HERBIVORY AND PATHOGEN DAMAGE ON CO-ASSOCIATED NATIVE AND INVASIVE PLANTS IN SURYAVINAYAK AREA, BHAKTAPUR, NEPAL

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DECLARATION

I hereby declare that this dissertation work entitled **"HERBIVORY AND PATHOGEN DAMAGE ON CO-ASSOCIATED NATIVE AND INVASIVE PLANTS IN SURYAVINAYAK AREA, BHAKTAPUR, NEPAL"** is my original work and all other information used are correctly recognized and acknowledged. I further declare that the work reported in this dissertation has not been submitted, either in part or in full, for any other academic award at this or any other university.

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LETTER OF RECOMMENDATION

This document confirms that **Ms. Sabita Twanabasu** has completed the dissertation entitled **"HERBIVORY AND PATHOGEN DAMAGE ON CO-ASSOCIATED NATIVE AND INVASIVE PLANTS IN SURYAVINAYAK AREA, BHAKTAPUR, NEPAL"** under our guidance. The research presented in this dissertation is original and conducted by the candidate. To the best of our knowledge, this work has not been submitted for any other academic qualification. We recommend the acceptance of this dissertation as a requirement for obtaining a MSc. Degree in Botany from Institute of Science and Technology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

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LETTER OF APPROVAL

The M. Sc. Dissertation entitled **"HERBIVORY AND PATHOGEN DAMAGE ON CO-ASSOCIATED NATIVE AND INVASIVE PLANTS IN SURYAVINAYAK AREA, BHAKTAPUR, NEPAL"** submitted to the Central Department of Botany, Tribhuvan University by Ms. Sabita Twanabasu has been accepted for the partial fulfillment of Master's Degree of Science in Botany.

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ABBREVIATIONS AND ACRONYMS

asl.	Above sea level
Cm	Centimeter
CZA	Czapex Dox Agar
ERH	Enemy Release Hypothesis
Ha	Hectare
IAPS	Invasive Alien Plant Species
IPBES	Intergovernmental Platform for Biodiversity and Ecosystem Services
M	Meter
Ml	Milliliter
MoFSC	Ministry of Forest and soil Conservation
NWH	Nobel Weapon Hypothesis
SCF	Suryabinayak Community Forest
SPSS	Statistical Package for Social Sciences

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ABSTRACT

Various kinds of pests and pathogens pose significant challenges to plants across various ecosystems. According to enemy release hypothesis, the invasive alien plant species (IAPS) often face less challenge from pests and pathogens compared to native plants because they lack their natural enemies in their introduced environments. Among IAPS in Nepal, *Ageratina adenophora* and *Lantana camara* are listed as high risk posed species. This study measured herbivory and pathogen damage on these two IAPS in Suryabinayak Community Forest of Bhaktapur district, Bagmati Province, Nepal. Similarly, the damage is compared to native plants *Adenostemma lavenia* and *Schima wallichi* which were co-associated with *A. adenophora* and *L. camara.* Herbivores were sampled and identified up to generic level. Phyllospheric fungi were also cultured, isolated and identified. Herbivory damage was found to be high in native plants comparing to the IAPS. Comparing pathogen damage between two IAPS, *L. camara* leaves were less damaged than that of *A. adenophora*. A total of 31 species of herbivores were found to be associated with the IAPS (30 species from *A. adenophora* and 5 species from *L. camara*). Altogether, 33 species of fungi were isolated from the phyllosphere of the IAPS (22 species from *A. adenophora* and 11 from *L. camara*). A positive correlation was found between herbivory and pathogen damages. The study revealed that the IAPS in the study site are less suffered by herbivore damage compared to natives, yet frequent and large number of herbivore visit suggests potential adaptation, signifying at the prospect of biological control agents.

Keywords: IAPS, native species, herbivores, phyllosphere fungi, natural enemy

शोध-सार

मिचाह प्रजातिका वनस्पतिहरुलाई उनीहरुको प्राकृतिक वासस्थान भन्दा बाहिर क्षेत्रहरुमा त्याहाँका किरा र रोगजनक जीवहरुले कम क्षति पुऱ्याउँदछ किनभने तिनीहरुसँग रैथाने किरा तथा अन्य जीवहरु अनुकुल भएका हुँदैनन् तर रैथाने वनस्पतीहरु भने उनीहरुको शत्र जीवहरुबाट बढी क्षतिग्रस्त हुन्छन् । कम क्षति हुने भएका कारण मिचाहा प्रजातिहरु रैथाने वनस्पतिहरु सँग बढि प्रतिस्पर्धात्मक हुन्छन् । यसै विषयसँग सम्बन्धित यो अध्ययन नेपालमा भएका बाह्य मिचाह *Ageratina adenophora* (कालोबनमारा) र *Lantana camara* (काँडे बनमारा) प्रजातिमा केन्द्रित छ । यी दुई मिचाहा प्रजातिहरुलाई रोग तथा किराहरुले पुर्‍याएको क्षतिलाई ती मिचाह प्रजातिहरु सँगै हुर्कीरहेका रैथाने *Adenostemma lavenia* र *Schima wallichi* (चिलाउने) प्रजातिहरुमा भएको क्षति संग तुलना गरिएको थियो । साथै मिचाहाहरुमा पाइएका किराहरु र तिनीहरुका पातमा पाइएका ढुसीहरुको संकलन तथा पहिचान गरिएको थियो । अध्ययनबाट रैथाने प्रजातिहरु रोग तथा किराहरुबाट बढी क्षतिग्रस्त भएको पाइए पनि विभिन्न ३१ प्रजातिका किराहरु र ३३ प्रजातिका ढुसीहरु मिचाह प्रजातिहरु संग आबद्ध भएको पाइइएको छ । साथै किराले गरेको क्षति संगै ढुसीहरुको क्षति पनि बढेको देखिन्छ । तसर्थ, मिचाहा प्रजातिहरुमा पनि विभिन्न किराहरु र ढुसीहरु अनुकुलन भइरहेका हुनसक्ने अनुमान गर्न सकिन्छ । यिनीहरुको विस्तृत अध्ययनबाट भविष्यमा मिचाहा प्रजातिहरुको जैविक नियन्त्रणका लागि उपयोगि किराहरु र ढुसीहरु पत्ता लगाउन सहज हुनेछ ।

शब्द कुन्जिहरुः मिचाह प्रजाति, रैथाने प्रजाती, किराहरु, ढुसी, शत्रु जीव

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CHAPTER - 1
INTRODUCTION

1.1 Background

Plants species introduced outside of their biogeographic distribution are considered as the alien plant species. Some of the introduced plants become naturalized and some of them become invasive, displacing native plants and threatening native biodiversity, altering ecosystem processes and services, and causing significant economic damage (Pimentel et al. 2005; Shah et al. 2020). They produce undesirable alterations in natural vegetation composition and environment by prolifically spreading (Gaertner et al. 2009; Barney et al. 2013). Many of them become quite widespread due to their ability to produce a large number of seeds over time in soil (Rejmanek and Richardson 1996).

The accomplishment of an exotic species in establishing, developing, and maintaining a population outside of its geographic area of origin is referred to as biological invasion and the plant species responsible are termed as the invasive alien plant species (IAPS) (Pimentel et al. 2005). The IAPS are considered as the major drivers of biodiversity loss and subsequent ecological alteration. According to the United Nations Intergovernmental Platform for Biodiversity and Ecosystem Services (IPBES), nearly one-fifth of the Earth's surface is at risk of biotic invasions, impacting native biodiversity and huge economic loss (IPBES 2019, Rai et al. 2020). The IAPS have got ability to change soil physicochemical and biological qualities besides altering species richness and composition (Callaway et al. 2004; Gaggini et al. 2017). Furthermore, the livelihood of the people is impacted by rapidly growing IAPS (Rai et al. 2012; Mazza et al. 2014).

With increased globalization of trade and human migration, the number of alien species has increased in all climatic areas from the tropics to the high mountains (Seebens et al. 2017). Thousands of plant species have been naturalized outside of their native distribution ranges (Van Kleunen et al. 2015). And large numbers of IAPS have been reported as the causes of extinctions of endangered species (Schmitz and Simberloff 1997; Pimentel et al. 2005).

1.2 IAPS in Nepal

In Nepal, biological invasion has been a challenging environmental problem, affecting soil quality, biodiversity, ecosystems, and so on (Shrestha et al. 2016). Nepal's topography exhibits wide climatic and elevation gradients. Because of such variations, wide varieties of exotic plants including IAPS have been introduced and growing aggressively in the country (Shrestha 2016).

Biological invasions have become a challenging for biodiversity conservation, ecosystem management and agriculture production in Nepal (MoFSC 2014). Many of the alien plants in the country are naturalized and over two dozen have become invasive species in different ecosystems (Shrestha 2016; Gulzar et al. 2024).

Majority of these species have come to Nepal from Americas and Europe through India via transportations and many of them are intentionally introduced as ornamental plants (Tiwari et al. 2005; Shrestha 2016). Most of them are confined to tropical and subtropical regions of Nepal (Terai, Siwalik and Mid Hills) with few of them like *Ageratina adenophora* which has reached near to the temperate zone (Shrestha 2016).

Among the IAPS in Nepal, *Ageratina adenophora* (Sprengel) R.M. King and H. Rob. and *Lantana camara* (L.) are highly problematic and widely distributed species throughout the country (Shrestha 2016; Shrestha et al. 2019). *Ageratina adenophora* which is native to central Mexico belongs to the family Asteraceae and has been established since long time ago (Tiwari et al. 2005). This has invaded along road sides, fallow lands, and forest margins to even inside the deep forest canopies (Thapa et al. 2016).

Lantana camara (L.) is native to Central and Southern America belongs to the family Verbenaceae (Sharma et al. 2007). It has been listed as one among the 100 most noxious invasive weeds of the world which has also been established since long time ago from western, central and eastern tropical to subtropical regions in Nepal (Lowe et al. 2000; Tiwari et al. 2005).

1.3 Invasion mechanisms

Several hypotheses have been proposed regarding mechanisms of alien plant invasion. Some of the key hypotheses are the propagule pressure hypothesis (Colautti et al. 2004), phenotypic plasticity (Geng et al. 2007), resource competition hypothesis (Vila and Weiner 2004) and enemy release hypothesis (Keane and Crawley 2002). According to the propagule pressure hypothesis, invasion is successful when there is a plentiful supply of plant propagules (Lockwood 2009). Pheonotypic plasticity hypotheses states that, because of morphological and physiological flexibility or fitness homeostasis, invasive species can sustain high fitness throughout a wide environmental range (Richards et al. 2006). According to the resource competition hypothesis, mutually negative interactions between two or more individuals induce a decline in population growth rate of native plants (Vila and Weiner 2004). There is competition between the species, which benefits invading species more than native species and eventually leads to IAPS invasion (Vila and Weiner 2004).

The Novel Weapon Hypothesis (NWH) was proposed by Callaway and Ridenour (2004). According to this theory, IAPS possess and utilize special chemical weapons that are potent allelopathic agents in novel environments. Allelochemicals have stronger competitive advantages to IAPS in their new introduced range. Many studies on role of allelochemicals in invasion success revealed that the chemicals are responsible to change soil microbial populations and nutrient dynamics to higher plants' germination, growth and development (Kourtev et al. 2002; Ehrenfeld 2003; Vander et al. 2007).

One of the most emerging and highly interesting hypotheses is the Enemy Release Hypothesis (ERH) which explains that the IAPS are less impacted by enemies (e.g., herbivores) than native species in the introduced places, because in the new geographical location, the IAPS are freed from their natural enemies like pests and pathogens (Keane and Crawley 2002). Pathogenic microorganisms (fungi, bacteria, and viruses) have co-evolved with herbivores and adapted to certain hosts to produce disease (Agarios 2005). Therefore, invasive species are thought to compete more successfully with native species in their invasive environment (Middleton 2019).

Several documentations support this idea. For example, Cincotta et al. (2009) tested the enemy release hypothesis by comparing percent leaf area loss, gall damage, fungal damage, and specific leaf area between a native sugar maple (*A. saccharum* L.) and invasive Norway maple (*Acer platanoides* L.). They found a significantly less leaf damage in invasive maple than sugar maple. Conversely, Agrawal and Kotanen (2003) found that invasive plants and congeneric native plant suffered same level of herbivore attack. Agrawal et al. (2005) found that the invading species are less vulnerable to herbivores, pathogens, and fungal attack than their congeneric native counterparts.

Costan et al. (2022) investigated enemy release hypothesis by conducting a large-scale field surveys of insect diversity and abundance in the native (United Kingdom) and introduced (New Zealand) ranges of *Rumex* species. They observed that *Rumex* plants had a lower diversity of insect herbivores in an introduced range, all insect specialists present in the native range were absent, and plants had lower levels of herbivore damage on both roots and leaves.

Williams et al. (2016) also tested whether the three exotic plant species, *Rubus phoenicolasius*, *Fallopia japonica*, and *Persicaria perfoliata* experienc less herbivory or pathogen attack than native species by comparing with their native congeners. They found that *F. japonica* and *R. phoenicolasius* received less leaf damage than their native congeners, and *F. japonica* also contained a lower diversity and abundance of invertebrate herbivores.

