

**Evaluation of Allelopathy of Selected Aromatic Plants of
Rawalakot, Azad Jammu and Kashmir**



By

Humayun Khurshid

Department of Environmental Science

Faculty of Basic and Applied Sciences

International Islamic University, Islamabad

(2016)



Evaluation of Allelopathy of Selected *Albizia* Species at
Ravizhok and Jammal Area

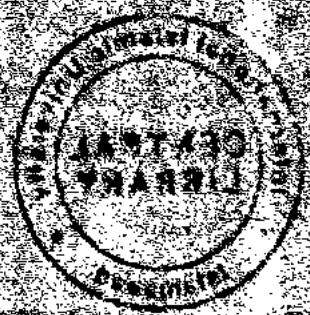
Accession No. *MS*



by
Hanyoung Kim

Department of Environmental Science
Faculty of Basic and Applied Sciences
International Islamic University, Islamabad

(2016)



**Evaluation of Allelopathy of Selected Aromatic Plants of
Rawalakot, Azad Jammu and Kashmir**



Researcher

Humayun Khurshid

186/FBAS/MSES/F13

Supervisor

Dr. Muhammad Ibrar Shinwari

Assistant Professor

Department of Environmental Science

Department of Environmental Science

Faculty of Basic and Applied Sciences

International Islamic University, Islamabad

(2016)

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

Acceptance by the Viva Voce Committee

Thesis Title: Evaluation of Allelopathy of Selected Aromatic Plants of Rawalakot, Azad Jammu and Kashmir.

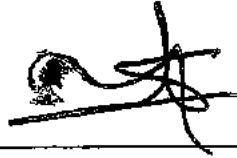
Name of Student: Humayun Khurshid

Registration No: 186 – FBAS/MSES/F13

Accepted by the Faculty of Basic & Applied Sciences, Department of Environmental Science, International Islamic University, Islamabad in partial fulfilment of the requirements for the Master Studies in Environmental Science.

Viva Voce Committee

Dean, FBAS



(Prof. Dr. Muhammad Sher)

Chairman, DES



(Prof. Dr. Muhammad Irfan Khan)

Supervisor



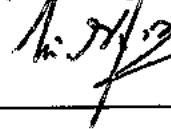
(Dr. Muhammad Ibrar Shinwari)

External Examiner



(Dr. Mir Ajab Khan)

Internal Examiner



(Prof. Dr. Muhammad Irfan Khan)

Date: 05-04-2016

A thesis submitted to the Department of Environmental Sciences, International Islamic University Islamabad as a partial fulfillment of the requirement for the award of the degree of Master Studies in Environmental Science.

DEDICATION

I dedicate this research to my family and friends whose appreciable efforts, prayers and support helped me in the completion of this final thesis for my Master's degree in Environmental Science.

DECLARATION

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

Date 04-04-2016



CONTENTS

1. INTRODUCTION.....	1
2. LITERATURE REVIEW	6
2.1 Allelochemicals.....	8
2.2 Factors Affecting Allelochemicals Production.....	9
2.3 Process of Actions of Allelochemicals.....	13
2.4 Allelopathy Assessment.....	17
2.5 Examples of Allelopathic Activity of Plant Species.....	19
2.5.1 <i>Chenopodium ambrosioides</i>.....	19
2.5.2 Allelopathic Influences of Artemisinin.....	20
2.5.3 Allelopathic Influences of Chenopodiaceae.....	21
2.6 Allelopathy as a Tool for Weed Control.....	21
2.7 Allelopathy as an Alternative and Eco-friendly Tool for Disease Control in Plants.....	23
3. RESEARCH METHODOLOGY	27
3.1 Study Area.....	27
3.2 Field Work.....	27
3.2.1 Sample Collections.....	28
3.3 Materials.....	28
3.4 Methods.....	28
4. RESULTS	32
5. DISCUSSION and CONCLUSION.....	46

5.1 Discussion46

5.2 Conclusion50

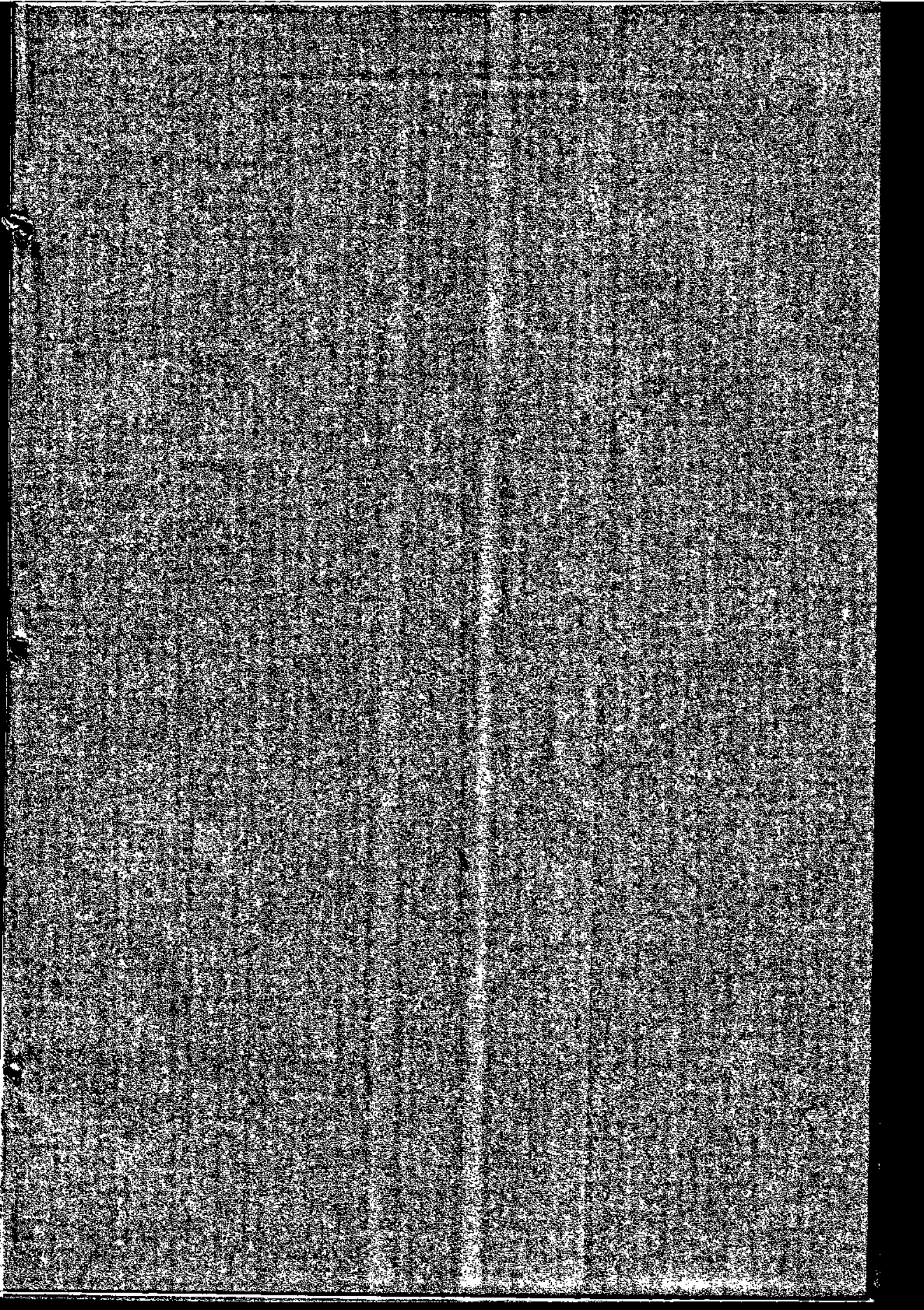
REFERENCES.....51

List of Figures

1. Fig 3.1. Dish Pack Method.....	29
2. Fig 4.1. Comparison of Radicle and Hypocotyl growth inhibition by Dish Pack method	35
3. Fig 4.1a. <i>Vicia sativa</i>	36
4. Fig 4.1b. <i>Vicia sativa</i> experiment picture.....	36
5. Fig 4.2a. <i>Allium sativum</i>	38
6. Fig 4.2b. <i>Allium sativum</i> experiment picture.....	38
7. Fig 4.3a. <i>Rosa damascena</i>	40
8. Fig 4.3b. <i>Rosa damascena</i> experiment picture.....	40
9. Fig 4.4a. <i>Trifolium repens</i>	42
10. Fig 4.4b. <i>Trifolium repens</i> experiment picture.....	42
11. Fig 4.5a. <i>Pinus wallichiana</i>	44
12. Fig 4.5b. <i>Pinus wallichiana</i> experiment picture.....	44

List of Tables

- 1. Table 2.1 Allelopathic suppression of pathogens, nematodes and diseases26**
- 2. Table 2.2 Allelochemicals showing defence against pathogens26**
- 3. Table 3.1 Botanical names, local names and family names of the tested plant species...30**
- 4. Table 4.1 Allelopathic Activity of Aromatic Plant Samples by Dish Pack Method.....32**



ACKNOWLEDGEMENTS

All praises and thanks to Almighty Allah, the creator of the universe and the most beneficent and merciful, who blessed me with an opportunity to work on this research project.

I would like to thank my respected supervisor Dr. Muhammad Ibrar Shinwari for his dynamic supervision, constructive criticism and overall help throughout the research work. I am also thankful to the Chairman and the Head of Department for providing the possible facilities to complete this research.

Humayun Khurshid

Abstract

A research to evaluate the allelopathic potential of 39 aromatic plant species of Rawalakot, Azad Kashmir was carried out using dish pack method. Leaf leachates of aromatic plant species were used against the lettuce seeds as the test species and growth was measured after 72 hours. In the study area, aromatic plants play a significant role among the farmers and almost all the people living in villages and remote areas. Some plant species have bioactive properties and these properties enable them to have allelopathic activity. Plant species showed certain inhibitory and stimulatory allelopathic effects on the growth of lettuce. *Vicia sativa* showed the greatest inhibitory effect on lettuce growth followed by *Allium sativum*, *Rosa damascena*, *Trifolium repens* and *Pinus wallichiana* among other top 5 species. These species have certain uses e.g. they produce food for humans and animals, used for ornamental purposes along with some medicinal uses and also for timber wood and fodder for the cattle. The results of this study suggest that allelopathic potential of the aromatic plants can serve as a tool for weed control, growth enhancer and as a result improving the environmental quality and sustainable development along with a lot of economic benefits for the local community.

1. Introduction

In this study an effort has been made to evaluate the allelopathic potential of 39 aromatic plant species of Rawalakot, Azad Jammu & Kashmir.

Allelopathy is an effect of a species on the growth of another species through the release of chemicals into the environment. This effect may be direct or indirect, positive or negative (Rice, 1984). Allelopathic interactions can be an important factor in the success of aromatic plant species as well as invasive plants. They may affect physiological processes and as a result may influence the structure of communities (Inderjit, 2003).

Allelopathy is a normal process and it involves beneficial and harmful biochemical interactions among all types of plants and also include other microorganisms. After the book "allelopathy" by Rice (1984) was published, attention in scientific community increased and its definition was reinforced later on. The effects of a plant species on another may be inhibitory or stimulatory and this is dependent upon the concentration of chemical compounds released from these plants. Certain chemicals decrease the growth of some plant species at small concentrations and they can increase the growth of other plant species at higher concentration. In allelopathy, the allelochemicals are released from dead or living tissues of donor plant and they are received by the target plant. Different organs of plants such as tissues, leaves, flowers, fruits, stems, roots, rhizomes, seeds, pollen are the common sources of allelopathic chemicals of donor plants (Weir *et al.*, 2004).

Aromatic plants are the plants in which aromatic substances are produced and released. They are used in making perfumes, in cooking, in the food, pharmaceutical and also in liquor industries. A large number of aromatic plants are the species of the *Lauraceae*, *Umbelliferae*, *Myrtaceae* and *Labiatae* families. Certain allelochemicals are synthesized during growth and developmental period of the plants. Plants release these chemicals at their early developmental

stages and even when plants are stressed and competing with neighboring plants for water, light, nutrients (Smirnoff and Hutchinson 1965). Crops that possess allelopathic activity can decrease the risk of major diseases that could reduce crop yields due to disease causing agents and as a result they reduce fungicide, bactericide and anti-viral application, so it saves the cost and helps to protect our environment (Huang and Chou, 2005). Certain phytochemicals have low or zero toxicity to beneficial insects and animals and have a variety of activity with varying and diverse site of action and they possess a high degradation rate. This shows that allelopathic activity is directly or indirectly involved in the management of diseases in crop production (Bais *et al.*, 2004a).

These chemicals can influence important physiological processes like respiration, photosynthesis, cell division, elongation, membrane fluidity, protein biosynthesis and also the activities of enzymes. Allelopathic compounds may also have an impact on tissue water status. Normally, they are more effective in mixtures in order to affect the targets (Rice, 1984 and Everts, 2002).

Active substances of aromatic plants have strong allelopathic properties and they can be safely used in agro-ecosystems. Certain medicinal plants inhibit the growth of selected weeds and several herbicidal allelochemicals from these plants have also been identified (Khan *et al.*, 2010). It is not much difficult to screen the allelopathic plants from medicinal plants because they are rich in metabolic compounds (Fujii *et al.*, 2004). It has been reported in many research works that the kind and concentration of secondary metabolites is different among certain plant species and these factors may be different even in the different parts of the same plant. These secondary metabolites mostly have qualitative and quantitative differences and this depends upon genetic drift, physiological conditions, season, harvesting time and analytical method sample preparation (Çirak *et al.*, 2008).

The anti-malarial drug artemisinin is obtained from the leaves of *Artemisia annua* and the release of this compound into forest soils may cause a risk in forest ecosystems like the effects on ectomycorrhizal fungal nutrient uptake and also in the areas where commercial cultivation of this plant is carried out. This drug is recommended for the initial treatment of malaria by the World Health Organization. This plant is grown on large scale in Southwest China for commercial use because of high artemisinin concentration in its leaves (Yang *et al.*, 2009). Certain plant species release secondary metabolites which are referred to allelochemicals, into soil environment for their safety from other plants and pathogenic microbes (Duke *et al.*, 2000).

Weeds are considered among the major threats to plants worldwide and the use of herbicides has increased to a large extent over the recent decades as a result increasing the cost and also environmental impacts of herbicides (Brethour and Weersink, 2001). Plants having allelopathic potential can be used as an alternative tool for weed control. To reduce the reliability on chemical weed control methods, several alternative strategies and techniques are under development to establish biological solutions and to reduce the harmful effects of herbicidal use in agricultural field (Xuan *et al.*, 2005).

It is hoped that this study will encourage further studies related to the field of allelopathy in order to develop new techniques for weed and disease control in crops and plants and also obtain certain other benefits.

Purpose of Study

Aromatic plants have a major influence on different components of the environment. Certain aromatic plants and their by-products have been used by human beings over the years. Rawalakot has a large variety of aromatic plant species and they are used for different purposes, e.g. source of food, fuel, ornamental uses, medicinal uses, furniture making etc. Many aromatic plant species can survive the conditions like pollution, salinity, excessive rainfall, drought and flower in these situations. Along with this, some of them are also avoided by herbivores and pathogens because they produce certain toxic and repellent compounds. These features show that these plants species might have some unstudied allelopathic potentials. Therefore, there is a need to increase the use of these species for other purposes as well, e.g. bio pesticides and bio fertilizers and for this purpose it is necessary to evaluate their allelopathic potential.

Aims and objectives

The aim of this research is to study the allelopathic potential of aromatic plants of Rawalakot, in order to contribute towards the knowledge on alternative methods of weed and disease control in crops and plants.

The objectives of the study include:

- Identification of aromatic plant species in Rawalakot.
- Screening and evaluation of allelopathic potential of the identified aromatic plants of Rawalakot.

Expected Outcomes

The research will provide an opportunity to assess the allelopathic potential of certain aromatic plant species. This study will also create awareness amongst farmers and rural community as well as a new scientific document will be developed as a baseline for other researchers. Also this study will provide the management options of weed control and disease control for government, local organizations and also NGOs for the future scenario in the light of current facts and figures.

2. Literature Review

Knowledge of interactions between plants has been gathered for more than two thousand years. Many examples are described by farmers, gardeners, botanists that suggest allelopathic interactions between plants. Destruction of weeds through chickpea (*Cicer arietinum*) plants was observed by Theophrastus (300 B.C.). It was reported by Romans in 1A.D. that farmland can be burnt or destroyed by barley, chickpea, bitter vetch (*Vicia ervilia*) and fenugreek (*Trigonella foenumgraecum*). He also explained that the shade of black walnut is heavy and black walnut and its residues may cause harm to man and any other in its vicinity. In 1600's many naturalists noted in the English literature that several plants do not grow properly in the presence of one another. The Japanese literature also gives examples of plants causing harm to others because of the release of toxic compounds with rainfall, mainly Japanese red pine (*Pinus densiflora*) (Rice, 1984).

