ALLELOPATHIC EFFECT OF SELECTIVE INVASIVE PLANTS ON GERMINATION OF *Triticum aestivum L.*

A PROJECT WORK SUBMITTED TO THE

DEPARTMENT OF BIOLOGY

CENTRAL CAMPUS OF TECHNOLOGY

INSTITUTE OF SCIENCE AND TECHNOLOGY TRIBHUVAN UNVERSITY

DHARAN, SUNSARI

NEPAL

FOR THE AWARD OF

BACHELOR OF SCIENCE (B.Sc.) IN BOTANY

BY

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12th June, 2023

RECOMMENDATION

This is to recommend that Ms. Renuka Shrestha, (Symbol No: 500080036, T. U. Registration NO:5-2-8-84-2018) has carried out project work entitled "**Allelopathic effect of selective invasive plants on germination of *Tritium aestivum L.*"** for the requirement to the project work in Bachelor of Science (B.Sc.) degree in Botany under my/our supervision in the Department of Biology, Central Campus of Technology, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal.

To my/ our knowledge, this work has not been submitted for any degree.

She has fulfilled all the requirements laid down by the Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal for the submission of the project work for the partial fulfillment of the Bachelor of Science (B.Sc.) degree.

....

Asst. Prof. Mr. Pramod Sen Oli
Supervisor
Department of Biology
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Tribhuvan University

12th June, 2023

DECLARATION

This project work entitled "**Allelopathic Effect of Selective Invasive Plants on Germination of *Triticum aestivum.L.*"** is being submitted to the Department of Biology, Central Campus of Technology, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal for the partial fulfillment of the requirement to the project work in Bachelor of Science (B.Sc.) degree in Botany. This project work is carried out by me under the supervision of Asst. Prof. Mr. Pramod Sen Oli in the Department of Biology, Central Campus of Technology, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal.

This work is original and has not been submitted earlier in part or full in this or any other form to any university or institute, here or elsewhere, for the award of any degree.

Signature
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[12th June, 2023]

LETTER OF FORWARD

[Date: 12th June, 2023]

On the recommendation of **asst. Prof. Mr. Pramod Sen Oli**, this project work is submitted by **Renuka Shrestha**, Symbol No. 500080036, T.U. Registration No 5-2-8-84-2018, entitled **"Allelopathic Effect of Selective Invasive Plants on Germination of Triticum aestivum"** is forwarded by the Department of Biology, Central Campus of Technology, for approval to the Evaluation Committee, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal.

He has fulfilled all the requirements for the project work by the Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal.

Asst. Prof. Mrs. Sanju Parajuli

Head of Department

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BOARD OF EXAMINATION AND CERTIFICATE OF APPROVAL

This project work (PRO-406) **"Allelopathic Effect of Selective Invasive Plants on Germination of Triticum aestivum L."** by Ms. Renuka Shrestha, Symbol No. 500080036 and T.U. Registration No. 5-2-8-84-2018 under the supervision of Asst. Prof. Mr. Pramond Sen Oli in the Department of Biology, Central Campus of Technology, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), is hereby submitted for the partial fulfillment of the Bachelor of Science (B.Sc.) degree in Botany. This report has been accepted and forwarded to the Controller of Examination, Institute of Science and Technology, Tribhuvan University, Nepal for the legal procedure.

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ABSTRACT

Allelopathy is the release of secondary compounds by a plant that affects the growth of surrounding plants in a stimulatory and inhibitory manner. An "invasive species" is a species that is foreign or non-native to the ecosystem under study and whose introduction hurts or is anticipated to harm the economy, the environment, or human health.

The current study's objective is to use various extracts to observe the allelopathic effects in plants of *Ageratum houstonianum* and *Ageratum conyzoides*. *Ageratum houstonianum* and *Ageratum conyzoides* plants were procured from the Dharan neighborhood and dried in a lab at room temperature for a month. The extract was then created using cold percolation on distilled water. All extracts' seed germination rates were identified.

In a laboratory experiment, the allelopathic effects of plant extract from *Ageratum houstonianum* and *Ageratum conyzoides* on the germination and seedling growth of Triticum aestivum were examined at concentrations of 2.5%, 5%, 7.5%, and 10%. The findings showed that the effects of water extract at different concentrations considerably affected all aspects of wheat seedling germination and growth when compared to controls. The effect of water extract of mixed (*Ageratum houstonianum* and *Ageratum conyzoides*) at 10% concentration was discovered to have the lowest germination percentage, germination index, root and shoot length, and seedling size in wheat. With increasing aqueous solution concentration, the shoot, roots, and shoot germination length were all decreased. The study found that increasing the quantity of *Ageratum houstonianum* and *Ageratum conyzoides* plant extracts hurt *Triticum aestivum* germination, shoot length, root length, and biomass output compared to the control.

Keywords: Allelopathic effect*, Ageratum houstonianum. L, Ageratum conyzoides. L,* Plant extract, *Triticum aestivum. L.*

शोधसार

एलेलोप्याथी भनेको बिरुवाद्वारा माध्यमिक यौगिकहरू निस्कने हो जसले वरपरका बिरुवाहरूको वृद्धिलाई उत्तेजक र निषेधात्मक तरिकाले असर गर्छ। एक "आक्रमणकारी प्रजाति" एक प्रजाति हो जुन अध्ययन अन्तर्गत पारिस्थितिकी तंत्र को लागी विदेशी वा गैर-स्थानीय हो र जसको परिचयले अर्थतन्त्र, वातावरण, वा मानव स्वास्थ्यलाई हानि पुऱ्याउँछ वा हानि पुऱ्याउँछ।

हालको अध्ययनको उद्देश्य एजेराटम हाउस्टोनियम र एजेरेटम कन्जोइड्सका बिरुवाहरूमा एलेलोप्याथिक प्रभावहरूको अनुसन्धान गर्न विभिन्न अर्कहरू प्रयोग गर्नु हो। एजेराटम हाउस्टोनियम र एजेरेटम कोनोजोइड बिरुवा धरान छिमेकीबाट खरिद गरी कोठाको तापक्रममा प्रयोगशालामा एक महिनासम्म सुकाइयो। निकासी त्यसपछि डिस्टिल्ड पानीमा चिसो पर्कोलेसन प्रयोग गरेर सिर्जना गरिएको थियो। सबै अर्कको बीउ अंकुरण दरहरू पहिचान गरियो

प्रयोगशाला प्रयोगमा, ट्रिटिकम एस्टिभमको अंकुरण र बिरुवाको वृद्धिमा एजेरेटम हाउस्टोनियम र एजेरेटम कन्जोइड्सबाट बिरुवाको निकासीको एलेलोप्याथिक प्रभावहरू 2.5%, 5%, 7.5%, र 10% को सांद्रतामा जाँच गरियो। विभिन्न सांद्रतामा पानीको निकासीको प्रभावले गहुँको बिरुवाको अंकुरण र वृद्धिका सबै पक्षहरूलाई नियन्त्रणको तुलनामा धेरै प्रभाव पारेको निष्कर्षले देखाएको छ।10% एकाग्रतामा मिश्रित (*Ageratum houstonium* and *Ageratum conyzoides*) को पानीको प्रभाव सबैभन्दा कम अंकुरण प्रतिशत, अंकुरण सूचकांक, जरा र अंकुरको लम्बाइ, र गहुँमा बिरुवाको आकार भएको पत्ता लाग्यो। बढ्दो जलीय घोल एकाग्रता संग, अंकुर को लम्बाई, जरा, र अंकुरण सबै घट्यो। अध्ययनले एजेराटम हाउस्टोनियम र एजेराटम कोनोजोइड बिरुवाको अर्कको मात्रा बढाउँदा ट्रिटिकम एस्टिभम अंकुरण, अंकुरको लम्बाइ, जराको लम्बाइ र नियन्त्रणको तुलनामा बायोमास उत्पादनमा नकारात्मक असर परेको पाइएको छ।

