POPULATION DENSITY, LIFE HISTORY TRAITS AND TRADITIONAL ECOLOGICAL KNOWLEDGE OF *Paris polyphylla* Sm. IN DOLAKHA, NEPAL

A Thesis submitted for the partial fulfillment of the requirement for Master of Science in Botany

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Submitted to
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DECLARATION

I hereby declare that the dissertation work "**POPULATION DENSITY, LIFE HISTORY TRAITS AND TRADITIONAL ECOLOGICAL KNOWLEDGE OF *Paris polyphylla* Sm. IN DOLAKHA, NEPAL"** is carried out by myself and has not been submitted elsewhere for any other academic degree. All the sources of information have been specifically acknowledged by reference wherever adopted from other sources.

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ABSTRACT

A number of medicinal and aromatic plants (MAPs) are threatened because of increasing cross border trade, habitat destruction and unsustainable harvesting. The life of people who depend on these resources and the knowledge associated with them are also under the threat. *Paris polyphylla* Sm. is one of those plants which has seen rapid rise in its global demand and concerns are being expressed about its declining wild populations. However, like many other medicinal plants, the data on population parameters and performance across the distribution range are largely lacking. Despite different studies identifying the need for recognition of the distribution pattern and biological traits of medicinal plants to assist conservation efforts, there has been very little empirical data and more focus has always been placed on the possibility of discovering novel wonder drugs. Therefore, in the present study, the population density and structure and variability in the performance of *P. polyphylla* between three different populations (lower temperate, mid temperate and upper temperate) across an elevation range (1800-3100 m asl) in Dolakha, Nepal has been assessed. The results showed that in all the population, the density of juveniles was lower than that of vegetative and reproductive adults. The mean density varied between 0.55 ± 0.05 and 1.4 ± 0.13 individuals/m^2 with the mid temperate population having the highest average density and the lower temperate having the least. Among different environmental variables, human disturbances (trampling and harvesting) and topographic parameters were the major factors contributing to variability in density and performance of the plant. The majority of the features were lowest in the upper temperate zone (at the highest elevation). The persistence and growth of *P. polyphylla* will depend on the effectiveness of measures adopted to control over-harvesting and premature harvesting of rhizome and also protection of younger life stages. In addition, *P. polyphylla* populations must be protected from other typical anthropogenic disturbances such as grazing and trampling.

Keywords: Population size and structure, Ethno ecological Knowledge, Anthropogenic disturbance, Plant performance

ACRONYMS

MAPs	Medicinal and Aromatic Plants
asl	above sea level
m	meter
WHO	World Health Organization
DoF	Department of Forest
GPS	Global Positioning System
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
TEK	Traditional Ecological Knowledge
SPSS	Statistical Program for Social Science
ANOVA	Analysis of Variance
MuMin	Multi Model Interference
ZIP	Zero Inflated Poisson
RRI	Relative Radiation Index

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CHAPTER I: INTRODUCTION

1.1 Background

Plants have been utilized to treat and cure ailments since antiquity. Medicinal and Aromatic Plants (MAPs) are used primarily by over 70% of the world's population to fulfill their healthcare requirements (WHO, 2002). Besides traditional uses, there has been a revived global enthusiasm in the utilization of MAPs as a foundation for pharmaceutical production. Because the majority of the species utilized are still taken from the wild, this has put a strain on natural resources (Barata *et al*., 2011). With the increased global interest in medicinal plants as potential sources of new pharmaceutical products, the preservation of medicinal plants and knowledge of their applications are essential for the future of human health care.

The conservation of a plant population is strongly reliant on information about its demographic state, genetic structure, reproductive behavior and biotic and abiotic interactions (Iriondo, 1996; Shrestha *et al*., 2011). Along with these, knowledge on the effects of harvesting on various vegetative and reproductive traits of species, response of different populations of the species to different harvesting regimes and assessment of harvest quantities that allows long-term survival are equally critical for sustainable management of MAPs (Ticktin, 2004). It also demands an awareness of how local people perceive, evaluate, and make decisions regarding the resources.

Studies of population dynamics and structure provide a critical foundation for understanding the future of plant populations and predicting the effects of various harvesting regimes on population performance (Rokaya *et al*., 2017). Demographic variables like as recruitment, mortality and growth can be used to characterize the dynamics of plant populations and the balance between these demographic features determines the structure of the population (Bharali *et al.*, 2012). For the prolonged survival of a population, continued regeneration is required (Thakuri, 2010). The regeneration potential of a species is determined by the density of seedlings and juveniles in the population (Bharali *et al.*, 2012). The future composition of species is influenced by the abundance of established seedlings and juveniles (Thakuri, 2010). Plant population structures that incorporate seedlings and juveniles would provide additional information on the species' status at an initial stage of regeneration.

Seed germination and seedling and juvenile establishment are dependent on availability of suitable habitat, water and the ability to respond to particular lighting conditions (Chapagain *et al*., 2019). Plants grow and live in restricted range of environmental variables such as elevation, canopy cover, accessibility to water and light etc. and variations in these elements influence the growth, morphology and restoration at various levels (Block and Treter, 2001; Duan *et al*., 2009). As a result, ecological factors can be utilized to assess a plant's population structure as well as its regeneration profile (Teketay, 1996).

The most important basic parameters needed to determine a plant species' ability to maintain its population is its abundance and attributes of an individual such as plant size and reproductive efficiency. Reproductive features are particularly crucial for a species' establishment and persistence after a disturbance. Number of seeds, its biomass and viability, growing season and disturbance characteristics all affect recruitment rates (Chambers *et al.,* 1990). Understanding the variety in such features is critical for determining the long-term viability of populations in ecosystem that have been disturbed by human activity. This type of natural variations in life history allows researchers to investigate its influence on population performance. It is also useful to investigate the causes and consequences of life-history variation (Kim and Donohue, 2011). Variation in plant life history and functional aspects such as blooming, seed mass, seed quantity and seed dormancy among different species has substantial ecological ramifications, as external factors exert significant selective pressures and have an important impact throughout the life cycle (Liu *et al*., 2017).

Plant life history and life cycle are influenced by the environment. Variability in biogeography, elevation and precipitation are assumed to have a significant impact on the evolution of plant species. To cope with challenging circumstances, maximize short term beneficial opportunities and deal with the unpredictability of when they will appear, organisms acquire a range of traits (Tonnabel *et al*., 2017). Elevation gradient might be an ideal element for examining the variation of functional features in plants in response to environmental influences because of acute fluctuations in abiotic factors and the subsequent selection pressure over a short distance in mountainous environments (Bresson *et al*., 2011). Disturbance regimes, such as harvesting, fire and grazing are also structured along elevation gradients, in addition to topographic variations (Chapagain *et al*., 2019).

In mountain ecosystems, increasing anthropogenic activities have caused and continue to develop a variety of new settings (Zhang *et al*., 2019). International trade in medicinal and aromatic plants (MAPs) has put tremendous strain on populations of particular alpine plants and their habitats in the Himalayas (Olsen, 2005; Pyakurel *et al*., 2018). Other factors such as animal grazing and fire, may also have an impact on the density of alpine plant populations (Niu *et al*., 2016). Plant population dynamics may be affected by harvesting whole plants or plant parts, which can alter reproduction, survival, and growth (Ghimire *et al*., 2008). The impacts of harvesting on plant populations, on the other hand, differs depending on environmental conditions, adaptability of plants, and processes like regeneration and colonization which are specific to species (Ticktin, 2004; Gaoue *et al*., 2013). High-altitude plants generate self-sustaining adaptation methods to cope with such severe environments. Production of spherical flowers with larger floral axes (Molau, 1993), long lasting sepals and functional root systems (Korner, 1999), production of large number of seeds (Korner, 1999), underground parts for vegetative propagation (Korner, 2003), as well as a higher fraction of underground biomass (Webber and May, 1977), all make a significant contribution to population persistence.

Overexploitation of resources and destructive collection methods have been highlighted as the major concerns to survival of medicinal plant species as it influences them directly or indirectly (David *et al*., 2014; Ouarghidi *et al*., 2017). Decline in reproduction, growth, and survival rates of the targeted species are primary environmental effects of unsustainable harvesting practices. Tolerance to harvesting varies and is dependent on various elements like the lifespan of the individual, the portion of the plant collected, availability of the species, the range of space where it grows, the rate at which the species grows etc. Slow-growing plants, for example, are particularly sensitive to intensive harvesting, but weedy plants are less vulnerable (Peters, 1996; Andel and Havinga, 2008).

Estimating the long-term viability of a population and impacts of collection of wild plants on rest of the ecosystem variables necessitates long-term research that might be difficult to isolate and monitor. Local people, on the other hand, frequently rely on local knowledge to manage wild plant harvesting successfully and sustainably (Schmidt and Ticktin, 2012; Tomasini and Theilade, 2019). As a result, indigenous people's participation in the management of natural resources and harvesting is considered critical for its effectiveness (Staddon *et al*., 2014). Study of local peoples' perception regarding the long-term viability of wild plant collection could be a potential way to understanding the sustainability of collection process for short-term studies. This involves, for example, collection procedures and conservation strategies, not just the particular procedures employed by active collectors, but also the assessment of plant populations and activities of other collectors. It could be used to detect early signals of alteration in species and population patterns, laying framework for rigorous conservation surveillance (Papageorgiou *et al*., 2020).

Paris polyphylla Sm. (Satuwa) is one of those medicinal plants whose global demand and market price has undergone rapid rise in recent past. It is one of the most exported medicinal plants in Nepal along with *Nardostachys grandiflora* and *Neopicrorhiza scrophulariiflora* (DOF, 2017). IUCN and CAMP have recognized it as Vulnerable (VU) species in Nepal (Bhattarai *et al*., 2002) and the Government of Nepal has given it a priority status for financial growth and study (DOF, 2017).

The rhizomes of *P. polyphylla* are traded extensively on a global scale each year. This rise in market demand has brought about the rise in price. In China, the cost of *P. polyphylla* rhizomes has risen by 400 times in the last four decades (Cunningham *et al.,* 2018). This trend of price rise has been infectious to neighboring countries including Nepal where the price has risen by almost hundred times from Rs.150 in 2006 (Pyakurel and Baniya, 2011) to Rs. 13000 in 2017 (DFO, 2017).

It is an important ingredient in several traditional Chinese and Tibetan medicinal herbal formulations and has been utilized to cure hepatic disease in China for many years (Lee *et al.,* 2005). It has also been an important part of folk medicine in Nepal and India and has been used to get rid of parasitic worms (Pande *et al.,* 2007) and as an antidote for the treatment of snake bite (KC *et al*., 2010). Currently, it is the major constituent raw material for some herbal medicines produced by 'Yunnan Baiyao' group (Long *et al.,* 2003).

With this rise in global demand and market price, there has been tremendous pressure on wild population of *P. polyphylla*. In last 15 years, the sizes of wild harvested *P. polyphylla* rhizomes sold in China are gradually decreasing and there is strong evidence for adding it to CITES Appendix II (Cunningham *et al.,* 2018). With this intensified demand, the knowledge gap about the status of populations has risen.

Despite concerns and assumptions about the decline in population of *P. polyphylla*, there are very little empirical data to support the assumption. So, there is an urgent need for assessing the trade's impact on the plant population of *P. polyphylla* by combining trade data (size and structure, traded quantities and their origin) and biological information at the species level such as reproductive biology, life history strategies, abundance, habitat suitability and reaction to environmental disturbances.

1.2 Rationale

Currently, many medicinal plants are threatened because of habitat destruction and unsustainable harvesting. With this, the life of people who depend on these resources and the knowledge associated with them are also under the threat. People are concerned about the rapidly declining wild population of *Paris polyphylla* (He *et al.*, 2006) but the data on population parameters are largely lacking (Cunningham *et al.*, 2018). Despite different studies identifying the need for recognition of the distribution pattern and biological traits of medicinal plants to assist conservation efforts, there has been very little empirical data and more focus has always been placed on the possibility of discovering novel pharmaceutical products. So, there is a need for obtaining up-to-date information on distribution pattern and population parameters such as population density and abundance, rate of decline and extent of occurrence in order to accurately identify the conservation status of *P. polyphylla*. In addition, there is also a need for documentation of traditional ecological knowledge (TEK) of local people.

1.3 Research Objectives

The broad objective is to assess the ecological status and conservation issues of *P. polyphylla* in Dolakha district, Nepal.

The specific objectives are:

- To study the current trade trend of *P. polyphylla* in Nepal.
- To document the traditional ecological knowledge regarding people's perception of its status and its uses.
- To analyze the variation in population density and structure of *P. polyphylla* subjected to different levels of anthropogenic disturbances across three different sites.

- To study the impact of environmental factors like elevation and canopy cover on the population density and rhizome biomass of *P. polyphylla*.

