IMPACT OF *AGERATUM HOUSTONIANUM* MILL. INVASION ON PLANT DIVERSITY AND CROPS IN DIFFERENT LAND USE TYPES OF KANCHANPUR, NEPAL

A DISSERTATION WORK SUBMITTED FOR THE

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THE MASTER'S DEGREE IN BOTANY (ECOLOGY UNIT)

BY

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LETTER OF APPROVAL

The M.Sc. Dissertation entitled "**Impat of *Ageratum houstonianum* Mill. invasion on plant diversity and crops in different land use types of kanchanpur, Nepal**" submitted by **Miss Sangita Singh** has been accepted for partial fulfillment of the requirement for Master's Degree in Botany.

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DECLARATION

I, Sangita Singh, hereby declare that the work enclosed here is entirely my own, except where states otherwise by reference or acknowledgement, and has not been published or submitted elsewhere, in whole or in part, for the requirement for any other degree or professional qualification. Any literature, data or works done by others and cited within this thesis has been given due acknowledgement and listed in the reference section.

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RECOMMENDATION LETTER

This is to certify that MSc Dissertation entitled “Impact of *Ageratum houstonianum* Mill. Invasion on Plant Diversity and Crops in Different Land Use Types of Kanchanpur, Nepal” has been carried out by **Miss Sangita Singh** under my supervision. This work has been completed by candidate’s original research work based on field sampling and lab work. To the best of our knowledge this thesis work has not been submitted for any other degree in any institution. We recommend this dissertation work to be accepted as a partial fulfillment for Master’s Degree in Botany, Department of Botany, Amrit Campus, Tribhuvan University.

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ABSTRACT

Invasive plant species means an alien species which become established in natural or semi-natural ecosystems and threatens native biological diversity. It has high resistant capacity, produce large number of seeds and can grow easily in different types of habitat. It can out-compete native species, reduces wildlife habitat potential, alter ecosystem processes and change soil properties and have great role in land cover change. *Ageratum houstonianum* is a native species of America that enters to Nepal. It has grown in different land use (agriculture, forest, grassland). Laljhadhi-Mohana Biological corridor (LMBC) is corridor between Suklaphanta national park and Dudhwa national park largest national park of India. Corridor is a pathway of plants and animals so invasive plants species can easily enters. From India to Nepal invasive species can easily enters through corridors and may impact native species diversity. To understand the impact of *A. houstoniunum* on species diversity, vegetation sampling was done. Altogether 120 plots of size 2m×2m were laid in each sampling site (invaded and non-invaded sites).Different quantitative parameters for vegetation analysis like frequency, density, coverage, IVI, Simpson Index, Shannon Wiener Index were calculated. Results showed that the diversity of non-invaded area was greater than the invaded area. To understand the impact of allelochemical effect of leaf to the crop plant, different concentrations (2, 10, 20, 30, 50%) were prepared. Seed germination of winter and summer crop plant was done in different concentrations of leaf extract and also in control to compare the impact. Germination was significantly inhibited (p=0.05) in higher concentrations (20, 20, 50%) than lower concentrations (2, 10%) of mustard crop. Measurement of radicle and plumule was taken. Growth rate was lower in high concentrations (20, 30, 50%) than low concentrations (2, 10%). Summer crop (maize and paddy) was not affected by allelochemical effect of leaf extract but winter crop (mustard and wheat) was highly affected by allelochemical effect of *A. houstonianum*.

Keywords: Allelochemical, corridor, invaded, non-invaded

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	: Analysis of variance
SPSS	: Statistical Package for Social Science
GoN	: Government of Nepal
IAS	: Invasive and Alien species
IVI	: Important Value Index
Fig	: Figure
gm	: Gram
no	: Number
sp	: species
cm	: Centimeter
Aa,b,c,d	: subset
DHM	: Department of Hydrology and Meterology

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CHAPTER 1: INTRODUCTION

1.1 Background

There has been an unparalleled increase in migration as a result of rising globalization and a growing human population (both intentional and unintentional) of species beyond their natural biogeographical range. In fact, invasive species are spreading at an alarmingly fast rate over the planet, posing one of the most serious dangers to global biodiversity. It has piqued scientists' curiosity in determining the scope of invasive species' presence and impact (Davis, 2009). Invasive alien species have encroached at many ecosystems, disrupting their structure and function while also diminishing native biodiversity (Borgmann and Rodewald, 2005). Out-compete native species or occupy the available niches in alien environment (Cowie, 1998a:1998b) and cause major economic loss in countries around the world, by decreasing growth and productivity of useful species (Pimentel e*t al.*, 2000).

Invasive Alien plant species (IAPS) are plants that are native to one location but have been brought to an area outside of their normal distribution range where they colonize and threaten biological diversity, ecosystems, and human well-being, and the processes are known as biological invasion (CBD, 2002) . The IAPS can display strong allelopathic properties (Rai and Tripathi, 1982), rapid vegetative growth, prolific seed production (Norbu, 2004), long-lived seeds, early maturation to sexually reproductive stage, phenotypic plasticity and ability to survive in wide range of environmental condition (Tiwari *et al.,* 2005). The IAPS can have a variety of detrimental effects on biodiversity, ecosystems, human health and livelihood, agriculture, and aquaculture, as well as economic losses (Rai and Singh, 2020). IAPS invasion is frequently linked to out-competing native species, resulting in biodiversity loss (Miththapala, 2007). These damage are aggravated by climate change (Kriticos *et al.*, 2003), pollution, habitat loss and human-induced disturbances (Kohli *et al.*, 2009). The IAPS can break plant-consumer interactions drive population decline and species extinction (Donlan *et al*., 2003).

Invasive species are those that have spread beyond of their natural range, causing harm to other species, communities, or entire ecosystems, as well as human health. Non-native, introduced, non-indigenous, exotic, and foreign species are some of the terms used to describe them. Non-natives, on the other hand, are not all destructive or invasive. Invasive alien species (IAS) are a subset of alien plant, animal, fungal, virus, and bacteria species whose establishment and spread endangers ecosystems, habitats, or economically or environmentally important species (McNeely *et al.*, 2011). According to Richardson *et al*. (2011), Invasive species are foreign species that have self-replacing populations through numerous life cycles, generate fertile progeny in large numbers over long distances, and may have negative consequences on the habitat they invade (impacts). Invasive species might be plants, animals, or microbes, and they can be found in practically any ecosystem. At least ten percent of the world's vascular plants (300,000) have the capacity to invade other ecosystems and have a direct or indirect impact on native biota (Lowe *et al.*, 2000).

In Nepal, at least 179 alien flowering plant species have become naturalized. There have been reports of 26 species, largely native to the tropical Americas, being invasive and having an impact on the ecosystem, particularly crop production (Shrestha *et al.*, 2019). Asteraceae is the largest family having 10 species of IAPS followed by two families Amaranthaceae and Caesalpiniaceae having two species each; and remaining 11 families are represented by single species (Shrestha, 2016). Among the IAPS in Nepal, four species (*Chromolaena odorata, Eichornia crassipes, Lantana camera* and *Mikania micarantha*) are included in world's 100 worst species (Lowe *et al*., 2000). All of these species are the most bothersome in various ecosystems in Nepal; for example, *C. odorata* and *M. micrantha* have been causing serious difficulties in the Terai Sal forest, and *Ageratina adenophora* has been causing serious problems in the subtropical lower forest of Central Nepal (Thapa *et al*, 2017). Shrestha (2016) listed following 21 Invasive species which are distributed in all three regions (Eastern, Central and Western) of Nepal: *Ageratina adenophora, Chromolaena odorata, eichhornia crassipes, Ipomea cornea, Lantana camera, Alternanthera philoxeroides, Parthenium hysterophorus, Ageratum conyzoides, Ageratum houstonianum, Amaranthus spinosus, Argemone Mexicana, Senna tora, Senna occidentalis, Hyptis suaveolens, Pistia stratiotes, Bidens pilosa, Mimosa pudica, Oxalis latifolia, Xanthium strumarium, Erigeron karvinskianus* and *Galinsuga quadriradiata*. An assessment of Invasive alien plant species (IAPs) was undertaken for the first time by IUCN Nepal during 2002-2003 in Nepal (GoN, 2019). Nepal's ecosystem is vulnerable to alien plants species because it disrupts various habitat and environmental factors (Kunwar, 2003).

Ageratum haustonianum Mill. is a 30 to 70cm tall annual ornamental herb. It is native to Southeastern Mexico, Central America, and is known as floss flower. It is now grown as an attractive flower and has become invasive as weeds. Traditional Chinese medicine uses the entire plant of *A. haustonianum* to take away heat and harmful substances. This plant is used as an antiphlogistic in Central America to reduce swelling and soreness in the throat (Nan Lu *et al.,* 2014). *A. houstonianum* contain essential oil. Essential oil of *A. houstonianum* is toxic (Pamo et al., 2004). The juice of *A. houstonianum* is used as an external wound healing treatment for skin injuries in traditional medicine (Shin *et al.,* 2007).

The existence of unique weapons such as allelopathic characteristics contributes to the success of alien invading plants (Rai and Tripathi, 1982) and its evolution as a result of increased competition (Callaway and Ridenour, 2004). The current research finds empirical parallels between the effectiveness of exotic human invasions and the employment of advance armaments by exotic plants (Callaway and Ridenour, 2004). Naturalized species are more invasive and free of natural revels –Enemy release hypothesis (ERH) (Torchin *et al.,* 2003). The most of alien species successfully naturalize and disrupt native species, resulting in ecological homogeneity, changes in hydrological features, and gene pools (Vitousek, 1989).

Allelopathy, a stimulatory or inhibitory effect generated by a plant on another species as a result of chemical discharge into the environment, is included in the latter mechanism (Putnam and Tang, 1986). Allelopathy is a biological phenomenon in which a plant produces one or more allelochemicals that affect other species' germination, growth, metabolism, development, survival, and reproduction (Hossain *et al.,* 2012). Allelochemicals affect the target organism and community in either a positive or negative way (Mali and Kanade, 2014). Weed can also affect the growth of plants by releasing allelochemicals into the developing environment (Edrisi and Farahbakhsh, 2011). Allelopathic qualities of several plants have been suggested as a possible cause of serious effects on growth and seedling dry weight of numerous plants in rangeland and farmland (Algandaby and Salama, 2018). The current usage of allelopathy for crop species in weed management in the agricultural system is now.

However, a cash crop cultivator can be used to broaden its duty (Wu *et al.*, 1999). Furthermore, cover crops that exude allele-chemicals or produce residues that breakdown to release allele-chemicals that are phytotoxic to weeds can be planted before cash crops (Batish *et al.*, 2006).

Invasive alien plant species (IAPS) displace native species, diminish biodiversity, change species composition, and have a negative impact on forest regeneration (Belnap *et al.*, 2005, Baret *et al.*, 2008). IAPS has taken over the vegetation and is threatening to extirpate local native species (Vargas *et al*., 2014). IAPS has expanded widely in Nepal, posing a severe threat to biodiversity conservation and forest management (MFSC 2014, Shrestha, 2019). Weed is a term used in agriculture to describe undesired plants that grow in crop fields and compete with crop plants due to their short vegetative phase but high reproduction potential (Cheng and Cheng 2015). Rice (1984) described allelopathy as one organism's direct or indirect inhibitory or positive effect on another through the creation of chemical substances. Allelopathic plants harm surrounding plants by spreading chemicals into the soil that can stifle plant development, nutrition uptake, and germination (Singh *et al.,* 2003). Invasive plants have the potential to alter soil parameters such as moisture and temperature (Belnap and Phillips, 2001: Zavaleta, 2000), pH (D’Antonia, 1993), soil organic matter, carbon, nitrogen and phosphorus content (Chapuis Lardy *et al*. 2006). It's crucial to look into the impact of invasive species on both species composition and soil properties since soil and plants are intertwined, and changes in vegetation caused by invasive species will eventually lead to changes in soil properties, which will lead to more changes in vegetation (Vitousek, 1989).

