EFFECTS OF HERBIVORY, PATHOGENS AND HABITAT TYPES ON INVASIVE ALIEN PLANT SPECIES IN CHOBHAR, KATHMANDU, NEPAL

A Dissertation Submitted for Partial Fulfillment of the Requirements for the Master's of Science in Botany

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LETTER OF RECOMMENDATION

This is to recommend that the dissertation work entitled "**Effects of Herbivory, Pathogens and Habitat Types on Invasive Alien Plant Species in Chobhar, Kathmandu, Nepal"** has been carried out by Miss Reetu Deuba under our supervision. This is her original research entire work and has been accomplished on the basis of candidate's original research. To the best of our knowledge, this dissertation work has not been submitted for any other academic degree in any institution. We therefore, recommend this dissertation to be accepted for the partial fulfillment of the M.Sc. in Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

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I, Reetu Deuba, hereby declared that this dissertation entitled "**Effects of Herbivory, Pathogens and Habitat Types on Invasive Alien Plant Species in Chobhar, Kathmandu, Nepal”** is my original work and all other sources of the information used are duly acknowledged. I have not submitted it or any of its part to any other universities for any academic degree.

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Reetu Deuba

Date: August, 2019

ABSTRACT

Invasive alien plant species (IAPS) in Nepal have become a great problem. They are responsible to cause severe damages to our ecosystem and contribute a huge amount of economic loss. It has been considered that the invasive species spreads rapidly because they are comparatively less damaged by herbivores and pathogens in the invaded range. This study aims to understand effects of herbivores, pathogens and habitats on invasive species *Ageratina adenophora*, *Ageratum conyzoides*, *Bidens pilosa*, *Parthenium hysterophorus* in Chobhar area of Kathmandu, Nepal. Additionally, the study compared density, frequency and coverge of these species. Ecological parameters were measured by sampling quadrats of size (1×1 m^2) in fallow land, forest and along road towards north and south aspects of the study area. Herbivory damages assessment was conducted by sampling plants from the quadrats sampled. Results showed that the herbivory damage differs among the IAPS and it depends on habitat type. In branches, *B. pilosa* was greately damaged in fallow land, *A. adenophora* in the forest, *A. conyzoides* in the road towards north aspect and *P. hysterophorus* in road towards south aspect. Similarly, in case of leaves, the highest damage was found in *B. pilosa* from sites fallowland and Road towards north aspects. In *A. conyzoides* and *A. adenophora* damages were found maximum in the road towards north aspect but in *P. hysterophoru*s the damage was severe in road towards south aspect. Moreover, a total of 13 fungi were isolated from the infected leaves of IAPS and identified. Further studies are recommended for additional information in herbivory damages and fungal identification. This work might be helpful to know the current status of herbivory damages on selected IAPS in Nepal.

Key-words: Biological control, fungal species, habitat, herbivory damages, IAPS.

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	-	Analysis of variance
asl	-	above sea level
df	-	degree of freedom
GIS	-	Geographical Information System
ERH	-	Enemy Release Hypothesis
SPSS	-	Statistical Package for Social Science
PDA	-	Potato Dextrose Agar
CDA	-	Czapek Dox Agar
MEA	-	Maltose Extract Agar
NA	-	Nutrient Agar
IAPS	-	Invasive Alien Plant Species
IAS	-	Invasive Alien Species
CDB	-	Central Department of Botany

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CHAPTER – I

INTRODUCTION

1.1Background

1.1.1 Definition of Invasive Alien Plant Species (IAPS)

Invasive alien plant species (IAPS) has become a great problem worldwide. A subset of naturalized species that has spread so far rapidly and extensively and caused significant negative impacts on ecosystem, human health, biodiversity, infrastructures and economy in the introduced range are called Invasive alien plant species (Pysek *et al*. 2004). IAPS is also defined as the species which are introduced on the new locations from their native range either intentionally or accidently and has a tendency to spread to cause damage to the environment (Tiwari *et al.* 2005). Literally, the word ’invasion’ means an act or process which affects something or someone in the way that is not welcomed. According to Tiwari *et al*. (2005) many species in Nepal got introduced unintentionally through trade, tourism and transport.

1.1.2 Impacts of IAPS

IAPS threat on native biological diversity is one of the highlighted issues throughout the world (Wilcove *et al.* 1998; Vitousek *et al*. 1997; Timsina *et al.* 2011; Shrestha 2016; Thapa *et al*. 2017). IAPS are of great concern because they are able to establish on many sites and spread quickly to the point of disrupting plant communities. They show high competitiveness and have ability to colonize new areas within short periods of time (Reichard and White 2001). They are considered as the main drivers of ecosystem change, and alteration of food chain in their introduced range (Gurevitch and Padilla 2004; Shabbir and Bajwa 2006). The problem of invasive species is prevalent both in developed as well as developing countries, but their impact is likely to be higher in developing countries like Nepal due to the lack of awareness and resources to manage and control them (Shrestha 2016).

Invasion of alien species (animals, pests, viruses, pathogens and plants) presents serious threat to biodiversity because of their negative effects on flora and fauna species, food web, habitat and also to ecosystem dynamics (Karki and Poudel 2014), resource availability and economic well-being of all environments (Boy and Wilt 2013; Grevitch and Padilla 2004).

Biological invasion, a widespread global change, has been challenging the conservation of biodiversity and natural resources (Simberloff *et al.* 2013). IAPS compete with native plants for nutrients, water and light (Witkowski 1991; Aderson 1996). They can have allelopathic effect on other species and also they are able to alter native ecosystem and diversity (Mack *et al*. 2000; Barton *et al.* 2007). Biological invasion is one of the main causes of biodiversity loss as the IAPS may cause species declining in the natural habitats to extinction (Bellard *et al*. 2016; Downey and Rirchardson 2016) and therefore it is considered as the second biggest threat after habitat destruction and ecosystem degradation (Vitousek *et al*. 1997; Reddy 2012).

With increasing globalization of trade along with human movement, the number of alien species has been increasing in all climatic regions and continents (Seebens *et al.* 2017) from tropics to high mountain (Pauchard *et al*. 2016). In the world, at least 13,168 species of plants have become naturalized outside their native distribution range (Van Kleunen *et al*. 2015). It has been declared that IAPS are responsible for at least 3 of the 24 known extinction of endangered species in the US (Schmitz and Simberloff 1997). Pimentel *et al*. (2005) mentioned that, around 80% of the endangered species are threatened as a result of invasion by alien species.

IAPS have caused damage costs equal to 53% of agricultural GDP in the USA, 31% in the UK and 48% in Australia (Perrings 2007). In addition, the damage costs reported in South Africa, India and Brazil were 96%, 78% and 112% respectively on agricultural GDP (Perrings 2007). Infact, the loss of agriculture production is more in developing countries than developed countries (Perrings 2007). Invasion of IAPS have also emerged threatening impacts in ecosystem, biodiversity and agriculture production of Nepal (MoFSC 2014). Nepal physiographic and climatic diversity is also supporting suitable habitat for IAPS. In addition, food security and agriculture sector is also more dependent on imported agriculture products, seeds and seedlings. Due to these scenario, Nepal's agriculture sector has been ranked third among the most threatened countries out of the 124 countries measured (Paini *et al*. 2016; Shrestha *et al.* 2017).

The IAPS are known to have impact on belowground and aboveground communities (Mack and Antonio 2003). They are known to release myriads of allelochemicals to their habitats and these chemicals get leached to the soils from aboveground and belowground parts or both (Dayakar *et al*. 2009; Balami *et al*. 2017) and alters the soil biophysical properties (Hinsinger *et al.* 2005).

Their positive impacts also are taken into consideration; ornamental value, manure etc, highlighting its positive aspects, there occur controversial statements, whether invasive species are friends or foe or pests or providence and weed or wonder etc (Rai *et al*. 2012).

1.1.3 IAPS in Nepal

In case of Nepal, biological invasion has emerged as new environmental problem, it directly impacts to soil community, biodiversity conservation, ecosystem etc (Shrestha *et al*. 2016). The topography of Nepal shows extreme altitudinal variation, such altitudinal differences and climatic changes in environmental condition makes it vulnerable for the Invasion of IAS. Many studies show that the number and abundance of IAPS in Nepal has been increasing since few decades (Shrestha 2016).

Tiwari *et al*. (2005) reported 166 naturalized and of them 21 are IAPS as a problematic with negative impact to local environment and the livelihood of the Nepalese people. In the recent years, the lists of IAPS and naturalized species have been frequently updated based on publications and field researches (Siwakoti 2012; Shrestha 2016).

A recent review has reported at least 179 plant species are naturalized (Shrestha *et al.* 2019) and of them 26 are IAPS (Shrestha *et al.* 2017) with negative impacts on environment and economy. Out of 26 IAPS in Nepal (Shrestha *et al*. 2017) four species namely *Chromolaena odorata* (L.) R. M. King and H. Rob, *Eichhornia crassipes* (Mart.) Solms, *Lantana camara L.* and *Mikania micrantha* Kunth, are included in 100 of the world’s worst invasive alien species (Lowe *et al*. 2000). In addition, *Parthenium hysterophorus* L. is another problematic species in urban areas, grasslands and residential areas. *Ageratum houstonianum* Mill., *Alternanthera philoxeroides* Mart., *Erigeron karvinskianus* DC., *Oxalis latifolia* Kunth., *Parthenium hysterophorus*, *Pistia stratiotes* L. etc have been considered highly problematic to farmers (Ranjit 2013; Ranjit *et al*. 2012; Shrestha *et al.* 2018; Siwakoti *et al.* 2016) and *Ageratum conyzoides* in agricultural lands.

Among the highly problematic IAPS in Nepal, *Ageratina adenophora* (Spreng.) King and H. Rob. is the most widely distributed species in forest ecosystem, i.e it is distributed in tropical to subtropical regions from east to west Nepal. It is known to have some sorts of negative impacts, like it inhibits native species growth and development as well as replaces native species. Some of the species such as *Chromolaena odorata* (Joshi 2006), *Ageratina adenophora* (Chhetri 1986; Thapa *et al*. 2017), *Mikania micrantha* (Sapkota 2007; Shrestha 2012) and *Parthenium hysterophorus* (Timsina *et al.* 2011) have been studied in Nepal in context of their distribution and ecological impacts. Biological invasion in Nepal has also become a challenging problem in biodiversity conservation, ecosystem and agriculture production (MoFSC 2014).

Plant invasion in mountain areas are likely to risk due to increase in trade, tourism activities, climatic change (Pauchard *et al*. 2016) and anthropogenic disturbances and can alter both native flora and fauna species composition inducing long term negative impacts (Alexander *et al*. 2016; Fei *et al*. 2014). Only a few IAPS *Ageratina adenophora*, *Galinsoga quadriradiata* has been reported from high mountains while the high himalayan areas has remained free from IAPS until now (Shrestha *et al*. 2016; Siwakoti *et al*. 2016).

Number of both naturalized species and IAPS are higher in eastern and central Nepal than that of in western Nepal (Bhattarai *et al*. 2014). Physiographic and climatic diversity of Nepal is not only supporting wide spectrum of organisms and ecosystems but also providing potential suitable habitats for the species native to anywhere in the world. To address the emerging threat of invasive alien species, Nepal’s National Biodiversity Strategy and Action Plan 2014-2020 has targeted a number of activities including impact assessments of selected invasive alien plant species (IAPS) and release of biological control agents against IAPS (MoFSC 2014).

1.1.4 Invasion Mechanism

Various mechanism have been postulated to understand the invasion success of alien plants, such as resource competition hypothesis, empty niche hypothesis, novel weapon hypothesis, enemy release hypothesis etc. (Holzmueller and Jose, 2009). It means invasive plants use multiple strategies to get dominance in their introduced range. Resource competition hypothesis states that there is mutually negative interactions among two or more individuals and such interaction causes reduction in growth rate at population level. There occur competition between the species, as a result invasive species get benefitted through competition than that of native and finally invasion of IAPS occurs. One of the most widely used hypothesis is ERH. Enemy Release Hypothesis assumes that an alien species when introduced into new land experience reduced impacts from their natural enemies which leads to the increase in the distribution and abundance of the alien species (Yang *et al*. 2010; Keane and Crawley 2002). ERH also states that exotic plants are less likely to be attacked by generalist herbivores in their introduced range (Scharer *et al.* 2011). IAS leave their specialist herbivores in their native land (Keane and Crawley 2002). Herbivores are specific to their host plant and co-evolved with their host plant. Although, the ERH remains a controversial topic in invasion ecology and no general consensus has been established on how to test it (Jeschke *et al.* 2012).

Callaway and Ridenour (2004) proposed Novel Weapon Hypothesis (NWH). According to this hypothesis, IAPS possess and release novel biochemical weapons that works as unusually powerful allelopathic agents in novel area. The allelochemicals provide greater competitive advantages in their new range than in their original range. Many research works on invasion success showed that the allelochemicals can modify the soil microbial communities (Vander *et al.* 2007; Kourtev *et al.* 2002) and soil nutrient dynamics (Ehrenfeld 2003) in the introduced range. Hence, release of phytotoxics allelochemicals is one of the potentially significant invasion mechanisms. This approach is known to be followed by number of IAPS for successful invasion (Boudiaf *et al*. 2013).

1.1.5 IAPS control and Management

The principles methods of controlling IAPS are chemical, mechanical and biological control (Wittenberg and cock 2001). Physical methods or mechanical methods includes hand-pulling, cutting and burning. Manual removal has been regarded as the most environment friendly method of IAPS control, but for this type of control, effort is required and can be applied only to relatively small area where labour groups or other bodies are available (Hobbs and Humphries 1995).

Chemical methods can be applied in controlling and managing herbaceous invasive plants in agriculture. The chemical methods of controls have several demerits such as the harmful chemicals often creates environmental and health problems for humans and non-target species. However, the chemical pesticides like glyphosate, Imazapyr, Imazapic, Triclopyr etc are most commonly used pesticides for weeds and invasive herbs control (Wills 2017).

Biological control methods seems safe, sound and effective methods. It involves the introduction of host-specific natural enemies (fungi, herbivores etc) of the IAPS from its original native range. Alien plants natural enemies may be able to survive, and even reproduce, but does not invade aggressively in its new habitat (Fowler *et al*. 2000). Therefore, biological control methods should be considered as an effective control methods than that of other methods. Thus, assume a key role in IAPS management programmes (Olckers *et al*. 1998).

On the basis of Pathogenicity test, Barton (2004) reported 26 species of fungi originating from 15 different countries have been used as classical biological control agents over weeds in 7 different countries. For eg. In *Parthenium hysterophorus*, *Puccinia abrupta* var. *partheniicola* and *Puccinia melampodi* were recognized as biological control agent. In case of *Ageratina adenophora* two agents (1) *Procecidochares utilis*, a stem gall fly and (2) *Passalora ageratinae*, leaf-spot fungal pathogens individually were released on *Ageratina adenophora* for effective biocontrol agent.

There are several studies carried out by many researchers concerning the biological control of IAPS globally but in case of Nepal limited works had been conducted. According to Balami *et al*. (2017), the level of herbivory damage in native *Alnus nepalensis*, in the place invaded by *Ageratina adenophora* in Nepal was measured and finally result showed that invasive plant experienced lower level of herbivory damage than native plants does. It means because of ERH (reduced herbivory damage) Invasive *A. adenophora* might have benefitted behind its successful invasion in Nepalese forest.

Biological control by herbivores according to Shrestha *et al.* (2011), an insect *Zygogramma bicolorata* fortuitous arrival had led to some degree of weed suppression (*Parthenium hysterophorus*), since 2009 in some locations around Hetaunda, Bharatpur, Butawal and Bhairahawa, Nepal. However, it is also reported that *Z. bicolorata* entered Nepal in the same way as *P. hysterophorus*, along road corridors from India. Another biological control agent, *Puccinia abrupt* var. *partheniicola*, the winter rust, is also present in Nepal. This winter rust had first encountered in Nepal at Kirtipur, Kathmandu valley in May 2011 (Shrestha 2012), but it has remained localized just in a few locations in the valley and the damage caused by this agent has been minimal. A concept have been developed that there might be hervivores affecting those IAPS which are naturalized since long time in Nepal. Identification of these insects bites and measurement of their damage to IAPS helps in effective biocontrol. IAPS also contains anti-herbivory chemicals which are allelopathic and anti-microbial in characteristics.

Overall, management strategies of IAPS through physical, chemicals and biocontrol method have been reported previously but implementation of particular method should be based on the mechanism of biological invasion. For example, if native species are heavily damaged by herbivores in comparision to IAPS invaded the control approach should be focused on how to control herbivores so that they could be less damaged by herbivores and could be able to compete with IAPS. On the other hand, scientific community should now be focused on new issue like IAPS herbivores interaction, IAPS soil microbe interaction, fungal interaction between IAPS etc. should also be revealed.

1.2 Justification

In Nepal there are 26 IAPS, which had been already reported as problematic species. Mostly, Invasive and naturalized alien species are highly problematic near the construction sites and human settlements and they are creating severe problem in the native species diversity, disturbance, composition, abundance, ecosystem and soil health as well as human health and livelihood. There are several studies conducted regarding IAPS distribution, diversity and impacts but there are very less information regarding particular invasion mechanisms. As there are several mechanism of invasion such as; ERH mechanism, allelopathic mechanism, novel weapon hypothesis mechanism, resource competition hypothesis mechanism etc. which mechanism of invasion in particular habitat is working should be explored.

Assessment on herbivory damage to IAPS can be one of the useful studies to know the status of herbivore adaptation and effectiveness of their damage on IAPS. It would give some sort of information regarding biological control method of IAPS. Herbivory damage assessment can be done in both IAPS and native plants and the damage severity can be compared to know whether the IAPS are less damaged and it is the cause of plant invasiveness. Testing Enemy Release Hyposthesis needs native congeners of IAPS. In case of absence of native congener the associated native species with IAPS can be taken for generating some basic outlines of herbiovry damage (Balami *et al*. 2017).

Moreover, before carrying out herbivory assessment it is always crucial to measure soil ecological studies of IAPS like density, frequency, coverage of IAPS and also, herbivory/pathogen damages assessment. Such studies are important to know the current status of IAPS and damage by herbivores. Herbivory assessment also helps to identify particular herbivore or pathogen. Since 1979 considerable progress has been made towards practical use of plant pathogens as safe and effective agents of weed management (Charudattan and Walker 1982; Kour *et al*. 2014). During long course of naturalization of IAPS some of the native herbivores and pathogens might have adapted to the IAPS. The study can be initiated from selecting some areas which are highly invaded since long time ago. In this study some areas of Kathmandu valley have been selected.

1.3 Research Questions

1.3.1 Major question:

1. What is the status of herbivory and pathogen damage in IAPS in Nepal?

1.3.2 Minor questions:

1. What is the ecological status of IAPS in Chobhar area of Kathmandu valley?
2. Is there any impact of habitat type in herbivores and pathogens on IAPS?

1.4 Objectives

1.4.1 General objective

To understand the effects of herbivory, pathogens and habitats on selected IAPS in Kathmandu valley.

