ALLELOPATHY OF MEDICINAL PLANTS AND ITS IMPACT ON CONSERVATION OF SOIL ENVIRONMENT

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ALLELOPATHY OF MEDICINAL PLANTS AND ITS IMPACT ON CONSERVATION OF SOIL ENVIRONMENT

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بسم الله الرحمن الرحيم

DEDICATION

I dedicate my work to My ***Beloved Parents, family members, friends***

&

Respected teachers

ACCEPTANCE BY THE VIVA VOCE COMMITTEE

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DECLARATION

I ***Khawaja Waqar Ali*** (9-FBAS/PHDES/F13), student of PhD in Environmental Science (session 2013-17), hereby declare that the matter printed in the thesis titled "Allelopathy of Medicinal Plants And its Impact on Conservation of Soil Environment" is my own work and has not been published or submitted as research work or thesis in any form in any other university or institute in Pakistan or abroad.

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Signature of Deponent
Khawaja Waqar Ali

FORWARDING SHEET BY RESEARCH SUPERVISOR

The thesis entitled "Allelopathy of Medicinal Plants And its Impact on Conservation of Soil Environment" submitted by Khawaja Waqar Ali in partial fulfillment of PhD degree in Environmental Science has been completed under my guidance and supervision. I am satisfied with the quality of student's research work and allow him to submit this thesis for further process to graduate with PhD Degree from Department of Environmental Science, as per IIU rules & regulations.

Date: 14-012-2017

_____

Dr. Muhammad Ibrar Shinwari,
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Those who bear the throne (of Allah) and those near to Him glorify and praise their Lord. They believe in Him and implore His forgiveness for those who believe… (Quran, 40:7).

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ABSTRACT

The taxonomic richness of medicinal plants in Pakistan can foster notable economic contributions through medicinal, industrial and environmental applications. The release of allelochemicals from medicinal plants have metamorphosed the discourse of research on medicinal plants. The allelopathy epitomizes stimulatory or inhibitory interactions among organisms due to their inherent ability to forego biological invasions and impede biological activities by release of certain chemicals. This study extended to the allelopathic effect of 221 species collected from different regions of Pakistan. The cardinal objective was to assess the allelopathic effect of medicinal plants collected from different regions of Pakistan using dish pack method. As the dish pack method allows the assessment of allelopathic effect due to volatile secondary metabolites released from different plants. Hence, the germination and growth of lettuce was tested against selected plant species. The findings of this study envisaged the *Boerhavia procumbens* for strong inhibitory effect and *Plectranthus rugosus* for strong stimulatory effect due to release of volatile allelochemicals. The results hereby envisions future applications of allelopathy in sustainable agriculture, industry, weed management, agro-environment conservation, food security and soil conservation.

The second objective of this study was to explore allelopathic effect of medicinal plants on the germination and seedling growth of lettuce using sandwich method. The 207 plant species had been tested using sandwich method to assess allelopathic effect of leachates of different parts of plants on lettuce seeds and provide baseline information for exploration of medicinal plants for economic benefits. The results of this study identifies 4 plants having strong inhibitory effect, 14 plants with medium inhibitory and 13 plants of low inhibitory effect on the lettuce seeds. *Boerhavia*

procumbens exhibited the strongest inhibitory allelopathic effect and *Viburnum* grandiflorum presented strong stimulatory effect on the growth of lettuce seeds.

The allelopathic effects of aqueous extract of different plant species on wheat (*Triticum aestivum* L.), Lettuce (*Lactuca sativa* L.) and maize (*Zea mays* L.) were also assessed in this study. We have selected nine plant species (*Boerhavia procumbens* Banks ex Roxb., *Jacaranda mimosifolia* D. Don, *Datura metel* L., *Asparagus adscendens* Roxb., *Plectranthus rugosus* Wall. ex Benth. *Parthenium hysterophorus* L., *Arisaema triphyllum* (L.) Schott, *Crotalaria medicaginea* DC. and *Amaranthus viridis* L) and apply their aqueous extract directly to crop seeds as well as through the soil medium to assess their allelopathic effect in both conditions. The results apprehended that the aqueous extracts of all the selected species revealed significant inhibitory effect on germination and growth of wheat, maize and lettuce as compared to the aqueous extracts applied to the soil. However, lettuce was more influenced from the inhibitory effect followed by maize and wheat in the soil irrigated with the aqueous extracts of selected species. Hence, the diversified allelopathic effects of plants have the potential to improve weed management, sustainable agriculture, food production as well as medicinal, industrial and environmental applications

CHAPTER 1

1. INTRODUCTION

The term allelopathy was used for the first time in 1937 by an Austrian scientist Hans Molisch, which means suffering or mutual harm in Greek. More specifically, allelopathy refers to the release of allelochemicals, which retard or enhance the growth of other plant species present in their vicinity (Niyazi *et al.,* 2017). Plant dynamics, growth, physiology, survival and community composition are also affected by allelochemicals (Bais *et al.,* 2006). Allelopathy can affect plant productivity, diversity, ecology, occurrence, growth, development, and succession. Aside from many adverse allelopathic effects of plant species, there are many selective beneficial and neutral effects (Niyazi *et al.,* 2017). These allelochemicals normally categorized as secondary metabolites which may be isoprenoids, phenolic, alkaloids, terpenoids, glucosinolates and flavonoids, etc.

Allelochemicals are normally present in all parts of the plants, including flowers, fruit, leaves, rhizomes, seeds, stems, seeds, pollen and roots (Mubashir and Shah, 2011). These are released by leaching, volatilization, residue decomposition, root exudation and other natural and agricultural processes (Stamp, 2003). According to Pinton *et al.* (2001) about 5-21 percent of the carbon comes from the soil (rhizosphere) through photosynthetically fixed root exudates. Meanwhile, a wide range of nutrients which are important for microorganisms also come in the rhizosphere by the release of root exudates (Bacilio-Jimenez *et al.,* 2003; Zhang *et al.,* 2017).

The plant biomass had an allelopathic effect on both plant and soil (Baličević *et al.,* 2015b). A notable inhibition of weed growth in paddy field is evident from dwarf lilyturf dry biomass. It can be employed as a natural herbicide in a rice field (Lin *et al.*, 2003). Similarly, dry biomass of common greater celandine, lovage, mallow, basil and chamomile had shown a higher inhibitory effect, retard weed growth as well as reduce seed germination (Baličević *et al.,* 2015a). The powder of *Nicitiana tabacum* L., *Crocus sativus* L., *Nerium oleander* L., *Ricinus communis* L., *Sorghum vulgare* L. and *Datura inoxia* Mill. had exhibited stronger inhibitory effect against redroot pigweed and effectively control the pigweed as a natural herbicide (Nekonam *et al.*, 2014). The dry biomass of fennel had reduced germination, seedling growth and root length of hoary cress. The inhibition rate was substantially higher in fresh residues of the fennel (Ravlić *et al.,* 2015).

Alike, the effect on the microorganism, soil, and environment, significant allelopathic impacts of medicinal plants has been reported recently. Medicinal plants are being used for curing different diseases as well as attributing the allelopathic effects (Shinwari, 2010). Different plant species are indicating variations in allelopathic activities in different habitats, as well as same plant species, pose different toxic effect (Gilani *et al.,* 2010; Celewicz-Gołdyn and Kuczyńska-Kippen, 2017). A large number of plant species among 387 Japanese medicinal plants have shown allelopathic potentials during multiple experiments (Fujii *et al.,* 2003).

1.1 Plant to plant allelopathic impacts of medicinal plants

Many researchers are working with medicinal plants for the search of new allelochemicals. The allelopathic effect can be inhibitory or stimulatory depending on the dynamic and static availability, identity of the active compound and the target species (Keating, 1999). The allelopathic medicinal plants are easy to screen as

compared to other plants, perhaps, due to their medicinal use (Fujii *et al.*, 2004; Yadav *et al.,* 2017). In plants with allelopathic activity, there are around 400,000 secondary metabolites and few of them have been examined yet (Swain, 1977). According to Wakdikar, (2004) there are only 12.5% medicinal plants and the unidentified compounds from them may have many promising growth inhibitors.

The essential oil of *Eucalyptus* has an allelopathic effect on growth, height and germination of Bermuda grass, *Cynodon dactylon* L. and *Amaranthus retroflexus* L. *(*Daneshmandi and Azizi, 2009*).* Essential oils reduce root length, germination percentage and shoot the length of the pigweed species (Rassaeifar *et al.,* 2013). *Cynodon dactylon* L. is a perennial plant and have an allelopathic effect on other plants (Christians, 2004). *Artemisia tridentata* Nutt. leaves and *Eucalyptus globulus* have also an allelopathic effect on seed germination of *Cynodon dactylon* L. (Bidarnamani *et al.,* 2015). Significant effects of rosemary oil have been documented on the germination of *Acroptilon repens* (L.) DC., *Amaranthus retroflexus* L., and *Portulaca oleracea* L. (Ramezani *et al.,* 2008). Allelochemicals from aqueous extracts of *Euphorbia guyoniana* Boiss. & Reut. and *Retama retam* completely inhibits the germination efficiency and radicle length of *Bromus tectorum* (Salhi *et al.,* 2011).

The aqueous extracts of sunflower *Helianthus ogrodowy* and *Helianthus annus* L. had affected the seedling growth and seed germination of the *Sinapis alba* L. (mustard plant). It is observed that increasing concentration of aqueous extracts had inhibited the seed germination (Rawat *et al.,* 2012). *Artemisia herba-alba* Asso. is being used as a folk medicine, but the seeds of *Artemisia herba-alba* Asso. contain phytotoxic chemicals. The germination and seedling growth of the *Anabasis setifera* Moq. had been decreased by the aqueous extracts from fruits of *Artemisis herba-alba* with the increase in concentration (Modallal and Al-Charchafchi, 2006). The inhibition of germination

and seedling growth was much more of immature fruit as compared to mature fruit. It is believed that phenolics are the possible agents for this allelopathic activity (Modallal and Al-Charchafchi, 2006). The metabolism and germination inhibition on other seeds by essential oils have also been reported. Germination of seeds of some common weed species like *Sonchus oleraceus* L., *Sinapis arvensis L.*, *Alcea pallida* waldst., *Amaranthus retroflexus* L., *Raphanus raphanistrum* L., *Centaurea salsotitialis* L. *and Rumex nepalensis* Spreng. has been affected by the essential oil from different plants like *Mentha spicata* L., *Pimpinella anisum* L., *Foeniculum vulgare* Mill., *Rosmaris officinalis* L., *Thymbra spicata* L., *Layandula stoechas* L., *Salvia officinalis* L., *Coriandrum sativum* L., *Origanum onites* L., *and Carum carvi* L. The high inhibitory effect of essential oil from *Thymbra spicata* L., *Origanum onites* L., *Carum carvi* L. and *Mentha spicata* L. is revealed against some weeds, when essential oil was used in low concentration (Azirak*et al.,* 2008). Different allelochemicals from plants like *Pityrogramma* ssp, *Helianthus annuus* L. (Maciás *et al.,* 1997), *Pluchea lanceolate* (DC.) C.B. Clarke, *Erica australis* L., *Astrophytum Notocactus*, *Neochilenia* Backeb., *Parodia* Speg., *Trifolium pretense*, *Oryza sativa* L., *Pancratium biflorum* Roxb. and *Lens culinaris* Medik. (Kong *et al.,* 2006) have a positive and negative impact on other plants.

1.2 Allelopathic effects of medicinal plants on soil rhizosphere

The presence of allelochemicals in the soil has a potential to affect different biotic and abiotic processes through recalcitrant of humic substances, sorption of organic matter, polymerization, microbial and chemical transformation into other compounds (Hättenschwiler and Vitousek, 2000; Meiners *et al.,* 2017). The degradation of organic acids in the rhizosphere is reduced by allelochemicals but stimulates the biodegradation of xenobiotics (Shaw *et al.,* 2006). The nature of the soil is being affected by the release of different phenolic compounds in the rhizosphere. The

knapweed, *Centaurea maculosa* Lam. affected the soil rhizosphere (Blair *et al.,* 2009). Both, type and concentration of these released allelochemicals affect plant physiology, growth rate and other functions of the plants (Cesco *et al.,* 2010). Some allelochemicals released from the roots of a plant reacts with soil organic matter or trapped in it. When soil of *C. ladanifer* L. was analyzed, five different types of allelochemicals were found in it (Sosa *et al.,* 2010).

Trigonella L., *Parasponia andersonii* Planch., *Sinorhizobium meliloti, Rhizobium leguminosarum,* and *Medicago melilotus* can affect biological processes of soil, the growth of the seedlings by adsorption and absorption in soil and nutrient bio-geocycles. Soil dynamics, growth, physiology, survival and community composition are also affected by allelochemicals (Bais *et al.,* 2006). Soil nutrient availability can be increased or decreased by root exudation due to soil chemicals and biological process. *Lupinus albus* L. excrete the citrate which reduces the pH level of soil, influences the solubility of metals (Fe and Mn) and mobilize the soluble phosphate (Weiner, 2001). The soil properties could be affected by on larger scale resulting in positive or negative resource availability to nearby plants. Root exudates can affect soil resource availability by organic acid secretion or by phytosiderophore secretion (Bais *et al.,* 2006).

Alliaria petiolate concentration and its mechanism of release were studied in soil. The results show reduced half-lives in nonsterile soils and longer time in sterile soils (Barto and Cipollini, 2009).

Cistus ladanifer L. produces aglycone unapproachable flavonoid to microorganisms because the molecules partly remain stuck in soil micropores or solid organic phase and moderately ruined (Sosa *et al.,* 2010). Rhizosphere has microbial communities which can degrade allelochemicals by biodegradation. The degradation process is higher in aged plants due to release of flavonoids. The increasing flavonoids

result in more microbial communities to degrade them in the soil rhizosphere (Shaw *et al.,* 2006). Roots mainly excrete organic material in the soil which acts as the growth substrate (Werner, 1998). The soil quality and plant fitness are markedly governed by rhizosphere functioning. As microbial development in the rhizosphere can help plant species to adopt stressed environment like water and nutrient deficiency (Bowen & Rovira, 1999).

Allelochemicals have an eminent impact on nutrients in soil, affecting the plant growth (Nannipieri and Paul 2009). Some secondary metabolites that are released in rhizosphere had the ability to protect plants from diseases, alter nutrient cycle, regulate plant growth and induce allelopathic effects (Garg and Geetanjali, 2007; Buer *et al.*, 2010). *Rhizobia* species, including *Azorhizobium, Mesorhizobium, Bradyrhizobium, Sinorhizobium, Rhizobium,* and *Sinorhizobium* releases the allelochemicals which have symbiotic effects on nutrient cycle and nitrogen fixation (Dakora, 2003). According to Hättenschwiler and Vitousek, (2000) the interaction of flavonoids with proteins can play an important role in the nutrient cycle, dynamics of soil organic matter and control soil-plant interactions. Some allelochemicals like flavonoids have both negative as well as positive effects on nitrogen processes. Effect on nitrogen processes is dependent upon the type of allelochemicals, fungi and microorganisms like symbiotic nitrogen-fixing bacteria (*Frankia* and *Rhizobium*) present in the soil (Hättenschwiler and Vitousek, 2000). Polyphenol protein complexes enhance nitrogen immobilization and produce nitrogen-containing organic compounds. These nitrogen-containing organic compounds are low molecular weight such as amino acids and are taken up by plants (Hättenschwiler and Vitousek, 2000). About 90-95% of total sulfur in soil occur as organic sulfur while the rest is in inorganic form and can be chemically transformed by both inorganic and biological processes (Stevenson, 1994). In soil, sulfur is present in

the form of sulfur amino acids, glucosinolates, proteins and peptides, alkali sulfates and sulfonates (Violante *et al.,* 2002).

Allelochemicals affect phosphorus cycle as well as mineralization, decomposition, and immobilization (Cross and Schlesinger, 1995). The flavonoids from roots produce stable complexes with aluminium and iron. Phosphates of iron and aluminium, thus increase the phosphorus availability to plants (Cesco *et al.,* 2010). *Vallisneria spiralis* L. and *Ceratophyllum demersum* L. have positive interactions with phosphorous cycle and boost available phosphorous (Dai *et al.,* 2017). It has been reported that the roots of the alfalfa plants release flavonoids which form ferric phosphate (Masaoka *et al.,* 1993). *Lantana camara* L. (Verbenaceae) has improved the nutrient cycle, including nitrogen, phosphorous and potassium in the Nairobi National Park, Kenya (Kamweya *et al.,* 2017).

1.3 Effect of allelopathy on soil microorganisms

The microbial abundance is much higher and directly attached to the roots of plants in the soil (Smalla *et al.,* 2006). The chemicals released from plants into rhizosphere affect microorganisms and making rhizosphere a complex environment (Mukerji *et al.,* 2006; Li *et al.,* 2017). Plant species, soil type, management practices, microbial interactions, and other environmental factors affect the diversity and composition of bacterial taxa in the rhizosphere (Hoitink and Boehm, 1999). Allelochemicals affect the microbial degradation of chemicals in soil (Schoefer *et al.,* 2003). Allelochemicals also attract pathogenic microbes, promote the growth of plants and microbial communities (Cook *et al.,* 1995). Plant growth promoting rhizobacteria is indirectly advantageous for plant growth as they control some biologically harmful microorganisms or root pathogens that inhibit plant growth due to parasitism, decreasing pollutant toxicity, including antibiotic and hydrogen cyanide production,

synthesis of extracellular enzymes to hydrolyze the fungal cell wall and competition for nutrients and niches within the rhizosphere (Bhattacharyya and Jha, 2012).

Positive, neutral, and negative associations are present between root-root, root-insect, and root-microbe. There are many examples of positive interaction, such as root colonization by plant growth promoting bacteria (PGPB) and bacterial biocontrol agents, the symbiotic association between mycorrhizal fungi and epiphytes. Negative interactions include invertebrate herbivory, parasitism among plants, competition among plants and pathogenesis by bacteria or fungi (Bais *et al.,* 2006). *Eucalyptus grandis* W. Hill has released volatile organic compounds in soil and exerted a toxic impact on *Eisenia fetida (*Zhiqun *et al.,* 2017)

Bacterial strains are affected by different allelochemicals like polyphenols. There was the increase in the growth rate of several bacterial species, induction of nodulation with gene transcription, promotion of chemotaxis (Aoki *et al.,* 2000). The legume-rhizobia has symbiotic interactions with biological activities of microorganisms in soil (Aoki *et al.,* 2000). The benzoic acid released from *Arachis hypogaea* L. (peanut) root exudates has induced the changes in the microorganism communities into the soil (Liu *et al.,* 2017).

A real example of diverse meanings for a chemical signal is the secretion of isoflavones by soybean roots, which attract a mutualist (*Bradyrhizobium japonicum*) and pathogens (*Phytopthora sojae*) (Morris *et al.,* 1998). Once allelochemicals are released in the rhizosphere many microorganisms are multiplied rapidly in response to allelochemicals. *Centaura maculsa* exudes catechin from its roots which are showing antimicrobial activities in plants (Veluri *et al.,* 2004). The flux of flavonoids from the roots of allelopathic plants to soil rhizosphere is stimulating the activity of heterotrophic bacteria and prevailing over the soil autotrophic microorganisms (Toal *et al.,* 2000).

Dalbergia sissoo Roxb. ex DC. showed the extent of diversity of microorganism and notable differences in the protein pattern of root nodule (Tilak *et al.,* 2005). The allelochemicals from tea monocropping, soybean monocropping, tea and soybean intercropping have significantly changed the microbial metabolic activities and functional diversity (Qin *et al.,* 2017).

Resin and other major components like diterpenoid kaurenoic acid released from *Pseudognaphalium vira vira* affected the soil bacteria in the vicinity of the plant (Urzua *et al.,* 1995). Plants also release enough quantity of organic material through exudations, sloughing of border cells and secretion of mucilage. The organic compounds affected the bacterial composition, community, activity and size is also affected by plant species (Nguyen, 2003; Hamilton and Frank, 2001; Innes *et al.,* 2004). The potato rhizosphere favors the growth of nematode biomass as compare to protozoa biomass (Griffiths, 1990). It is also known that many plant-feeding nematodes have a precise array of appropriate host plants. *Leucanthemum vulgare* Lam. has shown the strongest negative impact on the growth of nematodes and microorganism (Van Ruijven *et al.,* 2003).

Not only allelochemicals affect soil microorganisms, but also affect some fungal species. The yield and type of degradation products formed by the reaction of allelochemicals with fungi and bacteria depend on upon the allelochemicals used and bacterial or fungal species (Schoefer *et al.,* 2003).

Flavonoids are also cleaved by some rhizobial strains which result in phloroglucinol and protocatechuic acid via chalcone intermediate. β-oxidation of the side chain of cinnamic acid results in the formation of protocatechuic acid. They have a significant impact on the microorganisms. *Cytisus scoparius* (Humbert & Maire) Talavera has developed rapid allelopathic effect on ectomycorrhizal fungi by lowering the

colonization in soil, but surprisingly fungi stabilize the population over time (Grove *et al.,* 2017). The intercropping of garlic and cucumber has reduced the fungal community and increase the bacterial population. There was a significant improvement in the overall soil microbial environment (Du *et al.,* 2017). The *Dendroctonus ponderosae* (pine beetle) supports the soil fungal community. It has been found that the mortality of trees reflected the decline in the fungal population (Pec *et al.,* 2017). *Mimosa pigra, Vachellia nilotica, Parkinsonia aculeate, Jatropha gossypiifolia* and *Tamarix aphyllahave* shown no significant difference in fungal community structure, richness and diversity in the North Australian region *(*Raghavendra *et al.,* 2017*)*

1.4 Effect of allelopathy on environment

At the ecosystem level, non-indigenous plants have altered the geomorphological process, hydrological cycle, and biogeochemical process and at the community level, there is the change in resource competition (Gordon, 1998). The allelopathic medicinal plants regulate the carbon cycle as well as fix atmospheric carbon dioxide (Haase *et al.,* 2007). The allelopathic medicinal plants have an ecological role in biodiversity conservation. They affect the plant ecology through plant succession, structure of plant communities, growth, diversity, dominance, plant productivity, and occurrence (Ferguson and Rathinasabapathi, 2003). The plant species *Macrocystis* and *Saccharin*a are known as underwater forests and builds structural habitats within coastal ecosystem. They play a diverse role in ecological services (Tang & Gobler, 2011). The genus Ulvacan alleviate the pollution load and nutrient enrichment. They inhibit the growth of other harmful algal bloom through allelopathy (Tang & Gobler, 2011). Water chemistry has been significantly affected by some aquatic species like *E. crassipes, H. verticillata,* and *P. stratiodes*. Due to these species, there is an increase in watercolor, turbidity, and dissolved carbon dioxide level, but the

decrease in dissolved oxygen, phosphorus, and pH (Schmitz *et al.,* 1993). They also promote the expansion of coastal dead zone by developing anoxic condition (Smetacek & Zingone, 2013). *Microcystis aeruginosa*can inhibit the growth of green algae (*Chlorella pyrenoidosa, Scenedesmus quadricauda*) and a diatom (*Cyclotella meneghiniana*), and potentially treat the water and wastewater (Wang *et al.*, 2017). The allelochemicals from *Potamogeton crispus* L. can reduce the eutrophication and effectively improve water quality as well as restore the eutrophic lake *(*Zhou *et al.,* 2017).

1.4.1 Use of allelopathic plants as a natural herbicide

The medicinal plants affect the growth of weeds and have a potential to use them as natural herbicides (Sodaeizadeh *et al.,* 2009; Li *et al.,* 2009). It is important to note that the allelopathic effect of plant species on each other could be alternative to pesticides and open a new arena in environmental protection. The phytotoxins can affect shoot growth, germination, root growth, respiration, photosynthesis, metabolic production and results in cell death of the vulnerable plants (Weir *et al.,* 2004). Furthermore, natural allelochemicals can reduce the number of risks from synthetic pesticides, including persistency of pesticides, high cost and environmental consequences (Quaswm and Foy, 2001). *Sorghum bicolor Monech* L. has strong allelopathic potential and released phenolic compounds. Meanwhile, it has an ability for controlling weed under field condition. It is providing an opportunity to substitute the chemical pesticides as well alleviating their toxic impacts on the environment (Jabran, 2017). The allelopathic bioactive compounds can apply for weed control, growth regulation and pest management. They seem to be much safer than synthetic chemical and reduces the environmental pollution and health defects (Ashraf *et al.,* 2017). Plants like *Juglans nigra, Centaurea diffusa, Sorghum* species and *Centaurea*

maculosa produce phytotoxins (Bais *et al.,* 2002). In some species like *Asparagus officinalis, Cucumis sativa* and *Centaurea maculosa* autoinhibition from phytotoxic root exudates has been reconciled (Perry *et al.,* 2005). Carboline is a phytotoxin that is released by *Elytrigia repens* increases the herbivore resistance in neighboring plants (Glinwood, 2003). *Ophiopogon japonicas* (K) have an inhibitory effect on weed in rice and could be effective weed control (Lin *et al.,* 2003).