By releasing harmful essences, some plants can prevent other plants from germinating and developing. Allelochemicals or allelopathic chemicals are the essences, while allelopathy is the method (Rice, 1984). Allelopathy is any process in which secondary metabolites produced by plants, bacteria, viruses, and fungi have a favorable or negative impact on the growth and development of agricultural and biological systems (Torres et al., 1996). Allelochemicals are secondary metabolites produced by plants that influence the germination and growth of plants in their immediate proximity (Rehman, 2005). Angiosperms are said to have a plethora of allelopathic chemicals that are used in weed control techniques (Zhang, 2009). Bioassays under laboratory settings can detect allelopathic activity found in the extraction of a variety of higher plants and plant parts. Allelochemicals were first studied in the lab for seed germination and seedling development (Vyryan, 2002). Allelopathy was defined as the synthesis of allelochemicals by one plant to hinder the germination of other plants. Allelochemicals can be found in every organ of the plant, including roots, stems, rhizomes, leaves, fruits, and seeds, or in just one or two of these organs (Zeng *et al.,* 2008). Through root exudation, volatilization breakdown, and leaching of plant wastes, they are discharged into the soil (Chau, 1999).

The importance of allelopathy in biological weed control and crop yield has long been recognized, and numerous approaches for determining allelopathic activity have been proposed (Fujii *et al.,* 2004). In recent years, allelopathic determination of medicinal species has received a lot of attention (Li *et al.,* 2009). Allelopathy provides a problem for practical weed management strategies (Travlos *et al.,* 2007). Weeds are commonly connected with many crops and pose a significant danger to crop output in many cropping systems. Wheat weeds may reduce yields by up to 25%-30%, according to estimates (Chaudhary *et al.,* 2008). Numerous allelochemicals stymie seed germination, plant growth, and biomass production by disrupting a number of physiological systems within the plant body (Zohaib *et al.,* 2016). Photosynthesis, respiration, cell elongation and enlargement, metabolic activities, protein and amino acid synthesis, and enzyme activities are all important plant functions that are affected by allelochemicals (Shao-lin *et al.,* 2004). Allelopathic interactions can occur depending on the duration of the association, the density of weeds, the species of weeds, and management strategies. Allelopathic weeds have been described in the literature in enormous numbers; they harm crop plants from emergence through maturity, causing significant economic losses (Zohaib *et al.*, 2014).

1.2 Justification

Corridor design is a centripetal conservation tool to facilitate movement between fragmented patches. Increase in anthropogenic activity has caused degradation in forest connectivity, influencing animal movement to small degree Laljhadi-Mohana Biological corridor (Laljhadhi-Mohana Biological corridor (LMBC) is corridor between Suklaphanta national park and Dudhwa national park largest national park of India), is under pressure of anthropogenic change. *Ageratum houstonianum* is invasive species which has grown abundantly in this area. Few researches have been conducted there on invasive species which are responsible for loss of species. LMBC has problem created by invasive species. Land use is changing day by day. Invasive species has great role in land use change. This study will help to find out problem initiated by *A. houstonianum*. Main aim of this study is conservation of native species. This study will useful for agriculture and will be helpful for maintaining ecosystem.

1.3 Research Questions

This research will try to answer the following research questions:

i. Does *Ageratum houstonianum* have impact on species diversity in different land use types of LMBC?

ii. Does *Ageratum houstonianum* have allelopathic effect on germination of crop plants?

1.4 Objectives

The main objective of the study was to find out the impact of *Ageratum houstonianum* in species diversity and crop seed germination in different land use types of Laljhadi-Mohana Biological corridor (LMBC).

The specific objectives of the study were:

- To enumerate the diversity of associated plant species in different land use types of LMBC.
- To examine species diversity of plant species in invaded and non-invaded sites in different land use type of LMBC.
- To analyze the effect of *Ageratum houstonianum* on the seed germination of agriculture crops.

1.5 Limitations

i. Due to COVID lockdown there were difficulties in vegetation sampling and lab work.

ii. Only leaf was taken for preparation of different concentration of *A. houstonianum* .

iii. Vegetation sampling was done on only one season.

iv. Soil analysis was not done.

CHAPTER 2: LITERATURE REVIEW

At least 179 alien plant species have formed a self-replacing population (i.e. naturalized) in Nepal, with 26 species, largely native to the tropical Americas, being reported as invasive and having a negative influence on the ecosystem, particularly agriculture production. In agroecosystems, *A. houstoniunum* is the most problematic (Shresth *et al.,* 2017). Many exotic plants have economic value, since certain alien species, which are frequently farmed, can supply food, medicine, fuel, or fodder to local societies (Das, 2013). Exotic plants have been proven to have a competitive edge over native plants by using allelochemicals, positive plant-soil feedbacks, and high nutrient concentrations (Kulmatiski, 2006).

In the current environment, plant invasion has been regarded as one of the most problematic threats to consider for conserving world biodiversity (Powel *et al*., 2011). Plant invasion species are intruding on land, particularly in forests, where they are displacing native tree regeneration and replacing forest floor vegetation. They also hamper forest operations due to their bushy and spreading character (Kumar and Prasad, 2014).

Invaders exert new selection pressures on resident species, such as through resource rivalry or the use of innovative weaponry. Novel weapons have been found to aid invasion, although it is unclear if native species that coexist with invaders have adapted to withstand them. These resident species that can adapt to new selective agents can coexist with invaders, while others are at risk of extinction locally (Lyytinen *et al.,* 2019). Anthropogenic disruptions frequently alter biological communities and create chances for the invasion of non-native species (Calinger, 2015).

Invasive species richness is more affected by socioeconomic pressure than naturalized species richness (Assl *et al.*, 2019). Invasive species have different effects on the diversity and composition of resident vegetation, therefore management decisions based on impact must distinguish between them. The most severe effects on species diversity and evenness are exerted by tall invading species capable of producing populations with a cover far greater than that of native dominant species. Invaders with a high impact pose a major threat to the landscape because a strong impact on the community size is linked to a decline in species diversity at larger scales (Hejda *et al.,* 2009).

Nutrient enrichment can amplify the allelopathic effects of exotics on natives in an invaded habitat (Xio, 2019). There is no general metric for impact, and the pattern that emerges is dependent on the ecological metric used. Despite the fact that impact is highly context dependent, some species features, such as life form, size, and pollination syndrome, may provide a way to anticipate impact regardless of the habitat or geographical region invaded (Pysek *et al.,* 2011).

The genus *Ageratum* consist of about 40 spp. Found in tropical and subtropical countries, however, only two spp. *A. conyzoides* L. and *A. houstonianum Mill*. are well known (Kong *et al.,* 2004). The chemical composition of essential oil of *A. houstonianum Mill*. was investigated by GC and GC/MS. The essential oil was found to be a mixture of about 50 chemical compounds of which proceneI(23.34%) and proceneII(43.99%) and β-caryopyllene(9.18%) were identified as major constituents. Other chromene derivatives present in lower concentration have been identified and reported (Chandra, 1995). *A. houstonianum* had insecticidal constituents like proceneI, proceneII and β-caryopyllene. It's constituent compound have potential for development into natural insecticides or is used for control of insects (Lu *et al.,* 2014). Essential oil extracted from the leaves of *A. houstonianum* had high efficiency to control parasite (Pamo *et al.,* 2017).

In the seed germination experiment, seed germination and seedling survivorship of native species were determined in the presence and absence of eucalyptus leaf litter or living roots. In the seed germination experiment, seed germination rates of *Elaecarpus sylvestris* and *Tsoongiodendron odorum* were low in both the presence and absence eucalyptus treatment. The germination rate of *Delonix regia* was significantly inhibited by all eucalyptus treatment (Zhang *et al.,* 2009).

On the study of impact of three invasive species (*Prunus serotino Ehrh.* , *Quercus rubra L*. and *Robinia pseudoacacia* on natural regeneration diversity, species composition and density. Result showed an overlap of species on taxonomic diversity and functional richness. There is no impact on phylogenetic diversity and other functional diversity components. The natural regeneration of forest-forming tree species reached lower densities in invaded than non-invaded forest types (Dyderski *et al.,* 2020).

Trianthema portulacastrum L. had allelopathic effects and significant inhibitory effects on germination and seedling growth (Sutradhar *et al.,* 2017). Allelopathic effects of two dominant exotic weeds *A. houstonianum Mill.* and *Chromolaena odorata* showed strong derogative effects on crop plants (Pertin *et al.,* 2018).Invasive species (*Parthenium hysterophorus* and *Chromolaena odorata*) are responsible for inhibition of seedling germination. Leaf extract of these invasive inhibit radicle and plumule growth (Devi *et al.,* 2012).

Research conducted in the greenhouse of the National Research Centre, Giza Egypt showed leaf extract of two medicinal plants *Plectranthus amboinicus (Lour.*) and *Ocimum basilicum L.* inhibit the growth of weeds (EI-Rokiek, 2018). *Trianthema portulacastrum* l. had allelopathic effects and had significant inhibitory effect on germination and seedling growth (Sutradhar *et al*., 2017).Invasive Alien Plant Species had negatively affected tree regeneration and species richness of parsa National Park (Chaudhary *et al.,* 2020). *Argemone Mexicana L.* had allelopathic effects on seed germination and seedling growth of Sorghum bicolor (L.). It inhibits the growth of radicle and plumule (Alagesaboopathi *et al.,* 2013).Some associated weeds of wheat had allelopathic effect which inhibit wheat germinability and biomass production through release of allelochemicals and they are as threat to profitable crop production (Zohaib *et al.,* 2016).

Ipomea carnea is an invasive species. It had many phytochemicals like flavonoids, phenols, tannins and terpenoids. These chemicals are responsible for allelopathic properties which effect on germination of *Amaranthus spinosus* and *Casia fistula* (Jain *et al.,* 2017).*Ageratum conyzoides L.* is an invasive weed that has severely infested cultivated lands and interferes with the growth of crops. *A. conyzoides* exert allelopathic effect toward rice crop by releasing water soluble phytochemicals (Negi *et al.,* 2020). Garlic mustard (*Alliaria potiolata*), Amur honeysuckle (*Lonicere maackii*) and lesser celandine (*Ranunculus ficaria*) are three invasive of US. These species had allelopathic effect on germination and reproduction of *Arabidopsis thaliana*, leaf extracts of these species delayed the germination of agriculture species (*Brassica oleraceae*, *Lactuca sativa* and *Ocimum basilicum*). *Alliaria petiolata* extract were most harmful (Cipollini *et al.,* 2011). By the complete randomized design (CRD) with three replicate, allelopathic effect of *Lantana camara* and *Tithonia diversifolia* were tested. Result showed that germination percentage, radicles and plumule length decreased with increase in concentration of leaf extract. Reduction in length was more pronounced in extracts derived from *Lantana camera* than *Tithonia diversifolia* (Ngonadi *et al.,* 2019).*Chromolaena odorata* is a invasive weed species adversely affecting both crop and grazing lands in tropical and subtropical areas. Study was conducted to investigate the allelopathic effects of *Chromolaena odorata*. Leaf extract inhibited seed germination and seedling growth of the experimental crop and pasture species (Muzzo *et al.,* 2018).

Invasive alien plant species (IAPS) are a significant threat to agriculture resulting in crop loss and increased production cost. Because of their detrimental effects on floral and faunal organisms and their ecosystems, they pose a significant threat to biodiversity (Shah *et al*., 2020).Correlation between the proportion of invasive alien taxa and species diversity were tested. Result showed that the proportion of invasive taxa has a negative effect on the species diversity of all the analyzed syntax in the ruderal vegetation (Rendekova *et al.,* 2019).Invasive species can exhibit allelopathic effects on native species. The allelopathic effects of invasive species on seed germination and growth of native species may altered or even enhanced under conditions with diversified acid deposition. This study showed allelopathic effect of invasive species plant *Solidago canadensis* on seed germination and growth of native plants might be enhanced under increased and diversified acid deposition (Wang *et al.,* 2016).

Invasive plant species should be evaluated and prioritized for management according to their impacts which include reduction in native diversity, changes to nutrient pools and alteration of fire regimes (Barney *et al*., 2013).Several works related to allelopathy has been worked out in different part of world. Allelopathic effect of *Chromolaena odorata* on crop plants evaluated by Pertin (2018). Allelopathic effects of *Parthenium hysterophorus* on seedling germination was examined by Devi (2012). Sutradhar (2017) conducted a research on Allelopathic effects of *Trianthus portulacastrum L.* on seed germination and seedling growth. Another plant *Argemone mexicana* had allelopathic effects on seed germination and seedling growth (Alagesaboopathi, 2013).

Timsina *et al.*, (2010) conduct research on Impact of Parthenium hysterophorus L. invasion on plant species composition and soil properties of grassland communities in Nepal.

