STATUS OF *Chromolaena odorata* IN CHAPAKOT MUNICIPALITY AND ITS EFFECT ON GERMINATION OF SELECTED CROP AND NON-CROP PLANTS

A THESIS

Submitted for Partial Fulfillment of the Requirement of Master's Degree in Botany

BY

Roshan Adhikari

Symbol: 555/074

TU, Reg. No. 5-2-48-1486-2013

Batch: 2074

Department of Botany

Amrit Science Campus

Tribhuwan University Kathmandu, Nepal

2022

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I, Roshan Adhikari, 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 are listed in the reference section.

SIGNATURE

Roshan Adhikari
Department of botany
Amrit campus
Lainchaur, Kathmandu
February, 2022

Tribhuvan University
Institute of Science & Technology
AMRIT CAMPUS
P. O. Box No. 102, Thamel, Kathmandu, Nepal.
E-mail: amritcampus@ntc.net.np
☎ 4411637
4410408

Institute of Science & Technology
Amrit Campus

Ref No.:

Date: 13/02/2022

RECOMMENDATION

This is to recommend that the Master's thesis entitled **"Status of *Chromolaena odorata* in Chapakot Municipality and its effect on germination of selected crop and non-crop plants"** is carried out by Roshan Adhikari under my supervision. The entire work is based on original scientific investigations and has not been submitted for any other degree in any institutions. I therefore, recommend this thesis work to be accepted for the partial fulfillment of Master's Degree in Botany.

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Supervisor
Prof. Dr.Kanta Poudyal
Amrit Campus
Tribhuvan University
Kathmandu, Nepal
February, 2022

Tribhuvan University
Institute of Science & Technology
AMRIT CAMPUS
P. O. Box No. 102, Thamel, Kathmandu, Nepal.
E-mail : amritcampus@ntc.net.np
4411637
4410408

Institute of Science & Technology Amrit Campus

Ref No.: Date:

APPROVAL

The thesis work submitted by Roshan Adhikari entitled **"Status of *Chromolaena odorata* in Chapakot Municipality and its effect on germination of selected crop and non-crop plants"** submitted to Department of Botany, Amrit Campus, Tribhuvan University, TU Registration Number 5-2-48-1486-2013 has been accepted for the partial fulfillment of the requirement for Master's Degree in Botany.

Expert committee

External Examiner
Dr. Anjana Devkota
Department of Botany
Central Department of Botany
Tribhuvan University
Kathmandu, Nepal

Internal Examiner
Lect. Krishna Prasad Sharma
Department of Botany
Trichandra Campus
Tribhuvan University
Kathmandu, Nepal

Supervisor
Prof. Dr. Kanta Poudyal
Department of botany
Amrit Campus
Tribhuvan University
Kathmandu, Nepal

Program Coordinator
Lect.Dr. Laxmi Joshi Shrestha
Department of Botany
Amrit Campus
Tribhuvan University
Kathmandu, Nepal

Head of Department
Asso. Prof. Dr. Shila Singh
Department of Botany
Amrit Campus
Tribhuvan University
Kathmandu, Nepal

Date of oral examination: 23 March, 2022

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ABBREVIATIONS

ANOVA	Analysis of Variance
C	Coverage
D	Density
F	Frequency
FG	Final germination
GI	Germination Index
GP	Germination percentage
IP	Inhibitory percentage
IVI	Important Value Index
LSD	Least Significance Difference
MGT	Mean Germination time
MRT	Minimum Residence Time
RC	Relative coverage
RD	Relative density
RF	Relative Frequency
RG	Relative germination

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ABSTRACT

A worst weed named *Chromolaena odorata* has great impact on native plants and vegetation due to its fast invading characteristics. It is the worst invasive species in different part of the world including Nepal. This study tried to find out the status of *C. odorata* on Chapakot Municipality by concentric quadrat (30 quadrats) methods in which 5m radii for study of shrub and 1m radii for study of herbs and also tried to find out the impact of aqueous extract of leaves of *C. odorata* by petridish bio-assay. 100 g of dried grinded leaves mixed with 1ltr distilled water and filtrates were considering as 100% concentration and different concentration of 25%, 50%, 75% were made. Seeds of *Zea mays*, *Senna tora*, *Triticum aestivum*, *Eleusine caracona, and Ageratum conyzoides* were tested in term of germination in different concentration and seedling growth were also measured. IVI value of *Chromolaena odorata* was found higher compared to other plant species. The result showed that there was significant difference ($P<0.05$) on seed germination and seedling growth of crop and non-crop plants. Tested non-crop plants were affected severely as compared to crop plants.

Key words: *Allelopathy, Chromolaena odorata, concentration, germination, seedling growth*

CHAPTER 1: INTRODUCTION

1.1. Background

1.1.1. Diversity and status of *Chromolaena odorata*

Chromolaena odorata (L.) King and Robinson (Asteraceae) is a perennial shrub native to Central and South America (Ambika, 1980). This species has been considered one of the most invasive weeds globally that threaten agriculture and the environment in Central and Western Africa, tropical America, India, Nepal, Philippines, southern China, South Africa, eastern Indonesia, and Australia (Muniappan *et al.*, 2005). In most invaded countries, *C. odorata* can quickly establish and often form a dense scrambling thicket that grows through and smother plant crops, forestry, and native vegetation (Ambika, 1980). Mandal and Joshi (2014) reveal areas with high biotic pressure, high-temperature variation and open forest canopy. The absence of herbivory is the most favorable habitat for *C. odorata*. They also claimed that a higher level of phosphorus, potassium, magnesium, soil organic matter, and nitrogen and acidic soil in all invaded sites are possible reasons for the further invasion of *C. odorata*. The mechanisms underlying the invasion success of *C. odorata* are still not well understood. The "tens rule" hypothesizes that about 10% of all species transported to a new environment will be released or escape and become introduced species. Subsequently, 10% of those introduced species establish viable populations in the wild. Finally, about 10% of the established species become highly damaging. *C. odorata* depends on the combination of its high reproductive capacity, high relative growth rate, and capacity to inhibit native plant growth by fair competition and accumulation of native soil pathogens (Mangla *et al.*, 2008). Previous studies have shown that *C. odorata* produces a variety of allelochemicals, including flavonoids (Ngozi *et al.*, 2009), terpenoids (Duñg *et al.*, 1992), and alkaloids (Ambica, 1980; Biller, Boppré, *et al.*, 1994; Akinmoladun *et al.*, 2007).

C. odorata first recorded in Nepal in 1825 is locally called "Seto Banmara." It is found in most Terai, Siwalik, and That is, 1% of the number originally transported to the new environment is a highly damaging invader (Williamson and Brown, 1986). Hills areas. It invades many habitats, including roadsides, forests, agricultural fields, disturbed grasslands, and abandoned fields, causing significant economic and biodiversity losses (Shrestha 2016). *C. odorata* is mainly dispersed by wind. It has a Minimum Residence Time (MRT) of 190 years even though the *C. odorata* has not been reported from the Terai Region west of Karnali River (Shrestha 2016). In

Nepal, *C. odorata* is not found beyond 83°15' East longitude to the west as it requires a minimum rainfall of about 1200mm, and does not extend boyend 28°11 N to the North (Joshi *et al.*, 2005). The reduction of biodiversity and prevention of forest regeneration due to *C. odorata*, particularly in Terai, Siwalik, and Middle Mountain regions of Chitwan-Annapurna Landscape, Nepal. They have formed impenetrable and monodominant stands smothering other vegetation and preventing native tree seedling establishment. This situation had reduced forest regeneration and minimized the supply of forage to wild and domestic animals (Shrestha *et al.*, 2019).

1.1.2. Allelopathy

The phenomenon of plants influencing neighboring plants by releasing chemicals in the environment has been known as early as c. 370 BC. Greeks and Romans have used this knowledge in agriculture since c. 64 AD. However, it was not until 1937 that Hans Molish gave it a formal name, allelopathy (Mallik, 2008). Molish (1937) defined allelopathy as encompassing both stimulatory and inhibitory biochemical interactions among plants at all levels of complexity, including microorganisms. This term was introduced to define "any direct or indirect harmful or beneficial effect by one plant (including microorganisms) on another through the production of chemical compounds that escape into the environment" by Rice (1984).

Allelopathy is a phenomenon that describes the effect of biochemical produced by one organism on the growth, survival, development, and reproduction of other neighboring organisms which may be stimulatory or inhibitory (Reigosa *et al.*, 2006) to the counterpart species. Such biochemical interactions (beneficial or detrimental) can be used in plant cultivation management as growth regulators, herbicides, and insecticides. Allelopathy generally refers to the detrimental effect of the high plant of one species (the donor) on the germination, growth, and development of another species (the recipient) (Devi and Dutta, 2012) through the production of biochemicals known as allelochemicals. Allelochemicals are non-nutritional secondary metabolites produced by living organisms (i.e., plants) that have stimulatory or inhibitory effects upon the growth, health, behavior, or population biology of neighboring organisms (plants, insects, and microbes. In-plant to plant allelopathy, the more common inhibitor effects are visible upon plant functions such as respiration (Kohli *et al.*, 1997); photosynthesis (Patterson, 1981); water balance and stomatal function; stem conductance of water; xylem element flux; membrane permeability; cell division and development; protein synthesis; and enzyme activity alteration (Barkosky *et al.*, 2000). Within plants, allelochemicals may be distributed broadly among organs such as seeds, flowers, pollen, leaves, stems, and roots, or sometimes found in just one or two such locations. Their kingdom distribution is rather specific, with a particular class of allelochemical being often found confined to a limited range of plant families or genera (Haig, 2008). Allelochemicals, in general, are released into soils by leaf leaching, root exudation, or degradation of plant residues before any allelopathic effects become evident (Inderjit and Nilsen, 2003). Allelopathic interactions do not occur if the allelochemicals rapidly degrade after being released into the soil. Soil microbes play a significant role in mediating allelopathic interactions between organisms by transforming allelochemicals into less or more toxic chemicals (Inderjit, 2005; Kwong *et al.*, 2008; Achatz *et al.*, 2014). Biological invasion is causing profound current biodiversity loss in different parts of the world and involves different stages: introduction, establishment, naturalization, and fast dispersion outside the standard ranks. Invasion causes a reduction in the abundance of native species or the eradication of populations of a particular species. Exotic species have to exceed different biological filters to invade a new habitat. The importance of allelopathy in the invasion process may include the release of secondary chemical metabolites into the surrounding environment to inhibit the seedling establishment and other Ecophysiological attributes of native biota (Lorenzo *et al.*, 2013). The biological invasion has been considered an essential component of environmental changes and a leading cause of decline and loss of native biodiversity (Kohli *et al.*, 2004). The problem of invasive species is prevalent both in developed and developing countries like Nepal due to the lack of expertise and limited resources available for their management (Shrestha, 2016). In Nepal, 25 species of plants are reported as invasive; four species (*Chromoleana ordata, Eichhornia crassipes, Lantana camera,* and *Mikania Micrantha*) are included in the world's hundred worst invasive species (Shrestha, 2016).

The term germination in the seeds of higher plants denotes the outcrop of a root or shoots from the seed coat, while emergence is the visible penetration of the shoot above the soil surface (Hadas and Russo, 1974; Hadas, 1976; Arnold *et al.*, 1991). For that seed to germinate, it must be placed in environmental conditions favorable to this process (Crawford and Brändle, 1996). The length of time passed between the first seed to germinate, and the last, variation in germination speed and timing that the majority of seeds germinate, these all have impacts on diverse cultural operations like fertilizing, harvesting, and field maturity of crops (Roberts and Totterdell, 1981; Washitani and Saeki, 1986; Kader and Jutzi, 2001).

The proposed study site, Syangja is one of the District which has large number of people depends on the agriculture. The developmental activity like road, canal construction and unscientific agricultural production make this district vulnerable to encroachment of invasive species. Knowledge about the impact of *C. odorata* on native vegetation, agriculture field and pasture land of Syangja district is trivial (verbal communication).

1.2. Justification

Invasions by alien plants into unspoiled ecosystems are a cause for concern because the pristine systems are important stores of biodiversity. Indeed, the introduction of non-indigenous species into protected areas is a direct threat to conservation. Consequently, it is fundamental to document the status of invasive plants and its impacts on native communities and to determine if, and at what rate, native communities re-establish following the removal of invasive.

Previous work has not been done regarding status and allelopathic impact of *Chromolaena odorata* on the germination and seeding growth of wheat (*Triticum aestivum*), millet (*Eleusine caracona*) and maize (*Zea mays*) in Nepal. Hence, this study provides the information about the impact of *Chromolaena odorata* on germination and growth of major crop plants and other invasive species like *Ageratum conyzoides & Senna tora.*

1.3. Research Questions

The major research question of this research study was what does the status of *Chromolaena odorata* in Chapakot municipality and how does the concentration of aqueous extract of *Chromolaena odorata* effects on germination and seedling growth of some selected crops and non-crop plants?

1.4. Research Objectives

The general objective of the study was to find out the allelopathic effect of *Chromolaena odorata*. The specific objectives were:

i. To find out the Importance Value Index (IVI) and Species diversity indices of different species in study site.

ii. To assess the effects of aqueous extract of *C. odorata* in the crop plants and in non-crop plants on the rate of germination.

iii. To assess the effects of aqueous extract of *C. odorata* on average length of root and shoot of crop plants and invasive plants at the initial growth stage.

1.5. Hypothesis

Chromolaena odorata aqueous leaf extract has significant effect on seed germination and seedling growth of the crops and invasive species.

1.6. Limitations

The major limitations of the study were:

i. Only aqueous extract leaves of *Chromolaena odorata* were taken to find out the allelopathic impact in different species.

ii. Only five species (i.e. *Zea mays*, *Senna tora*, *Triticum aestivum*, *Eleusine coracana* and *Ageratum conyzoides*) were taken to find out the allelopathic impact of aqueous extract of *C. odorata*.

CHAPTER 2: LITERATURE REVIEW

2.1.Diversity

A study was done to find out the impacts of *Chromolaena odorata* on plant species richness, composition, and *Shorea robusta* seedlings in a tropical Sal Forest of Nepal. Results showed differences in native species richness and *S. robusta* seedling density between invaded and uninvaded plots. The invaded plots were associated with fewer species than uninvaded plots. Plot type (invaded and uninvaded), *C. odorata* density, and the cover showed the effect on vegetation composition. Moreover, some of the native species were replaced by *C. odorata* invaded sites Thapa *et al.* (2016).

The effects of the Triffid weed *C. odorata* on small and large mammals in Hluhluwe-iMfolozi Park and found that the invaded areas were not suitable habitats for small mammals. It was also found that small and large mammal species richness and diversity decreased with the increase in invasion duration, which shows that the more time *C. odorata* is left to establish, the more disturbance it causes to the habitat (Dumalisile, 2009). Agboola and Muoghalu (2015) evaluated the impact of *Chromolaena* and *Tithonia* and found an average number of plant species in *Chromolaena* and *Tithonia* invaded plots was reduced by 31.6 % and 25.4 %, respectively; Shannon-Wiener diversity was reduced by 13 % and 27 %, respectively; species evenness was reduced by 10.5 % and 24.9 % respectively. Sorensen similarity of an index between invaded and uninvaded plots for *Chromolaena* and *Tithonia* was 43.8 % and 32.6 %, respectively. Shackleton *et al.*, (2017) results indicate that *C. odorata* is a relatively new invader that already has severe negative impacts and threatens livelihoods and the environment. Impacts include reductions in native biodiversity and the amount of available forage for livestock, reduced crop and water yields, and impaired mobility. The continued spread of the species will increase additional negative impacts on poor rural communities.