LIST OF ACRONYMS AND ABBREVIATIONS

IP:	Invasive Plants
GPS:	Global Positioning System
SPSS:	Statistical Package for the Social Sciences
ANOVA	Analysis of variance
gm	grams
h	hours
Lat	Latitude
Long	Longitude

LIST OF SYMBOLS

°C	Degree Celsius
ml	milliliter
%	Percentage
mm	Millimeter

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CHAPTER 1

INTRODUCTION

1.1. Allelopathic

Allelopathy is the release of secondary chemicals by a plant that has an impact (both stimulatory and inhibitory) on the growth of nearby plants. The Greek terms allelon, which means "of each other," and pathos, which means "to suffer," are the sources of the term allelopathy, which was coined by Molisch in 1937. mean the harm that one does to the other(Y. N. Devi et al., 2014) The phrase, though, is now widely understood to refer to both the inhibitory and stimulatory actions of one plant (Reigosa et al., 2006) According to the Topics in Biology Laboratory Manuel, allelopathy refers to “the beneficial or harmful effects of one plant on another plant. Considering the beneficial properties of plants, not only to us, but the environment as well, it is important to understand the nature of Allelopathy and how it affects plant ecology. Native species' allelopathic potential may lead to biotic resistance to invasive plants, whereas allelochemicals emitted by foreign species may encourage the spread of invasive species(Koroma & Kamara, 2021).

These allelochemicals are divided into many categories based on their chemical properties. Phytochemicals, alkaloids, terpenes, and fatty acids are the five allelochemicals that are most frequently discovered in plants. most frequently occurring allelochemicals in plants include phenolics, alkaloids, terpenes, fatty acids, and indoles. Almost all plant tissues contain allelochemicals, including leaves, flowers, fruits, stems, roots, rhizomes, seeds, and pollen. Several Chemicals can be discharged in combination and may have additive or synergistic harmful effects(Putnam, 1986). Numerous invasive plants' mechanisms of success may be explained by allelopathy(Hierro & Callaway, 2003)

Numerous allelochemicals have been discovered and identified, and some of them play a role in the plant defense system. (D. Singh, 2019). It is proposed to use the allelopathic effects of legumes and cereals to control weed growth(Conklin et al., 2002) pests, and illnesses(Messiaen, 1992).

Allelochemicals, also known as allelochemicals, are substances produced by plants that have an allelopathic effect. They can be found in many plant components. including the seeds, flowers, pollen, rhizomes, leaves, stems, and roots. Root exudation, leaching from above-ground components, volatilization, and/or the breakdown of plant material are released into the environment(O. I. Devi et al., 2012).

1.2 Invasive Plant

A species that is considered an "invasive species" is. alien or non-native to the ecosystem being studied; and, whose introduction harms or is expected to impair the economy, the environment, or human health. An introduced species that overpopulates and damages its new environment is considered invasive or alien (Davis & Thompson, 2000). Invasive species hurt habitats and bioregions, harming the economy, the environment, and/or the ecology (Gorgens & Van Wilgen, 2004). Species that have colonized a region but are not native there are known as alien or naturalized species; those that pose a threat to local native species and biodiversity are frequently referred to as invasive species (Sandlund et al., 2001).

One of the biggest threats to native species and ecosystems around the world is invasive species. They pose a serious threat due to their capacity to expand quickly, competition, and quick colonization speeds(Basarkar & Saoji, 2013). Research on invasive plant management plays a crucial role in informing practitioners' choices. Practical but surprisingly scant information about specific ecosystems and invaders is produced by scientific research(Flory, 2010). Exotic plant invasions may harm native plant populations by diminishing the diversity of their native species(Levine et al., 2003).

Our objective is a detailed analysis of the processes underlying the reported effects of exotic plant invasions, not a blanket assessment of impacts. It's crucial to comprehend these processes to some of the most crucial issues in the study of biological invasions, such as why just a tiny percentage of established(Simberloff, 1981).

The effects of an invading species do not always persist during the course of the invasion, and both ecological and evolutionary processes have the potential to amplify or reduce the effects of invaders on the local population(Strayer, 2012).

1.3 Triticum aestivum L.

Plant interactions have been investigated in both managed and unmanaged ecosystems. Agriculture-based systems. Allelopathy can interfere with other crops and crops and weeds, which could impact plant production is profitability. There are documented crop and plant species exhibiting allelopathic activity (Inderjit & Dakshini, 1998)

. It was first introduced into Ethiopia during the Ethio-Somali war in 1978 and has since spread worldwide to every part of the world. Currently, it is the most prevalent weed in eastern Ethiopia (Netsere & Mendesil, 2012). The development of mechanized product processing methods in recent years and the history of agriculture have led to a significant expansion of land devoted to the main agricultural variety. Accordingly, almost 95% of the wheat produced contains the hexaploid species *Triticum aestivum* L., also known as "common," "bread," or "soft" wheat(Marcussen et al., 2014)

Triticum aestivum L., a hexaploid species commonly known as "common" or "bread" wheat, is the main species of wheat farmed worldwide. However, a tetraploid variety that is adapted to the hot, dry climate, T. turgidum var. durum, makes up roughly 35–40 mt of the entire world production conditions surrounding the Mediterranean Sea and other areas with comparable climates. This is frequently referred to as "pasta wheat" or "durum wheat" and is used to make pasta. Only a few tiny plots of land are used to grow other varieties of wheat, either for a cultural reason or to supply the growing market for healthy meals. Three of them are spelled (T. aestivum var. spelled), a cultivated variety of hexaploid wheat, emmer (tetraploid T. turgidum var. dicoccum), and einkorn (diploid T. monococcum var. monococcum). Compared to bread and durum wheat, spelled emmer, and most einkorn varieties are hulled (i.e., the glumes are securely closed over the grain and are not removed by threshing).

Possibly the most significant food crop in the world is wheat. According to the International Wheat Council (1994), 550 million metric tons (MMT) of wheat is produced worldwide. About 100 MMT of this is exchanged annually on the global market. This chapter will go through the main categories of consumer products and the specifications for flour quality, quantitative evaluation of flour quality, milling quality and its relationship to the usefulness of flour, intrinsic grain, and grain lot quality, and the applications of wheat for feed.

One of the most significant farming systems in Nepal is the wheat-rice system. wheat accounts for 22.5% of all grain production and takes up 23% of wheat cultivated on around 760,000 ha, with a projected production of 1,834,212 mt. Wheat production has increased from 1.5 Mt ha1 in the 1960s to 2.5. The growth can be attributed to the introduction of superior cultivars, irrigation, and incentives offered by growing markets. Farmers sell about 60% of their wheat harvest(Farnworth et al., 2019).

All around Nepal, women are actively engaged in wheat farming. However, little is known about their responsibilities and functions, the difficulties they encounter, and the chance the s they grab. Using the search terms, look for peer-reviewed literature. Only ten papers relating to "gender"/"social equity"/"wheat"/"Nepal" during a20 years were discovered. These studies only tangentially address women's roles in wheat(Jafry, 2016).

