EFFECT OF *Ageratina adenophora* INVASION ON LITTER DECOMPOSITION, FINE ROOT PRODUCTION AND MYCELIAL GROWTH OF SELECTED SAPROPHYTIC FUNGI

A dissertation submitted for the partial fulfillment of the requirements for the Master's Degree in Botany (Ecology)

BY

DEEPA GHARTI MAGAR

Exam Roll No: Bot. 819/076

TU Reg. No.: 5-2-37-315-2013

SUBMITTED TO

Department of Botany

AMRIT CAMPUS

Institute of Science and Technology

Tribhuvan University

Kathmandu, Nepal

29 May 2024

EFFECT OF *Ageratina adenophora* INVASION ON LITTER DECOMPOSITION, FINE ROOT PRODUCTION AND MYCELIAL GROWTH OF SELECTED SAPROPHYTIC FUNGI

A dissertation submitted for the partial fulfillment of the requirements for the Master's Degree in Botany (Ecology)

BY

DEEPA GHARTI MAGAR

Exam Roll No: Bot. 819/076

TU Reg. No.: 5-2-37-315-2013

SUBMITTED TO

Nepal Academy of Science and Technology (NAST)

Khumaltar, Lalitpur, Nepal

29 May 2024

A thesis grant supported by Nepal Academy of Science and Technology (NAST)

DECLARATION

I, Deepa Gharti Magar, hereby declare that this dissertation entitled **"Effect of *Ageratina adenophora* invasion on litter decomposition, fine root production and mycelial growth of selected saprophytic fungi"** is my original work and all other sources if the information used are duly acknowledge. I have not submitted it or any of its parts to any other universities for my academic award.

Deepa Gharti Magar
Department of Botany
AMRIT CAMPUS
Tribhuvan University
Kathmandu, Nepal
Date: May 29, 2024

Tribhuvan University
Institute of Science and Technology
AMRIT CAMPUS
P.O Box No: 102, Thamel, Kathmandu,
Nepal. E-mail: amritcampus@ntc.net.np

LETTER OF RECOMMENDATION

This is to certify that Ms. Deepa Gharti Magar has completed this thesis work entitled **"Effect of *Ageratina adenophora* invasion on litter decomposition, fine root production, and mycelial growth of selected saprophytic fungi"** as the partial fulfillment of Master's Degree in Botany under my supervision. To the best of my knowledge this work has not been submitted for any other degrees. Therefore, I recommend this thesis to be accepted for Master's Degree in Botany.

.....

Supervisor
Dr. Laxmi Joshi Shrestha
Assistant Professor
Department of Botany
AMRIT CAMPUS
Tribhuvan University
Kathmandu, Nepal

Date: May 29, 2024

Tribhuvan University
Institute of Science and Technology
AMRIT CAMPUS
P.O Box No: 102, Thamel, Kathmandu,
Nepal. E-mail: amritcampus@ntc.net.np

LETTER OF APPROVAL

The thesis work entitled **"Fffect of *Ageratina adenophora* invasion on litter decomposition, fine root production and mycelial growth of selected saprophytic fungi"** submitted to the Department of Botany, Amrit Campus, Tribhuvan University by **Ms. Deepa Gharti Magar** has been approved for the partial fulfillment of the requirements for a Master's Degree in Botany.

EXAMINATION COMMITTEE

External Examiner
Prof. Dr. Ram Kailash Prasad Yadav
Central Department of Botany
Tribhuvan University
Kritipur, Kathmandu

Internal Examiner
Mr. Hari Sharan Adhikari
Teaching Assistant
Department of Botany
Amrit Campus, Trbhuvan University
Lainchour, Kathmandu

Supervisor and Coordinator
Dr. Laxmi Joshi Shrestha
Assistant Professor
Department of Botany
Amrit Campus, Tribhuvan University
Lainchour, Kathmandu

Head of Department
Dr. Jaya Prakash Hamal
Assistant Professor
Department of Botany
Amrit Campus, Tribhuvan University
Lainchour, Kathmandu

Date of Oral Examination: June 30, 2024

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to my research supervisor **Asst. Prof. Dr. Laxmi Joshi Shrestha**, Coordinator, Department of Botany, Amrit Campus, Tribhuvan University, Nepal for her kind supervision, continuous encouragement and all kind of supports. I am highly indebted to **Dr. Sujan Balami**, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, China for his invaluable support, guidance, encouragement and cooperation during my work.

I am indebted to **Dr. Jaya Prasad Hamal**, Head of Department and **Dr. Shila Singh** (Former Head of Department), Department of Botany, Amrit Campus, Tribhuvan University, Kathmandu, Nepal for providing administrative and laboratory assistance.

I express sincere gratitude to **Prof. Dr. Mukesh Kumar Chhetri** and **Asst. Prof. Gyanu Thapa**, Coordinator of B.Sc. Program, Department of Botany, Amrit Science Campus, Kathmandu for continuous encouragement and all kind of support. I also would like to express my sincere thanks to **Assoc. Prof. L.B. Thapa**, Central Department of Botany, Tribhuvan University, Kathmandu, Nepal and **Ms. Bimala Gharti Magar**, Kirtipur for encouragement and support.

Nepal Academy of Science and Technology (NAST), Khumaltar, Lalitpur is acknowledgement for providing research grant (Grant No. 35/2080/81).

I would like to thank my special friends **Ms. Elina Rai** and **Simran Rai** during field and laboratory works. Last, but not least, I would like to express my sincere gratitude to parents, husband, daughter Luniva and family members for their constant suggestions, co-operations and best wishes.

Deepa Gharti Magar

ABSTRACT

Invasive alien plant species (IAPS) are the major causes of biodiversity and economic loss because their invasions have great negative impacts on native species habitats, ecosystem functioning and services, soil physicochemical and biological properties, and native diversity. This study aims to evaluate the effect of the *Ageratina adenophora* invasion on the litter decomposition, fine root production, and mycelial growth of selected saprophytic fungi. The study was carried out in the Champadevi community forest of Chandragiri Municipality, Kathmandu. The leaf litter and fine roots of native tree *Alnus nepalensis* were placed into soils invaded and uninvaded by *A. adenophora*. There were a total of 9 replicated sub-sites for each invaded and uninvaded site for the observation of the litter and root decomposition. This study revealed that the rate of decomposition of leaf litter of *A. nepalensis* was high in *A. adenophora* invaded sites; however, fine root decomposition does not significantly differ between invaded and uninvaded sites. Similar findings were found in the decompositions of green tea as in *A. nepalensis* leaf litter decompositions. However, like fine root decomposition, red tea decomposition was not different between invaded and uninvaded sites. While comparing the pH, uninvaded sites were comparatively more acidic than invaded sites; on the other hand, fine root production was higher in *A. adenophora* invaded sites than uninvaded sites. The nitrogen content of leaf litter and fine root of *A. nepalensis* was reduced by *A. adenophora* in the invaded sites, but the carbon content was not altered. However, *A. adenophora* root extract was found to be toxic for growth of fungi such as *Fusarium, Aspergillus,* and especially *Trichoderma*. The mycelial growth inhibition increased with increasing concentrations of *A. adenophora* root extract. Hence, *A. adenophora* was found to be responsible for accelerating *A. nepalensis* leaf litter decomposition, increasing fine root production, decreasing nitrogen content, and reducing the mycelial growth of saprophytic fungi.

Keywords: Fine roots, invasion, litter decomposition, saprophytic fungi

शोध-सार

Ageratina adenophora (कालो बनमारा) एक मिचाहा प्रजातीको वनस्पति हो । यो वनस्पति नेपालको रैथाने वनस्पति *Alnus nepalensis* (उत्तिस) को जंगल तथा उत्तिसका बेर्नाहरु उम्रिरहेको विभिन्न स्थानहरुमा फैलिरहेको छ । तसर्थ यो मिचाहा वनस्पतिले उत्तिसका झरेका पात तथा जराहरु कुह्याउने र नयाँ जराहरु बन्ने प्रकृयामा के भुमिका खेलिरहेको छ भन्ने तथ्य उजागर भएको छैन । तसर्थ यो तथ्य उजागर गर्नका लागि यस अध्ययनले कालो बनमारा भएको र नभएको स्थानहरुमा उत्तिसका सुकेका पात तथा जराहरुको कुहिने तथा नयाँ जराहरु बन्ने दर मापन गरी विश्लेषण गरेको छ । साथै पात तथा जराहरु कुहिनमा सहयोग गर्ने ढुसीहरुको बृद्धिमा कालो बनमाराले निकाल्ने रस (extract) को अशरको पनि मापन गरिएको छ । यसरि मापन गरिएका तथ्याङ्कहरुको विश्लेषण गर्दा कालो बनमारा भएको स्थानहरुमा नभएको स्थानहरु भन्दा उत्तिसका जराहरुको तुलनामा सुकेका पातहरु छिटो कुहिएका छन् भने उत्तिसका नयाँ जराहरु अत्याधिक बृद्धि भएका छन् । यसरि कुहिदै गर्दा नाइट्रोजनको मात्रा घटेको तथा अम्लता बढेको देखिन्छ । बनमाराको जराबाट पानिमा मिसिएर निस्किएको रसहरुले *Fusarium, Aspergillus* र *Trichoderma* जस्ता ढुसी प्रजातीहरुको बृद्धि घटेको देखिएको छ ।

शब्दकुञ्जि: उत्तिस, मिचाहा वनस्पति, बनमारा, ढुसी, खनिज तत्व

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AGNPP: Above ground net primary production

ANOVA: Analysis of Variance

FRB: Fine root biomass

FRDW: Fine root dry weight

FRDM: Fine root decomposition month

FRDRM: Fine root decomposition rate month

GTDM: Green tea decomposition month

GTDRM: Green tea decomposition rate month

IAPS: Invasive alien plant species

LN: Leaf nitrogen

LC: Leaf carbon

LDM: Leaf decomposition month

LDRM: Leaf decomposition rate month

PDA: Potato Dextrose Agar

PHM: pH month

RTDM: Red tea decomposition month,

RTDRM: Red tea decomposition rate month

RN: Root nitrogen

RC: Root carbon

SOC: Soil organic carbon

CHAPTER 1: INTRODUCTION

1.1 Background

Invasive alien plant species (IAPS) are the major causes for biodiversity and economic loss because their invasions have negative impacts on native species habitats, ecosystem nctioning and services, soil physico-chemical and biological properties, and native diversity (Wang *et al.,* 2018; Linders *et al.,* 2020; Shabani *et al.,* 2020). Habitats of native species invaded by IAPS have been turned into land of the monocultures of IAPS and the changes brought by IAPS are irreversible (Vander-Zanden and Olden, 2008).

In Nepal, there are more than 2 dozen of troublesome IAPS in Nepal (Tiwari *et al.,* 2005; Shrestha, 2016; Shrestha *et al.,* 2021) and the number and distribution of such species have been expanding throughout the country (Shrestha *et al.,* 2021). Most commonly, the IAPS are spreading from tropical to subtropical areas of the nation. Among the problematic IAPS, *Ageratina adenophora* (Kalo-Banmara in Nepali) is spreading throughout the country from tropical to lower temperate regions of Nepal (Poudel *et al.,* 2020). It is well known that this species is changing the composition, diversity, and richness of native species as well as the quality of the soil (Sapkota, 2007; Thapa *et al.,* 2016; Fu *et al.,* 2018).

In additions, the invasions of *A. adenophora* have negative impacts on soil nutrient dynamics (Kumar and Garkoti, 2021). The plants in an ecosystem contributes their litter and the decomposition of the litter contributes soil nutrients (Palm, 1995). The breakdown of litter returns organic materials and nutritional components to the soils (Chen and Twilley, 1999). It plays a crucial role in regulating the dynamics of the ecosystem, ecological production, nutrient cycling, and soil fertility. When *A. adenophora* invades a native ecosystem, it alters the native ecosystems nutritional dynamics and functioning (Kumar and Garkoti, 2021). Mainly, the leachates from fresh leaves, roots as well as from the litters of *A. adenophora* are phytotoxic to the growth and development of native species (Darji *et al.,* 2021).

Invasion of *A. adenophora* is highly severer in the montane forests in Nepal (Thapa *et al.*, 2016). The montane forest includes *Alnus nepalensis* as one of the dominant native tree species (Joshi and Garkoti, 2021). *A. nepalensis* is known for its symbiotic nitrogen fixation and it has capability to restore soil fertility in the degraded lands (Mishra *et al.*, 2018). It is one of the important forest type around Kathmandu valley, Nepal (Thapa *et al.*, 2016) but severe invasion of *A. adenophora* have been creating a problem in its regeneration and soil quality (Balami *et al.*, 2017*).*

Ageratina adenophora may also alter the production of fine roots in the forest. Fine roots with a diameter of less than 2 mm regulate the biogeochemical cycles of ecosystems. Fine roots vary with climate due to rising mean annual temperature and precipitation, fine root biomass, production, and turnover rate. With the availability of soil N and P, fine root biomass in the boreal forest drastically decreased (Yuan and Chen, 2010).

