1. INTRODUCTION

1.1 Background

Known with various names such as false ragweed, Star weed, Bitter weed, White top, Bastard feverfew etc., *Parthenium hysterophorus* L. is an annual weed found in tropical and subtropical regions (Tiwari *et al.*, 2005). The weed is likely to be mistaken with a number of other introduced weeds from the family including seedlings of cobbler's pegs, leaves of *Artemisia* spp, flowers of bishop's weed (*Ammi majus*) and hemlock (*Conium maculatum*) and the ragweed (*Ambrosia* spp) (Agriculture & Resource Management Council of Australia & New Zealand, 2000).

The noxious weed is an aggressive colonizer that colonizes in disturbed, bare areas along roadsides, heavily stocked areas around yards and watering points (Facts, 2004), in degraded natural ecosystem as well as in fallow lands and around settlements (Tiwari *et al.*, 2005). It has been identified as the native to the subtropics of both North and South America. The weed is now widely distributed in a number of tropical and subtropical countries like Australia, India, China, and Kenya (Navie *et al*, 1996; Facts, 2004). Its occurrence was first reported in Nepal in 1982 by Hara *et al.* (Tiwari *et al.*, 2005). A report suggested that the weed has been finding its place in Katmandu Valley from the year 1986 (Mishra, 1991; Adhikari and Tiwari, 2004).

The weed has been found toxic to human as well as cattle. Some of the report suggested that regular contact with the plant or its pollen could cause dermatitis, hay fever, and even asthma in humans (Bhowmik *et al*, 2007). It is also reported to have adverse effects to livestock and cattle and even can cause death if consumed in significant amount (CRC, 2003). The *Parthenium* weed is reported to have different kind of allelopathic impacts on different plants. Different biochemical studies of the plant suggested the presence of an active chemical '*Parthenin*', a sesquiterpene lactone (see photo plate- II) which is responsible for allergic reactions and strong allelopathy of the weed (Bhowmik *et al*, 2007). The allelochemicals released from the plant inhibits the germination and growth of pasture grasses, legumes, cereals, vegetables, other weeds, and even trees (Agriculture & Resource

Management Council of Australia & New Zealand, 2000). Its residues in soil atmosphere creates problem in reliable production and pasture establishment (Annonymous, 2004). Reduced pasture production by excluding beneficial forage plants, resulting in a monoculture; the weed causes heavy loss with estimated cost $ 16 million per year in Australia (Agriculture & Resource Management Council of Australia & New Zealand, 2000). This problematic weed, however, is reported to be replaced effectively by *Cassia uniflora* - a leguminous under shrub in some part of Karnataka and Maharashtra states of India as claimed by Joshi (1991).

The weed has not been used for any purpose in Nepal (Tiwari *et al.,* 2005). However, the weed is reported as good source of biogas and also can be used as green manure. Whole plant extracts can be used as flea repellant and also as herbicide (Agriculture & Resource Management Council of Australia & New Zealand, 2000). In addition, its pesticidal potential has been established in terms of ovicidal, anti-fleedant, and nemato-cidal effects (Datta and Saxena, 2001). Despite of being toxic its industrial uses are also accountable (Sastri and Kavathekar 1990). Interestingly, its medicinal properties have been reported as a remedy against hepatic amoebiasis (Sharma and Bhutani, 1988). This weed has also been used as folk medicines in the Caribbean and Central America (Navie *et al.* 1996).

Allelopathy generally refers to the bio chemical interaction between and among plant species. The interaction generally takes place by their soil released diffusates. Both positive as well as negative allelopathic interactions are possible among plants. The weed *Parthenium* is reported to exert negative allelopathic influence to its neighbouring plant species. Therefore, much attention has been paid to the allelopathic properties of this weed. Researches are being carried out worldwide in search of suitable control and management system. The general control methods like manual plucking, fencing, burning etc. have been found not much effective. Besides use of chemical herbicide to control it has a number of side effects on neighboring flora in the community. As an alternate, it would appear that certain plant essential oils could be effective in its control through inhibition / suppression of the viability / germinability of larger population of the seeds produced by *Parthenium hysterophorus* L. The present investigation has been carried out to elaborate the allelopathic

effects of this noxious weed in inhibition of seed germination of fast germinating plant species such as radish (*Raphanus sativus* L.) and to study the inhibitory potential of some plant essential oils, extracted from easily available aromatic plant species at the local level, in germination inhibition of the seeds of weed in question.

1.2 Objectives

a. Broader Objectives:

i) To study the allelopathic effects / interactions of *Parthenium* against seed germination of *Raphanus sativus* L.

ii) To study the seed germination behavior of *Parthenium* weed as affected by some plant essential oils.

b. Specific Objectives

1) To analyze the effect of seed and plant diffusates on seed germination of radish (*Raphanus sativus* L.).

2) To analyze the effect of seed and plant diffusates in germinating radish seeds as indicated by the level of glucose.

3) To analyze the effects of essential oils of *Cymbopogon citratus* (DC) Stapf., *Eucalyptus citriodara* Hook. and *Cinnamomum camphora* L. on seed germination and seedling growth of *Parthenium hysterophorus* L.

4) To find out an effective concentration of the essential oils for inhibiting *Parthenium* seed germination.

1.3 Hypothesis

a) Plant diffusates and seed diffusates from *Parthenium* is effective to inhibit the *Raphanus* seed germination.

b) The essential oils from *Cymbopogon citratus* (DC) Stapf., *Eucalyptus citriodara* Hook. and *Cinnamomum camphora* L. inhibit the seed germination and seedling length of *Parthenium.*

1.2 Rationale

Parthenium weed is a vigorous species that colonizes weak pastures with spares ground cover. The weed has prolific seed producing ability (15,000/ plant) and fast spreading in nature and therefore can colonize around the agricultural fields too (Agriculture & Resource Management Council of Australia & New Zealand, 2000). Although it is a problematic weed in Australia, India, Nepal and other subcontinents, it may become more prominent in other parts of the world in near future. Limited control practices are available for managing the species in various environments (Bhowmik *et al*, 2007). The weed is now spreading very rapidly in Kathmandu valley as well as in central Terai of Nepal where many of the fertile agricultural lands are under heavy infestation and still others are under threat of infestation. The weed is reported to cause Sevier crop loss infesting highly fertile land in central Queensland and its potential to infest Nepalese agricultural land therefore cannot be underestimated. In Nepal the weed seems to be infesting the maize field, rice field, wheat field and other crop fields. Farmers with their domestic cattle are experiencing various health hazards from the weed and therefore responding it as useless, harmful and an undesirable one. Therefore the high risk of heavy crop loss and other socio economic impact from the weed is ultimate if no proper control measure has been practiced. The findings of the study might be helpful in practicing the control strategy for the weed.

1.5 Limitations

Due to time and economic factors, the study suffers from following limitations.

- The study was carried out only for 12 months under limited laboratory resources of Central Department of Botany.
- The whole research was conducted in laboratory and field application was not trialed.
- Necessary chemical characterization of plant diffusates, seed diffusates and essential oils through GC – MS or any other way (s) could not be accomplished.
- Lots of political hindrance influenced the ease of the study.

2. LITERATURE REVIEW

There are numerous literatures about the plant *Parthenium hysterophorus* L. Various researches are being conducted world wide in different aspects of the weed. However no much works are conducted in Nepal. Hence only few Nepalese research papers were available for review. Some of the National and International literatures that were reviewed are as follows:

2.1 Weed status, Life cycle and Control

Haseler (1976) studied the seed nature and mode of dispersal of the weed *Parthenium* and proposed that the weed has prolific seed producing ability and can produce large amount of seeds (upto100, 000 per plant).

Navie *et al.* (1998) studied the nature of the seed, its germination, and its dispersal including their world wide distribution, ecology and allelopathy. They suggested 74% seeds viability after 2 years burial and predicted the half life of the seed to be about 6 yrs. They also reported that seed germination is dependent on high moisture and is inhibited by shading, plant competition or burial.

Agriculture & Resource Management Council of Australia and New-Zealand, Australian & New Zealand Environment & Conservation Council and Forestry Ministers, (2000) proposed a strategic plan for the *Parthenium hysterophorus* in response to growing community concern regarding the spread of the weed, both within Queensland and interstate and the effects of the weed in production areas, on the environment and on human health. The strategy was aimed for preventing its spread in new areas, raising awareness and commitment, reduce the impact and coordinate management at national level. The literature highlights the history of spreads, biology of the weed including its Phenology, uses, impacts on the productivity of cattle and crops, health hazards to human and cattle. Besides, the plan also superfluities different control measures including pasture management, bio control applied till date along with the socio economic factors affecting management.

CRC (2003) proposed the weed as highly problematic with its impact in rangelands and summer cropping areas of Queensland where it seriously affected the pastoral industry, costing farmers and grazers over $ 22 million a year in reduced production and management costs. The report suggests that more than 340 million *Parthenium* seeds can be present per hectare in the surface soil, compared to native 120,000 native grass seeds. The mode of seed dispersal by vehicles, machinery and animals, and pasture seed, stock feed and water is also explained. The weed is explained to be highly competitive one that can displace the neighbouring flora and responsible for high degree of allelopathy towards neighbouring crops decreasing their productivity. The report also highlights different control measures including mechanical control such as fencing, burning, overgrazing control, pasture spelling etc., biological control and chemical (Herbicide) control methods applied till date.