1.4 Significance of the Study

To thrive, many invasive species heavily rely on the allelopathic effects on native seed germination and seedling growth. Because of how quickly and aggressively they spread, invasive species have the potential to harm the environment. The reason invading species are dominant is due to their robust capacity for reproduction as well as their propensity for exponential development. The importance of this study helps us learn important things about how invasive plant species interact with one another and how it affects the productivity of the *Triticum aestivum L.* crop. Comparisons between the results of the germination effect using *Ageratum houstonianum L.* and *Ageratum conyzoides L.* would be useful in identifying the barriers to *Triticum aestivum L.* systematic growth. The results of this study may also be useful for agricultural professionals in identifying and reducing the negative effects on the germination of seeds are specific invasive plant species. Improved crop output and long-term agricultural practices may result from this. The results may also be useful in the field of managing alien species. This would be in favor of limiting the negative effects on the environment and the economy caused by the interaction between invasive plants and the life cycle of *Triticum aestivum L.*

1.5 Objectives

1.5.1 General Objective

- To study the allelopathic effect of selective invasive plants on germination of *Triticum aestivum* L.

1.5.2 Specific Objectives

- To compare the allelopathic effects of different invasive plants species on germination on *Triticum aestivum* L.
- To determine the does-dependent effects of invasive plants allelochemicals on *Triticum aestivum* L. germination.
- To investigate the allelopathic potential of selected invasive plants on *Triticum aestivum* L. germination.

CHAPTER 2

LITERATURE REVIEW

2.1 Allelopathic

Environmental degradation, such as acid deposition, has negatively impacted many locations in recent decades due to the continued expansion of human activity; this is especially dangerous in the three places with the worst acid rain on earth(Z. Zhang, 2005) (J.-E. Zhang et al., 2007). S. canadensis, one of the most infamous invasive species in China, had substantial allelopathic effects on the development of native species, and this trait was crucial to the invasion's success(Chen et al., 2005). Studies conducted in the past demonstrated that acid deposition increased the allelopathic potential of an invasive plant (Wedelia trilobata) and therefore promoted its invasiveness(Wang et al., 2012). According to the definition of allopathy, the direct negative or positive impact of one plant on another is caused by the chemical components that elude the surroundings(Brown et al., 1991). The word "allelopathy" was first used by Molisch (1937) to describe biochemical interactions between all plant kinds, including microorganisms. Today, allelopathy is recognized as more than just a trait that the plant kingdom has accumulated through the course of evolution(Putnam & Tang, 1986) Allelopathy is a very practical approach to weed control. Studies have shown Growing interest in plant allelopathy's ability to manage weeds in agroecosystems (Uludag et al., 2006). Allelochemicals' chemistry allows for direct or indirect weed management and can serve as Biological herbicides (Soltys et al., 2013). To get beyond the limitations of synthetic pesticides, alternative weed management solutions must be developed. Given the foregoing, allelopathy appears to be the most practicable weed management technique because it satisfies the requirements for eco-friendliness and is currently cost-effective in treating several weeds(Msafiri et al., 2013). Allelopathy occurs frequently in nature, and most alien plants can use its potential effects to their advantage to survive(Latif et al., 2017). The different allelochemicals found in plant shoots in the current investigation may be responsible for the aqueous extract of P. hysterophorus to impede germination and seedling growth(Oli et al., n.d.). Seed germination and seedling growth of B. pekinensis, L. sativa, and O. sativa have been examined after treatment with A. argyi powder extracts tossing toss the allelopathic effects of three different extracts of A. argyi. The outcomes demonstrated that the allelopathic Increases in inhibition were concentration-dependent(Li et al., 2021). Many different plant species produce secondary metabolites, some of which are essential in acting as defense mechanisms in the plant rhizosphere as allelopathic chemicals(Naeem et al., 2018). Two species of broomrape, P. ramosa and O. cumana, had their radicle development subjected to an examination of the allelopathic potential of buckwheat root exudates. The effects of buckwheat were compared to those of two sunflower cultivars, NR5 and P96, as well as the unfavorable control, GR24.(Fernández-Aparicio et al., 2021). Extract from Parthenium hysterophorus reduced the lengthening of C. iria's ’and radicle. With an increase in extract concentration, the degree of inhibition grew. A P. hysterophorus extract concentration of 50 g L-1 or higher decreased the coleoptile and Irian’s radicle length by 100% (Motmainna et al., 2021).

2.2 *Ageratum houstonianum L.*

Due to its blue inflorescence and thread-like florets, *Ageratum houstonianum* Mill. (Family: Asteraceae) is a perennial herb also known as "Floss Flower" or "Blue Mink?" In tropical and subtropical countries, it flourishes as a weed. On the biological characteristics of *A. houstonianum,* there is a dearth of information in the literature. Only a few data on the plant's antioxidant and antibacterial activities are known (Chandraker et al., 2020). There hasn't been any known metal-NP synthesis from this plant up to this point.

2.3 *Ageratum conyzoides L.*

Goat weed is the common name for *Ageratum conyzoides* L. It is a native of both North America and Central America. As a family member the non-woody plant family, Asteraceae, is widespread in tropic and subtropical regions of the world, including India(Okunade, 2002).

At a 20% leachate concentration, *Ageratum conyzoides* significantly reduced the germination of pea seeds in both kinds. There greatest inhibition in the 'Arpan variety's (39.6%) and 'Sapna' variety's (38.7%) seed germination at 40% leachate concentration(A. K. Singh, 2021).

Rice seed germination was greatly impacted by soaking in distilled water and weed extracts in water. The meanest maximum germination time (MGT) was discovered after soaking rice seeds in T. portulacastrum root and leaf extracts. However, rice seeds that were treated in distilled before seeding showed the shortest mean time to germination. Rice seeds were soaked in water extracts of D. Egyptian and E. indica's root, leaf, and entire plant in a manner comparable to but distinct from that of T. portulacastrum. In situations where seeds were pre-soaked in root and leaf extracts of T. portulacastrum before sowing, the maximum time required for 50% germination (T50) was discovered. In the treatment of distilled water, the minimum T50 was noted. When rice seeds were immersed in shoot and seed extracts from all the weeds, the outcomes were the same, but the same water extracts of T. portulacastrum were statistically similar to, but significantly different from, the root and leaf extracts of D. aegyptium and E. indica. The highest MGT and T50 values in the treatment where rice seeds were immersed in T. portulacastrum root extract suggest that there may be inhibitory chemicals in water extracts of T. portulacastrum roots that delayed the germination of rice seeds

(Mubeen et al., 2011a).

Extracts from fresh black mustard plant leaves, stems, flowers, roots, and combination solutions revealed inhibitory effects on seed germination. The degree of inhibition rose as extract concentration increased. All aqueous extracts significantly decreased seed germination as compared to distilled water control at the highest extract concentration (20 g kg1. Chung and Miller (1995) showed that the degree of inhibition increased with an increase in extract concentration, which supports this conclusion(Turk & Tawaha, 2003a).

The length of the wheat radicle was significantly reduced by all weed extracts at concentrations of 3 and 5%. Extracts from Ipomoea carnea, Alternanthera sessilis, and Malachra capital were most negatively impacted. The radicle growth was most impacted by ipomoea at all concentrations. However, all of the weed extracts showed a reduction in the length of the radicle. In practically all doses of weed extracts, the radicle's length shrank significantly(Joshi* & Joshi, 2016).

Throughout the Z. Mays germination and growth stages, the allelopathic effect of T. diversifolia was examined. A twofold effect of the allelochemicals in T. diversifolia was seen on this test species. Figure 1 displays the impact of T. diversifolia FSE on Zea mays radical length, plumule length, and percentage of germination. With time noticed gradual increases in germination in both the control and FSE-treated plants. The germination percentage differences between the FSE-treated and control plants were not found to be statistically significant ($p = 0.05$). Germination percentages for the FSE-treated and control plants were found to be 98.9 and 97.78%, respectively, after 14 days (Oyerinde et al., 2009).