Hence, herbivory or the feeding of animals on living plant parts is an important ecosystem phenomenon which has direct or indirect impact on primary production as well as vegetation shape and composition (Schowalter 2012). Herbivory damage can limit plant growth and survival and plants also have evolved with complex responses to avoid being consumed by herbivores or to survive and thrive in the face of herbivory harm (Liu et al. 2012). Defoliation, sap withdrawal, root damage, and galling of plant tissues by herbivores are the activities which can reduce existing plant biomass and cause resource redirection in plant tissues (Zhou et al. 2015). Also, such herbivory frequently lowers a plant's reserves and slows the rate of growth, development and regeneration (Bellows and Headrick 1999).

1.4 Herbivores as the biological control agents

Biological management is one of the safe and environmental friendly strategies that include the introduction of host specific natural enemies (invertebrates or pathogens) of the IAPS from its native habitat. The intentional introduction or manipulation of specific herbivorous such as insects to control the invasive plants is the practice of biological management of invasive plants and such agents are the biological control agents (Driesche and Hoddle 2009).

A gall fly named *Procecidochares utilis* is a natural enemy of invasive *Ageratina adenophora* (Crutwell 1974). This insect is one of the biological control agents recommended so far for the biological control of *A. adenophora.* Its introduction to Hawaii had a significant impact on the weed in the regions with low to moderate moisture levels (Bess and Haramoto 1972; Paudel et al. 2019).

Other insects *Procecidochare connexa, Aphis gossypii, Cremastobombycia chromolaene, Cionothrix praelonga* and fungal pathogens *Alternaria zinnia, Passalora perfolatia* are also reported as the effective bio-control agents of invasive *Chromoleaena odorata* (Davis et al. 2013). According to Koirala et al. (2011), the exotic rust *Puccinia spegazzinii* and its strain could be effective biological control agents of *Mikania micrantha*.

The species *Lantanophaga* (Platyptilia) *Pusillidactyla* (Walker) (Pterophoridae) are also recommended for biological control of *Lantana camara* after field observation in India and Burma (Muniappan et al. 1986). The insects *Ophiomyia lantanae* (fruit-mining fly), *Calycomyza lantanae* (agromyzid seed-fly), *Teleonemia elata* (leaf-sucking bug), *Teleonemia scrupulosa* (leaf-sucking bug) were also found to be effective agents for controlling *L. camara*. In South Africa, *Aceria lantanae* and *Ophiomyia lanatane* are improving control in frost-free areas on some varieties of *L. camara* (Shackleton et al. 2017).

These herbivores have impact on plant growth and fitness by (i) damaging plants' leaves, twigs, roots and internal parts) and (ii) modifying plants' physiological functions. Several herbivores influence the amounts of accessible nitrogen and other nutrients (Throop and Lerdau 2004), nutrients in leaves (Karban et al. 1997), photosynthetic activity (Zangerl et al. 2002; Nabity et al. 2009) and acquisition of atmospheric CO_2 and transpiration rate (Marlina et al. 2013).

Microbes in the phyllosphere also have significant role in plant growth and development. Phyllosphere is the habitat on the surface of plant leaves which includes leaf's outer layer and surrounding (Kirschner 2018). Bacteria and fungi in the phyllosphere are the inhabitants of leaves (Stone et al. 2018). Pathogenic microbes can disrupt the phyllosphere microbiome by targeting the plant immune system (Mandal and Jeon, 2023). They also change the leaf microbial population and increasing susceptibility to future infection (Seybold et al. 2020). Therefore, microbial composition and their interaction among them and with host plant is crucial to study to understand their role in plant growth, development and adaptation.

1.5 Statement of problem and research gap

Management of the alien plant invasion to reduce the loss of biodiversity, ecosystem changes, economic loss is the urgent necessity of today's world. There are number of researches done about the diversity and impacts of invasive plants which are suggesting for the sustainable and environment friendly management of IAPS. The sustainable and environmentally friendly method comprises the biological control methods. For the application of biological control method, first pathogens and herbivores damaging these plants should be explored but such assessments on herbivory and pathogen damage to the IAPS in Nepal have not been conducted well enough yet. Further, no work has been done investigating if Nepalese insects are also feeding on the IAPS.

Ageratina adenophora and *Lantana camara* have been naturalized IAPS in different habitats such as forest, shrub-lands, grass-lands and agro-ecosystem in Nepal. Enemy Release Hypothesis (ERH) explains that the IAPS introduced to the exotic area will experience lesser impact from the natural enemies which may account for the increase in diversity and abundance (Roy et al. 2011). Several studies have been conducted previously (Keane et al. 2002; Vila et al. 2005; Roy et al. 2011) to explain this hypothesis. It would be very interesting to test this hypothesis in different geographical areas, invaded ecosystems, plant communities, and climatic conditions because these factors may alter the abundance of the enemies of IAPS as well as the effectiveness of their damage to the host. Therefore, Nepalese ecosystems could be the best study areas to carry out such kinds of research as Nepal has extreme climatic variations ranging from tropical to alpine zone which may have direct or indirect effects on the IAPS enemies, their abundance, and damage level.

In the case of *A. adenophora* some of their natural enemies such as an insect *Procecidochares utilis* and a fungal pathogen *Passalora ageratinae* (for *A. adenophora*) have been detected in Nepal (Paudel et al. 2019). Somewhere it is mentioned that the effectiveness of damage by this fly to *A. adenophora* is not significant (Shrestha 2016) but its actual status (abundance, damage level etc.) has not been explored yet. In case of *L. camara* it is unknown whether its natural enemies are introduced in Nepal or not. No any assessments have been carried out till the date about the natural enemies (pests and pathogens) of *L. camara* in Nepal. Therefore, it is very urgent to conduct such studies to get this valuable information.

These invasive species have been naturalized and become invaded in Nepal since long time ago (Tiwari et al. 2005). During this long introduced history, some native insects and pathogens of our country might have adapted on eating them. Now, it is becoming an important subject of study to know whether native pests and pathogens of Nepal are damaging them or not. This will help to know about the potential biocontrol agents.

1.6 Research Hypothesis

i. Invasive *Ageratina adenophora* and *Lantana camara* are less damaging by herbivores and pathogen in comparison to associated native species.
ii. Many insects might be visiting or damaging invasive species as their invasion history is long enough.
iii. Phyllosphere region serves as the source of pathogen inoculum.

1.7 Research Questions

i. What kinds of herbivores are found in invasive *A. adenophora* and *L. camara*?
ii. What is the level of damage caused by herbivores in *A. adenophora* and *L. camara* and their co-associated native species in Nepal?

iii. What are the phyllospheric fungi present on the phyllosphere of *A. adenophora* and *L. camara*?

1.8 Research objectives

The general objective of this research work is to access the damage of herbivores and pathogens on the invasive plants *A. adenophora* and *L. camara*. The specific objectives are as follows:

i. To explore and identify herbivores present in *A. adenophora* and *L. camara*.
ii. To measure the level of damage caused by herbivores and pathogens in *A. adenophora* and *L. camara* and their associated native species.
iii. To explore and identify phyllosphere fungi of *A. adenophora* and *L. camara*.

1.9 Limitations of Study

i. Survey, sampling and identification of herbivores were limited to invasive species only.
ii. Herbivores were sampled during day time only. So, this study couldn't explore light sensitive herbivores.

CHAPTER - 2

MATERIALS AND METHODS

2.1 Study area

This study was carried out in the Suryabinayak Community Forest (SCF), Bhaktapur, Nepal (Figure 1). It lies between 27°37'N to 27°40'N latitude and 85°23'E to 85°29'E longitude with elevation range from 1345 to 2025 m above sea level. The area is popular as it has a famous Suryavinayak Ganesh temple. There exists a Suryabinayak hill occupied by a community managed forest named Suryabinayak Community Forest (SCF). This forest is extended about 300 ha area.

The SCF is dominated by *Schima-Castanopsis* plant community. The major tree species of the forest are *Schima wallichii, Castanopsis tribuloides, Alnus nepalensis, Grevellea robusta, Syzygium cumini, Rhododendron arboreum, Pyrus pashia, Fraxinus floribunda*, *Acer oblongum* and *Myrica esculenta.* This scared forest is home to several species of butterflies, insects, birds and other animals (Shrestha et al. 2018). Similarly, shrubs like *Rubus ellipticus, Osbeckia stellata* and herbs like *Adenostemma lavenia* and several other species are found in this forest but the forest margin, trails and road sides within the forest is highly invaded by *A. adenophora* and *L. camara.*

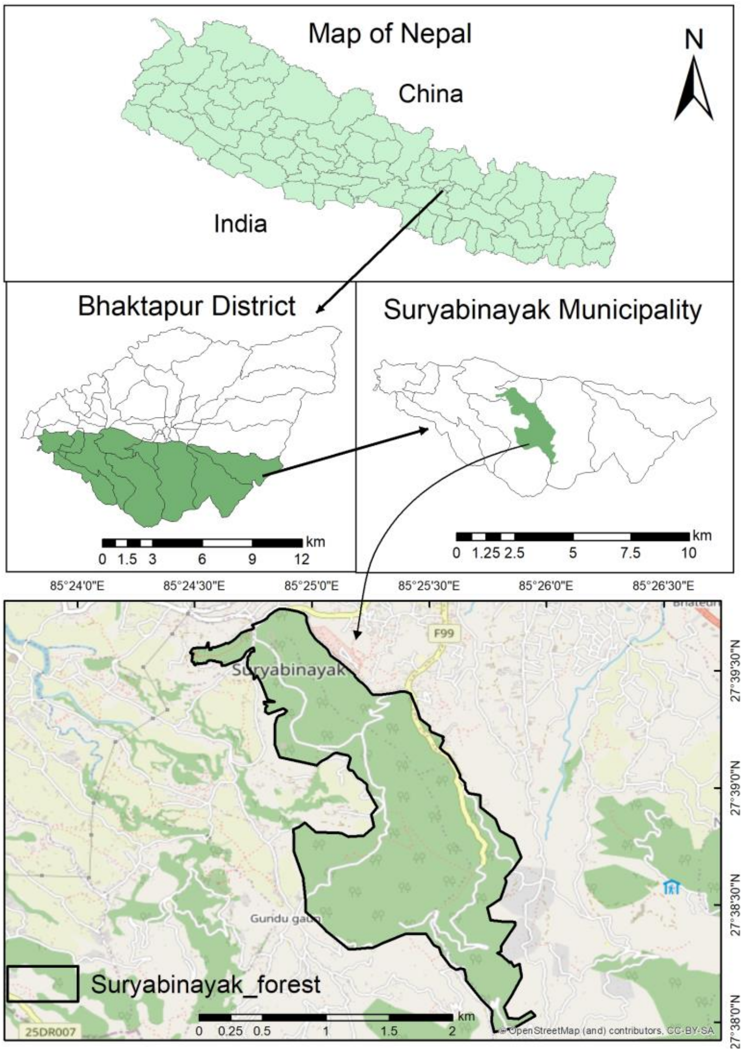

Figure 1*:* Map of study area showing the Suryavinayak Community Forest in Bhaktapur district, Nepal.

2.2 Study species

2.2.1 Invasive species

Ageratina adenophora and *L. camara* were two invasive species spread throughout the forest particularly along the roadside, trails and forest margins. Therefore, these two invasive species were selected for the study of herbivory and pathogen damage assessment.

Ageratina adenophora **(Spreng.) King & H. Rob**

Ageratina aadenophora is a perennial herbaceous plant belonging to family Asteraceae (Figure 2). The plant height ranges about 1-3m (Bess and Haramoto 1959). Leaves are dark green, opposite, serrate; flowers are white and borne terminally on the clusters. The seed is achene and are dispersed by many ways. This plant is native to Mexico and became invasive and spreading rapidly in southern and southern eastern Asia, eastern Australia, New Zealand and Southwestern Africa (Cronk and Fuller 1995).

This weed is called as 'Kalo Banmara' in Nepal. In Nepal, it is found at an elevation of 400-2600m in Tarai, Siwalik, hills, and low-mountains of eastern, central and western Nepal (Tiwari et al. 2005*)*. It mostly invades forest, shrub-land, grassland, and agro-ecosystems.

Figure 2. *Ageratina adenophora*

***Lantana camara* L.**

Lantana camara L. (Family: Lamiaceae), an ornamental shrub native to Americas, has spread as an intractable weed in many parts of the world (Sharma et al. 1988). The plant grows to a height of 2-3 m and spreads its branches to cover an area of about 1-2 m mature leaves are 5-9 cm long, ovate or oblong, cuneate, rounded or cordate at the base, and crenate and rugose above (Sharma et al. 1981). The flowers are subumbellate and fruits are drupe with greenish in the early stages and dark blue when ripen. The fruits are consumed by birds (Chopra et al. 1965).

It is called as ‘Kirne or Kande Banmara’ in Nepal where it has been distributed from tropical to subtropical regions throughout the country (Figure 3). It has invaded forest, grassland and agro-ecosystem (Shrestha 2016).

Figure 3. *Lantana camara*

2.2.2 Native species

Two species *Adenostemma lavenia* and *Schima wallichi* frequently associated with the invasive species were selected as the native species to compare the herbivory damage with invasive species.

Adenostemma lavenia var. *lavenia* (L.) Kuntze

Adenostemma lavenia var. *lavenia* (L.) Kuntze is the plant belonging to family Asteraceae (Figure 4). This plant is native to the tropical and subtropical range of Asia. It is a perennial herb and grows primarily in the subtropical biome. Its leaves are elliptic to lanceolate and opposite. The inflorescence terminal, loose, paniculate with its white flowers. It is reported from forests and thickets on slopes of 400 -2000 m asl in different parts of Nepal.