1.4.2 Specific objectives

1. To measure density, frequency and coverage of IAPS in different habitats.
2. To evaluate the damage caused by herbivores and plant pathogens in the selected IAPS.
3. To find out fungal pathogens from the fungal infected leaves of IAPS.
4. To find out the soil rhizospheric fungi associated with selected IAPS.

1.5 Limitations

1. Herbivory damages on branches and leaves were assessed.
2. Potato Dextrose Agar media was only used for isolation of fungi.
3. Rhizospheric fungi were only isolated from the IAPS invaded soil.
4. Identification of fungi was based on morphology of colony and fungal structure.

CHAPTER –II

LITERATURE REVIEW

2.1 Diversity and Distribution of IAPS

An assessment of IAPS in Nepal for the first time was undertaken by IUCN during 2002-2003 and reported total of about 219 alien species of flowering plants (Tiwari *et al.*2005; Siwakoti 2012; Sukhorukov 2014) and 64 species of animals (Budha 2015) are naturalized in Nepal out of them 21 were IAPS. Documentation of IAPS in Nepal has been started since 1958 and most of them were unpublished master thesis research (Poudel and Thapa 2012).

According to Shrestha (2016), 26 species had listed as invasive with the addition of five species to the list of Tiwari *et al*. (2005). Among 26 IAPS from Nepal, 4 species namely; *Chromolaena odorata, Eichhornia crassipes*, *Lantana camara* and *Mikania micrantha* are included in world's 100 worst invasive species (Lowe *et al.* 2000; Shrestha *et al*. 2017). *Chromolaena odorata*, *Ageratina adenophora*, *Mikania micrantha* and *Parthenium hysterophorus* are among most studied IAPS in forest and grassland ecosystem of Nepal (Timsina *et al*. 2011; Shrestha *et al*. 2011 and Thapa *et al*. 2017).

Dobremez (1976), as cited in Shrestha (2016) reported that Nepal lies at the cross-road of six floristic provinces of Asia and the floral elements of all provinces are represented in Nepal. Topography of Nepal lies in widest elevation gradients (59 m to 8848 m) and can provide suitable habitat and climatic condition for organisms to servive. In addition to elevation gradient the climatic variation in different altitude is also peculiar and hence, Nepal is a hot spot for alien invasion.

According to the recent report, there are at least 179 alien species of flowering plants are naturalized in Nepal (Shrestha *et al*. 2019), out of them 26 are IAPS (Shrestha *et al.* 2017), belonging to 15 families are reported till now in Nepal. Among them, *Ageratum haustonianum, Alternanthera philoxeroides, Erigeron karvinskianus*, *Oxalis latifolia*, *Parthenium hysterophorus* and *Spergula arvensis*, tended to be highly invaded along agro-ecosystem and troublesome to farmers having high toxicity and low palatability (Ranjit 2013; Siwakoti *et al*. 2016; Shrestha *et al.* 2017). The majority of alien plant species in Nepal are confined to low land particularly in central Nepal (Tiwari *et al*. 2005). Though the Nepalese scientific community was aware of the arrival of IAPS since long, scientific study of the problem get accelerated only after 2000 and Central Department of Botany, Tribhuvan University is playing a main role in IAPS related research in Nepal (Poudel and Thapa 2012).

Bhattarai *et al.* (2014) studied on the distribution patterns of IAPS in the himalayas of Nepal and found that invasive plant species richness is highest in the central phytogeographic region followed by central and eastern regions as native plants does. Their study supports the hypothesis that, high native diversity facilitates invasive plant species, that is, the native and invasive plant species may require similar natural conditions, but invasive plant species seem more dependent and influenced by anthropogenic disturbances.

Murphy *et al.* (2013) investigated on; *Mikania micrantha* and found that its invasion has been a serious problem in the forest and grassland of the Chitwan National Park, Koshi Tappu wildlife Reserve and many other areas in the Siwalik and Terai regions. It is a perennial climbing herbs and one of the most important IAPS in many Asian countries including Nepal (Zhang *et al.* 2004; Willis *et al*. 2008). This species in Nepal was first reported in 1963 from eastern region and has spread towards western side of the country (Tiwari *et al.* 2005).

Perrings (2007) found that IAPS have caused damage costs equal to 53% of agricultural GDP in the USA, 31% in the UK and 48% in Australia. In addition, the damage costs reported in South Africa, India and Brazil were 96%, 78% and 112% respectively on agricultural GDP. Infact, the loss of agriculture production is more in developing countries than developed countries (Perrings 2007). Invasion of IAPS have also emerged threatening impacts in ecosystem, biodiversity and agriculture production of Nepal (MoFSC 2014).

Nepal physiographic and climatic diversity is also supporting suitable habitat for IAPS invasion. In addition, food security and agriculture sector is also more dependent on imported agriculture products, seeds and seedlings. Due to these scenario, Nepal’s agriculture sector has been ranked third among the most threatened countries out of the 124 countries measured (Paini *et al*. 2016; Shrestha 2017).

According to Adkins *et al*. (2014) *Parthenium hysterophorus*, which is a aggressively growing weed can be effectively managed through existing biological control strategies with suppressive plants, and this approach is likely to work into the future in a changing climate, and in other areas around the globe where weed *parthenium* is becoming a problem. *Eichhornia crassipes* is another most problematic IAPS threatening mostly in the Ramsar sites and it has several negative effects to the aquatic lives as well as to the livelihood of wetland dependent local communities (Shrestha 2016). This species is also included in 100 of the world's worst IAPS (Lowe *et al*. 2000).

According to Kasara *et al.* (1998) weeds are unwanted plant or a plant with a negative importance or plant that compete with human for the soil. Agriculture weeds are also undesirable plants that invaded agriculture land, where they reduce the productive capacity of crops (Pimentel 1986).

Weeds species occur in high abundance in all agriculture lands along with abundance of numerous seeds production and efficient capacity to resist the adverse environmental condition. Weeds flora are common problem of crops field and cause trouble to the farmers, for the management because they have effective dispersal ability and persistent propagule like rhizome, tuber, seeds etc. (Thomson *et al*. 1997; Honnay *et al.* 2002). Thus, control measure of such weeds are major challenge to the agricultural fields (Pimentel 1986).

The Levine *et al.* (2004) reported that competition, herbivores and competitors diversity have strong effects on invader establishment and their performance. In the range land of Northern Himalayas (India), invasion by *Parthenium hysterophorus*, *Ageratum conyzoides* and *Lantana camara* significantly decrease species richness in the invaded areas (Kohli *et al.* 2004). Similarly, many other studies (Bimova *et al*. 2004) reported decrease in species richness by invasion.

With increasing globalization of trade along with human movement, the number of alien species has been increasing in all climatic regions and continents (Seebens *et al.* 2017) from tropics to high mountain (Pauchard *et al*. 2016). In the world, at least 13,168 species of plants have become naturalized outside their native distribution range (Van Kleunen *et al*. 2015). It has been declared that IAPS are responsible for at least 3 of the 24 known extinction of endangered species in the US (Schmitz and

Simberloff 1997). Pimentel *et al.* (2005) mentioned that, around 80% of the endangered species are threatened as a result of invasion by alien species.

2.2 Biological Invasion Mechanism

Alien Plant species are often transported outside of their native range, and some of these plants will naturalize without creating major problems (Thomas and Palmar 2015). Biological invasion happens when an organism arrives somewhere beyond its natural range of distribution (Williamson 1996). According to Vitousek *et al.* (1997), biological invasion is one of the well documented changes in biodiversity and environment globally. Rapid expansion of Invasive plants are threatening biodiversity, crop production and ecosystem health throughout the world (Moles *et al*. 2012).

Nowadays, most invasions happens from human actions, either intentionally or accidently. Invasion grows rapidly since the mid-twentieth century as scientific researcher have become increasingly aware of the many applied issue of managing invasive species, as well as fundamental ecological questions raised by biological invasions (Lowery *et al*. 2013).

Biological invasion is a global phenomenon that has the capacity to dramatically alter native community (Alvarez and Cushman 2002). More than 50% of the IAPS are considered as harmful around the world (Richardson *et al*. 2000). All the exotic species do not have the potential to naturalize and all the naturalized do not have the potential of invasion. Based on ‘tens rule” (Williamson 1996), probability of naturalized species become invasive approximately 100:10. This means 10% of the introduced species have chance to turn into naturalized and 10% of them have chance to turn into invasive i.e. the 0.1% of the introduced species are potential to turn into invasive species.

The Enemy Release Hypothesis predicts that specialist enemies of an alien (exotic) species will be not present in areas where it has been introduced. By explanation, specialist enemies that attack a single alien species (i.e. single-species specialists) do not occur outside the native range of their host. However, there are two main methods through which specialist enemies can be found in the exotic areas: host switching (divert) and co-introduction of enemies. If a plant species is introduced to a region that contains closely related native congeners, the specialist enemies of those congeners might switch to attack the exotic species (Connor *et al*. 1980; Jobin *et al*. 1996). Several studies have shown that specialist insect herbivores can switch to exotic congeners, although their impact is rarely measured (Bowers *et al.* 1992; Ros *et al*. 1993).

Aggrawal *et al.* (2005) worked over the ERH in 30 taxonomically paired native and introduced plants. They found that natives are more vulnerable to negative feedback from soil pathogens and benefited less from positive feedback from mycorrhiza and other beneficial microbes. The results of their experiment showed that introduced plants suffered less negative feedback than natives. For instance, *Artimesia* and *Geum* experienced both greater herbivory and stronger negative soil feedback than that of their introduced congeners.

Ashton *et al.* (2005) concluded that litter from the exotic species gets decomposed and release nitrogen significantly faster than litter from the native species. The result suggests that the invasion of exotic species alters decomposition and nutrient cycling in forest.

Balami *et al.* (2017) reported that; species richness of soil fungi was lower in the *A. adenophora* invaded soil compared to the uninvaded soil. *Ageratina adenophra* invaded soil accumulated higher frequency of pathogenic fungi than that of saprophytic fungi. This study concluded that the invasive *A. adenophora* modifies belowground soil fungi communities as one of the mechanisms involved in the successful invasion of *A. adenophora*.

Boudiaf *et al*. (2013) studied on *Acacia mearnsii* and showed that presence of an exotic *A. mearnsii* highly impacts soil properties, microbial functions and ectomycorrhizal community (structure and colonization rate) of natural habitats which lead to decrease of the early growth of *Quercus suber* (native) seedling.

The literature on the plant soil interaction strongly suggests that the introduction of a new plant species, such as invasive exotic, has the potential to change many components of the carbon, nitrogen, water and other cycles of ecosystem and finally invade into the new areas and also alter soil nutrients dynamics by differing from native species in biomass and productivity, tissue chemistry, plant morphology and phenology.

According to Fan *et al*. (2013) found that the induced resistance of *Alternanthera sessilis* (Native) was more efficient and sophisticated than that of the invasive congener after herbivores attack. The native herbivores can constrain the abundance and reduce the adverse effects of invasive species once they have successfully established (Levine *et al*. 2004).

2.3 Impacts of IAPS on ecological and socio-economic aspects

The problem of invasive species is prevalent both in developed as well developing countries, but their impact is likely to be higher in developing countries like Nepal due to the lack of resources and expertise (Shrestha 2016). Invasion of alien species (animals, pests, viruses, pathogens and plant) cause serious threat to biodiversity because of their negative effects on flora and fauna species, food web, their habitat and also to ecosystem dynamics (Karki and poudel 2014).

Biologicalinvasion has been considered as an important component and major cause of human-produced results that losses biodiversity (Vitousek *et al*. 1997; Pimentel *et al*. 2000). Its impacts have been predicted to increase even further under future climatic condition (Paini *et al.* 2016; Early *et al.* 2014). Biological invasion is a pervasive global change, challenging the conservation of biodiversity and natural resources (Simberloff *et al.* 2013).

IAPS compete with native plants for nutrients, water and light (Witkowski 1991; Aderson 1996) and can be allelopathic and alters native ecosystem and diversity (Mack *et al*. 2000; Day *et al.* 2003; Barton *et al*. 2007). It is one of the main causes of biodiversity loss especially from species extension to decline in their numbers (Bellard *et al*. 2016; Downey and Rirchardson 2016).

It has been affirmed that Invasive Alien Species are responsible for at least 3 of the 24 known extinction of endangered species in the US (Schmitz and Simberloff 1997). Pimentel *et al*. (2005) mentioned that around 80% of the endangered species are threatened as a result of invasion by alien species. According to the Invasive specialist

Group, out of the 100 species that have been identified as the worst invasive species in the world, 32 are invasive plants species (Lowe *et al*. 2000).

Invasive species caused damage costs equal to 53% of agricultural GDP in the USA, 31% in the UK and 48% in Australia (Perrings 2007), By contrast damage costs in South Africa, India and Brazil were 96%, 78% and 112% respectively on agricultural GDP (Perrings 2007), infact the loss of agriculture production is more in developing countries than developed countries. High economic loss due to IAS has been reported from many countries, one example in USA and an estimates indicates that invasive plants and animals entail US $20 billion direct economic losses each year in various sectors (Gould 2004).

Four species namely *Chromolaena odorata* (L.) R.M. King and H. Rob, *Eichhornia crassipes* (Mart.) Solms, *Lantana camara L.* and *Mikania micrantha* Kunth, are included in 100 of the world's worst invasive alien species (Lowe *et al*. 2000), because they cause problem in our forest and agriculture land.

2.4 Impacts of allelochemicals on soil microbial communities

Callaway and Aschehoug (2000) suggested that differential effects of roots exudates on the invasion *Centaurea diffusa*, noxious weed of North America have been correlated with invasion success.

Stinson *et al.* (2006) reported that *Alliaria petiolata*, a European invaders of North American forests, suppresses native plant growth by disrupting mutualistic association between native canopy tree seedlings and belowground arbuscular mycorrhizal fungi representing its antifuingal phytochemistry. Respective result explain an indirect mechanism by which invasive plants can impact native flora.

Diffuse knapweed (*Centaurea diffusa*), a Eurasian knapwood species that has invaded many natural ecosystem in western North America. This species releases the chemical 8-hydroxy quinoline from its roots, which has been demonstrated to be an antimicrobial agent (Vivanco *et al*. 2004).

Essential oil production from *Ageratina adenophora* had shown the presence of several organic compounds like sabinene and 1, 8-cineole (Padailia *et al*. 2010).

These compounds were found to have antibiosis properties to the higher plants and to the soil microbes (Inderjit *et al*. 2011).

2.5 Biological control of IAPS and assessment on herbivores and fungal pthogens

Biological control methods seems safe, sound and involve the introduction of host-specific natural enemies (invertebrates or diseases) of the IAPS from its original native range. Alien plants natural enemies may be able to survive, and even reproduce, but does not invade aggressively in its new habitat (Fowler *et al*. 2000). This methods should therefore be used as a means of increasing the efficacy in control methods and thus, assume as a key role in all integrated weed management programmes (Olckers *et al*. 1998). Since 1990's several agreements and technical guidelines were formulated to minimize the risk of invasive alien species. Nepal is also a signitary to the major international agreements related to IAPS (Shrestha *et al*. 2016).

There are several studies carried out by many researchers concerning the biological control of IAPS globally but in case of Nepal limited works had been conducted. According to Balami *et al*. (2017), the level of herbivory damage in native *Alnus nepalensis*, in the place invaded by *Ageratina adenophora* in Nepal was measured and finally result showed that invasive plant experienced lower level of herbivory damage than native plants does. It means because of ERH (reduced herbivory damage) Invasive *A. adenophora* might have benefited behind its successful invasion in Nepalese forest.

Thapa *et al*. (2017) found that *Ageratina adenophora* invaded soil and its litter inhibit *Schima wallichii* seedling growth when *Schima wallichii* densities were less. But vice-versa when *Schima wallichii* densities were higher, the inhibitory effect of *A. adenophora* on *S. wallichii* may be minimised either by removing litter and/or maintaining high seedling densities, it might get occurred because of allelochemicals in invasive.

Biological control by herbivores, according to Shrestha *et al.* (2011), an insects *Zygogramma bicolorata* fortuitous arrival had led to some degree of weed suppression (*Parthenium hysterophorus*). Since 2009, in some locations around

Hetauda, Bharatpur, Butwal and Bhairahawa, Nepal. However, it is speculated that *Z. bicolorata* entered Nepal in the same way as *P. hysterophorus*, along road corridors from India.

Puccinia abrupt var. *partheniicola*, the winter rust, is also present in Nepal. This winter rust had first encountered in Nepal at Kirtipur, Kathmandu valley in May 2011 (Shrestha 2012). The rust has remained localized just in a few locations in the valley and the damage caused by this agent has been minimal. This winter rust is highly host specific and naturally found in Central and South America infecting *P. hysterophorus* (Parmelee 1967).

Accordingly, Koirala *et al.* (2011) found that exotic rust *Puccinia spegazzinii* and its strain were the effective biological control released in *Mikania micrantha* in Chitwan Nepal for the very first time.

Globally, on the basis of pathogenicity test, Barton (2004) reported 26 species of fungi originating from 15 different countries have been used as classical biological control agents over weeds in 7 different countries. For eg. In *Parthenium hysterophorus*, *Puccinia abrupta* var. *partheniicola* and *Puccinia melampodi* were recognized as biological control agent. In case of *Ageratina adenophora* two agents (1) *Procecidochares utilis*, a stem gall fly and (2) *Passalora ageratinae*, leaf-spot fungal pathogens individually were released on *Ageratina adenophora* for effective biocontrol agent. Biological control of IAPS seems safe, sound and effective method, though the use of pathogenic fungi as classical biological control agents for weeds began in 1971.

According to Morris (1989), a leaf-spot pathogens *Phaeromularia* species was used for biological control of Crofton weed (*Ageratina adenophora)* in South Africa and this pathogens seems host specific. Similarly, for control of *Parthenium hysterophorus* in North East Maxico, 3 different pathogens were used as biological control agents (1) *Puccinia abrupt* (2) *Puccinia halstedii* and (3) *Entyloma compositarum* (Romero *et al*. 2001).

Guatimosim *et al*. (2015) reported ten pathogenic fungal pathogens from *Bidens pilosa* and *Bidens subalternans*, namely; *Cercospora bidentis*, *C. maculicola*, *Entyloma bidentis*, *E. compositarum*, *E. guaraniticum*, *Neoerysiphe cumminsiana*, *Plasmopara halstedii*, *Podosphaera xanthii*, *Uromyces bidentis*, and *U. bidenticola*. Three other species were found closely associated only with *B. subalternans*: *Colletotrichum bidentis***,** *Pseudocercosporella bidentis* and *Sphaceloma bidentis* and *Pseudocercosporella bidentis* is new to science from Brazil.

According to Agrawal *et al.* (2014) had enumerated about 26 fungal pathogens including *Alternaria* sp., *Cladosporium* sp., *Colletotrichum* sp., which were identified for biological control in South Africa for biological control. Several exotic plant pathogens as *Puccinia chondrillina* and *Entyloma ageratinae* has helped to control the weeds *Chondrilla junica* in Australia, *Ageratina riparia* in Hawaii respectively (Julien and Griffiths 1998).

Vila *et al*. (2005) tested prediction by comparing herbivore pressure on the native European and introduced North America populations of *Hypericum perforatum* and found that the *H. perforatum* are less damaged by insect herbivory and suffer less mortality than population in the native range.

