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ALLELOPATHIC EFFECTS OF *PARTHENIUM HYSTEROPHORUS* L.

I. EXUDATION OF INHIBITORS THROUGH ROOTS

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KEY WORDS

Allelopathy Exudation Growth inhibition Nodulation Parthenium Roots

SUMMARY

Association with *Parthenium hysterophorus* L. caused retarded growth and nodulation in bean (*Phaseolus vulgaris* var. 'Burpees Stringless') the inhibition decreasing with increasing distance from the weed. Leachate collected from Parthenium grown pots also caused similar inhibition in bean growth. The inhibitory nature of the root exudate was confirmed under sterile cultural conditions by its effect on wheat (*Triticum aestivum* var. 'UP301') seedling growth. At the rosette and flowering stage of the weed there was maximum exudation of inhibitors which remained active for about thirty days.

INTRODUCTION

Parthenium hysterophorus L. a tropical American weed has spread to almost all parts of India within two decades covering approximately 5 m hectares of land[5] including cultivated fields. The weed forms huge pure stands of several hectares in many situations and the ground studded with the cypsella and receptacles of the weed does not support any other vegetation. Such a situation was suggestive of, among other attributes like competitive ability, a possible allelopathic mechanism being operative. Several weeds like *Sorghum halopense*, *Salvia leucophylla* and *Artemisia californica* forming pure dense stands are also reported to exert allelopathic influence[1,7]. Preliminary trials conducted in our laboratory revealed the presence of growth inhibitors in the fruits and receptacles of the weed[12]. However, mere presence of inhibitors in the fruit or any part of the plant does not amount to allelopathy. Reports of the inhibitory nature of the root

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exudates of *Parthenium argentatum*[4] prompted the suspicion that the inhibitors in *P. hysterophorus* also got exuded to the substratum. Hence, studies were undertaken to test if such a mode of toxin release was operative in *P. hysterophorus* also.

MATERIALS AND METHODS

Influence of Parthenium association on bean

Seeds of bean (*Phaseolus vulgaris* L. var. 'Burpees Stringless') obtained from Standards Seeds Corporation, Bangalore were sown under field conditions at 10, 20 and 30 cm distance in six radial rows from healthy Parthenium plants which had just started flowering in five replications. Watering was done as required. The mean minimum and maximum temperature during the experimental period were 18 and 29°C respectively and mean relative humidity 63%. Seedlings emergence was noted and twenty day old seedlings were studied for nodule number, their fresh and dry weight and dry weight of shoot and root.

Effect of leachate from Parthenium grown soil on bean

Fortnight old Parthenium plants were transplanted one each to earthen pots of 30 cm diameter and 35 cm depth containing garden soil (2 part mud + 2 part horse dung manure + 1 part sand) and were grown for 30 days. When the plants were in the flowering stage the pots were drained. In the control set pots without Parthenium plants were used. To collect the exudate the pots were arranged in a row on a raised platform and water from a common source was made to trickle drop by drop into all the pots for forty minutes daily and the leachate that drained though the soil mass was collected through the out-let at the bottom of each pot.

Pots of 20 cm diameter and 30 cm depth filled with garden soil were seeded with bean and devided into three sets of five each. One of the sets was fed with leachate collected from Parthenium grown pots, the second with leachate from pots without Parthenium plants and the third with tap water not passed through the pots. This served as the control for any effect attributable to difference in the nutrient status of the drained water. Two bean plants were maintained in each pot. During the test period the mean minimum and maximum temperature were 20 and 25°C and mean relative humidity 68%. The emergence of bean seedlings was noted and 20-day old plants were studied as under the previous experiment.