Figure 4. *Adenostemma lavenia*

***Schima wallichii* (DC.) Korth.**

Schima wallichii is an evergreen, medium-sized to large tree belonging to the family Theaceae (Figure 5). It is a medium size tree having white flowers. Flowering and fruiting occur throughout the year, but usually it flowers in April-June. The seeds are shed in January to March of the following year.

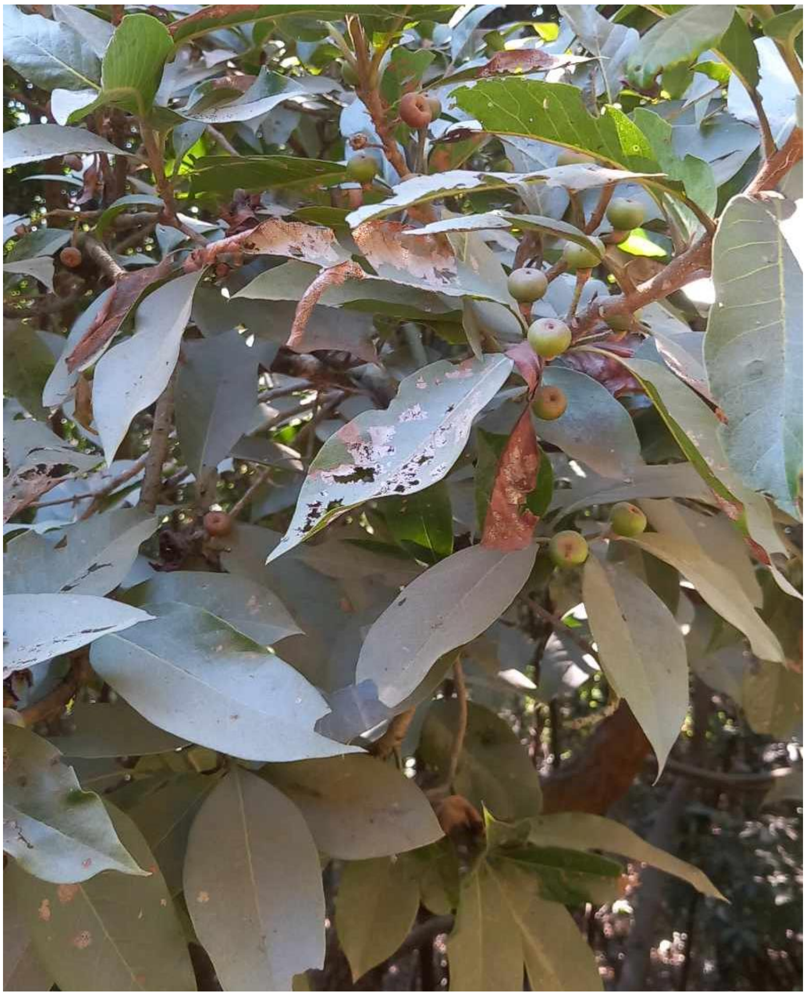

Figure 5. *Schima wallichi*

2.3 Herbivory and pathogen damage assessment

2.3.1 Plant sampling: *Ageratina adenophora* and *Adenostemma lavenia*

Herbivory damage assessment in *A. adenophora* and *A. lavenia* was carried out by sampling total 50 quadrats (plots) of size 1×1m^2 in the Suryabinayak Community Forest, Suryabinayak Municipality of Bhaktapur district, Nepal.

The quadrats were sampled along the roadside starting from the right and left side of the Suryabinayak temple up to the top of Suryabinayak hill. The distance between two quadrats was 20-50 m. From each quadrat a single individual of native and invasive plant located in the center of quadrat was selected for the study. In case of absence of species at the center, the nearest species from the center was selected for the study. Tree canopy cover was estimated in the quadrats by visual observation.

2.3.2 Plant sampling: *Lantana camara* and *Schima wallichi*

Herbivory damage assessment for *L. camara* and *S. wallichi* was carried out by sampling altogether 20 plants of each species. Altogether 20 quadrats of size 5×5 m^2 were sampled in study area where both native and invasive species were co-associated. Three branches (branch near the surface, branch at the middle of plant and branch at top of plant) of *L. camara* and sapling of *S. wallichii* (1 to 3m tall) were taken for damage assessment. The distance between two quadrats was 50 to 100 m. Tree canopy estimation was also done in each plot.

2.3.2 Damage assessment

The herbivory bite and pathogen damage (detectable change in color, spots) in the leaves were distinguished by visual observations. The damage such as chewing, mining, skeletonizing, sucking or galling were considered as the herbivory/insect damage and rust, leaf spots, viral leaf curl were considered as pathogen damage (Schuldt et al. 2017). Damaged on leaves by herbivores and pathogen were counted and measured in the form of damage percentage. The damage was categorized into herbivory and pathogen damage. Percentage of damaged leaves was calculated by using the formula:

$$\text{Percentage leaf damage} = \frac{\text{Total no. of damaged leaves in all branches}}{\text{Total number of leaves in all branches}} \times 100\%$$

2.4 Insect survey, collection and identification

Insect herbivores of *A. adenophora* and *L. camara* were collected through following methods described by Schauff (2001).

i. Hand Picking Method

The large sized pests such as beetles and grasshoppers were collected from each quadrat by hand picking method and directly trapped into the wide mouthed container and were preserved in vials containing 80% ethanol.

ii. Sweeping Method

Sweeping nets was made up of piece of a steel wire with rim of diameter 30-38 cm and light polyester fabric through which air can flow freely was used as net. Sweeping net were used for collecting flying insect pests. The net was swept along the plants in left right and up down motions.

iii. Beating Method

A beating sheet of 1×1m^2 was prepared with the help white cotton cloth. It was stretched across two diagonal pieces of wood joined at the center. It was placed under the plant and the foliage and branches were shaken. This method was particularly used for profusely branched *L. camara.*

iv. Insect identification

Photographs of insect pests were taken with the help of stereomicroscope and identified following Thapa (2000) and with the help of expert.

2.5 Phyllosphere fungi

Leaves of altogether 20 plant individuals of each *A. adenophora* and *L. camara* were sampled for the assessment of phyllosphere fungi. Leaves were transferred in sterile plastic bag to laboratory. They were cut into small pieces of about 1×1cm^2. After that the pieces were transferred to conical flask (250ml) containing 100ml of sterile water and shaken for about 20 minutes. After that 1ml of sample was taken out and poured in sterile petri dish. Then sterile molten Czapex Dox Agar (CZA) media was added to it and rotated in clockwise and anticlockwise direction. Each sample was replicated 3 times in the culture plates. Then the plates were incubated at 28±2°C for 7days in incubator.

After 7th day, the number of colonies formed in each plate, colony color, morphology, texture etc. were observed carefully and noted down. Then, pure culture was done in CZA media. Then photograph of pure culture was taken with the help of digital microscope. Fungi were identified following Watanabe (2010) and also with the help of expert.

2.6 Statistical analysis

The data were analyzed in the software SPSS (Statistical package for Social Sciences) version 29 (IBM Corp 2023). Individual sample T-test was carried out to compare herbivory damage between *A. adenophora* and *Adenostemma lavenia* and *L. camara* and *S. wallichi.* Pearson correlation coefficient was calculated to find correlation between herbivory damage, pathogen damage, tree canopy, elevation and galls. Statistically significant level was $p \leq 0.05$.

CHAPTER - 3

RESULTS

3.1. Herbivory damage

3.1.1. *Ageratina adenophora* and *Adenostema lavenia*

On comparing the herbivory damaged leaves between invasive *A. adenophora* and native *Adenostemma lavenia,* it was found that the herbivory damage was significantly high in native *A. lavenia* than the invasive species ($p < 0.001$). In native *A. lavenia* the damaged leaves were 51.66% while the damage was only 31.07% in invasive *A. adenophora* (Figure 6, Appendix 1 and Appendix 2).

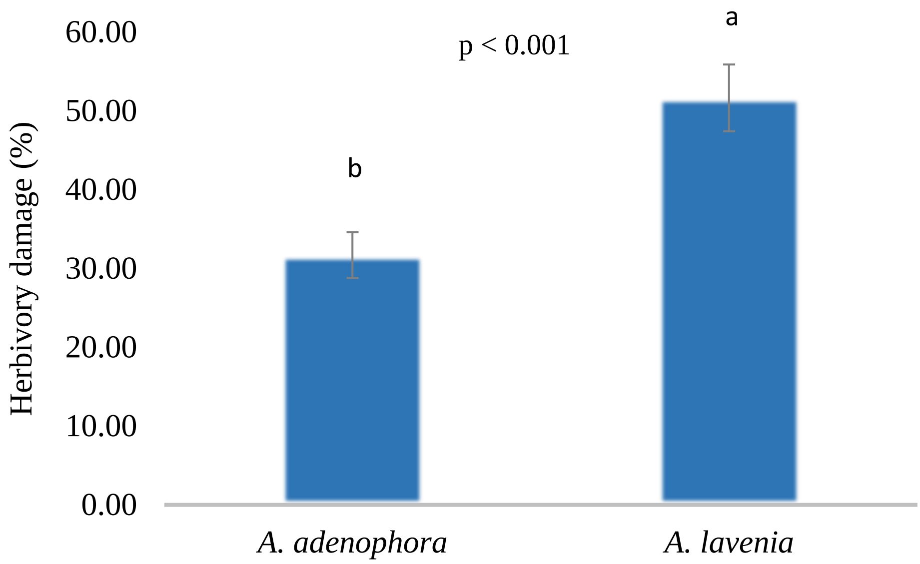

Figure 6. Herbivory damage in invasive *A. adenophora* and native *A. lavenia.* Different alphabets above error bar indicate significance differences ($p < 0.05$).

3.1.2. *Lantana camara* and *Schima* wallichii

On comparing the herbivory damaged leaves between invasive *L. camara* and native *S. wallichi,* it was found that the damage was significantly high in native *S. wallichi* than invasive *L. camara* ($p < 0.001$). The damaged leaves of *S. wallichi* were 79.74% while it was only 53.82% in invasive *L. camara* (Figure 7, Appendix 3 and Appendix 4).

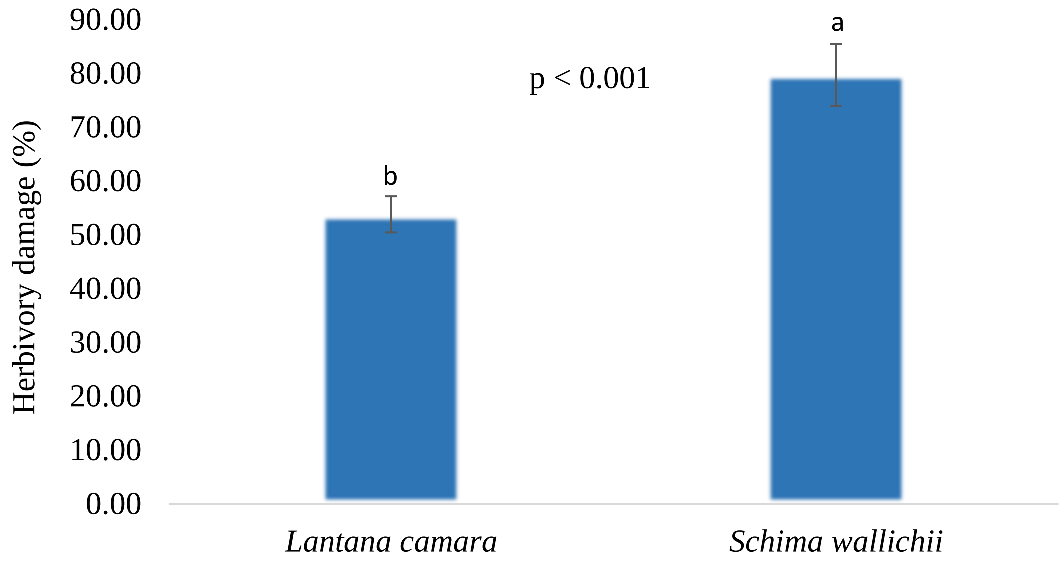

Figure 7. Herbivory damage in invasive *L. camara* and native *S. wallichi*. Different alphabets above error bar indicate significance differences ($p < 0.05$).

3.1.3 Pathogen damage in *A. adenophora* and *L. camara*

Pathogen damage was compared in between two invasive species *A. adenophora* and *L. camara*. It was found that pathogen damaged leaves were significantly higher in invasive *A. adenophora* (17.01%) than invasive *L. camara* (9.31%) (Figure 8, Appendix 1 and Appendix 3).