CHAPTER II: LITERATURE REVIEW

2.1 Trade of *Paris poplyphylla* in Nepal

The trade of *Paris polyphylla* in Nepal was inconsistent and confined to limited quantities before 2000 (Olsen, 2005). Amatya and Sayami (1998) reported a trade record of 250 kg *Paris* rhizome from Jumla and limited amount from Baitadi district in 1997. The large-scale trade of its rhizomes started only after 2010 in Nepal with the growth of its use in China (Cunningham *et al*., 2018). Pyakurel *et al.*, (2018) in a study carried out in Baitadi district of western Nepal found the total trade volume of *P. polyphylla* in the district to be 2085 kg in the year 2014/15 and had the highest cumulative value of NRs. 7.7 million. Kunwar *et al*. (2020) based on trade record of Hamro Ban issues reported that the average quantity of collection and trade for *P. polyphylla* rhizome during the course of 18 years (2000–2017) totaled roughly 15 tons per year. He *et al.* (2018) reported it to be one of the seven Nepali herbal remedies that China imports the most. Despite the growth in demand and supply, there is still limited data and documentation of the medicinal plant trade in Nepal (Kunwar *et al*., 2020).

2.2 Traditional Ecological Knowledge

The rhizome of *Paris polyphylla* has been traditionally utilized for various medicinal purposes. The underground part of *P. polyphylla* is frequently used in Nepal for the cure of intestinal worm infestation, for treatment of muscle stiffness, for treatment of indigestion and also for the treatment of pulmonary diseases (Bhattarai and Ghimire 2006).

The people of Panchase protected forest, Nepal have been utilizing *P. polyphylla* for treatment of fever, indigestion, skin disease, disease related to immune system, ear, eyes, nose and throat, diseases of animals, treatment of poisoning, respiratory diseases since long. They considered the plant as being useful like honey. Members of Gurung community used it to alleviate the harmful effect of alcohol and narcotics while it was used by local practioners with the belief of treating witchcraft related injuries. *P. polyphylla* rhizomes were used to perform different religious rituals and also in the funeral ceremony (Pokharel *et al.*, 2019).

Bhattarai (2018) found it to be useful in the treatment of Gastritis, ulcer, wounds, diarrhea and menorrhea and body pain in an ethnobotanical study carried among the Tharu community of Illam.

Kunwar *et al*. (2020) reported that the underground part of *P. polyphylla* has traditionally been utilized in Nepal for general healthcare as a component to counteract the effects of poison and also as a remedy to cure parasitic intestinal worms. In a study carried out across Nepal, *P. polyphylla* was found to be used for various purposes among which stomachache, cuts/wounds and fever were the most common diseases cured. Based on Relative Importance Level (RIL), it was most frequently used to cure parasitic intestinal worms (0.21), followed by treatment of microbial infection (0.18), treatment of fever (0.14) and as an antivenom (0.14).

Chaudhary *et al.* (2017), in a study carried out about the traditional practices of people of Kailash sacred landscape, Nepal found that the dry paste of rhizome was effective in the treatment of dizziness, boils, tumors, gallstones, wounds, paralysis, and gastritis.

Subedi (2017), in an ethnobotanical study conducted in Panchase protected forest, Nepal, found it to be useful for the treatment of pyrexia, gastrointestinal disorder, injuries, cough, hemorrhoids, poisoning and congestion of blood vessels.

Malla *et al*. (2015) reported the consumption of juice of *P. polyphylla* rhizome for the cure of gastric and menstruation pain in a study carried out in Parbat district of western Nepal. Moreover, it was served as stimulant while the powder of the rhizome was used in physical injuries and to cure parasitic worm infestation.

Paul *et al.* (2015) studied about the indigenous medicinal uses of *P. polyphylla* in the Indian Himalayan region and found its use to be restricted to certain areas. The underground part was particularly used to cure fever by some ethnic groups of mountainous regions while its paste was used as an antidote against venom.

Pfoze *et al.* (2013) found the rhizome of *P. polyphylla* being applied for the cure of burns, cuts, diarrhea, dysentery, fever stomachache and wounds, in a study carried out in Manipur, India.

K.C. *et al.* (2010) reported the use of *P. polyphylla* by local inhabitants of Ghandruk, Kaski. According to the study, the plant has been used traditionally for centuries to treat a variety of ailments in livestock, including burns, wounds, headaches, and fever caused by poisonous plants (bikh) that livestock eat. Moreover, some individuals utilized it as a jaundice remedy.

In Nepal, the powdered rhizome of *P. polyphylla* was found to be effective for treating narcotic side effects, fever, food poisoning, snakebites, and bug bites in two separate investigations by Dutta (2007) and Baral and Kurmi (2006). The study also mentioned its application to the treatment of both internal and external wounds, as well as in the treatment of diarrhea and dysentery in animals.

Gurung (2002) found the rhizome of *P. polyphylla* to be useful in the treatment of constipation, snake bite, cuts/wounds, cholera and gastritis in a study carried out among the people of Chitre and Bhadaure VDC of Kaski district about the medicinal plants and their traditional uses.

It is a significant Chinese traditional remedy. The roots and rhizomes of *P. polyphylla* are known as Rhizoma Paridis, and it is frequently used in traditional Chinese medicine to cure diseases related to blood, to cure fever, as an antidote and to cure inflammation (Cheng *et al.,* 2008). It's used to cure severe hemorrhage, congestion of blood vessels, pathogenic invasion and in recent years, it has been utilized to treat cancer as well (Xiao *et al.,* 2009). The subterranean components are used to treat various ailments brought about by accidents, sprains, spasms, and cramps (Liang, 2000). Entire plant is useful to cure fever, while the underground components are useful to reduce pain, to cure inflammation (removes heat), to cure fever, to cure cough and to purify blood (Yung, 1985; Duke and Ayensu, 1985). An extraction of rhizome is useful for cure of arthritis, appendicitis, lymphadenopathy, sinusitis, parotitis, epidemic Japanese B encephalitis, ulcers, diphtheria, and other conditions. It reduces pain, reduces swelling, and treats boils, carbuncles, sore throats, and acute pain. In traditional Chinese medicine, it is a key medicinal plant for cure of hepatic, abdominal, nasal, pulmonary, larynx, and chest cancer (Vassilopoulos, 2009).

2.3 Population Size and Structure

Regarding population trends in *Paris polyphylla*, there are comparatively limited empirical data available. The limited studies have shown the population density of *P. polyphylla* in the wild to be relatively low and a continuous decline in its natural population has been reported (Kunwar *et al*., 2020).

In a study by Tariq *et al*. (2021), carried out in Bageshwar district of Uttarakhand state, India, the density of *P. polyphylla* was found to range from 0.50 plants/m^2 to 2.00 plants/m^2. The low density and decline in population of *P. polyphylla* in the study area was attributed to a number of human-caused factors like extensive and unauthorized extraction, grazing, encroachment etc.

Kunwar *et al*. (2020) found the average density of *P. polyphylla* to be very low (0.078/m^2) in a study carried out across Nepal. The plant was found in majority of mid-hill and mountainous districts at elevations ranging from 1500 m to 3500 m. The study also found that premature harvesting, habitat degradation and illegal trade driven by the high demand for *P. polyphylla* had impacted its sustainability and made it vulnerable to extinction.

Lepcha *et. al.* (2019), in a study carried out in Sikkim Himalaya, India, reported the density of *P. polyphylla* to be varying in a range of 0.45 plants/m^2 to 3.89 plants/m^2 and the frequency to be varying in a range of 36% to 76%. The study further revealed the plant as a threatened species in the study area and was deemed to be in immediate danger of going extinct. The unauthorized removal of its underground part was the main threat to its existence.

Pokharel *et al.* (2019) reported a low presence of *P. polyphylla* plant in a study conducted in Nepal's Panchase protected forest (at only two sites out of nine sites studied). At Naulesim, 11 plants were found in one location and 5 in another, but at Jaljala, 2 plants were found in one location and 1 in another. The plants were not found in other regions, despite the fact that the rest of the sites were suitable. According to the locals, the plant's availability had fallen at a rapid rate, and it is now extremely rarely available. The study considered peoples' activities such as premature harvesting, illegal trade, unscientific extraction of forest products, tourism, grazing, construction activities and forest fire as the primary reason for reduced availability of *P. polyphylla.*

Paul *et al.* (2015) reported the population density of *P. polyphylla* in Arunachal Pradesh to be low, in a range of 0.42 plants/ m^2 to 1.48 plants/ m^2. The study found the wild populations of plant at risk of extinction because of excessive harvesting and limited spontaneous regeneration of the plant and accordingly been listed as an endangered species.

K.C. *et al.* (2010), in a study conducted to document the conservation issues, geographic range and reproductive ecology of *P. polyphylla* in Ghandruk Village Development Committee, Kaski reported the mean population density to be as low as 1.78 individuals/m^2. The study identified trampling, overexploitation, premature harvesting (before seed maturity), unsustainable extraction of rhizome (extraction of whole rhizome without leaving any part), reduced number of viable seed production, prolonged dormancy period and poor seedling growth to be the primary concerns to the recovery of the plant.

Similarly, Bhattarai and Ghimire (2006) observed density of 2.2 individuals/m^2 in Kaski. Some other studies found relatively low densities, such as 0.21 plants per square meter in Rasuwa (Gurung, 2007) and 0.2 plants per square meter in Dhankuta (Paudel *et al.*, 2019).

2.4 Effect of Environmental Variables on Plant Performance

There are very limited studies regarding the response of the *Paris polyphylla* plant to environmental conditions.

Tariq *et al.* (2021) found that rainfall, altitude and associated plants were the most significant environmental factors affecting the plant performance in a study carried out in Bageshwar district of Uttarakhand state, India.

Kunwar *et al.* (2020) found temperature and precipitation to be the most influential factors affecting plant performance in a study carried out in 51 districts across Nepal. It was found to be growing at elevation ranging from 1500 m to 3500 m in Nepal in habitats with extensive canopy cover of *Rhododendron*, *Quercus* and *Laurus* forests. The bamboo groves and grassy or rocky slopes with dense canopy cover had sparse population.

Lepcha *et al.* (2019), in a study carried out in Sikkim Himalaya, India, found precipitation, slope and aspect to be the most influential factors affecting the plant performance and *Arisaema*, Himalayan Sarcococca and ferns to be the common plants occurring with *P. polyphylla*.

Deb *et al.* (2015) studied the morphological and physiological characteristics, community interactions and response to artificial conditions of *P. polyphylla* in

Nagaland, India, in four different habitats. The study found that *P. polyphylla* exhibits vigorous development and reproduction in areas that are unaltered and have a canopy coverage of at least 80%. The plant thrives in moist soil that is rich in humus, and is commonly associated with *Quercus semecarpifolia*, *Taxus* species, *Daphne bholua*, *Rhododendron*, *Smilax aspera* etc. The study also found out that more than half of the plants of a particular population were in vegetative stage without production of flowers and fruits. The study considered light as an important factor during seed germination and also found out that seed yield is greatly reduced when the canopy cover is lesser.

K.C. *et al.* (2010) found the distribution of *P. polyphylla* being restricted to certain areas in the studied populations, notably in wet, shaded areas beneath the thick cover of mixed broad-leaved woodland. The plant was found between 1900-2900 m altitudes and was most commonly associated with *Viburnum erubescens, Arisaema*, fern, and *Sarcococca coriaceae*.

2.5 Phytomedicinal Properties of *Paris polyphylla*

The use of *Paris polyphylla* is no longer limited to indigenous community but is intensified because of its phytomedicinal properties. In China, it is extensively utilized as a component of many highly effective natural supplements such as primary care for physical injuries (Long *et al*., 2003). According to many research, it is said to show possible physiological effects. China has conducted research on the pharmacological activity of the plant with an emphasis on its anticarcinogenic and antibacterial properties.

2.5.1 Anticancer activity

The anticancer activity of *Paris polyphylla* has been thoroughly examined in China. Steroid saponins were discovered to be the primary anti-tumor active ingredients according to phytochemical and pharmaceutical investigations (Zhang *et al.*, 2007). The bioactive compounds like Polyphyllin and Rhizoma Paridis Saponin (RPS) extracted from *P. polyphylla* have shown potential anticancer activities against Lung cancer and Liver cancer (Zhang *et al.*, 2014; Lin *et al.*, 2015). Similarly, extract of *P. polyphylla* is also found to have potential bio-active compounds showing anti-cancer activity against Ovarian, Cervical and Breast cancer (Wang *et al.*, 2016; Zhang *et al*., 2014; Cozzi, 2003).

2.5.2 Antimicrobial activity

Paris polyphylla is reported to have antibacterial and antifungal activities. The rhizome of *P. polyphylla* has demonstrated antibacterial action against *Bacillus dysentery, B. paratyphi*, *B. typhi*, *Staphylacoccus*, *Escherichia coli*, *Streptococcus*, *Meningococcus* etc. (Sharma *et al.*, 2015). *Candida albicans*, *Saccharomyces cerevisiae*, and *Cladosporium* were significantly inhibited by some phytochemicals extracted from *P. polyphylla* var. *yunnanensis* (Sharma *et al.*, 2015).

