ALLELOPATHIC ACTIVITY ASSESSMENT IN SELECTED PLANTS COLLECTED FROM NURSERIES OF ISLAMABAD.

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Dedicated to My Family

FORWARDING SHEET BY RESEARCH SUPERVISOR

The dissertation entitled **"Allelopathic Activity Assessment in Selected Plants Collected from Nurseries of Islamabad."** 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 Master of Science degree from department of Environmental Science, as per IIU rules and regulations.

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Abstract:

The present study was focused on exploring the allelopathic potential of 150 exotic ornamental plant species belonging to 62 families, on *Lactuca sativa* L. (Lettuce plant) by using dish pack method. The specimens were collected from local plant nurseries of Islamabad. *Passiflora alata* curtis (Passifloraceae) Pandanus *tectorius* Parkinson ex Du Roi (Pandanaceae), *Solanum betaceum* Cav. (Solanaceae), *Polyscias paniculata* 'variegata' (Araliaceae), *Terminalia ivorensis* A. Chev. (Combretaceae) and *Pittosporum tobira* Thunb. (Pittosporaceae). *Passiflora alata* curtis (Passifloraceae) commonly known as Passion fruit has the strongest inhibition effect of 100% against the Lettuce seeds ggermination. On the other hand, *Melaleuca bracteata* F. Muell., *Leucophyllum frutescents* (Berland.) I.M. Johnst., *Gladiolus grandif lora* L., *Hydrangea macrophylla* (Thunb.) Ser., Cryptanthus *bromelioides* var. tricolor M.B. Foster and *Syngonium podophyllum* Schott had strong elongation effect (lower inhibition) on the growth of Lettuce seedlings. The results of this study can be used for future research for identification and characterization of allelochemicals found in these plants; the plants which had showed inhibitory effect can be used for the control of weeds as an alternative of herbicides/weedicides. Those plants which have stimulatory effect or minimum inhibition can be used as growth promoters in agriculture and horticulture. It is suggested that ornamental plants should not be planted or sold commercially before their risk assessment and screening for allelopathic potential.

CHAPTER 01

INTRODUCTION

1.1 Introduction:

German plant physiologist Hans Molisch (1937) coined the word *Allelopathy*, a term derived from two Greek words; *allelon* and *pathos. Allelon* mean 'of each other' while *pathos* refers to 'to suffer'. This is a natural phenomenon in which plants, micro-organisms, viruses, and fungi produce such chemicals (phytotoxins), which upon their release in the environment affect the growth and development of biological ecosystems. These chemicals produced by a plant or its residues are called *Allelochemicals* that can change the growth, development and normal functioning of the plant, resulting in the decline of its productivity in an agro-ecosystem (Young, 1986; Chou, 1999). They take part in plant-herbivore, plant-plant and plant-insect interactions or chemical communication (Weir *et al.*, 2004), as well as in microbe-plant and microbe-microbe interactions (Singh *et al.*, 2003). These interactions can obstruct the structure of the ecosystem and its dynamics (De Albuquerque *et al.*, 2010).

Allelopathy shows the adverse effects on plants and other organisms (Rizvi *et al.*, 1992). Different scientists gave different definitions of Allelopathy. Rice (1984) defined Allelopathy as; "any direct or indirect harmful or beneficial effect of one plant (including microorganisms) on another, through the production of chemical compounds that escape into the environment" (Rice, 1984). International Allelopathy society defines allelopathy as; "any process involving secondary metabolites produced by plants, microorganisms, viruses and fungi that influence the growth and development of agricultural and biological systems (Anonymous, 1996).

Allelochemicals are mostly secondary metabolites of low molecular weight, excreted by plants (Ma *et al.*,2011; Sharma and Deykota, 2015). They are generally considered as nonnutritive chemicals or waste material produced during the decomposition of microorganisms (Makoi and Ndakidemi, 2012; Cheng, 2015). Allelochemicals are present in almost every part of the plant; roots, shoots, leaves, seeds, fruit, flowers, stems and even pollen (Putnam and Tang, 1986; Sharma and Deykot a, 2015; Gatti *et al.,* 2004). Different plants species release allelochemicals into the environment through different pathways. Commonly known pathways are;

i. Exudation from the leaves followed by deposition on them and later washing off when it rains;
ii. Exudation of volatile allelochemicals from leaves, stem *etc.* of plants;
iii. Exudation through roots (Chon *et al.*, 2006).
iv. Deterioration of plant materials (litter fall or dead roots) (Putnam and Tang, 1986; Fujii, 2004; Mubeen, 2011).

The role of Allelopathy is crucial in ecology as allelochemicals play an important role in the process of biological invasion. Although these are non-essential constituents and do not affect the normal functioning of a plant directly yet, the harmful allelochemicals are involved in defense of plants against certain pathogens, herbivores and other associated plants species (Fraenkel, 1959; Stamp, 2003). Some invasive plant species are perceived to be successful because they produce such novel compounds that serve as mediators of the new plant-plant interactions or allelopathic agents (Callaway, 2004).

Intentional and unintentional movement of various plant species, beyond their natural habitat and geographical barriers have been facilitated due to increased travelling and trade, as well as progressive tourism. Some of these exotic species show great adaptability to their environment and can rapidly grow and efficiently spread in the new environment. This has adversely affected the economy, society, and ecological system of the local areas. Presently, allelopathy is the most important element that influences the invasion and proliferation of exotic plants (Chengxu, 2011).

Human-assisted influx of exotic plant species is sometimes a million times more than the natural rate. Species so introduced in a particular region are named as *exotics* or *aliens* and they are in several thousands (Khan, 2010). Bio-invasion is among the main environmental problems of the world and a major threat to biodiversity (Catalán, 2013). The dislocation of the various species including micro-organisms as well as plants and animal species from one region to another due to human action is accepted as one of the most severe threats to Earth's ecosystem and biodiversity. Bio-invasion is biological pollution, which produces more adverse effects on environment than other forms of pollution (Khan, 2010). After the habitat loss biological invasion is second major threat to biodiversity at world level (Reddy,2008). According to the Cartagena Protocol, habitat loss due to introduction of exotic species in an area is the main cause of biodiversity loss. After habitat loss, habitat invasion produced by exotic species is the second primary threat to endangered species According to the surveys, nearly 20% of endangered vertebrates are vulnerable to the adverse impact of exotic species. An additional danger to biodiversity is habitat modification, which arises due to the introduction of exotic species (Alder, 2000). Ornamental plants (plants that are grown exclusively for their aesthetic values or for decorative purposes), whether indoor and outdoor plants are chosen for their size, shape, color, smell and flowers rather than their environmental effects and ecological services (Lecomte,2016).

Non-native species have been rapidly spreading into the new environments causing severe human accelerated changes in the environment and have resulted in extensive economic and environmental hazards (Hastings, 2006). Consequently, native plants have been excluded by the invasive species in spite of the fact that mostly they are not dominant in their natural habitats (Callaway and Aschehoug, 2000). Exotic plants, which are introduced deliberately due to their ornamental importance or accidently, have the ability to compete with the native flora of that area by decreasing the survival, growth and biomass of native vegetation (Catalán, 2013). The main corridor for plant invasions throughout the world is Ornamental horticulture (Dehnen-Schmutz, 2007).

Allelopathy can increase the competitive success of the introduced plants in an area by releasing phytotoxins in the environment. These phytotoxins can disturb the growth and life supporting processes of other plants (Callaway, 2002). That is why allelopathy is considered as one of the most significant factors for the success of exotic flora (Filemon and Patrick, 2014). Exotic plants effectively compete with the native species in the terrestrial ecosystems. They decrease their chances of survival by decreasing their growth rate and the available biomass (Levine,2003; Hager, 2004; Qin *etal.*, 2013). A theory states that allelopathy responsible for the success of some of the invasive species.

Ornamental plants have been gaining an economic importance in the international market and their demand is rapidly increasing worldwide. Major portion of the industry comprises of the cut flowers followed by the flowering pot plants, flower bulbs, nursery crops, trees and other propagation materials. This market flourishes in the Western Europe, the United States, Japan, Netherlands, Italy, Colombia, Israel, Denmark, Belgium, Germany and many other countries (Lawson, 1996). Ecological communities have been altered greatly due to the invasive plants and animals. They influence the proper functioning and health of the affected ecosystems (Ameen, 1999).

Pakistan is blessed with a variety of plants, which is attributed to its diverse climatic conditions, and wide range of phytogeography of the country. Total flora of Pakistan comprises of 6000 species (Shinwari, 2000) with a majority of medicinal plants. Many plants have arrived here accidently or brought purposefully like, beautification of a particular area or the horticultural purposes, *etc*. Islamabad is a well-planned city in which native flora is almost intact, well developed, abundant with a variety of species and further been enriched by the introduction of abundant ornamental plants and trees (11 million marks in 1982). Some of the most common plant species that were introduced in Pakistan in the past, have become invasive and have started affecting the native habitat, flora and ecosystems of Pakistan. Previously, the invasion of newly panted species in the capital city was unnoticed but later on, the experts took it seriously and research work was initiated to find out their harmful effects. Plant invasion may be through the import of seeds or their disposal by water, air or through different from the adjoining areas. Some of the most common invasive exotic species include; *Lantana camara* L., *Ailanthus altissima* (Mill.) Swingle, *Broussonetia papyrifera* L. *Bromus unioloides* Kunth *and Sylibum marianum* L. To make the capital city green and to prevent the process of soil erosion, *Broussoneyia papyrifera L.*, a native species to South Asia was introduced and planted in Islamabad in 1960s. It is a most problematic species in Northern Pakistan. Due to its fast growth rate, vegetative propagation and seeds dispersal by birds it has invaded in many areas. It has both social and ecological adverse effects (Khan *etal.,* 2010).

According to a study of "Pakistan Medical Council" 1995, approximately 45.5% of the patients in Islamabad and Rawalpindi were related to pollen allergy caused by Paper Mulberry (Marwat, 2010). *Ailanthis altissima* is a species native to central China. In Swat and Hazara, Forest department started the plantation of these trees along roadside as a common source of firewood. Now, it has become an invasive species of the wastelands and has replaced the local vegetation. It shows a prolific reproduction and rapid growth. *Lantana Camara L.*, native to Mexico and the United States of America was introduced initially as an ornamental plant in the capital territory of Islamabad, especially along the Margalla hills which contains allelochemicals like Lantadenea. *Prosopis juliflora* (Sw.) DC.; a native species of Northern Mexico and the USA was introduced in Sindh and the Punjab province for afforestation of deserts (sand dune stabilization) in 1950s and 1960s (Marwat, 2010; Qureshi, 2014). This tree has not become an invasive species and it could be seen in various ecosystems like in agricultural fields, rivers sides, coastal belts, and mountainous slopes especially along the roadsides.

Allelopathy is an interaction among plant species or between plants and microorganisms in which a plant produces secondary metabolites (allelochemicals). These secondary metabolites are capable of altering the chemistry of rhizosphere and have effect on other neighboring plants. The use of synthetic herbicides or weedicides for the weed management possess certain environmental hazards. Allelochemicals can be used as an alternative of synthetic herbicides for weed management as these have low cost and are also environment friendly (Macias *etal.*, 2001; Abbas *etal.*, 2017; Jabran, 2017)

Biodiversity is the integrity of genes, comprising of various ecological processes which consists of four major components; species diversity, genetic diversity, ecosystem diversity and the diversity of ecological processes (Kilic and Arslan, 2010; Haq, 2010). Species diversity indicates the diversity of certain species in a certain region or all over the world (Bulut and Yilmaz, 2010). Pakistan has a very rich and diverse flora due to the diversity in its climate and ecological regions, soil conditions and multiple ecological regions. Among 270,000 species of vascular plants present on the earth (Walter and Gillet, 1998), more than 6000 species exist in Pakistan (Ahmad, 2003; Qureshi ,2014) while at least 189 pteridophytes (ferns and their allies); 153 Sino-Himalayan and 36 Euro Siberian are present in Pakistan. Liverworts, Algae, lichens and mosses are poorly known. Nearly 87 genera and 3,383 species of fungi have been reported from Pakistan (Shah and Baig, 2001; Shinwari and Maryum, 2010). According to an analysis of the flora of Pakistan, at gene level a high rate of diversity is present, since the number of species per genus is much lower than the global average. The flora includes elements of six phytogeographic regions; Saharo-Sindian, the Mediterranean, Indian, Sin-Japanese, Euro-Siberian and Irano-Turanian region. At least 189 pteridophytes; 153 Sino-Japanese and 36

Euro-Siberian species are present in Pakistan. Among the flowering plants, four monotypic genera; Spiroseris, Douepia, Wendelboa and Suleimania and about 400 species (7.8%) are endemic to Pakistan. Most endemics are of Irano-Turanian and Sino-Japanese origin. Northern and Western mountains of Pakistan comprise of almost 80% of Pakistan's endemic flowering plants (Shinwari and Maryum, 2010).

Due to population explosion, deforestation, unplanned urbanization, and overexploitation of natural resources, Pakistan is under tremendous ecological pressure. (Alam and Ali, 2009). Natural forests are limited (less than 4 %) which are rapidly declining at a rate of 4–6 % per year (Ibrar, 2003). This rapid degradation of forests clearly indicates a severe decline in the number of species and loss of genetic diversity of population (Afzal, 2001). Conserving biological diversity in forest and agricultural ecosystem is of great importance as the production of goods and services depends on the basic ecological services provided by biodiversity. Similarly, biodiversity improves the resilience of a system to bear the external pressures like natural disasters; floods, droughts etc. or management issues (Fisher *et al.*,2006).

Invasive species are a major threat for the environment, economy and health. These are generally exotic species which are introduced in an area either deliberately or sometime unintentionally. Over the past couple of centuries, a large number of exotic plants have naturalized in various part of world and have become alien species. After the habitat destruction this biological invasion also called biological pollution is major threat to biodiversity. Invasive species are found in all kinds of ecosystems globally and include all kinds of living organisms (Raghubanshi,2005) but plants, mammals and insects comprise the most common types of invasive in terrestrial environments (Hoenicka and Fladung, 2006). Plants species are considered as the worst invaders due to their huge biomass. In back history colonization and exploration were the main causes for the spread of invasive alien species. Luckily, in Pakistan the invasive alien species are not as problematic or established as in some other countries.

Unluckily, there is very limited work has done in context of biological invasion (Qureshi, 2014).

The process of biological invasion by alien invasive species needs breaking some natural berries like environmental, physical and biological in different phases of invasion; Entry/Escape, Establish, Expand, Explode and Entrench (Williams, 1997). The degree to which plant get naturalized and spread in an area depends on some factors like; propagules pressure, properties of ecosystem i.e. physical, chemical and biological diversity of that area in which a specie is introduces and also the properties of exotic species itself. Whenever an exotic species finds favorable condition it easily get established in that area (Lonsdale, 1999).

Invasive alien species is a global issue which requires international cooperation including the capacity building of staff, strengthening the legislation regarding invasive alien species, cross sectoral collaboration, checking cross boundary transit of invasive alien species, taking initiatives and improving techniques, checking of consignments, mass awareness of key stakeholders, informed decisions, detailed scientific inventory, scientific research and putting in place proper quarantine regulations. The ability of some plants to affect the other neighboring plants has been well recognized since antiquity. The pioneer work related to allelopathy is attributed to Theophrastus (ca. 300 B.C), who was a successor of Aristotle who observed the injurious effects of cabbage on a vine and proposed that these effects were due to the "odours" from the cabbage plants (Willis, 1985).

1.2 Problem Statement:

Plants introduced by various nurseries without environmental risk assessment may cause serious health issues as well as ecological and economic damages in Pakistan. These invasive plants introduced due to their ornamental value, having an invasive potential, can bloom and survive under the worst environmental conditions of pollution, water, scarcity, salinity, *etc.* In addition to this, mostly herbivores as well as pathogens avoid them due to the frequent production of noxious compounds (repellents). All of these properties suggest that these species might possess a hitherto allelopathic potential, which should be explored by converting these threats into opportunities.

1.3 Aim:

The aim of the proposed study is to evaluate allelopathic potential/ Phytotoxicity found in the nurseries of Islamabad and development of a baseline data.

1.4 Objectives:

1. Identification of ornamental exotic/ invasive plants in use at nurseries of Islamabad.
2. To study, the phytotoxicity through assessment of the allelopathic potential of plants collected from nurseries of Islamabad.

1.5 Significance of Study:

Assessment of allelopathic potential of ornamental plants will result in identification of allelopathic plants and the effect of phytotoxins released in the environment by them. Plants having negative allelopathic effect can be further studied in-depth to find out more about their allelochemicals and their possible social, ecological and economic effects. The proposed study will result in the development of a base line data of phytotoxic ornamental exotic/invasive plants and it will trigger the research work leading towards national data base development of phytotoxic plants and integration with international database.

CHAPTER 02

LITERATURE REVIEW

Invasive alien species colonize the environment, act as mediators of change and threaten native biodiversity of any ecosystem. Invasive or exotic species are the non-native species, which have been deliberately or unintentionally introduced into different habitats. Mostly exotic species are introduced in an area by importing their seeds, eggs, spores, or other biological material capable of propagating that species. The introduction of these species likely to cause social, economic and environmental harm or harm to human health (Rashid *et al.*, 2014).

Allelopathy is a branch of chemical ecology that deals with the study of effects of secondary metabolites produced in plants or microorganisms on the growth, development and distribution of other living organisms (plants and microorganisms) in their ecosystem (Einhellig,1995; Cheng,2015). In early 20th century, allelopathy was mainly focused on agricultural productivity (de-Albuquerque, 2011). The study of allelopathy increased in the 1970s and has undergone rapid development since the mid-1990s, becoming a popular topic in botany, ecology, agronomy, soil science, horticulture, and other areas of inquiry in recent years. The allelopathic interaction can be one of the significant factors contributing to species distribution and abundance within plant communities and can be important in the success of invasive plants (Zheng *etal.*,2015). After 1960, plant ecology was included in allelopathy due to the contributions of Muller, who described a unique pattern of herb exclusion by adjacent chaparral vegetation, *Salvia leucophylla* (Muller, 1966). Muller and his students contributed their efforts to allelopathy research on several chaparral shrubs (Muller, 1969) and concluded that allelopathy plays a significant role in the California fire cycle phenomenon of chaparral vegetation. Muller indicated that the luxuriant growth of herbaceous plants in the first growing season following a fire was due primarily to the denaturation of the allelopathic compounds from *Adenostoma fasciculatum* Hook. and Arn (Chou,2010).

Since the past few decades, the studies on allelopathy in plants have been developed and allelopathic crops are now cultivated in fields as a cover crop, crop rotation, green manure etc. Allelopathy is a dynamic process and it is beyond just a donor and receptor plant or organism. The advancement in research and new approaches have identified many allelochemicals and helped in understanding their synthesis, release and their fate in soil. Modern methods and new approaches have helped identify potential allelochemicals and have led to a better understanding of the biosynthesis, release and dynamics of these allelochemicals in the soil. Genetic studies are just getting underway and gene identification and manipulation are seen as the next step in the establishment of allelopathic traits as important tools in weed management programs (de Albuquerque *etal.*, 2011).

