Allelopathic Potential of Selected Weeds on Germination and Growth of Lettuce

By

Sidra Tul Ain

Department of Bioinformatics and Biotechnology

Faculty of Basic and Applied Sciences

International Islamic University Islamabad

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Researcher

Sidra Tul Ain

MSBT5-93

Supervisor
Dr. Shamim Akhtar
Assistant Professor

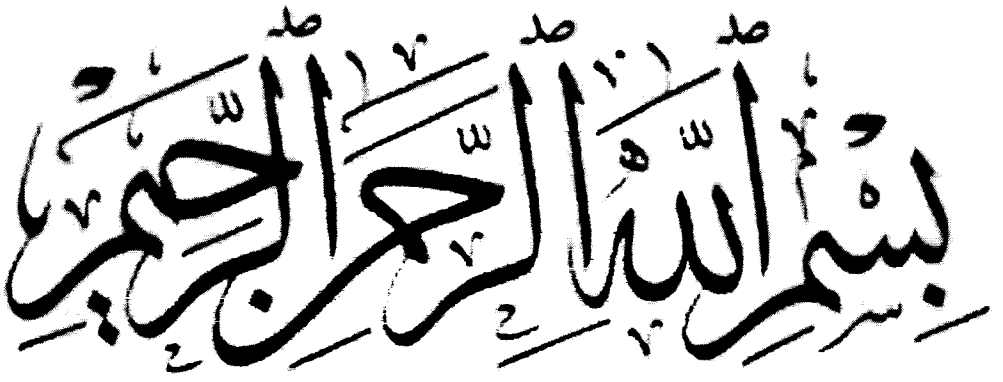
Co-Supervisor
Dr. Naveeda Riaz
Associate Professor

Department of Bioinformatics and Biotechnology

Faculty of Basic and Applied Sciences

International Islamic University Islamabad

(2015)



Department of Bioinformatics and Biotechnology

International Islamic University Islamabad

Dated: _____

FINAL APPROVAL

It is certificate that we have read the thesis submitted by Ms. Sidra Tul Ain and it is our judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the M.S Degree in Biotechnology.

COMMITTEE

External Examiner

Dr. Naazneen Bangash
Assistant Professor
COMSATS Islamabad

Internal Examiner

Dr. Sobia Tabassum
Assistant Professor
International Islamic University, Islamabad

Supervisor

Dr. Shamim Akhter
Assistant Professor
University of Gujrat

Co-Supervisor

Dr. Naveeda Riaz
Associate Professor
International Islamic University Islamabad

Chairperson

Dr. Asma Gul
Chairperson
International Islamic University Islamabad

Dean, FBAS

Dr. Arshad Zia
International Islamic University, Islamabad

A thesis submitted to Department of Bioinformatics and Biotechnology, International Islamic University, Islamabad as a partial fulfillment of requirement for the award of the Degree of MS Biotechnology

DEDICATION

This Humble effort is

Sincerely Dedicated to My

"Affectionate Parents"

Whose Love is More Precious

Whose Prayers will Never Die

And

To My Loving Husband and kids

DECLARATION

I hereby declare that the work present in the following thesis is my own effort, except where otherwise acknowledged and that the thesis is my own composition. No part of the thesis has been previously presented for any other degree.

Date 15-3-2019

Sidra Tul Ain

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ACKNOWLEDGEMENT

All praise is for **ALLAH** Lord of all worlds, the most Affectionate, the Merciful, who taught writing by the pen, taught man what he knew not. After the Almighty Allah, all praises and thanks are for the Holy Prophet Muhammad (PBUH) who is forever a model of guidance and knowledge for humanity.

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Last but not the least, I am not able to select the words to extend my heartfelt thanks for my loving Parents and husband who always prayed for my success and they helped a lot during my thesis research. May Allah bless them all (Ameen).

LIST OF TABLE

ABSTRACT

Allelopathy is an important aspect in plant interactions. It is the inhibitory or stimulatory effect of one plant species on another plant species by releasing certain chemicals. The research work was planned to check the allelopathic potential of Parthenium hysterophorus, Cannabis sativa, Euphorbia helioscopa, Lantana camara and Xanthium strumarium in the growth and activity of lettuce. Lettuce was used as a test plant due to its various properties including easy handling and growth conditions. Different methods including sandwich method, Aqueous extract method and soil incorporation methods were used to assess the allelopathic potential of different plant species against the lettuce. Maximum activity was reported with cannabis sativa by using sandwich method. The allelopathic activity was concentration dependant. Maximum activity was recorded with higher concentrations of Parthenium hysterophorus and cannabis sativa. The main aim of the present study of plants is to conduct research that can be used allelopathic plants as high value crops for farmers. This is a preliminary research using various screening methods to found plants with allelopathic potential. Once the bioactivity of the plant is established, it could be viable economic opportunities for the farmers to apply it as bio herb.

Chapter No. 1

INTRODUCTION

Allelopathy is the chemical inhibition or stimulation of microorganism or plant on other plants by releasing chemical compounds into the surroundings. This phenomenon maintains balance of different plant communities to natural ecosystems. It is characterized by the plants genetic during its growth and well known production of secondary metabolites. The concentration of compounds effects the inhibitory and stimulatory effects (Bhowmik, 2003). Allelopathy is well considered natural tool for biological weed control (Cheema and Khaliq, 2000). Allelochemicals develops many new tools which combat the evolution of resistance of herbicide in weeds (Anjum and Bajwa, 2005). Research on allelopathy was initiated in Pakistan in early seventies (Akhtar *et al.,* 2014). The allelopathic extent of inhibition on seedling growth and crops' germination differs from species of weed to weed (Mehmood *et al.,* 2014).

All around the world Plant breeders are devoting their resources and time for breeding to get better yield, resistance and adaptation against different diseases and pests. In fact, research prefers historical cultivars with better ability of weed suppression than modern cultivars (Lemerle *et al.,* 2001; Vandeleur and Gill, 2004; Wicks *et al.,* 2004; Wolfe *et al.,* 2008). It has been estimated that 581-650 plant species 12% of the flora) are endangered or threatened but also been suggested that this number would increase (Nasir, 1991). The plants' over-exploitation, habitat destruction, foundation/initiation of species of alien and pollution are the key causes for this danger.

Lettuce (*Lactuca sativa*) is a herbaceous plant of family Asteraceae, main and most various of flowering plant family. Main weeds that interferes with lettuce includes common purslane (*Portula caoleracea*), barnyard grass (*Echinochloa colonum*), smooth pigweed (*Amaranthus hybridus*), common lambsquaters (*Chenopodium album*) and shepherd's purse (*Capsella bursa-pastoris*) (Santos *et al.*, 2003; Haar and Fennimore, 2003; Fennimore and Umeda, 2003).

Parthenium has some allelopathic chemicals which are water soluble such as parthenin and a lactone of pseudo guanolide nature in different parts of the weed. The allelopathic nature of *Parthenium* shown by many researchers (Kohli *et al.*, 2006 ; Singh *et al.*, 2003). Parthenin, a sesquiterpene lactone, is the active secondary metabolite of *Parthenium* (Hernández *et al.*, 2011). *Parthenium* in higher concentration is also harmful for humans and animals health (Evans, 1997).

Cannabis sativa also contain the well-known include thujone menthol, terpenoids cannabinoids, and camphor (Ameh *et al.*, 2010). Bonner (1950), developed and reinforced the thought of allelopathy Evenari (1961) Among the worst weeds *Lantana camara* is considered as one of the worst specie of the world (Ranalli, 1999). Evaluation of allelopathic activity of *Parthenium hysterophorus*L, *Lantana camara, Xanthium strumarium, Cannabis sativa* L and *Euphorbia helioscopa* on lettuce (*Lactuca sativa* L.) germination and growth that could be further used in agroecosystem, is the key aim of the study which has been going on.

Objectives of Study

- To analyze the allopathic potential of different weeds on germination and growth of lettuce
- To evaluate various screening methods to find plants with allelopathic potential
- To screen out bioactivity of the plants and their economic opportunities for the farmers

Chapter No. 2

REVIEW OF LITERATURE

Aqueous extracts of *Xanthium strumarium* reduced early growth, germination, and *Lactuca sativa*'s dry weight, *Pennisetum americanum* and *Brassica compestris*. *Parthenium hysterophours* is either well known Allelopathic plant. Residues and extracts of these plants significantly reduced the weight of root and shoot dry and germination of various plants (Inam *et al.*, 1987). Biradar *et al.*, (2006) described that the high level of N, K and P in *Parthenium* compost. *Parthenium* compost contains at least two times more phosphorus nitrogen, potassium, and then farm yard manure. High concentration of macro and micro nutrients in composted *Parthenium* may increase crop production (Kishor *et al.*, 2010).