2.2.Allelopathy

The Allelopathic effects of entire shoot extract, plant part extracts, and shoot residue of parthenium (*Parthenium hysterophorus* L.) on corn (*Zea mays* L.), ryegrass (*Lolium multiflorum* Lam.), wheat (*Triticum aestivum* L.), velvetleaf (*Abutilon theophrasti* Medik.), and soybean [*Glycine max* (L) Merr.] growth was examined which showed a strong correlation between extract concentration and increased toxicity to test species (Mersie and Singh, 1987).

The effects of shoot or root extracts were studied by Jha and Dhakal (1990), where 15 g crushed shoot or 3 g crushed root placed in 100 ml water for 24 h followed by filtering or shoot or root water (15 g entire shoot or 3 g intact root soaked in 100 ml water and removed after 24 h), from *Ageratum conyzoides, Alysicarpus vaginalis, Centella Asiatica, Desmodium triflorum, Eclipta alba, Fimbristylis dichotoma, F. littoralis, Hedyotis corymbosa* and *Oxalis corniculata* on seed germination and seedling growth of rice and wheat. Seed germination was inhibited in rice and wheat by *A. conyzoiodes* and *F. dichotoma*; *F. dichotoma* also inhibited shoot and root growth (Jha and Dhakal, 1990).

Ageratum conyzoides, C. odorata, and *Lantana camara* are common weed species in Cameroon whose Essential oil extracts were tested for efficacy on the morality of the maize grain weevil, *Sitophilus zeamais* (Coleoptera, Curculionidae), which showed the significant insect mortality with all the essential oils used. The study by Bouda *et al.* (2001) showed that mortality of *S. zeamais* increased with the concentration of the essential oils and the duration of exposure of the weevils on the treated substrates.

Allelopathic effects of *C. odorata* aqueous leaf extract and residues incorporated in the soil on the growth and water status of *Lycopersicon esculentum* Mill were studied. Significant growth reductions in *L. esculentum* were observed from additions of *C. odorata* aqueous – leaf extract at concentrations as low as 1g fresh weight in 40ml of water. Decreases in leaf water potential accompanied a reduction in growth. Incorporation of *C. odorata* leaf material into the soil in which *L. esculentum* Mill seedlings were germinated and grown caused significant depression in growth over the 2-week test period with the addition of 2g residue to 80g soil. Allelochemicals released from *C. odorata* plants and residues are suggested to explain yield reductions in crops in fields where *C. odorata* plants are present. One mechanism of toxic action on seedlings involved interference with water balance (Onwugbuta-Enyi, 2001).

Seeds of three crops, i.e., corn (maize) (var. ACR89DMR), cowpea (*Vigna unguiculata* (L.), Walp var. Ife brown), and soybean (var. Tax 923-2E) and two weeds – Siam weed and tridax (*Tridax procumbens* L.) were used for the experiment. Weed seed germination was inhibited by siam weed extract more than crop seed germination (Adetayo *et al.*, 2005).

The allelopathic potential of two cultivars of sunflower (*Helianthus annuus* L.) Lech and Ogrodowy was studied. Leaf aqueous extracts of sunflower at 2.5, 5, and 10 % (m/v) concentrations were applied to determine their effect on mustard (*Sinapis alba*) seed germination and seedlings' growth under laboratory conditions, increasing the concentration of aqueous extracts of sunflower inhibited seed germination, up to the almost complete failure of germination (Bogatek *et al.*, 2006).

Allelopathic effects of aqueous extract of leaves of *Parthenium hysterophorus* were studied on seed germination and seedling growth of three cereal crops (*Oryza sativa* L., *Zea mays* L., and *Triticum aestivum* L.), three cultivated crucifers (*Raphanus sativus* L., *Brassica campestris* L., and *Brassica oleracea* L.) and two wild species of family Asteraceae (*Artemisia dubia* Wall ex. Besser and *Ageratina adenophora* (Spreng) King and HE Robins). Seed germination of all crucifer species was inhibited entirely at >2% leaf extract of *Parthenium hysterophorus*, but in other species, except maize, complete failure of seed germination was recorded only at >6% in *T. aestivum* and *A. adenophora;* at 10% in *O. sativa* and *A. dubia*. Seed germination of *Z. mays* was not completely inhibited, but it was low at a high extract concentration. The extract had a strong inhibitory effect on seedlings' root elongation in cereals and shoot elongation in crucifers and wild Asteraceae (Maharjan *et al.*, 2007).

Suwal *et al.* (2010) have revealed that aqueous extracts of *C. odorata* may contain water-soluble allelochemicals that exert inhibitory effects on the germination and seedling growth of paddy and barnyard grass by Petri dish bioassays. Viable seeds of paddy and barnyard grass in different concentrations of aqueous extracts (2, 4, 6, 8, and 10 %) of leaf, stem, and root of *Chromolaena odorata* were germinated at room temperatures. The leaf extract had a strong inhibitory effect on seed germination and linear growth of paddy and barnyard grass. The inhibition order was leaf> stem> root on both tested seeds.

The allelopathic effect of the aqueous leaf extract of *Parthenium hysterophorus* and *C. odorata* on the seed germination, radicle, and plumule growth of the seedlings of *Z. mays* L. was studied by applying five different treatments (2, 4, 6, 8, and 10%) of two plant leaf extracts viz., *P. hysterophorus* and *C. odorata*. Results exhibited that seed germination on account of allelopathic inhibition was found in all levels of leaf extract. However, the highest degree of inhibition in radicle and plumule growth of the test plant, i.e., *Z. mays* (L.), was observed in 10% concentration, i.e., 2.08cm and 7.82cm in *P. hysterophorus* and 1.39cm and 2.36cm in *C. odorata* respectively (Devi and Dutta, 2012).

The study revealed these invasive weeds' significant inhibition of seed germination due to their allelopathic potential. However, PRH increased the germination percentage of cotton seeds among the test crops while maize and soybean had no significant effect. A significant reduction of the plumule length of maize, cotton, and soybean seedlings was observed due to the allelopathic effect of *C. odorata.* Among the test crops, maize radicle length was more inhibited by the aqueous leaf extract of *C. odorata.* Maize suffered most from the allelopathic effect of *C. odorata* among the test crops (Masum *et al.*, 2012).

The ten herbaceous species comprised five natives and five non-native invasive species, all common in most habitats in southern China. *C. odorata* leaf and root extracts inhibited the seed germination, root length, and shoot length of most target species at different concentration levels. This inhibitory effect generally increased with increased extract concentration and was more pronounced with the leaf extract than the root extract. There were more obvious inhibitory effects on the five native species than the non-native ones, suggesting that the latter had co-evolved with *C. odorata* in their native range (South America) and were relatively less susceptible to allelopathic compounds released by *C. odorata*. These results suggested that allelopathy may contribute to *C. odorata* becoming dominant in invaded plant communities in southern China (Hu and Zhang, 2013).

The allelopathic impact of aqueous extract (2.5 and 5%) and residues (2 and 4%) of five aquatic weeds, namely *Alternanthera philoxeroides, Alternanthera sessilis, Conyza stricta, Polygonum barbatum, and Echinochloa crusgalli* was investigated on the germination and early growth of wheat (*Triticum aestivum*). The aqueous extracts and residues of all tested weeds at different concentrations suppressed the germination/emergence percentage, mean germination/emergence time, germination/emergence index, and early seedling growth of wheat(Abbas *et al.* 2014).

A study was conducted to determine the allelopathic effects of the aqueous leaf extract and leaf debris (incorporated into the soil) of *C. odorata* and *M. micrantha* on three bioassay weed species, namely *Eleusine indica*, *Cyperus iria*, and *A. conyzoides* under laboratory and greenhouse conditions. The study concluded that the leaf extracts of *C. odorata* and *M. micrantha* significantly reduced all seedling growth parameters of the three bioassay species. *C. odorata* and *M. micrantha* show allelopathic properties when used as a cover crop and mulch by controlling the growth of *A. conyzoides*, followed by *C. iria* and *E. indica* (Sahid and Yusoff, 2014).

Laboratory and soil cultured experiments were conducted to evaluate: the allelopathic activities of the fresh shoot aqueous extracts of *Tithonia diversifolia* (FSET) and *Chromolaena odorata* (FSEC) at concentrations 50%, 80%, and 100% on the germination, radicle, and plumule growth of *Hibiscus sabdariffa* plants, investigate the effects of 100%FSET and 100%FSEC on the growth, chlorophyll pigments, ascorbic acid and protein contents of this plant. Seed germination and juvenile seedling growth were reduced with the lowest concentration of the different extracts. The radicle growth was more inhibited than the plumule growth. The plant extracts had a concentration-dependent reduction of the seedling growth of the target crop (Otusanya *et al.*, 2015).

Aqueous extract of leaf, stem, and root of *Imperata cylindrica* and *Chromolaena odorata* at concentrations of 0, 5, 10, and 15% on germination and seedling growth *Centrosema pubescens* were investigated. The experimental design used was a completely randomized design with three replications. The study showed that plant species, plant parts, and their concentration had a significant effect ($p < 0.05$) on parameters measured. Aqueous extracts of both plants at all concentration levels inhibited germination percentage, germination rate, shoot, and root growth of *C. pubescent. These* effects were directly proportional to the concentration of extract. *C. odorata* extract was more inhibitory than *I. cylindrica* extract, and the leaf extracts showed the most inhibitory effect (Rusdy *et al.*, 2015).

Evaluation of the effect of different concentrations of leaf extract of *Parthenium hysterophorus* L. on seed germination, seedling growth, and fresh weight of *Phaseolus mungo* has been done. In this study allelopathic effect of leaf extract of different concentrations (2%, 4%, 6%, 8% and 10%) were compared with control treatment. After seven days of incubation at room temperature, the aqueous leaf extract of various concentrations of *P. hysterophorus* on seed germination, root & shoot length, R/S ratio, inhibition (-) or Stimulation (+) percent, relation elongation of root & shoot, and fresh weight of root & shoot of *P. mungo* have no significant inhibitory effect. This study disclosed that higher concentrations of leaf extract have irregularly affected the growth of P. mungo than lower concentrations (Shikha and Jha, 2016).

Allelopathic effect of *Parthenium hysterophorus* L. on seed germination and seedling growth of two crops, wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*), was studied at the concentrations level of 0%, 1%, 5%, and 10% leaf, stem, flower and root extracts *of P. hysterophorus*. Seed germination of wheat and barley was inhibited entirely at 10% of all *P. hysterophorus*, especially on flower extract. The seed germination, plumule, radicle length production were reduced with increasing concentration of the aqueous solution (Tessema and Tura, 2018).

Chimouriya *et al.'s* (2018) experiment showed significant inhibitory effects of aqueous extracts on seed germination, growth, and dry biomass of seedlings. The germination percentage, seedling vigor index, hypocotyl, extreme length, and seedling biomass are significantly reducing with an increase in the concentration of extract al $p<0.05$. The highest inhibition was found in Karma variety treated with dry aqueous extract of *Adhatoda vasica* and *Eupatorium adenophorum*, i.e., germination percentage by 34.49 and 27.6%, hypocotyl length by 71.04 and 67.9%, extreme length (63 and 59%), and seedling vigor index by 75.44 and 69.3% respectively. Dry aqueous extracts were more phytotoxic than fresh aqueous extracts in this experiment.

A study aims to identify volatile chemical components in the essential oil of *C. odorata*. The leaf samples of *C. odorata* were collected from the Radha Krishna Community Forest, Chitwan district of Nepal. The essential oil from the leaves of *C. odorata* was extracted, and the chemical composition of the oil was analyzed by gas chromatography (GC) and GC–mass spectrometry (GC–MS). A total of 19 chemical components from the oil were identified. The major components identified were linalool (21.64%); β-pinene (9.43%); 1, 3-cycloheptadiene (8.92%); β-cubebene (7%); cinnamaldehyde (5.30%) and caryophyllene oxide (4.94%). o-methoxy cinnamaldehyde and isoeugenyl acetate were not listed by previous studies as the components in the essential oils of *C. odorata*. The components identified by this study have allelopathic effects on native plant species, anti-herbivory properties, and medicinal values (Thapa *et al.*, 2021).

Most of the studies related to the allelopathic impact of *C. odorata* are focused on crop plants, but the present study focused both on crop plants and non-crops plant species. Thus, this study will help fulfill this gap as the study site was less explored. Therefore, this study could be pioneer research for this site.

Chapter 3: MATERIALS AND METHOD

3.1. Study area

3.1.1 Geomorphology

Geographically, Chapakot municipality of Syangja district of Nepal lies in 27.54^0 to 27.90^0N and 83.45^0 to 83.82^0E. This municipality has 120.59 km^2 areas and estimate terrain elevation above sea level is 300- 1100 m and there is Tanahu district in the East, Galyang municipality in the west, Harinas & Biruwa rural municipality in North and Palpa district in the South. Brahmin, Kshatriya, Magar, Kumal are the major ethnic groups. It lies in sub-tropical region and *Shorea robusta, Castanopsis indica, Schima wallichii* are the major dominating plant species in the forest of municipality. The temperature lies between 6^0C-35^0C. Kaligandaki River and Jyagdi River are the major water sources for agriculture in that locality.

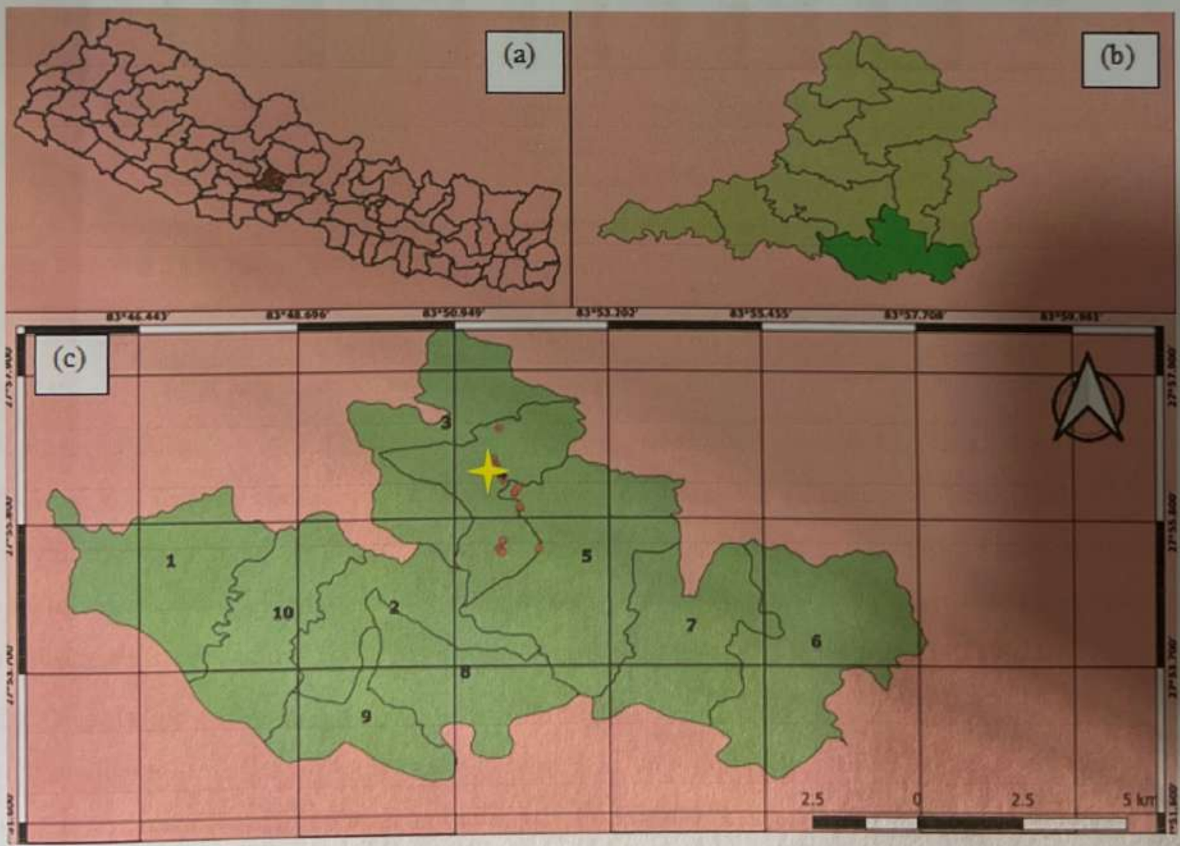


Figure 1. Study area showing (a) map of Neapal, (b) map of Syangja district and (c) Chapakot Municipality

3.1.2. Climate

The study area has typical warm temperate monsoon. Based on the record of Syangja station, the area has cold and dry winter (October to February), pre-monsoon dry summer (March to May) and monsoon summer (June to September). The average maximum and minimum temperatures of 30 years (1989-2018) climatic data showed 27.51°C and 15°C respectively and average precipitation were 2850.13mm (Department of Hydrology and Metrology, 2019) (Figure 2).