Different hypotheses were proposed for studying the success of exotic plant in their new non-native habitats. Many studies showed that the invasive success of exotic plants may be attributed due to the presence of strong allelochemicals in them and their effect on other native plant species. The "*novel weapons*" states that some plants contains such powerful allelochemicals that enable them to compete with other plants in new environments (Ni *etal.*,2012). *Centaurea maculosa* Lam. get established in non-native environment in because its strong allelochemicals and their effect on the native plant species in North America as compared to that of in Europe. The other hypothesis is "*evolution of increased competitive ability*", which proposes the escape from the enemies in their natural habitat and evolution of more competitive ability, which may be the reason for the success of dominant plants invaders in introduced regions (Bossdorf ,2013). *Centaurea maculosa* Lam. species have evolved to be greater and better competitors in their non-native habitat in North America than that in their

European native range. These two hypotheses highlighted main points for the comparison of natural competitive abilities to evolved competitive abilities of plants. The composition of native plant communities that are being invaded by invasive species in a region are of great importance for the success of invasive species than evolution of increased size and competitive ability (He *et al.*,2009). *"Natural enemies" hypothesis* states that the one of the main reason for the success and establishment of exotic invasive species in non-native ecosystem is due to the lack of their predator or consumers in new environments and they have escaped from their natural enemies in their native range which have controlled their population size and growth. But the failure of some control methods for invasive plants species; bio-controls, least consumer effect on population of invasive species and weak top-down control in natural many ecosystems indicate that some other processes may be attribute with the success of exotic species. Allelopathy (the release of allelochemicals that effect the growth and development of neighboring plants) is considered as an important process for the establishment of invasive species. It is more important in non-native areas than the native areas as, plants species in native zone are used to the allelochemicals while in non-native regions plants are naïve to these allelochemicals released by invasive species. The effects of allelopathy have been tested on native plants species, allelochemicals have been tested in different resources Allelopathic effects have been tested on native species, allelochemicals have been tested in fluctuating resource circumstances, different models have been used for approximation of comparisons of resource and allelopathic effects. The experimental techniques have been used to improve chemical effects (Herro and Ragan, 2003).

Since last some decades, exotic plants have been introduced in Pakistan. Many of these plants have now become the alien invasive species in many areas. These invasive plants were introduced for meeting needs of timber, fodder and fire wood (Hussain and Zarif, 2003). In Pakistan, alien invasive species are not as abundant as in other part of world. However, the effects of these exotic species on indigenous biodiversity have not been yet reported in Pakistan (Qureshi *et al.,* 2014; Shinwari and Shinwari, 2010). There is no database available for the invasive species yet that could give comprehensive information about the effect of exotic plant species on native species composition and diversity. Least data is reported regarding biological invasion of plant in literature due to inadequate research studies. About 700 plant alien species have been recorded as a result of these insufficient research studies (Khatoon and Ali, 1999). *Broussonetia papyrifera* (L.) L'Hér. ex Vent., *Prosopis juliflora* (SW.) DC., *Parthenium hysterophorus* L. and *Lantana camara* L. were declared as alien invasive species having considerable impacts in land (Hussain, 2003).

On 17th September,1999, in NARC, Islamabad a consultative workshop was arranged for the identification, evaluation and ranking of exotic plants that have threatened the biodiversity in natural ecosystems and in also in agricultural lands. The main importance was given to those exotic plants that had already gotten established in various ecosystems like, parks, reserves, national forests, range lands and also wildlife areas. 14 exotic plants including trees, shrubs and some aquatic plants, were declared as alien invasive species (Rashid *et al.,* .2014).

Allelopathic assessment of 38 shared invasive plants species and weeds of Pakistan and Japan at Plant Chemical Ecology Laboratory at National Institute of Agro-Environmental Science, Japan from January 2008 to March 2009. Three methods; Plant-box, Sandwich and Dish-packs were used for examination of root exudates, leaves litter and volatile allelochemicals. The results of the study showed that *Melilotus officinalis* (L.) Lam. had strong inhibition against *Lactuca sativa* L. the other allelopathic plants were *Melilotus alba, Datura stramonium* (Sw.) DC. and *Mirabilis jalapa* L. While *Rumex crispus* L., *Plantago lanceolata, Rumex conglomerates and Trifolium pratense* showed minimum inhibition effect. The results obtained can open new avenues for further research and exploration of the allelochemicals (Shinwari *et al.*,2013a).

In Pakistan 20 medicinal plants were collected from Pakistan and their phytotoxicity was examined by using plant-box method for roots exudates, sandwich method for leaf leachates and dish-pack method for volatile allelochemicals. *Lactuca staiva* L. was used as a test plant. In plant box method *Tagetes minuta* L. had the strong negative allelopathic effect. The other plants that showed inhibitory effect were *Setaria verticillate* (L.) P. Beauv and *Mirabilis jalapa* L. In sandwich method, *Pyrus pashia* Buch-Ham. ex D. Don had strongest effect on growth of radical. *Solanum surattense* Burm. f. and *Solanum villosum* Mill. had also showed inhibitory effects. *Tagetes minuta* L. showed strong inhibition against lettuce in dish-pack method. *P. juliflora* and *L. camara* aslo had negeative allelopathic effect (Shinwari ,2013 **b**).

Invader species are a great threat to local flora. Eight invader species of Pakistan were screened for their allelopathic activity through sandwich method. Toxic (inhibitory) and non-toxic (stimulatory) effects were assessed by recording their effect on germination and growth of lettuce in dishpack method. Radicle and plumule growth of lettuce were recorded. Except *Eutcalyptus glabra* all species resulted in inhibitory effects. *Xanthium strumarium* L. and *Cannabis sativa* L. showed strong inhibitory effects on radicle and plumule growth of lettuce. Maximum inhibition was recorded at highest concentration; even growth of lettuce was stopped with 50 mg leaves concentration of *C. sativa* (Akhtar *et al.*,2014).

Allelopathic interaction of wheat and little seed canary grass (*Phalaris minor* Retz.) was studied by using wheat cultivars and promising lines (Faisalabad-08, Lasani-08, Shafaq-06, Sehar-06, Miraj-08, Farid-06, Chakwal-50, V-04178, V-05066 and V-05082) through *the equal-compartment-agar method.* Results revealed that wheat cultivars had differential allelopathic inhibition activity against little seed canary grass through the production of phenolic compounds. Maximum inhibition in root length (54%), shoot length (59%), root dry weight (60%) and shoot dry weight (55%) of little seed canary grass was recorded when grown in association with wheat. Significant increase in production of total soluble phenolics was also observed in root and shoot of all wheat cultivars when grown in association with little seed canary grass as compared to when grown alone. In conclusion, cv. Shafaq-06 was found strongly allelopathic against little seed canary grass (Kashif *et al.,* 2015).

Adaptations of crops to forthcoming climate change was focused in a research study carried out in Japan. Leaf liter of 160 medicinal plants were subjected to evaluation of their allelopathic effects using the Sandwich method. *Lactuca sativa* L. was used as a test plant material in the bioassay. Maximum inhibition activity was shown by *Melia azedarach* (Meliaceae) followed by, Tylophora *tanakae* (Ascepiadaceae), *Cinchona* sp. (Rubiaceae), *Flueggea virosa* (Phyllanthaceae), *Hibiscus acetosella* (Malvaceae), *Justicia procumbens* (Acanthaceae), *Terminalia chebula* (Combretaceae), *Hibiscus syriacus* (Malvaceae), *Lycium chinense* (Solanaceae) and *Elaeocarpus japonicas* (Elaeocarpaceae). Minimum growth inhibition (maximum growth stimulation) was shown by *Ligustrum japonicum* (Oleaceae), *Vitex rotundifolia* (Lamiaceae) and *Alpnia intermedia* (Zingiberaceae). Results thus obtained may be used as a foundation for the research and investigation of novel allelochemicals (Shinwari *et al.,* 2017).

The antifungal effects of 52 dried samples of spices and herbs was measured by using the dish pack method, against phytopathogenic fungus, *Fusarium oxysporum*. Black zira *(Bunium persicum)* showed the strongest effect, followed by cumin and cardamom. Seven volatile compounds, γ-terpinene, limonene, *p*-cymene, β-pinene, α-pinene, cuminaldehyde, and myrcene were identified among which, cuminaldehyde and *p*-cymene showed the strongest antifungal activities against *F. oxysporum*.and another fungus species, *Verticillium dahliae*, and foliar phytopathogenic fungi, *Botrytis cinerea* and *Alternaria mali*.

Cuminaldehyde is the main antifungal compound detected in black cumin (Sekine *et al.*, 2007).

Methods for assessment of Allelochemicals.

Bioassays using petri dishes, amendments of plants residues, competition test plant-box, sandwich and dish-pack methods, are mostly used for the assessment of allelopathic plants.

Bioassay with Petri dishes is the most common technique and is a component of elaborate investigation in allelopathy research. In a bioassay different extracts, essential oils and isolated or commercial chemicals from plant material are tested on test plants for observing effect on seeds germination and their growth. There are different types of extracts which are prepared as required but mostly aqueous and hydro-alcoholic are used. Although all parts of plant contain allelochemicals but mostly leaves of plant are used for the preparation of extracts. A filter paper is placed at the bottom of petri dish and seeds of test plant are than placed on this filter paper for the assessing the effect of different concentrations of extract solution on their germination and growth. These petri dishes are than place in incubator or BOD chamber either in the dark or with a programmed photoperiod depending on the photoblastism of the seed tested. Sometimes, a small modification is made in this method by adding solid agar medium with extracts or isolated substances. Germination velocity and percentage as well as shoot and root length are the variables commonly evaluated in these bioassays (de Albuquerque,2011). The phytotoxicity of *Vulpia myuros* (L.) C.C. Gmel residues extracts on wheat (*T. aestivum* L. cv. Vulcan) was evaluated in a laboratory by using bioassays with petri dishes experiment. The delay in seeds germination and inhibitory effect on seedlings, as well as the reduction in coleoptile and roots growth was on seedlings was observed (An *et al.*, 1997). Similarly, by using same experiment it was found that the organic extracts of *Petiveria aliacea* L. had strong phytotoxic effect on seed germination and initial growth of lettuce as compared to the aqueous extracts (Pérez-Leal *et al.*,2005).

Competition test is used for studying allelopathy. It can be seen as the removal from the environment of common resources, while the allelopathy consists of the adding allelochemicals chemicals in environment (Olofsdotter,2002). Various methods have been used for studying competition; additive, substitutive, systematic and neighborhood. Generally additive method is used. In this protocol the density of one plant is kept same and other is change for the identification of allelopathic plants. In this density-response protocol suggested by Weidenhamer in 1989, number of donor species in kept constant while the number of targeted species are change (generally reduced) for assessing effect of allelochemicals (Weidenhamer *etal.*, 1989) Kruse,2000). Plants mostly show stimulator effect on low doses while on high doses plants have negative effect, their growth is reduced and a stage comes when plant dies. As density of plant increases the dose per plant decreases and plants start competing for allelochemicals. When the number of target plants are reduced there is sufficient availability of chemicals as the dose per plant increases (Sinkkonen,2005; Zeng ,2008; Haynes and Millar, 2012).

A study has been conducted by using density-response method for phytotoxicity analysis of lettuce seedlings grown in soil mixed with powder from *Macaranga tanarius* L. with lower seed density strong inhibitory allelopathic effect was observed on the growth of plants. Which was attributed to leaves decomposition. Many chemicals were identified in *M. tanarius* plant leaves. Among these chemicals, abasic acid had the strong negative effect on growth of lettuce and plants seedlings (Tseng *et al.*, 2003).

Rhizosphere soil or Residual toxicity in soil is a protocol for the assessment of allelochemicals in soil, released from the roots of allelopathic plants. Soil is collected from the rhizosphere of potential allelopathic plants. This soil is than used as medium for growing test plants. Pure soil is used as a control. The Aqueous extracts of these soils can also be used in Petri dish bioassays (Kruse,2000; Fujii,2005 August). A study was conducting for evaluation of soil toxicity by same methods. Soil was collected from the fields where for 5 years *Tithonia diversifolia* Hemsl. A. Gray (Mexican sunflowers) were grown. 6 test plants were grown on this soil and it was found that the seed germination and growth of seedlings were affected by the residual material as compared to the plants that are grown in normal soil (Tongma *et al.*,2001). During the analysis of phytotoxicity of soil it was found that the paddy soil infested with barnyard grass had severe negative effect on the growth of weeds and crops grown on that soil. Different aqueous and EtOH solutions from resin retained compounds were used. It was found that aqueous solutions had strong inhibitory effects on growth of rice and barnyard grass. (Khanh *et al.*,2008).

In allelopathy, hydroponics is also used. It is used for two main purposes; as a path way for the delivery of allelochemicals to the test plant and for the collection of root exudates for bioassays or for the identification of allelochemicals (de Albuquerque,2011). By continuous root exudates trapping system in hydroponic setup, the allelopathic potential of watermelon was assessed. Laboratory bioassays and high-performance liquid chromatography were used for quantification of phytotoxins in different plant tissues and root exudates collected using XAD-4 resin. Roots exudates of watermelon had inhibitory effect on lettuce and watermelon growth. The inhibition rate increased with the increase in concentration of root exudates. Inhibitory effects of various plant tissues were demonstrated using the bioassay tests. Frulic acid and ρ-hydrobenzoic acid were the dominant components found using HPLC and the amounts of allelochemicals found in the plant extracts were correlated with the bioassay results using the Petri dish test. The results indicate that the allelopathic potential of watermelon may play an important role in the phenomenon referred to as 'soil sickness' (Hao *etal.*, 2007).

Amendment of plant material in soil is a method that has been used for allelopathic research. In this method, plant matter is added in soil in different quantities for assessing allelopathic effect of allelochemicals released in soil after decomposition on test plants grown. To prevent the microbial contamination soil or substrate is sterilized. Due to its success, this method has been used in green house and field experiments (Kruse,2000; de Albuquerque,2011). The assessment by mixing plant residues of *Parthenium hysterophorus* L., it was observed that 40 $g.kg^{-1}$ of soil reduced the size and dry mass of *Brassica oleracea* L., *B. campestris* L. *and B. rapa* L. These results were credited to the release of water soluble phenolics into the soil by *P. hysterophorus* residues (Singh *et al.*, 2005). Using similar protocol, *A. conzoides* residues added in soil and its effects on growth and nodulation of *Cicer arietinum* L. was studied and it was confirmed that 10 $g.kg^{-1}$ material added was sufficient to cause significant reductions in all analyzed variables. Soil amendment with *Chenopodium murale* L. residues reduced the growth, nodulation and macromolecule content of two legume crops *(C. arietinum and Pisum sativum* L.) (Batish *etal.*, 2006; Batish,2007).

In 1992, Plant-box method was developed by Professor Youshiharu Fujii (Fujii, 1992). This method is used for the assessment of root exudates from potential allelopathic plants. It is based on the principle of dose-response to check the relationship between the concentration of chemicals released from roots and their effect or inhibition caused on test plants. Agar media is used to provide a medium for dispersion of chemicals from roots of donor plant to the test plants. Donor plants are cultivated in sand and stagnant water having nutrients for one to two months. Donor plant is put into cellulose (or nylon) tube and is placed at the corner of sterilized apparatus. Plant-box is kept in ice and is filled with autoclaved and cooled agar. After gelatinizing the agar media, seeds of test plants (usually lettuce (Great Lakes 366) are used due to their high sensitivity) are concentrically placed in the area around the donor plant and the container is sealed with clear wrap to prevent evaporation. The boxes are placed in a black vinyl pot to darken the roots of the target seedlings and kept in a BOD chamber. In control plant box filled with agar, having seeds but no donor plant is used. After a given period of time, length of radicle is measured and compared with that of the control (Fuji, 1994; Nishihara,2005; Fujii,2007; Fujii,2009). Plant box method was used for the assessment of allelochemicals in *Tamarindus indica* L.(Parvez,2003). This method was used for the identification of 7 ground cover plants (Shiraishi *et al.*,2002). Same method was used for the confirmation of the effect allelochemical {L-DOPA [L-3-(3,4-dihydroxyphenyl) alanine]} released from the roots of *Mucuna pruriens* L. (D.C). (Nishihara,2005).

Sandwich method (Fujii,1994) is used for the assessment of allelochemicals from leaf leachates. It works on the same principle of Plant-box method, allelochemicals moves from leaves to plant and agar media provide a pathway for their movement. First leaved of plants are collected and dried in oven at 60 °C for 24hours. These are than stored in bags for experiments. Six-well multi-dish plastic plates are used in which autoclaved agar at 115°C for 15mins and cooled at 45 °C is added. It is called Sand-method as a layer of 5ml of agar is added first at the base of one well in dish and then 10mg or 50 mg leaf litter is added on that layer. After this another 5ml of agar is added which form double layers. At the end 5 seeds of lettuce are placed on top layer. Then dishes are covered with lids, sealed with tape and cover with Aluminum foil and kept in incubator for 3days at 25 °C. after 3 days the length of radicle and hypocotyl are measured and compared with control. The control does not contain leaves material but agar and seeds only (Fujii,1994;2003 ;2004).

Sandwich method was used for the screening of 239 medicinal plant species. *Lactuca stiva* L. was used as a test plant. Top ranked species that showed strong allelopathic effect or inhibit the growth of lettuce were; *Artabotrys odoratissimus* R., *Annona cherimola* Mill. Dialium *guineensis* wild. T. *indica*, *Emblica pectinata* and *Hevea brasiliensis* L. In sandwich method 6 multi-well dishes (Nalge Nunc International K.K.) were used. In these dishes the dried leaves (at 25° for 24 hours) material was placed in between the two layers of autoclaved agar media at 40°C to 45°C. On the top layer of agar medium lettuce seeds were vertically placed for assessment of leaf leachate allelopathic effect. After placing seeds, dishes were covered with lids, sealed and placed in BOD chamber for 3days. Agar substrate is alone used as a control (Fujii, 1994 and Fujii *et al.*, 2003). Sandwich method was used for screening of 71 plants were screened by using Sandwich method for determination of their allelopathic potential. 7 species showed strong allelopathic effect against the lettuce seedlings by more than 50% (Shiraishi *et al.*, 2002). 160 medicinal plants were collected from Research Center for Medicinal Plants Resources, Tanegashima, Japan. Sandwich method was used for the evaluation of their allelopathic effects on Lettuce. *Melia azedarach* (Meliaceae), *Tylophora tanakae* (Ascepiadaceae), *Cinchona sp.* (Rubiaceae), *Flueggea virosa* (Phyllanthaceae), *Hibiscus acetosella* (Malvaceae), *Justicia procumbens* (Acanthaceae), *Terminalia chebula* (Combretaceae), *Hibiscus syriacus* (Malvaceae), *Lycium chinense* (Solanaceae) and *Elaeocarpus japonicas* (Elaeocarpaceae) were top ranked plants that had inhibitory effect. On the other hand, *Ligustrum japonicum* (Oleaceae), *Vitex rotundifolia* (Lamiaceae) and *Alpnia intermedia* (Zingiberaceae) had low inhibition of strong stimulatory effect on growth of Lettuce (Shinwari *et al.*, 2017).

