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Evaluation of allelopathic potential of selected plant species on *Parthenium hysterophorus*

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Abstract

The phytotoxicity of shoot leachates of selected plant species was assessed on germination, and on shootcut and seedling bioassays of *Parthenium hysterophorus*. Shoot leachates of selected plant species were effective in inhibiting germination of *Parthenium* seeds, with *Azardirachta indica* the most effective. Shoot growth was inhibited most, and shoots damaged the most, by leachates of *Solanum nigrum*.

Keywords: Biochemical interaction, leachates, bioassay

Introduction

Congress grass, *Parthenium hysterophorus* L., also known as Peterson's Curse or Santa-Maria Feverfew, is an obnoxious weed which can cause a total habitat change in native grasslands, the understorey of open woodlands and along rivers and flood plains (Chippendale & Panetta 1994). One of the major detrimental effects of *Parthenium* weed and a reason for its aggressiveness is its allelopathic effect on other plants. Many researches have documented the potential of allelopathic plants to reduce pathogens and affect weed emergence. The most common effects of allelochemicals may occur through leaching, volatilization, root exudations and decay of the fallen parts either through biotic or abiotic means (Anaya *et al.* 1990).

In recent years, creating competition between native and alien species has gained momentum as a control method. Numerous plants are reported to possess allelopathic potential and efforts have been made to use them in weed control (Knox 2008). The chemical exudates from allelopathic plants are proposed to play a major role in the allelopathic mode of action. Higher plants release a diversity of allelochemicals into the environment, including phenolics, alkaloids, long-chain fatty acids, terpenoids and flavonoids (Rice 1984, Chou 1995). However, these compounds are available in low concentration in most allelopathic plants. Positive and negative allelopathic effects have been reported of *Parthenium* on many agricultural crops and other plant species (Oudhia *et al*. 1997, Aggarwal & Kohli 1992) and it inhibits the surrounding herbaceous vegetation (Nath 1988, Srivastava *et al.* 1985).

There are hundreds of secondary metabolites in the plant kingdom and many are known to be phytotoxic (Einhelling 2002). Allelopathic effects of these compounds are often observed to occur early in the life cycle, causing inhibition of seed germination and/or seedling growth. The compounds exhibit a wide range of mechanisms of action, on DNA (alkaloids), photosynthetic and mitochondrial function (quinones), and phytohormone activity, ion uptake and water balance (phenolics). In the course of an extensive survey carried out to assess the distribution of *Parthenium* in India in the years 1987-1990 (Aneja 1991), it was observed that *Parthenium* does not grow in proximity to particular plant species. This suggests a natural antagonism of these species towards *Parthenium*.

The first observation that antagonistic competitor plants could replace *P. hysterophorus* and therefore had potential for biological control appears to have been made by Singh (1983), who noted that *Cassia uniflora* moved into areas previously occupied by *Parthenium* in the Maharashtra state of India. In order to manage this obnoxious weed in an ecologically safe and effective manner, therefore, we are searching for plants with an antagonistic potential via the mechanism of allelopathy. Here we evaluate the allelopathic potential of shoot leachates of selected plant species in order to assess their feasibility as possible antagonist plants for the future management of *Parthenium*.

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Materials & Methods

The treatments consist of four factors (i) shoot leachates of seven selected plant species; and (ii) mode of treatment (pre- or post); then (iii) treated at two concentrations (50 and 100%) of the leachate, or the control distilled water; and using (iv) leachate obtained by soaking for three different durations (3, 6 or 9 days). These were variously applied to seeds, cut shoots or seedlings.

The shoots of *Azadirachta indica* Juss., *Solanum nigrum* L., *Calotropis procera* Willd., *Cymbopogon citratus* L., *Withania somnifera* L., *Cassia occidentalis* L. and *Ocimum sanctum* L. were collected haphazardly from various sites. Seeds of *Parthenium hysterophorus* were obtained from a heavily infested *Parthenium* field.