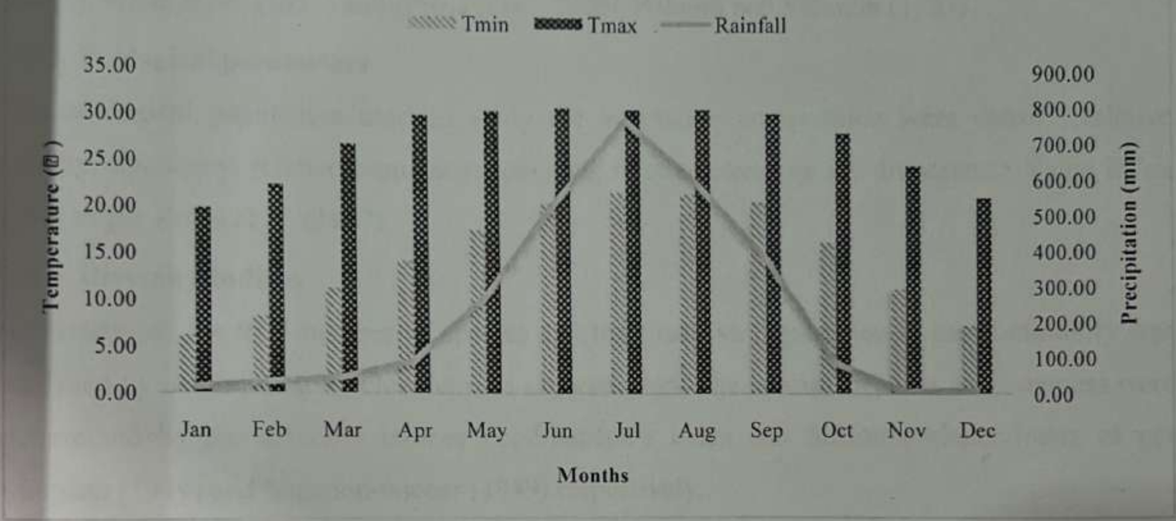


Figure 2. Average minimum-maximum temperature and precipitation of Chapakot Municiapility recorded at Syangja station between 1989-2018 (Department of Hydrology and Metrology, 2019)

3.2. Method

3.2.1. Species characters of *Chromolaena odorata*

Cromolaena odorata (L) King& Robinson (Syn. *Eupatorium conizoids* M. Vahi., *Eupatorium odoratum* L.; Family- Asteraceae; Common name- Cromoleana; Local name- Seto Banmara is perennial herb spreading profusely branched brown stem which is woody near the base and has opposite leaves. Flowers are cluster in more or less terminal and auxiliary pendiculate flat-topped inflorescence fruit is achene. The leaves has pungent odor when crushed (Gautier, 1992).

3.2.2. Vegetation sampling and plant identification

For the collection of field data sample site was selected within 450 m-650 m elevation zone as shown in the location map of study area (Figure 1). The entire sample site was located within the distribution range of *Chromoleana odorata* avoiding the tree species. The sample plot laid out in Gillang village, Kamti village, Lumdi dobhan of Chapakot municipality. The vegetation sampling was conducted along the transect line in sample site during January of 2021. Plots were laid out 10 m apart from the trail to avoid the direct anthropogenic disturbances. The minimum distance of each plot was 10 m apart from each other. Total 30 concentric circular sample plots were laid in which 5m radii for the study of shrubs and 1m radii for the study of herb. Data were taken carefully from each plot. Most of the plants were identified during field survey. Local name of the plants was identified with consultation of local people. All the plants present in each plot were recorded and some unidentified plants were identified by using relevant literatures such as Malla *et al.*, (1985) and (Press *et al.*, 2000), Polunin and Stainton (1987).

3.3.3. Ecological parameters

The ecological parameters used to study the vegetation composition were density, relative density, frequency, relative frequency, coverage, relative coverage and Importance Value Index (IVI) as per Zobel *et al.* (1987).

3.3.4. Diversity Indices

Diversity i.e. the total number of species and their relative importance in the community was analyzed by determining species richness and evenness. The species richness and evenness were determined by the diversity indices i.e. Simpson's index and Shannon-wiener index as per Simpson (1949) and Shannon-wiener (1949) respectively.

Simpson's index (D) = $1 - \left(\frac{\sum n(n-1)}{N(N-1)}\right)$

Where n= the total number of individual of particular species and N= the total number of all species.

Simpson's index of Diversity = 1-D

Shannon-Wiener index (H) = $-\Sigma$ Pi (ln Pi)

Where, Pi = $\frac{\text{total number of individual}}{\text{total number of all species}}$

Pi = Proportion of individual species

3.3.5. Vegetation analysis

A) Density and Relative density

Density is the total number of individuals of particular species counted in all the plots of specific sites. It is expressed in number per hectare for large species and for small species number of individual per square meter (Zobel *et al.*, 1987).

It was calculated as,

$$\text{Density} = \frac{total\ number\ of\ individual\ species\ occured}{Total\ number\ of\ quadrat\ studied} \times \frac{1}{area\ of\ quadrat}$$

Relative Density (RD) = $\frac{Density\ of\ individual\ species}{Totai\ density\ of\ all\ species}$ × 100%

B) Frequency and Relative Frequency

Frequency is the number quadrat in which species occurred and owned in term of percentage (Zobel *et al.*, 1987).

Frequency (F) = $\frac{No.of\ quadrat\ in\ which\ species\ occured}{Total\ number\ of\ quadrat\ studied}$ × 100%

Relative Frequency (RF) = $\frac{Friquency\ of\ individual\ species}{Total\ frequency\ of\ all\ species}$ × 100%

C) Coverage and Relative Coverage

Coverage of plant is the amount of surface area occupied by a plant estimated in quantitative terms within the quadrat. It is based upon the size and distribution of individuals and often measured characteristic of composition of plant (Zobel *et al.*, 1987).

Coverage (C) = $\frac{Total\ coverage\ of\ individual\ species}{Total\ number\ of\ quadrat\ studied}$

Relative coverage (RC) = $\frac{Coverage\ of\ individual\ species}{Total\ coverage\ of\ all\ species}$ × 100%

C) Importance Value Index (IVI)

IVI represents the total number of the relative value of Frequency, Density and Coverage of Species. It gives idea of the relative importance of a species in a community (Zobel *et al.*, 1987).

IVI= Relative Frequency +Relative Density +Relative Coverage

3.3.6. Laboratory method

Laboratory method for evaluation of effect of *Chromolaena ododrata* was based on Muzzo *et al.*, (2018). Detail methods used by Muzzo *et al.*, (2018) is described below:

a) Preparation of *Chromolaena odorata* aqueous leaf extract

Chromolaena odorata leaves sample were analyzed at the Amrit Science Campus Lab in September 24 – October 10. First, the *C. odorata* leaves were air dried at room temperature (30°C ± 4) for seven days. The dried leaves of *C. odorata* were milled through a 2 mm sieve. 100 g of powder extracts were added to 1 liter of distilled water in plastic buckets, vigorously stirred and kept for 24 hrs. at room temperature and then filtered through double-layered muslin cloth. The filtrates were served as a stock solution of 100 % concentration. By subsequent dilution with distilled water leaf extracts of 25, 50 and 75 % concentrations were prepared and stored in conical flasks at 4? until required. However, distilled water used as control (0%) during the experiment.

b) Germination of species

Seeds of crop plants *Tricicum aestivum* (wheat), *Zea mays* (maize), *Eleusine coracana* (finger millet) and invasive species *Ageratum conyzoides* and *Senna tora* were collected mention the time and stage of maturity of seeds. These seeds were surface sterilized by dipping them into 0.5 % aqueous solution of sodium hypochlorite and rinsed several times with distilled water. Petri dishes of 10 cm double layered with filter papers. In each selected species, 10 uniform seeds per specie were sequentially place in separate petri dishes and watered with 10 ml of each prepared concentration and labelled. The petri dishes were kept in a growth chamber at room temperature (28°C) until the final germination count after 10 days of sowing. The seeds were considered germinated after the emergence of the root.

c) Experimental design

The experiment was set in a Completely Randomized Design (CRD) with three replicates. Daily data for seed germination were recorded while the root and shoot lengths were measured in 2 days' gap after germination were recorded. Five observations were done for the calculation of seedling growth. Furthermore, computation of seed germination and seedling growth indices were done as described below.

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

$$\text{Germination Index (GI)} = \frac{\text{No.of germinated seeds}}{\text{Days of first count}} + \ldots + \frac{\text{No.of germinated seeds}}{\text{Days of final}}$$

Rate of germination (RG): were estimated using modified Timpson's index of (Khan & Ungar, 1984) by firstly calculating

Mean Germination time (MGT) = $\sum (N \times D)/n$

Where N is the number of seeds which in D-day grow, n is the total number of seeds grown and D is the number of days from the date of germination. The reversing of MGT at the end of this period RG was obtained.

d) Seedling growth indices

Inhibitory percentage (IP) =100- $\frac{\text{\% FG with } C.odorata \text{ aqueous leaf extract}}{\text{\% FG without } C.ododrata \text{ aqueous leaf extract}} \times 100$

Where, FG is final germination percentage.

d) Statistical Analysis

Shapiro-Wilk Test was done for the normality test of data and it was found that the data were normal. The data for germination and seedling growth indices were used to find out the significance difference along species, along observation and along concentration by using ANOVA test. To find out the association within group LSD (Least Significance Difference) and correlation was used at 5% confidence limit. All the statistical analysis was done using SPSS software version 20.0.

Chapter 4: RESULTS

4.1.Importance Value Index (IVI)

4.1.1. Herbs:

In this study, 26 species of herb were found. Among them *Chromoleana ododrata* was found to have highest IVI value of 60.54 followed by *Capillipedium parviflorum* (35.58), *Imperata cylindrica* (34.77), *Ageratum haustonianum* (19.43), *Bidens pilosa* (18.13), *Spermacoce alata* (13.11). Similarly, *Cissampelos pareira* (1.13), *Achyranthus aspera* (1.17) and *Iryngium foetidium* (1.34) were among the species having low IVI value (Figure 3).

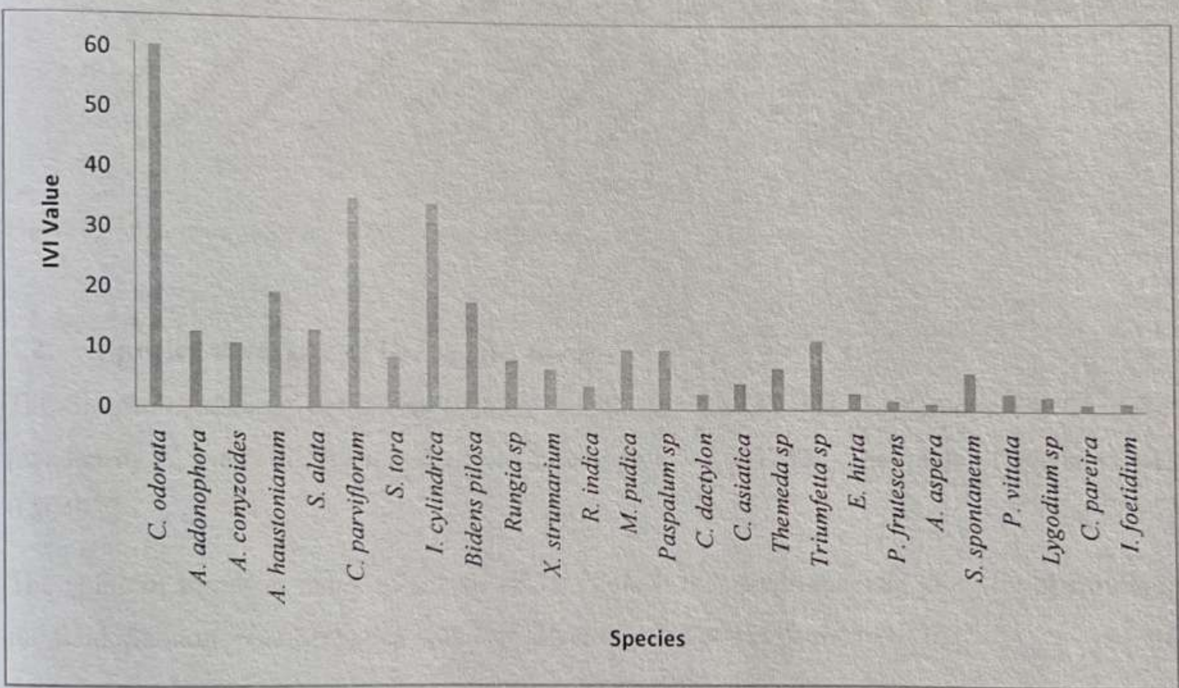


Figure 3. Importance Value Index (IVI) value of herbs

4.1.2. Shrubs:

In this study, 13 species of shrub were found. The highest IVI value of 87.04 was found to be of *Artimisia vulgaris* which was followed by the species *Clerodendrodom sp.* (46.55), *Woodfordia fructicosa* (34.91), *Colebrokea oppositifolia* (33.26), and *Pogostemon bengalensis* (23.44). Similarly, the species with lowest IVI were *Lantana camera* (2.11), *Calotropis gigantea* (4.83) and *Ziziphus sp.* (5.36) (Figure 4).

