Production Profile of Enzyme

The bacterial culture was grown under the pre-optimized conditions in the production medium and cellulase activity was assayed after every 3 h till 48 h post inoculation to determine the overall growth profile of KCPS-76.

Effect of Substrate Concentration on Cellulase Production

The effect of substrate concentration on extracellular cellulase production by bacterial strain KCPS-76 was determined by varying the concentration of Carboxymethyl cellulose from 0.25 to 2.0 % and the results were recorded.

Effect of Nitrogen Source and Concentration

Various organic (peptone, soya meal, tryptone, malt extract and beef extract) and inorganic nitrogen sources (urea, NH₄Cl, (NH₄)₂SO₄, NaNO₃ and NH₄(NO₃) with conc. range 0.2-1.6% were optimized to enhance the production of enzyme and growth of the selected bacterial isolate.

Effect of Temperature

To study the effect of incubation temperature on cellulase production, the bacterial culture was grown at different temperatures ranging from 20 to 50 °C in 50ml of optimized production medium with other conditions remaining unchanged. The optimum temperature was selected for further studies based on the activity of the extracellular enzyme.

Effect of pH

The effect of pH on cellulase production was investigated by cultivating the organism in 50 ml of production medium

under different pH ranging from 5.5–9.0 at 35°C for 24 h. The cellulase activity was measured and the optimum pH was selected for further studies.

Statistical Analysis

The statistical analysis of data obtained during the optimisation of process parameters for cellulase production by using classical one variable at a time method. Data presented on the average of three replicates (\pm SD) are obtained from three independent experiments.

Optimization of Reaction Conditions for Cellulase Production by KCPS-76

Since apart from the culture conditions, the reaction conditions also plays a vital role and affects the enzyme activity, therefore the enzyme was subjected to varied reaction conditions (Buffer pH and molarity, temperature, incubation time, substrate concentration) to find out the optimum conditions for its activity and the results were recorded in each case.

Isolation of Ethanol Fermenting Microorganism

Ethanol fermenting organisms were isolated from the local brewery of Kinnaur, in order to ferment the cellulosic waste for the production of ethanol.

RESULTS AND DISCUSSION

During the course of investigation, a total of 80 bacterial isolates were isolated to pure cultures and screened for the cellulase activity by congo red staining as shown in Figure 1. A clear zone was found around the cellulase producing isolates. Similar method was also used by some other workers for the selection of best cellulase producing bacteria on the basis of clear zone diameter (Ahmed et al., 2013; Rasul et al., 2015; Gautam & Sharma, 2015).

Secondary Screening

During the secondary screening, out of 80 isolates 47 isolates were positive for cellulase activity and the maximum activity was obtained by the isolate LCPS-25 (i.e. 1.26 ± 0.13 U/ml)

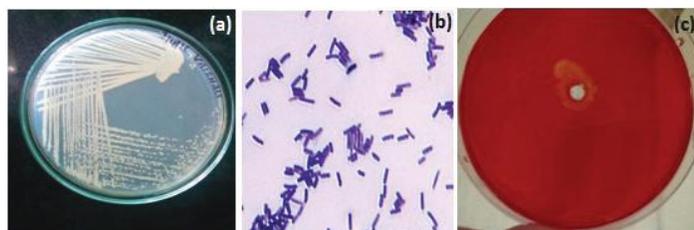


Figure 1: (a) KCPS-76 by streak plate method, (b) Gram's staining, and (c) Congo red staining.