Facts (2004) explained *Parthenium* as vigorously spreading weed that colonises weak pastures with sparse ground cover. The report suggests that the weed will readily colonise disturbed, bare areas along roadsides and heavily stocked areas around yards and watering points. The weed is explained to produce flowers and seeds throughout the year throughout its life and dies around the late autumn. Buried seeds last longer than the seed on soil surface with 50 percent viability even after the six years of burial at 5 cm below the surface. The life cycle of the weed is explained as: *Most germination is reported to occur during spring to early summer. Seedling growth takes place after one-two weeks which are fast in spring/summer and slow in winter. The mature plant will arise within/after weeks to the germination. Flowering occurs after the maturation. The flower lasts for 2-3 months, unless curtailed by frost and drought*. The same report further suggests that the weed costs cropping industries several million dollars per year. Besides, the origin of the weed, its spread and different control measures including biological controls by different pests and rust fungi are also explained.

Joshi *et al.* (2005) studied reproductive efficiency and biomass allocation of *Parthenium* weed at eight different places of Kathmandu valley. They reported that the weed is occurring with an average density 11-47 plants m^{-2} having maximum density in Chovar. On the same study it is also reported that the vegetative allocation of the weed is comparatively higher

than that of the reproductive allocation in all study sites with the average reproductive allocation ranging from 0.69-2.15 g.

Tiwari *et al.* (2005) described the weed as only species in Nepal under genus *Parthenium* growing within the range of 75-1350 m. They mentioned that the weed is reported for the first time in Nepal by *Hara et al.,* 1982 and explained the first herbarium record at Trisuli, 2000 ft. on date 6 July, 1967. The literature highlights the ecological characters of the weed including allelopathy and distribution. In the report the weed is suspected to introduce in Nepal via India two decades ago and has spread rapidly along roadsides, fallow lands and agricultural lands. The report explained the profuse occurrence of the weed in central and western Nepal being not so common in Jhapa district of Eastern Nepal.

Bhowmik *et al.*, 2007 mentioned the general account of the weed in a review paper. They focused on the weed *Parthenium* as an invasive species having adverse impact on crop production, animal husbandry and human health. They also explained the potential allelopathic hazards from the weed on developing countries like Nepal. The literature also highlights the current chemical, biological and other management practices applied till date.

2.2 Botany of *Parthenium* weed

Lindley (1838) in *Flora Medica* describes the plant as follows: "*The whole plant is bitter and strong-scented, reckoned tonic, stimulating and anti-hysteric. It was once a popular remedy in ague. Its odour is said to be peculiarly disagree to bees and that insects may be easily kept at a distance by carrying a handful of the flower heads.*"

Grierson and Long (2001), Facts (2004) explained the weed as a member of Asteraceae family belonging to the genus *Parthenium*. It is an annual weed with deep tap root and erect stem that becomes woody with age. It is highly branched with maximum branching at its top half and may reach to a height of two meters. The leaves are pale-green ranging in size from 5-15 x 0.4-5cm, deeply lobed, pubescent, acute with decurrent base; petiole 3-4 cm. long. Inflorescence a lax panicle of numerous small heads; peduncles 4-10mm. long. Heads radiate, 4-8mm. across. Receptacle convex, small. Involucres campanulate. Involucral bracts green, 5, elliptic to ovate, acute, 3-4 x 1-2mm., pubescent. Ray florets 5, with 2 seriate disc

florets on either side; corolla white to light yellow, ovate, orbicular, bilobed, obtuse. Style 1mm. long with stout, obtuse stigma. Disc florets many; corolla light yellow, narrowly campanulate, 1.5-2mm.long, 5 lobed ovate- acute. Stamens 5, anthers linear. Style 1-5mm. puberulous, entire. Achenes flattened, 2mm long. Papus of 2 broad, strongly reflected awns, 0.3-0.5mm long, puberulous along the side.

2.3 Allelopathic Impacts

Allelopathy concerns the effects of one plant on another (including microorganisms) due to chemicals released by them, or due to the breakdown of their metabolites (Wills, 1994). Many studies have been conducted in regard to the allelopathy of the weed *Parthenium*. The weed is reported to have inhibitory effects on neighbouring herbaceous vegetation. The allelopathic effect of the weed is well established in the early growth and physiology of *Ageratum conizoides*, different *Brassica sp, Eragrostis tef*, grain sorghum, many agricultural crops, legumes and crucifers. Phytotoxicity of the weed in a number of agricultural and non agricultural plants including many aquatic species has also been explained. A number of water- soluble allelochemicals including phenolic acids and Sesquiterpenes has been identified from the weed. Among them parthenin, caffeic acid and ρ-coumeric acid were isolated as the chief components from the root exudates and leaves of the plant. Many studies suggested these chemicals as the most responsible factors for high degree of allelopathy of the weed. Furthermore, many reports explained *C. uniflora*, as one of the solution for minimizing *Parthenium* infestation. The phenolic leachates from different parts of *C. uniflora*, especially from germinating seeds significantly inhibited the germination of *Parthenium* seeds and retarded the growth of their seedlings. Some of the researches that have conducted to establish the allelopathic nature of the weed were reviewed and summerised below.

Kanchan (1975) studied the different types of growth inhibitors from the weed *Parthenium* and reported that a number of compounds including water- soluble allelochemicals- phenolic acids and Sesquiterpene lactones.

Kanchan (1978) studied the agro physiological impact due to allelopathic influence of *Parthenium* and reported that the different allelochemicals from the weed has negative impact on neighboring herbaceous vegetation.

Kanchan and Jayachandra (1979, 1980 a) - studied the type of allelochemicals present in root exudates and other plant parts like leaves. They reported the higher concentrations of parthenin, caffeic acid and ρ-coumeric acid as chief components on root than in leaves.

Nath (1981) studied the effect of *Parthenium* weed extracts from its different parts on seed germination and growth of many crops. He reported the inhibitory effect of the extracts on most of the crops especially during germination and seedling development.

Picman and Picman (1984) suggested in their report that in *P. hysterophorus*, the water soluble plant metabolites play an important role not only in allelopathy and defense against herbivorous predators and diseases but also as autotoxins in population regulation and the timing of the germination processes.

Rice (1984) had studied the effects of most of the organic compounds of allelopathic nature in different concentrations. He mentioned that these organic compounds that are inhibitory at some concentrations are stimulatory to the same processes in very small concentrations.

Jarvis *et al*. (1985) studied The allelopathic potential of *Parthenium* through the release of phytotoxic substances such as, ferulic, caffeic, vanillic, chlorogenic, *p*-coumaric and *p*-hydroxybenzoic acids, parthenin, ambrosin and coronopilin and found their inhibiting property in the germination and growth of several crop plants and multi-purpose trees.

Shrivastava *et al.* (1985) studied the effect of *Parthenium* extracts on the seed germination and seedling growth of barley, pea and wheat. They discovered that aqueous extracts of leaves and inflorescences from the weed inhibited the germination and seedling growth of these crops but the extracts from roots and stems were less inhibitory.

Patil & Hedge (1988) isolated and purified parthenin from leaves of *P. hysterophorus* and studied its allelopathic and cytotoxic effects against wheat. They demonstrated that this

compound significantly decreased the germination of wheat seeds and adversely affected seedling growth.

Joshi (1991) studied the effects of *Cassia uniflora*, a leguminous undershrub on the *Parthenium hysterophorus* and found that the phenolic leachates from different part of the plant are effective to inhibit the seedling growth of *Parthenium*.

Bhatt *et al.* (1994) investigated a more cryptic effect of *Parthenium* extracts and found that almost 90 % pollen sterility could be induced in radish (*Raphanus sativus* L.) when seeds were pre - treated in varying concentrations and then subsequently field sown.

Mehta *et al.* (1995) studied the effect of foliar leachates from *Parthenium hysterophorus* in pollen tetrad and pollen sterility in Radish (*Raphanus sativus*). They reported significant reduction in pollen germination or pollen tube formation of the crop in the presence of different concentrations of the leachates.

Pandey (1996) studied the phytotoxicity of Sesquiterpene lactone, from the weed *Parthenium*, towards the different aquatic plant species and suggested high degree of potential to influence aquatic ecosystem thereby inhibiting the growth and development of different algae and other aquatic flora.

Adkins and Sowrby (1996) studied the allelopathic impact of the weed *Parthenium* in the neighboring herbs and shrubs plant and found that the weed is able to replace the nearby floral species thereby checking the growth and development in considerable extent.

Beekman *et al.* (1997) studied the Structure-cytotoxicity relationship of some Phelenanolide-type Sesquiterpenes. They found that these compounds exhibit specific structure-activity relationship at low concentrations and are very toxic to living cells.

Oudhia *et al.* (1999) studied the allelopathic effects of weeds on germination and seedling vigor of hybrid rice. They found that the different aqueous extract from the weeds *Parthenium hysterophorus* and *Lantana camara* produced negative as well as positive allelopathic responses on germination and seedling vigor of rice seeds.

Dayan *et al.* (1999) studied several biologically active compounds separately. They reported in their study about the phytotoxic potential of Sesquiterpene lactones which inhibited the germination growth and development of many neighbouring plants at low concentration.

Tefera (2002) studied Allelopathic effects of *Parthenium hysterophorus* extracts on seed germination and seedling growth of *Eragrostis tef*. He found that aqueous extracts from stem, root and flower have stimulatory effect on shoot length at all concentration levels. He also proposed that the leaf extract has inhibitory effect on the tef- seed germination process.