Parthenium's leaves and flowers aqueous extracts inhibited germination of seeds and caused complete failure of germination of seeds of teff when the leaf extract concentration of *Parthenium* weed was 10% (Tefera, 2002). In India, 90% reduction in forage production and yield drop of 40% in agricultural crops has been reported due to allelopathic effect of *Parthenium* (Singh *et al.*, 2003). In Ethiopia, sorghum grain yield was decreased from 40 to 90% due to both competition effect and allelopathic effect of *Parthenium* in the field (Tamado *et al.*, 2002).

The allelopathic actiity of medicinal plants was observed on the growth and germination of lettuce by Anjum *et al.* (2010). *B. papyrifera and Albezzia lebbeck* proved inhibitory effects on the hypocotyls and radicle growth by using sandwich method, when lettuce was used as a test plant. Despite, stimulatory effects at 5mg leaves concentrations, *P. hysterophours* reduced the growth and germination of lettuce. Among various parts screened, leaves showed more Allelopathic response towards the germination and growth of *Phalaris*

minor and Avena fatua (Anjum *et al.,* 2010; Aslam *et al.,* 2014). Higher concentrations of *Digeria muricata* significantly affected the germination of other test species (Aziz and Shaukat, 2014).

Cannabis sativa can fit well to crop rotation to control pests and not related to conventional food crops (Ranalli, 1999), so evaluation of allelopathic activity *Cannabis sativa L.* on lettuce becomes the important ambition of study. Different protocols are used for allelopathic assays. These assays include Sandwich (Fujii *et al.*, 2003). The aqueous extracts were tested individually on three selected weed species. The aqueous extracts of both the leaf and seed of *Leucaena leucocephala* inhibit germination and was concentration dependent. Rice germenination is delayed by aqueous extracts prepared from different plant parts of *Alternanthera* species. *Alternanthera philoxeroides* and *A. sessilis* inhibited rice germination by 9-100% and 4-49%, respectively. Rice seed germination was decreased by increasing concentration of aqueous leaf extracts of both weed species (Mehmood *et al.,* 2014).

To study the allelopathic effects of the aqueous extract of the leaf and seed of *Leucaena leucocephala* a laboratory experiment was conducted. The aqueous extracts were individually tested on three selected species of weed. The allelopathic effects of the leaf and seed extracts on shoot length, germination, root length and fresh weight of each of the selected weed species were determined (Ishak *et al.,* 2014).

The negative effects of leaf tissue and extracts of *Conocarpus lancifolius* in the germination, the growth traits, biochemical and physiological processes of bean and corn plants. Corn (monocot) was much more tolerant to the leaf extract than bean (dicot) plant. The inhibitory effects were correlated with increased concentration of leaf extract. In 10% leaf extract, there was no seed germination in both crops. Thirty-one compounds were detected in the leaf extract and most of them were phenolic compounds. The allelopathic effects of the leaf

extract with regards to germination were associated with inhibition by the seeds as well as the solute potential of extracts (Al Shatti *et al.,* 2014).

Fuji *et al.,* 2003 stated that 239 medicinal plant species were screened for allelopathic activity using the Sandwich method and using Lettuce as a test plant. Out of 239 species tested, 223 species caused inhibitory and 17 caused promoted responses to lettuce radical growth, respectively. The results presented could be used as an information for further research on the elucidation of chemicals involved in allelopathy phenomenon in nature. Such information could help future researchers to isolate new and potent bioactive chemicals from natural products.

For allelopathic effect *Lantana camara* was screened on (water hyacinth). When 4% aqueous leachate (w/v) of *L. camara* were tested, length of water hyacinth was retarded. Seedlings died after 22 days under the experimental conditions. Different concentrations of *Lantana* used, leachate concentrations from 1-4% were highly toxic to *Eichhornia crassipes* plants. Young *Lantana* twigs was more poisonous as compared to the leachate from mature twigs (Saxena, 2000).

Studies investigate the allelopathic effect of stem, leaf, root, and water extracts of fruits and infested soil of *Euphorbia helioscopia* L. on the germination of seeds and seedling growth of wheat, lentil chickpea were conducted in a completely randomized way with 4 replications. Water extracts prepared by soaking of stem, root, leaf, and fruit.

Dried parts of plant *E. helioscopia* in water (1:20 w/v) for a time period of 24 h. Seedling emergence, seedling vigor index, and total dry weight of chickpea, wheat, and lentil seedlings were reduced significantly when these crops were grown in soil taken from an *E. helioscopia* infested field compared to soil collected from an area free of any vegetation. *E. helioscopia* infested soil also significantly decreased the root length of lentil, and wheat and shoot length of lentil as compared to the control soil. Water extracts of various organs of *E.*

helioscopia significantly decreased the seedling growth of test crops and vigor index. Leaf extract had shown a greater inhibitory effect than the other extracts of plant organs. Water extracts from the stem, root, leaf, and fruit of *E. helioscopia* resulted in a reduction in the germination of seeds (chickpea and lentil only) and germination index but the leaf extract increased the mean germination time in all test crops.

To check the allelopathic effects of *Xanthium strumarium* it was determined under the laboratory conditions on *Parthenium hysterophorus*. Leaf extracts of *Xanthium* reduced the length of *Parthenium*. Germination suppressions in *P. hysterophorus* and reductions in the root, shoot length and plant dry weights of the weed were higher with 26% *X. strumarium* leaf extract treatment (Sinha and Singh, 2004).

Chapter No. 3

MATERIALS AND METHODS

All the research work was carried out at Applied Biotechnology and Genetic Engineering lab (ABGE) Department of Biotechnology and Bioinformatics, International Islamic University Islamabad.

3.1 Materials

3.1.1 Plant Material

Fresh leaves and leaf debris of *Parthenium hysterophorus*, *Lantana camara, Xanthium strumarium, Cannabis sativa* and *Euphorbia helioscopia,* were collected from various localities of Wah Cant. The collected material was screened for the allelopathic potential, using lettuce as a test plants. Dried leaves were subjected to Sandwich method and Aqueous extract method. Soil incorporation method was used to determine the effect of leaf debris on the growth of test plant in field like conditions.

3.2 Equipment

3.2.1 Glassware

Culture tubes (25 x 150 mm), Petri plates and beakers (Pyrex, Germany), and conical flasks (100 ml) were used as glassware for the media preparation and socking of seeds on sandwich and aqueous method.

3.2.2 Machinery

Drying oven (DHG-9053A), Electronic balance (shimadzu), Microwave oven (Orient), Shaker (HY-4 speed adjusting multipurpose vibrator).

3.3 Methodology

3.3.1 Sandwich Method

Sandwich method was used to investigate leaching substances from leaves

3.3.2 Agar Media Preparation

Agar powder was used as a growth meida. Thegelling temperature of agar media was 30-31°C. Agar solution (0.75%w/v) was prepared an autoclaved at 121°C for15 minutes. Three different concentrations of leaves (5, 10 and 50mg) were placed in multi-dishes (10 cm^2 area per each dish) in triplicate (Fujii *et al.*, 2003).

3.3.3 Surface Sterilization of Seeds

Mature *Parthenium hysterophorus, Lantana camara, Xanthium strumarium, Cannabis sativa* and *Euphorbia helioscopa,* seeds were surface sterilized by 1% sodium hypochlorite solution used by thorough washing with distal water.

3.3.4 Growth Conditions

Growth conditions including concentration, exposure of seeds to sterilizing agent, pH of media, temperature of growth room, photoperiod and light intensity were optimized.

3.3.5 Seedling Production

Surf sterilized seeds were placed in multidishes. Agar medium was added to each treatment, as a result dried plant material was rise up. The agar medium was solidified in each replication at room temperature. Again 5 ml of agar medium was added to each replication of treatment and solidified. Five lettuce seeds were placed in each replication of treatment. All petri plates were sealed and were placed in plastic box having moistened filter paper at the bottom of each petri plate. Petri plates were covered with aluminum foil and incubated in complete darkness at room temperature for 72 h. Radicle and hypocotyls elongation of seedlings was determined and percentage of elongation was calculated.

3.3.6 Radical elongation and Hypocotyl elongation

Five plants were taken from each treatment. Plants were washed at the end of experiment and were divided into radical and hypocotyl. After that radical and hypocotyl length of each plant was measured.

3.3.7 Aqueous extract method

This method was used to determine the solubility of allelochemicals in water.

3.3.8 Oven dried leaves

Oven-dried leaves (10g) were soaked in 100ml water in a flask and agitated for 24 h on an orbital shaker (150rpm) at room temperature. The extract was strained through two layers of cheese cloth and then two layers of Whatmann's filter paper.

3.3.9 Seedling Production

Five lettuce seeds were placed in each glass petri dish containing filter paper. The test solution (2.5ml) of different concentrations (five) was poured in each Petri dish. In control Petri dishes, distilled water was used. All Petri dishes were incubated at room temperature for 72 h. (Nasir *et al.*, 2005).