Under Stress Allelopathic Potential. Peanut, redroot amaranth, ryegrass, and cucumber all experienced growth and development inhibition by volatiles from *A conyzoides.* Under varying nutritional conditions, plant development was considerably inhibited. competition from B. pilosa and a lack. There were no appreciable variations in allelopathic potential in response to nutrient deficiency (control), physical injury, or 2,4-D therapy. Under conditions of nutrient deficit and B. pilosa competition, the allelopathic potential of A. conyzoides L. was increased(Kong et al., 2002).

The maximum germination index (GI) was seen when rice seeds were soaked in distilled water, while the least GI was seen when rice seeds were immersed in T. portulacastrum extracts from the leaves, roots, and entire plant. Before sowing, rice seeds were treated with D. aegyptium seed and shoot extracts that were statistically distinct from one another. Pre-sowing soaking in E. indica root extract differed noticeably from all other water extracts of the same weed. D. aegyptium and E. indica's root and leaf extracts were identical but dissimilar from those of T. portulacastrum. No statistically significant differences existed between any of the weeds' shoot extracts. Rice seeds soaked with T. portulacastrum leaf extract and whole plant extract both significantly reduced the percentage of rice that germinated, whereas distilled water treatment resulted in a larger proportion. E. indica root and shoot extracts differed noticeably from one another and other extracts of the same plant. The lowest GI and germination percentage were seen after pre-sowing soaking in T. portulacastrum leaf extract, indicating the possible presence of allelochemicals that may have diminished the vigor of the rice seeds and the germination process. The minimal GI and germination % of rice when, when treated with Xanthium leaf leachate, was similarly reported by Tanveer et al. in 2008(Mubeen et al., 2011b).

The seeds of the test species completely failed to germinate in 10% aqueous extract, except Zea mays. *Triticum aestivum L.* did not germinate even at 6% and 8%. as well as *Ageratina adenophora*. The crucifer species (Raphanus sativus, Brassica campestris, and Brassica oleracea) showed severe germination inhibition; there was no germination at concentrations of more than 2%(Maharjan et al., 2007).

A. conyzoides' allelochemicals serve a variety of purposes and exhibit remarkable bioactivities when applied to insects. Allelochemicals' insecticidal activity may be one of the most crucial biological processes. The first is ageratochromene. Ageratum genus antiallatotropins isolated. Ageratochromene and its derivatives have hormonal effects that are antijuvenile. The use of *A. conyzoides* volatile oil on cowpea seed shows insecticidal action against weevils. At 2.5 to 10 l/9.5 g beans, significant oviposition deterrence and total prevention of adult insect emergence were visible, with no negative physiological consequences(Kong et al., 2004).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Study Area

The sub-metropolitan Nepalese city of Dharan is the subject of this thesis study. It is situated in the Sunsari region. Dharan is located at 26°47.745'N latitude and 87°18.512'E longitude on the northern slopes of the Mahabharat Range. The city stretches southward toward the Terai region, with an elevation of roughly 1148 feet (349 meters). Dharan, a city in Nepal's east, is known for its stunning green hills and lush woods, which are home to a wide variety of plants and animals. Tourists travel to the city from all across Nepal and other countries because of its natural beauty and rich cultural history. The diverse population of Dharan also contributes to the city's vibrant atmosphere by enhancing the social fabric with a tapestry of traditions and customs.

3.1.2 Map of Study Area

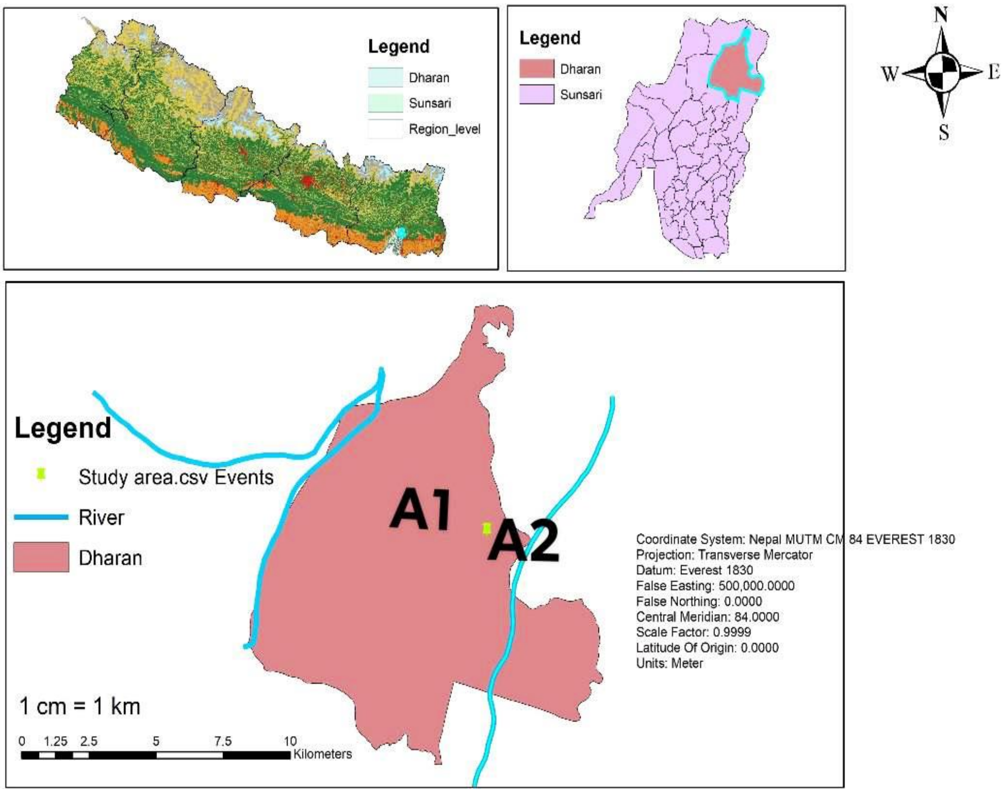

Figure 3: Map showing Study area: A1 *"Ageratum houstonianum"* and A2 "*Ageratum conyzoides*"

3.1.3 Climatic condition

Cool and dry with temperatures ranging from 10°C to 25°C (50°F to 77°F)

3.1.4 Equipment used

Various glass wares and electronic appliances were used and are listed in APPENDIX I.

3.2 METHODS:

3.2.1Data collection procedure and data analysis plan

The data and information gathered were subjected to quantitative analysis, which involves Processing, tabulating, and utilizing different statistical methods such as MS-Excel 2016, and IBM SPSS Statistics 25. The results were inferred and presented using tables and bar diagrams as necessary.

3.2.2 Collection of plants samples

On September 20, 2022, the Dharan hosted the performance of the current piece. *A. houstonianum* and *A. conyzoides* were gathered in their natural state for this experiment. and give them four weak to dry.

3.2.3 Preparation of Sample Extracts

The organic extraction was performed by using a cold percolation. Firstly, the leaves were washed with tap water and then with distilled water. The leaves were allowed to shade dry for a month at room temperature before being ground into a fine powder. 50 grams, 37.5 grams, 25 grams, and 12.5 grams of powdered plants each were combined with 500 ml of distilled water to create the aqueous extract, which was then left out at room temperature for 24 hours.