1.5 Problem Statement

- The secondary metabolites released from medicinal plants can affect shoot growth, germination, root growth, respiration, photosynthesis, metabolic production and results in cell death of the vulnerable plants.
- At the same time , the investigation of secondary metabolites can be used as bio pesticides and have a potential to reduce the number of risks from synthetic pesticides, including persistency of pesticides, high cost and environmental consequences.
- The allelopathic potential of medicinal plants of Pakistan are still unexplored. Few baseline data available.

1.6 Objectives of Research

The objectives of this study were:

- To assess the allelopathic potential of selected medicinal plants through bioassay.
- To study the impact of selected medicinal plants for their allelopathy on the soil environment.

1.7 Significance of Study

Allelochemicals have both negative as well as many beneficial effects on plant productivity, diversity, ecology, occurrence, growth, development, and succession.

This study attempted to explore both negative allelopathic effects comprising of reduction root length, inhibition of seed germination and seedling growth as well as positive effects (stimulation of growth). Meanwhile, it had endeavored the application of selected medicinal plants in the soil environment conservation and as a natural herbicide. This study may also create awareness amongst farmers and rural communities as well as new scientific document will develop as baseline for next coming researchers. According to identified problems, appropriate recommendations will be provided. Also, this study will provide management options for government, NGOs and local level organizations and likely future scenario in light of current facts and figures predicted.

1.8 Hypothesis

The allelochemicals exudate from medicinal plants into the soil affect the associated plants and soil environment**.**

1.9 Limitations

Although, this study covers the proposed objectives comprehensively. The study lacks the isolation ofsecondary metabolites which may be isoprenoids, phenolic, alkaloids, terpenoids, glucosinolates and flavonoids, etc. as well as their characterization using analytical techniques due to lack of time and some resources.

CHAPTER 2

2. REVIEW OF LITERATURE

Allelochemicals are released by different mechanisms, including root exudation, volatilization and decomposition of residues into the soil rhizosphere. Allelopathic plants have diverse ecological role, such as regulation of soil biota, nutrient chelation, and plant defense through low cost biological control of insects, diseases and pests, decomposition of residues improving nutrients and enhancing the crop diversification by reducing weed infestation. Wheat is used as an allelopathic plant which releases numerous chemicals affecting the soil environment (Aslam *et al*., 2016).

2.1 Allelopathic effects of different plants on other plants and agricultural crops / weeds

It has been reported that the introduced plants have the ability to compete the native plants by a belowground allelopathic effect on soil. An introduced crucifier, *Raphanus sativas* had shown an allelopathic effect on the *Lupinus nanus* legume and soil community in both field and greenhouse experiment. The *R. sativus* soils had decreased the density of rhizobium nodules on *L. nanus* roots which were small and non-beneficial. Meanwhile, *R. sativus* soils had shown no effect on the on fungal density, but decreased the fungivores nematode abundance and net positive effect on *L. nanus* biomass. The parasitic/mutualistic relationship between *L. nanus* and its rhizobial association might be altered due to *R. sativus* introduction (Pearse *et al*., 2014).

The pharmacological and toxicological properties of *Acacia concinna* are well known and it has been used as a traditional medicine. To investigate the allelopathic

activity of *Acacia concinna* the pod was extracted with aqueous menthol. It has been observed that *Acacia concinna* pod extract inhibited the growth of roots and shoots of *Lactuca sativa, Brassica napus, Lepidium sativum, Medicago sativa, Lolium multiflorum, Phleum pretense, Echinochloa crus- gallis* and *Vulpia myuros*. At application 1 and 3 mg dry weight equal extract/ml, correspondingly. With the increase of pod extract there has been a decrease in growth of plants. It has been observed that the test plants show more inhibition in growth as compared to shoots. *Acaccia concinna* pods and its extracts can be applied for weed management and control (Boonmee and Kato-Noguchi, 2017).

A big threat for crop production is the use of synthetic chemicals due to non – degradability, toxicity and negative effect on the environment. The ethanolic extracts of *Commiphora stocksiana* Engl bark and *Onosma bracteatum* (leaves and flowers) are evaluated for checking their allelopathic potential in contradiction of *Brassica napus* which select as a model plant.*Onosma bracteatum* leaves show a full allelopathic tendency towards seed germination efficiency of *Brassica napus* while the flowers of *Onosma bracteatum* and an extract of *Commiphora depict* the partial allelopathic effect against model plant.The plant architecture is also adversely influenced by the presence of extracts. On the basis of finding it shows that the seedling of *Brassica napus* shows toxicity and allelopathic effect is due extract of the plant which produce oxidative stress. Besides, *Onosma bracteatum* can be used as a natural herbicide in the form of allelopathic compounds isolated from this plant species or in the form of extract which is the replacement of harmful synthetic herbicide (Ali *et al*., 2017).

Sunflower (*Helianthus annuus* L.) due to their allelopathic effect can be used for controlling weeds in different crops as well as in sunflower. Due the presence of phenolic compounds and terpenes which are important allelochemicals present in

sunflower. The strong allelopathic potential shows the residues of sunflower plant which can be used for controlling weeds under many agricultural settings either mixing it into the soil or by scattering it in the form of a layer over the soil. Due to their allelopathic effect different genotypes of sunflower can be cultivated for controlling of weeds in sunflower (Quan and Liang, 2017).

The allelopathic potential of rue, fennel, sage seed and plant biomass on weed specie *Lepidium draba* (hoary cress) is checked by a series of experiments. Effect of plants evaluated through dry and fresh plant residues in soil and seed germination in petri dishes. The seedling length and germination of hoary cress is greatly affected by fennel seeds having a highest inhibitory rate and reduce germination up to 34.9%. However, extracts in petri dishes depict various effects, such as dry plant biomass reduced the 100% seedling growth and germination and fennel reduced the length of root up to 22.7%. The result shows that the allelopathic inhibitory effect was greater by incorporation of fresh rue residues which can also be used for weed control in agricultural setup as a natural bio herbicide (Ravlic *et al*., 2016).

To study the impact of essential oils from medicinal plants as bioherbicides, an experiment was conducted based on CRD (completely randomized design) with three replications. Seeds of three summer crops including tomato (*Solanum lycopersicum*), lettuce (*Lactuca sativa*) and pepper (*Piper longum*) were exposed to essential oils of *Pimpinella anisum* (anise), *Rosmarinus officinalis* (rosemary) and *Thymus vulgaris* (thyme) at concentrations of (25 and 50% diluted and undiluted). After five days calculation the result shows that the allelopathic effect on germination percentage was variable due to differences in the composition and different allelopathic effect of the studied essential oils. Furthermore, several essential oils have a greater inhibitory effect

on weeds and it can be used as a natural bioherbicide on a large scale in agricultural cropping system (Shokouhian *et al*., 2016).

To check the allelopathic effect of medicinal plants on the plumule length of mungbean (*Vigna radiate*), the leaf extracts of *Astragalus tribuloides*, *Ocimum sanctum* and *Calotropis procera* were applied in vivo conditions. Results showed that alkaline and alcoholic aqueous acidic extract of *Ocimum sactum, Astragalus tribuloides* and *Calotropis procera* showed a different length of mungbean in vivo conditions. The all extracts of *Ocimum sanctum* have no effect in contrast with control, *Calotropis procera* produce relatively greater as compared to control followed by acidic, aqueous but alkaline extract showed the negative impact on mungbean plumule length. While the alkaline alcoholic extract of *Astragalus tribuloides* showed positive effect as compared to aqueous acidic extract on the plumule mungbean. Therefore, the different extracts of these medicinal plants can be used as a growth regulator on the plumule of mungbean (Gupta, 2016).

Elinonurus muticus essential oil has large variability in the chemical constituent and it is a native grass from Brazil. To evaluate the allelopathic effect of *Elinonurus muticus* essential oil, four wild populations were collected which is rich in citral. Mass spectrometry and gas chromatography had been performed for chemical analysis. Onion and lettuce seeds were used to evaluate the phytotoxic activity. The results demonstrate the possibility of chemotypes are present in the studied populations which have chemical variability based on their geographic origin. Furthermore, *Elinonurus muticus* essential oil, have a negative effect on the growth and germination of onion and lettuce (Füller *et al*., 2017).

To check the allelopathic effect on below ground and above ground part, aqueous extract of four medicinal plants, *Asperagus racemosus, Valeriana wallichii,*

Picrorhiza kurroa and *Ocimum sactum* were applied on some traditional food and oilseed crops to check their effect on their seedling growth and germination. The aqueous extract of medicinal plants reduced significantly the germination, radicle and plumule growth of selected oil seed crops and pulse in bioassay. The results showed that the medicinal plants have allelochemicals which have an inhibitory effect on oil seed crops and food (Rawat *et al*., 2016).

Weed management through use of allelopathic effect seems to be attracted in recent years. Aqueous extract of medicinal plants of *Mentha longifolia* (Habek mint) and *Achilla wilhelmsii* (Yarrow) was collected to investigate its allelopathic effect on germination of plantain. The results depict that extract of Habek mint and Yarrow of aerial parts have significant impact on shoot and root fresh, dry weight, germination percentage, seed vigor and root and shoot length. It has shown that with the decrease in aqueous extract concentration, the rate of germination and percentage increased. Minimum rate of germination and percentage was belonged to 100% concentration of aqueous extract. The aerial parts of the Habek mint and Yarrow can be used as a bioherbicide for germination and growth of plantain (Behzadi, 2016).

To investigate the allelopathic effect of Neem (*Azadirachta indica*), aqueous extract of leaves was applied on crops like Wheat, Cow pea, Jowar and Mung. Leaf extract of neem inhibits the germination of all observed crops. Inhibition is more observed in Jowar, Mung and Cow pea. With the decrease in concentration, wheat germination was increased. Results show that inhibition is variable, not connected with concentration which is interesting. The lowest root length was recorded in Cow pea treatment, while the maximum root length was recorded in Mung treatment. Acetone of Neem plants also shows the allelopathic effect on the germination and growth of shoots and roots of the crops. Protein synthesis, plant water relation, chlorophyll

production, permeability, respiration and hormonal balance are influenced by the allelochemicals which are the basic plant processes (Kasarkar and Barge, 2016).

Astragalus herb root aqueous extracts were reported to exhibit autotoxic activity on their own seedlings and allelopathy activity against other plants. From the rhizosphere soil extract of cultivated *A. hoantchy*, ten compounds were isolated and clarified by spectroscopic analysis. These isolated compounds were quantified by HPLC (high performance liquid chromatography) in the rhizosphere soil. Results indicated that at higher concentrations, the crude menthol extract of rhizosphere soil considerably repressed the growth of *L. sativa* and *A. hoantchy*. Findings show that these compounds have allelopathic and autotoxic potential that affect the growth of other plants and *Astragalus* plants themselves (Guo *et al*., 2016).

To evaluate the allelopathic effect of *Rosmarinus officinalis L.* (Ros) and *Origanum onites* L. (Ori) essential oils on germination and seedling growth of durum wheat, germination index, seedling root and shoot length, germination percentage and seedling fresh weight of cultivars were measured. Results indicate that the essential oil's allelopathic effect depended on oil practiced doses, oil types and cultivars. Results also showed that with the increase of dose, the inhibition rate will also increase. Rose oil has a low allelopathic effect as compared to Ori oil on seedling growth and germination of durum wheat. Therefore, due to their allelopathic activity of these plants they could be used as allelopathic agents for the control of weeds (Atak *et al*., 2016).

Lantana camara have both stimulatory and inhibitory biochemical interaction between plants. The allelopathic effects of *Lantana camara* can be checked with different weeds, shrub, crops and trees under both field and laboratory conditions to evaluate its allelopathic effect. The results showed that the *Lantana camara*

allelochemicals had an allelopathic inhibitory effect on the growth, germination and metabolism of weeds, crops, vegetables and bryophytes (Mishra, 2015)

To check the allelopathic effect of invasive species *Solidago gigantean* Ait. on germination of weed species *Amranthus retroflexus* L. (redroot pigweed) and *Abutilon theophrasti* Med. (velvetleaf) and on the initial growth crops like barley, carrot and coriander experiments were taking place under lab conditions. The extracts were applied in both petri dish bioassay and in pots with soil. The results show that all the concentrations of extracts have an allelopathic effect on germination and growth of crops in petri dishes. Fresh weight and root length of barley is greatly reduced by the highest concentration of extract applied. Therefore, it is concluded that the goldenrod biomass extract had an allelopathic effect on both weeds and crops (Baličević *et al*., 2015b).

Allelopathic effect of different plants was checked against wheat species (*Avena fatua, Triticum aestivum* and *Rumex crispus*) and associated weeds. The aqueous extracts of *Datura alba* and *Parthenium hysterophorus* showed significant effect on weeds of *Triticum aestivum* while *Phragmites australis* aqueous extract showed moderate effect. The results showed that the aqueous extract *Datura alba* and *Parthenium hysterophorus* reduced shoot length, dry and fresh biomass and seed germination as compared to other treatments. The findings also showed that the plants contain allelochemicals which retard the growth, biomass and germination of other species. Therefore, these plants can be used as a bioherbicide for weed management (Afridi and Khan, 2015).

To check the allelopathic effect of *Aristolochia clematitis* L. on weed specie *Trripleuro spermun* L. extracts were collected from fresh and dry biomass of the plant. A water extract was applied in different concentrations in both petri dishes and in pots

with soil. Fresh biomass extract shows no significant effect germination it had reduced the weed seedling length. Extract in petri dishes from dry biomass showed inhibitory effect. The results showed that the *Aristolochia clematitis* show allelopathic effect of extract in both fresh and dry mass and its inhibitory effect depend on the concentration of water extract (Baličević *et al*., 2015c).

To assess the allelopathic effect of *Sonchus oleraceus* on the seedling growth and germination of *Trifolium alexandrinum*, three weed species (*Melilotus indicus, Brassica nigra* and *Chenopodium murale*) and *S. oleraceus* itself were applied, four different concentrations of aqueous extract of Sonchus oleraceus were applied. It has been observed that all concentrations of the plant extract retard the seedling growth and germination of *Chenopodium murale*. The lowest concentration of extract partially retards the seedling growth and germination of *M. indicus, B. nigra* and *S. oleraceus* while higher concentrations completely retard the growth. It has also been observed that alkaloids and phenols were present in higher amounts in *S. oleraceus* dry matter which was observed through phytochemical analyses. The results show that the aqueous extract of *Sonchus oleraceus* has an allelopathic effect on some weeds and it can be used a bioherbicide (Gomaa *et al*., 2014).

Sorghum bicolor L. (Sorghum) having a strong allelopathic effect and the allelochemical namely sorgoleone which is synthesized in the roots of the sorghum. Phenolic compounds which act as allelochemicals are found in the aerial parts of the *Sorghum bicolor* L. Due to their allelopathic activity sorghum is used under field conditions for controlling weeds. The allelopathic sorghum can also be used as a cover crop, allelopathic sorghum mulch, intercropping of allelopathic sorghum with other crops and the addition of sorghum in a crop rotation. Therefore, allelopathic sorghum

can be used as a bioherbicide for controlling weeds in same crop and with other crops (Jabran, 2017)

In Japan *Ophiopogon japonicas* K. (dwarf lilyturf) has been used as a medicinal plant as well as a cover crop to inhibit the growth of weeds. To evaluate the allelopathic effect of dwarf lilytruf on controlling weeds in transplanted rice experiments was conducted. The aqueous extract which is collected from the underground parts of the *Ophiopogon japonicas* K. retard the seedling growth and germination of three weed species, namely *Bidens biternata* L. (bur-Marigold), *Monocharia vaginalis* P. (monchoria) and *Cyperus difformis* L. (smallflower umbrella). While on low concentration it will stimulate the seedling growth of weed specie *Echinochloa crusgalli* L. (barnyardgrass) and at high concentration, it will inhibit the growth. The result shows that the dwarf lilyturf dry powder considerably retard the growth of the weeds in paddy field without harming the growth of transplanted rice. Therefore, *Ophiopogon japonicas* can be used as a natural herbicide in rice field for controlling weeds (Lin *et al*., 2003).

Experiments were conducted to evaluate the allelopathic effect of *P. harmala* stem, leaf and root extract on growth and germination of *Convolvulus arvensis* L. and *Avena fatua* L. and also identification of allelochemicals which are responsible for this activity. The results show that the high reduction in root length, germination and chlorophyll content in *A. fatua* whereas the inhibitory effect on seedling dry weight and shoot length was more seen in *C. arvensis*. It has also been observed that a great number of phenolic acids were present in *P. harmala* extracts. High phenolic acids were present in leaf extracts as compared to the root or stem extracts. Four phenolic acids were present in root and stem extract while seven phenolic acids were present in leaf extracts. Due to the more presence of phenolic acids in leaf extracts it has stronger inhibitory

effects. From the results it is concluded that the *P. harmala* can be used as a natural herbicide for controlling weeds instead of using harmful synthetic herbicide which pollutes the soil environment (Sodaeizadeh *et al*., 2009).

Phytoplankton species competition and succession is due to allelopathic interaction is regarded is one of the most important contributing factor. To evaluate the allelopathic effect of *Microcystis aeruginosa* on a diatom (*Cyclotella meneghiniana*) and two common green algae (*Chlorella pyrenoidosa, Scenedesmus quadricauda*) by adding exudates in co-culture tests from different growth stages. It has been observed that the exudates of *M. aeruginosa* from stationary phase and exponential growth phase ominously retard the growth of *C. meneghiniana, S. quadricauda and C. pyrenoidosa*. The *M. aeruginosa* presence, particularly suppressed the growth in co-cultures for all tested species. The results show that the *M. aeruginosa* allelopathic effect contribute a greater role in the competitive success of phytoplankton species (Wang *et al*., 2017).

The production of harmful chemicals produced by plants known as allelochemistry which affect the growth and other characteristics of susceptible plant species is an important aspect from point of scientific investigation. It has been proved by recent advances in plant biology that plant to plant communication and plant invasion is greatly influence by the alllelochemistry in the rhizosphere. To understand the molecular and biochemical changes which are brought by the allelochemicals on susceptible plant species recent progress has been made. It will also helpful to understand the complex mechanism to defend the plant species against toxic insult (Weir, 2004).

The ecosystem and agro-ecosystem aspects is significantly influenced by the allelopathic activities. The formation, release, effect on other plant species and environmental fate of allelochemicals depend on environment, genetic factors and their

interaction. This allelopathy phenomena help in developing applications in both agricultural and natural systems. Various studies show the environmental and genetic characteristic that controls the release and formation of allelochemicals in agro-ecosystem. The use of allelochemicals in association with their potential to use in mulches or no-tillage cropping system through cover crops can be a novel application. It has been concluded that the increase in allelopathic information will help in crop management system through weed and pest control which are used as formation of natural herbicide and direct use in the agricultural system (Muzell *et al*., 2016).

To evaluate the allelopathic effect of invasive species *Solidago dignity* Ait. (Giant goldenrod) on initial growth and germination of *Tripleurospermun inodorum* (L.) *C. H. Schultz* (scentless mayweed) and wheat species. Under laboratory conditions, experiments were conducted to investigate the effect of water extracts in pots with soil and in petri dish bioassay. The results show that the germination of wheat was slightly reduced in petri dish bioassay while it inhibits the growth of *T. inodorum* seedling growth and germination at all concentration, especially affected at higher concentration. So *Solidago giganetea* can be used as a natural herbicide to control weeds, especially in wheat cropping system (Ravlić *et al*., 2015).

To check the alleloapathic effect of six medical and cultivated and aromatic plants, chamomile (*Matricaria chamomilla* L.), lemon balm (*Melissa offinalis* L), common mallow (*Malva sylvestris* L.), basil (*Ocimum basilicum*), lovage (*Levisticum officinale* Koch.) and celandine (*Chelidonium majus* L.) on germination and growth of weed species *Tripleurospermum inodorum* (L.) *C.H. Schultz* (scentless mayweed), four experiments were conducted under laboratory conditions. The results showed that the weed seed germination was significantly inhibited in petri dishes with lovage seeds. The less allelopathic effect was seen in pots with soil. The extract of fresh plant shows

inhibitory effect on growth and germination of weed with the extract collected from common mallow and chamomile plants. Extracts collected from dry biomass of common mallow, basil, chamomile, lovage and greater celandine retard the seedling growth and germination of weed at higher concentrations. It has been concluded from all the experiments that extracts from dry plant biomass overall had a highest inhibitory effect, a reduction in weed growth and seed emergence when combined with soil while greater celandine and lovage has the highest allelopathic effect (Baličević *et al*., 2015a).

To evaluate the allelopathic effect and phytochemical study of four medicinal plants *Woodfordia fructicosa*, *Eclipta prostrate*, *Ageratum conyzoides* and *Cannabis sativa*, laboratory tests were conducted. By filtering paper method, aqueous extracts were collected from the root and stem of medicinal plants and applied on two test seeds *Pisum sativum* (pea) and *Triticum aestivum* (wheat). For checking allelopathic effect, seedling growth, germination and biomass production were observed while in phytochemical study checking, qualitative or absence or presence and quantitative or crude amount of the phytochemicals were estimated. The result shows that with the increase of the concentration of the aqueous extract of plants will increase the effect on seedling growth and germination of pea and wheat. It has also been observed that the saponi, phenol, alkaloid, tannin, flavonoid essential oil and glycoside were present in plant extracts (Sharma *et al*., 2015).

The screening of phytochemicals present in a specified medicinal plant through the recognition of bioactive principles is valuable and may lead to new discovery. Twenty-five medicinal plants were selected to check primary phytoconstituents to connect their presence with bioactive of the plants. It was found that the phenolics, tannins, steroids, alkaloids, flavonoids, glycosides, saponins and cardiac were present in the medicinal plants by using standard methods. The results show that the alkaloids

were present in 16 plants while nineteen out of twenty-five plants showed the presence of flavonoids. These phytochemicals play an important role in controlling different activities when released into the soil and it can also be used for better management of cropping system (Nandagoapalan *et al*., 2016).

To evaluate the allelopathic effect of leaf extracts of *Jatropha curcas* on early seedling growth and germination of wheat specie (cv. Inqlab-91), extracts were applied in different concentrations as seed soaking for five hours before sowing seeds in the plots. The results show that the at low concentrations of extracts applied significantly enhanced the germination index, shot fresh and dry weight, shoot length and root dry, fresh weight and root area of the wheat CV. Inqlab-91. Therefore *J. curcas* can be used as a growth stimulator in the fields of the wheat to obtain a good yield of the crop production (Khattak *et al*., 2015).

To investigate the allelopathic effect of *Thuja orientalis* L. on different weed species, essential oils isolated from cones and needles of the plant and analyzed by gas chromatography-mass spectrometry and chromatography- flame ionization method. It was found that twenty-one compounds were present in a needle and cone oils which differ quantitatively. The results show that both oils rich with monterpene hydrocarbons and the major constituents were *α – cedrol, α – pinene and β- phellandrene.* Herbicidal properties checked against three weed specie in crops: *Phalaris paradoxa* L., *Sinapis arvensis* L. and *Lolium rigidum* Gaud. The results showed that *T. orientalis* had a strong inhibitory effect on the selected weed species without harming the crops. Therefore, it can be used as a natural herbicide for controlling these invasive weeds in different cropping system (Ismail *et al*., 2015).

To evaluate the allelopathic effect of *Nicitiana tabacum* L., *Crocus sativus* L., *Nerium oleander* L., *Ricinus communis* L., *Sorghum vulgare* L. and *Datura inoxia* Mill.

On the growth and germination of redroot pigweed (*Amaranthus retroflexus*) experiments were conducted under greenhouse and laboratory conditions. Aqueous extract and powered of these plants was collected. The results show that the all the extracts have a significant effect on weight, germination and seedling length of pigweed plants. The greenhouse results show the significant inhibitory effect on the leaf area, amount of chlorophyll, height and dry weight of pigweed. The inhibitory effect of the powder plant application depends on dosage. The higher the concentration, the greater the inhibitory effect. It has been concluded that the extract and powder of the above tested plants can be used against redroot pigweed and it can also be used as a natural herbicide (Nekonam *et al*., 2014).