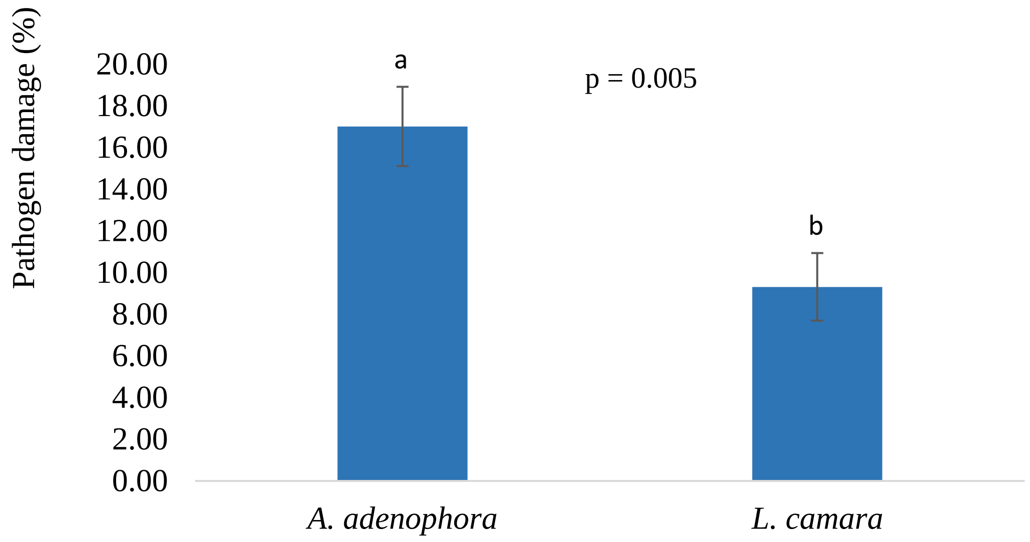

Figure 8. Pathogen damage in invasive *A. adenophora* and invasive *L. camara*. Different alphabets above error bar indicates significance differences ($p < 0.05$).

3.1.4 Relationship among galls, herbivory damage, pathogen damage, tree canopy and elevation in *A. adenophora*

Correlation among the galls, herbivory damage, pathogen damage, tree canopy and elevation in *A. adenophora* was studied. It was found that herbivory damage is negatively correlated with number of galls in *A. adenophora*. Herbivory damage in *A. adenophora* decreased formation of galls by 17% (p = 0.367) (Table 1, Appendix 1).

Pathogen damage also decreased the number of galls by 5% but this data was also not significant (p = 0.771). Herbivory damage increased the pathogen damage by 56% (p = 0.002). Relationship of tree canopy and number of galls was not significant (p = 0.143). Similarly, the tree canopy did not change the herbivory and pathogen damages with p value 0.648 and 0.332, respectively (Table 1, Appendix 1).

Relationship of elevation with number of galls, herbivory and pathogen damage and tree canopy was also not significant. Although, on increasing elevation an increasing trend of number of galls (p = 0.195), herbivory damage (p = 0.847), pathogen damage (p = 0.918) and tree canopy (p = 0.184) was found (Table 1, Appendix 1).

Table 1: Correlation among galls, herbivory damage, pathogen damage, tree canopy and elevation in *A. adenophora*

		Number of galls	**Herbivory damage**	**Pathogen damage**	**Tree canopy**
Herbivory damage	Correlation	-0.177			
	p value	0.367			
Pathogen damage	Correlation	-0.058	0.563**		
	p value	0.771	**0.002**		
Tree canopy	Correlation	0.284	0.090	0.191	
	p value	0.143	0.648	0.332	
Elevation	Correlation	-0.252	-0.038	-0.020	-0.258
	p value	0.195	0.847	0.918	0.184

**. Correlation is significant at the 0.01 level (2-tailed)

3.1.5 Relationship among herbivory damage, tree canopy and elevation in *Adenostemma lavenia*

Correlation among herbivory damage, tree canopy and elevation in *A. lavenia* was studied. Herbivory damage showed negative correlation with tree canopy in *A. lavenia* but insignificant (p = 0.216) (Table 2, Appendix 2).

With increasing elevation, herbivory damage showed increasing trend (p = 0.097) and tree canopy showed decreasing trend (p = 0.17) but the relationship was not significant (Table 2).

Table 2. Pearson's correlation among herbivory damage, tree canopy and elevation in *Adenostemma lavenia*

		Herbivory damage	**Tree canopy**
Tree canopy	Correlation	-0.241	
	p value	0.216	
Elevation	Correlation	0.320	-0.267
	p value	0.097	0.170

**. Correlation is significant at the 0.01 level (2-tailed)

3.1.6 Relationship among herbivory damage, pathogen damage, tree canopy and elevation in *L. camara*

The relationship of herbivory and pathogen damage were positive i.e., pathogen damage increased with herbivory damage by 61% (p = 0.004) in *L. camara*. Herbivory damage and pathogen damage did not show significant negative or positive relation with tree canopy (p > 0.05). On increasing elevation herbivory and pathogen damage were also not affected significantly (p > 0.05) (Table 3).

Table 3. Correlation among herbivory damage, pathogen damage, tree canopy and elevation in *L. camara*

		Herbivory damage	**Pathogen damage**	**Tree canopy**
Pathogen damage	Correlation	0.618**		
	p value	**0.004**		
Tree canopy	Correlation	-0.129	-0.165	
	p value	0.587	0.488	
Elevation	Correlation	0.063	-0.174	0.026
	p value	0.792	0.463	0.913

**. Correlation is significant at the 0.01 level (2-tailed)

3.1.7 Relationship among herbivory damage, tree canopy and elevation in *S. wallichii*

Correlation among herbivory damage, tree canopy and elevation in *S. wallichi* is shown in Table 4 (Appendix 4). It was found that herbivory damage is negatively correlated with tree canopy in *S. wallichi*. Herbivory damage decreases with tree canopy increase by 29% (p = 0.210). But this data is not significant.

With increase of elevation, it was found that herbivory damage increases and tree canopy decreases. Herbivory damage increase with increase in elevation in *S. wallichi* by 35% (p = 0.127) and tree canopy decreases by 32% (p = 0.160). But this data is also not significant.

Table 4. Correlation among herbivory damage, tree canopy and elevation in *S. wallichii*

		Herbivory damage	**Tree canopy**
Tree canopy	Correlation	-0.293	
	p value	0.210	
Elevation	Correlation	0.353	-0.326
	p value	0.127	0.160

**. Correlation is significant at the 0.01 level (2-tailed)

3.2 Herbivores found in invasive *A. adenophora* and *L. camara*

Total 31 herbivores belonging to 6 different order were found (Table 5, Appendix 5). Coleoptera was the most dominant order with 13 genera followed by hymenoptera (6), Hemiptera (5). Lepidoptera was the order with least number of herbivores in this study i.e, only 1 genus.

Table 5. Herbivores found in invasive *A. adenophora* and *L. camara*

SN	Order	Genera
1	Coleoptera	13
2	Hymenoptera	6
3	Hemiptera	5
4	Diptera	4
5	Homoptera	2
6	Lepidoptera	1
	Total	**31**

3.3 Herbivores richness in invasive species

Herbivores richness in invasive species *A. adenophora* and *L. camara* was compared and shown in Figure 9. Total 30 species were recorded in *A. adenophora* and 5 species were recorded in *L. camara.*

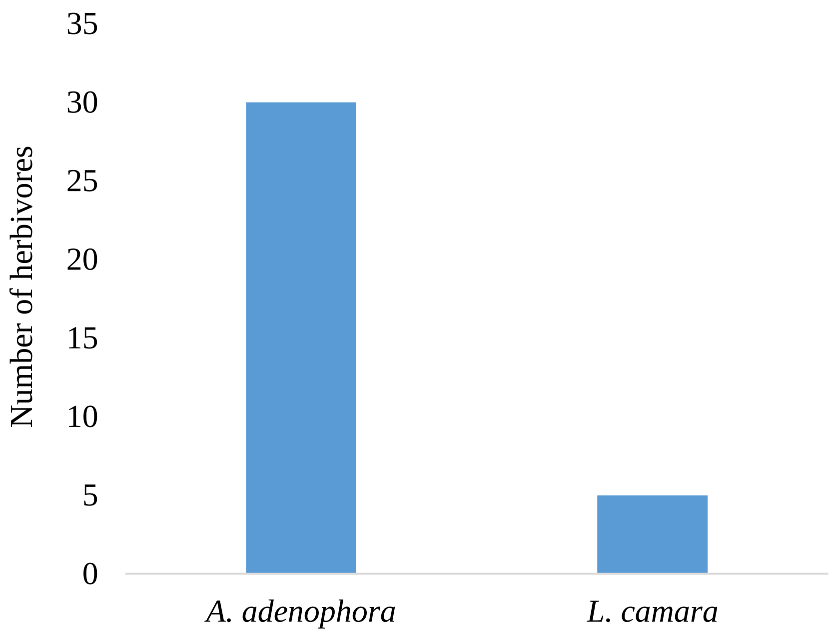

Figure 9. Herbivores richness in *A. adenophora* and *L. camara*

3.5. Frequency of herbivores in invasive species

Frequency of herbivores in invasive species *A. adenophora and L. camara* shown in Figure 10 (Appendix 6) below. *Lagria* was the most frequent species in *A. adenophora* followed by *Graphocephala* and *Formica* while *Alcidodes* was the most frequent species in *L. camara.*

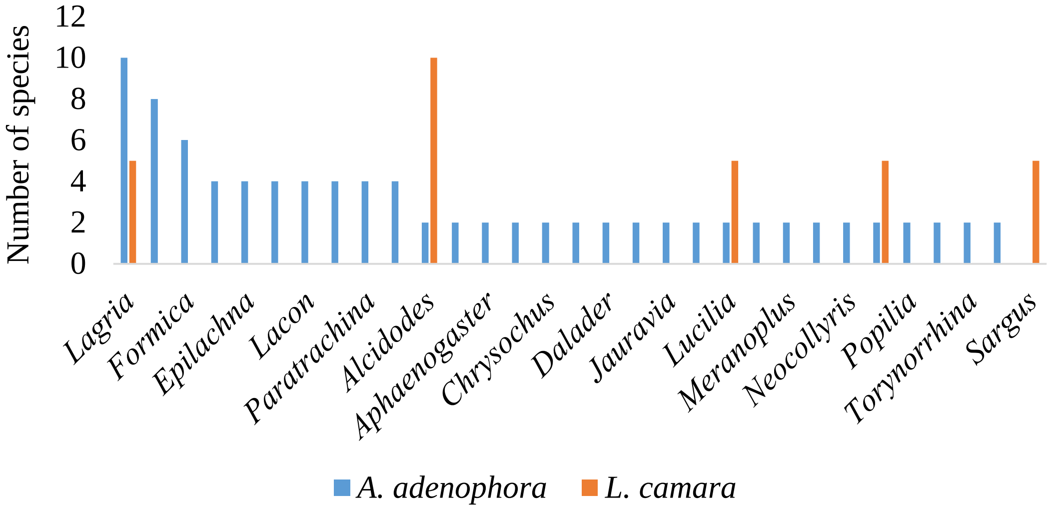

Figure 10. Frequency of herbivores in *A. adenophora* and *L. camara*

3.6 Phyllosphere fungi

3.6. 1 Fungal species richness

Altogether 22 species of fungi were isolated from phyllosphere of invasive plants *Ageratina adenophora* and *Lantana camara* (Appendix 7). They belong to 7 classes, 12 order, 14 families, 17 genera (Table 6).

Table 6. Fungal species found in phyllosphere of *A. adenophora* and *L. camara*

S.N.	Class	Order	Family	Genus
1	Agaricomycetes	1	1	1
2	Dipodascomycetes	1	1	1
3	Dothideomycetes	3	3	5
4	Eurotiomycetes	1	1	2
5	Leotiomycetes	1	1	1
6	Mucoromycetes	1	1	1
7	Sordariomycetes	4	6	6
	Total	**12**	**14**	**17**

Maximum number of fungi were isolated from *A. adenophora* (19 species) (Appendix 7). Altogether 9 species were recorded from *L. camara* (Appendix 7). Six fungi were present in phyllosphere of both invasive plants. They are *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Cladosporium* sp., *Fusarium oxysporum, F. solani* and *Penicillium chrysogenum.* Similarly, 13 species were present in *A. adenophora* only while 3 species (*A. nidulans, Ceratobasidium* sp. and *Nigrospora* sp.) were present in *L. camara* only. Fungal species richness in phyllosphere of *A. adenophora* and *L. camara* is shown in Figure 11 and list of the fungal species and their host plant is shown in Appendix 7.