Fine roots help in absorption of water and nutrients (Yuan and Chen, 2010). Fine roots have also been known as the most significant factor contributing to below-ground C fluxes in forest ecosystems and as being short-lived, producing up to 75% of the yearly net primary output (Gill and Jackson, 2000). Fine roots are important to the cycling and accumulation of carbon and nutrients in forest ecosystems (Ostonen, 2003).

Antifungal activity of *A. adenophora* on selected fungi may be beneficial or pathogenic to soil fungi. The effect of extract root of *A. adenophora* to soil fungi species and then it takes benefit from its antifungal activities against either pathogenic or be benificial in native soil. Previous study showed that *A. adenophora* is associated with high nutrient accumulations in invaded soil (Sun *et al.*, 2021). Various secondary metabolites such as quinic acid derivatives and sesquiterpenes that are found in *A. adenophora* (Zhang *et al.*, 2013) possesses antibiosis properties that may influence fungal or bacterial growth and development (Zhang *et al.*, 2013).

However, enough literature is deficient about the impact dynamics of *A. adenophora* on *Alnus nepalensis* forest ecosystems. Many studies have focused the impacts of *A. adenophora* on native species and some of the studies have focused on the impacts on the belowground communities as well (Thapa *et al.*, 2020; Balami *et al.*, 2017). Hence,

it is crucial to know the impacts of *A. adenophora* on soil nutrient dynamics in the *A. nepalensis* forest.

1.2 Justifications

Ageratina adenophora is the highly problematic invasive plants in lower montane forests of Nepal. The invasion of *A. adenophora* is known to change soil fungal community and soil nutrients. Leaf and fine root litters are major source of carbon in soil, the decomposition of which is mediated by soil nutrient status and saprophytic fungi. Due to changes in soil nutrient dynamics and saprophytic fungal growth, it is expected that the *A. adenophora* invasion will change the rate of decomposition of leaf and fine root. However, the information is largely scarce.

Litter quality may also affect the decomposition process. One of the important way to know the impacts of *A. adenophora* invasion on soil nutrition dynamics understanding of how the invasion affects the native plants litter or roots decomposition processes. Fine roots regulate the biogeochemical cycles of ecosystems. It is important to understand how ecosystems react to invasion. It is expected that root extract of *A. adenophora* may alter the activity of saprophytic fungi. Therefore, this study aims to evaluate the decomposition rate and nutrient loss of native *A. nepalensis* litter and fine roots between *A. adenophora* invaded and uninvaded areas. Additionally, fine root production in *A. adenophora* invaded and uninvaded areas and antifungal activity of root extract of *A. adenophora* on selected saprophytic fungi.

1.3 Research Questions

- What is the effect of *Ageratina adenophora* on *Alnus nepalensis* leaf litter and fine root decomposition?
- What is the effect of *A. adenophora* on nutrients of *A. nepalensis* leaf litter and fine root decomposition?
- Is there any variations in the process of *A. nepalensis* leaf litter and fine root decomposition between *A. adenophora* invaded and uninvaded sites?
- What is the effect of *A. adenophora* on fine root productions?
- What is the effect of *A. adenophora* on soil fungi that are involved in litter decomposition?

1.4 Research Objectives

Main objective of the study is to evaluate the effect of *A. adenophora* invasion on the litter decomposition, fine root production and mycelial growth of selected saprophytic fungi. The specific objectives are:

- To determine the rate of fine-root and leaf litter decomposition of native tree *Alnus nepalensis* in *A. adenophora* invaded and uninvaded sites.
- To determine the nutrient loss from fine root and leaf litters of *A. nepalensis* in invaded and uninvaded sites.
- To determine the effect of litter quality in decomposition rate in *A. adenophora* invaded and uninvaded sites using standard tea bag method.
- To evaluate the variation in fine root production in *A. adenophora* invaded and uninvaded sites.
- To determine the antifungal activity of root extract of *A. adenophora* on selected saprophytic fungi.

1.5 Limitations of the study

- There are large numbers of fungal species involved in decomposition of leaves and involved in fine root production, this study selected only three important saprotrophs.
- Due to the weight loss of litter in different months, other physiochemical properties of the litter were not performed.
- There might be a role of bacteria and actinomycetes in litter decomposition, but isolation of them was not performed due to their specialized nutritional requirements, phenotypic similarity, dormant or viable but non-culturable (VBNC) state under unfavorable conditions, etc.

CHAPTER 2: LITERATURE REVIEW

2.1. Effect of *Ageratina adenophora* on litter decompositions

In an ecosystems plant provides litter, and the litter breakdown releases nutrients into the soil (Palm, 1995). Organic materials and nutrient content are returned to the soils through the decomposition of litter (Chen and Twilley, 1999). Plant litters are important for controlling soil fertility, nutrient cycling and ecological health. The nutrient dynamics and functioning of a native environment are changed when A. *adenophora* invades it (Kumar *et al.*, 2021). The growth and development of native plant species primarily inhibited by the leachates from *A. adenophora* fresh leaves, roots, and litters (Darji *et al.*, 2021).

In contrast to the uninvaded soil, *A. adenophora* invaded soil exhibited a lower pH and higher levels of organic matter, total nitrogen, phosphorus, and potassium (Darji *et al.*, 2021). Rothstein *et al.* (2004) have reported that invasive plant species leads to the fast decomposition of liters than native species within the ecosystem. The most successful invasive plant species are quick colonists and grow fast (Van kleunen *et al.*, 2010).

The quicker decomposition of invasive plants causes an increased rate of carbon release and nutrient cycling in the invaded ecosystems. The invasive plant has a higher rate of nitrogen intake (Helsen *et al.*, 2018). Liao *et al.* (2008) reported that Invasive alien plant species might be responsible for the enhanced litter decomposition in invaded ecosystems.

Invasive alien plant species (IAPS) are the primary drivers of biodiversity loss ecosystem functioning, soil physico-chemical, biological properties, and native diversity (Wang *et al.*, 2018; Linders *et al.*, 2020). The invasion of *A. adenophora* reduces the cover of trees and a lower richness of native species. In *Schima–Alnus* forests, invasion *A. adenophora* on soil and the airborne effects of leaf litter have the potential to affect to native tree regrowth (Thapa *et al.*, 2020).

Helsen *et al.* (2018) reported that low litter quality and possible allelopathic impact of invaded plant species causes reducing litter decomposition rates. Similarly, Zhang *et*

al. (2016) found that exotic plant invasion could change the carbon cycles in ecosystems and higher labile carbon decomposition abundance were found in *A. adenophora* invaded soil suggesting a possible increase in carbon availability. Similarly, increasing in litter decomposition in invaded ecosystems may be a result of a few very significant invasive species (Liao *et al.*, 2008).

2.2. Effect on fine root productions

Yuan and Chen (2012) found that fine root production determines the biogeochemical cycles in terrestrial ecosystems and makes up the majority of belowground production. McCormack *et al.* (2015) reported the fine roots absorb essential soil nutrients and mediate biogeochemical cycling in terrestrial ecosystems. Li *et al.* (2022) claim that fine root decomposition has a major impact on the biogeochemical cycle in forests.

The formation and breakdown of fine roots, which supply plants with nutrients, are among the ecological processes mentioned by Violita *et al.* (2016). Carbon content, C/N ratio, and nitrogen were the main factors affecting the breakdown of fine roots. The management of the biogeochemical cycles in forest ecosystems is dependent on fine roots (Cai *et al*., 2019).

The primary sources behind the carbon and nutrient cycles in temperate forests are litter fall and fine root dynamics. Litter fall, productivity, and fine root biomass were investigated greater amount of fine roots produced (An *et al.*, 2017). Similarly, Lukac (2012) suggested that fine roots are crucial to the carbon, nitrogen, and water cycles in forests with continuous growth and dieback.

Finér *et al*. (2011) stated FRB (Fine root biomass) variation affected by various environmental factors such as latitude, mean annual precipitation, elevation, and temperature or forest stand parameters such as life form, age, basal area, and density. Klopatek (2007) reported the changes in soil carbon and nitrogen inputs from fine root development and litter fall. Yuan and Chen (2010) stated that fine root biomass production, and turnover are significantly influenced by environmental conditions.

2.3. Antifungal activity of *Ageratina andenophora*

Ageratina adenophora root extract contains different compounds leads to showing the strong inhibitory effect against the growth of every fungal strain (Zheng *et al*., 2018). Poudel *et al.* (2020) studied that phytochemicals present in *A. adenophora* were steroids, tannins, triterpenes, coumarins, and saponins could alter the microbial communities in the soil.

Similarly, Das and Devkota (2018) reported the antifungal activity and phytochemical profiles of *A. adenophora*. Balami *et al*. (2019) reported that inhibitory effects of *A. adenophora* alter the variety and composition of soil fungus that leads to changes in native plant species, ecosystem functioning, and soil qualities. Furthermore, a study conducted by Wan-Xue *et al*. (2010) found the inhibitory effect of *A. adenophora* on the soil microbial population.

Similarly, Balami *et al*. (2017) reported the *A. adenophora* invasion affects the variability, species richness, and species or community composition of soil fungus. They found that in the *A. adenophora*-invaded soil, the species richness of soil fungus was lower than in the uninvaded soil. *Adenophora* is therefore linked to a decreased species richness of saprophytic soil fungi and high occurrence frequency of pathogenic soil fungi. Thapa *et al.* (2020) found the effects of *A. adenophora* in invaded soil below ground and the airborne effects of leaf litter might prevent the regrowth of native trees in *Schima-Alnus* forests.

Hence, invasive alien plant species are the primary major drivers of biodiversity loss, physiochemical and microbial communities (Wang *et al.,* 2018). *A. adenophora* invasion could lead to the breakdown of litter, releasing nutrients into the soil. Invasive alien plants cause faster decomposition of litter than native species in the ecosystem (Van Kleunen *et al.,* 2010). The formation of fine roots supplies nutrients to the plants in the forest (Violita *et al.,* 2016). The invasion of *A. adenophora* could alter fine production in the forest as well. The root extract of *A. adenophora* contains different biochemical that can change the microbial community in the soil and compete with other native plants (Balami *et al.,* 2019).

CHAPTER 3: MATERIALS AND METHODS

3. 1 Study area

The study was conducted in Champadevi Community Forest, Chandragiri Municipality, Kathmandu, Nepal from June 2022 to May 2023. The forest was lies in 27°65′55.12″N and 85°24′68.54″E. The elevation of the study site ranges from 1300 to 1700 masl. The average annual temperature of the study area was 16 °C and annual precipitation 2800 mm. The forest was dominated by the *Alnus nepalensis*, *Schima wallichii*, *Symplocos* sp. and highly invaded by *A. adenophora*.

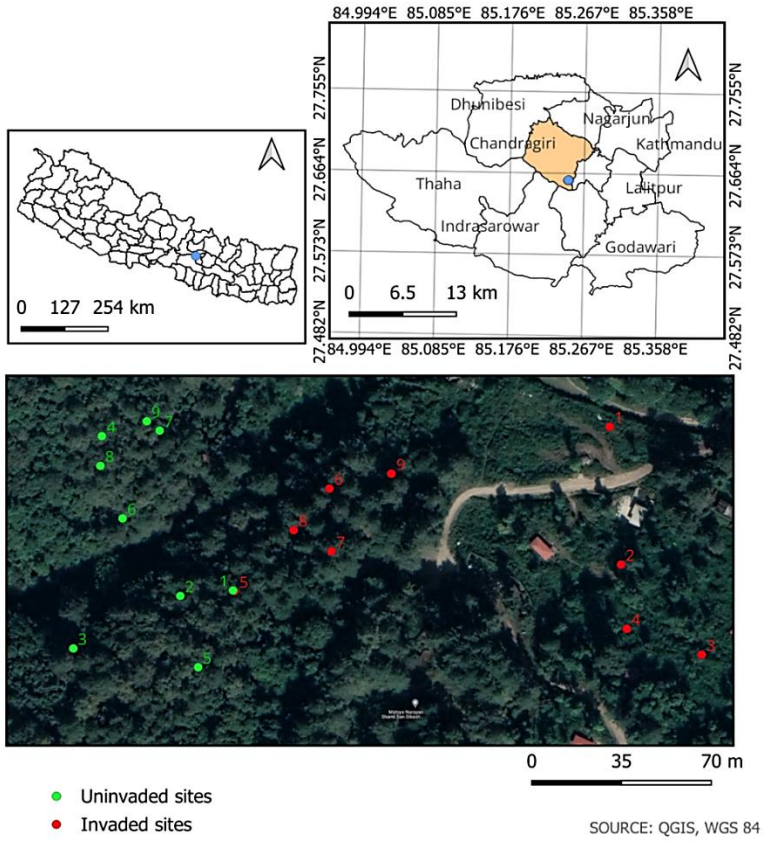


Fig.1: Map of the study area

3.2 Experimental Design

3.2.1 Leaf litter and fine root collection

The fallen fresh leaf litter of *Alnus nepalensis* was collected from the *Alnus* forest (Champadevi Community Forest) and air-dried at room temperature for a few days until the weight of the dried litter remained constant. Similarly, fine roots of *A. nepalensis* (≤ 1 mm in diameter) were also collected by digging out to a depth of 20 cm. The collected fine root samples were washed in tap water to remove soil particles and air-dried for a few days until the weight remained constant.