In 1800's, agronomists started noting the problems with the repeated cropping of some perennials. In 1804 it was discovered that clover was failing in some areas of England where it was being cultivated regularly due to sickness of soil, which increase with the time. De Candolle firstly did experiments and said that soil sickness, may be the result of toxicity linked with root exudates. In 1881, it was observed that vegetation under black walnut was sparse in the pasture settings, the reason of this may be high mineral requirement of the tree, or poisonous moisture that is dripping off the tree itself (Stickney and Hoy, 1881). It is worth noting that several species that have powerful medicinal effects on humans are found to have strong allelopathic effects also. Interest in allelopathy field increased again in the 20th century, with the development of new techniques of bioassay, extraction, chemical isolation and identification (Willis, 1997).

Certain papers were published from 1948 to 1965 that described certain allelochemicals from plant residues and also the significance of the microbial interaction after these residues decompose (Putnam and Weston, 1986).

In the 21st century, publications in the allelopathy field have increased largely as physiologists, soil scientists, weed scientists and natural products chemists continue to research this challenging area (Macias *et al.*, 2002).

International Allelopathy Society was formed in 1994. This is organized for scientific purpose, mainly to promote the cooperation and collaboration among the scientists in allelopathy field. Allelopathy research can bring different disciplines together to increase success in sustainable agriculture. Many international conferences have been organized to promote enthusiasm in allelopathic research. Allelopathic effects merely extend beyond plant suppression, they include vegetation patterns, seed preservation, germination of fungal spores, nitrogen cycle, mutualistic associations, crop productivity and also plant defence. Researches have demonstrated critical cases of seed and growth reduction by allelochemicals recently, that effect vegetation patterns, rate, sequences in plant succession, weed abundance, crop productivity and problems in replanting fruit and other crops as well (El-Darier and Tammam, 2009).

2.1 Allelochemicals

Chemicals which are released from plants and impose allelopathic influences are called allelochemicals. Most of these chemicals are classified as secondary metabolites from acetate pathway and range in structure from simple hydrocarbons to complex polycyclic aromatics e.g. phenolic compounds, tannins, flavonoids, alkaloids, steroids and glycosides. Almost all the classes of secondary plant products are implicated in allelopathic interference (Weston and Duke, 2003). The term "allelochemical" is related to the role of the compound, but not related to the actual chemical identity, since depending on an organism or some environmental parameters, one compound can sometimes act as an allelochemical and at other times or places can play other roles. There is a large number of secondary metabolites in the plant kingdom and many are found to be phytotoxic (Einhellig *et al.*, 2002). Chemicals need to be transferred from one organism to another for allelopathic process to take place. Many phytotoxic substances suspected of causing germination and inhibition in growth have been identified from plant tissues and soils. The phytotoxic activity of such chemicals outside the plant is changed by biological abiotic factors occurring in the soil (Kobayashi, 2004).

Plants or organisms that release such compounds are "donor species", and those influenced in their growth are called "target species". Allelopathy covers plant-plant, plant-microorganisms, plant-virus, plant-insects, and plant soil-plant chemical interactions. These effects might be stimulatory or inhibitory, it depends on the identity of the active compound, static and dynamic availability, persistence and fate of organics in the environment and varies on particular target species (Inderjit and Keating, 1999).

These chemicals may be produced by different parts of plants including roots, rhizomes, stems, leaves, flowers, Inflorescences, fruits, and seeds (Rice, 1984). They are produced in above or below ground plant parts or can be produced in both to cause effects in a

wide range of plant communities. The donor plants generally store the chemicals in the plant cell in an inactive form, e.g. water soluble glycosides, polymers, lignins and salts. These allelochemicals are released through cleavage by plants enzyme and also some environmental stress (Weston, 1996). Chemicals in higher plants are released by volatilization (significant under arid or semi-arid conditions), leaf or stem leachates (chemicals release by rainfall, dew or irrigation from the aerial parts of plants), root exudation (release of chemicals through roots by certain processes including diffusion, vesicle transport, ion channels) and decomposition by microorganism or other mechanisms, and are carried away by wind and water (Inderjit and Keating, 1999).

Concentrations of these chemicals in the donor plant can also vary over time and in the plant tissue produced. For example, Foliar and leaf litter leachates of *Eucalyptus* species are more toxic as compared to the bark leachates to certain food crops. These chemicals are generally secondary plant products and the also breakdown products from decomposing plant tissues. Allelochemicals are normally stored in the vacuoles and prevent bad effects on the producing plant, but are mostly leached out of the tissue (Hall and Henderlong, 1989). The inhibitory materials can be auto inhibitory or hetero inhibitory, some may be highly selective and their effect is dependent upon the concentration. It has been found that phytotoxic effects are density-dependent. Phytotoxicity is greatest at low plant densities and growth reductions due to resource competition are greatest at high plant densities (Weidenhamer *et al.*, 1989).

2.2 Factors affecting allelochemicals production

The amount in which chemicals are produced in the donor plant is the result of the interaction between plant's genetic factors and environmental factors. Climatic factors have a big influence on the production of such chemicals. Some chemicals may be influenced by the duration, amount and intensity of light. Greatest quantities are produced when there is an

exposure to the ultraviolet and long- day photoperiods. Increased amount of allelochemicals was reported by Rice (1984) from the plants exposed to higher ambient temperatures. Water plays significant role in allelopathy and acts as a solvent and also a carrier of these chemicals and leachates from an aerial plant parts and in the soil. Activity in the soil by microorganisms is sensitive to the moisture level of soil (Rizvi *et al.*, 1992).

Composition and concentration of allelochemicals change with the age, plant organs and among different plant species. Habitat may also play a significant role in the expression of allelopathy. Naturally, the allelopathic potential of a plant is likely to change with the site because of its climatic conditions. Allelopathic potential is changed by seasonal conditions such as air, soil temperature and soil moisture. It was found that allelopathic inhibition of germination, growth of aspen (*Populus tremula*) was most significant in May, June, and September (Qasem and Foy, 2001).

The isolation and identification of chemicals from producer plants with biological activity do not show that these compounds interfere in nature through allelopathy (Weston, 2000). Transformation, retention and transport of these chemicals in the soil and physicochemical and biological components of soil have effect on the fate of allelopathic chemicals, ultimately on allelopathy in the soil. Physicochemical factors affect the quantity and quality of compounds. Particularly, soil texture influences the expression of allelopathy in natural systems. Soil biological and chemical characteristics i.e. texture, nutrients, microorganisms, organic matter, moisture and pH have their influences on allelopathic expression. They influence adsorption and transport in soil and metabolism of allelochemicals (Kobayashi, 2004). Soil pH has an important role to play in the uptake and immobilization of inorganic ions and on the resultant accumulation of nutrients, and higher pH can increase microbial activity (Inderjit and Dakshini, 1994).

The chemical characters of soil mostly change after addition of plant debris, roots, leaves of donor plants and these changes have been proved to change the action of allelopathic chemicals. Allelopathic interactions in soil environments depend to a large extent on the turnover rate of allelochemicals in soil rhizosphere and their interactions with clay, organic matter and certain factors that may change the physio-chemical and biotic characteristics of the soil (Blum *et al.*, 1999). In the recent time research by Blum and his laboratory has shown that soil texture, soil pH, organic compounds, available nutrients are also very important in affecting the uptake of allelochemicals and also their ability to persist in the presence of soil microorganisms (Blum, 1998). Soil moisture dynamics may also effect the phytotoxicity of allelochemicals. Studies by Blum suggest that increased evapotranspiration and lower soil moisture also results in decreased plant phytotoxicity of allelochemicals in the soil solution (Blum *et al.*, 2002).

Microorganisms in the soil also play a significant role in allelopathy because they have the potential to change the effects, to degrade toxic compounds or to produce the toxic ones (Inderjit, 2003). They may influence the availability of nutrients, by the release of chemical compounds bound to soil particles. These compounds may be present in free, reversibly bound, or irreversibly bound forms. In general, the first two forms are considered important from the standpoint of allelopathy. However, bound forms may also be important. Allelopathic plant species increase the production of phytotoxic metabolites when they are subjected to environmental stresses factors e.g. mineral deficiency, extreme temperatures, moisture stress, extreme light levels, herbicides, fungicides, insecticides and plant growth regulators (Einhellig, 1989).

Allelochemicals need to be taken up by the receptor plant to have a direct effect (Willis, 1985). They are mainly absorbed by the target plant through the roots, by active or passive transport and they move through the xylem by mass flow. An allelochemical, when taken up,

interferes with certain physiological processes of the receptor plant. Different studies have shown that the response to allelochemicals can be concentration dependent. Those allelochemicals that inhibit the growth of certain species at a concentration might stimulate the growth of the same species at different concentrations. It is necessary to identify the concentration at which a specific response occurs if this interaction is to be used in weed management programme. Along with this, various parts of the plant may differ in their allelopathic potential. The metabolites leached from plants consist of different substances like mineral nutrients, carbohydrates, amino acids, and other organic compounds. These substances may inhibit or sometimes stimulate plant growth, depending on different concentrations, the leachability, season, and also the age of the plant. More recently it was proposed that complex biochemicals may not be the only substances which are used by plants to interfere with one another and it has also been suggested that inorganic elements may be used in allelopathic manner. They reported that elements including heavy metals and salts in soils can occur by hyperaccumulation, litter decomposition and by altering rhizosphere chemistry (Chon & Kim, 2002).

The phytotoxic activity of the chemicals released from incorporated residues can be affected by abiotic and biotic factors like physicochemical and microbiological soil properties (Popa *et al.*, 2008). So a phytotoxic compound may be inactivated, become more activated or converted to some new toxins by soil microorganisms (Kobayashi, 2004). Composition profile and quantity of allelochemicals depend on the time after incorporation into the soil. In most cases, it was found that the phytotoxic potential of decaying plant residues was maximum at the early stages of decomposition (Xuan *et al.*, 2005). As decomposition carries on, phytotoxicity decreases or even disappears. This can be explained by the fact that quantity and activity of phytotoxins are affected by biotic and abiotic processes in the soil (Sampietro *et al.*, 2007).

2.3 Process of Actions of Allelochemicals

The mode of action of a chemical can be divided into direct and indirect action. Alteration of soil properties, nutritional status and an altered population or activity of micro-organisms are the effects that represent the indirect action. The direct action includes the biochemical and physiological effects of allelochemicals on certain significant processes of plant growth and metabolism. The mechanism and modes of allelochemicals action were initially described and after that they have been continuously reviewed. Different mechanisms of action for allelochemicals are involved in the inhibition and modification of plant growth and development (Einhellig *et al.*, 1986). These are the sites or processes which are targets for allelochemicals : cell division , production of plant hormones and their balance , membrane stability and permeability , germination of pollen , mineral uptake , movement of stomata , pigment synthesis , photosynthesis , respiration , amino acid synthesis , nitrogen fixation , specific enzyme activities , inhibition of nitrifying bacteria, N₂ fixing bacteria, plant –water relations, modification of DNA and RNA and complexities of nutrients and conduction tissues (Wink *et al.*, 1998).

These chemicals can be selective in their actions or plants can be selective in their response. These things are complicated further by the presence of more than an active compound from a single plant. E.g. sorghum species contains cyanogenic glycosides, tannins, flavonoids, quinines and phenolic acids. All of these have certain inhibitory functions and most of them produce several biological lesions (Einhellig, 1995).

The effects of the extracts from most *Artemisia* species are because of presence of a mixture of compounds, mainly terpenoids and also include coumarins and polyacetylenes as well. Certain observations have shown that glandular hair-like trichomes on the leaf surface produce enormous quantities of camphor, camphene, cineole and significant quantities of

artemisinin and arteether among others, camphor is the most active in seed inhibition (Weston & Duke, 2003).

The initial biochemical effect of allelochemicals seems is on the synthesis of protein mediated by RNA and DNA. Certain allelochemicals have also been shown to inhibit mitosis in plant roots, e.g. coumarin can completely block mitosis in onion roots within a few hours after treatment. Volatile terpenes from *Salvia leucophylla* are inhibitors of mitosis in cucumber seedlings. These compounds also have effects on the division of a large number of bacterial species isolated from soil (Einhellig, 1995).

Germination of cereals depends on certain activities that regulate starch break down necessary for supplying substrates to respiratory metabolism. Eucalyptus leaf leachates decreased α -amylase activity in seeds of finger-millet (*Eleusine coracanta*), that result in inhibition of germination (Padhy *et al.*, 2000).

Glyoxylate cycle plays a vital role in the mobilisation of triacylglycerides in the germination process of fat storing seeds. During the early stages of germination, the enzymes of glyoxylate cycle such as isocitratelase (ICL) increase their activity because of maximum lipid metabolism in the storage tissue of germinating seeds (McLaughlin and Smith, 1994). Data obtained by certain research works suggests the effect of allelopathic compounds on the activity of isocitratelase and also on gene expression. It was also reported that the observed decrease in enzymatic activity is a secondary effect of allelochemicals related to protein damage. Therefore, effects of allelochemicals on seed germination appear to be mediated through a disruption of normal cellular metabolism rather than through damage of organelles. Reserve mobilisation, a process which usually takes place rapidly during early stages of seed germination seems to be delayed or decreased under allelopathy stress conditions (Muscolo *et al.*, 2001).

Disturbance in the process of photosynthesis is one of the most commonly observed physiological effect of different allelochemicals. Because of this action of allelopathic compounds, they have properties to be used in inorganic agriculture e.g. in sustainable weed management as natural herbicides. Decrease in chlorophyll content and reduction of carotenoid concentration was detected in lettuce seedlings when treated with artemisinin. Photosynthesis rate reduced in leaves of white mustard (*Sinapis Alba*) plants exposed to sunflower (*Helianthus annuus*) allelochemicals, with the reduction in transpiration rate that suggested limited CO₂ diffusion into chloroplast due to stomata closing (Gniazdowska and Bogatek, 2005).

Inhibition of seedling growth in allelopathy stress conditions can be a result of decreased ion up take. Root comes first in contact with allelochemicals in the rhizosphere, thus the effect of allelochemicals on ion up take is specifically important. Certain other studies with whole plants and cell cultures have shown a reduction in the uptake of macro and micronutrients in the presence of phenolic acids. The phenolic acids suppress absorption of phosphate, potassium, nitrate, and magnesium ions, and overall changes in tissue content of mineral ions are among the effects on plants growth (Einhellig *et al.*, 2002). Toxicity from vanillic and *p*-coumaric acids was found when barley seedling were deficient in P or N and concluded that toxicity of these phenolic compounds is dependent on nutrient concentrations. Therefore, effects of allelochemicals on ion uptake may be a result of the decreased respiration rate and insufficient amount of ATP synthesized in root cells (Gniazdowska and Bogatek, 2005).

Mostly, volatile compounds are released from plants in drought areas and water- born phytotoxins such as flavonoids, alkaloids, and phenolic compounds are released from plants in humid areas. Allelochemicals may also persist in the soil and affect neighbouring and also the plants in successions. The effects of these allelochemicals are often observed in the early life cycle that cause Inhibition of seed germination and seedling growth. The compounds have a

wide range of mechanisms of actions, alkaloids effects on DNA, quinons effects photosynthetic and mitochondrial functions, while phenolics effect phytohormonal activity, ion uptake, and water balance (Chou, 1999).

There are several secondary metabolites that act as plant allelochemicals including phenolic, terpenoid, flavonoid, and alkaloids. Phenolic compounds such as phydroxybenzoic, vanillic, *p*-coumaric, syringic and ferulic acids are a main category of such chemicals. They have been identified as allelopathic agents in natural and agroecosystems. They affect seed germination, seedling growth, chlorophyll content, respiratory activity, enzymes activities and also cell division (Inderjit, 2003). There is a little information on the interaction of phenols and other compounds on how they affect germination and seedling growth but there is evidence that a mixture of phenols increases their inhibitory action. It can be used as both pre- and post-emergent herbicide and is phytotoxic towards a number of weedy species (Li *et al.*, 1993).

The most common phenolic compounds in allelopathy are the derivatives of cinnamic and benzoic acids, coumarins, tannins and other polyphenolic complexes, and certain flavonoids. The level of production and release of these compounds varies among different plants (Macias *et al.*, 2004). Certain phenolic acids and tannins appear to have quite similar mechanisms of action, they inhibit the plant growth by multiple physiological effects that create a generalized cytotoxicity (Einhellig, 1996).