Another batch of mixed extracts was created by combining 6.25 + 6.25, 12.5 + 12.5, 18.75 + 18.75, and 25 + 25 g of powder of both the plant samples with 500 ml of distilled water, then letting it sit at room temperature for 24 hours. After filtering the plant extracts, different concentrations of the crude extracts—i.e., 2.5%, 5%, 7.5%, and 10% solutions were obtained. For *Ageratum houstonianum*, the treatments were categorized as Ah1(2.5%), Ah2(5%), Ah3(7.5%), and Ah4(10%), and for *A. conyzoides*, AC1(2.5%), Ac2(5%), Ac3(7.5%), and Ac4(10%). The plants treated with distilled water are represented by the control (CN).

3.2.4 Pre-treatment of *Triticum aestivum L.*

The pretreatment of the seed was done by rinsing them with distilled water and then sterilizing them by washing them in absolute ethanol. The sterilization process was repeated twice.

3.2.5 Process of Seed Germination

Sterile Petri plates were set on a laboratory table. Then sterilized filter paper was placed within the Petri plates. 10 pretreated seeds were placed on a Petri plate. This process was repeated for 45 sets of Petri plates. The seeds were left to germinate and then kept under observation for three days. After observing for three days, the number of germinated seeds was noted. The observation process was continued for 21 days. The aqueous plant extract was added in case the solution dried in each Petri plate. The temperature was also noticed each day.

CHAPTER 4

RESULTS AND DISCUSSION

Ageratum houstonianum L. and *Ageratum conyzoides L.* plants were gathered from the Dharan neighborhood. For a month, the plants were dried in a lab at room temperature. The plant extract was made using a cold percolation method in distilled water. Then an analysis of allelopathy was done.

4.1 Extraction of *Ageratum houstonianum*, *Ageratum conyzoides*, and mixed in different concentration

Ageratum houstonianum		*Ageratum conyzoides*		Mixed	
Extract (%)	Wt. of powder + Volume of water	Extract (%)	Wt. of powder + Volume of water	Extract (%)	Wt. of powder + Volume of water
2.5%	12.5gm+500 ml	2.5%	12.5gm+500 ml	2.5%	6.25gm+6.25gm+500ml
5%	25gm+500ml	5%	25gm+500ml	5%	12.5gm+12.5gm+500ml
7.5%	37.5gm+500 ml	7.5%	37.5gm+500 ml	7.5%	18.75gm+18.75gm+500 ml
10%	50gm+500ml	10%	50gm+500ml	10%	25gm+25gm+500ml

4.1.*1 Ageratum houstonianum* and *Ageratum conyzoides*

12.5gm of each powder was consumed together with 500 ml of distilled water .2.5% extract was produced. To make 5% of the extract, 25gm, and 500ml of distilled water were mixed with both powders. To make 7.5% of the extract, 37.5gm, 500ml of powder, and distilled water were combined. To make 10% of the extract, 50gm, 500ml of powder, and distilled water were combined. and 10 milliliters of pure, distilled water for the concentration.

4.1.3 Mixed Extract

By mixing 6.25g+6.25g and 500ml of distilled water, 2.5% of the extract was produced.500ml of distilled water and 12.5g+12.5g were combined to create 5% of the extract.7.5% of the extract was produced by combining 18.75 g+18.75 g with wit500 ml of distilled water. 500ml of distilled water and 25g+25g were combined to create 10% of the extract. for command 10 ml of pure water

The effect of whole plants (*Ageratum houstonium L.* and *Ageratum conyzoides L.* and mixed) on seed germination of *Triticum aestivum L.* is summarized in **(Table 1)**

Table 1: Number of growths of seedlings in average

Variety	Treatment (%)	0	3 days	6 days	9 days	12 days	15 days	18 days	21 days
Ageratum houstonianum	control	o	4	7	7.6	7.6	7.6	7.6	7.6
	2.5%	0	4.3	7.3	7.3	7.3	7.3	7.3	7.3
	5%	0	1	1.6	2.3	2.3	2.3	2.3	2.3
	7.5%	0	0	0.6	0.6	0.6	0.6	0.6	0.6
	10%	0	0.3	1.3	1.3	1.3	1.3	1.3	1.3
Ageratum conyzoides	control	0	4.6	7	7	7	7	7	7
	2.5%	o	4	7	7	7	7	7	7
	5%	0	0	0.6	0.6	0.6	0.6	0.6	0.6
	7.5%	0	0	0	0	0	0	0	0
	10%	0	0	0	0	0	0	0	0
mixed	Control	0	8.3	8.3	8.3	8.3	8.3	8.3	8.3
	2.5%	0	4.6	6.3	6.3	6.3	6.3	6.3	6.3
	5%	0	0	0	0	0	0	0	0
	7.5%	0	2.6	2.6	2.6	2.6	2.6	2.6	2.6
	10%	0	0.6	0.6	0.6	0.6	0.6	0.6	0.6

4.1.4. Ageratum houstonianum L.

On day 3, 4 seedlings, 7 seedlings, 9 seedlings, 7.6 seedlings, 12.7.6, and so on till day 21 on control on day 3, 4.3 seedlings were growing on day 6, 7.3 seedlings were growing on day 9, and the same thing continued on day 21 following the application of

2.5% extract. Following the application of 5% extract, 1 seedling began to grow on day 3, 1.6 seedlings on day 6, 2.3 seedlings on day 9, and the same thing continued up until day 21. After the 7.5% extract was applied, there were no seedlings on day 3, 0.6 seedlings on day 6, and nothing at all on day 21. Following the 10% extract application, 0.3 seedlings appeared on day 3, 1.3 seedlings appeared on day 6, and nothing appeared on day 21.

4.1.5. Ageratum conyzoides L.

On the control plant, there were 4.6 seedlings on day 3, 7 on day 6, and so on through day 21.4 seedlings appeared on day 3, 7 seedlings sprouted on day 6, and nothing appeared on day 21 after the application of the 2.5% extract. Following the application of the 5% extract, no seedlings grew on day 3, just 0.6 seedlings sprouted on day 6, and nothing occurred on day 21. On day 3, day 6, and day 21 following the application of the 7.5% extract, no seedlings developed. Following the application of the 10% extract, no seedlings appeared on days 3, 6, and 21.

4.1.6. Mixed extract

There were 8.3 seedlings on the control on day 3, 8.3 on day 6, and so on through day 21. There were 4.6 seedlings on the extract plant of 2.5% on day 3, 6.3 on day 6, and so on through day 21. On days three, six, and so on through day twenty-one, there were no seedlings on the 5% extract plant. On the 7.5% extract plant, there were 2.6 seedlings on day 3, 2.6 on day 6, and so on through day 21. There were 0.6 seedlings on the 10% extract plant on day 3, 0.6 on day 6, and so on through day 21.

The present study revealed that treatment of 2.5% extract inhibits the growth of seeds by mixed extract, *Ageratum conyzoides*, and then *Ageratum houstonianum*. Similarly revealed that treatment of 5% extract inhibits the growth of seeds by mixed extract, *Ageratum conyzoides*, and then *Ageratum houstonianum*. Similarly demonstrate that treating seeds produced by *Ageratum conyzoides*, mixed extract, and *Ageratum houstonium* with 7.5% extract reduces their growth. Studies show that 10% extract treatment of seeds from *Ageratum houstonianum*, combined extract, and *Ageratum conyzoides* decreases the growth of the seeds.

According to the author,(Khan, Muhammad Ayyaz, 2008) Alternanthera, which came from the five various doses of weed extracts, impacted the growth of the wheat plumule.

most. The length of the wheat plumule was significantly reduced in all of the weed extracts. shown that Eucalyptus camaldulensis L has an adverse influence on the development of wheat seedlings.

According to the author,(Ghodake et al., 2012) Wheat germination was examined for the allelopathic effect of Euphorbia species, and inhibition in Root-shoot length and germination rate. Wheat seed germination, radicle, plumule length, and biomass have all decreased.