According to research on the antimicrobial properties of *P. polyphylla*, root extract showed inhibitory action against *Staphylococcus aureus* only while aerial component extracts inhibited the growth of *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella fleixinerai* (Chhetri, 2012).

The literature review showed that the plant has enormous significance and medicinal value. Being one of the most desired plants, it also faces numerous threats. So, there is a need for recognition of its geographical range, distribution pattern, its present status in terms of its abundance, biological characteristics and documentation of Traditional Ecological Knowledge (TEK) associated with it to guide conservation activities.

CHAPTER III: MATERIALS AND METHODS

3.1 Study Area

3.1.1 Geography and climate

The present study was carried out in Dolakha district (85˚50’ E to 86˚32’ E longitude and 27˚28’ N to 28˚0’ N latitude (DDC/LGP, 1999). It is a mountainous district in Central Nepal with its geographical boundaries linked with China to the north, Ramechhap district to the east and south, and Sindhupalchowk district to the west. The altitude ranges from 762m asl to 7134m asl. The climatic condition in the district ranges from sub-tropical to Alpine climate. The average maximum temperature of the district is 16˚ in June while the average minimum temperature is 3˚ in January. The average annual rainfall was 204.4 mm with maximum in July (369.9 mm) and minimum in November (6.99 mm). The study was confined to three places; Lapilang Babare area under Kalinchowk Gaupalika, Khare and Gaurishankar area under Gaurishankar Gaupalika. Lapilang Babare area lies outside the Gaurishankar conservation area and is referred to as outside GCA (OGCA) hereafter while Khare and Gaurishankar are a part of it and is reffered to as GCA hereafter.

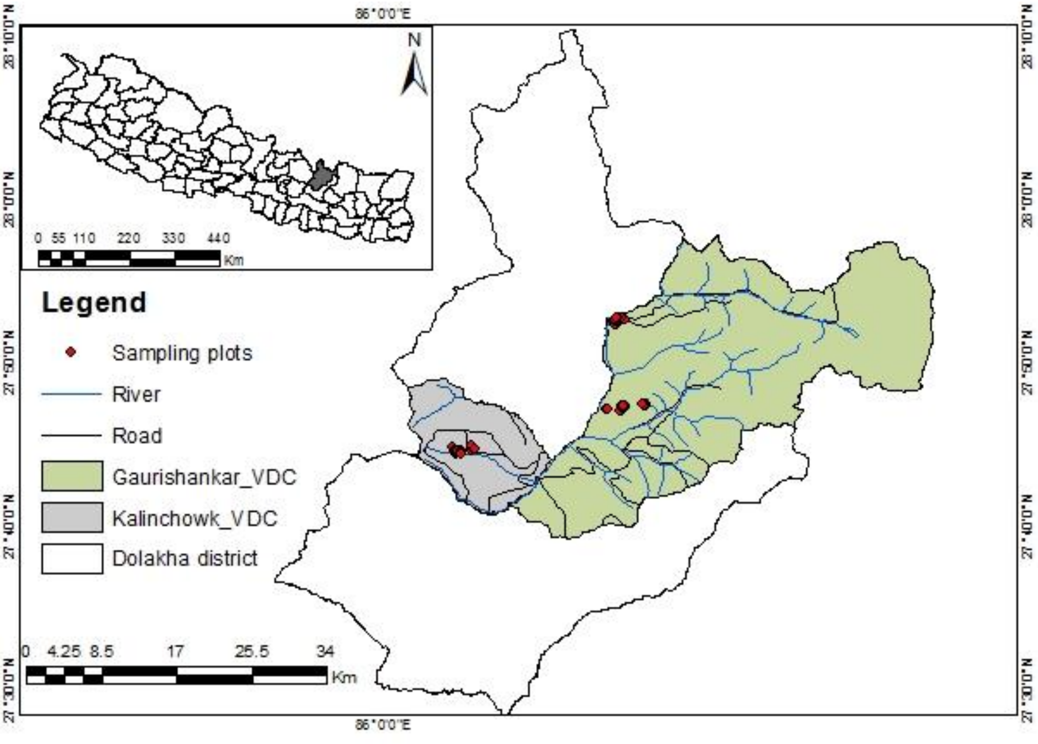


Figure 1: Map of the study area

3.1.2 Vegetation and plant diversity

Because of diverse climatic condition in the study area, it comprises of diverse vegetation type. The lower temperate region has *Pinus roxburghii* forest, *Schima-Castanopsis* forest, *Alnus nepalensis*, *Quercus semecarpifolia* forest and mixed broad-leaved forest while upper temperate region has *Pinus wallichiana*, *Rhododendron*, *Quercus lanata*, *Abies* and *Birch-Rhododendron* forest. Despite the fact that the study was limited to a narrow altitudinal range, the region comprises of various forest patches of tree species, such as *Alnus nepalensis*, *Schima wallichii*, *Lyonia*, *Quercus semecarpifolia*, *Pinus* and *Symplocos pyrifolia*, that forms a thick canopy. *Berberis asiatica*, *Daphne bholua*, and *Edgeworthia gardneri* form the well-developed shrub layers, while *Centella asiatica*, *Eupatorium adenophorum*, *Fragaria nubicola* and *Strobilanthes atropurpureus* are among the herbaceous plants.

3.1.3 Socio-economy and culture

The study area is home to people from several ethnic groups like Janajatis, Chetries, Dalits, Brahmin, Thakuri, Sanyasi etc. The mountainous region is occupied by people of Tibetan origin such as Sherpa and Yolmopa, whereas the hilly region is inhabited by Tamang, Thami, Sunuwar and indigenous people such as Surel and Jirel. Hinduism and Buddhism are two most commonly followed religions. The plant has abundant supply of water resources, serving as a catchment area for the Bhotekoshi, Sunkoshi, and Tamakoshi rivers, which serve as a source of water for some of the country's major hydropower projects. With popular tourist destinations including valleys, lakes, and monuments that are significant from both an aesthetic and religious perspective, it has a lot of promise. The main tourist destinations in the study area include Kalinchowk, Gaurishankar, Rolwaling Valley, Tso Rolpa Lake, etc.

3.2. Study Species

Paris polyphylla, commonly known as Satuwa in Nepal, is one of the vital medicinal plants which in recent years, have received recognition for its phytomedicinal properties. It is an erect, perennial, rhizomatous herbaceous plant with spider-like flowers that produce long, thread-like, yellowish green petals. The genus name comes from the word 'pars,' which refers to the plant's symmetry. The species name polyphylla is derived from the words 'poly' (many) and 'phyla' (leaves) (Shah *et al.*, 2012). *P.*

polyphylla, also called as "love apple" in English, belongs to the Melanthiaceae family (Long *et al*., 2003).

Figure 2: Study species

3.2.1. Taxonomic description

Paris polyphylla is an herbaceous plant with a perennating underground rhizome and greenish erect stem that bears no branches. The aerial stem is slender, glabrous, up to 1m tall with a diameter of 0.9 -2.4 cm. It has 2 to 3 whorls of leaves, each having 4 to 9 leaves. Leaves are **s**imple, arranged in whorls, petiolate, lanceolate, smooth margin, 7-13 × 1.4-3.4 cm, smooth. Single terminal flowers with short pedicel, yellowish green, tepal 3-5, sepaloid. Stamens 10, short and free. Ovary 5 × 6 mm; style 1.7-4.5 mm; stigma lobes usually 4, recurved at tips. Fruit globular; Seeds are reddish orange; a mature fruit contains 50-60 seeds.

3.2.2. Distribution and habitat

Paris polyphylla is native to China, Taiwan, the Indian Subcontinent, and Indochina (Ji *et al.*, 2006). Bhutan, China, north-eastern India, Laos, Myanmar, Nepal, Thailand, Vietnam, and Pakistan are all home to the species (Liang and Soukup, 2000; Shah *et al*., 2012). It can be found at elevations ranging from 1800 to 3500 meters across Nepal (DoF, 2017). It is a shade-loving plant that grows well in forests with canopy closure of more than 80% (Deb *et al.*, 2015). It is widely found in temperate mixed broad-leaved forest in Nepal, where it grows alongside plants such as *Daphne bholua*, *Rhododendron* sp., *Berberis asiatica*, *Aesculus indica*, *Quercus semecarpifolia*, *Neolitsea cubeba*, *Leucas* sp., *Impatiens sulcata* and others. It grows primarily in bamboo groves, grassy or rocky slopes, stream banks, mixed conifer forests, and scrub thickets in China. *P. polyphylla* flourishes in dense forests with little human influence. It thrives in a humus-rich, well-drained environment (Deb *et al.,* 2015).

3.2.3 Reproductive phenology

The rhizome of *P. polyphylla* is dormant for around 3-4 months. In the months of March and April, the rhizome sprouts. In the months of April and May, it blooms. Fruiting takes place in June/July, and seeds are ready to harvest in October. The majority of the plant dies out by the beginning to middle of November and all seeds are disseminated. It's a slow-germinating herb that can take up to seven months to sprout from seed (K.C. *et al.,* 2010).

3.3 Method

3.3.1 Trade trend

An attempt has been made to summarize the formal data of export of *P. polyphylla* from Nepal. Two sources of data were reviewed to study the trade trend of *P. polyphylla* in Nepal. The amount of *P. polyphylla* collected for trade was based on data from *Hamro Ban* volumes (2010-2017) compiled and published by Department of Forests (DoF) on annual basis while the price trend was based on data collected from Asia Network for Sustainable Agriculture and Bioresources (ANSAB).

3.3.2 Traditional ecological knowledge

Household surveys using semi-structured questionnaires were conducted during April - September, 2019 and April - September, 2020 among 304 persons (128 persons in the unprotected site, i.e., outside GCAP and 176 persons from the protected site i.e., GCAP). Besides, one-on-one and group interviews were also carried out in the course of the study. Local MAP users, leaders, local traders, resort landlords, local travel escorts, and livestock herders operating in the study area served as informants. Before beginning the interview, we explained the purpose of our study and acquired informed consent from the participants. The informants were also told that they might withdraw their agreement at any time throughout the session. Written notes were used to record the responses of the informants.

Among 304 people interviewed, 76 (involved in harvesting) were interviewed in detail to assess the ethnoecological knowledge about *Paris polyphylla*. They were classified into six social groups, three each from GCAP and outside GCAP (Table 7). The studies were carried out to evaluate differences in level of understanding among various groups and classes in the chosen areas of GCAP and outside GCAP. In this process, a total of 76 informants (involved in harvesting practices) were interviewed which included 32 informants from outside GCAP and 44 residing inside GCAP. In order to further measure the depth of their information regarding *P. polyphylla*, we inquired each person about habitat types, traits of various populations, sizes and densities of different populations in relation to each other, population trends, the factors of population change, risks, protection measures, and practices that could assure the species' survival and long-term management. We also inquired about the purpose of collection (whether it was for traditional use or for trade), its traditional uses, time and region of harvesting, for how long have they been using, the transfer of information about its traditional use from one generation to other, use of any particular device for collection, market value, nearby retailers, post-harvest processing and contribution to the livelihood from the informants. A scale with three general response categories was used to code the information related to ecological factors. In accordance with Ticktin and Johns (2002), a distinction between lack of any information (0) and some fundamental information (1) was made. Whenever more specific details were provided, a higher score (2) was given. Table 3 provides characteristics for each score. Variation of information possessed by different social categories were evaluated using Kruskal-Wallis test.

3.3.3 Habit characteristics, population size and structure

This field work was carried out from April 2019 to October 2020 to understand the population dynamics of *Paris polyphylla* in parts of Dolakha district. Sampling was carried out in three sites (Lapilang-Babare, Khare and Gaurishankar area) at an altitudinal range of 1800 m to 3100 m. The entire distribution range of *P. polyphylla* in the region was sampled. A (3m ×3m) plot was established and each plot was divided into nine 1m x 1m subplots, five of which (the center and four corner subplots) were systematically chosen for biological measurements. A total of 81 (3m ×3m) plots, with 405 (1m ×1m) sub-plots were placed. We used a GPS device to record latitude and longitude in each plot, and a clinometer and compass to determine slope and aspect. The cover (percentage) of trees, shrubs, herbs, grasses, lichens, bryophytes, litter, bare ground, solid rock, and scree in each subplot was visually estimated (Pauli *et al.*, 2015). As a proxy for microclimate at the site, the relative radiation index (RRI) was used.