Dishpack method is another method that is used for the assessment of volatile allelochemicals from the leaves of plants (Fujii,2000; Fujii,2005). 6-wells multi-dish is used in dish-pack as used in sandwich method.100mg of dried weight of leaf material is added in 4th well. Leaves are cut into small fragments of about 2mm^2. 7.0ml of distilled water is added in this sample well. In other remaining wells a filter paper is placed at the bottom of each well on which seven seeds are placed. 7.0 ml is added in all wells and after covering with lid, dish is sealed with plastic tape and warped in Aluminum foil. A dish without leaves is used as a control. Then both sample and control dishes are place in a BOD chamber or Incubator at 25^{0}C for three days. After three days the number of seeds germinated are counted and growth of radicle and hypocotyl of 5 out of 7 *Lactuca Staiva* L. seedlings is measured (Shinwari,2008; Shinwari and Fujii,20013b)

251 plant species collected from the Sino-Japanese Floristic Region were screened for allelopathic plant species. Sandwich method and dish pack methods were used for screening collected plants. Lettuce seeds were used at test plants for assessment of leaf leachates and volatile allelochemicals. 84 species showed inhibitory effect on lettuce radicle elongation in sandwich bioassay, *Photinia glabra* (Thunb.) Franch. and Sav. (0% radicle elongation). In the dish pack bioassay, *Photinia glabra, Liquidambar styraciflua* L., and *Cinnamomum camphora* (L.) J. Presl (90.6%, 61.4%, and 50.2% respectively) were among the species depicted strong inhibitory effect on lettuce radicle growth. Nine species promoted lettuce radicle growth, *Aesculus turbinata* Blume and *Quercus gilva* Blume showed the highest growth stimulatory effect (33.0% and 16.1% respectively) (Appiah ,2015).

75 medicinal plants collected from Iran were screened by using Dish-pack method for assessment of volatile allelochemicals. Lettuce was used as test plant. The strongest inhibition was 60% shown by Saffron and followed by *Dracocephalum kotschyi*, *Solanum nigrum* and *Artemisia aucheri.* Safranal was identified as the main chemical by Headspace Gas Chromatography-Mass Spectrometry analyses of saffron. Moreover, the EC50 of safranal was evaluated as 1.2 μg/L (ppb) (Mardani *etal.*,2015).

118 plants collected from Iran in 2009 were assessed by using dishpack method for their allelopathic potential. Results of bioassay with (*Lactuca sativa* L. seed) revealed that 23 species including *Achillea wilhelmsii* K. Koch and *Achillea filipendulina* Lam. elicited growth inhibitory action. Furthermore, a high suppression (83–95 %) in radicle elongation was observed in lettuce exposed to: flowers of *Achillea nobilis* L., *Lavandula vera* DC., and *Perovskia abrotanoides* Kar.; fruit of Ruta graveolens L.; seeds of *Bunium persicum* B. Fedtsch. and

Trachyspermum copticum (L.) Link; leavesof *Achillea biebersteinii* Afan., *Pulicaria gnaphalodes* (Vent.) Boiss., *Ziziphora clinopodioides* Lam., *Zataria multiflora* Boiss.; gum of *Ferula foetida* Regel; and stigma of *Crocus sativus* L. 14 species exhibited growth-promoting properties; the greatest promoting effect was exhibited by the leaves of *Cardaria draba, Verbascum speciosum* and *Urticadioica*, eliciting 69 %, 56 %, and 53 %, respectively (Amini *et al.,* 2014)

In Malaysia, a laboratory research was carried out for the evaluation 8 common weeds of conducted study was conducted to evaluate the allelopathic potential of eight common weed species namely, *Ageratum conyzoides, Tridax procumbens, Cyperus iria, Fimbristylis miliacea, Eleusine indica, Imperata cylindrica, Lygodium flexuosum* and *Nephrolepis biserrata* of different morphological characteristics (broadleaves, sedges, grasses and ferns). The allelopathic study of these weeds was carried out by testing the leaf litter leachate through the sandwich method and the volatile compounds of these weeds through the dish pack method with three replicates for each donor species. Among the eight-weed species tested, showed the strongest growth inhibition was shown by *Ageratum conyzoides* on lettuce radicle elongation (86%) in the sandwich bioassay followed by *Tridax procumbens* (71%). In the dish pack bioassay *Lygodium flexuosum* (fern) demonstrated maximum inhibition on the growth the radicle. Two weed species (*Nephrolepis biserrata* and *Fimbristylis miliacea* were) exhibited enhanced on the growth radicle in dish pack bioassay. The results presented can be utilized as a yardstick for further research in exploring the allelochemicals and for the development of new bioactive chemicals from natural products in weed control strategies (Nurul Ain *et al.,* 2016).

Chapter 03

MATERIAL AND METHODS

4.1 Study Area:

Islamabad, the capital territory of Pakistan is located on the Potohar Plateau at about 457-610 m above sea level at the altitude of 33° 49' N and 72° 24' E) in the North-East. The total area of the region is about 906.50 Square kilometers and the climate is humid, subtropical. Summers in the capital city are hot and humid accompanied by monsoon season. Temperature ranges from 25 °C-35 °C while winters are cold and temperature ranges between 16.6 °C-3.4°C. Summer starts from April, ends in September and winter stats from October and ends in March. The average rainfall is about 1143mm, annually. The average annual humidity, rate is about 55%. (CDA official, website). The original vegetation of Islamabad is, subtropical scrub forest type, (Champion, 1965), which has been modified considerably by the introduction of a large number of ornamental plants, and cultivated trees (Ahmad and Khattak, 2001). The flora of the area is an extension of the Mediterranean type (Stewart, 1957).

4.2 Materials

- *Lactuca stavia* L. Seeds (TAKI II Seed)
- 6 Wells Multi-dishes
- Glass beakers
- Distilled water (Automatic water still HWSFA30)
- Filter paper (Whatman No.1)
- Biosafety cabinet (RSBCA 115 MSC Class: II)
- BOD incubator (NB – 2201 LF)

- Oven (BJPX-SUMMER)
- Agar powder
- Latex Gloves
- Cellophane tape
- Aluminum oil
- Adjustable air-displacement pipette (Z58141N)
- Sterilized syringes
- Paper bags
- Graph sheet

4.3 Methods

4.3.1 Dish-pack Method:

Dish pack method (Fujii, 2000; Shinwari,2013 a; Shinwari, 2013 b; Appiah ,2015), was used for the screening allelopathic plants. This method was preferred since it is an easy and quick method commonly used for the detection of volatile allelochemicals. In Dish Pack method, multi-well plastic cultured dishes with six wells (36.0mm x18.0 mm) were used. The range of distance between the wells was 41mm, 58mm, 82mm and 92 mm from the source well. 100mg oven (KERN-PCB350-3) dried (60 °C for 24 hours) leaves litter was added in source well with 0.75ml of distilled water. In remaining five wells, filter paper was placed with 7 lettuce seeds with 0.75ml of distilled water. After placing lettuce seeds and adding distilled water, covered with their lids and airtight well with adhesive tape. The main purpose of sealing sides of the dishes was to avoid the moisture and loss of volatile allelochemicals. These dishes labeled properly, wrapped with aluminum were subjected to dark conditions. These were then place in an incubator (NB-2201LF) at 25 °C for three days. In control sample dish, the source well was left empty. In other wells, filter paper was placed (as it was done in plant sample dishes) and 7 lettuce seeds were added with 0.75 ml of Distilled water. Afterwards, it was placed in an incubator with other dishes after taping and covering it with Aluminum foil.

One control and 15 plants samples were taken. The number of seed germination, along with hypocotyl and Radicle length were measured and recorded after the completion of three days of incubation. These lengths of both Hypocotyl and Radicles were then compared with that of the control length to determine the allelopathic potential of plant.

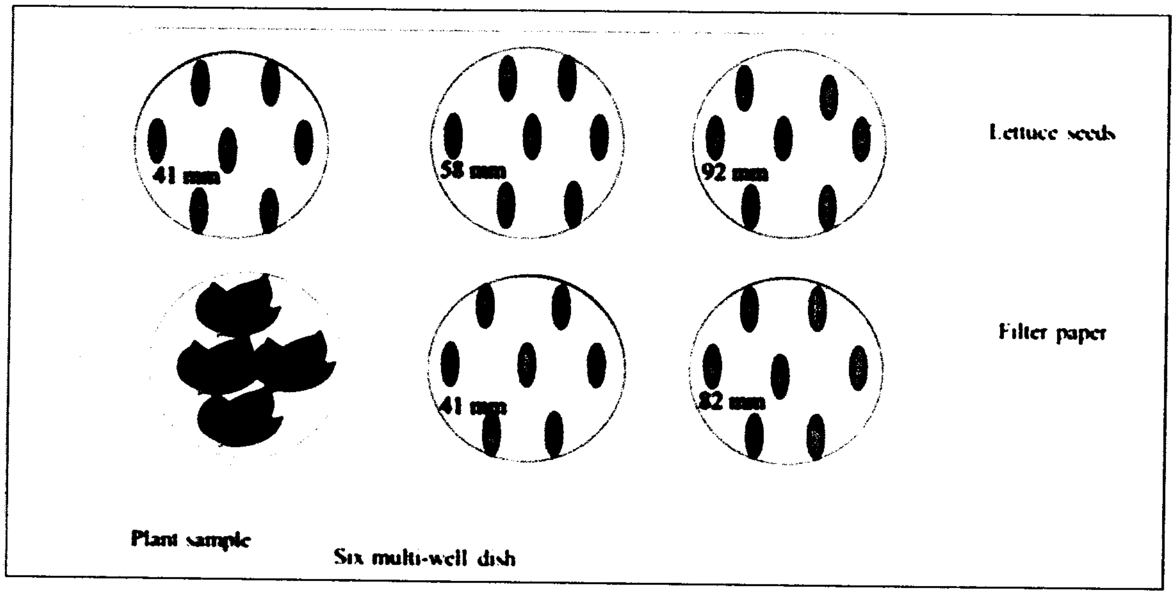

Figure 1: Multi-well plastic plate used to test for plant allelopathy through volatile substances.
Source Fujii, Y. (2005). Dish pack method: a new bioassay for volatile allelopathy

4.3.1 Plants Collection

Exotic ornamental plant specimens were collected from different nurseries of Islamabad. The main areas like Peshawar Mor, PTV-2 Road and Chak Sahzad road were focused due to major nurseries/plants business. 150 plants were sampled. Leaves of exotic ornamental plants collected in separate labelled paper bags. These collected specimens were identified through literature review, personal observation and interviewing nurseries owners/workers. Freshly collected leaves of each species were packed in separate paper bags and dried in oven at 60°C for 24 hours (BJX-Summer). The ecology laboratory of Department of Environmental Science, International Islamic university was used for allelopathic assessment of plants.

4.3.1 Test Plant

The volatile allelopathic chemicals were assessed in regard with seed the germination, mortality and hypocotyl elongation of *Lactuca stavia* L. (Lettuce), a test plant mostly used for the assessment of allelopathic potential of plants. *Lactuca stavia* L. (Takii Seeds Corp., Japan) was used due to its good response against allelochemicals and fast germination (Ervin and Wetzel, 2003; Shinwari, 2013 and Mardani, 2016). Lettuce seeds bought from a local seeds supplier in Islamabad were used.

4.4 Allelopathic Activity Evaluation:

The allelopathic activity can be expressed as the inhibition rate calculated from following equation;

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

4.5 Data Analysis

The data was analyzed for Mean and Standard Deviation values by using Microsoft Excel 2016.

Chapter 04

RESULTS AND DISCUSSON

4.1 Results:

Table 1. shows the elongation percentage of radicle and hypocotyl of *Lactuca sativa L.* seedlings tested against all plant species. The data was statistically analyzed. Values of Mean and standard deviation of the percentage values were calculated and required criteria of standard deviation were evaluated. The criteria of *, **, ***, **** in Table. 2 indicates the radicle elongation which is lower than the difference of the mean value and 1(σ), 1.5(σ), 2(σ),2.5(σ), *i.e.* the values of SDV are 40, 35, and 25, respectively.

For the assessment of allelopathic potential, 150 plant specimens belonging to 62 families were collected from various nurseries of Islamabad. Table 1. shows the percentage effects on growth (Elongation or Inhibition) on the hypocotyl and radicle of *Lactuca sativa* L. grown in multi-well dish packs with dried leaves of different exotic plants species. The negative and positive effect on the growth of Lettuce plant are shown in Table 1. The values of radicle growth of lettuce show negative effect as compared to values of respective parallel control. The results of present study showed that among 151 plant species, six plant species had strong allelopathic effect on the growth of lettuce seedlings.

The statistically analyzed data showed that out of 150 species, six plants have strong inhibition rate against the lettuce seedlings. These top six plant species are; *Passiflora alata* curtis (Passifloraceae), *Pandanus tectorius* Parkinson ex Du Roi (Pandanaceae), *Solanum betaceum* Cav. (Solanaceae), *Polyscias paniculata* 'variegata' (Araliaceae), *Terminalia ivorensis* A. Chev. (Combretaceae) and *Pittosporum tobira* Thunb. (Pittosporaceae). *Passiflora* *alata* curtis (Passifloraceae) commonly known as Passion fruit has the strongest inhibition effect of 100% (80-100%) against the Lettuce seedlings. There are 4 plant species that have inhibition rate between 40-59%; *Pandanus tectorius* Parkinson ex Du Roi; *Solanum betaceum* Cav.; *Polyscias paniculata* 'variegata'; *Terminalia ivorensis* A. Chev. 27 species have 20-39 %, below 19% there are 56 plants species having potential inhibitory effect. There are 62 plant species that have stimulatory effect on the Lettuce seedlings including top six ranking species i.e. *Melaleuca bracteata* F. Muell.; *Leucophyllum frutescens* (Berland.) I.M. Johnst.; *Gladiolus grandiflora* L. *Hydrangea macrophylla* (Thunb.) Ser. *Cryptanthus bromelioides var. tricolor* M.B. Foster and Dracaena *reflexa* Lam.

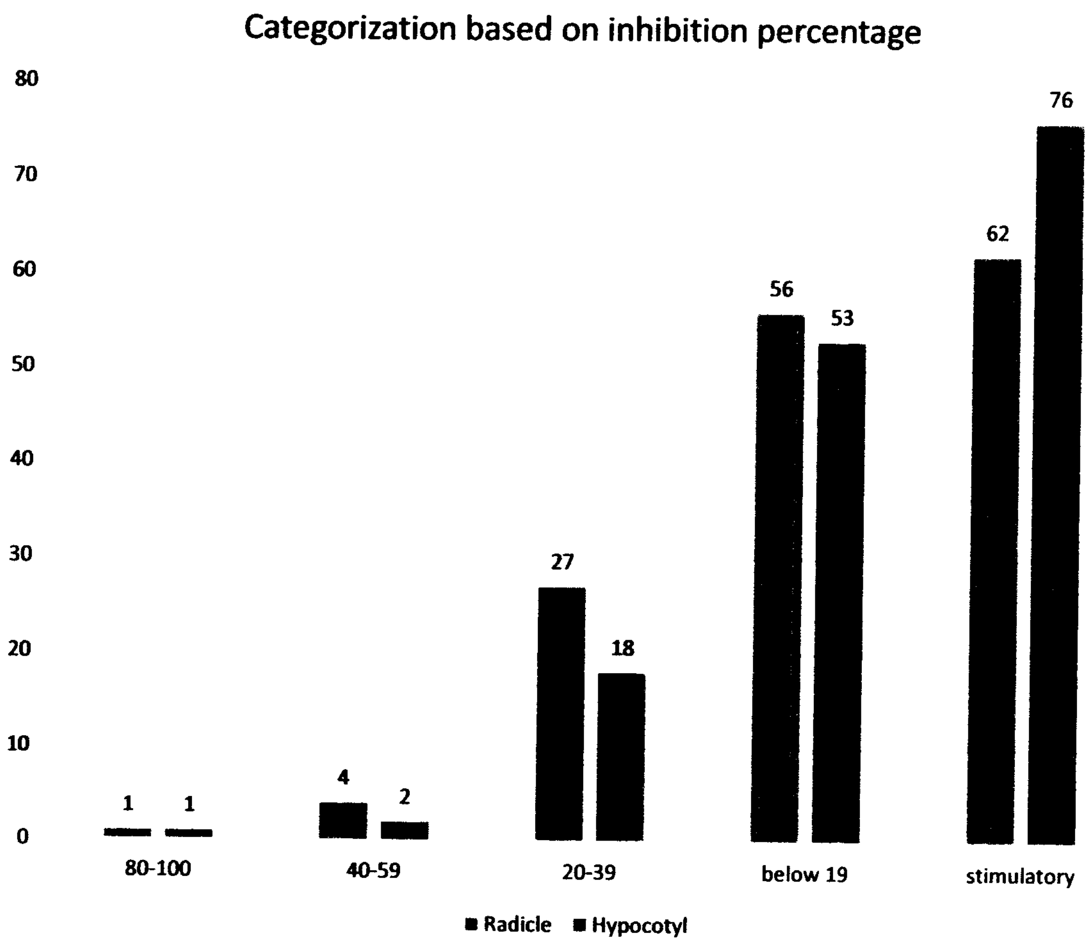

Figure 2: Categorization based on inhibition percentage on Lettuce seedling

Table 1: Allelopathic potential of Exotic ornamental plants collected from different nurseries of Islamabad.