As compared to the phenolic acids, the mechanisms of flavonoid action are less understood. Certain allelopathic flavonoids are effective inhibitors of energy metabolism, they block mitochondrial and chloroplast functions (Macias *et al.*, 2004). The concentration of a phenolic acids required to inhibit seed germination is normally higher than the concentration required to inhibit growth in whole seedlings. Along with many symptoms, a decrease in photosynthesis efficiency is a common effect of allelopathic phenolics. sorgoleone, a *p*-

benzoquinone in sorghum root exudates was found to inhibit the oxygen evolution of soybean leaf disk and isolated pea chloroplasts that resulted in growth reduction and photosystem II electron transferee reaction (Gonzalez *et al.*, 1997).

2.4 Allelopathy assessment

As a first step, laboratory bioassay is used to investigate the allelopathy. Bioassays are useful and necessary for studying the allelopathic potential of plant or soil extracts and also for evaluating the activity of the extracts during purification and identification of allelopathic compounds. Nearly all reports on allelopathy describe some type of bioassay method used to demonstrate allelopathic activity (Macias *et al.*, 2000).

Mainly, there are two kinds of measurements used for testing biological activity of allelopathic compounds: the measurement of specific biological activity (e.g., inhibition of photosynthesis) or measurements of some aspects of growth (e.g., germination, root dry weight). The most commonly used bioassay to test allelopathic activity in an extract is the technique of seed germination in Petri dishes on filter paper, sand, soil or agar. Percent germination has generally been used to measure the effects of allelopathic compounds. It's a rapid method for a large number of samples. Recording germination is a commonly used measurement but several investigations have shown that this is not the most sensitive parameter (Leather & Einhellig, 1988). Root length is often shown more sensitive than the germination bioassay, possibly because radicle elongation occurs by cell extension only. Germination includes both cell extension and cell division. Roots are not as easy to measure as germination and the design of the bioassay method can affect the growth of the roots (Wardle *et al.*, 1993).

Bioassays for such studies mostly comprise of seed germination, seedling growth, radicle length, seedling fresh weight, photosynthetic activity test and may detect potential allelopathic effects under controlled laboratory conditions (Aliotta *et al.*, 2006). Although

bioassays using water extracts from plants are helpful to prove the existence of allelochemicals in them, these effects mostly disappear under field conditions because of adsorption to soil particles, decomposition and leaching. Because of the criticisms directed to the aqueous extract bioassays, screening bioassays using intact plant seedlings have been developed. Plants at the seedling stage are also used in allelopathy studies (Wu *et al.*, 2000).

Different species are used in bioassays to assess allelopathic activity. Standard indicator species, like lettuce, radish and duckweed are recommended for the preliminary testing of allelopathic activity due to their availability and high sensitivity to allelopathic actions (Fujii, 1992).

2.5 Examples of Allelopathic Activity of Plant Species

2.5.1 *Chenopodium ambrosioides*

The chinampa farming system is recognized as one of the most intensive systems of agriculture in terms of total annual production per unit of land. Chinampas are essentially raised fields surrounded by water and they provide an example of an efficient and self-sustaining agricultural system which has been functioning for hundreds of years in the valley of Mexico. A most intriguing characteristic of the chinampa farming system is the absence of herbicides used for weed control. This suggests the presence of built-in mechanisms of weed control, that may include allelopathy, the interference in the metabolism and/or growth of a plant through substances produced and released into the environment by another plant (Rice, 1984).

It was found in a study of the chinampa ethnoflora that chinampa farmers grow *Chenopodium ambrosioides* only when the crops are ready to be harvested. According to the chinampa farmers, this plant doesn't let other plants to grow. Furthermore, the analysis of the chinampa vegetation indicated that *C. ambrosioides* distribution is clumped and that interspecific associations with crop plants are negative. *Chenopodium ambrosioides* is a widespread plant species native of tropical America. It is an annual or short-lived perennial herb that has been used for centuries as condiment, traditional purgative for intestinal worms and several other medicinal purposes. Its biological activity is widespread and it has been shown to affect viruses, fungi, nematodes and insects (Verma and Baranwal, 1983). Phytotoxic effects of this plant haven't been tested yet. Its widespread biological activity gives information about the traditional use and management of the species, and the vegetation analysis. Allelopathic interference may be significant in the places where this species is grown. For understanding the ecological basis of weed control in the chinampa agroecosystem, this study was conducted with the following objectives: To determine the possible allelopathic potential

of *Chenopodium ambrosioides*; to isolate and identify its active compounds; and to assess whether the active compounds from *Chenopodium ambrosioides* would produce allelopathic effects on crop species (Peterson *et al.*, 1989).

2.5.2 Allelopathic Influences of Artemisinin

Artemisinin is an anti-malarial drug extracted from the leaves of *Artemisia annua*. Its release into forest soils may produce a potential risk for forest ecosystems, i.e. effects on ectomycorrhizal fungal nutrient uptake, in areas where commercial and continual cultivation of this plant is practiced. Therefore, growth, proton and oxalate efflux, and nutrient uptake (nitrogen, phosphorus and potassium) of three isolates of *Suillus luteus* (S. luteus 1, S. luteus 13, and S. luteus 11) and of one isolate of *Suillus subluteus* (S. subluteus 12) were compared in culture solutions with different nominal artemisinin concentrations. The results proved that artemisinin influenced significantly the growth of all studied fungi. With 25mg artemisininL⁻¹ added, fungal biomass was decreased by 78.6% (S. luteus 1), 96.7% (S. luteus 13), 77.8% (S. luteus 11) and 86.8% (S. subluteus 12) compared with the control (without artemisinin). This explains why ectomycorrhizal fungal sporocarps in forests are consistently not found near cultivated fields of this plant. The amount of proton efflux by the fungal isolates also reduced as nominal artemisinin concentrations increased, which indicates the limited ability of ectomycorrhizal fungi to mobilize nutrients from soil minerals. However, nominal artemisinin significantly increased the rate of fungal oxalate efflux, suggesting membrane damage and the abnormal opening of anion channels on hyphae cell membranes. Nominal artemisinin also reduced the uptake of nitrogen, phosphorus and potassium by the fungal isolates. Therefore, artemisinin released from large *Artemisia annua* plantations may affect ectomycorrhizal fungal growth, nutrition and functions in forest ecosystems in Southwest China (Li *et al.*, 2014).

Artemisinin is recommended by WHO as a drug for the initial treatment of malaria. *Artemisia annua* is now grown on a large scale for commercial use in Southwest China, due to

high concentrations of artemisinin in the leaves. Several plant species release secondary metabolites, usually referred as allelochemicals, into soil environments to protect themselves against other plants and pathogenic microorganisms (Duke *et al.* 2000).

2.5.3 Allelopathic influences of Chenopodiaceae species on seed germination

Certain classes of allelopathic substances are produced naturally by most plant species. These compounds are usually synthesized in the leaves, which fall to the ground during periods of stress. Rain assists leaching of allelopathic substances into the soil, where they can affect the germination and growth of other plants. The effects of allelopathy on germination and growth of plants may occur through a number of processes such as reduced mitotic activity in roots and hypocotyls, suppressed hormone activity, reduced rate of ion uptake, inhibited photosynthesis and respiration, and inhibited protein formation, decreased permeability of cell membranes and inhibition of enzyme action. The results from the research suggest that allelopathy can be a possible mechanism to control the timing of germination and seedling establishment (Jefferson & Pennacchio, 2003).

2.6 Allelopathy as a tool for Weed Control

Weeds are among the major threats to crop productions worldwide and herbicide use has increased too much over recent decades. Most of that growth has been driven directly by increased labour costs and indirectly by available and effective alternative weed controls (Brethour and Weersink, 2001). To decrease the reliance on chemical weed managements, some other techniques are under development to discover biological solutions to reduce the negative impacts of herbicide and insecticide use in agriculture (Xuan *et al.*, 2005). Many plants produce certain secondary metabolites and also show allelopathic activity e.g. growth inhibitory effects on other plants. Certain plant species provide excellent weed control in intercropping or as soil additives. Allelopathic substances have tendency to be used as

herbicides or templates for new synthetic herbicide classes (Duke *et al.*, 2000). These allelochemicals are prone to qualitative and quantitative variations and it depends upon genetic drift and ploidy level, physiological conditions, season, harvesting time and analytical method sample preparation (Çirak *et al.*, 2008). Therefore, the determination of the optimum harvest time is important to obtain maximum natural production and to assess the viability of a plant as a potential crop (Taylor and Staden, 2001).

Because of increase in herbicide-resistant weed biotypes and environmental concerns about the safety of synthetic herbicides, appreciable efforts have been put into introducing alternative weed-management strategies and decreasing the dependence on synthetic herbicides. Use of certain plants with strong allelopathic properties for weed control has shown very good results. Cell growth in plants is dependent upon normal mitotic processes, then DNA synthesis, mitosis and cytokinesis occur. Cell division is a continuous process that occurs in plant meristematic regions (Singh *et al.*, 2002). Mitotic activity (a measurement of actively dividing cells known as the mitotic index), alterations in the mitotic phase, and individual cell aberrations are the main parameters by which plant growth can be evaluated. Cytogenetic assays for evaluation of these parameters are normally carried out using the *Allium* test. Use of this approach as a screening system accurately predicted the herbicidal activity of unknown compounds. Characterized by rather homogenous meristematic cells, very large chromosomes and a basic chromosome number of only sixteen, the *Allium cepa* (the common onion) is ideal for use in bioassays. *Allium* is a rapid, reliable, and inexpensive system by which the toxic effects of certain chemical compounds may be monitored and classified for potential environmental pollution effects (Leme and Marin, 2008).

2.7 Allelopathy as an Alternative and Eco-friendly Tool for Disease Control in Plants

Allelopathy takes place when plant release some specific chemicals which affect other species in its vicinity. It is a natural phenomenon and it has been observed for more than 200 years and the phenomenon reports as early as 300 BC document that many crop plants inhibited the growth of other plants. The term Allelopathy is derived from two Greek words: 'allelon', meaning 'of each other', and 'pathos', meaning 'to suffer'. This old concept was known to classical researchers in the Greek and Roman era. Negative impacts of crop plants on other plants were observed by Theophrastus and by Pliny II, while De Candolle recognized allelopathy to be soil sickness. The term 'allelopathy' was first used by Austrian plant physiologist Molisch, who defined it as the chemical interaction between plants and microorganisms. Allelopathy is a process whereby secondary metabolites synthesised by fungi, viruses, microorganisms and plants influence biological and agricultural systems which can be either stimulatory or inhibitory (Singh *et al.*, 2012).

The chemicals released are normally secondary plant metabolites or by-products of the principal metabolic pathways in plants. They are non-nutritional and may be synthesised in any plant part including leaves, stems, roots, bark, seeds, etc. Under helpful environmental conditions, these chemicals are released into the environment by the processes of volatilization, root exudation, decomposition and leaching, as a result affecting the growth of adjacent plants (Bais *et al.*, 2004a). Not all such chemicals are involved in vital physiological events within the plant system. Allelopathy involves the synthesis of plant bioactive compounds known as allelochemicals which are capable of acting as natural disease controller and can solve problems like resistance development in races of pathogens, health defects and soil and environmental pollution caused by the use of synthetic agrochemicals (Singh *et al.*, 2012).

When allelopathic crops are used as cover crops, intercrops, or grown in rotational sequences, they can combat disease pathogens and additionally build up fertility and organic matter status of soil, so reduce soil erosion and improve farm yields. So, allelopathy can be exploited profitably in a lot of ways. Allelopathy is process that involves secondary metabolites that effect the growth and development of agricultural and biological systems, including beneficial and harmful effects. Allelopathy also refers to the positive and negative effects of one plant on another plant, through the release of chemicals from plant parts by leaching, root exudation, volatilization, residue decomposition and certain other processes in both natural and agricultural systems. Micro-organisms including fungi, bacteria, viruses, and nematodes are an important part of agro ecosystem (Gomez *et al.*, 2003).

Control of disease-causing organisms is an important part in every crop production system. The goal of allelopathy for disease control has a great importance to increase crop production. This method is very useful in order to control diseases in various types of crops in an effective manner. It is dependent upon the chemical constitution of allelochemicals. Allelopathic interactions include plant-plant, plant microorganisms, plant-virus, and plant-soil-plant chemical interactions (Singh *et al.*, 2012).

Plant diseases have bad effects on many crops e.g. cereals, oilseeds, and especially vegetables. Certain soil-borne diseases cause extreme loss to crop production by disturbing the crop stand and decreasing product quality. Number of cultural practices like burning infected plant debris and using resistant cultivars have been used but diseases still cause abundant losses in crop productions. Plant allelopathy mechanism can be applicable as a component of integrated disease management program. Chemical disease control for most diseases is either unavailable or ineffective so plant pathogens can be suppressed using allelopathic crops in a number of ways. Intercropping is a reliable allelopathic technique to biologically control soil-borne disease (Gomez *et al.*, 2003). Root exudation releases allelochemicals into the

rhizosphere, effectively causing influence on the interactions between neighbouring plants and microbes. Canola tissues containing high levels of the glucosinolate 2-phenylethyl (2-PE) is proved to be toxic against a large number of cereal pathogens e.g. plant parasitic nematodes. Intercropping creates a microclimate which helps to reduce disease intensity (Singh *et al.*, 2012).

Table 2.1 Allelopathic suppression of pathogens, nematodes and diseases

Allelopathic source	Application mode/ rate	Pathogen/ disease suppression
Barley (<i>Hordeum vulgare</i> L.) + potato	Grown in rotation	55.1% reduction in inoculum intensity of <i>Rhizoctonia solani</i> (JG Kühn)
Turnip (<i>Brassica rapa</i> L.) + potato	Grown in rotation	56.2% reduction in inoculum intensity of <i>Rhizoctonia solani</i> (JG Kühn)
Indian mustard (<i>Brassica juncea</i> L.) + potato	Grown in rotation	45.5% reduction in inoculum intensity of <i>Rhizoctonia solani</i> (JG Kühn)
Rice (<i>Oryza sativa</i> L.)	Root exudates (1.5 mL)	37% reduction in germination of <i>Fusarium oxysporum</i> f. sp. <i>niveum</i> spores
Rice	Root exudates (20 mL)	71.88% reduction in spore reproduction of <i>Fusarium oxysporum</i> f. sp. <i>Niveum</i>

Source: (Singh *et al.*, 2012).

Table 2.2 Allelochemicals showing defence against pathogens

Crop name	Allelochemicals	Protected crop	Defence against pathogen
Potato	α -tomatine (<i>Solanum</i> , <i>lycopersicon</i>)	Tomato	<i>Corium rolfsii</i> <i>Fusarium oxysporum</i>
<i>L. pumilio</i>		Tomato	<i>Pseudomonas solanacearum</i> . <i>Alternaria solani</i> .
Potato	solanine, chaconine and solanidine		
<i>Cicerarietrum</i>	Alkaloid		<i>Mycosphaella blight</i>
Cow pea	Phenolic, alkaloid	Groundnut	<i>Cercospora</i> (Leaf spot)
Sorghum	Hydrocyanic acid		<i>Helminthosporium turcicum</i> and <i>Fusarium moniliforme</i> .
Watermelon and Rice	Phenolic acids, sugars and free amino acids	Watermelon	<i>Fusarium oxysporum</i> f. sp. <i>niveum</i>
Lettuce	Phenolics	Cucumber	<i>Fusarium oxysporum</i> f. sp. <i>radialis-cucumerinum</i>

Source: (Singh *et al.*, 2012).

3. Research Methodology

3.1 Study area

Rawalakot is a town located in district Poonch, Azad Kashmir and the district headquarters of Poonch division. Rawalakot is located at latitude 33°51'32.18"N, longitude 73°45'34.93"E and an elevation of 5374 feet. It is approximately 120 km (75 mi) from the city of Rawalpindi.

Rawalakot features a subtropical highland climate due to high altitude. It has mild to warm temperatures during the spring and autumn seasons, humid temperatures during summer and cold to snowy weather in the winter. The temperature can rise up to 38 °C during the summer season and drop to -5 °C during the winter. Snowfall occurs in December, January, and February and sometimes in March as well. Most of the rainfall occurs during the monsoon season.

Rawalakot has a variety of aromatic plant species which are used for certain purposes, e.g. source of food, ornamental purpose, medicinal uses etc. This research evaluates the allelopathic potential of some aromatic plant species of Rawalakot.

3.2 Field Work

The study trips were made in April and May, 2015. The field work was based on the sample collection of aromatic plant species from the different villages of Rawalakot.