Tamado *et al.* (2002) studied the interference by the weed *Parthenium hysterophorus* with grain sorghum in terms of weed density and competition duration. They reported severe yield loss by the weed density and duration of competition, peaking at 97 %.

Singh *et al.* (2002) studied the effect of Parthenin from the *Parthenium* weed on the germination of *Ageratum conizoides* and reported that very low concentration of the chemical can inhibit the germination and seedling development of the plant.

Batish *et al.* (2005) studied the phytotoxic effect of the *Parthenium hysterophorus* residues on three *Brassica* species and found that the residues from the weed can exert an allelopathic influence on the early growth of the *Brassica* crops by releasing the water soluble phenolics into the soil.

Regina *et al.* (2007) investigated the involvement of Parthenin in overall phytotoxicity of decomposing leaf material in a South African population of *P. hysterophorus* and suggested that the release of Parthenin during decomposition of leaf material has a potential to play a leading role for allelopathy in *P. hysterophorus*; however, its significance in a natural setting will very much rely on the amount of leaf material accumulated on soil surfaces and the concentration of Parthenin in residues.

2.4 Essential oils as Herbicide

Essential oils of aromatic plants are being explored to find out possible herbicides. Lots of researches are in progress. Some the literatures reviewed as per objective of this study.

Isman (2000) studied the utility of essential oils of aromatic plants and analyzed their possible herbicidal activities. He mentioned that these essential oils could be the good herbicides since they do not persist in soil or contaminate ground water and causes little or no mammalian toxicity.

Tworkoski (2002) studied the different essential oils as herbicides and reported that these oils can also be used as viable weed control technology under organic farming systems. He also reported that these essential oils in proper concentrations are able to suppress the germination and seedling development of different agricultural herbs.

Fandohan *et al.* (2004) studied the effect of different essential oils on the growth of *Fusarium verticillioides* and also analyzed the major compounds present in the oils they used. They reported that the essential oil from *Cymbopogon citratus* contains 16 compounds including citral (neral and geranial) (47%) and myrcene (28%). Similarly oil from *Eucalyptus citriodara* contains 15 compounds citronellal being the chief compound (66%).

Singh, *et al*. (2005) studied the effect of volatile oils from *Eucalyptus citriodora* on seed germination of *Parthenium* weed and concluded that the oil at very low concentration has weed suppressing ability and checks the germination and seedling development of the weed.

2.5 Glucose – Test

Somogyi (1951) described two copper reagents for quantitative determination of sugar, the one for colorimetric and the for iodometric methods. He found both of these reagents appropriate for the determination of sugar content in biological samples.

Nelson (1944) described the photometric method for the estimation of glucose with copper reagents and Arsenomolybdate reagents. He found that the optical density of the colour developed is proportional to the glucose taken and is stable over a long period of time.

3. MATERIALS AND METHODS

There are two aspects of the present allelopathic interaction studies. The first one is to analyse the effect of different *Parthenium* diffusates on Radish seed germination and the other is to analyse the effect of essential oils on *Parthenium* seed germination. The materials used and the methods adopted are described bellow with appropriate sub-headings.

3.1 Source of seeds

Parthenium plant sets seed throughout the year that ripens within a few weeks time. The seeds were collected at local level in fallow field condition in the month of August. The well dried fruits enclosing seeds were harvested by simple hand picking carefully covering hands with gloves, nose with mask and eyes with the sunglass so as to avoid the risk of allergy. The seeds were then sun / shed dried for about a weak. After proper drying of the seeds it was beaten gently with the help of a stick to separate the dried fruits from the plants. The fruits were winnowed gently and the healthy seeds were obtained, cleaned for dusts, and stored in cool / dry place.

Seeds of radish (*Raphanus sativus* L.) var. Thulo Mula, belonging to previous harvest, was obtained from the Kalimati vegetable market in Kathmandu. The seeds used in experiments were manually selected for similarity for healthiness.

3.2 Extract Preparations

Different extracts were prepared following separate methods and used in experiments. The methods applied, to obtain these extracts in laboratory, are summarized below.

3.2.1 Plant Diffusates (PD)

The rhizospheric soil, 15 cm below the surface, from pure stand of flowering maturity of *Parthenium* bush, was collected in the evening of a hot sunny day with no rain fall in the week or so. The soil sample (10 gm.) was steeped for 24 hrs in distilled water (100 ml.) at 25^0C (ambient) and filtered. Double filtration was carried out; the first through clean muslin cloth and the second using Whatman No.1filter paper. The obtained filtrate (85 ml

approximate) was considered as the 100% PD and used as stock. The stock was then diluted with distilled water to prepare the different concentrations ranging from 20%-100%. These concentrations were used in the experiments.

3.2.2 Seed Diffusates (SD)

The seed diffusates was obtained from plain agar (2 %) plate planted with *Parthenium* seeds. The *Parthenium* seeds were imbibed for 36 hrs in distilled water before being sown into the plates. Each of the plate (9 cm dia.) contained 50 seeds at equidistance. The plates were incubated at 25 ºC and left for a week. Moisture (2 ml sterilized distilled water per plate) was added at every 4^{th} day of incubation till the complete emergence of seedlings. At 90 % germination (approx.) the seedlings were removed from the plates. The agar gel from all the plates were then taken out and placed into a beaker (100 ml) containing 50 ml. sterilized distilled water and mixed thoroughly. After a period of 24 hrs the slurry was well stirred and centrifuged. The supernatant was used as the source of seed diffusates or the allelopathic chemicals. It was used as 100 % SD stock and diluted to obtain different concentrations ranging from 20 – 100 % as required in the experiments. These concentrations were applied in the experiment as SD (Seed Diffusates).

3.2.3 Essential oils

The essential oils from Lemon grass (*Cymbopogon citratus* (DC) stapf.), Camphor (*Cinnamomum camphora* L.) and Eucalyptus (*Eucalyptus citriodara* Hook.) was extracted using clevenger's oil extracting method. For each extraction of essential oil the leaves of Lemon grass, Eucalyptus and Camphor were shade dried for two days separately. Fifty grams of each leaf sample was cut finely so that to expose maximum surface of the internal tissues for maximum oil yield. The finely cut pieces were put into Clevenger's oil extracting apparatus together with 500 ml. water. The extraction was then run for about three to five hours for a good yield. The oil gets deposited at the nozzle of the apparatus which was obtained by opening its valve. The collected oil was dehydrated over anhydrous sodium sulphate and stored in 4 ºC in a refrigerator. The dehydrated oil is considered to be pure. The same process was carried out for all leaves from three different sources.

The stock was then prepared from so obtained pure oil. For preparation of stock, 4 ml. of 80% acetone was mixed with 1 ml. of pure oil. Dilution of the stock was done to prepare different concentrations of oils using 80% acetone. For obtaining 10 ml. of 4 – 20 ml l^{-1} concentration range, 0.2 ml -1ml stock was diluted with 9.8 ml- 9 ml acetone separately. Similarly, same concentration ranges of each essential oil were prepared.

3.3 Experiments

Different extracts prepared were used for the experiment in different ways as according to the objective of the research.

3.3.1 Radish seed germination

Sixty seeds of *Raphanus sativus* were soaked separately in each of the above prepared concentrations of plant diffusates (PD) and seed diffusates (SD) overnight. They were plotted at equidistant places in the Petri dishes containing moist filter paper and the respective concentrations (1.5 ml) were irrigated from one corner of each plate. Two replicas were made such that each plate contains only 30 seeds. A control group was made by soaking 60 seeds in distilled water and plating them in two Petri dishes each containing 30 seeds. The system was left at 25 ºC in dark place for 12 hours with frequent hourly observation. The seed germination data was then taken after the arrival of steady state of germination kinetics in the control group.

3.3.2 *Parthenium* seed germination

Prior to the treatment the seeds were imbibed for 36h.in distilled water. Fifty-four plastic disposable Petri dishes with three stacked filter papers of 9 mm. size were taken. Each of the plate was then provided with 3 ml. distilled water so that to moisten the filter papers. One and half milliliter from each concentration of each essential oil (Eucalyptus, Lemon grass, and Camphor) was kept in the Petri dishes with three replicas. The imbibed seeds of *Parthenium* were plotted in the Petri plates such that each plate contains 20 seeds at equidistant places. The whole set was then kept in an incubator at the temperature of about

25° ± 1° C. The required data were taken after the arrival of steady state of germination kinetics in the control group.

3.3.3 Determination of glucose level

The glucose production in *Raphanus* seeds in the presence of allelochemicals (plant diffusates and seed diffusates) was indirectly analyzed by detecting the amount of glucose present in germinating seeds. The sugar content on those seeds was estimated by Somogyi's and Nelson colorimetric method, 1951. The principal of the method is that the glucose in test solution reduces the blue colour of alkaline copper reagent. The level of glucose then was estimated by measuring the absorbance value of all test solutions and standard glucose solution of known strength (as reference) at 560 nm after the completion of colouring reaction. The value of glucose content and percent reduction in its level per seed was calculated using the formula:

$$\text{a) Glucose prod. / Seed} = \frac{A_{560} \text{ of test sol}^{n.} \text{ X Std. glucose conc. /unit } A_{560}}{\text{No. of seeds}} \text{ X dil. Factor*}$$

$$\text{b) \% Reduction of glucose / Seed} = 100 - \frac{\text{Glu. Content / seed on respective media}}{\text{Glu. Content / seed in D/W media}} \text{ X100}$$

3.4 Statistical test

A factorial design was adopted and correlation analysis was adopted to find out the relations between two variables where necessary. Similarly, ANOVA analysis was also carried out to find out the level of significance. The degree of probability ($P < 0.05$ or $P > 0.05$) has been incorporated into figures.