With relation to aspect, latitude, and inclination, the RRI was determined for each plot. It is a measure of how much solar radiation is present at a specific area at midday. It was determined using the formula: $\text{RRI} = \cos(180^{o} - \Omega) \times \sin(\beta) \times \sin(\Phi) + \cos(\beta) \times \cos(\Phi)$, where Ω aspect (slope azimuth in degrees), Φ = latitude (degrees) and β = slope (degrees) (Oke, 1987; Vetaas, 1992). Individuals of *P. polyphylla* were counted and divided into three stages based on their size and reproductive status. The stage classes were: juveniles (Jv; plant height < 10 cm and no. of leaves < 5), adult vegetative (Adv, plant height > 10 cm and no. of leaves > 5) and adult reproductive (Adr; plants bearing flower of fruit). The number of individuals belonging to various classes in each subplot were counted to determine the structure and density. Anthropogenic disturbances included animal droppings, trampling, harvesting, and fire. To indicate the level of disturbance, a five-point ordinal scale (0-4) was used. A score of '0' meant there was no disturbance, while a score of '4' meant there was heavy disturbance.

3.3.4 Vegetative and reproductive traits

We grouped elevation bands into three wider ecotone populations, namely lower temperate (1800-2200 m), mid temperate (2200-2600 m) and upper temperate (2600-3000 m). Fifteen of the most vigorous plants from each of the population were uprooted to keep an account of plant biomass, determine vegetative traits like plant height, diameter, leaf number, size and biomass of leaf as well as reproductive characteristics (size of fruit and seeds). For lower temperate region, single plant was uprooted from each plot (plot no. 1-5) at each site (Lapilang-Babare, Khare and Gaurishankar area). Similarly, for mid temperate region, single plant was collected from each plot from plot no. 10-14 from each site and for upper temperate region, from plot no 19-23 from each site. The fresh weight of the aerial and under-ground portion was assessed in the field. The plant parts were then dried at room temperature, wrapped in newspaper and brought to the laboratory for dry biomass assessment. We harvested fruit from each of these individuals in the field, measured their diameter, length, and fresh weight, and stored the fruits for dry biomass assessment later.

Figure 3: Sampling

3.3.5 Laboratory study

All of the air-dried plant materials were oven dried (at 60° C for 72 hours) and their biomass was quantified to a precision of 0.01 g. The number of seeds in each individual, as well as its weight per fruit was measured.

3.4. Statistical Analysis

We used a mixed-modeling method with a random effect of plot to investigate variation of *P. polyphylla* density and rhizome biomass using a clustered sample design with five sub-plots per plot. At the study site, direct field observations verified that the plant was rare and sparsely dispersed. As a result, for the analysis of plant density, methods specifically developed to handle count data with excess zeros were examined. Simple transformations like square root and log were unable to transform our data to meet standard assumptions of normality. The large number of zeros in the data prohibited these adjustments from achieving their goal of symmetrizing the data and reducing mean-variance dependence. For the analysis of plant density, among multiple modeling approaches, the most suitable was found to be Mixed Zero-Inflated Poisson (mixed ZIP) models based on the Akaike Information Criterion (AIC) (Hall, 2000) while the impacts of environmental variables on rhizome biomass was analyzed using generalized linear mixed effects model (GLMM) assuming Gaussian distribution.

The independent variables such as population, tree canopy cover, relative radiation index (RRI), shrubs coverage, herbs coverage, harvesting, trampling, droppings of livestock and fire were used to quantify the relationship between environmental factors, plant abundance at different stages of development and rhizome biomass. The plot's random effect was included in all models.

For the analysis of effect of environmental variables on plant density, we constructed thirteen different alternative models using the glmmTMB package (Appendix 1), then used the MuMIn package (Barton, 2018) in R (R core team,2021) to create an average model based on a set of top three alternative models (chosen based on delta AIC value). MuMIn created the average model using three models with AIC values smaller than 357.22 for juvenile, 434.7 for adult vegetative and 571.42 for adult reproductive stage.

Density (of a specific stage of *P. polyphylla*) = a + b(Population) + c_1Tree canopy$_{mn}$ + c_2 Herb cover$_{mn}$+ c_3Harvesting$_{mn}$ + c_4 Trampling$_{mn}$ + (1|Plot), where a, b (population), and c_1 - c_4 are fixed parameters, m = 1-81 is the plot (added as a random effect) and n=1-5 is the sub-plot. Three categories of population are lower temperate, mid temperate and upper temperate.

To calculate the density, the number of plants belonging to a particular stage inside the sub-plot were enumerated. Similarly, for the analysis of effect of environmental variables on rhizome biomass, different models were created (Appendix II) and for each model, the Akaike information criterion (AIC) was determined. The model having the least AIC value was considered as the best model.

Other life history features, unlike plant density and structure, were researched using population-level sample individuals. We sampled fifteen individuals from each population for the measurement of vegetative and reproductive characteristics. We checked if the values for each of these characteristics satisfied the assumptions of normality before proceeding with the analysis. Neither of the characteristics satisfied the basic assumption of normality, with the exception of plant height and biomass. As a result, we used Kruskal Wallis tests to look at how these attributes varied across the three groups. Because logarithmic treatment of the data produced statistical normality in the cases of plant height and biomass, we used one-way ANOVA in these parameters.

CHAPTER IV: RESULTS

4.1 Trade Trend

Based on the trade reports considered for the present research, *P. polyphylla* is found to have been traded in Nepal since 2000. However, it wasn't until 2010 that its trade in Nepal really took off (Cunningham *et al*., 2018). According to the official data of DoF, 6,686 kg of *P. polyphylla* rhizome were harvested and traded in Nepal in 2010.The trade volume was at its peak during the period of 2011- 2013 where 57.8, 41.6 and 45.7 tons were traded respectively. The trade volume declined thereafter and about 18 tons were traded in 2017. Official trade statistics of DOF of past 8 years (2010 -2017) revealed that Nepal, on average, supplied 24,750 kgs of *P. polyphylla* rhizomes per annum. Of the total quantity traded, about 75% is exported to China and the remaining 25% to India and local markets.

There were very limited instances of *P. polyphylla* being commercially traded till 2000 (Olsen, 2005). Virtually no monetary value existed before 2000. However, because of unprecedented growth of Chinese and international phytomedicinal market, the significance of *P. polyphylla* from Nepal has risen quickly in the recent times. After 2010, along with the expanding sales volume, the market value of *P. polyphylla* rhizome has also risen sharply. In 2010, the market price of high-quality *P. polyphylla* rhizomes was NRs. 700 per Kg but in 2017, the price reached upto NRs. 10,000 per kg, representing an increase of 1300%. However, the market demand, timing, and place of trading all have an impact on how irregular and volatile local market prices can be. Since 2017, the price has declined gradually. In 2020, the market price of *P. polyphylla* was NRs. 2000 per kg as low as in 2011.

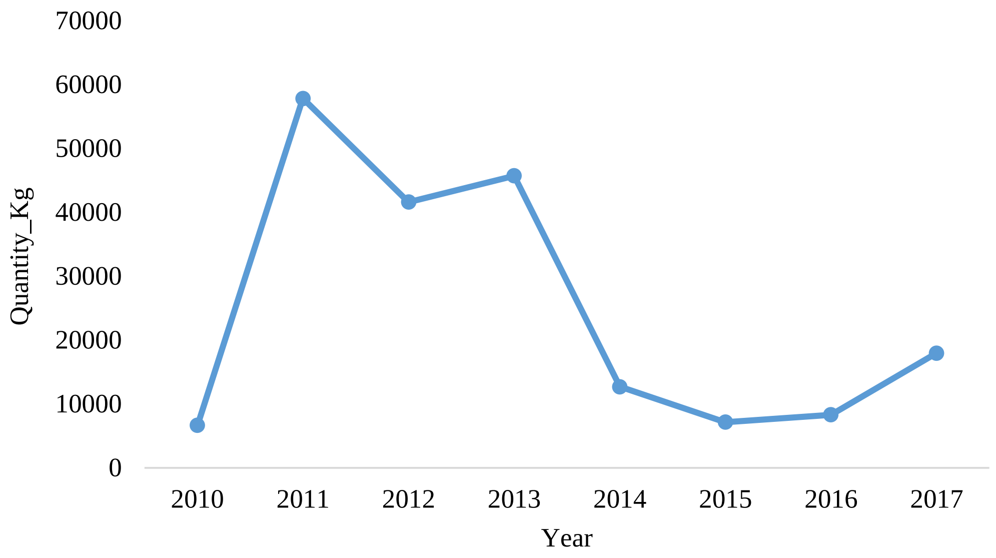


Figure 4: Total amount of *Paris polyphylla* rhizomes exported from Nepal.

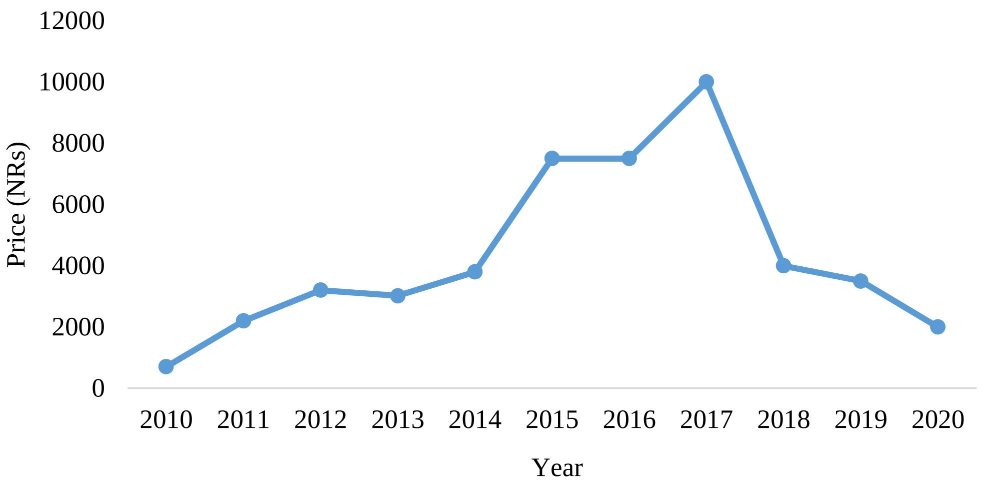


Figure 5: Market price of *Paris polyphylla* in Nepal from 2010 to 2020

4.2 Ethnoecological Knowledge

4.2.1 Knowledge on uses of *Paris polyphylla*

The results revealed that the majority of the respondents were familiar with the medicinal and commercial uses of *P. polyphylla*. Among 304 people interviewed, 66.45% people were aware of its medicinal uses while 70.4% were aware of its commercial uses. 11.18% people were unaware of its medicinal or commercial values.

Table 1: Use category of *Paris polyphylla.* based on interview of local people of Dolakha, Nepal

Category	**No. of respondents**	**Percentage (%)**
Medicinal uses (only)	56	18.42
Commercial uses (only)	68	22.37
Both	146	48.03
None	34	11.18

4.2.2 Medicinal uses of *Paris polyphylla*

The rhizome of *P. polyphylla* was found to be used for various medicinal purposes traditionally. Among 202 people knowing the medicinal uses, majority of people (84.1%) used it for alleviating alcohol intoxication and narcotic effects followed by its use in the treatment of Fever and Headache (71.3%). 58.4% people used it in the treatment of external injury, fracture and strains while 41.1% people used it for treating Diarrhea and indigestion. 11.4% of the respondents believed that it can be used in the treatment of snake bite.

Table 2: Medicinal uses of *Paris polyphylla* based on interview of local people of Dolakha, Nepal

Use category	**No. of respondents**	**Percentage (%)**
External Injury, fracture and strains	118	58.4
Fever, headache	144	71.3
Diarrhea and indigestion	83	41.1
Snake bite	23	11.4
Alleviating alcohol intoxication and narcotic effect	170	84.1

4.2.3. Variation in ethnoecological knowledge

The awareness and information of local people about the biology and ecology of *Paris polyphylla* varied considerably among social categories. The local representatives of GCAP and representatives of community forest outside GCAP obtained significantly higher scores than the other users (Table 3). However, the local traders and non-specialists from GCAP and outside GCAP obtained similar scores (Table 3).

The scores for information about the number of populations known, types of habitats, distribution range, population characteristics, and flowering and fruiting times varied just slightly. However, scores differed significantly among groups regarding the knowledge about population size and seed production. Only few people responded to these questions.

Table 3: Mean scores of ecological knowledges about *Paris polyphylla* for different user categories in GCA and outside GCA (OGCA) sites.

Variables	**Local Traders OGCA**	**Non-Special OGCA**	**Members of community forest OGCA**	**Local Traders GCA**	**Non-special GCAP**	**Representat-ives GCA**	**χ^2**	**p-value**
No. of populations known	1.37	1.27	1.67	1.33	1.4	1.85	9.71	0.08
Characteristics of different populations	0.62	0.73	1.44	0.8	0.86	1.42	9.58	0.08
Habitat Character	1.5	1.53	2	1.67	1.6	2	10.7	0.058
Population size	0.75^a	0.93^a	1.57^c	1.4^b	1.27^b	1.71^c	14.6	0.012
Flowering time	1.62	1.53	2	1.73	1.68	2	7.57	0.18
Fruiting time	1.62	1.47	2	1.67	1.54	2	10.6	0.06
Seeds per plant	0.88^a	0.6^a	1.33^b	0.46^a	0.45^a	1.14^b	16.7	0.05
Overall Mean	1.20	1.15	1.71	1.29	1.25	1.73		

Mean score in the same row with same superscript letter are not significantly different at p = 0.05 level (pairwise comparison by Kruskal-Wallis test)

Each score has the following characteristics: 0 = lack of knowledge of general population characteristics, 1 = some populations, habitat types, population sizes, range of distribution, and some general plant characteristics, 2= precise knowledge of variety of population characteristics.