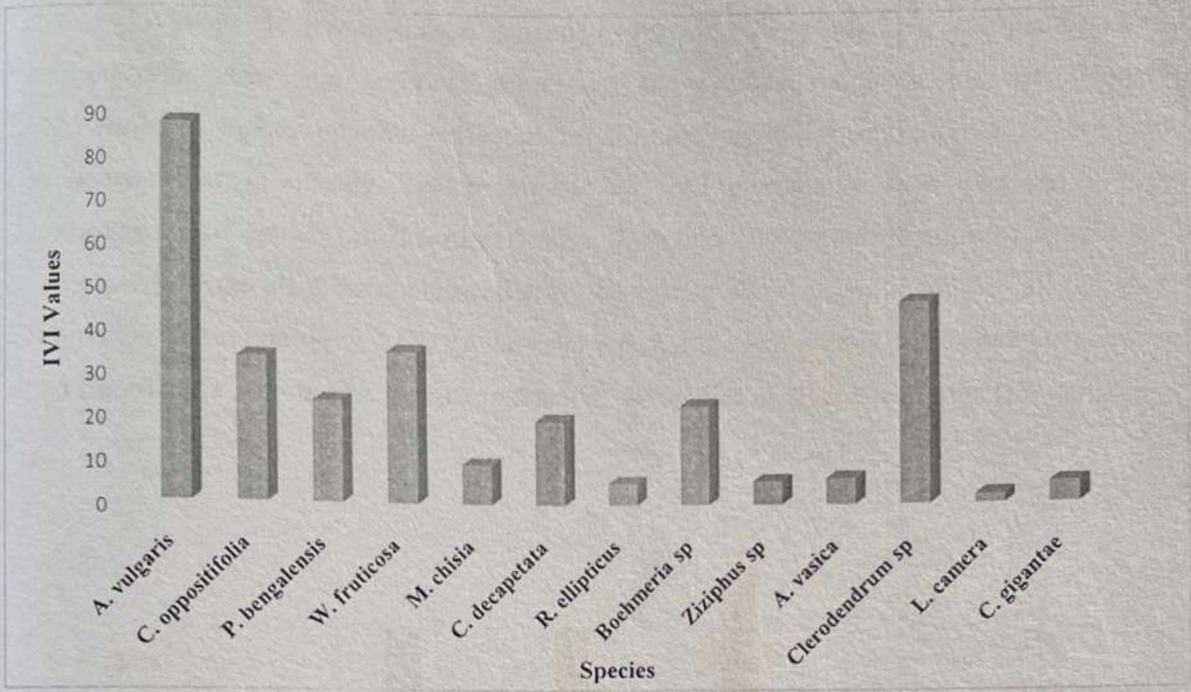


Figure 4. Importance value index (IVI) value of shrubs.

4.2. Species diversity of herbs and shrubs

The Simpson index of herbs was 0.0899. It indicated low diversity of herbs in the Chapakot invaded by *C. odorata*. Shanon-Weinner index value was 2.72031. The value of evenness was 0.804938.

The value of Simpson index of shrubs was 0.2245. It indicated moderate diversity of shrubs in the field. Shanon-Weinner index was 1.86. Evenness value was found to be 0.72.

Table 1. Simpson's and Shannon-weinner index value of herbs and shrubs.

SN	Simpson's index		Shanon-Weinner index	
	Herb	**Shrub**	**Herb**	**Shrub**
1	0.0899	0.2245	2.72031	1.86

4.3. Effects on germination

Extract of leaf of *C. odorata* had different allelopathic effect at different concentration in the species studied. The maximum inhibition of *A. conyzoides* (23% germination in aqueous extract of 25% concentration) seedling was observed in extract of the leaf followed by *E. caracona*

(63%, 7%, 10%, 3% germination in aqueous extract of 25%, 50%, 75%, 100% concentration respectively). Inhibition of germination increased as the concentration was increased. However, in *Z. mays,* the highest inhibitory effect occurred in the middle concentration (40% germination in aqueous extract of 50% concentration). No seed germination was observed in 100% concentration of extract on *S. tora* and 50%, 75% and 100% concentration of extract on *A. conyzoides*. While all other concentration of the extract showed germination of all the species (Figure 5). From One-Way ANOVA test showed that the leaf extract of *Chromolaena odorata* had significant difference on germination among all species of all concentration (P= 0.00).

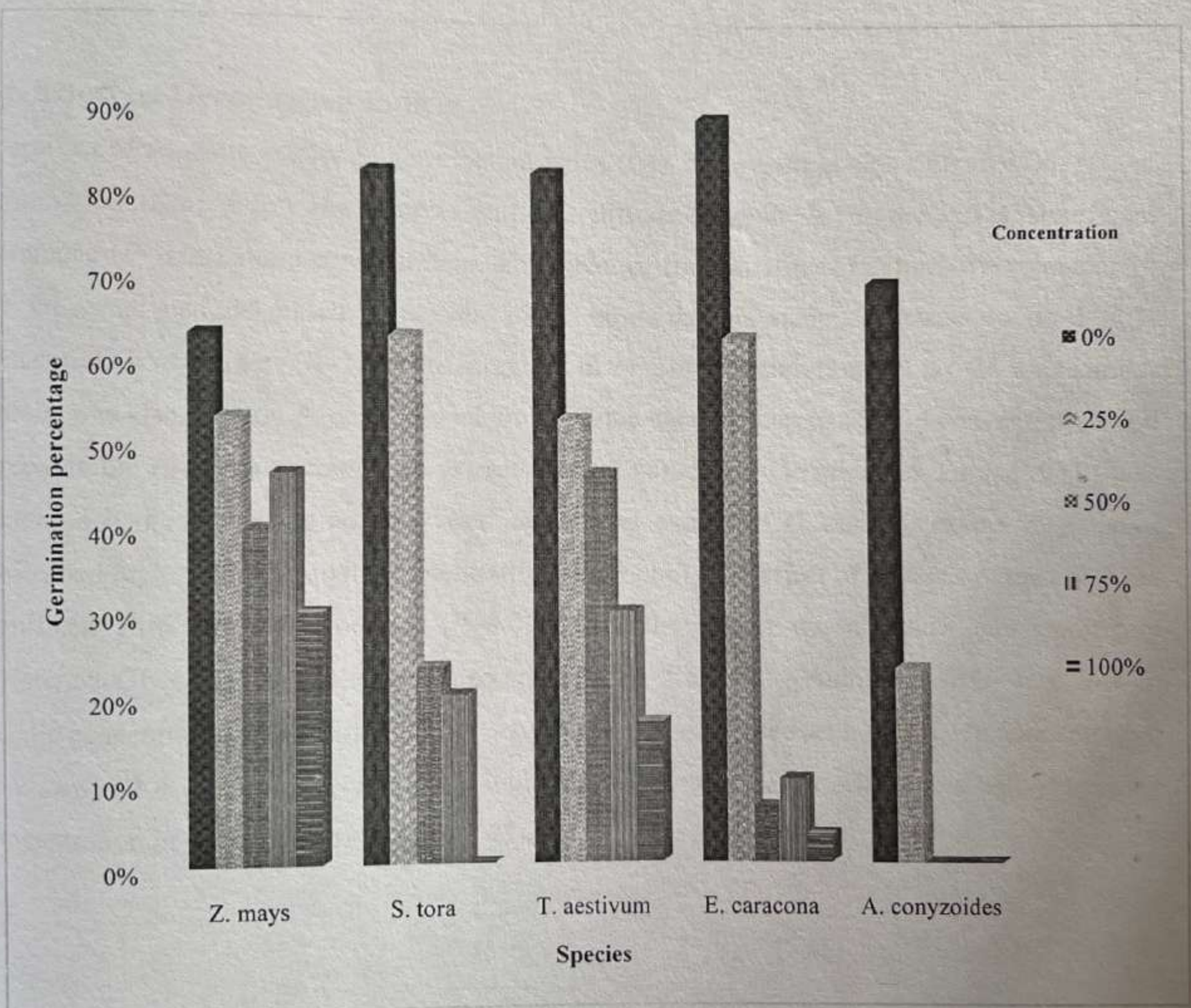


Figure 5. Pattern of Final Germination

One-way ANOVA test within the species, showed that there was significant difference within and between groups species (P = 0.00). However, from LSD, it showed that there was significant difference between *A. conyzoides* and all other species ($p < 0.05$) and *E. caracona* with *Z. mays*, *T. aestivum* and *A. Conizoides* ($p < 0.05$) while all other species didn't show any significant difference (Appendix VI).

One-way ANOVA test showed that there was no any significant difference (P=0.525) along the observation. The Post-hoc LSD test also confirmed that there was no any significant difference along all the 6 observations in all species (Appendix VI).

Also, ANOVA test between and within the groups of crops and invasive species resulted in significant difference (P = 0.01). The crops were *Z. mays*, *T. aestivum*, and *E. caracona* whereas the invasive species were *S. tora* and *A. conyzoides*.

4.4. Effect on Germination Indices

The effect of aqueous extract on germination index (GI), mean germination time (MGT), rate of germination (RG) didn't show any significant difference while in IP there was significant difference (P=0.00) along concentration. The effect of concentration of extract when increased, the GI value had decreased. However, in *Z. mays,* the GI value decreased up until 50% concentration of extract (GI=2.75) but increases in 75% of concentration (GI = 4.2). This similar pattern was also seen on *E. caracona* where GI value decreases up to 50% of concentration and increases on 75% concentration of extract (Figure 6a). Mean Germination Time (MGT) was increased while increasing concentration of aqueous extract of *C. odorata* except in *Z. mays* which had high MGT in middle concentration (Figure 6b). The effect of aqueous extract showed significant difference only on RG (P=0.01) while the extract doesn't show any significant difference GI, MGT and inhibitory percentage (IP). Relative germination (RG) was low in middle concentration of *Z. mays* and *E. caracona.* It was decreased with increasing concentration in *S. tora* and *A. conyzoides* (Figure 6c). Inhibitory percentage (IP) was increased with increasing concentration of aqueous extract (Figure 6d).

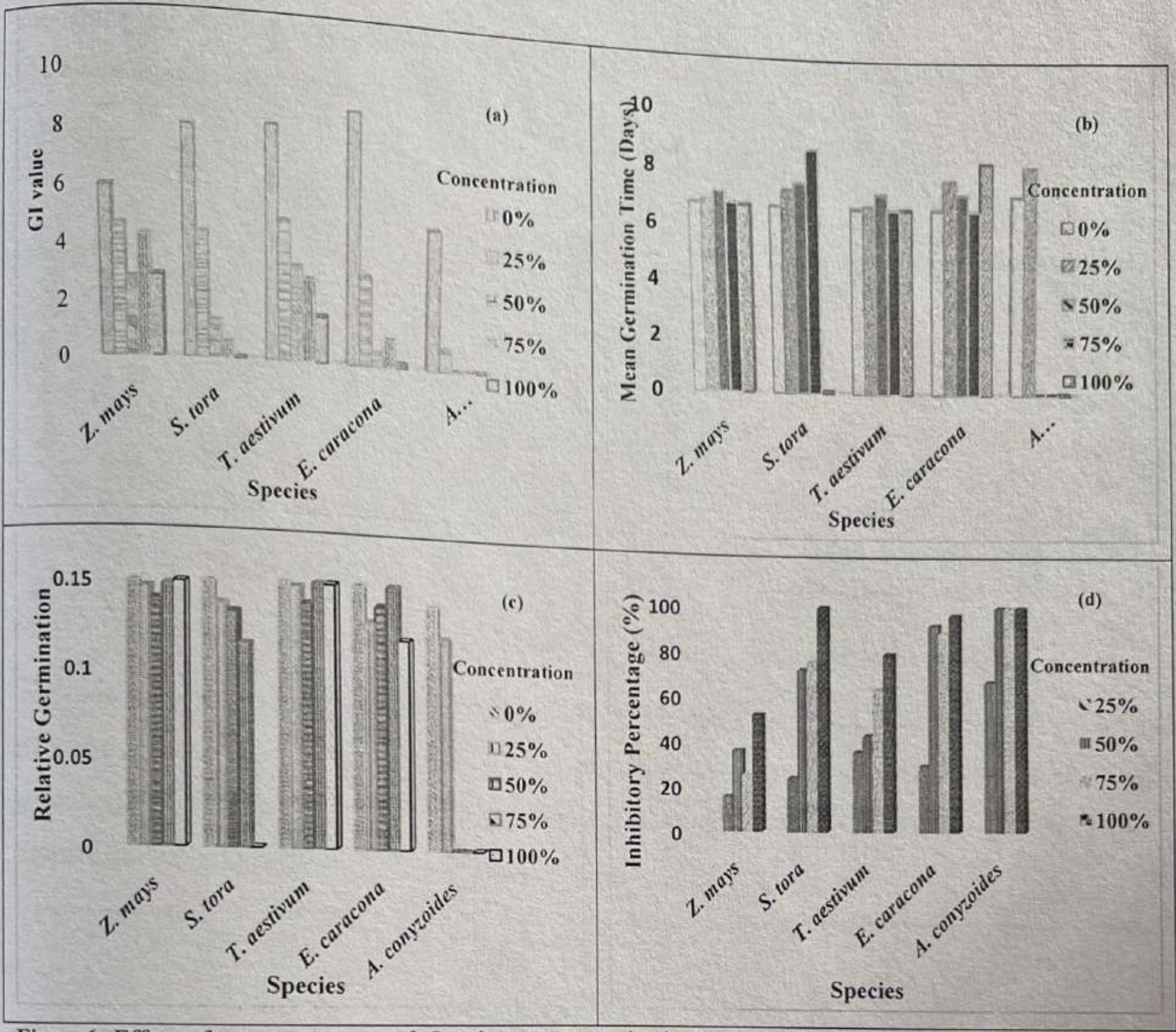


Figure6. Effect of aqueous extract of *C. odorata* on germination index(a), Mean germination time (b), Relative germination (c) and Inhibitory percentage (d) of different species

4.5. Effect on growth of Root and Shoot Length

4.5.1. Effect of aqueous extract along concentration

The extract of leaf of *C. odorata* had different allelopathic effect at different concentration. In *Z. mays,* the inhibitory effect on root length was maximum (1.12 cm) in 100% of concentration followed up by the middle concentration (2.60 cm). (Figure 7a). While in the shoot length, the maximum inhibition was seen on 100% followed by 50%. Remaining 25% and 50% showed similar results (Figure 7b).

The post–Hoc LSD test along concentration showed that there was no significant difference on root length of 50% to 75% and 100% concentration. Similarly, post–Hoc LSD test along concentration showed that shoot length of other concentration was not significant except with 0% concentration (Appendix IV and V).

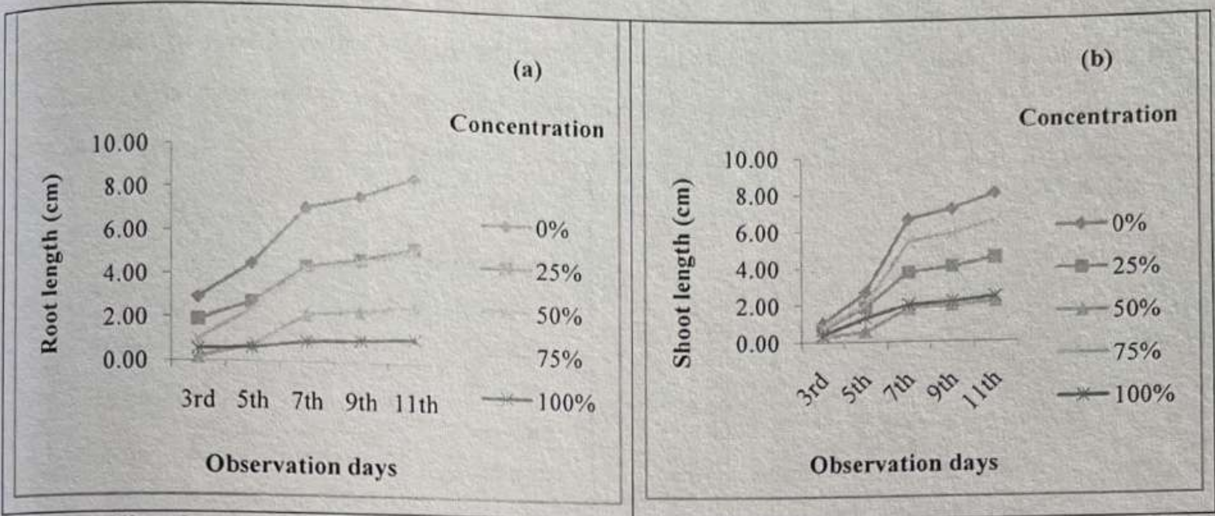


Figure 7. Effect of aqueous extract of *Chromolaena odorata* on Growth Pattern of root length(a) and shoot length(b) of *Z. mays*

In *Senna tora*, root growth (Figure 8a) and shoot growth (Figure 8b) were suppressed due to effect of aqueous extract of *C. odorata*. Root growth (0.28 cm) was more suppressed than shoot growth (0.72 cm) of *S. tora*. When the concentration of aqueous extract was increased, the suppression of root and shoot growth also increased.