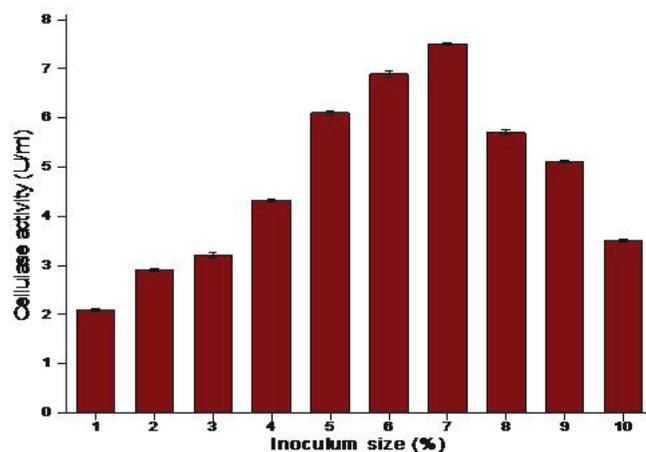


Figure 2: Optimization of inoculum size for the production of cellulase.

carrying Gram negative character. LCPS-25 isolate was isolated from cow dung sample collected from Billing village of Lahaul. KCPS-76 (Gram positive) showed maximum activity of 2.3 ± 0.09 U/ml and was collected from Kalpa (Kinnaur). KCPS-76 was further selected for the production parameter optimisation for cellulase.

Optimisation of Production Conditions of Cellulase from KCPS-76

Inoculum age and Inoculum size

The results demonstrated that the age of inoculum has a strong relation with the growth of the organism and cellulase production by it. It was found that the culture that was inoculated with 24 h inoculum age resulted in maximum cellulase activity of 4.76 ± 0.02 U/ml. Afterwards, Inoculum sizes ranging from 1 to 10% of 24 h old were studied to observe the impact over the cellulase production as shown in Figure 2. It was found that the cellulase production increased with the inoculum

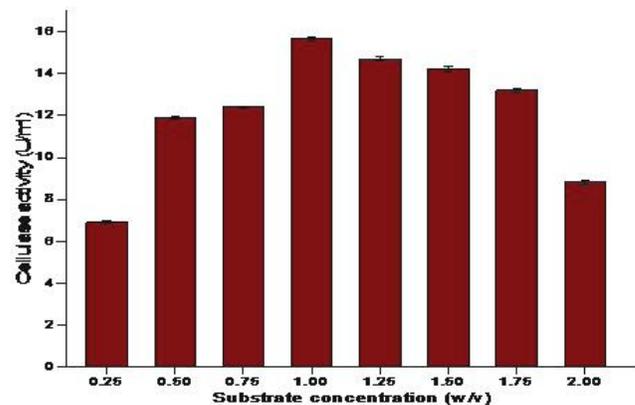


Figure 2: Effect of CMC concentration on cellulase production.

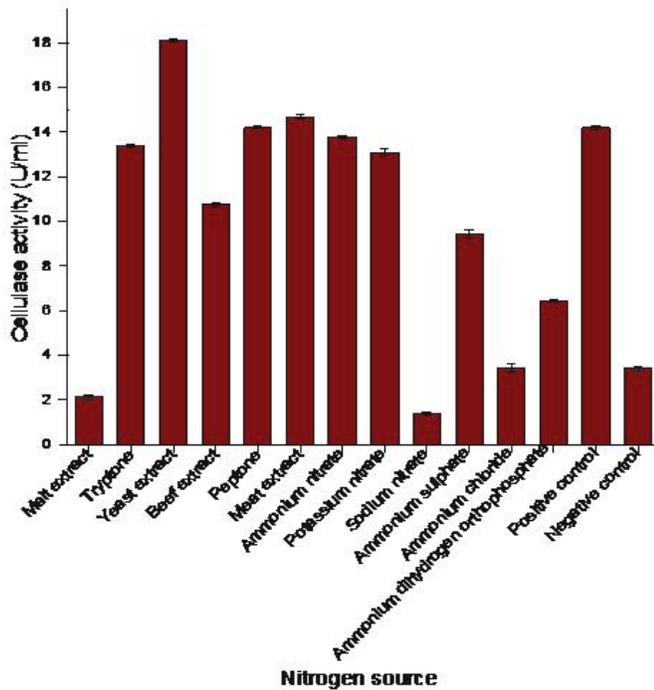


Figure 4: Effect of nitrogen sources on the cellulase production.

size up to 7%, with maximum activity of 7.5 ± 0.033 U/ml. Acharya and Chaudhary (2011) reported to have maximum cellulase activity with 2% (v/v) inoculum size.