Buccellato *et al.* (2012) found that 2 agents *Procecidochares utilis*, a stem gall fly and *Passalora ageratinae*, a leaf-spot fungal pathogens were released in South Africa for control of weeds but according to their findings, both agents reduce stem height and percentage of live leaves but there was no synergistic effect of two agents together. While in case of Nepal, Stem galling fly *Procecidochares utilis* stone and Leaf spot fungus *Passalora ageratinae* Crous and A.R wood from *Ageratina adenophora* (Winston *et al.* 2014; Shrestha *et al*. 2016). However, these biological control agents were introduced accidently from India.

Kelaniyangoda and Ekanayake (2010) found fungal infection at different growth stages of the *Parthenium*; rosette, preflowering and flowering stages, suppressed the plant growth, especially the leaf and flower production. According to them, the fungus *P*. *melampodii* which is host specific and can be effectively used as a potential bio-control agent against the *Parthenium* weed.

Liu and Stiling (2006) found that the insect herbivores fauna richness was significantly greater in the native plants than the Invasive plants and the reduction is twisted disproportionally towards specialists and insects feeding on reproductive parts. They also found that the herbivores damages level was less on the introduced invasive congeners than on the native plants.

Herbivores affect plant growth and fitness, not only by damaging organs and tissues (e.g., leaves, phloem, roots and twigs) but also by altering physiological traits. For instance, herbivory often affects the concentrations of available nitrogen and other important nutrients in foliage (Karban *et al.* 1997), significantly decreases photosynthetic activity (Zangerl *et al.* 2002; Nabity 2009) and increases leaf conductance, transpiration rate, and intercellular CO2 concentration (Marlina *et al.* 2013) of the remaining intact tissue.

To minimize damage, plants have developed resistance strategies against herbivores. Resistance strategies should reduce the preference or performance of herbivores and include constitutive resistance (permanently expressed irrespective of herbivore attacks) and induced resistance expressed only after herbivore attacks (Farfan *et al.* 2007). The biotic resistance hypothesis proposes that native enemies have a greater impact on exotic plants than on native plants (Paker and Hay 2003).

IAPS also contains anti-herbivory chemicals which are allelopathic and anti-microbial in characteristics. They have two main strategies to resist herbivory. They may employ constitutive defences, which are expressed continuously or induced defences and stimulated only after attack from an herbivory (Karban and Baldwin 1997). Such Induced defence have been demonstrated in over 100 plant species and elevate defensive responses when herbivores are present (Karban and Baldwin 1997; Agrawal and Rutter 1998).

CHAPTER – III

MATERIALS AND METHODS

3.1 Study Areas

Current study was conducted at three sites of Kathmandu valley that are Chobhar, Champadevi and Tribhuvan University area, Kirtipur. In the Chobhar area of Kirtipur, Kathmandu, Nepal ecological survey, herbivory damage assessment and sampling of rhizospheric soils were carried out. The Chobhar lies in Kathmandu district in the Bagmati Zone of Central Nepal and part of Kirtipur Municipality. Chobhar is situated at $27^{o}27'$ N and $85^{o}28'$ E and 1310 to 1346 m elevation above the sea level. The climate of the area is mild and generally warm and temperate.

Kathmandu-Hetauda road goes through Chobhar area. The road makes a half circle to the Chobhar area from northern side to southern side. Towards southern side there is a big area of fallowland and small forest near the site of Himal Cement Factory previously. All the subsites (along the road towards north and south facing slope, fallow land and the forest) were invaded by four types of major invasive alien species that are *Ageratina adenophora, Ageratum conyzoides, Bidens pilosa and Parthenium hysterophorus*. The field survey was conducted in September to November, 2018.

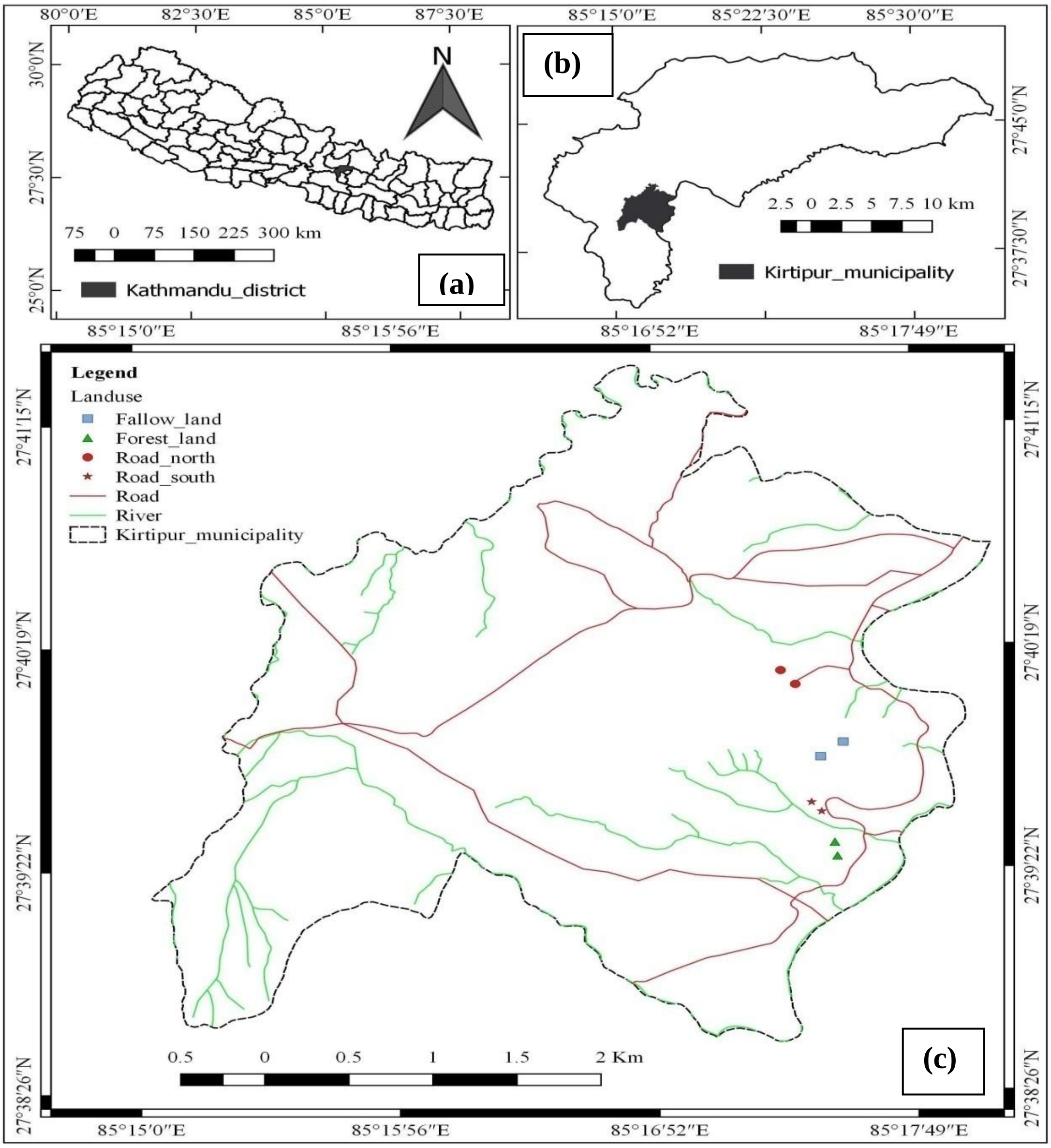

Figure 1: Diagrammatic representation of study area (Map prepared using QGIS), from where ecological survey, herbivory damages assessment and rhizospheric soil and fungal infected leaves of IAPS were collected.

Collection of fungal infected leaves of IAPS for fungal pathogens isolation was carried out from three sites; Chobhar, Champadevi and its adjoining areas of Kirtipur, Kathmandu. The Champadevi community forest is located at southwest part of Kathmandu valley (27^{o}42'06" N and 85^{o}19'14" E). The climate of the area is hot and humid in summer and dry in winter. Annual mean temperature is approximately 18^{o}C. Average annual precipitataion is about 1343 mm. Elevation: 2229 m. One of the worst invasive alien species *Ageratina adenophora* had found severely invaded entire forest of Champadevi upto the 2300 m at the top of the hills.

On the late winter (May, 2019) the winter rust *Puccinia abrupt* var. *partheniicola* was found highly spread in *Parthenium hysterophorus* around Tribhuvan University area, Kirtipur, Kathmandu. At the same time, the severity of infection of the pathogen was measured. The Tribhuvan University is located at an elevation of 1320 m near to Nayabazar Kirtipur.

Entire study comprises 3 experiments, field experiment on assessment of herbivory damages, density, frequency, coverage and collection of rhizospheric soil sample and collection of fungal pathogens infected IAPS leaves. Second experiment was laboratory experiment on culture, isolation and identification of soil rhizospheric fungi, soil parameters and fungal pathogens from IAPS leaves. Third experiment was green house experiment for pathogenicity test of fungal pathogens identified from IAPS leaves by Koch's Postulates.

3.2 Climate

The study area lies in the sub-tropical zone. The climatic data recorded at the nearest weather station shows that the hottest month was June (Maximum temperature 26.1°C) and the coldest month January (minimum temperature 3.5°C) (Figure 2). The monthly mean temperature of the area ranges from 10.67°C to 23.25°C with annual mean temperature of 16.72° C. The monthly precipitation was minimum in November (4.87 mm) and maximum in July (389.95 mm) with annual mean precipitation of 1612.12 mm (Figure 2). Out of the total annual precipitation, 75% rainfall was observed during June to September.

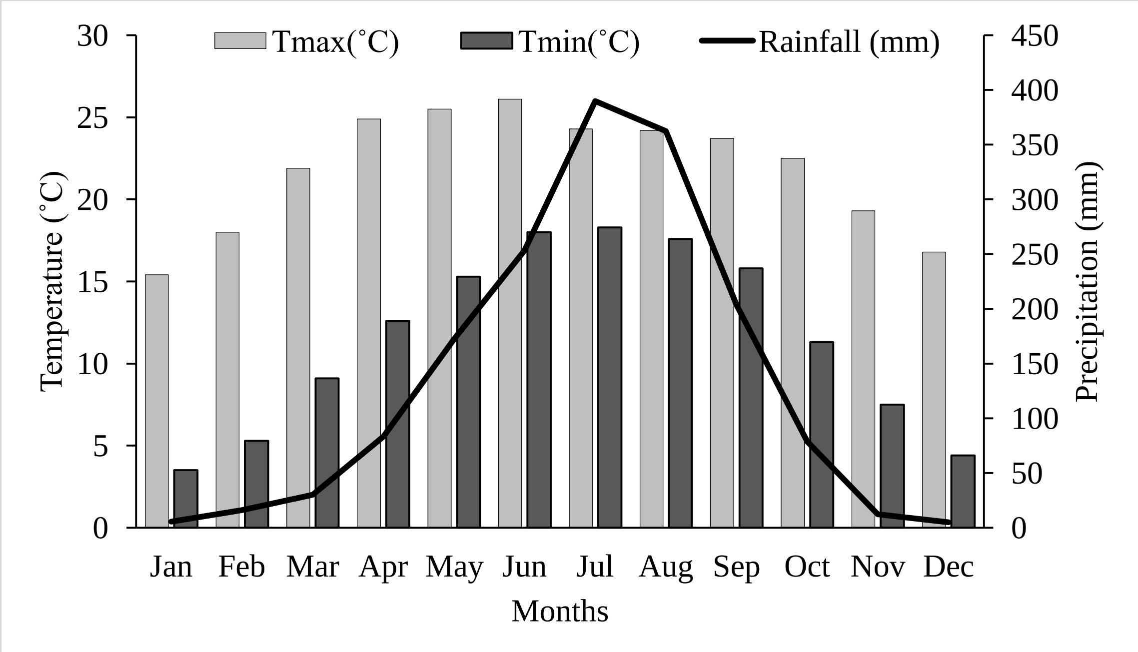

Figure 2 Ten years (2009-2018) mean monthly maximum and minimum temperature (ºC) and Precipitation (mm) recorded at Panipokhari (Kathmandu) the nearest weather station; which is located at around 4 km far from the study area. (Source: Department of Hydrology and Meterology/GoN 2018).

3.3 Field Assessment

During the field survey 4 subsites (forest, fallowland, road site towards north and south facing slopes of Chobhar area) were selected. These sites were found highly invaded by IAPS. The major IAPS were *Ageratina adenophora, Ageratum conyzoides, Bidens piolsa* and *Parthenium hysterophorus*.

3.3.1 Sampling process

Ecological parameters like; density, frequency and coverage were measured. All these parameters were measured by sampling quadrats of size 1×1m². In the road sides the quadrates were sampled along the road. The distance between two quadrats was 10m. In the forest and fallowland, five transects were made and in each transect 5 quadrates were sampled. A total there were 25 quadrates at each study site.

3.3.2 IAPS density

Density is the total number of species present in the each and every sampled plot. Numer of IAPS present in each quadrat was counted. Average density was calculated from all the sampled plots. The density value thus obtained for each quadrates studied is to be expressed as individual per quadrat.

3.3.3 IAPS frequency

Similarly, frequency stands for the presence or absence of total number of IAPS in the plot studied. It was calculated by using formula;

$$\text{Frequency (\%)} = \frac{\text{No.of quadrates in which selected invasive plant species occurred}}{\text{Total number of quadrates studied}} \times 100$$

3.3.4 IAPS coverage

Coverage, it is defined as the area occupied by the IAPS in each plot. It was measured in percentage (%) by the visual estimation (observation) starting from >5, 5, 10, 15, 20% and so on.

3.3.5 Assessment on Herbivory damages in IAPS

Herbivory damages were also measured in Chobhar area. Plants were selected from the plots sampled for density, frequency and cover estimation. Herbivory damage on the selected IAPS (*Ageratina adenophora, Ageratum conyzoides, Bidens pilosa, Parthenium hysterophorus*) was measured as the percent damage on leaves and branches.

From each quadrat, three individual plants of each IAPS were selected from the three corners of the triangle made inside each quadrates to count number of healthy and herbivory infected branches and leaves. Herbivory damages in branches and leaves include guilds as; necrotic spots, insects bites, leaf spots other than fungal pathogens, leaf rolling, leaf curling, galls, tumor and blights were taken to measure as herbivory damages. The damage percent of leaves and branches was calculated by following formula:

$$\text{Percent damage (\%)} = \frac{\text{No. of branches or leaves damaged}}{\text{Total no. of branches/leaves present}} \times 100$$

3.3.6 Collection of fungal pathogens from IAPS

During field survey, Chobhar, Champadevi and adjoining areas were visited to search pathogens associated with IAPS. The IAPS infected by fungal pathogens were collected. About 25-30 infected leaves were collected in sterile bag and brought in the CDB lab and preserved in freeze (at 4°C for about 15 hrs). Symptoms of fungal pathogens were rust, leaf spots, anthracnose, mildew etc.

3.3.7 Measurement of infection severity of *Puccinia abrupt* var. *partheniicola*

On the late winter (May, 2019) the winter rust *Puccinia abrupt* var. *partheniicola* was found highly spread in *Parthenium hysterophorus* around Tribhuvan University, Kirtipur area. At the same time, its severity assessment was done. A total of 50 quadrats were sampled along road side. The size of the quadrat was $1{\times}1m^2$ and distance between two quadrats was about 5m to 10 m. First, the total plants present in the each quadrate were counted and at the same time total infected plants were counted.

Percentage of severity infection in plants can be calculated as;

$$\text{Disease Severity (\%)} = \frac{\text{Total number of infected plants present in quadrats}}{\text{Total number of plants present in quadrats}} \times 100$$

Then, in the infected plants counted, infection severity classess was categorized as:

1. Less infected plants (infection <25%),

2. Moderately infected plants (infection >25% and less than 50%)

3. Highly infected plants were measured (infection >50%).

3.3.8 Collection of rhizospheric soil samples

During the field work in the Chobhar area, four IAPS were selected for collection of the rhizospheric soil. The soil was collected by uprooting the IAPS and the soil around the roots was brushed using sterile brush. The IAPS were *Bidens pilosa*, *Parthenium hysterophorus*, *Ageratina adenophora* and *Ageratum conyzoides*. Five individual plants of each species were uprooted at distance of 15/20m. The soils samples were then mixed to make composite sample for each species. A total 25 individual plant of each IAPS were uprooted and each composite sample had rhizospheric soil of 5 individual IAPS. Soil samples were collected in the sterile plastic bag and transported to CDB Lab. In this way all invasive species soil sample were collected and stored in freeze (at 4^{o}C) for one day.

3.4 Laboratory Experiment

3.4.1 Culture, isolation and identification of fungi in infected leaves

Fungal infected leaves collected from the field were brought to the CDB Lab which was preserved in the freeze at 4^{o}C and next day, leaves collected were washed with sterilized distilled water in order to remove dust and the adherent soil particles. Some pathogens such as rusts were not washed but observed directly in the compound microscope to identify.

The infected parts of the leaves were cut into 1-1.5 cm fragments, surfaces were sterilized with 70% ethanol for 1-2 minutes and finally, were rinsed in sterile distilled water for 2 to 3 times. These fragments were transferred on to the Potato Dextrose Agar (PDA) medium (potato; 200 g, agar-agar; 20 g, dextrose; 20 g, distilled water; 1000ml). Petriplates were supplemented with streptomycin antibiotic and were incubated at 25±2^{o}C (Aneja *et al.* 2014) for 7 days.

3.4.2 Isolation of fungus from rhizospheric soil

Fungi from the rhizospheric soil were cultured by using soil plate methods (Warcup 1950). About 0.05 gm of soil samples was scattered on bottom of sterile petri-plate and molten cooled PDA media was added, which was rotated gently to disperse the soil particles in the medium. 1% of the amoxicillin solution was added to the medium before pouring it into the petri-plate for preventing the bacterial growth. The plates were incubated at 26±1^{0}C for 7 days for the proper growth and development of fungus. Fungal colonies were examined under stereoscopic microscope. The fungal colonies were sub-cultured for the identification and record the diversity of fungus present in the soil.

3.4.3 Identification of fungi

The identification of the fungi was made in situ (if possible) otherwise its pure culture in PDA plates was prepared for later identification. Fungal colony characters were observed and temporary slides were prepared for microscopic observation by Scotch tape method. Microscopic and macroscopic morphological criterion was considered for identification process. The fungi grown were isolated, pure cultured and observed under stereoscopic microscope. The fungi were identified using standard literature: Barnett and Hunter (1960), Watanable (2010), Gilman (1975), Illustrated genera of Imperfect Fungi by Barnett, H. L. (1960), Booth (1971), Mallikarjuna and Jayapal (2015), consulting supervisor and co-supervisors and senior scientists. Photographs of all fungi were taken and identified genera and species are described in Appendix I and Photoplates 1, 2 and 3.