According to other literature review, impact of some invasive species like *Parthenium hysteriphorus*, *Lantana camera* was studied. Few researches on *Ageratum houstonianum* have been conducted but alleopathic effect on crop plants are meager. Hence a study on effects of *Ageratum houstonianum* on plant diversity at different land use types and crops has been proposed in this study.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study area

3.1.1 Geographic location

The study was conducted in different land use type of Purnabas municipality of Kanchanpur district which is located in LMBC. The study area is located between 28°01’34’’ N and 80°30’0’’ E. Altitude is 290m.

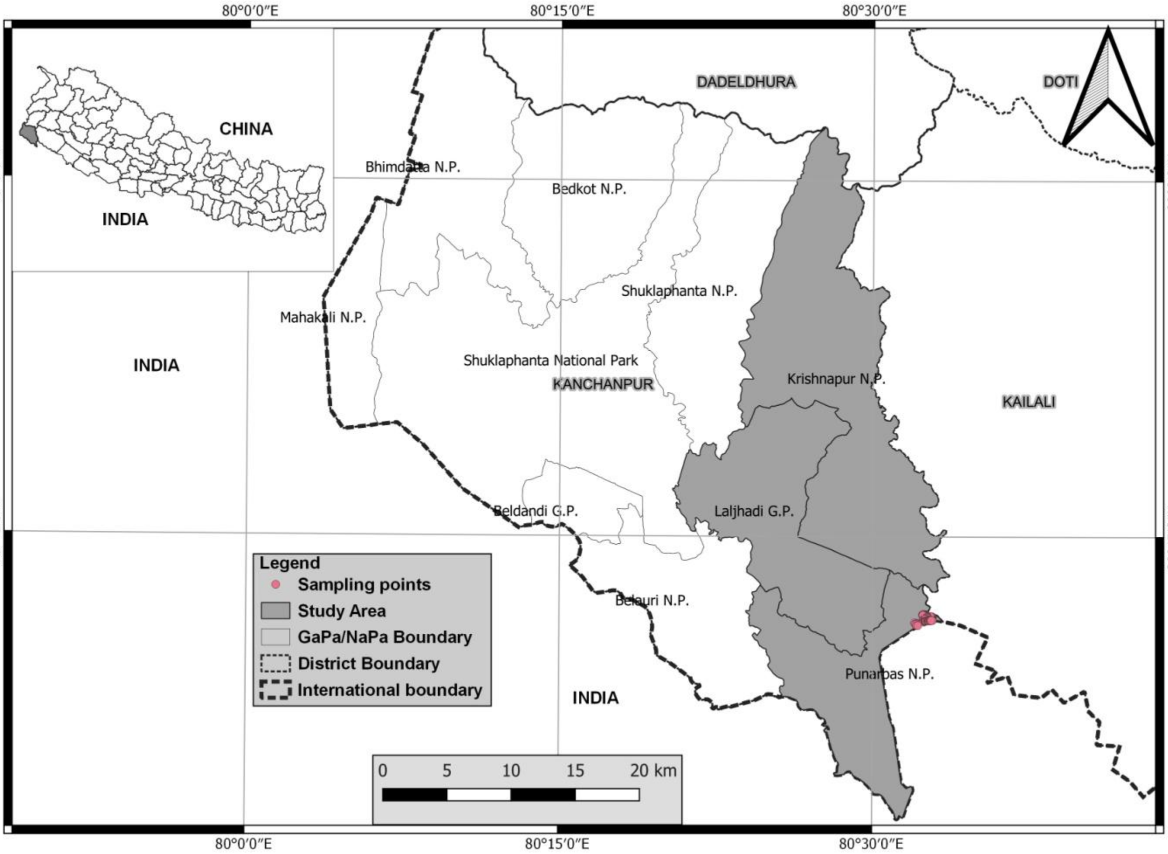


Figure 3. 1: Map of Nepal showing Kanchanpur district

3.1.2 Climate

The study area is characterized tropical climate. The mean yearly maximum and minimum temperature of the area is 30.3˚C and 18.09˚C respectively. The area experiences the maximum average monthly temperature during May with 37.3° C and minimum during January with 7.85°C.The average annual rainfall of the area is 168.31 mm and area receives the highest rainfall 644.14 mm in July.

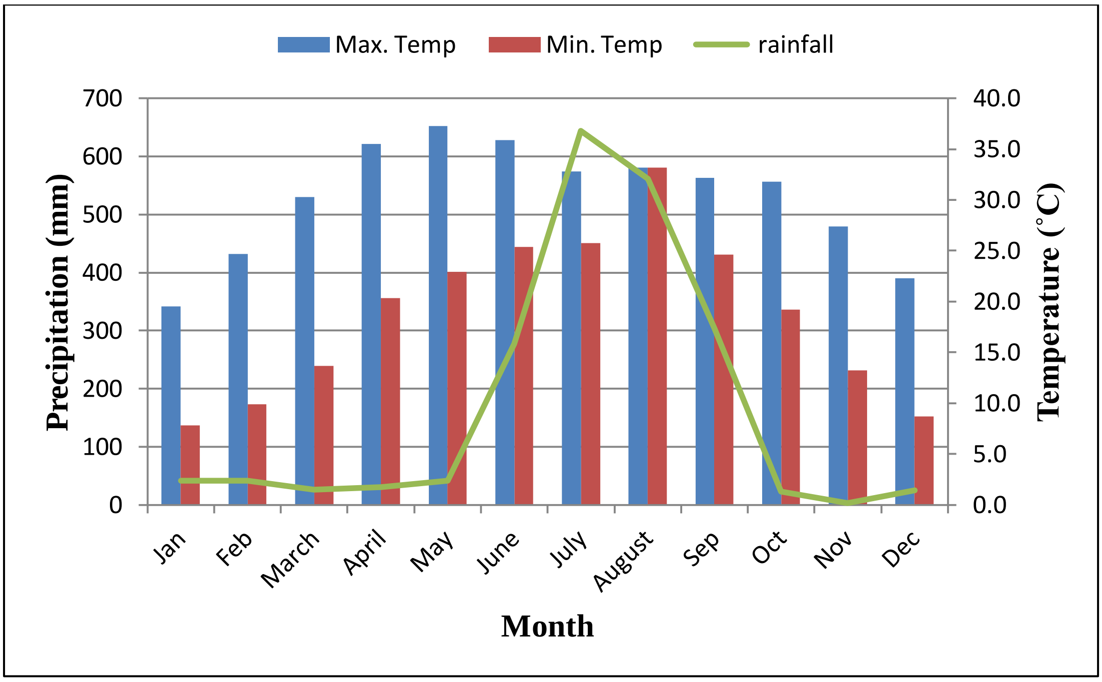


Figure 3. 2: Ten years (2010-2020) climatic graph showing Average monthly temperature and Rainfall of study area. (Source: DHM, 2021).

3.1.3 Sampling site and Vegetation

Research has been conducted in Purnabas municipality which lies in LMBC corridor. Different land use type (Agriculture, road site, abandoned agriculture and Forest) of Purnabas municipality was research area. Where agricultural land is seasonal fallow land, abandoned agricultural land referred to as the process of cessation of farming activities, both with intentional and unintentional giving away of land for natural encroachment, road site is area in which high vehicle movement and anthropogenic movement occurs. Vegetation of forest is characterized by *Shorea robusta.*

3.2 Study design

3.2.1 Field sampling and data collection

Three field visits were made for the research within the study area. The first preliminary visit was carried out during February 2020. Vegetation sampling for herbs was done on September 2020 to January 2021. Sampling was done in different land use types (Agriculture, Abandoned Agriculture, road site and forest). Vegetation sampling was done by systematic sampling method using quadrat methods in different land use type. There were two sampling site i.e. invaded area and non-invaded area (plot having less than 10% coverage of *Ageratum sp*. was considered as non invaded area and vice versa). In each invaded and non invaded site was selected and 30 qudrats of 2m×2m were laid randomly at each land use type (Agricultural, abandoned agricultural, road site and forest). Total 120 quadrats were laid for data collections.

3.2.2 Plant Identification

Most of the plant species were identified in the field by using the field guide book 'Plant Resources of Kailali'. The plant species that were not identified in the field were collected, tagged and pressed in herbarium press. The local names of the specimens were recorded by consulting local villagers as far as possible. The specimens were identified by taxonomist in the Department of Botany, Amrit Campus.

3.3 Vegetation Analysis

The field data was used to calculate IVI by using Relative frequency, Relative density, and Relative coverage. Diversity index was calculated by following Zobel *et al*. (1987).

Frequency and Relative frequency

Frequency is the proportion of sampling units containing the species.

$$\text{Frequency} = \frac{\text{Number of quadrats in which an individual species occured}}{\text{Total number of plots sampled}} \times 100$$

$$\text{Relative Frequency (RF \%)} = \frac{\text{Frequency of individual species occured}}{\text{Sum of the frequencies of all species}} \times 100$$

Density and Relative density

$$\text{Density} = \frac{\text{Total number of species occured}}{\text{Total number of quadrats studied} \times \text{area of quadrat}}$$

$$\text{Density (stem/ha)} = \frac{\text{Total number of individuals of species in all plots}}{\text{Total number of plot studied} \times \text{size of plot (m}^2\text{)}} \times 10000$$

Relative density can be obtained by comparing the density of occurrences of all of the species present.

Relative Density (RD %) = $\frac{\text{Density of individual species}}{\text{Total density of all species}} \times 100$

Relative coverage

Relative coverage (RC %) = $\frac{\text{Coverage of individual species}}{\text{Coverage of sll species}} \times 100$

Important Value Index (IVI)

IVI shows the complete or overall picture of ecological importance of the species in a community. The IVI of each species was calculated as:

IVI = RF+RD+RC

Where,

RF= Relative frequency of individual species

RD= Relative density of individual species

RC= Relative coverage of individual species

Species richness and Species Diversity

The number of species per unit area of interest has been used as measure of species richness of that particular area i.e.

Species richness (R) = S

Where,

S = Number of species within the area of interest

Species diversity refers to the number of species and their relative abundance in a particular location. Shannon Diversity Index (H') and Simpson's Dominance Index were used as measure of species diversity in this study.

Diversity Index (H')

The Shannon index (Shannon, 1948) is one of the most employed variables for the estimation of species diversity; for its determination is employed the formulation:

$H' = -\sum Pi.\ln(Pi)$

Where,

H' = Species Diversity Index

Pi = proportion of the species Pi = ni / N

N = total importance value of plants

ni = importance value of each species

Simpson's Diversity Index

Simpson's diversity index given by Simpson (1949) is often used calculation of plant diversity within a habitat. Within a sample area all plants of all species are counted. The diversity was then calculated using the following equation

$$D = \sum (ni/N)\ 2$$

Where,

D = Simpson's Dominance Index

N = total importance value of plants

ni = importance value of each species

3.4 Allellopathic effect

Allellopathic effect was examined following the protocol of Muzzo *et al*. (2018).

Preparation of leaf extract

Leaf of matured *Ageratum houstonianum* were collected from study field. Collected *A. houstonianum* leaves were dried in room temperature (30˚±4) for 7 days and thereafter in an oven at 70˚c for 48 hour. The dried leaves were milled through a 2mm sieve. A hundred (100) g of powder extracts was added to 1 liter of distilled water in plastic buckets, stirred and was kept for 24 hrs at room temperature and then filtered through double-layered muslin cloth. The filtrates was served as a stock solution of 100% concentration. By subsequent dilution with distilled water leaf extract of 2, 10, 20, 30 and 50% concentrations were prepared and was stored in conical flasks required. However, distilled water was used as control (0%).

Preparation of selected crops

Experiment was conducted on two different seasons (Winter and Summer). Experiment was conducted on August 2020 for summer crop plants. Summer crop plants (paddy and maize) were taken. Experiment was conducted on January 2021 for winter crop germination. Winter crop (mustard and wheat) were taken for experiment. These seeds were surface sterilized by dipping them into 0.5 % aqueous solution of sodium hypochlorite and was rinsed several times with distilled water. Fifteen petri dishes of 10cm double layered with Whatman Number 1 filter papers per species per 5 concentrations levels from the leaf extracts were used while control in each species, 10 uniform seeds per species were placed in separate petri dishes and watered with 5 ml of each prepared concentration and was labeled. The petri dishes were kept at room temperature until the final germination was counted. The seeds were considered germinated upon the emergence of the root. The experiment was set in a completely randomized design with three replicates. Daily data for seed germination was recorded while the root and shoot length was measured 10 days post sowing.