3.2.1 Sample Collection

The sample collection was carried out during the blooming period of the plants species and the specimens were preserved and identified. The leaves samples were dried by putting in the incubator for 24 hours at 60°C. The plant samples were then deposited in the lab of IIU, Islamabad for the research.

3.3 Materials

Materials used in the lab for the experiment include: filter papers, aluminum foil, beakers, lettuce seeds, crushed leaves of the plant samples, scotch tape, distilled water, syringe, face mask, marker, scissors etc.

3.4 Method

Dish pack method was used for the analysis of allelopathic potential of aromatic plant species. It is a newly developed method to analyze volatile allelochemicals. Volatile compounds from leaves are mostly diffused by wind and usually difficult to be concentrated enough to have potent inhibitory activity in the field. In dish pack method, crushed leaves of the plant species are put into one of the well in a 6-well-multi-dish. One filter paper and 0.7ml of distilled water is added into the other 5 wells, and 7 seeds of lettuce are put on the filter paper. Each side of the multi-dish is sealed with aluminum foil and put into the incubator. The growth of test plants is measured after 72 hours.

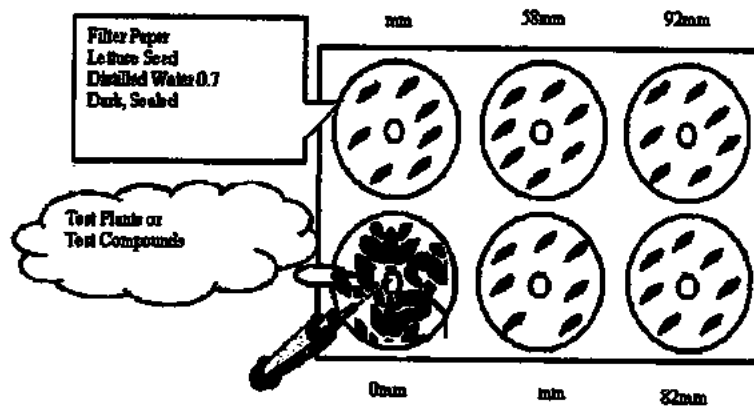


Fig. 3.1 Dish Pack Method

7A-16415

Table 3.1: Botanical names, local names and family names of the tested plant species.

No	Botanical Names	Local Names	Family Names
1.	<i>Vicia sativa</i> Linn.	Phali	Papilionaceae
2.	<i>Allium sativum</i> Linn.	Thoom	Alliaceae
3.	<i>Rosa damascena</i> Miller	Gulaab	Rosaceae
4.	<i>Trifolium repens</i> Linn.	Sree	Papilionaceae
5.	<i>Pinus wallichiana</i> Jackson	Raar	Pinaceae
6.	<i>Elaeagnus umbellata</i> Thunb.	Giyanni	Elaeagnaceae
7.	<i>Campsis grandiflora</i> Schumann (Thunberg)	Bail	Bignoniaceae
8.	<i>Ranunculus muricatus</i> Linn.	Maleen	Ranunculaceae
9.	<i>Robinia pseudoacacia</i> Linn.	Keekar	Mimosaceae
10.	<i>Prunus domestica</i> Linn.	Plum	Rosaceae
11.	<i>Rosa brunonii</i> Lindley	Safaid Gulaab	Rosaceae
12.	<i>Dryopteris stewartii</i> Fraser-Jenk	Kaki	Dryopteridaceae
13	<i>Ficus palmata</i> Forssk	Phawara	Moraceae
14	<i>Cedrus deodara</i> (D. Don) G. Don	Dayaar	Pinaceae
15	<i>Morus alba</i> Linn.	Toot	Moraceae
16	<i>Melia azedarach</i> Linn.	Dareek	Meliaceae
17	<i>Diospyros virginiana</i> Linn.	Amlook	Ebenaceae
18	<i>Rumex acetosa</i> Linn.	Halfri	Polygonaceae
19	<i>Prunus persica</i> (Linn.) Batsch	Aarwari	Rosaceae
20	<i>Coriandrum sativum</i> Linn.	Dhania	Apiaceae
21	<i>Punica granatum</i> Linn.	Daroona	Punicaceae
22	<i>Populus nigra</i> Linn.	Safeda	Salicaceae
23	<i>Mentha piperita</i> Linn.	Poodeena	Lamiaceae
24	<i>Prunus domestica subsp. Pomariorum</i> Linn.	Aaloo Bukhara	Rosaceae
25	<i>Prunus avium</i> Linn.	Loochi	Rosaceae

26	<i>Pseudocystidia sinensis</i> (Dumont de Courset) Koehne	Bai dana	Rosaceae
27	<i>Hibiscus syriacus</i> Linn.	Safaid Patti	Malvaceae
28	<i>Cannabis sativa</i> Linn.	Bhang	Cannabaceae
29	<i>Dahlia pinnata</i> 1 Cavanilles	Aaloo Phool	Asteraceae
30	<i>Berberis lycium</i> Royle	Sumbal	Berberidaceae
31	<i>Antirrhinum majus</i> L.	Qasni Phool	Scrophulariaceae
32	<i>Dahlia pinnata</i> 2 Cavanilles	Aaloo Phool	Asteraceae
33	<i>Prunus americana</i> Marshall	Khoobani	Rosaceae
34	<i>Dryopteris affinis</i> (Lowe) Fraser –Jenkins	Kangi	Dryopteridaceae
35	<i>Aesculus indica</i> (Wall. ex Cambess.) Hook.	San	Hippocastanaceae
36	<i>Mentha piperita</i> 2 Linn.	Bareena	Lamiaceae
37	<i>Brassica juncea</i> (Linn.) Czernohorsky	Oaar	Brassicaceae
38	<i>Viburnum grandiflorum</i> Wall. ex DC.	Jammar	Caprifoliaceae
39	<i>Thuja occidentalis</i> Linn.	Saroo/Mor Pankhi	Cupressaceae

4. Results

Extension of hypocotyl and radicle for all the tested plant species is given in the table 4.1. This shows that pattern variations of rates have been figured and the principles of variations in the patterns have been assessed.

Table4.1: Allelopathic Activity of Aromatic Plant Samples by Dish Pack Method

Botanical Names	Family	Radicle	Hypocotyl	Ranking
1. <i>Vicia sativa</i> Linn.	Papilionaceae	64.144	90.277	***
2. <i>Allium sativum</i> Linn.	Alliaceae	67.689	81.433	**
3. <i>Rosa damascena</i> Miller	Rosaceae	74.013	105.902	**
4. <i>Trifolium repens</i> Linn.	Papilionaceae	74.421	89.939	**
5. <i>Pinus wallichiana</i> Jackson	Pinaceae	76.349	84.016	*
6. <i>Elaeagnus umbellate</i> Thunb.	Elaeagnaceae	79.545	65.744	*
7. <i>Campsis grandiflora</i> Schumann (Thunberg)	Bignoniaceae	76.530	110.869	*
8. <i>Ranunculus muricatus</i> Linn.	Ranunculaceae	111.842	104.166	
9. <i>Robinia pseudoacacia</i> Linn.	Mimosaceae	111.842	116.319	
10. <i>Prunus domestica</i> Linn.	Rosaceae	104.046	105.5	
11. <i>Rosa brunonii</i> Lindley	Rosaceae	95.375	94	
12. <i>Dryopteris stewartii</i> Fraser-Jenk	Dryopteridaceae	107.658	95.5	

13. <i>Ficus palmata</i> Forssk	Moraceae	83.815	90	
14. <i>Cedrus deodara</i> (D. Don) G. Don	Pinaceae	85.585	87.475	
15. <i>Morus alba</i> Linn.	Moraceae	88.511	115.345	
16. <i>Melia azedarach</i> Linn.	Meliaceae	92.124	91.463	
17. <i>Diospyros virginiana</i> Linn.	Ebenaceae	101.156	101.626	
18. <i>Rumex acetosa</i> Linn.	Polygonaceae	87.899	96.938	
19. <i>Prunus persica</i> (Linn.) Batsch	Rosaceae	103.693	98.360	
20. <i>Coriandrum sativum</i> Linn.	Apiaceae	109.375	94.262	
21. <i>Punica granatum</i> Linn.	Punicaceae	100.852	100.409	
22. <i>Populus nigra</i> Linn.	Salicaceae	103.591	117.391	
23. <i>Mentha piperita</i> Linn.	Lamiaceae	86.647	92.213	
24. <i>Prunus domestica</i> subsp. <i>Pomariorum</i> Linn.	Rosaceae	98.011	108.606	
25. <i>Prunus avium</i> Linn.	Rosaceae	89.133	77.356	
26. <i>Pseudocydonia sinensis</i> (Dumont de Courset) Koehne	Rosaceae	99.431		
27. <i>Hibiscus syriacus</i> Linn.	Malvaceae	87.025	93.478	
28. <i>Cannabis sativa</i> Linn.	Cannabaceae	112.341	102.173	
29. <i>Dahlia pinnata</i> L Cavanilles	Asteraceae	102.848	100	
30. <i>Berberis lycium</i> Royle	Berberidaceae	101.265	95.652	

31. <i>Antirrhinum majus</i> L.	Scrophulariaceae	120.535	127.232	
32. <i>Dahlia pinnata</i> 2 Cavanilles	Asteraceae	117.283	89.130	
33. <i>Prunus americana</i> Marshall	Rosaceae	95.679	102.173	
34. <i>Dryopteris affinis</i> (Lowe) Fraser-Jenkins	Dryopteridaceae	91.049	78.260	
35. <i>Aesculus indica</i> (Wall. ex Cambess.) Hook.	Hippocastanaceae	106.481	102.173	
36. <i>Mentha piperita</i> 2 Linn.	Lamiaceae	102.071	103.603	
37. <i>Brassica juncea</i> (Linn.) Czernohorsky	Brassicaceae	105.029	117.117	
38. <i>Viburnum grandiflorum</i> Wall. ex DC.	Caprifoliaceae	112.426	128.378	
39. <i>Thuja occidentalis</i> Linn.	Cupressaceae	96.153	94.594	
Mean		95.473	98.547	
SD		13.939	13.133	
Mean-1SD		81.534	85.413	
Mean-1.5SD		74.565	78.847	
Mean-2SD		67.595	72.280	
Mean-2.5SD		60.626	65.713	

Table 4.1 gives the details about the percentage growth rate, compared to that of the control; stronger inhibitory activity in the radicle: *M-1(σ), ** M - 1.5(σ), ***M-2(σ).

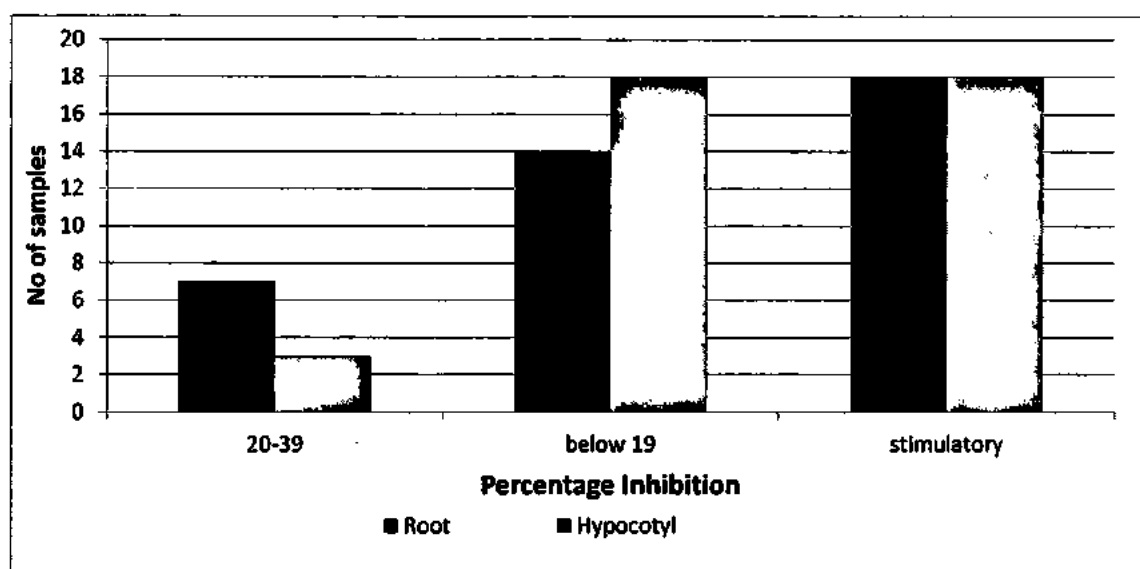


Figure 4.1: Comparison of Radicle and Hypocotyl growth inhibition in lettuce by Dish Pack method.

4.1. Description of Top Five Allelopathic Aromatic Plants

1. *Vicia sativa*

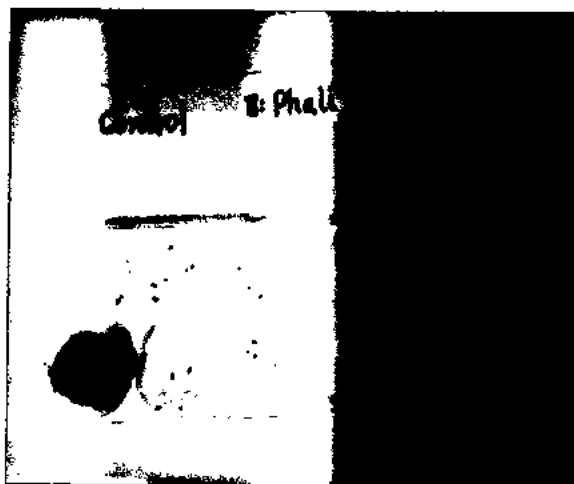
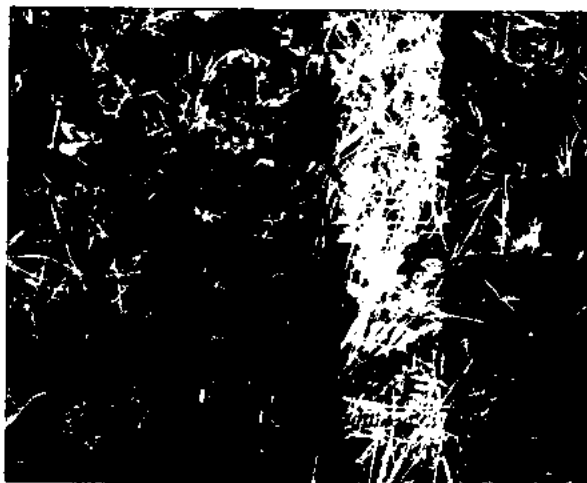


Figure 4.1a. *Vicia sativa*

Figure 4.1b. Experiment Picture

Common Name: Vetch

Local Name: Phali

Flowering Period: June – August

Habitat: Fields, yards, wasteland, with fodder crops etc.

Habit: Herb

Origin: It is native to Southern Europe and also cultivated in Mediterranean, west and central Asia, China, eastern Asia, India and also in USA in many temperate, subtropical and tropical regions.

Description:

Vicia sativa is an annual herb and it can grow up to 2 metres tall. Its stem is four-angled, it is sometimes hairy, can be branched and unbranched, can also be climbing or decumbent (trailing along the ground). Its leaves are composed of 3-8 pairs of opposite leaflet and a terminal 2-3 branched tendril that assists in the climbing habit. Flowers of this plant are papilionaceous (resembling the garden pea flower). They occur in the axils between the leaf stalk and the main stem, can be single or in clusters of up to 3. It has a fruit which is sub-cylindrical pod up to 7

cm long and contains up to 12 seeds. The seeds are round and can be black to brownish in colour.

Potential Uses:

It is widely cultivated for its high quality forage and grain for livestock. Common vetch is a valuable cover crop, it is also used as green manure. It helps with the suppression of spring weeds and is a beneficial cover crop in vineyards and orchards. Weed suppression is increased when the legume is associated with a cereal companion crop (Sattell *et al.*, 1998).

Environmental Importance:

Common vetch is a mostly grown as a forage crop and outside cultivation it is often considered as a weed. It belongs to the legume family. It can fix nitrogen from the air due to symbiotic relationship with bacteria in the root nodules. Common vetch is high in protein.

Its residues are succulent and decompose quickly. They act as green manure. The best stage is full bloom and residues are easily ploughed down with a disc harrow. Residues provide a moisture-conserving mulch in strip-tillage systems. Because of Nitrogen fixation, common vetch lowers the overall C: N ratio of mixed pasture and speeds up decomposition (Sattell *et al.*, 1998).