According to the author,(Bora et al., 1999) discovered that some plant seeds' ability to germinate was inhibited by leaf extracts of Acacia auriculiformis The proportion of crops to extract concentration was obvious.

According to the author(Turk & Tawaha, 2003b), They claimed that leaf extracts from allelopathic plants generated the most inhibiting impact. How much of a reduction gradually increased as extract concentration went from 4 to 20 g. Leaf extract decreased germination to 26.0, 28.6, 36.7, 43.9, and 56.1% at the respective 4, 8, 12, 16, and 20 g kg1.

Table 2: ANOVA table of comparison of plant extract

Multiple Comparisons								
Dependent Variable				Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
shoot length	Tukey HSD	10%Ah	2.5% Ah	-17.5056*	1.16067	0.000	-20.8722	-14.1389
			5%Ah	-2.3056	1.16067	0.297	-5.6722	1.0611
			7.5%Ah	-3.2222	1.16067	0.066	-6.5889	0.1444
			Control	-14.2333*	1.16067	0.000	-17.6000	-10.8667
		2.5% Ah	10%Ah	17.5056*	1.16067	0.000	14.1389	20.8722
			5%Ah	15.2000*	1.16067	0.000	11.8333	18.5667
			7.5%Ah	14.2833*	1.16067	0.000	10.9167	17.6500
			Control	3.2722	1.16067	0.060	-0.0944	6.6389
		5%Ah	10%Ah	2.3056	1.16067	0.297	-1.0611	5.6722
			2.5% Ah	-15.2000*	1.16067	0.000	-18.5667	-11.8333
			7.5%Ah	-0.9167	1.16067	0.932	-4.2833	2.4500
			Control	-11.9278*	1.16067	0.000	-15.2944	-8.5611
		7.5%Ah	10%Ah	3.2222	1.16067	0.066	-0.1444	6.5889
			2.5% Ah	-14.2833*	1.16067	0.000	-17.6500	-10.9167
			5%Ah	0.9167	1.16067	0.932	-2.4500	4.2833
			Control	-11.0111*	1.16067	0.000	-14.3778	-7.6445
		Control	10%Ah	14.2333*	1.16067	0.000	10.8667	17.6000
			2.5% Ah	-3.2722	1.16067	0.060	-6.6389	0.0944
			5%Ah	11.9278*	1.16067	0.000	8.5611	15.2944
			7.5%Ah	11.0111*	1.16067	0.000	7.6445	14.3778
Root length	Tukey HSD	10%Ah	2.5% Ah	-8.8444*	1.15828	0.000	-12.2041	-5.4847
			5%Ah	-0.2611	1.15828	0.999	-3.6208	3.0986
			7.5%Ah	-0.3611	1.15828	0.998	-3.7208	2.9986
			Control	-10.3756*	1.15828	0.000	-13.7353	-7.0159
		2.5% Ah	10%Ah	8.8444*	1.15828	0.000	5.4847	12.2041
			5%Ah	8.5833*	1.15828	0.000	5.2236	11.9430
			7.5%Ah	8.4833*	1.15828	0.000	5.1236	11.8430
			Control	-1.5311	1.15828	0.680	-4.8908	1.8286
		5%Ah	10%Ah	0.2611	1.15828	0.999	-3.0986	3.6208
			2.5% Ah	-8.5833*	1.15828	0.000	-11.9430	-5.2236
			7.5%Ah	-0.1000	1.15828	1.000	-3.4597	3.2597
			Control	-10.1144*	1.15828	0.000	-13.4741	-6.7547
		7.5%Ah	10%Ah	0.3611	1.15828	0.998	-2.9986	3.7208
			2.5% Ah	-8.4833*	1.15828	0.000	-11.8430	-5.1236
			5%Ah	0.1000	1.15828	1.000	-3.2597	3.4597
			Control	-10.0144*	1.15828	0.000	-13.3741	-6.6547
		Control	10%Ah	10.3756*	1.15828	0.000	7.0159	13.7353
			2.5% Ah	1.5311	1.15828	0.680	-1.8286	4.8908
			5%Ah	10.1144*	1.15828	0.000	6.7547	13.4741
			7.5%Ah	10.0144*	1.15828	0.000	6.6547	13.3741

Based on observed means.
The error term is Mean Square (Error) = 6.037.

*. The mean difference is significant at the .05 level.

Table 3: Levene's test between extracts

Levene's Test of Equality of Error Variances					
		Levene Statistic	df1	df2	Sig.
shoot length	**Based on Mean**	**4.794**	**14**	**30**	**0.000**
	Based on Median	**0.862**	**14**	**30**	**0.603**
	Based on the Median and with adjusted df	**0.862**	**14**	**6.668**	**0.617**
	Based on trimmed mean	4.308	14	30	0.000
Root length	Based on Mean	6.621	14	30	0.000
	Based on Median	0.949	14	30	0.523
	Based on the Median and with adjusted df	0.949	14	7.308	0.559
	Based on trimmed mean	5.794	14	30	0.000
Tests the null hypothesis that the error variance of the dependent variable is equal across groups.					
a. Design: Intercept + Extract * Plant + Extract + Plant					

Levene's test variants depending on several measurements, including mean, median, trimmed mean, and adjusted degrees of freedom (df), are listed in the table. The significance level (Sig.) denotes the likelihood that the reported results were obtained by chance.

For shoot length:

- Based on Mean: The significance level is 0.000 (p 0.001) and the Levene statistic is 4.794 with 14 degrees of freedom (df1) and 30 degrees of freedom (df2). This shows that the error variance among the various extracts differs significantly.
- Based on Median: The significance level is 0.603 and the Levene statistic is 0.862. Based on the median measure, the test does not detect a significant difference in the error variance.
- Based on the Median and with adjusted df: With 14 df1 and 6.668 df2 and a significance level of 0.617, the Levene statistic is 0.862. When the presumption of equal variances is broken, the adjusted degrees of freedom are employed. Based on the median measure and adjusted df, the test does not detect a significant difference in the error variance in this instance.

- Based on trimmed mean: The significance level is 0.000, and the Levene statistic is 4.308. Based on the trimmed mean measure, the test detects a substantial difference in the error variance.

For root length:

- The Levene's test results for root length show substantial variations in error variance based on mean and trimmed mean measures, but no significant differences based on median measures. This pattern is similar to that for shoot length.

In conclusion, the Levene's test shows that there are differences between the different extracts in the error variances of shoot length and root length. This shows that not all extract groups have identical levels of these dependent variables' variability. "Intercept + Extract * Plant + Extract + Plant" is how the exact statistical model that was employed for the analysis is characterized.

Table 4 shows the average shoot length and root length. *Ageratum houstonianum*, *Ageratum conyzoides*, and combined extracts' allelopathic effects on seedling germination and growth were examined. 10% extract on *Ageratum houstonianum* demonstrates that no root germination was detected, and the shoot length was found to be minimal.7.5% at and 10% extract on *Ageratum conyzoides* demonstrates that no root germination and shoot germination. *Ageratum conyzoides* and *Ageratum houstonianum* combined at 5% extract show no sign of root or shoot germination.