3.3.10 Soil Incorporation Method

This method was used to determine the effect of leaf debris on the growth of test plant under field-like conditions.

3.3.11 Preparation of Pots

The mixture of soil+ sand (300g, 64 % sand and 36% soil) each was mixed with 0.16%, 1.14% and 2.3% of dried leaves. In control seeds were sown in the mixture of sand + soil. Twenty lettuce seeds were sown in each pot. The experiment was run in replica of three in green house. Pots were watered daily. Emergence was recorded after 4 days of planting and after 7 days, each pot was thinned to the five healthiest plants. All plants were harvested after

2 weeks and the soil was carefully washed from the roots. Radicle, hypocotyl length and fresh and dry weight of roots was measured (Nasir *et al.,* 2005).

3.3.12 Root, Shoot length

From each treatment, 3 plants were harvested. At the end of experiment, plants were divided into root and shoot after washing. Root and shoot length of each plant was measured.

3.3.13 Fresh weight

To measure fresh weight, plants were uprooted at the end of the experiment.

3.3.14 Dry weight

Separated roots and shoots after recording fresh weights, were placed in oven at 70°C for 72 hour and then reweighed to estimate their dry mass.

Following treatments were used

Treatments	Concentration's
T0	Well-watered control lettuce
T1	Leuttce + 5mg (*Parthenium hysterophorus*, *Cannabis sativa, Euphorbia helioscopia,*, *Lantana camara,*and *Xanthium strumarium)*
T2	Leuttce + 10mg (*Parthenium hysterophorus*, *Lantana camara, Xanthium strumarium, Cannabis sativa* and *Euphorbia helioscopia,)*
T3	Leuttce + 50mg *(Parthenium hysterophorus*, *Lantana camara, Xanthium strumarium, Cannabis sativa* and *Euphorbia helioscopia)*

3.4 Data Analysis

All the data was analysed statistically by two factorial Analysis of Variance (ANOVA) using Statistix software version 9.0 at 5% level of significance.

Chapter No. 4

RESULTS AND DISCUSSION

4.1 SANDWICH METHOD

Application of allopathic *Parthenium hysterophorus*, *Lantana camara*, *Xanthium strumarium*, *Cannabis sativa*, *Euphorbia helioscopia*, shows significant results in inhibition of lettuce seeds as compared to control seeds. The effect of Allelopathy was determined by three different (sandwich, aqueous extract, and incorporation of leaf material in to soil) methods using CRD (Completely Randomized Design).

4.1.1 Radical length

4.1.2 Percentage length

Result revealed that a great decrease in radicle lengths was recorded when allelopathic activity of *Parthenium hysterophorus* was investigated. Inhibition rate was concentration dependent. Maximum inhibition was recorded at 50 mg leaf leachates extracts. At 5 and 10 mg leaf leachates 45 and 21% lengths were recorded (Table 1). Radicle length of lettuce under the effect of 5mg concentration of *Parthenium hysterophorus* were reduced up to 40-60% (Rao &Mamta, 2013).

Cannabis sativa radical lengths of *Lettuce* were also concentration dependent (Table 1). Significant decrease in radicle length was recorded at higher concentrations. No germination was observed at higher concentration (Table 1). Percentage inhibition was 100% at higher concentration (Table 1), *C. sativa also* resulted in 69% plumule inhibition at 5mg leaves concentration (Shiraishi *et al.*, 2005). The inhibitory effect of different concentrations of extract of *C. sativa* on seed germination might be due to imbalance in metabolism controlled by various enzyme activities (Oyun, 2006).

Low concentration when *Euphorbia helioscopia* were shown Stimulatory effects on an experimental species (Table 1). Percentage length was 101% at 5 mg leachate concentrations (Table 1). However, inhibitory activities were shown at higher concentrations that are 89 activities and 53% activity at 10 and 50 mg leaf respectively (Table 1).

There was a tremendous decrease in radical lengths of *Lettuce was recorded* when *Lantana camara* was screened for its allelopathic activity (Table 1). The rate of reduction was very high when contrasted to control, however, there was slight decrease in radicle length with increased in concentration that is 74, 53 and 46% (Table 1).

All concentrations of *Xanthium strumarium* resulted an inhibitory activity. Maximum inhibition was recorded at 50 mg leaf leachates. Percentage lengths of radicle were 53, 27 and 9% at different concentrations when *Xanthium* was screened for its allelopathic activity (Table 1). The results are in accordance with the findings of (Fujii *et al.*, 2003). About 239 medicinal plants of different families were screened for their allelopathic potential by sandwich method, 223 species resulted in inhibitory effects towards the *Lettuce* seedlings while 17 species were stimulatory in response. With 10 mg or 50 mg concentration of leaves radicle length inhibition was more than 80% in 19 species, about 60-79% in 16 species, 40-59% in 43 species and 20-39% in 72 species. In remaining 73 species length inhibition was 0.3- 19% (Fujii *et al.*,2003).

Table 1: Length of radical of *Lettuce* (%) under the effect of leaves of different species by sandwich method

Species	Control	5mg	10mg	50mg
	Length (%)	Length (%)	Length (%)	Length (%)
Parthenium hysterphorus	100	45	21	15
Cannabis sativa	100	26	21	0
Euphorbia helioscopia	100	101	89	53
Lantana camara	100	74	53	46
Xanthium strumarium	100	53	27	9

4.1.3 Comparative analysis of weed species

In terms of radicle length all the species with their concentrations used were significantly different ($P<0.05$) (Table 2). Maximum length was recorded in *Euphorbia helioscopia* (22 mm), while the lowest length was recorded in Cannabis that is 5.3 mm. So the maximum inhibition was showed Cannabis that showed its strong allelopathic potential. Radicle lengths of other species were 14.3 (Lantana), 13.3 (Parthenium), and 10.65 mm in Xanthium (Table 2).

4.1.4 Comparative analysis of treatments

All the treatments were also significantly different in terms of radicle length ($P<0.05$). Maximum length was recorded in Control treatment that is (17.5 mm), whereas minimum length was recorded in 50 mg leaves (6.22 mm). So the results revealed that Radicle length was decreased with an increased in concentration (Table 2). The results were agreed with the result of Fujii et al., 2004. At 50mg concentration of leaves of *Cymbopogon citrates* and *Derris scandens* and showed the maximum inhibition a gainst Lettuce seedling length by sandwich method (Fujii et al., 2004).

4.1.5 Comparative analysis of interaction

A significant interaction was recorded between concentrations and species ranging from Zero mg (Cannabis, 50 mg) to 29 mm (Parthenium, control) (Table2).

Among all experiments species radicle length was concentration dependent. Minimum length was recorded at maximum concentration (50 mg) while Maximum length was recorded with control treatment. *Euphorbia helioscopia* shows Stimulatory effects at 5 mg leaf extract as compared to control of other species, but radicle length was decreased at higher concentrations (Table 2). In other species reduction was increased with an increased in

concentration (Table 2). Pervez et al., (2003) reported strong allelopathic effect of *Tamarinda indica* leaf leachates on a range of weeds and crops by sandwich method.

Table 2: ANOVA table for radical length (mm) of *Lettuce* seedlings under the effect of different weeds by sandwich method

Treatment	Species Means					
	Partheniu m	***Cannabis***	***Euphorbia***	***Lantana***	***Xanthium***	**Means**
Control	29 A	14.3 Ef	25.3 Bc	21 b-d	19.3 d	17.5A
5mg	13.3 Gh	3.8 k-m	25.6 B	15.6 Gh	13 hi	14,26 B
10mg	6.3 Jk	3.1 k-m	22.6 Cd	11.3 Gh	7 j	10.06C
50mg	4.6 j-l	0.00 M	13.6 Gh	9.6 Hi	3.3 lm	6.22 D
Means	13.3 C	5.3 E	22 A	14.3 B	10.65 D	

*Any two means carrying the same letter(s) in a column or row are non- significantly different at P=0.05 by Duncan's Multiple Range Test

4.2 Hypocotyl length

4.2.1 Percentage length

Under the effect of *Parthenium hysterophorus* the rate of inhibition was increased. The percentage length of hypocotyl was increased by increasing in concentration that is 50mg (Table 1). Percentage length of hypocotyl was 72% when 5mg leaf leachates were used, but a gradual decrease in percentage length was noticed at higher concentrations that are 53 and 46% respectively (Table 3). The radical and hypocotyl length was observed lower than the control. Parthenium residues have inhibitory effects on the growth and germination of lettuce (Akhtar et al., 2014) .Extract from the shoot parts of *C. sativa* has a higher inhibitory effect to lettuce germination and seedling growth than extract from roots (Akhtar et al., 2014).