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* The value of dilution factor in the equation is 500 since the extract was diluted 500 times (see Appendix-II)

4. RESULTS

The results obtained from overall research are summarized below within respective headings:

4.1 Germination of *Raphanus* seeds

The germination of 24 hour water imbibed *Raphanus* seeds followed the normal sigmoidal growth pattern in hourly basis. The maximum germination of those imbibed seeds was achieved inbetween 5-10 h. These seeds were found germinating by 91.66% after 10 hours of treatment in normal distilled water (fig.1). A stationary phase was achieved under the observation of 12 hours (Appendix-I, Table-1). Therefore the maximum germination of 24 hour water imbibed *Raphanus* seed will arive within 10-12 hrs. in laboratory conditions. About 91.66% seed viability and fast germination in laboratory was reported and thought to be suitable for experimentation.

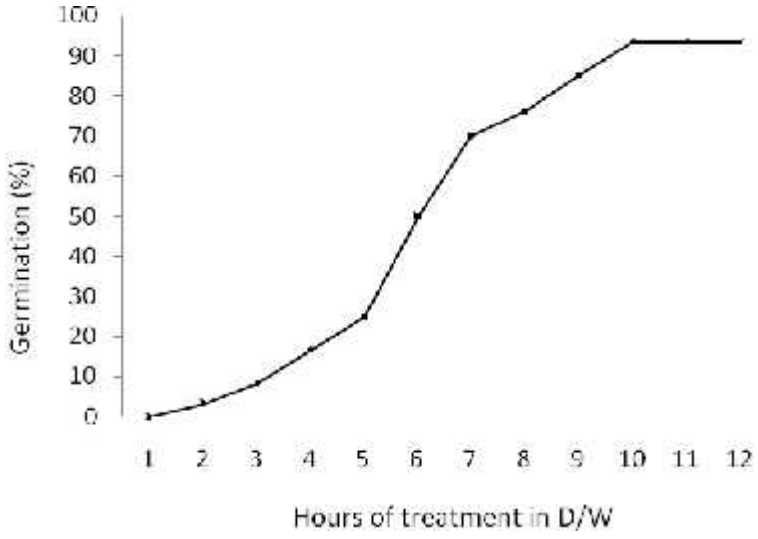

Fig. 1. Hourly germination of water imbibed *Raphanus* seeds in distilled water.

The germination experiment of *Raphanus* seeds in the presence of rhizospheric plant diffusates and seed diffusates clearly shows the low germination in their different concentrations.

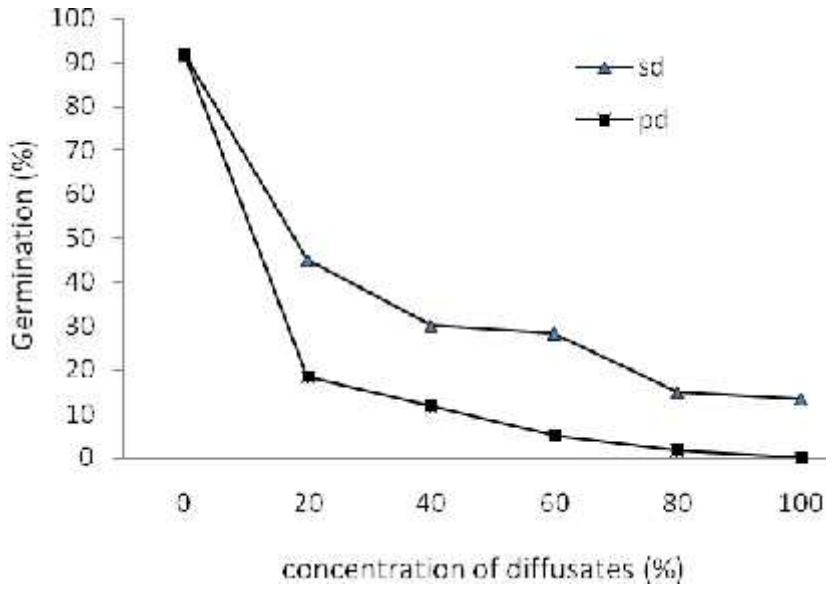

Fig. 2. Germination of water imbibed*Raphanus* seeds at different concentrations of plant diffusates.

Both diffusates are found influencing the normal germinationn pattern where they were able to retard and minimize the germination of those seeds. The graph in fig. 2 shows the germination of *Raphanus* seeds in both diffusates. The graph clearly visualises the low germination of *Raphanus* seeds in PD reaching to nil at its 100 % concentration in comparision to the SD. (Appendix-I, Table 2 and 3).

The correlation analysis revealed the correlation factor - 0.889 for SD with 5% level of significance. This indicates that there undoubtly exist a negative correlation between the SD concentrations and seed germination of *Raphanus*. Furthermore the negative correlation with correlation factor - 0.784 between PD concentrations and seed germination also lead to conclude its inhibitory effect on those seeds during their germinations.

The significant negative correlation coefficients (- 0.889 for SD and - 0.784 for PD) values (Table- 1 and 2) reveal that the germination of *Raphanus* seed goes on decreasing along with the increase in concentratuions of both allellochemicals. In other words the concentrations plays negative role in the germination process of those seeds and hence the inhibition

suddenly increases along with the increase in concentratons. The maximum inhibition for both is found at 100 percent concentrations of each diffusates.

Table- 1. Correlation between concentration of SD and % seed germination of *Raphanus.*

		CONC	PSG IN SD
CONCENTRATION	Pearson correlation	1.000	- 0.889*
	Sig. (2-tailed)	-	.018
	N	6	6
PERCENT SEED GERMINATION IN SD	- 0.889		1.000
	0.18		-
	6		6

*Correlation is significant at the 0.05 level (2-tailed).

Table- 2. Correlation between concentration of PD and % seed germination of *Raphanus.*

		CONC	PSG IN PD
CONCENTRATION	Pearson correlation	1.000	- 0.784
	Sig. (2-tailed)	-	.065
	N	6	6
PERCENT SEED GERMINATION IN PD	- 0.786		1.000
	0.065		-
	6		6

4.2 Germination of *Parthenium* seed

The daily seed germination pattern of *Parthenium* seed in water and acetone was examined and found the germination percentage reaching to 95% and 85% in water and acetone (80%) respectively after 15 days (Appendix- I, Table-4). The difference is not so much larger and near about 85% seeds were under germinating condition in acetone (80%). Therefore acetone (80%) was used as a solvent of essential oils for the experiment. The graph in fig. 3

gives the idea of the germination pattern of those seeds in water and acetone. The germination pattern is smooth and the percentage is higher in water than that of acetone.

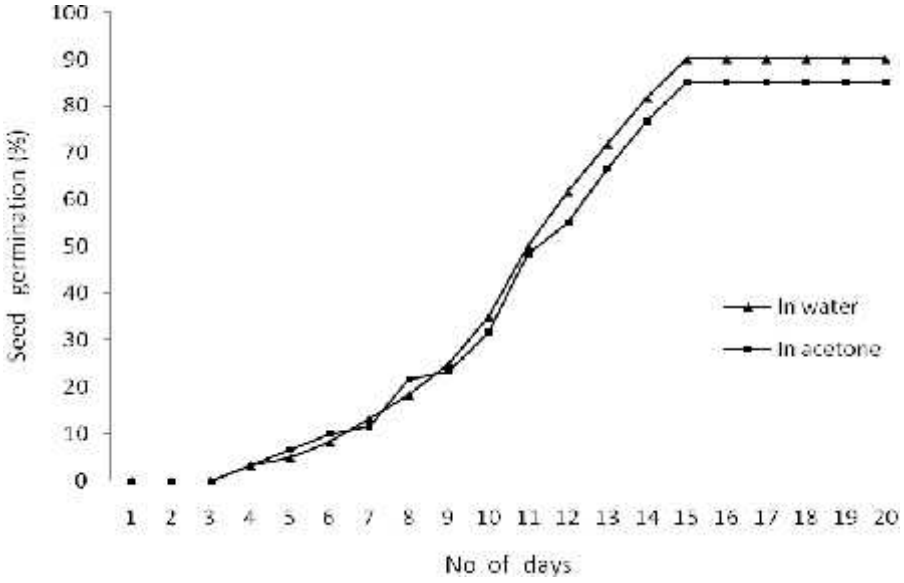

Fig. 3. Daily seed germination pattern of *Parthenium* in Water and Acetone.

The effect of essential oils in different concentrations (0 to 20 ml l^{-1}) from the plants *Cinnamomum camphora* (Camphor tree), *Eucalyptus camaldulensis* (Eucalyptus), *Cymbopogon citratus* (Lemon grass) was analyzed in the seed germination and seedling length of *Parthenium* weed. Lemon grass at 8 ml l^{-1}, *Cinnamomum* and Eucalyptus oil each at 12 ml l^{-1} inhibited the germination of *Parthenium* seeds completely (Fig.4). In the case of *Cinnamomum* oil, only 11.67 % and 1.67 % seeds germinated at 4 ml l^{-1} and 8 ml l^{-1} concentrations respectively. None of the seeds, however, germinated at and above 12 ml l^{-1} concentrations of any of the oils. The summary of the seed germination count is given in Appendix- I, Table -5.

