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ALLELOPATHIC EFFECTS OF PARTHENIUM HYSTEROPHORUS ON GERMINATION OF RAPHANUS SATIVA SEEDS

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The term 'allelopathy' was coined to describe the effect of the one plant on neighbouring plants. The word Allelopathy has been derived from two Greek words *Allelon* meaning 'each other' and *Pathos* meaning 'to suffer' i.e. the injurious effects of one plant upon another However, Molisch (1937) coined this term which refers to all biochemical interactions (stimulatory and inhibitory) among plants, including micro-organisms. It represents the plant - against plant an aspect of the broader field of chemical ecology. Some authors have used the term in a more restricted sense to describe only the harmful effects o f one higher plant upon another. Rice (1984) reported many cases of allelopathy which either directly or indirectly involved microbes. Allelochemicals which inhibited the growth of some species at certain concentrations may stimulate the growth of same or different species at lower concentrations. The term allelopathy generally refers to the detrimental effects of higher plants of one species (the donor) on the germination, growth or development of another species (the recipient). In the crop fields, at any given time there are at least more than one plant species growing together. These species may be weeds, while in sole cropping there may be crops and weeds mixed. In crop mixtures or intercropping systems, the major plant species are crops with which some weeds may also be present. When the two plant species grow together they interact with each other either inhibiting or stimulating their growth or yield through direct or indirect allelopathic interactions. Allelopathy is generally associated with interactions between living plants and have been observed in agricultural lands from centuries Generally inhibitory effects are considered to be the principal effects of chemicals (allelochemicals) released by the plants on the neighbouring plants.

Crops exert allelopathic effects on other crops and weeds They may inhibit (+ve effect) or stimulate (-ve allelopathic effect) the germination and growth of weeds in agro-ecosystems. The commonality of weed species within a crop ecosystem throughout a wide geographical area indicate that crops biochemically promote certain weeds and inhibit others.

One crop may inhibit or stimulate the germination, growth and yield of associated crop growing with it (Crop mixture or intercropping) or of following crop (monoculture or crop rotations) through release of leachates or washing from germinating seeds or from decomposing crop residues. Germinating seeds of barley inhibited seed germination of white mustard *(Sinapis arvensis)*, while the seed leachate of wheat, oat, maize, vetch and prosomillet helped increasing seed germination (Prutenskaya 1972). Seed and root leachates of barley inhibited seed germination and seedling growth of wheat and tobacco but leachates of living plants were more inhibitory than dead ones (Overland 1966). The ascorbic acid in the root extracts of *Raphanus* stimulated the seedling growth of pearl millet (Sharma and Singhvi 1981). Many phytotoxic compounds from crop residues or their extracts are released which inhibit the growth of other plants. Collison and Conn (1925) concluded that two mechanisms are associated with phytotoxicity of plant residues, (a) toxic compounds which act quickly and are usually inactivated through their adsorption by colloidal matter in the soil, and (b) stimulation of microbial population which immobilizes much of nitrogen making it unavailable to the plants. This review is confined to the first mechanism l e. inhibitory /stimulatory effects of compounds released from crop residues. Allelochemicals refer mostly to the secondary metabolites produced by plants and are byproduct of primary metabolic processes (Levm 1976). They have an allelopathic effect on the growth and development of the same plant or neighbouring plants. The term allelochemicals include, (a) plantbiochemicals that exert their physiological / toxicological action on plants (allelopathy, autotoxicity or phytotoxicity), (b) plant biochemicals that exert
their physiological/ toxicological action on micro-organism (allelopathy or phytotoxicity) and (c) microbial biochemicals that exert their physiological/ toxicological action on plants (allelopathy and phytotoxicity). Secondary compounds are metabolically active in plants and micro-organisms, their biosynthesis and biodegradation play an important role in the ecology and physiology of the organism in which they occur.