To investigate the allelothic effect of *Matricaria chamomilla* L. (chmomile) on the initial growth and germination of weed specie *Cardaria draba* L. (hoary cress), experiments were conducted under laboratory conditions. To determine the effect of chomamile water extracts collected from dry and fresh chamomile biomass in concentrations of 5% and 10%. The results showed that there was no significant impact on the growth while it showed inhibitory effects on weed seed germination. The extracts from dry and fresh chamomile biomass showed inhibitory effects on fresh weight, germination, shoot and root length of hoary cress. It has also been observed that the 10% concentration of the chamomile biomass completely inhibits the root and shoot length and fresh weight of weed. It has been concluded that chamomile can be used as a natural herbicide for controlling the weeds in agricultural cropping system (Baličević *et al*., 2014).

Experiments were conducted to check the allelopathic effects of *Eclipta alba* weed on seedling growth and seed germination weed plants (*Cassia sophera* L., *Cassis tora* L.) and crop plants (*Oryza sativa* L., *Phaseolus aureus* L.). Aqueous extract of leaf,

root and stem were applied in different concentrations under laboratory conditions. The results show that the aqueous extract from leaf, stem and root have no effect on seed germination of test plants. While the aqueous extract from all parts show an inhibitory effect on dry weight, root and shoot length of the test plants. Furthermore, the dry biomass shows the strong allelopathic effect on weeds as compared to crops. It has been concluded that the dry biomass of these plants can be used for control of weeds which are growing in crops (Gulzar and Siddiqui, 2014).

2.2 Allelopathic effect of various plants on soil

The invasive *Acacia dealbata* had changed the soil characteristics due to the release of allelopathic compounds. The seedling growth of *Acacia dealbata* in native soil had been affected negatively by its leachates and found no interaction between *Acacia dealbata* leachate and soil microbiota. Soil microbiota had shown a positive effect on the initial growth of *Acacia dealbata* but decreased the seedling growth (Lorenzo and Rodríguez-Echeverría, 2012).

It has been assessed that *M. littorea* had no competition with *C. edulis*. But, the litter of invasive *C. edulis* had ability to stay on soil surface for many years and suppress the early plant growth and native plant germination process due to the release of allelopathic substances. The invasive species colonize the sand dunes in a much better manner than native species. The results highlighted that the dune restoration projects invaded by *C. edulis* were failed and future restoration projects should take into account the long lasting effects of *C. edulis* (Novoa *et al*., 2012).

It has been studied the allelopathic effect of *Rehmannia glutinosa* L. continuous monoculture pattern of rhizosphere and change in the diversity of microflora. The structure of fungal community in monoculture rhizosphere soil was entirely different

from control soil. Furthermore, *R. glutinosa* has ability to decrease the diversity of fungal community in soil (Zhang *et al*., 2011).

The degree of interaction with the roots define the magnitude of the effect of intraspecific variation in plant on the soil organism. The root glucosinolate profile had faced a high degree of intraspecific variation in white cabbage (*Brassica oleracea* var.capitate). The decomposer organisms, including earth worms and Collembola were not affected while root-feeding nematodes were affected by intraspecific variation. The lack of glucosinolate gluconasturtiin in Badger Shipper favors the abundance of the root-feeding nematodes due to rapid degradation of glucosinolates in soil. The low biomass of root-feeding nematodes as compared to other soil organisms restricts the effect on the higher tropic level organisms. The belowground herbivores were predominantly affected by variation in root chemistry (Kabouw *et al*., 2010).

The involvement of arbuscular mycorrhizal fungi interacts with soil biota and changes the dynamics of abundance. The mycorrhizal abundance of surrounding soil was reduced by *Brassica nigra* genotype along reduction in the growth of heterospecific competing due to the allelopathic chemicals (Lankau *et al*., 2011).

Allelopathic effect plays a vital role on mineral content (both macro and micro elements) of the soil. An experiment was conducted to check the allelopathy of *Prosopis juliflora* on *Acacia ehrenbergiana* for both species through mineral analysis of both rhizosphere soil and leaf extract. It was examined that the decreased in all mineral content in combined soil. Maximum mineral uptake is present in Fe^{+2} and Ca^{+2} in Prosopis juliflora invasive plant. Mg^{+2} leads to more reduction due to severe competition on essential elements. It also shows that macro and microelements may be responsible, successful establish and dominance of *Prosopis juliflora* in invading the area (El-Shabasy, 2017).

Two populations of autumn olive found throughout the Midwestern United States, which is highly invasive, nitrogen fixing shrub were studied. Soils were evaluated for moisture, microbial community composition, moisture, pH and enzyme activity. The result shows that the autumn olives significantly affected dominant taxa of archaeal and bacterial communities, specified ammonia- oxidizing microorganisms. These variations in microbial communities were associated local density of autumn olive shrubs. The conclusions show that soil microbial community can be affected by the presence of autumn olive though it has presented in Our findings suggest that autumn olive can affect soil microbial community composition, even when it is present in comparatively low densities (Malinich *et al*., 2017)

Due to several soil borne diseases three is the problem of seedling establishment is found in tomato and one of these diseases is root rot which is caused by *Fusarium oxysproum*. To control this disease, many chemical methods are present, but the use of these chemicals will lead soil and water pollution and deplete the soil micro-environment. Leaf extract of six different plants (Neem, Lantana, White musale, Wood apple, Periwinkle and White cedar) was applied on tomato. The result shows that the priming with the Perwinkle, wood apple, leaf, Neem and White musale extract has a positive effect on tomato growth even in the presence of pathogen. Therefore, the use of these plants can be as a natural growth promoter in tomato where there is the problem of these pathogens and also conserve soil from the depletion of these chemicals which are used to control these pathogens (Prabha *et al*., 2016).

During the secondary metabolism of various parts of plants essential oils are naturally produced which are complex volatile compounds. A large of plants have been explored worldwide with medicinal properties and used for the purpose of extraction of essential oils due to their antimicrobial activities against the fungal, bacterial and viral

pathogens. Essential oils become more précised in their mode of action due to the presence of a large number of phenols, antimicrobial compounds, alkaloids and other terpenes derivatives against the abundant diversity of pathogenic microorganisms. Therefore, essential oils can be used as good replacement or substitution against harmful microorganisms. So the essential oils from medicinal plants play a vital role in inhibition of pathogenic microorganisms and can be used as a natural herbicide against pathogenic microorganisms to conserve the soil environment (Akthar *et al*., 2014).

2.3 Allelopathic effect of some plants on soil microorganism

The activity of Coumarin has been demonstrated against weeds in in- vitro conditions. In each experimental system and treatment, plant growth (mass and length of root and shoot), fungal and bacterial genetic diversity and soil microbial activity and mass has been determined. The results show that the coumarin can be used as a promising natural herbicide and have a potential to apply in sustainable agriculture (Niro *et al*., 2016).

The relationship among microbes control the occurrence of unsafe algal blooms which intimidate water quality. These interactions can be influenced by the release of allelochemicals. The research has been conducted to investigate the seasonal changes among bloom forming cyanobacterium *Microcystis aeruginosa* and benthic microbial assemblages. The result shows that the allelopathy and phosphorus (P) competition by the microbial muster differ seasonally and inhibit growth of *M. aeruginosa.* It has been observed that the strong interaction was present per unit biomass of the microbial assembly in winter as compared to summer. This shows that in summer condition the recruitment of cyanobacteria is inhibited. The allelopathy can be applied to adjust the aquatic ecosystem function and structure by implying allelopathy of microbial assembly (Wu *et al*., 2017).

Tagetes minuta exhibits a wide range of biological activities against nematodes, insects, microbes and it is also an aromatic plant. Experiments were conducted to check the allelopathic effect of *T. minuta* on invasive weeds – *Phalaris minor* Retz. *Chenopodium murae* L. and *Amaranthus virdis* L. The results showed that the volatile oil of *T. minuta* considerably reduced the growth, respiratory ability, germination and chlorophyll content of weeds in a dose dependent manner. It had been concluded that the volatile oil of *T. minuta* had a strong allelopathic effect on weed species and it can be used as a bio herbicide for weed management (Arora *et al*., 2015).

To investigate the phytochemical and antimicrobial activities of *Adiatum capillus veneris*, extracts from the form the roots, leaves and stems of *Adiatium capillus veneris* was extracted with ethyl acetate, methanol, hexane and ethanol and screened for their antimicrobial activity against five fungal and ten MDR bacterial strains. The phytochemical analysis showed the presence of alkaloids, terpenoids, saonins, flavonoids, tannins, steroids, cardiac glycosides and sugar reducing agents. The results show that the methanol, water and ethanol extracts of stems, leaves and roots showed significant antifungal and antibacterial activities against most of the MDR bacterial and fungal strains. It has been concluded that the *Adiatum capillus veneris* can be used against harmful bacteria and fungi present, which damage the crop production (Ishaq *et al*., 2014).

2.4 Anti-microbial activities of allelopathic plants

The use of the plants as antifungal as an alternative to synthetic fungicide is an important aspect which is currently in the spotlight. To check the antifungal activity, chemical analysis of 16 essential oils was determined. Through in vitro microdilution method antifungal properties were evaluated against 21 fungi which were isolated from herbal drugs. The results show that the all oils show antifungal effect against all fungi

used. It has also been observed that oil containing mainly monoterpene alcohols had been more effective and inhibited all fungi tested. Furthermore, it had been decreased the total number of fungi, in situ, using essential oil was evaluated (Stević *et al*., 2014).

The secondary products present in plants comprise of different metabolites and reflects the beneficial effects of medicinal plants. The medicinal actions of plants are considered as a unique phenomenon attributed to a taxonomically distinct and specific plant species having a consistent release of combination of secondary products. The screening of the plant species was carried out by ethnobotanical approach, known as a common technique to study antifungal and antimicrobial activities in pharmacology. Several plants have shown antifungal and antibacterial activities (Saranraj and Sivasakthi, 2014).

To evaluate antimicrobial and antioxidant activity of *Arisaema jacquemontii* roots the methanolic extract has been utilized. Three types of assays, including *nitroblue tetrazolium* (NBT), ferric reducing power tests and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) had shown substantial antioxidant activity with values 62.17± 0.17 % (NBT), reduced ferricyanide complex (Fe^{3+}) to ferrous form and 64.16±0.19 (DPPH). The broth dilution method demonstrated the minimum inhibitory concentration (0.24-0.41 mg/ml) and antibacterial activity by inhibiting the growth of bacteria (Gram-negative and Gram-positive). The growth inhibition of mycelium revealed antifungal activity. The flavonoid and phenolic content in plant extract was clearly correlated with positive antioxidant and antimicrobial activities (Baba and Malik, 2015).

The ethanolic and aqueous extract from leaves of *Moringa olefera* were applied to perform phytochemical analysis to determine antifungal activity through well diffusion method using *Candida albicans, Candida tropical* and *Saccharomyces*

cerevisiae strain. Many allelochemicals including saponins, alkaloids, tannins, flavonoids and steroids were in both extracts during phytochemical screening. Moreover, it was also observed that the antifungal activity of *the Moringa olefera leaf* was strong against *Saccharomyces cerevisiae*, strong against *Candida tropical, but* no activity was detected against *Candida albicans (*Patel, 2014).

The bioactive compounds present in medicinal plants are causing substantial endophytic microbes. The endophytic bacteria were isolated from *Hypericum perforatum* and *Ziziphora capitata,* these medicinal plants were collected from the Chatkal Biosphere Reserve of Uzbekistan. A notable activity against bacterial and fungal pathogens was evident from *Hypericum perforatum* extract, but *Ziziphora capitate* did not express any antimicrobial activity. The analysis through Matrix-assisted laser desorption ionization (MALDI) and time-of-flight mass spectrometer revealed endophytes from *H. perforatum* belong to Bacillus, Pantoea, Achromobacter, Enterobacter, Stenotrophomonas, Pseudomonas, Arthrobacter and Erwinia and from Z. capitate belong to Bacillus, Pantoea, Erwinia, Achromobacter and Pseudomonas. The antagonistic isolates potentially control the tomato root rot resulted from *Fusarium oxysporum*, stimulate growth, and cost-effective biological control under greenhouse conditions (Egamberdieva *at al*., 2017).

CHAPTER 3

3. RESEARCH METHODOLOGY

3.1. Study Area

The plant samples were collected from Islamabad and its vicinity (Baragali, Margalla Hills, Murree, and Barahkoh), Rawalpindi, Azad Jammu and Kashmir (AJK) (Rawalakot and Bagh). The experimental work was performed at Ecology Laboratory, Environmental Laboratories Complex, Department of Environmental Science, International Islamic University, Islamabad and National Agriculture Research Centre (NARC), Islamabad.

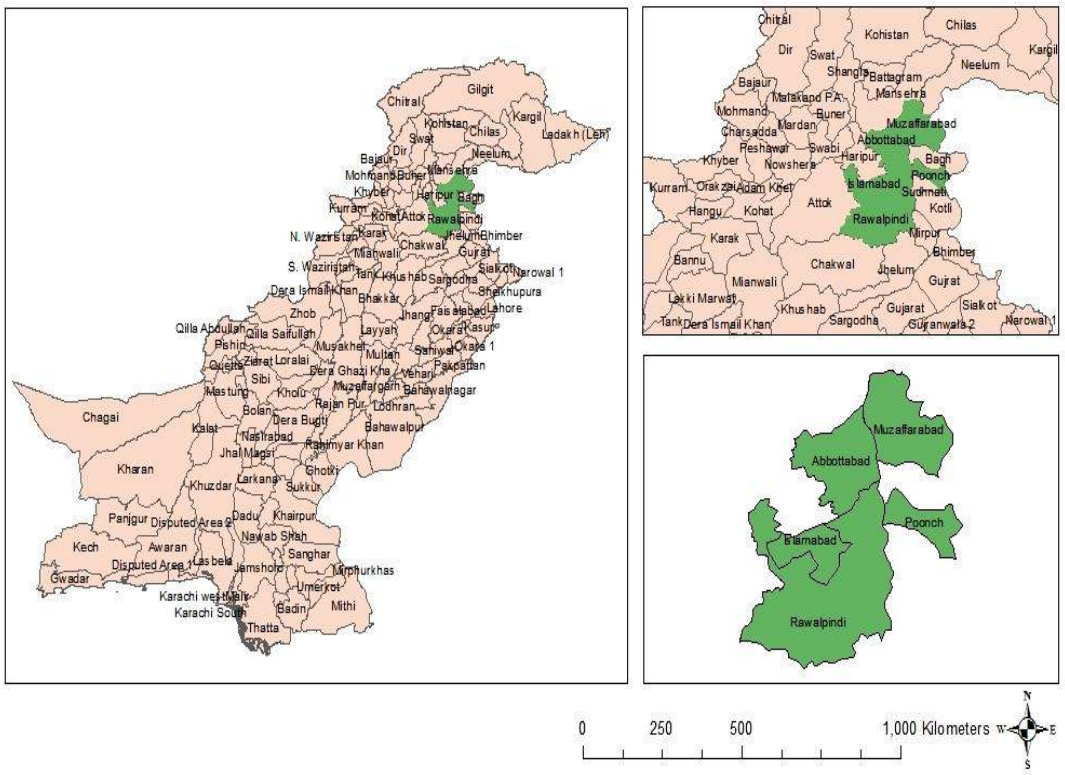

Figure 3.1 Map of the study areas

3.2. Materials And Instruments

- Plant samples
- *Lactuca sativa* Seeds (TAKI II Seed G – LEO 1)
- *Triticum aestivum* seeds (Pakistan 2013)
- *Zea mays* seeds (Islamabad Gold)
- Petri dishes
- 6 Well multidishes
- Muslin cloth
- Funnel
- Beakers
- Reagent bottles
- Electric grinder
- Optoelectronical shaker
- Distilled water fully automatic (HWSFA30)
- Filter paper (Whatman No.1)
- Biosafety cabinet (RSBCA 115 MSC Class: II)
- B.O.D. Incubator (NB – 2201 LF)
- Oven (BJPX-SUMMER)
- Vertical autoclave fully automatic (CLASSIC 1050)
- Loading balance (KERN PCB 350 - 3)
- Haier refrigerator (HRF – 420 FDX)
- Ceramic Tweezer
- Agar, powder (Code 01059-85)
- Chemicals (NaOH, $HgCl_2$)
- Gloves

- Cellophane tape
- Aluminium foil
- Adjustable air-displacement pipette (Z58141N)
- Sterilized syringes
- Polythene bags
- Soil (NARC)
- Paper bags and newspapers
- Logarithmic graph

3.3. Research Methods

3.3.1. Sample collection and preparation

A total of 230 medicinal plants was collected from various locations enriched with plant species. The study focused on collection of medicinal plants from Islamabad and its vicinity Rawalpindi, Azad Jammu and Kashmir (AJK) (Rawalakot and Bagh). The fresh samples were collected, separately placed in paper bags, labelled. The samples were transported to Ecology Laboratory, Environmental Laboratories Complex for further experimentation. A herbarium sheet of each plant sample was developed for the identification and then oven dried at 60°C for 48 hours. The samples were stored in air tight box to avoid contamination and ensure chain of custody. An analysis of the allelopathic potential of collecting plant species was performed through leaf litter analysis using Sandwich and Dish Pack method (Fuji, 2004; Shinwari *et.al*, 2013; Appiah *et al*., 2015).

3.3.2. Sandwich Method

The agar medium was prepared in a beaker by adding 7.5 g agar powder in 1000 ml of distilled water. The beaker was placed in an autoclave at a temperature of 120 °C, 115 pa pressure for 20 minutes as well as cooled down to 40 °C (Appiah et al., 2015). The dried plant sample (10 or 50 mg) was added in 5 ml plus 5 mlagar, two layers in each well of a multi-well plastic plates (six wells. Furthermore, 5 seeds of Lettuce (*Lactuca Sativa* Var.) were vertically placed in each well. The plates were concealed with plastic tape, labelled, and wrapped with aluminum foil for incubation in dark condition (Fujii et al., 2004) (Fig. 3.2). The sandwich method is known as productive and reported a technique to evaluate to allelopathic effect under laboratory condition. This study screened 207 medicinal plants as well as determine allelopathic activity of leachates from collected donor plant's leaves and a control (only Lettuce in agar). The experiments were replicated three times to ensure accuracy and average values represent reported data. An incubator (BGPX/Summer) containing multi-plastic tubes were used at 25 °C for 72 hours for incubation, which followed by hypocotyl and radicle length measurement (Shiraishi et al., 2002; Fujii *et al*., 2003; Fujii *et al*., 2004; Morikawa *et al*., 2012; Shinwari et al., 2010; Appiah *et al*., 2015).

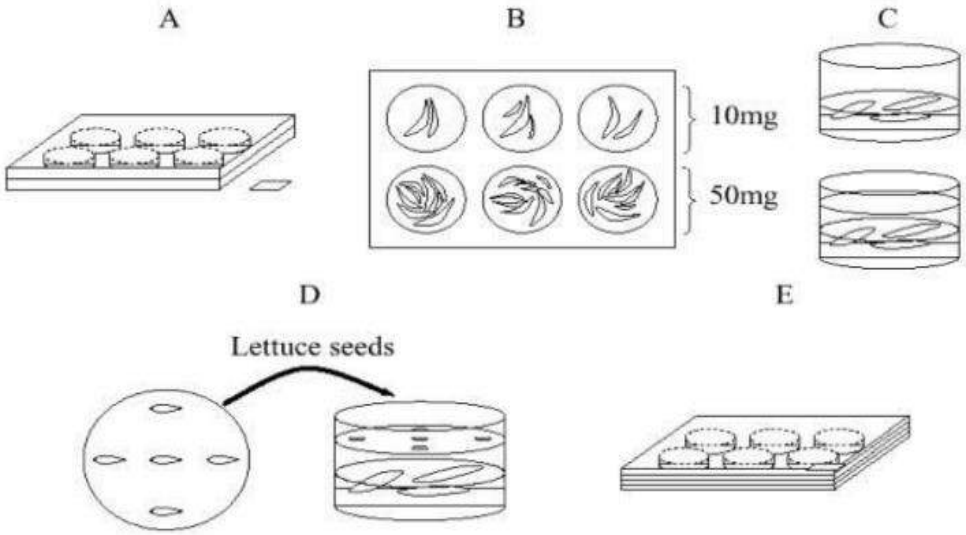

Figure 3.2. Sandwich Method to evaluate allelopathic activity of leachates from the plant's leaves

3.3.3. Dish pack method

The presence of volatile allelochemicals released from plant species has achieved attention in recent research. The dish pack method has been adopted as a valuable technique to determine the presence of volatile allelochemicals from plants. This study screened 221 medicinal plant species and evaluated them for possible allelopathic effects due to volatile substances. The experimentation was carried out in multi-well plastic dishes (6 well) using distilled water, plant material and lettuce seeds. The distances between the well containing plant sample and other wells were 41, 58, 82 and 92mm respectively (Fig. 3.3). The distilled water (0.75 ml) was enumerated in each wells having filter paper except source well, which contain only 200 mg of oven-dried plant material. Alike sandwich method, control sample did not behold any plant sample. In each well, seven lettuce seeds were implied in the filter paper. The multi-well dishes were wrapped with aluminum foil, tightly sealed with cellophane tape (circumvent of volatile substances) and kept in incubator at 25°C for 72 hours. The hypocotyl and radicle lengths were recorded after the incubation to assess growth

inhibition and stimulation as well as determine the degree of inhibition (Fujii *et al*., 2005; Amini *et al*., 2014; Halimshah *et al*., 2016; Nurul *et al*., 2016; Gilani *et al*., 2017)

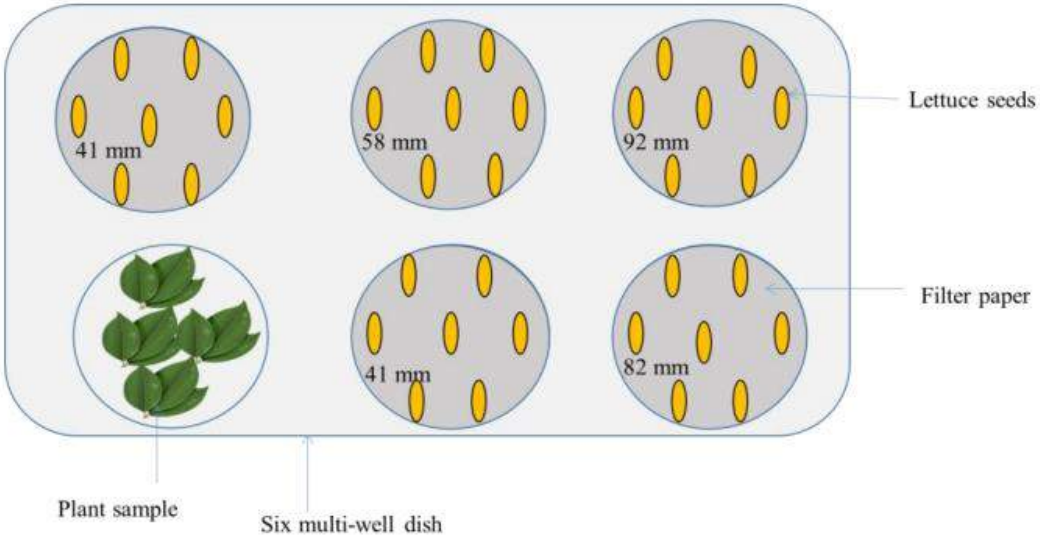

Figure 3.3 View from top of Multi-well plastic plate used to test for plant allelopathy through volatile substances

3.3.4. Statistical analysis

The results have three replications, randomized design and acquire statistical analysis. This study had evaluated the means, standard deviation, variance (SDV) and standard deviation (SD) using Microsoft Excel 2016. The equations used are given below:

$$\text{Inhibitory } \% = 100 - \frac{\text{Average length of treatment radicle/hypocotyl}}{\text{Average length of control radicle/hypocotyl}}$$

$$\text{Elongation}\% = \frac{\text{Average length of treatment radicle/hypocotyl}}{\text{Average length of control radicle/hypocotyl}}$$

3.3.5. Method of aqueous extract preparation and application on crops

Fresh and clean leaves of nine plants (*Boerhavia procumbens* Banks ex Roxb., *Jacaranda mimosifolia* D. Don , *DaturaDatura metel* L., *Asparagus adscendens* Roxb. , *Plectranthus rugosus* Wall. exBenth., *Parthenium hysterophorus* L., *Arisaema triphyllum* (L.) Schott, *Crotalaria medicaginea* DC. and *Amaranthus viridis* L) were collected from different regions of Pakistan (Islamabad, Baragali, Murree, Rawalakot Azad Jammu and Kashmir). The distilled water was used to wash the leaves followed by dry them in the shade, and grinding into fine powder by electronic grinder. Aqueous extract was prepared by mixing distilled water (1000 ml) with dry powdered plant biomass (100 g) of each plant and retained at room temperature for 24 hours (Norsworthy, 2003). The debris was removed by filtration through muslin cloth and filter paper. The final concentration of 10% (100 g/l) was prepared by the dilution with distilled water. The final aqueous extract was applied directly on wheat, maize and lettuce seeds in petri dishes

The sterilized filter papers (Whatman No.1) were placed in sterilized petri dishes for wheat, maize, lettuce and control respectively. The seeds (sterilized with 0.1% with $HgCl_2$) of *Zea mays* L. (Islamabad Gold), *Triticum aestivum* L. (Pakistan 2013) and *Lactuca sativa* L. were obtained from National Agricultural Research Centre (NARC). During the experiment, 15 seeds of *Triticum aestivum* L. and *Lactuca sativa* L. and 12 seeds of *Zea mays* L. were placed in petri dishes followed by direct exposure of aqueous extract of each selected plant species. Meanwhile, the control was germinated by distilled water. Finally, the petri dishes were placed in an incubator at a temperature (24 °C) for eight days. The calculations of growth, including germination percentage, shoot and root length after 8 days (Kasarkar and Barge, 2016; Norsworthy, 2003).