Sr. No	Scientific Name	Common Name	Origin	Family	R	H	Criterion‡
					Extension	(%) †	
1.	*Passiflora alata* curtis	Sweet passion fruit	Brazil	Passifloraceae	0.00	0.00	****
2.	*Pandanus tectorius* Parkinson ex Du Roi	Variegated screw pine	Philippines to Pacific	Pandanaceae	43.24	75.17	***
3.	*Solanum betaceum* cav.	Tree tomato/tamarillo	Andes, Peru, Ecuador, And Colombia S. America	Solanaceae	56.67	89.45	**
4.	*Polyscias paniculata* variegata	Aralia	Mauritius	Araliaceae	58.04	84.00	**
5.	*Terminalia ivorensis* A. Chev.	Black afara, brimestonewood	Guinea-Bissau to Westren Vameroon	Combretaceae	59.46	62.94	**
6.	*Pittosporum tobira* (Thunb.) W.T. Aiton	Japanese pittosporum	China, Japan	Pittosporaceae	62.17	55.56	**
7.	*Tabernaemontana corymbosa* (variegated)	Variegated pinwheel jasmine	Horti. Origin	Apocynaceae	62.50	81.13	*
8.	*Pedilanthus thithymaloides* L. variegatus	Variegated Zigzag Plant	Horti. Origin	Euphorbiaceae	63.22	90.09	*
9.	*Cyathula prostata* cultivar	Iresine	Brazil	Amaranthaceae	63.89	96.64	*
10.	*Elaeocarpus sylvestris* (Lour.) Poir.	Elaeocarpus	Chinajapan, Korea, Taiwan and Vietnam	Elaeocarpaceae	67.57	68.18	*
11.	*Hippeastrum* Herb.	Amaryllis	Peru	Amaryllidaceae	67.71	72.85	*
12.	*Quisqualis indica* L.	Rangoon creeper, Combretum indicum	Malaya, Southeast Asia and West Tropical Africa	Combretaceae	68.53	101.24	*
13.	*Hibiscus acetosella* Welw. ex Hiern	African rosemallow/red shield	Africa	Malvaceae	68.97	74.32	*
14.	*Dracaena sanderiana* sander	Lucky bamboo golden	Africa	Asparagaceae	69.62	86.96	*
15.	*Asplenium nidus* L.	Osaka fern, bird's-nest fern	Hawaii, Other Parts of Polynesia and Africa	Aspleniaceae	70.27	76.92	*
16.	*Neoregelia Flandria*	Bromeliad	Horti. Origin	Bromeliaceae	70.27	97.90	*

17.	*Philodendron bipinnatifidum* schott ex endl.	Horsehead philodendron	South America	Araceae	70.27	71.68	*
18.	*Cordyline fruticosa* (L.) A. Chev.	Red dracaena	Eastern Asia	Asparagaceae	71.07	99.17	*
19.	*Impatiens balsamina* L.	Touch-me-not, balsom	India, Myanmar	Balsaminaceae	71.07	115.70	*
20.	*Codiaeum variegatum* (L.) ex A. Juss.	Croton variegatus	S. India, Ceylon and Malaya	Euphorbiaceae	72.78	93.48	*
21.	*Antigonon leptopus* Hook. and Arn.	Mexican creeper	Mexico	Polygonaceae	72.92	76.00	*
22.	*Cestrum diurnum* L.	Day jessamine	W. Indies	Solanaceae	72.92	66.23	*
23.	Magnolia champaca (L.) Baill. Ex Pierre	Machalia/champak	India, South China	Magnoliaceae	72.97	89.16	*
24.	*Murraya paniculata* (L.) Jack	Marwa/orange jasmine	South America	Rutaceae	74.44	107.41	
25.	*Alternanthera bettzickiana* (Regel) G. Nicholson	Green Hedge	South America	Amaranthaceae	75.89	98.00	
26.	*Dypsis lutescens* (H. Wendl.) Beentje and J. Dransf.	Golden cane palm	Madagascar	Arecaceae	76.72	66.99	
27.	*Rosa hybrid* 'Iceberg'	Rose white vein	Rosaceae	Rosaceae	77.41	101.24	
28.	*Bryophyllum pinnatum* (Lam.) Oken	Miracle leaf, setawar kampung	Tropical Africa	Crassulaceae	78.65	112.96	
29.	*Dieffenbachia* Schott	Diffen plant/ Dumb cane	Brazil	Araceae	78.87	60.00	
30.	*Magnolia coco* (Lour.) Dc.	Magnolia coco/ Dwarf magnolia	China	Magnoliaceae	79.73	87.41	
31.	*Monstera deliciosa* Liebm.	Monstera, indian ivy	Mexico And Guatemala	Araceae	80.06	82.64	
32.	*Solandra maxima* (Sessé and Moc.) P.S. Geen	Solandra gutata, hawaiian lily	Mexico And Central America.	Solanaceae	80.73	81.13	
33.	*Bougainvillea spectabilis* Willd.	Bougainvillea	Brazil	Nyctaginaceae	81.22	97.11	
34.	*Rosa grandiflora* 'Tournament of Roses'	Rose Pink	-	Rosaceae	81.22	109.50	
35.	*Cycas revoluta* Thunb.	Kangi palm, sago palm	Japan	Cycadaceae	81.46	103.31	

36.	*Ficus longifolia* schott	Ficus	Brazil	Moraceae	81.46	80.58	
37.	*Plumbago auriculata* Lam.	Plumbago, forget-me-not	South Africa	Plumbaginaceae	82.03	82.78	
38.	*Ruscus reticulatus* Thunb.	Butcher's broom	Northern Africa, western Asia, Europe	Asparagaceae	82.26	101.01	
39.	*Dracaena braunii* Engl.	Lucky bamboo	Cameroon, Africa	Asparagaceae	82.28	82.61	
40.	*Aeonium ciliatum* Webb andBerthel.	Aeonium	Canary Islands	Crassulaceae	82.43	90.91	
41.	*Cryptanthus bivittatus* (Hook.) Regel	Pink starlite earth star	Brazil	Bromeliaceae	82.87	86.78	
42.	*Vriesea* Lindl.	Vriesea	Mexico, Westindies And America	Bromeliaceae	83.33	126.13	
43.	*Aloe barbadensis* Mill.	Aleo vera	Northern Africa	Asphodelaceae	83.76	99.17	
44.	*Buddleja* L.	Butterfly bush	East Asia, America and Africa	Scrophulariaceae	83.76	107.44	
45.	*Pittosporum tobira* var. sukuraii Gowda	Japanese pittosporum variegated	Japan, Korea And China	Pittosporaceae	84.72	102.94	
46.	*Plectranthus scutellarioides* (L.) R. Br.	Coleus	Tropical and Subtropical Asia To Northern Australia	Lamiaceae	84.77	121.62	
47.	*Begonia × erythrophylla* hérincq	Beef steak begonia	Horticulture Origin	Begoniaceae	85.94	87.75	
48.	*Ficus pumila* L.	Creeper/creeping ficus	China and Japan, S.E. Asia	Moraceae	85.94	74.50	
49.	*Juniperus virginiana* L.	Juniper grey owl	Eastern North America	Cupressaceae	86.11	84.03	
50.	*Spiraea japonica* L. F.	Japenese spiraea	Japan, Kore and China	Rosaceae	86.11	92.44	
51.	*Beaumontia grandiflora* Wall.	Nepal trumpet flower/ Easter lily vine, herald's trumpet	Eastern Himalayas	Apocynaceae	86.29	90.91	
52.	*Ficus elastica* Roxb. Ex Hornem	Rubber plant	India, Nepal, Burma, Malayan Archipelago, Java	Moraceae	86.67	105.50	
53.	*Parthenocissus tricuspidata* (Siebold and Zucc.) Planch.	Boston ivy/ Virginia creeper, boston ivy	Japan, China	Vitaceae	88.54	104.30	
54.	*Russelia equisetiformis* Schltdl. and Cham.	Coral plant, firecracker plant	Mexico	Plantaginaceae	88.54	87.75	
55.	*Aglaonema* Schott	Aglaonema	Tropical Asia	Araceae	88.61	84.78	

56.	*Laurus nobilis* L.	Bay leaf	Southern Mediterranean Region	Lauraceae	88.71	116.16	
57.	*Wisteria sinensis* (Sims) Dc.	Wisteria	China	Fabaceae	89.08	121.62	
58.	*Rhaphidophora tetrasperma* Hook. F.	Mini monstera	Malaysia	Araceae	89.89	95.04	
59.	*Lantana camara var. Flava* (Medik.) Moldenke	Lanatana camara yellow	America	Verbenaceae	90.52	85.59	
60.	*Elettaria cardamomum* (L.) Maton	Elaichi	India	Zingiberaceae	91.15	76.16	
61.	*Begonia rex putz*	Bigonia	N.E.India	Begoniaceae	91.27	89.87	
62.	*Cordyline terminalis* (L.) Kunth	Goodluck plant	Tropical Asia And Australia	Asparagaceae	91.67	110.09	
63.	*Juniperus squamata* Lamb.	Blue star juniper	Afghanistan to China and Taiwan	Cupressaceae	91.67	96.64	
64.	*Trachelospermum jasminoides* (Lindl.) Lem.	Star jasmine	China, Japan.	Apocynaceae	91.67	88.24	
65.	*Chlorophytum comosum* 'vittatum' (Thunb.) Jacques	Spider plant, spider ivy	Africa	Liliaceae	92.26	90.00	
66.	*Franciscea uniflora* Pohl	Yesterday, today and tomorrow	South America	Solanaceae	92.45	86.09	
67.	*Bismarckia nobilis* Hildebrandt and H. Wendl.	Silver bismarck palm	Madagascar	Arecaceae	92.70	105.37	
68.	*Schefflera arboricola* (Hayata) Merr.	Dwarf umbrella-tree	Taiwan	Araliaceae	93.33	84.86	
69.	*Schefflera arboricola* 'variegata'	Dwarf umbrella-tree	Horti. Origin	Araliaceae	93.33	77.98	
70.	*Viburnum sect. Tinus* Maxim.	Viburnum	America	Adoxaceae	93.33	91.74	
71.	*Phoenix canariensis* Wildpret	Phoenix palm	Canary Islands	Arecaceae	93.55	136.36	
72.	*Zamia furfuracea* Aiton	Cardboard palm, sago cycas, abottabadi palm	Mexico	Zamiaceae	93.55	141.41	
73.	*Lantana camara var. Aculeata* (L.) Moldenke	Lantana red	America	Verbenaceae	93.75	76.00	
74.	*Strelitzia reginae* Aiton	Bird of paradise	South Africa	Strelitziaceae	93.92	101.31	

75.	*Justicia brandegeeana* Wassh. and L.B. Sm.	Shrimp (mexican shirmp)	Northeast Mexico	Acanthaceae	94.44	88.24	
76.	*Carissa carandas* L.	Karonda	Indo-Malaysia	Apocynaceae	95.24	70.00	
77.	*Aspidistra elatior* Blume	Bar-room plant, cast-iron plant	China, Japan	Asparagaceae	95.51	88.84	
78.	*Campsis radicans* (L.) Bureau	Golden bells, trumpet creeper	Southeastern United States	Bignoniaceae	95.51	112.96	
79.	*Canna × generalis* L.H. Bailey	Kalee, canna varigated	Horti. Origin	Cannaceae	95.51	121.90	
80.	*Datura suaveolens* Humb. and b Bonpl. ex willd.	Angel's trumpet/ Datura yellow flowers	Brazil	Solanaceae	97.66	89.40	
81.	*Dahlia coccinea* Cav.	Dahlia	Mexico	Asteraceae	97.83	92.11	
82.	*Jasminum sambac* L.	Motia	India	Oleaceae	97.83	95.86	
83.	*Yucca aloifolia* variegata	Spanish dagger	Horti. Origin	Agavaceae	97.83	107.14	
84.	*Chamaedorea elegans* Mart.	Cane palm, good-luck palm	Mexico	Arecaceae	97.88	88.24	
85.	*Lonicera japonica* Thunb.	Honey suckel	Japan And Korea	Caprifoliaceae	98.39	111.11	
86.	*Philodendron scandens* K. Koch and Sello	Sweet heart climber, Heart-leaf philodendron	Mexico, Central America and South America	Araceae	99.38	116.54	
87.	*Araucaria heterophylla* (Salisb.) Franco	Arukaria	Norfolk Island (East of Australia)	Araucariaceae	99.70	74.00	
88.	*Anthurium andraeanum* Linden	Anthurium/ falmingo flower	Southwestern Colombia	Araceae	100.00	103.15	
89.	*Euonymus fortunei* (Turcz.) Hand. -mazz.	Euonymus	China, Japan, And Korea.	Celastraceae	100.00	117.65	
90.	*Canna indica* L.	Canna lilly	Tropical America And W. Indies	Cannaceae	100.26	96.03	
91.	*Chrysanthemum morifolium* Ramat.	Gul-e-daowdi	China	Asteraceae	100.26	86.09	
92.	*Brachychiton acerifolius* (A. Cunn.) Macarthur and C. Moore	Brachychiton	Eastern Australia	Malvaceae	101.12	109.50	
93.	*Begonia rex-cultorum*	Begonia rex	Hort. Origin	Begoniaceae	101.27	106.52	
94.	*Gardenia jasminoides* J. Ellis	Gardenia	China	Rubiaceae	101.27	104.35	

95.	*Duranta erecta* 'Gold Edge'	Golden Dew Drop	Florida, the Caribbean south to Brazil	Verbenaceae	101.61	116.16	
96.	*Livistona rotundifolia* (Lam.) Mart.	Table palm	South-East Asia	Arecaceae	101.61	106.06	
97.	*Schinus terebinthifolia* Raddi	Brazilian peppertree	Brazil	Anacardiaceae	102.53	101.24	
98.	*Elaeodendron australe* Vent.	Red-fruited olive plum	Australia	Celastraceae	102.70	83.92	
99.	*Viburnum lentago* L.	Snowball/nannyberry viburnum	Eastern North America	Adoxaceae	102.70	101.40	
100.	*Cymbidium finlaysonianum* Wall. Ex lindl.	Orchid	Vietnam, Malaysia, Philippines	Orchidaceae	102.78	92.44	
101.	*Duranta erecta* variegata	Duranta (white)	West Indies	Verbenaceae	102.78	100.84	
102.	*Rhapis excels* (Thunb.) A. Henry	Ladyfinger palm	China	Arecaceae	103.17	101.31	
103.	*Hoya acuta* variegata	Hoya	India, Burma, China, Australia	Apocynaceae	104.43	104.35	
104.	*Ixora coccinea* L.	Jungle geranium	India	Rubiaceae	104.43	115.22	
105.	*Caryota mitis* Lour.	Fishtail palm	Burma To Malay Peninsula, Java, Philippines	Arecaceae	104.50	107.84	
106.	*Brachychiton populneus* (Schott and Endl.) R. Br.	Kurrajong	Australia	Sterculiaceae	104.84	101.01	
107.	*Furcraea foetida* (L.) Haw.	Furcaria	South Brazil	Asparagaceae	104.84	103.54	
108.	*Codiaeum variegatum 'Bravo'*	Croton plant	Indonesia and other Eastern Pacific	Euphorbiaceae	104.89	130.63	
109.	*Dioon spinulosum* Dyer ex Eichler	Giant dioon	East Asia, America and Africa	Zamiaceae	104.89	105.86	
110.	*Beaucarnea recurvata* Lem.	Beaucarnea	Southeastern Mexico	Asparagaceae	105.34	121.90	
111.	*Aspidistra guangxiensis* S.C. Tang and Y. Liu	Aspidestra	China, India, Japan, Laos, Thailand, And Vietnam.	Asparagaceae	105.82	76.80	
112.	*Spiraea virginiana* Britton	Spiraea	North Carolina and West Virginia	Rosaceae	106.45	108.59	
113.	*Euonymus japonicus* Thunb.	Euonymus	China, Japan And Korea	Celastraceae	106.94	115.55	
114.	*Osmanthus fragrans* (Thunb.) Lour.	Osmanthus	Temperate Himalaya, Sikkim, China, Japan.	Oleaceae	107.59	86.96	

115.	*Tecoma stans* (L.) Juss. Ex kunth	Techonma yellow	South America	Bignoniaceae	108.15	112.96	
116.	*Sophora secundiflora* (Ortega) Lag. Ex dc.	Sophora	Texas and Mexico	Fabaceae	108.63	98.00	
117.	*Pyrostegia venusta* (Ker Gawl.) Miers	Golden shower	Brazil, Argentina, Bolivia And Paraguay.	Bignoniaceae	110.25	99.62	
118.	*Salix babylonica* L.	Weeping willow	Northern China	Salicaceae	110.25	103.38	
119.	*Jasminum multiflorum* (Burm. f.) Andrews	Indian Chambeeli/ Star Jasmine	India, Thailand and Vietnam	Oleaceae	111.29	136.36	
120.	*Hamelia patens* Jacq.	Hamelia	America	Rubiaceae	111.61	94.00	
121.	*Dracaena fragrans* (L.) Ker Gawl.	Dracaena/ corn plant	West Africa	Asparagaceae	111.80	110.90	
122.	*Hoya kerrii* Craib	Sweetheart hoya	Thailand, Laos and Cambodia	Apocynaceae	112.34	104.35	
123.	*Dracaena marginata* Lam.	Dragon tree	Madagascar, Mauritius	Asparagaceae	112.43	101.31	
124.	*Epipremnum aureum* (Linden and André) G.S. Bunting	Money plant	Solomon Islands	Araceae	113.33	126.15	
125.	*Oxalis triangularis* A. St.-Hil	False shamrock	Brazil	Oxalidaceae	113.33	119.27	
126.	*Magnolia grandiflora* L.	Chinese magnolia	Southern United States	Magnoliaceae	113.35	95.86	
127.	*Citharexylum spinosum* L.	Fiddle wood	West Indies	Verbenaceae	114.52	131.31	
128.	*Begonia rex hybrid*	Bigonia	Hort. origin	Begoniaceae	115.08	117.65	
129.	*Jacaranda mimosifolia* D. Don	Jacaranda	South America	Bignoniaceae	115.17	100.00	
130.	*Excoecaria cochinchinensis* Lour.	Chinese croton	Southern Indochina To Peninsular Malaysia	Euphorbiaceae	118.01	109.02	
131.	*Nephrolepis exaltata* (L.) Schott	Lale fern, Boston fern	Central America And the Caribbean	Nephrolepidaceae	118.33	121.56	
132.	*Phalaenopsis* Blume	Moon orchid	Borneo, Indonesia, Philippines Papua New Guinea and Australia (Queensland)	Orchidaceae	120.25	136.96	
133.	*Chamaedorea cataractarum* Mart.	Cat palm	Mexico	Arecaceae	120.37	147.06	
134.	*Ficus microcarpa* L. F.	Bonsai tree	China and Eastren Asia	Moraceae	120.54	92.00	