Demonstration of allelopathic action in sand culture

Sand of mesh size 120 was washed as described by Hewitt[6] and taken in 500 ml Corning beakers and autoclaved at 15 lb pressure for 15 minutes. Parthenium plants were grown in the sterilised sand media and were fed daily with 50 ml of quarter strength Hoagland solution. After 30 days growth, the plants were removed carefully from the sand and wheat (*Triticum aestivum* L. var. 'UP 301') grains obtained from National Seeds Corporation were sown in them. Grains sown in sterilised sand in which no Parthenium plant was grown served as control. The set was replicated five times with five test plants in each container. Addition of nutrient solution was continued as before for ten days. During the period the mean minimum and maximum temperatures were 25– and 29°C respectively and the mean relative humidity 65%. Ten day old seedlings were harvested and dry weight of leaves and roots determined.

In a parallel set processed as above the sterilised sand in the Parthenium grown and control series was leached with 100 ml distilled water, concentrated to one forth the volume under reduced pressure and tested on the growth of wheat seedlings on blotters in 9 cm petri-dishes under laboratory light conditions and at a mean maximum and minimum temperature of 26°C and 18°C respectively and the mean relative humidity 65%. The dry weight of 72 h old seedlings was determined.

Leaching of inhibitors at different stages

Parthenium seedlings two in each were raised in pots with garden soil and were removed at the four-leaf, rosette and flowering stages. Bean seeds two in each were sown in these pots. Pots containing garden soil which were not used for growing Parthenium were used in the control. The set was replicated five times. During the experimental period the mean minimum and maximum temperatures were 18 and 29°C and mean relative humidity 63%. Twenty day old bean seedlings were studied for parameters as before.

Life of the inhibitors

Parthenium plants grown in five replications of two each were removed from the plots at the flowering stage and on 1,7, 15 and 30 days after removal, bean seeds were sown in the soil. Twenty day old plants, two per pot, grown under conditions mentioned in the preceding experiment were harvested and studied for parameters as mentioned earlier except nodule fresh weight.

Table 1. Inhibition of nodulation and root and shoot dry weight in *Phaseolus vulgaris* L. var. 'Burpees Stringless' grown with *Parthenium hysterophorus* L.

Exp. No.	Test species	Nodule number per plant	Nodule fresh weight (mg)	Nodule dry weight (mg)	Root dry weight (mg)	Shoot dry weight (g)
1.2	*Bean*					
	Radial distance from Parthenium (cm)					
	10	14* (1.2)	45* (4.5)	150* (2.5)	600* (0.02)	6** (0.3)
	20	22** (2.5)	60** (5.2)	251** (7.5)	950** (0.02)	7** (0.5)
	30	34** (4.5)	1050** (25.5)	500** (10.5)	1300** (0.50)	10** (1.2)
	200 (control)	43 (5.6)	1685 (30.3)	553 (12.5)	1400 (0.5)	11 (2.4)

Figures in parentheses refer to standard error.
*, ** Significantly different from the control respectively at 0.01, 0.05 level or better.

Table 2. Inhibition of nodulation and root and shoot dry weight in 20 day-old plants of *Phaseolus vulgaris* L. var. 'Burpees Stringless' fed with *Parthenium hysterophorus* L. – grown soil leachate

Treatment	Nodule number per plant	Nodule fresh weight (mg)	Nodule dry weight (mg)	Root dry weight (mg)	Shoot dry weight (g)
Control (Tap water)	20 (2.5)	142 (10.5)	49 (8.5)	760 (0.05)	9 (1.5)
(Control pot leachate)	21 (3.5)	140 (9.5)	50 (6.5)	780 (0.06)	9 (1.5)
Parthenium grown pot leachate	14** (3.5)	50* (8.5)	33* (4.5)	490* (0.09)	7** (1.5)

Figures in parentheses refer to standard error.
* ,** Significantly different from the control respectively at 0.01, 0.05 level or better.