Germination indices

Germination percentage was calculated by following formula.

$$\text{Germination percentage (GP)} = \frac{\text{Total number of seed germinated}}{\text{Total number of seed sown}} \times 100$$

3.5 Statistical Analysis

The statistical analysis was performed by using MS excel 2013, SPSS version 16. ANOVA were conducted to access the impact of concentration on crop seed germination

CHAPTER 4: RESULT

4.1. Vegetation Structure

Altogether 30 species were recorded associated with *A. houstonianum* at four different land-use types of Laljhadi-Mohana biological corridor. Diversity of plants associated with *A. houstonianum* was different at different land use type of corridor. Most common associated species found in all land use types with *A. houstonianum* were *Cynodon* sp., *Crysopogan* sp., *Acmella paniculata, Bidens* sp.*, Mimulus tinella*.(Table 4.1)

Table 4.1: Plant species associated with *Ageratum houstonianum* in different land use types

S.N.	**Scientific Name**	**Family**	**Occurrence in Land use type**			
			Agricultural field	Abandoned Agricultural field	Road site	Forest
1	*Ageratum houstonianum*	Asteraceae	+	+	+	+
2	*Lens esculenta*	Fabaceae	+			
3	*Brasica nigra*	Brassicaceae	+			
4	*Cynodon* sp.	Poaceae	+	+	+	+
5	*Vicia angustifolia*	Fabaceae	+			
6	*Lathyrus aphaca*	Fabaceae	+			
7	*Salvia plebeian*	Lamiaceae	+			
8	*Crysopogan* sp.	Poaceae	+	+	+	+
9	*Sida cardifolia*	Malvaceae	+		+	+
10	*Oxalis corniculata*	Oxalidaceae		+		+
11	*Evolvus nummularis*	Convolvulaceae		+		+
12	*Sida rhombifolia*	Malvaceae		+		+
13	*Eragrostis tinella*	Poaceae		+	+	
14	*Barleria crestata*	Anthacaceae	+		+	+

15	*Mimulus tinella*	Phrymaceae	+		+
16	*Bidens sp.*	Asteraceae	+	+	
17	*Cynodon pentadactyla*	Poaceae	+		+
18	*Acmella paniculata*	Asteraceae	+		+
19	*Bothriochloa* sp.	Poaceae		+	
20	*Axonopus compressus*	Poaceae		+	
21	*Ludwigia perennis*	Onagraceae		+	
22	*Imperata sp.*	Poaceae		+	+
23	*Elephantopus scaber*	Asteraceae			+
24	*Centella asiatica*	Apiaceae			+
25	*Chlerodendron visosum*	Lamiaceae			+
26	*Acmella ulignosa*	Asteraceae	+		
27	*Spermaceae alata*	Rubiaceae			+
28	*Sonchus asper*	Asteraceae			+
29	*Settaria gluaca*	Poaceae			+
30	*Boerhavia* sp.	Nyctaginaceae			+

4.1.1. Community structure in Agriculture Fields

Altogether eight plant species were associated with *A. houstonianum* invaded agricultural fields, which were mostly cultivated with *Lens esculenta*. IVI of the cultivated crop *Lens esculenta* scored highest and next to it the invasive weed *A. houstonianum* scored highest among the weeds (figure 4.1).

At agricultural fields where percentage cover of *A. houstonianum* was less than 10% was considered as non-invaded plots. IVI of the cultivated crop *Lens esculenta* at non invaded fields was highest and was more than that in the invaded agricultural fields. *Brasica nigra* scored second highest IVI among the weeds in non-invaded fields (figure 4.2).

Figure 4.1: IVI of plant species in invaded agricultural field with *Lens esculenta* as crop

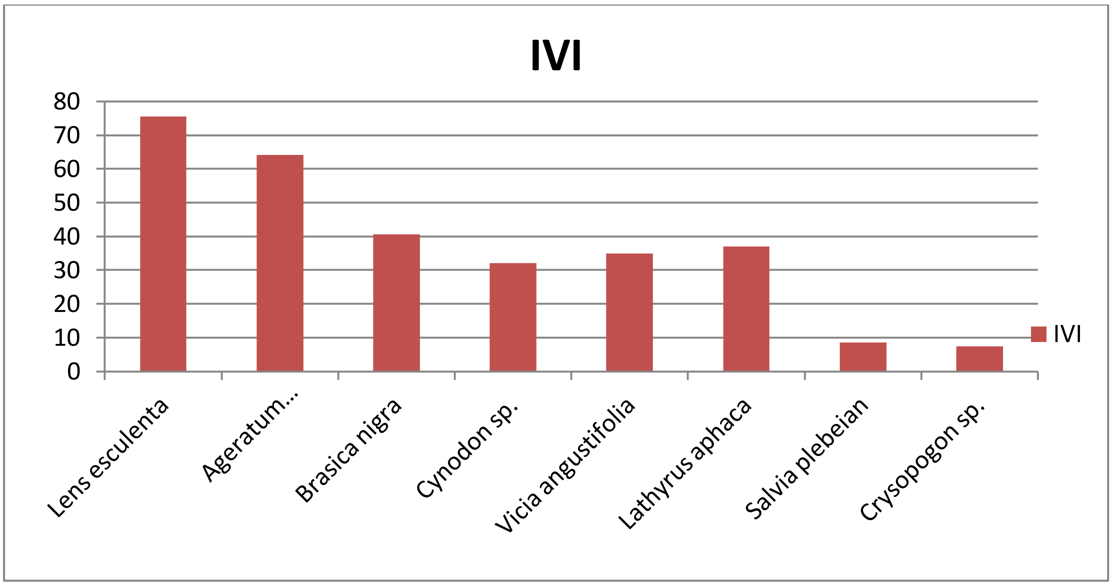
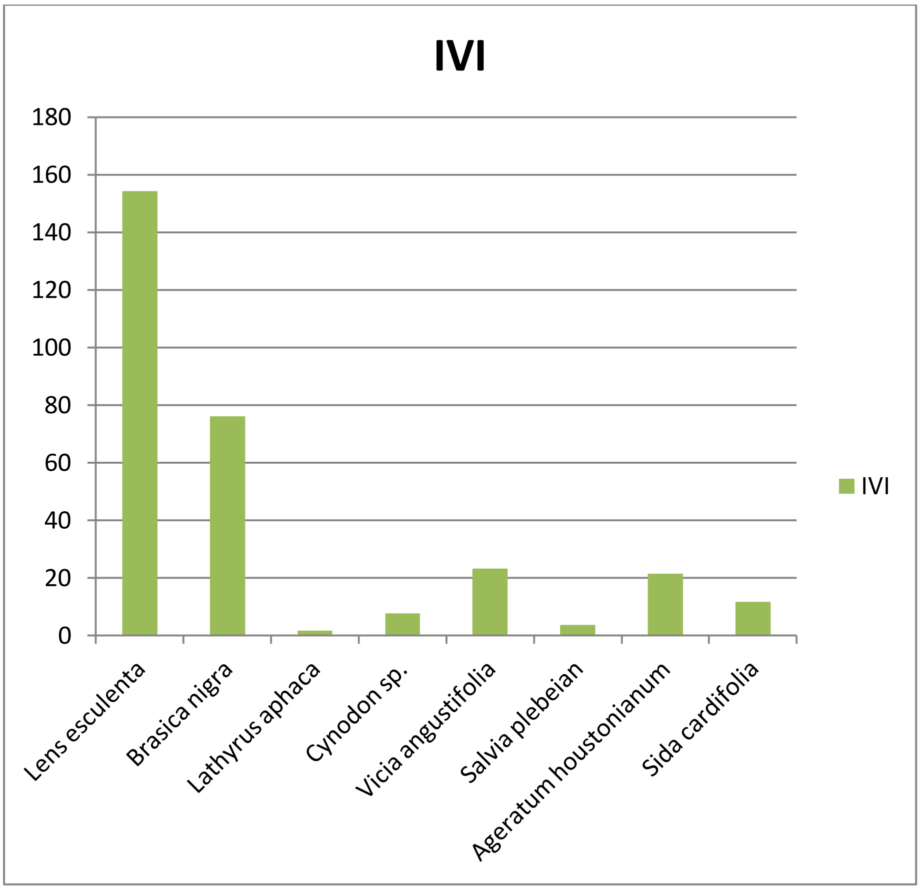


Figure 4.2: IVI of plant species in non-invaded agricultural field with *Lens esculenta* as crop



4.1.2. Community structure in Abandoned Agriculture

All together 11 plant species were recorded from *A. houstonianum* associated Abundant Agriculture fields. IVI of invasive weed *A. houstonianum* scored highest and next to it *Eragrostis tinella* scored highest IVI in invaded abundant agriculture area (figure 4.3).

At non invaded abundant agriculture area where percentage coverage was less than 10%, *Eragrostis tinella* scored highest IVI followed by *Oxalis corniculata*, *Bidens sp*. (figure 4.4)

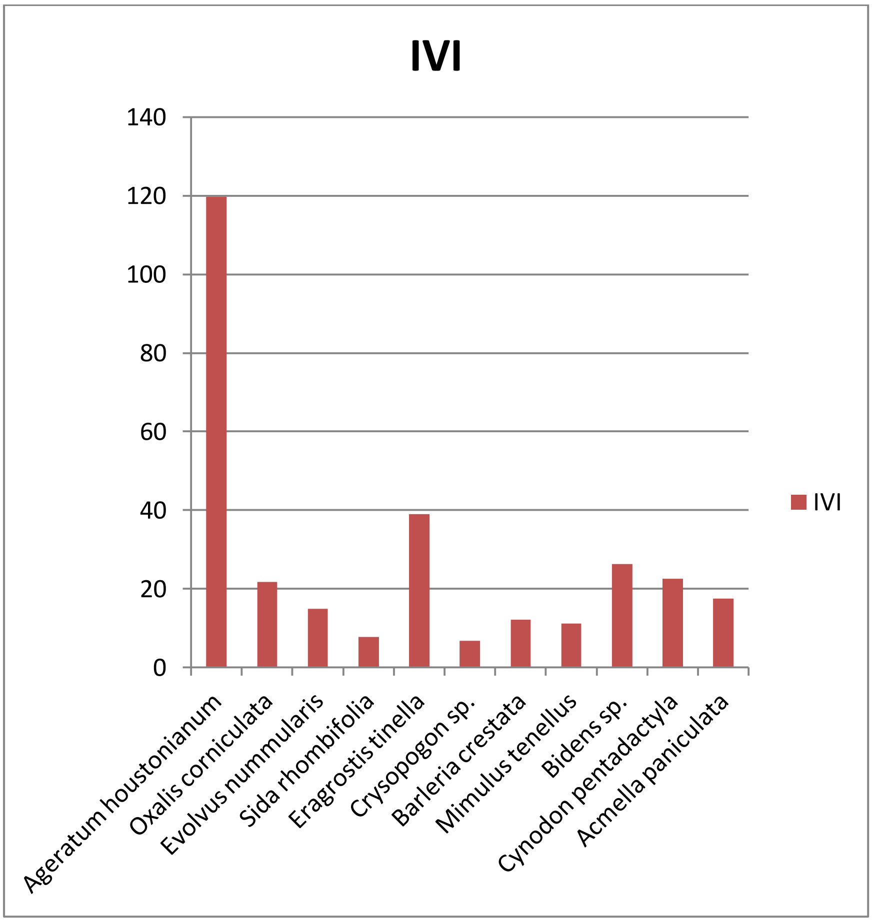


Figure 4.3: IVI of plant species in invaded Abandoned agricultural field.