Plant parts known to contain allelochemicals are -

i) Roots and rhizomes - In general, they contain fewer and less potent or smaller amounts of allelochemicals than leaves, but some times it may be the reverse also.

ii) Stems - They contain allelochemicals and are some times the principal sources of toxicity.

iii) Leaves - They are the most important sources of allelochemicals. Specific inhibitors in leaves have been demonstrated by many workers.

iv) Flowers / inflorescence and Pollen - Although studies on flowers or inflorescence are limited, there is growing evidence that the pollen of com and *Parthenium* have allelopathic properties.

v) Fruits - Many fruits are known to contain toxins and have been proved to be inhibitory to microbial growth and seed germination.

vi) Seeds - Seeds of many plant families or species have been found to inhibit seed germination and microbial growth.

Leaching - Leaching is the removal of substances from plants by the action of aqueous solvents such as rain, dew, mist and fog (Tukey Jr. 1970), although earlier workers such as Bonner (1960) have used the term excretion for thisphenomenon. All plants seen to be leachable, although the degree depends on type of tissue, stage of maturity and type, amount and duration of precipitation (Tukey Jr. 1970) A large diversity of allelopathic compounds are leached, both organic and inorganic such as phenolic acids, terpenoids and alkaloids (Bomer, 1960, Tukey and Mecklenburg 1964, Tukey Jr 1970) The leaching of mineral nutrients, carbohydrates and phytohormones, may be beneficial for growth of associated species. Under field conditions, the occurrence of the allelopathic phenomenon depends on the amount of ramfall during late spring or early summer, presumably at a critical stage for crop growth and development. Leached material may fall to the soil beneath, from where they can be absorbed directly through roots by either (a) the same plant from which they were leached or (b) adjacent plants of the same or different species. Besides, leached materials may also be intercepted before they reach the soil by stems, branches and foliage of the same or adjacent plants.

Leaf and Root extract - The aqueous extract of leaf and root contains many allelochemicals. These allelochemicals are dissolved or precipitated in water and seeds and plants are treated. The extract also contains some phenolic acid and alkaloids and these types of allelochemicals exhibit a significant inhibitory effect to the germination and growth of the treated plants or seeds. Inhibitory activity was found mainly in the ethanol and water extracts. Under the premise enunciated it was considered pertinent to examine more closely the allelopathic effects of weeds on the germination and growth of seedlings emerged from the treated seeds. For this purpose common species of weed viz *Parthenium hysterophorus* L was selected to find their effects on *Raphanus sativa.* The findings are expected to help the agriculturists in a

big way exploiting allelopathy for eliminating detrimental effects and utilizing the beneficial ones for boosting up crop production.

Review:

Lethal allelopathic effects of *Parthenium* on many agricultural crops have been reported (Narwal 1994, Oudhia *etal.* 1997a, b, Oudhia and Tripathi 1998, Oudhia *et al.* 1999, Vyas and Oudhia 1999). The allelopathic potential of *Parthenium* leaves was studied on germination and seedling vigour of sunflower. The positive (inhibitory) allelopathic effects of different quantities of *Parthenium* leaves was observed which were due to the presence of lethal allelochemicals in Parthenium leaves. Presence of many lethal allelochemicals like parthenin, coronopiUin, caffeic acid, P-coumaric acid, alkaloids and sesquiterpene lactones from different parts of *Parthenium* have been reported (Narwal 1994).

Parthenium hysterophorus, a newly introduced major weed was studied on pulse crops (Sasikumar *et al.* 2002). The leachates of leaf, stem, flower and roots of *Parthenium* were found to contain phenolic compounds viz. caffeic, p-coumaric, ferulic, p-hydroxy benzoic and vanillic acids. The influence of identified phenolics and leachates was investigated on the germination, seedling length, dry matter, vigour index and nitrogenase activity of cowpea, blackgram, greengram, horsegram and pigeonpea In bioassays, the mixture of these phenolic acids as well as individual compounds inhibited the germination and vigour index of all test crops. The leachates from different parts of *Parthenium* significantly reduced the germination in all crops blackgram and greengram. Likewise, seedling length was also significantly inhibited of all the crops.