Afridi and Khan, (2015) tested the allelopathic effects of aqueous extract of *Parthenium hysterophorus, Phragmites australis, Datura alba*and *Oryza sativa* on wheat and weeds in soil medium (pot experiment). However, we have performed the experiment in 6 well multi dishes using the same procedure. During the experiment, 10 seeds of each species (Seeds *Zea mays* L., *Triticum aestivum* L. and *Lactuca sativa* L.) were planted in 6 well multi dishes with soil. The multi dishes were watered with aqueous extract of each selected plant species, while the control was irrigated with distilled water for eight days at room temperature in overall three time replications. The calculations of germinated seeds were performed on a daily basis while hypocotyl and the radicle length of the seeds were calculated over 8 days and compared with the control.

CHAPTER 4

4. RESULTS AND DISCUSSION

4.1. Allelopathic effect of medicinal plants using dish pack method

Medicinal plants have served humanity for cure of diseases for centuries (Hussain *et al*., 2014). Besides the treatment of diseases, they have a potential of natural herbicides, weed management, sustainable and climate adaptive conservation of biodiversity due to release of bioactive compounds (Appiah *et al*., 2015). Many secondary metabolites are released from medicinal plants which resulted in frequent allelopathic activities (Fujii *et al*., 1990). The seedling growth of lettuce was inhibited due to strong allelopathic effects of medicinal plants, including the families Myrtaceae, Apocynaceae, Guttiferae, Annonaceae, Leguminosae, Rubiaceae and Euphorbiaceae (Fujii *et al*., 2003). Moreover, the phytotoxic effects of several medicinal plant species on germination and seedling growth of other plant species have been reported in previous studies. The factor utilization of allelopathic effect can control weed management and hamper transformation from adverse effects to beneficial effects (Fujii *et al*., 2003; Han *et al* 2008; Islam and Kato-Noguchi 2013; Sodaeizadeh *et al*., 2009; Appiah *et al*., 2015).

The allelochemicals from the medicinal plant restricts the growth of other plants due to their disruption potential in hormonal balances, water uptake rate and physiological process (Cheng and Cheng, 2015). The aqueous extracts of *Picrorhiza kurroa, Ocimum sanctum, Asperagus racemosus* and *Valeriana wallichii* (medicinal plants) had decreased the seed germination of oilseed crops due to a strong inhibitory effect of allelochemicals (Rawat *et al*., 2016). The release of allelochemicals follows

numerous mechanisms, including leaching from aerial parts, decomposition of plant biomass into soil, exudation from roots and volatilization (Latif *et al*., 2017). The dish pack method can effectively identify the allelopathic effects of volatile compounds from plants on lettuce (Appiah *et al*., 2015). The medicinal plants (221) were collected from different regions of the country, identified their families, genera, and specie as well screen them prior to assess allelopathic effects. The allelopathic effect of 221 medicinal plants were also tested and enlisted them based on their allelopathic effect.

The lettuce seeds were exposed to volatile compounds released from 221 plants to assess inhibitory or stimulatory effect in 6 well multi-dishes contained the dry leaves (oven dried) and distilled water. Additionally, the control was grown without plant material in a dish. The detail and allelopathic effect of all selected plants (221) is demonstrated in Table 4.1. The results revealed that the volatile compounds of different plants had exhibited inhibitory or stimulatory effect on the germination and growth of the hypocotyl and radicle of the lettuce in a different manner. The seedling growth of lettuce was completely inhibited by one specie, 3 species reflected strong inhibition, 8 species indicated medium inhibition, 18 species revealed least inhibitory effect and 112 species shown insignificant allelopathic effects. Contemporary, 64 species least promoted the growth while 15 species presented strong stimulatory allelopathic effect on lettuce seeds. The complete inhibitory effect was noticed by *Boerhavia procumbens* Banks ex Roxb. (Nyctaginaceae) while strong inhibitory effect was observed by *Parthenium hysterophorus* L., *Arisaema triphyllum* (L.) Schott. and *Crotalaria medicaginea* DC. followed by medium inhibitory effect from *Amaranthus viridis* L., *Xanthium strumarium* L., *Phalaris minor* Retz., *Ficus microcarpa* L.f., *Woodfordia fruticosa* (L.) Kurz, *Plantago lanceolate* L., *Oxalis corymbosa* DC and *Tabernaemontana corymbosa* (variegated) .However, *Ficus virgate* Reinw. ex Blume, *Helicteris isora* Linn.,

Debregeasia salicifolia (D. Don) Rendle, *Fragaria vesca* L., *Adiantum capillus*-veneris L., *Polygonum persicaria* L., *Jasminum sambac* L., *Ficus carica* L., *Plantago major* L., *Mentha arvensis* L., *Ficus religiosa* L., *Rumex hastatus* D.Don, *Viburnum cotinifolium* D.Don, *Geranium pusillum* L.and *Plectranthus rugosus* Wall. ex Benth.exhibited strong stimulatory allelopathic effect on lettuce radicle growth as copmpared to control. It is notable to mention that *Plectranthus rugosus* had indicated an exceptional stimulatory allelopathic effect on radicle growth of lettuce.

Table 4.1 Allelopathic potential assessment of 221 medicinal plants using dish pack method from Pakistan

Sr. No.	Species Name (Scientific)	Family	Extension (%) †		Crite-rion‡
			Radicle	Hypocotyl	
1.	*Boerhavia procumbens* Banks ex Roxb.	Nyctaginaceae	0	0	****
2.	*Parthenium hysterophorus* L.	Asteraceae	48.36	93.85	***
3.	*Arisaema triphyllum* (L.) Schott.	Araceae	49.64	95.057	***
4.	*Crotalaria medicaginea* DC.	Fabaceae	49.90	89.68	***
5.	*Amaranthus viridis* L.	Amaranthaceae	52.71	113.79	**
6.	*Xanthium strumarium* L.	Asteraceae	52.95	113.71	**
7.	*Phalaris minor* Retz.	Poaceae	53.67	70.59	**
8.	*Ficus microcarpa* L. f.	Moraceae	55.73	104.16	**
9.	*Woodfordia fruticose* (L.) Kurz	Lythraceae	56.12	99.82	**
10.	*Plantago lanceolate* L.	Plantaginaceae	58.03	82.47	**
11.	*Oxalis corymbosa* DC	Oxalidaceae	59.02	114.54	**
12.	*Tabernaemontana corymbosa* (variegated)	Apocynaceae	60.39	72.22	**
13.	*Urtica dioica* L.	Urticaceae	61.75	85.91	*
14.	*Cirsium arvense* (L.) Scop.	Asteraceae	62.37	99.66	*
15.	*Phoenix sylvestris* (L.) Roxb.	Arecaceae	63.41	109.71	*
16.	*Taraxacum officinale* L.	Asteraceae	63.98	104.59	*
17.	*Pentanema divaricatum* Cass.	Asteraceae	65.36	96.95	*
18.	*Delphinium aquilegifolium* (Boiss.)Bornm.	Ranunculaceae	65.47	78.17	*
19.	*Trichodesma indicum* (L.) Lehm.	Boraginaceae	65.76	107.14	*
20.	*Acacia modesta* Wall.	Fabaceae	66.39	89.409	*

21.	*Achillea millefolium* L.	Asteraceae	66.78	97.90	*
22.	*Mallotus philippensis* (Lam.) Mull. Arg	Euphorbiaceae	67.05	101.30	*
23.	*Berberis lyceum* Royle	Berberidaceae	67.68	89.35	*
24.	*Plantago ovata Forssk.*	Plantaginaceae	68.50	66.31	*
25.	*Beaucarnea recurvata* lem.	Asparagaceae	68.77	103.67	*
26.	*Lantana camara* L.	Verbenaceae	69.36	122.43	*
27.	*Asparagus recemosus* Willd.	Asparagaceae	69.97	114.20	*
28.	*Salvia moorcroftiana* Wall. ex Benth.	Lamiaceae	70.59	81.39	*
29.	*Viola odorata* L.	Violaceae	70.96	132.11	*
30.	*Jasminum officinale* L.	Oleaceae	72.17	74.82	*
31.	*Commelina benghalensis* L.	Commelinaceae	73.35	74.15	
32.	*Dicliptera bupleuroides* Nees	Acanthaceae	74.15	93.75	
33.	*Solandra maxima* (Sesse &Moc.) P.S. Green	Solanaceae	74.34	97.58	
34.	*Murraya paniculata* (L.) Jack	Rutaceae	74.43	107.40	
35.	*Berberis vulgaris* L.	Berberidaceae	74.43	107.93	
36.	*Mirabilis jalapa* L.	Nyctaginaceae	74.60	147.88	
37.	*Flacourtia indica* (Burm. f.) Merr.	Salicaceae	75	90.82	
38.	*Solanum surattense* Burm. f.	Solanaceae	76.08	89.62	
39.	*Rosa brunonii* Lindl.	Rosaceae	76.63	95.36	
40.	*Myrsine Africana* L.	.Primulaceae	76.67	96.66	
41.	*Aloe barbadensis* mill.	Asphodelaceae	77.65	106.57	
42.	*Barleria cristata* L.	Acanthaceae	77.68	95.56	
43.	*Bryophyllum pinnantum* (Lam.) Kurz	Crassulaceae	78.65	112.96	
44.	*Convolvulus arvensis* L.	Convolvulaceae	79.42	105.51	
45.	*Oxalis sect. Corniculatae* R. knuth	Oxalidaceae	79.69	111.20	
46.	*Duranta erecta*L.	Verbenaceae	79.84	88.02	
47.	*Canna X generalis* L.H. Bailey	Cannaceae	79.84	84.50	
48.	*Salix babylonica* L.	Salicaceae	79.92	89.35	
49.	*Androsace sarmentosa* Wall	Primulaceae	80.35	98.79	
50.	*Melilotus indicus* (L.) All.	Fabaceae Lindl.	80.62	107.25	
51.	*Ranunculus repens* L.	Ranunculaceae	81.10	102.89	
52.	*Cascabela thevetia* (L.) Lippold	Apocynaceae	81.15	95.07	
53.	*Amaranthus hybridus* L.	Amaranthaceae	81.22	94.10	

54.	*Silene vulgaris* (Moench) Garcke	Caryophyllaceae	81.84	92.78	
55.	*Euphorbia helioscopia* L.	Euphorbiaceae	82.17	73.79	
56.	*Geranium wallichianum* D.Don ex Sweet, Geran.	Geraniaceae	82.42	101.17	
57.	*Carrisa opaca* Stapf ex Haines	Apocynaceae	82.50	88.33	
58.	*Micromeria biflora* (Buch – Ham.Ex D. Don) Benth	Lamiaceae	82.96	96.15	
59.	*Sida alba* L.	Malvaceae	83.17	132.89	
60.	*Dodonaea viscosa* (L.) Jacq.	Sapindaceae	83.33	91.50	
61.	*Quisqualis indica* L.	Combretaceae	83.76	96.83	
62.	*Zanthoxylum armatum* DC.	Rutaceae	83.81	80.93	
63.	*Robinia pseudoacacia* L.	Fabaceae	84.06	120.40	
64.	*Opuntia monacantha* Haw.	Cactaceae	84.16	99.166	
65.	*Schefflera arboricola* Hayata	Araliaceae	84.26	88.54	
66.	*Sida cordata* (Burm. f.) Bross. Waalk	Malvaceae	84.27	105.50	
67.	*Cyperus rotundus* L.	Cyperaceae	84.66	110.62	
68.	*Taverniera cuneifolia* (Roth) Arn.	Fabaceae	84.74	103.97	
69.	*Oenothera rosea* L' Hér. ex Aiton	Onagraceae	84.82	97.93	
70.	*Cassia fistula* L.	Fabaceae	85.24	92.013	
71.	*Cornus macrophylla* Wall.	Cornaceae	85.56	115.97	
72.	*Lagerstroemia indica* L.	Lythraceae	85.67	111.11	
73.	*Saccharum spontaneum* L.	Poaceae	85.87	90.40	
74.	*Bauhinia variegate* L.	Fabaceae	86.01	125.89	
75.	*Broussonetia papyrifera* (L.) L'Hér. ex Vent	Moraceae	86.06	103.29	
76.	*Quercus dilatata* Lindl.	Fagaceae	86.30	84.18	
77.	*Geranium* L.	Geraniaceae	86.38	112.67	
78.	*Conyza Canadensis* (L.) Cronquist	Asteraceae	86.38	91.54	
79.	*Asparagus adscendens* Roxb.	Asparagaceae	86.66	95	
80.	*Acacia nilotica* (L.) Willd. ex Delile	Fabaceae	86.73	105.94	
81.	*Psammogeton biternatum* Edgew.	Apiaceae	86.83	91.32	
82.	*Verbascum thapsus* L.	Scrophulariaceae	86.83	96.86	
83.	*Dioscorea deltoidea* Wall. ex Griseb.	Dioscoreaceae	86.86	90.17	
84.	*Glycine clandestine* J.C. Wendl.	Fabaceae	86.86	95.53	
85.	*Vicia sativa* L.	Fabaceae	87.5	96.66	
86.	*Rosa indica* L.	Rosaceae	87.69	86.26	
87.	*Eurphobia royleana* Boiss.	Euphorbiaceae	87.83	91.46	

88.	*Beaucarnea recurvata* lem.	Asparagaceae	89.65	106.11	
89.	*Duranta erecta* (Variegata)	Verbenaceae	89.88	87.03	
90.	*Dichanthium annulatum* (Forssk) Stapf	Poaceae	89.92	96.33	
91.	*Justicia adhatoda* L.	Acanthaceae	90	95	
92.	*Asphodelus tenuifolius* Cav.	Asphodelaceae	90.25	98.85	
93.	*Jatropha integerrima* Jacq.	Euphorbiaceae	90.31	109.15	
94.	*Indigofera tinctoria* L.	Fabaceae	90.77	101.19	
95.	*Carthamus oxyacantha* M. Bieb.	Asteraceae	91.13	122.82	
96.	*Euphorbia cotinifolia* L.	Euphorbiaceae	91.29	129.62	
97.	*Duchesnea indica* (Andrews) Teschem	Rosaceae	91.51	98.79	
98.	*Datura stramonium* L.	Solanaceae	92.02	93.75	
99.	*Coriandrum sativum* L.	Apiaceae	92.13	108.88	
100	*Duranta plumieri* Jacq.	Verbenaceae	92.69	100	
101	*Rubus fruticosus* L.	Rosaceae	93.01	102.04	
102	*Polypodium virginianum* L.	Polypodiaceae	93.22	62.5	
103	*Poa annua* L.	Poaceae	93.28	98.48	
104	*Digera muricata* (L.) Mart	Amaranthaceae	93.68	100.76	
105	*Ranunculus arvensis* L.	Ranunculaceae	93.75	96.08	
106	*Chenopodium ambrosiodes* L.	Amaranthaceae	93.81	110.46	
107	*Dalbergia sissoo* Roxb. ex DC.	Fabaceae	94.24	105.63	
108	*Cassia fistula* L.	Fabaceae	94.28	105.07	
109	*Elettaria cardamomum* (L.) Maton	Zingiberaceae	94.97	109.53	
110	*Lantana indica* Roxb.	Verbenaceae	95	106.66	
111	*Campsis radicans* (L.) Bureau	Bignoniaceae	95.51	112.96	
112	*Aesculus indica* (Wall. Ex Cambess.) Hook	Sapindaceae	95.69	95.28	
113	*Artemisia scoparia* Waldst. & Kit.	Asteraceae	95.72	100	
114	*Pteris cretica* L.	Pteridaceae	95.72	94.61538	
115	*Abies pindrow* (Royle ex D. Don)	Pinaceae	95.98	104.59	
116	*Dioscorea sect. Seriflorae* Burkill & Perrier	Dioscoreaceae	96.01	90.65	
117	*Nerium oleander*L.	Apocynaceae	96.74	109.23	
118	*Withania somnifera* (L.) Dunal	Solanaceae	96.85	117.95	
119	*Scrophularia altaica* Murray	Scrophulariaceae	96.98	105.50	
120	*Olea ferruginea* Wall ex Aitch.	Oleaceae	97.22	91.46	
121	*Indigofera heterantha* Wall. ex Brandis	Fabaceae	97.31	105.91	
122	*Salvia nemorosa* L.	Lamiaceae	97.45	100.79	

123	*Punica granatum* L.	Lythraceae	97.50	104.16	
124	*Clematis obvallata* (Ohwi) Tamura	Ranunculaceae	97.67	93.52	
125	*Sisymbrium Sect Irio* DC.	Brassicaceae	98.21	102.89	
126	*Fagonia indica* Burn. f.	Zygophyllaceae	98.28	100.55	
127	*Sida cordifolia* Linn.	Malvaceae.	98.28	95.94	
128	*Bougainvillea spectabilis* Willd.	Nyctaginaceae	98.31	77.77	
129	*Sonchus asper* (L.) Hill	Asteraceae	98.64	93.84	
130	*Dryopteris ramose* (C. Hope)C.Chr.	Dryopteridaceae	98.95	92.68	
131	*Hedera helix* L.	Araliaceae	98.96	103.09	
132	*Lythrum salicaria* L.	Lythraceae J.	99.10	110.62	
133	*Carissa carandas* L.	Apocynaceae	99.12	90.82	
134	*Ficus polita* Vahl.	Moraceae	99.18	95.48	
135	*Argyrolobium roseum* Jaub.	Fabaceae	99.28	100	
136	*Euphorbia pulcherrima* Willd. ex Klotzsch	Euphorbiaceae	99.39	117.05	
137	*Gallium aparina* L.	Rubiaceae	99.54	102.63	
138	*Origanum vulgare* L.	Lamiaceae Martinov	99.57	103.57	
139	*Sargeretia thea* (Osbeck) M.C. Johnston	Rhamnaceae	100	94.16	
140	*Woodfordia floribunda* salisb.	Lythraceae	100	105	
141	*Verbena tenuisecta* Briq.	Verbenaceae	100.09	102.99	
142	*Buxus wallichiana* Baill.	Buxaceae	100.19	110.70	
143	*Vernonia anthelmintica* (L.) Willd.	Asteraceae	100.45	92.37	
144	*Tecoma stans* (L.) Juss. ex Kunth	Bignoniaceae	100.78	107.39	
145	*Saxifraga rotundifolia* L.	Saxifragaceae	100.79	89.68	
146	*Nasella tenuissima* (Trin.) Barkworth	Poaceae	101.14	120.84	
147	*Salvia aegyptiaca* L.	Lamiaceae	101.83	106.92	
148	*Saussurea heteromalla* (D.Don)Hand –Mazz	Asteraceae	102.04	90.82	
149	*Otostegia limbata* (Benth.) Boiss	Lamiaceae	102.45	89.17	
150	*Oxalis corniculata* L.	Oxalidaceae	102.51	117.10	
151	*Rosa* × *centifolia* L.	Rosaceae	102.52	101.85	
152	*Bergenia ciliate* Stemb.	Saxifragaceae	102.67	85.88	
153	*Peganum harmala* L.	Nitrariaceae	103.58	108.01	
154	*Oxalis repens* Thunb.	Oxalidaceae	103.81	105.35	
155	*Cuphea hyssopifolia* Kunth	Lythraceae	103.93	96.29	
156	*Asplenium laetum* Sw.	Aspleniaceae	104.24	111.32	

157	*Anisomeles indica* (L.) Kuntze	Lamiaceae	104.69	92.20	
158	*Ranunculus muricatus* L.	Ranunculaceae	105.05	95.86	
159	*Rhynchosia minima* (L.)DC.	Fabaceae	105.36	99.53	
160	*Punica granatum* L.	Lythraceae	106.41	105.21	
161	*Chrysanthemum morifolium* ramat.	Asteraceae	106.41	102.30	
162	*Elettaria cardamomum* (L.) Maton	Zingiberaceae	106.55	100.69	
163	*Leucas capitata* Desf.	Lamiaceae	106.87	94.09	
164	*Tagetes erecta* L.	Asteraceae	107.14	92.53	
165	*Typha minima* Funck ex Hoppe	Typhaceae	107.14	87.65	
166	*Polygonum plebeium* R. Br.	Polygonaceae	108.05	79.46	
167	*Bougainvillea glabra* Choisy	Nyctaginaceae	108.14	92.59	
168	*Cestrum diurnum* L.	Solanaceae	108.63	103.87	
169	*Calotropis procera* (Aiton) R. Br.	Apocynaceae	108.63	105.63	
170	*Potamogeton lucens* Linn.	Potamogetonaceae	108.89	97.56	
171	*Buxus papillosa* C.K. Schneid.	Buxaceae	109.32	130.11	
172	*Withania coagulans* (Stocks) Dunal	Solanaceae	109.50	99.62	
173	*Artemisia scoparia* Waldst. & Kit.	Asteraceae	109.55	112.96	
174	*Phyla nodiflora* (L.) Greene	Verbenaceae	109.73	101.47	
175	*Viburnum grandiflorum* Wall. ex DC.	Adoxaceae	110.11	99.48	
176	*Cynodon dactylon* (L.) Pers.	Poaceae	110.16	85.71	
177	*Juglans regia* L.	Juglandaceae	110.86	94.50	
178	*Hibiscus rosa-sinensis* L.	Malvaceae	110.95	107.40	
179	*Tagetes patula* L.	Asteraceae	111.30	98.21	
180	*Fragaria nubicola* (Hook.f.) Linndl. Ex. Lacaita	Rosaceae	111.53	107.38	
181	*Mentha longifolia* (L.) Huds.	Lamiaceae	111.89	84.44	
182	*Ficus benghalensis* L.	Moraceae	112.09	99.65	
183	*Solanum erianthum* D.Don	Solanaceae	112.61	120.23	
184	*Euphorbia hirta* L.	Euphorbiaceae	112.99	95.56	
185	*Kickxia ramosissima* Janch.	.Plantaginaceae	113.71	105.35	
186	*Ficus palmata* Forssk	Moraceae	113.93	94.61	
187	*Ligustrum lucidum* W.T. Aiton	Oleaceae	113.94	100.69	
188	*Acacia catechu* (L. f.) Willd	Fabaceae Lindl.	114.62	92.20	
189	*Erigeron Canadensis* L.	Asteraceae	115.88	110.61	

190	*Maytenus royleanus* (Wall.) Cuford in Senk Biol	Celastraceae	116.66	117.59	
191	*Rumex nepalensis* Barker & C.H. Wright	Polygonaceae	116.81	98.79	
192	*Jacaranda mimosifolia* D.Don	Bignoniaceae	117.14	110.65	
193	*Vetiveria zizanioides* (L.) Nash	Poaceae	117.36	99.63	
194	*Cissampelos pareira* L.	Menispermaceae	117.64	105.08	
195	*Datura innoxia* Mill.	Solanaceae	117.80	105.69	
196	*Aloe vera* (L.) Burm. f.	Asphodelaceae	118.52	109.75	
197	*Oxalis oregana* Nutt.	Oxalidaceae	118.64	100	
198	*Rubus hispidus* L.	Rosaceae	118.64	93.18	
199	*Nyctanthes arbor-tristis* L.	Oleaceae	118.85	101.56	
200	*Pinus wallichiana* A.B. Jacks.	Pinaceae	119.04	95.47	
201	*Cymbopogon nardus* (L.) Rentle	Poaceae	119.70	100.89	
202	*Cuscuta californica* Hook. & Arn.	Convolvulaceae	119.76	94.63	
203	*Callistemon citrinus* stapf.	Myrtaceae	121.19	92.98	
204	*Clematis gouriana Roxb. ex* DC.	Ranunculaceae	121.21	98.42	
205	*Strobilanthes glutinosus* Nees in Wall.	Acanthaceae	122.29	91.53	
206	*Elaeagnus angustifolia* L.	Elaeagnaceae	127.70	108.26	
207	*Ficus virgata* Reinw. ex Blume	Moraceae	127.86	111.97	
208	*Helicteris isora* Linn.	Steruliaceae	127.86	127.60	
209	*Debregeasia salicifolia* (D. Don) Rendle	Urticaceae	128.09	102.58	
210	*Fragaria vesca* L.	Rosaceae	128.78	98.42	
211	*Adiantum capillus-veneris* L.	Pteridaceae	129.06	103.61	
212	*Polygonum persicaria* L.	Polygonaceae	132.03	90.55	
213	*Jasminum sambac* L.	Oleaceae	140.69	104.33	
214	*Ficus carica* L.	Moraceae	142.85	119.81	
215	*Plantago major* L.	Plantaginaceae	142.85	96.45	
216	*Mentha arvensis* L.	Lamiaceae	145.02	99.40	
217	*Ficus religiosa* L.	Moraceae	147.77	94.61	
218	*Rumex hastatus* D.Don	Polygonaceae	149.35	107.28	
219	*Viburnum cotinifolium* D.Don	Adoxaceae	150.43	108.26	
220	*Geranium pusillum* L.	Geraniaceae	154.76	92.51	
221	*Plectranthus rugosus* Wall. ex Benth.	Lamiaceae	232.50	101.66	

	Mean		94.92	100.13	
	SD		22.48	13.43	
	Mean-SD		72.43	86.69	
	Mean-1.5SD		61.19	79.97	
	Mean-2SD		49.95	73.25	
	Mean-2.5SD		38.70	66.53	

† Table 4.1.Percentage of growth rate compared with the control; ‡ stronger inhibitory activity in the radicle: *M-1 (σ), **M-1.5 (σ), *** M-2 (σ), and **** M-2.5 (σ). σ (SD) : standard deviation of radicle. M: mean of radicle.