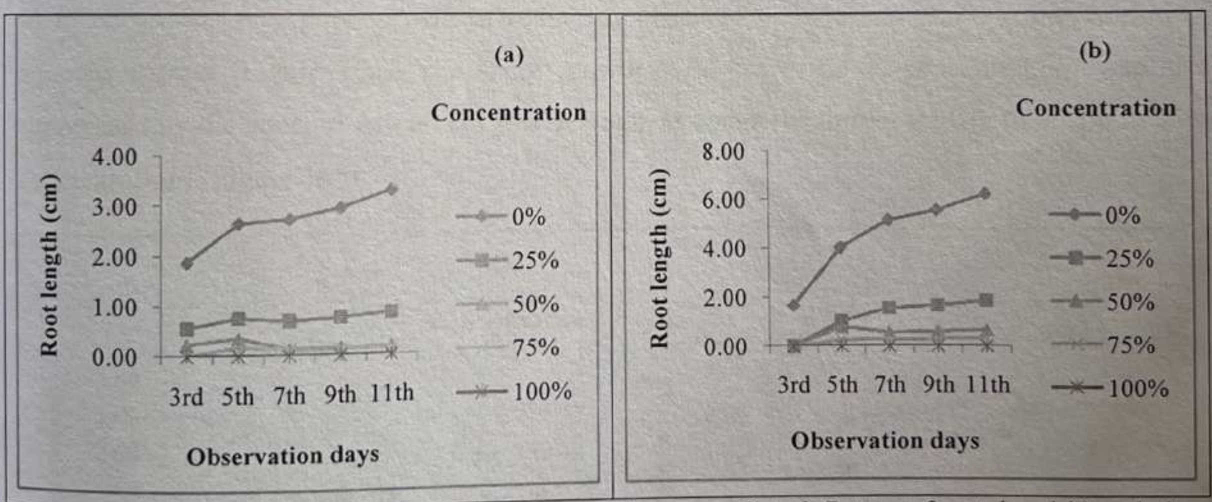


Figure 8. Effect of aqueous extract of *Chromolaena odorata* on Growth Pattern of root length(a) and shoot length(b) of *S. tora*.

In *T. aestivum*, it was observed that the concentration of aqueous extract was increased, the suppression of root growth (2.16 cm) (Figure 9a) and shoot growth (2.66 cm) (Figure 9b) also increased. The root growth was affected more than that of shoot growth.

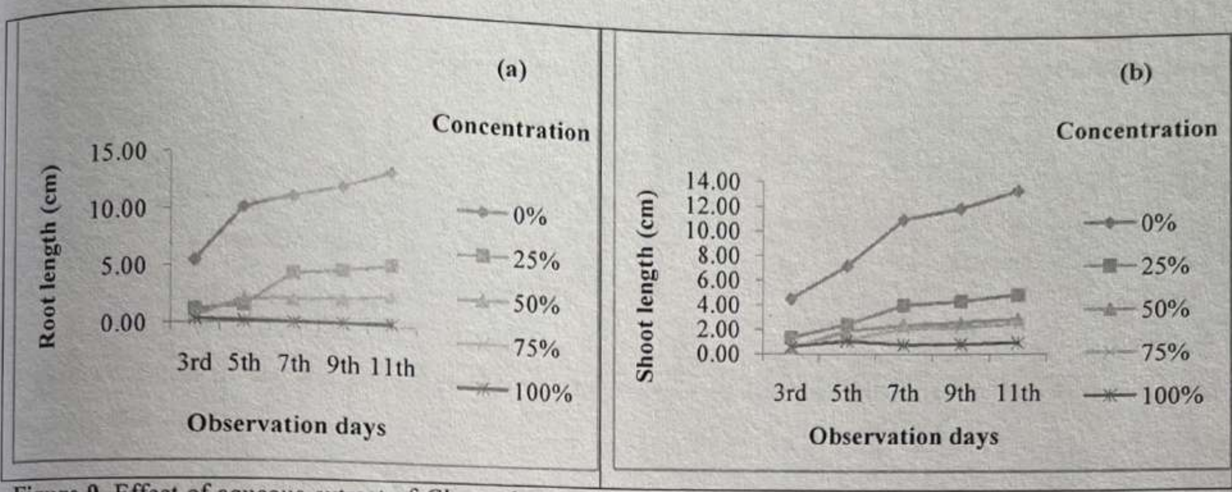


Figure 9. Effect of aqueous extract of *Chromolaena odorata* on Growth Pattern of root length (a) and shoot length (b) of *T. aestivum*

In *E. caracona,* very poor growth of roots was observed in 75% and 100% concentration of aqueous extract (Figure 10a). The shoot growth (1.64 cm in 25 % concentration) was also suppressed by the aqueous extract but less in length as compared to root growth (0.69 cm in 25% concentration) (Figure 10b).

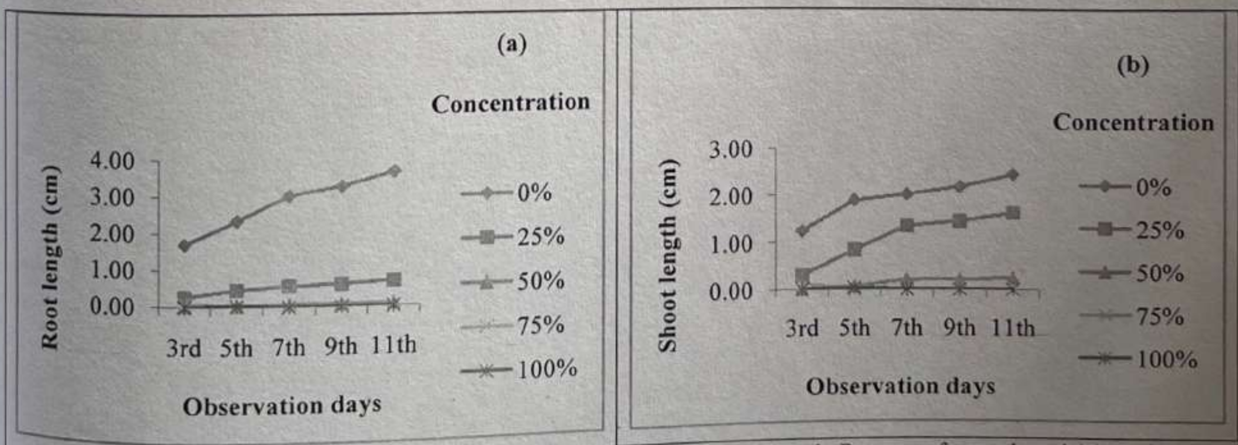


Figure 10. Effect of aqueous extract of *Chromolaena odorata* on Growth Pattern of root length(a) and shoot length(b) of *E. coracana*

In *Ageratum conyzoides*, no growth of roots was observed in 50%, 75% and 100% concentration of aqueous extract (Figure 11a). Moreover, the shoot growth (0.63 cm) as suppressed by the aqueous extract but less in length as compared to root growth (0.42 cm) (Figure 11b).

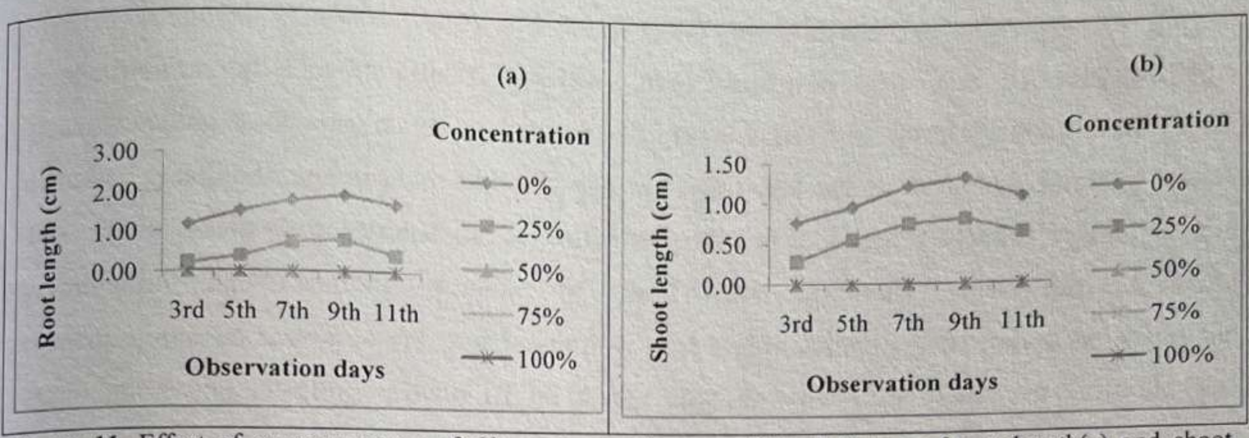


Figure 11. Effect of aqueous extract of *Chromolaena odorata* on Growth Pattern of root length(a) and shoot length(b) of *A. conyzoides*

4.5.2. Effect of aqueous extract along Species

From One-Way ANOVA test, it shows that the leaf extract of *Chromolaena odorata* has significant difference on root and shoot length along all species ($P= 0.00$) (Appendix IV and V). As compared to other species, the growth of root and shoot on other species were more suppressed than that of *Z. mays*. Post-hoc LSD test along species showed that there was no significant difference between root length of *Z. mays* and *T. aestivum*, *S. tora* and *E. caracona*, and *S. tora* and *A. conyzoides*. Similarly, there was no significant difference between shoot length of *Z. mays* and *T. aestivum*, *S. tora* and *E. caracona*, *A. conyzoides* and *E. caracona* (Appendix IV and V).

Chapter 5: DISCUSSION

Chromolaena odorata, often forming a dense stand, grows through native vegetation in most invaded countries (Ambika, 1980) and prevents the establishment of other species due to competition and allelopathic effects. Shrestha (2016) found it in most Terai, Siwalik, and Hills areas, invading a diverse range of habitats, including roadsides, forests, agricultural fields, disturbed grasslands, and abandoned fields, causing significant economic and biodiversity losses. The present study demonstrated the role of allelopathy of *C. odorata* aqueous extract on the germination and seedling growth of the *Triticum aestivum, Zea mays, Eleusine coracana, Ageratum conyzoides,* and *Senna tora*. The higher extract concentration inhibited the germination and seedling growth of all tested crop and non-crop plants in general. The allelopathic effect of the *C. odorata* showed a tremendous inhibitory effect on the invasive species, i.e., *A. conyzoides* and *S. tora*, and a more negligible effect on the crop plant.

This study also attempts to find out species diversity and Importance Value Index (IVI) of herbs and shrubs in the invaded area by *C. odorata.*

5.1.Species diversity and Importance Value Index (IVI):

Twenty-six species of herbs and 13 species of shrubs were found in the field study, where *Chromolaena odorata* was found as the dominant species with an IVI value of 60.54 in the field (Figure 3). Among 26 species of herb and 13 species of shrubs, 9 herbs and 1 species of shrub were listed as invasive species in Nepal (Shrestha *et al*., 2017). This study showed that the study field was affected by invasion and *C. odorata* had high IVI in that community. This result supported by Shrestha *et al.*, (2017) stated that the negative impacts of biological invasion on biodiversity, natural ecosystems, agriculture, and the economy appeared to be increasing in Nepal. Higher IVI of *C. odorata* might be due to the allelochemicals (Akinmoladun *et al*., 2007; Ambica, 1980; Biller *et al.*, 1994), high reproductive capacity, and open canopy (Mangla *et al.*, 2008).

5.1.Effect of Extract on the germination:

The aqueous extracts of the leaves of *Chromolaena odorata* had been found to possess an inhibitory effect on the germination of *Tricicum aestivum, Zea mays, Eleusine coracana, Ageratum conyzoides,* and *Senna tora*. The inhibitory effect was found in all species of all concentrations. In this concern, Chiang *et al.* (2003) mentioned that the effect of plant extracts on seed germination came from the various chemical constituents present in these plants. Chemical constituents (secondary metabolites) can affect seed germination and plant productivity. Inhibition effects typically result from a combination of allelochemicals that interfere with various physiological processes in the receiving plant (Islam *et al.*, 2018). Some flavonoids had an allelopathic effect as they could increase the levels of reactive oxygen, causing inhibition of germination (Kumar *et al.*, 2010). This study showed that low aqueous concentration had a low inhibitory effect. Common inhibitory effect in such aqueous concentration might be due to the low amount of allelochemicals substance which could not inhibit germination physiology of seeds.

There was a highly significant inhibitory effect on the germination of the seeds of the *A. conyzoides* in comparison to other seeds. The inhibitory effect increased with the increasing concentration except in *Z. mays*, where 50% concentration showed more inhibition than 75% concentration on germination (Figure 5). The effect of aqueous extract did not completely inhibit the germination of Z. mays. A similar result was found by Maharjan *et al.*, (2007). Increasing inhibitory power on germination of *Z. mays* with increasing mulch level of *C. odorata* was observed by, an aqueous extract of *C. odorata* was more inhibitory in 50% concentration could be due to high permeability of the allelochemicals through the seed coat in moderate concentration Usuah *et al.*, (2013).

There was no single germination of *A. conyzoides* in 25%, 75%, and 100% concentration of aqueous extract of leaves of *C. odorata* (Figure 5). Similar results were found by Nornasuha and Ismail, (2013). Aqueous leaf extract and leaf debris of *C. odorata* and *M. micrantha* incorporated into the soil showed significant effects on total germination and germination indices and seedling growth of *A. conyzoides* both in the laboratory and greenhouse. In the Lab Experiment, the *C. odorata* leaf extract caused a more significant reduction in total germination, germination indices, and seedling growths of *A. conyzoides*. The Lower value of IVI of *A. conyzoides* in the field indicated that lower presence which might be due to allelopathic impact of *C. odorata* (Figure 3), indicating the low presence of *A. conyzoides* in the field invaded by *C. odorata*. This study showed a significant difference in seed germination of crop plants and invasive plants, as germination in the invasive plant was more inhibited than crop plants. Similar results were observed by Adetayo *et al.* (2005), where *C. odorata* extract inhibited seed germination more in weeds than in crops. Akobundu (1987) further listed factors like soil temperature, soil moisture regime, alternate wetting and drying of soil, soil nitrate level to affect seed germination. Although the effect was slight on crop seeds and more on weed seeds, this report is corroborated by the findings of Oke, (1988), where *C. odorata* extract inhibited the seed germination of cowpea, soybean, and tridax. The inhibitory effect of *C. odorata* extract on germination in this study was in agreement with the findings of Bhowmik and Doll, (1982) and Johnson and Coble, (1986). They observed that many weed species exhibit allelopathic effects on germination.

No germinations were observed in *Senna tora* by 100% concentration (Figure 5) of aqueous extract due to the higher amount of allelochemicals in higher concentrations. The present study also showed that phytotoxicity of the plant extracts was concentration-dependent because there was an increment in inhibition with an increasing concentration of extract. These results agreed with the findings of Fariba *et al.*, (2007), who stated that allelochemicals stimulated or inhibited plant growth depending on their concentration.