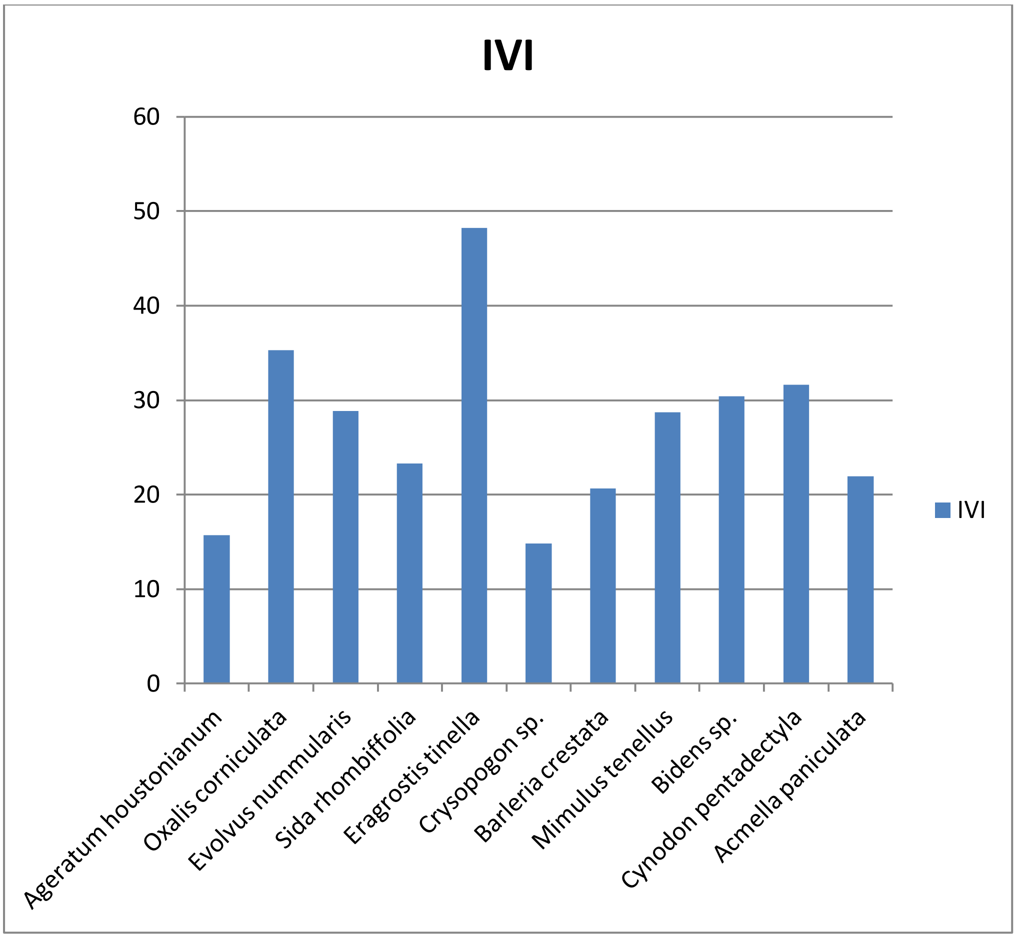


Figure 4.4: IVI of plant species in non-invaded Abandoned agricultural field

4.1.3. Community structure in Road site

Altogether eleven plant species were associated with *A. houstonianum* invaded road site area. Invasive weed *A. houstonianum* had highest IVI followed by *Bothriochloa sp, Sida cordata* (figure 4.5).

Plant associations were similar in both invaded and non invaded site. *Imperata sp.* scored highest IVI and next to it another plant species *Cynodon sp.* scored highest IVI (figure 4.6).

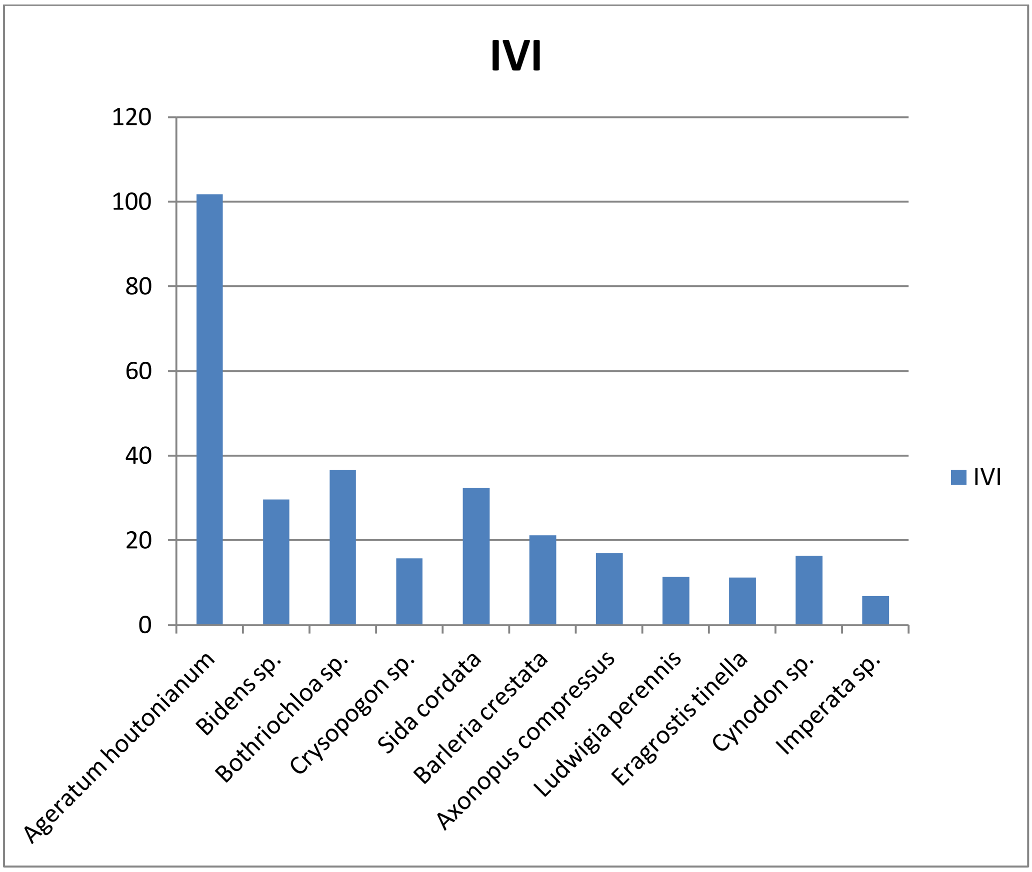


Figure 4.5: IVI of plant species in Invaded area of road site

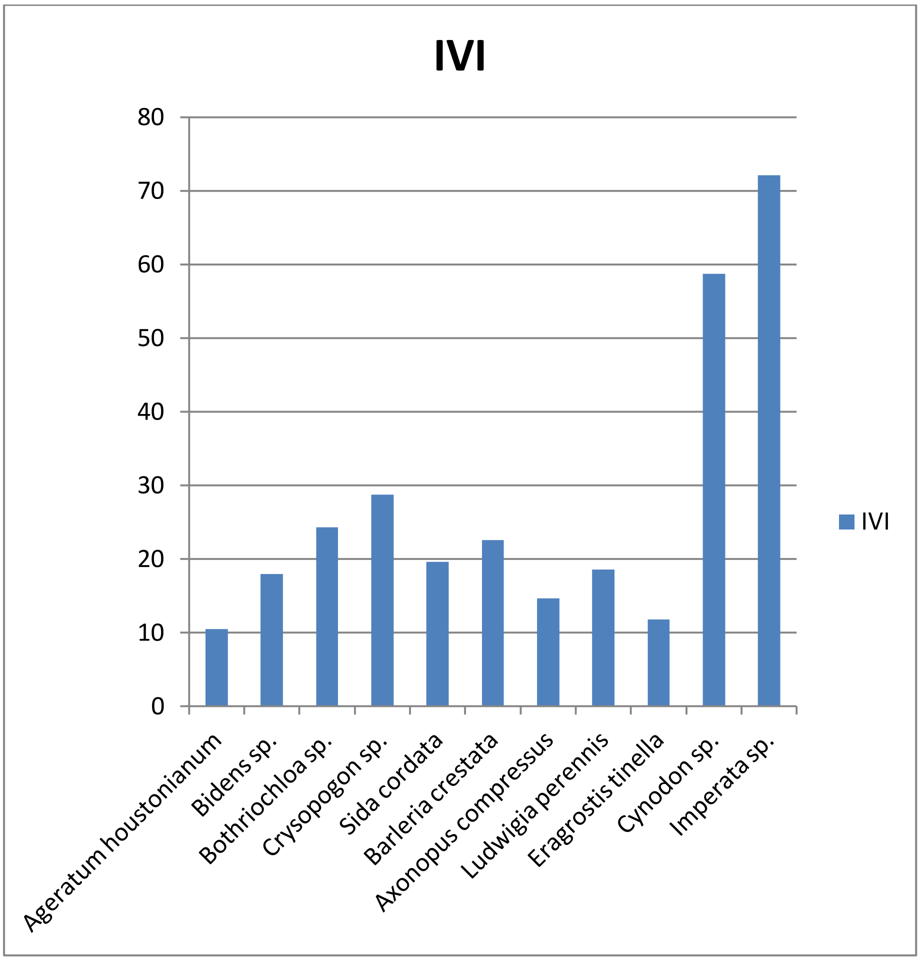


Figure 4.6: IVI of plant species in Non-invaded area of road site

4.1.4. Community structure in Forest

Altogether eighteen plant species were recorded from *A. houstonianum* invaded forest area. Invasive weed scored highest and next to it *Imperata sp*. scored highest IVI followed by *Evolvus nummularius* (figure 4.7).

Altogether twenty species were recorded from *A. houstonianum* non-invavded area where percentage coverage of *A. houstonianum* is less than 10%. *Imperata sp* scored highest IVI in non-invaded area and next it *cynodon sp*. scored highest IVI (figure 4.8).