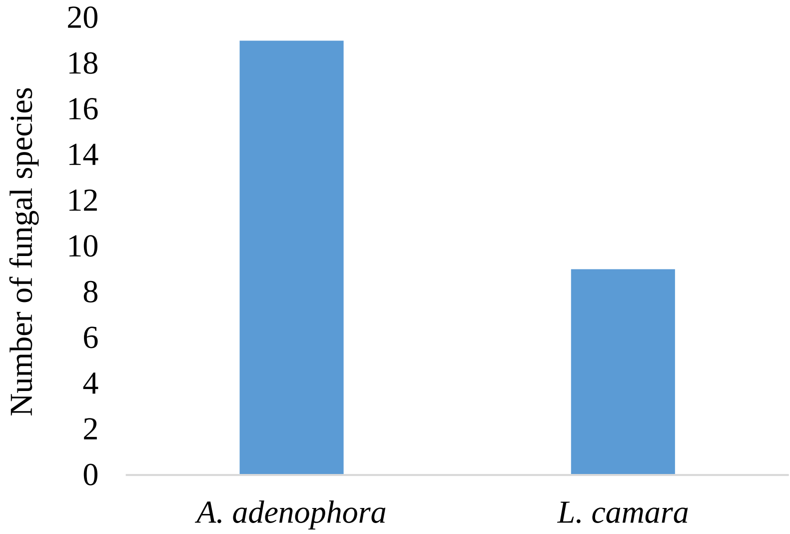

Figure 11. Fungal species richness in *A. adenophora* and *L. camara*

3.6.1.1 Fungal species richness in *A. adenophora*

Total 19 species of fungi were isolated from the phyllosphere of invasive plant *A. adenophora.* They belong to 6 class, 10 order and 13 families (Figure 12) Dothidiomycetes was the dominant Class with 6 fungal species, Eurotiales and Hypocreales were dominant order (4 species) and Aspergilliaceae was the dominant family (4 species) in *A. adenophora.*

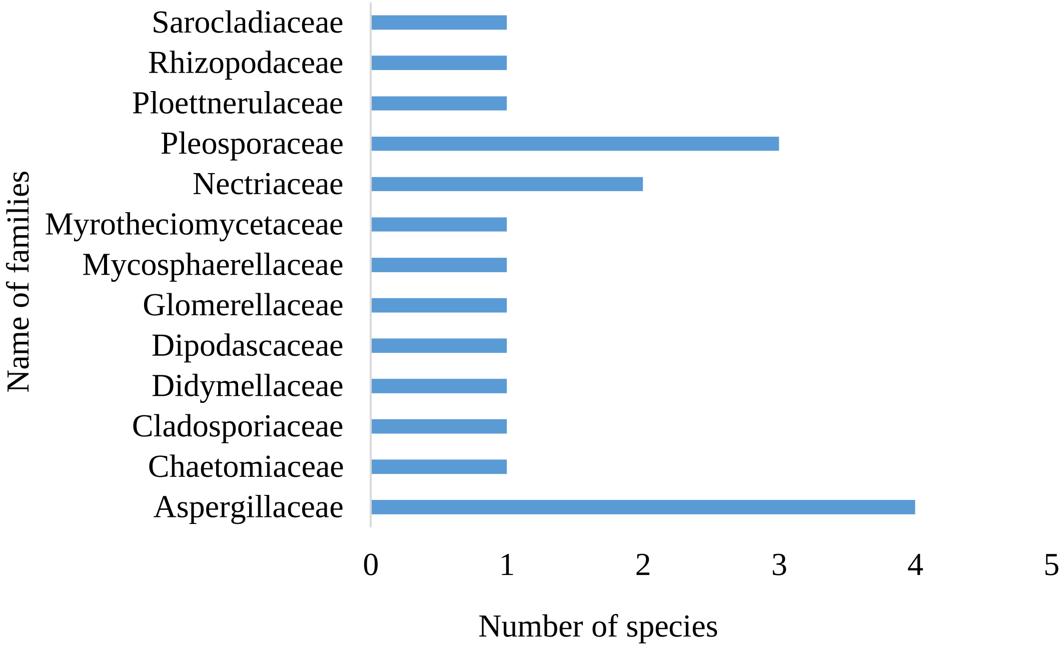

Figure 12. Fungal species belonging to different families *A. adenophora*

3.6.2.1 Fungal species richness in *L. camara*

Altogether 9 different species of fungi were isolated from phyllosphere of *L. camara*. They belong to 4 classes, 6 order and 6 families (Figure 13). Eurotiomycetes is the dominant class (5 species), Eurotiales is the dominant order (5 species) and Aspergillaceae was the dominant family (5 species).

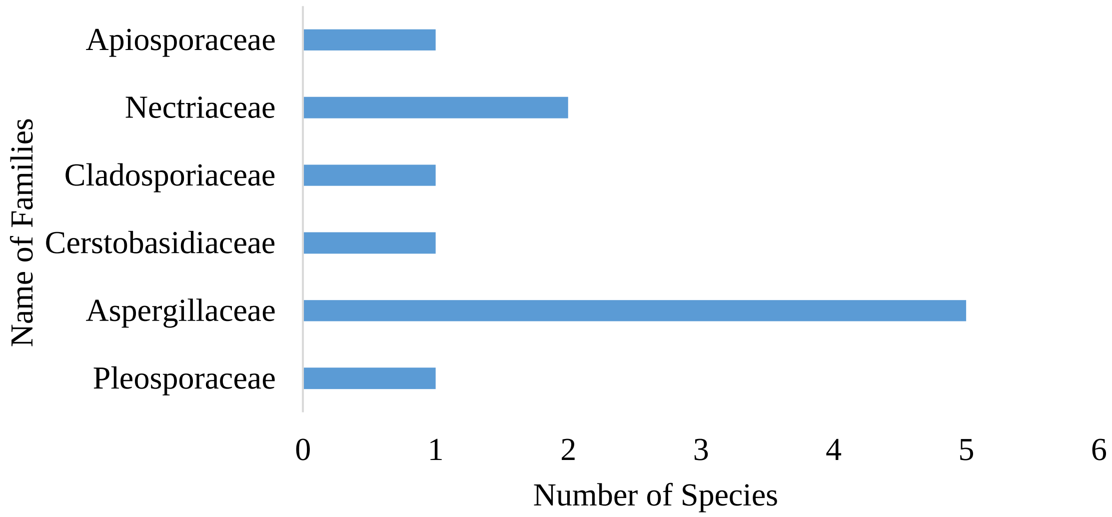

Figure 13. Fungal species belonging to different families *L. camara*.

3.6.2 Frequency of fungi in *A. adenophora* and *L. camara*

A total 22 fungal species were identified from the Phyllosphere of *A. adenophora* and *L. camara* (Appendix 7). The mean frequency of all identified Phyllosphere fungi was calculated as shown in Figure 14. Among the fungi isolated, *Aspergilus fumigatus* was the most frequent species in both plants with frequency of 63.33% in *A. adenophora* and 81.67% in *L. camara*.

In *Ageratina adenophora, Fusarium oxysporum* (43.33%) was the second frequent species followed by *F. solani* (25.00%), *A. flavus* (21.67%). *Cercospora* sp. *Chaetomium* sp. and *Sarocladium* sp. were equally frequent with frequency of (20.00%). Frequency of other fungi are *Curvularia lunata* (13.33%), *Didymella* (11.67%), *Alternaria alternate* (10.00%), *Colletotrichum gloeosporioides* (10.00%), *Trichothecium roseum* (8.33%). *Cladosporium cladosporioides*, *Cylindrosporium* sp., *Penicillium chrysogenum* and *Rhizopus* sp. has frequency 6.67%. *Geotrichum candidum* and *Alternaria* sp. were the least frequent species with frequency of 1.67%.

In *Lantana camara, A. flavus* (36.67%) was the second frequent species followed by *A. niger* (25.00%) and *F. solani* (40.00%), *A. niger* (38.33%), *Ceratobasidium* sp. (20.00%), *F. oxysporum* (16.67%), *Nigrospora* sp. (16.67%). *Alertnata alternata*, *A. nidulans* and *Cladosporium* has frequency of 11.67%. *Penicillium chrysogenum* was the least frequent species with frequency of 6.67%.

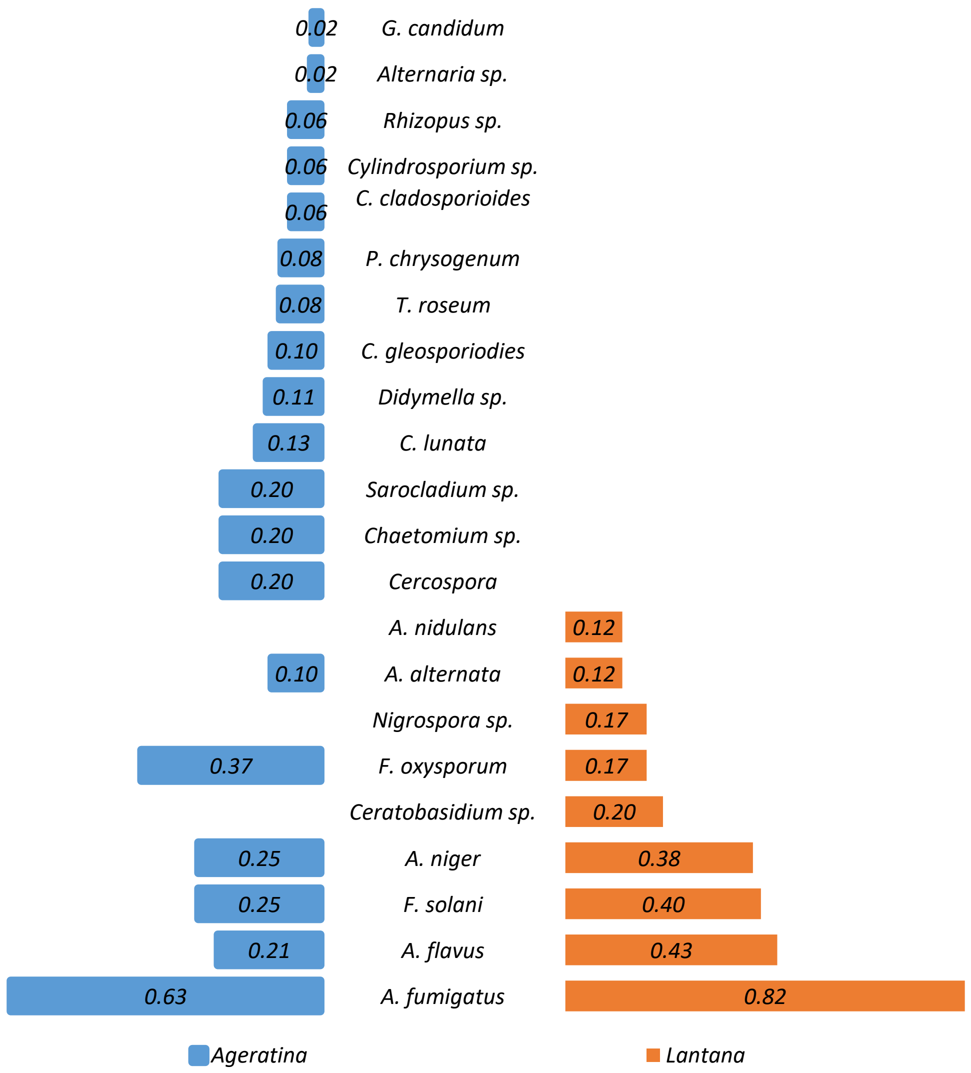

Figure 14. Frequency of fungi in *A. adenophora* and *L. camara*

CHAPTER-4

DISCUSSION

4.1. Herbivory damage between invasive and native species.

Herbivory damage was compared between invasive *A. adenophora* and *L. camara* with native *Adenostemma lavenia* and *S. wallichi*, respectively. The result showed comparatively high herbivory damage in both native species than the invasive ones (Figure 6 and 7). This shows that the invasive species in the study site are comparatively less stressed by herbivores whereas the native species might be greatly stressed by the herbivores. According to enemy release hypothesis, when plant species are introduced to an exotic environment, the way they are controlled by their herbivores and natural enemies' decreases, leading to increased spread and abundance (Kaene and Crawley 2002). The result of this study supports this hypothesis.

Balami and Thapa (2017) compared herbivory damage in native *Alnus nepalensis* and invasive *A. adenophora*. *Ageratina adenphora* showed around 66% less leaf damage than *A. nepalensis.* In this study, *A. adenphora* revealed around 20% less herbivory damage than *Adenostemma lavenia* (Figure 6) and *L. camara* showed around 26% less herbivory damage (Figur 7). Balami and Thapa (2017) concluded that the invasive *A. adenophora* suffered less herbivory damage than the native *Alnus nepalensis*. Their study predicted that some pests or parasites may have adapted to feeding on *Ageratina* during a long period of introduction and establishment, maybe co-evolving with native plants, and concluded that native herbivores rarely switch to invasive even after a lengthy duration of invasion. Similarly, Carpenter and Cappuccino (2005) have also studied on herbivory damage between alien and native plant species in Ottawa, Canada and revealed that exotics species were less affected by herbivores.

Research has shown that exotic plant species have smaller herbivore communities in their introduced area than in their native location (Agarwal and Kotanen 2003). This reduced herbivory damage may provide an advantage to exotic plants over native plants, as anticipated by the traditional "enemy release" concept for introduced organism's success (Keane and Crawley 2002, Torchin et al. 2003).

4.2 Relationship between herbivory damage and pathogen damage

Herbivory damage in invasive plant was positively correlated with pathogen damage in *A. adenophora* (Table 1) and *L. camara* (Table 3). The result showed that when the level of herbivory increases in invasive plant, it also increased the level of pathogen damage. It can be expected that, herbivory can weaken plants, making them more susceptible to pathogens. When herbivores feed on plants, they can cause physical damage to plant tissues, which provides entry points for pathogens. Additionally, the stress caused by herbivory may weaken the plant's immune system, making it less able to defend against pathogen attacks.

Herbivores may act as vectors or facilitate the entry of pathogens by breaking down foliar tissue and plant defensive barriers (Rodriguez et al. 2023). The result in this study indicated positive relation between herbivory and pathogen damage. Schuldt et al. (2017) also discovered positive relation between herbivory and pathogen damage.

4.3 Herbivory damage, pathogen damage, tree canopy and elevation

Table 1 shows that there is negative relationship between herbivory damage and gall formation in *A. adenophora* but the relation was not significant. However, it can be expected that if the herbivores damage the plant, there might be chance of reduction in reproductive activities of gall fly. This may be also linked with the nutrition supply by the damaged plants for appropriate development of larvae of the gall fly. Studies regarding the relationship between herbivory damage and reproductive behavior of gall fly are deficient. Therefore, it is essential to carry out the comprehensive study to compare herbivores feeding and reproductive behaviors. Similar was the case of pathogen damage and number of galls (Table 1).