The ANOVA table below suggests that the treatments are significant whereas the sources of essential oils are not significant at 5% level. This indicates that irrespective of the sources of essential oils their concentrations are effective in decreasing the seed germination of the wed *Parthenium*. The general effects of essential oils on seed germination are given on the fig.5.

The graph clearly visualizes the sharp declination in the marginal mean value along with the increase in concentrations of essential oils.

Table- 3. ANOVA chart for *Parthenium* seed germination at different oil concentrations.

Source of Variation	Sum of square	Degree of freedom	Mean square	Variance Ratio
Total	17628.96	17	-	-
Concentrations	17569.66	5	3513.93	742.9**
Plants	12.05	2	6.025	1.27 ns
Error (Residual)	47.25	10	4.73	-

** = Significant at 5% level, ns = Not significant at 5% level.

Thus, all essential oil tested affected seed germination significantly ($P < 0.05$). Effects of plant sources were, however, not significant ($P > 0.05$). Finally, the concentration responses were found as 20 ml l^{-1} = 16 ml l^{-1} = 12 ml l^{-1} > 8 ml l^{-1} > 4 ml l^{-1} ($P < 0.05$; LSD = 3.96). (Appendix- III, Table- 1).

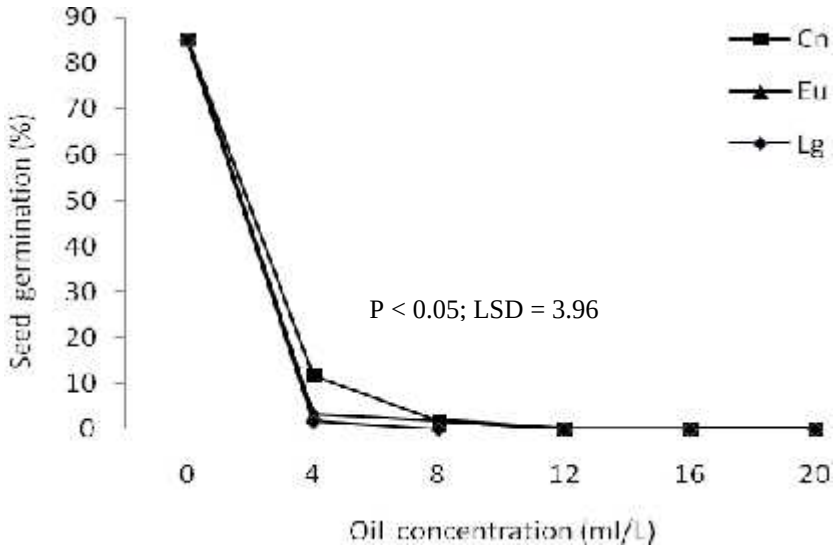

Fig. 4. Effects of the different concentrations with different sources of essential oils on Seed germination.

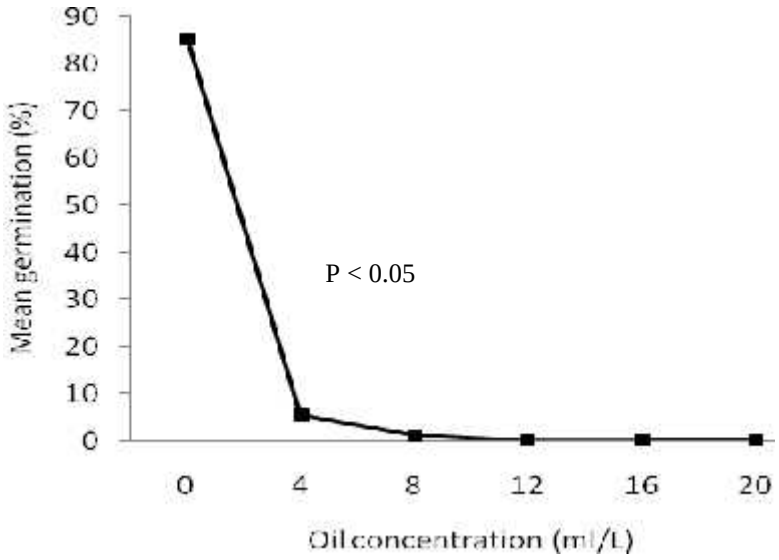

Fig. 5. General effects of essential oils on seed germination of *Parthenium* weed.

The effect of essential oils on seedling length of the weed *Parthenium* was analyzed simultaneously with that of the seed germination. Results were found corresponding to that of the germination count *i.e.* the former results.

Among all the essential oils the oil from lemongrass is found very sensitive to low concentration in comparison to others. All the essential oils were found successful to affect the seedling development and its length. The seedling lengths for 4 ml l^{-1} of Lemon grass, 8 ml l^{-1} of *Cinnamomum* and 8 ml l^{-1} of Eucalyptus oil showed similar values (Appendix- I, Table-6).

The ANOVA table below suggests that the treatments are significant at 5% level whereas the sources (plants) are not significant at the same level. This indicates that irrespective of the sources of essential oils their concentrations are effective in decreasing the seedling length of the weed *Parthenium*. This interpretation is same to that of the former since the trend of the effect of the essential oils is same. Furthermore the graph in fig.6 shows the comparative effect of all concentrations of essential oils on seedling length. The sharp decrease in germination by all essential oils was also seen in the seedling length. The sharp declination of graph for marginal mean value in fig. 7 also supports the result.

Table- 4. ANOVA chart for *Parthenium* seedling length at different oil concentrations.

Source of Variation	Sum of square	Degree of freedom	Mean square	Variance Ratio
Total	884.93	17	-	-
Concentration	881.21	5	176.24	660.07**
Plants	1.05	2	0.525	1.966 ns
Error (Residual)	2.67	10	0.267	-

** = Significant at 5% level, ns = Not significant at 5% level.

Thus, entire essential oil significantly ($P < 0.05$) affected seedling length irrespective to differences in their sources similar to as before. Effects of plant sources were, however, again not significant ($P > 0.05$). Finally, the concentration responses were found as 20 ml l^{-1} = 16 ml l^{-1} = 12 ml l^{-1} > 8 ml l^{-1} > 4 ml l^{-1} ($P < 0.05$; LSD = 0.94). (Appendix-III, Table-2). This trend is completely similar to that of the former one.

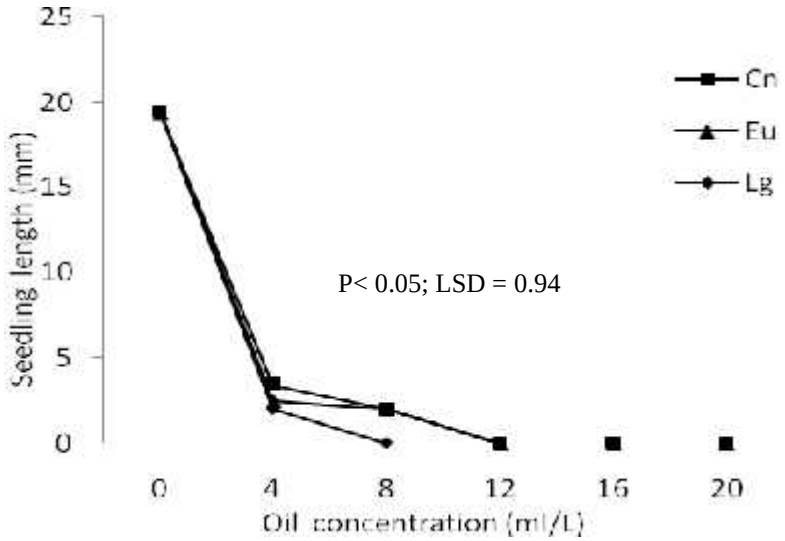

Fig. 6. Effects of the different concentrations with different sources of essential oils on Seedling length of *Parthenium.*

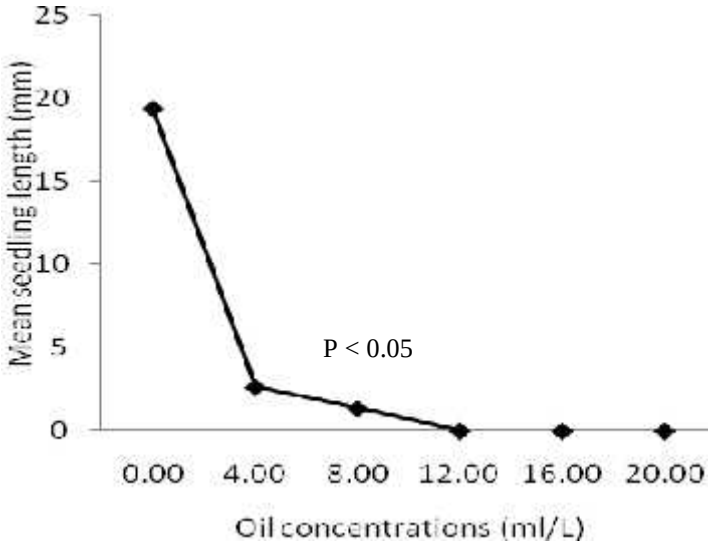

Fig. 7. General effects of essential oils on Seedling length of *Parthenium*.

4.3 Glucose level

The analysis of glucose content in *Raphanus* seeds grown in plant and seed allelochemicals revealed that these seed exihibits high degree of shortage of glucose at the time of germnation in comparision to that of the normally grown seeds. The glucose produced per seed in distilled water grown seeds is 0.335 nanogram which is decreased by 55% reaching to 0.151nanogram/seed in the seeds grown in 100 % plant diffusates. Further, the value is decreased by 37% reaching to 0.211nanogram/seed in case of the seeds grown in 100% seed diffusates. The figures 8 and 9 shows the glucose levels and simoultaneous reduction percentage of it respectively.(Appendix- I, Table- 8).