3.4.4 Determination of soil parameters

- **Soil pH**

Soil pH was determined by using Fischer's digital pH meter in 1:2 ratio of soil and water (ASTM G51-95, 2012). Ten gram of soil was taken in a beaker and 20ml of distilled water was added to the beaker containing soil and the soil water suspension was stirred with a glass rod thoroughly for about 5 minutes and then left it for half an hour. The pH meter was turned on and allowed to warm up for 15 minutes. Before measuring the soil pH, the pH meter was caliberated using the buffer solution of known pH (pH 4, 7 and 9). The electrode was dipped into the beaker containing soil water suspension and the pH value was noted after waiting for 30 seconds.

- **Soil EC**

EC of the soil was measured by using Fischer's digital EC meter in 1:2 ratio of soil and water (ASTM G187-05, 2005). Ten gram of sieved soil was taken in a beaker and 20 ml of distilled water was added to it and the soil water suspension was stirred with a glass rod for about 5 minutes. The EC meter was turned on and allowed to warm up. Before measuring the EC of the soil meter was washed with distilled water. The electrode of the EC meter was dipped into the beaker containing soil water suspension and the value of EC was noted.

3.5 Statistical analysis

Data were entered in MS Excel 2010 and were analysed using SPSS (version 23). Damages shown by herbivores in *Bidens pilosa* was analysed using One-way ANOVA while in case of *Ageratina adenophora, Ageratum conyzoides* and *Parthenium hysterophorus* herbivory damages data were analysed using T-test. Density and coverage of IAPS from every sites were analysed using One-way ANOVA. Similarly, frequency data and rhizospheric fungi data were entered in MS Excel 2010 and graph were prepared.

CHAPTER – IV

RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Density of IAPS in different sites

Ageratina adenophora had the highest density in the Chobhar area in the forest (27 plants per plot). In case of *Ageratum conyzoides* the highest density was found along the road side towards north aspect while it was absent in the fallow-land. The density of *Bidens pilosa* was the highest (28 plants per plot) in the road side along north aspect. Fallowland was the site where the density of *B. Pilosa* was the least i.e. 4 plants per plot. The density of *Parthenium hysterophorus* was the highest in road side along the south aspect. The least density of *P. hysterophorus* was measured in the forests and road side along the north aspect (**Figure 3**). **Table 1** shows statistical details.

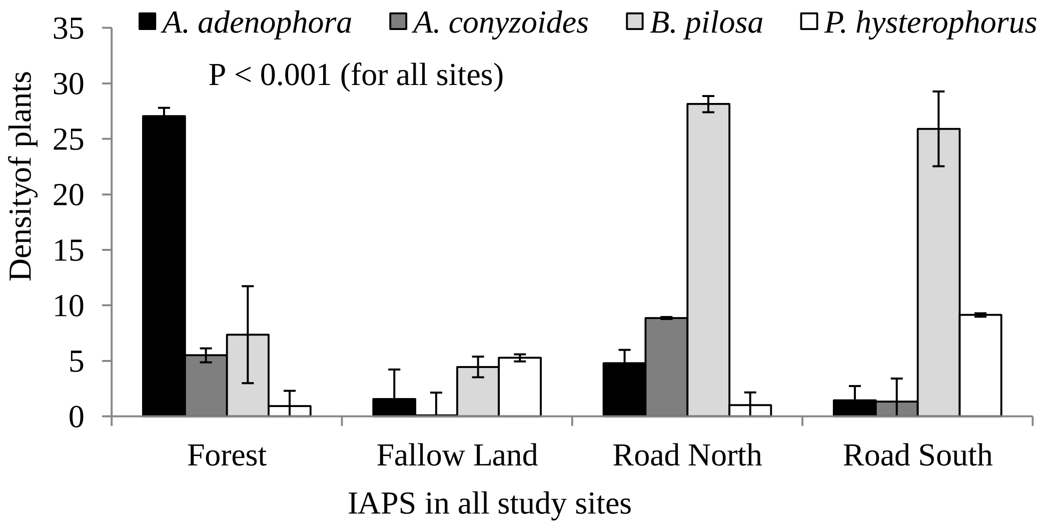

Figure 3: Density of IAPS in different study sites

Table 1: Test statistics for density of IAPS in different study sites

Test statistics	Non-parametric (Kruskal-wallis) test. forest	Fallowland	RoadNorth	RoadSouth
chi-square	51.45	57.27	42.38	55.68
df	3.00	3.00	3.00	3.00
P value	P<0.001	P<0.001	P<0.001	P<0.001

4.1.2 Frequency of IAPS in different sites

Almost all species are present in all the study sites of Chobhar area. In case of *Ageratina adenophora* the highest frequency was found in forest (100%) and least frequency in the fallowland followed by the road north and road south side as shown in **figure 4**. Similarly, highest frequency in road north and lowest in fallowland was found in case of *Ageratum conyzoides*. In *Bidens pilosa*, maximum frequency were found in road towards north aspect (100%) and forest (100%) followed by road towards south aspect (95%) and fallowland (91%). Finally, in *Parthenium hysterophorus*, highest frequency was found in road towards south aspect (85%) and least frequency in road towards north aspect (18%) followed by forest and fallowland (**Figure 4**).

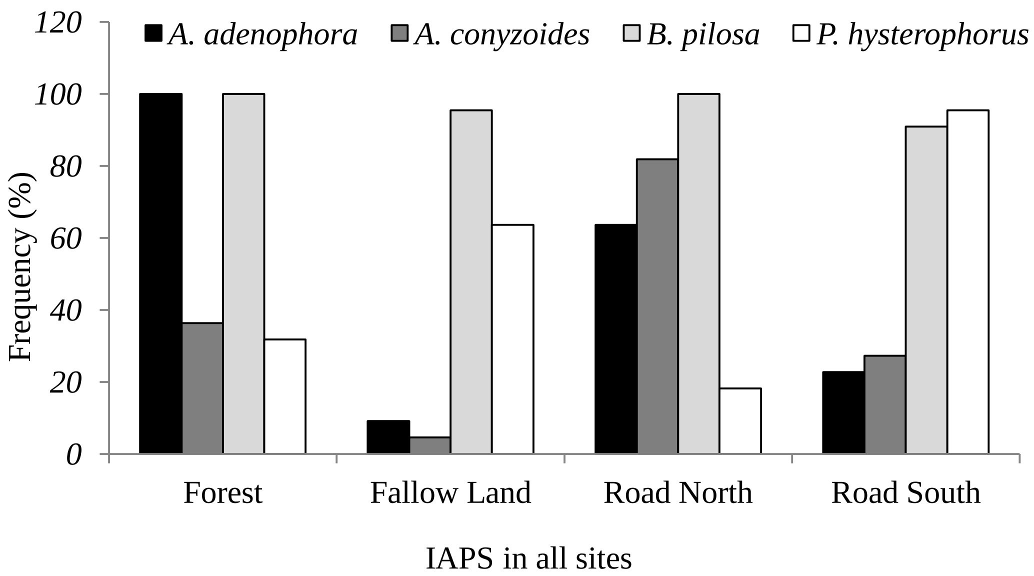

Figure 4: Frequency of IAPS in different study sites

4.1.3 Coverage of IAPS in different sites

In *Ageratina adenophora*, the highest coverage was found in forest (38%) and least coverage in fallowland followed by road towards north and south aspect (**Figure 5**). Similarly, in case of *Ageratum conyzoides*, maximium coverage was found in forest in comparison to the coverage found in fallowland, road towards north and south aspects. While talking about *Bidens pilosa*, maximum coverage was found in road towards north aspect (34%) followed by road towards south aspect (31%) and minimum coverage was found in forest followed by fallowland (8%). Finally, the highest coverage was found in road side south aspect (18%) followed by fallowland and least in road side north aspect in *Parthenium hysterophorus* (**Figure 5**). According to sites and species, data are not normal. Therefore, Non-parametric test (Kruskal wallis test) was applied (P<0.001) (**Table 2**).

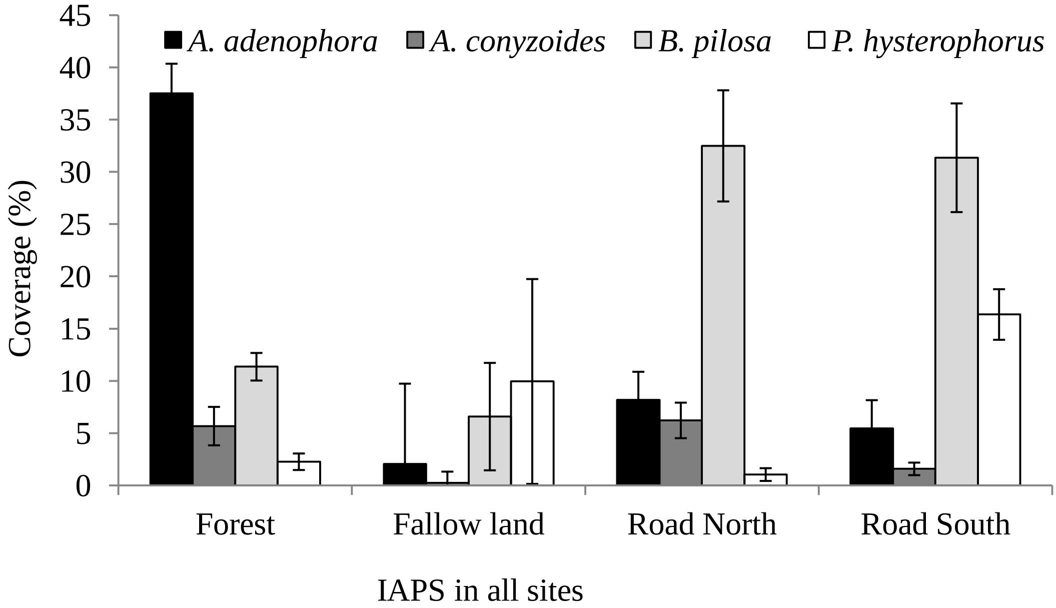

Figure 5: Coverage of IAPS in different study sites.

Table 2: Test statistics for coverage of IAPS in different study sites

	Forest Kruskal-wallis test	**Fallowland**	**Road Towards North Aspect**	**Road Towards South Aspect**
Chi-Square	61.113	41.302	47.112	46.730
df	3	3	3	3
Asymp. Sig.	P<0.001	P<0.001	P<0.001	P<0.001

4.1.4 Relationship between densities of different IAPS

Regression analysis showed that there is relationship in between density of *Bidens pilosa* with the density of *Ageratina adenophora* in forest (P = 0.822) and fallowland (P = 0.131) shows relationship between density of *B. pilosa* and *P. hysterophorus* (**Figure 6**). In road towards north aspect, there was no relation between densities of *Bidens pilosa* and *Ageratum conyzoides* (P=0.446). Similarly, in road towards south aspect, there was no relation between densities of *Bidens pilosa* and *Parthenium hysterophorus* (P=0.124) (**Figure 6**).

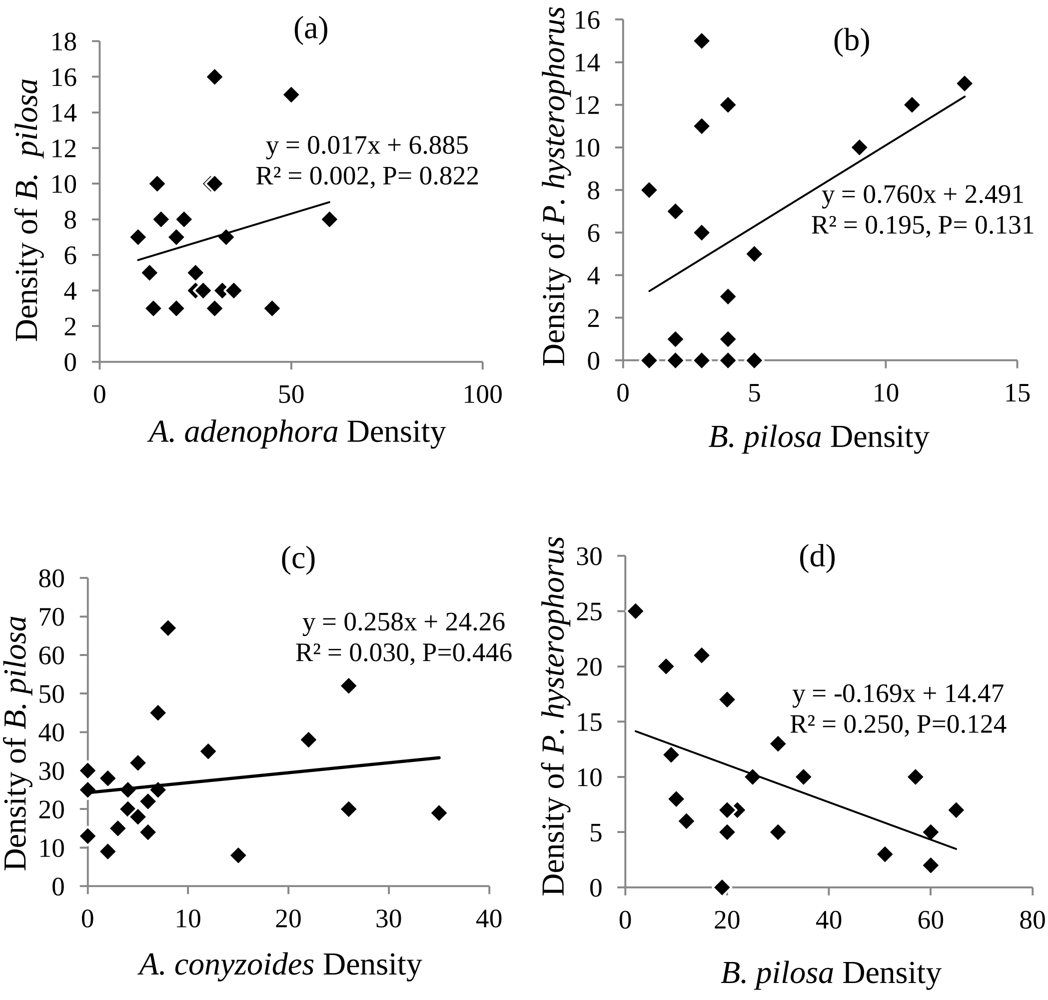

Figure 6: Relationship in between the density of selected IAPS from Selected sites. **(a)** *Bidens pilosa* and *Ageratina adenophora* in forest site **(b)** *Bidens pilosa* and *Parthenium hysterophorus* in fallowland **(c)** *Ageratum conyzoides* and *Bidens pilosa* in road towards north aspects **(d)** *Bidens pilosa* and *Parthenium hysterophorus* in road towards south aspects

4.1.5 Relationship between coverage of different IAPS

There was negative relationship between *Ageratina adenophora* and *Bidens pilosa* coverage i.e. the cover of *Bidens pilosa* decreases on increasing the cover of *Ageratina adenophora* (**P = 0.020, Figure 7**). In fallowland there was no positive or negative relationship between coverage of *Bidens pilosa* and *Parthenium hysterophorus* (**P = 0.0764**). Similarly, in **Figure 7**(c), there was no positive or negative relationship between coverage of *Bidens pilosa* and *Ageratum conyzoides* (**P = 0.101**). In the road side towards south aspect, there was negative relationship between *Parthenium hysterophorus* and *Bidens pilosa* coverage i.e. the cover of *Parthenium hysterophorus* decreases on increasing the cover of *Bidens pilosa* (**P =0.004, Figure7**).

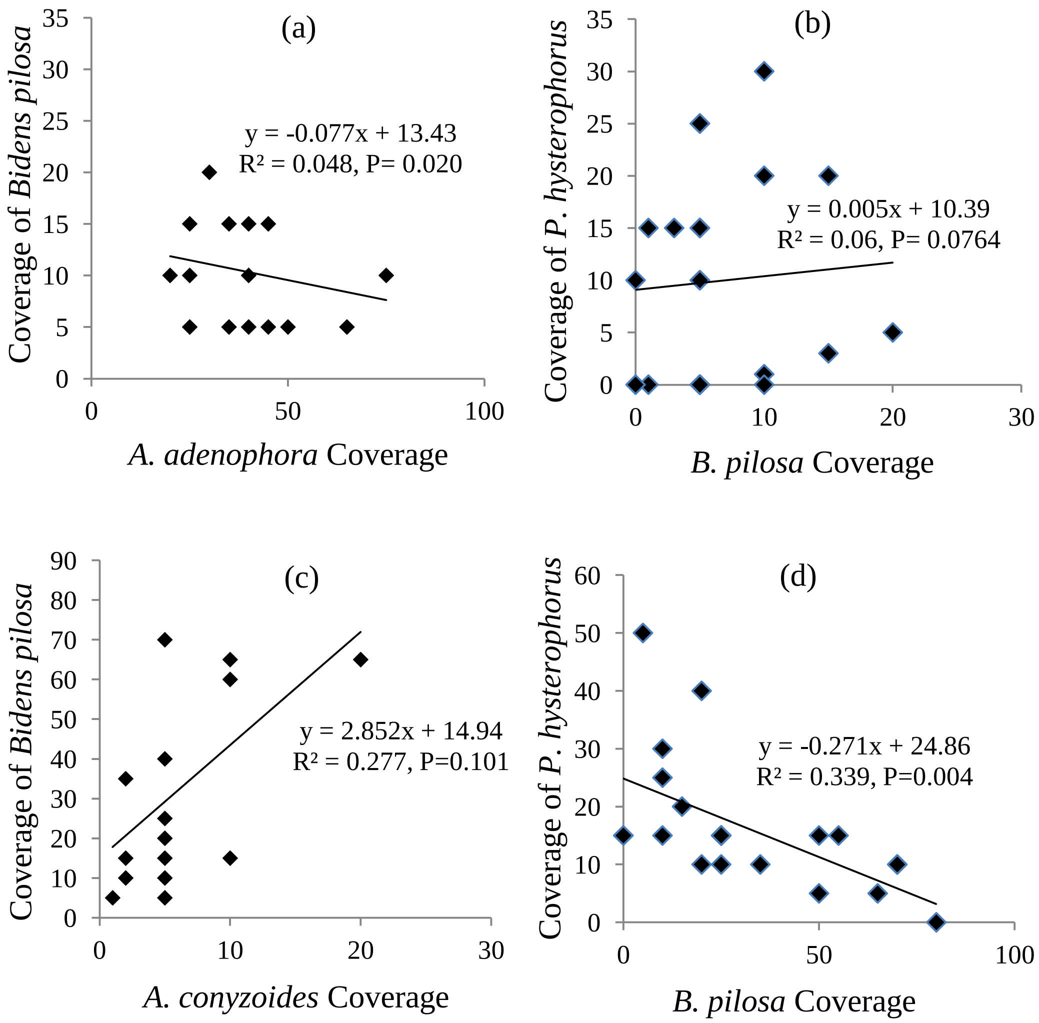

Figure 7: Relationship between the coverage of **(a)** *Bidens pilosa* and *Ageratina adenophora* in forest site **(b)** *Bidens pilosa* and *Parthenium hysterophorus* in fallowland **(c)** *Ageratum conyzoides* and *Bidens pilosa* from road side towards north aspects **(d)** road side towards south aspect *Bidens pilosa* and *Parthenium hysterophorus*

4.1.6 Herbivory damages severity of IAPS

IAPS were found in all sites but the damaged level differed from species to species. Herbivory damage also varied with sites (habitat). **Figure 8** shows the infection (in %) of both branches and leaves from the study sites. In some site, herbivory damage was found too least while in some sites damaged found was higher. In *B. pilosa* highest damaged severity was found in fallowland and least in forest side, while in *A. conyzoides* higest in road towards north aspects and absence in case of fallowland. Highest damaged severity was found in forest and least in fallowland in *A. adenophora*. Finally, *P. hysterophorus* in forest had highest and road towards south aspects have least damaged severity by herbivores (**Figure 8 a**).