CMC Concentration

Production of cellulase by KCPS-76 was studied by growing it in medium (pH-7, temperature 30 °C, 48 h production time) with different concentration of CMC (0.25 to 2.5% w/v). Of different concentrations of CMC used, an increasing trend in the activity of cellulase was observed till 1% concentration, with maximum enzyme activity of 15.65 ± 0.096 U/ml (Figure 3). Effect of substrate varies with the type of microorganism, and habitat, etc. Similar observations were also made by some other workers in past. The maximum enzyme activity of $3.028 \mu\text{g}/\text{mg}$ protein was achieved from *Bacillus* sp. when CMC (1%) was utilized as carbon source by Das et al. (2010). Singh et al. (2014) showed similar results with maximum cellulase production from *Bacillus* sp. in basal medium supplemented with CMC (2%) as a substrate at 40°C after 72 h of incubation.

Nitrogen Sources and Nitrogen Concentration

Nitrogen is one of the major cell proteins and stimulation of cellulase activity by ammonium sulphate salt might be due to their direct entry in protein synthesis (Mandals, 1975). The use of organic nitrogen sources as compared

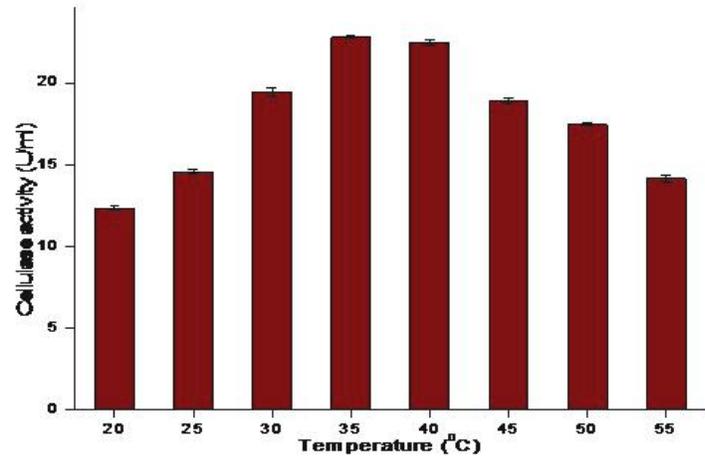


Figure 5: Effect of temperature on the cellulase production.

to inorganic sources for maximum cellulase production was found to be more suitable for maximum cellulase production (Ariffin et al. 2008; Ray et al. 2007). Effect of various organic nitrogen and inorganic nitrogen sources were studied to evaluate their role in cellulase production. The activity (18.2 ± 0.043 U/ml) recorded as shown in Figure 4 showed that the yeast extract emerged as the most appropriate nitrogen source with the concentration of 0.6% w/v for cellulase production with maximum enzyme.

Temperature

To study the effect of temperature on the production of cellulase by isolate KCPS-76, it was grown at different temperature ranging from (25 to 70 °C). The results indicated that enzyme production increased with increasing temperature and was maximum at 35 °C with an enzyme activity of 22.78 ± 0.089 U/ml as shown in Figure 5. In similar study for the production of cellulase by *Bacillus subtilis* and *Bacillus circulans*, the temperature was 35 °C and 40 °C and pH in the range of 7.0–7.5 was found to be optimum Ray et al. (2007); Shaikh et al. (2013). A very high cellulase production of 104.68 U/ mL was reported with *Bacillus* sp. BSS3 at pH 9, 37°C with 1% CMC (Padilha et al., 2015).

