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Effect of *Parthenium hysterophorus* L. Root Extracts on Seed Germination and Growth of Maize and Barley

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ABSTRACT

We studied the allelopathic effect of *Parthenium hysterophorus* on the germination and shoot growth of maize (*Zea mays*) and barley (*Hordeum vulgare*) in the laboratory. Both cereal species were negatively affected by the aqueous root extracts of parthenium. As the concentration of parthenium weed root extracts increased, so the germination, shoot length and root length of maize and barley decreased. Experiments, which were conducted under two different temperature regimes, indicated that both species reacted differently to the same concentrations as a direct influence of temperature. These preliminary findings indicate that a more extensive study is required to fully explore the allelopathic potential of parthenium weed extracts under various factors so that these may be used effectively against different weeds.

Keywords: allelopathy, *Hordeum vulgare*, parthenium, weed, *Zea mays*

INTRODUCTION

There is increasing interest in plant allelopathy to protect crops from detrimental effects of allelochemicals. Similarly, the use of allelochemicals against weeds, an environmentally friendly approach to suppress the germination and growth of many weeds, is gaining popularity. Due to drawbacks in other methods of weed control and increasing reliance on herbicides there is an increasing threat to biodiversity and the environment. Therefore, plant-derived chemicals are considered comparatively safer to the environment. The idea that plants affect neighboring plants by releasing chemicals in the environment has been known since 370 BC (Willis 1985, 1997). Plant-derived chemicals may proved to be an effective tool of weed management in the future as many plant-derived allelochemicals cause germination failure and reduce biomass in many weeds and crops (Khan *et al.* 2004, 2005). Over the past two decades, much more work has been done on plant-derived compounds as environmentally safe alternatives to herbicides for weed control (Duke *et al.* 2002). These chemicals could be used for weed management directly or their chemistry could be used to develop new herbicides. Some allelopathic compounds that are reported to play a role in weed management are: allyl isothiocyanate (black mustard, *Brassica nigra*), fatty acids (buck wheat, *Fagopyrum Esculentum*), isoflavonoids and phenolics (clovers, *Trifolium* spp.; sweet clover, *Melilotus* spp.), phenolic acids and scopoletin (oat, *Avena sativa* L.), hydroxamic acids (cereals), phenolic acids, dhurrin, sorgoleone (sorghum, *Sorghum bicolor*) (Weston 1996). Crop residues can provide selective weed control through their physical presence on the soil surface and through the release of allelochemicals (Weston 1996; Inderjit and Keating 1999). Putnam (1983, 1988) termed allelochemicals as "nature's own herbicides". Duke *et al.* (2002) discussed that natural compounds have several benefits over synthetic compounds. For example, natural compounds may have a novel structure due to the diversity of molecular structures. This diversity is because synthetic chemists have been biased toward certain types of chemistry.

Globally parthenium weed (*Parthenium hysterophorus* L.) is considered a noxious weed. In India, parthenium weed causes yield losses of up to 40% in several crops (Khosla and Sobti 1979) and it is reported to reduce forage production by up to 90% (Nath 1981). Chippendale and Panetta (1994) reported that in Queensland, parthenium weed commonly dominates cultivated and other disturbed areas and its presence in cropped lands can almost double cultivation costs and restrict the sale and movement of contaminated produce.

Adkins (1996) reported that in Australia it has become widespread in grazing land from central Queensland to northern New South Wales. It causes direct losses to the grazing industry (about $A 14-18 million per annum) and is a human health hazard, causing allergic rhinitis and contact dermatitis. The inhibitory effects of *P. hysterophorus* on germination of many crops have been reported (Narwal 1994). The widespread occurrence of this weed may be attributed to its aggressive behaviour, very high seed production potential and suppressive effects on neighbouring plants through allelopathic interactions (Evans 1997) and drastically retards the growth of many species (Tefera 2002). *P. hysterophorus* can form dense, pure stands underneath plant residues, and seeds accumulate and render the soil unsuitable for other vegetation (Kohli and Batish 1994). Xuan *et al.* (2005) reported that allelopathic properties of plants can be exploited successfully as a tool for pathogen and weed reduction. For over a decade, preliminary surveys of a few hundred allelopathic plants in the Southeast Asia and Japan ecosystems were made, and more than 30 species, including crops which exhibited the greatest allelopathic potential, were selected and examined for their impacts on the emergence of pathogens and weeds. Primary conclusions were that the application of these plant materials at 1–2 tons ha^{-1} can reduce weed biomass by about 70%, and increase rice yield by about 20%. Khan *et al.* (2005) reported that