135.	*Callistemon citrinus* Stapf	Bottle brush	S.E. Australia	Myrtaceae	120.97	108.59	
136.	*Plumeria obtusa* L.	Gulchin/white champa	Southern Mexico And W. Indies	Apocynaceae	122.02	98.00	
137.	*Yucca aloifolia* L.	Dagger plant, aloe yucca	Southern USA, Caribbean Islands, Mexico	Agavaceae	122.67	114.66	
138.	*Hibiscus rosa-sinensis* L.	Hibiscus	South China	Malvaceae	123.33	116.97	
139.	*Ficus maclellandii* King	Amastel king	Northeast India, South China, Thailand and Malaysia	Moraceae	124.22	110.90	
140.	*Aucuba japonica* Thunb.	Japanese aucuba	Korea, Japan And China	Garryaceae	124.34	102.94	
141.	*Asparagus densiflorus var. Sprengeri* Kunth	Sprengeri fern	North America	Asparagaceae	126.40	136.36	
142.	*Ficus elastica var. variegata* Roxb. ex Hornem.	Rubber plant varigated	India, Nepal, Burma, Malayan Archipelago, Java	Moraceae	126.67	133.03	
143.	*Tabernaemontana divaricata* (L.) R.Br. ex Roem.andSchult.	Chandni	Norther India, Myanmar and Thailand	Apocynaceae	126.67	116.97	
144.	*Gervillea robusta* A. Cunn.ex R.Br.	Silver oak, Australian silky oak	Australia	Proteaceae	128.88	97.74	
145.	*Dracaena reflexa* Lam.	Song of India	Mexico	Araceae	130.75	114.86	
146.	*Cryptanthus bromelioides var. tricolor* M.B. Foster	Tricolor earthen star	Hort. Origin	Bromeliaceae	131.33	136.96	
147.	*Hydrangea macrophylla* (Thunb.) Ser.	Hydrangea, snowball flower	Eastern Asia	Hydrangeaceae	138.33	123.85	
148.	*Gladiolus grandiflora* L.	Sword lily	South Africa	Iridaceae	140.86	134.30	
149.	*Leucophyllum frutescens* (Berland.) I.M. Johnst.	Silervry	Mexico	Scrophulariaceae	143.68	137.39	
150.	*Melaleuca bracteata* F. Muell.	Melaleuca/black tea tree	Western Australia	Myrtaceae	151.39	105.04	

† Table 2: Details about the percentage growth rate as compare to that of the control; ‡stronger inhibitory activity in the radicle: *M–1(σ), ** M – 1.5(σ), ***M–2(σ), and **** M – 2.5(σ)

Mean	***94.62***	***98.59***	***5.38***	***1.38***
St. Dev (SD)	21.60	21.22	21.60	21.20
Mean-SD	73.02	77.37	-16.22	-19.82
Mean-1.5SD	62.22	66.76	-27.03	-30.42
Mean-2 SD	51.41	56.15	**-37.83**	**-41.02**
Mean-2.5 SD	40.61	45.54	-48.63	-51.62

4.2 Discussion:

Current research focuses on the allelopathic effect of 150 plant species tested against Lettuce plant (*Lactuca stavia* L.) collected from different commercial plants nurseries of Islamabad and adjacent areas. Out of 150 plants species the top ranked six plants having the strong allelopathic effect. *Passiflora alata* Curtis is top ranked with 100% inhibitory effect on radical and hypocotyl. It lies in 80-100% range. Followed by, *Pandanus tectorius* Parkinson ex Du Roi, *Solanum betaceum* cav., *Polyscias paniculata* 'variegata', and *Terminalia ivorensis* Chev. were in the range of 40-59% and *Pittosporum tobira* (Thunb.) W.T. Aiton within 20-39% range.

Passiflora alata Curtis, liana commonly known as, Sweet Passion fruit, showed 100% inhibition effect (Radicle and Hypocotyl) against the Lettuce seedlings. It is a perennial vine belonging to Passifloraceae family, that has beautiful flowers and palatable sweet fruit. It is native to South America and Brazil and is world widely cultivated as a fruit plant due to its sweet taste, is used as ornamental plant and is also a vital part of traditional medicines (Boeira, 2010; Pacheco *et al.,* 2012). *P. alata* leaves contain polyphenols that are potent antioxidants. Its leaves have been traditionally used in American states as a remedy for anxiety and nervousness (Rudnick, 2007). It has been reported that the *P. alata* seeds embryo extracts had certain chemicals that had adversely affected the germination of Lettuce (de Freitas ,2016). This shows that this plant contains potential allelochemicals that can inhibit the growth and development of other plants. A research conducted on the volatile composition of *P. alata* support this postulate that *P. alata* has allelochemicals that are responsible for its severe effects on other neighboring plants. In this study about 45 volatile allelochemicals were identified (Mamede, 2017).

The second strongest allelopathic potential was found in *Pandanus tectorius* Parkinson ex Du Roi. It is also called *Pandanus baptistii* Misonne, *Pandanus tectorius* cv "baptistii" (Rauch and

Weissich, 2009) Pandanus or variegated Screw Pine, belongs to Pandananceae family. It is naturally found in coastal areas, near coasts forests of some south Asian countries including South India and Sri Lanka, south east Asia and in tropical Australia (Lim,2012). The author has reported the use of *P. tectorius* in Vietnam for the cure of diabetes, hypertension, hepatitis and for kidney problems (nephropathy) (Loi,2004). Seven aldehyde compounds were extracted from *P. tectorius* fruits showed significant inhibition for α-glucosidase which is more than the standard drug acarbose which is anti-diabetic (Mai *et al.*,2015). Least information is reported regarding the allelopathic potential of this plant in literature. However, in present study *P. tectorius* has shown the negative effect on the germination of seeds and the growth of Lettuce. Which shows that the plant has strong allelopathic potential.

The third in ranking found was *Solanum betaceum* cav. commonly known as tree tomato or Tamarillo, belongs to Solanaceae family. It is native to South America (Andes, Peru, Ecuador, and Colombia). *S. betaceum* commonly known as tree tomato or Tamarillo, belongs to *Solanaceae* family. It is native to (Andes, Peru, Ecuador, and Colombia) South America. Its fruit is used as an alternative of tomato and is rich in vitamins and iron. It has flourished and has become a marketable crop in its native range and also in some other countries like New Zealand (Acosta-Quezada *et al.,* 2011 and Durant *et al.,* 2013). The fruit of *S. betaceum* contains antioxidants and are very effective against the lipid oxidation (Castro-Vargas *et al.,* 2013). In current study it has shown inhibitory effect against the test plant used. However, no significant work has been reported related to the allelopathic potential of this plant.

The next species followed by were; *Polyscias paniculata* 'variegata' is a variety of *Polyscias paniculata* (DC.) Baker of Araliaceae family. *P. paniculata* is native to Mauritius. It is a critically endangered species and is included in IUCN red list (Florens and Strahm, 2000) There is no data found regarding the allelopathic potential of *P. paniculata* "varigata".

Terminalia ivorensis Chev. belongs to Combretaceae family. It is native to Andes, Peru, Ecuador, Colombia and S. America. It is generally called Black Afara tree. *T. ivorensis* is a medicinal plant and in Ivorian traditional medicine it is used for the treatment of different type of diseases; diabetes, cough, hypertension *etc*. The bark of this plant is used as anti-malarial agent. The plant has antioxidant, anti-fungal and cytotoxic actives reported in two studies (Ponou *et al.,* 2010; Sitapha *et al.,* 2013). The ethanolic and aqueous extracts of this plant had significant effect against the bacteria. It contains certain phytochemicals; tannins, saponins, flavonoids, polyphenols and some quantities of alkaloid. It was suggested that the bark of this plant contain these chemicals and it can be used for the treatment of microbial borne diseases and further many useful compounds can be extracted from this (Coulibaly *et al.,*2014). In the current study *T. ivorensis* shown the inhibitory effect against the Lettuce seedlings.

Pittosporum tobira (Thunb.) W.T. Aiton is an aromatic, evergreen plant belonging to Pittosporaceae. It is native to Japan, Korea and China. It is mostly used for ornamental purposes. In some previous studies it has been mentioned that it contains sesquiterpenoids, carotonoids and tepenoids. A Saponin from *P. torbira* leaves shown antibiotic potential (Ramalhete *et al.,* 2008). The essential oils from the flowers of this plant had anti-cancer potential. The author suggested that it can be used in health care against cancer (Sun *et al.,* 2017). *P. tobira* is a toxic plant but in China it is used as a medicine for dysentery, rheumatism and dentition (Wiart, 2006). The larvicidal effects of essential oils extracted from the leaves of *P. torbira* against the Aedes aegypti (a yellow fever mosquito). These essential oils were very effective against this mosquito and had the potential for using as insecticide (Chung *et al.,*.2010; Wermuth, 2011). However, *P. torbira* effect on other plants is least reported in literature but the result of the current study shows that the leaves of this plant contain volatile allelochemicals that have significant adverse effects on the seed germination and growth of lettuce seedlings. This shows that it has allelopathic potential and which can be further studied in future.

Allelopathy is not only about the inhibitory effects of allelochemicals on growth of other plants but it also includes the elongation or stimulatory effect on the growth of other plants. The results shown in Table 1. represent that there are five plant species; *Melaleuca bracteata* F. Muell., *Leucophyllum frutescents* (Berland.) I.M. Johnst., *Gladiolus grandiflora* L., *Hydrangea macrophylla* (Thunb.) Ser. and Cryptanthus *bromelioides* var. tricolor M.B. Foster have growth stimulatory effect on the lettuce seedlings.

Melaleuca bracteata F. Muell, of Myrtaceae family is native to Western Australia commonly known as Black tea tree or Melaleuca shown highest elongation effect or lowestinhibition effect against Lettuce seedlings. Generally, *Melaleuce* genus is rich with antioxidants and due to this the plants of this genus are used world widely in food, medicines and especially in cosmetics manufacturing. A study was conducted for the determination of antioxidants present in *M. bracteata*. It is found that *M. bracteata* contains two main antioxidants; methyl eugenol (86.86%) and *trans*-cinnamic acid methyl ester (6.41%) (Hou *et al.*,2016c and Siddique *et al.,* 2017). In South Africa the leaves of M. *bracteata* are used for healing of wounds and for the treatment of skin diseases (Hutching *et al.,* 1996). Although a lot of work has been done on *Melaleuce* genus but only a few reports have been found related to *M. bracteata.* The essential oil extracted for the *M. bracteata* had inhitory effect on the seed germination and growth of weed species (Almarie *et al.,* 2016). Results of Table.1 indicated that *M. bracteata* has positive effect on the seed germination and development of *L. stiva.*

The second ranked plant having stimulatory effect was *Leucophyllum frutescens* (Berland.) I.M. Johnst. of Scrophulariaceae family commonly known as Texas ranger, purple sage or Texas silver leaf. It is native to Mexico and is mostly used as ornamental plant as hedge in houses, gardens and along road side green patches (Hunt *et al.,* 2015). The methanol extracts of *L. frutescens* are effective against the liver disorders as they contain the hepatoprotective compounds (Balderas-Renteria *et al.*, 2007). The anti-microbial activity of *L. frutescens* against *Staphylocous auresus* and *Escherichia coli* (*E. coli*) (Menchaca *et al.,* 2013). Phytotoxicity of tetrahydro-furofuran extracted from the leaves of *L. frutescens*. It showed the inhibitory effect against the test plants used i.e. *L. stiva* and *A. stolnifera* (Rimando *et al.,* 1999). On the basis of the results of current study in Table.1 *L. frutescens* showed high stimulatory effect against the seed germination and growth of *L. stiva*. which contradict with the past report.

Stimulatory effect was found in *Gladiolus grandiflora* L. It belongs to Iridaceae family commonly called gladiolus. It is an herbaceous plant having beautiful flowers. It is native to South Africa. It is most commonly used as ornamental plant in gardens but also cultivated for cut flowers (Negi *et al.*, 2014). It has great importance in flower market, in Pakistan after rose it the second most highly selling flower (Riaz *et al.*, 2007 and Nadeem *et al.*, 2011). The results of current research exhibited that *G. grandiflora* had high stimulatory effects on the germination of seed and growth of *L. stiva* seedlings. It indicates the presence of certain volatile allelochemicals that can be used to enhance the growth of other plants. Least literature was found regarding the allelopathic potential of *G. grandiflora,* which shows the need for further in-depth research for the allelochemicals identification and characterization.

Plumeria obtusa L. is the next stimulatory species belongs to Apocynaceae family, is native to Tropical America. It is commonly known as Gul-e- cheen, white champa or Araliya. *Plumeria obtusa* and *Plumeria rubra* are mostly found in Pakistan (Perry and Metzger, 1980) and are grown in parks, gardens, in front of houses and also along roadsides due to their beautiful flower and fragrance (ornamental importance). The leaves extract of *P. obutsa had* anti-microbial, anti-bacterial and anti-fungal potential. It was suggested that due to presence of anti-microbial compounds *P. obutsa could* be a cost-effective treatment of infectious diseases (Rawani *et al.,* 2011; Ali *et al.,*2013). Leaves are used for treatment of wounds and skin borne diseases (Wong *etal.,* 2013). On the basis of the results in Table 1. *P. obutsa* has stimulatory or positive effect on the Lettuce seedlings. It shows that it contains beneficial allelochemicals that can be used for betterment of growth of other plants or crops. However, different secondary metabolites were reported in previous reports but least information was found about effect on plant growth. It is found that more research is needed for its allelopathic potential assessment and about secondary metabolites effect on neighboring plants.

Other species having stimulatory effects were, *Hydrangea macrophylla* Thunb.Ser. of hydrangeaceae family and native to Northeast Asia (Dilshara *et al.,* 2013). It is mostly used as ornamental plant due to beautiful flowers. This plant has special ability to change its flowers colour according to pH of soil (Savona *et al.,* 2012). In Chinese folk medicine, it is considered as a toxic plant. Food poising due to cyanogenic glycosides are reported in this plant in Japan. Two well-known cyanides were extracted from this plant that proves that this plant is toxic. Its toxicity should be assessed before use for the treatment of patients (Nakamura *et al.,* 2009). In Chinese traditional medicine the flowers of *H. macrophylla* are for the treatment of various diseases; dysphoria, heart hot fright, malaria. It is also reported that *H. macrophylla* also possess (Ling *et al.,*2013). Least literature is reported about the allelopathic potential or phytotoxicity of *H. macrophylla*. In the present study *H. macrophylla* showed positive effect on the *L. stiva* seeds germination and growth.

Cryptanthus bromelioides var. *tricolor* M.B. Foster of Bromeliaceae family is a variegated plant of horticulture origin. It is mostly used as ornamental plant due to its beautiful leaves, having green, yellowish white and red colour in them (Mathews and Rao, 1982; Pickens, 2006). Least literature is reported related to any other use of plant. In the current study *C. bromelioides* var. *tricolor* has stimulatory effect on the Lettuce.

CHAPTER05

CONCLUSIONS AND RECOMENDATIONS

The current research was conducted for the assessment of allelopathic potential of 150 exotic ornamental plants, collected from local plant nurseries of Islamabad, the capital territory of Pakistan. *Lactuca sativa L.* (Lettuce) was used as a test plant for assessing the effects of volatile allelochemicals. Exotic plants are introduced in Pakistan from different countries especially from Thailand and Holland. It has been found that there is no screening process for the risk assessment of plants imported from different countries. Plants are imported on the basis of their ornamental value; flowers size, colour, fragrance, shape etc. Furthermore, research can be conducted on screened plants for identification of allelochemicals. *Passiflora alata* Curtis (Passifloraceae), *Pandanus tectorius* Parkinson ex Du Roi (Pandanaceae), *Solanum betaceum* Cav. (Solanaceae), *Polyscias paniculata* ′variegata′ (Araliaceae), *Terminalia ivorensis* A. Chev. (Combretaceae) and *Pittosporum tobira* Thunb. (Pittosporaceae) have been found as the top-rated plants that showed the strong inhibition against the test plants used. *Melaleuca bracteata* F. Muell., *Leucophyllum frutescents* (Berland.) I.M. Johnst., *Gladiolus grandif lora* L., *Hydrangea macrophylla* (Thunb.) Ser., Cryptanthus *bromelioides* var. tricolor M.B. Foster and *Syngonium podophyllum* Schott have strong stimulatory effect (lower inhibition) on the growth of Lettuce seedlings. The results of this study can be used as benchmark data for carrying out further research for the identification of allelochemicals. The allelochemicals having inhibition effect, can be used for weeds management and also as an alternative of synthetic herbicides. The plants that showed stimulatory effect have beneficial allelochemicals that can be used as growth promoters of other plants both in agriculture and horticulture.

On the basis of results of current study, it is suggested that any plant that have to be introduced from other countries in Pakistan should be assessed for its allelopathic potential prior to its plantation on large scale or commercial sale. This initial screening of plants may be very helpful and time saving for carrying out further in-depth research. Endemic plants species should be promoted for ornamental purposes instead of importing all and sundry plants from other countries that might poses severe threat to native flora of Pakistan. As it is cleared from some past incidents that some plants had been introduced in 80s for ornamental purpose in Pakistan but at present they have become problematic and are causing severe social, economic and ecological effects.

References

Abbas, T., Zahir, Z. A., Naveed, M., and Kremer, R. J. (2017). Limitations of Existing Weed Control Practices Necessitate Development of Alternative Techniques Based on Biological Approaches. *Advances in Agronomy.*

Acevedo-Rodríguez, P. and Strong, M.T. (2005). Monocotyledons and Gymnosperms of Puerto Rico and the Virgin Islands. Contributions from the United States National Herbarium **52**: 1-415.

Acosta-Quezada, P. G., Martínez-Laborde, J. B., and Prohens, J. (2011). Variation among tree tomato (Solanum betaceum Cav.) accessions from different cultivar groups: implications for conservation of genetic resources and breeding. *Genetic Resources and Crop Evolution*, **58**(6), 943-960.

Ahmad, S. (2012). A Study of Poisonous Plants of Islamabad Area, Pakistan. In *Series B: Biological Sciences* (p. 129).

Ali, N., Ahmad, D., Bakht, J., Shah, S., and Ullah, F. (2013). Antimicrobial activity of leaves extracted samples from medicinally important Plumeria obtusa. *Journal of Medicinal Plants Research*, **7**(17), 1146-1153.

Almarie, A., Mamata, A., and Rukunudina, I. (2016). Chemical composition and herbicidal effects of Melaleuca bracteata F. Muell. essential oil against some weedy species. *Int J Sci Eng Res*, **7**(1), 507-512.

Ameen, M. (1999, May). Development of guiding principles for the prevention of impacts of alien species. In *a consultative workshop in advance of the 4th meeting of SBSTTA to the CBD organized by IUCN Bangladesh at Dhaka on May* (Vol. **25**, p. 1999).

Appiah, K. S., Li, Z., Zeng, R., Luo, S., Oikawa, Y., & Fujii, Y. (2015). Determination of allelopathic potentials in plant species in Sino - Japanese floristic region by sandwich method and dish pack method, **4**(4), 381–394. https://doi.org/10.14419/ijbas.v4i4.5147

Balderas-Renteria, I., Camacho-Corona, M. R., Carranza-Rosales, P., Lozano-Garza, H. G., Castillo-Nava, D., Álvarez-Mendoza, F. J., and Tamez-Cantú, E. M. (2007). Hepatoprotective effect of Leucophyllum frutescens on wistar albino rats intoxicated with carbon tetrachloride. *Annals of hepatology*, **6**(4), 251-254.