Effect of Medium pH

The isolate KCPS-76 when grown in the optimal medium at a pH range of 4.0- 12.0, in order to select the optimal pH for the growth medium for cellulase production as reported in Figure 6. Maximum enzyme activity of 23.87 ± 0.097 U/ml was observed at pH 9. A very high cellulase production of 7.83 U/ml was reported with *Bacillus* sp. Y3 at pH 7, 37°C with 1% CMC (Lugani et al. 2015). Sethi et al. (2013) reported the maximum production of cellulase at a pH range of 9–11.

Table 1: Reaction parameter optimisation for cellulose

S. No.	Reaction Parameters	Optimised Value
1.	Buffer system and pH	Citrate buffer (pH-6)
2.	Buffer Molarity	0.5M
3.	Substrate Conc.	1.5%
4.	Temperature	55 °C
5.	Incubation Time	15 min

Optimisation of Reaction Parameters

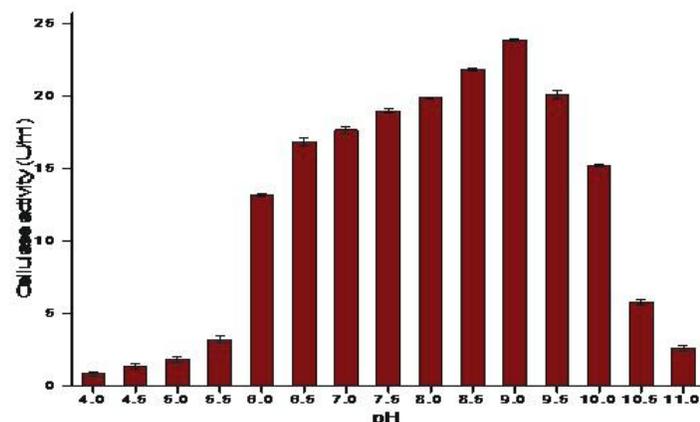
The maximum activity obtained after optimisation of production parameters and reaction parameters was 36.2 ± 0.13 U/ml, which was 13.9 fold from the initial one. Hence, LCPS-76 is a potential microbe for the degradation lignocellulosic waste from agro-waste residues (*ref.* Table 1).

Isolation of C-6 Fermenting Microbes

Total 7 isolates were isolated from the local brewery of Kinnaur District. Among them, 4 isolates showed positive results for fermentation mechanism and EFS-3 was considered as potentially active microbe for fermentation of C-6 sugars.

CONCLUSION

The present study is a sincere effort for exploring microflora for novel strains from the Himalayan region with high catalytic efficiency of cellulases. The selected bacterial isolate KCPS-76 isolated from District Kinnaur (H.P.) emerged as a potential isolate for increased cellulase production which can also be utilised for the lignocellulosic waste degradation. Optimization of production parameters of CPS-76 led to 13.9 fold increase in activity. Stability and activity of cellulase at broad range of pH i.e. from 6.0 to 10.5 and temperature (30-55 °C) makes it a suitable candidate for various applications

**Figure 6:** Effect of pH on the cellulase production.

including bioethanol production, fruit juice clarification, in detergent industries etc. Also the isolated ethanol fermenting strain from local brewery of Kinnaur can be a potential and novel isolate for C-6 sugar fermentation.

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Some Prominent Recommendations

Excerpts from the 1st Himalayan Researchers Consortium–2018

- Uniform methodologies needs to be followed for analyzing biodiversity elements across the Indian Himalayan Region;
- More focus on field studies are essential to understand the patterns of diversity in different ecosystem
- Sharpening of objectives are essential to deliver better output;
- Essentiality of collaborative work among the Fellows towards achieving desirable Global Biodiversity Targets;