METHODOLOGY AND MATERIALS

Plant Materials -. *Parthenium hysterophorus*

Family – Asteraceae

Parthenium hysterophorus is the most unwanted weed in Indian subcontinent, and collected from the fields of Uttar Pradesh. *Parthenium* is commonly known as Congress weed, carrot weed, Gajar ghas, False rag weed, Fever few, Chatak Chandani, Ramphool etc. (Oudhia et al. 2000, Oudhia 2000a). *Parthenium* probably originated around the Gulf of Maxico and has spread to USA, Africa and India (Haseler 1976). In Australia and India *Parthenium* has achieved the status of "Worst weed".

Preparation of leaf extract

Parthenium leaf extract was prepared from oven dried (at 80°c for 24 hr) leaves. Leaves after cleaning were chopped into small pieces and oven dried. Dried leaves 10 g were soaked in 50 ml. o f 50 per cent alcohol for 12 hr. Then it was filtered and after evaporation o f alcohol volume was made upto 100 ml. with double distilled water. From this stock solution graded solutions were prepared by adding double distilled water.

Preparation of root extract

Fresh roots of selected weed plants were collected. These roots were washed with tap water for removal of soil particles and then air-dried for a few minutes. Then the roots were cut into small pieces and 10 gm of roots were taken. Distilled water (200 ml) was taken in a beaker and 10 gm of prepared roots were added. The water was allowed to boil for 15-20 minutes and the volume o f water was reduced to 100 ml. Then water was allowed to cool for few minutes and filtered through cotton. This solution was used as a stock solution. From this stock, different concentrations (25, 50 and 100 per cent) of the root extract were prepared by diluting with distilled water.

Preparation of leachate

Root leachate of the selected weed plants were used in this experiment. Fresh roots were collected and washed with tap water for removal of soil particles and air dried for a few minutes. Cleaned roots (10 gm) were taken and cut into small pieces. The cut pieces of roots were placed in to a beaker and 100 ml of distilled water was added and allowed to stay for 24-72 hour for the exudation of allelochemicals. Then the solution was filtered through cotton and used as leachate. This leachate was used as stock solution and different concentrations were made by adding distilled water.

Sterilization of treated seeds

The treated crop seeds were sterilized with Hgdh (0.01%) solution for 5 minutes. After treatment the seeds were allowed to dry and used in the laboratory experiments.

Seething growth

After germination, the root lengths and shoot lengths were measured. For each treatment there were three replications, from each replication 5 no. of seedlings were measured randomly.

Critical difference (Cd) :

The analysis of variance table gives only a broad indication of performance of the concentration and time as well as their interactions on growth rate. But in order to get the clearer appraisal of the specific phenomena of the different treatment combinations as well as the different levels o f the main factors, the calculation o f the CD is necessary. CD was calculated as follows :

$$CD = \sqrt{\frac{ErrorMSSx2}{n}}$$ x 't' value at 5% or 1% level for Error Degrees of Freedom.

Where, n = Total unit / individual unit.

Where, n - Total unit / individual unit. The calculated CD was utilized in testing the difference between the three mean values as significant or not.

RAPHANUS SATIVUS (RADDISH)

Germination of *Raphanus Sativus* (RADDISH) seeds

The effect of *Parthenium* leaf extract on the germination of seeds has been recorded as per cent of germination.The histograms were drawn from the means . The extract inhibited the germination of *Rapahanus* seeds and the intensity o f increased with the rise in concentrations and also with the passage of time. Thus, after 120 hr the mean percentage of germination was recorded as 66.01, 63.06, 48.97 and 31.18 at the concentrations of 0,25, 50 and 100 per cent of the extract As the effect became so spectacular after 120 hr, the experiment was not continued beyond that period. The leaf extract initially inhibited germination of *Trigonella* seeds. But with the passage of time the inhibitory effect disappeared and after 96 hr there was cent per cent germination at all the concentrations .Germination of *Brassica* seeds were also inhibited by all the concentrations of the extract. However, with the passage of time the intensity of inhibition somewhat decreased but it was not alleviated to the stimulatory level. The analysis o f variance of the data reveals the effect of *Parthenium* leaf extract to be highly significant establishing its inhibitory and stimulatory effect on seeds germination. The time effect was also found to be highly significant which indicates that the percentage of germination varied during different time intervals. The interaction between time and concentrations was also significant.