Similarly, **Figure 8**(b) shows that all selected IAPS leaves from all sites were damaged by herbivores but the damaged level differes from aspects and habitat types. In *A. adenophora* highest damages was found in road side towards north aspect followed by road side towards south aspect and forest while least damages was found in fallowland. Highest damages in leaves of *Ageratum conyzoides* was found in road side towards north aspect and was absent in fallowland. Similarly, in case of *Bidens pilosa* highest was found in road side towards north aspect and least in forest. Finally, *Parthenium hysterophorus* leaves damages was found highest in road side towards north aspect and forest. And least leaves damage was found in road side towards north aspects (**Figure 8b)**.

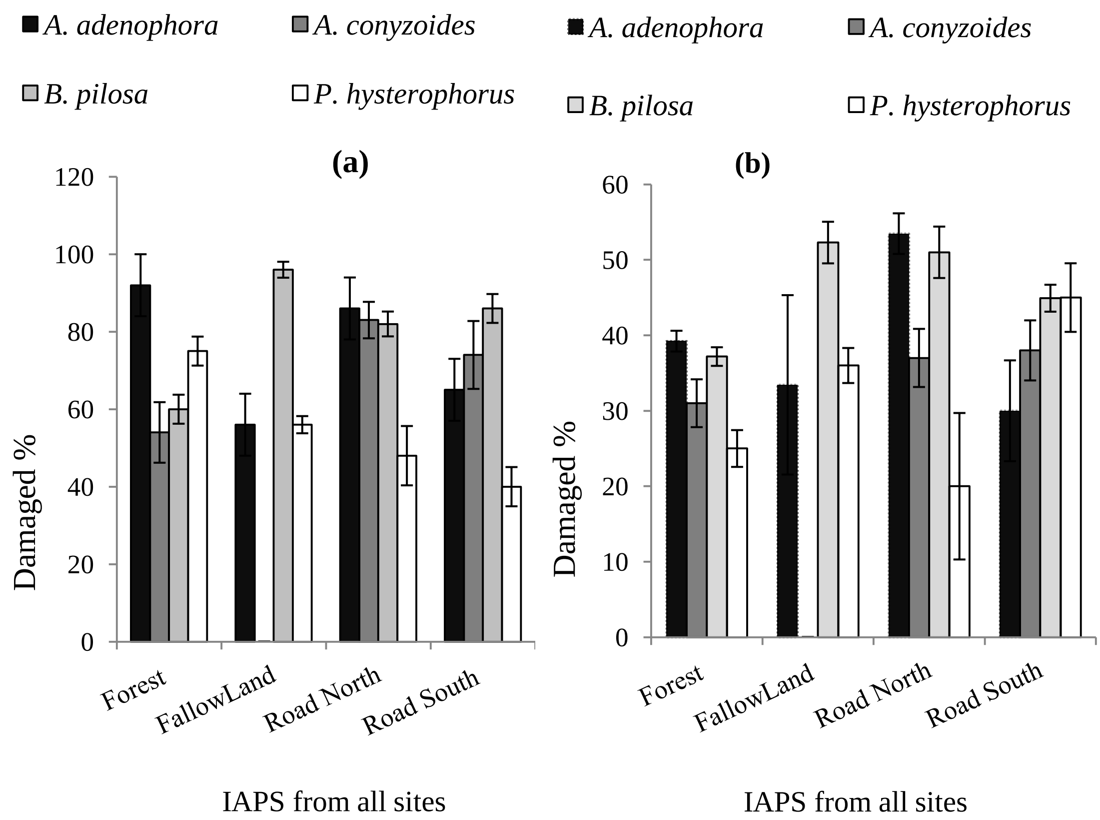

Figure 8:(**a**) Infected branches (in %) and (**b**) Infected leaves (in %)

4.1.6.1 Herbivory Damages in *Bidens pilosa*

Bidens pilosa is found in all sites studied. In branches, the herbivory damages severity was the least in forest (64%) comparing to road sites (both north and south aspect) and fallowland (**Figure 9**, **Table 3**, **P<0.001**). Similarly, the leaf damages were greater in both road side (north aspect) and fallowland than the road side (south aspect) and the forest (**Figure 9**). Damage in road towards north and fallowland are significantly different from the aspect road towards south and forest (**Table 3**, **P<0.001**).

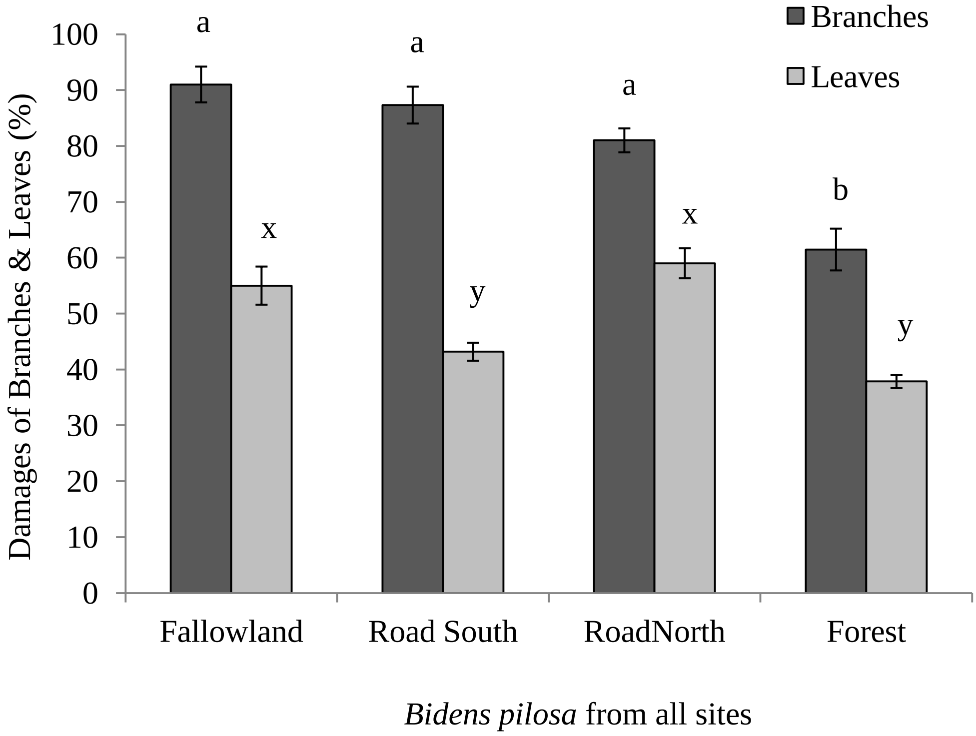

Figure 9: Herbivory damages in *Bidens pilosa.* The letters above error bar indicates significant differences.

4.1.6.2 Herbivory damages in *Ageratina adenophora*

Ageratina adenophora damages severity was found in 2 sites (road sites towards north aspect and forest), in other remaining sites data were not sufficient for analysis so were neglected. In case of branches, highest herbivory damage was found in the forest (94%) than that of road site north aspect i.e 90% but there was no significant differences (**Figure 10, Table 4**). Similarly, damages in case of leaves, forest had the least damages (39%) while road (north aspects) had the highest damages (53%) but statistically, the damage was not significant (**Figure 10, Table 4**)

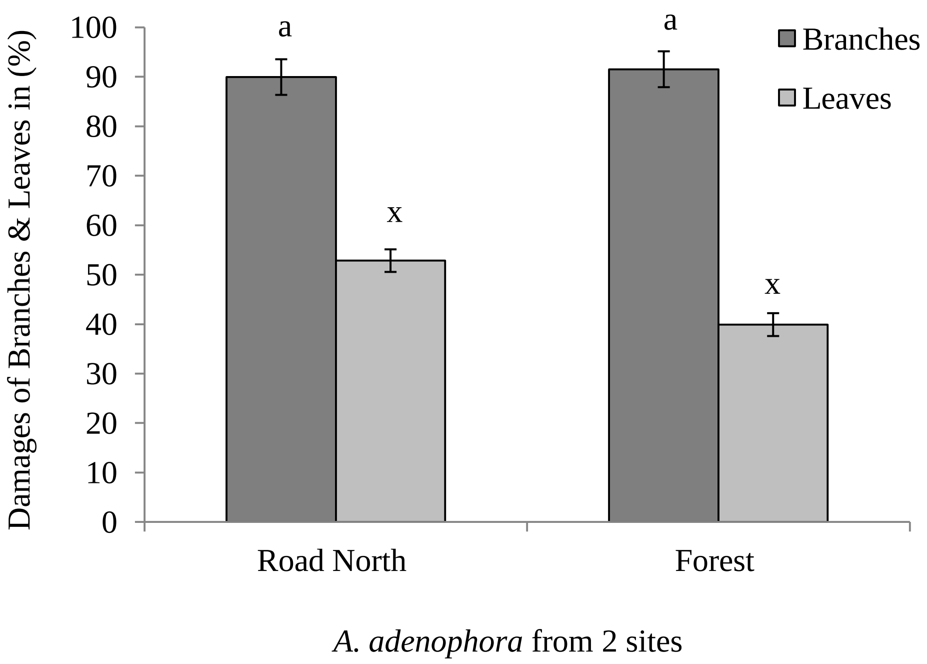

Figure10: Herbivory damages in *Ageratina adenophora***.** The letters above error bar indicates significant differences.

4.1.6.3 Herbivory damages in *Ageratum conyzoides*

Ageratum conyzoides was found in 2 sites, in road sites (north and south aspects). In other 2 sites data were inappropriate and it was neglected. The highest damage was found in road towards north aspects than in road towards south aspects in branches (**Figure 11**). Similarly, damages severity in leaves was the highest in road towards north aspects (38%) than in road towards south aspects (36%) but significant differences was not found. Statistically there was significant differences found in case of branches (**Figure 11, Table 4**).

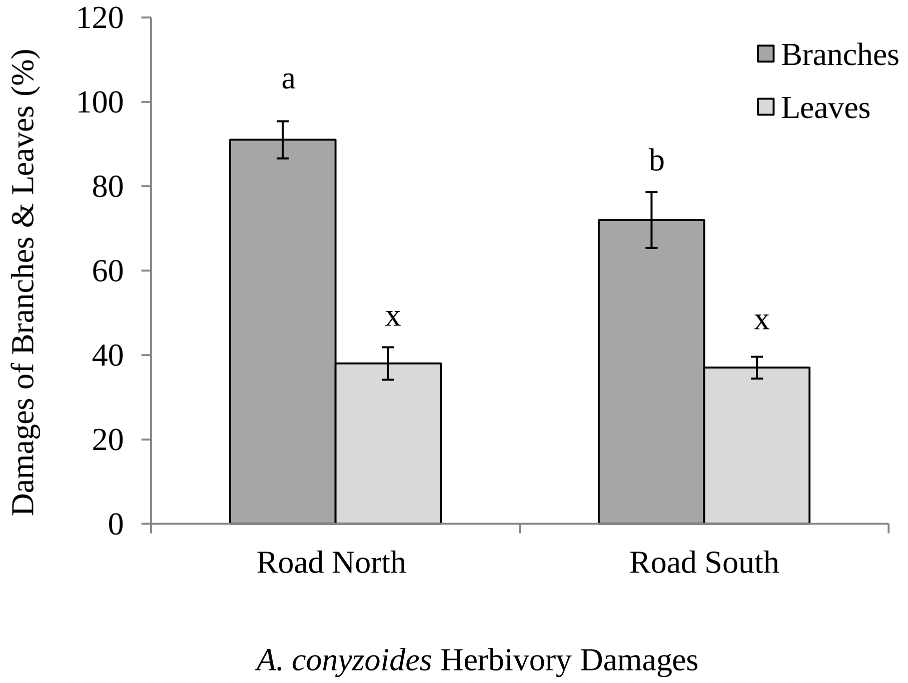

Figure 11: Herbivory damages in *Ageratum conyzoides* in road side. The letters above error bar indicates significant differences.

4.1.6.4 Herbivory damages in *Parthenium hysterophorus*

In case of *Parthenium hysterophorus*, the damages severiry in branches was found the highest in road towards south aspects (70%) than that of fallowland (52%). Similarly, in leaves, the highest damages was found in road towards south aspects (42%) than that of fallowland (35%). Statistically, there was significant differences found in both branches and in leaves (**Figure 12, Table 4**).

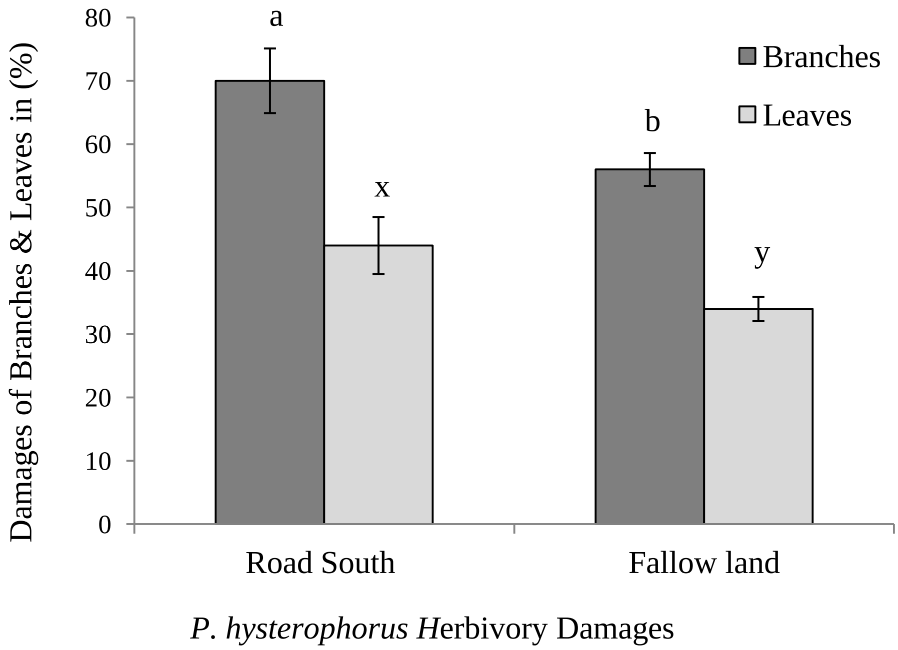

Figure 12: Herbovory damages in *P. hysterophorus*. The letters above error bar indicates significant differences.

Table 3: Statistical analysis of infected branches and leaves in IAPS

Invasive Plants along with infected parts	**Mean Square (MS)**	**Df**	**F**	**P- value**
Bidens pilosa (Branches, One-way Anova and Leaves, Kruskal wallis test**)**				
Branches	3450.613	3	15.758	P<0.001
Leaves	37.081(chi-square)	3	17.413	P<0.001

Table 4: Statistical analysis of infected branches and leaves in IAPS

Invasive Plants along with infected parts	t-value	Df	F	P-value
Ageratina adenophora (Branches and Leaves, Mann-whitney/independent sample t-test)				
Branches	0.999	42	0.079	P=0.780
Leaves	3.406	42	0.075	P=0.785
Ageratum conyzoides **(**Branches/Leaves= mann-whitney/independent sample t-test**)**				
Branches	1.146	42	13.405	P=0.002
Leaves	1.023	42	3.864	P=0.266
Parthenium hysterophorus (Branches and Leaves, independent sample T-test**)**				
Branches	2.248	42	21.389	P=0.001
Leaves	2.154	42	8.111	P=0.007

4.1.7 Soil pH of all species

In the present study, pH of the soil samples ranged from 6.5 to 8. Soil pH of *Ageratina adenophora* invaded soil was of 7.2. Similarly, in *Bidens pilosa* invaded soil pH was 7.5. In the soil collected from *Parthenium hysterophorus* the pH was 7.9. In *Ageratum conyzoides* invaded soil pH was 7.4. Statistically, the pH value was found highest in the rhizospheric soil of *Parthenium hysterophorus* (pH 7.88) followed by *Bidens pilosa* and *Ageratum conyzoides* and least pH was found in soil collected from *Ageratina adenophora* (pH 7.14). The difference was significant. *Parthenium hysterophorus* soil had pH significantly higher than soil of *B. pilosa*, *A.*

conyzoides and *A. adenophora*, while pH of *B. pilosa* soil and *A. conyzoides* soil was similar (**Figure13**). Statistically, least pH value was found in rhizospheric soil *A. adenophora* (**Figure 13, Table 5**).

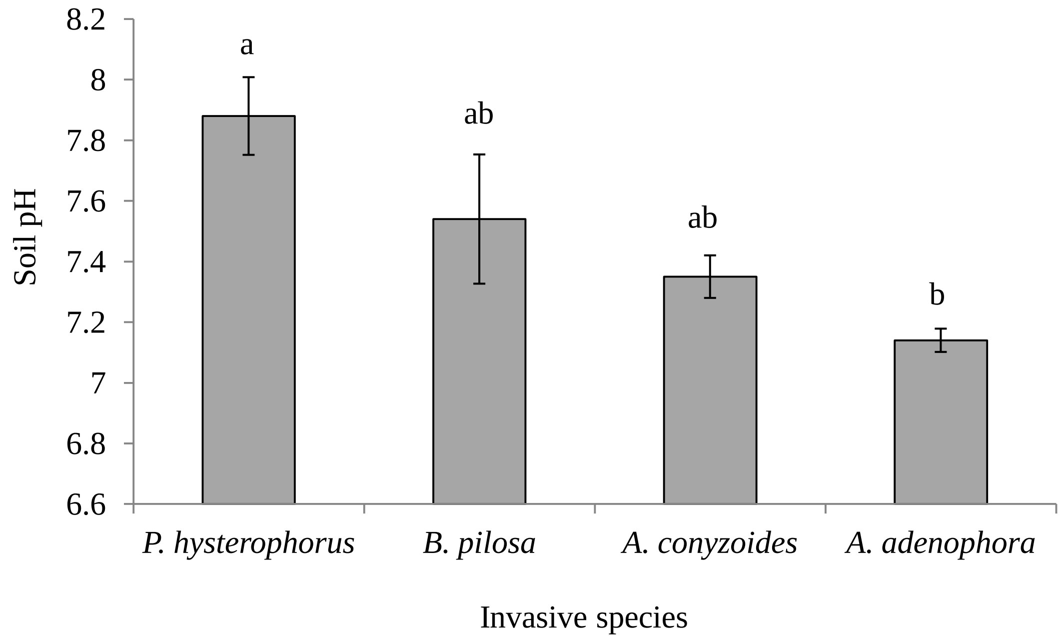

Figure 13: Soil pH of IAPS. The letters above error bar indicates significant differences.