Climate Change Studies

- Researcher should highlight the effect of Climate Change and Anthropogenic Activities.
- Need for Dietary analysis of small carnivores for two seasons (Summer & Winter).
- Study area has been extensively studied earlier gaps regarding information on Small Carnivores and Riverine Systems needs to be further expedited.
- Identify dog and wolf scats in the study area and further analyze the diet niche overlap with red fox.
- Species response to the changing land use.
- Identification of nematode up to the species level is essential.
- Processing of soil and extraction of nematode.
- Need for Analysis of specificity of feeding habit of Dragon fly.
- Work on the Isolation of Himalayan microbes fermenting to the level of yeast.
- Devise a vulnerability index for individual species.
- Experimentation to be done in natural condition, instead of the control condition for pollination as well as the climate change study.
- Collection of other ecological data such as weather parameters and anthropogenic disturbances needs to be collected.
- Identify Potential indicator species for monitoring habitat alteration and effect of climate change in Indian Himalayan Region.
- 'Climate Envelope Modelling' can be used to predict where such moth species are projected to occur in future.
- Spatial analyses of the richness pattern with major climatic (annual mean temperature & annual precipitation) and vegetation classes need to explored in GIS platform.

Authenticity of Target Species

- The authenticity of target species needs to be verified from Botanical Survey of India (BSI) through submitting the proper herbarium specimens. The authenticity and taxonomy of the targeted alien species, researcher needs to deposit the voucher herbarium specimens in BSI.
- Consult BSI and Forest Research Institute (FRI) for authenticating plant names.

Long-Term Monitoring

- *Long-Term Monitoring (LTM) Sites needs to be established for assessing the species diversity in different altitude.*
- *Conduct Monitoring surveys in historically prominent collection localities to see whether species assemblages have changed over past 150 years.*
- *Work towards building an Institutional mechanism for long-term monitoring in Sikkim.*
- *Identify Potential indicator species for monitoring habitat alteration and effect of climate change in Indian Himalayan Region.*
- *Morphometric traits' variation needs to be sought along altitudinal gradient across different Himalayan biogeographic zones.*
- *Spatial analyses of the richness pattern with major climatic (annual mean temperature & annual precipitation) and vegetation classes need to explored in GIS platform.*

Permission for Data Collection and Sampling

- *Constraints of the researcher regarding the no sampling permission in (e.g. Sikkim) may be brought to the notice of the NMHS-PMU & MoEF&CC.*

Data Collection and Analysis with Statistical Methods and Tools

- *Uniform methodologies needs to be followed for analyzing biodiversity elements across the Indian Himalayan Region.*
- *More focus on field studies are essential to understand the patterns of diversity in different ecosystem.*
- *In the context of "Methods of Biodiversity Data Analysis", diversity analysis is required in the field as well as available network in the world. Essentiality of collaborative work among the Fellows towards achieving desirable Global Biodiversity Targets.*
- *Consider the recent published literature for improving the research methodologies.*
- *Recommendation of the specific tree species for polluted environment should be based on a particular habitat and altitude specific.*
- *Need to specifically work on Phylogenetic analysis.*
- *The researcher need to network with other researchers / institutions for data collection on other taxa/faunal group within the permanent plots.*

IUCN Standard Criteria for Assessing the Threaten Index

- *UCN criteria for evaluating the rarity index of the threatened plants to be followed.*

Nine-fold Cycle of Data Processing

1. *Baseline Data Collection*
2. *New Data Collection*
3. *Data Co-relation and Analysis*
4. *Data Processing into Information*
5. *Information Processing into Policy Drafts and Decision-making*
6. *Policy Drafts Processing into Implementation Policy and Framework*
7. *Policy-based Demonstrative Framework and Participation of Stakeholders*
8. *Impacts and Results*
9. *Assessment Remarks and Long-Term Strategic and Implementation Framework.*