Allelopathic action *Cannabis sativa* was active in, as at concentration there is no germination of test plant (Table 3) Percentage length were 42 and 28% at 5mg and 10 mg leaf extract (Table.3). Results revealed that *Euphorbia helioscopia* was less active as compared to other species. *Euphorbia helioscopia* shown Stimulatory effects at low concentration of leaf extract (5mg leaf extract). Percentage hypocotyl length was 86% However, reduction in hypocotyl length was observed at higher concentration (10mg leaf extract). Percentage length was 66% at 50mg leaf extract (Table 3).

Inhibitory action was recorded in case of *Lantana camara* it retarded the hypocotyl length of lettuce plant at different concentrations. The percentage lengths at different concentrations were 84, 66 and 54% respectively (Table 3).

Xanthium strumarium also shown high rate of inhibition with different concentrations that is 5,10 and 50mg (Table 3). Percentage lengths were 33, 25 and 5% respectively when 5, 10 and 50 mg leaves leachates were used (Table 3).

4.2.2 Comparative analysis of different species

All the species and their concentrations used were significantly different ($P<0.05$) in terms of hypocotyl length (Table 4). Maximum hypocotyl length was recorded in *Euphorbia* (14 mm), whereas lowest hypocotyl length was recorded in *Cannabis* (3 mm).

The seedling length was minimum udder the active action of *Cannabis.* At maximum concentration germination of *Lettuce* seedlings was stopped. Among other species including *Parthenium, Euphorbia, Lantana* and *Xanthium* allelopathic activity was recorded with 9.6, 14, 12.68 and 6.05 mm respectively. In case of *Parthenium, Lettuce* seedlings showed decline in hypocotyl length (Table 4). The results were in agreement with the findings of Fujii *et al.*, 2003. While screening 239 medicinal plants for their allelopathic activity 100% hypocotyl length inhibition of *Lettuce* seedlings was recorded in two species belonging to family Fabaceae and Myrtaceae.

4.2.3 Comparative analysis of treatments

Treatments also resulted in significant difference. Control treatment showed maximum length 15mm, while 50 mg leaves showed minimum length (5mm) (Table 4). So a tremendous decrease in hypocotyl length was recorded with increasing concentration (Table 4).

4.2.4 Comparative analysis of interaction

A significant interaction was recorded between concentrations and species ranging from Zero Mm *Cannabis* in 50 mg leaves) to 15 mm (control for *euphorbia)* (Table 4). When hypocotyl length in control was compared to the length of hypocotyl under different treatments, reduction in length was recorded with increased in concentration.

Maximum inhibition was recorded at higher concentration (Table 4). Incase if *Euphorbia* hypocotyl length was increased at 5 mg concentration of leaf extract, when

compared to control (Table 4). However, hypocotyl length was decreased with increased in concentration. In other species inhibition of plumule length was concentration dependent, where maximum inhibition was recorded at 50 mg leaf extract concentration (Table 4).

Table 3: Length of Hypocotyl of *Lettuce* (%) under the effect of leaves of different species by sandwich method

Species	Control	5mg	10mg	50mg
	Length (%)	Length (%)	Length (%)	Length (%)
Parthenium hysterphorus	100	72	41	26
Cannabis Sativa	100	42	28	0
Euphorbia helioscopia	100	86	73	66
Lantana camara	100	84	66	5.4
Xanthium strumarium	100	33	25	.5

Table 4: ANOVA table for hypocotyl length (mm) of *Lettuce* seedlings under the effect of different weeds by sandwich method

Species Means						
Treatment	*Parthenium*	*Cannabis*	*Euphorbia*	*Lantana*	*Xanthium*	**Means**
Control	16 a-c	7 Jk	15 c-f	16.6 ab	13.6 c-f	17.05 A
5mg	11.60 e-i	3 l-n	15 b-d	14 c-e	4.6 Lm	11.5B
10mg	6.6 K	2 m-o	14 c-f	11 f-i	2.5 Lm	8.2 C
50mg	4.3 L	0.00 O	12 e-h	9 h-j	5 O	5.95D

*Any two means carrying the same letter(s) in a column or row are non- significantly different at P=0.05 by Duncan's Multiple Range Test

4.3 AQUEOUS EXTRACT METHOD

4.3.1 Radicle Length

4.3.2 Percentage length

An overall reduction in percentage length was recorded at different concentrations. Reduction rate was more pronounced at higher concentration.

Results raveled that the allelopathic effect of *Parthenium hysterophorus* was most active as compared to other species, where no germination was observed at higher concentration (Table 5). Percentage lengths of radicle of *Lettuce* were 73, 21, 11, 5.5 and zero% at 2, 10, 20, 30 and 40% aqueous extracts of *Parthenium.* (Table 5). The aqueous extracts of *C. sativa* and *Parthenium hysterophorus* were highly effective in reducing germination *growth* of lettuce. Growth was significantly reduced when 50mg aqueous extract concentration was used. While this reduction is more pronounced in case of *Parthenium hysterophorus*as compared to *C. sativa.* Radicle length of lettuce under the effect of 5mg concentration of *C. sativa* and *Parthenium hysterophorus* were reduced up to 40-60% (Rao &Mamta, 2013).

The inhibitory effect of cannabis sativa in radical length of leuttce showed in the (table 5). Percentage lengths were 78, 72 and 11% at 2, 10 and 20% extract respectively. No growth was recorded at 30 and 40% aqueous extract (Table 5).

When *Euphorbia helioscopia* was screened for its allelopathic activity, high concentration shows maximum inhibition. Percentage lengths were 82, 76, 66, 52 and 51% at 2, 10, 20, 30 and 40% respectively (Table 5).

The result reveled *Lantana camara* showed inhibitory effects towards the radicle length of *Lettuce*. Percentage lengths were 73, 54, 47, 39 and 35% at 2, 10, 20, 30 and 40% aqueous concentrations respectively (Table 5).

Apllicaton of aqueous extracts of *Xanthium strumarium* were showed to, rate of inhibition was increased with increased in concentration (Table 5). 2% extract *Xanthium strumarium* of showed stimulatory effect that the Percentage length was 109%. Percentage lengths with other concentrations were 96, 93, 72 and 51% at 10, 20, 30 and 40% aqueous extract concentrations (Table 5). Aqueous extracts of *Xanthium strumarium* caused inhibitory effects towards *Lettuce* (Inam *et al.*, 1987). Five different aqueous extract concentrations of leaves of *Tetrapleura tetraptera* were tested against *Lycopersicon esculantum, Abelmoschus esculantum, Amaranthus spinosus* and *Solanum melongena* (Amoo *et al.*, 2008). The root length of all tested species was significantly reduced at higher extract concentrations (Amoo *et al.*, 2008). *Capsicum annum* resulted in significant inhibitions at all treatments of aqueous extracts of *Tetrapleura tetraptera* applied (Amoo *et al.*, 2008). The level of inhibition was in direct proportion to the concentration (Amoo *et al.*, 2008).

4.3.3 Comparative analysis of species

All the species and their concentrations used were significantly different ($P<0.05$) in terms of radicle length (Table 6). *Parthenium* showed minimum radicle length (5.7mm). Radicle length was 9.65 in *cannabis*, 12.2 in *Euphorbia*, 13.4 mm in *Lantana*, and12.5 in case of *Xanthium.* The results are in accordance with those of Singh *et al.*, 2003. They examin that extracts prepared from burnt residues of *Parthenium* inhibited seedling length and dry weight of *Raphanus sativus* (Radish) and *Cicer arietinum*(chick pea).

4.3.4 Comparative analysis of treatments

Treatments resulted in significant difference. The maximum radicle length was recorded in control treatment (19.1mm) while the lowest radicle length (4.84 mm) was observed in higher concentration that is 40% aqueous extract material (Table 6). The radicle length was concentration dependent. Radicle length was 15.72, 12.06, 8.2, 4.8 and 5.9 mm at

2, 10, 20, 30 and 40% aqueous extract (Table 6). High concentrations of aqueous extract of *Parthenium hysterophorus* resulted in higher decrease of seedling lengths of all tested species (Sajjan *et al.*, 1997).

4.3.5 Comparative analysis of interaction

A significant interaction was observed between species and treatments it is ranging from zero mm (*Cannabis* at 30% and 40% aqueous extract concentration and *Parthenium* at 40% concentration) to 23 mm in *Lantana* control (Table 6). Along with other species allelopathic activity was depend on concentration. Maximum inhibition was recorded at 40% aqueous concentration (Table 6). At 2% aqueous extract *Xanthium* proved to be stimulatory. Among other species radicle length was concentration dependent. *Xanthium* at higher concentration radicle length was decreased (Table 6).