2. *Allium sativum*

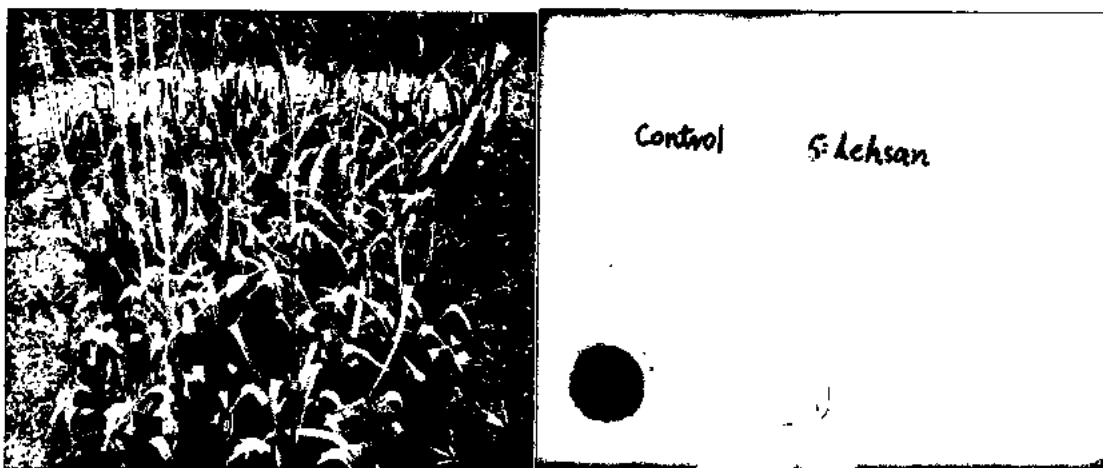


Figure 4.2a. *Allium sativum*

Figure 4.2b. Experiment Picture

Common Name: Garlic

Local Name: Thoom/Lehsan

Flowering Season: Its cloves come out in late autumn for an early summer crop. It can also be planted in early spring season but the production may decrease.

Habitat: Cultivated beds

Habit: Herb

Origin: Asia

Description:

It is a bulbous plant, can grow about 1.2 meter. It produces hermaphrodite flowers and its pollination is carried out by bees and some other insects.

Potential Uses

Its bulb can be used raw or cooked. It is used all over the world and mainly in southern Europe for the flavouring in a number of foods. Garlic is very nutritious and healthy addition to the diet but flavour is extremely strong so it is mostly used in low quantities for the flavour in salads and also in cooked foods. Its leaves can also be used in salads and can be cooked

also. It can be used for medical purposes as well, e.g. antiseptic, anti-inflammatory, tonic, anti-microbial etc.

3. *Rosa damascena*



Figure 4.3a. *Rosa damascena*

Figure 4.3b. Experiment Picture

Local Name: Gulaab

Common Name: Rose

Flowering Season: April – May

Origin: Middle East

Habit: Deciduous shrub

Habitat: This plant grows best at moderate temperatures with a flow of humid air during flowering.

Description:

It is a perennial plant and it can live as long as 50 years. Plant can reach the height of 2.5 to 3 meters. Its stems are provided with certain prickles of unequal size. Its leaf has 5 to 7 leaflets. Some parts are rich in vitamin C.

Potential Uses:

It is mostly used as an ornamental plant grown for flowers in the garden and indoors as well. It also has an important use for commercial production of perfumes and cut flower crops. It is also used as landscape plant and for the purposes like game cover and slope stabilization. It is used for several medicinal purposes also.

Environmental Importance:

Air filtration, anti-microbial, has a pleasant odour etc.

4. *Trifolium repens*

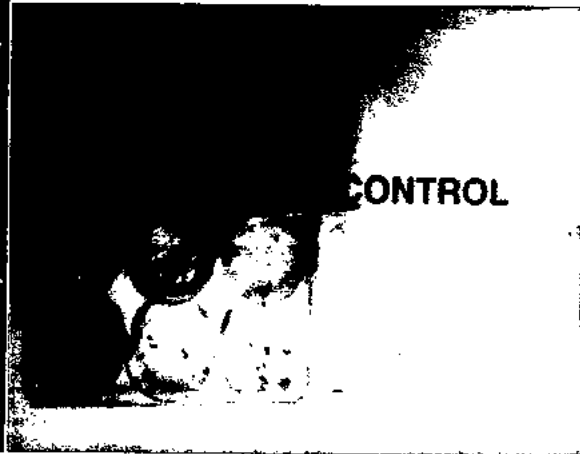


Figure 4.4a. *Trifolium repens*

Figure 4.4b. Experiment Picture

Common Name: White Clover

Local Name: Sree

Origin: Europe

Flowering Period: May to June

Habit: Herbaceous perennial

Habitat: Lawns, grassy areas, fields etc.

Description:

It is a dwarf and mat-forming perennial that mostly grows to 4 inches tall and spreads to 12 inches or more by stems which root along the ground at the nodes. It has trifoliate (3-parted), green leaves and the flowers are white that bloom in spring season. Its leaves and flowers appear on different stalks from the creeping stems. Its flowers are attractive to the bees. It is a nitrogen fixing plant and used in crop rotation.

Potential Uses:

It is use as a lawn substitute, in meadows etc. It is also added to grass seed for lawns or grassy areas where grass doesn't grow alone, used as forage also.

Environmental Importance:

This plant has the ability to compete with weeds. It is moderate during early but has improved ability with time. Also has good nitrogen fixing ability.

5. *Pinus wallichiana*

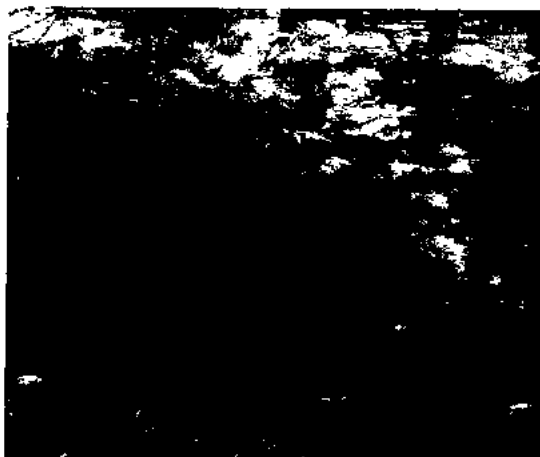


Figure 4.5a. *Pinus wallichiana*

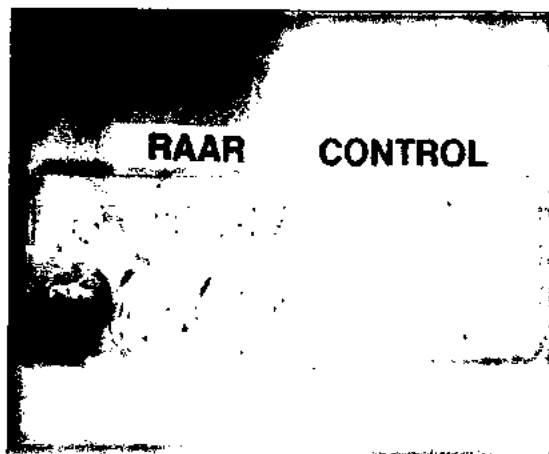


Figure 4.5b. Experiment Picture

Local Name: Raar

Common Name: Pine tree

Habit: Coniferous trees

Habitat: Mountain screes, Glacier forelands

Flowering Season: They are evergreen trees.

Origin: Himalayan Region

Description:

Pines are evergreen, coniferous resinous trees that can grow from 30-50 m tall. It mostly grows in mountain valleys at high altitudes. Temperate climates with dry winters and wet summers are favourable for this plant.

Potential Uses:

Its wood is hard, durable and resinous. It has a lot of commercial importance due to the utilization of its timber and wood pulp. Also used for commercial production of turpentine. Used for planting in parks and gardens. Its wood has a lot of use in high-value carpentry items, e.g. window frames, furniture, floors, roofing and panelling.

Environmental Importance:

Pine trees provide habitat and also food source wildlife. It has high resistance to air pollution and tolerates it much better than many other species.

5. Discussion and Conclusion

Discussion

Certain hindrance and stimulation is displayed by the allelopathic effect of the 39 aromatic plant species used in this research.

Inhibition in the radicle of lettuce was found, as a result of the allelopathic effect of 7 species from number 20 to 39 and 14 species out of 1-19 also caused inhibition in the radicle of the lettuce. Inhibition in the hypocotyl of lettuce was measured as a result of the effect of 3 species from number 20-39. From number 1-19, 18 species caused the inhibition in the hypocotyl of lettuce. From all 39 species, 18 were found to have stimulatory effect on the growth.

Species showing the strongest allelopathic effect on the growth of lettuce include *Vicia sativa* followed by *Allium sativum*, *Rosa damascena*, *Trifolium repens* and *Pinus wallichiana*.

In 2013, an experiment was performed in order to assess the allelopathic effect of water extracts of *Vicia sativa* on the germination and seedling growth of mungbean (*Vigna radiata*) and mashbean (*Vigna mungo*) at different concentrations viz. 0, 1, 2, 3 and 4%. The results of the experiment showed that the water extracts of *Vicia sativa* inhibited almost all of the germination and seedling growth properties of these crops at certain concentrations compared to the control. Lowest germination percentage, germination index, root and shoot length and seedling dry weight of both crops was observed because of the effect of water extract of *Vicia sativa* at 4% concentration. Its effect was approximately same with the concentration of extract at 3%. Presence of Ferulic acid, 4-Hydroxy-3-Methoxybenzoic acid and p-Coumaric acid was found after water extracts were analyzed. This study shows that *Vicia sativa* has an inhibitory influence on the germination and seedling growth of mungbean and mashbean which occurs as

a result of the presence of phenolics. So, *Vicia sativa* can decrease the production of crops (Zohaib *et al.*, 2014).

The essential oil of *Rosa damascena* petals was evaluated in the research to find out its antibacterial effects against three strains of *Xanthomonas axonopodis*. The essential oil of *Rosa damascena* showed certain antibacterial properties and it may be a potential control agent in the management of the disease caused in tomato and pepper plants (Basim and Basim, 2003).

Garlic (*Allium sativum*) stalk is a by-product and the waste of garlic production but now it is considered as a useful resource. It is important to utilize this resource in an effective manner to reduce the environmental pollution and also for the improvement of sustainable agriculture. The allelopathy of decomposed garlic stalk was investigated in a research at certain ratios (0:100, 1:100, 3:100 and 5:100) using lettuce as the allelopathic test plant. According to the results of the research, it was found that lower concentration improved lettuce plant growth and increase in the concentration reduced the growth. The highest ratio of stalk to soil (5:100) significantly improved the root length and fresh weight of lettuce but a reduction was found in the shoot length and fresh weight. Lower ratio of stalk to soil caused an increase in protein and chlorophyll content and the activity of plant enzymes. Increase in the concentration resulted in an increase in the activity of soil urease and sucrose. This research serves as a scientific basis for allelopathic study of decomposed garlic stalk and its application (Han *et al.*, 2013).

In order to assess the allelopathic activity of *Trifolium repens* on grasses, the effects on the germination rate, germination energy, germination index, vigor index, shoot height, root height, dry weight, fresh weight of *Lolium perenne*, *Festuca rubra*, *Festuca arundinacea*, *Poa pratensis* and *Agrostis stolonifera* from the decomposed liquids from *Trifolium repens* were studied in this research. Bio-assay results showed that the decomposed liquids resulted in an improvement in both seed germination and seedling growth of all test turf grasses at low

concentration (0.011 g·mL⁻¹) and significantly inhibited the growth at high concentration (0.090 g·mL⁻¹) (P<0.05). The decomposed liquids decreased the seed germination of turf grasses and inhibited the growth of seedling. Presence of phenolics in the decomposed liquids was found after the analysis of allelochemicals. The study shows that the decomposed liquids of *Trifolium repens* have strong allelopathic potential and inhibit the normal growth of turf grasses. Allelopathy of decomposition is one of the reasons due to which *Trifolium repens* invades the turf grass (Liang *et al.*, 2011).

Aqueous methanol extracts from pine needles caused an inhibition in the growth of roots and shoots of *Lepidium sativum*, *Lactuca sativa*, *Medicago sativa*, *Lolium multiflorum*, *Pheleum pratense*, *Digitaria sanguinalis* and *Echinochloa crus-galli*. Increase in the concentration caused an inhibition which suggests that the pine needles have certain allelopathic properties and have growth inhibitory substances. The aqueous methanol extract was purified and an inhibitory substance was isolated and determined by spectral data as 9 α , 13 β -epidioxyabeit-8(14) en-18-oic acid. This substance resulted in the inhibition of root and shoot growth of cress and *Echinochloa crus-galli* seedlings at concentrations more than 0.1 mM. The endogenous concentration of the substance was 0.13 mol/kg pine needle. The results from the research suggest that 9 α , 13 β -epidioxyabeit-8(14) en-18-oic may have a contribution to the inhibitory effect of the pine needles and may have a significant role in the allelopathy of pine (Kato *et al.*, 2009).

The results of this study agree with the previous studies where these species were found to have allelopathic effects on the growth of test species. Some of the effects are concentration dependent. This study found out *Vicia sativa* having an inhibitory effect on the growth of lettuce which agrees with the study performed by (Zohaib *et al.*, 2014), where water soluble phenolics of *Vicia sativa* caused inhibition on the germination and seedling growth of pulse

crops. Results also agree with the findings of allelopathic effects of decomposed garlic (*Allium sativum*) stalk on lettuce, where an increase in concentration resulted in an increase in inhibition (Han *et al.*, 2013). Moreover, the results also agree with the research on decomposed liquids of *Trifolium repens* inhibited the growth of turf grasses (Liang *et al.*, 2011). This study also shows similarity with the findings of the study on pine needles that caused inhibition in the root growth of certain plant species due to presence of allelochemicals (Kato *et al.*, 2009).

Conclusion

According to results obtained from this research, it can be concluded that the tested plant species have certain allelopathic properties. The interactions between the allelochemicals of leaf leachates of the plant species and test species (lettuce) revealed that some species have strong inhibitory effect on the growth, i.e. they cause reduction in radicle and hypocotyl elongation of lettuce and some species increased the growth rate because of their stimulatory effect. It was evident from the results that *Vicia sativa* (common vetch) has the strongest inhibitory potential among all the tested species. Most of the species already have certain uses e.g. sources of food, ornamental uses, few are used for their medicinal properties, timber wood etc. This study suggests that the aromatic plant species may be used for the biological weed control because of their strong inhibitory effects and also the utilization of certain species as a catalyst to improve the crop production due to their positive effects on growth. Along with this, certain species may also be used as natural medicines because of strong anti-microbial properties. It may also be concluded that further researches are required on their mechanism of action and separation, purification and identification of bioactive substances to improve the possibilities of using them effectively as bio herbicides and growth enhancers and also for other suitable purposes in the future.

References

- Aliotta, G., Cafiero, G., & Otero, A. M. (2006). Weed germination, seedling growth and their lesson for allelopathy in agriculture. In *Allelopathy* (pp. 285-297). Springer Netherlands.
- Bais, H. P., R. Fall and J. M. Vivanco. (2004 a). Biocontrol of *Bacillus subtilis* against infection of *Arabidopsis* roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. *Plant Physiol.*, 134:307-319.
- Basim, E., & Basim, H. (2003). Antibacterial activity of *Rosa damascena* essential oil. *Fitoterapia*, 74(4), 394-396.
- Blum, U. (1998). Effects of microbial utilization of phenolic acids and their phenolic acid breakdown products on allelopathic interactions. *Journal of Chemical Ecology*, 24(4), 685-708.
- Blum, U., King, L. D., & Brownie, C. (2002). Effects of wheat residues on dicotyledonous weed emergence in a simulated no-till system. *Allelopathy Journal*, 9(2), 159-176.
- Blum, U., Shafer, S. R., & Lehman, M. E. (1999). Evidence for inhibitory allelopathic interactions involving phenolic acids in field soils: concepts vs. an experimental model. *Critical Reviews in Plant Sciences*, 18(5), 673-693.
- Brethour, C., & Weersink, A. (2001). An economic evaluation of the environmental benefits from pesticide reduction. *Agricultural Economics*, 25(2-3), 219-226.
- Chon, S. U., & Kim, J. D. (2002). Biological activity and quantification of suspected allelochemicals from alfalfa plant parts. *Journal of Agronomy and Crop Science*, 188(4), 281-285.
- Chou, C. H. (1999). Roles of allelopathy in plant biodiversity and sustainable agriculture. *Critical Reviews in Plant Sciences*, 18(5), 609-636.
- Çirak, C., Radušienė, J., & Arslan, B. (2008). Variation of bioactive substances in *Hypericum montbretii* during plant growth. *Natural product research*, 22(3), 246-252.
- Duke, S. O., Dayan, F. E., Romagni, J. G., & Rimando, A. M. (2000). Natural products as sources of herbicides: current status and future trends. *Weed Research-Oxford*, 40(1), 99-112.
- Duke, S. O., Romagni, J. G., & Dayan, F. E. (2000). Natural products as sources for new mechanisms of herbicidal action. *Crop Protection*, 19(8), 583-589.
- Einhellig, F. A. (1989). Interactive effects of allelochemicals and environmental stress. *Phytochemical Ecology: allelochemicals, mycotoxins and insect pheromones and allomones*, 9, 101-117.
- Einhellig, F. A. (1995). Mechanism of action of allelochemicals in allelopathy.
- Einhellig, F. A. (1996). Interactions involving allelopathy in cropping systems. *Agronomy Journal*, 88(6), 886-893.
- Einhellig, F. A., Putnam, A. R., & Tang, C. S. (1986). Mechanisms and modes of action of allelochemicals. *The science of allelopathy*, 171-188.
- Einhellig, F. A., Reigosa, M. J., & Pedrol, N. (2002). The physiology of allelochemical action: clues and views. *Allelopathy: from molecules to ecosystems*, 1-23.