5.2. Effects on germination indices

The effect of aqueous extract on Germination Index, Mean Germination Time, and Relative Germination did not show any significant difference, while in IP, there was a significant difference among different concentrations (Figure 6). A similar result was obtained by (Muzzo *et al.*, 2018). The GI for species reduced as the concentration increased, agreeing with (Ramamoorthy & Paliwal, 1993). However, IP increased while concentration level increased in the present study. Similar results were reported when the sorghum plant was treated with *Trianthema portulacastrum* (Randhawa *et al.*, 2002), *Ammi majus, Guiera senegalensis*, and *Salix* species (Hassan *et al.*, 2012), and *C. odorata* tested with *Sorghum Vulgare* (Muzzo *et al.*, 2018). In *Z. mays* GI decreased up to 50% concentration and increased in 75% (Figure 6) that might be due to seeds responses to allelochemicals varies with the concentration.

5.3. Seedling growth

These results showed that when the extract concentration increased, inhibition of both roots and shoot length decreased in all tested species, i.e., *Tricicum aestivum, Zea mays, Eleusine coracana, Ageratum conyzoides,* and *Senna tora* (Figures 7-11). Ogbe *et al.*, (1994) reported a robust inhibitory effect of leaf extract of Chromolaena odorata on Z. mays seedling.

Eze & Gill (1992) reported that *C. odorata* contained many allelochemicals, especially in the leaves, which inhibited the growth of many plants in nurseries and plantations. Ambica (1980) reported that the leaves of *Chromolaena odorata* contain many allelopathic chemicals, which could be responsible for reducing the growth of root, shoot, and seedling growth of other species. Hoque *et al.*, (2003) found that different concentrations of *C. odorata* leaf aqueous extract significantly inhibited the germination, root and shoot elongation, and development of lateral roots of *Cicer arietinum*, *Brassica juncea*, *Cucumis sativus*, *Phaseolus mungo*, *Raphanus sativus*, and *Vigna unguiculata* which showed similarities with present study result.

The root length of tested species (*Tricicum aestivum*, *Zea mays*, *Eleusine coracana*, *Ageratum conyzoides* and *Senna tora)* seed decreased than shoot length in all extracts (25%, 50%, 75%, 100%) (Figure 7-11). Such type of inhibitory effect was reported by Chung *et al.*, (2002) in their experiment on the root length of paddy seed and barnyard grass seed conducted to test 23 known allelochemicals, including cinapic acid. Their results indicated that roots might have been affected more than stems and leaves because they were continuous contact with the extracts. Inhibition of growth was found in all species. However, responses among species were different, which might be due to higher concentration and the selectivity of allelochemicals on the target plants (Duke, 2003).

Shahrokhi *et al.*, (2012) reported that aqueous extract of *Amaranthus retroflexus* had the most significant inhibitory effect on the root length of the wheat seedling. The present results agreed with those of Angiras *et al.*, (1987) that radicle growth in maize was more inhibited by aqueous extracts of *Echinochloa crusgalli* and *Cyperus rotundus* than shoot growth which was because the root is the first organ that absorbs the allelochemicals from the environment. Besides, root tissue had more excellent permeability than shoot tissue (Nishida *et al.*, 2005). The decrease in root length compared to shoot length at higher concentrations (25%, 50%, 75%, and 100%) could be due to a high level of root inhibiting allelochemical substances. Early growth inhibition might be due to the high allelochemicals present in the current study.

Chapter 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The results of this study are in agreement with the null hypothesis which stated that *Chromolaena odorata* aqueous leaf extract has significant effect on seed germination and seedling growth of the selected cereal crops and invasive species. The present study had shown that aqueous extract of *C. odorata* could play differing physiological role on the growth of *Tricicum aestivum* (wheat), *Zea mays* (maize), *Eleusine coracana* (finger millet), *Ageratum conyzoides* and *Senna tora* plants, by inhibiting germination and juvenile seedling growth dose dependently. The result also indicated that the aqueous extracts of *C. odorata* and the growth of the roots were more retarded than the shoots. High inhibition of germination and seedling growth was pronounced at 75% and 100% concentration levels. Therefore, aqueous extract of *Chromolaena odorata* can use for control of *Senna tora* and *Ageratum conyzoides* in the high concentration.

6.2 Recommendations

These results were obtained under laboratory conditions. The evaluation of the allelochemicals and their isolation, identification, release, and movement under field conditions warrant further research.

REFERENCES

Abbas, T., Tanveer, A., Khaliq, A., Safdar, M. E., and Nadeem, M. (2014). Allelopathic effects of aquatic weeds on germination and seedling growth of wheat. *Herbologia, 14*(2), 11-25.

Achatz, M., Morris, E. K., Müller, F., Hilker, M., and Rillig, M. C. (2014). Soil hypha-mediated movement of allelochemicals: arbuscular mycorrhizae extend the bioactive zone of juglone. *Functional Ecology, 28*(4); 1020-1029.

Adetayo, O., Lawal, O., Alabi, B., and Owolade, O. (2005). Allelopathic effect of siam weed (*Chromolaena odorata*) on seed germination and seedling performance of selected crop and weed species. Paper presented at the Proceedings of the 4th World Congress on Allelopathy, *"Establishing the Scientific Base"*, Wagga Wagga, New South Wales, Australia.

Agboola, O. O., and Muoghalu, J. I. (2015). Changes in species diversity, composition, growth and reproductive parameters of native vegetation invaded by *Chromolaena odorata* and Tithonia diversifolia in Osun State, southwest Nigeria. *Futa Journal of Research in Sciences, 11*(2); 217-230.

Akinmoladun, A. C., Ibukun, E., and Dan-Ologe, I. (2007). Phytochemical constituents and antioxidant properties of extracts from the leaves of *Chromolaena odorata. Scientific Research and Assays, 2*(6); 191-194.

Akobundu, I. O. (1987). *Weed science in the tropics. Principles and practices*: John Wiley.

Ambica, S. (1980). Influence of light on seed germination in Eupatorium odoratum L. *Indian Forester, 106*(9); 637-640.

Ambika, S. (1980). Suppression of plantation crops by *Eupatorium* weed. *Current Science, 49*(22); 874-875.

Angiras, N., Singh, S. D., and Singh, C. (1987). Allelopathic Effects of Important Weed Species on Germination and Growth of Maize and Soybean Seedlings1. *Indian Journal of Weed Science, 19*(1and2); 57-65.

Barkosky, R. R., Einhellig, F. A., and Butler, J. L. (2000). Caffeic acid-induced changes in plant–water relationships and photosynthesis in leafy spurge Euphorbia esula. *Journal of Chemical Ecology, 26*(9); 2095-2109.

Benech Arnold, R., Fenner, M., and Edwards, P. (1991). Changes in germinability, ABA content and ABA embryonic sensitivity in developing seeds of *Sorghum bicolor* (L.) Moench. induced by water stress during grain filling. *New Phytologist, 118*(2); 339-347.

Bhowmik, P., and Doll, J. (1982). Corn and Soybean Response to Allelopathic Effects of Weed and Crop Residues 1. *Agronomy Journal, 74*(4); 601-606.

Biller, A., Boppré, M., Witte, L., and Hartmann, T. (1994). Pyrrolizidine alkaloids in *Chromolaena odorata*. Chemical and chemoecological aspects. *Phytochemistry, 35*(3); 615-619.

Bogatek, R., Gniazdowska, A., Zakrzewska, W., Oracz, K., and Gawronski, S. (2006). Allelopathic effects of sunflower extracts on mustard seed germination and seedling growth. *Biologia Plantarum, 50*(1); 156-158.

Bouda, H., Tapondjou, L., Fontem, D., and Gumedzoe, M. (2001). Effect of essential oils from leaves of *Ageratum conyzoides, Lantana camara* and *Chromolaena odorata* on the mortality of *Sitophilus zeamais* (Coleoptera, Curculionidae). *Journal of Stored Products Research, 37*(2); 103-109.

Chiang, L., Chiang, W., Liu, M., and Lin, C. (2003). In vitro antiviral activities of *Caesalpinia pulcherrima* and its related flavonoids. *Journal of Antimicrobial Chemotherapy, 52*(2); 194-198.

Chimouriya, S., Shrestha, I., Piya, S., Lamichhane, J., and Gauchan, D. (2018). Allelopathic effects of Adhatoda vasica and *Eupatorium adenophorum* on germination and growth behavior of Capsicum annum. *International Journal of Innovative Science and Research Technology, 3*; 362-368.

Chung, I., Kim, K., Ahn, J., Chun, S., Kim, C., Kim, J., and Kim, S. (2002). Screening of allelochemicals on barnyardgrass (*Echinochloa crus-galli*) and identification of potentially allelopathic compounds from rice (*Oryza sativa*) variety hull extracts. *Crop Protection, 21*(10); 913-920.

Crawford, R., and Brändle, R. (1996). Oxygen deprivation stress in a changing environment. *Journal of Experimental Botany, 47*(2); 145-159.

Devi, O., and Dutta, B. (2012). Allelopathic effect of aqueous extract of *Parthenium hysterophorus* and *Chromolaena odorata* on the seed germination and seedling vigour of *Zea mays* L. vitro. *Academic Journal of Plant Science, 5*; 110-113.

Duke, S. O. (2003). Ecophysiological aspects of allelopathy. *Planta, 217*(4); 529-539.

Dumalisile, L. (2009). *Effects of Chromolaena odorata on mammalian biodiversity in Hluhluwe-iMfolozi Park, South Africa* (Doctoral dissertation, University of Pretoria).

Duñg, N. X., Bien, L. K., and Leclercq, P. A. (1992). The constituents of the leaf oil of Chromolaena odorata (L.) RM King and H. Robinson from Vietnam. *Journal of Essential Oil Research, 4*(3); 309-310.

Eze, J., and Gill, L. (1992). Chromolaena odorata-a problematic weed. *Compositae newsletter, 20*; 14-18.

Fariba, M., Javad, K., and Mohammad, A. (2007). Allelopathic potential of *Trifolium resupinatum* L. and *Trifolium alexandrum* L. *Weed Biology Management, 7*; 178-183.

Gautier, L. (1992). Taxonomy and distribution of a tropical weed: *Chromolaena odorata* (L.) R. King and H. Robinson. *Candollea, 47*(2); 645-662.

Hadas, A. (1976). Water uptake and germination of leguniinous seeds under changing external water potential in osmotic solutions. *Journal of Experimental Botany, 27*(3); 480-489.

Hadas, A., and Russo, D. (1974). Water Uptake by Seeds as Affected by Water Stress, Capillary Conductivity, and Seed-Soil Water Contact. I. Experimental Study 1. *Agronomy Journal, 66*(5); 643-647.

Haig, T. (2008). Allelochemicals in plants Allelopathy in sustainable agriculture and forestry *Springer* (pp. 63-104):.

Hassan, M. M., Daffalla, H. M., Yagoub, S. O., Osman, M. G., Gani, M. E. A., and Babiker, A. (2012). Allelopathic effects of some botanical extracts on germination and seedling growth of Sorghum bicolor L. *Journal of Agricultural Technology, 8*(4); 1423-1469.

Hooker, J. (1882). Flora of British IndiaVolume III. *M/S Bisen Singh Mahendra Pal Singh, New Connaught Place, Dehradun and M/S Periodical Experts*; 630-632.

Hoque, A., Ahmed, R., Uddin, M., and Hossain, M. (2003). Allelopathic effect of different concentration of water extracts of *Acacia auriculiformis* leaf on some initial growth parameters of five common agricultural crops. *Pakistan journal of agronomy, 2*(2); 92-100.

Hu, G., and Zhang, Z. (2013). Allelopathic effects of *Chromolaena odorata* on native and non-native invasive herbs. *Journal of Food, Agriculture & Environment, 11*(1); 878-882.

Inderjit, and Nilsen, E. T. (2003). Bioassays and field studies for allelopathy in terrestrial plants: progress and problems. *Critical Reviews in Plant Sciences, 22*(3-4); 221-238.

Inderjit, S. (2005). Invasive plants: Ecological and agricultural aspects: *Springer Science & Business Media.*

Islam, A. M., Yeasmin, S., Qasem, J. R. S., Juraimi, A. S., and Anwar, M. P. (2018). Allelopathy of medicinal plants: current status and future prospects in weed management. *Agricultural Sciences, 9*(12); 1569-1588.

Jha, S., and Dhakal, M. (1990). Allelopathic effects of various extracts of some herbs on rice and wheat. *Journal of the Institute of Agriculture and Animal Science, 11*; 121-123.

Johnson, W. C., and Coble, H. D. (1986). Effects of three weed residues on weed and crop growth. *Weed Science, 34*(3); 403-408.

Joshi, C., De Leeuw, J., Skidmore, A. K., Van Andel, J., Lekhak, H. D., and Van Duren, I. C. (2005). Remote sensing and GIS for mapping and management of invasive shrub Chromolaena odorata in Nepal. In *Proceedings of Eighth AGILE Conference on Geographic Information Science, Estoril, Portugal* (Vol. 71).

Kader, M., and Jutzi, S. (2001). Drought, heat and combined stresses and the associated germination of two sorghum varieties osmotically primed with NaCl. *Phytopathology, 3*; 22-24.

Khan, M. A., and Ungar, I. A. (1984). The effect of salinity and temperature on the germination of polymorphic seeds and growth of *Atriplex triangularis* Willd. *American Journal of Botany, 71*(4); 481-489.

Kohli, R., Batish, D., and Singh, H. (1997). Allelopathy and its implications in agroecosystems. *Journal of Crop Production, 1*(1); 169-202.

Kohli, R. K., Dogra, K. S., Batish, D. R., and Singh, H. P. (2004). Impact of invasive plants on the structure and composition of natural vegetation of northwestern Indian Himalayas1. *Weed Technology, 18*(sp1); 1296-1300.

Kumar, D., Kumar, N. M., Akamatsu, K., Kusaka, E., Harada, H., and Ito, T. (2010). Synthesis and biological evaluation of indolyl chalcones as antitumor agents. *Bioorganic and medicinal chemistry letters, 20*(13); 3916-3919.

Kwong, K.-L., Wong, P.-K., Lau, S. S., and Qiu, J.-W. (2008). Determinants of the distribution of apple snails in Hong Kong two decades after their initial invasion. *Malacologia, 50*(1-2); 293-302.

Lorenzo, P., Hussain, M. I., and González, L. (2013). Role of allelopathy during invasion process by alien invasive plants in terrestrial ecosystems *Allelopathy* (pp. 3-21): *Springer*.

Maharjan, S., Shrestha, B. B., and Jha, P. K. (2007). Allelopathic effects of aqueous extract of leaves of *Parthenium hysterophorus* L. on seed germination and seedling growth of some cultivated and wild herbaceous species. *Scientific World, 5*(5); 33-39.

Mallik, A. U. (2008). Allelopathy: advances, challenges and opportunities *Allelopathy in sustainable agriculture and forestry* (pp. 25-38): Springer.

Mandal, G., and Joshi, S. P. (2014). Invasion establishment and habitat suitability of *Chromolaena odorata* (L.) King and Robinson over time and space in the western Himalayan forests of India. *Journal of Asia-Pacific Biodiversity, 7*(4); 391-400.

Mangla, S., Inderjit, and Callaway, R. M. (2008). Exotic invasive plant accumulates native soil pathogens which inhibit native plants. *Journal of Ecology, 96*(1); 58-67.

Masum, S., Ali, M., Mandal, M., Haque, M., and Mahto, A. (2012). Influence of *Parthenium hysterophorus, Chromolaena odorata* and PRH on seed germination and seedling growth of maize, soybean and cotton. *Bangladesh J, 3*(1and2); 83-90.