Effect of *Parthenium* leaf extract on germination of *Raphams* seeds

Conc %	% Germination (after hrs)			Total for Conc.	Mean % of Germination
	72	96	120		
0	**49.00**	**58.60**	**64.10**	**171.70**	**57.23**
25	**54.50**	**60.50**	**61.10**	**176.0**	**58.67**
50	**36.60**	**49.80**	**46.50**	**132.9**	**44.3**
100	**05.40**	**21.30**	**29.90**	**56.6**	**18.87**

Shoot length

The experimental arrangements were the same as in the case of preceding experiments. The means of shoot length were calculated (Table 29 A, B, C) and the histograms were drawn using the means (Fig.29).

The extract proved to be inhibitory on growth of shoots in all the test crops. But the lowest concentration (25%) exhibited slight promontory effect on *Trigonella*. The inhibition was gradual with increase of extract concentrations. The mean lengths for different concentrations of the leaf extract for *Raphanus* and *Brassica* were 3.51, 2.83, 2.15, 1.13 cm after 120 hr. and 3.15, 2.99, 2.42, 2.19 cm after 144 hr respectively. The lengths of the *Trigonella* shoots were recorded as 4.31, 4.47, 3.81, 3.32 cm at concentrations of 0, 25, 50 and 100 per cent after 96 hr respectively.

On statistical analysis (Appendix Tables 29 D, E, F) the effect of the

Parthemium leaf extract emerged as highly significant establishing its inhibitory effect in the growth of shoot lengths during different time periods. The highly significant time effect is indicative of such a variation. The interaction between time and concentrations are also found to be significant establishing differential growth rate elicited by the extract during different time intervals.

Conc %	Shoot length (after hrs)			Total for Conc.	Mean of Shoot length
	72	96	120		
0	**1.01**	**1.66**	**3.44**	**6.11**	**2.04**
25	**0.98**	**1.37**	**2.56**	**4.91**	**1.64**
50	**0.56**	**1.12**	**2.10**	**3.78**	**1.26**
100	**0.12**	**0.49**	**1.12**	**1.73**	**0.58**

Root length

Effect of *Parthenium* leaf extract on root growth of the young seedling was examined. The means of growth were tabulated (Tables 30 A, B, C) and histograms were drawn from the mean.

The mean-tables and the histograms reveal the highly inhibitory effect of the extract on the test crop seedlings. The mean root lengths of *Raphartus* for concentrations of 0, 25, 50 and

100 per cent concentrations of the extract were recorded as 6.73, 4.29, 2.94, 1.04 cm after 120 hr. The mean root lengths of *Trigonella* were 3.42,3.05, 3.05,1.79 cm after 96 hr and in case of *Brassica* the root lengths were 3.64, 2.62, 1.69, 1.25 cm after 144 hr of treatment. The effect of retardation of growth gradually increased from lower to higher concentrations and also with the passage of time. Whatever may be the magnitude of inhibition caused by the extract, root length continued to go in comparison to their respective controls. On statistical analysis (Appendix Tables 30 D, E, F) the inhibitory effect of *Parthenium* leaf extract turned out to be highly significant. The effect o f time.was also found to be highly significant. This establishes the differential rate of growth during different time periods. The interaction between the effect of extract and time was also found to be highly significant establishing differential rate of inhibition during different time intervals under varying concentrations of the extract.