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water extracts of *Eucalyptus camaldulensis*, *Prosopis juliflora* and *Acacia nilotica* significantly affected the germination and growth of wheat and wild oat. Inam and Hussain (1988) reported that *Silybum marianum* aqueous extracts from leaves, stems, inflorescence and roots, rain leachates, litter and soil collected from underneath significantly decreased the germination and early growth of mustard (*Brassica* sp.), cucumber, wheat and sorghum in various bioassays.

In Pakistan, this weed species can be found in many districts in irrigated as well as in rain-fed areas and is rapidly spreading (Shabir and Bajwa 2006). Presently parthenium weed can be found along roadsides and even in agricultural crop like maize in North-west, Pakistan. This weed has taken the shape of a noxious weed and is becoming a threat to crop production, animal husbandry and human health due to its strong allelopathic effects. Amin *et al.* (2007) reported that the water extracts of parthenium weed plants significantly decreased the germination and growth of wheat and associated weeds. Meo and Khan (2005) concluded that the morphology of mature pollen grains of *P. hysterophorus* collected from Islamabad and Rawalpindi indicated that it is an allergy-causing weed and that pollen grains are radially symmetrical, isopolar, trizonocolporate, non-lacunate and echinate. They further suggested to check and eradicate its distribution in Rawalpindi and Islamabad due to its significant allergenic potential.

Keeping in view the importance of the allelopathic potential of *P. hysterophorus*, we conducted experiments under laboratory conditions with the objective of investigating the allelopathic status of parthenium weed by monitoring the growth response of *Zea mays* and *Hordeum vulgare* to water extracts from the roots of *P. hysterophorus* L. at various levels.

MATERIALS AND METHODS

Two runs of laboratory-based experiments were conducted during July, 2007 (first run) and October, 2007 (second run) in Weed Research Laboratory, Department of Weed Science, NWFP Agricultural University Peshawar, Pakistan, to investigate the allelopathic effects of root extracts (water) of *P. hysterophorus* on seed germination, and growth of maize (*Z. mays* cv. 'Azam') and barley (*H. vulgare* cv. 'Frontier-87'). The seeds of crops were kept in shade till its use. These varieties are commercial and grown on a large area in North-west, Pakistan. *P. hysterophorus* plants were collected from an infested area at the flowering stage in February, 2007 and shade dried. The roots were ground and the ground dry powder of *P. hysterophous* roots was soaked for 24 hours in tap water at room temperature without shaking. Ground powder were soaked in water at 10, 50 and 100 g L^{-1} and these extracts were used as concentrations (treatments) after filtered through a double layer of muslin cloth followed by a Whatman no. 1 filter paper. The experiment was laid out in Completely Randomized Design at room temperature (28 ± 2 and 20 ± 2°C during July and October, respectively). Ten healthy seeds of each crop were placed in 10-cm diameter Petri dishes on blotting paper and moistened with 5 ml of extracts. Tap water was used as the control. There were four treatments i.e. 0% (control), 10, 50 and 100 g L^{-1}, replicated three times. Five mL of the same concentration of root extract was applied to Petri dishes when needed, except for the control. The water extracts were placed in a cool and dark place to avoid photochemical decomposition and the Petri dishes were randomized after every two days to avoid any environmental effects. After 10 days, the effect of different concentrations of root extracts was measured on seed germination percentage, shoot length plant^{-1} (cm) and root length (cm) plant^{-1} of the two test species (i.e. *Z. mays* and *H. valgare*), using an electronic balance and graduated scale. The entire experiment was repeated once. As also observed in a previous study (Amin *et al.* 2007), there was no fungus attack therefore no fungicide was applied to the seeds. Temperature data was recorded by fixing a thermometer in the laboratory. The data collected were subjected to analysis of variance using MSTATC computer software. Means were separated using least significance difference (LSD) test at P<5% (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Germination percentage of maize