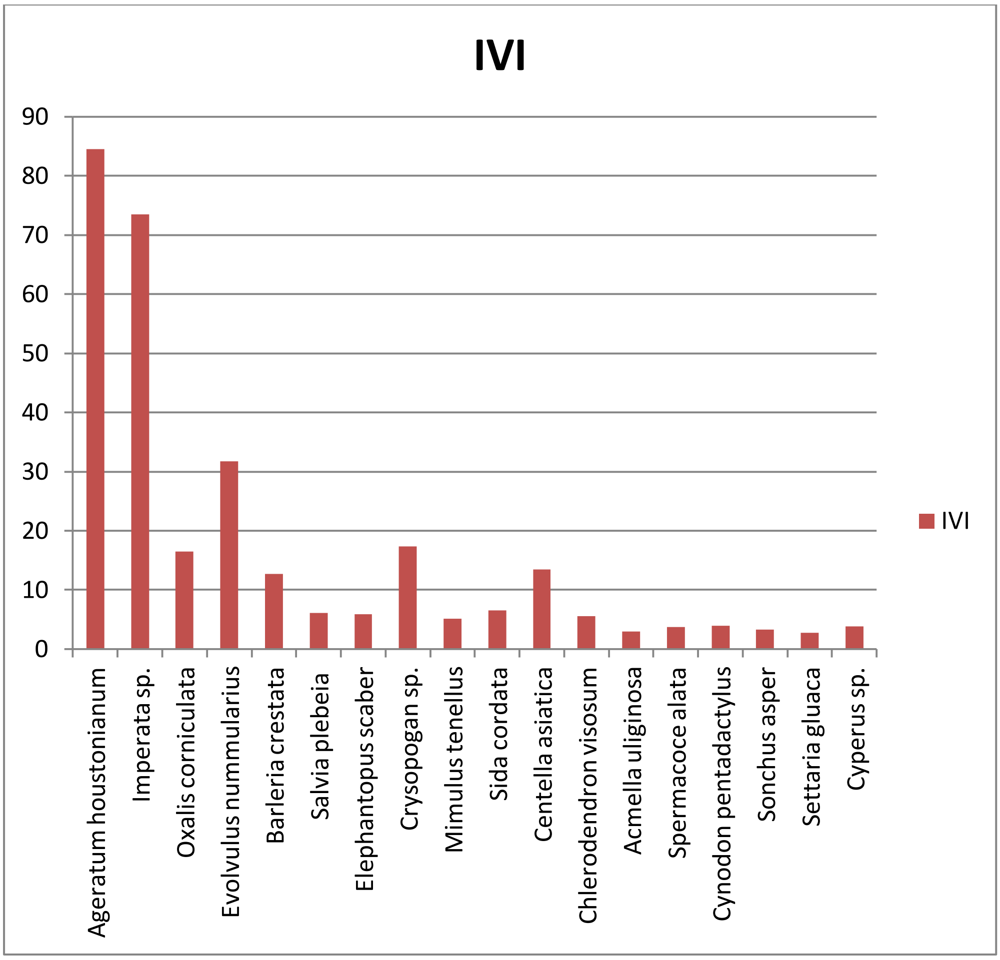


Figure 4.7: IVI of plant species in Invaded area of Forest

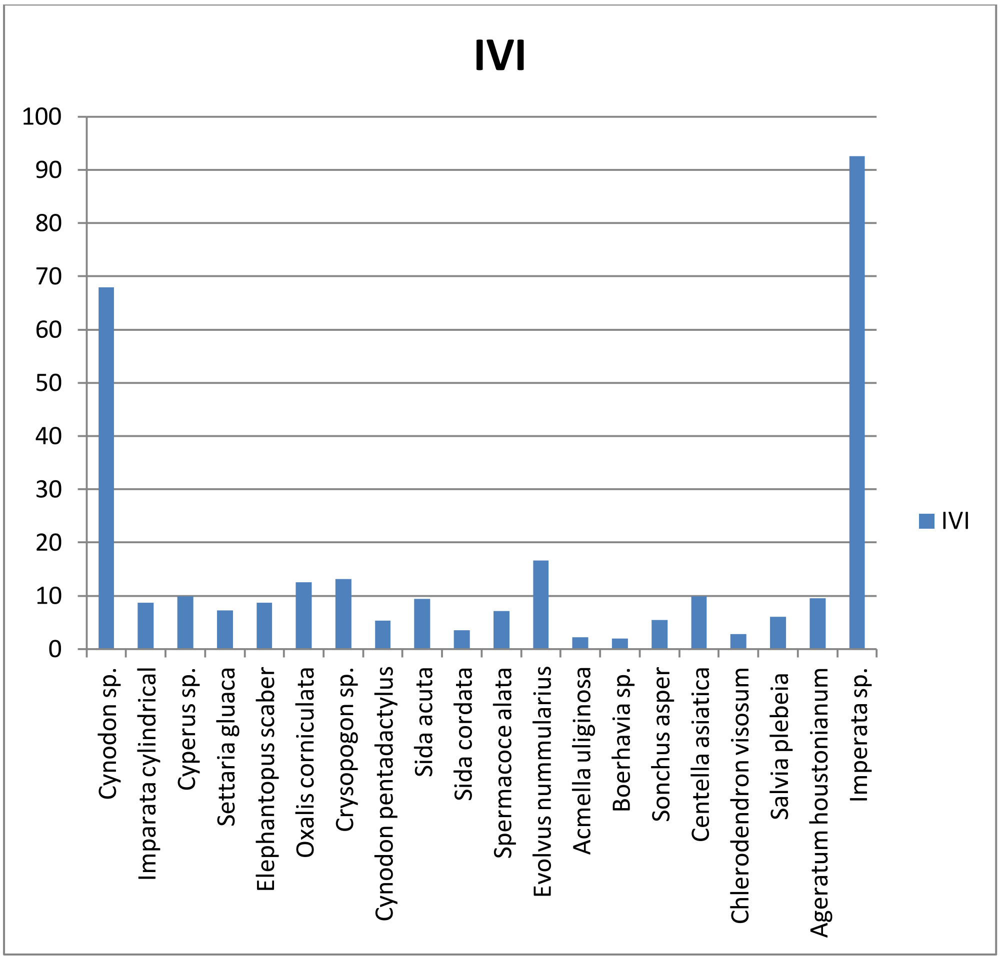


Figure 4.8: IVI of plant species in Non-invaded area of Forest

4.1.5. Diversity indices

Diversity indices were compared in invaded and non invaded area. In agriculture area both diversity indices Simpson index of diversity and Shannon wiener diversity index were high in invaded agriculture area. Evenness was high in invaded area of agriculture field. But in Abundant agriculture area both diversity index were high in non-invaded area and evenness is also higher in non-invaded area. In road site both diversity index were high in non-invaded area and evenness also high in non-invaded area. In forest area, Simpson index of diversity was high in invaded area and Shannon wiener diversity index was high in non-invaded area. Evenness was high in non-invaded area of forest in comparison to invaded area.

Table 4.2: Diversity indices of different Area

S.N.	Index	Agriculture		Abundant Agriculture		Road site		Forest	
		Invaded	Non-invaded	Invaded	Non-invaded	Invaded	Non-invaded	Invaded	Non-invaded
1	Simpson index of diversity	0.81	0.44	0.55	0.9	0.56	0.79	0.73	0.55
2	Shannon wiener diversity index	1.74	0.79	1.24	2.31	1.36	1.85	1.54	2.42
3	Evenness	0.84	0.38	0.51	0.96	0.56	0.77	0.53	0.80

4.2. Allelopathic effect of *Ageratum houstonianum* on seed germination

A. houstonianum had negative impact on seed germination of winter crops but there is no harmful impact on summer crops. Similar result found in growth of radicle and plumule length. Growth of winter crop inhibited by *A. houstonianum* leaf extracts.

4.2.1 Allelochemical effect of *A. houstonianum* on seed germination of summer crop plants

Different concentration of leaf extract showed different result. In summer crop plants *A. houstonianum* had less impact on seed germination. In paddy, germination was same in all the concentrations. In maize, germination was less in 2% concentration used as compare to other concentrations. In 30% concentration, germination rate was higher.

Table 4.3: Allelochemical effect of *A. houstonianum* on germination of summer crop plants

Summer crops	**Concentrations**	**Germination% (Mean ± standard deviation)**
Paddy	Control	70.00±0.00 a
	2%	63.33±15.27a
	10%	66.66±23.09a
	20%	63.33±20.81a
	30%	73.33±20.81a
	50%	60.00±10.00a
Maize	Control	40±20.00ab
	2%	36.66±11.54a
	10%	43.33±23.09ab
	20%	63.33±15.27ab
	30%	70.00±10.00b
	50%	50.00±17.32ab

(Same letter after means ISD in a column do not differ significantly at P=0.05 according to one way ANOVA followed by Duncan's multiple range test.)

4.2.2 Allelochemical effect of *A. houstonianum* on seed germination of winter crop plants

In winter crop plants, germination was inhibited by leaf extract of *A. houstonianum*. In wheat, germination was similar in 2% and 10% concentration as compared to control. Germination was less in 20% concentrations as compared to control and germination is highly inhibited in 30 and 50% concentrations. In mustard, germination was less in all the concentrations used as compared to control, higher inhibition was observed in 50 percent concentration.

Table 4.4: Allelochemical effect of *A. houstonianum* on germination of winter crop plants

Winter crop	**Concentrations**	**Germination% (Mean ± Standard deviation)**
Wheat	Control	100±0.00b
	2%	100±0.00b
	10%	100±0.00b
	20%	96.66±5.77ab
	30%	90±10.00a
	50%	90±0.00a
Mustard	Control	73.33±15.27b
	2%	63.33±35.11b
	10%	43.33±28.86ab
	20%	46.66±25.16ab
	30%	46.66±11.54ab
	50%	13.33±5.77a

(Same letter after means ±SD in a column for each crop do not differ significantly at P=0.05 according to one way ANOVA followed by Duncan's multiple range test.)

4.2.3 Allelochemical effect of *A. houstonianum* on growth of Plumule and Radicle of Paddy

This result shows growth of plumule and radicle of crop plant was highly affected by different concentrations of leaf extract of *A. houstonianum*. In paddy, growth of plumule was less in all concentrations of leaf extract as compared to control. Growth is sigmificantly (p=0.05) inhibited in 2% and 10% concentration of leaf extract. Growth of radicle is less in 2, 10 and 30% concentrations of leaf extract as compared to control. Growth of radicle is high in 50% concentration of leaf extract.

Table 4.5: Allelochemical effect of *A. houstonianum* on Plumule and Radicle length of Paddy

Parts of Paddy	Concentrations	Mean ± Standard deviation
Plumule length(cm)	Control	4.004±0.915b
	2%	3.21±1.01a
	10%	3.19±1.28a
	20%	3.8±0.94ab
	30%	3.53±0.81ab
	50%	3.51±0.88ab
Radicle length(cm)	Control	3.83±1.77ab
	2%	2.93±1.35a
	10%	3.37±2.09a
	20%	3.83±1.32ab
	30%	3.36±1.04a
	50%	4.55±1.92b

(Same letter after means ± SD in a column do not differ significantly at P=0.05 according to one way ANOVA followed by Duncan's multiple range test.)

4.2.4 Allelochemical effect of *A. houstonianum* on growth of Plumule and Radicle of maize

Growth of Plumule of maize seedlings was less in 10% concentrations and growth is high in 2%, 20%, 30% and 50%. Growth of radicle is similar in all concentrations.

Table 4.6: Allelochemical effect of *A. houstonianum* on Plumule and radicle length of Maize

Parts of maize	Concentrations	Mean ± Standard deviation
Plumule length(cm)	Control	4.67±2.45ab
	2%	5.84±2.38abc
	10%	3.35±0.85A
	20%	5.92±3.01abc
	30%	6.29±3.25bc
	50%	7.65±1.66c
Radicle length(cm)	Control	6.29±4.85a
	2%	6.92±1.98a

	10%	5.12±1.88a
	20%	7.00±3.56a
	30%	8.14±2.84a
	50%	5.92±2.35a

(Same letter after means ± SD in a column do not differ significantly at P=0.05 according to one way ANOVA followed by Duncan's multiple range test.)

4.2.5 Allelochemical effect of *A. houstonianum* on growth of Plumule and Radicle of Wheat

Growth of plumule of wheat seedling is similar in 2, 10 and 30% concentrations of leaf extract of *A. houstonianum* as compare to control. Growth rate of plumule was decreased in 30% and 50% concentrations of leaf extract. Growth of radicle is highly affected by different concentrations of leaf extract. Growth of radicle is higher in control. In addition of different concentration of leaf extract growth rate of radicle is significantly inhibited (p=0.05%). Growth of radicle is highly inhibited in 50% concentration of leaf extract.

Table 4.7: Allelochemical effect of *A. houstonianum* on Plumule and Radicle length of Wheat

Part of Wheat	**Concentrations**	**Mean ± Standard deviation**
Plumule length(cm)	Control	5.41±1.63c
	2%	5.23±1.00c
	10%	5.19±1.29c
	20%	5.23±1.02c
	30%	4.25±0.89b
	50%	1.86±0.75a
Radicle length(cm)	Control	7.36±2.06d
	2%	6.29±1.58c
	10%	6.3±1.8c
	20%	5.75±1.37c
	30%	3.98±1.10b

50%	2.03±0.56a

(Same letter after means ± SD in a column do not differ significantly at P=0.05 according to one way ANOVA followed by Duncan's multiple range test.)

4.2.6 Allelopathic effect of *A. houstonianum* on growth of Plumule and Radicle of Mustard

Growth of plumule and radicle is higher in control. Growth of plumule and rdicle is less in 2, 10, 20 and 30% concentrations. Growth of plumule and radicle is highly inhibited by 50% concentrations of leaf extract.

Table 4.8: Allelochemical effect of *A. houstonianum* on plumule and radicle length of Mustard

Parts of Mustard	Concentrations	Mean ± Standard deviation
Plumule length(cm)	Control	1.57±0.46d
	2%	1.35±0.808cd
	10%	1.00±0.65bc
	20%	0.83±0.36abc
	30%	0.56±0.47ab
	50%	0.32±0.15a
Radicle length	Control	2.56±1.34c
	2%	1.66±0.85bc
	10%	1.73±1.37bc
	20%	1.01±0.53ab
	30%	0.88±0.65ab
	50%	0.37±0.22a

(Same letter after means ± SD in a column do not differ significantly at P=0.05 according to one way ANOVA followed by Duncan's multiple range test.)

CHAPTER 5: DISCUSSION

5.1. Impact of *Ageratum houstonianum Mill.* on plant diversity

The result of this study demonstrates a strong effect of *Ageratum houstonianum* on species diversity. These findings are generally consistent with other studies on invasive plants species which indicates strong effect of invasive species. Strong impact on the community scale is associated with reduction in species diversity at higher scale; invaders with a high impact represent a serious hazard to landscape (Hejda *et al.,* 2009). The results demonstrate large differences in the vegetation composition of invaded and non-invaded sites. *Eragrostis tinella, Bothrichloa sp.* and *Imperata sp.* had highest IVI on both invaded and non-invaded area. These plants species belongs to the Poaceae family. Poaceae family has highest potential of pollen emission (Aboulaich *et al.*, 2008). Poaceae family can be explained by its capacity of long-distance dispersal, effective establishment biology, ecological flexibility, resilience to disturbance and the capacity to modify environments (Linder *et al.,* 2018).