Table 5: Length of radicle of *Lettuce* (%) under the effect of leaves of different species by aqueous extracts method

Plant species	Treatments (aqueous extracts)					
	T1 (control)	T2 (2%)	T3 (10%)	T4 (15 %)	T5 (20 %)	T6 (40%)
	Length (%)	**Length (%)**	**Length (%)**	**Length (%)**	**Length (%)**	**Length (%)**
Parthenium hysterphorus	100	73	21	11	5.5	0
Cannabis sativa	100	78	72	11	0	0
Euphoria helioscopia	100	82	76	66	52	51
Lantana camara	100	73	54	47	39	35
Xanthium strumarium	100	109	96	93	72	51

Table 6: ANOVA table for radicle length (mm) of *Lettuce* seedlings under the effect of different concentrations of weeds by aqueous extract method

Treatment	Species Means					
	Partheni um	*Cannabis*	*Euphorbi a*	*Lantana*	*Xanthium*	**Means**
Control	18 b-e	22 Ab	17 c-g	23 ab	15.5 d-i	19.1A
2%	13.3 j-p	17.3 b-f	14 e-k	17 c-g	17 c-h	15.72 B
10%	3.8 Q	16 c-h	13 g-n	12.5 h-o	15 e-j	12.06 C
20%	2.1 Q	2.6 Q	11.5 k-p	11 i-p	14 e-l	8.2 D
30%	1 Q	0.0 Q	9 m-p	9 n-p	10.8 k-p	5.96 E
40%	0.0 Q	0.0 Q	8.8 n-p	8.2 op	7.2 op	4.84 E
Means	5.7 AB	9.65 C	12.2B	13.4 AB	12.05AB	

*Any two means carrying the same letter(s) in a column or row are non- significantly different at P=0.05 by Duncan's Multiple Range Test

4.4 Hypocotyl Length

4.4.1 Percentage length

Under the effect of aqueous concentrations of different species, percentage length of *Lettuce*, was calculated and compared to control. As concentration was increased a tremendous increase in inhibition was recorded (Table 7). Five different concentrations 2, 10, 20, 30 and 40% of stock solution were used.

Results showed that *Parthenium hysterophorus* is well known for its growth inhibitory action. Its allelopathic nature where 81% length was recorded at 2% extract concentration. Percentage lengths were 30, 21and 1.8% respectively at 2, 10, 20 and 30% aqueous extract. No germination was recorded at 40% extract (Table 7).

Cannabis sativa showed strong allelopathic action at higher concentration. Germination of test plant was stopped at maximum concentrations and also decreased than control (Table 5). Hypocotyl's percentage length was 77, 64 and 15% at 2, 10 and 20% aqueous extract and it was decreased with increased in concentration. Root and shoot lengths of *Lycoris radiata* leaves completely inhibited of test species in Aqueous extract of at higher concentration.

Allelopathic activity of *Euphorbia helioscopia* was less as compared to the other weeds. It shows 93,87, 85, 84 and 78% When 2, 10, 20, 30 and 40% aqueous extract concentrations were used. (Table 7).

Small decrease in percentage length was recorded when *Lantana camara* was used as an experimental plant. But, rate of inhibition was increased with increased in concentration. Recorded Percentage length activity was 85% at minimum concentration (2% aqueous extract), 79% at 10% extract, 70% at 20% extract, 50% at 30% extract and percentage length was 30% at 40% aqueous extract concentration (Table 7). The results are in accordance to the findings of Ismail and Chong (2002). They examine that the germination Chinese cabbage and tomato reduced at higher concentrations of the aqueous extract of *Mikania*.

Xanthium strumarium showed length inhibitory effects on test plant (Table 7). Percentage lengths were 75, 68, 67, 61 and 45% at 2, 10, 20, 30 and 40% aqueous extracts (Table 7). Similar results are found as made by Iqbal *et al.*, 2004.That *Ophiopogon japonicus* leaves showed negative effects on seed germination of Timothy (*Phelum pretense*), Alfalfa (*Medicago sativa*), and *Lettuce* (*Lactuca sativa*).

4.4.2 Comparative analysis of species

All the species and their concentrations used were significantly different ($P<0.05$) in terms of hypocotyl length (Table 8). Among the species, the highest length (14.1 mm) was recorded for *Euphorbia*, while the lowest length (6.4 mm) was recorded for *Parthenium* and *cannabis* (Table 8). Among other species hypocotyl lengths were 6.4, 14.1, 11.5, and 11.13, in *Cannabis, Eucalyptus, Lantana*, and *Xanthium* respectively (Table 8).

4.4.3 Comparative analysis of treatments

The treatments were also significantly different. The maximum hypocotyl length (15.84 mm) was observed for the control treatment, while the lowest length (5.3 mm) was recorded for 40% aqueous extract used as treatment. These lengths were concentration dependent. At 2% extracts 13.12 mm length was recorded. The hypocotyls lengths were 12.55, 10.46 and 6.5 mm at 10, 20 and 30% aqueous extract concentrations (Table 8).

4.4.4 Comparative analysis of interaction

A significant interaction was recorded between species and treatments, that is ranging from zero mm (*Cannabis* and *Parthenium* at 40% aqueous extract concentration and *Cannabis* at 30% concentration) to 16 mm (*Euphorbia* in control treatment) (Table 8). Among all species showed inhibitory effects. Hypocotyl lengths depend upon the concentrations of species. As the concentration was increased length inhibition was also increased (Table 8).

Table 7: Length of hypocotyle of Lettuce (%) under the effect of leaves of different species by aqueous extract method

Plant species	Treatments (Aqueous extracts)					
	T1 (control)	T2 (2%)	T3 (10%)	T4 (20 %)	T5 (30 %)	T6 (40%)
	Length (%)	**Length (%)**	**Length (%)**	**Length (%)**	**Length (%)**	**Length (%)**
Parthenium hysterphorus	100	81	30	21	1.8	0
Cannabis sativa	100	77	64	15	0	0
Euphorbia helioscopia	100	93	87	85	84	78
Lantana camara	100	85	79	70	50	30
Xanthium strumarium	100	75	68	67	61	45

Table 8: ANOVA table for plumule length (mm) of *Lettuce* seedlings under the effect of various concentrations of weeds by aqueous extract method

Treatments (Aqueous extract)	Species Means					
	Parthenium	*Cannabis*	*Euphorbia*	*Lantana*	*Xanthium*	**Means**
Control	16.3 a-h	15 d-m	16 a-i	16 c-m	15.9 b-k	15.84 A
2%	13.3 f-n	11.6 g-o	15 c-l	13.7 f-n	12 i-o	13.12 B
10%	5 q-s	9.6 l-p	14 d-m	12.7 g-o	11 j-p	12.55 C
20%	3.5 r-t	2.2 st	13.7 f-n	11.2 j-p	10.8 m-p	10.46 D
30%	0.33 T	0.0 t	13.6 e-n	8.7 o-q	9.9 n-q	6.5 E
40%	0.00 T	0.0 t	12.5 g-o	7 p-r	7.2 Pq	5.3D
Means	6.4 D	6.4 D	14.1 B	11.55 C	11 13 C	

INCORPORATION OF LEAF MATERIAL IN TO SOIL

4.5 Root Length

4.5.1 Percentage length

Percentage length of root was concentration dependent. Highest inhibition was recorded by *Cannabis* where reduction in length had led to cessation of length at higher concentration (Table 9).

Parthenium hysterophorus showed high root inhibition. That maximum inhibition was recorded at 6.9 g leaf material (Table 9). Percentage lengths of root of *Lettuce* were 55, 41 and 12% at 1.8, 3.4 and 6.9 g of leaf material (Table 9).

In case of *Cannabis sativa* percentage lengths of root were 88, 0 and zero%. That the root lengths were decreased with increased in the concentration (Table 9).

Euphorbia helioscopia showed stimulatory effect at 1.8g concentration. Percentage length of root of *Lettuce* was 113% as compared to control. At 3.4 g leaf material 100% length was recorded. Percentage length was 95% at 6.9 g concentration of leaf material (Table 9).

Results revealed that the inhibitory effect of *Lantana* towards the length of *Lettuce* root. Lengths were 79, 70 and 62% at different concentrations as compared to control (Table 9). Length inhibition of tested species were noticed when *Lantana camara* use as allelopathic agent (Sahid and Sugau, 1993).

Xanthium strumarium was also allelopathically active. Percentage lengths were 89, 84 and 56% respectively (Table 9). Different the test species showed emergence sensitivity towards the different concentrations of dried leaf tissues of *L. radiata* in to soil. High concentrations resulted no emergence (Iqbal *et al.*, 2006).

4.5.2 Comparative analysis of species

All the species and their concentrations used were significantly different ($P<0.05$) in terms of root length (Table 10). The highest root length was recorded for the *Euphorbia* (23.5 mm), whereas *Cannabis* proved to be most inhibitory with 11.75 mm root length (Table 10). Root lengths were 15.1, 18.7, and 20.6for *Parthenium*, *Lantana* and *Xanthium* respectively (Table 10).

4.5.3 Comparative analysis of treatments

All the treatments used were significantly different at $P<0.05$. The highest root length was recorded for control treatment (25.2 mm), while the minimum length was recorded (11 mm). By incrementing concentration, root length decreased (Table 10).