Therefore, both the allelochemicals from the plant are effective in reducing the glucose level during germination of *Raphanus* seeds. The low glucose production per *Raphanus* seed grown in plant and seed diffusates of *Parthenium* resembles with the inhibition of germination of those seeds on different concentratios of the both diffusates as found earlier. Also the high glucose production on the distilled water grown *Raphanus* seeds matches with the high germination (91.66 %) of those seeds.

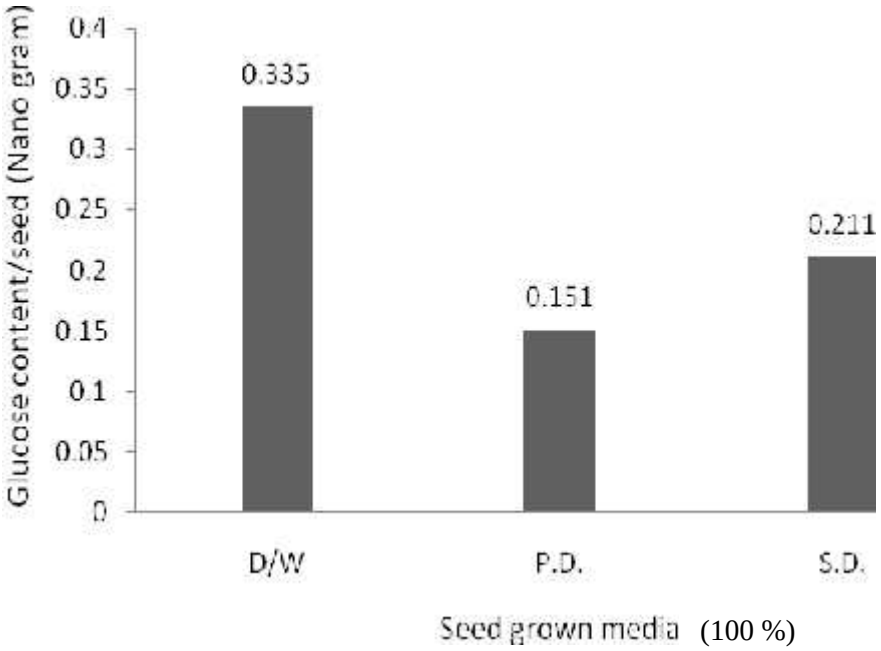

Fig. 8. Glucose content per *Raphanus* seed (in nanogram) grown in 100% concentration of three different media.

5. DISCUSSION

The imbibed seeds of *Raphanus sativus* start to respire and the germination process proceeds after the synthesis of glucose inside it. The *Raphanus* seeds are one of the fastly germinating seeds and were found to germinated within 10-12 h. at 25° C in laboratory (Appendix- I, Table- 1). The seeds are light sensitive and hence was kept in dark place (Gaurdia *et al*., 1987) upto the emmergence of seedligs.

Possible reason for less inhibitory effect of seed diffusates might be the low percentage of parthenin content in it. The seeds were soaked in water for 36 h. for imbibition prior to germination while preparing the seed diffusates. This lead to washing off the parthenin possibly in much amount. In other hand the plant diffusates was prepared from rhizospheric soil which was collected in the dry season when there was no shower from a long time. Various reports suggests high concentration of parthenin in the root of the plant(Kanchan and Jayachandra 1979, 1980 a) Furthermore, leaf residues are believed to deliver large amounts of Parthenin to soils during decomposition (Regina *et al*., 2007). Obviously the accumulation of parthenin and probably the other associated chemicals were at high conentration on the soil. Thus the high effectiveness of the plant diffusates in inhibiting the *Raphanus* seed germination due to high concentration of the parthenin is possible.

The low germination of *Raphanus* seeds in the presence of seed and plant diffusates of *Parthenium* is probably due to the effect of the chemical parthenin, a sesquiterpene lactone which is supposed to be present in the plant and obviously in its diffusates. The chemical is responsible for strong inhibitory influence of the weed to many neighbouring plants (Adkins and sowerby, 1996) and strong competitiveness with crop plants (Tamado *et al*., 2002). Among several biologically active compounds, the phytotoxic potential of sesquiterpenes has long been established (Dayan *et al.*, 1999). Furthermore, an allelopathic influence of water soluble phenolics released by the plant into the soil is reported on the early growth of *Brassica* crops (Batish *et al*., 2005) and seed germination and seedling growth of *Eragrostis tef* (Tefera, 2002). Recently, Regina *et al.* (2007) investigated the level of involvement of Parthenin in overall phytotoxicity of decomposing leaf material in a South African population of *P. hysterophorus*. Results showed that the contribution of Parthenin is highly

dependent on its concentration within extract solutions and varied between 16% and 100% of overall phytotoxicity of leaf extracts. Besides, Parthenin treatments are proven to delay germination and stimulate root growth at low doses in various experiments.

The late germination of *parthenium* seeds in water starting after 4 days of innoculation might be due to the hard seed coat, insufficient temperature (25° C) and humidity in laboratory. Normally these seeds requires near about 30° C in *in vitro* condition along with high humidity and rain fall. The mobilization of seed germinating prcursors perhaps starts lately on those seeds possibly as a result of some sorts of autotoxic effects. Varios reports are in the favour of autotoxic effect of parthenin during its own seed germination. The seed will not germinate immidiately or within few days of dispersal till the parthenin present on the seed scales will not washed off to some extent (Oudhia *et al.*, 1999).

Essential oils are considered as antibacterial, antifungal, insecticides and inhibitory agent to the biological systems. Also these essential oils are popularly known to have antifungal property which might be due to the presence of some characteristic compounds responsible for fungi toxicity. The adverse effects of essential oils on seed germination and seedling length clearly indicates the herbicidal property of these oils towards *Parthenium*. Herbicidal activities of volatile oils of *Eucalyptus citriodora* Hook. against *Parthenium hysterophorus* L. has been reported recently by Singh *et al*. (2005). Foliar damage as well as suppression in seed germination of the *Parthenium* plant by the application of the Eucalypt oil at low concentration is also been established on the same report.

These results may be interpreted in terms of different kinds of terpenoid especially monoterpenes and di-terpenes present in plant essential oils and their probable effects on seed germination process. It is reported that these terpenoid are group of compounds with variable biological activities. Active at low concentrations these compounds exhibit specific structure-activity relationship (Fandohan *et al.*, 1997). Possible roles of these terpenes in regulating hydrolytic enzymes including amylases, *de novo* synthesized in germinating seeds, can not be overruled. Works in the line of further chemical characterization of the used essential oils are speculated to justify the finding of the present investigation.

The glucose production per *Raphanus* seed grown in plant diffusates is comparatively lower than that of the seed grown in seed diffusates of *Parthenium*. This finding also matches with the higer inhibitory role of plant diffusates than seed diffusates on *Raphanus* seed germination. Possible reason for the low glucose production in *Raphanus* seeds grown in the plant and seed diffusates of *Parthenium* might be due to the reduction in the starch and fat mobillisation during their germination. Possible roles of these allelochemicals present in both types of diffusates of *Parthenium* in regulating hydrolytic enzymes including amylases, *de novo* synthesized in germinating seeds, can not be overruled. Works in the line of further chemical characterization of these allelochemicals and their function in germinating seeds are speculated to justify the finding of the present investigation.

6. CONCLUSION

The overall study leads to conclude that

I) The plant released and seed released diffusates from invasive alien weed *Parthenium hysterophorus* has allelopathic impact on the *Raphanus sativus* in laboratory conditions. Increase in concentrations of both diffusates there is decrease in *Raphanus* seed germination. The diffusates not only inhibits the germination of *Raphanus* seeds but minimizes the glucose level produced during their germination too.

II) The essential oils of Lemongrass (*Cymbopogon citratus*), Eucalyptus (*Eucalyptus citriodora*) and Camphor (*Cinnamomum camphora*) are strictly inhibitory in their different concentrations for seed germination and seedling length of the invasive alien weed *Parthenium hysterophorus* in laboratory conditions. Therefore these essential oils can be used as good herbicides in controlling the *Parthenium* populations.

APPENDIX - I

Table- 1. Hourly germination of *Raphanus* seeds in distilled water.

Hours of treatment in distilled water	Germinated seeds (%)
1	0
2	3.33
3	8.33
4	16.66
5	25.00
6	50.00
7	70.00
8	76.66
9	85.00
10	93.33
11	93.33
12	93.33

Table- 2. Germination of imbibed *Raphanus* seeds in different concentrations of plant diffusates at 12 hours.

Concentrations (%)	R_1	R_2	Total	% germination	% Inhibition
0 (Control)	28/30	27/30	55/60	91.66	8.33
20	6/30	5/30	11/60	18.33	81.66
40	4/30	3/30	7/60	11.66	88.33
60	0/30	3/30	3/60	5	95
80	1/30	0/30	1/60	1.66	98.33
100	0/30	0/30	0/60	0	100

R_1 and R_2 stands for 1^{st} and 2^{nd} replicas.

Table- 3. Germination of imbibed *Raphanus* seeds in different concentrations of seed diffusates at 12 hrs.