Statistical analysis of the data showed that different concentrations and times had non-significant effect on germination percentage of maize (**Table 1**). Means of the concentrations showed that maximum germination was recorded in the control and decreased with the increasing concentration. Parthenium weed extract slightly inhibited maize seed germination, inhibition being more pronounced in the second run that was conducted in October 2007 probably due to the difference in temperature. Overall with the increasing concentration the germination of maize decreased in both runs. The present findings confirm the presence of allelochemicals in the root extracts of parthenium. Einhelling (1995) reported that allelophatic inhibition typically results from a combination of allelochemicals which interfere with several physiological processes in the receiving plant or microorganism. Thus these extracts can be effectively used for suppressing many weed species. However, still there is a lack of information how to use this as a bioherbicide under field conditions. The present findings revealed that parthenium weed can be used as a source of bioherbicides in the future although there is still a lack of research in this branch of science. More research will lead us to better understand and interpret results. The parthenium weed extracts may act differently in different locations depending on the origin of the collection. Several researchers have reported that allelochemicals in this plant are origin-dependent. Variations in the composition of sesquiterpene lactones were observed as a result of the origin of *P. hysterophorus* populations (Picman and Towers 1982). Similarly, in an investigation of aerial plant parts two major classes of putative water-soluble allelochemicals, phenolic acids and sesquiterpene lactones, were isolated (Kanchan and Jayachandra 1980). Hence the infestation of parthenium weed accumulates toxic chemical substances in the soil and thus adversely affects subsequent crops.

Shoot length of maize

Shoot length data shown in **Table 2** indicates that concentration means were significantly (P≤0.05) while time means and their interaction were non-significantly affected. Among the concentration means maximum shoot length was recorded in control followed by all other concentrations. However these concentrations were statistically at par with each other. Analogous results were reported by Amin *et al.* (2007). They reported that with increasing concentration of parthenium weed extract, the shoot length of weeds decreased significantly. The inhibition may not be same for all species as the inhibitory magnitude of plants is species-de-

Table 1 Effect of parthenium root extracts on germination (%) of maize.

Concentration (g L^{-1})	Time: Run I	Time: Run II	Concentration means
0	86.66	80.00	83.33
10	90.00	86.66	83.33
50	66.66	46.66	56.66
100	70.00	36.66	53.33
Time means	78.33	62.50	

Table 2 Effect of parthenium root extracts on shoot length (cm) of maize.

Concentration (g L^{-1})	Time: Run I	Time: Run II	Concentration means
0	4.53	2.50	3.52 a
10	1.77	2.67	2.22 b
50	1.73	1.49	1.61 b
100	1.75	0.65	1.20 b
Time means	2.44	1.83	

LSD value for concentration means = 1.114

Values followed by different letters are significantly different at P≤ 0.05 level according to LSD test.

pendent with varietal differences (Xuan *et al.* 2003; Hong *et al.* 2004). Overall the data indicated that with increasing concentration of parthenium weed root extracts the shoot length decreased in both the first and the second run although non-significantly. The present results suggest that a higher concentration of parthenium weed extract is more harmful than lower concentrations. Thus the areas where the parthenium weed densities are higher can pose a serious problem for succeeding crops. The dense stands of parthenium weed will be able to accumulate more allelochemicals in the soil and thus pollute the soil environment. Singh *et al.* (2003) reported that with increasing concentration of parthenium weed, the inhibition of radish and chickpea also increased. Stimulatory effect of parthenium extracts on many crops and weeds present interesting phenomena that draw the attention of scientists. Amin *et al.* (2007) reported that the response of different species to parthenium weed extracts was different. The phenomenon of low dose stimulation and high dose inhibition are known as hormesis. In a similar study, Belz *et al.* (2007) reported some stimulatory effect of parthenium on other plant species and reported that at present, it remains speculative what caused the observed stimulatory effects. They further added that the observed stimulatory effects of leaf extracts of *P. hysterophorus* were probably mostly attributable to parthenin, although in general the hormetic effect was less pronounced for parthenin in leaf extracts. Hence a parthenium infestation should be discouraged in croplands as it can negatively affect the germination of many crop species. Even during a short extraction period, the contribution of nutrients to the observed hormetic effects at low extract concentrations cannot be excluded, since low levels of nutrients can promote the root growth of test plant species as well (Belz and Hurle 2004).