4.2.4 Local peoples' perceptions on status of *Paris polyphylla*

Most of the people (90.78%) thought that the abundance of *Paris polyphylla* has declined at present as compared to that of five years earlier. However, 1.32% of the people believed it to be more common. 3.95% people found no change and the remaining 3.95% people did not know the status (Table 4). Among people who believed the population to be declining, 47.82% perceived overextraction as the major cause of decline, followed by premature harvesting (28.99%) and habitat destruction (7.24%).

15.95% of who believed the population to be declining were unaware of its underlying causes (Table 5).

Table 4 : Knowledge on population status of *Paris polyphylla* as perceived by local people of Dolakha, Nepal

Category	No. of respondents	Percentage (%)
Declining	69	90.78
Increasing	1	1.32
No change	3	3.95
Unknown	3	3.95
Total	76	100

Table 5 *:* Reasons for decline in population as perceived by local people

Category	No. of respondents	Percentage (%)
Overextraction	33	47.82
Premature harvesting	20	29
Habitat destruction	5	7.24
Unaware	11	15.95
Total	69	100

4.2.5 Harvesting practices of people

A majority of people (88.15%) harvested the rhizome of *Paris polyphylla* prematurely before the seed dispersal while only 11.85% people harvested it at maturity (Table 6). 80.26% people remove the whole rhizome while harvesting and 19.74% leave a part of rhizome during harvesting (Table 7). This showed that the majority of people are involved in unsustainable harvesting practices.

Table 6 : Harvesting practices of local people of Dolakha, Nepal

Month of Harvesting	No. of respondents	Percentage (%)
May-June (Pre-mature)	52	68.42
July-August (Pre-mature)	15	19.73
September-October	9	11.85
Total	76	100

Table 7 : Harvesting treatment by local people of Dolakha, Nepal

Harvesting treatment	**No. of respondents**	**Percentage (%)**
Removal of whole rhizome	61	80.26
Removal of a part of rhizome	15	19.74
Total	76	100

4.3 Habit Characteristics

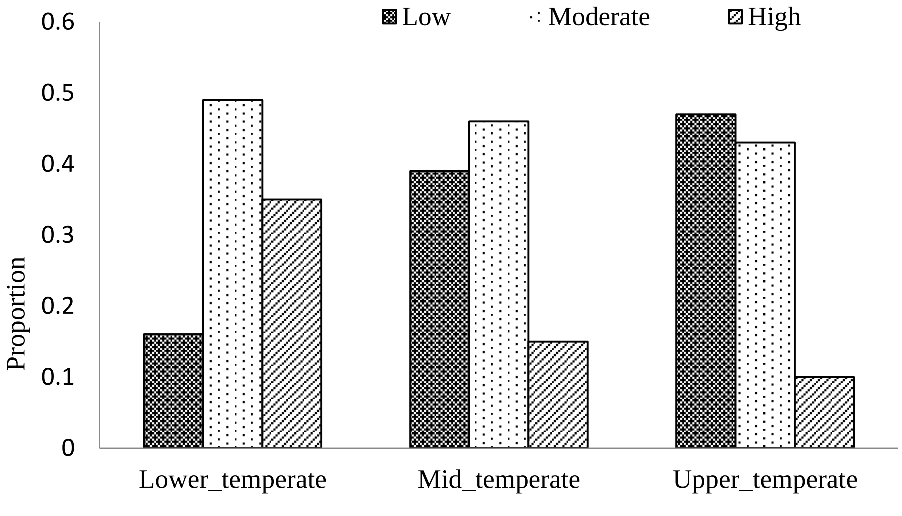
Paris polyphylla was recorded from moist shady habitat in forest with tree canopy closure of about 70% in the temperate region (1800-3000 m) of Dolakha, with main vegetation type being temperate mixed broad-leaved forest associated with *Alnus nepalensis*, *Quercus semecarpifolia*, *Daphne bholua*, *Rhododendron* sp., *Berberis asiatica*, *Aesculus indica*. It thrived in the deep forest, where the soil was humus-rich and human influence was negligible.

The habitat characteristics of *P. polyphylla* differed in various aspects among three populations. The lower temperate region was characterized by dense herb and shrub cover, high anthropogenic disturbance, relatively higher soil and litter depth and lower elevation. The upper temperate region was characterized by harsh environmental conditions but less anthropogenic disturbance. When compared to the lower and upper temperate regions, the mid temperate zone has intermediate characteristics.

Tree canopy was highest at mid temperate region (71.44%) and lowest at Upper temperate region (61.59%). Herb cover and shrub cover were higher at lower elevation and reduced significantly at higher elevations. The maximum bryophyte coverage was found in the Upper Temperate Region while soil depth and litter depth declined as elevation increased. The intensity of anthropogenic disturbance differed among three populations with lower temperate region experiencing highest disturbance and the upper temperate region the least (Figure 6).

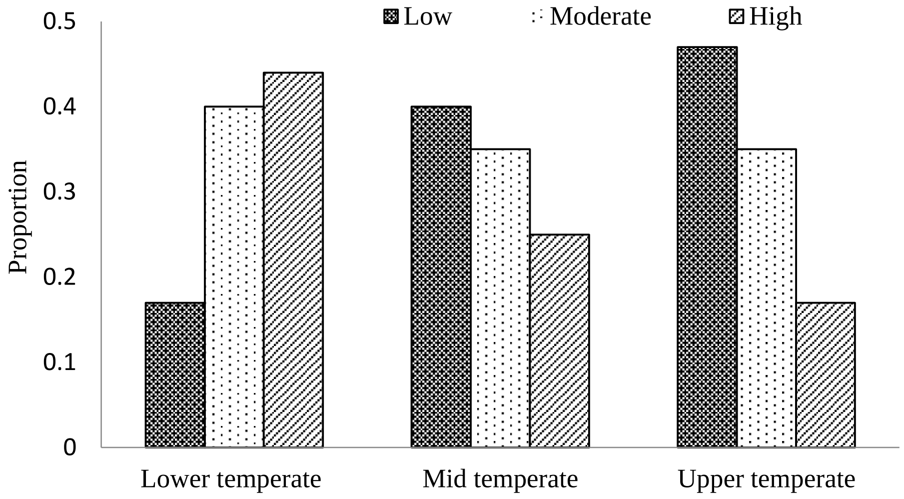
Table 8: Habitat characteristics of *P. polyphylla* in three study populations of Dolakha, Nepal. Values are specified as mean ± SE.

Habitat attributes	Lower temperate	Mid temperate	Upper temperate
Tree canopy (%)	66.97 ± 0.57	71.44 ± 0.6	61.59 ± 0.82
Shrub cover (%)	18.02 ± 0.7	18.84 ± 0.77	12.93 ± 0.67
Herb cover (%)	52.36 ± 1.31	39.07 ± 1.34	28.87 ± 1.16
Bryophyte covers on soil (%)	4.13 ± 0.27	5.61 ± 0.35	8.9 ± 0.39
Lichen cover on soil (%)	0.87 ± 0.07	1.07 ± 0.1	0.88 ± 0.07
Soil depth (cm)	12.72 ± 0.19	10.86 ± 0.32	9. 43 ± 0.15
Litter depth (cm)	5.77 ± 0.04	5.21. ± 0.05	4.63 ± 0.05
Solid rock cover (%)	4.3 ± 0.63	4.93 ± 0.7	3.87 ± 0.6
Elevation (m asl.)	2041.41 ± 9.71	2413.26 ± 10.88	2760.37 ± 10.23
Slope (degrees)	30.11 ± 0.53	27.89 ± 0.56	28.89 ± 0.57
Relative radiation index (RRI)	0.81 ± 0.01	0.72 ± 0.01	0.73 ± 0.01



Scale: 0-1=low, 2=moderate, 3-4=high

Figure 6: Frequency of varying levels of harvesting in three populations of *P. polyphylla* in Dolakha, Nepal.



Scale: 0-1=low, 2=moderate, 3-4=high

Figure 7: Frequency of varying levels of trampling in three populations of *P. polyphylla* in Dolakha, Nepal.

4.4 Population Density and Structure

The densities of seedlings and juveniles, vegetative adults, and reproductive adults were found to be significantly different in the three populations (Table 9). In all groups, the density of seedlings and juveniles was observed to be lower than that of vegetative and reproductive adults. The overall density of *P. polyphylla* across elevations was hump shaped, with a maximum density of 1.4 ± 0.13 plants/m^2 in the mid-temperate population while the lowest density (0.56 ± 0.05 plants/m^2) was observed in the lower temperate region. The density of juvenile, adult vegetative and adult reproductive in the whole study area was found to be 0.2 ± 0.03, 0.25 ± 0.05 and 0.54 ± 0.06 respectively. The overall density of the plant combining all stages was found to be 0.99 ± 0.06.

Table 9: Population density (m^{-2}) of various life stages of *Paris polyphylla* in different populations of Dolakha, Central Nepal.

Population	**Juvenile**	**Adult vegetative**	**Adult reproductive**	**Total**
Lower temperate	0.10 ± 0.02 ^{a}	0.13 ± 0.03 ^{a}	0.33 ± 0.05 ^{a}	0.55 ± 0.05 ^{a}
Mid temperate	0.30 ± 0.05 ^{b}	0.38 ± 0.06 ^{b}	0.72 ± 0.08 ^{b}	1.4 ± 0.13 ^{b}
Upper temperate	0.21 ± 0.04 ^{b}	0.24 ± 0.05 ^{a}	0.59 ± 0.07 ^{b}	1.03 ± 0.1 ^{b}
Overall density	0.2 ± 0.03	0.25 ± 0.05	0.54 ± 0.06	0.99 ± 0.06
χ^2 value	9.52	12.52	13.01	19.89
p-value	0.05	0.02	0.01	< 0.001

Densities are stated as mean ± SE χ^2 and p-values were based on Kruskal-Wallis test, df =2, n = 135. At p = 0.05; the values for each stage category do not differ significantly among populations with the same superscript letter.

4.5 Variation in Vegetative and Reproductive Traits

Vegetative and reproductive characters differed significantly among populations. The plants in the lower temperate population were the most vigorous in terms of plant height and stem girth (Table 10). The mean plant height in lower temperate, mid temperate and upper temperate region were respectively 42.47± 0.9 cm, 38.73 ± 0.78 cm and 28.55 ± 0.76 cm while the mean stem girth were 3.9 ± 0.15 mm, 3.4 ± 0.14 mm and 2.6 ± 0.13 mm respectively. The upper temperate population had a lower dry mass of rhizome and aboveground plant components compared to other populations (Table 10). The average dry mass of rhizome was 9.03 ± 0.22, 7.5 ± 0.21 and 6.5 ± 0.15 in the lower temperate, mid temperate and upper temperate population respectively. The biomass of underground plant parts was greater than that of aerial plant parts in all the populations. The dry biomass ratios for underground and aerial plant components differed significantly between the populations investigated and were found to be highest in the upper temperate population (Table 10). It was found to be 1.89 ± 0.04, 1.59 ± 0.03 and 1.55 ± 0.03 in the upper temperate, mid temperate and lower temperate region respectively.

The number and size of leaves also differed among populations. The average leaf size was found to be 44.92 ± 0.85, 36.7 ± 1.39 and 21.05 ± 0.67 cm^2 respectively in the lower temperate, mid temperate and upper temperate region while the number of leaves were found to be 10.93 ± 0.18, 9.4 ± 0.27 and 8.2 ± 0.39 respectively.

There was significant difference in the size of fruits between the populations (χ^2 =29.51, $p < 0.001$) with the lower temperate population having the highest mean value of 3.87 ± 0.27 cm^3 the upper temperate population having the lowest mean value of 1.48 ± 0.12 cm^3 (Table 10). There was significant difference in the number of seeds produced per plant among the three populations (χ^2 = 24.42, $p < 0.0001$ with the lower temperate population having highest mean value of 52.93 ± 1.6 seeds per plant and the upper temperate population with lowest mean of 35.33 ± 1.52 seeds per plant.

The upper temperate population was often characterized by restricted growth, lower biomass, fewer and smaller leaves, and fewer and smaller reproductive structures as compared to the lower and mid temperate populations.