3.2.2 Preparation of litter and fine root bags

Leaf litter bag: Dried leaf litter of *Alnus nepalensis* (5 g) was packed in a nylon bag of size 13×18 cm^2 (mesh size 2 mm).

Fine root bag: Similarly, dried fine root samples of *Alnus nepalensis* (1 g) were packed in polypropylene bags (5.5 × 5 × 5.5 cm^3, Tetrahedral). The red tea bags and green tea bags were used as reference.

3.2.3 Decomposition experiment

The packed leaf litter bags and fine root bags were placed in the soil surface of uninvaded (*Alnus* forest) and *Ageratina adenophora* invaded sites. The number of litter bags were also similar as the fine root bags. A total of nine leaf litter bags, eighteen tea bags (9 green tea bags + 9 red tea bags) and nine fine root bags were placed in *A. adenophora* invaded and uninvaded site. Altogether, there were nine replicates in each sites. The site was selected randomly in the similar landscape. To evaluate the effects of litter quality, standard tea bag method was used (Didion *et al.*, 2016).

A plot of 1.5x1.5 m^2 was designed and a total of 18 plots (9 plots in invaded and 9 plots in uninvaded) were made. The leaf litter bags and fine root bags were buried in soil at depth around 5 cm. In the interval of 2, 3 and 4 months, the samples were serially digger out and further weight loss rate, changes in nutrients, soil properties analysis were done. All the methods were followed according to Bocock *et al.* (1960); Witkamp and Olson (1963).

3.2.4 Decomposition rate

The leaf litter and fine root samples contaminated with soil particles, which were removed by gently rinsing with tap water and brush. Cleaned litter samples were oven-dried for 3 days at 80°C. Then, the final weight was taken. Weight loss and decomposition rate was analyzed by measuring the litter weight loss. The weight loss and weight loss rate was determined by using following equations (Darmawan *et al.,* 2021).

$$\text{Weight loss (WL)} = \frac{Original\ weight - Final\ weight}{Original\ weight} * 100$$

$$\text{Weight loss rate (WLR)} = \frac{WL}{Days\ in\ field}$$

3.2.5 Determination of physicochemical properties of litter

Soil pH

The soil pH was measured using a digital pH meter. Before pH measurement, the pH meter was calibrated with two buffer tablets of pH 4 and pH 9. Calibration was performed at the beginning of each measurement. Each buffer tablet was dissolved in 100 ml of distilled water to make the suitable buffer solutions. The sample soil solution was prepared by making the solution in a 1:2 ratio with distilled water. 10 g of soil from each plot (invaded and uninvaded) was mixed with 20 ml of distilled water for measuring the pH. For every single sample, replication was done three times (Zobel *et al.,* 1987*).*

Litter carbon

Carbon content was calculated by following method Walkley and Black (De Vos *et al.,* 2007, Zobel *et al.*, 1987). Similarly, nitrogen content was determined by following method Kjeldahl (1883). For the determination of carbon and nitrogen, among the nine plots, the three nearest plots were mixed into one of each month. So that the final three leaf litters and three fine root samples were prepared from both invaded and uninvaded sites from each month. Carbon and nitrogen content were tested at Soil Water and Air Testing Laboratories Pvt. Ltd., Babarmahal, Kathmandu, Nepal.

For the determination of organic carbon, 0.5-gram soil samples were taken in a 500-ml flask. Then, 10 ml of potassium dichromate ($1NK_2Cr_2O_7$) and 20 ml of sulfuric acid (H_2SO_4) were added, shaken well, and allowed to stand on a sheet of asbestos for about 39 minutes in a ventilated area. 200 ml of distilled water was then added, along with 10 ml of 85% phosphoric acid and 1 ml of diphenylamine as an indicator solution. Run 1N ferrous sulfate slowly from a burette with constant stirring until the solution is purple or blue. Continuously added ferrous-sulfate solution until the color becomes greenish. 0.5 ml of 1N potassium dichromate was added, and the titration was completed by adding more ferrous sulfate until the last trace of blue disappeared. The organic matter content was calculated using the following formula:

Organic matter content % = $\frac{V1-V2}{W} \times 0.003 \times 100$

V_1= Volume of $K_2CR_2O_7$

V_2= Volume of $FeSO_4$

W= Weight of soil taken

Litter Nitrogen

Nitrogen content was determined by following main three steps.

1. Digestion: 1 gm of messed litter samples were heated in the presence of sulfuric acid. The acid breaks down the organic substance via oxidation, releasing reduced nitrogen in the form of ammonium sulfate. Potassium sulfate was added to increase the boiling point of the medium. Catalysts like mercury and copper are also used in the digestion process. The sample was fully decomposed when we obtained clear and colorless solutions.

$$\text{Organic carbon} + H_2SO_4 \rightarrow \text{(Digest) } Cu^{+2} + (NH_4)_2SO_4$$

2. Distillation: A small amount of sodium hydroxide was added to the solution during distillation in order to convert the ammonium salt into ammonia. Following distillation, the vapors are captured in a unique solution made of water and hydrochloric acid (HCl).

$$(NH_4)_2SO_4 + 2NaOH \rightarrow Na_2SO_2 + 2H_2O + NH_3$$

3. Titration: The amount of nitrogen or ammonia in the sample was ascertained by back titration, as some HCl neutralized as the ammonia dissolved in the acid-

trapping solution. A standard base solution, such as NaOH, was used to back titrate the acid residue.

$$B(OH)_2+H_2O+Na_2CO_3 \rightarrow NaHCO_3+CO_2+H_20$$

$$NH_3+HCl \rightarrow NH_4Cl$$

The percentage of nitrogen can be determined using the given formula,

$$\text{Nitrogen \%} = \frac{1.4 \times N \times V}{W}$$

Where,

V = Acid used in titration (ml)

N = Normality of standard acid

W = Weight of sample (g)

3.3 Fine root productions

Fine root production was determined by the soil core method. The size of the soil core was 10 cm x 10 cm x 10 cm. Soil core was done in triplicate in each plot, both in invaded and uninvaded areas. After collecting the soil core, fine roots were manually sorted and then oven dried at 80 °C for 3 days until a constant weight was obtained. The method was followed by Persson (1980).

Fine root production = Biomass of fine roots

3.4 Antifungal activity of root extract of *Ageratina adenophora*

3.4.1 Test fungi

Three different saprophytic fungi, namely *Aspergillus* sp., *Trichoderma* sp., and *Fusarium* sp., that were frequently encountered in the soil were selected to test the antifungal activity as they are more likely to be relevant in the study area. These fungal species were chosen based on their role as decomposers in forests. The proposed species was isolated from the forest soil by the serial dilution method (Aneja, 2003).

3.4.2 Extract preparation

Ageratina adenophora roots were collected from the Champadevi community forest and then air-dried. A fine powder of air-dried *A. adenophora* root was prepared. Twenty grams of root powder were soaked in sterile distilled water for 72 hours and then filtered through five layers of muslin cloths. The filtrate was then centrifuged to remove debris

and again filtered through Whatman No. 1 filter paper. The stock solutions were stored at 4 °C until use. A total of four concentrations of root extract were made: the stock solution (100%) and diluted extracts at concentrations of 10%, 30%, 50%, and 100% (Balami *et al.,* 2019).

3.4.3 Fungal treatment

Antifungal activity of *A. adenophora* root extract was done following the poison food techniques (Grover and Moore, 1962). The root extracts of *A. adenophora* was amended on the Potato Dextrose Agar (PDA) plates such that each PDA plate contained root extract and PDA in ratio 1:4. Test fungi were cultured on Petri-plates containing *A. adenophora* extracts (control (0%), 10%, 30%, 50% and 100%). During the treatment 5 mm^2 sized actively growing mycelial block of each fungus was aseptically inoculated on the PDA. Each treatment was done in triplicates. The plates were incubated at 25°C. After a week, the maximum and minimum diameter of colony of each test fungus was recorded. Mean value of the diameter of mycelial growth for each treatment was calculated. The inhibition percentage was calculated by using the following formula (Grover & Moore, 1962).

$$\text{Inhibition \%} = \frac{\text{Radial growth in control} - \text{radial growth in treatment}}{\text{Radial growth in control}} \times 100\%$$

3.5 Statistical Analysis

Decomposition rate, nutrient parameters and fine root production between *A. adenophora* invaded and uninvaded sites were compared using independent sample T-test. Effects of *A. adenophora* root extract on radial growth of fungal species were compared using Two-way Analysis of Variance (ANOVA). The analyses were carried out using R Statistical Programming (R Core Team, 2022).

CHAPTER 4: RESULTS

4.1. Weight loss of *A. nepalensis* leaf litter

The weight loss of *A. nepalensis* leaf litter was not significantly different until 60 days (Month-2) between invaded and uninvaded sites. The weight loss was found to be different in 3rd and 4th months, respectively i.e. the weight loss of the leaf litter was significantly low in the uninvaded sites compared to the invaded sites (Fig. 2A). In the 3rd month (Month-3), the weight loss was 70.20% in invaded site where as the weight loss was 39.86% in the uninvaded site. Similarly, the weight loss in Month-4 was 83.96% and 56.86% in invaded and uninvaded sites, respectively (Fig. 2A).

Calculating the rate of weight loss per day, the result was found to be similar to the weight loss percent as shown in Fig. 2A. There was no significant difference in the rate for Month-2 while the rate was significantly low in the uninvaded sites than invaded sites in the Month-3 and Month-4 (Fig. 2B). The rate of weight loss was 0.5-0.6% per day until the Month-2. After that, the rate remained almost constant to 0.4% per day in the uninvaded sites whereas in the invaded sites the rate increased upto 0.7%and 0.8% per day (Fig. 2B).

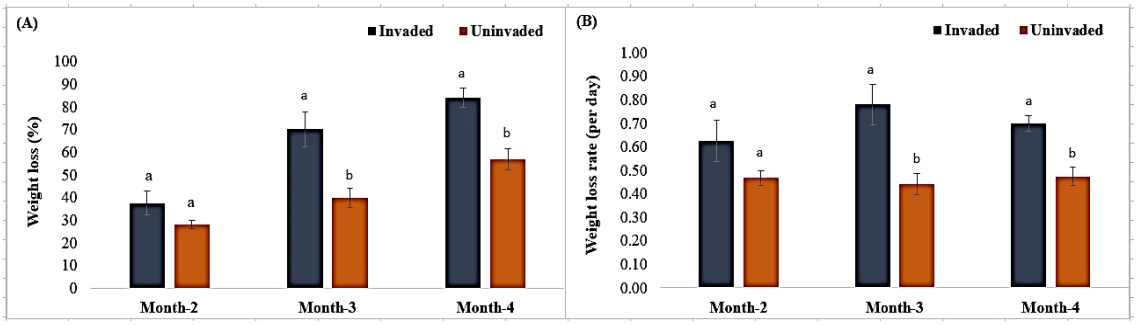


Fig. 2: Weight loss percent (A) and weight loss rate (B) of leaf litter of *Alnus nepalensis* in *A. adenophora* invaded and uninvaded sites. Different letters 'a-b' above error bar indicate significant differences between invaded and uninvaded sites at each month ($p < 0.05$).

4. 2. Weight loss of *A. nepalensis* fine root

Fine root weight loss of *A. nepalensis* was not significantly different until 60 days (Month-2), 90 days (Month-3) and 120 days (Month-4) in between invaded and uninvaded sites (Fig. 3A). Similarly, rate of weight loss per day, the result was found to be similar to the weight loss percent as shown in Fig. 3A. There was no significant difference in the rate for Month-2 and Month-4 while there were changes in weight loss rate in Month-3 i.e. 0.26% in invaded and 0.33% in uninvaded site (Fig. 3B) respectively.