Simpson index of diversity of forest and agriculture plot of invaded area was higher. Higher the value of Simpson's index of diversity, higher will be the species diversity. This might be due to the fact that species diversity was calculated including *A. houstonianum*. Simpson diversity index accounts proportion of species in a sample where as Shannon diversity index is based on randomness present at a site and consider both species richness and equability in distribution in sample (He and Hu, 2005). Shannon wiener diversity index was higher in all non-invaded area and lower in invaded area. This result demonstrates that the diversity of invaded area was lower due to the impact of *A. houstonianum.* This result contrasts with the conclusions of other plant species. Invasive species have negative impacts on species diversity (Kohli *et al.,* 2004). Similar result was found in another invasive plant *Argemone Mexicana. Argemone Mexicana* had significant effect on cultivated agricultural field and has allelopathic impact on the growth of wild plants species (Hasan *et al.,* 2014).

In agriculture field, diversity and evenness of invaded site was higher and diversity of non-invaded site was lower. This might be due to *Ageratum houstonium* invasion.

Result shows that in invaded area all species have similar IVI. But in non-invaded area only *Lens esculenta* and *Brasica nigra* had highest IVI. This finding can be agreement with Empty Niche Hypothesis (ENH) proposed by Catford (2009). In abandoned agriculture area evenness and diversity were higher in non-invaded area. In invaded area diversity and evenness were lower. This might be due to impact of *A. houstonianum* in abandoned agriculture. Invasive species may cause changes in ecological services by impacting hydrological cycle, recycling of nutrients, conservation and regeneration of soils, pollination of crops and seed dispersal (McNeely *et al.,* 2001). Diversity of road site was less in invaded area and higher in non-invaded area. Similar study was conducted at Assam along with their harmful impacts. Total 18 invasive alien species of 10 families were recorded from road site area of the study site. Harmful effects were identified on native plants, crop production, live stock, grazing, human health, water drainage etc (Das *et al.,* 2013). In this study, Simpson's diversity is higher in invaded area of forest as compare to non-invaded area. Similar result showed by *Parthenium hysterophorus.* The plant density including *Parthenium hysterophorus* differed significantly between plot types. The density was highest in non-invaded plots. There were fewer species in non-invaded plots than transitional and invaded (Timsina *et al.,* 2010). Previous investigator discover that Shannon's diversity index and evenness showed that non-invaded plots had significantly more diversified species than invaded area of forest. Diversity indices reduced significantly in invaded quadrats indicated that native plant species become less diverse due to *Xanthium strumarium* invasion. The degree of *X. strumarium* invasion affected on species richness resulted to reduce diversity indices significantly in invaded quadrats (Iqbal *et al.,* 2020).

5.2. Allelopathic effect of *A. houstonianum* on crop plants

Different concentration of leaf extract showed different result. In summer crop plants *A. houstonianum* had less impact on seed germination. In paddy, germination was same in all the concentrations. In maize, germination was less in 2% concentration in comparison to other concentrations. In 30% concentration, germination rate was higher in maize and indicted inhancement of germination with *A. houstonianum* leaf extracts. *A. houstonianum* is medicinally used plant. It contain proceneII(62.68%), proceneI(13.21%) and β-caryophyllene(7.92%). These compounds have potential to control insects and bacteria (Lu X. N. *et al.,* 2014). The chemical found in *A. houstonianum* might have promoted the seed germination in some plants.

In winter crop plants, germination was inhibited by leaf extract of *A. houstonianum*. In wheat, germination was similar in 2% and 10% concentration as compared to control. Germination was less in 20% concentrations as compared to control and germination is highly inhibited in 30% and 50% concentrations. In mustard, germination was less in all the concentrations used as compared to control, higher inhibition was observed in 50 percent concentration. The result was consistent with another result showed by some invasive species. Aqueous extracts of leaves of different invasive species *Hyptis sauveolens, Ricinces communis, Alternanthera sessills, Ipomea cornea, Malachra capitata* and *Cymbopogon citrus* had allelochemical effects which are responsible to inhibit the germination of *Vigna radiata* (Joshi *et al.,* 2015).

One of the most notorious invasive species *Chromolaena odorata* showed strong allelopathic effects on growth of seedling (Muzzo *et al*., 2018). *Ageratum conyzoides* exert allelopathic effect towards rice crop by releasing water soluble phytochemicals. It showed significant reduction in seed germination and seedling growth with increasing concentrations (Negi *et al.,* 2020). This study achieved similar result. Growth of plumule and radicle of crop plant was highly affected by different concentrations of leaf extract of *A. houstonianum*. In paddy, growth of plumule was less in all concentrations of leaf extract as compared to control. Growth is highly inhibited in 2% and 10% concentration of leaf extract. Growth of radicle was reduced in 2, 10 and 30% concentrations of leaf extract as compared to control, but growth of radicle is high in 50% concentration of leaf extract.

Previous investigator discover that *Parthenium hysterophorus* and *Chromolaena odorata* had highest degree of inhibition in radicle and plumule growth of the *Zea mays* was observed in 10% concentration (Devi *et al*., 2012). This study showed similar result. In maize, growth of plumule was less in 10% concentrations. Growth is high in 2%, 20%, 30% and 50%. This could be due to the fact that *A. houtonianum* is invasive as well as medicinal plant. *A. houstonianum* is responsible to inhibit the growth of bacteria which are responsible for promotion of growth of seedling. One of the study showed *A. houstonianum* had antibacterial activity. The antibacterial activity of AH-CuNPs was studied against gram-negative bacteria, E. coli and significant (Chandraker *et al*., 2020)growth inhibition was recorded. Growth of radicle is similar in all concentrations.

In wheat, growth of plumule is similar in 2, 10 and 30% concentrations of leaf extract of *A. houstonianum* as compare to control. Growth rate of plumule was decreased in 30% and 50% concentrations of leaf extract. Growth of radicle is highly affected by different concentrations of leaf extract. Growth of radicle is higher in control. In addition of different concentration of leaf extract growth rate of radicle is highly inhibited. Germination of radicle is highly inhibited in 50% concentration of leaf extract. The result was consistent with another invasive plant *Argemone mexicana L.* It had allelochemical effect which is responsible for inhibition of germination and inhibits the root and shoot length of wheat (Shelar *et al.,* 2018). And leachates and residues of weeds inhibit wheat germinability and biomass production through release of allelochemicals (Zohab *et al*., 2010).

In mustard, growth of plumule and radicle is higher in control. Growth of plumule and rdicle is less in 2, 10, 20 and 30% concentrations. Growth of plumule and radicle is highly inhibited by 50% concentrations of leaf extract. This result has similar results with another study. Leaf aqueous extract contain water soluble allelochemicals which could inhibit seed germination. Extract of *Argemone Mexicana L.* is responsible for inhibition of germination and reduce plumule and radicle length of Sorghum (Alagesaboopath *et al.,* 2013). There were many studies which showed invasive species were responsible for inhibition of growth of germination and reduce shoot and root length.

Thus according to the results, invasive plants could release allelochemicals into the invaded ecosystems and then trigger allelopathic effects on the seed germination and growth of native plant. Invasive plants should be evaluated and prioritized for management according to their impacts, which include reduction in native diversity, change to nutrient pools, and alteration of fire regimes (Barney, 2013).

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

Ageratum houstonianum was recorded in all land-use types of Laljhadi-Mohana biological corridor. Native plants which are environmentally important are threatened by *A. houstonianum.* Diversity is highly affected by *A. houstonianum* invasion. It has reduced biodiversity in agricultural land, abandoned agricultural field, road site and forest land, and thus it has affected biodiversity at the corridor. Plant diversity was higher in non-invaded area and lower in invaded area. Germination of winter crop plants is inhibited by leaf extract of *A. houstonianum*. But in summer crop plants there was no harmful impact. This result shows *A. houstonianum* may promotes the germination of summer plants. In winter crop plants, growth of shoot length and root length is inhibited by different concentrations of leaf extract.

6.2. Recommendations

Following are recommendation from this study

i. Further research on other invasive species is recommended in LMBC.

ii. IAs monitoring and management at corridor is recommended.

iii. Education and awareness program to the local people is most important to overcome the problem of invasive species.

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APPENDICES

Appendix I: List of plant species recorded in study area with their families.

S.N.	Name of Plant species	Family
1.	*Ageratum houstonianum Mill.*	Asteraceae
2.	*Lens esculenta*	Fabaceae
3.	*Brasica nigra*	Brassicaceae
4.	*Cynodon* sp.	Poaceae
5.	*Vicia angustifolia*	Fabaceae
6.	*Lathyrus aphaca*	Fabaceae
7.	*Salvia plebeian*	Lamiaceae
8.	*Crysopogan* sp.	Poaceae
9.	*Sida cardifolia*	Malvaceae
10.	*Oxalis corniculata*	Oxalidaceae
11.	*Evolvus nummularis*	Convolvulaceae
12.	*Sida rhombifolia*	Malvaceae
13.	*Eragrostis tinella*	Poaceae
14.	*Barleria crestata*	Anthacaceae
15.	*Mimulus tinella*	Phrymaceae
16.	*Bidens* sp.	Asteraceae
17.	*Cynodon pentadactyla*	Poaceae
18.	*Acmella paniculata*	Asteraceae
19.	*Bothriochloa* sp.	Poaceae
20.	*Axonopus compressus*	Poaceae
21.	*Ludwigia perennis*	Onagraceae
22.	*Imperata cylindrica*	Poaceae
23.	*Elephantopus scaber*	Asteraceae
24.	*Centella asiatica*	Apiaceae
25.	*Chlerodendron visosum*	Lamiaceae
26.	*Acmella ulignosa*	Asteraceae
27.	*Spermaceae alata*	Rubiaceae
28.	*Sonchus asper*	Asteraceae
29.	*Settaria gluaca*	Poaceae
30.	*Boerhavia* sp.	Nyctaginaceae

Appendix II: Relative density, Relative Frequency,Relative Coverage and IVI of plant species in invaded agricultural field with *Lens esculenta* as crop

S.N.	Name of plan species	Relative density (%)	Relative frequency (%)	Relative coverage (%)	IVI
1	*Lens esculenta*	31.05	19.82	24.59	75.46
2	*Ageratum houstonianum*	19.34	19.82	24.94	64.1
3	*Brasica nigra*	20.35	2.18	18.16	40.69
4	*Cynodon dactylon*	6.54	19.82	5.62	31.98
5	*Vicia angustifolia*	10.04	14.54	10.27	34.85
6	*Lathyrus aphaca*	9.94	14.54	12.49	36.97
7	*Salvia plebeian*	2.34	3.96	2.21	8.51
8	*Crysopogon* sp.	0.36	5.28	1.68	7.32

Appendix III: Relative density, Relative Frequency,Relative Coverage and IVI of plant species in non-invaded agricultural field with *Lens esculenta* as crop

S.N.	Name of plant species	Relative Density	Relative Frequency	Relative coverage	IVI
1	*Lens esculenta*	70.72	23.81	59.7	154.23
2	*Brasica nigra*	24.6	23.81	27.64	76.05
3	*Lathyrus aphaca*	0.04	1.58	0.17	1.79
4	*Cynodon dactylon*	0.45	6.34	0.82	7.61
5	*Vicia angustifolia*	2.3	15.71	5.29	23.3
6	*Salvia plebeian*	0.07	3.17	0.41	3.65
7	*Ageratum houstonianum*	1.14	17.46	2.88	21.48
8	*Sida cardifolia*	0.65	7.93	3.11	11.69

Appendix IV: Relative density, Relative Frequency,Relative Coverage and IVI of plant species in invaded Abandoned agricultural field

S.N.	Name of plant species	Relative density	Relative Frequency	Relative Coverage	IVI
1	*Ageratum houstonianum*	66.15	12.93	40.58	119.66
2	*Oxalis corniculata*	4.09	8.6	9.09	21.78
3	*Evolvus nummularis*	2.22	10.34	2.4	14.96
4	*Sida rhombifolia*	1.03	5.17	1.52	7.72
5	*Eragrostis tinella*	9.75	12.93	16.4	39.08
6	*Crysopogon* sp.	1.22	3.44	2.05	6.71
7	*Barleria crestata*	1.85	6.03	4.34	12.22
8	*Mimulus tenellus*	1.9	6.8	2.52	11.22
9	*Bidens pilosa*	5	12.93	8.38	26.31
10	*Cynodon pentadactyla*	3.8	12.93	5.86	22.59
11	*Acmella paniculata*	2.92	7.75	6.8	17.47