To produce the leachates, the upper part of shoot tips were collected, and 100 g soaked in 500 ml double-distilled water under aseptic conditions for 3, 6 and 9 days and placed in a conical flask and kept in a refrigerator at $8 \pm 1\ ^0$C. The aqueous leachates were filtered through three layers of muslin cloth/cheese cloth to remove debris. The filtrate was then re-filtered through one layer of Whatman no.1 filter paper. Leachates of 50% and 100% concentration were prepared with sterilized distilled water and used for bioassay.

For germination assays, viable seeds of *P. hysterophorus* were thoroughly washed with tap water to remove dirt and dust, and rinsed with mild detergent solution for 5-7 minutes. The seeds were surface-sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for 10 min and again washed with sterilized distilled water 4-7 times. For each treatment combination there were six replicates of 10 seeds each. Three factors were applied: leachate (seven levels - the test plant species), concentration (three levels, control, 50% and 100%), and mode of treatment (two levels, pre- or post-treated). Pre-treated groups were soaked in 10 ml of the relevant leachate/concentration combination for 4 h, and then placed in Petri dishes on filter paper moistened with distilled water. Post-treated groups had no advance treatment but were simply placed in Petri dishes on filter paper moistened with the relevant leachate/concentration combination. The relative humidity was 82 ± 1 %. Petri dishes were covered and placed in sealed polythene bags to prevent loss of volatilization of leachate, and kept undisturbed for 10 d at 25 ± 2 °C. Thus pre-treated groups were exposed to the leachate/concentration combination only for 4 hr, whereas post-treated groups were exposed for 10 d.

To test effects on shoot growth, 5-cm shoots of *Parthenium* with one or two inflorescences were taken, washed in tap water, dipped in 1% NaOCl solution for 3 min and the tips immediately washed in sterilized distilled water to remove any residual trace of the chemical. An inclined cut was made at the tip, and the shoots placed in test tubes containing 10 ml of shoot leachate, with cotton buds and aluminium foil to make airtight. The effect of the leachate was observed after 24, 48 and 72 h at room temperature. Phytotoxic damage was recorded on a scale (0-5, where 0 = no effect; 1 = slight chlorosis and necrosis; 3 = acute chlorosis and marked necrosis/drooping of entire twig; 4 = falling of flowers and leaves/high necrosis and chlorosis; 5 = acute chlorosis and very high necrosis leading to death of the whole shoot).

The impact on seedlings of *Parthenium* was assessed by raising seedlings in plastic pots containing sterilized soil, sand and peat (1:1:1) at room temperature (25 ± 1°C). These seedlings were sprayed with shoot leachate and assessed after 24, 48 and 72 h. Phytotoxic damage was recorded on a scale (0–4, where 0 = no effect; 1 = slight chlorosis; 2 = marked chlorosis; 3 = drooping of seedling; 4 = death of seedling).

Statistical analysis of the two- or three-way factorial design used Analysis of variance, with the 5% level of significance.

Results

The shoot leachates of selected plant species reduced the germination of *Parthenium hysterophorus*. Among pre-treated seeds, the maximum inhibition was observed for *Cassia occidentalis*, where only 10% germinated in both 50% and 100% concentrations (Table 1), followed by *Solanum nigrum* and *Ocimum sanctum* at 30% germination at the higher concentration. Reasonably high inhibition of germination occurred for shoot leachates of *Ocimum sanctum* (50% concentration) and *Azadirachta indica* (100% concentration). The other species moderately inhibited the germination of *P. hysterophorus* seeds at the higher concentration. Therefore, the order of severity is *C.occidentalis* > *S.nigrum* > *O.sanctum* > *A.indica* > *W.somnifera* > *C.procera* > *C.citratus*.

Table 1: Effect of shoot leachates of selected plant species at different concentrations on percent germination of *Parthenium hysterophorus* seeds (either pre- or post-treated). n=6 for each group. The negative control group of distilled water had a germination of 90%. C.D. = critical difference at the 5% level of significance.