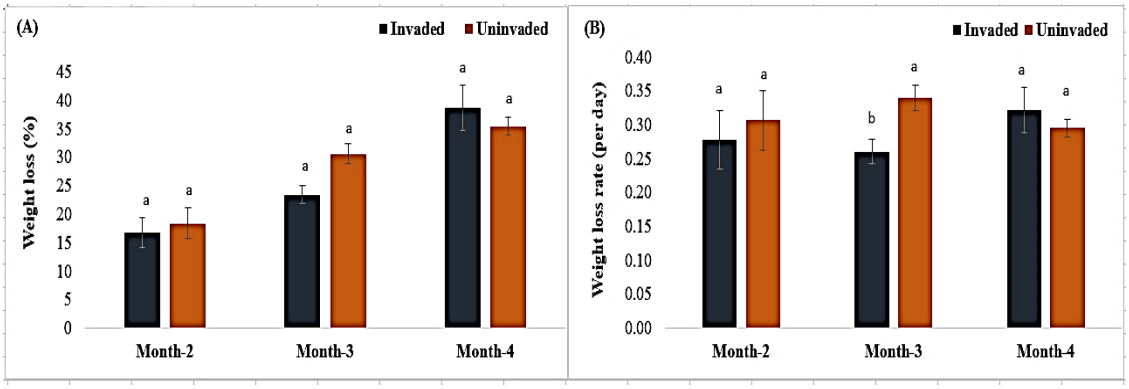


Fig. 3: Weight loss percent (A) and weight loss rate (B) of *Alnus nepalensis* fine root in *A. adenophora* invaded and uninvaded sites. Different letters 'a-b' above error bar indicate significant differences between invaded and uninvaded sites at each month ($p < 0.05$).

4.3 Weight loss of Green tea

The weight loss of green tea was not significantly different until 60 days (Month-2) between invaded and uninvaded sites. However, weight loss was found to be significantly different in 3rd and 4th months, respectively i.e. the weight loss of the green tea was significantly low in the uninvaded sites compared to the invaded sites (Fig. 4A). In the 3rd month (Month-3), the weight loss of green tea was 81.79% in invaded site where as the weight loss was 73.51% in the uninvaded site. Similarly, the weight loss of green tea in Month-4 was 85.99 % and 77.18 % in invaded and uninvaded sites, respectively (Fig. 4A).

Calculating the rate of weight loss per day, the result was found to be similar to the weight loss percent as shown in Fig. 4B. There was no significant difference in the rate

for Month-2 while the rate was significantly low in the uninvaded sites than invaded sites in the Month-3 and Month-4 (Fig. 4B). The rate of weight loss was 1.20-1.40 % per day until the Month-2. After that, the rate remained almost constant to 1% per day in the invaded sites whereas in the uninvaded sites the rate decreased up to 0.8 and 0.9% per day (Fig. 4B) while in Month-4, in invaded site the weight loss rate was found 0.7% and in uninvaded site around 0.7% respectively (Fig. 4B).

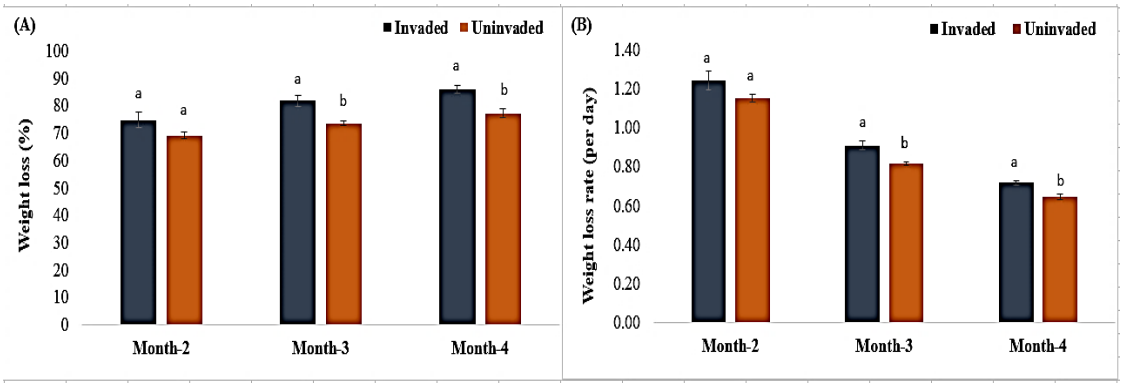


Fig. 4: Weight loss percent (A) and weight loss rate (B) of green tea in *A. adenophora* invaded and uninvaded sites. Different letters 'a-b' above error bar indicate significant differences between invaded and uninvaded sites at each month ($p < 0.05$).

4.4 Weight loss of red tea

In the case of red tea, significantly no difference was found in weight loss of all months (Month-2, Month-3 and Month-4) in invaded and uninvaded sites (Fig. 5A). Red tea weight loss was significantly in increasing order. Weight loss was found slightly higher in invaded site in comparison to uninvaded sites.

Similarly, in the weight loss rate, there was no significant changes were found in all the months of invaded and uninvaded sites (Fig. 5B). The weight loss rate was found significantly higher in Month-2 i.e. in the range of 0.7 to 0.8% while in Month-3 in the range of 0.6% in Month-4 in the range of 0.5% respectively (Fig. 5B).

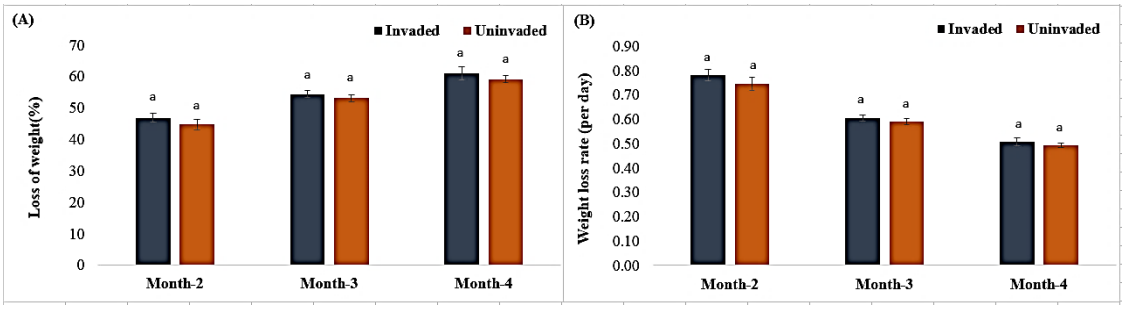


Fig. 5: Weight loss percent (A) and weight loss rate (B)of red tea in *A. adenophora* invaded and uninvaded sites. Different letters 'a-b' above error bar indicate significant differences between invaded and uninvaded sites at each month ($p < 0.05$).

4.5 Soil pH

Soil pH changes were found to be higher in *Ageratina adenophora* invaded sites in comparison to uninvaded sites, as shown in Fig. 6. The mean pH values of invaded and uninvaded soil in month-2 were 5.51 and 4.51, respectively, whereas in month-4, 5.47 and 4.53, respectively.

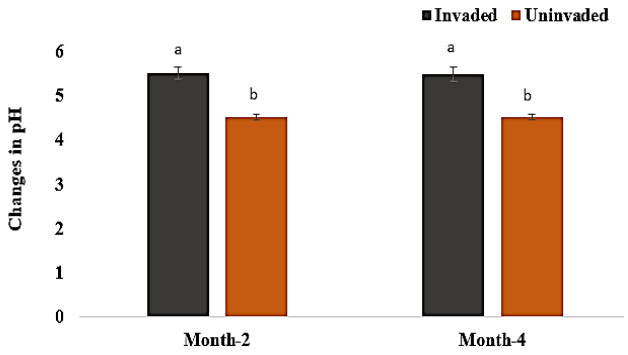


Fig. 6: Changes in soil pH in *A. adenophora* invaded and uninvaded sites. Different letters 'a-b' above error bar indicate significant differences between invaded and uninvaded sites at each month ($p < 0.05$).

4.6 Fine root productions

Fine root production was found higher in *A. adenophora* invaded sites in comparison to uninvaded site. Dry weight of fine roots was found at around 1.4 g in invaded site while 0.8 g in uninvaded sites (Fig. 7).

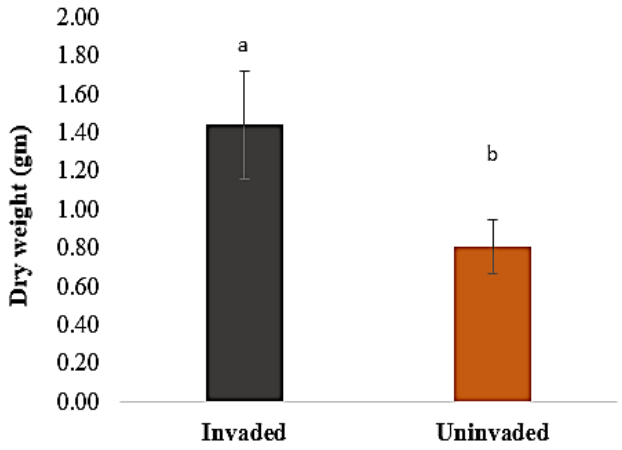


Fig. 7: Dry weight of fine roots of *A. nepalensis* in *A. adenophora* invaded and uninvaded sites. Different letters 'a-b' above error bar indicate significant differences between invaded and uninvaded sites at each month ($p < 0.05$).

4.7. Nutrient content of litters

4.7.1. Carbon and nitrogen content of leaf litter of *Alnus nepalensis*

The nitrogen content of *A. nepalensis* leaf litter was not significantly different until 90 days (Month-2 and Month-3) between *A. adenophora* invaded and uninvaded sites. The nitrogen content was found to be significant different in 4th month, i.e. the nitrogen content of leaf litter was significantly low in the invaded sites compared to the uninvaded sites (Fig. 8A). In the 4th month (Month-4) the nitrogen content of leaf litter was 2.31% in invaded site where as it was 2.95% in the uninvaded sites respectively (Fig. 8A).

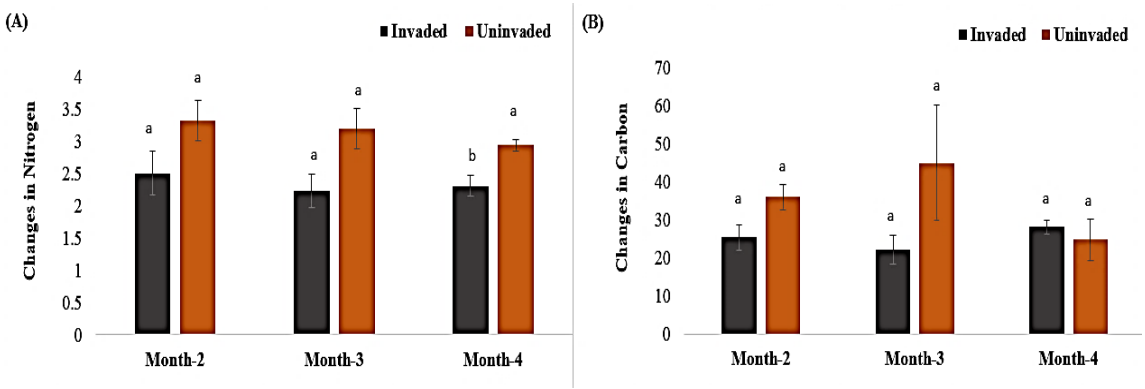


Fig. 8: Nitrogen content (A) and carbon content (B) of leaf litter of *Alnus nepaensis* in *A. adenophora* invaded and uninvaded sites. Different letters 'a-b' above error bar indicate significant differences between invaded and uninvaded sites at each month ($p < 0.05$).

In the case of carbon, significantly no difference was found in carbon content of all months (Month-2, Month-2, and Month-3) as shown in Fig. 8A. The result showed that carbon content was significantly low in the invaded sites than uninvaded sites. Similar results were also obtained in month-3 while carbon content was found high in uninvaded site in comparing to invaded site. In the Month-4 (Fig. 8B), carbon content was found significantly high in invaded site comparison to uninvaded site (Fig. 8B).

4.7.2 Carbon and nitrogen content of fine root of *Alnus nepalensis*

The nitrogen content of *A. nepalensis* fine root was not significantly different until 90 days (Month-2 and Month-3) between *A. adenophora* invaded and uninvaded sites. The nitrogen was found to be different in 4th months, i.e. the nitrogen content of fine root was significantly low in the invaded sites compared to the uninvaded sites (Fig. 9A). In the 4th month (Month-4) the nitrogen content of fine root litter was 1.77% in invaded site where as the 2.02% in the uninvaded sites respectively (Fig. 9A).