4.1.1. Discussion

Boerhavia procumbens Banks ex Roxb. is a valuable medicinal plant in Pakistan due to its substantial treatment potential against numerous diseases, including constipation, blood purifiers, jaundice, internal inflammation and dysmenorrhea (Qureshi & Bhatti, 2008; Hameed *et al*., 2011). It has been pointed out the *B. procumbens* as a threat to sustainable agriculture (Nasim and Shabbir, 2012). Although, the allelopathic effects of *B.diffusa* are reported in some studies (Ikuenobe *et al*., 2003; Jaryan *et al*., 2013; Gbashinbo *et al*., 2016) but the allelopathic effects of *B. procumbens* are seldom reported. The results of this study showed that *B. procumbens* had strongest inhibitory potential among 221 species on lettuce seedling growth due to release of volatile compounds.

Parthenium hysterophous L. is rapidly spreading invasive weeds across the Pakistan. It has been imposed various adverse effects on biodiversity, crop production, livestock and human health due to release of allelochemicals (Shabbir and Bajwa, 2006). The stronger inhibitory allelopathic effect of *P. hysterophous* has been reported on germination, root and shoot length of grass pea (*Lathyrus sativus* L.) (Thakur, 2017).

P. hysterophous had a stronger inhibitory effect on seedling growth and germination of lettuce (Wakjira *et al*., 2005). Similarly, the drastic inhibition of germination and seedling growth of onion has been reported due to the allelopathic effect of *Parthenium hysterophous* (Wakjira, 2009). The cost incurring has been highlighted due to adverse effects of *P. hysterophous* on forestry, roadsides, agriculture, fisheries, human health and wetlands (Masum *et al*., 2015).

Arisaema triphyllum (L.) Schott had an effective response against earthworm invasions due to release of its allelochemicals and maintained its abundance (Bohlen *et al*. 2004, Hale *et al*. 2006). A study has been conducted on 48 species to assess their resilience against climate change and global warming. The results had indicated that only *A. triphyllum* had presented the resilience against climate change as well as increased its abundance in the changing climate scenario (Pucko *et al*., 2011). The release of calcium oxalate has been reported from *A. triphyllum* which had a potential to change soil properties and pose serious health effects like kidney stone Saupe, (2017).

Crotalaria medicaginea DC.had low pharmacological activity, strong inhibitory effect on the growth of *Staphylococcus aureus* and *Proteus vulgaris* as well as possessing diverse antimicrobial activity against many bacteria (Devendra *et al*., 2012). *Crotalaria* species had a potential to control soil erosion, fix nitrogen, amend soil properties and biomass of plants. Additionally, they suppress the nematode communities (Wang *et al*., 2002).

The inhibitory allelopathic effect of *Amaranthus viridis* L. has reported on wheat, maize and rice (Ali *et al*., 2005). The suppression of root-knot nematodes has been reported due to the allelopathic effect of *A. viridis* at inoculum rates and amendment in soil properties which eventually inhibit the plant growth (Begum *et al*.,

2003). The significant reduction has been noticed in germination and seedling growth of wheat, corn and pearl millet due to the allelopathic effect of *A.viridis* aqueous extract (Hussain *et al*., 2003). The leaves of *Amaranthus viridis* had strongly inhibited the germination, hypocotyl and radicle length of lettuce due to release of secondary metabolites (Cândido *et al*., 2010).

Xanthium strumarium L. had reduced the germination and growth of different plant species. However, the strong inhibitory effect was observed in *Amaranthus retroflexus*L and *Papaver somniferum* L (Kadioglu, 2004). The insignificant allelopathic effect of aqueous extract of *Xanthium strumarium* has been observed on lentil (*Lens culinaris* Medic.) in low concentration. However, a negative allelopathic effect of *Xanthium strumarium* was noted in higher concentrations and it reduced the chlorophyll, seedling dry weight, germination and plumule length of Lentil (Benyas *et al*., 2010). Similarly, undistributed pattern of toxicity and inhibitory allelopathic effect of *X. strumarium* had been reported on lentils, corn sesame, canola and chickpea. The aqueous extract of *X. strumarium* inhibits the shoot length, germination and root length (Shajie and Saffari, 2007). The presence of many secondary metabolites including tannins, anthraquinones, phenols, reducing sugars, flavonoids, alkaloids, terpinoids sterols, saponins, phenols and glycosides have been confirmed in the extract of *X. strumarium* (Suradkar *et al*., 2017). The extracts of *Xanthium strumarium* have been prepared in water, petroleum ether and methanol. The extract in petroleum ether more suppressed the growth of *Echinocloa spp*. (rice weed) (Kumari *et al*., 2016). The extract of *X. strumarium* had decreased the germination, chlorophyll content, dry weight, hypocotyl, and radicle length of maize due to the allelopathic effect (Jalali *et al*., 2013).

The release of isoxaflutole from the *Phalaris minor* has been attested and reported the phytotoxicity to biomass, shoot and chlorophyll of wheat as well as its

potential to change the soil chemistry (Kaur and Bhowmik, 2004). The reduction in dry weight, root and shoot length of wheat has been highlighted due to release of phenolic compounds and secondary metabolites from *Phalaris minor* Retz (Kashif *et al*., 2015). Similarly, the inhibitory effect on germination and seedling growth has also been observed of wheat due to release of secondary metabolites from *P. minor* (Rather *et al*., 2016).

The numerous allelochemicals including 2- propyl phenol has been isolated from *Ficus microcarpa* which inhibited the growth of *Chlorella pyrenoidosa* (Jiang *et al*. 2014). Many chalcones which exist in the roots of *Ficus microcarpa* had exhibited the allelopathic effect on soil properties and other plants (Yang *et al*., 2010). The leaf extract of *Ficus microcarpa* L. f. has been applied to *Scenedesmus quadricauda* and the results confirmed the strong inhibitory effect on the growth of *Scenedesmus quadricauda* due to allelochemicals released from the leaves of the *F. microcarpa* (Guo *et al*., 2011). The *Ficus microcarpa* has been recognized as an invader species in Hawaii and identified its inhibitory effect on the distribution of secondary forest species such as *Erythrina sandwicense* (Starr *et al*., 2003).

The *Woodfordia fruticose* (L.) Kurz had a strong inhibitory allelopathic effect on the germination of *Triticum aestivum* and *Pisum sativum* seeds as well as root and hypocotyl growth due the release of flavonoids, alkaloid, saponins, terpinoids and tannins in different proportions (Devkota, 2013). The phytotoxic and allelopathic effects of *W. fruticosa* has been studied on germination and growth of radish. The *W. fruticose* apprehends inhibitory allelopathic effect on the germination, seedling growth and dry biomass of radish (Khan *et al*., 2011). The antibacterial activity of methanolic extract of *W. fruticose* has been determined due to allelochemicals (Mishra *et al*., 2013).

The strongest inhibitory allelopathic effect of *W. fruticose* stem extract on the germination of wheat and pea has been reported (Sharma and Devkota, 2015).

The leaf extract of *Plantago lanceolate* L. exhibited the negative allelopathic effect on the germination of *Descoronia sofia, Sylibum marianum* and *Chinicus benidictus* (Arouiee, 2010). The allelopathic effect of *P. lanceolate* has been reported on seed germination time, seed inhibition, seed vigor index and seed germination index of sunflower, wheat and maize. The finding of the research expressed inhibitory effect on germination of wheat maize and sunflower (Khan *et al*., 2012).

Apart from the inhibitory allelopathic effect, plants have stimulatory effect on the growth of other plants. The stronger stimulatory allelopathic effect of *Plectranthus rugosus* (Lamiaceae)was observed on growth and germination of lettuce followed by *Geranium pusillum (*Geraniaceae*), Viburnum cotinifolium (*Adoxaceae*), Rumex hastatus (*Polygonaceae*), Ficus religiosa* (Moraceae), *Mentha arvensis* (Lamiaceae), *Plantago major* (Plantaginaceae), *Ficus carica* (Moraceae), Jasminum sambac (Oleaceae) and *Polygonum persicaria (*Polygonaceae*)* (Table 4.2).

The twenty-four compounds have been identified from the *Plectranthus rugosus* Wall. ex Benth.. These secondary metabolites likely to affect positively or negatively the other plants in the vicinity. However, the results of this study indicated the stronger stimulatory allelopathic effect on the germination and growth of lettuce (Irshad *et al*., 2012). The very low inhibitory effect of *Geranium pusillum* has been observed on sunflower, lacy phacelia and white mustard. Alike, the findings of this study also confirmed the stimulatory allelopathic effect of *Geranium pusillum* L.on lettuce in a dishpack method. The *Viburnum cotinifolium* and *Rumex hastatus* D.Don had also presented stimulatory allelopathic effect on lettuce (Wrzesińska *et al*., 2016). The low phytotoxicity and inhibitory allelopathic effect of *R. hastatus* has been reported

on other plants (Hameed *et al*., 2009). It also been noticed the positive cytotoxicity of *R. hastatus* on *Artemia salina* (Hussain *et al*., 2010). The stimulatory allelopathic effect of *Ficus religiosa* L has been reported on the wheat growth (Iqbal *et al*., 2017). The indole-3-acetic acid has been isolated from *Ficus religiosa* L and proven its ability to promote plant growth. The findings of this study had also provided the evidence of stimulatory allelopathic effect of *Ficus religiosa*L on germination and growth of lettuce (Nimnoi and Pongsilp, 2009).

The secondary metabolites have not only the ability to inhibit plant growth, but also can stimulate the growth of many plants. This ability likely to enhance tolerance of plants against several biotic (diseases) and abiotic (climate change) stresses. Additionally they can play an effective role in agro-environment conservation, biodiversity conservation, environmental sustainability, and food security.

4.2. Evaluation of medicinal plants allelopathy using sandwich method

The services to human beings provided by medicinal plants are acknowledged and well documented since antiquity. Plant interacts with the environment through chemical pathways known as allelopathy (Aslani *et al*., 2014). The interactions occur due to the release of secondary metabolites which are wonderful natural complex molecules and difficult task to formulate them synthetically (Kumar *et al*., 2011). The natural complex compounds have potential for beneficial utilization in the industry as a raw material, medicines and agriculture (Morris, 1999). The researchers are more interested, exploring and wisely using bioactive compounds or allelochemicals for a bio-friendly products and eco-friendly products as well as their effective application in managing agricultural productivity, health and environmental sustainability (Bibi *et al*., 2011; Khanh *et al*., 2005; Albuquerque *et al*., 2011; Farooq *et al*, 2013).

The exploration and effective utilizations of allelochemicals require substantial prior investigation of allelopathic effects of medicinal plants. The sandwich method is recognized as an effective technique developed by Fujii (1994) to investigate the allelopathic effects of leachates (dry leaves of plants). The objective of this study was to investigate the allelopathic effects of medicinal plants of lettuce using sandwich method. The study had attempted the identification of allelopathic effect of 207 plant species collected from different regions of Pakistan. The current research particularly focused collection, screening, identification, and allelopathic investigation of medicinal plants. The allelopathic effects of some medicinal plants used in this study were less or seldom reported previously. Hence, this study will share, the more knowledge and contribute to effective utilization of medicinal plants in agriculture and industry in addition to medicinal use.

The growth and germination of lettuce seeds portray the allelopathic effect in sandwich method due to leachates released from different parts of medicinal plants. The results demonstrated that 4 plants exhibited strong inhibitory effect, 14 plants presented medium inhibitory and 13 plants showed the low inhibitory effect on the lettuce seeds. Contemporary, the 7 plants substantially stimulate the growth of lettuce seeds in sandwich method. The detail of plant species, their families and allelopathic effects is illustrated in table 4.2. *Boerhavia procumbens* Banks ex Roxb., *Jacaranda mimosifolia* D.Don, *Datura metel* L.and *Asparagus adscendins* Roxb.revealed strong inhibitory effect on the germination and seedling growth. The results pointed out the medium inhibitory effect on14 species, including*Berberis vulgaris* L, *Urtica dioica* L., *Tagetes erecta*L, *Calotropis procera* (Aiton) R. Br., *Hedera helix* L, *Plectranthus rugosus* Wall. ex Benth., *Tabernaemontana corymbosa* (variegated), *Centaurea iberica* Trevir. ex Spreng., *Buxus papillosa* C.K. Schneid., *Datura innoxia* Mill., *Cyperus rotundus* L., *Hibiscus rosa-*

sinensis L., *Sisymbrium Sect Irio* DC.and *Clematis gouriana* Roxb. ex DC.. However, *Buxus wallichiana* Baill., *Cirsium arvense* (L.) Scop., *Sida cordata*(Burn. f.) Bross, *Xanthium strumarium* L., *Geranium* L., *Delphinium aquilegifolium* (Boiss.) Bornm., *Withania somnifera* (L.) Dunal, *Trichodesma indicum* (L.) Lehm., *Digera muricata*(L.) Mart, *Solanum erianthum* D.Don, *Solanum surattense* Burm. f., *Typha minima* Funck ex Hoppeand *Potamogeton lucens* Linn.expressed low inhibitory effect on the germination and growth of lettuce seeds. Meanwhile, the stimulatory allelopathic effects were recorded from the dry leaves of *Viburnum grandiflorum* Wall. ex DC., *Maytenus royleanus*(Wall.) Cuford in Senk Biol, *Phoenix sylvestris* (L.) Roxb., *Woodfordia fruticose* (L.) Kurz, *Beaucarnea recurvate*lem.*Poa annua* L., and *Viola odorata* L.on lettuce germination and seedling growth (hypocotyl and radicle length).

Table 4.2. Allelopathic potential assessment of 207 medicinal plants using sandwich method from Pakistan

Sr. No.	**Species Name (Scientific)**	**Family**	**Extension （%）**		**Criter-ion**
			Radicle 10mg	**Hypocotyl 10mg**	**Radicle 10mg**
1.	*Boerhavia procumbens* Banks ex Roxb.	Nyctaginaceae	0	0	***
2.	*Jacaranda mimosifolia* D.Don	Bignoniaceae	2.67	8.064	***
3.	*Datura metel* L.	Solanaceae	4	3.22	***
4.	*Asparagus adscendens*Roxb.	Asparagaceae	4.93	12.94	***
5.	*Berberis vulgaris* L.	Berberidaceae	5.76	11.76	**
6.	*Urtica dioica*L.	Urticaceae	7.40	15.29	**
7.	*Tagetes erecta*L.	Asteraceae	8	16.12	**
8.	*Calotropis procera* (Aiton) R. Br.	Apocynaceae	9.82	35.42	**
9.	*Hedera helix*L.	Araliaceae	11.56	43.42	**
10.	*Plectranthus rugosus* Wall. ex Benth.	Lamiaceae	12.42	32.46	**

11.	*Tabernaemontana corymbosa*(variegated)	Apocynaceae	13	12.90	**
12.	*Centaurea iberica* Trevir. ex Spreng.	Asteraceae	13.06	37.59	**
13.	*Buxus papillosa* C.K. Schneid.	Buxaceae	13.29	58.28	**
14.	*Datura innoxia* Mill.	Solanaceae	13.68	36.99	**
15.	*Cyperus rotundus* L.	Cyperaceae	14.28	31.78	**
16.	*Hibiscus rosa-sinensis* L.	Malvaceae	14.66	22.58	**
17.	*Sisymbrium Sect Irio* DC.	Brassicaceae	15	28.43	**
18.	*Clematis gouriana*Roxb. ex DC.	Ranunculaceae	15.73	28.24	**
19.	*Buxus wallichiana* Baill.	Buxaceae	17.6	54.92	*
20.	*Cirsium arvense* (L.) Scop.	Asteraceae	18.57	82.35	*
21.	*Sida cordata* (Burn. f.) Bross.	Malvaceae	20.091	41.55	*
22.	*Xanthium strumarium* L.	Asteraceae	21.22	51.12	*
23.	*Geranium* L.	Geraniaceae	21.25	30.01	*
24.	*Delphinium aquilegifolium* (Boiss.) Bornm.	Ranunculaceae	23.69	65.14	*
25.	*Withania somnifera* (L.) Dunal	Solanaceae	23.75	37.91	*
26.	*Trichodesma indicum* (L.) Lehm.	Boraginaceae	24.04	67.22	*
27.	*Digera muricata* (L.) Mart	Amaranthaceae	24.50	70.87	*
28.	*Solanum erianthum* D.Don	Solanaceae	24.81	54.30	*
29.	*Solanum surattense* Burm. f.	Solanaceae	25.51	42.04	*
30.	*Typha minima*Funck ex Hoppe	Typhaceae	27.27	42.85	*
31.	*Potamogeton lucens*Linn.	Potamogetonaceae	27.39	28.57	*
32.	*Malva parviflora* L.	Malvaceae	27.75	57.14	
33.	*Lagerstroemia indica* L.	Lythraceae	28	29.03	
34.	*Parthenium hysterophorus* L.	Asteraceae	28.80	58.06	
35.	*Argyrolobium roseum* Jaub.	Fabaceae	29.09	63.09	
36.	*Conyza Canadensis* (L.) Cronquist	Asteraceae	29.33	45.16	
37.	*Leucas capitate* Desf.	Lamiaceae	29.86	60.10	
38.	*Rumex nepalensis* Barker & C.H. Wright	Polygonaceae	30.05	75.42	
39.	*Ranunculus muricatus* L.	Ranunculaceae	31.21	72	
40.	*Conyza Canadensis* (L.)Cronquist	Asteraceae	31.25	37.91	
41.	*Oxalis sect. Corniculatae* R. Knuth	Oxalidaceae	31.61	89.15	
42.	*Silene vulgaris*(Moench) Garcke	Caryophyllaceae	32.09	55.29	

43.	*Justicia adhatoda*L.	Acanthaceae	32.69	56.64	
44.	*Ranunculus arvensis* L.	Ranunculaceae	32.94	98.28	
45.	*Oxalis repens*Thunb.	Oxalidaceae	33.83	64.90	
46.	*Arisaema triphyllum* (L.) Schott.	Araceae	34.22	77.45	
47.	*Pteris cretica* L.	Pteridaceae	34.31	60.19	
48.	*Bougainvillea glabra* Choisy	Nyctaginaceae	34.66	48.38	
49.	*Artemisia scoparia* Waldst. & Kit.	Asteraceae	34.80	57.28	
50.	*Oxalis corniculata* L.	Oxalidaceae	35.05	60.24	
51.	*Duchesnea indica* (Andrews) Teschem	Rosaceae	35.26	85.71	
52.	*Achillea millefolium* L.	Asteraceae	35.74	34.68	
53.	*Rosa brunonii* Lindl.	Rosaceae	35.83	83.42	
54.	*Cannabis sativa* L.	Cannabaceae	36.06	90.75	
55.	*Vicia sativa* L.	Fabaceae	36.21	56.81	
56.	*Acacia nilotica* (L.) Willd. ex Delile	Fabaceae	36.72	82.72	
57.	*Quisqualis indica* L.	Combretaceae	37.5	75.82	
58.	*Jasminum officinale* L.	Oleaceae	38.27	87.15	
59.	*Dioscorea deltoidea*Wall. ex Griseb.	Dioscoreaceae	38.78	83.87	
60.	*Otostegia limbata* (Benth.) Boiss	Lamiaceae	39.11	56.79	
61.	*Rhynchosia minima* (L.) DC.	Fabaceae	39.70	85.43	
62.	*Oxalis oregana*Nutt.	Oxalidaceae	39.86	72.28	
63.	*Tecoma stans* (L.) Juss. ex Kunth	Bignoniaceae	40	56.87	
64.	*Indigofera tinctoria* L.	Fabaceae	40.74	74.86	
65.	*Sida cordata* (Burm. f.) Bross. Waalk	Malvaceae	42.15	87.37	
66.	*Dalbergia sissoo*Roxb. ex DC.	Fabaceae	42.5	45.81	
67.	*Ficus microcarpa* L. f.	Moraceae	42.66	84.77	
68.	*Geranium pusillum* L.	Geraniaceae	42.69	70.05	
69.	*Flacourtia indica* (Burm. f.) Merr.	Salicaceae	42.93	80.62	
70.	*Amaranthus hybridus* L.	Amaranthaceae	43.34	80.92	
71.	*Ranunculus repens* L.	Ranunculaceae	43.35	83.42	
72.	*Canna* x *generalis* L.H. Bailey	Cannaceae	43.75	58.45	
73.	*Rubus fruticosus* L.	Rosaceae	43.82	106.14	
74.	*Convolvulus arvensis* L.	Convolvulaceae	44.36	82.11	
75.	*Bauhinia variegate* L.	Fabaceae	45.26	64.70	

76.	*Berberis lyceum* Royle	Berberidaceae	45.62	68.20	
77.	*Androsace sarmentosa* Wall	Primulaceae	45.66	93.71	
78.	*Ipomoea carnea* Jacq.	Convolvulaceae	45.71	102.25	
79.	*Rubus hispidus* L.	Rosaceae	45.98	89.24	
80.	*Cornus macrophylla* Wall.	Cornaceae	46.09	65.88	
81.	*Commelina benghalensis* Linn.	Commelinaceae	46.57	76.62	
82.	*Mirabilis jalapa* L.	Nyctaginaceae	46.66	53.22	
83.	*Broussonetia papyrifera* (L.) L'Hér. ex Vent	Moraceae	46.73	77.10	
84.	*Mallotus philippensis* (Lam.) Mull. Arg	Euphorbiaceae	47.14	86.70	
85.	*Jasminum sambac* L.	Oleaceae	47.19	77.96	
86.	*Oxalis corymbosa* DC	Oxalidaceae	47.34	102.25	
87.	*Amaranthus viridis* L.	Amaranthaceae	47.36	90.06	
88.	*Verbena tenuisecta* Briq.	Verbenaceae	47.36	70.19	
89.	*Olea ferruginea* Wall ex Aitch.	Oleaceae	47.45	92.14	
90.	*Adiantum capillus*-veneris L.	Pteridaceae	47.46	86.01	
91.	*Bougainvillea spectabilis* Willd.	Nyctaginaceae	47.5	58.45	
92.	*Chrysanthemum morifolium* ramat.	Asteraceae	47.87	79.76	
93.	*Erigeron canadensis* L.	Asteraceae	47.90	86.70	
94.	*Withania coagulans* (Stocks) Dunal	Solanaceae	48	81.48	
95.	*Ficus polita* Vahl.	Moraceae	48.10	62.65	
96.	*Taraxacum officinale* L.	Asteraceae	48.14	84.91	
97.	*Polygonum plebeium* R. Br.	Polygonaceae	48.53	77.72	
98.	*Justicia adhatoda*L.	Acanthaceae	48.75	82.79	
99.	*Dodonaea viscosa* (L.) Jacq.	Sapindaceae	48.88	65.84	
100.	*Euphorbia pulcherrima*Willd. ex Klotzsch	Euphorbiaceae	48.97	82.70	
101.	*Cynodon dactylon* (L.) Pers.	Poaceae	49.33	78.18	
102.	*Tagetes patula*L.	Asteraceae	49.33	69.35	
103.	*Debregeasia salicifolia* (D. Don) Rendle	Urticaceae	49.38	84.70	
104.	Salix babylonica L.	Salicaceae	49.71	83.42	
105.	*Cuphea hyssopifolia* Kunth	Lythraceae	50	63.19	
106.	*Vernonia anthelmintica* (L.) Willd.	Asteraceae	50.20	76.13	
107.	*Plantago ovata* Forssk.	Plantaginaceae	50.22	79.22	
108.	*Chenopodium ambrosiodes* L.	Amaranthaceae	50.27	87.39	