Plants	pre-treated seeds			post-treated seeds		
	50%	100%	mean ± se	50%	100%	mean ± se
Azadirachta indica	60	40	63.3 ± 2.3	20	0	36.7 ± 2.1
Solanum nigrum	30	30	50.0 ± 1.1	63.3	56.7	70.0 ± 1.0
Calotropis procera	80	53.3	74.4 ± 1.0	46.7	46.7	61.1 ± 2.2
Cymbopogon citratus	76.7	63.3	76.7 ± 1.8	46.7	26.7	54.4 ± 1.8
Withania somnifera	56.7	50	65.6 ± 1.3	50	23.3	54.4 ± 1.7
Cassia occidentalis	10	10	36.7 ± 2.8	70	70	76.7 ± 1.5
Ocimum sanctum	40	30	53.3 ± 1.7	50	50	63.3 ± 2.2
Mean	50.5	39.5	60.0 ± 1.9	49.5	39.0	59.5 ± 1.9
		df	F	C.D.		
Control vs Concentration treatments		1	87.87***	9.7		
Concentrations (50% vs 100%)		1	35.55***	9.5		
Plants x Treatment x Concentrations		6	2.39*	13.5		

Among post-treated seeds, the maximum inhibition of germination of *Parthenium* was observed in *A.indica* at the higher concentration (Table 1), where no germination was observed at all; at 50% concentration, only 20% of seeds germinated. From the data of Table 1, the order of severity is *A. indica* > *C.citratus* > *W.somnifera* > *C.procera* > *O.sanctum* > *S.nigrum* > *C.occidentalis*. The highest germination of *Parthenium* seeds was observed in the control (90%). Overall, post-treatment was slightly more effective than pre-treatment; 100% concentration is significant over 50% concentration.

Phytotoxic damage inflicted on shoots of *Parthenium* by the shoot leachates of *S.nigrum* was the highest, followed by *C.citratus* (Table 2). The damage was most pronounced in the strongest leachate applied for the longest time at the highest concentration. Distilled water (control) caused no damage at all.

Maximum phytotoxic damage to seedlings (Table 3) was observed in shoot leachates of *S.nigrum*, followed by *C.citratus*. However, the severity of *A.indica* was much more pronounced after 48 h as compared to *C.citratus* at 50% (C_1) concentration but the data does not vary significantly(P>0.05). Distilled water (control) caused no damage at all.

Table 2: Effect of shoot leachates of selected plant species on shoots of *Parthenium hysterophorus* in a shoot-cut bioassay (assessed on a 0-5 scale). The leachates were obtained by soaking for 3, 6 or 9 days, and applied two concentrations (50% and 100%), and assessed after 24, 48 and 72 h. The negative control group had distilled water. C.D. = critical difference at the 5% level of significance.

Plant species	soaking time	after 24 h 50%	after 24 h 100%	after 48 h 50%	after 48 h 100%	after 72 h 50%	after 72 h 100%	Mean
Azadirachta indica	3 days	0.33	0.33	1.00	1.00	1.67	2.00	1.05 ± 0.23
	6 days	0.33	0.33	2.00	2.00	2.00	2.00	1.44 ± 0.35
	9 days	0.67	1.00	2.00	2.00	2.00	3.00	1.77 ± 0.61
Solanum nigrum	3 days	1.00	2.00	2.00	2.33	2.00	3.00	2.05 ± 0.85
	6 days	1.00	2.00	2.00	2.00	3.00	3.00	2.16 ± 0.81
	9 days	2.00	2.00	2.00	3.00	2.00	3.00	2.33 ± 0.75
Calotropis procera	3 days	0.00	0.00	0.33	0.33	1.00	1.00	0.44 ± 0.31
	6 days	0.00	0.00	0.67	1.00	1.00	1.00	0.61 ± 0.28
	9 days	0.00	0.00	1.67	1.67	2.00	2.00	1.22 ± 0.21
Cymbopogon citratus	3 days	0.00	0.67	1.00	1.00	1.67	2.00	1.05 ± 0.22
	6 days	0.33	1.00	1.00	1.00	2.00	2.00	1.22 ± 0.71
	9 days	0.67	1.00	1.67	2.00	3.00	3.67	2.00 ± 0.78
Withania somnifera	3 days	0.00	0.00	0.67	1.00	1.00	1.00	0.61 ± 0.60
	6 days	0.00	0.00	1.00	1.00	1.00	1.00	0.66 ± 0.50
	9 days	0.67	1.00	1.67	2.33	2.00	3.00	1.77 ± 0.61
Cassia occidentalis	3 days	0.33	0.33	0.67	0.67	1.33	1.67	0.83 ± 0.42
	6 days	0.33	0.33	0.67	0.67	1.67	1.67	0.89 ± 0.30
	9 days	0.33	0.67	0.67	1.00	1.67	2.00	1.05 ± 0.20
Ocimum sanctum	3 days	0.00	0.00	0.00	0.00	0.33	0.33	0.11 ± 0.05
	6 days	0.00	0.33	0.00	0.33	0.33	0.33	0.22 ± 0.10
	9 days	0.33	0.33	0.33	0.33	1.00	1.00	0.55 ± 0.39
Mean		0.39	0.63	1.09	1.26	1.60	1.88	1.14 ± 0.80
Control				0.00 ± 0.00				