Table 3. Growth of seedlings of *Triticum aestivum* L. var. 'UP301' in *Parthenium hysterophorus* L. – grown medium

Exp. no.	Medium	Root dry weight (mg)	Leaf dry weight (mg)
I.1.4	Control (Sterile sand)	26 (2.5)	18 (4.5)
	Treatment	16* (4.5)	15** (3.6)
I.1.4a	Control (Sterile sand extract)	3 (0.5)	3 (0.5)
	Treatment (Extract from Parthenium grown sand)	2** (0.6)	2** (0.8)
I.1.5	Control (Non-sterile soil)	28 (1.5)	20 (2.0)
	(Sterile soil)	27 (2.1)	20 (2.3)
	Parthenium grown soil		
	Non-sterile	22** (2.5)	18** (4.5)
	Sterilised	16* (4.5)	15** (3.5)

Figures in parentheses refer to standard error.
* ,** Significantly different from the control at 0.01, 0.05 level or better respectively.

RESULTS

Parthenium association caused retarded growth and nodulation in bean, the inhibition decreasing with increasing distance from the weed (Table 1). Leachate drained from Parthenium grown pots caused significant reduction in all the parameters studied in bean plants. Further, there was no significant difference in the performance of bean plants fed with tap water and leachate of garden soil without Parthenium (Table 2). The electrical conductivity of Parthenium grown pot leachate was 0.17×10^{-5} Mhos, that of control pot leachate 0.22×10^{-5} Mhos and of tap water 0.08×10^{-5} Mhos the difference between the former two cases being insignificant as to cause any difference in growth. The root and shoot dry weight of wheat seedlings raised in Parthenium grown sand medium as well as in leachate of the same medium were inhibited significantly (Table 3). Soil from which Parthenium plants were removed at the rosette and flowering stage caused greater inhibition of nodulation and root and shoot growth than that from which the weed was removed at the four leaf stage (Table 4). The inhibitors remained active for 15 days and with 30 days interval the inhibition was insignificant except in shoot dry weight (Table 5).

Table 4. Nodulation and root and shoot dry weight of 20 day-old plants of *Phaseolus vulgaris* L. var. 'Burpees Stringless' seedlings grown in medium that supported the growth of *Parthenium hysterophorus* L. up to different stage

Medium	Nodule number per plant	Nodule fresh weight (mg)	Nodule dry weight (mg)	Root dry weight (mg)	Shoot dry weight (g)
Control soil	22 (10.5)	461 (25.0)	170.0 (18.5)	140 (0.5)	11 (2.9)
Soil – after removing the weed at:					
4 leaf stage	21* (7.5)	460 (20.5)	170.0 (14.5)	140 (0.5)	11 (0.6)
Rosette stage	14* (2.5)	300** (190.0)	95* (14.8)	540* (0.1)	7* (1.8)
Flowering stage	14* (3.0)	290** (25.0)	90* (12.5)	510* (0.3)	7* (1.8)

Figures in parentheses refer to standard error.
*, ** Significantly different from the control at 0.01, 0.05 level or better respectively.

Table 5. Nodulation and root and shoot dry weight of 20 day-old plants of *Phaseolus vulgaris* L. var. 'Burpees Stringless' grown in medium left fallow for different periods after removing *Parthenium hysterophorus* L.

	Nodule number per plant	Nodule dry weight (mg)	Root dry weight (mg)	Shoot dry weight (g)
Control	40 (25.0)	195 (26.5)	1 (0.20)	10 (1.3)
Fallow period (days)				
1	13** (2.5)	100** (8.5)	0.5 (0.15)	6* (1.6)
7	17** (2.5)	125** (12.5)	0.6 (0.16)	8** (1.3)
15	20** (3.5)	115** (15.5)	0.6 (0.05)	8** (2.2)
30	38 (4.5)	190 (22.5)	0.9 (0.11)	9 (1.8)

Figures in parentheses refer to standard error.
* ,** Significantly different from the control respectively at 0.01, 0.05 level or better.

DISCUSSION

The exudation of inhibitory substances from Parthenium root is evidenced by the inhibition in nodule number, their fresh and dry weight and root and shoot dry weight in bean plants fed with water drained through Parthenium grown pots. As this experiment was conducted with non-sterile soil it was likely that microorganisms in the soil had altered the nature of root exudates making them inhibitory and thus the results did not prove the inhibitory nature of the root exudate itself. However, results of the experiments using sterilised sand clearly testify the nature of the Parthenium exudates.