109.	*Carthamus oxyacantha* M. Bieb.	Asteraceae	50.61	76.69	
110.	*Euphorbia cotinifolia* L.	Euphorbiaceae	50.66	45.16	
111.	*Salvia aegyptiaca* L.	Lamiaceae	50.90	94.04	
112.	*Saussurea heteromalla* (D.Don)Hand –Mazz	Asteraceae	51.02	65.88	
113.	*Quercus dilatata*Lindl.	Fagaceae	51.23	67.03	
114.	*Vetiveria zizanioides* (L.) Nash	Poaceae	51.52	92.47	
115.	*Phyla nodiflora* (L.) Greene	Verbenaceae	51.54	85.54	
116.	*Acacia modesta* Wall.	Fabaceae	51.55	75.72	
117.	*Clematis obvallata* (Ohwi) Tamura	Ranunculaceae	51.71	91.32	
118.	*Lantana indica* Roxb.	Verbenaceae	51.85	84.09	
119.	*Sida alba* L.	Malvaceae	51.87	94.03	
120.	*Sonchus asper* (L.) Hill	Asteraceae	52.23	96.38	
121.	*Woodfordia floribunda* salisb.	Lythraceae	52.26	102.59	
122.	*Polygonum persicaria* L.	Polygonaceae	52.43	109.60	
123.	*Polypodium virginianum* L.	Polypodiaceae	52.44	72.42	
124.	*Aesculus indica* (Wall. Ex Cambess.) Hook	Sapindaceae	52.46	110.61	
125.	*Campsis radicans* (L.) Bureau	Bignoniaceae	52.5	63.19	
126.	*Duranta plumieri* Jacq.	Verbenaceae	52.5	55.29	
127.	*Zanthoxylum armatum* DC.	Rutaceae	52.88	82.30	
128.	Aloe barbadensis mill.	Asphodelaceae	53.08	96.08	
129.	*Schefflera arboricola* Hayata	Araliaceae	53.33	104.66	
130.	*Jatropha integerrima* Jacq.	Euphorbiaceae	53.75	91.62	
131.	*Rumex nepalensis* Spreng.	Polygonaceae	54.32	88.63	
132.	Elettaria cardamomum (L.) Maton	Zingiberaceae	54.54	92.85	
133.	*Cascabela thevetia* (L.) Lippold	Apocynaceae	54.66	61.29	
134.	*Rosa indica*L.	Rosaceae	54.66	61.29	
135.	*Cymbopogon nardus* (L.) Rentle	Poaceae	54.84	110.75	
136.	*Ficus carica* L.	Moraceae	55.14	84.70	
137.	*Plantago lanceolate* L.	Plantaginaceae	55.14	78.82	
138.	*Fragaria nubicola* (Hook. f.) Lindl. ex Lacaita	Rosaceae	55.49	96	
139.	*Geranium wallichianum* D.Don ex Sweet, Geran.	Geraniaceae	56.17	102.79	
140.	*Plantago major* L.	Plantaginaceae	56.17	93.78	
141.	*Euphorbia helioscopia* L.	Euphorbiaceae	56.32	93.23	
142.	*Cissampelos pareira* L.	Menispermaceae	56.36	111.90	
143.	*Lantana camara* L.	Verbenaceae	57.39	82.79	

144.	*Aloe vera* (L.)Burm. f.	Asphodelaceae	57.57	88.095	
145.	*Asplenium laetum* Sw.	Aspleniaceae	57.62	97.38	
146.	*Callistemon citrinus* Stapf.	Myrtaceae	58.18	111.90	
147.	*Myrsine Africana* L.	.Primulaceae	58.22	76.54	
148.	*Nerium oleander* L.	Apocynaceae	58.66	65.84	
149.	*Acacia nilotica* (L.) Willd. ex Delile	Fabaceae	60.26	79.79	
150.	*Salvia moorcroftiana* Wall. ex Benth.	Lamiaceae	60.45	77.48	
151.	*Dryopteris ramose* (C. Hope) C.Chr.	Dryopteridaceae	60.69	109.71	
152.	*Sargeretia thea* (Osbeck) M.C. Johnston	Rhamnaceae	61.16	109.63	
153.	*Nasella tenuissima* (Trin.) Barkworth	Poaceae	61.33	93.26	
154.	*Beaucarnea recurvate* lem.	Asparagaceae	61.42	79.09	
155.	*Indigofera heterantha* Wall. ex Brandis	Fabaceae	61.42	90.39	
156.	*Psammogeton biternatum* Edgew.	Apiaceae	61.58	95.28	
157.	*Micromeria biflora* (Buch-Ham. ex D.Don) Benth.	Lamiaceae	62.54	86.74	
158.	*Artemisia scoparia* Waldst. & Kit.	Asteraceae	62.66	61.29	
159.	*Duranta erecta* (Variegata)	Verbenaceae	62.66	72.58	
160.	*Origanum vulgare* L.	Lamiaceae	62.71	109.94	
161.	*Lythrum salicaria* L.	Lythraceae	62.85	148.87	
162.	*Cassia fistula* L.	Fabaceae	64	70.96	
163.	*Nyctanthes arbor-tristis* L.	Oleaceae	64.60	89.15	
164.	*Duranta erecta* L.	Verbenaceae	65.33	79.03	
165.	*Ligustrum lucidum* W.T. Aiton	Oleaceae	66.66	62.90	
166.	Fragaria vesca L.	Rosaceae	67.41	124.29	
167.	*Juglans regia* L.	Juglandaceae	67.48	94.11	
168.	*Murraya paniculata* (L.) Jack	Rutaceae	67.5	66.35	
169.	*Cestrum diurnum* L.	Solanaceae	68	79.03	
170.	*Opuntia monacantha* Haw.	Cactaceae	68.26	121.24	
171.	*Oenothera rosea*L' Hér. ex Aiton	Onagraceae	68.78	172.57	
172.	*Saccharum spontaneum* L.	Poaceae	68.92	97.38	
173.	*Rosa × centifolia* L.	Rosaceae	69.33	79.032	
174.	*Verbascum Thapsus* L.	Scrophulariaceae	69.80	94.623	
175.	*Phalaris minor* Retz.	Poaceae	69.92	101.98	

176.	*Strobilanthes glutinosus*Nees in Wall.	Acanthaceae	71.16	93.785	
177.	*Bryophyllum pinnantum*(Lam.) Kurz	Crassulaceae	71.25	77.40	
178.	*Cyperus rotundus*L.	Cyperaceae	71.83	99.24	
179.	*Solandra maxima* (sessé & moc.) P.s. green	Solanaceae	73.00	112.13	
180.	*Fagonia indica* Burn. f.	Zygophyllacea e	73.06	125.38	
181.	*Punica granatum* L.	Lythraceae	73.25	89.41	
182.	*Ficus benghalensis* L.	Moraceae	74.01	115.18	
183.	*Elaeagnus angustifolia* L.	Elaeagnaceae	74.15	107.34	
184.	*Saxifraga rotundifolia* L.	Saxifragaceae	74.28	99.24	
185.	*Ficus palmata* Forssk	Moraceae	74.66	131.60	
186.	*Pinus wallichiana* A.B. Jacks.	Pinaceae	76.40	101.69	
187.	*Anisomeles indica* (L.) Kuntze	Lamiaceae	76.80	115.02	
188.	*Abies pindrow* (Royle ex D. Don)	Pinaceae	78.39	108.37	
189.	*Asparagus recemosus* Willd.	Asparagaceae	79.77	120.43	
190.	*Helicteris isora* Linn.	Steruliaceae	81.09	118.07	
191.	*Mentha arvensis* L.	Lamiaceae	82.39	102.82	
192.	*Salvia nemorosa* L.	Lamiaceae	82.39	106.21	
193.	*Ficus virgate* Reinw. ex Blume	Moraceae	82.47	97.59	
194.	*Ficus religiosa* L.	Moraceae	82.71	117.31	
195.	*Rumex hastatus* D.Don	Polygonaceae	83.14	126.55	
196.	*Viburnum cotinifolium* D.Don.	Adoxaceae	85.18	102.79	
197.	*Euphorbia royleana* Boiss.	Euphorbiaceae	86.4	93.26	
198.	*Elettaria cardamomum* (L.) Maton	Zingiberaceae	87.03	139.66	
199.	*Carissa opaca* Stapf ex Haines	Apocynaceae	88.64	120.43	
200.	*Bergenia ciliate* Stemb.	Saxifragaceae	90.12	128.49	
201.	*Viola odorata* L.	Violaceae	92.78	110.84	
202.	*Poa annua* L.	Poaceae	96.29	159.77	
203.	*Beaucarnea recurvate* lem.	Asparagaceae	96.57	117.91	
204.	*Woodfordia fruticose* (L.) Kurz	Lythraceae	98.76	102.35	
205.	*Phoenix sylvestris* (L.) Roxb.	Arecaceae	106.17	120	
206.	*Maytenus royleanus* (Wall.) Cuford in Senk Biol	Celastraceae	115.58	121.38	

207.	*Viburnum grandiflorum* Wall. ex DC.	Adoxaceae	168.53	160.45	
	Mean		49.73	78.82	
	SD		22.11	28.52	
	Mean-1SD		27.62	50.29	
	Mean-1.5SD		16.56	36.03	
	Mean-2SD		5.511	21.76	
	Mean-2.5SD		5.54	7.506	

Criterion shows strong inhibitoryeffect on the radicle by deviation value: *M-1 (SD), **M-1.5 (SD), *** M-2 (SD) and****M-2.5 (SD). SD: standard deviation of radicle. M: mean of radicle.

4.2.1. Discussion

Boerhavia procumbens Banks ex Roxb. exhibited the highest inhibition on seeds germination and seedling growth of lecttuce among 207 species. It has numerous medicinal uses, but its allopathic effects are least reported in literature. However, it has been considered as a threat to sustainable agriculture due to its vast distribution and impact on crop production (Nasim and Shabbir, 2012). *Jacaranda mimosifolia* D.Don releases the secondary metabolites such as flavonoids, flavones, triterpenes, steroids and iridiods from flowers and leaves (Gambaro *et al*., 1988; Binutu and Lajubutu, 1994). *J. mimosifolia*, posed inhibitory effect on the growth of wheat (Mongelli *et al*., 1997) and *P. aeruginosa* (Gambaro *et al*., 1988; Binutu and Lajubutu, 1994). The high anti-microbial activity of *J. mimosifolia* has been reported against different bacteria (Rojas *et al*., 2006). The inhibitory allelopathic effect of *J. mimosifolia* has been noticed on the growth of lettuce (Nawaz *et al*., 2013). The *J. mimosifolia* has been enlisted among the plants which threatened theNgorongoro Conservation Area due to the release of allelochemicals (Witt *et al*., 2017).

The presence of cardiac glycosides, amino acids, alkaloids, tannins, flavonoids, saponins, carbohydrates and phenols has been reported in aqueous and methanolic extract of *Datura mete L.* It has the ability to control the noxious weeds, particularly

Parthenium hysterophorus L (Al-Snafi, 2017). The aqueous extract of *Datura metel* L had inhibited the early seedling growth and germination of *Parthenium hysterophorus* L in a laboratory bioassay (Ramachandran, 2017). Effectively controlled of pathogens (cause crop diseases) has been reported by the application of aqueous and methanolic leaf extract of *Datura metel* under greenhouse condition (Kagale *et al*., 2004). The allelopathy of *D. metel* has been tested on *P. hysterophorus* and suggested the use of *Datura metel* for management of *P. hysterophorus* (Chaudhary and Iqbal, 2013). Although, reported the release of phytochemicals including phenols, saponins, sterols, ketones, tri-terpenoids, glycosides, steroids, nitrogenous constituents and alipathic compounds from *Asparagus adscendens* (Thakur, 2016 ; Mamta and Shukla, 1995). However, the allelopathic effect of *Asparagus adscendens* are least reported, but the current study indicated the inhibitory allelopathic effect of *Asparagus adscendens* Roxb.on germination and growth of lettuce.

The strong inhibitory allelopathic effect of *Berberis vulgaris* L. has been recorded among 68 species on lettuce seedling growth and germination in sandwich method (Amini *et al*., 2016). Similarly, the allelopathic effect of *B. vulgaris* among 178 Caucasian plant species has been reported on lettuce growth in sandwich method (Mardani *et al*., 2016). The rusting and damage to stems of cereal and wheat from *B. vulgaris* has been reported due to release of allelochemicals (Peterson *et al*., 2005). However, the finding of this study evident the inhibitory allelopathic effect of *B. vulgaris* on lettuce germination and seedling growth.

The inhibitory allelopathic effect of *Urtica dioica* L has been identified on the growth of oat (Bojović *et al.*, 2015).The methanolic extract of *U. dioica* exhibited stronger inhibitory effect on the germination and growth of radish, among 13 medicinal plants collected from the Margalla Hills Islamabad(Khan *et al*., 2011). The aqueous

root extract of *U. dioica* effected the arbuscular mycorrhizal fungi in the maize roots which eventually decreases the growth and dry biomass of maize (Džafić *et al*, 2010). The significant decreased in growth and root weight of *Hordeum vulgare L.*during the application of aqueous leaves and root extracts of *U. dioica* has been reported due to release of allelochemicals (Dziamski and Stypczynska, 2015).

The flowers of *Tagetes erecta* remarkably inhibited the germination and seedling growth of lettuce (Xiaoyong *et al.,* 2017). Similarly, the release of a phytotoxic compound (phenols and flavnoids) has been reported from *Tagetes erecta* L.and reduction in germination and development (Santos *et al*., 2015). The suppression of parthenium has been reported due to the allopathic effect of *Tagetes erecta* (Duary and Mukherjee, 2013). The aqueous extract of *T. erecta* reduced the transpiration and respiration as well as decrease the seedling growth and germination of *Brassica oleracea* (Dragomir and Nicolae, 2014). The inhibition of seedling growth and germination of *Amaranthus tricolor* L. has been observed due to the allelochemicals released from the ethanolic extract of *T. erecta* (Nguyen *et al*., 2016).

Calotropis procera (Aiton) R. Br.had decreased seedling growth, inhibited the seed germination and significant change in biomass of Acacia species due to the release of allelochemicals (Alshahrani *et al*., 2017).

The aqueous extract of *C. procera* contains phytotoxic compounds and inhibits the seed germination, reduce seedling biomass, root length and shoot length of cultivated crops particularly wheat (Yasin *et al*., 2012). The allelopathic effect of *C. procera* dry leaf aqueous extract has been investigated and observed that there was delay in germination, less germination percentage, decrease in radicle length of Cucumber, Barley, Fenugreek, Wheat and Alssana at higher concentrations of *C. procera* (Al-Zahrani and Al-Robai, 2007). The stronger inhibitory allelopathic effect

of *C. procera* aqueous extract has been reported on the germination and growth of *Parthenium hysterophorus* (Mishra, 2015). The reduction in plumule length of mung bean by leaf alcoholic extract of *C. procera* has been identified in vivo conditions Gupta, (2016). *C. procera* inhibited the growth of wheat, tomato and cucumber due to release of allelochemicals (El-Khatib *et al*., 2016). The phytotoxic effect of *C. procera* has been observed on wheat and mustard at higher aqueous extract concentration and noted the inhibitory effect on seed germination and seedling length (Aslam *et al*., 2016).

Hedera helix L. is among rapidly invading species into forest ecosystems in the United States. It has exhibited the negative allelopathic effect on the growth, distribution and density of *Coreopsis lancelota* (Biggerstaff and Beck, 2007). The changed in soil traits, reduction in root and shoot length and germination of native riparian plant species has been identified due to the release of allelochemicals from *H. helix* (Le and Sonu, 2000). Alike, the decrease in the number of native plant species has also been reported due to the allelopathic effect of *H. helix.* Additionally, it has threatened many endangered species and soil properties (Binggeli, 2005). The growth and germination of beans and corns was strongly inhibited by the allelochemicals from the *H. helix* (Marian *et al*., 2017). The decrease in moisture content for neighboring plants, growth suppression, and reduction in density of *Hydrophyllum tenuipes* and *Vancouveria hexandra* has been reported due to allelochemicals released from *Hedera helix* (Copp, 2014).

The plants have ability to stimulate the growth of other plants through allelopathic pathways. The results presented strong stimulatory allelopathic effect of *Viburnum grandiflorum* Wall. ex DC. on germination and growth of lettuce. Similarly, the allelochemicals from *Maytenus royleanus* (Wall.) Cuford in Senk Biol promoted the growth of lettuce. The stimulatory growth due to certain secondary metabolites has been

pointed out from medicinal plants can induce tolerance against diseases and abiotic stresses. *Phoenix sylvestris* (L.) Roxb.and*Woodfordia fruticosa* (L.) Kurzexhibited the stimulatory allelopathic effect on the germination and growth of lettuce (Shinwari *et al*., 2017). Alike, *Beaucarnea recurvate, Poa annua, Viola odorata* and*Bergenia ciliate* had also shown stimulatory allelopathic effect on germination and growth of lettuce in the current study.

4.3. Allelopathy of medicinal plants (nine) aqueous extract on *Triticum aestivum* L.*, Lactuca sativa* L. and *Zea mays* L. in direct application and soil medium

The plants are blessed with an inherent ability to impede biological activities of other organisms, present in their vicinity due to allelopathy (ability to release certain chemicals). The allelopathy constitutes both positive and negative natural interactions among organisms. Hence, bioactive secondary metabolites released from donor organisms are accomplishing this phenomenon. These bioactive secondary metabolites are agenized as allelochemicals and becoming part of environment through different mechanisms, including leaching, root exudation, decomposition of plant residue and volatilization (Appiah *et al*., 2015).

The introduction of different plant species has successfully mediated the new plant-plant interaction as well as commute the function of allelopathic effects due to novel compounds released from the plants (Callaway *et al*., 2004). The allelochemicals have diverse effects on growth and development of vulnerable plant species conceding of shoot elongation, decrease in radicle length, absence of root hairs, maculated seeds, staining of seeds, increased number of root hairs, dry weight reduction and necrosis (Bhadoria, 2011). Additionally, the plant allelochemicals have altered interactions among organisms (microbes), community dynamics into the soil environment and determine the development of plants in the soil (Meiners *et al*., 2017; de Moraes Gomes *et al*., 2017). Meanwhile, they affect cellular structure, metabolism, photosynthesis,

enzyme activity, nutrient absorption and hormonal regulation of target plant (Ferreira and Áquila, 2000). The researchers are exploring the allelopathic interactions of plants with other plants and soil rhizospheres by application of vegetation extracts. The extraction of allelochemicals from selected plant species using aqueous or alcoholic solvent comprehends the elaboration of allelopathic effects (Mendez *et al*., 2011; de Moraes Gomes *et al*., 2017). The aqueous extract of *Azadirachta indica* A. Juss leaves has been applied on Wheat, Mung, Jowar and Cow pea and observed significant allelopathic effect on shoot length, biomass, root and germination stage (Kasarkar and Barge, 2016). The suppression of germination and subsequent growth of *A. fatua, H. annus, Zea mays* and *R. dentatus* has been noted by aqueous extract of *Euphorbia helioscopia* (Anwar *et al*., 2017). The aqueous extracts of selected nine plant species (*Boerhavia procumbens* Banks ex Roxb., *Jacaranda mimosifolia* D. Don, *Datura metel* L., *Asparagus adscendens* Roxb. , *Plectranthus rugosus* Wall. exBenth., *Parthenium hysterophorus* L., *Arisaema triphyllum* (L).Schott, *Crotalaria medicaginea* DC. and *Amaranthus viridis* L) were applied directly to crop seeds as well as through the soil medium to assess their allelopathic effect in both conditions.

4.3.1. Allelopathic effect on wheat, maize and lettuce in aqueous extracts of selected (nine) plant species

The different species had shown different allelopathic effects on wheat, maize and lettuce. The results had shown a minimum growth of hypocotyl 1.5 mm and maximum length 11 mm by application of *Boerhavia procumbens* aqueous extract on wheat. However, three seeds did not hamper germination (Fig.4.1 and 4.13). Meanwhile, the radicle length was noted, ranged from 6-41mm in case of *Boerhavia procumbens* aqueous extract on wheat (Fig.4.2). The aqueous extract of *Jacaranda mimosifolia* had shown a significant inhibitory effect on seed germination and only six seeds were germinated but all lengths for below 2mm. The minimum hypocotyl length

was 0.5 mm and maximum length of 1 mm was recorded during the exposure of wheat to aqueous extract of *Jacaranda mimosifolia* (Fig.4.1 and 4.13). On the other hand, lowest radicle length of 0.5 mm and highest of 2.5 mm was recorded (Fig. 4.2). The results had demonstrated the germination of 14 seeds of wheat during the application of aqueous extract of *Datura metel* (Fig.4.13). The results exhibited 0.5 mm (minimum) and 13 mm (maximum) hypocotyl length while radicle length varies from 1.5-17 mm during the application of aqueous extract of *Datura metel* (Fig. 4.1 and 4.2). The wheat had represented hypocotyl growth (2.5 – 16 mm), radicle length (9 – 29 mm) and germination of 13 seeds during exposure of *Asparagus adscendens* aqueous extract to wheat in petri dishes (Fig. 4.1, 4.2 and 4.13). Contemporary, the aqueous extract of *Plectranthus rugosus* simulate exceptionally growth of both hypocotyl (16 – 35 mm) and radicle (33 – 63 mm) length in all seeds (Fig. 4.1, 4.2 and 4.13). The aqueous extract of *Parthenium hysterophorus* had indicated inhibitory allelopathic effect in all seeds of wheat, including no germination in 8 seeds (Fig. 4.13). The aqueous extract of *Parthenium hysterophorus* inhibits hypocotyl length to 2.5 mm (maximum) and radicle length to 6 mm (maximum) during the experiment (Fig. 4.1 and 4.2). Alike *Plectranthus rugosus*, the aqueous extract of*Arisaema triphyllum* expressed elongation of both hypocotyl and radicle in all seeds (Fig. 4.1, 4.2 and 4.13). Similarly, the aqueous extract of *Crotalaria medicaginea* exhibited the positive allelopathic effect on germination of wheat (13 seeds). The radicle length varies from 2 – 36 mm while hypocotyl length varies from 1 – 22 mm (Fig. 4.1, 4.2 and 4.13). The results had evinced most inhibitory effect on wheat germination in case of *Amaranthus viridis*. It is important to denote that only 5 seeds were germinated while hypocotyl and radicle lengths were restricted to 1.5 mm (maximum) and 4 mm (maximum) respectively during the application of aqueous extract of *Amaranthus viridis* on wheat (Fig. 4.1, 4.2 and 4.13).

The aqueous extracts of selected species had confronted inhibitory allelopathic effect on lettuce. The results had shown that aqueous extract of *Boerhavia procumbens* inhibits the hypocotyl and radicle length of 5 mm and 4 mm respectively in ten lettuce seeds (Fig. 4.3, 4.4 and 4.13). The results had reflected the very strong inhibitory effect of aqueous extracts of *Jacaranda mimosifolia, Parthenium hysterophorus, Crotalaria medicaginea* and *Amaranthus viridis* on lettuce seeds and suppressed the entire growth of the hypocotyl and radicle in all seeds (Fig. 4.3, 4.4 and 4.13). Besides *Jacaranda mimosifolia, Parthenium hysterophorus, Crotalaria medicaginea* and *Amaranthus viridis*, the aqueous extracts of *Arisaema triphyllum* and *Asparagus adscendens* had also completely inhibited the radicle growth (Fig.4.4). Meanwhile, the different hypocotyl lengths were recorded in *Arisaema triphyllum* (1.5 – 6 mm) and *Asparagus adscendens* (0.5 – 7 mm) (Fig. 4.3). The aqueous extract of *Datura metel* had showed the radicle growth ranged from 1.5 mm to 11 mm while the radicle growth varies from 0.5 mm to 2 mm (Fig. 4.3 and 4.4). The results demonstrated the positive allelopathic effect of *Plectranthus rugosus* on lettuce seeds as compared to other tested species. The maximum hypocotyl length of 21.5 mm and radicle length 4 mm were noted after the experiment (Fig. 4.3 and 4.4).