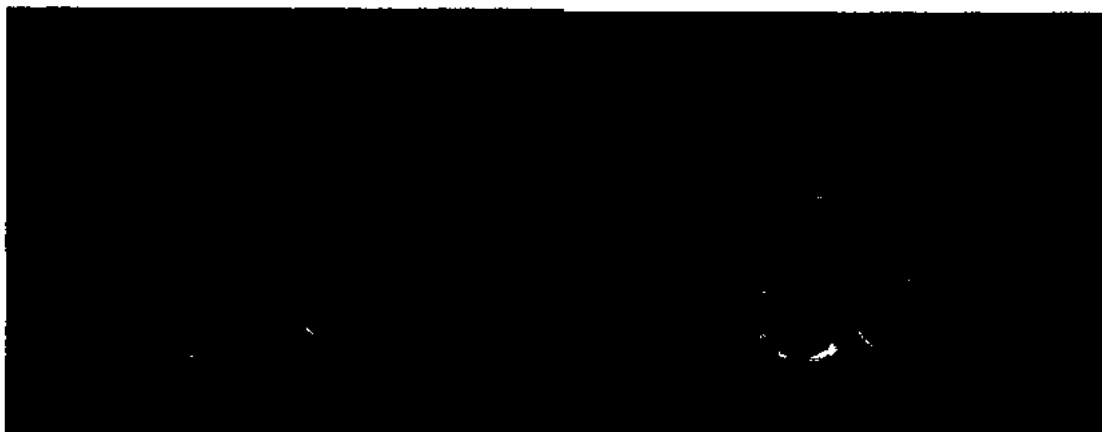
- El-Darier, S. M., & Tammam, A. M. (2009). Potentially Phytotoxic Effects and Oxidative Stress of *Achillea santolina* L. on two Economically Important Field Crops in Egypt. In *International Conference on Plants and Environmental Pollution (ICPEP)* (pp. 6-11).
- Everts, K. L. (2002). Reduced fungicide applications and host resistance for managing three diseases in pumpkin grown on a no-till cover crop. *Plant Dis.*, 86:1134-1141.
- Foy, C. L. Inderjit. (2001). Understanding the role of allelopathy in weed interference and declining plant diversity. *Weed Technol.*, 15, 873-878.
- Fujii, Y. (1992). The potential biological control of paddy weeds with allelopathy: Allelopathic effect of some rice varieties. In *Proceedings International Symposium on Biological Control and Integrated Management of Paddy and Aquatic Weeds in Asia* (pp. 305-320).
- Fujii, Y., Shibuya, T., Nakatani, K., Itani, T., Hiradate, S., & Parvez, M. M. (2004). Assessment method for allelopathic effect from leaf litter leachates. *Weed Biology and Management*, 4(1), 19-23.
- Gniazdowska, A., & Bogatek, R. (2005). Allelopathic interactions between plants. Multi-site action of allelochemicals. *Acta Physiologiae Plantarum*, 27(3), 395-407.
- Gómez-Rodríguez, O., Zavaleta-Mejía, E., González-Hernández, V. A., Livera-Munoz, M., & Cárdenas-Soriano, E. (2003). Allelopathy and microclimatic modification of intercropping with marigold on tomato early blight disease development. *Field Crops Research*, 83(1), 27-34.
- Gonzalez, V. M., Kazimir, J., Nimbai, C., Weston, L. A., & Cheniae, G. M. (1997). Inhibition of a photosystem II electron transfer reaction by the natural product sorgoleone. *Journal of Agricultural and Food Chemistry*, 45(4), 1415-1421.
- Hall, M. H., & Henderlong, P. R. (1989). Alfalfa autotoxic fraction characterization and initial separation. *Crop science*, 29(2), 425-428.
- Han, X., Cheng, Z. H., Meng, H. W., Yang, X. L., & Ahmad, I. M. R. A. N. (2013). Allelopathic effect of decomposed garlic (*Allium sativum* L.) stalk on lettuce (*L. sativa* var. *crispa* L.). *Pakistan Journal of Botany*, 45, 225-233.
- Huang, H. C., & C. H. Chou. (2005). Impact of plant disease biocontrol and allelopathy on biodiversity and agricultural sustainability. *Pl. Pathol. Bull.*, 14:1-12.
- Inderjit, & Dakshini, K. M. M. (1994). Allelopathic effect of *Pluchea lanceolata* (Asteraceae) on characteristics of four soils and tomato and mustard growth. *American Journal of Botany*, 799-804.
- Inderjit, D. SO, (2003). Ecophysiological aspects of allelopathy. *Planta*, 217(4), 529-539.
- Inderjit, K., & Keating, L. (1999). Allelopathy: principal and practice. John promises for biological control in: advance in agronomy, (eds). Sparks D. *Academic Press*, 67, 141-231.
- Jefferson, L. V., & Pennacchio, M. (2003). Allelopathic effects of foliage extracts from four Chenopodiaceae species on seed germination. *Journal of Arid Environments*, 55(2), 275-285.
- Kato-Noguchi, H., Fushimi, Y., & Shigemori, H. (2009). An allelopathic substance in red pine needles (*Pinus densiflora*). *Journal of plant physiology*, 166(4), 442-446.

- Khan, Z. R., Midega, C. A., Bruce, T. J., Hooper, A. M., & Pickett, J. A. (2010). Exploiting phytochemicals for developing a 'push-pull' crop protection strategy for cereal farmers in Africa. *Journal of Experimental Botany*, **61**(15), 4185-4196.
- Kobayashi, K. (2004). Factors affecting phytotoxic activity of allelochemicals in soil. *Weed biology and management*, **4**(1), 1-7.
- Leather, G. R., & Einhellig, F. A. (1988). Bioassay of naturally occurring allelochemicals for phytotoxicity. *Journal of Chemical Ecology*, **14**(10), 1821-1828.
- Leme, D. M., & Marin-Morales, M. A. (2008). Chromosome aberration and micronucleus frequencies in *Allium cepa* cells exposed to petroleum polluted water—a case study. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, **650**(1), 80-86.
- Li, H. H., Inoue, M., Nishimura, H., Mizutani, J., & Tsuzuki, E. (1993). Interactions of trans-cinnamic acid, its related phenolic allelochemicals, and abscisic acid in seedling growth and seed germination of lettuce. *Journal of chemical ecology*, **19**(8), 1775-1787.
- Li, Q., Yuan, L., & Huang, J. (2014). Allelopathic effects of artemisinin on ectomycorrhizal fungal isolates in vitro. *Pedobiologia*, **57**(4), 271-276.
- Liang, J., Cheng, Z. H., Xu, P., & Meng, Q. L. (2011). Allelopathy of *Trifolium repens* Decomposed Liquids on Turfgrasses [J]. *Acta Agrestia Sinica*, **2**, 015.
- Macías, F. A., Castellano, D., & Molinillo, J. M. (2000). Search for a standard phytotoxic bioassay for allelochemicals. Selection of standard target species. *Journal of agricultural and food chemistry*, **48**(6), 2512-2521.
- Macías, F. A., López, A., Varela, R. M., Torres, A., & Molinillo, J. M. (2004). Bioactive apocarotenoids annuionones F and G: structural revision of annuionones A, B and E. *Phytochemistry*, **65**(22), 3057-3063.
- Macías, F. A., Varela, R. M., Torres, A., Galindo, J. L., & Molinillo, J. M. (2002). Allelochemicals from sunflowers: chemistry, bioactivity and applications. In *Chemical Ecology of Plants: Allelopathy in Aquatic and Terrestrial Ecosystems* (pp. 73-87). Birkhäuser Basel.
- McLaughlin, J. C., & Smith, S. M. (1994). Metabolic regulation of glyoxylate-cycle enzyme synthesis in detached cucumber cotyledons and protoplasts. *Planta*, **195**(1), 22-28.
- Muscolo, A., Panuccio, M. R., & Sidari, M. (2001). The effect of phenols on respiratory enzymes in seed germination. *Plant Growth Regulation*, **35**(1), 31-35.
- Padhy, B., Patnaik, P. K., & Tripathy, A. K. (2000). Allelopathic potential of Eucalyptus leaf litter leachates on germination and seedling growth of finger millet. *Allelopathy Journal*, **7**(1), 69-78.
- Peterson, G. S., Kandil, M. A., Abdallah, M. D., & Farag, A. A. A. (1989). Isolation and characterisation of biologically-active compounds from some plant extracts. *Pesticide science*, **25**(4), 337-342.
- Popa, V. I., Dumitru, M., Volf, I., & Anghel, N. (2008). Lignin and polyphenols as allelochemicals. *Industrial crops and products*, **27**(2), 144-149.
- Putnam, A. R., Weston, L. A., & Tang, C. S. (1986). Adverse impacts of allelopathy in agricultural systems. *The science of allelopathy*, 43-56.

- Qasem, J. R., & Foy, C. L. (2001). Weed allelopathy, its ecological impacts and future prospects: a review. *Journal of crop production*, 4(2), 43-119.
- Rice, E.L. (1984). *Allelopathy*. Academic Press, London.
- Rizvi, S. J. H., Haque, H., Singh, V. K., & Rizvi, V. (1992). A discipline called allelopathy. In *Allelopathy* (pp. 1-10). Springer Netherlands.
- Sampietro, D. A., Sgariglia, M. A., Soberón, J. R., Quiroga, E. N., & Vattuone, M. A. (2007). Role of sugarcane straw allelochemicals in the growth suppression of arrowleaf sida. *Environmental and experimental botany*, 60(3), 495-503.
- Sattell, R., Dick, R., Luna, J., McGrath, D. M., & Peachey, R. E. (1998). *Common vetch (Vicia sativa L.)*. Corvallis, Or. Extension Service, Oregon State University.
- Singh, A. K., Kumar, P., Nidhi, R., & Gade, R. M. (2012). Allelopathy-A Sustainable Alternative and Eco-Friendly Tool for Plant Disease Management. *J. Plant Disease Sci*, 7(2), 127-134.
- Singh, H. P., Batish, D. R., & Kohli, R. K. (2002). Allelopathic effect of two volatile monoterpenes against bill goat weed (*Ageratum conyzoides L.*). *Crop protection*, 21(4), 347-350.
- Smirnoff, W. A. and Hutchinson, P. M. (1965). Bacteriostatic and bacteriocidal effects of extracts of foliage from various plant species on *Bacillus thuringiensis var thuringiensis* Berliner. *J. Invert. Pathol.* 7: 273 -280.
- Stickney, J. S., & Hoy, P. R. (1881). Toxic action of black walnut. *Trans. Wis. State Hort. Soc*, 11, 166-167.
- Taylor, J. L. S., & Van Staden, J. (2001). The effect of age, season and growth conditions on anti-inflammatory activity in *Eucomis autumnalis* (Mill.) Chitt. Plant extracts. *Plant Growth Regulation*, 34(1), 39-47.
- Verma, H. N., & Baranwal, V. K. (1983). Antiviral activity and the physical properties of the leaf extract of *Chenopodium ambrosoides L.* *Proceedings: Plant Sciences*, 92(6), 461-465.
- Wardle, D. A., Nicholson, K. S., & Rahman, A. (1993). Influence of plant age on the allelopathic potential of nodding thistle (*Carduus nutans L.*) against pasture grasses and legumes. *Weed Research*, 33(1), 69-78.
- Weidenhamer, J. D., Hartnett, D. C., & 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., & Vivanco, J. M. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. *Current opinion in plant biology*, 7(4), 472-479.
- Weston, L. A. (1996). Utilization of allelopathy for weed management in agroecosystems. *Agronomy Journal*, 88(6), 860-866.
- Weston, L. A. (2000). Are laboratory bioassays for allelopathy suitable for prediction of field responses? *Journal of Chemical Ecology*, 26(9), 2111-2118.
- Weston, L. A., & Duke, S. O. (2003). Weed and crop allelopathy. *Critical Reviews in Plant Sciences*, 22(3-4), 367-389.
- Willis, R. J. (1985). The historical bases of the concept of allelopathy. *Journal of the History of Biology*, 18(1), 71-102.

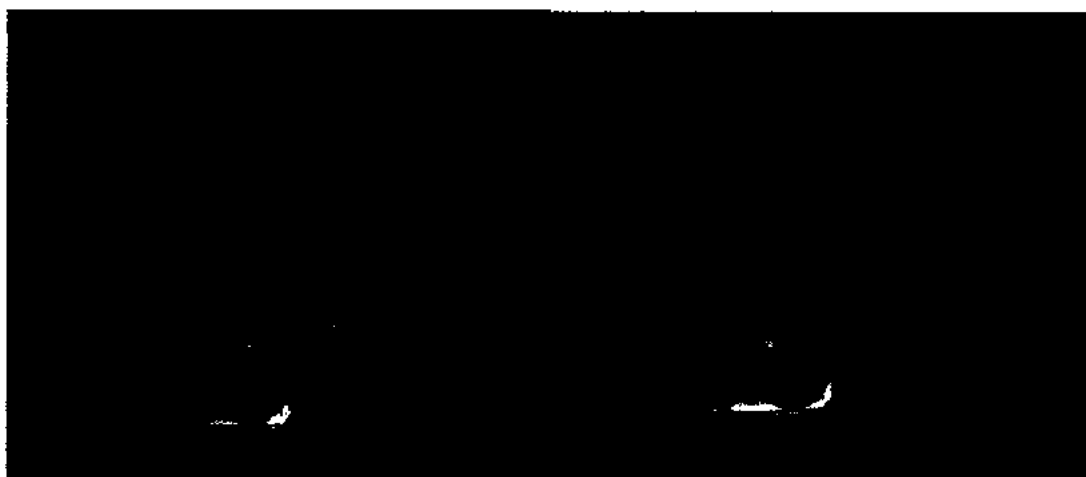
- Willis, R. J. (1997). The history of allelopathy. 2. The second phase (1900–1920), the era of SU Pickering and the USDA Bureau of soils. *Allelopathy Journal*, 4(1), 7-55.
- Wink, M., Schmeller, T., & Latz-Brüning, B. (1998). Modes of action of allelochemical alkaloids: interaction with neuroreceptors, DNA, and other molecular targets. *Journal of Chemical Ecology*, 24(11), 1881-1937.
- Wu, H., Pratley, J., Lemerle, D., & Haig, T. (2000). Laboratory screening for allelopathic potential of wheat (*Triticum aestivum*) accessions against annual ryegrass (*Lolium rigidum*). *Crop and Pasture Science*, 51(2), 259-266.
- Xuan, T. D., Shinkichi, T., Khanh, T. D., & Chung, I. M. (2005). Biological control of weeds and plant pathogens in paddy rice by exploiting plant allelopathy: an overview. *Crop Protection*, 24(3), 197-206.
- Xuan, T. D., Tawata, S., Khanh, T. D., & Chung, I. M. (2005). Decomposition of allelopathic plants in soil. *Journal of agronomy and crop science*, 191(3), 162-171.
- Yang, X., Van der Kraan, P. M., Bian, Z., Fan, M., Walboomers, X. F., & Jansen, J. A. (2009). Mineralized tissue formation by BMP2-transfected pulp stem cells. *Journal of dental research*, 88(11), 1020-1025.
- Zohaib, A., Ehsanullah, T. T., Abbas, T., & Rasool, T. (2014). Influence of water soluble phenolics of *Vicia sativa* L. on germination and seedling growth of pulse crops. *Scientia*, 8(3), 148-151.