Bossdorf, O. (2013). Enemy release and evolution of increased competitive ability: at last, a smoking gun! *New Phytologist*, **198**(3), 638-640.

Callaway, R. M. (2002). The detection of neighbors by plants. *Trends in Ecology and Evolution*, **17**(3), 104-105.

Callaway, R. M., and Aschehoug, E. T. (2000). Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science*, **290**(5491), 521-523.

Castro-Vargas, H. I., Benelli, P., Ferreira, S. R., and Parada-Alfonso, F. (2013). Supercritical fluid extracts from tamarillo (Solanum betaceum Sendtn) epicarp and its application as protectors against lipid oxidation of cooked beef meat. *The Journal of Supercritical Fluids*, **76**, 17-23.

Champion, S. H., Seth, S. K., and Khattak, G. M. (1965). Forest types of Pakistan. *Forest types of Pakistan.*

Cheng, F., and Cheng, Z. (2015). Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Frontiers in plant science*, **6**.

Chou, C. H. (2010). Biochemical Interactions Among Plants: Allelopathy as Ecosystem Regulator. *Medical and Health Sciences,* Volume V, **187**.

Chung, I. M., Seo, S. H., Kang, E. Y., Park, W. H., and Moon, H. I. (2010). RETRACTED: Larvicidal effects of the major essential oil of Pittosporum tobira against Aedes aegypti (L.). *Journal of enzyme inhibition and medicinal chemistry*, **25**(3), 391-393.

Cornejo, J. J., Munoz, F. G., Ma, C. Y., and Stewart, A. J. (1999). Studies on the decontamination of air by plants. *Ecotoxicology*, **8**(4), 311-320.

Coulibaly, K., Zirihi, G. N., Guessennd-Kouadio, N., Oussou, K. R., and Dosso, M. (2014). Antibacterial properties studies of trunk barks of terminalia ivorensis (Combretaceae), a commercial and medicinal specie, on some methicillin-resistant Staphylococci spp strains. *African health sciences*, **14**(3), 753-756.

de Albuquerque, M. B., dos Santos, R. C., Lima, L. M., de Albuquerque Melo Filho, P., Nogueira, R. J. M. C., Da Câmara, C. A. G., and de Rezende Ramos, A. (2011). Allelopathy, an alternative tool to improve cropping systems. A review. *Agronomy for Sustainable Development*, **31**(2), 379-395.

Dehnen-schmutz, K., Touza, J., Perrings, C. and Williamson, M. (2007), The Horticultural Trade and Ornamental Plant Invasions in Britain. Conservation Biology, **21**: 224–231.

Dilshara, M. G., Jayasooriya, R. G. P. T., Lee, S., Jeong, J. B., Seo, Y. T., Choi, Y. H., ... and Kim, G. Y. (2013). Water extract of processed Hydrangea macrophylla (Thunb.) Ser. leaf attenuates the expression of pro-inflammatory mediators by suppressing Akt-mediated NF-κB activation. *Environmental toxicology and pharmacology*, **35**(2), 311-319.

Einhellig F. A. (1995). "Allelopathy-current status and future goals," in Allelopathy: Organisms, Processes, and Applications, eds Inderjit A., Dakshini K. M. M., Einhellig F. A., editors. (Washington, DC: American Chemical Society Press;), 1–24.

Ervin, G. N., and Wetzel, R. G. (2003). An ecological perspective of allelochemical interference in land–water interface communities. *Plant and Soil*, *256*(1), 13-28.

Fischer, J., Lindenmayer, D. B., and Manning, A. D. (2006). Biodiversity, ecosystem function, and resilience: ten guiding principles for commodity production landscapes. *Frontiers in Ecology and the Environment*, **4**(2), 80-86.

Florens, D. (TPTNC) and Strahm, W. 2000. *Polyscias paniculata*. The IUCN Red List of Threatened Species 2000: e. T32696A9718852.

Fujii, Y. (1992). The potential biological control of paddy weeds with allelopathy: Allelopathic effect of some rice varieties. In *Proceedings of the International Symposium on Biological Control and Integrated Management of Paddy and Aquatic Weeds in Asia (Tsukuba, Japan, 23 October 1992)* (pp. 305-320). National Agricultural Research Center.

Fujii, Y. (1994). Screening of allelopathic candidates by new specific discrimination, and assessment methods for allelopathy, and the identification of L-DOPA [L-3, 4-dihydroxyphenylalanine] as the allelopathic substance from the most promising velvetbean (Mucuna pruriens). *Bulletin of the National Institute of Agro-Environmental Sciences (Japan).*

Fujii, Y. (2000). Development of new bioassay for volatile allelochemicals: Dish-pack method. *J. Weed Sci. Technol.*, **45**, 80-81.

Fujii, Y. (2005). Dish pack method: A new bioassay for volatile allelopathy. *Thymus*, **2**(3), 23.

Fujii, Y. (2009). Overview of Research on Allelochemicals. In *Survey of Plant Natural Resources and isolation of allelochemicals in Monsoon Asia" under the 2009 NIAES International Symposium entitled "Challenges for Agro-Environmental Research in Monsoon Asia" W3-01* (pp. 1-5).

Fujii, Y., and Hiradate, S. (Eds.). (2007). *Allelopathy: new concepts and methodology*. CRC Press.

Fujii, Y., Furubayashi, A., and Hiradate, S. (2005, August). Rhizosphere soil method: a new bioassay to evaluate allelopathy in the field. In *Proceedings of the 4th World Congress on Allelopathy Establishing the Scientific Base. Edited by JDI Haper, M. An, and JH Kent. Charles Sturt University, Wagga, New South Wales, Australia* (pp. 490-492).

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

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

Hager, H. A. (2004). Competitive effect versus competitive response of invasive and native wetland plant species. *Oecologia*, **139**(1), 140-149.

Hao, Z. P., Wang, Q., Christie, P., and Li, X. L. (2007). Allelopathic potential of watermelon tissues and root exudates. *Scientia Horticulturae*, **112**(3), 315-320.

Haq, F. U., Ahmad, H., and Alam, M. (2010). Species diversity of vascular plants of Nandiar valley western Himalaya, Pakistan.

Hastings, A., Hall, R. J., and Taylor, C. M. (2006). A simple approach to optimal control of invasive species. *Theoretical population biology*, **70**(4), 431-435.

Haynes, K., and Millar, J. (Eds.). (2012). *Methods in Chemical Ecology Volume 2: Bioassay Methods*. Springer Science and Business Media.

He, W. M., Feng, Y., Ridenour, W. M., Thelen, G. C., Pollock, J. L., Diaconu, A., and Callaway, R. M. (2009). Novel weapons and invasion: biogeographic differences in the competitive effects of Centaurea maculosa and its root exudate (±)-catechin. *Oecologia*, **159**(4), 803-815.

Hierro, J. L., and Callaway, R. M. (2003). Allelopathy and exotic plant invasion. *Plant and soil*, **256**(1), 29-39.

Hoenicka, H., and Fladung, M. (2006). Biosafety in Populus spp. and other forest trees: from non-native species to taxa derived from traditional breeding and genetic engineering. *Trees*, **20**(2), 131-144.

Hunt, W. F., Lord, B., Loh, B., and Sia, A. (2015). Selection of plants that demonstrated nitrate removal characteristics. In *Plant selection for bioretention systems and stormwater treatment practices* (pp. 7-20). Springer Singapore.

Hutching, A., Scott, A. H., Lewis, G., and Cunningham, A. B. (1996). Zulu medicinal plants. *An inventory University of Natal press, pietermaritzburg*, 53-54.

Ibrar, M. (2003). *Conservation of indigenous medicinal plants and their traditional knowledge found in moist temperate Himalayas Pakistan* (Doctoral dissertation, Quaid-i-Azam University, Islamabad).

Jabran, K. (2017). Allelopathy: Introduction and Concepts. In *Manipulation of Allelopathic Crops for Weed Control* (pp. 1-12). Springer International Publishing.

Jan, A., and Ali, S. I. (2009). Conservation status of Astragalus gilgitensis Ali (Fabaceae): acritically endangered species in the Gilgit District, Pakistan. *Phyton (Horn)*, **48**(2), 211-223.

Kashif, M. S., Cheema, Z. A., and Farooq, M. (2015). Allelopathic Interaction of Wheat (*Triticum aestivum*) and Littleseed Canarygrass (*Phalaris minor*). *International Journal of Agriculture and Biology*, **17**(2).

Kato-Noguchi, H., and Ino, T. (2005). Possible involvement of momilactone B in rice allelopathy. *Journal of plant physiology*, **162**(6), 718-721.

Khan, M. A., Qureshi, R. A., Gillani, S. A., Ghufran, M. A., Batool, A., and Sultana, K. N. (2010). Invasive species of federal capital area Islamabad, Pakistan. *Pakistan Journal of Botany*, **42**(3), 1529-1534.

Khanh, T. D., Xuan, T. D., Chung, I. M., and Tawata, S. (2008). Allelochemicals of barnyardgrass-infested soil and their activities on crops and weeds. *Weed biology and management*, **8**(4), 267-275.

Khatoon, S., and Ali, S. I. (1999). Alien Invasive Species in Pakistan, University of Karachi. *Pers. Comm., Pakistan.*

Kilic. M. and O. S. Arslan 2010. Turkey's Forests and Biodiversity. Workshop on International Symposium on Biology of Rare and Endemic Plant Species. (Biorare Symposium) May 26- 29, 2010, Fethiye-Muğla, Turkey.

Kruse, M., Strandberg, M., and Strandberg, B. (2000). Ecological effects of allelopathic plants-a review. *NERI Technical Report*, **315**.

Lawson, R. H. (1996, March). Economic importance and trends in ornamental horticulture. In *IX International Symposium on Virus Diseases of Ornamental Plants* **432** (pp. 226-237).

Lecomte, C., Alabouvette, C., Edel-Hermann, V., Robert, F., and Steinberg, C. (2016). Biological control of ornamental plant diseases caused by Fusarium oxysporum: a review. *Biological Control*, **101**, 17-30.

Liang, Q., Li, G. Q., and Xu, W. H. (2013). Chemical composition of essential oil from Hydrangea macrophylla flower. *Chemistry of Natural Compounds*, **49**(2), 365-366.

Lim, T. K. (2012). *Edible medicinal and non-medicinal plants* (Vol. **1**, pp. 656-687). New York, NY, USA: Springer.

Lin, W. X., He, H., Shen, L., Chen, X., Ke, Y., Guo, Y., and He, H. (2004). A proteomic approach to analysing rice allelopathy on barnyard grass (Echinochloa crus-galli L.). In *Proceedings of the 4th International Crop Science Congress, Brisbane, Australia (http://www. cropscience. org. au/icsc2004/poster/2/4/1/1414_xionglw. htm–access in October 01, 2008).*

Loi, D. T. (2004). Medicinal plants and remedy of Vietnam. *Hanoi: Publisher of Medicine.*

Lonsdale, W. M. (1999). Global patterns of plant invasions and the concept of invasibility. *Ecology*, **80**(5), 1522-1536.

Ma, L., Wu, H., Bai, R., Zhou, L., Yuan, X., and Hou, D. (2011). Phytotoxic effects of Stellera chamaejasme L. root extract. *African Journal of Agricultural Research*, **6**(5), 1170-1176.

Macek, T., Kotrba, P., Svatos, A., Novakova, M., Demnerova, K., and Mackova, M. (2008). Novel roles for genetically modified plants in environmental protection. *Trends in biotechnology*, **26**(3), 146-152.

Macías, F. A., Molinillo, J. M., Galindo, J. C., Varela, R. M., Simonet, A. M., and Castellano, D. (2001). The use of allelopathic studies in the search for natural herbicides. *Journal of Crop Production*, **4**(2), 237-255.

Mai, D. T., Le, T. D., Nguyen, T. P., Phan, N. M., Nguyen, H. A., Nguyen, T. T. P., and Tran, L. Q. (2015). A new aldehyde compound from the fruit of *Pandanus tectorius* Parkinson ex Du Roi. *Natural product research*, **29**(15), 1437-1441.

Makoi, J. H., and Ndakidemi, P. A. (2012). Allelopathy as protectant, defence and growth stimulants in legume cereal mixed culture systems. *New Zealand Journal of Crop and Horticultural Science*, **40**(3), 161-186.

Mamede, A. M., Soares, A. G., Oliveira, E. J., and Farah, A. (2017). Volatile Composition of Sweet Passion Fruit (*Passiflora alata Curtis*). *Journal of Chemistry*, **2017**.

Mardani, H., Kazantseva, E., Onipchenko, V., and Fujii, Y. (2016). Evaluation of allelopathic activity of 178 Caucasian plant species. *International Journal of Basic and Applied Sciences*, **5**(1), 75-81.

Mardani, H., Sekine, T., Azizi, M., Mishyna, M., and Fujii, Y. (2015). Identification of safranal as the main allelochemical from saffron (*Crocus sativus*). *Natural product communications*, **10**(5), 775-777.

Marwat, K. B., Hashim, S., and Ali, H. (2010). Weed management: a case study from north-west Pakistan. *Pakistan Journal of Botany*, **42**, 341-353.

Mathews, V. H., and Rao, P. S. (1982). In vitro plant regeneration in lateral bud explants of Cryptanthus bromelioides var. Tricolor MB Foster. *Plant cell reports*, **1**(3), 108-110.

Menchaca, M. D. C. V., Morales, C. R., Star, J. V., Or, A., Morales, M. E. R., Nuntilde, M. A., and Gallardo, L. B. S. (2013). Antimicrobial activity of five plants from Northern Mexico on medically important bacteria. *African Journal of Microbiology Research*, **7**(43), 5011-5017.

Miller, D. A. (1996). Allelopathy in forage crop systems. *Agronomy Journal*, **88**(6), 854-859.

Mubeen, K., Nadeem, M. A., Tanveer, A., and Zahir, Z. A. (2011). Allelopathic effect of aqueous extracts of weeds on the germination and seedling growth of rice (*Oryza sativa* L.). *Pakistan Journal of Life and Social Sciences*, **9**(1), 87-92.

Muller, C. H. (1966). The role of chemical inhibition (allelopathy) in vegetational composition. *Bulletin of the Torrey Botanical Club*, 332-351.

Nadeem, M., Khan, M. A., Riaz, A., and Ahmad, R. (2011). Evaluation of growth and flowering potential of rosa hybrida cultivars under Faisalabad climatic conditions. *Pakistan* Journal of *Agricultural* Sciences, **48**(4), 283-288.

Nakamura, S., Wang, Z., Xu, F., Matsuda, H., Wu, L., and Yoshikawa, M. (2009). The absolute stereostructures of cyanogenic glycosides, hydracyanosides A, B, and C, from the leaves and stems of Hydrangea macrophylla. *Tetrahedron Letters*, **50**(32), 4639-4642.

Negi, R., Kumar, S., and Dhiman, S. R. (2014). Evaluation of different cultivars of gladiolus (*Gladiolus grandiflorus* L.) suitable for low hills of Himachal Pradesh. *Indian Journal of Scientific Research and Technology,***2**(6), 6-11.

Ni, G. Y., Zhao, P., Huang, Q. Q., Hou, Y. P., Zhou, C. M., Cao, Q. P., and Peng, S. L. (2012). Exploring the Novel Weapons Hypothesis with invasive plant species in China. *Allelopathy Journal*, **29**(2).

Nishihara, E., Parvez, M. M., Araya, H., Kawashima, S., and Fujii, Y. (2005). L-3-(3, 4-Dihydroxyphenyl) alanine (L-DOPA), an allelochemical exuded from velvetbean (Mucuna pruriens) roots. *Plant Growth Regulation*, ***45***(2), 113-120.

Nurul Ain, M. B., Nornasuha, Y., and Ismail, B. S. (2016, November). Allelopathic assessment of selected common weeds in Malaysia. In *AIP Conference Proceedings* (Vol. **1784**, No. 1, p. 060039). AIP Publishing.

Olofsdotter, M., Jensen, L. B., and Courtois, B. (2002). Improving crop competitive ability using allelopathy—an example from rice. *Plant Breeding*, ***121***(1), 1-9.

Pacheco, G., Garcia, R., Lugato, D., Vianna, M., and Mansur, E. (2012). Plant regeneration, callus induction and establishment of cell suspension cultures of Passiflora alata Curtis. *Scientia horticulturae*, **144**, 42-47.

Pallewatta, N., Reaser, J. K., and Gutierrez, A. T. (2003). Invasive alien species in South-Southeast Asia: national reports and directory of resources. *Invasive alien species in South-Southeast Asia: national reports and directory of resources.*

Parvez, S. S., Parvez, M. M., Fujii, Y., and Gemma, H. (2003). Allelopathic competence of Tamarindus indica L. root involved in plant growth regulation. *Plant growth regulation*, **41**(2), 139-148.

Perry, L. M., and Metzger, J. (1980). *Medicinal plants of east and southeast Asia: attributed properties and uses*. MIT press.

Pickens, K. A., Wolf, J., Affolter, J. M., and Wetzstein, H. Y. (2006). Adventitious bud development and regeneration in Tillandsia eizii. *In Vitro Cellular and Developmental Biology-Plant*, **42**(4), 348-353.

Ponou, B. K., Teponno, R. B., Ricciutelli, M., Quassinti, L., Bramucci, M., Lupidi, G., ... and Tapondjou, L. A. (2010). Dimeric antioxidant and cytotoxic triterpenoid saponins from Terminalia ivorensis A. Chev. *Phytochemistry*, **71**(17), 2108-2115.

Putnam, A. R., and Duke, W. B. (1978). Allelopathy in agroecosystems. *Annual review of phytopathology*, **16**(1), 431-451.

Putnam, A.R. and Tang, C.S. 1986. "The Science of Allelopathy;" John Wiley and Sons, New York, 317 pp.

Qin, R. M., Zheng, Y. L., Valiente-Banuet, A., Callaway, R. M., Barclay, G. F., Pereyra, C. S., & Feng, Y. L. (2013). The evolution of increased competitive ability, innate competitive advantages, and novel biochemical weapons act in concert for a tropical invader. *New Phytologist*, **197**(3), 979-988.

Qureshi, H., Arshad, M., and Bibi, Y. (2014). Invasive flora of Pakistan: a critical analysis. *Journal of Biodiversity and Environmental Sciences,* **4,** 407-424.

Radosevich, Steven R., Jodie S. Holt, and Claudio Ghersa. *Weed ecology: implications for management*. John Wiley and Sons, 1997.

Ramalhete, C., Lopes, D., Mulhovo, S., Rosário, V. E., and Ferreira, M. J. U. (2008, October). Antimalarial activity of some plants traditionally used in Mozambique. In *Workshop Plantas Medicinais e Fitoterapêuticas nos Trópicos. IICT/CCCM,* **29***, 30 e 31 de Outubro de*.