Table 10: Variation in vegetative and reproductive traits of *Paris polyphylla* among three populations

Traits	**Low**	**Mid**	**High**	**χ^2/ F value***	**p-value**
Plant height (cm)	42.47± 0.9 ^{a}	38.73 ± 0.78 ^{b}	28.55 ± 0.76 ^{c}	37.78*	< 0.01
Stem diameter (mm)	3.9 ± 0.15 ^{a}	3.4 ± 0.14 ^{a}	2.6 ± 0.13 ^{b}	23.51	< 0.01
Leaf length (cm)	13.76 ± 0.18 ^{a}	12.66 ± 0.18 ^{b}	9.4 ± 0.19 ^{c}	142.10	< 0.01
Leaf breadth (cm)	3.26 ± 0.05 ^{a}	2.89 ± 0.08 ^{b}	2.24 ± 0.05 ^{c}	67.71	< 0.01
Leaf area (cm^2)	44.92 ± 0.85 ^{a}	36.7 ± 1.39 ^{b}	21.05 ± 0.67 ^{c}	141.06	< 0.01
Leaf number	10.93 ± 0.18 ^{a}	9.4 ± 0.27 ^{b}	8.2 ± 0.39 ^{c}	21.52	< 0.01
Fruit volume (cm^3)	3.87 ± 0.27 ^{a}	2.94 ± 0.23 ^{b}	1.48 ± 0.12 ^{c}	29.51	< 0.01
Fresh weight of fruit (g)	2.31 ± 0.07 ^{a}	1.86 ± 0.07 ^{b}	1.73 ± 0.05 ^{b}	22.24	< 0.01
Dry weight of fruit (g)	0.46 ± 0.01 ^{a}	0.36 ± 0.01 ^{b}	0.33 ± 0.01 ^{b}	25.29	< 0.01
Dry weight of seed (g)	0.16 ± 0.01 ^{a}	0.13 ± 0.01 ^{b}	0.11 ± 0.01 ^{b}	25.08	< 0.01
No. of seeds per plant	52.93 ± 1.6 ^{a}	41.26 ± 1.99 ^{b}	35.33 ± 1.52 ^{b}	24.42	< 0.01
Volume of rhizome (cm^3)	16.38 ± 0.8 ^{a}	11.52 ± 1.04 ^{b}	6.72 ± 1.96 ^{c}	35.10	< 0.01
Fresh weight of rhizome (g)	26.61 ± 0.70 ^{a}	22.20 ± 0.61 ^{b}	19.21 ± 0.47 ^{c}	29.68*	< 0.01
Dry weight of rhizome (g)	9.03 ± 0.22 ^{a}	7.5 ± 0.21 ^{b}	6.5 ± 0.15 ^{c}	29.04*	< 0.01
Above ground biomass (g)	5.8 ± 0.14 ^{a}	4.74 ± 0.1 ^{b}	3.47 ± 0.1 ^{c}	37.76	< 0.01
Ratio of biomass (Underground to aerial)	1.55 ± 0.03 ^{a}	1.59 ± 0.03 ^{a}	1.89 ± 0.04 ^{b}	26.70	< 0.01

At p = 0.05, the values for each stage category do not differ significantly among population with same superscript letter.

4.6 Effect of Environmental Factors on Plant Density and Biomass

The habitat characteristics of the three studied populations varied in different aspects (Table 8). The lower temperate population was located in area with high herb cover, high canopy coverage and with loamy soil. The herb cover, soil depth and litter depth gradually decreased towards higher elevation. The alpine population had least canopy coverage, soil depth and litter depth with more of silty soil. Compared to the other two populations, the lower temperate population was more influenced by anthropogenic pressure (Fig. 6).

Mixed zero-inflated Poisson (mixed ZIP) regression models found out five factors that with a pronounced relationship to plant density (Table 11). Tree canopy exhibited positive impact on the density of all stages of *P. polyphylla* (Pearson correlation, r = 0.369, 0.4, 0.654 and 0.745 for juvenile, adult vegetative, reproductive and total density respectively; $p < 0.001$) while the density of juvenile and reproductive stage was significantly affected by population.

Herb cover, harvesting and trampling exhibited a significant negative effect on the density of *P. polyphylla*. Herb cover showed negative impact on density of juvenile and adult vegetative stages (Pearson correlation, r = -0.377 and -0.179 respectively, $p < 0.001$). Harvesting scores were found to be negatively related to the density of adult vegetative and reproductive stages (Pearson correlation, r = -0.654 and -0.202 respectively, $p < 0.001$) while trampling scores were found to be negatively related to the density of juvenile stage (Pearson correlation, r = -0.412, $p < 0.001$).

Generalized Linear Mixed Effect models (GLMM) identified two variables that affected rhizome biomass (Table 12). Population had negative parameter estimates indicating that the rhizome biomass gradually decreased with increasing elevation. The rhizome biomass was found to be sensitive to environmental variations along the elevation gradient. The lower population was also associated with high disturbance. Harvesting also negatively affected rhizome biomass. The unsustainable harvesting practices of people could be attributed to its negative imp

Table 11: Effect of environmental factors on plant density

Stage	Average Model	Zi component	Count Component						
			Intercept	Low	Mid	Tree Canopy	Herb Cover	Harvesting	Trampling
Juvenile	Full	-5.752 (0.413)	-2.628 (1.432)	3.433* (1.652)	0.498 (1.219)	0.605* (0.249)	-0.555 *** (0.464)	-0.783 (0.531)	-2.003*** (0.507)
	Conditional	-5.752 (0.413)	-2.628 (1.432)	3.433* (1.652)	0.498 (1.219)	0.605* (0.249)	-0.555 *** (0.464)	-0.783 (0.531)	-2.003*** (0.504)
Adult Vegetative	Full	-6.298 (0.043)	-3.697** (1.351)	-0.679 (1.504)	-2.137 (1.299)	0.666** (0.247)	-0.298** (0.419)	-1.633*** (0.677)	-1.322 (0.491)
	Conditional	-6.298 (0.043)	-3.697** (1.351)	-0.679 (1.504)	-2.137 (1.299)	0.666** (0.247)	-0.298** (0.419)	-1.633*** (0.677)	-0.702 (0.480)
Adult Reproductive	Full	-5.080 (0.298)	-9.224*** (1.19)	-1.304** (1.561)	-2.894* (1.156)	0.427*** (0.213)	0.378 (0.396)	-0.073 (0.006)	-0.389 (0.0015)
	Conditional	-5.080 (0.298)	-9.224*** (1.19)	-1.304** (1.561)	-2.894* (1.156)	0.427*** (0.213)	0.378 (0.396)	-0.847** (1.162)	0.564* (0.942)

Table 12 : Effect of environmental variations on rhizome biomass

Explanatory variable	**Estimate**	**Standard Error**
Intercept	36.2525 ***	1.5262
Population	-3.6997 ***	0.4277
Harvesting	-2.6204 ***	0.5157

CHAPTER V: DISCUSSION

5.1 Trade Trend

The trade of *P. polyphylla* in Nepal was inconsistent and confined to limited quantities before 2000 (Olsen, 2005). However, its large-scale trade started post 2010 only (Cunningham *et al.,* 2018). The significance of Nepalese *P. polyphylla* has increased significantly in recent years with the growth of the Chinese and international phytomedicinal markets. Its rhizomes are now commonly used in conventional medicinal practices in China and Nepal (Acharya and Rokaya, 2005). It is one of the seven Nepali herbal remedies that China imports the most (He *et al.,* 2018). Official trade statistics of DOF of past 8 years (2010 -2017) revealed that Nepal, on average, supplied 24,750 kgs of *P. polyphylla* rhizomes per annum. However, these official trade records of medicinal plants are restricted and underestimated (Ghimire *et al*., 2016). The export of medicinal herbs from Nepal to China is significantly undervalued by government of Nepal (He *et al.*, 2018). Sometimes, the transactions made in the market are twice the trade data recorded in the government accounts (Pyakurel *et al.,* 2018). Despite China being Nepal's one of the largest importer and large amount of *Paris* rhizome being sent to China (He *et al.*, 2018), there is currently little information and paperwork available about the trade of medicinal plants in Nepal. Due to lax enforcement at Nepal's notably porous borders, several MAPs, including *P. polyphylla*, are illegally exported (Larsen *et al.,* 2000).

The accelerated demand of *P. polyphylla* has not only contributed to the large-scale unregulated harvesting from the natural population resulting in its decline but also to rapid rise in its market price. The demand for *P. polyphylla* is primarily centered in China. The stock value of *P. polyphylla* rhizomes in China has increased by four hundred times in a span of less than four decades since 1980s (Cunningham *et al.,* 2018). The price of *P. polyphylla* rhizome has increased dramatically in Nepal as well. From 2010 to 2017, the market price of *P. polyphylla* rhizomes increased from NRs. 700 per kg to NRs. 10,000, representing an increase of 1300% (DoF, 2017).

5.2 Ethnoecological Knowledge

The rhizome of *P. polyphylla* has been traditionally utilized for various medicinal purposes. In the present study, it was used in the treatment of fever and headache

(71.30%), alleviation of alcohol intoxication and narcotic effects (84.10%), treatment of external injury, fracture and strains (58.4%), Diarrhea and indigestion (41.10%) and snake bite (11.40%). The findings resembled with the previous studies. Kunwar *et al*. (2020) found that stomachache, cuts/wounds and fever were the most frequent ailments treated by *P. polyphylla*. Similarly, Baral and Kurmi (2006), Dutta (2007), and K.C. *et al*. (2010) reported its use in the treatment of fever, food poisoning, as a remedy for snake bites and poisonous bugs, to reduce effects of drugs and also to cure internal and external wounds, among other things. Some other studies report more extensive use of the plant in China where it is an important folk medicinal herb. The rhizomes are used to treat injuries like as fractures, convulsions, and strains caused by falls (Liang, 2000). In traditional Chinese medicine, it is a key medicinal plant for cure of hepatic, abdominal, nasal, pulmonary, larynx, and chest cancer (Vassilopoulos, 2009 and K.C. *et al.*, 2010).

The results showed that 90.78% of people thought that the abundance of *Paris polyphylla* has declined at present as compared to that of five years earlier. The perception of respondents regarding declining population of *P. polyphylla* was supported by the recent studies by Cunningham *et al*. (2018), Deb *et al*. (2015), Kunwar *et al*. (2020), who suggested a continuous decline in its subpopulations. Negi *et al*. (2014) discovered that overharvesting is depleting the species' wild population in India. He *et al*. (2006, 2007) found the situation to be comparable to China where substantial decline in its abundance have been documented due to overexploitation and habitat fragmentation. K.C. *et al*., (2010) reported a negative effect on the abundance of *P. polyphylla* even from the domestic consumption in Kaski, Nepal.

Among people who believed the population to be declining, 47.82% perceived overextraction as the major cause of decline, followed by premature harvesting (29%) and habitat destruction (7.24%). 15.95% of who believed the population to be declining were unaware of its underlying causes. In a recent study, Pokhrel *et al*. (2019) estimated the Relative Threat Factor Severity Index (RTFSI) for *P. polyphylla* in Nepal. Illegal collecting received the highest RTFSI score (0.90) followed by unscientific harvesting (0.83). Similarly, Semwal *et al*., (2007) and Tariq *et al.*, (2016) found that the low density of *P. polyphylla* was related to anthropogenic factors such as large-scale and unrestricted harvesting, grazing, trampling, and so on. Over-exploitation due to high demand, illegal trading, insufficient trade surveillance, and permeable borders all lead to the species' unsustainable harvesting (Kunwar *et al*., 2020). Indiscriminate extraction of entire rhizome before seed maturation is accountable for lower regeneration of the plant in its natural habitat (Paul *et al*., 2015). Its population decline is also attributed to cattle grazing that is not controlled. The consumption of seeds and fruits by birds and animals, and the entire plant by herbivorous animals further affect the natural regeneration process (Paul *et al.*, 2015).

5.3 Variation in Population Density and Structure

The population density of *Paris polyphylla* in wild was relatively low and there has been a continuous decline (Cunningham *et al.*, 2018; Kunwar *et al*. 2020). The mean density of *P. polyphylla* (0.99 plants/m^2) in the present study area is comparable with previous findings. For example, density of 1.78 individuals /m^2 was observed across four study sites in the Ghandruk of Kaski district (K.C. *et al.*, 2010). Similarly, Bhattarai & Ghimire (2006) observed density of 2.2 individuals/m^2 in Kaski. In some more researches, density was relatively low i.e., 0.21 plants per square metre in Rasuwa (Gurung, 2007), 0.2 plants per square metre in Dhankuta (Paudel *et al*., 2019). The density was reported to be even lower (0.078 individuals/m^2) in a recent study by Kunwar *et al*. (2020) that was conducted in 51 of Nepal's 77 districts. The mean density of *P. polyphylla* in the present study was relatively high in comparison to recently reported values. However, because the sampling plots were chosen based on subjective criteria, the existing data reflected the maximum density.