4.5.4 Comparative analysis of interaction

A significant interaction was recorded between species and the treatments used to range from 0mm (*Cannabis,* 6.9g) to 29 mm (*Parthenium*, control) (Table 10).

Table 9: Length of root of *Lettuce* (%) under the effect of leaves of different species by incorporating leaf material in to soil

Species	Control	1.8g Leaf material	3.4g Leaf material	6.9g Leaf material
	Length (%)	Length (%)	Length (%)	Length (%)
Parthenium hysterphorus	100	55	41	12
Cannabis sativa	100	88	0	0
Euphorbia helioscopia	100	113	100	95
Lantana camara	100	79	70	62
Xanthium strumarium	100	89	84	56

Table 10: ANOVA table for root length (mm) of *Lettuce* seedlings under the effect of various concentrations of various weeds by incorporation of leaf material in to soil

Treatments	Species Means					
	Partheni um	*Cannabis*	*Euphorbi a*	*Lantana*	*Xanthium*	**Means**
Control	29 c-e	25 b-e	23 b-d	24 b-d	25 a-c	25.2 A
1.8g leaf material	16 h-j	22 c-e	26 Ab	19 d-g	22 b-e	19.6B
3.4g leaf material	12 Jk	0.0 l	23 b-d	17 e-i	21.5 c-e	14.7 C
6.9g leaf material	3.5 L	0.0 l	22 c-f	15 f-j	14 ij	11 D
Means	15.125 D	11.75 D	23.5 A	18.75 B	20.62 B	

*Any two means carrying the same letter(s) in a column or row are non- significantly different at P=0.05 by Duncan's Multiple Range Test

4.6 Shoot Length

4.6.1 Percentage length

An overall decrease in percentage length was recorded when concentration of leaf material was increased.

The activity of *Parthenium hysterophorus* proved to be inhibitory towards the length of shoot of *Lettuce*. Percentage lengths were 83, 70 and 29% at 1.8, 3.4 and 6.9g material respectively (Table 11).

Cannabis sativa also seemed to be nearly inhibitory towards the shoot length of *Lettuce*. Percentage length was 77% (23% inhibition) at 1.8 g leaf material concentration, when compared to control (Table 11). At 3.4 g it show100% inhibition (0% length) was recorded. At same time no percentage length was recorded at 6.9 g leaf material, therefore 100% inhibitions (Table 11). The leaf material of *O. japonicus* in to soil also resulted maximam length inhibition of alfalfa at all applied concentrations (Iqbal *et al.*, 2004).

Percentage lengths in case of *Euphorbia helioscopia* were 70, 59 and 59% at 1.8, 3.4 and 6.9 g leaf material as compared to control (Table 11).

Leaf material of *Lantana camara* was also very inhibitory towards the length of test plant. Rate of inhibition was concentration dependent. Percentage lengths of shoot were 88, 60 and 59% with increased concentrations as compared to control (Table 11).

Xanthium strumarium had shown maximum inhibition at high concentration. Percentage lengths were 87, 76 and 61% at different concentrations when compared to control (Table 11). The results were similar to the findings of Nasir *et al.*, 2005. Chinese cabbage's root and shoot length was restricted to the different concentrations of *Robinia pseudo-acacia.*

4.6.2 Comparative analysis of species

All the species and their concentrations used were significantly different ($P<0.05$) in terms of shoot length (Table 12). The maximum shoot length was recorded in *Euphorbia* (27mm) control, while the minimum length was recorded in *Cannabis* (0 mm) 6.9g (Table 12).

4.6.3 Comparative analysis of treatments

All the treatments used were significantly different in terms of shoot length ($P<0.05$). the maximum length of shoot was recorded for control treatment 20.4 mm, while the lowest length was recorded for 6.9 g a leaf that is 9.5 mm (Table 12).

4.6.4 Comparative analysis of interaction

All the species and their concentrations used were significantly different ranging from zero mm (*Cannabis*, 6.9g) to 27mm (*euphorbia* control). Amongst all the species, drop in shoot length was recorded at different concentrations (Table 12). Reduction in shoot length of *Lettuce* was concentration dependent. Maximum inhibition was found when 6.9 g leaf material was used as treatment with all the species. Maximum shoot lengths were recorded in control treatment, however minimum length was recorded at maximum concentration, therefore maximum allelopathic activity was recorded at 6.9 g leaf material concentration (Table 12).

Table 11: Length of shoot of *Lettuce* (%) under the effect of leaves of different species by incorporation of leaf material in to soil

Species	Control	1.8g Leaf material	3.4g Leaf material	6.9g Leaf material
	Length (%)	Length (%)	Length (%)	Length (%)
Parthenium hysterphorus	100	83	70	29
Cannabis sativa	100	77	0	0
Euphorbia helioscopia	100	70	59	59
Lantana camara	100	88	60	59
Xanthium strumarium	100	87	76	61

Table 12: ANOVA table for shoot length (mm) of *Lettuce* seedlings under the effect of various concentrations of different weeds by incorporation of leaf material in to soil

Treatments	Species Means					
	Partheniu m	*Cannabis*	*Euphorbi a*	*Lantana*	*Xanthium*	**Means**
Control	15.5 a-d	15 g-l	27 A	25 ab	19.5 c-g	20.4 A
1.8g leaf material	11 c-f	12 k-n	19 d-i	22 a-d	17 e-j	16.2 B
3.4g leaf material	11 e-k	0.0 p	16 f-l	15.5 g-l	15 f-l	11.5 C
6.9g leaf material	4.5 O	0.00 p	16 f-l	15 g-l	12 l-n	9.5 D
Means	10.5 C	6.75 E	19.5 B	19.37B	15.87 C	

4.6.5 Root fresh and dry weights

Root fresh weights were recorded in milligrams

4.6.6 Percentage activity

Fresh weights showed 100% activity in control. When compared to control so there was a gradual decrease in fresh weights of *Lettuce* roots under different treatments. *Cannabis* proved to be inhibitory as it leaf material at higher concentration completely retarded the root fresh and dry weights (Table 13and 14).

Maximum inhibitory activity was shown by *Cannabis sativa* in terms of root fresh weights. Root fresh weight was 52% at 1.8 g leaf material. No fresh weight was recorded with 3.4 and 6.9 g leaf material concentration. This showed 48, 100 and 100% inhibitory activity. Root dry weights also resulted in inhibitory activity. That was 36% at 1.8 g leaf material. However, zero% concentrations dry weight activity was recorded at higher (Tables 15 and 16). Incorporation of leaf material of *Mikania micrantha* resulted in reduction in height and seedling fresh weights (Ismail and Mah, 1993).

Euphorbia helioscopia had shown 100% fresh weight activity at 1.8 g leaf material. Root dry weight activity was 105% as compared to control that was 100%. Activity of root fresh weights was 100 and 93% though activity of dry weight was increased at 3.4g leaf material as compared to control. Percentage activity of dry weight was 62% at 6.9g of leaf material, therefore 38% inhibitions (Table 15 and 16).

Lantana camara also showed inhibitory effect in terms of root fresh and dry weight activity. When compared to control percentage activity of root fresh weight was 80, 76 and 64% at 1.8, 3.4 and 6.9g leaf material. Percentage activity of dry weights was 75, 55 and 38% respectively. (Tables 13 and 15).

That means 7, 65 and 39% inhibition. Percentage activity of root dry weights was 52, 33 and 35% respectively as compared to control. Percentage inhibitions were 48, 52 and 65% respectively (Table 13 and 15).

There was a reduction in root fresh and dry weights of *Lettuce* seedlings when *xanthium* applied as a test plant compared to control. Percentage activity among different species was concentration had shown (Tables 13 and 15). Percentage activity was 83% at 1.8g leaf material, 75% inhibition was recorded at 3.4 g of leaf material (25% inhibition) and 41% activity was recorded at 6.9g leaf material (59% inhibition). Percentage activities of root dry weights were 83, 72 and 50% respectively (Tables 13 and 15).

4.6.7 Comparative analysis of different species

All the species and their concentrations used were significantly different ($P<0.05$) in terms of root fresh weight (Table 14). The maximum fresh weight of root was recorded in case of *Euphorbia* 5.1 mg), while minimum fresh weight was recorded in *Parthenium* (1.92 mg). Among other species root fresh weights were 3.6,4, and 4.5, mg in case of, Cannabis, *Lantana* and *xanthium* respectively (Table 14).

All the species and their concentrations used were significantly different ($P<0.05$) in terms of root dry weights (Table 16). The maximum dry weight was recorded for *Euphorbia* (3.45 mg), whereas minimum dry weight was recorded for *Parthenium* (0.35 mg). Among other species root dry weights were 0.8, 1.25 and 1.26mg in *Cannabis*, *Euphorbia*, *Lantana*, and *Xanthium* respectively (table 16).