Rashid, M., Abbas, S. H., and Rehman, A. (2014). The status of highly alien invasive plants in Pakistan and their impact on the ecosystem: a review

Rashid, M., Abbas, S. H., and Rehman, A. (2014). The status of highly alien invasive plants in Pakistan and their impact on the ecosystem: a review. *Innovare Journal of Agricultural Science,* **2**,1-4.

Rauch, F. D., and Weissich, P. R. (2009). *Small Trees for the Tropical Landscape: A Gardener's Guide*. University of Hawaii Press.

Rawani, A., Pal, S., and Chandra, G. (2011). Evaluation of antimicrobial properties of four plant extracts against human pathogens. *Asian Pacific Journal of Tropical Biomedicine,* **1**(1), S71-S75.

Reddy, C. S. (2008). Catalogue of invasive alien flora of India. *Life Science Journal*, **5**(2), 84-89.

Riaz, T., Khan, S. N., and Javaid, A. (2007). Scenario of gladiolus production in Punjab, Pakistan. *Pakistan Journal of Botany,* **39**(7), 2389-2393.

Rice, E.L., 1984. *Allelopathy*, 2nd edition. Academic Press, New York, USA.

Rimando, A. M., Dayan, F. E., Mikell, J. R., and Moraes, R. M. (1999). Phytotoxic lignans of Leucophyllum frutescens. *Natural toxins*, **7**(1), 39-43.

Rizvi, S. J. H., Haque, H., Singh, V. K., and Rizvi, V. (1992). A discipline called allelopathy. In *Allelopathy* (pp. 1-10). Springer Netherlands.

Rudnicki, M., de Oliveira, M. R., da Veiga Pereira, T., Reginatto, F. H., Dal-Pizzol, F., and Moreira, J. C. F. (2007). Antioxidant and antiglycation properties of Passiflora alata and Passiflora edulis extracts. *Food Chemistry*, **100**(2), 719-724.

Savona, M., Sacco, E., and Ruffoni, B. (2012). Improving micropropagation performances in Hydrangea spp.: temporary immersion shoot culture and induction of morphogenetic events. *Acta horticulturae*, **961**, 457-464.

Sekine, T., Sugano, M., Majid, A., and Fujii, Y. (2007). Antifungal effects of volatile compounds from black zira (Bunium persicum) and other spices and herbs. *Journal of chemical ecology*, **33**(11), 2123-2132.

Shah, M., and Baig, K. J. (2001). Threatened Species Listing in Pakistan: Status, Issues ad Prospects.

Sharma, S., and Devkota, A. (2015). Allelopathic potential and phytochemical screening of four medicinal plants of Nepal. *Scientific World*, **12**(12), 56-61.

Shinwari, D., and Ibrar, M. (2008). The Study of Advanced Taxonomic and Conservation Techniques Applying on Plant Species with Toxic or other Biologically Functional Ingredients Implied to Market as well as Environment.

Shinwari, M. I., and Fujii, Y. (2013 b). Allelopathic activity of medicinal plants and weeds from Pakistan. *Allelopathy Journal*, **32**(2), 223.

Shinwari, M. I., and Shinwari, M. I. (2010). Botanical diversity in pakistan; past present and future. *World Environment*, **85**.

Shinwari, M. I., Iida, O., Shinwari, M. I., and Fujii, Y. (2008). Evaluation of allelopathic activity of 160 Japanese medicinal plants by sandwich method. *Journal of Weed Science and Technology*, **53**, 73.

Shinwari, M. I., Iida, O., Shinwari, M. I., and Fujii, Y. (2017). Evaluation of Phytodiversity For Allelopathic Activity and Application to Minimize Climate Change Impact: Japanese Medicinal Plants. *Pakistan Journal of Botany*, **49**, 139-144.

Shinwari, M. I., Shinwari, M. I., and Fujii, Y. (2013a). Allelopathic evaluation of shared invasive plants and weeds of Pakistan and Japan for environmental risk assessment. *Pakistan Journal of Biotechnology*, **45**, 467-474.

Shiraishi, S., Watanabe, I., Kuno, K., and Fujii, Y. (2002). Allelopathic activity of leaching from dry leaves and exudate from roots of ground cover plants assayed on agar. *Weed biology and management*, **2**(3), 133-142.

Singh, H. P., Batish, D. R., and Kohli, R. K. (2003). Allelopathic interactions and allelochemicals: new possibilities for sustainable weed management. *Critical reviews in plant sciences*, **22**(3-4), 239-311.

Singh, H. P., Batish, D. R., Kaur, S., Setia, N., and Kohli, R. K. (2005). Effects of 2-benzoxazolinone on the germination, early growth and morphogenetic response of mung bean (Phaseolus aureus). *Annals of applied biology*, **147**(3), 267-274.

Singh, K. P. (2005). Invasive alien species and biodiversity in India. *Current Science*, **88**(4), 539.

Sinkkonen, A. (2005). Modeling the effect of density-dependent chemical interference upon seed germination. *Nonlinearity in biology, toxicology, medicine*, **3**(2), nonlin-003.

Sitapha, O., Elisee, K. K., and Joseph, D. A. (2013). Antifungal activities of Terminalia ivorensis A. Chev. bark extracts against Candida albicans and Aspergillus fumigatus. *Journal of Intercultural Ethnopharmacology*, **2**(1), 49-52.

Song, B., Xiong, J., Fang, C., Qiu, L., Lin, R., Liang, Y., and Lin, W. (2008). Allelopathic enhancement and differential gene expression in rice under low nitrogen treatment. *Journal of chemical ecology*, **34**(5), 688-695.

Stamp, N. (2003). Out of the quagmire of plant defense hypotheses. *The Quarterly Review of Biology*, **78**(1), 23-55.

Stewart, R. R. (1958). *The Flora of the Rawalpindi District, West Pakistan*. Gordon college.

Sun, Y.F., Wink, M., Wang, Z.Q., Lu, H.F., Xia, P.G., Liu, H.T., Wang, S.X., Sun, Y. and Liang, Z.S. (2017). The Essential Oils from Pittosporum tobira Flowers Display Antiproliferative Properties and Inducing Apoptosis on Lung Carcinoma Cells.

Thapar, R., and Singh, N. B. (2006). Effects of leaf-residues of Croton bonplandianum on growth and metabolism of Parthenium hysterophorus L. *Allelopathy Journal*, **18**(2), 255-266.

Tongma S., Kobayashi K., Usui K. (2001) Allelopathic activity of Mexican sunflower [Tithonia diversifolia (Hemsl.) A. Gray] in soil under natural field conditions and different moisture conditions, *Weed Biology and Management*. **1**, 115–119.

Tsanuo, M. K., Hassanali, A., Hooper, A. M., Khan, Z., Kaberia, F., Pickett, J. A., and Wadhams, L. J. (2003). Isoflavanones from the allelopathic aqueous root exudate of Desmodium uncinatum. *Phytochemistry*, **64**(1), 265-273.

Tseng, Mei-Huims, Yueh-Hsiung Kuo, Yih-Ming Chen, and Chang-Hung Chou. "Allelopathic Potential of Macaranga tanarius (L.) Muell. –Arg." *Journal of chemical ecology* **29**, no. 5 (2003): 1269-1286.

Walter, K. S., and Gillett, H. J. (Eds.). (1998). *1997 IUCN Red List of threatened plants*. IUCN.

Weidenhamer, J. D., Hartnett, D. C., and Romeo, J. T. (1989). Density-dependent phytotoxicity: distinguishing resource competition and allelopathic interference in plants. *Journal of Applied Ecology*, 613-624.

Weir, T. L., Park, S. W., and Vivanco, J. M. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. *Current opinion in plant biology*, **7**(4), 472-479.

Wermuth, C. G. (Ed.). (2011). *The practice of medicinal chemistry*. Academic Press.

Wiart, C. (2006). *Medicinal plants of the Asia-Pacific: drugs for the future?* World Scientific.

Williams, P. A. (1997). *Ecology and management of invasive weeds*. Dept. of Conservation.

Willis, R. J. (1985). The historical bases of the concept of allelopathy. *Journal of the History of Biology*, **18**(1), 71-102.

Wong, S. K., Lim, Y. Y., and Chan, E. W. (2013). Botany, uses, phytochemistry and pharmacology of selected Apocynaceae species: A review. *Pharmacognosy Communications*, **3**(3), 2.

Young, C. C. (1986). Autointoxication of *Asparagus Officinalis* L.

Zeng, R. S., Mallik, A. U., and Luo, S. M. (Eds.). (2008). *Allelopathy in sustainable agriculture and forestry* (pp. 189-282). New York, NY: Springer.

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

Zhou, Y., Yu, L., Zhang, J., & Lu, Y. (2008). Molecular approaches in improving the rice allelopathy. *Allelopathy Journal*, **22**(2), 275-281.

ANNEXURE-1

Top Ranked Plants Having Stimulatory Effects

Figure 1: *Passiflora alata* curtis

Figure 2: Strong inhibition of *Passiflora alata* curtis Lettuce.

Figure 3: *Pandanus tectorius* Parkinson ex Du Roi

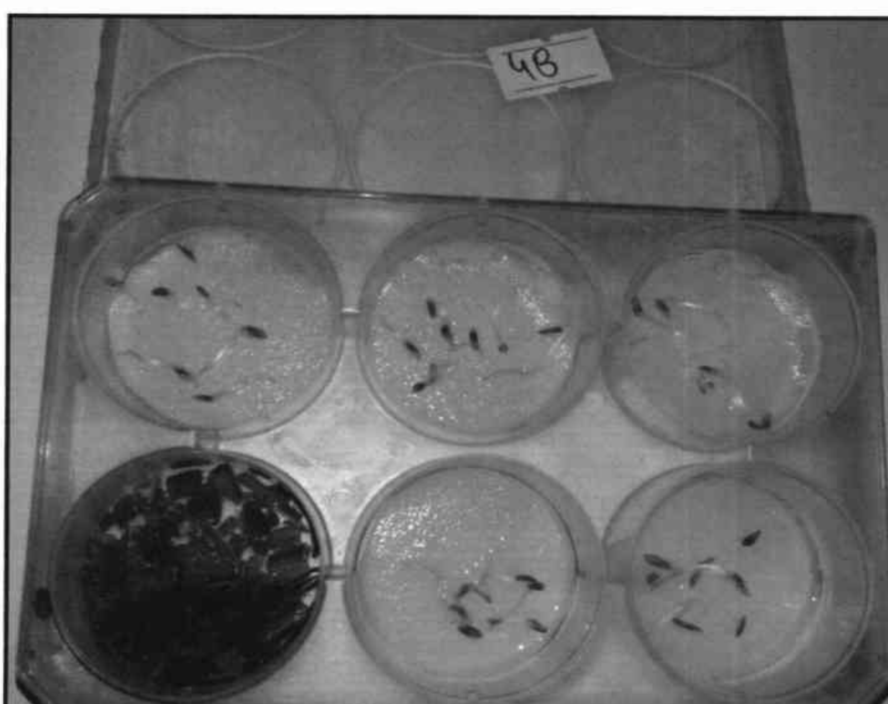

Figure 4: Growth of *Pandanus tectorius* Parkinson ex Du Roi in dishpack experiment.

Figure5: ***Solanum betaceum*** **cav.**

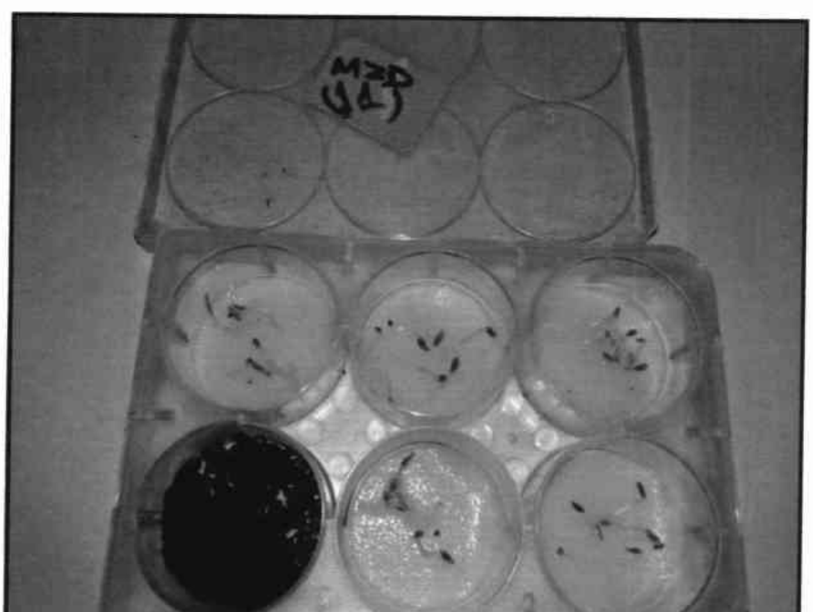

Figure 6: Growth of *Solanum betaceum* cav. in dishpack experiment.

Figure 7: *Polyscias paniculata* variegata

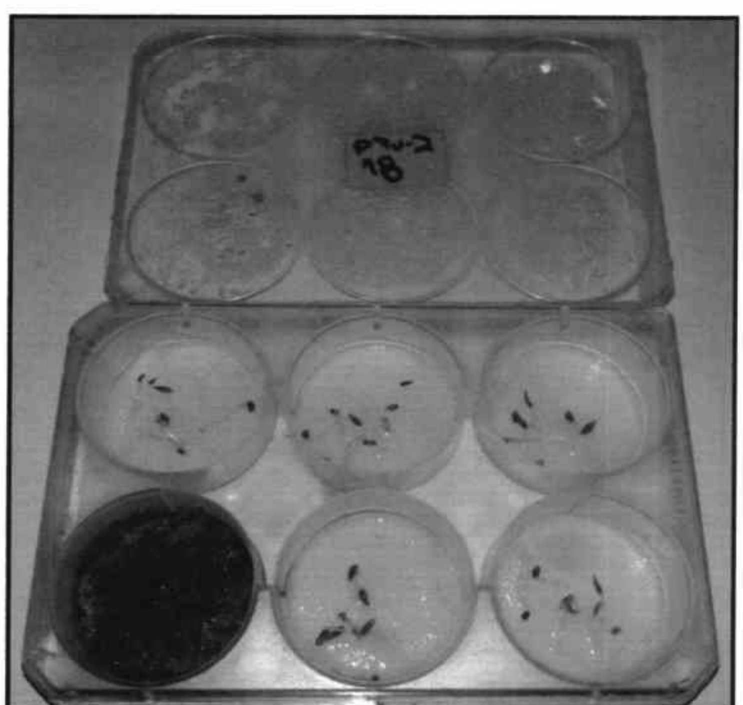

Figure 8: Growth of *Polyscias paniculata* variegata. in dishpack experiment

Figure 9: *Terminalia ivorensis* A. Chev.

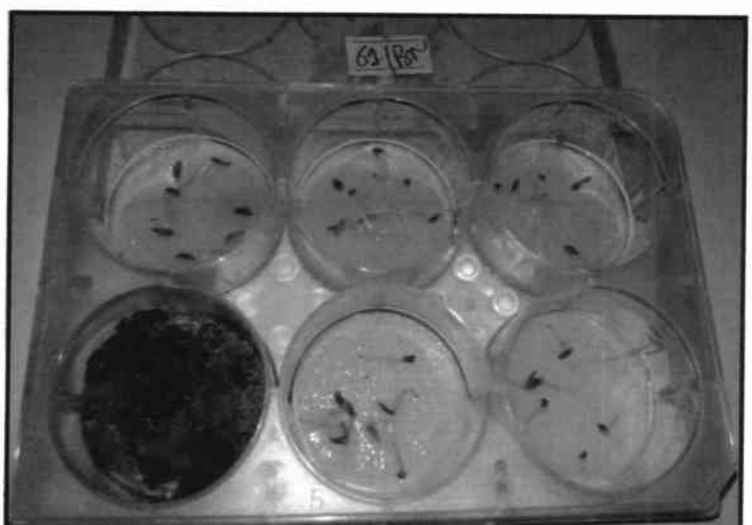

Figure 10: Growth of *Terminalia ivorensis* A. Chev in dishpack experiment

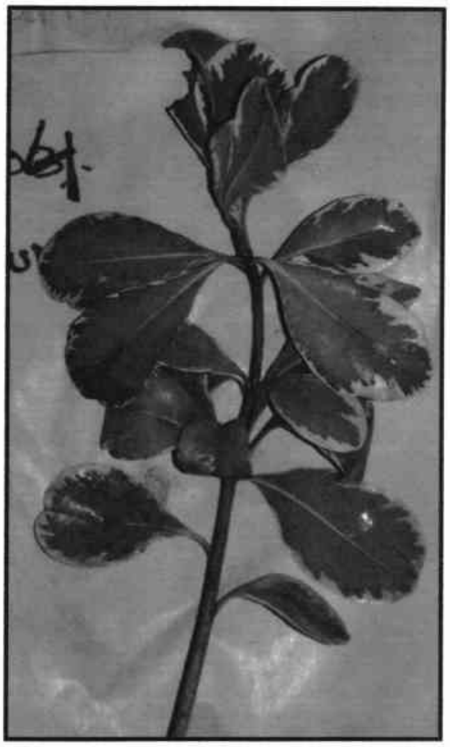

Figure 11: *Pittosporum tobira* (Thunb.) W.T. Aiton

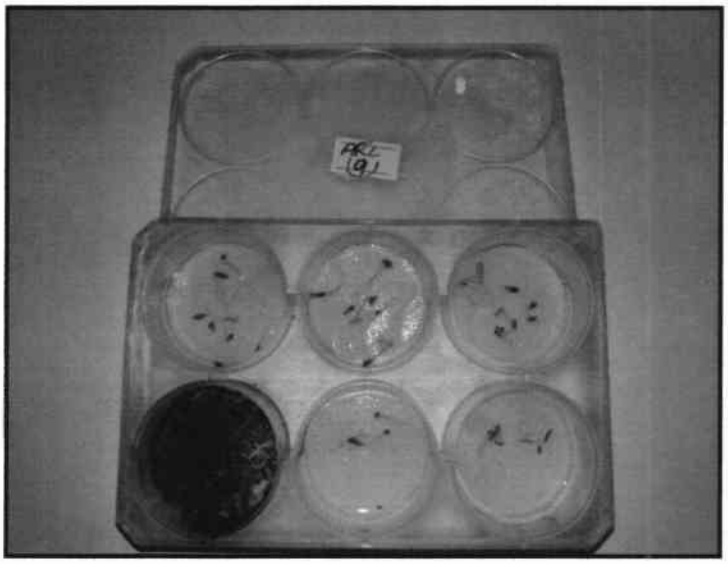

Figure 12: Growth of *Pittosporum tobira* (Thunb.) W.T. Aiton. in dishpack experiment

ANNEXURE-II

Top Ranked Plants Having Stimulatory Effects

Figure 13: *Melaleuca bracteata* F. Muell.