Table 5: One-way ANOVA of soil pH of IAPS

One-way Anova	
P-value	0.008
F	5.653
Df	3
Mean square	0.483

4.1.8 Soil EC of all species

In the present study, Electrical Conductivity (EC) of the rhizospheric soil samples ranged from -20 to -63 milliSiemens per meter (mS/m) in each samples of each plots. EC of rhizospheric soil from *Bidens pilosa* was -41.4 mS/m while in the soil from *Ageratum conyzoides*, the EC was -34mS/m. Similarly, in *Ageratina adenophora* the average EC value was -21.2mS/m. And finally, the EC value was found the highest in *Parthenium hysterophorus* i.e -62.2 mS/m. After data analysis, ANOVA results showed that, statistically there were significant differences. Statistically, EC of the rhizospheric soil collected from *P. hysterophorus* was the highest followed by *B. pilosa*, *A. conyzoides* and *A. adenophora*. The EC of the soil from *A. conyzoides* and *A. adenophora* was the significantly lower (**Figure 14, Table 6**).

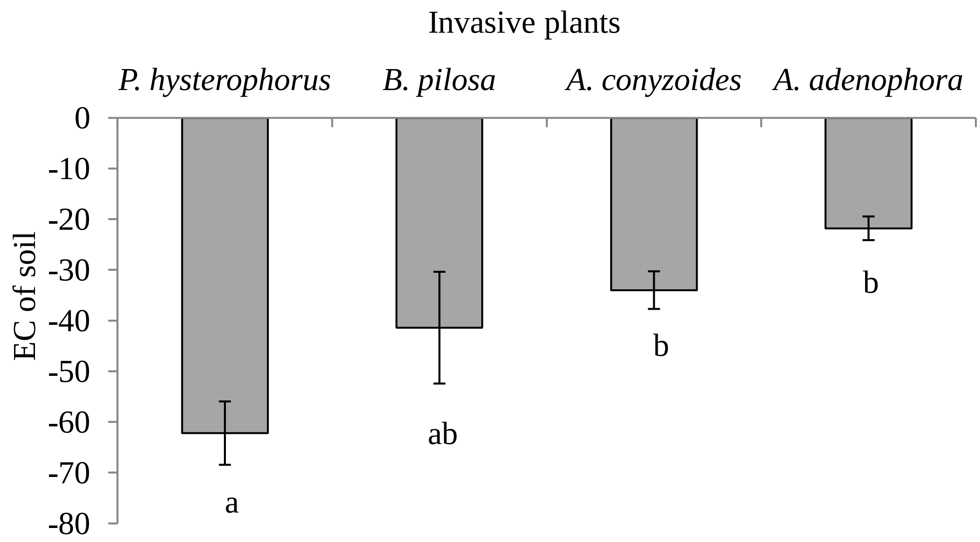

Figure 14: Average soil EC value of all selected IAPS. The letters above error bar indicates significant differences.

Table 6: One-way ANOVA of soil EC in all IAPS

One-way Anova	
P-value	0.005
F	6.394
Df	3
Mean square	1436.583

4.1.9 Frequency of rhizospheric soil fungi

A total 11 fungal species were identified from the rhizospheric soil of selected IAPS. The mean frequency of all rhizospheric fungi was calculated as shown in **Figure 15**.

Among the fungi isolated, *Aspergilus niger*, *Fusarium oxysporum*, *Mucor* sp., *Penicillium notatum*, *Rhizopus stolonifer*, *Gliocladium* sp., *Chaetomium* sp., *Alternaria alternata* and *Curvularia lunata* were the most frequent fungi identified from the rhizospheric soil of *Ageratina adenophora* (**Figure 15, Table 7**).

The frequency of *Alternaria alternata* was the highest (80%) in *Ageratina adenophora* followed by *Fusarium oxysporum* (67%), *Aspergilus niger* (60%), *Gliocladium* (53%), *Curvularia lunata* (53%), *Mucor* (47%), *Penicillium notatum* (33%), *Chaetomium* (33%) and *Rhizopus stolonifer* (20%). Nine fungi were isolated from *Ageratum conyzoides* in which *Gliocladium* had highest (93%) frequency followed by *Absidia* (73%), *Mucor* (67%), *Rhizopus stolonifer* (60%), *Aspergilus flavus* (47%), *Penicillium notatum* (47%), *Chaetomium* (47%) and least frequency (13%) was of *Aspergilus niger.*

In *Bidens pilosa, Mucor* showed the highest frequency (87%) followed by *Fusarium oxysporum* (80%), *Rhizopus stolonifer* (77%), *Absidia* (74%), *Alternaria alternata* (62%), *Aspergilus niger* (54%), *Penicillium notatum* (52%), *Curvularia lunata* (50%) and *Aspergilus flavus* (14%). Total 10 fungi were isolated from *Parthenium hysterophorus*, in which *Absidia* sp., *Fusarium oxysporum* and *Penicillium notatum* comprises highest frequency (67%) followed by *Mucor* (60%), *Curvularia lunata* (60%), *Alternata alternata* (53%), *Rhizopus stolonifer* (60%), *Gliocladium* (47%), *Aspergilus niger* (40%) and *Aspergilus flavus* had least frequency (27%) (**Figure 15, Table 7**).

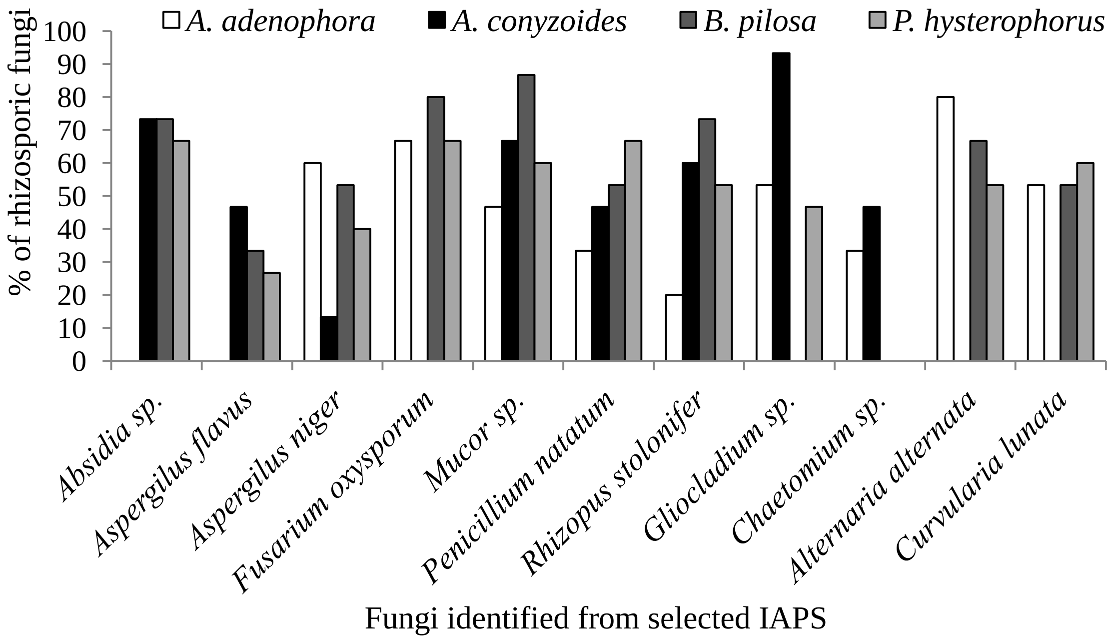

Figure 15: Frequency of all rhizospheric soil fungi

Table 7: Fungi isolated from rhizospheric soil of selected IAPS.

S.N.	Names of IAPS	Family	Rhizospheric soil fungi isolated from IAPS
1	*Ageratina adenophora*	Asteraceae	*Aspergilus niger*, *Fusarium oxysporum*, *Mucor* sp., *Penicillium notatum*, *Rhizopus stolonifer*, *Gliocladium* sp., *Chaetomium* sp., *Alternaria alternata* and *Curvularia lunata.*
2	*Ageratum conyzoides*	Asteraceae	*Gliocladium* sp., *Absidia* sp., *Mucor* sp., *Rhizopus stolonifer*, *Aspergilus flavus*, *Penicillium notatum*, *Chaetomium* sp., *Aspergilus niger.*
3	*Bidens pilosa*	Asteraceae	*Mucor* sp., *Fusarium oxysporum*, *Rhizopus stolonifer*, *Absidia* sp., *Alternaria alternata*, *Aspergilus niger*, *Penicillium notatum*, *Curvularia lunata* and *Aspergilus flavus*.
4	*Parthenium hysterophorus*	Asteraceae	*Absidia* sp., *Fusarium oxysporum*, *Penicillium notatum*, *Mucor* sp., *Curvularia lunata*, *Alternata alternata*, *Rhizopus stolonifer*, *Gliocladium* sp., *Aspergilus niger* and *Aspergilus flavus.*

The description of these fungi with classification is based on Alexopoulos and Mims (1979) (Appendix I).

4.1.10 Fungi isolated from IAPS leaves

Table 8: Fungi isolated from IAPS leaves

S. N.	Preliminary identification	Class	Host plant and parts (leaves)	Symptoms	Colony characters
1	*Fusarium oxysporum*	Deuteromycetes	*P. hysterophorus*	Leaf spot	Reddish white
2	*Alternaria sp.*	Deuteromycetes	*P. hysterophorus*	black spot	Blackish grey color
3	*Alternaria alternata*	Deuteromycetes	*P. hysterophorus*	Black Leaf spot	Blackish color
4	*Fusarium solani*	Deuteromycetes	*P. hysterophorus*	Leaf spot	White color
5	*Curvularia lunata*	Deuteromycetes	*P. hysterophorus*	Leaf Discolor	Black color
6	*Curvularia lunata*	Deuteromycetes	*A. adenophora*	Black rust	No culture (Black color)
7	*Cercospora partheniicola*	Deuteromycetes	*P. hysterophorus*	Leaf spot	Black color
8	*Colletotrichum capsici.*	Deuteromycetes	*P. hysterophorus*	Anthracnose	white to brown
9	*Passalora ageratinae*	Ascomycetes	*A. adenophora*	Leaf spot	brown to light black
10	*Geotrichum candidum*	Ascomycetes	*A. adenophora*	Leaf spot	Light yellowish color
11	*Cladosporium cladosporoides*	Deuteromycetes	*A. adenophora*	Black rust	No culture (Black color)
12	*Chaetomium anguipilium*	Ascomycetes	*P. hysterophorus*	Leaf color light yellowish	Light yellowish color
13	Winter rust *(Puccini abrupt* var. *partheniicola)*	Basidiomycetes	*P. hysterophorus*	Light black color leaf (rust)	No culture (Black color)

The description of these fungi identified is given in **Appendix I**. The classification followed is based on the Alexopoulos and Mims (1979). Figures of fungi are shown in photoplates 1, 2 and 3.

4.1.11 Severity infection of *Puccinia abrupt* var. *partheniicola in Parthenium hysterophorus* around TU area

Almost all the plants per plots were found severily infected by winter rust (*Puccinia abrupt*).Total of about 94 % plants per plot were found infected by the rust fungi in TU area, Kirtipur. Among them, 24 % plants per plots were found highly infected, 50% plants per plots were found moderately infected and 20% plants per plots were found less infected (**Figure 16**).

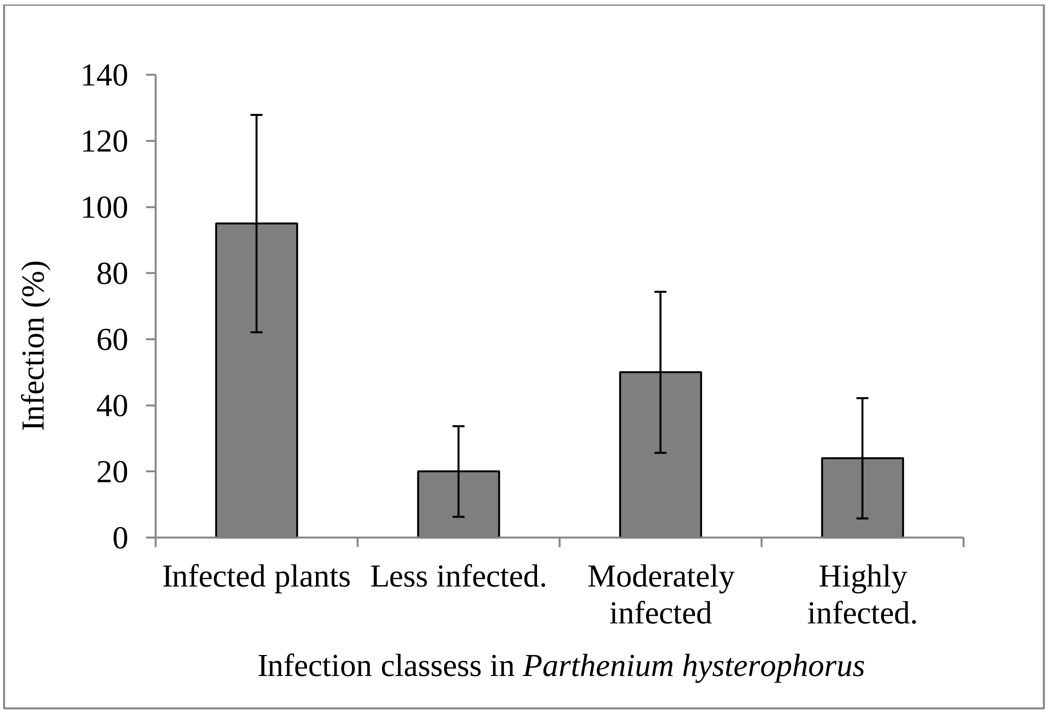

Figure 16: Infection severity of rust fungi in *P. hysterophorus* around Tribhuvan University area, Kirtipur.

4.2 DISCUSSION

4.2.1 Density, Frequency and Coverage of selected IAPS

Comparing densities of selected four invasive species fallowland shows low density than other habitat (sites) such as forest and roadside. As the fallowland of the study area was highly disturbed, this may be the reason of low density of IAPS. On the other sites including fallowland not all IAPS are equally dense. For example, in the forest there was high density of *Ageratina adenophora* and other IAPS have less than 10 plants per plot (Figure 3).

Ageratina adenophora invasion takes place in the new range (habitat), exhibits vegetative means of reproduction and allelopathy (Wan *et al.* 2010; Fabbro *et al.* 2014; Thapa *et al.* 2017). It indicates that the forest of Chobhar area is prone to invasion of *Ageratina adenophora*. It might be because of disturbance and canopy gap of the forest. Thapa *et al*. (2016) found that the higher canopy reduces the density of *Ageratina adenophora*.

In forest, *Ageratina adenophora* requires direct sunlight for growth and reproduction and therefore they are sensitive to the shade of canopies (Thapa *et al.* 2016). Similarly, high tree canopy cover lowers availability of light on the ground surface which is less favourable for growth and development of invasive plants (Norbu 2004). It shows that forest is suitable for invasion by *Ageratina adenophora*. High density of *Ageratina adenophora* in forest indicates that it might have been invaded the forest before other IAPS and its higher density replaces other IAPS. One of the reason behind high density of *Ageratina adenophora* in forest might be reduced herbivory.

In case of road side there was high density of *Bidens pilosa*. It means *Bidens pilosa* favours road side than forest and fallowland. But the clear effect of aspects is seen i.e. *Ageratum conyzoides* was very less in southern aspects than the northern aspect. Similarly, *Parthenium hysterophorus* was densely present in southern aspect than that of northern aspect (Figure 3). In the present study, *P. hysterophorus* was found highly in southern aspect and fallow land than forest and northern aspects, it might due to disturbance, habitat suitability etc.

According to shrestha *et al*. (2015), distribution of *P. hysterophorus* spread and established along road side and fallowland of urban and pre-urban areas, which is similar to our findings. The habitat and aspect has great influence in the distribution of species (Pysek *et al*. 2012). Here, results showed that the IAPS distribution vary on the basis of aspects and habitat type.

Frequency of four IAPS in all sites was not equally present but in *Bidens pilosa* frequency was found almost similar in all the habitat types (Figure 4). It might be due to its annual growing nature of seeds, suitable habitat, canopy gap, highly disturbance tolerance nature of seeds in comparison to other IAPS etc and also it might had invaded our land types (i.e. study sites) before other IAPS and its higher frequency and invasion replaces other IAPS as discussed above. It proves that all sites are vulnerable and prone to *B. pilosa*.

According to Tiwari *et al*. (2005) *B. pilosa* had entered Nepal since 1910 while the *Ageratina adenophora* had introduced in 1952. This history shows that *B. pilosa* was introduced first in Nepal and it can be expected that the first migrant should invade ecosystems first. In this study *Ageratina adenophora* frequency was found the highest in forest. Several studies confirmed that canopy gap induces *Ageratina* invasion in the forests (Thapa *et al*. 2017). Here, high frequency of *Ageratina* in the forest might be due to canopy gap. Simlarly, Norbu (2004) showed that high canopy reduces invasive plant frequency and coverage. The frequency of *A. adenophora* is low in fallowland which might be due to high level of disturbances (Figure 4).

In *Ageratum conyzoides*, highest frequency was found in northern aspect than that of southern aspect, forest and fallowland. It means *A. conyzoides* favours southern aspects for its growth and development but the clear effect of aspects i.e. *Parthenium hysterophorus* frequency was found densely in southern aspect than northern aspect. The aspects have great influence in frequency of species (Pysek *et al*. 2012). Results showed that IAPS frequency is variable on the basis of habitat type except *B. pilosa* which has almost similar frequency in all the habitat types (Figure 4).

Statistically, it shows that the coverage of IAPS ($P>0.001$) depends on habitat types and aspects (Figure 5, Table 2). Comparing coverage of four IAPS the fallowland shows low coverage of all the IAPS than other sites such as forest and roadsides. As the fallowland was highly disturbed by human activities, animals, natural calamities etc so that plant cannot grow, this may be reason behind the low coverage of the IAPS.

On the other hand, coverage of IAPS was not equally dense in all the study sites including fallowland. For instance in forest there was the highest cover of *Ageratina adenophora* than other IAPS present. It indicates that forest is vulnerable to invasion by *A. adenophora*. It might be because of suitable habitat for *A. adenophora*, disturbances, canopy gap of forest. High tree canopy cover lowers availability of light on the ground surface which is less favourable for growth and development of invasive plants i.e. *A. adenophora* (Thapa *et al*. 2016).

While in road side there we found, *B. pilosa* occupied with high coverage almost similar in both northern than southern aspect. It means *B. pilosa* prefer roadside for its growth than forest and fallowland. But, *A. conyzoides* was very less in southern aspect than northern aspects. The clear effect of aspect was seen i.e. *P. hysterophorus* was dense in southern in comparison to that of northern aspect. Hence, the aspects have great influence in the coverage of species present. Finally, result showed that the coverage of IAPS was not similar.

Cover and density of one IAPS have effect on another. Our results show that *Bidens pilosa* cover has negative effect on *Ageratina adenophora* cover (Figure 7a). Similarly, *Parthenium hysterophorus* and *Bidens pilosa* has the relationship between them (Figure 7d). It might be due to allelochemical effect of one species to the another (Thapa *et al*. 2016).

4.2.2 Herbivory damages in different sites

Assessment of herbivory damage and measurement of its severity in the IAPS would be helpful to know the relations between herbivores and IAPS introduced. In world and in case of Nepal, less works on herbivory damages in IAPS have been done. Herbivory damages assessment on IAPS based on different aspects and habitat type is the novel work which is carried out in this study.