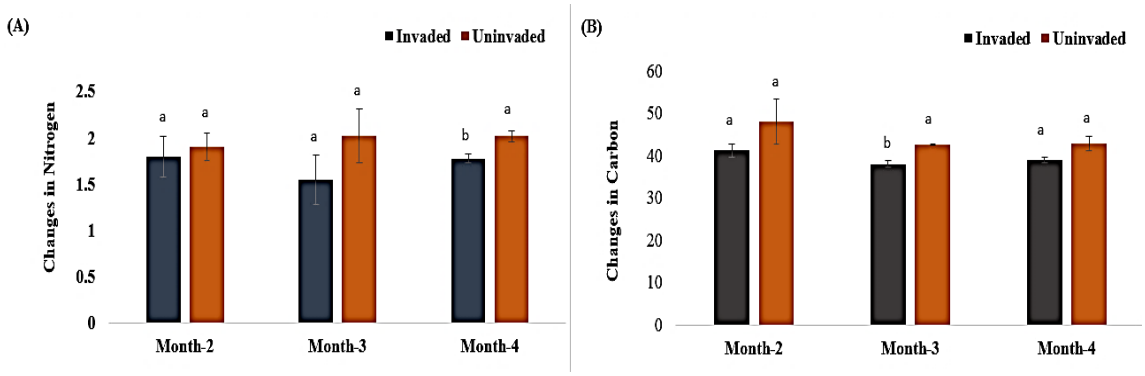


Fig. 9: Nitrogen content (A) and carbon content (B) of fine root litters of *A. nepaensis* in *A. adenophora* invaded and uninvaded sites. Different letters 'a-b' above error bar indicate significant differences between invaded and uninvaded sites at each month ($p < 0.05$)

In case of carbon, significantly no difference was found in carbon content in all months (Month-2, Month-3, and Month-4) as shown in Fig. 9B. Carbon content was significantly low in *Ageratina adenophora* invaded sites than uninvaded sites. The carbon content found was 38.03% in invaded site while 42.58% found in uninvaded site. In the Month-4, there was significantly no change in invaded and uninvaded site (Fig. 9B).

4.8 Antifungal activity of root extract of *Ageratina adenophora*

The radial growth of fungal hyphe was decreased by *Ageratina adenophora* root extract. The radial growth was high in control but increasing root extract concentrations the raidal growth was also dereased (Fig. 10). The radial growth of *Fusarium* in control was 4.87cm and in other concentratons it was less than 3.17cm, 2.92cm, 2.78cm, and 1.66cm at concentrations 10%, 30%, 50% and 100% respectively.

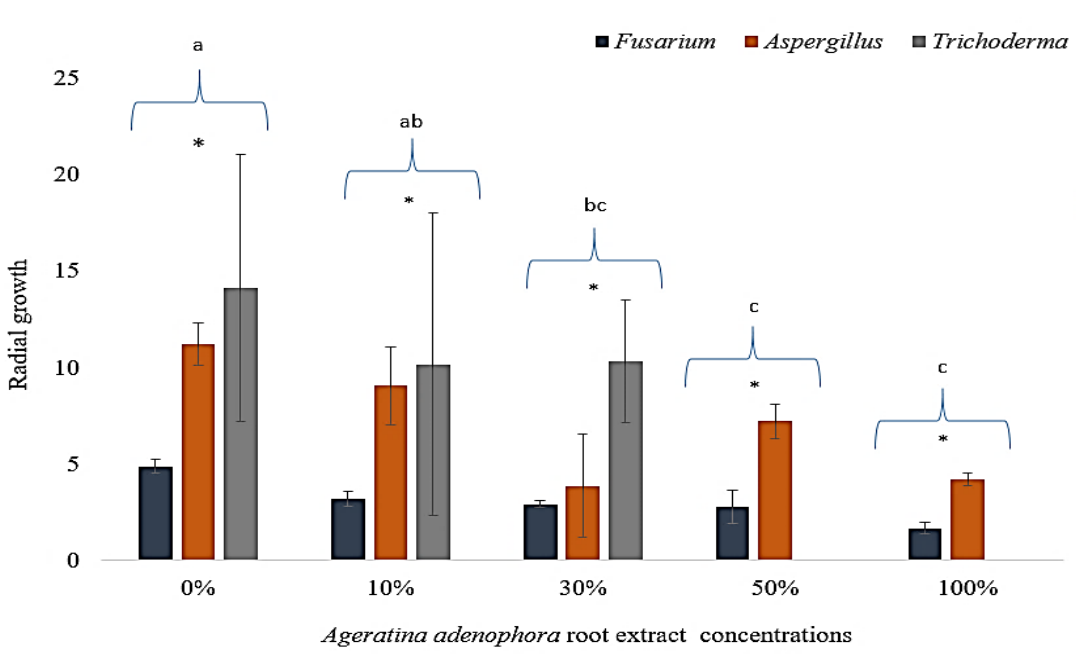


Fig. 10: Effect of *A. adenophora* root extract on radial growth of fungal species. Different letters 'a, b, c' above error bar indicate significant differences among extract concentrations and '*' indicates significant differences among fungal species ($p < 0.05$).

Similarly, in *Aspergillus*. significantly higher effect was seen than *Fusarium* ie 11.21cm, 9.04cm, 3.85cm, 7.22cm, 4.2cm in different concentration 10%, 30%, 50% and 100% respectively. Likewise, in *Trichoderma* sp, the radial growth was high in control and significantly, high in 10% and 30% while at 50% but in 100% radial growth was not seen. In all three fungal species, the radial growth was significantly reduced at concentrations 50% and 100%.

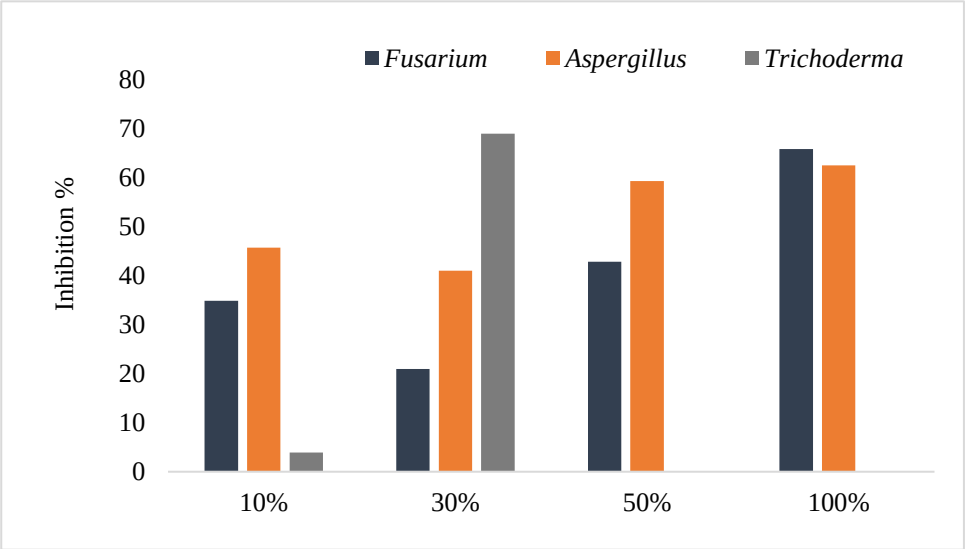


Fig.11: Inhibition of *A. adenophora* root extract on radial growth of fungi

Root extract of *Ageratina adenophora* showed the higher, significantly higher and significant effect on the selected saprophytic fungi. Fungal mycelial growth was inhibited on the different root extract concentrations of *A. adenophora*. Among the three fungal species, high growth inhibition was recorded in *Trichoderm*a (Fig.11) while significantly similar inhibition was recorded in *Fusarium and Aspergillus* (Fig.11).

In *Fusarium*, low growth inhibition percentage was found at 30 % concentration i.e. around 20-25% while highest inhibition percentage was found at 100 % concentration around 70%. At 50% and 10% concentrations, significantly similar inhibition was recorded. Similarly, in *Aspergillus*, low inhibition percentage was found at 30% i.e. at range of 40-43% while high inhibition percentage was found at 50% and 100% i.e. at the range of 60-62% and 65-70% respectively. At 10% around 45-50%, inhibition percentage was recorded. In *Trichoderma*, highest inhibition percentage was found at 50 % and 100% where fungal mycelial growth was not found (Fig.11). Similarly, at 30% concentration around 70% inhibition was recorded while at 10% low inhibition percentage was recorded around 5%.

CHAPTER 5: DISCUSSION

5.1 Weight loss of *Alnus nepalensis* leaf litter

The high weight loss percentage and weight loss rate were found in *Ageratina adenophora* invaded sites in comparison to uninvaded sites (Fig. 2A and B). It might be due to the fact that *A. adenophora* may release allelopathic chemicals into the soil, inhibiting the growth of other plant species and altering the microbial communities responsible for decomposition (Sun *et al.,* 2021). Chemicals found in leaf leaches and root can directly affect the breakdown of organic matter, including plant litter (Jiao and Huang, 2024).

Zhao *et al.* (2019) also gave similar results, such as that *A. adenophora* may have different nutrient uptake patterns compared to native vegetation. This can altered nutrient cycling processes in invaded areas, potentially leading to faster decomposition rates of plant leaves due to increased nutrient availability for decomposers.

5.2 Weight loss of *Alnus nepalensis* fine root

The decomposition of fine root of *A. nepalesis* was not significantly different in weight loss percentage and weight loss rate between *Ageratina adenophora* invaded and uninvaded sites, which might be due to the *A. adenophora* release allelochemicals can interfere with the activity of decomposer organisms such as fungi and bacteria, slowing down the decomposition process (Thapa *et al*., 2020). The pH of the soil was slightly acidic at the *A. adenophora* uninvaded site. This might be one of the reasons for the slow decomposition of root litter. *A. adenophora* invasion could alter soil properties such as moisture content, pH, and nutrient availability, creating conditions less favorable for decomposer organisms.

Fine-root decomposed more slowly due to factors such as lignin content and microbial accessibility. Additionally, the burying of roots in the soil can limit the access of decomposer organisms (Walela *et al.,* 2014). However, in many cases, leaves tend to decompose more rapidly than roots due to their higher nutrient content and easier accessibility to decomposer organisms (Guo *et al.,* 2021).

As Couteaux *et al.* (1995) gave a similar concept on the decomposition of litters is influenced by several key elements, including the climate, the quality of the litter, and the kind and quantity of the decomposing organisms. Li *et al.* (2022), fine root decomposition played a significant biogeochemical cycle in forest. The rate of fine root decomposition was measured by litterbags and undamaged cores. They found that the nitrogen turnover and fine-root degradation happen far more quickly than what litterbag studies.

5.3 Weight loss of Green tea and Red tea

The weight loss of the green tea was found to be considerably lower in the uninvaded areas than in the invaded sites (Fig. 4A and B). It might be due to green tea contains high levels of catechins (Yong Feng, 2006) which are a type of polyphenol with antioxidant properties. These tea catechins are more reactive and susceptible to degradation compared to the polyphenols present in red tea. The minimal processing and higher content of reactive compounds in green tea make it decompose faster than red tea (Gramza, *et al.*, 2005). The high decomposition of green tea was found in *A. adenophora* invaded site in comparison to uninvaded sites it might be due to invasion of *Ageratina adenophora*.

Weight loss percentage and weight loss rate of red tea in between the invaded and uninvaded sites was not significantly different (Fig. 5A and B). The slow decomposition of red tea it might be due to it undergoes a full oxidation process. This oxidation process reduces the reactivity of the tea leaves, making them more stable compared to the less oxidized green tea leaves. Comparatively, decomposition of red tea was significantly high in *A. adenophora* invaded site than uninvaded site.

The result showed similarity with result obtained by Keuskamp *et al.* (2013) such as weight loss was significantly higher in green tea in comparison to red tea. The green tea is considered a high-quality litter due to its high nutritional content and soluble carbon content, while red tea is considered a low-quality litter due to its low nutrient content, particularly nitrogen, and high lignin content higher values of one or both of them indicate a slower rate of decomposition.

5.4 Fine root productions

The fine root production was high in *Ageratina adenophora* invaded soil in comparison to uninvaded soils. This indicates that *A. adenophora* is competing with *Alnus nepalensis* by producing a large number of fine roots. Fine roots are responsible for nutrient uptake. In *A. adenophora* invaded soils, nitrogen was used at a high rate, which led to nutrient shortages for *A. nepalensis* and other native plants. The invasion of *A. adenophora* can lead to reduced fine root production in native species due to competition, allelopathy, and changes in soil properties and microbial communities. At the same time, *Ageratina adenophora* may increase its fine root production to enhance its competitive advantage and establish itself in new environments.

5.5 Nutrient content of *Alnus nepalensis* leaf litter

A significant difference was found in the nitrogen content of the leaf litter between the invaded and uninvaded sites (Fig. 8A). The lower nitrogen content in *Ageratina*-invaded sites is likely a result of complex interactions between *Ageratina* invasion, soil properties, nitrogen cycling, allelopathic effects, pH, organic matter, and nutrient availability (Zhao *et al.,* 2019). As a result, there would be less nitrogen available for uptake by *Alnus nepalensis,* leading to a lower nitrogen content in its tissues (Lu *et al.*, 2017). It may alter the composition of soil microbial communities responsible for nitrogen fixation, nitrification, and denitrification, leading to reduced nitrogen availability for *Alnus nepalensi* (Zhao *et al*., 2019).

Likewise, no significant difference was found in carbon content. Comparatively, carbon content was lower at invaded sites than at uninvaded sites (Fig. 8B). It might be due to the *A. adenophora* invasion, which might alter carbon storage and decomposition rates (Xia *et al.,* 2024). Some plant species may have higher litter quality or root exudates that promote carbon sequestration (Panchal *et al.*, 2022). *A. adenophora* invasion can alter microbial communities in the soil, affecting decomposition rates and carbon cycling processes (Li *et al.*, 2022). *A. adenophora* litter may have different qualities compared to native vegetation, leading to variations in decomposition rates and carbon input into the soil (Zhao *et al.*, 2019).