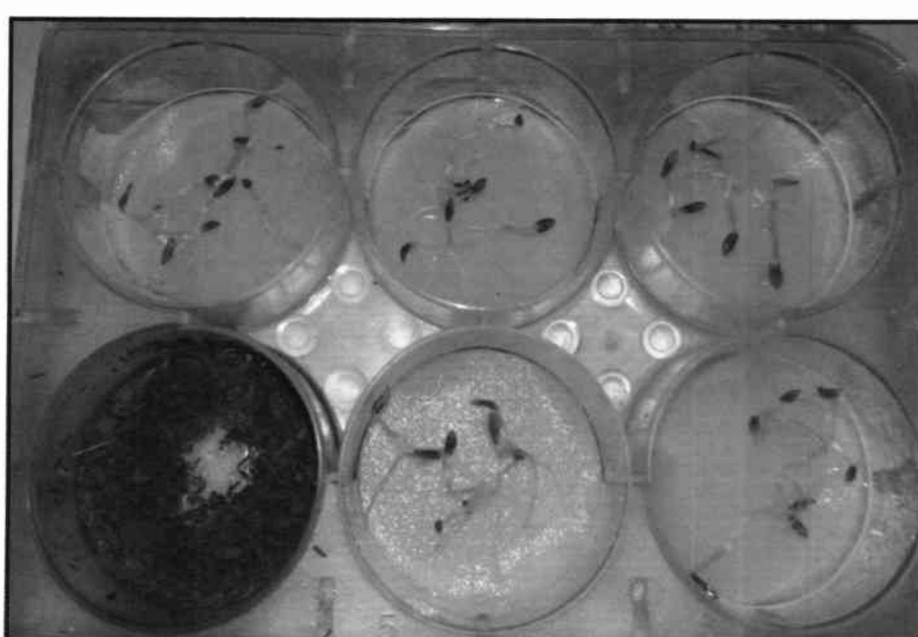

Figure 14: Growth of *Melaleuca bracteata* F. Muell. In dish-pack experiment.

Figure 15: *Leucophyllum frutescens* (Berland.) I.M. Johnst.

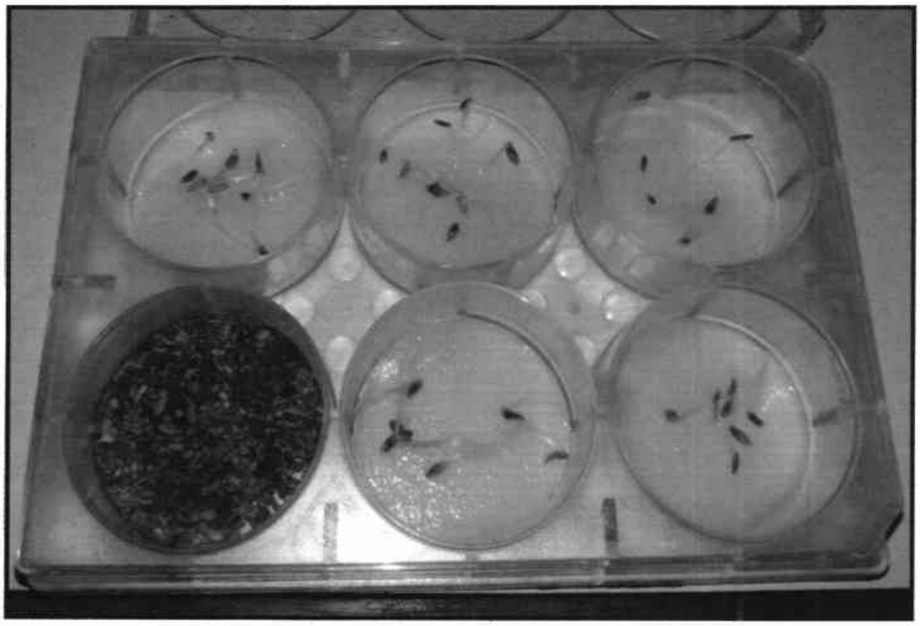

Figure 16: Growth of *Leucophyllum frutescens* (Berland.) I.M. Johnst.Dish-pack experiment.

Figure 17: *Gladiolus* L.

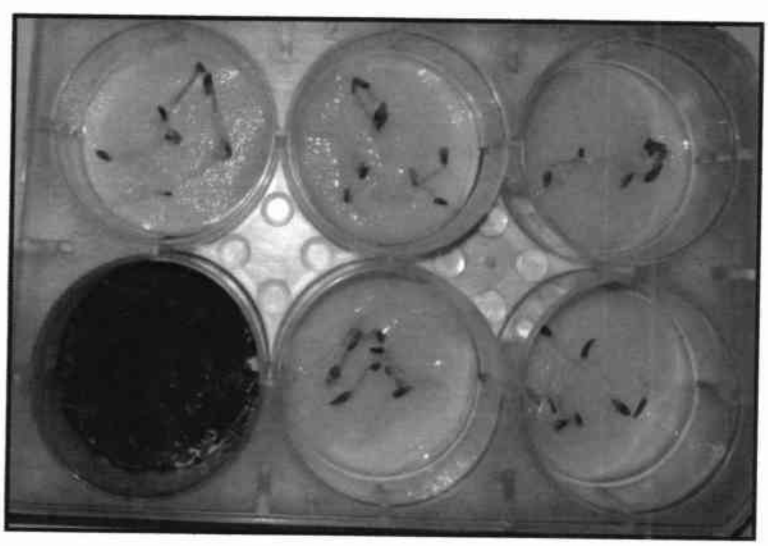

Figure 18: Growth of *Gladiolus* L. in dish-pack experiment.

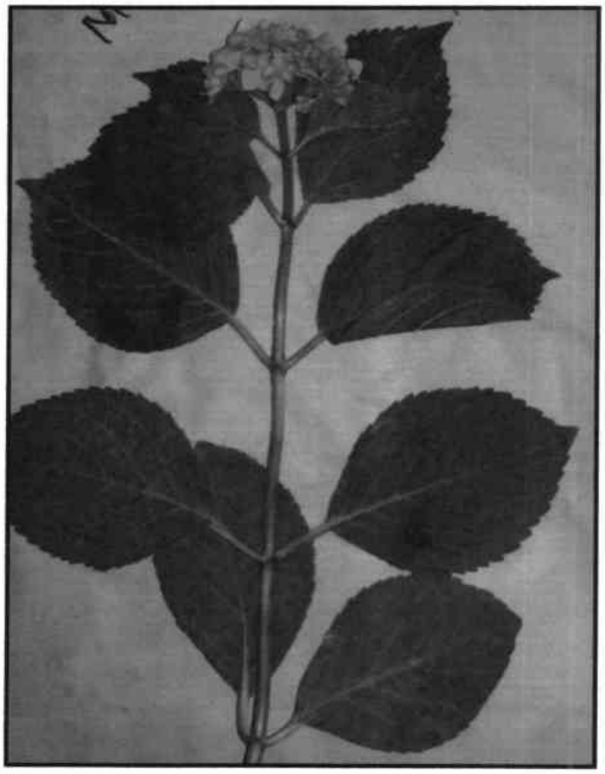

Figure 19: *Hydrangea macrophylla* (Thunb.) Ser.

Figure 20: Growth of *Hydrangea macrophylla* (Thunb.) Ser. in dish-pack experiment.

Figure21: *Cryptanthus bromelioides var. tricolor* M.B. Foster

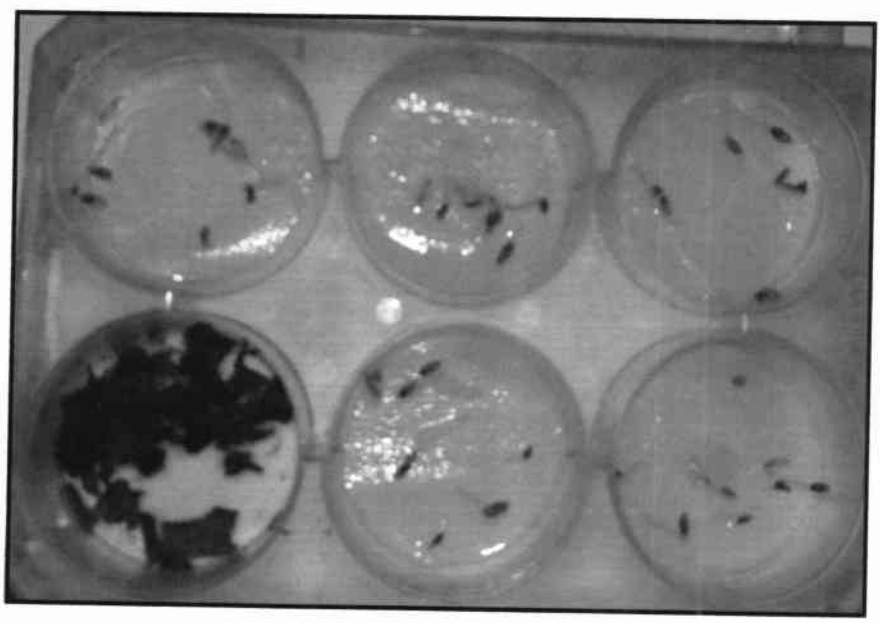

Figure 22: Growth of *Cryptanthus bromelioides var. tricolor* M.B. Foster in **dish-pack experiment.**

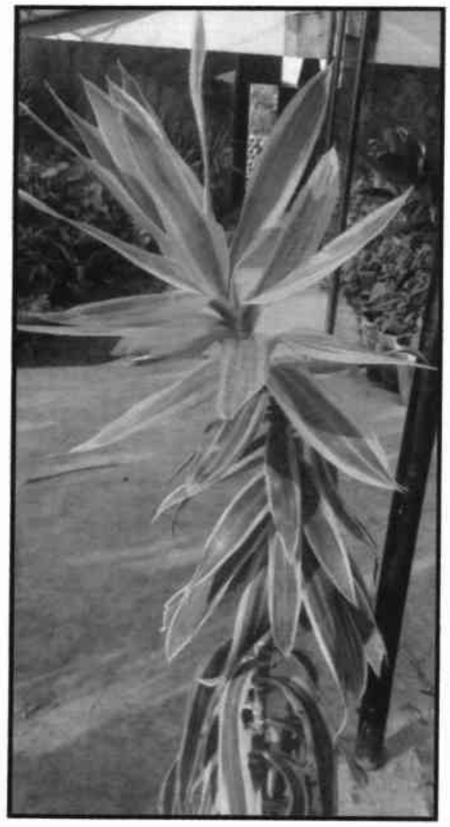

Figure 23: ***Dracaena reflexa*** Lam.

Figure 24: ***Growth of Dracaena reflexa*** Lam. in d***ish-pack experiment.***

ANNEXURE III

Review of Allelopathic Effect of Studied Plants

Name of plant	Family	Test Plant	Bioassay Used	Application/Use	Allelopathic Effect	Reference
Passiflora alata	Passifloraceae	*Lactuca Sativa* L.	Dishpack Method	Remedy for anxiety and nervousness.	Inhibitory	Mamede, 2017; de Freitas ,2016; Pacheco *et al.*, 2012; Boeira, 2010; Rudnick, 2007
Pandanus tectorius Parkinson ex Du Roi	Pandananceae	*Lactuca Sativa* L.	Dishpack Method	Effective for Diabetes, Hypertension, Hepatitis and for kidney problems	Inhibitory	Mai *et al.*,2015; Lim,2012; Loi,2004
Solanum betaceum cav.	*Solanaceae*	*Lactuca Sativa* L.	Dishpack Method	Contains antioxidants and are very effective against the lipid oxidation	Inhibitory	Acosta-Quezada *et al.*, 2011; Castro-Vargas *et al.*, 2013; Durant *et al.*, 2013
Polyscias paniculata ´variegata´	Araliaceae	*Lactuca Sativa* L.	Dishpack Method	-	Inhibitory	Florens and Strahm, 2000
Terminalia ivorensis Chev.	Combretaceae	*Lactuca Sativa* L.	Dishpack Method	Anti-malarial antioxidant, anti-fungal and cytotoxic actives	Inhibitory	Coulibaly *et al.*,2014; Sitapha *et al.*, 2013; Ponou *et al.*, 2010
Pittosporum tobira (Thunb.) W.T. Aiton	Pittosporaceae	*Lactuca Sativa* L.	Dishpack Method	Anti-cancer potential. Antibiotic Dysentery, rheumatism and dentition	Inhibitory	Sun *et al.*, 2017; Wermuth, 2011; Chung *et al.*,.2010; Ramalhete *et al.*, 2008; Wiart, 2006
Melaleuca bracteata F. Muell	Myrtaceae	*Lactuca Sativa* L.	Dishpack Method	Contain Anti-oxidants, treatment of wounds and skin diseases	Stimulatory	Siddique *et al.*, 2017; Hou *et al.*,2016c; Almarie *et al.*, 2016; Hutching *et al.*, 1996
Leucophyllum frutescens (Berland.) I.M. Johnst	Scrophulariacea	*Lactuca Sativa* L.	Dishpack Method	Anti-microbial Effective against the liver disorders	Stimulatory	Hunt *et al.*, 2015; Menchaca *et al.*, 2013; Balderas-Renteria *et al.*, 2007; Rimando *et al.*, 1999

Gladiolus grandiflora L.	Iridaceae	*Lactuca Sativa* L.	Dishpack Method	-	Stimulatory	Negi *et al.*, 2014 Riaz *et al.*, 2007 and Nadeem *et al.*, 2011
Plumeria obtusa L	Apocynaceae	*Lactuca Sativa* L.	Dishpack Method	Anti-microbial, anti-bacterial and anti-fungal	Stimulatory	Rawani *et al.*, 2011; Ali *et al.*,2013; Wong *etal.*, 2013; Perry and Metzger, 1980
Hydrangea macrophylla Thunb.Ser.	hydrangeaceae	*Lactuca Sativa* L.	Dishpack Method	Dysphoria, heart hot fright, malaria anti-allergic, anti-fungal and anti-oxidative potentials	Stimulatory	Ling *et al.*,2013; Dilshara *et al.*, 2013; Savona *et al.*, 2012; Nakamura *et al.*, 2009
Cryptanthus bromelioides var. *tricolor* M.B. Foster	Bromeliaceae	*Lactuca Sativa* L.	Dishpack Method	-	Stimulatory	Pickens, 2006; Mathews and Rao, 1982

ANNEXURE IV

Summary of Allelopathic Assessment found in Literature Review

Name of plant	Family	Test Plant	Bioassay Used	Allelopathic Effect	Reference
Melilotus officinalis (L.) Lam.	Fabaceae	*Lactuca Sativa* L.	Plant-box, Sandwich and Dish-packs	Inhibbitory	Shinwari *et al.*,2013a
Tagetes minuta L.	Asteraceae	*Lactuca Sativa* L.	Plant box method Dish-pack method	Inhibbitory	Shinwari *et al.*, 2013b
Xanthium strumarium L.	Asteraceae	*Lactuca Sativa* L.	Sandwich method	Inhibbitory	Akhtar *et al.*,2014
Cannabis sativa L.	Cannabaceae	*Lactuca Sativa* L.	Sandwich method	Inhibbitory	Akhtar *et al.*,2014
Melia azedarach L.	Meliaceae	*Lactuca Sativa* L.	Sandwich method	Inhibbitory	Shinwari *et al.*, 2008
Ligustrum japonicum	Oleaceae	*Lactuca Sativa* L.	Sandwich method	Stimulatory	Shinwari *et al.*, 2008
Triticum aestivum L. (cultivar Shafaq-06)	Poaceae	*Phalaris minor* Retz	*The equal-compartment-agar method.*	Inhibbitory	Kashif *et al.*, 2015
Petiveria aliacea L.	Petiveriaceae	*Lactuca Sativa* L.	Bioassay with Petri dishes	Inhibitory	Pérez-Leal *et al.*,2005
Vulpia myuros (L.) C.C. Gmel	Poaceae	*Triticum aestivum* L.	Bioassay with Petri dishes	Inhibbitory	An *et al.*, 1997
Macaranga tanarius (L.) Müll.Arg.	Euphorbiaceae	*Lactuca Sativa* L.	Density-response	Inhibbitory	Tseng *et al.*, 2003
Citrullus lanatus (Thunb.) Matsum. & Nakai	Cucurbitaceae	*Cirtrullus lanatus &* *Lactuca Sativa* L.	ContinuousRoot Exudates Trapping System (Hydroponics)	Inhibitory	Hao *etal.*, 2007
Parthenium hysterophorus L.	Asteraceae	*Brassica oleracea* L., *B. campestris* L. *and B. rapa* L.	Amendment of plant residues	inhibitory	Singh *et al.*, 2005
Ageratum conyzoides L.	Asteraceae	*Cicer arietinum*	Amendment of plant residues	inhibitory	Batish *etal.*, 2006
Chenopodium murale L.	Amaranthaceae	*C.arietinum and Pisum sativum L.)*	Amendment of plant residues	inhibitory	Batish,2007
Tamarindus indica L	Fabaceae	*Echinochloa crusgalli Mill. ; Trifolium repens L.);* *Lactuca sativa L.* *Raphanus sativus L.*	Plant box Soil agar sandwich method	Inhibbitory	Parvez,2003
Mucuna pruriens (L.) DC. var. *utilis*	Faboideae	*Lactuca Sativa* L.	Plant-box Method	Inhibitory	Nishihara,2005

Artabotrys odoratissimus R.	Annonaceae	*Lactuca Sativa* L.	Sandwich Method	Inhibitory	Fujii *et al.*, 2003
Photinia glabra (Thunb.) Franch. and Sav.	Rosaceae Juss.	*Lactuca Sativa* L.	Sandwich Method	Inhibitory	Appiah ,2015
Aesculus turbinata Blume	Sapindaceae	*Lactuca Sativa* L.	Sandwich Method	stimulatory	Appiah ,2015
Crocus sativus L.	Iridaceae	*Lactuca Sativa* L.	Sandwich Method	Inhibbitory	Mardani *etal.*,2015
Ageratum conyzoides L.	Asteraceae	*Lactuca Sativa* L.	Sandwich Method	Inhibbitory	Nurul Ain *et al.*, 2016
Lygodium flexuosum (L.) Sw.	Lygodiaceae	*Lactuca Sativa* L.	Dish pack Method	Inhibbitory	Nurul Ain *et al.*, 2016
Nephrolepis biserrata (Sw.) Schott	Nephrolepidaceae	*Lactuca Sativa* L.	Dish pack Method	stimulatory	Nurul Ain *et al.*, 2016
Melia azedarach L.	Meliaceae	*Lactuca Sativa* L.	Sandwich Method	Inhibbitory	Shinwari *et al.*, 2017
Ligustrum japonicum Thunb.	Oleaceae	*Lactuca Sativa* L.	Sandwich Method	Stimulatory	Shinwari *et al.*, 2017