Root length of maize

Like shoot length, root length of maize was non-significantly affected by different concentrations and time. However, keeping in view the allelopathic character of parthenium, the root length of maize decreased with the increasing concentrations (**Table 3**). In the first run, the temperature was favourable for seed germination of maize therefore it might be the reason that values for maize are greater during first run as compared to second run. Allelochemicals can affect germination in a variety of ways and can be broadly classified as plant phenolics and terpenoids, which show great chemical diversity and are involved in a number of metabolic and ecological processes and parthenium weed residues are known to exhibit a high degree of phytotoxicity (Kanchan and Jayachandra 1980), which is largely attributed to the presence of water-soluble allelochemicals including phenolics and the sesquiterpene lactone parthenin (Picman and Picman 1984).

However, it seems that not only the concentrations but the temperature always plays an important role in allelopathy. Kohli *et al.* (1996) observed that even if no inhibition in germination by leaf extracts occurred, the initiation of germination was considerably delayed for species such as *Cyamopsis tetragonoloba* (L.) Taub. or *Trifolium alexandrinum* L. Delayed germination was also reported from investigations with infested soils (Kohli and Batish 1994). Thus it can be concluded from the present results that parthenium weed extracts are harmful for crop germination and any accumulation in the soil can adversely affect the germination of crops in the soil where parthenium weed stands are present. Parthenium weed effect on seed germination is a complex phenomenon and needs to be fully explored so that these allelochemcials can be selectively used for weed control or for stimulation of crop growth and germination.

Table 3 Effect of parthenium root extracts on root length (cm) of maize.

Concentration ($g\ L^{-1}$)	Time: Run I	Time: Run II	Concentration means
0	8.16	3.93	6.05
10	3.55	4.92	4.23
50	4.07	2.91	3.49
100	2.64	1.73	2.18
Time means	4.60	3.37	

Germination of barley

Data pertaining germination of barley indicated that concentration means were non-significantly while run and their interation was significantly ($P<0.05$) affected (**Table 4**). Still there was consistent affect of different concentrations on the germination percentage of barley. During second run, the temperature was lower therefore barley germination was greater as compared to first run. However, under higher temperature the allelopathic effects of concentration were greater, which provides an opportunity to control weeds through allelochemicals by exploring various temperature regimes. At highest concentrations the germination percentage of barley during the first run ceased completely and was greatly suppressed. Thus it can be concluded that at higher concentrations the inhibitory effect of parthenium weed extract is greater. Heavy infestation of parthenium weed in an area might thus accumulate allelochemicals to a greater degree in the soil, which would adversely affect crops. Similarly allelochemicals present in the soil can affect the subsequent crops and weed germination. However, further extensive studies should be undertaken to explore the allelopathic potential of parthenium weed on other crops. Singh *et al.* (2003) reported that the extracts prepared from unburnt (UR) and burnt (BR) residues of *P. hysterophorus* affected the growth of two winter crops, radish and chickpea: both unburnt UR and BR were toxic, inhibiting completely the seedling length and reducing the dry weight of the test crops. Meanwhile, it has been established that *P. hysterophorus* releases phytotoxic compounds by root exudation, leaching from vegetative parts of living plants and decaying plant residues (Kanchan and Jayachandra 1980) as well as from the achenecomplex (Picman and Picman 1984; Reinhardt *et al.* 2004).