Annexures:



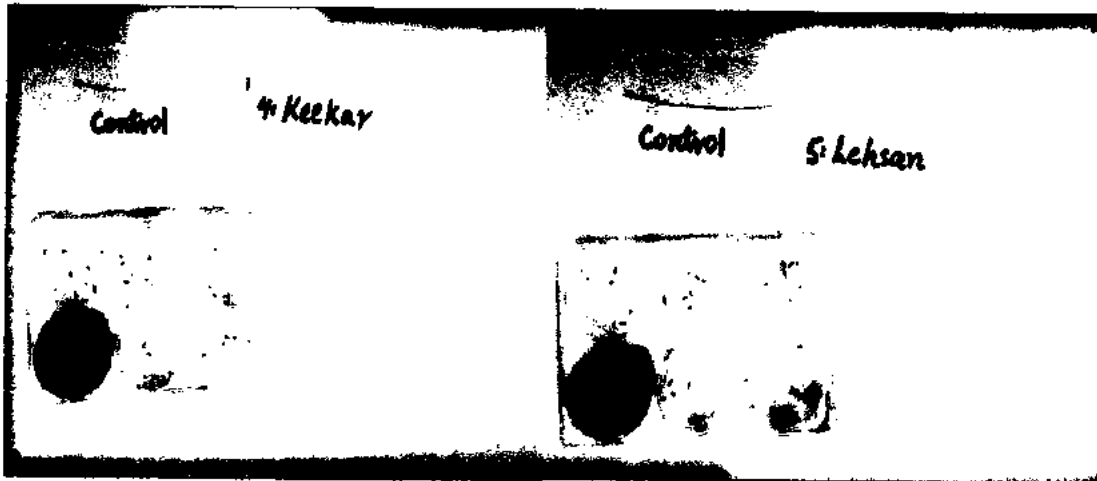
Control

Rosa damascena



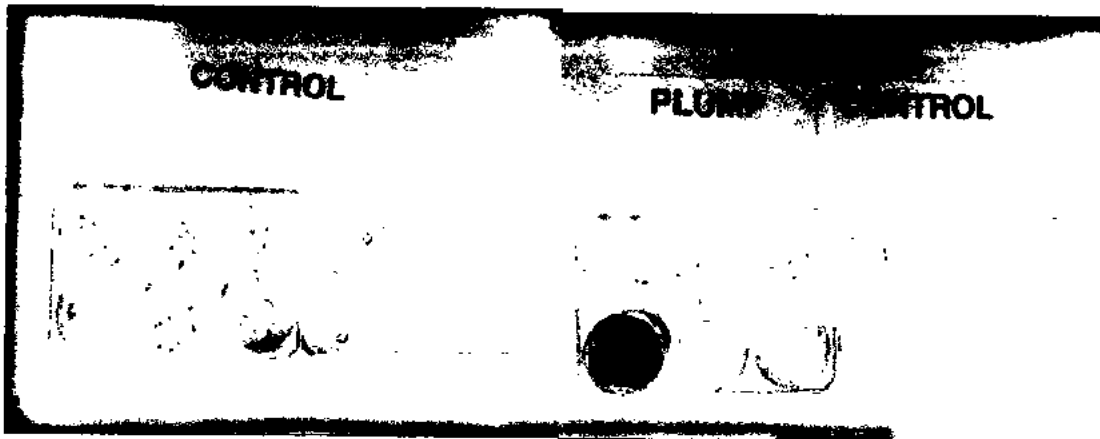
Ranunculus muricatus

Vicia sativa



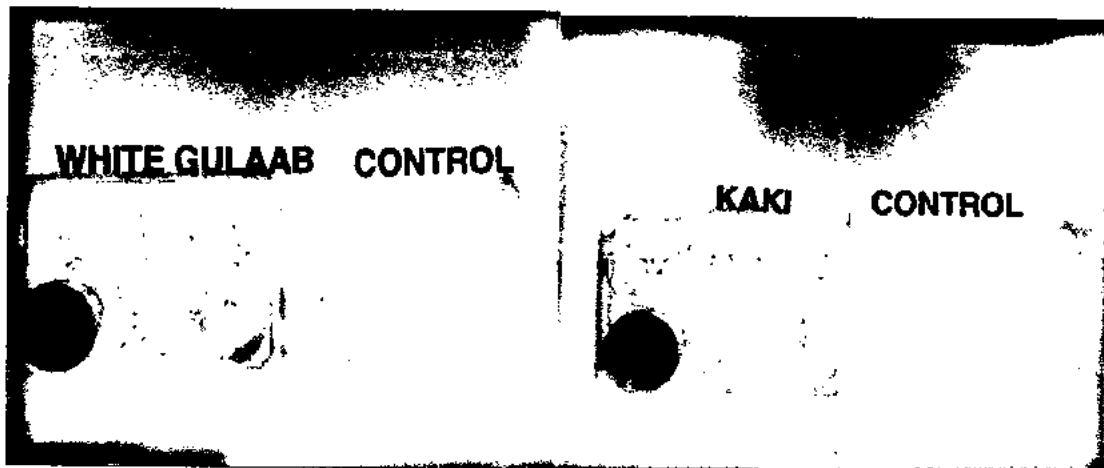
Robinia pseudoacacia

Allium sativum



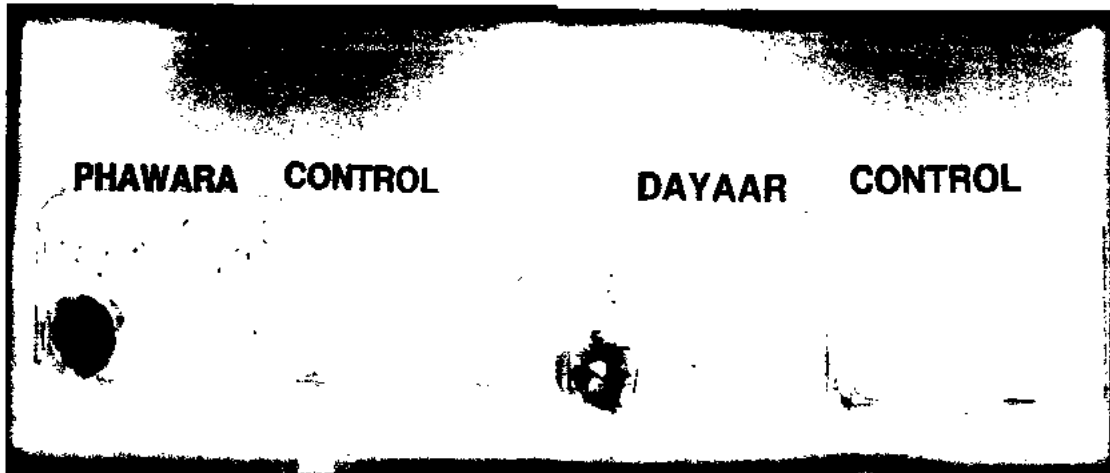
Control

Prunus domestica



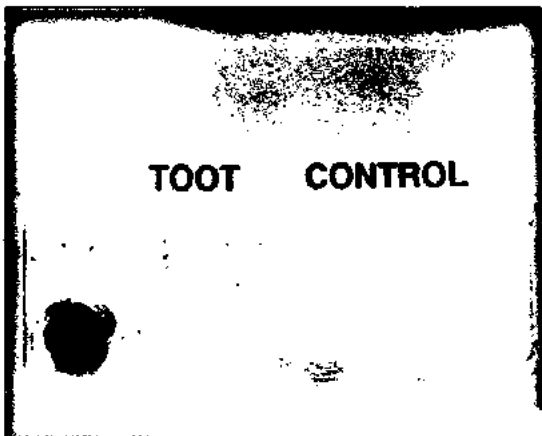
Rosa brunonii

Dryopteris stewartii

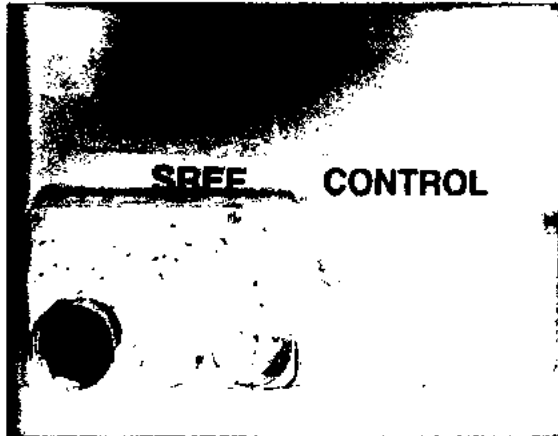


Ficus palmata

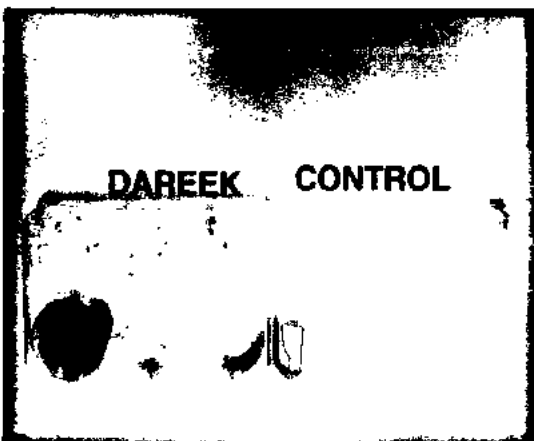
Cedrus deodara



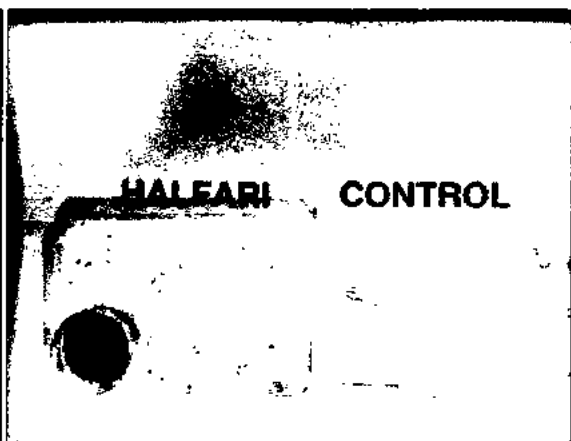
Morus alba



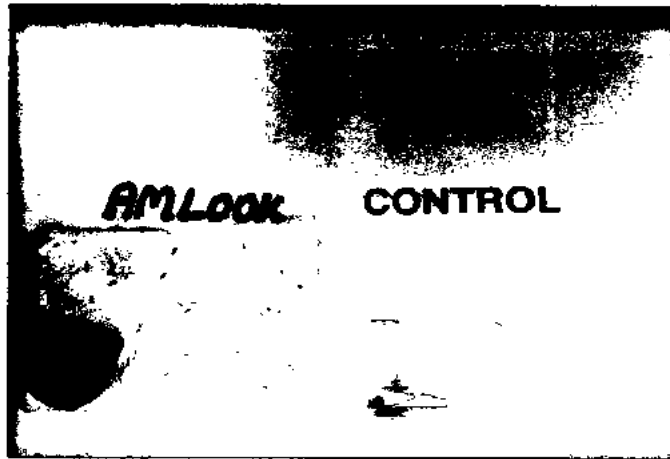
Trifolium repens



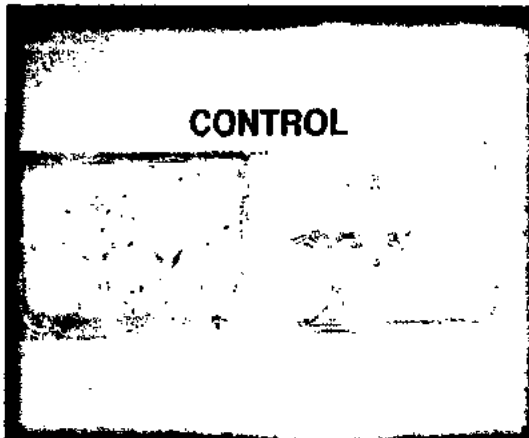
Melia azedarach



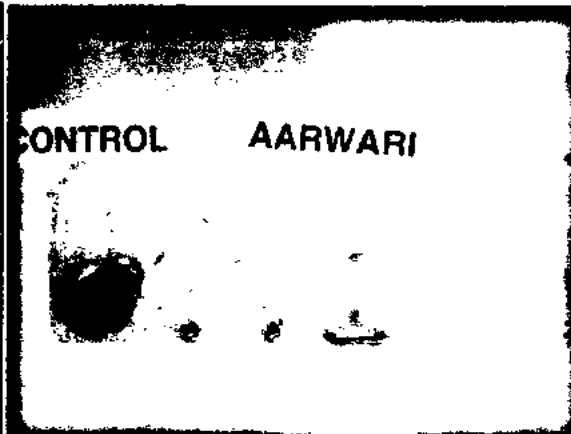
Rumex acetosa



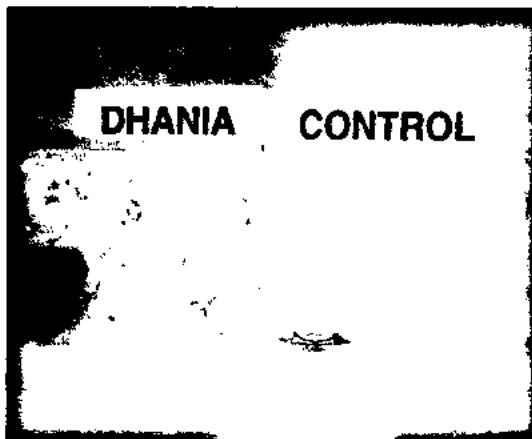
Diospyros virginiana



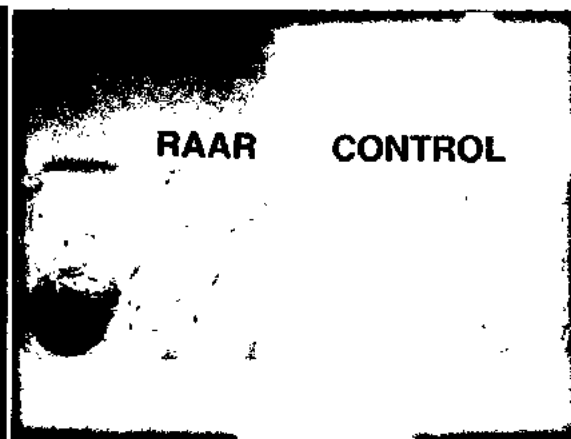
Control



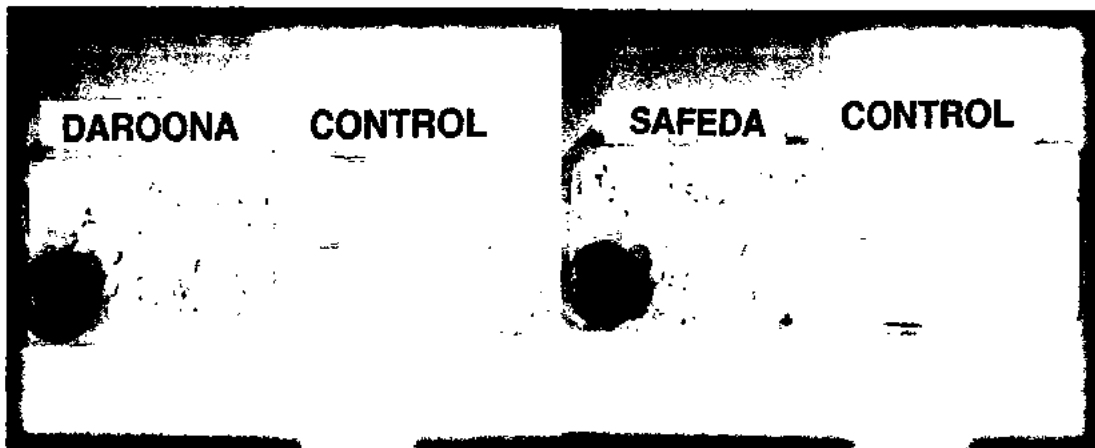
Prunus persica



Coriandrum sativum

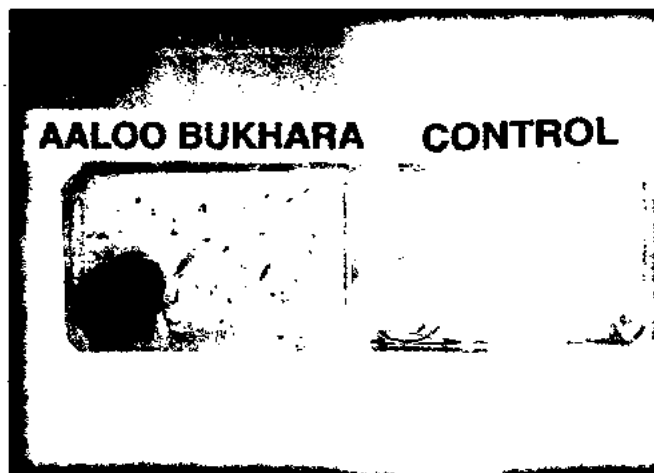


Pinus wallichiana

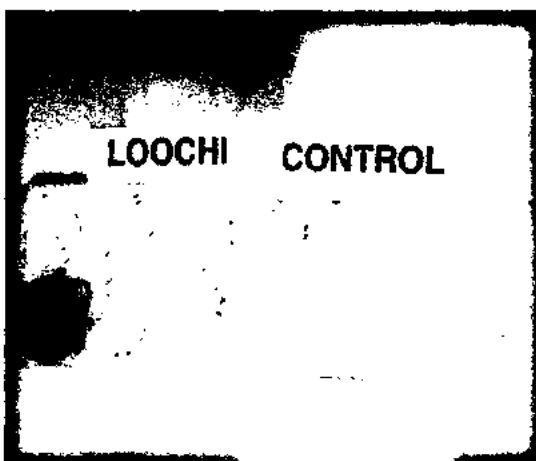


Punica granatum

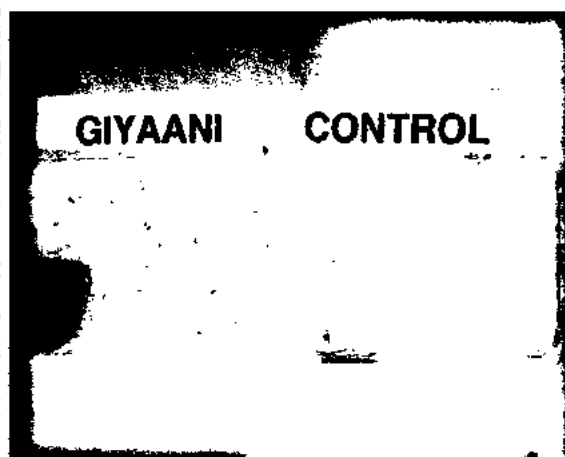