With increase in elevation, herbivory damage and pathogen damage decreased in *A. adenophora* and *L. camara* but the difference was not significant (Table 1 and Table 3). As the data is not significant, this study cannot conclude the direct effect of elevation on herbivory damage. Some of the previous studies highlighted that there was increased insect herbivory damage in the high elevations (Kooyers et al. 2017).

With increase in elevation, number of gall formation is decreased by 25% (Table 1) in *A. adenophora* but this data is not significant. Poudel et al. (2020) experimented elevation dependent stem galling of *A. adenophora* by gall fly *Procecidochares utilis* and concluded that elevation had significant effect on both size and abundance of gall. According to them, elevation is a significant abiotic element that impacts the size and abundance of *P. utilis* stem galls, and the galls that *P. utilis* forms serve as nutrient sinks.

Table 2 shows herbivory damage is negatively correlated with tree canopy and elevation in *Adenostemma lavenia* but the data is not significant. Similar result is seen in case of *L. camara* (Table 3) and *S. wallichi* (Table 4). All these data are not significant. But it can be expected from these results that tree canopy might decrease the herbivory damage. Gossner et al. (2014) studied differential response of herbivores and herbivory under tree canopy in temperate forest and concluded that arthropod herbivory is generally greater under tree canopy. Previous studies regarding the relation between herbivory damage with tree canopy and elevation in these plants is lacking. So, further studies regarding relation between herbivory damage with tree canopy and elevation should be carried out.

4.4 Herbivores species richness in *A. adenophora* than *L. camara*

On comparing the species richness in *A. adenophora* and *L. camara*, large number of herbivores were found in *A. adenophora* than *L. camara.* It is quite interesting to know why large number of herbivores visited *A. adenophora.*

It can be expected that the variation is may be due to habit of plant. *A. adenophora* which is herb with soft leaf and smooth texture while *L. camara* is a shrub with comparatively thick and rough leaf surface. Differences in leaf texture, thickness, and other physical characteristics can influence herbivory rates (Peeters et al. 2002). Plants with softer leaves or thinner cuticles may be more susceptible to herbivore damage than those with tougher, thicker leaves (Malishev et al. 2015).

Another factor behind this could be the chemical composition of these invasive plants. *L. camara* contains toxic compounds such as caryophyllene II oxide and aromadendrene II oxide which act as bio pesticides against defoliator herbivores

(Murugesan et al. 2016). These compounds make the plant less appetizing and potentially toxic to many herbivores, reducing the number of insects that feed on it. In contrast, *A. adenophora* might have a chemical composition that is less deterrent to herbivores. This indicates that *L. camara* are the potential bio-control agent for insect herbivores than *A. adenophora.* But, literatures regarding the comparative assay of insecticidal properties of *A. adenophora* and *L. camara* is lacking.

Lagria, *Graphocephala* and *Formica* were the frequent herbivores in *A. adenophora* (Figure 10). *Lagria* sp. is a genus of beetle, belonging to family Tenebrionidae is a significant pest crop. *Lagria* feeds on nectar and pollens of plant (Zhou 2001). *Lagria* is the most frequent insect in *A. adenophora* (10%) and second frequent insect in *L. camara* (5%) (Figure 10). It will be interesting to study that why beetle *Lagria* visited frequently *A. adenophora* than *L. camara,* despite of having colored, scented and nectarious flower in *L. camara.* Beetles generally prefer off white and heavily scented flower (Cook et al. 2004). This might be the reason behind the more frequent visit of *Lagria* in *A. adenophora* which has off white colored flower than *L. camara* which has bright and colored flowers.

In an experiment carried out in south-west China, Westwood, a polyphagous ant that is a natural enemy of *A. adenophora*, killed the entire plant by interrupting the nutrition exchange between shoots and roots (Niu et al. 2010). *Formica* species were also recorded frequently associated with leaf and stem of plants. So, further studies must be carried out to measure damage severity caused by this species in *A. adenophora.*

Procecidochares utilis is one of the seven biological control agents for *A. adenophora* that have been suggested so far (Wan et al. 2010). However, in areas with abundant rainfall, the fly had no effect on the weed (Bess and Haramoto 1972; Paudel et al. 2019). Several galls which might be formed by these plants in stem and leaf of *A. adenophora* was observed during field study but this gall fly was not present inside the gall formed, only the eggs were present inside the gall formed. So, seasonal exploration must be carried out to trap this gall fly.

Alcidodes were the frequent herbivores in *L. camara* (10%) and least frequent herbivore in *A. adenophora* (2%) (Figure 10). *Alcidodes* is a genus of weevils in the family Curculionidae. It prefers to reside inside the stem and mainly feeds on vascular tissue and foliage (Chavan and Saxena, 2019). *A. adenophora* which is herb has comparatively softer stem than shrub *L. camara* which has strong vascular tissue. So, this might be the reason behind frequent visit of *Alcidodes* in *L. camara* than *A. adenophora.*

The species *Lantanophaga* (Platyptilia) *Pusillidactyla* (Walker) (Pterophoridae) are also recommended for biological control of *L. camara* after field observation in India and Burma (Munippan et al. 1986). *Ophiomyia lantanae* (fruit-mining fly), *Calycomyza lantanae* (agromyzid seedfly), *Teleonemia elata* (leaf-sucking bug), *Teleonemia scrupulosa* (leaf-sucking bug) were used for controlling *L. camara* but most failed as it has several varieties or forms resulting in complicating the introduction and establishment of exotic insects. In South Africa, *Aceria lantanae* and *Ophiomyia lanatane* are improving control in frost-free areas on some varieties of *L. camara* (Shackleton et al. 2017). But these species were not recorded during field study.

The frequency of the insect herbivores is not more than 10% in both invasive plants (Figure 10). This study was carried out during day time only and only recorded the herbivores of day time. So, this study has not included light sensitive insects which might be the potential herbivores. Detailed study must be carried out during night time also to trap light sensitive insect herbivores. Further, herbivory damage was observed higher in native species than invasive species. But, very few herbivores were seen around the native plants during field exploration in day time. So, it can be expected that herbivores that feeds on native plants might be light sensitive which might be voracious leaf eaters during night time.

4.5 Phyllosphere fungi richness in *A. adenophora* than *L. camara*

Phyllosphere refers to the aboveground parts of plant that harbors diverse communities of microorganisms including fungi. Microbes often establish an endophytic or epiphytic life cycle on the phyllosphere that helps the host plant and enables functional communication with the surroundings (Yadav et al. 2020).

Maximum number of fungi was isolated from phyllosphere of *A. adenophora* than *L. camara* (Figure 11). It might be due to the strong chemical composition in leaves of *L. camara* which forms a less suitable habitat for fungal colony. Futher, herbivores are the potential carriers of fungal pathogens. This study results showed less herbivory and pathogen damage in *L. camara* than *A. adenophora.* So, this might be the reason behind less fungi in phyllosphere of *L. camara.* Similarly, physical characteristics such as moisture content in leaf, texture of leaf, and structure of leaf can also impact on fungal colonization (Gutschick 1999). Leaf of *A. adenophora* comparatively contain more moisture and has smooth leaf texture than *L. camara.* So, this might also indicate the reason behind a smaller number of fungi in phyllosphere of *L. camara.*

Aspergillaceae is the dominant family in the phyllosphere of both invasive *A. adenophora* and *L. camara.* This indicates that Aspergillaceae is the common air borne fungi in the phyllosphere of both invasive plants. Even *Aspergillus* is the most common fungal species in the phyllosphere of both plants, there is no any known disease caused by these fungi in plants. So, it may indicate that *Aspergillus* fungi only make host to these plants but don't cause disease to them.

Chen et al. (2020), characterized the air borne fungal communities of invasive *A. adenophora* and evaluated their potential to cause disease to the plant. This study found Cladosporiaceae as the frequent fungus present in the canopy air of *A. adenophora* mainly represented by genus *Cladosporium.* Species of Didymellaceae cause leaf spot disease and stem lesions in *A. adenophora* (Chen et al. 2022). In this study also, leaf spots were recorded in *A. adenophora.* Detailed study is required to confirm this leaf spot recorded were due to fungal pathogen *Didymella* sp. found in phyllosphere of *A. adenophora.*

Passalora ageratinae, a leaf spot fungus, was reported to reduce plant height and the number of leaves and flowers by lowering photosynthetic rate, transpiration, and chlorophyll content (Zhang et al. 2008; Poudel et al. 2019). The joint action of gall fly and pathogen *P. ageratinae* was suggested to be a useful agent for biological control of *A. adenophora* (Buccellato et al. 2012). Symptoms of disease caused by this fungus, *P. ageratinae* were observed in the leaves of the plant, but this study couldn't identify this fungus in phyllosphere isolation of fungi. So, this fungus might be incorporated into the host tissue and endophyte culture should be carried out to conclude that symptoms of visible leaf spots were caused by *P. ageratinae.*

Four fungal species namely, *Septoria* sp. (Trujillo and Norman 1995), rust fungi *Prospodium tuberculatum* (Tomley and Riding 2002), leaf spot pathogen *Passalora lantanae* (Breeyen 2003) and rust fungi *Puccinia lantanae* had been released as a classical bio control agent for *L. camara* in different part of the world (Thomas et al. 2021). In an assessment conducted by Thomas et al. (2021), *Puccinia lantanae* was concluded as a potential biocontrol agent to cause considerable leaf damage in *L. camara.* Leaf rust of *L. camara* which might be caused by this fungus, was observed but *P. lantanae* was not isolated from the phyllosphere of this plant.

Aspergillus fumigatus, Fusarium oxysporum, A. niger, F. solani, A. flavus, Alternaria alternata were recorded from phyllosphere of both fungi. But fungi like *Cercospora,* sp., *Chaetomium* sp., *Sarocladium* sp., *Curvularia lunata* sp., *Didymella* sp. etc were particularly host specific and recorded only from the phyllosphere of *A. adenophora.* Similarly, *Nigrospora* sp., *Ceratobasidium* sp. and *A. nidulans* were host specific to *L. camara* only. Along with the 17 fungi isolated by Ojha et al. (2019), *Aspergillus* sp. were dominantly present in polluted air of industrial area while *Nigrospora* sp. were present in both polluted and unpolluted sites.

CHAPTER – 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1. Herbivory damage was high in native species than invasive species. Both *Adenostemma lavenia* and *S. wallichi* shows greater herbivory damage than invasive plants *Ageratina adenophora* and *Lantana camara* respectively. This concludes that native plants are more damaged and less competitive with invasive plants.

2. Pathogen damage was more in *A. adenophora* than *L. camara.* Herbivory damage increases the susceptibility to pathogen damage in both *A. adenophora* than *L. camara.* This implies that joint action of herbivores and pathogen might be effective to control invasive plants.

3. Total 31 herbivores belonging to 6 different order were found associated with *A. adenophora* and *L. camara* in this study. Maximum number of herbivores were found in *A. adenophora* i.e., 30 species and only 5 species of herbivores were recorded from *L. camara.*

4. A total 33 fungal species were identified from the Phyllosphere of *A. adenophora* and *L. camara*. Maximum number of fungi was isolated from phyllosphere of *A. adenophora* than *L. camara*.

5. Overall, herbivory damage was less in invasive plants than native ones and herbivory damage increases the susceptibility to pathogen damage. So, controlling and management of destructive herbivores may reduce pathogenic diseases in native plants On the other hand, adaptation of herbivores on invasive plants can be beneficial for controlling and management of invasive plants.

5.2 Recommendations

1. The result of this study shows positive correlation between herbivory and pathogen damage. So, further studies focused on herbivory with pathogen damage should be carried out as to know the effectiveness of the damage for controlling invasive plants.

2. This study was only focused in exploration of phyllosphere fungi. So, studies focused in culture of endophytic fungus should be carried out to explore endophytic fungi and their role in invasive plants.

3. Detailed exploration should be carried out to explore the herbivores associated with the native species. It will help to find out the reason behind the more herbivory damage in native species.