Table 4: Showing root and shoot length average

variety	Treatment (%)	Root length	Shoot length
Ageratum houstonianum	control	9.32cm	15.5cm
	2.5%	7.7cm	19.7cm
	5%	0.7cm	6.3cm
	7.5%	0.23cm	2cm
	10%	0	1.1cm
Ageratum conyzoides	control	9cm	16cm
	2.5%	15.3cm	20.5cm
	5%	0.6cm	4.6cm
	7.5%	0	0
	10%	0	0
Mixed	control	13.3cm	15.65cm
	2.5%	4cm	16.45cm
	5%	0	0
	7.5%	1.3cm	11.7cm
	10%	0.5cm	3cm

Table 5: Comparision of root and shoot length

Levene's Test of Equality of Error Variances					
		Levene Statistic	**df1**	**df2**	**Sig.**
shoot length	**Based on Mean**	**4.794**	**14**	**30**	**0.000**
	Based on Median	**0.862**	**14**	**30**	**0.603**
	Based on the Median and with adjusted df	**0.862**	**14**	**6.668**	**0.617**
	Based on trimmed mean	4.308	14	30	0.000
Root length	Based on Mean	6.621	14	30	0.000
	Based on Median	0.949	14	30	0.523
	Based on the Median and with adjusted df	0.949	14	7.308	0.559
	Based on trimmed mean	5.794	14	30	0.000
Tests the null hypothesis that the error variance of the dependent variable is equal across groups.					
a. Design: Intercept + Extract * Plant + Extract + Plant					

According to ANOVA, there is a significant difference (mean) between the germination of the seed of *Triticum aestivum* between extracts Signiant of studied plants.

Table 6: Root and shoot length effect

Tests of Between-Subjects Effects						
Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	shoot length	2566.136[a]	14	183.295	30.236	0.000
	Root length	1205.116[b]	14	86.080	14.258	0.000
Intercept	shoot length	3509.484	1	3509.484	578.910	0.000
	Root length	773.602	1	773.602	128.139	0.000
Extract * Plant	shoot length	333.820	8	41.727	6.883	0.000
	Root length	222.533	8	27.817	4.608	0.001
Extract	shoot length	2222.727	4	555.682	91.663	0.000
	Root length	965.990	4	241.497	40.001	0.000
Plant	shoot length	9.590	2	4.795	0.791	0.463
	Root length	16.592	2	8.296	1.374	0.269
Error	shoot length	181.867	30	6.062		
	Root length	181.116	30	6.037		
Total	shoot length	6257.487	45			
	Root length	2159.834	45			
Corrected Total	shoot length	2748.003	44			
	Root length	1386.232	44			
a. R Squared = .934 (Adjusted R Squared = .903)						
b. R Squared = .869 (Adjusted R Squared = .808)						

Table 7:Tests of between effects of root and shoot length

Tests of Between-Subjects Effects						
Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	shoot length	2566.136[a]	14	183.295	30.236	0.000
	Root length	1205.116[b]	14	86.080	14.258	0.000
Intercept	shoot length	3509.484	1	3509.484	578.910	0.000
	Root length	773.602	1	773.602	128.139	0.000
Extract * Plant	shoot length	333.820	8	41.727	6.883	0.000
	Root length	222.533	8	27.817	4.608	0.001
Extract	shoot length	2222.727	4	555.682	91.663	0.000
	Root length	965.990	4	241.497	40.001	0.000
Plant	shoot length	9.590	2	4.795	0.791	0.463
	Root length	16.592	2	8.296	1.374	0.269
Error	shoot length	181.867	30	6.062		
	Root length	181.116	30	6.037		
Total	shoot length	6257.487	45			
	Root length	2159.834	45			
Corrected Total	shoot length	2748.003	44			
	Root length	1386.232	44			
a. R Squared = .934 (Adjusted R Squared = .903)						
b. R Squared = .869 (Adjusted R Squared = .808)						

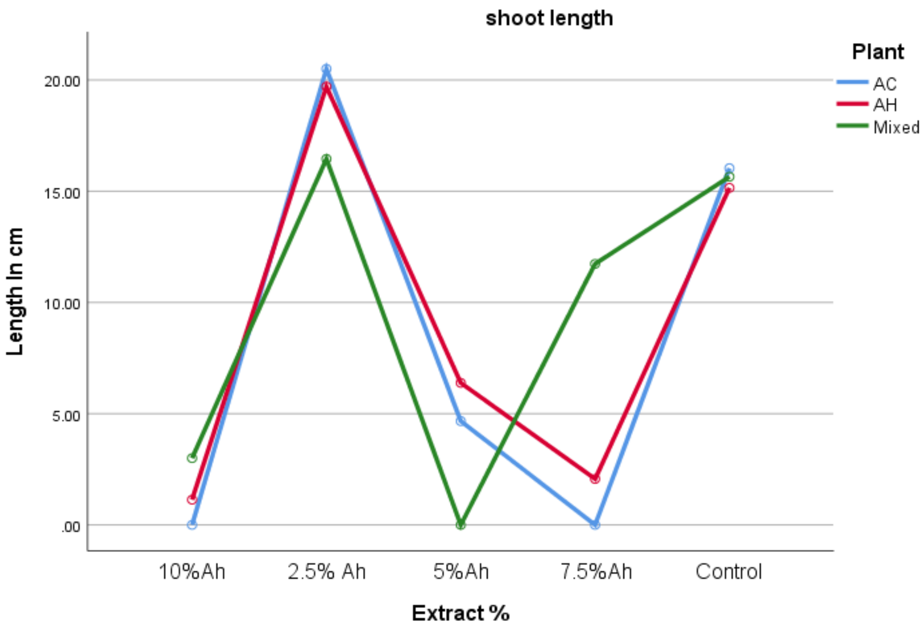

Figure 1: Showing length of shoot length of *Triticum aestivum*

Figure 1 shows the shoot length of Triticum aestivum in different extracts was found that at 2.5% extracts of all three extracts are effective for the growth of the root as it has less allelopathic effect.

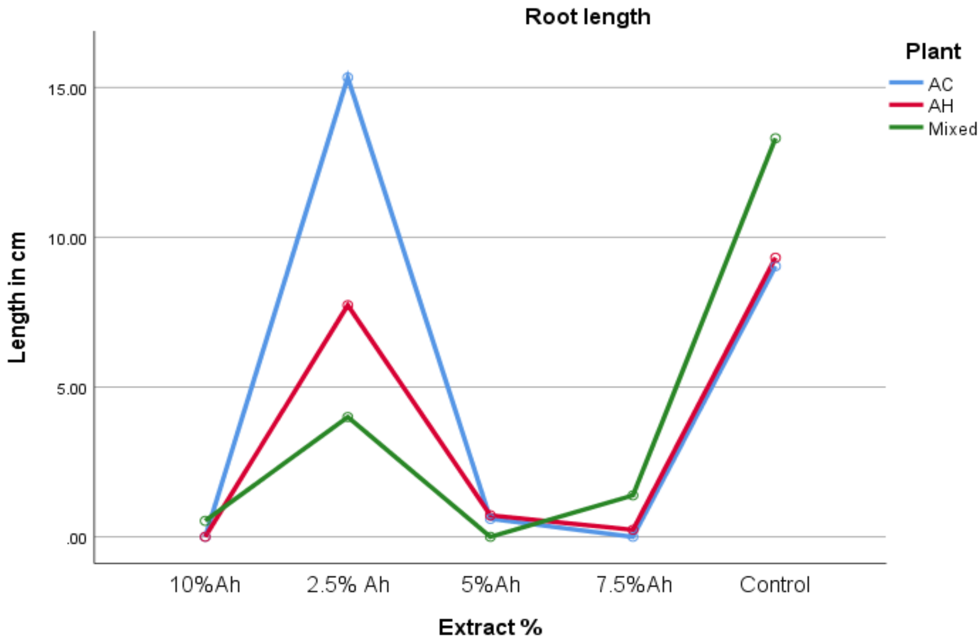

Figure 2: showing root length of *Triticum aestivum*

Figure 2 shows the relation between root length and the vantage of the extract. At 2.5% extract of all three extracts, the root length is found to be greater.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Allelopathy has been mentioned in numerous invasion case studies, but its prevalence and significance in plant communities are still little understood. The majority of the species we evaluated had some potential for allelopathy. There was no obvious pattern among the various living forms, however, that would allow one to foretell which invading species would be most likely to display allelopathy. We support the use of this or comparable techniques to screen and rank invasive species based on their relative allelopathic potential. (A. K. Singh, 2021)(Pisula & Meiners, 2010)

As a result, we were able to show that *Ageratum houstonianum*, *Ageratum conyzoides*, and mixed extract have detrimental impacts on crops, including lower seed germination and the emergence of *Triticum aestivum*. Allelopathic activity will differ when reseeding wheat depending on the quantity of wheat residue and the stage of growth when tillage occurs. In comparison to *Ageratum houstonium* and *Ageratum conyzoides*, the combined extract is considerably better.