Populus nigra



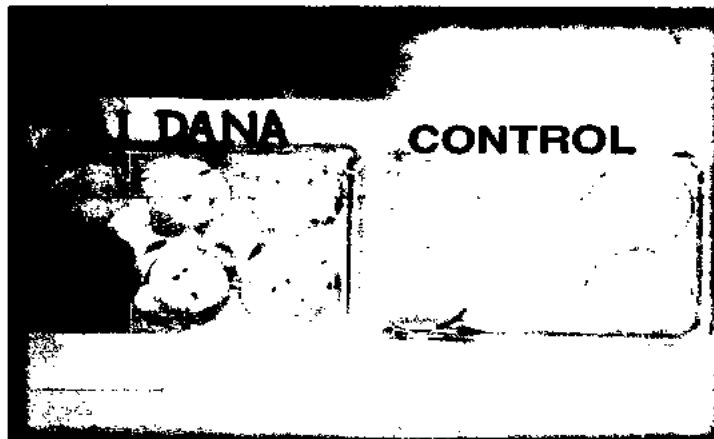
Prunus domestica subsp. Pomariorum



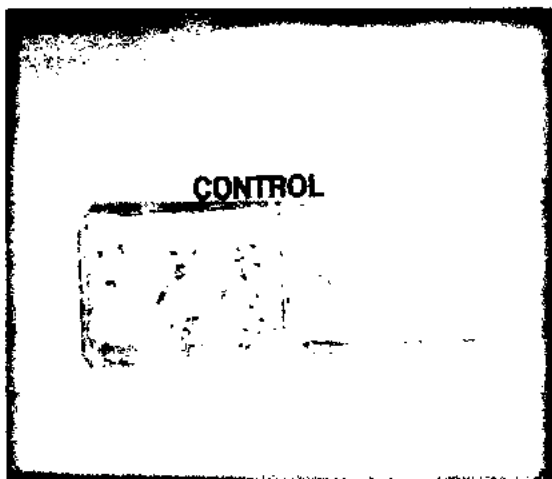
Prunus avium



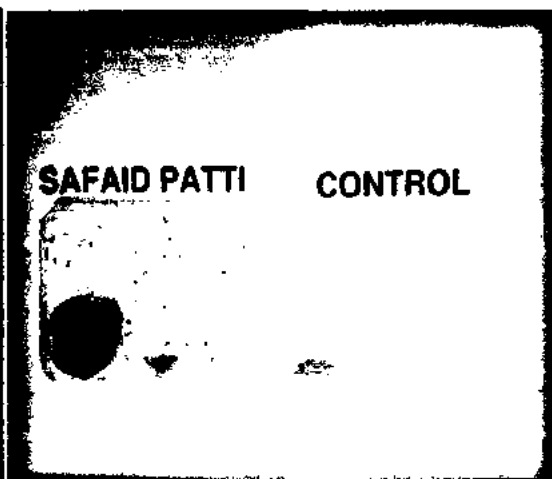
Elaeagnus umbellata



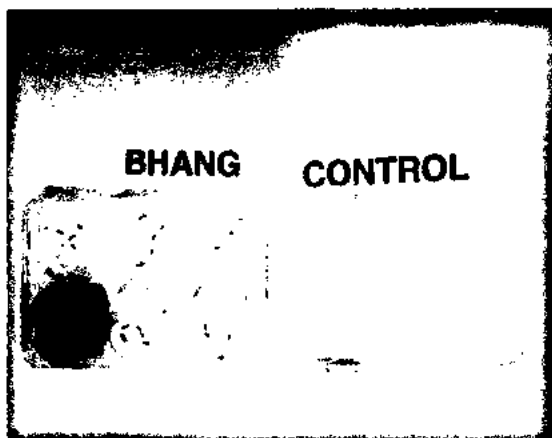
Pseudocydonia sinensis



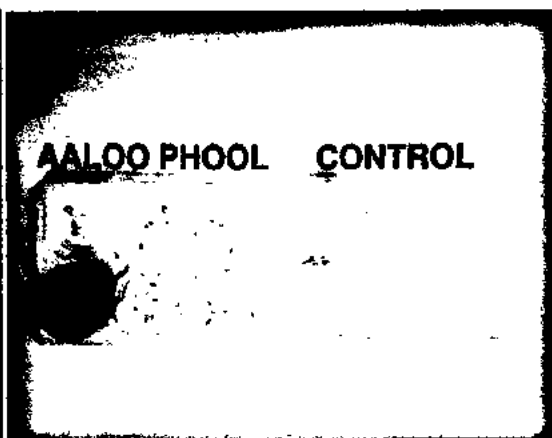
Control



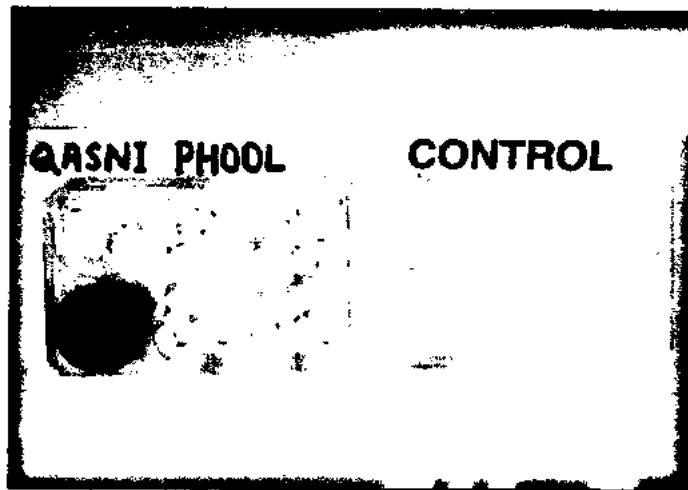
Hibiscus syriacus



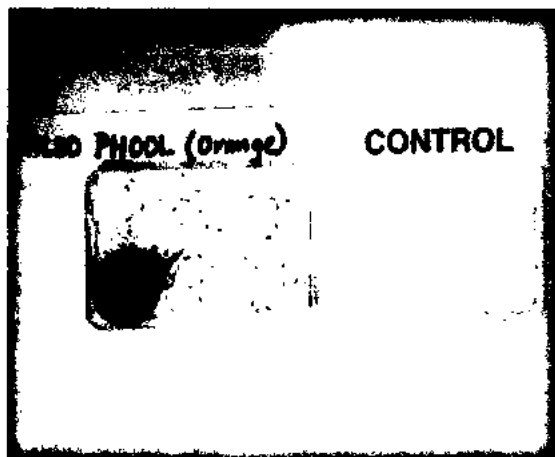
Cannabis sativa



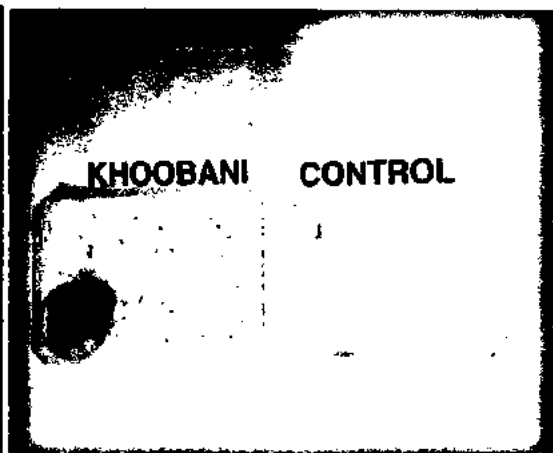
Dahlia pinnata 1



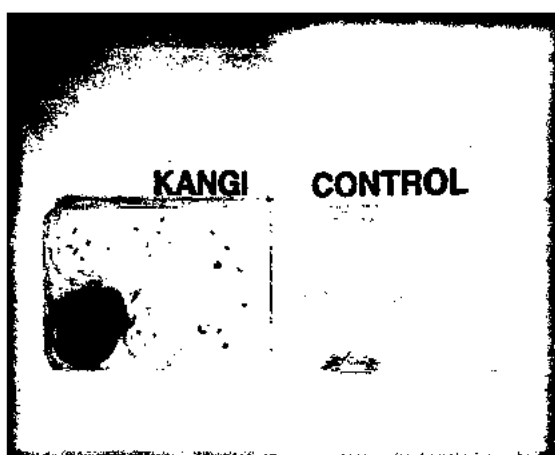
Antirrhinum majus



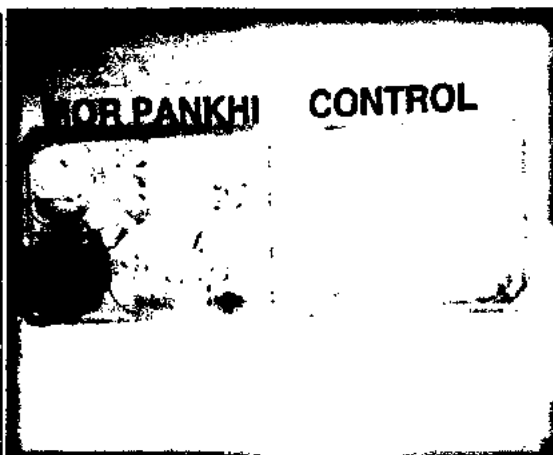
Dahlia pinnata 2



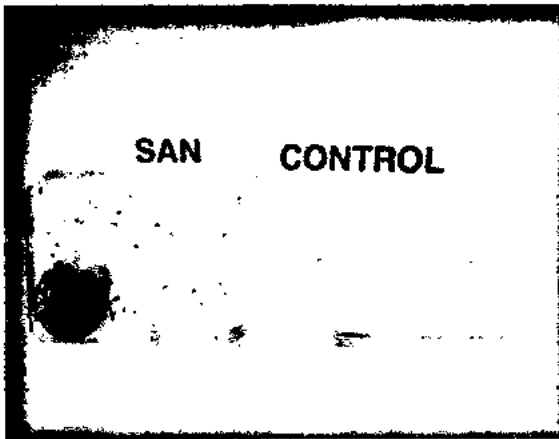
Prunus americana



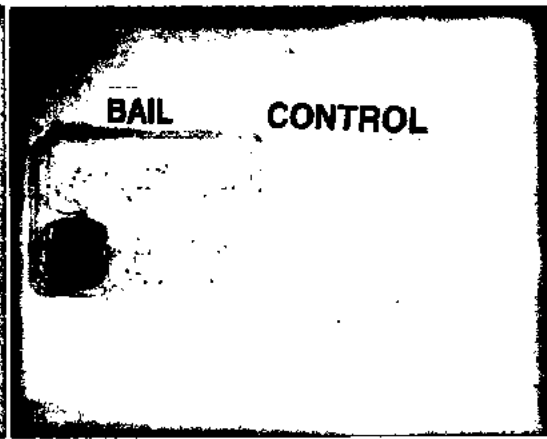
Dryopteris affinis



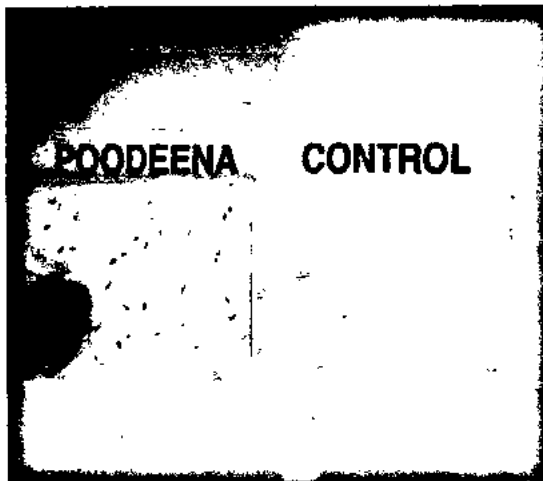
Thuja occidentalis



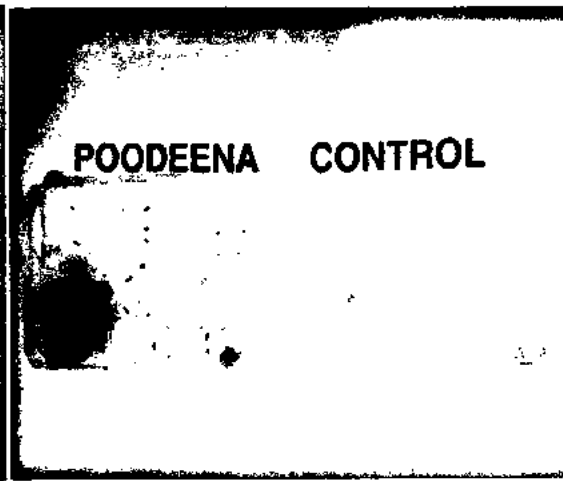
Aesculus indica



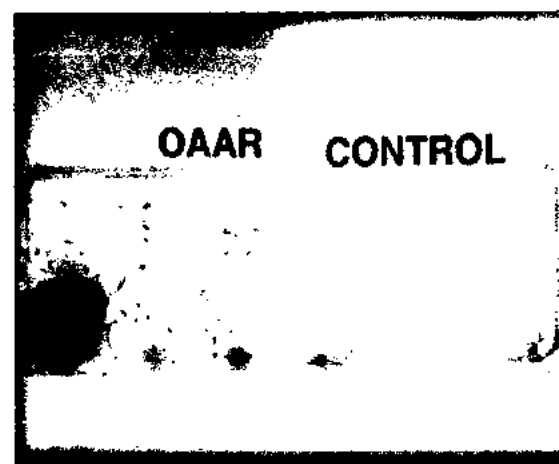
Camphis grandiflora



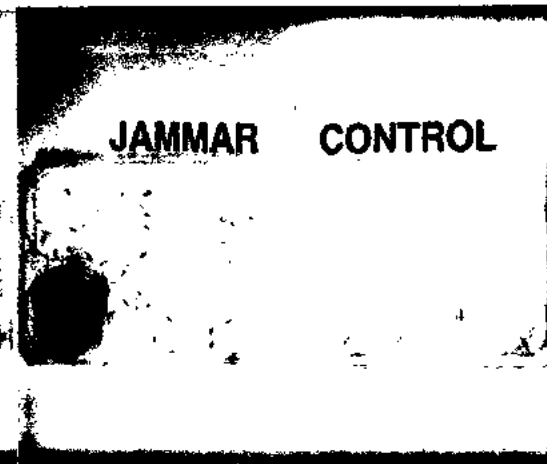
Mentha piperita 1



Mentha piperita 2



Brassica juncea



Viburnum grandiflorum