4. Explorative study must be carried out during night time also to trap light sensitive insect herbivores.

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APPENDICES

Appendix – 1

Details of measuring parameters (herbivory and pathogen damage, gall number in *A. adenophora*) in sampling plots with gradient of elevation and tree canopy

Plot Number	Herbivory damage (%)	Gall number	Pathogen damage (%)	Elevation	Tree canopy cover (%)
1	24.05	9.00	12.66	1412	68
2	26.79	4.00	19.64	1433	84
3	33.33	1.00	13.33	1440	75
4	50.0	3.00	8.33	1444	72
5	31.91	0.00	10.64	1448	45
6	27.78	1.00	16.67	1438	70
7	25.00	3.00	25.00	1445	43
8	33.33	6.00	27.78	1451	75
9	13.04	4.00	13.04	1452	78
10	22.22	1.00	8.33	1456	68
11	31.03	2.00	17.24	1459	32
12	26.92	0.00	15.38	1463	79
13	35.00	1.00	30.00	1441	84
14	30.00	1.00	20.00	1440	20
15	19.05	3.00	16.67	1430	35
16	18.18	0.00	4.55	1425	53
17	15.00	1.00	0.00	1433	10
18	66.67	1.00	41.67	1432	66
19	20.83	1.00	12.50	1434	36
20	36.00	1.00	32.00	1426	61
21	38.46	1.00	34.62	1424	28
22	40.63	1.00	40.63	1433	34
23	22.73	2.00	0.00	1429	13
24	41.67	3.00	8.33	1427	33
25	44.44	3.00	11.11	1345	80

26	15.63	3.00	3.13	1388	80
27	10.81	4.00	0.00	1392	64
28	15.09	0.00	20.75	1405	80
29	58.33	0.00	33.33	1415	65
30	25.81	0.00	11.29	1424	86
31	22.22	0.00	22.22	1450	53
32	50.00	0.00	50.00	1453	65
33	27.78	0.00	16.67	1453	51
34	50.00	0.00	50.00	1453	74
35	27.78	1.00	16.67	1460	65
36	33.33	0.00	5.56	1464	74
37	22.50	2.00	20.00	1471	71
38	41.67	1.00	33.33	1472	39
39	42.86	2.00	28.57	1478	78
40	31.25	0.00	0.00	1483	71
41	71.43	0.00	21.43	1487	52
42	46.43	1.00	25.00	1495	67
43	35.00	0.00	20.00	1496	55
44	37.50	2.00	15.63	1508	58
45	75.00	2.00	41.67	1410	69
46	19.23	0.00	15.38	1525	37
47	18.00	2.00	12.00	1528	76
48	18.52	0.00	18.52	1535	64
49	25.00	1.00	4.17	1544	31
50	40.24	0.00	29.27	1563	59

Appendix - 2

Details herbivory damage in *Adenostemma lavenia* in sampling plots with gradient of elevation and tree canopy

Plot number	Herbivory damage (%)	Elevation	Tree canopy cover (%)
1	26.47	1412	68
2	34.38	1433	84
3	66.67	1440	75
4	55.56	1444	72
5	50.00	1448	45
6	60.00	1438	70
7	45.45	1445	43
8	62.50	1451	75
9	36.11	1452	78
10	40.00	1456	68
11	53.85	1459	32
12	30.00	1463	79
13	30.77	1441	84
14	38.46	1440	20
15	42.86	1430	35
16	25.64	1425	53
17	27.08	1433	10
18	58.33	1432	66
19	70.00	1434	36
20	42.86	1426	61
21	31.25	1424	28
22	57.14	1433	34
23	54.17	1429	13
24	41.67	1427	33
25	33.33	1345	80
26	33.33	1388	100
27	90.00	1392	64
28	58.82	1405	80

29	21.43	1415	65
30	53.33	1424	86
31	61.54	1450	53
32	100.00	1453	65
33	42.86	1453	51
34	50.00	1453	74
35	70.00	1460	65
36	54.55	1464	74
37	25.00	1471	71
38	100.00	1472	39
39	66.67	1478	78
40	16.67	1483	71
41	52.00	1487	52
42	90.91	1495	67
43	83.33	1496	55
44	68.18	1508	58
45	100.00	1410	69
46	80.00	1525	37
47	42.86	1528	76
48	55.56	1535	64
49	100.00	1544	31
50	31.25	1563	59

Appendix-3

Details of measuring parameters (herbivory and pathogen damage in *L. camara*) in sampling plots with gradient of elevation and tree canopy

Plot no	Herbivory damage (%)	Pathogen damage(%)	Elevation	Tree canopy Cover (%)
1	73.17	9.76	1452	20
2	59.42	20.29	1469	70
3	64.55	16.36	1432	60
4	56.71	44.51	1428	0
5	61.98	49.59	1427	0
6	52.70	41.89	1414	35
7	60.49	30.86	1382	74
8	57.89	23.68	1387	52
9	62.38	55.45	1249	0
10	34.15	34.15	1411	90
11	54.95	43.96	1398	35
12	93.22	79.66	1392	73
13	31.58	1.75	1258	66
14	29.73	2.70	1451	37
15	48.61	16.67	1446	24
16	42.03	20.29	1410	70
17	41.00	26.00	1424	60
18	55.77	32.69	1418	53
19	56.44	32.67	1421	47
20	39.82	23.89	1426	26

Appendix- 4

Details herbivory damage in *Schima wallichi* in sampling plots with gradient of elevation and tree canopy

Plot	Herbivory damage (%)	Elevation	Tree canopy
1	66.67	1362	70
2	100	1413	85
3	7.14	1412	78
4	51.61	1408	80
5	100	1423	78
6	90.48	1427	84
7	96.3	1467	82
8	72.41	1468	72
9	96.15	1487	69
10	65.22	1467	39
11	96.36	1457	42
12	97.37	1460	43
13	93.33	1459	1
14	100	1465	33
15	100	1455	38
16	88.57	1477	46
17	72.73	1442	76
18	32.14	1428	71
19	68.75	1501	76
20	90.24	1506	63

Appendix -5

List of herbivores in invasive *A. adenophora* and *L. camara*

SN	Genus	Order	Family	*A. adenophora*	*L camara*
1	*Alcidodes*	Coleoptera	Curculionidae	+	+
2	*Anthicus*	Coleoptera	Anthicidae	+	_
3	*Aphaenogaster*	Hymenoptera	Formicidae	+	_-
4	*Camponotus*	Hymenoptera	Formicidae	+	_
5	*Cetonia*	Coleoptera	Scarabaeidae	+	_
6	*Chrysochus*	Coleoptera	Chrysomelidae	+	_
7	*Cosmoscarta*	Homoptera	Cercopidae	+	_
8	*Dalader*	Hemiptera	Coreidae	+	_
9	*Epilachna*	Coleptera	Coccinellidae	+	_
10	*Episyrphus*	Diptera	Syrphidae	+	_
11	*Euthenes*	Hemiptera	Tessaratomidae	+	_
12	*Formica*	Hymenoptera	Formicidae	+	_
13	*Graphocephala*	Homoptera	Ciccadelidae	+	_
14	*Jauravia*	Coleoptera	Coccinellidae	+	_
15	*Lacon*	Coleoptera	Elateridae	+	_
16	*Lagria*	Coleoptera	Tenebrionidae	+	+
17	*Lema*	Coleoptera	Chrysomelidae	+	_
18	*Leptocorisa*	Hemiptera	Alydidae	+	_
19	*Lucilia*	Diptera	Calliphoridae	+	+
20	*Lygus*	Hemiptera	Miridae	+	_
21	*Meranoplus*	Hymenoptera	Formicidae	+	_
22	*Mimela*	Coleoptera	Scarabaeidae	+	_
23	*Neocollyris*	Coleoptera	Carabidae	+	_
24	*Orygia*	Lepidoptera	Erebidae	+	+
25	*Paratrachina*	Hymenoptera	Formicidae	+	_
26	*Plecia*	Diptera	Bibionidae	+	_
27	*Popilia*	Coleoptera	Scarabaeidae	+	_
28	*Sargus*	Diptera	Stratiomyidae	_	+
29	*Tetramorium*	Hymenoptera	Formicidae	+	_
30	*Torynorrhina*	Coleoptera	Scarabaeidae	+	_
31	*Zelus*	Hemiptera	Reduviidae	+	_

'+ ' = Present, '-' = Absent

Appendix- 6

Frequency of Herbivores in invasive species *A. adenophora* and *L. camara*

Insect	***A. adenophora***	***L. camara***
Lagria	10	5
Graphocephala	8	0
Formica	6	0
Camponotus	4	0
Epilachna	4	0
Episyrphus	4	0
Lacon	4	0
Leptocorisa	4	0
Paratrachina	4	0
Plecia	4	0
Alcidodes	2	10
Anthicus	2	0
Aphaenogaster	2	0
Cetonia	2	0
Chrysochus	2	0
Cosmoscarta	2	0
Dalader	2	0
Euthenes	2	0
Jauravia	2	0
Lema	2	0
Lucilia	2	5
Lygus	2	0
Meranoplus	2	0
Mimela	2	0
Neocollyris	2	0
Orygia	2	5
Popilia	2	0
Tetramorium	2	0
Torynorrhina	2	0
Zelus	2	0
Sargus	0	5

Appendix – 7

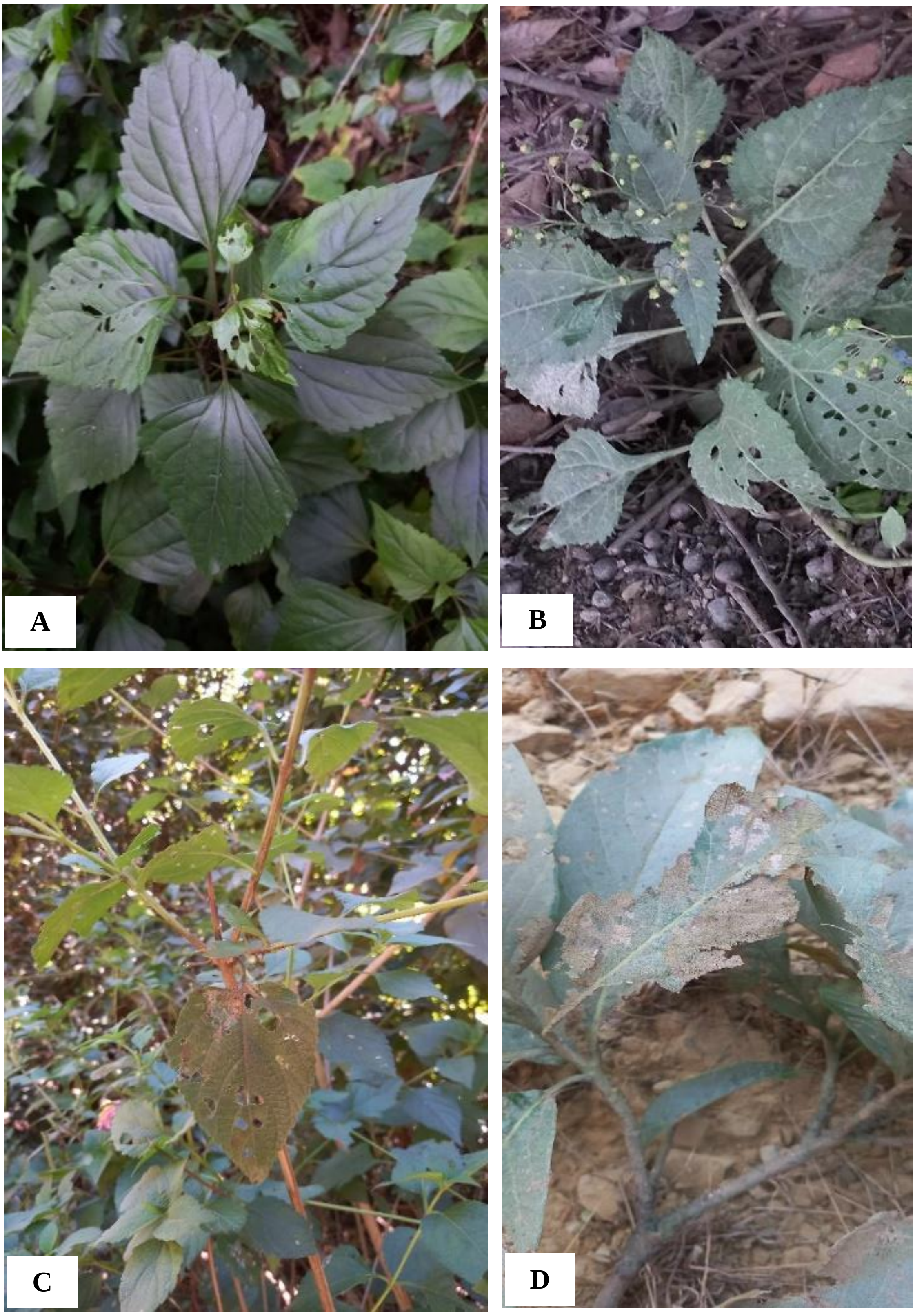
Taxonomic details of identified fungi of *A. adenophora* and *L. camara*

SN	Name of Fungal species	Phylum	Classs	Order	Family	Host
1	*Alternaria alternata* (Fr.) Keissl.	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Aa, Lc
2	*A*lternaria sp.	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Aa
3	*Aspergillus flavus* Link	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Aa, Lc
4	*Aspergillus fumigatus* Fresen.	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Aa,Lc
5	*Aspergillus nidulans(*Eidam) G. Winter	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Lc
6	*Aspergillus niger*Teigh.	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Aa,Lc
7	*Cercospora* sp.	Ascomycota	Dothideomycetes	Mycospharellales	Mycosphaerellaceae	Aa
8	*Ceratobasidium sp.*	Basidiomycota	Agaricomycetes	Cantharellales	Cerstobasidiaceae	Lc
9	*Chaetomium* sp.	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	Aa
10	*Cladosporium cladosporioides* (Fresen.) G.A. de Vries	Ascomycota	Dothideomycetes	Cladosporilaes	Cladosporiaceae	Aa, Lc
11	*Colletotrichum gleosporiodies(Penz.) Penz. & Sacc.*	Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	Aa