5.6 Nutrient content of *Alnus nepalensis* fine root

A significant difference was found in the nitrogen content of fine roots between the invaded and uninvaded sites, with invaded sites having a much lower nitrogen content (Fig. 9A). It might be due to the fact that *Ageratina adenophora* might utilize a high rate of nitrogen and compete with *Alnus nepalensis* for nutrients, including nitrogen, leading to reduced nitrogen availability for the native species (Pachhai, 2019). Likewise, *A. adenophora* could alter pH, organic matter, and nitrogen availability through processes like allelopathy or nutrient cycling (Weidenhamer and Callaway, 2010).

The present study revealed a similarity with the result obtained by See *et al.* (2019), as fine root decomposition is different from that predicting leaf decomposition, despite the fact that the chemical drivers of fine root decomposition are similar to those of leaf decomposition. Goebel *et al.* (2011) suggested that root turnover was fastest in the finest roots of the root system. The higher C: N ratio decomposed more rapidly.

5.7 Antifungal activity

The root extract of *Ageratina adenophora* found to be toxic for the radial growth of fungi (Fig.11). On increasing the concentration of root extract of *A. adenophora* the toxicity also increased. It might be due to *Adenophora* root extracts are rich in various bioactive compounds such as saponins, flavonoids, and phenolic acids. These compounds can have antifungal properties, inhibiting their growth at a cellular level (Poudel *et al.,* 2020).

Likewise, combination of multiple bioactive compounds in the root extract can have a synergistic effect, where the combined action of these compounds is greater than the sum of their individual effects (Vaou *et al.,* 2022). The present study shows similarity with results obtained by Balami *et al.* (2019) as found as all types of extracts showed inhibitory effect on fungal growth. The degree of inhibition varied with fungal species, extract type and concentration. The inhibitory activities of *A. adenophora* to the soil fungi could bring changes in soil fungal diversity and their composition.

Ageratina adenophora root extract contains various chemicals compounds that might be responsible for changing soil microbial community. Balami *et al.* (2017) suggested

that *A. adenophora* changed the species composition of soil fungi in invaded soil. This causes the replacement of saprophytic fungi and the accumulation of pathogenic fungi. Consequently, *A. adenophora* is linked to a higher frequency of pathogenic soil fungal occurrence and a reduced species richness of saprophytic soil fungi.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Leaf decomposition rate was high in *Ageratina adenophora* invaded site but fine root decomposition not significantly vary between invaded and uninvaded sites. Similar results were found in decomposition of green tea. However, like root decomposition red tea decomposition was no different between invaded and uninvaded sites. While comparing the pH, uninvaded site was comparatively acidic than invaded site on the other hand fine root production was high in *A. adenophora* invaded site than uninvaded site.

The nitrogen content of leaves and fine roots of *Alnus nepalensis* reduced by *A. adenophora* in the invaded sites but carbon content was not altered however; *A. adenophora* root extract was found to be toxic for growth of fungi such as *Fusarium*, *Aspergillus* and especially *Trichoderma*. The mycelial growth inhibition increased with increasing the concentrations of *A. adenophora* root extract.

6.2 Recommendations

Overall, *Ageratina adenophora* was found to be responsible for accelerating *Alnus nepalensis* leaves decompositions, increasing fine root productions and decreasing nitrogen content. *A. adenophora* roots are toxic for saprophytic fungi.

1. As *Ageratina* was found to be responsible factor for accelerating *A. nepalensis* leaf decompositions. The nutrients dynamics might be altered in invaded sites but the nutrients are used up by *A. adenophora* for successful growth. Hence controlling of *Ageratina* would be beneficial to remain nutrient load in the soil for native plants.
2. The results showed that how nitrogen in *A. adenophora* invaded site indicates *Ageratina* responsible to reduced soil nitrogen, therefore its removal from invaded sites could increase the nitrogen content.
3. Removal of *A. adenophora* recommended for abundance and growth of saprophytic fungi like *Fusarium*, *Aspergillus* and *Trichoderma*.

REFERENCES

An, J. Y., Park, B. B., Chun, J. H., & Osawa, A. (2017). Litterfall production and fine root dynamics in cool-temperate forests. *PLoS One*, *12*(6), e0180126.

Aneja, K. R (2003). Experiments in Microbiology Plant Pathology and Biotechnology, 4th ed., New Age International Publishers, New Delhi.

Balami, S., Thapa, L. B., & Jha, S. K. (2017). Effect of invasive *Ageratina adenophora* on species richness and composition of saprotrophic and pathogenic soil fungi. *Biotropia*, *24*(3), 212-219.

Balami, S., Thapa, L. B., & Jha, S. K. (2019). Effects of invasive *Ageratina adenophora* on mycelial growth of some important soil fungi. *Songklanakarin Journal of Science & Technology*, *41*(2), 465-470.

Bocock, K. L., Gilbert, O., Capstick, C. K., Twinn, D. C., Waid, J. S., & Woodman, M. J. (1960). Changes in leaf litter when placed on the surface of soils with contrasting humus types: I. Losses in dry weight of oak and ash leaf litter. *Journal of Soil Science*, *11*(1), 1-9.

Cai, H., Li, F., & Jin, G. (2019). Fine root biomass, production and turnover rates in plantations versus natural forests: effects of stand characteristics and soil properties. *Plant and Soil*, *436*, 463-474.

Chen, R., & Twilley, R. R. (1999). A simulation model of organic matter and nutrient accumulation in mangrove wetland soils. *Biogeochemistry*, *44*(1), 93-118.

Couteaux, M. M., Bottner, P., & Berg, B. (1995). Litter decomposition, climate and liter quality. *Trends in ecology & evolution*, *10*(2), 63-66.

Darji, T. B., Adhikari, B., Pathak, S., Neupane, S., Thapa, L. B., Bhatt, T. D., & Bishwakarma, K. (2021). Phytotoxic effects of invasive *Ageratina adenophora* on two native subtropical shrubs in Nepal. *Scientific Reports*, *11*(1), 1-9.

Darmawan, A. A., Ariyanto, D. P., Basuki, T. M., & Syamsiyah, J. (2021, July). Measuring of leaf litter decomposition rate and flux of carbon dioxide in various land cover in Gunung Bromo Education Forest, Karanganyar. In *IOP Conference Series: Earth and Environmental Science*, 824, 012055, IOP Publishing.

Das, R. K., & Devkota, A. (2018). Antifungal activities and phytochemical screening of two invasive alien species of Nepal. *Studies in Fungi*, *3*(1), 293-301.

De Vos, B., Lettens, S., Muys, B., & Deckers, J. A. (2007). Walkley–Black analysis of forest soil organic carbon: recovery, limitations and uncertainty. *Soil Use and Management*, *23*(3), 221-229.

Didion, M., Repo, A., Liski, J., Forsius, M., Bierbaumer, M., & Djukic, I. (2016). Towards harmonizing leaf litter decomposition studies using standard tea bags—a field study and model application. *Forests*, *7*(8), 167.

Finér, L., Ohashi, M., Noguchi, K., & Hirano, Y. (2011). Factors causing variation in fine root biomass in forest ecosystems. *Forest Ecology and Management*, *261*(2), 265-277.

Fu, D., Wu, X., Huang, N., & Duan, C. (2018). Effects of the invasive herb *Ageratina adenophora* on understory plant communities and tree seedling growth in *Pinus yunnanensis* forests in Yunnan, China. *Journal of Forest Research*, *23*(2), 112-119.

Gill, R. A., & Jackson, R. B. (2000). Global patterns of root turnover for terrestrial ecosystems. *The New Phytologist*, *147*(1), 13-31.

Goebel, M., Hobbie, S. E., Bulaj, B., Zadworny, M., Archibald, D. D., Oleksyn, J., & Eissenstat, D. M. (2011). Decomposition of the finest root branching orders: linking belowground dynamics to fine-root function and structure. *Ecological Monographs*, *81*(1), 89-102.

Gramza, A., Korczak, J., & Amarowicz, R. (2005). Tea polyphenols-their antioxidant properties and biological activity-a review. *Polish Journal of Food and Nutrition Sciences*, 2005, 219-235.

Grover, R. K. & Moore, J. D. (1962). Toximetric studies of fungicides against brown rot organism. Sclerotina fruticola. *Phytopathology*, 52, 876-880

Guo, L., Deng, M., Yang, S., Liu, W., Wang, X., Wang, J., & Liu, L. (2021). The coordination between leaf and fine root litter decomposition and the difference in their controlling factors. *Global Ecology and Biogeography*, *30*(11), 2286-2296.

Helsen, K., Smith, S. W., Brunet, J., Cousins, S. A., De Frenne, P., Kimberley, A., & Graae, B. J. (2018). Impact of an invasive alien plant on litter decomposition along a latitudinal gradient. *Ecosphere*, *9*(1), e02097.

Jiao, Y., & Huang, J. (2024). Allelopathic effects of aqueous extracts from uncomposted and composted Mexican devil (*Ageratina adenophora*) plants on

forest fungal growth and soil nitrogen and phosphorus mobilization. *Weed Science*, *72*(1), 76-85.

Joshi, R. K., & Garkoti, S. C. (2021). Influence of Nepalese alder on soil physico-chemical properties and fine root dynamics in white oak forests in the central Himalaya, India. *Catena*, *200*, 105140.

Keuskamp, J. A., Dingemans, B. J., Lehtinen, T., Sarneel, J. M., & Hefting, M. M. (2013). Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems. *Methods in Ecology and Evolution*, *4*(11), 1070-1075.

Kjeldahl, J. (1883). Neue Methode zur Bestimmung des Stickstoffs in organischen Korpern. Z. *Analytical Chemistry*, *22*, 366-382.

Klopatek, J. M. (2007). Litterfall and fine root biomass contribution to nutrient dynamics in second-and old-growth Douglas-fir ecosystems. *Plant and soil*, *294*, 157-167.

Kumar, M., & Garkoti, S. C. (2021). Functional traits, growth patterns, and litter dynamics of invasive alien and co-occurring native shrub species of chir pine forest in the central Himalaya, India. *Plant Ecology*, *222*(6), 723-735.

Li, X., Zheng, X., Zhou, Q., McNulty, S., & King, J. S. (2022). Measurements of fine root decomposition rate: Method matters. *Soil Biology and Biochemistry*, *164*, 108482.

Liao, C., Peng, R., Luo, Y., Zhou, X., Wu, X., Fang, C., Chen, J., & Li, B. (2008). Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. *New Phytologist*, *177*, 706–714.

Linders, T. E., Bekele, K., Schaffner, U., Allan, E., Alamirew, T., Choge, S. K., Eckert, S., Haji, J., Muturi, G., Mbaabu, P. R., & Eschen, R. (2020). The impact of invasive species on social-ecological systems: relating supply and use of selected provisioning ecosystem services. *Ecosystem Services*, *41*, 101055

Lu, Y., Ranjitkar, S., Harrison, R. D., Xu, J., Ou, X., Ma, X., & He, J. (2017). Selection of native tree species for subtropical forest restoration in Southwest China. *PloS One*, *12*(1), e0170418.

Lukac, M. (2012). "Fine root turnover," in Measuring Roots, ed. S. Mancuso (Berlin: Springer), 363–373.

McCormack, M. L., Dickie, I. A., Eissenstat, D. M., Fahey, T. J., Fernandez, C. W., Guo, D & Zadworny, M. (2015). Redefining fine roots improves understanding

of below-ground contributions to terrestrial biosphere processes. *New Phytologist*, *207*(3), 505-518.

Mishra, J., Fatima, T., & Arora, N. K. (2018) Role of secondary metabolites from plant growth-promoting rhizobacteria in combating salinity stress. In: Egamberdieva D, Ahmad P (eds) *Plant Microbiome*, Stress Response. Springer, Singapore, pp 127–163.

Ostonen, I. (2003). Fine root structure, dynamics and proportion in net primary production of Norway spruce forest ecosystem in relation to site conditions. Tartu University Press.

Pachhai, S. (2019). Response of Nitrogen Fixing Non-leguminous *Alnus nepalensis* D. Don and Leguminous *Glycine max* (L.) Merr. to Selected Invasive Species. MSc. Dissertation submitted to Central Department of Botany, Tribhuvan University, Nepal.

Palm, C. A. (1995) Contribution of agroforestry trees to nutrient requirements in intercropped plants. *Agroforestry System*, 30, 105–124

Panchal, P., Preece, C., Peñuelas, J., & Giri, J. (2022). Soil carbon sequestration by root exudates. *Trends in Plant Science*, *27*(8), 749-757.

Persson, H. (1980). Fine-root production, mortality and decomposition in forest ecosystems. *Vegetatio*, *41*, 101-109.