A concept have been developed that there might be hervivores affecting IAPS which are naturalized since long time in Nepal. In accordance with enemy release hypothesis, there are natural enemies of IAPS (i.e. *Ageratina adenophora*) in its native range, such as tephritid gall fly, Lepidopteran stem borer and curculionid feeding on leaves (Osborne 1924; Balami *et al*. 2017).

All the selected IAPS were damaged by herbivores but the damages levels diffrers from IAPS and habitat (Figure 7(a) and (b). In *Bidens pilosa*, herbivory damage was found in all the study sites (Figure 8). The highest level of herbivory damages in branches was found in fallowland followed by road towards south and north aspects and least in forest. Similarly, herbivory damage in leaves was found the highest in fallowland and road towards north aspect and less damage in forest and road towards south aspect. It might be because fallowland and road towards north aspect are favourable habitat than other sites for the breeding, growth and development of herbivores in IAPS.

In *Ageratina adenophora*, there we found highest damages in both sites (90%), statistically there was no differences in herbivory damages found in forest and road side north aspects in Branches and leaves (Figure 9). Similarly, In case of *Ageratum conyzoides*, the highest damage in road side noth aspect was found than south aspect in branches. On the other hand, damages level in leaves was found almost similar in both sites in *Ageratum conyzoides* (Figure 10). In *Parthenium hysterophorus* statistically, herbivory damages (in branches and leaves) from road side south aspects was found the highest and fallowland was found the least. It concludes that the invasive alien species are also damaged by herbivores but the damage level is affected by aspect and habitat types.

According to Shrestha *et al.* (2011), an insect *Zygogramma bicolorata* fortuitous arrival had led to some degree of weed suppression (*Parthenium hysterophorus*), since 2009 in some locations around Hetauda, Bharatpur, Butawal and Bhairahawa, Nepal. However, it is also reported that *Z. bicolorata* entered Nepal along road corridors from India. Buccellato *et al.* (2012) reported *Procecidochares utilis*, a stem gall fly in *Ageratina adenophora* for effective biocontrol agent which seems safe, sound and effective method, for weeds control began in 1971. These herbivores might have damaged IAPS on the field and have damaging effects too.

For eg. In case of *Ageratina adenophora*, its invasion in Nepal dates back to 1950s (Tiwari *et al.* 2005). It would be interesting to hypothesize that some pests or parasites might have adapted on feeding to *A. adenophora* including other IAPS whose invasion period seems long. During this course of long time of introduction and establishment of IAPS some herbivores might have adapted on them. We cannot assure that the bites belong to only its natural enemy (for eg. *Procecidochares utilis*), they might also belong to other insects that are co-evolved pests of native plants. Identification of these insects bites and measurement of their damage to IAPS helps in effective biocontrol and helps to know current status of IAPS. We recommend further studies for its confirmation.

4.2.3 Effect of pH, EC and Fungal frequency in IAPS

Soil pH denotes soil's acidity and alkalinity and its measure of hydrogen ions (H+) in soil solution. Higher the H+ ion concentration lowers the pH value and vice-versa (Alvarez *et al*. 1988). Similarly, EC is the electrical conductivity that measures soil salinity, nutrition present in the soil, organic matter etc beneficial for soil and plants. Soil pH and EC are correlated with the fungi of IAPS. Soil physiochemical properties have obvious effect on the soil biota and plants. Soil pH is an important controlling factors of the abundance and diversity of fungal species (Rousk *et al*. 2010). In this present study, the records on Deuteromycetes are relatively large as compared to others. Fungi of this group produce sufficiently high spores (Webster and Roland 2007) hence the soil dilution method accompany with dilution of these spores.

In *Ageratina adenophora* the highest frequency of *Alternaria alternate* was found in which the pH and EC value was low. While in *Parthenium hysterophorus*, *Aspergilus flavus* have lowest frequency with high pH and EC value it might be because, this fungi need low pH and EC. *Absidia* and *Aspergilus flavus* are absent in *Ageratina adenophora* because of low pH and EC value, it might be because these two fungi requires high pH and EC in the soil. In case of *Ageratum conyzoides*, pH and EC value was found lowest (pH 7.3, Figure 13) and *Gliocladium* shows highest fungal frequencies followed by *Absidia*, *Mucor*, *rhizopus* etc and *Aspergilus niger* had the lowest frequency (Figure 14).

In *Bidens pilosa Mucor* sp. occupy highest frequency followed by *Fusarium oxysporum*, *Rhizopus stolonifer*, *Alternaria alternata*, *Absidia*, *Curvularia*, *Aspergilus niger* and *penicillium* and lowest frequency of *Aspergilus flavus* was with lowest pH and EC value in comparison with other IAPS (Figure 13, 14 and 15). It indicates that frequency of fungal species in IAPS might get depends on pH and EC. However, it is also possible that these fungal communities might have involved in changing pH and EC of the soil.

Comparing fungal frequency of selected IAPS, it was found that *Absidia*, *Aspergilus flavus*, *Mucor*, *Penicillium notatum*, *Rhizopus stolonifer*, *Gliocladium*, *chaetomium*, have higher frequency in all selected IAPS (Figure 15) but *Absidia* and *Aspergilus* were absent in *Ageratina adenophora*. While another species *Gliocladium* was absent in *Bidens pilosa* and *Chaetomium* was absent in both *Bidens Pilosa* and Parth*enium hysterophorus* (Figure 15). This indicates that all the fungal species having higher frequencies and those that are absent can be sensitive to the selected IAPS, accumulation of fungal species in the rhizospheric soil of IAPS might be because of their affinity towards allelochemicals compounds present in the IAPS (i.e some allelochemicals attracts fungi).

Van der Putten (2007) concluded that invasive plants, pathogenic and symbiotic soil microbes will have strongest effects on the abundance of individual species, community diversity and ecosystem functioning. They also proposed that the understanding, predicting and counteracting consequences of enhanced global homogenization of natural communities through IAPS will require future studies on how pathogenic, symbiotic and decomposer soil microbes interact, how they are influenced.

4.2.4 Fungi identified from IAPS and severity of *P. abrupta* var. *partheniicola*

During survey conducted in 2018 in Chobhar Kirtipur and its adjoining area, over 25-30 diseased specimens were examined for the fungal pathogens (Table 8). A Total of 13 fungal pathogens were identified from IAPS. *Alternaria alternata*, *Alternaria* sp., *Fusarium oxysporum*, *Fusarium solani*, *Colletotrichum capsici*, *Curvularia lunata, Cercospora partheniicola*, *Chaetomium anguipilium* and winter rust *Puccinia abrupt* var. *partheniicola* were identified from diseased leaves of *Parthenium hysterophorus*.

Similarly, from *Ageratina adenophora* 4 fungal species namely *Passalora ageratinae*, *Geotrichum candidum*, *Curvularia lunata* and *Cladosporium cladosporoides* were isolated and identified (Table 8). Some species were identified live on *Parthenium hysterophorus* and *Ageratina adenophora* leaves without culture and other species after growing on PDA media (Table 8). Similar results were obtained by Aggarwal *et al.* (2014) in *P. hysterophorus* in India but the winter rust *Puccinia abrupt* var. *partheniicola* was not identified by them.

But according to Shrestha (2012) winter rust *Puccinia abrupt* var. *partheniicola* were identified in *P. hysterophorus* in Nepal. Buccellato *et al.* (2012), a leaf-spot pathogen *Passalora ageratinae* was identified from *Ageratina adenophora* as similar to our finding.

Identification of fungal pathogens from leaves of IAPS helps in biological control of IAPS. Further works must be done in order to test its host specificity for biological control of IAPS. *Puccinia abrupt* var. *partheniicola* and *Passalora ageratinae* were the fungi which were already identified in Nepal (Shrestha 2012; Winston *et al.* 2014; Shrestha *et al*. 2016). While other fungi identified are new to Nepal. These fungal species identified could be further used to test biological control of *Parthenium hysterophorus* and *Ageratina adenophora.*

Similarly, *Parthenium hysterophorus*, one of the highly problematic invasive weeds found around our roadside, fallowland, agroecosystem etc. Around roadside of Tribhuvan University and its adjoining area of Kirtipur, these weeds are found highly spread covering our roadside area, our walking way and we found this weeds, highly infected by the winter rust (i.e. *Puccinia abrupt* var. *partheniicola* which is found host specific). This winter rust is highly host specific and naturally found in central and South America destroying *P. hysterophorus* (Parmelee, 1967).

Puccinia abrupta var. *partheniicola* is analogous to that of *P. chondrillina,* which produces only uredinia and telia; neither pycnia nor aecia have been reported (Hasan and Wapshere, 1973). *Puccinia abrupt* var *partheniicola,* autoecious species evidence from a few North American records (Arthur, 1929). Our results showed that, identification of fungus and measurement of its infection severity level in weeds, might be helpful and one of the better contribution towards biological control of the *P. hysterophorus* so that the population of *P. hysterophorus* might be reduced to some economic level.

CHAPTER V

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

- From the result it can be concluded that the differences in density, frequency, and coverage of IAPS differs on habitat and aspect type.
- One kind of IAPS have effect on density and coverage of anther IAPS.
- Herbivory damages in branches and leaves of IAPS are also differs on habitat and aspects types. In branches, *B. pilosa* was greately damaged in fallow land, *A. adenophora* in the forest, *A. conyzoides* in the road towards north aspect and *P. hysterophorus* in road towards south aspect.
- Similarly, herbivory damages in leaves, the highest damage was found in *B. pilosa* from sites fallowland and Road side north aspects. In *A. conyzoides* and *A. adenophora*, damages was found maximum in the road towards north aspect but in *P. hysterophoru*s the damage was severe in road towards south aspect.
- Altogether, 11 rhizospheric soil fungi in IAPS were identified using PDA culture.
- A total 13 fungi are found associated to the selected IAPS
- EC and pH showed impacts on fungal frequency.
- *Parthenium hysterophorus* was found highly infected by winter rust (*Puccinia abrupt* var. *partheniicola*) and its infection severity measurement in plants showed that 94% plants per plots was found infected by winter rust.

5.2. RECOMMENDATION

- Besides four IAPS studied in the study site other IAPS density, frequency, coverage and Herbivory damages from agro-ecosystem, abundant land etc are also to be measured for which appropriate way to find the population and to find its control measure.
- Control and management of current IAPS would be beneficial to protect native and endangered forest elements.
- Herbivory damage should be monitored and the assessment should be continued as the monitoring and assessments would be helpful for developing new biological control strategies for existing IAPS.
- Herbivores identification works should be carried out in order for the biological control of IAPS.
- Assessment on soil microbes gives new ideas about plant-soil microbial interactions and feedback mechanisms which should be continued in future studies.
- Further more, fungal identification works need to be done using different media like CDA, PSA, MEA, NA etc including PDA in order for identification and biological control methods for IAPS.
- There is an urgent need to control and manage IAPS in the study site as the scenario indicates the level of invasion would be severe in future.

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Appendix I: Description of the fungi.

1. *Alternaria* Nees.

The colony of *Alternaria* sp. on PDA is green with white margins. The mycelium is branched and septate and the aerial hyphae are undeveloped. On the living leaves of *P. hysterophorus,* symptoms are characterized as dark brown, irregular marginal spots. Conidiophores are pale brown; simple or branched; bearing conidia at the apex and apical fertile parts and solitary, rarely in groups, septate, light brown conidia mostly solitary, dark brown, smooth.

2. *Alternaria alternata* (Fr.) Keissl.

The symptoms appeared as a small. Black or brown spots scattered on leaves. The spots became irregular in shape with the increment in size, the symptoms or spots became irregular in shape. Several such spots makes the plants dry. After culture the mycelium become grey green in colour with white margins and becomes black at maturity. Conidiophores are straight or curved; short to long; simple to branch. Conidia are ovcalvate; long; ellipsoid; small in size.

3. *Fusarium solani* Mart.

Colonies are white and cottony and slimy. However, it becomes blue-green or bluish brown at mature phase. It has aerial hyphae supporting conidiophores. Conidiophores branch into thin, elongated. Macroconidia are shorter than those that produce microconidia, where macroconidia are slightly curved, hyaline and broad having 3 to 4 septa.

4. *Fusarium oxysporum* Snyder and Hansen.

Conidiophores hyaline, simple, short or not well differentiated from hyphae, bearing spores masses at the apexes. Conidia hyaline, of two kinds: macroconidia boat-shaped and microconidia ellipsoidal, 1-celled. Chlamydospores brown, globose, usually solitary.

5. *Curvularia lunata* (Wakker) Boedijn.

Symptoms appeared on the leaves are brown to black leaf spots scattered all over the leaf and on the PDA culture. *Curvularia* sp. appears as shiny velvety grey, fluffy growth on the colony surface with septate conidiophores. Conidiophores arises from sub-cuticular stromata, erect, inflated at the base, dark brown, septate, conidia are olive brown, usually curved, rounded at base, 2 central cells etc.

6. *Cercospora parthenicola* Fresen.

This is a genus of ascomycete fungi. Most species have no known sexual stage. The spots or symptoms begins as pale green spots on the upper surface of the *Parthenium hysterophorus* leaf. These spots gradually enlarge, turn brown in colour. The mycelium well developed, branched, intercellular and septate. Conidiophores are hyaline to dark brown, septate, straight etc.

7. *Colletotrichum capsici* Corda.

Soft White color colony appeared on the PDA. Light yellowish or whitish like color spots appeared on the leaves of *Parthenium hysterophorus*. Light coloured perithecia, Dark brown ellipsoidal ascospores seen.

8. *Passalora ageratinae* Crous & A.R. Wood.

Colony dirty black and dirty whitish color seen on PDA media after 7 to 8 days of culture. Conidia septate and elongated..

9. *Geotrichum candidum* Link.

Light white yellowish, yeast-like colony appeared on PDA media. In the centre of colony round swollen thick small button like structure appeared.The hyphae colour appears to be hyaline or lightly pigmented. Conidia appear arthrosporous, terminal or intercalary.

10. *Cladosporium cladosporoides* (Pers.) Link.

Brown or Black colour colony appears on PDA. Conidia are dark-pigmented that are formed in simple or branching chains, being smooth, one to four celled. Conidiophores are erect, straight or flexuose, unbranched or branched.

11. *Chaetomium anguipilium* Kunze ex Fries.

Colony found fast growing, cottony and white in colour initially. Mature colony becomes grey to olive in color. Septate hyphae, asci and ascospores. Ascospores were found olive brown in color and lemon shaped.

12. *Puccinia abrupt* Diet. & Holw.var *partheniicola* (Jackson) Parmelee.

Brown black rust appeared during winter season. Black colour colony appeared on PDA media. This winter rust appeared black on the leaves of *P. hysterophorus*. They are found live on leaves i.e. without culture. Brown color rounded uredinospores were found. This uredinospores were found without culture.

13. *Rhizopus stolonifer* Ehrenberg.

Colonies dark black color appears in petriplate containing PDA media. Mycelium of two kinds, one submerged in the substratum and the other aerial, constituting the arching filamentous or stolon. This stolon presents from place to place the nodes on which the rhizoids occur. At the point the sporangiophores arises. They may be single but in groups of two or three or more.

14. *Absidia* van Tieghem.

Colony white color as that of mucor sp. Mycelium formed as in *Rhizopus* by frequently branched stolons, sporangiophores straight, rarely single, more often in groups. Rhizoids originate elsewhere from the stolon except at the origin of sporangiophores. Sporangia apparently equal, pyriform, erect. Columellae hemispheric or conic.

15. *Mucor* Micheli.

White color colony appears in PDA media. Mycelium widespread in and on the substratum, without rhizoids, richly branched, sporangiophores springing singly from the mycelium but usually forming a thick turf, erect, either unbranched with terminal sporangia or branched with, like sporangia on the branch ends. Columella always present, of various shapes, colourless. Spores spherical or ellipsoidal.

16. *Penicillium notatum* Thom.

Colony colour light sky blue with water droplet like body in centre of colony surrounded by white margin. It reproduces by forming dry chains of spores (or conidia) from brush-shaped conidiophores. The conidia are blue to blue-green. Conidiophores are septate.

17. *Gliocladium* Coda.

Colony light yellowish, dirty greenish type. Conidiophores hyaline; erect; granular and rough on the surface; branched; bearing spore masses on phialides at the apex; phialides verticillate or penicillate; gradually tapering towards apex. Conidia hyaline; pinkish in mass; spindle-shaped; ellipsoidal or boat-shaped; 1-celled.

18. *Aspergilus niger* van Tieghem.

Black coloured colony appeared; reverse dirty white. Conidiophores mostly arise directly from the substratum; smooth; septate or non-septate varying size. Conidial heads fuscous; blackish brown; purple brown in every shade to carbonous black. Conidia globose with dark brown coloured colony.

19. *Aspergilus flavus* Link.

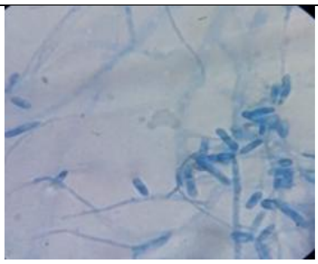
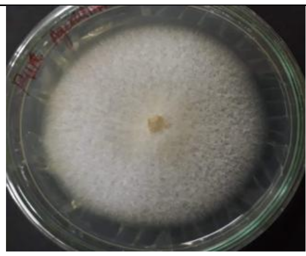
Light green colour colony with ridges and dirty white margin and reverse in PDA. Heads in colony vary from small with a few chains of conidia to large columnar masses or both mixed in same area.

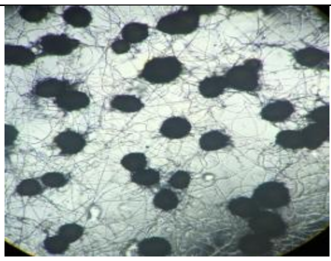
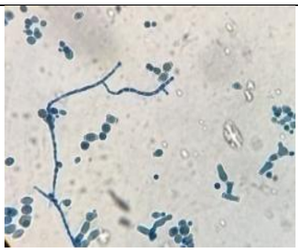
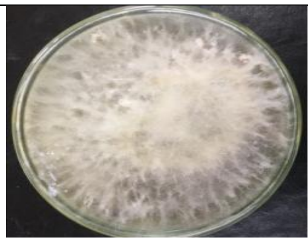
Table 9: Classification of fungi isolated from rhizospheric soil of IAPS

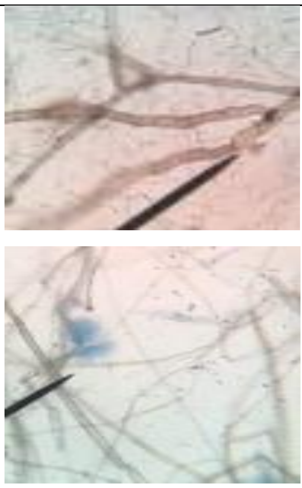
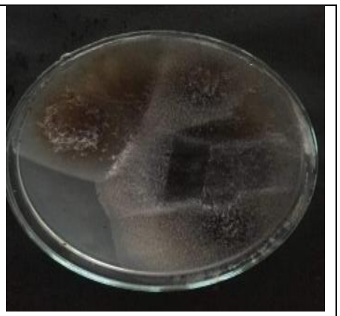
(Alexopoulos and Mims, 1979).