In India, the highest density of 1.48 individuals / m^2 and lowest of 0.42 individuals /m^2 have been reported from Arunanchal Pradesh (Paul *et al*., 2015) and in Sikkim, it varied from 0.45 to 3.81 plants/m^2 (Lepcha *et al.,* 2019). The wild stocks of the plant are declining because of overharvesting in India (Negi *et al*., 2014). A similar problem exists in China, where overharvesting and habitat fragmentation have resulted in dramatic population decline (He *et al*., 2006, 2007).

Population density was the lowest in lower temperate region (Table 8), which lies closer to the human settlement area. The lowest density in this region can be caused by the intense pressure from the human population and trampling. The increased population at higher elevations can be attributed to habitat inaccessibility and reduced human exploitation. Relatively smaller size of *P. polyphylla* found in the higher elevation could also have contributed to their higher density than found at lower elevation. The destructive effect of trampling and harvesting of rhizome by local people prior to fruiting seemed to be the cause of a fall in its abundance and hence very low density at some sites. The lower percentage of younger life phases (seedlings and juveniles) in the examined populations and the correspondingly high proportion of adults (vegetative and reproductive) clearly show that *P. polyphylla*'s recruitment capacity is generally poor. The species is slow-growing and the seeds can stay dormant for several years (Chauhan, 2021).

Despite the fact that *P. polyphylla* has a high average seed production (74.35/m^2), seed viability is poor (29%) (K.C. *et al*., 2010). The plants do not produce flowers in the first year and require a minimum of three years to reach reproductive maturity (Cunningham *et al*., 2018). Anthropogenic factors like overextraction and premature collection may diminish regeneration potentiality and growth of plants in alpine habitats (Tonnabel *et al*., 2017) and as a result, diminish the population density. Life history characteristics such as prolonged regeneration time, prolonged seed dormancy and high habitat specialization are some of the characteristics that render this plant more vulnerability to threats caused by human activities.

5.4 Variation in Vegetative and Reproductive Traits

P. polyphylla showed a range of life history traits in response to specific environmental conditions in lower temperate, mid temperate and high temperate habitats. Between the populations, there was a lot of diversity in terms of characteristics (Table 10). The declining trend in plant height, stem girth, leaf area, and plant biomass seen in plants from lower temperate to high temperate populations (Table 10) revealed that plant size decreases as elevation increases. The lower temperate (lower elevation) population of *P. polyphylla* showed stronger vegetative and reproductive vigor like production of larger fruits and higher number of seeds per individual. Plants may function better primarily because of suitable abiotic conditions, such as temperature and moisture (Billings, 1973; Körner, 2003). Facilitation (positive interactions) is a process prevalent at high elevations that helps plants deal with the hostile environment. It also improves plant performance in habitats with superior abiotic conditions (Callaway *et al*., 2002). Thus, the enhanced vegetative vigor of *P. polyphylla* seen at lower altitude is most likely due to the availability of favorable abiotic conditions that promote plant growth.

Plant development is often restricted at higher elevations due to harsh climatic conditions (Körner, 2003). The reduction in plant height is mostly related to corresponding decline in temperature and short growing period in alpine area (Brown *et al.*, 2003). The poor availability of nutrients in the soil, thin soil profile etc. could be related to the stunted growth of plant in high altitude. Plants from the upper temperate population had the highest dry biomass ratio for below and above ground plant part (Table 10). A significant fraction of underground biomass has been regarded as an adaptive response to harsh climatic conditions on numerous occasions (Webber & May, 1977). The fruit size and no of seeds were larger in individuals from lower temperate region as compared to other populations. Seed production by high-altitude species is temporally and geographically varied and is influenced by climatic factors (short, chilly growth seasons) (Chambers, 1995). The decreasing seed number and seed mass with increasing elevation can be explained as its response to stressful environment.

5.5 Effect of Environmental Factors on Plant Density and Biomass

The mixed ZIP model revealed that harvesting and population (elevation) had significant negative impact on adult plant density. The Commercial collectors want larger mature plants and harvest the whole rhizome prematurely before seed maturity. The probability of seed formation for future regeneration is reduced when the entire plant is removed before seed maturation (Sheldon *et al.*, 1997). The mixed ZIP model analysis further revealed that herb cover exhibited a significant negative impact on the density of juvenile and adult vegetative plants, which could be a result of herb cover limiting the amount of area available for seedling establishment, growing competition, and hence influencing growth and development. Similarly, trampling also had significant negative impact on the density of juvenile as well as adult plant. Plants are damaged by regular trampling by wild animals and humans, which inhibits them from blossoming, ripening seeds, and disseminating their seeds (Chardon *et al.*, 2018). Mixed ZIP model also revealed positive significance of tree canopy on density of all the life stages of *P. polyphylla*. It showed preference for growing in dense forest with canopy cover of around 70% with minimal human interventions. The result is supported by the findings of Deb *et al.* (2015) and K.C. *et al.* (2010) in which the species preferred to grow under moist, shady old growth forests with over 80% canopy cover.

CHAPTER VI: CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Paris polyphylla has enormous value as important traditional medicine for rural communities. It faces numerous threats as a highly desired medicinal plant species that is found in dense forest of temperate region. The density and performance (vegetative and reproductive characters) of *P. polyphylla* varied significantly among the populations. Anthropogenic disturbances and topographical factors were the most crucial factors which determined the density and abundance of *P. polyphylla*. The lower temperate population was subjected to the most intense anthropogenic pressure, which declined as elevation increased. The existing harvesting technique was detrimental because it involved uprooting the entire plant, and it was most likely the main factor for the lower temperate population's decreased recruitment and density. Despite the fact that adults in the lower temperate population have better vegetative performance and produce more flowers and fruits per reproductive adult, the loss of younger plants due to increased disturbance is not compensated. The majority of the features were lowest in the upper temperate zone (at the highest elevation), indicating that the plant was exposed to tough climatic circumstances. The persistence and growth of *P. polyphylla* will depend on the effectiveness of measures adopted to control over-harvesting and premature harvesting of rhizome and also protection of younger life stages. In addition, to ensure completion of flowering, fruiting, and distribution of viable seeds, *P. polyphylla* populations must be protected from other typical anthropogenic disturbances such as grazing and trampling.

6.2 Recommendation

Based on the result of the present study, following recommendations are made:

- Awareness among locals about conservation and sustainable harvesting of the plant should be raised.
- Locals should be encouraged for the commercial farming of the plant so that the pressure on wild population is reduced
- Despite the ban on commercial collection of the plant in the district, the population continues to decline. Hence, effective implementation of the ban with the involvement of the people is recommended.

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APPENDICES

Appendix I

Alternative models prepared for analyzing effect of environmental variables on plant density

Model 1

$\text{Density}_{mn} = a + (1|\text{Plot})$

Model 2

$\text{Density}_{mn} = a + b(\text{Population}) + (1|\text{Plot})$

Model 3

$\text{Density}_{mn} = a + b(\text{Population}) + c_1\text{Tree canopy}_{mn} + (1|\text{Plot})$

Model 4

$\text{Density}_{mn} = a + b(\text{Population}) + c_1\text{Tree canopy}_{mn} + c_2\text{ herb cover}_{mn} + (1|\text{Plot})$

Model 5

$\text{Density}_{mn} = a + b(\text{Population}) + c_1\text{Tree canopy}_{mn} + c_2\text{ herb cover}_{mn} + c_3\text{harvesting}_{mn} + (1|\text{Plot})$

Model 6

$\text{Density}_{mn} = a + b(\text{Population}) + + c_1\text{ herb cover}_{mn} + c_2\text{harvesting}_{mn} + (1|\text{Plot})$

Model 7

$\text{Density}_{mn} = a + b(\text{Population}) + + c_1\text{Tree canopy}_{mn} + c_2\text{harvesting}_{mn} + (1|\text{Plot})$

Model 8

$\text{Density}_{mn} = a + b(\text{Population}) + c_1\text{harvesting}_{mn} + (1|\text{Plot})$

Model 9

$\text{Density}_{mn} = a + b(\text{Population}) + + c_1\text{Tree canopy}_{mn} + c_2\text{harvesting}_{mn} + c_3\text{RRI}_{mn} + (1|\text{Plot})$

Model10

$\text{Density}_{mn} = a + b(\text{Population}) + + c_1\text{Tree canopy}_{mn} + c_2\text{harvesting}_{mn} + c_3\text{herb cover}_{mn} + c_4\text{trampling}_{mn} + (1|\text{Plot})$

Model 11

$\text{Density}_{mn} = a + b(\text{Population}) + c_1\text{harvesting}_{mn} + c_2\text{trampling}_{mn} + (1|\text{Plot})$

Model12

$\text{Density}_{mn} = a + b(\text{Population}) + c_1\text{Tree canopy}_{mn} + c_2\text{harvesting}_{mn} + c_3\text{trampling}_{mn} + (1|\text{Plot})$

Model13

$\text{Density}_{mn} = a + b(\text{Population}) + c_1\text{harvesting}_{mn} + c_2\text{herb cover}_{mn} + c_3\text{trampling}_{mn} + (1|\text{Plot})$

Where, a, b (Population) and $c_1 \ldots c_{13}$ are fixed model parameters, m=1…66 is the plot (random effect) and n=1…5 is the sub-plot. Population has three categories (lower temperate, mid temperate and upper temperate). Density is expressed as the number of individuals in a given stage category counted within a 1 m^2 sub-plot.

Appendix II

Set of models prepared for analysis of effect of environmental variables on biomass

Model 1

$\text{Rhizome\_biomass}_{mn} = a + (1|\text{Plot})$

Model 2

$\text{Rhizome\_biomass}_{mn} = a + b(\text{Population}) + (1|\text{Plot})$

Model 3

$\text{Rhizome\_biomass}_{mn} = a + b(\text{Population}) + c_1\text{Tree canopy}_{mn} + (1|\text{Plot})$

Model 4

$\text{Rhizome\_biomass}_{mn} = a + b(\text{Population}) + c_1\text{Tree canopy}_{mn} + c_2\text{ herb cover}_{mn} + (1|\text{Plot})$

Model 5

$\text{Rhizome\_biomass}_{mn} = a + b(\text{Population}) + c_1\text{Tree canopy}_{mn} + c_2\text{ herb cover}_{mn} + c_3\text{harvesting}_{mn} + (1|\text{Plot})$

Model 6

$\text{Rhizome\_biomass}_{mn} = a + b(\text{Population}) + + c_1\text{ herb cover}_{mn} + c_2\text{harvesting}_{mn} + (1|\text{Plot})$

Model 7

$\text{Rhizome\_biomass}_{mn} = a + b(\text{Population}) + + c_1\text{Tree canopy}_{mn} + c_2\text{harvesting}_{mn} + (1|\text{Plot})$

Model 8

$\text{Rhizome\_biomass}_{mn} = a + b(\text{Population}) + c_1\text{harvesting}_{mn} + (1|\text{Plot})$

Model 9

$\text{Rhizome\_biomass}_{mn} = a + b(\text{Population}) + + c_1\text{Tree canopy}_{mn} + c_2\text{harvesting}_{mn} + c_3\text{RRI}_{mn} + (1|\text{Plot})$

Model10

$\text{Rhizome\_biomass}_{mn} = a + b(\text{Population}) + + c_1\text{Tree canopy}_{mn} + c_2\text{harvesting}_{mn} + c_3\text{herb cover}_{mn} + c_4\text{trampling}_{mn} + (1|\text{Plot})$

Model 11

$\text{Rhizome\_biomass}_{mn} = a + b(\text{Population}) + c_1\text{harvesting}_{mn} + c_2\text{trampling}_{mn} + (1|\text{Plot})$

Model12

$Rhizome\_biomass_{mn} = a + b(Population) + c_1 Tree\ canopy_{mn} + c_2 harvesting_{mn} + c_3 trampling_{mn} + (1|Plot)$

Where, a, b (Population) and $c_1 \ldots c_{13}$ are fixed model parameters, m=1…81 is the plot (random effect) and n=1…5 is the sub-plot. Population has three categories (lower temperate, mid temperate and upper temperate).

Appendix III

Questionnaire for traditional ecological knowledge

Informants' details:

Name.................... Gender................................ Age....................

Occupation.............................. Education...............................

Residence..Ethnicity.............................

Religion.................................... History of living ....

1. Do you know Satuwa?

2. What are the uses of Satuwa?

3. Do you use it? If yes, for what purpose do you use it? Medicinal or commercial?

4. Which part of the plant is used? For what purpose is it used?

5. Do you know about its availability? If yes, how many populations do you know?

6. What are the characteristics of different population and size of population?

7. What are the characteristics of its habitat?

8. About phenology- Time of Germination

   Flowering and fruiting time

   Rate of germination of seed

   Seed production per plant

9. Do you harvest it? Are you involved in its trading?

10. When do you harvest? Which part do you harvest? Do you leave some part for regeneration?

11. What is the status of population of Satuwa? Is it increasing or decreasing or constant as compared to 5 years ago? If decreasing, what are the causes of decline?