Mersie, W., and Singh, M. (1987). Allelopathic effect of parthenium (*Parthenium hysterophorus* L.) extract and residue on some agronomic crops and weeds. *Journal of Chemical Ecology, 13*(7); 1739-1747.

Molish, H. (1937). The influence of one plant on other: allelopathy. *Jena: Gustav Fischer*.

Muniappan, R., Reddy, G., and Lai, P.-Y. (2005). Distribution and biological control of *Chromolaena odorata Invasive plants: ecological and agricultural aspects* (pp. 223-233): Springer.

Muzzo, B., Mwilawa, A., Maleko, D., and Mtengeti, E. (2018). Allelopathic effect of *Chromolaena odorata* aqueous leaf extracts on seed germination and seedling growth of selected crop and pasture species in Tanzania. *International Journal of Botany Studies, 3*(5); 41-48.

Ngozi, I. M., Jude, I. C., and Catherine, I. C. (2009). Chemical profile of *Chromolaena odorata* L.(King and Robinson) leaves. *Pakistan Journal of Nutrition, 8*(5); 521-524.

Nishida, N., Tamotsu, S., Nagata, N., Saito, C., and Sakai, A. (2005). Allelopathic effects of volatile monoterpenoids produced by *Salvia leucophylla*: inhibition of cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings. *Journal of chemical ecology, 31*(5); 1187-1203.

Nornasuha, Y., and Ismail, B. (2013). *Comparative allelopathic effects of Chromolaena odorata (L.) King & Robinson and Mikania micrantha HBK on Ageratum conyzoides*. Paper presented at the The role of weed science in supporting food security by 2020. Proceedings of the 24th Asian-Pacific Weed Science Society Conference, Bandung, Indonesia, October 22-25, 2013.

Ogbe, F., Gill, L., and Iserhien, E. (1994). Effects of aqueous extracts of *C. odorata* L. on radical and plumule growth and seedling height of maize, *Z. mays* L. *Compositae Newsletters, 25*, 31-38.

Oke, A. (1988). The allelopathic potential of *Chromolaena odorata* on seed germination and seedling growth of vigina unguiculata var. K59 and *Zea mays* var DMR-SRW and germination of *C. odorata* and Tridax procumbens weed seeds. B. *Agric. Tech.(Crop production) Project Report*.

Onwugbuta-Enyi, J. (2001). Allelopathic Effects of *Chromolaena Odorata* L.(RM King and Robinson–(Awolowo Plant')) Toxin on Tomatoes (*Lycopersicum esculentum* Mill). *Journal of Applied Sciences and Environmental Management, 5*(1), 69-73.

Otusanya, O., Ogunwole, A., and Tijani, M. (2015). Allelopathic effect of *Tithonia diversifolia* and *Chromolaena odorata* on the germination, growth and chlorophyll accumulation of Hibiscus sabdariffa (L.). *International Journal of Botany and Research, 5*(3); 1-14.

Patterson, D. (1981). Effects of allelopathic chemicals on growth and physiological responses of soybean (Glycine max). *Weed Science, 29*(1); 53-59.

Polunin, O. and Stainton, A. (1987). Flowers of the Himalaya. Oxford University Press, New Delhi, India.

Press, J. R., Shrestha, K. K., and Sutton, D. A. (2000). *Annotated checklist of the flowering plants of Nepal*. Natural History Museum Publications.

Ramamoorthy, M., and Paliwal, K. (1993). Allelopathic compounds in leaves of *Gliricidia sepium* (Jacq.) Kunth ex Walp. and its effect on *Sorghum vulgare* L. *Journal of chemical ecology, 19*(8); 1691-1701.

Randhawa, M., Cheema, Z., and Ali, M. A. (2002). Allelopathic effect of sorghum water extract on the germination and seedling growth of *Trianthema portulacastrum*. *International Journal of Agriculture and Biology, 4*(3); 383-384.

Reigosa, M. J., Pedrol, N., and González, L. (2006). *Allelopathy: a physiological process with ecological implications*: Springer Science & Business Media.

Roberts, E., and Totterdell, S. (1981). Seed dormancy in Rumex species in response to environmental factors. *Plant, Cell & Environment, 4*(2); 97-106.

Rice, E. L. (1984). *Allelopathy*, 2nd ed. New York: Academic Press.

Rusdy, M., Riadi, M., Sari, A. M., and Normal, A. (2015). Comparative allelopathic effect of *Imperata cylindrica* and *Chromolaena odorata* on germination and seedling growth of Centrosema pubescens. *Int. J. Sci. Res. Pub, 5*(4); 1-5.

Sahid, I., and Yusoff, N. (2014). Allelopathic effects of*Chromolaena odorata*'(L.) King and Robinson and'*Mikania micrantha*'HBK on three selected weed species. *Australian Journal of Crop Science, 8*(7); 1024-1028.

Shackleton, R. T., Witt, A. B., Nunda, W., and Richardson, D. M. (2017). *Chromolaena odorata* (Siam weed) in eastern Africa: distribution and socio-ecological impacts. *Biological Invasions, 19*(4); 1285-1298.

Shahrokhi, S., Darvishzadeh, M., Mehrpooyan, M., and Farboodi, M. (2012). Comparison of allelopathic effects of *Amaranthus retroflexus* L. different organs extracts on germination and initial growth of Alvand and Zarrin wheat cultivars. *International journal of Agronomy and Plant Production, 3*(11); 489-494.

Shikha, R., and Jha, A. (2016). Evaluation of effect of leaf extract of *Parthenium hysterophorus* L. on seed germination, seedling growth and fresh weight of *Phaseolus mungo*. *American Journal of Research Communication, 4*(2); 86-103.

Shrestha, B., Siwakoti, M., and Ranjit, J. (2017). *Status of invasive alien plant species in Nepal.* Paper presented at the Conservation and utilization of agricultural plant genetic resources in Nepal (Joshi BK, KC HB, Acharya AK.(eds). Proceedings of 2nd National Workshop, 22-23 May 2017 Dhulikhel.

Shrestha, B. B. (2016). Invasive alien plant species in Nepal. *Frontiers of botany, 2016*; 269-284.

Shrestha, B. B., Shrestha, U. B., Sharma, K. P., Thapa-Parajuli, R. B., Devkota, A., and Siwakoti, M. (2019). Community perception and prioritization of invasive alien plants in Chitwan-Annapurna Landscape, Nepal. *Journal of environmental management, 229*; 38-47.

Suwal, M. M., Devkota, A., and Lekhak, H. (2010). Allelopathic effects of *Chromolaena odorata* (L.) King & Robinson on seed germination and seedlings growth of paddy and barnyard grass. *Scientific World, 8*(8); 73-75.

Tessema, S. S., and Tura, A. M. (2018). Allelopathic property of Parthenin on seed germination and seedling growth of wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*). *International Journal of Chemical and Biochemical Sciences, IJCBS, 14*(2018); 23-27.

Thapa, L. B., Kaewchumnong, K., Sinkkonen, A., and Sridith, K. (2016). Impacts of invasive *Chromolaena odorata* on species richness, composition and seedling recruitment of *Shorea robusta* in a tropical Sal forest, Nepal. *Songklanakarin Journal of Science and Technology, 38*(6); 683-689.

Thapa, L. B., Pathak, S., Pal, K. B., Miya, T. M., Darji, T. B., Pant, G., and Pant, R. R. (2021). Chemical Constituents of the Essential Oil of Invasive *Chromolaena odorata* leaves in Central Nepal. *Journal of Nepal Chemical Society, 42*(1); 132-137.

Usuah, P., Udom, G., and Edem, I. (2013). Allelopathic effect of some weeds on the germination of seeds of selected crops grown in Akwa Ibom State, Nigeria. *World Journal of Agricultural Research, 1*(4); 59-64.

Washitani, I., and Saeki, T. (1986). Germination responses of *Pinus densiflora* seeds to temperature, light and interrupted imbibition. *Journal of Experimental Botany, 37*(9); 1376-1387.

Williamson, M., and Fitter, A. (1996). The varying success of invaders. *Ecology, 77*(6); 1661-1666.

Zheng, Y. L., Feng, Y. L., Zhang, L. K., Callaway, R. M., Valiente-Banuet, A., Luo, D. Q., and Silva-Pereyra, C. (2015). Integrating novel chemical weapons and evolutionarily increased competitive ability in success of a tropical invader. *New Phytologist, 205*(3); 1350-1359.

Zobel, D. B., Jha, P. K., Behan, M. J. and Yadav, U. K. R. (1987). A Practical Manual for Ecology. Ratna Book Distributors, Kathmandu, Nepal

Appendices

Appendix I: Data sheets used in field sampling

Date: ……………Investigator: ………………………District : ……………..
Municipality: ………………… Locality: …………………… Altitude(m) :………….
Aspect :…………………..Slope:………………..Latitude:…………………………
Longitude:…………………………Disturbance level:(0 – 3):…..
Plot No: Quadrat No: Quadrat size:

For shrub

S.N.	Plant species	Local name	No. of species	Coverage	Remarks

For herb

S.N.	Plant species	Local name	No. of species	Coverage	Remarks

Appendix II: Plant community attributes of study area

Herb	Frequency	RF	Density	RD	Coverage	RC	IVI
Chromoleana odorata	100.00	11.54	3.90	14.26	1625	34.74	60.55
Ageratina adonophora	40.00	4.62	1.03	3.76	207	4.43	12.80
Ageratum conyzoides	43.33	5.00	1.12	4.11	79	34.74	10.80
A. haustonianum	60.00	6.92	1.97	7.21	248	5.30	19.43
Spermacoce alata	23.33	2.69	1.27	4.65	270	5.77	13.12
Capillipedium parviflorum	80.00	9.23	3.58	13.10	620	13.26	35.59
Senna tora	36.67	4.23	0.68	2.48	93	1.99	8.70
Imparata cylindrica	73.33	8.46	4.78	17.48	413	8.83	34.77
Bidens pilosa	63.33	7.31	1.80	6.59	198	4.23	18.13
Rungia sp	33.33	3.85	0.69	2.52	95	2.03	8.40
Xanthium strumarium	23.33	2.69	0.52	1.90	109	2.33	6.92
Reinwardia indica	16.67	1.92	0.28	1.01	50	1.07	4.00
Mimosa pudica	43.33	5.00	1.00	3.64	75	1.60	10.25
Paspalum sp	40.00	4.62	1.10	4.03	81	1.73	10.38
Cynodon dactylon	13.33	1.54	0.27	0.97	13	0.28	2.79
Centella asiatica	16.67	1.92	0.56	2.05	25	0.53	4.51
Themeda sp	23.33	2.69	0.45	1.63	135	2.89	7.21
Triumfetta sp	46.67	5.38	1.11	4.07	110	2.35	11.81
Euphorbia hirta	13.33	1.54	0.23	0.85	22	0.47	2.86
Perilla frutescens	6.67	0.77	0.11	0.39	19	0.41	1.56
Achyranthus aspera	6.67	0.77	0.05	0.19	10	0.21	1.18
Saccharum spontaneum	23.33	2.69	0.31	1.12	120	2.57	6.38
Pteris vittata	13.33	1.54	0.24	0.89	26	0.56	2.99
Lygodium sp	13.33	1.54	0.13	0.47	19	0.41	2.41
Cissampelos pareira	6.67	0.77	0.05	0.19	8	0.17	1.13
Iryngium foetidium	6.67	0.77	0.12	0.43	7	0.15	1.35
Shrubs	**Frequency**	**RF**	**Density**	**RD**	**Coverage**	**RC**	**IVI**
Artimisia vulgaris	63.33	22.09	0.10	40.10	405.00	24.85	87.04

Colebrookea oppositifolia	36.67	12.79	0.02	8.39	197.00	12.09	33.27
Pogostemon bengalensis	23.33	8.14	0.02	6.71	140.00	8.59	23.44
Woodfordia fruticosa	40.00	13.95	0.02	6.54	235.00	14.42	34.91
Maesa chisia	13.33	4.65	0.00	1.17	55.00	3.37	9.20
Caesalpenia decapetata	23.33	8.14	0.01	4.19	115.00	7.06	19.39
Rubus ellipticus	6.67	2.33	0.00	0.84	30.00	1.84	5.00
Boehmeria sp	26.67	9.30	0.02	7.89	93.00	5.71	22.89
Ziziphus sp	6.67	2.33	0.00	1.51	25.00	1.53	5.37
Adhatoda vasica	6.67	2.33	0.00	1.51	35.00	2.15	5.98
Clerodendrum sp	30.00	10.46	0.05	20.13	260.00	15.95	46.55
Lantana camera	3.33	1.16	0.00	0.34	10.00	0.61	2.11
Calotropis gigantae	6.67	2.33	0.00	0.67	30.00	1.84	4.84

Appendix III: Plot Characteristics

quadrat no	Latitude	longitude	Aspect	slope	Altitude	Disturbance
1	27.9397	83.8605	63°NE	24°	464.6	1
2	27.9407	83.8605	258°W	14°	460	2
3	27.9407	83.86	205°SW	18°	460	2
4	27.9736	83.8605	221°SW	21°	470	1
5	27.9411	83.86	170°S	31°	480	1
6	27.9513	83.86	19°N	21°	480	2
7	27.863	83.8955	62°NE	19°	480	1
8	27.9441	83.8586	162°S	22°	480	2
9	27.9438	83.5888	200°S	19°	470	2
10	27.9438	83.8586	179°S	20°	470	2
11	27.9427	83.8591	107°E	22°	500	1
12	27.9425	83.8594	60°NE	21°	480	1
13	27.9422	83.8591	80°E	18°	490	2
14	27.9388	83.8611	132°SE	17°	490	2

15	27.9416	83.8594	75°E	22°	490	3
16	27.9227	83.8602	287°W	24°	470	2
17	27.9227	83.86	273°W	24°	460	1
18	27.9225	83.86	220°SW	18°	470	1
19	27.9222	83.8608	203°SW	19°	470	1
20	27.9219	83.8608	287°W	20°	460	0
21	27.9227	83.8697	283°W	26°	470	0
22	27.9247	83.8611	254°W	27°	460	1
23	27.9247	83.8608	274°W	24°	470	3
24	27.9241	83.8338	255°W	27°	470	3
25	27.926	83.8616	224°SW	28°	480	1
26	27.9358	83.8638	266°W	24°	490	1
27	27.9361	83.8638	270°W	26°	490	2
28	27.9372	83.8647	61°NE	25°	500	0
29	27.9325	83.8652	259°W	23°	580	0
30	27.9327	83.865	284°W	31°	570	1

Appendix IV: Post Hoc test (Shoot length)

Dependent Variable: Shoot length					
Post hoc test of allelopathic impact along concentration					
	(I) concentration	(J) concentration	Mean Difference (I-J)	Std. Error	Sig.
LSD	0%	25%	2.692898*	0.1133893	0.027
		50%	3.782929*	0.1133893	0.019
		75%	3.348506*	0.1133893	0.022
		100%	3.605467*	0.1133893	0.02
	25%	0%	-2.692898*	0.1133893	0.027
		50%	1.090031	0.1133893	0.066
		75%	0.655608	0.1133893	0.109
		100%	0.912569	0.1133893	0.079
	50%	0%	-3.782929*	0.1133893	0.019

		25%	-1.090031	0.1133893	0.066
		75%	-0.434423	0.1133893	0.163
		100%	-0.177462	0.1133893	0.362
	75%	0%	-3.348506*	0.1133893	0.022
		25%	-0.655608	0.1133893	0.109
		50%	0.434423	0.1133893	0.163
		100%	0.256961	0.1133893	0.265
	100%	0%	-3.605467*	0.1133893	0.02
		25%	-0.912569	0.1133893	0.079
		50%	0.177462	0.1133893	0.362
		75%	-0.256961	0.1133893	0.265

Post hoc test of allelopathic impact along species

LSD	*Zea mays*	*Senna tora*	1.982503*	0.1095445	0.035
		Triticum aestivum	-0.359130	0.1095445	0.188
		Eleusine caracona	2.631005*	0.1148913	0.028
		Ageratum conyzoides	3.055277*	0.1148913	0.024
	Senna tora	*Zea mays*	-1.982503*	0.1095445	0.035
		Triticum aestivum	-2.341634*	0.1095445	0.030
		Eleusine caracona	0.648501	0.1148913	0.112
		Ageratum conyzoides	1.072774	0.1148913	0.068
	Triticum aestivum	*Zea mays*	0.359130	0.1095445	0.188
		Senna tora	2.341634*	0.1095445	0.030
		Eleusine caracona	2.990135*	0.1148913	0.024
		Ageratum conyzoides	3.414408*	0.1148913	0.021

Eleusine caracona	*Zea mays*	-2.631005*	0.1148913	0.028
	Senna tora	-0.648501	0.1148913	0.112
	Triticum aestivum	-2.990135*	0.1148913	0.024
	Ageratum conyzoides	0.424272	0.1200000	0.175
Ageratum conyzoides	*Zea mays*	-3.055277*	0.1148913	0.024
	Senna tora	-1.072774	0.1148913	0.068
	Triticum aestivum	-3.414408*	0.1148913	0.021
	Eleusine caracona	-0.424272	0.1200000	0.175

*. The mean difference is significant at the 0.05 level.