Poudel, A. S., Shrestha, B. B., Joshi, M. D., Muniappan, R., Adiga, A., Venkatramanan, S., & Jha, P. K. (2020). Predicting the current and future distribution of the invasive weed *Ageratina adenophora* in the Chitwan–Annapurna Landscape, Nepal. *Mountain Research and Development*, *40*(2), R61.

Poudel, R., Neupane, N. P., Mukeri, I. H., Alok, S., & Verma, A. (2020). An updated review on invasive nature, phytochemical evaluation, & pharmacological activity of *Ageratina adenophora*. *International Journal of Pharmaceutical Science and Research, 11*, 2510-2520.

Rothstein, D. E., Vitousek, P. M., & Simmons, B. L. (2004). An exotic tree alters decomposition and nutrient cycling in a Hawaiian montane forest. *Ecosystems*, 7, 805–814.

Sapkota, L. (2007). Ecology and management issues of *Mikania micrantha* Banko Janakari in Chitwan national park, Nepal. *17*(2), 27-39.

See, C. R., Luke McCormack, M., Hobbie, S. E., Flores-Moreno, H., Silver, W. L., & Kennedy, P. G. (2019). Global patterns in fine root decomposition: climate,

chemistry, mycorrhizal association and woodiness. *Ecology Letters*, *22*(6), 946-953.

Shabani, F., Ahmadi, M., Kumar, L., Solhjouy-fard, S., Tehrany, M. S., Shabani, F., Kalantar, B. & Esmaeili, A. (2020). Invasive weed species' threats to global biodiversity: Future scenarios of changes in the number of invasive species in a changing climate. *Ecological Indicators*, *116*, 106436.

Shrestha, B. B. (2016). *Invasive alien plants Species in Nepal*. *In: Frontiers of Botany*, Eds.: P.K. Jha, M. Siwakoti and S. Rajbhandary, Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, pp. 269-284.

Shrestha, H. S., Adhikari, B., & BB, S. (2021). *Sphagneticola trilobata* (Asteraceae): first report of a naturalized plant species for Nepal. *Rheedea*, 31, 77-81.

Sun, Y. Y., Zhang, Q. X., Zhao, Y. P., Diao, Y. H., Gui, F. R., & Yang, G. Q. (2021). Beneficial rhizobacterium provides positive plant–soil feedback effects to *Ageratina adenophora*. *Journal of Integrative Agriculture*, *20*(5), 1327-1335.

Thapa, L. B., Kaewchumnong, K., Sinkkonen, A., & Sridith, K. (2016a). Impacts of invasive *Chromolaena odorata* on species richness, composition and seedling recruitment of *Shorea robusta* in a tropical Sal forest, Nepal. *Songklanakarin Journal of Science and Technology*, 38, 683–689.

Thapa, L. B., Kaewchumnong, K., Sinkkonen, A., & Sridith, K. (2020). Airborne and belowground phytotoxicity of invasive *Ageratina adenophora* on native species in Nepal. *Plant Ecology*, *221*(10), 883-892.

Tiwari, S., Siwakoti, M., Adhakari, B., & Subedi, K. (2005). An inventory and assessment of invasive alien plant species of Nepal. Kathmandu (NP): IUCN-The World Conservation Union.

Van Kleunen, M., Weber, E., & Fischer, M. (2010). A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters*, *13*(2), 235-245.

Vander Zanden, M. J., & Olden, J. D. (2008). A management framework for preventing the secondary spread of aquatic invasive species. *Canadian Journal of Fisheries and Aquatic Sciences*, *65*(7), 1512-1522.

Vaou, N., Stavropoulou, E., Voidarou, C., Tsakris, Z., Rozos, G., Tsigalou, C., & Bezirtzoglou, E. (2022). Interactions between medical plant-derived bioactive compounds: focus on antimicrobial combination effects. *Antibiotics*, *11*(8), 1014.

Violita, V., Triadiati, T., Anas, I., & Miftahudin, M. (2016). Fine root production and decomposition in lowland rainforest and oil palm plantations in Sumatra, Indonesia. *Hayati Journal of Biosciences*, *23*(1), 7-12.

Walela, C., Daniel, H., Wilson, B., Lockwood, P., Cowie, A., & Harden, S. (2014). The initial lignin: nitrogen ratio of litter from above and below ground sources strongly and negatively influenced decay rates of slowly decomposing litter carbon pools. *Soil Biology and Biochemistry*, *77*, 268-275.

Wang, C., Zhou, J., Liu, J., Jiang, K., Xiao, H., & Du, D. (2018). Responses of the soil fungal communities to the co-invasion of two invasive species with different cover classes. *Plant Biology*, *20*(1), 151-159.

Wan-Xue, L., Hong-Bang, N., Fang-Haoo, W., & Bo, L. (2010). Effects of leachates of the invasive plant, *Ageratina adenophora* (Sprengel) on soil microbial community. *Acta Ecologica Sinica*, *30*(4), 196-200.

Weidenhamer, J. D., & Callaway, R. M. (2010). Direct and indirect effects of invasive plants on soil chemistry and ecosystem function. *Journal of Chemical Ecology*, *36*, 59-69.

Witkamp, M., & Olson, J. S. (1963). Breakdown of confined and non-confined oak litter. *Oikos*, 138-147.

Xia, Y., Feng, J., Zhang, H., Xiong, D., Kong, L., Seviour, R., & Kong, Y. (2024). Effects of soil pH on the growth, soil nutrient composition, and rhizosphere microbiome of *Ageratina adenophora*. *The Journal of Life and Environment Sciences*, *12*, e17231.

Yong Feng, W. (2006). Metabolism of green tea catechins: an overview. *Current Drug Metabolism*, *7*(7), 755-809.

Yuan, Z. Y., & Chen, H. Y. (2010). Fine root biomass, production, turnover rates, and nutrient contents in boreal forest ecosystems in relation to species, climate, fertility, and stand age: literature review and meta-analyses. *Critical Reviews in Plant Sciences*, *29*(4), 204-221.

Yuan, Z. Y., & Chen, H. Y. (2012). A global analysis of fine root production as affected by soil nitrogen and phosphorus. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1743), 3796-3802.

Zhang, L., Xiaochi, M. A., Hong, W. A. N. G., Shuwei, L. I. U., Siemann, E., & Jianwen, Z. O. U. (2016). Soil respiration and litter decomposition increased

following perennial forb invasion into an annual grassland. *Pedosphere*, *26*(4), 567-576.

Zhang, M., Liu, W. X., Zheng, M. F., Xu, Q. L., Wan, F. H., Wang, J., & Tan, J. W. (2013). Bioactive quinic acid derivatives from *Ageratina adenophora*. *Molecules*, *18*(11), 14096-14104.

Zhao, M., Lu, X., Zhao, H., Yang, Y., Hale, L., Gao, Q., & Wan, F. (2019). *Ageratina adenophora* invasions are associated with microbially mediated differences in biogeochemical cycles. *Science of the Total Environment*, *677*, 47-56.

Zheng, G., Luo, S., Li, S., Hua, J., Li, W., & Li, S. (2018). Specialized metabolites from *Ageratina adenophora* and their inhibitory activities against pathogenic fungi. *Phytochemistry*, *148*, 57-62.

Zobel, D. B., Jha, P. K., Behan, M. J., & Yadav, U. K. R. (1987). A practical manual for ecology. Ratna Book Distributors, Kathmandu, Nepal.

APPENDICES

A. List of materials, media, chemicals and reagents:

a) Equipments:

1. Autoclave
2. Incubator
3. Hot air oven
4. Refrigerator
5. Weighing machine
6. Weighing machine
7. Biological Safety Cabinet
8. Grinder
9. Mortar and Pestle

b) Glass Wares:

1. Beakers
2. Petri dishes
3. Conical Flask
4. Volumetric flask
5. Volumetric flask
6. Test tubes
7. Pipettes
8. Measuring Cylinders

d) Chemicals and Reagents:

1. Ethanol
2. Potassium dichromate
3. Ferrous sulphate
4. 85% phosphoric acid
5. Sulphuric acid (H2SO4)
6. Diphenylamine indicator
7. Hydrochloric acid6
8. Bradford's Reagent
9. Sodium Hydroxide
10. Potato Dextrose Agar

e. Miscellaneous

1. Innoculating loop and wire
2. Labeling tape
3. Dropper
4. Cotton
5. pH paper
6. Forceps
7. Parafilm Tape
8. Nylon litter bag
9. Permanent Marker
10. Aluminum foil
11. Tea bags
12. Distilled Water

B. Composition of Potato DextroseAgar :

The culture media used were from Hi-Media Laboratories Pvt. Limited,Bombay, India (All composition are given in gram per liter and 25° C (Temperature).

S.No	Ingredients	gram/liter
1	Potato infusion form	200
2	Dextrose	20
3	Agar	15

Final pH at 25°C 5.6±0.2

Preparation: As directed by manufacturer, 39 g of the medium was dissolved in 1000 mL of distilled water and then boiled to dissolve completely. The medium was autoclaved at 121° C (15 lbs. pressure) for 15 minutes. The sterilized medium was then poured in sterilized Petri- dishes and was allowed to cool.

C. Study site details

Table 1: Altitudes and co-ordinates of plots of *Ageratina adenophora* invaded sites

Sites	Altitude	Latitude	Longitude
1	1545m	27°39′23.89 ″ N	85°14′54.23″E
2	1506m	27°39′22.13″N	85°14′54.39″E
3	1567m	27°39′20.98″N	85°14′55.52″E
4	1517m	27°39 ′ 21.31″N	85°14′54.47″E
5	1362m	27°39′21.79″N	85°14′48.97″E.
6	1342m	27°39′23.1″N	85°14′50.3″E,
7	1325m	27°39′22.3″N	85°14′50.33″E
8	1583m	27°39′23.36″N	85°14′47.09″E
9	1342m	27°39′23.29″N	85°14′51.17″E

Table 2: Altitudes and co-ordinates of plots of *Ageratina adenophora* uninvaded sites

Sites	Altitudes	Latitudes	Longitudes
1	1586m	2°39′21.79″N	85°14′48.97″E
2	1588m	27°39′21.73″N	85°14′48.21″E
3	1605m	27°39′21.06″N	85°14′46.71″E
4	1580m	27°39′23.77″N	85°14′47.11″E
5	1592m	27°39′20.82″N	85°14′48.46″E
6	1578m	27°39′22.72″N	85°14′47.4″E
7	1516m	27°39′23.84″N	85°14′ 7.92″E
8	1583m	27°39′23.36″N	85°14′47.09″E
9	1581m	27°39′23.96″N	85°14′47.74″E

D. Statistical Analysis

Table 3: Statistical analysis of decomposition of leaves and fine roots of *Alnus nepalensis*

Sites		N	Mean	Std. Deviation	Std. Error Mean
LDM2	invaded	9	37.5756	15.91104	5.30368
	uninvaded	9	28.0667	5.47673	1.82558
LDM3	invaded	9	70.2067	23.21348	7.73783
	uninvaded	9	39.8696	12.25907	4.08636
LDM4	invaded	9	83.9615	12.43534	4.14511
	uninvaded	9	56.8659	13.87416	4.62472
LDRM2	invaded	9	.6263	.26518	.08839
	uninvaded	9	.4678	.09128	.03043
LDRM3	invaded	9	.7801	.25793	.08598
	uninvaded	9	.4430	.13621	.04540
LDRM4	invaded	9	.6997	.10363	.03454
	uninvaded	9	.4739	.11562	.03854
FRDM2	invaded	9	16.6963	7.78447	2.59482
	uninvaded	9	18.3926	7.92142	2.64047
FRDM3	invaded	9	23.4185	4.71620	1.57207
	uninvaded	9	30.5926	5.01378	1.67126
FRDM4	invaded	9	38.6074	11.90220	3.96740
	uninvaded	9	35.3815	4.67862	1.55954
FRDRM2	invaded	9	.2783	.12974	.04325
	uninvaded	9	.3065	.13202	.04401
FRDRM3	invaded	9	.2602	.05240	.01747
	uninvaded	9	.3399	.05571	.01857
FRDRM4	invaded	9	.3217	.09919	.03306
	uninvaded	9	.2948	.03899	.01300
GTDM2	invaded	9	74.6645	8.46318	2.82106
	uninvaded	9	69.1222	3.48986	1.16329
GTDM3	invaded	9	81.7960	6.02693	2.00898
	uninvaded	9	73.5131	2.64267	.88089
GTDM4	invaded	9	85.9916	3.95377	1.31792
	uninvaded	9	77.1886	4.81091	1.60364
GTDRM2	invaded	9	1.2444	.14105	.04702
	uninvaded	9	1.1520	.05816	.01939
GTDRM3	invaded	9	.9088	.06697	.02232
	uninvaded	9	.8168	.02936	.00979
GTDRM4	invaded	9	.7166	.03295	.01098
	uninvaded	9	.6432	.04009	.01336
RTDM2	invaded	9	46.9414	4.20301	1.40100
	uninvaded	9	44.7829	4.87454	1.62485
RTDM3	invaded	9	54.3857	3.32495	1.10832
	uninvaded	9	53.1568	3.29222	1.09741
RTDM4	invaded	9	60.9563	6.21730	2.07243
	uninvaded	9	59.2262	3.27731	1.09244