12. Do you think it requires conservation? If yes, what measures can be adopted?

Appendix IV

Demographic characteristics of respondents

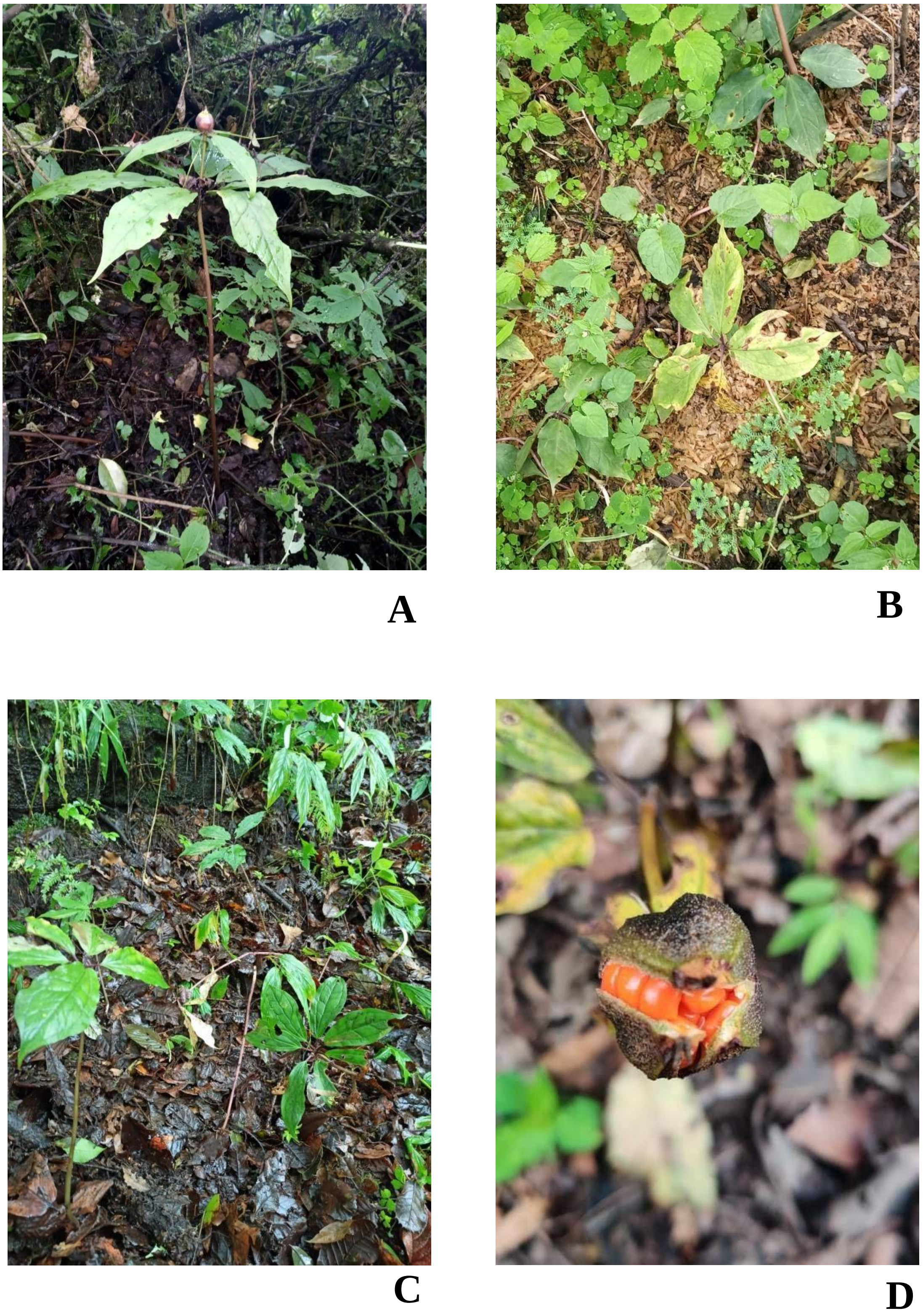
Demographic characteristics		Number (%)
Total number of the people interviewed		304
Gender		
	Male	211 (69.4)
	Female	93 (30.6)
Literacy		
	Illiterate	58 (19.08)
	Literate	246 (80.92)
Age		
	30-40	51 (16.78)
	40-50	114 (37.5)
	50-60	80 (26.3)
	>60	59 (19.41)

Appendix V

Categorization of people interviewed in detail

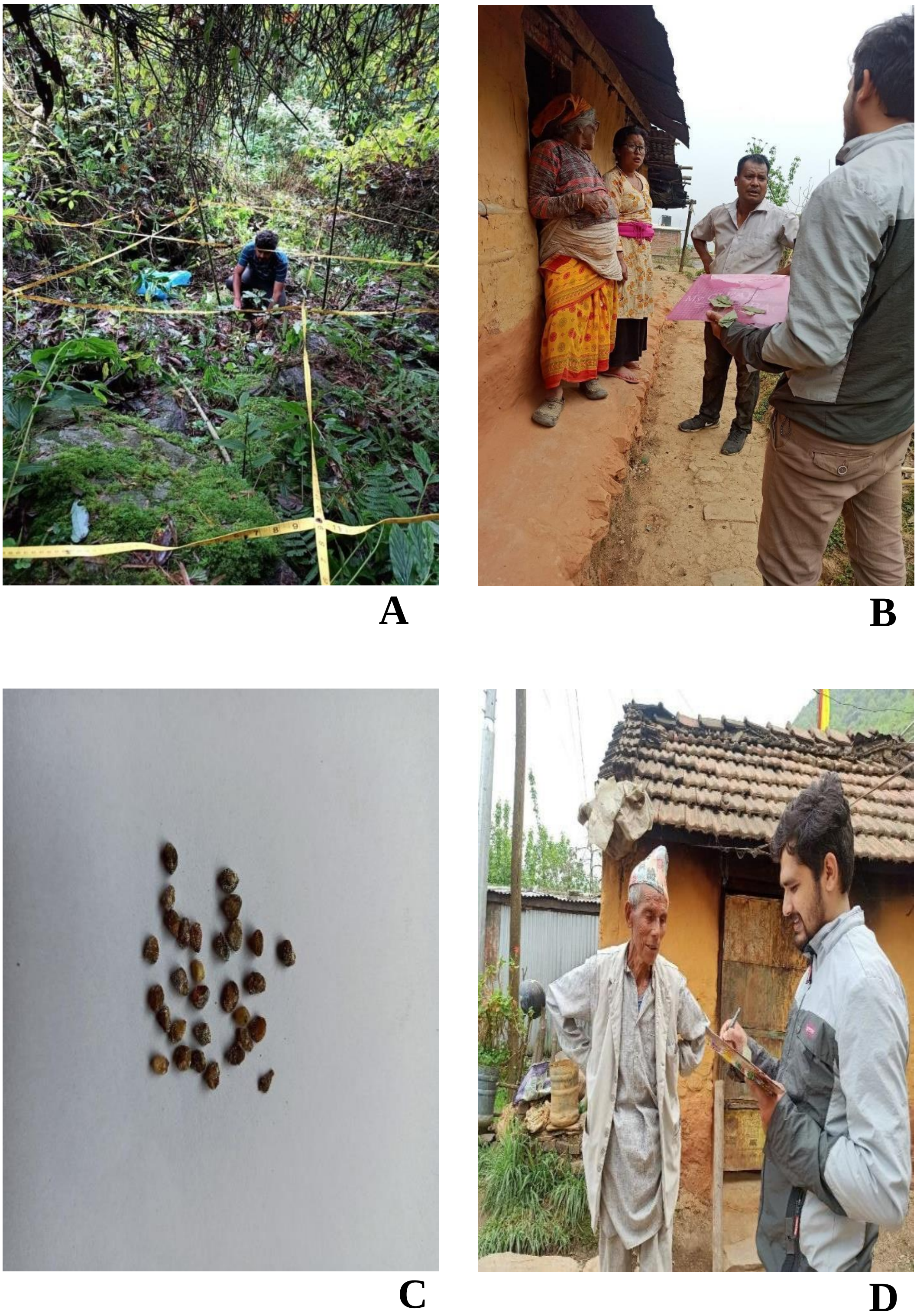
Site	Social group	Major profession	No of people	Age range	Sex
GCAP	Local Traders	Leaders/Traders/Hotel owners/Tourist Guide	15	30-50	All male
	Non specialist	Farmers/ Animal Husbandry	22	20-50	15 male 7 female
	Local representatives	Teachers/Leaders	7	30-55	6 male 1 female
Outside GCAP (LB)	Local Traders	Leaders/Traders/Hotel owners	8	25-55	All male
	Non specialist	Farmers/ Animal Husbandry	15	20-50	9 male 6 female
	Representatives of community forest	Teachers/Leader/Farmer	9	30-50	7 male 2 female

PHOTO PLATE I



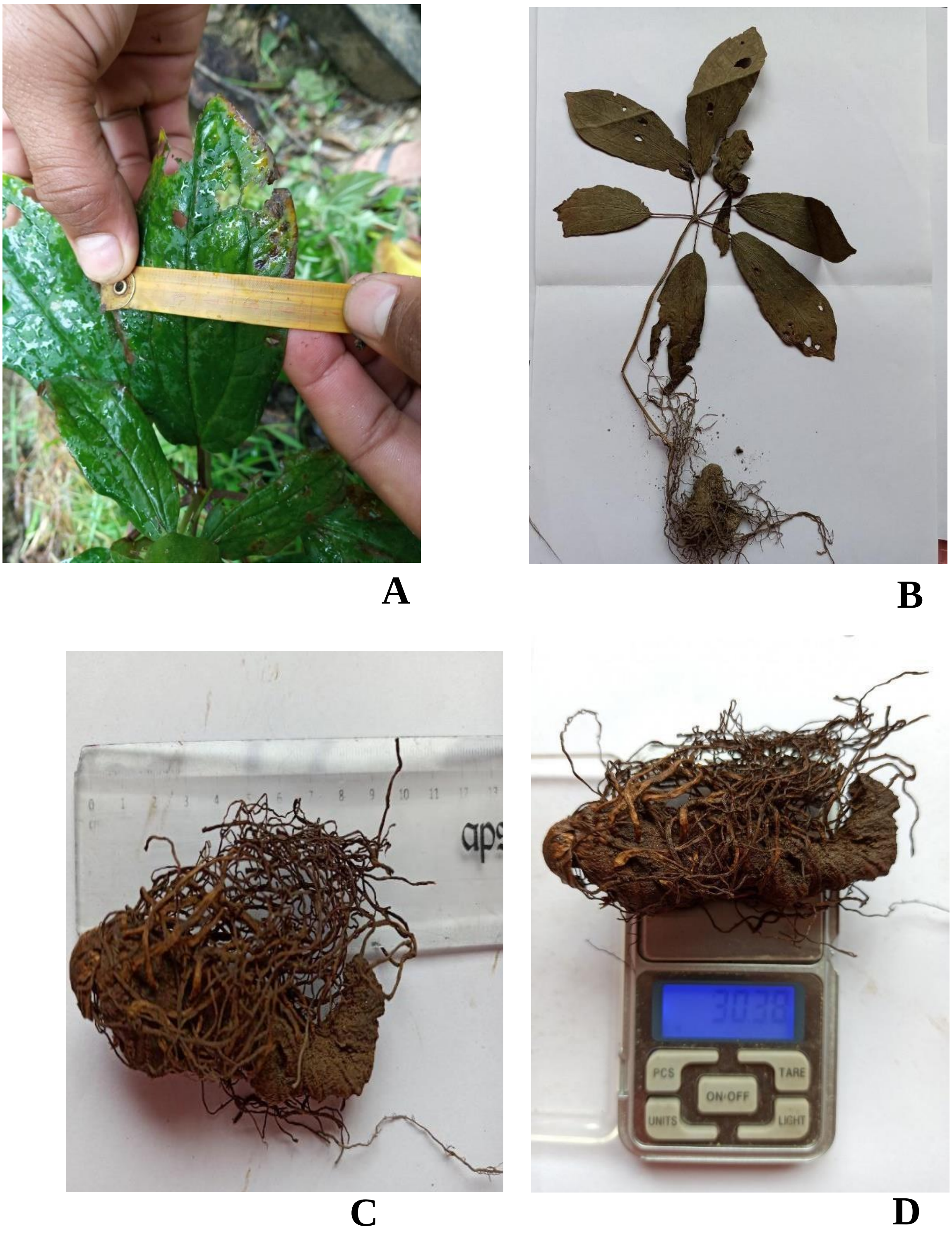
A. *Paris Polyphylla* plant B. Juvenile stage C. Adult Vegetative D. Fruiting stage.

PHOTO PLATE II



A. Sampling. B. Interview Survey C. Seed of *Paris polyphylla* D. Interview Survey

PHOTO PLATE III



A. Measurement of leaf B. Dry Specimen of *Paris polyphylla* plant C. Measurement of Rhizome D. Weight of Rhizome.