Appendix V: Post Hoc test (Root length)

Dependent Variable:	Root length				
Post hoc test of allelopathic impact along concentration					
			Mean Difference (I-J)	Std. Error	Sig.
LSD	0%	25%	2.954082*	0.0661438	0.014
		50%	4.094500*	0.0661438	0.010
		75%	3.972751*	0.0661438	0.011
		100%	4.056981*	0.0661438	0.010
	25%	0%	-2.954082*	0.0661438	0.014
		50%	1.140418*	0.0661438	0.037
		75%	1.018669*	0.0661438	0.041
		100%	1.102900*	0.0661438	0.038
	50%	0%	-4.094500*	0.0661438	0.010
		25%	-1.140418*	0.0661438	0.037
		75%	-0.121749	0.0661438	0.317
		100%	-0.037519	0.0661438	0.672
	75%	0%	-3.972751*	0.0661438	0.011
		25%	-1.018669*	0.0661438	0.041

		50%	0.121749	0.0661438	0.317
	100%	100%	0.084231	0.0661438	0.424
		0%	-4.056981*	0.0661438	0.010
		25%	-1.102900*	0.0661438	0.038
		50%	0.037519	0.0661438	0.672
		75%	-0.084231	0.0661438	0.424

*. The mean difference is significant at the 0.05 level.

Dependent Variable: Root length

Post hoc test of allelopathic impact along species

			Mean Difference (I-J)	Std. Error	Sig.
LSD	*Zea mays*	*Senna tora*	2.647168*	0.0639010	0.015
		Triticum aestivum	-0.062584	0.0639010	0.507
		Eleusine caracona	2.642285*	0.0670199	0.016
		Ageratum conyzoides	2.976498*	0.0670199	0.014
	Senna tora	*Zea mays*	-2.647168*	0.0639010	0.015
		Triticum aestivum	-2.709751*	0.0639010	0.015
		Eleusine caracona	-0.004882	0.0670199	0.954
		Ageratum conyzoides	0.329331	0.0670199	0.128
	Triticum aestivum	*Zea mays*	0.062584	0.0639010	0.507
		Senna tora	2.709751*	0.0639010	0.015
		Eleusine caracona	2.704869*	0.0670199	0.016
		Ageratum conyzoides	3.039082*	0.0670199	0.014
	Eleusine caracona	*Zea mays*	-2.642285*	0.0670199	0.016
		Senna tora	0.004882	0.0670199	0.954
		Triticum aestivum	-2.704869*	0.0670199	0.016
		Ageratum conyzoides	0.334213	0.0700000	0.131
	Ageratum conyzoides	*Zea mays*	-2.976498*	0.0670199	0.014
		Senna tora	-0.329331	0.0670199	0.128
		Triticum aestivum	-3.039082*	0.0670199	0.014

		Eleusine caracona	-0.334213	0.0700000	0.131

*. The mean difference is significant at the 0.05 level.

Appendix VI: Post hoc test (Germination)

Dependent Variable: Germination percent

Post hoc test of allelopathic impact along concentration

			Mean Difference (I-J)	Std. Error	Sig.
LSD	0%	25%	33.34075%*	4.49107%	0.000
		50%	55.33692%*	4.45223%	0.000
		75%	54.78136%*	4.45223%	0.000
		100%	63.67025%*	4.45223%	0.000
	25%	0%	-33.34075%*	4.49107%	0.000
		50%	21.99617%*	4.52710%	0.000
		75%	21.44061%*	4.52710%	0.000
		100%	30.32950%*	4.52710%	0.000
	50%	0%	-55.33692%*	4.45223%	0.000
		25%	-21.99617%*	4.52710%	0.000
		75%	-0.55556%	4.48857%	0.902
		100%	8.33333%	4.48857%	0.065
	75%	0%	-54.78136%*	4.45223%	0.000
		25%	-21.44061%*	4.52710%	0.000
		50%	0.55556%	4.48857%	0.902
		100%	8.88889%*	4.48857%	0.050
	100%	0%	-63.67025%*	4.45223%	0.000
		25%	-30.32950%*	4.52710%	0.000
		50%	-8.33333%	4.48857%	0.065
		75%	-8.88889%*	4.48857%	0.050

*. The mean difference is significant at the 0.05 level.

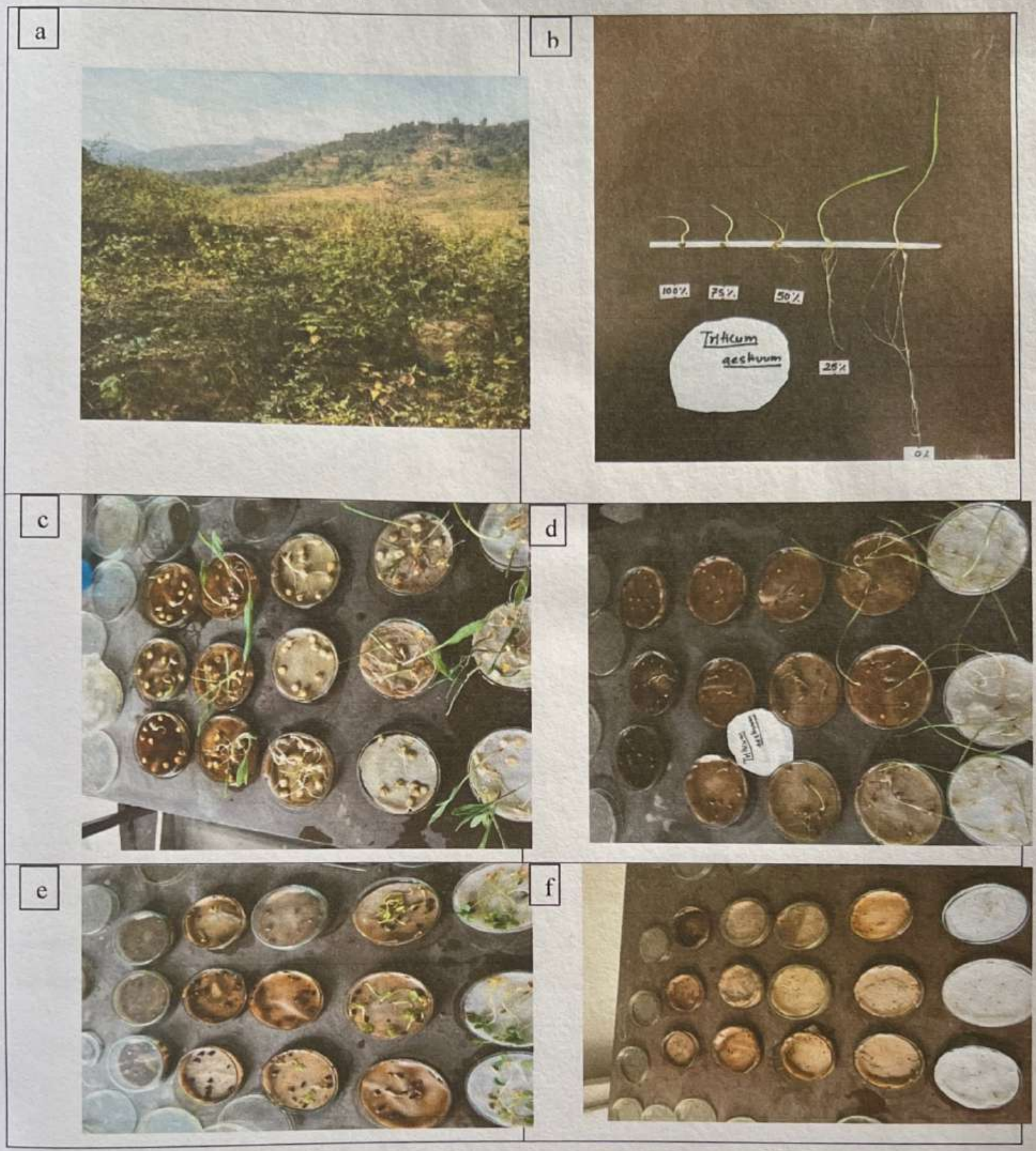
Dependent Variable: Germination percent

Post hoc test of allelopathic impact along species

			Mean Difference (I-J)	Std. Error	Sig.
LSD	*Zea mays*	*Senna tora*	11.66667%	6.98503%	0.097
		Triticum aestivum	-1.00000%	6.98503%	0.886

	Eleusine caracona	13.88889%*	6.98503%	0.049
	Ageratum conyzoides	29.77778%*	6.98503%	0.000
Senna tora	*Zea mays*	-11.66667%	6.98503%	0.097
	Triticum aestivum	-12.66667%	6.98503%	0.072
	Eleusine caracona	2.22222%	6.98503%	0.751
	Ageratum conyzoides	18.11111%*	6.98503%	0.010
Triticum aestivum	*Zea mays*	1.00000%	6.98503%	0.886
	Senna tora	12.66667%	6.98503%	0.072
	Eleusine caracona	14.88889%*	6.98503%	0.035
	Ageratum conyzoides	30.77778%*	6.98503%	0.000
Eleusine caracona	*Zea mays*	-13.88889%*	6.98503%	0.049
	Senna tora	-2.22222%	6.98503%	0.751
	Triticum aestivum	-14.88889%*	6.98503%	0.035
	Ageratum conyzoides	15.88889%*	6.98503%	0.024
Ageratum conyzoides	*Zea mays*	-29.77778%*	6.98503%	0.000
	Senna tora	-18.11111%*	6.98503%	0.010
	Triticum aestivum	-30.77778%*	6.98503%	0.000
	Eleusine caracona	-15.88889%*	6.98503%	0.024

Appendix II: Selected photo plates



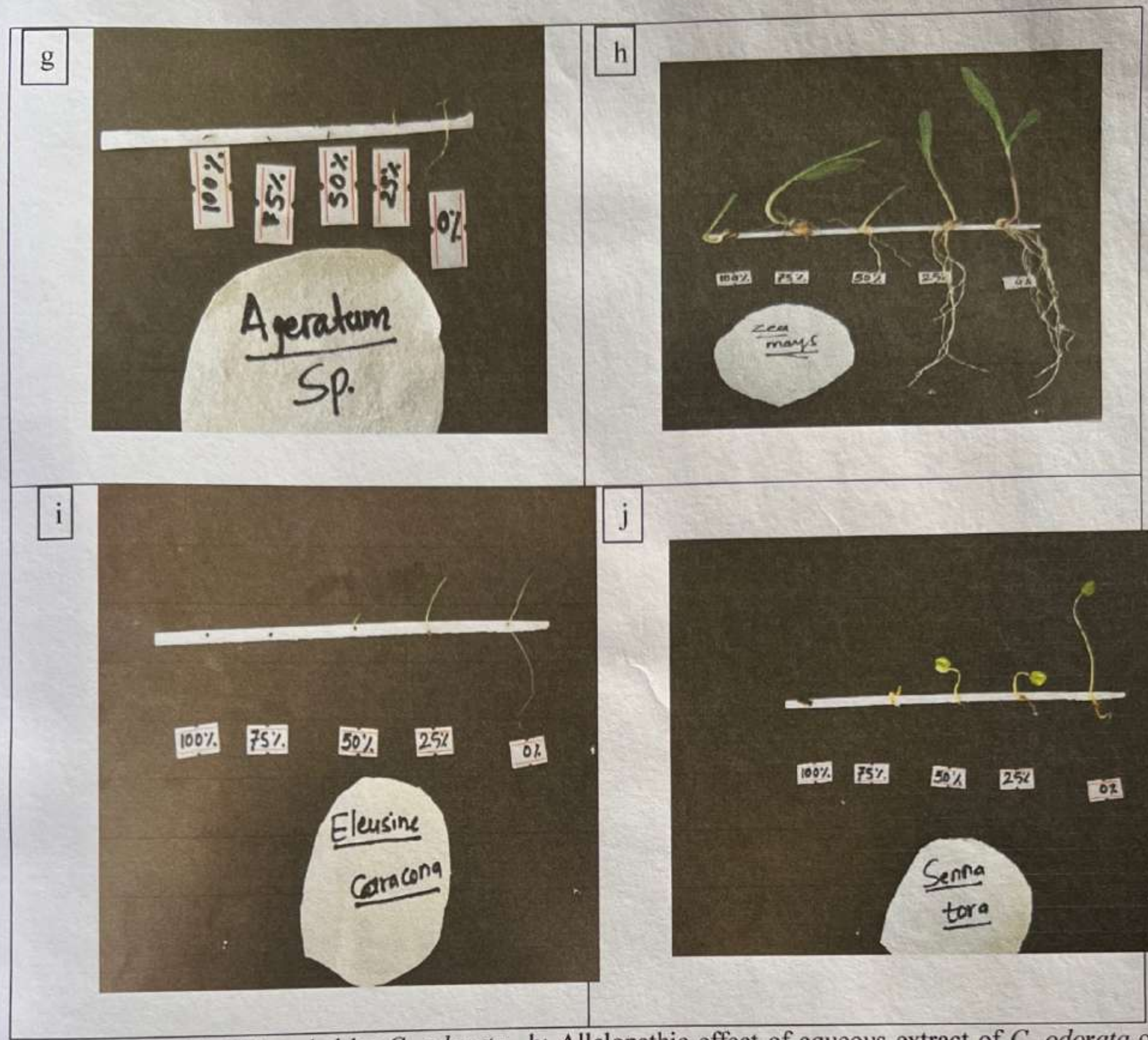


Photo plate a: Field invaded by *C. odorata*., b: Allelopathic effect of aqueous extract of *C. odorata* on seedling growth of *T. aestivum*., c: Allelopathic effect of aqueous extract of *C. odorata* on germination of *Z. mays,* d: *T. aestivum, e: S. tora,*f: *E. coracana, g:* Allelopathic effect of aqueous extract of *C. odorata* on seedling growth of *A. conyzoides*, h: *Z. mays.*, i: *E. coracana.*, j:*S. tora.*,