The maize seeds had responded to aqueous extracts of selected species in a diverse mechanism, including total inhibition to mixed response. The exposure of *Boerhavia procumbens* aqueous extract resulted in germination of 5 maize seeds, 0.5 – 3 mm hypocotyl length and 5 – 16 mm radicle length (Fig. 4.5, 6.6 and 4.13). However, six seeds were germinated in *Jacaranda mimosifolia* aqueous extract medium with a hypocotyl length of 1 – 5.5 mm and a radicle length of 1.5 – 5 mm (Fig. 4.5, 4.6 and 4.13). The *Datura metel* aqueous extract supported the germination of 8 seeds which was maximum after the *Asparagus adscendens* (9 seeds germinated) (Fig.4.13). The

maximum hypocotyl length was recorded 6.5 mm while radicle length was extended to 12 mm in aqueous medium of *Datura metel* (Fig. 4.5 and 4.6). The *Asparagus adscendens* aqueous extract had introduced stimulatory allelopathic effect as compared to other selected plant species. The hypocotyl and radicle lengths were protracted to 16 mm (maximum) and 39 mm (maximum) respectively in aqueous extract of *Asparagus adscendens* (Fig. 4.5 and 4.6). Unlike to *Asparagus adscendens*, only 5 maize seeds exposed to aqueous extract of *Plectranthus rugosus*were germinated and expressed inhibitory allelopathic effect (Fig. 4.13). The aqueous extract of *Plectranthus rugosus* restricted the maximum hypocotyl and radicle growth to 2 mm and 14 mm respectively (Fig. 4.5, 4.6 and 4.13). The results had attributed unpredictable allelopathic effect in *Arisaema triphyllum* and *Crotalaria medicaginea* because of strong inhibitory effect in about 50% seeds and strong elongatory effect in remaining seeds (Fig. 4.13). The hypocotyl lengths were noted differently in *Arisaema triphyllum* (0.5 – 7 mm) and *Crotalaria medicaginea* (4 – 42 mm) aqueous extracts. However, the radicle lengths of 4.5 – 29 mm and 4 – 41 mm were recorded in aqueous extracts of *Arisaema triphyllum* and Crotalaria medicaginea respectively (Fig.4.5 and 4.6). The results had rendered significant inhibitory effect (no seed germination) on maize in aqueous extracts of *Parthenium hysterophorus* and*Amaranthus viridis* (Fig. 4.5, 4.6 and 4.13).

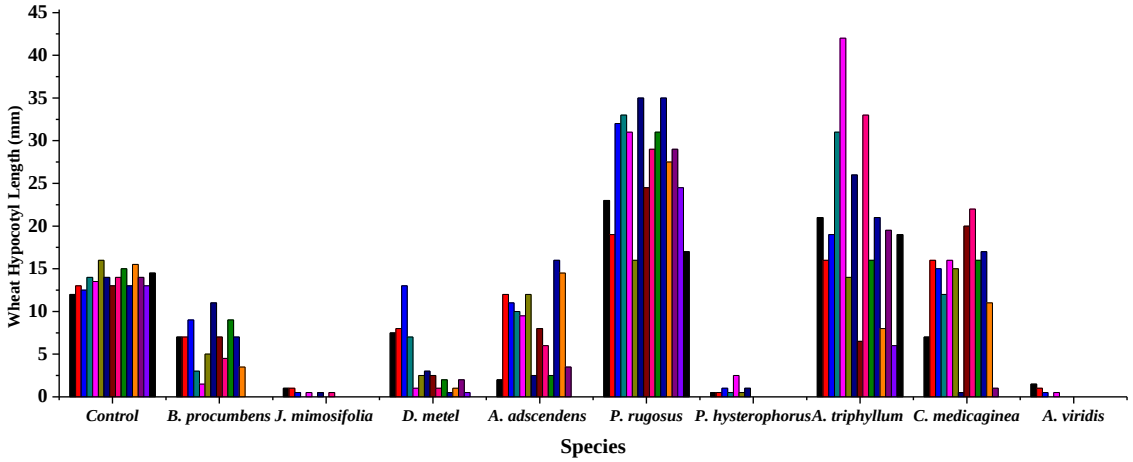

Figure 4.1 Effect of plant extracts samples on wheat hypocotyl length (mm)

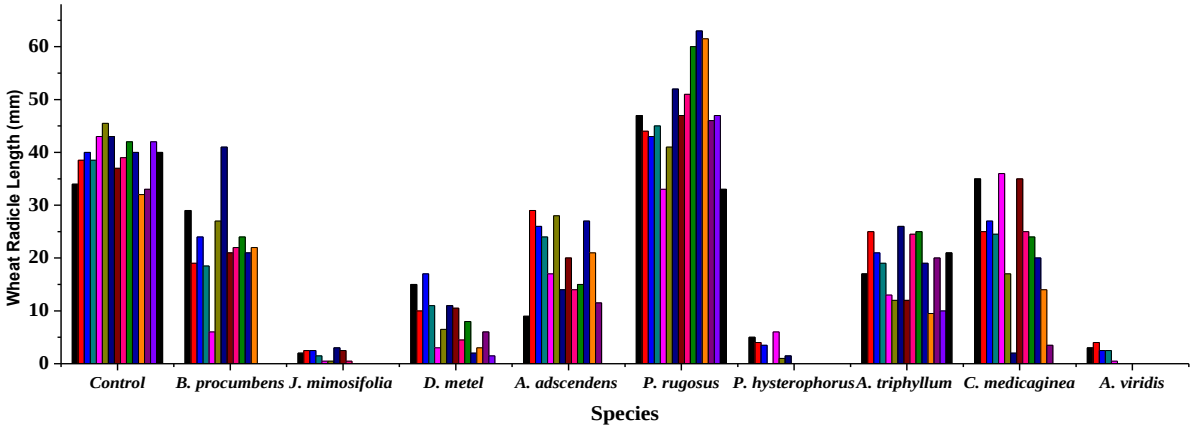

Figure 4.2 Effect of plant extracts samples on wheat radicle length (mm)

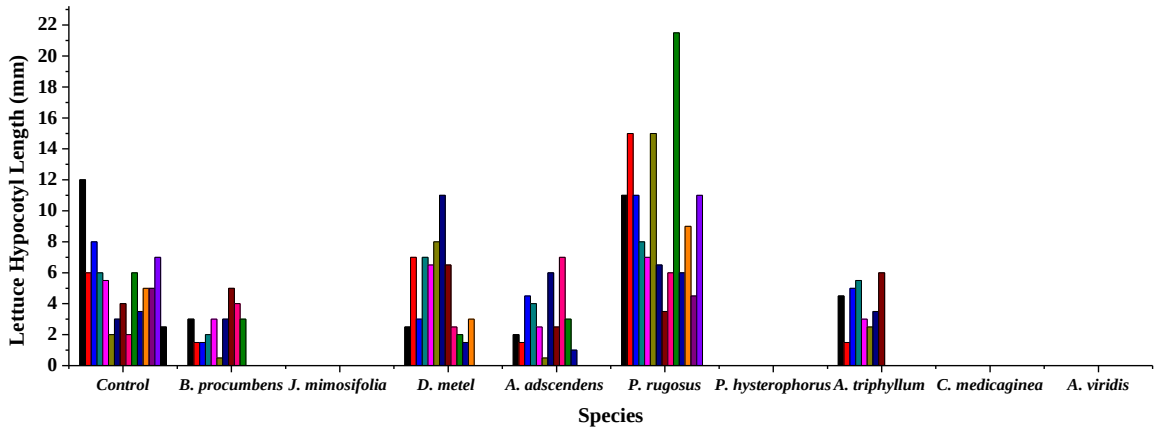

Figure 4.3 Effect of plant extracts samples on lettuce hypocotyl length (mm)

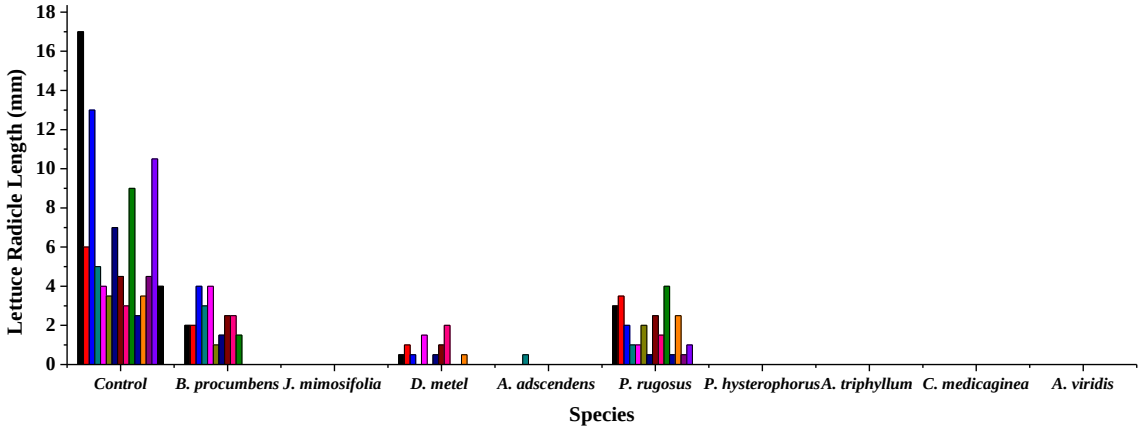

Figure 4.4 Effect of plant extracts samples on lettuce radicle length (mm)

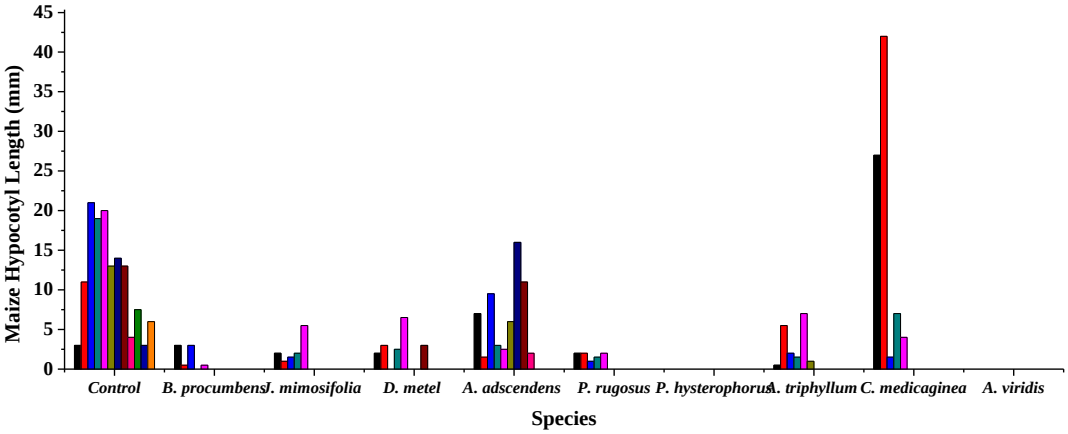

Figure 4.5 Effect of plant extracts samples on maize hypocotyl length (mm)

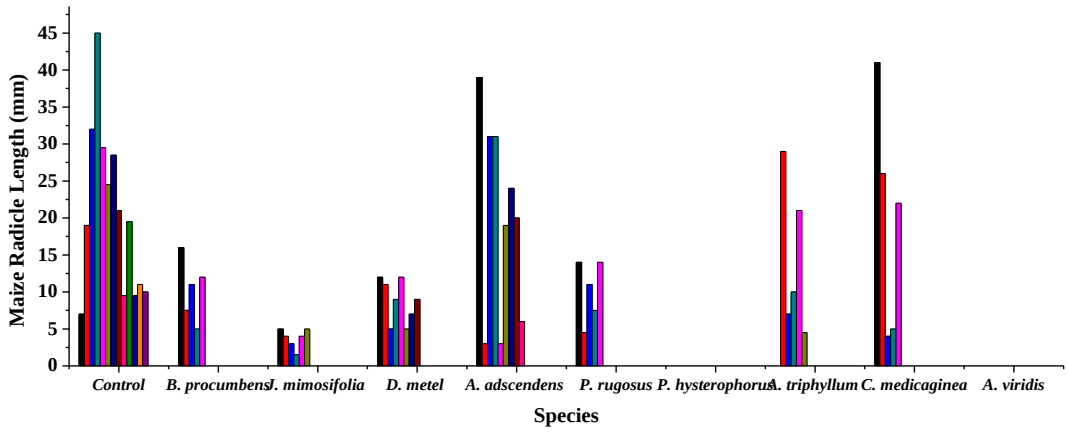

Figure 4.6 Effect of plant extracts samples on maize radicle length (mm)

4.3.2. Allelopathic effect on wheat, maize and lettuce in aqueous extracts of selected (nine) plant species in soil medium

The allelopathic effect of aqueous extracts of selected plants on wheat, maize and lettuce in soil were different from direct seed germination in aqueous extract. It is evident from the results that 9 seeds were germinated, showed 19 – 41 mm hypocotyl length and 33 – 61 mm radicle length in the soil irrigated by aqueous extract of the *Boerhavia procumbens* (Fig. 4.7, 4.8 and 4.14). The maize seeds had represented maximum hypocotyl length (35 mm), maximum radicle length (67 mm) and 60% seed germination in the soil watered by aqueous extract of the *Jacaranda mimosifolia* (Fig. 4.7, 4.8 and 4.14). However, five seeds of maize were germinated in the soil containing aqueous extract of *Datura metel* (Fig. 4.14), expressed the hypocotyl length of 18 – 40 mm as well as radicle length 17.5 – 57 mm in the same medium (Fig. 4.7 and 4.8). The results exhibited six seed germination of maize, 41.5mm (maximum) hypocotyl length and 52 mm (maximum) radicle length in the presence of aqueous extract of *Asparagus adscendens* in the soil (Fig. 4.7, 4.8 and 4.14). The soil environment irrigated with *Plectranthus rugosus* affirmed the germination of 8 seeds, hypocotyl length ranged from 7mm to 29.5 mm and radicle length varies from 19 mm to 51 mm (Fig. 4.7, 4.8 and 4.14). The aqueous extract of*Parthenium hysterophorus*inhibits 60% seed germination in soil along with different hypocotyl length (31 – 40 mm) and radicle length (35 – 75 mm) (Fig. 4.7, 4.8 and 4.14). Alike *Plectranthus rugosus*, 8 seeds were germinated, hypocotyl length ranged from 15 mm to 24 mm and radicle length ranged from 17 mm to 43 mm in the soil irrigated with aqueous extract of *Arisaema triphyllum* (Fig. 4.7, 4.8 and 4.14). Contemporary, the aqueous extracts of *Crotalaria medicaginea* and *Amaranthus viridis* exhibited inhibitory allelopathic effect on the soil. The results corroborated the hypocotyl lengths from 10 – 11.5 mm and 15 – 31 mm in *Crotalaria medicaginea* and *Amaranthus viridis* respectively, whereas, the radicle lengths from 31

– 39 mm and 20 – 40 mm in *Crotalaria medicaginea* and *Amaranthus viridis* respectively (Fig. 4.7 and 4.8).

The soil environment irrigated with plant extracts except *Parthenium hysterophorus* had asserted stimulatory allelopathic effect on wheat germination. The seed germination rate was 100% in aqueous extracts of all selected species except *Parthenium hysterophorus* (40%) (Fig. 4.14). The results proved the hypocotyl length in the range of 31 – 40 mm and radicle length ranged from 35 - 65 mm in the soil watered with aqueous extract of *Parthenium hysterophorus* (Fig. 4.9 and 4.10). However, *Boerhavia procumbens* aqueous extract irrigated soil indicated maximum hypocotyl length of 38 mm and radicle length of 55 mm (maximum) (Fig.4. 9 and 4.10). The results presented hypocotyl length (21 – 37 mm) and radicle length (17 – 34 mm) in the soil containing aqueous extract of *Jacaranda mimosifolia* (Fig. 4.9 and 4.10). The irrigation of *Datura metel* aqueous extract to soil expressed hasten the allelopathic effect, presented the hypocotyl length (24 – 39 mm) and radicle length (24 – 39 mm) (Fig. 4.9 and 4.10). The results pointed minimum hypocotyl length of 27 mm and maximum length of 36 mm while 28.5 – 53.5 mm radicle length were recorded in the soil irrigated with aqueous extract of *Asparagus adscendens* (Fig. 4.9 and 4.10). The aqueous extract of *Plectranthus rugosus* represented wheat hypocotyl length (19 – 31.5 mm) and radicle length (31 – 48 mm) in the soil (Fig. 4.9 and 4.10). The soil irrigated with the aqueous extract of *Arisaema triphyllum e*xhibited hypocotyl length from 15 – 32 mm and radicle length from 31 – 42 mm (Fig. 4.9 and 4.10). Unlike the direct application of aqueous extract of *Crotalaria medicaginea*, the application of aqueous extract of *Crotalaria medicaginea* into the soil expressed stimulatory allelopathic effect and maximum hypocotyl (25 mm) and radicle (41.5 mm) lengths were recorded (Fig. 4.9 and 4.10). Alike *Crotalaria medicaginea*, the aqueous extract of *Amaranthus*

*viridis*indicated positive allelopathic effect, maximum wheat hypocotyl length (46.5 mm) and maximum wheat radicle length (37 mm) in the soil (Fig. 4.9 and 4.10).

The aqueous extracts of selected plant species had shown mixed elongatory and inhibitory effects on lettuce seeds in soil. The lettuce seeds had indicated 60% germination rate, 15 mm hypocotyl length (maximum) and 8.5 mm radicle length (maximum) in the soil irrigated with aqueous extract of *Boerhavia procumbens* (Fig. 4.11, 4.12 and 4.14). The aqueous extract of *Jacaranda mimosifolia* had confronted inhibitory allelopathic effect, 60% seed germination rate, hypocotyl length (6.5 – 11 mm) and radicle length (3 – 5.5 mm) of lettuce seeds in the soil (Fig. 4.11, 4.12 and 4.14). The results expressed sixty percent germination rate, inhibitory allelopathic effect, hypocotyl length (6.5 – 16 mm) and radicle length (3.5 – 7 mm) of lettuce seeds in the soil watered with aqueous extract of *Datura metel* (Fig. 4.11, 4.12 and 4.14). The lettuce seeds exhibited hypocotyl length in the range of 6 mm to 17 mm and radicle length varies from 1 mm to 5 mm (more inhibitory effect on radicle) in the soil containing aqueous extract of *Asparagus adscendens*(Fig. 4.11 and 4.12). The inhibitory allelopathic effect on lettuce was observed in the soil having an aqueous extract of *Plectranthus rugosus* along with 80% seed germination rate, hypocotyl length (5 – 11 mm) and radicle length (1.5 – 5.5 mm) (Fig. 4.11, 4.12 and 4.14). The results demonstrated inhibitory allelopathic effect on lettuce seeds during the application of *Parthenium hysterophorus* aqueous extract in soil. The germination rate, maximum hypocotyl length and maximum radicle length were recorded 90%, 15 mm and 4.5 mm respectively (Fig. 4.11, 4.12 and 4.14). It had been noted that aqueous extract of *Arisaema triphyllum* endured inhibitory allelopathic effect as well as comprehended 100% germination rate, hypocotyl length (5 – 19 mm) and radicle length (1 – 13 mm) in the soil environment (Fig. 4.11, 4.12 and 4.14). The results had attested the

suppression of both hypocotyl and radicle length to 16 mm and 4 mm respectively in the soil irrigated with aqueous extract of *Crotalaria medicaginea* (Fig. 4.11 and 4.12). The soil containing aqueous extract of *Amaranthus viridis* had manifested 80% seed germination rate, maximum hypocotyl length (11 mm) and inhibitory allelopathic effect on lettuce radicle length (1.5 – 3 mm) (Fig. 4.11, 4.12 and 4.14).

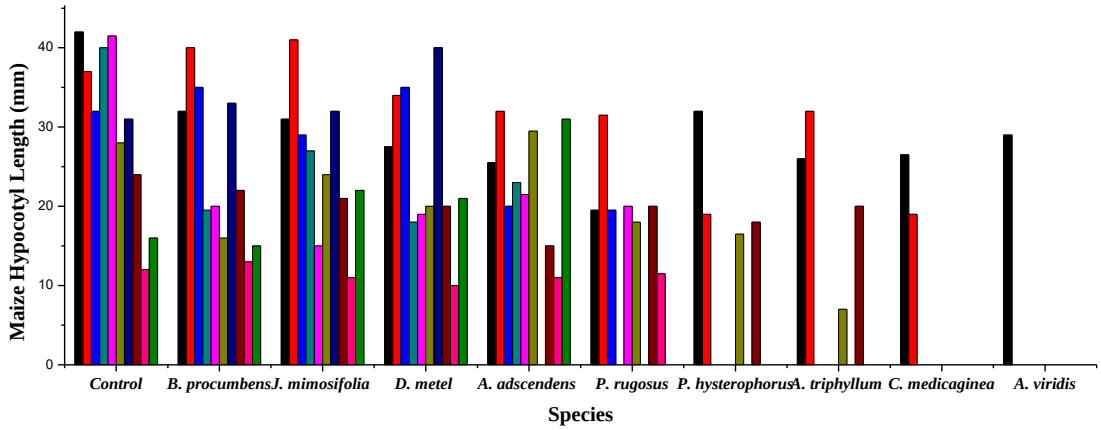

Figure 4.7 Effect of plant extracts samples on maize hypocotyl length (mm) in soil

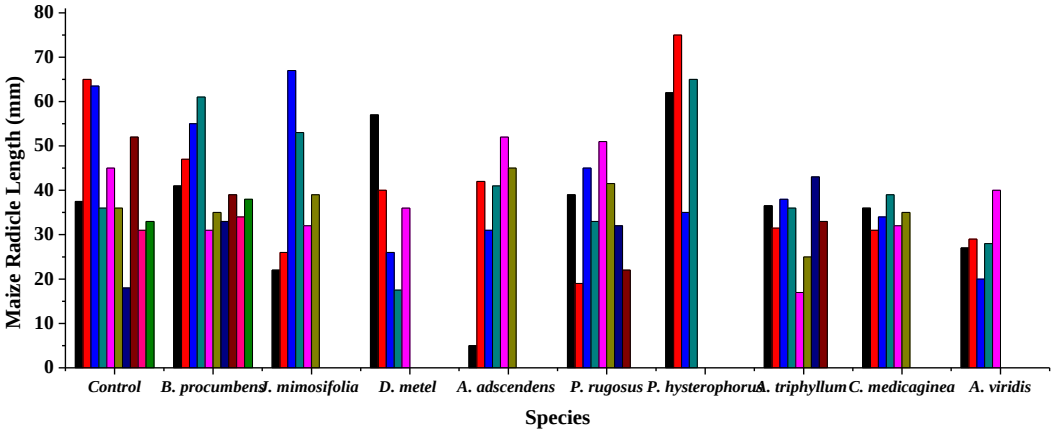

Figure 4.8 Effect of plant extracts samples on maize radicle length (mm) in soil

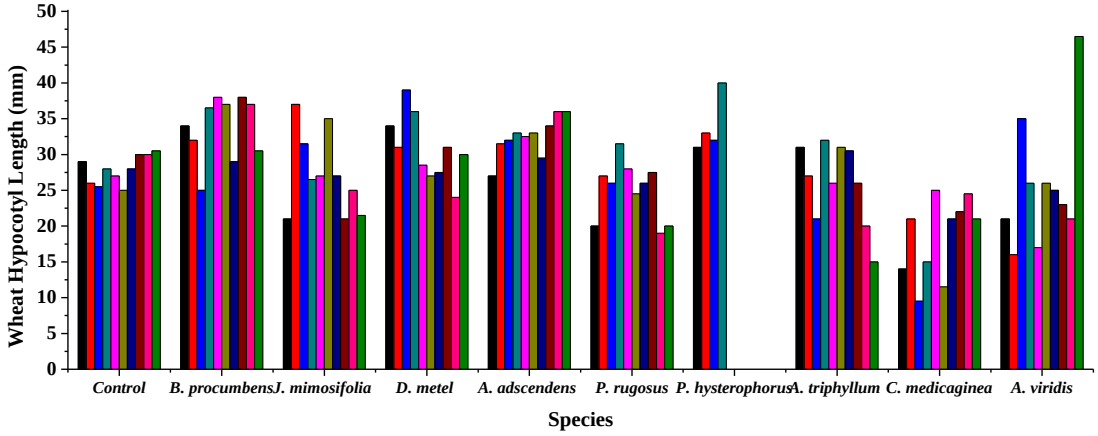

Figure 4.9 Effect of plant extracts samples on wheat hypocotyl length (mm) in soil