Concentrations (%)	**R_1**	**R_2**	**Total**	**Germination (%)**	**Inhibition (%)**
0 (Control)	28/30	27/30	55/60	91.66	8.33
20	16/30	11/30	27/60	45	55
40	10/30	8/30	18/60	30	70
60	8/30	9/30	17/60	28.33	71.67
80	3/30	6/30	9/60	15	85
100	4/30	4/30	8/60	13.33	86.67

R_1 and R_2 stands for 1^{st} and 2^{nd} replicas.

Table- 4. Daily germination pattern of *Parthenium* seeds in Water and Acetone.

No of days	**Seed germination in water (%)**	**Seed germination in Acetone (%)**
1^{st}	0	0
2^{nd}.	0	0
3^{rd}.	0	0
4^{th}.	3.33	3.33
5^{th}.	5.00	6.66
6^{th}.	8.33	10
7^{th}.	13.33	11.69
8^{th}.	18.33	21.66
9^{th}.	25.00	23.33
10^{th}.	35.00	31.66
11^{th}.	50.01	48.33
12^{th}.	61.66	55.00
13^{th}.	71.81	66.68
14^{th}.	81.66	76.66
15^{th}. - 20^{th}.	90	85.00

Table- 5. Germination count of *Parthenium* seeds in various concentrations of three different essential oils at 20$^{th.}$ day.

Oil type	Replica plate	No. of germinated seeds				
		4 ml.L^{-1}	**8 ml.L^{-1}**	**12 ml.L^{-1}**	**16 ml.L^{-1}**	**20 ml.L^{-1}**
Lemon grass (*Cymbopogon citratus*)	R_1	1/20	0/20	0/20	0/20	0/20
	R_2	0/20	0/20	0/20	0/20	0/20
	R_3	0/20	0/20	0/20	0/20	0/20
Total	–	1/60 **(1.67%)**	0/60 **(0%)**	0/60 **(0%)**	0/60 **(0%)**	0/60 **(0%)**
Cinnamomum (*Cinnamomum camphora*)	R_1	2/20	0/20	0/20	0/20	0/20
	R_2	1/20	0/20	0/20	0/20	0/20
	R_3	4/20	1/20	0/20	0/20	0/20
Total	–	7/60 **(11.67%)**	1/60 **(1.67%)**	0/60 **(0%)**	0/60 **(0%)**	0/60 **(0%)**
Eucalyptus (*Eucalyptus citrodora*)	R_1	1/20	0/20	0/20	0/20	0/20
	R_2	1/20	0/20	0/20	0/20	0/20
	R_3	0/20	1/20	0/20	0/20	0/20
Total		2/60 **(3.33%)**	1/60 **(1.67%)**	0/60 **(0%)**	0/60 **(0%)**	0/60 **(0%)**

Table- 6. Seedling length (in mm.) of germinated seeds of *Parthenium* in different concentrations of three essential oils on 20 th. day.

S. N.	**Acetone (Control)**			**Lemon grass**			**Cinnamomum**						**Eucalyptus**					
-	-	-	-	**4 ml.L^{-1}**			**4 ml.L^{-1}**			**8 ml.L^{-1}**			**4 ml.L^{-1}**			**8 ml.L^{-1}**		
-	**R_1**	**R_2**	**R_3**	**R_1**	**R_2**	**R_3**	**R_1**	**R_2**	**R_3**	**R_1**	**R_2**	**R_3**	**R_1**	**R_2**	**R_3**	**R_1**	**R_2**	**R_3**
1	6	5	21	0	2	0	0	0	6	0	0	0	0	0	0	0	0	0
2	21	17	19	0	0	0	2	0	0	0	0	0	0	0	3	0	0	2
3	-	16	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	19	20	-	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0
5	18	19	23	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
6	21	-	23	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
7	-	18	20	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
8	19	18	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	21	17	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	22	-	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	23	16	-	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
12	23	15	19	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
13	23	-	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	-	16	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	22	21	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	22	22	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	23	21	21	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0
18	23	23	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	19	-	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	18	18	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ASL of GS		1252/54 = 19.39		2/1 = 2			24/7 = 3.428			2/1 = 2			5/2 = 2.5			2/1 = 2		

ASL of GS = Average seedling length of germinated seeds.

Table- 7. Absorbance values of *Raphanus* seed extract grown on different media.

S.N.	**Standard glucose** (10^{-8} gm/ml.)	**Extract of P.D. grown seeds.**	**Extract of S.D. grown seeds.**	**Extract of D/W grown seeds**.
1	0.015	0.044	0.062	0.9
2	0.016	0.045	0.063	0.11
3	0.014	0.046	0.064	0.10
Mean	**0.015**	**0.045**	**0.063**	**0.10**

Table-8: Glucose level produced in the germinating *Raphanus* seeds in different media after 12 hours.

Seed grown media	**Glucose content/seed (x10^{-9}or Nanogram)**	**Reduction in glucose level (%)**
Seeds grown in D/W	0.335	0
Seeds grown in P.D.	0.151	55 (approx)
Seeds grown in S.D.	0.211	37 (approx).

APPENDIX - II

Glucose estimation (Somogyi's and Nelson Method, 1951).

a) Preparation of sodium salt of C_{10}

a) Hundred mili Mole decanoic acid was prepared in chloroform.

b) The chloroform was evaporated

c) Hundred milli mole of NaOH was added.

d) 0.1% tween-20 (in water) was added to make the final volume 10ml.

e) The mixture was diluted to 100 fold to get 1mM (milli Mole) sodium salt of decanoic acid.

b) Preparation of Somogyi's alkaline copper Reagent

Name of chemical	Amount (per liter of D/W)
Anhydrous Na_2Co_3	*24gms.*
Sodium potassium tartarate	*12gms.*
$NaHCo_3$ (Sodium bi carbonate)	*16gms.*
$CuSO_4.5H_2O$ (Copper sulphate)	*04gms.*
Na_2So_4 (Sodium sulphate)	*180gms.*

The above mentioned chemicals were mixed and diluted to 1litre distilled water.

c) Preparation of Arsenomolybdate reagent

To prepare Arsenomolybdate reagent 25 gms. of Ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$ was dissolved in 450ml of water and 21 ml. of conc. H_2SO_4 and mixed properly. Again 3 gms of $Na_2HAsO_4.7H_2O$ was dissolved in 25ml distilled water in another container. The solutions were then mixed and placed in an incubator at 27 ºC for 24-48 hours before use.

d) Glucose -Test

a) The Raphanus seeds were treated whole night in the allelochemical released from plant *in vivo* i.e. plant diffusates (P.D.) and allelochemical released by seed *in vitro* (S.D.).

b) Ten seeds from each treatment were kept in different Petri dishes provided with distilled water soaked moist filter paper.

c) The sets were left for 10-12 hours at 25ºC in dark place.

d) After the germination of Raphanus seeds, the system was stored in 4 ºC in a refrigerator.

e) Next day, for each treatment following set of experiment was conducted.

- 10 Raphanus seeds were boiled in 2ml water for three minutes.
- Those seeds were homogenized with 5ml. of water in a mortar-pestle.
- The content was centrifuged at 3000 rpm for 5 minutes and the supernatant was taken discarding the pellet.
- The supernatant was diluted with distilled water to a final volume of 50ml.
- One ml of this solution was mixed with 1ml of Somogyi's alkaline copper Reagent in a test tube.
- The solution was then placed in boiling water bath for 10 minutes.
- The solution in tubes was cooled in ice cold water for 5 minutes.
- One ml of Arsenomolybdate reagent was added and the mixture was finally diluted to 10ml with distilled water.
- Absorbance value at560 nm was taken using water as blank.

f) The amount of glucose content per seed was estimated with the help of the absorbance value of freshly prepared D-glucose solution ($1x10^{-8}$gm/ml.) at560nm wavelength using comparative unitary method.

APPENDIX - III

Calculation for ANOVA

The statistical test for correlation analysis and ANOVA analysis was carried out using SPSS 11.5 windows version. The results are tabulated below.

Table- 1. Percent seed germination of *Parthenium* in different concentrations of three essential oils.

Plants	**Control**	**4 ml.L^{-1}**	**8 ml.L^{-1}**	**12 ml.L^{-1}**	**16 ml.L^{-1}**	**20 ml.L^{-1}**	**Total**
Lemon grass	85	1.67	0	0	0	0	**86.67**
Cinnamomum	85	11.67	1.67	0	0	0	**98.34**
Eucalyptus	85	3.33	1.67	0	0	0	**90.00**
Grand total	**255**	**16.67**	**3.34**	**0**	**0**	**0**	**275.01**
Average	**85**	**5.56**	**1.11**	**0**	**0**	**0**	**-**

No. of data = 18, $\sum x = 275.01$

Here,

N = 18, $\sum x = 275.01$

a) Correction factor (C_f) = $(\sum x)^2 / N = (275.01)^2 / 18 = 4201.69$

b) Total sum of squares about the mean (TSS) = $(n_1)^2 + (n_2)^2 + (n_3)^2 + \ldots\ldots + (n_{18})^2 - C_f$.

Where, n = a single data.

$= (85)^2 + (1.67)^2 + (0)^2 + \ldots..+ (0)^2 - 4201.69$

$$= 21830.65 - 4201.69$$

$$TSS = 17628.69$$

c) Sum of squares for treatments (SST) = $1/3\left[(255)^2 + (16.67)^2 + (3.34)^2 + (0)^2 + (0)^2 + (0)^2\right] - C_{f.}$

$$= 1/3\left(65314.0445\right) - 4201.69$$

$$SST = 17569.658$$

d) Sum of squares of plants about mean (SSP) = $1/6\left[(86.67)^2 + (98.34)^2 + (90)^2\right] - C_{f.}$

$$= 1/6\left(25282.44\right) - 4201.69$$

$$SPP = 12.05$$

Hence,

Residual (error) = TSS – SST – SSP = 17628.96 – 17569.658 – 12.05

$$= 47.25$$

The treatments were found significant with variance ratio 742.9. We analyze only the significant case.