	df	F value	C.D.
Control v/s Concentration treatments	1	498.4***	0.642
Plants × Days × Hours × Concentrations	24	6.4***	0.905

Table 3: Effect of shoot leachates of selected plant species on shoot damage of *Parthenium hysterophorus* in a seedling bioassay (assessed on a 0-4 scale). The leachates were obtained by soaking for 3, 6 or 9 days and applied at two concentrations (50% and 100%), and assessed after 24, 48 and 72 h. The negative control group had distilled water. C.D. = critical difference at the 5% level of significance.

		after 24 h		after 48 h		after 72 h		
Plant species	soaking time	50%	100%	50%	100%	50%	100%	Mean
Azadirachta indica	3 days	1.33	1.33	2.00	2.00	2.67	3.00	2.06 ± 1.00
	6 days	1.33	1.33	3.00	3.00	3.00	3.00	2.44 ± 0.92
	9 days	1.67	2.00	3.00	3.00	3.00	4.00	2.78 ± 1.02
Solanum nigrum	3 days	2.00	3.00	3.00	3.33	3.00	4.00	3.06 ± 1.85
	6 days	2.00	3.00	3.00	3.00	4.00	4.00	3.17 ± 1.72
	9 days	3.00	3.00	3.00	4.00	3.00	4.00	3.33 ± 1.89
Calotropis procera	3 days	1.00	1.00	1.33	1.33	2.00	2.00	1.44 ± 0.85
	6 days	1.00	1.00	1.67	2.00	2.00	2.00	1.61 ± 0.72
	9 days	1.00	1.00	2.67	2.67	3.00	3.00	2.22 ± 1.05
Cymbopogon citratus	3 days	1.00	1.67	2.00	2.00	2.67	3.00	2.06 ± 1.02
	6 days	1.67	2.00	2.00	2.00	3.00	3.00	2.28 ± 1.28
	9 days	1.67	2.00	2.67	3.00	4.00	4.00	2.89 ± 0.70
Withania somnifera	3 days	1.00	1.00	1.67	2.00	2.00	2.00	1.61 ± 0.23
	6 days	1.00	1.00	2.00	2.00	2.00	2.00	1.67 ± 0.15
	9 days	1.67	2.00	2.67	3.33	3.00	4.00	2.78 ± 1.05
Cassia occidentalis	3 days	1.33	1.33	1.67	1.67	2.33	2.67	1.83 ± 0.18
	6 days	1.33	1.33	1.67	1.67	2.67	2.67	1.89 ± 0.23
	9 days	1.33	1.67	1.67	1.67	2.67	3.00	2.06 ± 1.03
Ocimum sanctum	3 days	1.00	1.00	1.00	2.00	2.67	1.33	1.11 ± 0.09
	6 days	1.00	1.33	1.00	1.00	1.33	1.33	1.22 ± 0.75
	9 days	1.33	1.33	1.33	1.33	2.00	2.00	1.56 ± 0.63
Mean		1.41	1.63	2.09	2.27	2.60	2.86	2.15 ± 1.10
Control					0.00 ± 0.00			

	df	F value	C.D.
Control v/s Treatment	1	1015.8***	0.605
Plants × Days × Hours × Concentrations	24	6.7***	0.852