4.6.8 Comparative analysis of different treatments

All the treatments used were significantly different. The maximum fresh weight was recorded in control treatment (5.9 mg), while minimum fresh weight was shown by 6.9 mg concentration (1.9 mg). All the treatments used were significantly different from each other.

The maximum dry weight was recorded with control treatment (2 mg) while minimum dry weight was recorded for 6.9 g (.91mg) (Table 16).

4.6.9 Comparative analysis of interaction

A significant interaction was recorded between species and their concentrations in terms of root fresh weight ranging from zero mg (*Cannabis*, 6.9g) to 3.5mg (*euphorbia* control) (Table 16). Same sequence observed in dry weight. In current research the results revealed that the application of lower concentratin of weeds shows less inhibitory effect on fresh and dry weight of the lettuce seedling. Similar result were notice that *C. sativa* and *Parthenium hysterophorus*. At 5mg leaves have strong inhibitory effects on the root and hypocotyls growth of lettuce. (Anjum *et al.*, 2010).

It can be concluded from the present study that *Parthenium hysterophours* and *Xanthium strumarium* have strong Allelopathic potential and can be further checked for their herbicide potential.

Table 13: Fresh weights of roots of *Lettuce* (%) under the effect of leaves of different species by incorporating leaf material in to soil

Species	Control	1.8g Leaf material	3.4g Leaf material	6.9g Leaf material
	Activity (%)	Activity (%)	Activity (%)	Activity (%)
Parthenium hysterphorus	100	73	35	20
Cannabis sativa	100	52	0	0
Euphorbia helioscopia	100	100	93	62
Lantana camara	100	80	76	64
Xanthium strumarium	100	83	75	41

Table 14: ANOVA table for fresh weights (mg) of roots under the effect of various concentrations of different invader species by incorporation of leaf material in to soil

Treatment	Species Means					
	Parthenium	*Cannabis*	*Euphorbia*	*Lantana*	*Xanthium*	**Means**
Control	3.4 e-k	9.5 bc	5.8 d-g	5 e-j	6 e-i	5.9 A
1.8g leaf material	2.5 i-o	5 f-m	5.8 d-f	4 g-o	5 e-i	4.46 B
3.4g leaf material	1.2 m-q	0.0 q	5.4 e-h	3.8 h-o	4.5 f-l	2.9 C
6.9g leaf material	0.6 o-q	0.0 q	3.6 g-n	3.2 i-o	2.5 j-p	1.98 D
Means	1.925 EF	3.62 DE	5.15 B	4.00CD	4.50 C	

*Any 2 means carrying the same letter(s) in a column or row are non- significantly different at P=0.05 by Duncan's Multiple Range Test

Table 15: Dry weights of roots of *Lettuce* (%age) under the effect of leaves of various species by incorporating leaf material in to soil

Species	Control	1.8g Leaf material	3.4g Leaf material	6.9g Leaf material
	Activity (%)	Activity (%)	Activity (%)	Activity (%)
Parthenium hysterphorus	100	37	23	12
Cannabis sativa	100	36	0	0
Euphorbia helioscopia	100	105	102	85
Lantana camara	100	75	55	38
Xanthium strumarium	100	83	72	50

Table 16: ANOVA table for dry weights (mg) of roots under the effect of various concentrations of different invader species by incorporation of leaf material in to soil

Treatment	Species Means					
	Parthenium	*Cannabis*	*Euphorbia*	*Lantana*	*Xanthium*	**Means**
Control	0.8 e-j	1.8 Ab	3.5 c-i	1.8 c-f	1.8 c-g	2 A
1.8g leaf material	0.3 Ij	1.5 c-h	3.7 c-i	1.5 d-j	1.5 c-h	1.7 B
3.4g leaf material	0.2 Ij	0.0 J	3.6 c-i	1 e-j	0.99 d-j	1.158 BC
6.9g leaf material	0.1 Ij	0.0 J	3 e-j	0.7 f-j	0.75 e-j	0.91 C
Means	0.35 C	0.825 B	3.45 B	1.25 B	1.26 B	

*Any two means carrying the same letter(s) in a column or row are non- significantly different at P=0.05 by Duncan's Multiple Range Test

Chapter No. 5

SUMMARY

For evaluation of the allelopathic effect of weeds on seedling growth of lettuce, experiments were performed at Applied Biotechnology & Genetic Engineering lab (ABGE) Department of Biotechnology & Bioinformatics, International Islamic University Islamabad. The seeds of lettuce got from National Agriculture Research center (NARC), Islamabad. There are 3 different concentrations (5mg. 10mg and 50mM) of weeds (*Parthenium hysterophorus*, *Cannabis sativa, Euphorbia helioscopia, Lantana Camara* and *Xanthium strumarium)* were applied on lettuce seeds by using three different methods i.e sandwich method, aqueous extract method and soil incorporation method. Seeds growth and biomass of seedlings significantly reduced under weeds effect.

The results of Sandwich method concluded that *Cannabis sativa* had strong Allelopathic activity at all concentrations. Significant decline in hypocotyl and radicle length was observed at all concentrations as compared to control between different species. Rate of inhibition was concentration dependent. However, *Euphorbia helioscopia* was unable to show significant allelopathic activity. *Euphorbia helioscopia* showed stimulatory effects at low concentration.

Significant allelopathic activity was also recorded in aqueous extract method, it showed that *Cannabis sativa* and *Parthenium hysterophorus* had strong allelopathic activity as compared to other weeds. At higher concentration of aqueous extract of these species 100% allelopathic activity was recorded. *Xanthium strumarium* showed stimulatory effects at minimum concentration of extract. However, allelopathic activity increased with increased in concentration.

In incorporating leaf material in to soil it was concluded that significant reduction was recorded by all species at all concentrations except *Euphorbia* at 1.8g of leaves concentration. *Cannabis sativa* was most active in allelopathic activity. So, it can be concluded that *Cannabis sativa* and *Parthenium hysterophorus* are strong inhibitors of *Lettuce* seeds growth.

The main aim of the present study of plants was to conduct research that can be used allelopathic plants as high value crops for farmers. This is a primary research using different selection methods to get plants with allelopathic potential. The application of bioactivity. after its establishment, of the plants as bio herbicide could be very feasible opportunity for the farmers.

Chapter No. 6

REFERENCES

Akhtar, S., Bangash, N., Asghar, R., Munir, M., & Khalid, N. (2014). Allelopathic assessment of selected invasive species of Pakistan. *Pakistan J Bot, 46*, 1709-13.

Al-Shatti, A. H., Redha, A., Suleman, P., & Al-Hasan, R. (2014). The Allelopathic Potential of Conocarpus lancifolius (Engl.) Leaves on Dicot (Vigna sinensis L.), Monocot (Zea mays L.) and Soil-Borne Pathogenic Fungi. *American Journal of Plant Sciences, 5*(19), 2889

Ameh S.J., Obodozie O., Inyang U. S., Abubakar M.S., Garba M. (2010). Current phytotherapy - A perspective on the science and regulation of herbal medicine. Journal of Medicinal Plants Research. 4(2): 72-81

Amoo, S. O., Ojo, A. U., & Van Staden, J. (2008). Allelopathic potential of Tetrapleura tetraptera leaf extracts on early seedling growth of five agricultural crops. *South African Journal of Botany, 74*(1), 149-152.

Anjum, A., Hussain, U., Yousaf, Z., Khan, F., & Umer, A. (2010). Evaluation of allelopathic action of some selected medicinal plant on lettuce seeds by using sandwich method. *Journal of Medicinal Plants Research, 4*(7), 536-541.

Anjum, T., & Bajwa, R. (2005). A bioactive annuionone from sunflower leaves. *Phytochemistry, 66*(16), 1919-1921.

Aslam, F., Khaliq, A., Matloob, A., Abbas, R. N., Hussain, S., & Rasul, F. (2014). Differential allelopathic activity of Parthenium hysterophorus L. against canary grass and wild oat. *J Anim Plant Sci, 24*, 234-244.

Aziz, S., & Shaukat, S. S. (2014). Allelopathic potential of Digera muricata, a desert summer annual. *Pak. J. Bot, 46*(2), 433-439.

Bhowmik, P. C. (2003). Challenges and opportunities in implementing allelopathy for natural weed management. *Crop protection, 22*(4), 661-671.

Biradar, D. P., Shivakumar, K. S., Prakash, S. S., & Pujar, B. T. (2010). Bionutrient potentiality of Parthenium hysterophorus and its utility as green manure in rice ecosystem. *Karnataka Journal of Agricultural Sciences, 19*(2).

Bonner, J. (1950). The role of toxic substances in the interactions of higher plants. *The Botanical Review, 16*(1), 51

Cheema, Z. A., & Khaliq, A. (2000). Use of sorghum allelopathic properties to control weeds in irrigated wheat in a semi arid region of Punjab. *Agriculture, Ecosystems & Environment, 79*(2-3), 105-112.