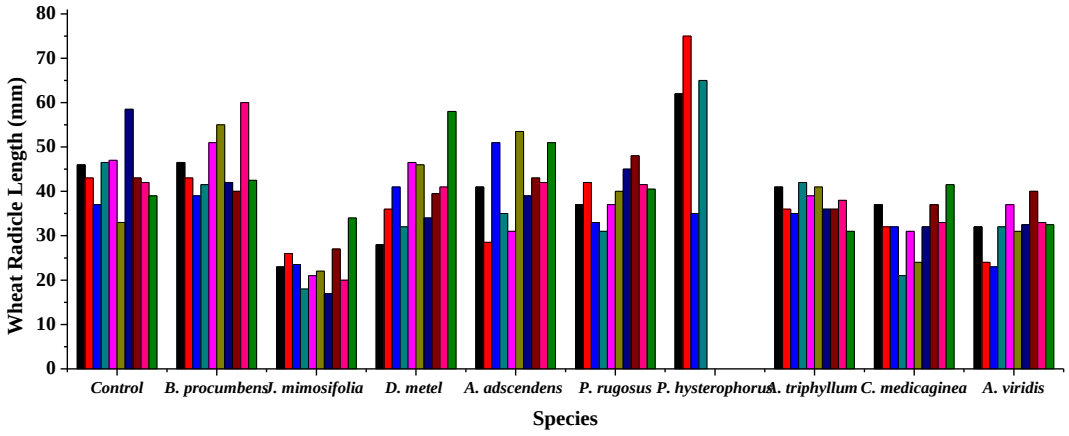

Figure 4.10 Effect of plant extracts samples on wheat radicle length (mm) in soil

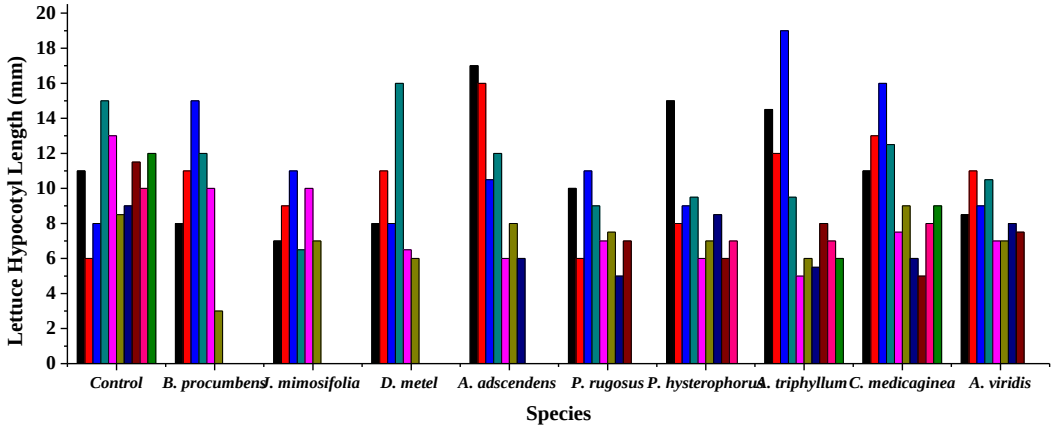

Figure 4.11 Effect of plant extracts samples on lettuce hypocotyl length (mm) in soil

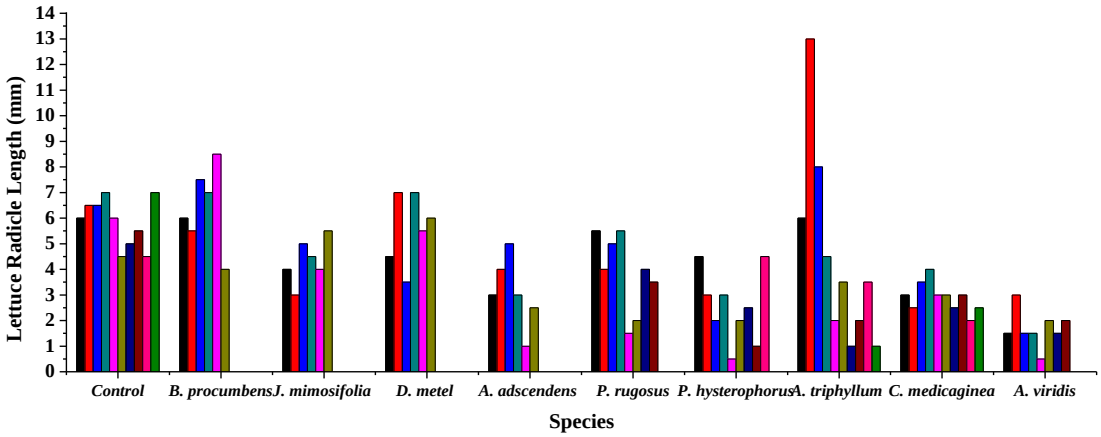

Figure 4.12 Effect of plant extracts samples on lettuce radicle length (mm) in soil

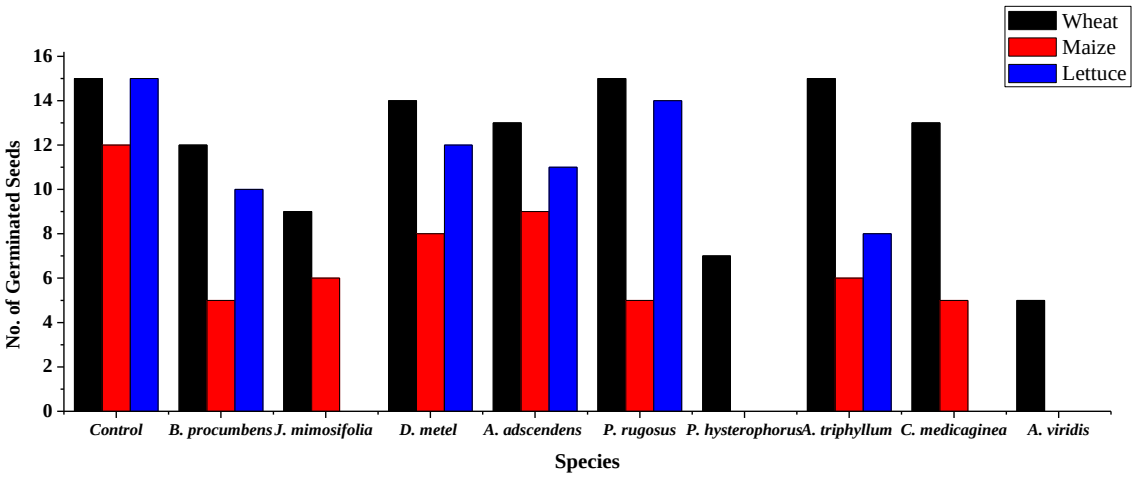

Figure 4.13 Effect of plant extracts samples on wheat, maize and lettuce seed germination rate

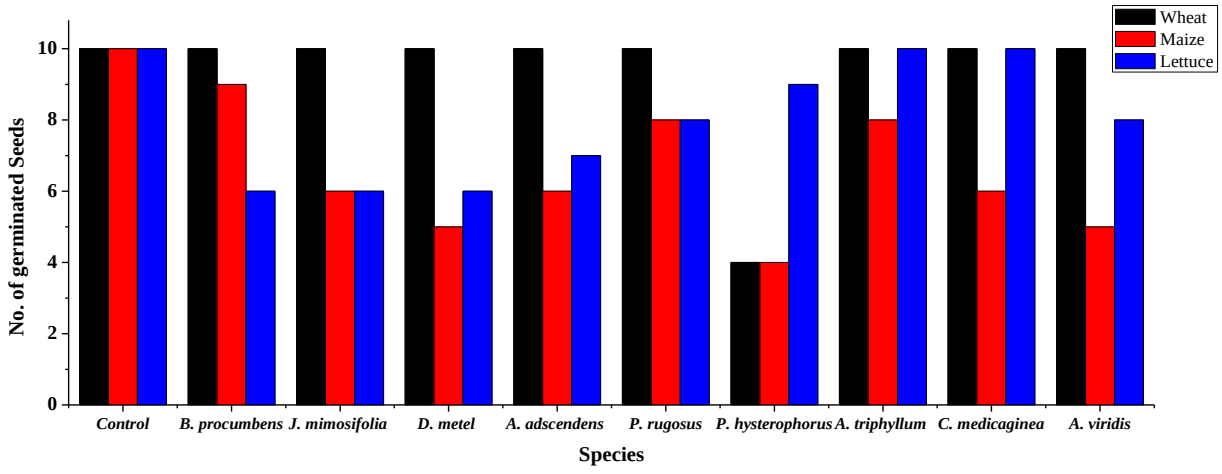

Figure 4.14 Effect of plant extracts samples on wheat, maize and lettuce seed germination rate in soil

4.3.3. Discussion

The *Boerhavia procumbens* Banks ex Roxb.has threatened wheat crop and livelihood of local farmers due to its potential allelopathic effects in the Bannu, Pakistan (Jan *et al*., 2012). Contemporary, *B. procumbens* did not affect soil fertility, particularly nitrification process and stimulate plant growth in the soil irrigated with aqueous extract of *B. procumbens* (Mahmood *et al*., 2014). The results of this study also attested the argument of Mahmood *et al*. (2014) in soil (Fig. 9 and 10). *Jacaranda mimosifolia* has been used for the treatment of acidic soil as well as tested its allelopathic effect on wheat growth is the soil (Njogu, 2012). The study noted variation in growth of wheat when exposed to leaf extract of *Jacaranda mimosifolia* D.Don.

The *Datura* species released phyto-toxins into the soil, which affect the growth of other plants (Unger, 1994). The allelopathic effects of *Datura stramonium L.* and*Datura innoxia* on wheat, maize and lettuce are reported in several studies (Kalistović, 2017; Abad *et al*., 2017; Gella *et al*., 2013; Arouiee, 2010; Sun et al., 2005). However, the allelopathic effects of *Datura metel* L. on wheat, maize and lettuce are least reported. The tenuazonic has been isolated acid from *Datura stramonium, Datura innoxia* and *Datura metel* L., tested its effects on wheat, lettuce, rye and green gram. The results confirmed a reduction in the chlorophyll content of all host plants (Janardhanan and Husain, 1984). The *Asparagus adscendens* Roxb. has been rated high value medicinal plant, plenty of benefits and endangered plants in the Himalayan mountains along with the release of a variety of compounds including vitamins, proteins, tannins, saponins and alkaloids (Thakur and Sharma, 2015). The release of many steroids, essential oil, phytoecdydteriods, triterpenoids, saponins and glycosides has also been reported from the *A. adscendens (*Dinan *et al*., 2001). The root extract of

Asparagus adscendens has been applied to soil and determined the marked reduction in population of *F. udum* (Singh and Rai, 2000).

The different twenty-three compounds had been characterized from *Plectranthus rugosus*Wall. ex Benth.using gas chromatography-mass spectrometry (GC-MS). The identified compounds contained 83% essential oils while spatulenol, β-caryophyllene and germacene D were main constituents (Irshad *et al*., 2012). GLC, mass spectrometry and NMR has also been applied to characterize essential oils from *Plectranthus rugosus* extract and identified p-cymene, alpha-cadinol, myrcene, beta phellandrene, limonene, alpha-pinene caryophyllene oxide, alpha-phellandrene, caryophyllene and delta-cadinene as some major constituents (Weyerstahl et al., 1983). However, the allelopathic effect of *P. rugosus* is seldom reported in the literature but these organic compounds released from *P. rugosus* had expressed allelopathic effect on lettuce, maize and wheat germination in the current study as discussed above. The positive allelopathic has been observed effect of *Arisaema triphyllum* (L.) Schott.on neighboring plants (*Alliaria petiolata*) in a field experiment (Evans *et al*., 2016).

The allelopathic effects of *Crotalaria medicaginea* DC., *P. hyslerophorus*, *X stramonium* and *C. mata* have been studied on different food crops, including maize. The results of their study, presented less phyto-toxicity and insignificant allelopathic effect of *C. medicaginea* on maize (Bhatt *et al*., 1994). The Isorhamnetin, Quercittrin and Acacetin along with two new allelochemicals3,5,7,3,4-pentahydroxy-6-methoxyflavone-3-O-α-L-rhamnopyranosyl-7-O-β-D-glucopyranosyl-(1,4)-O-β-D-xylopyranoside and 3, 5, 7-trihydroxy-8,4-dimethoxyflavone-5-O-β-D-galactopyranosyl-7-O-α-Lrhamnopyranosyl-(1,3) arabinopyranoside have been isolated and characterized from *C. medicaginea* using spectroscopic analysis (Yadava and Vishwakarma, 2014). The suppression has been noted in maize production in the

presence of *Crotalaria medicaginea* and some other weeds in their study (Dangwal *et al*., 2011).

The aqueous extract of *Amaranthus viridis* L. had retarded the germination and seedling growth of canola and rye-grass during direct application in petri-dishes due to some toxic and allelopathic compounds released from aqueous extract (Sultana *et al*., 2012). Besides, *A. viridis* had affected the soil properties and modified the native plant growth. The invasion inhibited the rhizobial development, AM fungi and *Acacia* growth (Sanon *et al*., 2009). The inhibitory allelopathic effect of aqueous extract of *A. viridis* has been reported on germination of *Parthenium hysterophorus* (Mishra, 2015). The inhibitory allelopathic effect of shoot and root of *Amaranthus viridis* has been observed on seed germination of sorghum, wheat, and maize (Dharmaraj *et al*. 1988). Similarly, the inhibitory allelopathic effect of *Amaranthus viridis* has also been noted on germination and growth of wheat (Dalvi, 2010; Ghodake *et al*. (2012).

Parthenium hysterophorus L. is well known for its significant allelopathic effect. Mishra and Nautiyal, (2012) recorded the inhibition in root and shoot length due to the presence of *P. hysterophorus.* The allelopathic effect of *P. hysterophorus* has also been assessed on maize in the laboratory. The aqueous root extract of *P. hysterophorus* had negatively affected the germination and shoot length of maize and exhibited the significant decrease in shoot length and germination of maize (Rashid *et al*., 2008). The high capacity of *P.hysterophorus* has been reported to distribute, adapt climatic conditions and induce a large number of seeds in soil and allelopathic effect. It had decreased the diversity of plant species, crop yield (maize and wheat) and adversely affected the animal and human health in Ethiopia (Abdulkerim-Ute and Legesse, 2016). The essential oils from the leaves of *P. hysterophorus* has been identified and characterized them by GLC and GLC-MS as well as reported the

inhibitory allelopathic effect of *P. hysterophorus* on lettuce seed germination, radicle and hypocotyl growth (Wakjira *et al*., 2009). The phytotoxicity of *P. hysterophorus* has been studied through the application of aqueous extract of *P. hysterophorus* on peanut and soybean in a laboratory based experiments. The suppression of germination of soybean and peanut due to phyotoxicity of *P. hysterophorus* has been recorded (Sorecha and Bayissa, 2017).

CHAPTER 5

5. CONCLUSIONS AND RECOMMENDATIONS

The examination of 221 plant species for allelopathic effect using dishpack method divulged the strong inhibitory alepathic effect from *Boerhavia procumbens, Parthenium hysterophorus* and *Arisaema triphyllum* on lettuce. Consequently, *Plectranthus rugosus, Geranium pusillum and Viburnum cotinifolium* had exhibited the strong stimulatory effect on the germination of lettuce. The findings of the study affirmed the stronger inhibitory effect of*Boerhavia procumbens* and stimulatory effects of *Plectranthus rugosus, Viburnum cotinifolium and Rumex hastatus*which was least reported in the previous studies. Additionally, this study had focused on both inhibitory and stimulatory effects instead of only inhibitory effects.

The present study concludes that *Boerhavia procumbens* exhibits the strongest inhibitory allelopathic effect and *Viburnum grandiflorum* presents strong stimulatory effect on the growth of lettuce seeds in sandwich method. The study identified least reported allelopathic effect of many plants particularly *Plectranthus rugosus* (inhibitory effect), *Viburnum grandiflorum* (stimulatory effect), *Maytenus royleanus* (stimulatory effect), *Phoenix sylvestris* (stimulatory effect), *Woodfordia fruticose* (stimulatory effect), *Beaucarnea recurvate* (stimulatory effect), *Poa annua* (stimulatory effect), *Viola odorata* (stimulatory effect) and*Bergenia ciliate* (stimulatory effect). Hence, the exploration of allelopathic effects likely to improve immunity against diseases, soil properties, survival of species, distribution, weed management, sustainable agriculture, and compatibility with climate change.

The selected plant species had exhibited diverse allelopathic effects on germination and growth of maize, lettuce and wheat. The direct application of aqueous extracts of *Amaranthus viridis, Jacaranda mimosifolia and Parthenium hysterophorus* reflected significant inhibitory effect on wheat hypocotyl and radicle. However, the lettuce hypocotyl growth was complete (100%) inhibited in aqueous extracts of *Amaranthus viridis, Crotalaria medicaginea, Jacaranda mimosifolia and Parthenium hysterophorus. The aqueous extracts of Amaranthus viridis, Crotalaria medicaginea, Jacaranda mimosifolia, and Parthenium hysterophorus* in addition with *Arisaema triphyllum* inhibited 100% radicle growth of the lettuce. Meanwhile, maize hypocotyl and radicle growth were completely (100%) inhibited by aqueous extracts of *Amaranthus viridis* and *Parthenium hysterophorus* but other species also reflected the negative allelopathic effect.

Contemporary the aqueous extracts exhibited less allelopathic effects in the soil as compared to direct application of plant extracts to maize, lettuce and wheat. The aqueous extracts of*Amaranthus viridis, Crotalaria medicaginea, Jacaranda mimosifolia Datura metel*and *Asparagus adscendens*indicated both inhibitory and stimulatory effect on germination and growth of maize in soil irrigated with the plant extracts. Furthermore, the soil watered with aqueous extracts supported the germination and growth of wheat except *Parthenium hysterophorus.* However, the germination and growth (hypocotyl and radicle) of lettuce seeds were inhibited by *Jacaranda mimosifolia Datura metel, Asparagus adscendens, Amaranthus viridis and Boerhavia procumbens* in irrigated soil.

Overall, the selected species had ability to disturb the ecosystem functions and services as well as hamper threat to biodiversity. Based on the results, it is strongly recommended to:

- Effective utilization of the species for the emerging dynamics of biological control (bioherbicide) of weed, crop and weed management as well as environmental sustainability by avoiding the toxic effects of synthetic pesticides.
- Explore both inhibitory and stimulatory allelopathic effects to improve tolerance of the plants against environmental changes particularly climate change, soil conservation, sustainable weed management, biodiversity conservation, industrial applications, sustainable agriculture, food security and environmental sustainability.
- Isolate allelochemicals for industrial application, bio herbicides, interactions of allelopathy with biodiversity conservation and adaptability with climate change for long-term environmental sustainability.

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ANNEXURE I

Top Ten Plants Pictures of Dishpack Method

1. ***Boerhavia procumbens*** **Banks ex Roxb.**

2. ***Parthenium hysterophorus*** **L.**

***3. Arisaema triphyllum* (L.) Schott.**

***4. Crotalaria medicaginea* DC.**

***5. Amaranthus viridis* L.**

***6. Xanthium strumarium* L.**

***7. Phalaris minor* Retz.**

***8. Ficus microcarpa* L. F.**

***9. Woodfordia fruticosa* (L.) Kurz**

***10. Plantago lanceolate* L.**

ANNEXURE II

Top Ten Plants Pictures of Sandwhich Method

1. ***Boerhavia procumbens*** **Banks ex Roxb.**

2. ***Jacaranda mimosifolia*** **D.Don**

***3. Datura metel* L.**

***4. Asparagus adscendens* Roxb.**

***5. Berberis vulgaris* L.**

***6. Urtica dioica* L.**

***7. Tagetes erecta* L.**

***8. Calotropis procera* (Aiton) R. Br.**

***9. Hedera helix* L.**

***10. Plectranthus rugosus* Wall. ex Benth.**

ANNEXURE III

Review of Allelopathic Effect of Studied Plants.

Name of plant	Family	Test Plant	Bioassay Used	Allelopathic Effect (S=Stimulatory) (I=Inhibitory)	Type of Extract Used	References
*Boerhavia procumbens*Banks ex Roxb.	Nyctaginaceae	*Lactuca sativa* L.	Sandwich Method	Inhibitory		(Jan *et al*., 2012) (Mahmood *et al*., 2014)
		Lactuca sativa L.	Dish pack Method	Inhibitory		
		Lactuca sativa L. *Triticum aestivum* L. *Zea mays* L.		Inhibitory	Aqueous Extract	
*Jacaranda mimosifolia*D. Don	Bignoniaceae	*Lactuca sativa* L.	Sandwich Method	Inhibitory		(Njogu, 2012) (Witt *et al*., 2017) (Nawaz *et al*., 2013)
		Lactuca sativa L. *Triticum aestivum* L. *Zea mays* L.		Inhibitory	Aqueous Extract	
Datura metel L.	Solanaceae	*Lactuca sativa* L.		Inhibitory		(Kalistović, 2017) (Abad *et al*., 2017) (Gella*et al*., 2013)
		Lactuca sativa L. *Triticum aestivum* L. *Zea mays* L.	Sandwich Method	Inhibitory	Aqueous Extract	

*Asparagus adscendens*Roxb.	Asparagaceae	*Lactuca sativa* L.		Inhibitory		(Dinan*et al*., 2001) (Singh and Rai, 2000)
		Lactuca sativa L. *Triticum aestivum* L. *Zea mays* L.	Sandwich Method	Inhibitory (*Lactuca sativa* L. *Triticum aestivum* L) Stimulatory (*Zea mays* L.)	Aqueous Extract	
*Plectranthus rugosus*Wall. ex Benth.	Lamiaceae	*Lactuca sativa* L.	Sandwich Method	Inhibitory		(Irshad*et al*., 2012)
		Lactuca sativa L.	Dish pack Method	Stimulatory		
		Lactuca sativa L. *Triticum aestivum* L. *Zea mays* L.		Inhibitory (*Zea mays* L.) Stimulatory (*Triticum aestivum* L. and *Lactuca sativa* L.)	Aqueous Extract	
Parthenium hysterophorus L.	Asteraceae	*Lactuca sativa* L.	Dish pack Method	Inhibitory		(Mishra and Nautiyal, 2012) (Shabbir and Bajwa, 2006). (Thakur, 2017) (Rashid *et al*., 2008)
		Lactuca sativa L. *Triticum aestivum* L. *Zea mays* L.		Inhibitory	Aqueous Extract	

Arisaema triphyllum (L.) Schott.	Araceae	*Lactuca sativa* L.	Dish pack Method	Inhibitory		(Evans *et al*., 2016) (Bohlen *et al*., 2004) (Hale *et al*., 2006). (Puckoet *al*., 2011)
		Lactuca sativa L. *Triticum aestivum* L. *Zea mays* L.		Inhibitory (*Lactuca sativa* L. and *Zea mays* L.) Stimulatory (*Triticum aestivum* L.)	Aqueous Extract	
Crotalaria medicaginea DC.	Fabaceae	*Lactuca sativa* L.	Dish pack Method	Inhibitory		(Vishwakarma, 2014) (Dangwalet *al*., 2011) (Wang *et al*., 2002).
		Lactuca sativa L. *Triticum aestivum* L. *Zea mays* L.		Inhibitory (*Lactuca sativa* L. and *Zea mays* L.) Stimulatory (*Triticum aestivum* L.)	Aqueous Extract	
Amaranthus viridis L.	Amaranthaceae	*Lactuca sativa* L.	Dish pack Method	Inhibitory		(Hussain *et al*., 2003) (Cândidoet *al*., 2010)
		Lactuca sativa L. *Triticum aestivum* L. *Zea mays* L.		Inhibitory	Aqueous Extract	