RTDRM2	invaded	9	.7824	.07005	.02335
	uninvaded	9	.7464	.08124	.02708
RTDRM3	invaded	9	.6043	.03694	.01231
	uninvaded	9	.5906	.03658	.01219
RTDRM4	invaded	9	.5080	.05181	.01727
	uninvaded	9	.4936	.02731	.00910

Table 4: Statistical analysis of pH of Month first(Month-2) and Month last (Month-4)

String		N	Mean	Std. Deviation	Std. Error Mean
PHM2	Invaded	9	5.5130	.42135	.14045
	uninvaded	9	4.5096	.17585	.05862
PHM4	Invaded	9	5.4733	.48363	.16121
	uninvaded	9	4.5259	.18217	.06072

Table 5: Statistical analysis of dry weight of fine roots

String		N	Mean	Std. Deviation	Std. Error Mean
FRDW	invaded	10	1.4380	.89083	.28171
	uninvaded	10	.8041	.43800	.13851

Table. 6: Statistical analysis of nutrients (carbon and nitrogen) content of leaves and fine root of *Alnus nepalensis*

String		N	Mean	Std. Deviation	Std. Error Mean
LN2	invaded	3	2.5100	.59152	.34152
	Uninvaded	3	3.3267	.53379	.30818
LN3	invaded	3	2.2333	.44163	.25497
	uninvaded	3	3.2033	.55148	.31840
LN4	invaded	3	2.3100	.28000	.16166
	uninvaded	3	2.9533	.15567	.08988
LC2	invaded	3	25.5033	5.69064	3.28550
	uninvaded	3	36.0033	5.98732	3.45678
LC3	invaded	3	22.1633	6.51725	3.76274
	uninvaded	3	45.0567	26.05130	15.04073
LC4	invaded	3	28.1567	3.09015	1.78410
	uninvaded	3	24.8433	9.65215	5.57267
RN2	invaded	3	1.7967	.38423	.22184
	uninvaded	3	1.9033	.25482	.14712
RN3	invaded	3	1.5500	.46487	.26839
	uninvaded	3	2.0233	.49743	.28719
RN4	invaded	3	1.7767	.08505	.04910

	uninvaded	3	2.0200	.10000	.05774
RC2	invaded	3	41.1833	2.52042	1.45517
	uninvaded	3	48.0067	9.28699	5.36185
RC3	invaded	3	38.0367	1.47548	.85187
	uninvaded	3	42.5833	.09292	.05364
RC4	invaded	3	38.9900	1.28339	.74097
	uninvaded	3	42.7933	3.01074	1.73825

Table 7: Statistical analysis of radial growth of fungi

Concentration	**Fungi**	**Mean**	**Std. Deviation**	**N**
10 %	*Fusarium*	3.1778	.39487	3
	Aspergillus	10.1667	1.99360	3
	Trichoderma	9.0444	7.83449	3
	Total	7.4630	5.19063	9
30 %	*Fusarium*	3.8556	.18359	3
	Aspergillus	10.3333	2.66604	3
	Trichoderma	2.9222	3.17461	3
	Total	5.7037	4.06504	9
50 %	*Fusarium*	2.7889	.86303	3
	Aspergillus	7.2222	.89463	3
	Trichoderma	.0000	.00000	3
	Total	3.3370	3.21487	9
100 %	*Fusarium*	1.6667	.28868	3
	Aspergillus	4.2000	.32146	3
	Trichoderma	.0000	.00000	3
	Total	1.9556	1.84421	9
Control	*Fusarium*	4.8778	.35642	3
	Aspergillus	11.2111	1.08747	3
	Trichoderma	14.1222	6.91185	3
	Total	10.0704	5.38763	9
Total	*Fusarium*	3.2733	1.18034	15
	Aspergillus	8.6267	3.01107	15
	Trichoderma	5.2178	7.06814	15
	Total	5.7059	4.92248	45

Table 8: Independent sample t- test of decompositions of leaves and fine roots of *Alnus nepalensis*

		Levene's Test for Equality of Variances		t-test for Equality of Mean		
		F		**t**	**df**	**p-value**
LDM2	Equal variances assumed	8.263	.011	1.695	16	.109
LDM3	Equal variances assumed	5.762	.029	3.467	16	.003
LDM4	Equal variances assumed	.097	.760	4.363	16	.000
LDRM2	Equal variances assumed	8.263	.011	1.695	16	.109
LDRM3	Equal variances assumed	5.762	.029	3.467	16	.003
LDRM4	Equal variances assumed	.097	.760	4.363	16	.000
FRDM2	Equal variances assumed	.132	.721	-.458	16	.653
FRDM3	Equal variances assumed	.014	.907	-3.127	16	.007
FRDM4	Equal variances assumed	3.648	.074	.757	16	.460
FRDRM2	Equal variances assumed	.132	.721	-.458	16	.653
FRDRM3	Equal variances assumed	.014	.907	-3.127	16	.007
FRDRM4	Equal variances assumed	3.648	.074	.757	16	.460
GTDM2	Equal variances assumed	6.538	.021	1.816	16	.088
GTDM3		8.713	.009	3.776	16	.002
GTDM4	Equal variances assumed	.000	.992	4.241	16	.001
GTDRM2	Equal variances assumed	6.538	.021	1.816	16	.088
GTDRM3	Equal variances assumed	8.713	.009	3.776	16	.002

GTDRM4	Equal variances assumed	.000	.992	4.241	16	.001
RTDM2	Equal variances assumed	.174	.682	1.006	16	.329
RTDM3	Equal variances assumed	.020	.889	.788	16	.442
RTDM4	Equal variances assumed	1.055	.320	.738	16	.471
RTDRM2	Equal variances assumed	.174	.682	1.006	16	.329
RTDRM3	Equal variances assumed	.020	.889	.788	16	.442
RTDRM4	Equal variances assumed	1.055	.320	.738	16	.471

Table 9: Independent samples t-test pH of Month first(Month-2) and Month last (Month-4)

		Levene's Test for Equality of Variances		**t-test for Equality of Means**		
		F	**Sig.**	**t**	**df**	**p-value**
PHM2	Equal variances assumed	5.711	.030	6.593	16	.000
PHM4	Equal variances assumed	15.845	.001	5.500	16	.000

Table. 10: Independent samples t-test of dry weight of fine roots

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	t	df	p-value
FRDW	Equal variances assumed	1.946	.180	2.019	18	.059

Table. 11: Independent smples t-test of nutrients (carbon and nitrogen) content of leaves and fine roots of *Alnus nepalensis*

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	**Sig.**	**t**	**df**	p-value
LN2	Equal variances assumed	.117	.749	-1.775	4	.151
LN3	Equal variances assumed	.054	.827	-2.378	4	.076
LN4	Equal variances assumed	.557	.497	-3.478	4	.025
LC2	Equal variances assumed	.001	.979	-2.202	4	.092
LC3	Equal variances assumed	7.029	.057	-1.477	4	.214
LC4	Equal variances assumed	3.431	.138	.566	4	.601
RN2	Equal variances assumed	.631	.472	-.401	4	.709
RN3	Equal variances assumed	.075	.797	-1.204	4	.295
RN4	Equal variances assumed	.003	.956	-3.211	4	.033
RC2	Equal variances assumed	7.358	.053	-1.228	4	.287
RC3	Equal variances assumed	4.663	.097	-5.327	4	.006
RC4	Equal variances assumed	1.417	.300	-2.013	4	.114

Table 12: Radial growth of fungi

	Fungi	**N**	**Subset**	
			1	**2**
Tukey HSD[a,b]	*Fusarium*	15	3.2733	
	Trichoderma	15	5.2178	
	Aspergillus	15		8.6267
	Sig.		.192	1.000

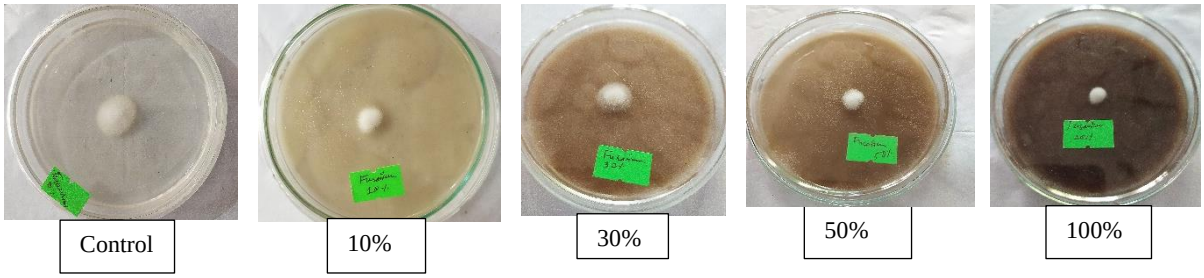
PHOTOPLATES

Dried leaf and fine root litters of *Alnus nepalensis*

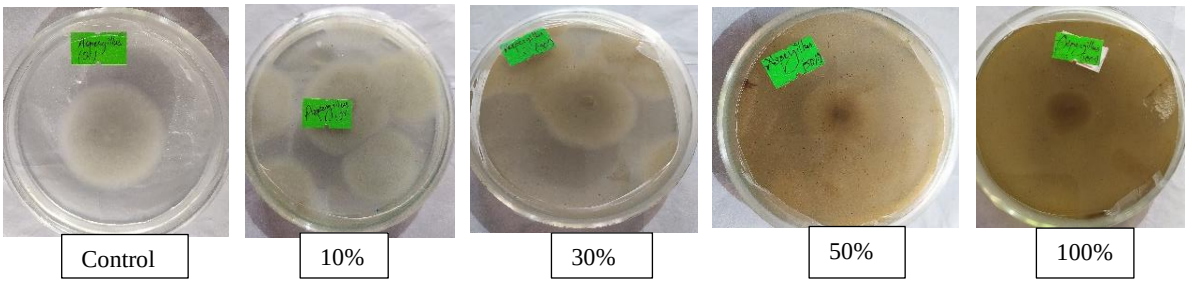
Placement of litters in *Ageratina adenophora* invaded and uninvaded sites

Measurement of pH

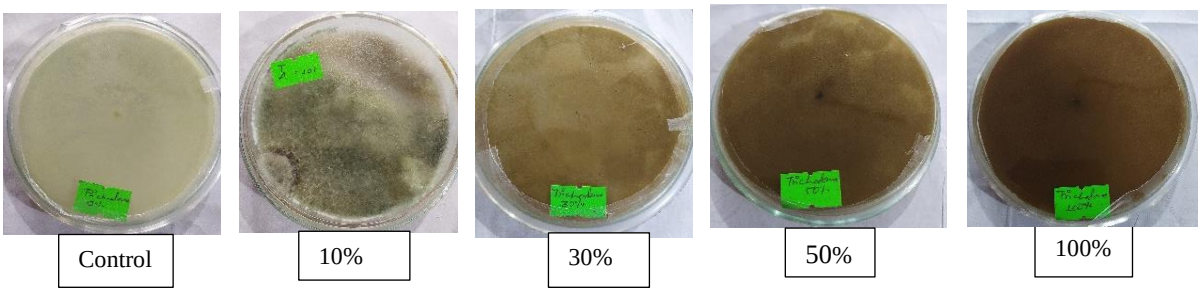
Culture of fungi in Biological Safety Cabinet



Effect of *Ageratina adenophora* root extract on the mycelial growth of *Fusarium*



Effect of *Ageratina adenophora* root extract on the mycelial growth of *Aspergillus*



Effect of *Ageratina adenophora* root extract on the mycelial growth of *Trichoderma*

Effect of Ageratina adenophora invasion on litter decomposition, fine root production and mycelial growth of selected saprophytic fungi

ORIGINALITY REPORT

19%
SIMILARITY INDEX

PRIMARY SOURCES

1	elibrary.tucl.edu.np Internet	493 words — 3%
2	www.thaiscience.info Internet	299 words — 2%
3	www.researchgate.net Internet	122 words — 1%
4	Xiaogai Ge, Lixiong Zeng, Wenfa Xiao, Zhilin Huang, Xiansheng Geng, Benwang Tan. "Effect of litter substrate quality and soil nutrients on forest litter decomposition: A review", Acta Ecologica Sinica, 2013 Crossref	90 words — 1%
5	www.coursehero.com Internet	89 words — 1%
6	Guadalupe, Sarah Siemers. "The Impact of Culture in Help-Seeking Behaviors Related to Autism Spectrum Disorders.", Oakland University, 2020 ProQuest	75 words — 1%
7	byjus.com Internet	66 words — < 1%