Evans, H. C. (1997). Parthenium hysterophorus. A review of its weed status and the possibilities for biological control. *Biocontrol News and Information, 18*, 89N-98N.

Evenari, M., & Carr, D. J. (1961). Chemical influences of other plants (allelopathy). In *External Factors Affecting Growth and Development/Aussenfaktoren in Wachstum und Entwicklung*(pp. 691-794). Springer, Berlin, Heidelberg.

Farooq, M., Jabran, K., Cheema, Z. A., Wahid, A., & Siddique, K. H. (2011). The role of allelopathy in agricultural pest management. *Pest management science, 67*(5), 493-506.

Fennimore, S. A., & Umeda, K. (2003). Weed control in glyphosate-tolerant lettuce (Lactuca sativa). *Weed technology, 17*(4), 738-746.

Fisher, R. F., Woods, R. A., & Glavicic, M. R. (1978). Allelopathic effects of goldenrod and aster on young sugar maple. *Canadian Journal of Forest Research*, *8*(1), 1-9.

Fujii, Y., Parvez, S. S., Parvez, M. M., Ohmae, Y., & Iida, O. (2003). Screening of 239 medicinal plant species for allelopathic activity using the sandwich method. *Weed Biology and Management*, *3*(4), 233-241.

Fujii, Y., Shibuya, T., Nakatani, K., Itani, T., Hiradate, S., & Parvez, M. M. (2004). Assessment method for allelopathic effect from leaf litter leachates. *Weed Biology and Management*, *4*(1), 19-23.

Haar, M. J., & Fennimore, S. A. (2003). Evaluation of integrated practices for common purslane (Portulaca oleracea) management in lettuce (Lactuca sativa). *Weed Technology*, *17*(2), 229-233.

Inam, B., Hussain, F., & Bano, F. (1987). Allelopathic effects of Pakistani weeds Xanthium strumarium L. *Pakistan Journal of Scientific and Industrial Research (Pakistan)*.

Iqbal, Z., Furubayashi, A., & Fujii, Y. (2004). Allelopathic effect of leaf debris, leaf aqueous extract and rhizosphere soil of Ophiopogon japonicus Ker-Gawler on the growth of plants. *Weed Biology and Management*, *4*(1), 43-48.

Iqbal, Z., Nasir, H., Hiradate, S., & Fujii, Y. (2006). Plant growth inhibitory activity of Lycoris radiata Herb. and the possible involvement of lycorine as an allelochemical. *Weed biology and management*, *6*(4), 221-227.

Ishak, M. S., & Sahid, I. (2014). Allelopathic effects of the aqueous extract of the leaf and seed of Leucaena leucocephala on three selected weed species. In *AIP Conference Proceedings* (Vol. 1614, No. 1, pp. 659-664). AIP.

Ismail, B. S., & CHONG, T. V. (2002). Effects of aqueous extracts and decomposition of Mikania micrantha HBK debris on selected agronomic crops. *Weed Biology and Management*, *2*(1), 31-38.

Ismail, B. S., & Mah, L. S. (1993). Effects of Mikania micrantha HBK on germination and growth of weed species. *Plant and Soil*, *157*(1), 107-113.

Kishor, P., Ghosh, A. K., Singh, S., & Maury, B. R. (2010). Potential use of parthenium (Parthenium hysterophorus L.) in agriculture. *Asian J Agric Res*, *4*, 220-225.

Kohli, R. K., Batish, D. R., Singh, H. P., & Dogra, K. S. (2006). Status, invasiveness and environmental threats of three tropical American invasive weeds (Parthenium hysterophorus L., Ageratum conyzoides L., Lantana camara L.) in India. *Biological Invasions*, *8*(7), 1501-1510.

Lemerle, D., Verbeek, B., & Orchard, B. (2001). Ranking the ability of wheat varieties to compete with Lolium rigidum. *Weed Research*, *41*(3), 197-209.

Mehmood, A., Tanveer, A., Nadeem, M. A., & Zahir, Z. A. (2014). Comparative allelopathic potential of metabolites of two Alternanthera species against germination and seedling growth of rice. *Planta Daninha*, *32*(1), 1-10.

Nasir, H., Iqbal, Z., Hiradate, S., & Fujii, Y. (2005). Allelopathic Potential of Robinia pseudo-acacia L. *Journal of Chemical Ecology*, *31*(9), 2179-2192.

Nasir, Y. J. (1991). Threatened plants of Pakistan. *Plant Life of South Asia*, 229-234.

Oyun, M. B. (2006). Allelopathic potentialities of Gliricidia sepium and Acacia auriculiformis on the germination and seedling vigour of maize (Zea mays L.). *American Journal of Agricultural and Biological Science*, *1*(3), 44-47.

Parvez, S. S., Parvez, M. M., Nishihara, E., Gemma, H., & Fujii, Y. (2003). Tamarindus indica L. leaf is a source of allelopathic substance. *Plant Growth Regulation*, *40*(2), 107-115.

Ranalli, P. (1999). Agronomical and physiological advances in hemp crops. *Advances in hemp research*, 61-84.

Rao, F. A. A. R., & Mamta, K. (2013). Allelopathic Effect of Aqueous Extracts of Neem (Azadiracta indica) and Eucalyptus (Eucalyptus citroides) on the Growth and Germination of Wheat (Triticum aestivum var-desi). *Journal of Environmental Science and Engineering*, *1*(1), 42-45.

Sahid, I. B., & Sugau, J. B. (1993). Allelopathic effect of Lantana (Lantana camara) and Siam weed (Chromolaena odorata) on selected crops. *Weed Science*, *41*(2), 303-308.

Sajjan, A. S., Hiremath, S. M., & Badanur, V. P. (1997). Allelopathic effect of eucalyptus on germination and seedling characteristics in some crops. *Annals of Plant Physiology*, *11*, 54-57.

Santos, B. M., Dusky, J. A., Stall, W. M., Bewick, T. A., & Shilling, D. G. (2004). Mechanisms of interference of smooth pigweed (Amaranthus hybridus) and common purslane (Portulaca oleracea) on lettuce as influenced by phosphorus fertility. *Weed science*, *52*(1), 78-82.

Saxena, M. K. (2000). Aqueous leachate of Lantana camara kills water hyacinth. *Journal of Chemical Ecology*, *26*(10), 2435-2447.

Sharma, G. P., Raghubanshi, A. S., & Singh, J. S. (2005). Lantana invasion: an overview. *Weed Biology and Management*, *5*(4), 157-165.

Shiraishi, S., Watanabe, I., Kuno, K., & Fujii, Y. (2005). Evaluation of the allelopathic activity of five Oxalidaceae cover plants and the demonstration of potent weed suppression by Oxalis species. *Weed biology and management*, *5*(3), 128-136.

Singh, H. P., Batish, D. R., Pandher, J. K., & Kohli, R. K. (2003). Assessment of allelopathic properties of Parthenium hysterophorus residues. *Agriculture, ecosystems & environment*, *95*(2-3), 537-541.

Sinha, N. K., & Singh, S. J. (2004). Allelopathic effects of Xanthium strumarium on Parthenium hysterophorus. *Indian Journal of Plant Physiology*, *9*(3), 313-315.

Tamado, T., Schutz, W., & Milberg, P. (2002). Germination ecology of the weed Parthenium hysterophorus in eastern Ethiopia. *Annals of applied biology*, *140*(3), 263-270.

Tanveer, A., Rehman, A., Javaid, M. M., Abbas, R. N., Sibtain, M., Ahmad, A. U. H & Aziz, A. (2010). Allelopathic potential of Euphorbia helioscopia L. against wheat (Triticum aestivum L.), chickpea (Cicer arietinum L.) and lentil (Lens culinaris Medic.). *Turkish Journal of Agriculture and Forestry*, *34*(1), 75-81.

Tefera, T. (2002). Allelopathic effects of Parthenium hysterophorus extracts on seed germination and seedling growth of Eragrostis tef. *Journal of Agronomy and Crop Science*, *188*(5), 306-310.

Vandeleur, R. K., & Gill, G. S. (2004). The impact of plant breeding on the grain yield and competitive ability of wheat in Australia. *Australian Journal of Agricultural Research*, *55*(8), 855-861.

Wicks, G. A., Nordquist, P. T., Baenziger, P. S., Klein, R. N., Hammons, R. H., & Watkins, J. E. (2004). Winter wheat cultivar characteristics affect annual weed suppression. *Weed technology*, *18*(4), 988-998

Wolfe, M. S., Baresel, J. P., Desclaux, D., Goldringer, I., Hoad, S., Kovacs, G,& Van Bueren, E. L. (2008). Developments in breeding cereals for organic agriculture. *Euphytica*, *163*(3), 323.