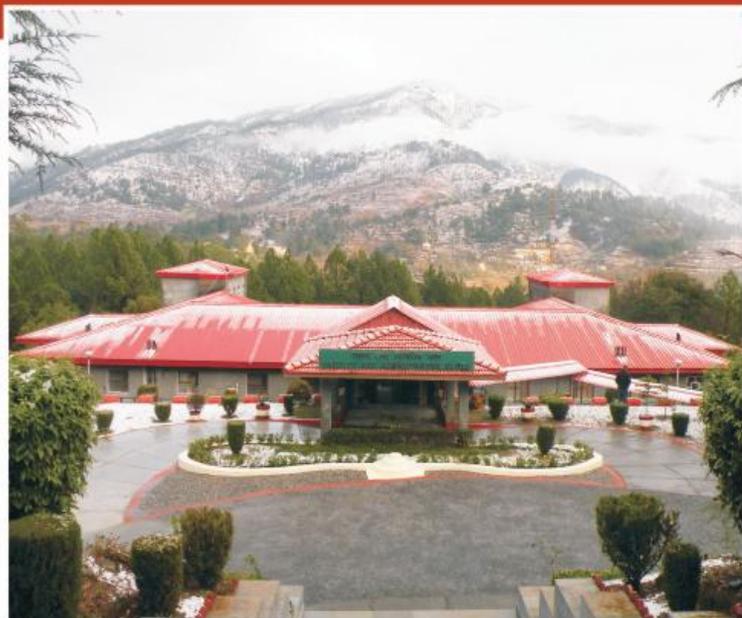
Indian Himalaya



1st Himalayan Researchers Consortium-2018 Group Photograph



About the Nodal Institute – GBPNIHESD



G.B. Pant Institute of Himalayan Environment and Development (GBPIHED) was established in 1988-89, during the birth centenary year of Bharat Ratna Pt. Govind Ballabh Pant, as an autonomous Institute of the Ministry of Environment, Forests & Climate Change (MoEF&CC), Govt. of India, which has been identified as a focal agency to advance scientific knowledge, to evolve integrated management strategies, demonstrate their efficacy for conservation of natural resources, and to ensure environmentally sound development in the entire Indian Himalayan Region (IHR). The Institute attempts to maintain a balance of intricate linkages between socio-cultural, ecological, economic and physical systems that could lead to sustainability in the IHR. To achieve this, the Institute follows a multidisciplinary and holistic approach in all its Research and Development programmes with emphasis on interlinking natural and social sciences. In this effort, particular attention is given to the preservation of fragile mountain ecosystems, indigenous knowledge systems and sustainable use of natural resources.

A conscious effort is made to ensure participation of local

inhabitants for long-term acceptance and success of various programmes. Training, environmental education and awareness to different stakeholders are essential components of all the R&D programmes of the Institute.

The Institute has been upgraded recently to a National-level institute namely "**G.B. Pant National Institute of Himalayan Environment and Sustainable Development (GBPNIHESD)**". The Institute has a decentralized set up across the Indian Himalaya, with its headquarters at Kosi-Katarmal, Almora, and at present five other Regional Centres (RCs) are operational at Srinagar (Garhwal RC), Mohal-Kullu (Himachal RC), Tadong-Gangtok (Sikkim RC), Itanagar (NE RC) and Mountain Division (at MoEF&CC, New Delhi).

About the National Mission on Himalayan Studies (NMHS)

Realizing the significance of the Himalaya for "Ecological Security of the Nation" and necessity as "Climate Regulator for much of Asia", the Government of India attached highest priority to protect unique but highly fragile Himalayan ecosystem and launched the "National Mission on Himalayan Studies (NMHS)" in year 2015-16 as a Central Sector (CS) Grant-in-Aid Scheme. The NMHS targets to provide much needed focus through holistic understanding of system components and their linkages, in addressing the key issues relating to sustainable conservation, management and development of natural resources in the Indian Himalayan Region (IHR). The mission is to launch and support innovative studies and related knowledge interventions to find scientifically sound solutions and best practices. The Mission strategy is to focus on enhancing livelihood of local communities in line with the National Environment Policy, 2006 of the Government of India.



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