Schreiner and associates[17,18] showed by testing the water extract of the soil in contact with wheat, oat, corn and cowpea on the growth of wheat, that the root exudates of several plants were inhibitory. Pickering[10,11] proved the inhibitory effects of water drained through soil supporting grass seedlings on the growth of apple seedlings. Lonner and Galston[4] clearly demonstrated the inhibitory nature of root exudates of *Parthenium argentatum* and identified the inhibitor as transcinnamic acid. There are several other reports of the inhibitory nature of root exudates[1,2,3,8,9,13,14,15,19]. Rice[15] showed the inhibitory effect of root exudates of *Ambrosia psilostachya*, *Euphorbia supina*, *Helianthus annuus* and three grass species on nodulation in legume when grown with the weeds. It is interesting to point out here that *Ambrosia psilostachya* belongs to the same subtribe as that of *P. hysterophorus*.

The inhibition in different growth parameters of bean grown with Parthenium is obviously the result of competition and allelopathy. In many studies on growth inhibition the effects have been attributed solely to competition without analysing the contribution by allelopathy. Rice[16] suggested that a comprehensive investigation should be undertaken to assess the magnitude of weed-crop chemical interaction under field conditions. Bell and Koeppe[2] experimenting on giant fox-tail (*Setaria faberii*) and corn interference, separated out the interference due to allelopathy from competition and stated that when competition was removed from the giant fox-tail-corn interference, the inhibition of corn growth dropped from 90 to 35%. They compared the 'single-pot-experiment' where corn plants were grown around the edge of pots in which dense population of giant fox-tail was maintained in the centre with the 'stair-step-experiment' where competition was totally eliminated. In the 'single-pot-experiment' good supply of nutrient solution was maintained and shade factor was also eliminated. The authors held that differences in growth were due to differential uptake of nutrients. But under abundant supply of nutrients the question of differential uptake of nutrients attributable to competition does not arise. Further, the authors have no data in support of the differential uptake which, even if

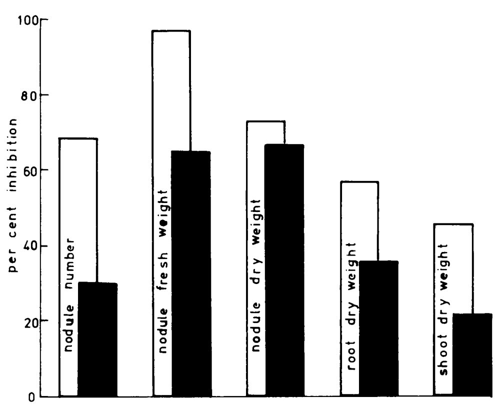

Fig. 1. Growth inhibition in 20 day-old plants of bean (*P. vulgaris* L. var. Burpees Stringless) attributable to allelopathy ■ and combined effect of allelopathy and competition □ due to *P. hysterophorus*.

it was there, could have been attributed to allelopathic effects. On these grounds, it may be stated that the inhibition due to competition claimed by the authors is open to question. In the present study also, keeping in mind the limitations of the method a comparison could be made of the results of experiments where bean plants were grown with Parthenium at different distances with experiments where bean plants were grown with the pot leachate of Parthenium. Here, the inhibition in the former experiment could be attributable to allelopathy + competition and in the latter to allelopathy. Such a comparison revealed that inhibition due to allelopathy was 30, 65, 67, 36 and 22% respectively in nodule number, nodule fresh weight, nodule dry weight and root and shoot dry weight (Fig. 1).

In Parthenium, high rate of exudation of inhibitors is attained at the rosette stage itself. The data of the experiments on life of the inhibitors revealed that after removing Parthenium plants, the inhibitors exuded to the soil had become almost ineffective by about 30 days (Table 5). However, under natural conditions the inhibition would be lasting as the exudation from the living Parthenium plant continues till the plant dries up.

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