S.N	Rhizospheric soil fungi	Classes
1	*Mucor sp.*	Zygomycetes
2	*Fusarium oxysporum,*	Deuteromycetes
3	*Rhizopus stolonifer*	Zygomycetes
4	*Alternaria alternata*	Deuteromycetes
5	*Aspergilus flavus.*	Deuteromycetes
6	*Curvularia lunata*	Deuteromycetes
7	*Aspergilus niger*	Deuteromycetes
8	*Penicillium notatum*	Deuteromycetes
9	*Absidia sp.*	Zygomycetes
10	*Chaetomium anguipilium.*	Ascomycetes
11	*Gliocladium sp.*	Deuteromycetes

Photo plates 1: Fungi isolated from infected leaves of IAPS.

Fungal species	**Plants Leaves**	**Pure culture**
 Figure 1: *Colletotrichum capsici*	 *P. hysterophorus* leaf infected	

 Figure 2: *Chaetomium anguipilium*	 *P. hysterophorus* Leaf infected	 Pure culture
 Figure 3: *Geotrichum candidum*	 *A. adenophora* leaf infected	 Pure culture
 Figure 4: *Fusarium oxysporum*	 *P. hysterophorus* leaf infected	 Pure culture
 Figure 5: *Curvularia lunata*	 *P. hysterophorus* leaves infected	 Pure culture

 Figure 6*: Cercospora partheniicola*	 *P. hysterophorus* leaves infected	 Pure culture

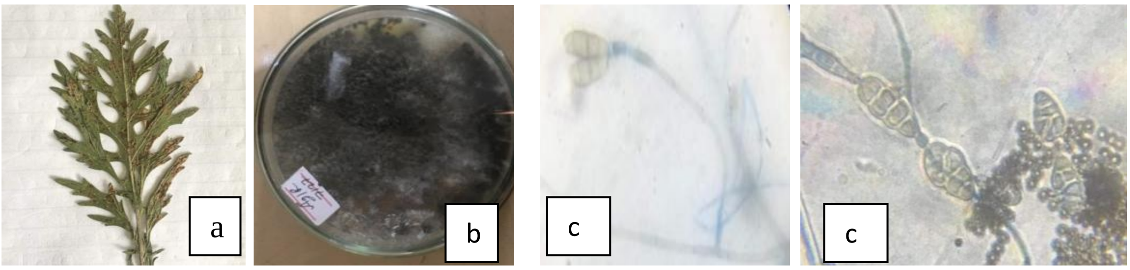


Figure 7: a (*Parthenium hysterophorus* leaf infected), b (pure culture) and c *(Alternaria* sp.)

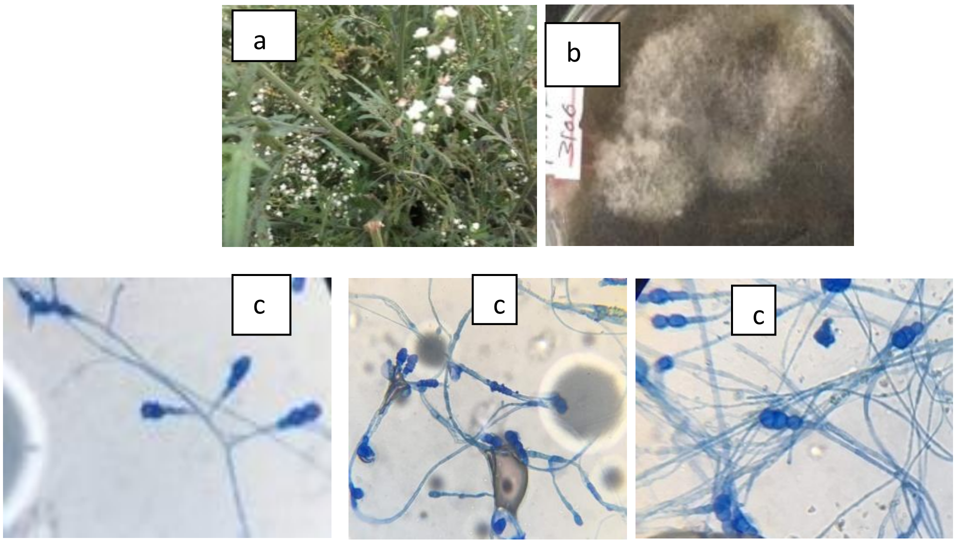


Figure 8: a (*P. hysterophorus* leaf infected), b (pure culture) and c (unidentified sp.)

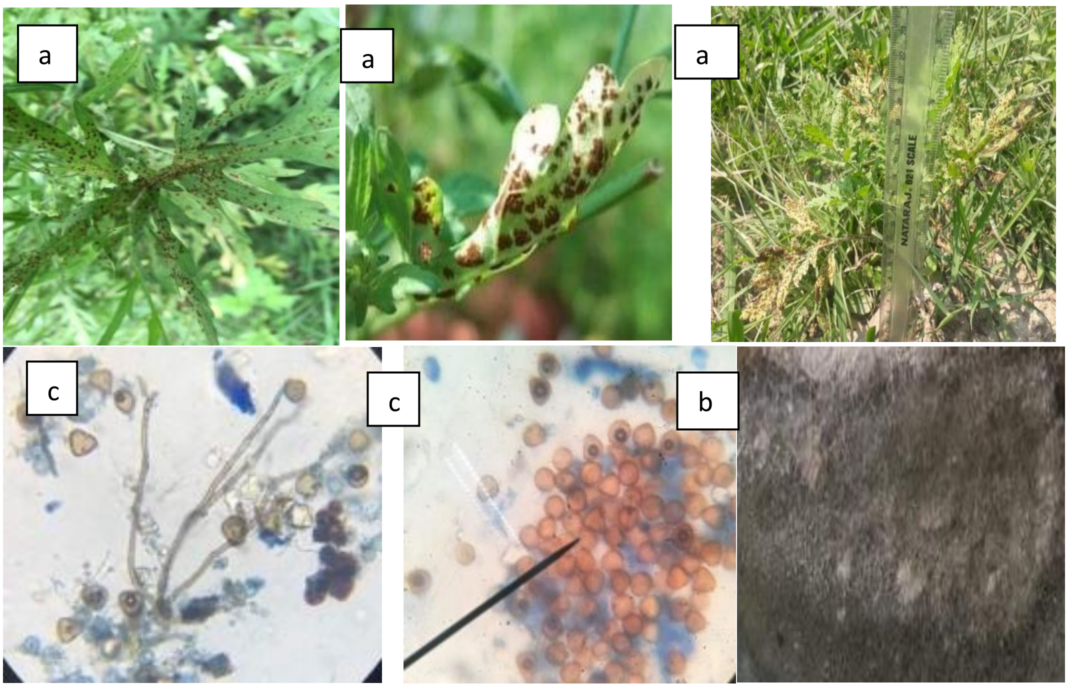


Figure 9: a (*P. hysterophorus* leaf infected), b (pure culture) and c (*Puccinia abrupt* var. *partheniicola*)

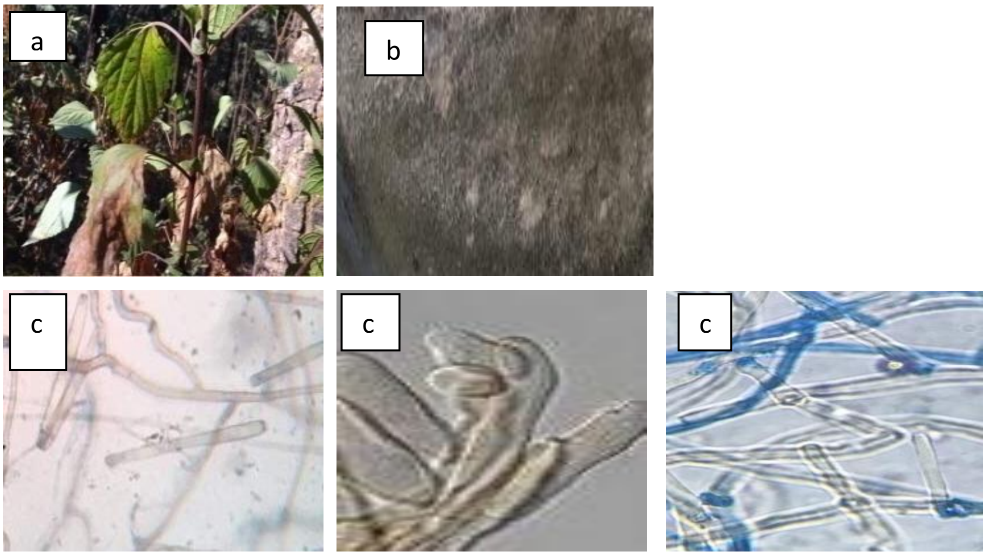


Figure 10: a (*Ageratina adenophora* leaves infected), b (Pure culture) and c (*Passalora ageratinae* isolated from *Ageratina adenophora* leaves).

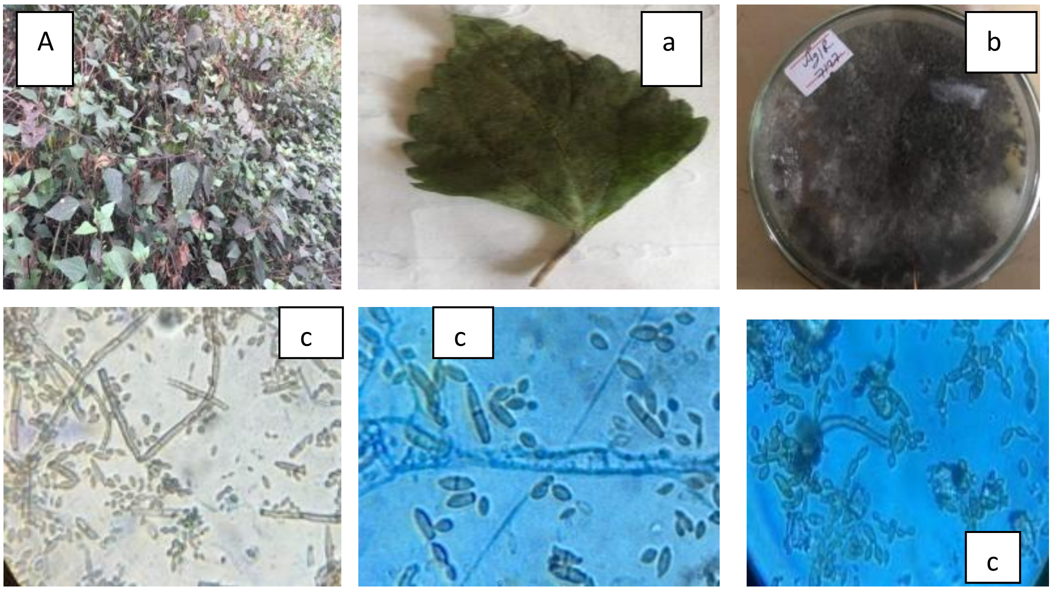


Figure 11: A (*Ageratina adenophora* leaves infected by winter rust), a (Single leaf infected by winter rust), b (Pure culture) and c (Mixture of fungi i.e. *Curvularia lunata* and *Cladosporium cladosporioides* spores isolated from winter rust of *Ageratina adenophora* leaves)

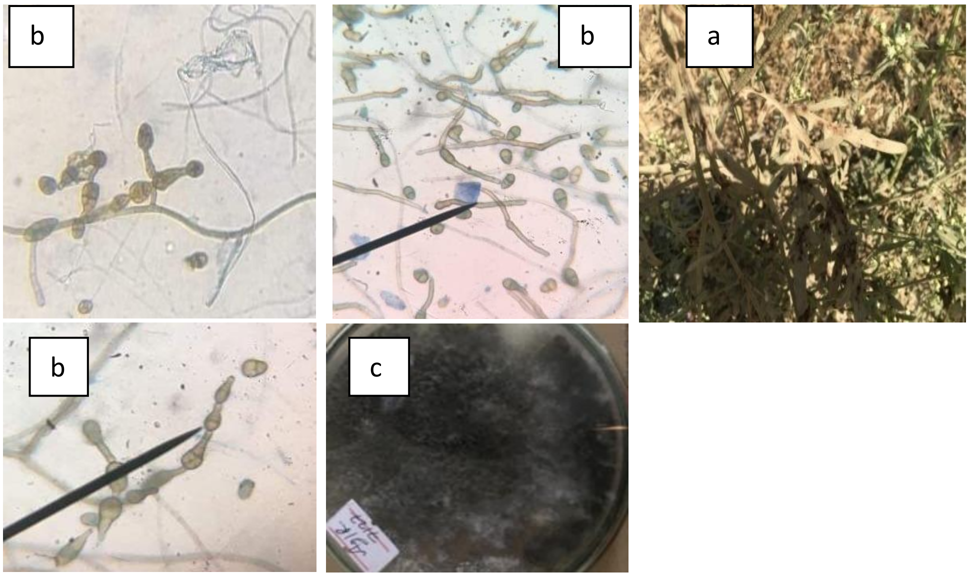


Figure 12: a (*Parthenium hysterophorus leaf*), b (*Alternaria alternata*) and c (Pure culture)

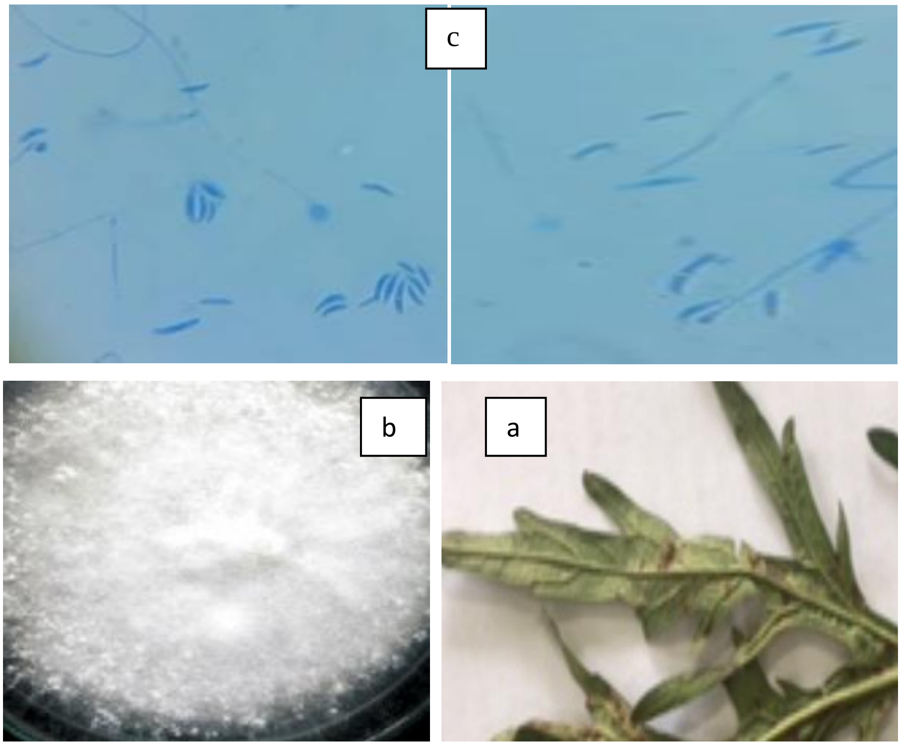


Figure 13: a (*P. hysterophorus* leaf), b (pure culture) and c (*Fusarium solani*)

Photo plates 2: Fungi isolated from Rhizospheric soil of IAPS.

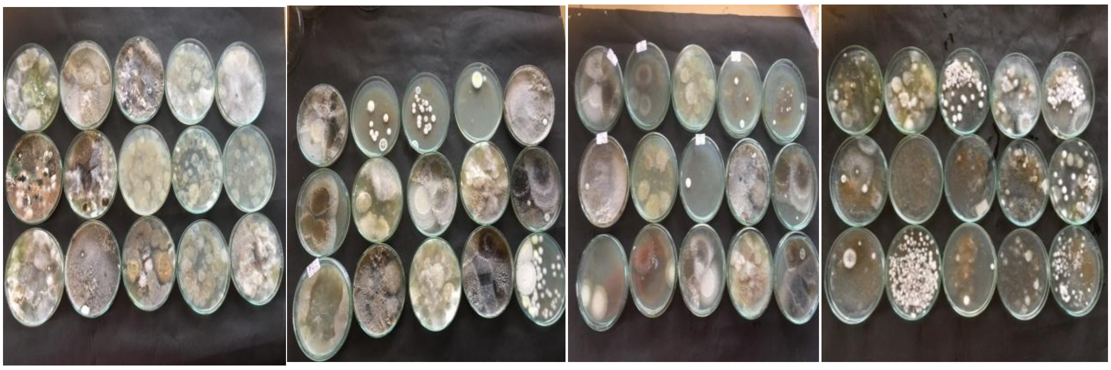
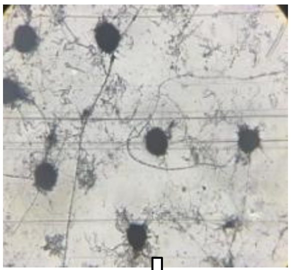
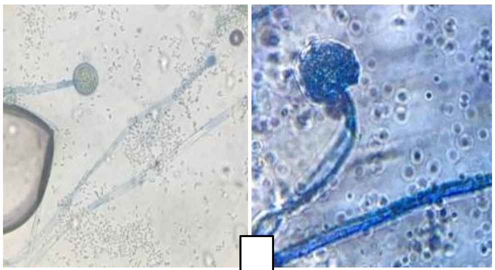


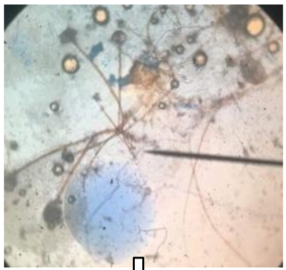
Figure 1: Petriplates showing culture of fungi isolated from rhizospheric soil of selected IAPS



Chaetomium anguipilium



Absidia sp.



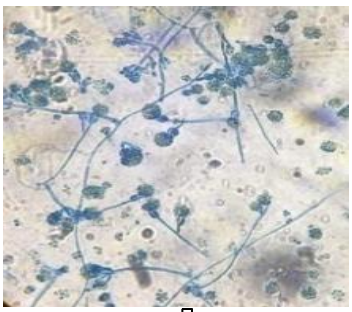
Rhizopus stolonifer

Penecillium notatum

Fusarium oxysporum

Alternaria alternata

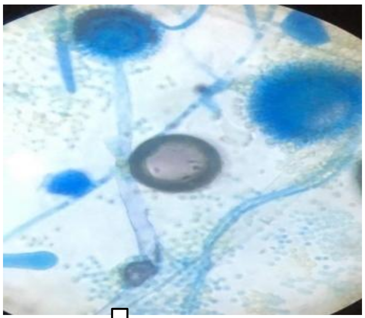
Curvularia lunata



Gliocladium sp.

Mucor sp.

Aspergilus niger



Aspergilus flavus

Photo plates 3: Photographs taken during laboratory work and field work.