Germination of *Raphanus* seeds treated with *Parthenium* root extract:

The effect of *Parthenium* root extract on the germination of tested crop seeds was observed and the data were tabulated (Tables 31 A, B, C). The histograms were drawn from the means. The mean-tables and the histograms exhibit the effects o f *Parthenium* root extract to be highly inhibitory. Percentage of germination decreased with the increase of extract concentration in all the tested crop seeds. The

germination percentages at the concentrations of 0,25, 50 and 100 per cent in *Raphanus* and *Brassica* were recorded as 70.00, 54.45, 51.11, 50.01, and 91.67, 81.67, 76.67, 75.11 after 120 hr. For *Trigonella* seeds the germination percentages were 100.00, 95.56, 94.45, 94.45 and 92.22 after 96 hr of treatment for the same range of concentrations respectively. On statistical analysis of the data the highly inhibitory effect of *Partheinum* root extract turned out to be highly significant. The highly significant time reveals the fact that the germination percentage varied during different time periods. The interaction between the

two factors (effect x time) also emerged as highly significant establishing differential percentages of germination elicited by the extract during different time intervals.

Conc %	root length (after hrs)			Total for Conc.	Mean of root length
	72	96	120		
0	**0.87**	**2.44**	**5.60**	**8.91**	**2.97**
25	**0.59**	**1.27**	**2.48**	**4.34**	**1.45**
50	**0.51**	**1.20**	**2.12**	**3.83**	**1.28**
100	**0.20**	**0.62**	**1.48**	**2.30**	**0.77**

Discussion and Conclusions:

Parthenium plant extract at lower concentration (25%) induced the germination than control on *Raphanus* seeds after 144 hr of treatment. But at higher concentrations of the extract, less germination percentage was recorded. The root extract of *Parthenium* inhibited the germination of *Raphanus* seeds and the intensity increased with the increased concentrations.

Effect of *Parthenium hysterophorus* leaf extract, root extract and root leachate

Germination of seed

The leaf extract o f *Parthenium* significantly inhibited the germination of *Raphanus,* seeds within the time lag of 144 hr. of observations. Interestingly, the intensity of inhibition increased with the rise of leaf extract concentration. Thus at 100 per cent of concentration the germination percentage was very poor as compared to control. The root extract of *Parihemrum* inhibited the germination of *Raphanus,* seeds at all the concentrations of the applied. The rate of inhibition gradually increased with rise of extract concentrations. The leachate prepared from the *Parthenon* roots was effective in decreasing the germination of *Raphanus* seeds. In this case, higher concentration (100%) proved to be more inhibitory.

In this experiment the root extract of *Parthenium* was most effective than the root leachate on germination of seeds. *Parthenium* shoot leachate produced less inhibitory effects as compared to *Parthenium* shoot extract on germination of ground nut. The root and shoot , leaf and inflorescence extracts of *Parthenium* reduced the seed germination and seedling of *Raphanus*.

Growth of seedlings

Parthenium leaf extract significantly inhibited the shoot growth of *Raphanus* seedlings. The extract proved to be highly effective as an inhibitor for root growth of *Raphanus* seedling. The inhibition became

intense with the increased concentrations of the extract. The root extract of *Parthenium* also inhibited the shoot length of *Raphanus* seedlings. The gradual inhibition was observed in *Raphanus* seedlings. The root length of treated seedlings was significantly inhibited with the extract concentration from lower to higher. The root leachate of *Parthenium* was prepared after 72 hr and 24 hr and was applied to *Raphanus*. In both the cases the shoot length was inhibited of the seedlings by the leachate. The root length of seedlings also decreased with increased concentrations of the leachate. The ratio of inhibition was almost the same in both

types of leachate. The results obtained in the present experiment support the view that parthenin extracted from *hysterophorus* reduced the seed germination and
seedling growth.

The aqueous extracts significantly reduced germination, seedling length and seedling vigour index of all crops and increasing extract concentrations resulted in stronger decreases.

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