Appendix V: Relative density, Relative Frequency,Relative Coverage and IVI of plant species in non-invaded Abandoned agricultural field

S.N.	Name of plant species	Relative Density	Relative Frequency	Relative Coverage	IVI
1	*Ageratum houstonianum*	4.9	7.03	3.78	15.71
2	*Oxalis corniculata*	12.06	9.3	13.96	35.32
3	*Evolvus nummularis*	9.88	10.9	8.07	28.85
4	*Sida rhombiffolia*	7.44	9.37	6.47	23.28
5	*Eragrostis tinella*	18.41	11.7	18.1	48.21
6	*Crysopogon* sp.	4.8	5.4	4.65	14.85
7	*Barleria crestata*	6.95	5.4	8.29	20.64
8	*Mimulus tenellus*	9.99	10.15	8.58	28.72
9	*Bidens pilosa*	9.69	10.93	9.8	30.42
10	*Cynodon pentadectyla*	9.5	11.7	10.4	31.6

11	*Acmella paniculata*	6.31	7.8	7.8	21.91

Appendix VI: Relative density, Relative Frequency, Relative Coverage and IVI of plant species in Invaded area of road site

S.N.	Name of Plant species	Relative Density	Relative Frequency	Relative coverage	IVI
1	*Ageratum houtonianum*	54.42	11.36	36.03	101.81
2	*Bidens pilosa*	5.67	11.36	12.55	29.58
3	*Bothriochloa* sp.	7.52	11.36	17.67	36.55
4	*Crysopogon* sp.	1.91	9.84	3.99	15.74
5	*Sida cordata*	18.54	9.84	4.05	32.43
6	*Barleria crestata*	3.14	10.6	7.37	21.11
7	*Axonopus compressus*	2.45	9.09	5.37	16.91
8	*Ludwigia perennis*	1.53	6.81	3.06	11.4
9	*Eragrostis tinella*	1.04	6.81	3.31	11.16
10	*Cynodon* sp.	2.54	9.09	4.74	16.37
11	*Imperata cylindrica*	1.19	3.78	1.81	6.78

Appendix VII: Relative density, Relative Frequency,Relative Coverage and IVI of plant species in Non-invaded area of road site

S.N.	Name of Plant species	Relative Density	Relative Frequency	Relative coverage	IVI
1	*Ageratum houstonianum*	1.78	6.31	2.38	10.47
2	*Bidens pilosa.*	5.63	7.26	5.09	17.98
3	*Bothriochloa* sp.	6.1	10.52	7.71	24.33
4	*Crysopogon* sp.	7.98	10.52	10.26	28.76
5	*Sida cordata*	4.13	9.47	6.04	19.64
6	*Barleria crestata*	4.27	11.57	6.76	22.6
7	*Axonopus compressus*	2.16	8.42	4.05	14.63
8	*Ludwigia perennis*	3.56	9.47	5.56	18.59

9	*Eragrostis tinella*	1.64	7.26	2.86	11.76
10	*Cynodon dactylon*	26.77	10.52	21.47	58.76
11	*Imperata cylindrical*	35.93	8.42	27.76	72.11

Appendix VIII: Relative density, Relative Frequency,Relative Coverage and IVI of plant species in Invaded area of Forest

S.N.	Name of plant species	Relative Density	Relative Frequency	Relative coverage	IVI
1	*Ageratum houstonianum*	32.53	12.06	39.91	84.5
2	*Imperata cylindrica.*	39.52	12.06	21.86	73.44
3	*Oxalis corniculata*	4.3	8.29	3.9	16.49
4	*Evolvulus nummularius*	9.78	11.26	10.7	31.74
5	*Barleria crestata*	2.18	8.04	2.5	12.72
6	*Salvia plebeia*	0.57	4.8	0.77	6.14
7	*Elephantopus scaber*	0.92	4.02	0.91	5.85
8	*Crysopogan* sp.	3.08	5.6	8.7	17.38
9	*Mimulus tenellus*	0.81	3.2	1.11	5.12
10	*Sida cordata*	0.99	4.02	1.54	6.55
11	*Centella asiatica*	3.21	5.6	4.64	13.45
12	*Chlerodendron visosum*	0.6	4.02	0.96	5.58
13	*Acmella uliginosa*	0.15	2.4	0.38	2.93
14	*Spermacoce alata*	0.18	3.2	0.33	3.71
15	*Cynodon pentadactylus*	0.34	3.2	0.38	3.92
16	*Sonchus asper*	0.13	2.4	0.72	3.25
17	*Settaria gluaca*	0.2	2.4	0.19	2.79
18	*Cyperus* sp.	0.39	3.2	0.24	3.83

Appendix IX: Relative density, Relative Frequency,Relative Coverage and IVI of plant species in Non-invaded area of Forest

S.N.	Name of plant species	Relative Density	Relative frequency	Relative coverage	IVI
1	*Cynodon dactylon*	28.01	10.46	29.49	67.96
2	*Imperata cylindrical*	2.15	3.48	3.03	8.66
3	*Cyperus* sp.	2.24	5.81	1.82	9.87
4	*Settaria gluaca*	1.29	4.65	1.29	7.23
5	*Elephantopus scaber*	1.55	4.65	2.51	8.71
6	*Oxalis corniculata*	2.58	6.97	3.048	12.598
7	*Crysopogon* sp.	3.18	5.81	4.19	13.18
8	*Cynodon pentadactylus*	1.12	2.32	1.9	5.34
9	*Sida acuta*	1.98	5.81	1.67	9.46
10	*Sida cordata*	0.68	2.32	0.53	3.53
11	*Spermacoce alata*	1.29	4.65	1.21	7.15
12	*Evolvus nummularius*	4.13	8.13	4.34	16.6
13	*Acmella uliginosa*	0.25	1.16	0.76	2.17
14	*Boerhavia* sp.	0.43	1.16	0.38	1.97
15	*Sonchus asper*	0.86	3.48	1.06	5.4
16	*Centella asiatica*	2.15	5.81	1.98	9.94
17	*Chlerodendron visosum*	0.086	1.16	1.52	2.766
18	*Salvia plebeia*	1.12	3.48	1.44	6.04
19	*Ageratum houstonianum*	1.72	5.81	2.057	9.587
20	*Imperata* sp	43.1	12.79	36.67	92.56

Appendix X: Plot characterstics

latitude	Longitude	Altitude	highest elevation
28˚,41',34''	80˚32'51"	167m/549f	177m/580f
28˚41'35''	80˚32'52"	180m/593f	"
28˚41'357''	80˚32'51"	174m/571f	
28˚41'38''	80˚32'52"	170m/560f	180m/590f
28˚41'36''N	80˚32'50"E	169m/557f	"
28˚41'36''	80˚32'51"E	181m/595f	181m/593f
28˚41'32''	80˚32'49"	177m/582f	
28˚41'31''	80˚32'49"	132m/434f	181m/593f
28˚41'30''	80˚32'51"	174m/572f	188m/616f
28˚41'34''	80˚32'51"	170m/559f	"
28˚41'34"	80˚32'50"	180m/591f	190m/623f
28˚41'34''	80˚32'49"	181m/596f	198m/649f
28˚41'31''	80˚32'46"	176m/578f	"
28˚41'30''	80˚32'49"	161m/529f	198m/649f
28˚41'30'	80˚32'44"	168m/552f	"
28˚41'29''	80˚32'43"	149m/490f	"
28˚41'30''	80˚32'42"	154m/606f	"
28˚41'30''	80˚32'41"	182m/598f	203m/666f
28˚41'31''	80˚32'48"	176m/579f	"
28˚41'29''	80˚32'40"	209m/687f	209m/685f
28˚41'29'	80˚32'39"	187m/608f	"
28˚41'30''	80˚32'38"	185m/608f	"
28˚41'31''	80˚32'38"	155m/510f	"
28˚41'30'	80˚32'48"	174m/572f	"
28˚41'29''	80˚32'36"	162m/533f	"
28˚41'29''	80˚32'36"	175m/575f	"
28˚41'30''	80˚32'35"	156m/513f	"
28˚41'30''	80˚32'34"	170m/559f	"
28˚41'29''	80˚32'34"	168m/552f	"
28˚41'28''	80˚32'33"	170m/559f	"
28˚41'39''	80˚32'48"	173m/569f	"
28˚41'39''	80˚32'49"	168m/555f	"
28˚41'38''	80˚32'49"	182m/598f	"
28˚41'37''	80˚32'49"	171m/562f	"

28°41'37"	80°32'49"	177m/582f	"
28°41'37'	80°32'48"	161m/529f	"
28°41'37"	80°32'47"	163m/448f	"
28°41'37"	80°32'47"	165m/542f	"
28°41'38"	80°32'46"	167m/572f	"
28°41'39"	80°32'47"	168m/555f	"
28°41'42"	80°32'35"	180m/591f	"
28°41'43"	80°32'35"	182m/598f	"
28°41'42"	80°32'33"	154m/507f	"
28°41'43"	80°32'33"	139m/459f	"
28°41'43"	80°32'32"	162m//534f	"
28°41'43"	80°32'32"	177m/582f	"
28°41'42"	80°32'31"	155m/509f	
28°41'42"	80°32'30"	177m/582f	
28°41'43"	80°32'29"	209m/686f	242m/793f
28°41'44"	80°32'28"	167m/549f	"
28°41'44"	80°32'28"	253m/833f	285m/935f
28°41'44"	80°32'27"	161m/529f	"
28°41'44"	80°32'26"	201m/660f	"
28°41'44"	80°32'26"	147m/483f	"
28°41'44"	80°32'25"	190m/624f	"
28°41'44"	80°32'25"	167m/549f	"
28°41'43"	80°32'24"	188m/618f	"
28°41'44"	80°32'24"	147m/484f	"
28°41'42"	80°32'24"	172m/565f	"
28°41'42"	80°32'25"	169m/555f	"
28°41'39"	80°32'37"	170m/560f	170m/557f
28°41'37"	80°32'38"	182m/598f	185m/606f
28°41'36"	80°32'38"	174m/572f	"
28°41'34"	80°32'38"	171m/562f	"
28°41'32"	80°32'36"	176m/578f	"
28°41'32"	80°32'31"	174m/572f	"
28°41'31"	80°32'26"	170m/559f	"
28°41'29"	80°32'25"	171m/562f	"
28°41'32"	80°32'35"	175m/577f	"
28°41'32"	80°32'30"	178m/584f	"

28˚41'26''	80˚32'25"	173m/569f	200m/656f
28˚41'25''	80˚32'27"	179m/588f	"
28˚41'27''	80˚32'29"	174m/572f	"
28˚41'27''	80˚32'30"	166m/546f	"
28˚41'27''	80˚32'32"	179m/588f	"
28˚41'28''	80˚32'33"	187m/615f	"
28˚41'28''	80˚32'34"	162m/533f	"
28˚41'28''	80˚32'34"	161m/529f	"
28˚41'28''	80˚32'37"	155m/510f	"
28˚41'29''	80˚32'39"	233m/765f	233m/764f
28˚41'28''	80˚32'40"	158m/519f	"
28˚41'28'''	80˚32'41"	158m/519f	"
28˚41'29''	80˚32'43"	175m/585f	"
28˚41'29''	80˚32'43"	178m/585f	"
28˚41'29''	80˚32'45'"	179m/588f	"
28˚41'28''	80˚32'46"	150m/493f	"
28˚41'29''	80˚32'48"	163m/536f	"
28˚41'29''	80˚32'52"	188m/618f	"
28˚41'29''	80˚32'49"	163m/536f	"
28˚41'21''	80˚32'9"	262m/862f	290m/951f
28˚41'22''	80˚32'5"	208m/683f	"
28˚41'23''	80˚32'1"	210m/690f	290/951f
28˚41'21''	80˚32'5"	219m/719f	"
28˚41'19''	80˚32'4"	214m/705f	"
28˚41'19''	80˚32'4"	221m/727f	"
28˚41'16''	80˚32'7"	188m/618f	"
28˚41'16''	80˚32'10"	210m/689f	"

PHOTOPLATE

Germinating seeds in petridish

Plumule and Radicle of mustard seed

Measuring radicle of maize

Pouring leaf extract