Discussion

Some plants are already known to have potential in combating *Parthenium hysterophorus.* Anjum *et al.* **(**2005) concluded that an aqueous extract of *Imperata cylindrica* may restrict germination and seedling growth. The herbicidal potential of leaf leachates of plants such as *Cymbopogon citratus*, *Withania somnifera* and *Calotropis procera* have been assessed before; the effects of *C.citratus* were pronounced (Knox & Paul 2007). Foliar leachates of *Cassia* and then *Rumex* were the most effective in reducing levels of various leaf chemicals (Jaggi *et al.* 2008). Aqueous extracts of *Ocimum americanum* significantly inhibited the germination and seedling growth of *Parthenium* (Singh & Thaper 2002). The allelochemicals present in *Azadirachta indica* have inhibitory effects on germination and seedling growth of *Parthenium* and *Amaranthus* (Sindhu *et al.* 2004). Aqueous foliar leachates of *Azadirachta indica, Prosopis juliflora, P. cineraria* inhibit seed germination of *Parthenium* by more than 95 percent (Dhawan *et al.*1994). Several other plants such as *Tephrosia purpurea, Amaranthus spinosus, A.viridis, Hyptis savolensis, Sida cordifolia* and other species of *Cassia* have been found to suppress *Parthenium* (Anonymous 1992, Thapar & Singh 2003). In an experiment by Senthil *et al.* (2004), complete inhibition of *Parthenium* seed germination occurred using aqueous foliar extracts of *Cassia occidentalis, Andrographis paniculata, Abutilon indicum* and *Hyptis suaveolens.* All the species tested, inhibited the early seedling growth and fresh weight of the seedlings, with the greatest effect by *Cassia sericea* and *Hyptis suaveolens*.

Allelopathic properties can be used successfully as a tool for weed reduction. During field surveys in some sites *Cassia occidentalis, Calotropis procera, Chenopodium album, Croton bonplandianum, Abutilon theophrasti* and *Acacia arabica* replaced *Parthenium* in patches. Mamatha & Mahadevappa (1988, 1992) based on their preliminary surveys reported that *Cassia sericea*, *C. tora*, *Tephrosia purpurea* and *Croton bonplandianum* restricted *Parthenium* invasion in many states in India. Joshi & Mahadevappa (1986) reported that *Cassia occidentalis* has successfully displaced the weed in Dharwad and surrounding areas without any human intervention. Native ecofriendly weeds such as *Croton bonplandianum* and *Amaranthus spinosus* may do the same (Singh & Thaper 2002, Swain *et al*. 2004). Weeds such as *Achyranthes aspera, Datura stramonium, Calotropis procera, Cassia occidentalis,* etc., are commonly found in close to *Parthenium,* but the main competitor was *C. occidentalis* (Knox *et al*. 2006).

The mode of action might be by inhibiting respiration (Rice 1984) and energy transfer (Moreland & Novitzky 1985) responsible for ATP synthesis, or perhaps inhibiting gibberellin and IAA-induced growth. Chlorosis and necrosis cause loss of chlorophyll from leaves. Tanak *et al*. (1993) reported that mycotoxins cause photobleaching of chloroplasts, and allelochemicals may do the same. Polyphenolic compounds and other allelochemicals are known to change the permeability of plasma membrane, leading to cell leakage (Abbas *et al*. 1992). Inhibition of photosynthetic process depletes food reserves, and then proteins and other compounds can serve as respiratory substrates (Taiz & Zeiger 1998: 228-9). There was further decrease in carbohydrate and protein contents which might cause the death of plant. The depletion of carbohydrate and protein in higher concentration of allelochemicals indicate the magnitude of inhibition of assimilatory and biosynthetic processes.

We conclude that particular species (*Cassia occidentalis, Azadirachta indica, Solanum nigrum)* are good candidates for future field experiments to determine whether plant competition can control *Parthenium hysterophorus*.

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