Here,

$$t_{\alpha,\, df=10}\,(t_{cal.}) = \sqrt{\frac{2 \times \text{Residual mean square}}{\text{No. of replications}}}$$

$$= \sqrt{\frac{2 \times 4.73}{3}}$$

$$t_{cal.} = 1.776$$

Again from table,

$$P_{0.05;\, df = 10} = 2.228$$

Hence,

Least significance difference (LSD) = $P_{0.05;\, df = 10} \times t_{\alpha,\, df=10}$

= 2.228 x 1.776

LSD = 3.96

Table- 2. Average seedling length (in mm.) of germinated seeds in different concentrations of three essential oils.

Plants	Control	4 ml.L^{-1}	8 ml.L^{-1}	12 ml.L^{-1}	16 ml.L^{-1}	20 ml.L^{-1}	Total
Lemon grass	19.39	2	0	0	0	0	**21.39**
Cinnamomum	19.39	3.43	2	0	0	0	**24.82**
Eucalyptus	19.39	2.5	2	0	0	0	**23.89**
Grand total	**58.17**	**7.93**	**4**	**0**	**0**	**0**	**70.1**
Average	**19.39**	**2.643**	**1.33**	**0**	**0**	**0**	**-**

No. of data = 18, $\sum x = 70.1$

Here,

N = 18, $\sum x = 70.1$

a) Correction factor (C_f) = $(\sum x)^2 / N = (70.1)^2 / 18 = 4914.01$

b) Total sum of squares about the mean (TSS) = $(n_1)^2 + (n_2)^2 + (n_3)^2 + \ldots\ldots + (n_{18})^2 - C_f$.

Where, n = a single data.

$= (19.39)^2 + (2)^2 + (0)^2 + \ldots..+ (0)^2 – 273$

$= 1157.93 – 273$

TSS = 884.93

c) Sum of squares for treatments (SST) = $1/3 \left[(58.17)^2 + (7.93)^2 + (4)^2 + (0)^2 + (0)^2 + (0)^2\right] - C_f$.

$= 1/3 \left(3462.634\right) – 273$

$$SST = 881.21$$

d) Sum of squares of plants about mean (SSP) = $1/6\left[(21.39)^2 + (24.82)^2 + (23.89)^2\right] - C_{f.}$

$$= 1/6\,(1644.3)^2 - 273$$

$$SPP = 1.05$$

Hence,

Residual (error) = TSS – SST – SSP = 884.93 – 881.21 – 1.05

$$= 2.67$$

The treatments were found significant with variance ratio 660.07. We analyze only the significant case.

Hence,

$$LSD = P_{0.05;\ df = 10} \times \sqrt{\frac{2 \times \text{Residual mean square}}{\text{No. of replications}}}$$

$$= 2.228 \times \sqrt{\frac{2 \times 0.267}{3}} \qquad \left[\text{Since, } P_{0.05;\ df = 10} = 2.228\right]$$

$$= 0.94 \text{ (approx.)}$$

Hence,

Least significance difference (LSD) = 0.94

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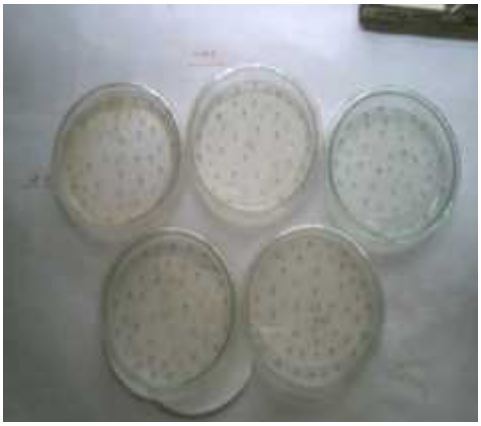
PHOTO PLATE- I

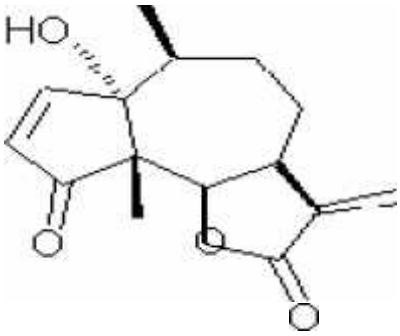
A. Parthenium infestation in maize field.

B. Monoculture vegetation of *Parthenium*



C. *Parthenium* seeds in agar plate.

D. Plant diffusates (soil extract).



E. Chemical structure of Parthenin

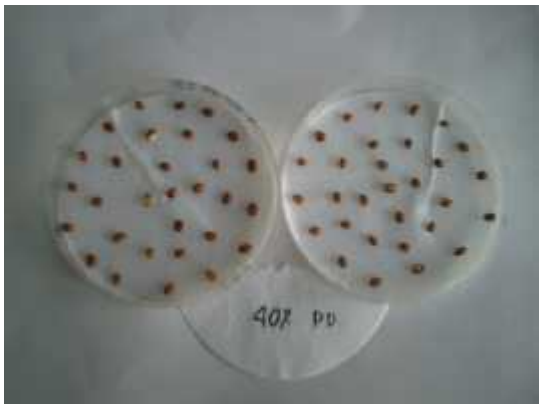
F. Plating of Raphanus seeds in plant and seed diffusates of Parthenium.

PHOTO PLATE-II

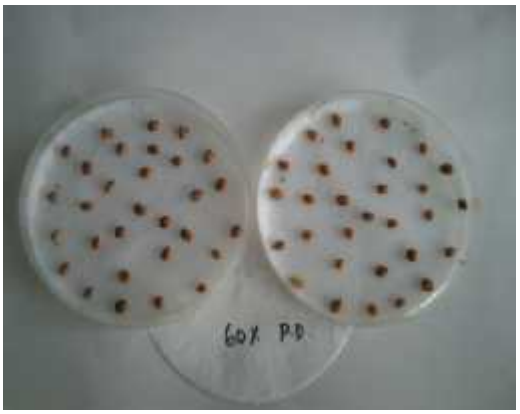
Time : At 12 hrs.

Control (D/W) treated seeds.

20% PD treated seeds.

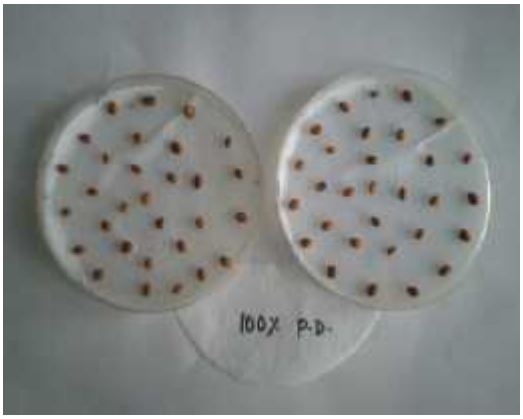


40% PD treated seeds.



60% PD treated seeds.

80% PD treated seeds.



100% PD treated seeds.

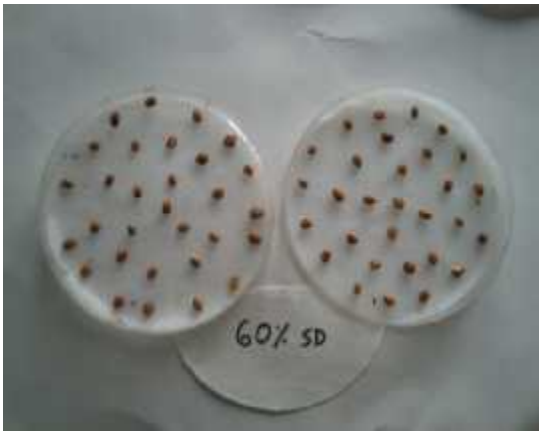
PHOTO PLATE- III

Time: At 12 hrs.

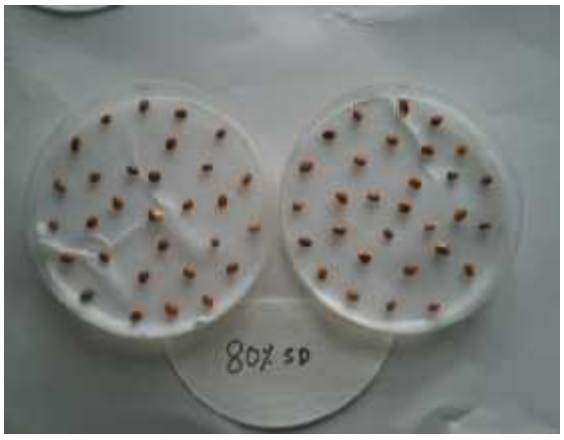
Control (D/W) treated seeds.

20% SD treated seeds.

40% SD treated seeds.



60% SD treated seeds.



80% SD treated seeds. 100% PD treated seeds.

PHOTO PLATE- IV

A. Raphanus seeds in100%. PD, SD and D/W

B. Colouring reaction of glucose with Somogyi's copper reagent

C. Clevenger's oil extracting apparatus.

D. Herbarium specimen of

E. Herbarium specimen of

F. Herbarium specimen of

Eucalyptus citrodora *Cinnamomum camphora*. *Cymbopogon citratus*