12	*Curvularia lunata* (Wakker) Boedijn	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Aa
13	*Cylindrosporium* sp.	Ascomycota	Leotiomycetes	Helotiales	Ploettnerulaceae	Aa
14	*Didymella* sp.	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae	Aa
15	*Fusarium oxysporum*Schltdl.	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Aa,Lc
16	*Fusarium solani* (Mart.)Sacc.	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Aa, Lc
17	*Geotrichum candidum* Link.	Ascomycota	Dipodascomycetes	Dipodascales	Dipodascaceae	Aa
18	*Nigrospora* sp.	Ascomycota	Sordariomycetes	Xylariales	Apiosporaceae	Lc
19	*Penicillium chrysogenum* Thom.	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Aa,Lc
20	*Rhizopus* sp.	Mucoromyceta	Mucoromycetes	Mucorales	Rhizopodaceae	Aa
21	*Sarocladium* sp.	Ascomycota	Sordariomycetes	Hypocreales	Sarocladiaceae	Aa
22	*Trichothecium roseum* (pers.) Link.	Ascomycota	Sordariomycetes	Hypocreales	Myrotheciomycetaceae	Aa

Aa = *Ageratina adenophora*, Lc = *Lantana camara*

PHOTOPLATES



Photoplate 1: Herbivory damaged leaves of (A) *Ageratina adenophora*, (B) *Adenostemma lavenia*, (C) *Lantana camara* and (D) *Schima wallichi*

Photo plate 2: Counting of damaged leaves of (A) *A. adenophora*, (B) *L. camara*. Sampling of herbivores (C, D, E)

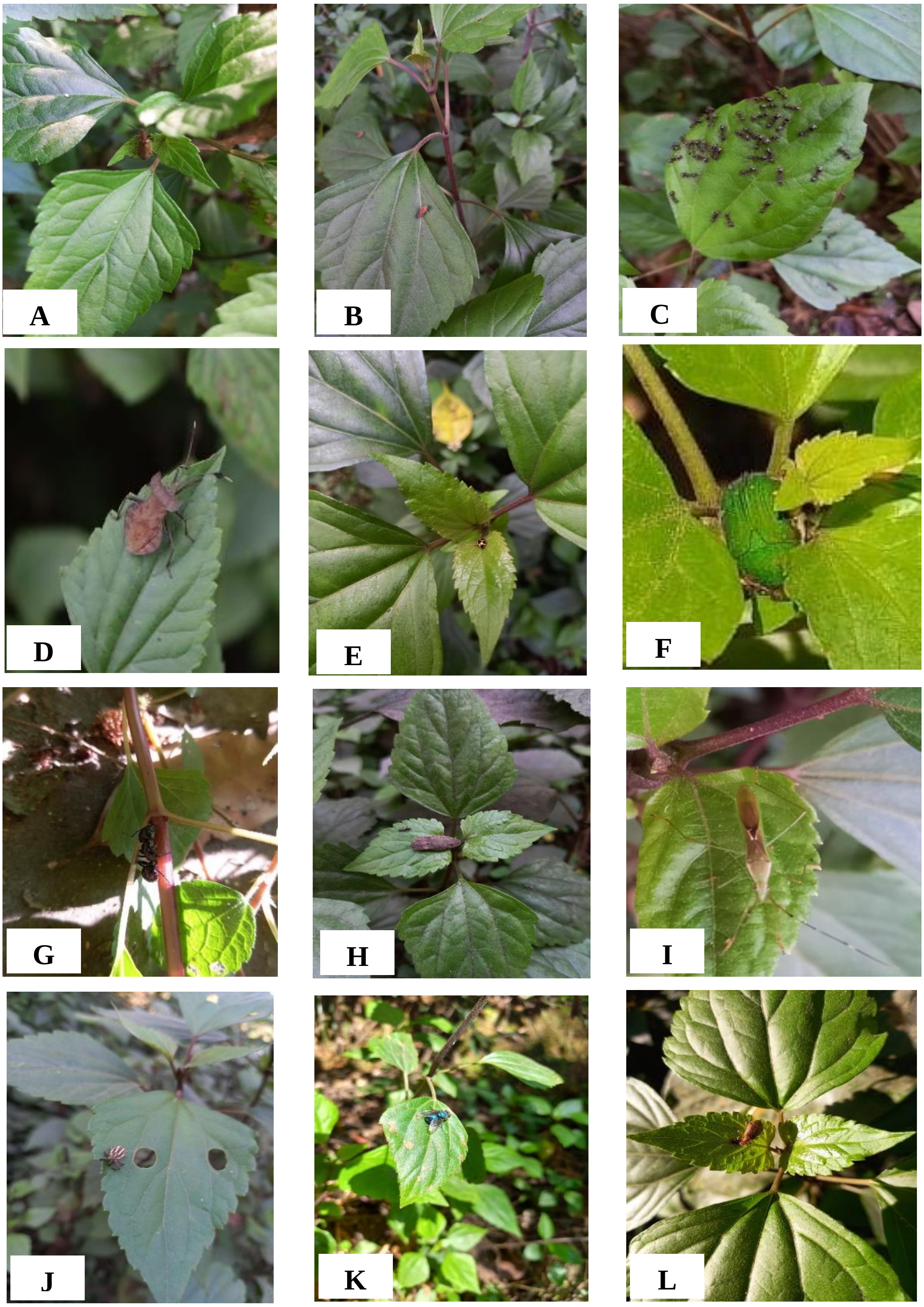


Photo plate 3: Some herbivores associated with *A. adenophora* (A) *Lagria* sp. (B) *Graphocephala* sp. (C) *Formica* sp. (D) *Dalader* sp. (E) *Epilachna* sp. (F) *Mimela* sp. (G) *Camponotus* sp. (H) *Lacon* sp. (I)*Leptocorsia* sp. (J) *Alcidodes* sp (K) *Lucilia* sp. (L) *Anthicus* sp.

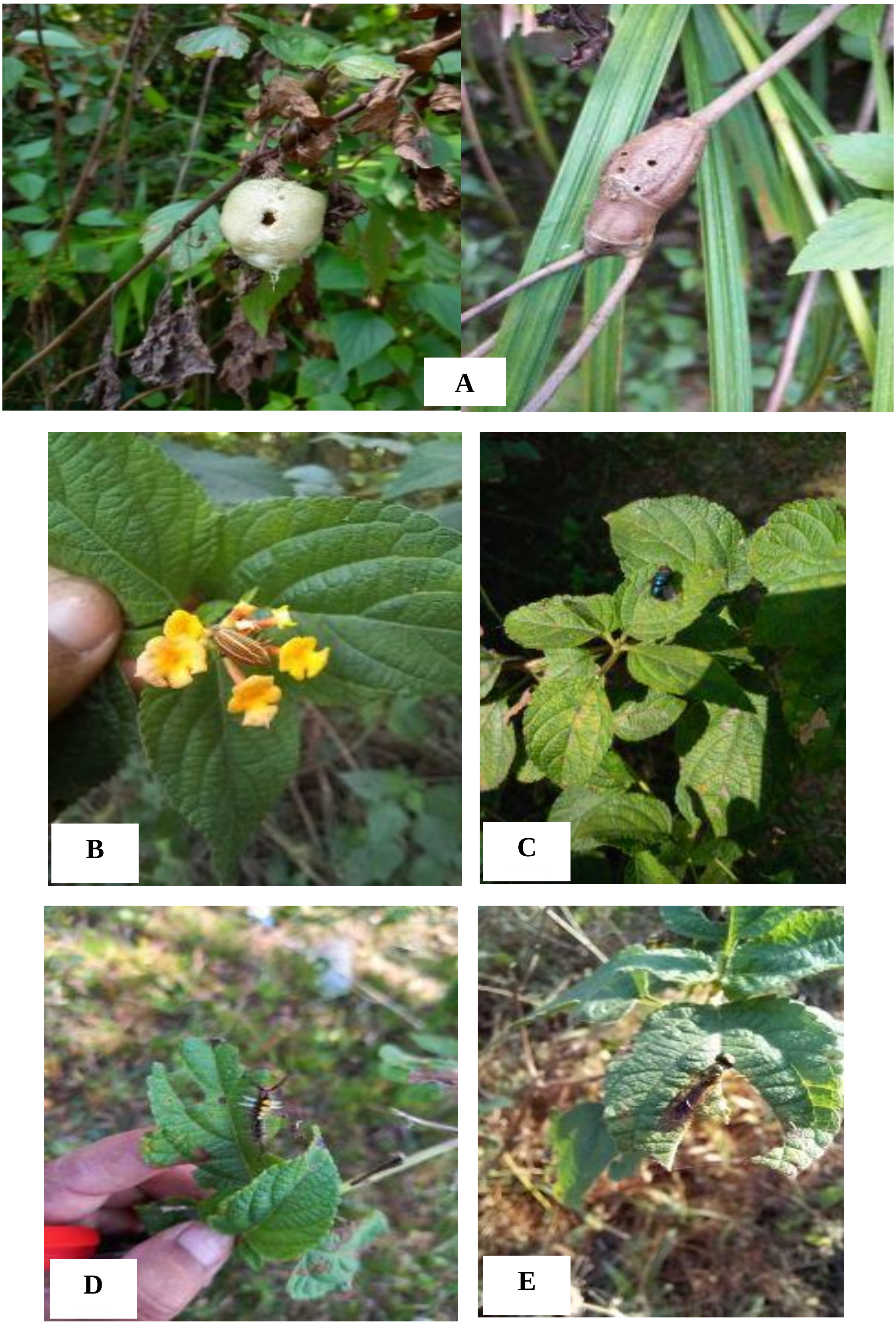


Photo plates 4: (A) Gall formation in *Ageratina adenophora* , Some berbivores associated with *L. camara* (B) *Alcidodes* sp. *Lucilia* sp. (D) *Sargus* sp. (E) *Orygia* sp.

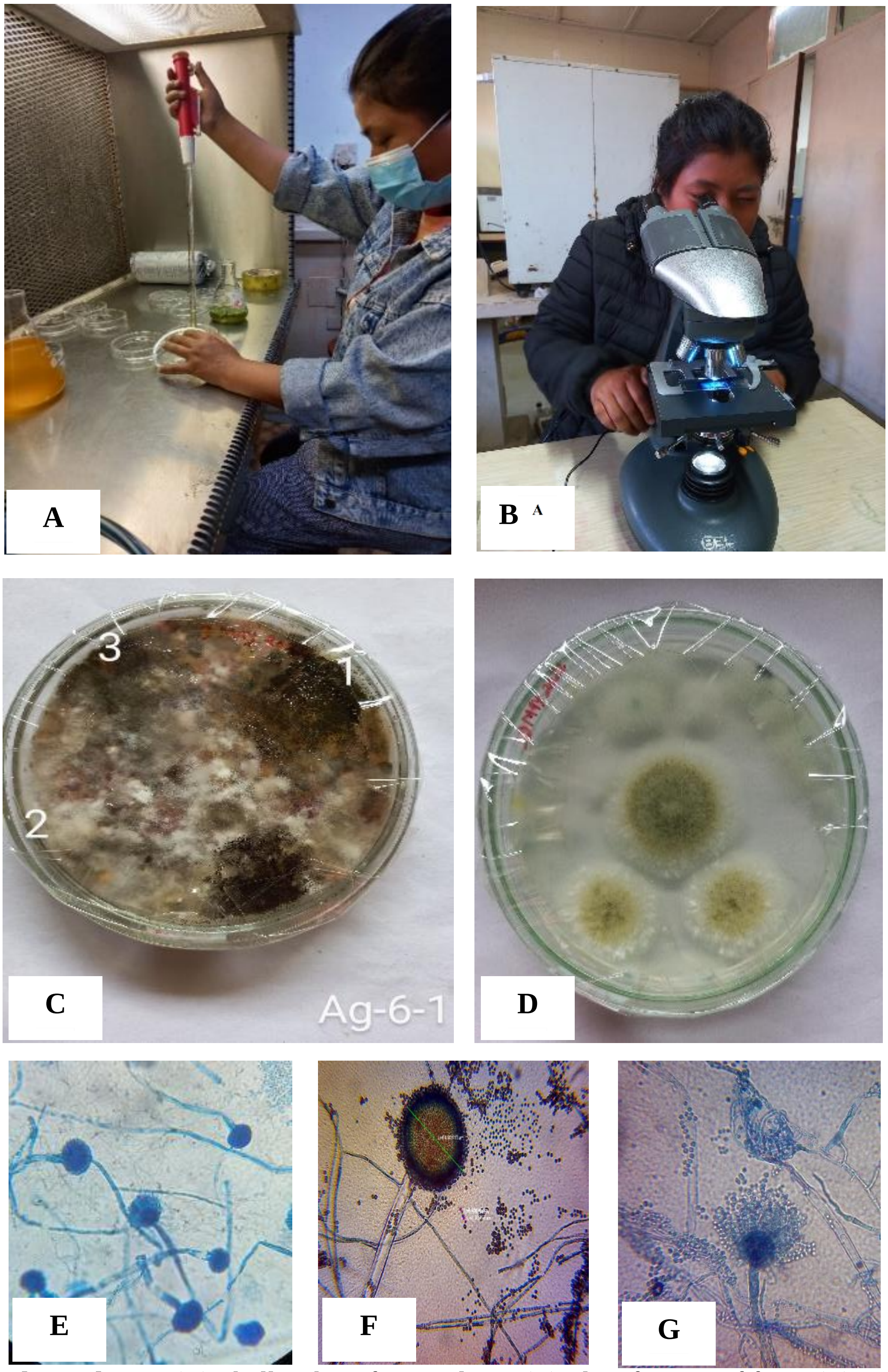


Photo plate 5: (A) Phyllosphere fungi culture, (B) Identification of fungi (C) Colony counting, (D) Pure culture (E) *Aspergillus fumigatus*, (F) *Aspergillus niger* (G) *Aspergillus flavus*