5.2 Limitation

- Phytochemicals were not analyzed.
- The present work has been conducted for the partial fulfillment of the requirement for the bachelor’s degree in BSc botany. It was accomplished within an academic year. Hence depth study was not possible.

5.3 Recommendations

- Phytochemical analysis is recommended for further study.
- Study of the effect on other crops is suggested.

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APPENDIX I

Equipment

Physical apparatus	Specification
Electric balance	Phoenix instrument
Electric grinder	Crompton
Refrigerator	LG
Thermometer	-
Hot air oven	Faithful

Glassware	Specification
Beaker	Borosilicate
Measuring cylinder	Corning
Funnel	Borosilicate
Petri dish	-
Glass rod	-
Conical flask	Borosilicate

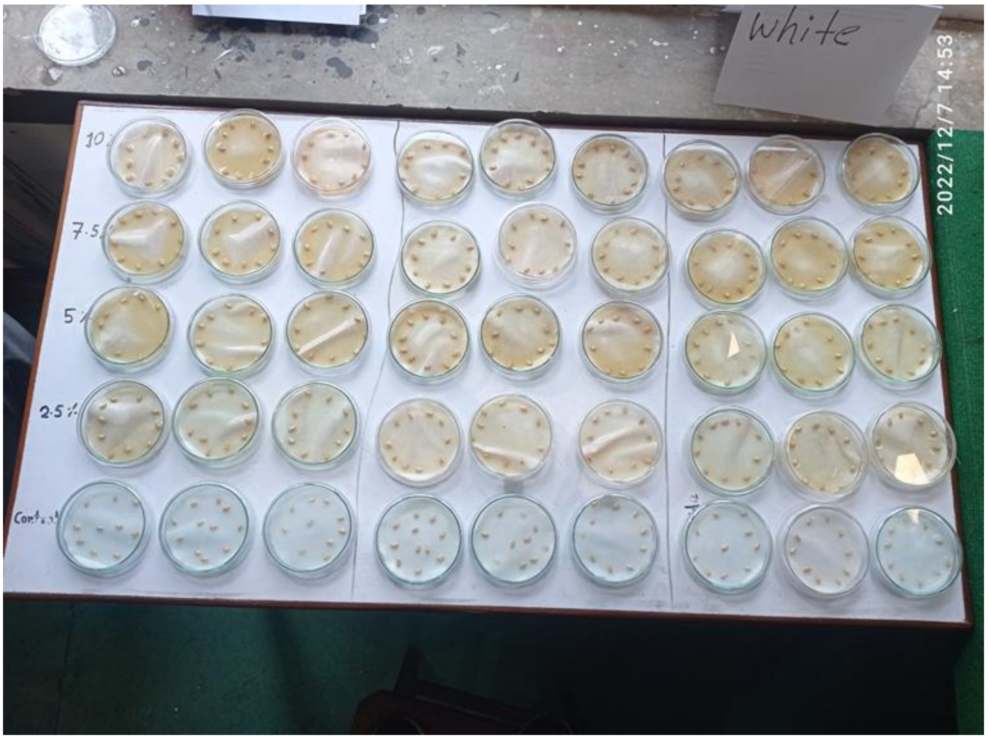
APPENDIX II

Photographs

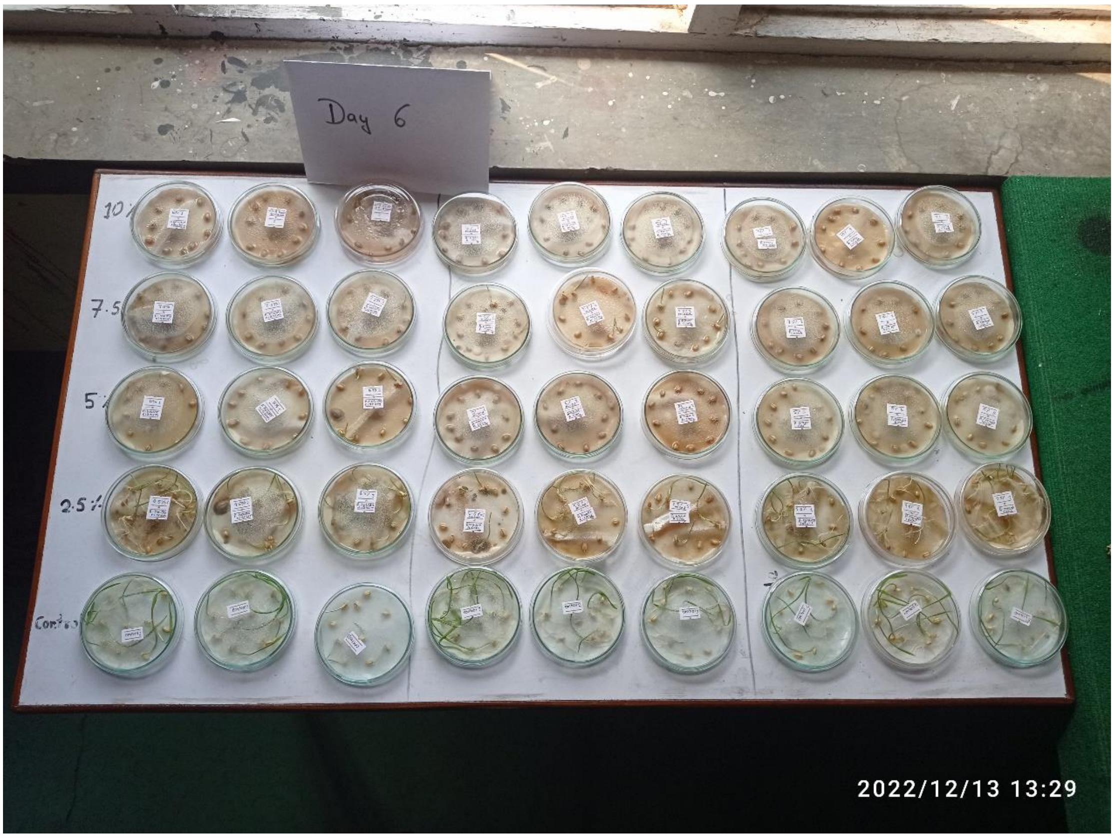
Photograph 1*: Ageratum houstonianum*

Photographs 2:Extract preparation of plant material

Photographs 3:Extracts



Photographs 4:Seed germination day 1



Photograph 5: Seed germination on day 6

Photographs 6:Seed germination on day 9

Photograph 7: Seed germination on day 12

Photographs 8: Germinated plant

Photographs 9: Measuring germinated root and shoot

Photographs 10:Measuring

Photographs 11:Measuring