Table 4 Effect of parthenium root extracts on germination (%) of barley.

Concentration ($g\ L^{-1}$)	Time: Run I	Time: Run II	Concentration means
0	40.00 bc	93.33 a	66.66
10	23.33 c	96.66 a	60.00
50	0.00 d	83.33 a	41.66
100	0.00 d	50.00 b	25.00
Time means	15.83 b	80.83 a	

LSD value for interaction means = 18.70
LSD value for time means = 26.46
Values followed by different letters are significantly different at $P\leq 0.05$ level according to LSD test.

Shoot length of barley

Concentration means and run means were significantly ($P<0.05$) while interaction of run and concentration was non-significant for shoot length of barley (**Table 5**). Maximum shoot length was recorded in control and then decreased with the increasing concentrations of parthenium root extracts. Similarly the effect of concentration during first run was greater as compared to second run. These results depicted that temperature also plays a vital role in manipulating the effect of allelochemicals on the seed germination. By fully exploring this nature of allelochemicals and seed interaction a meaningful weed control method could be developed. We conclude that the effect of parthenium weed root extracts was more inhibitory in first run that was conducted in July 2007. Analogous results were reported by many researchers. The inhibitory magnitude of plants is species-dependent with varietal differences (Xuan *et al.* 2003; Hong *et al.* 2004). Overall the data indicated that with increasing concentration of parthenium weed root extracts the shoot length decreased in both the first and the

Table 5 Effect of parthenium root extracts on shoot length (cm) of barley.

Concentration ($g\ L^{-1}$)	Time Run I	Time Run II	Concentration means
0	6.93	7.51	7.22 a
10	2.18	7.35	4.77 b
50	0.00	4.03	2.01 c
100	0.00	2.02	1.01 c
Time means	2.27 b	5.23 a	

LSD value for concentration means = 1.808
LSD value for time means = 2.56
Values followed by different letters are significantly different at P≤ 0.05 level according to LSD test.

second run. Amin *et al.* (2007) reported similar results. They reported that with increasing concentration of parthenium weed extract, the shoot length of weeds decreased significantly. The present results suggest that a higher concentration of parthenium weed extract is more harmful than lower concentrations. Thus the areas where the parthenium weed densities are higher can pose a serious problem for succeeding crops. The dense stands of parthenium weed will be able to accumulate more allelochemicals in the soil and thus pollute the soil environment. Singh *et al.* (2003) reported that with increasing concentration of parthenium weed, the inhibition of radish and chickpea increased. The increasing concentration and decreasing trend in barley traits was consistent throughout the study. However the temperature effect was more pronounced in the present study which suggests further exploration of allelochemicals under a series of experiments and various temperatures. However, in the second run barley produced longer shoots, which might be due to the fact that October is more favourable for barley. As there are various chemicals found in parthenium weed and their mode of action and inhibition potential differ under different conditions therefore detailed and more extensive studies will be helpful in formulating a weed management package where parthenium weed extracts are used as a component. Species-specific differences in the sensitivity to aqueous extracts of fresh or dry leaf material of *P. hysterophorus* were reported in previous studies (Kohli *et al.* 1996).

Root length of barley

Statistical analysis of the data indicated that concentration means, run means and their interaction had significant ($P<0.05$) effect on the root length of barley (**Table 6**). At a higher concentration of parthenium root extracts root length of barley ceased during the first run and was greatly suppressed during the second run. Similarly, a higher concentration had a great influence on the root length of barley. As roots are the primary parts that regulate the plant body therefore any negative response of a crop to allelochemicals provides evidence to be vigilant. However by using these allelochemicals against weeds can provide a novel method of weed control in the future and parthenium weed could be used as a bioherbicide for effectively killing other weeds. Similarly, the stimulatory effect on seed germination can also be explored to use this weed as a resource of plant growth regulators. However, much more work is needed to use parthenium root extract under field conditions on a commercial scale for crop production and crop protection as well. Since a higher concentration of parthenium weed extract retarded the growth of the test species, a parthenium weed infestation should be prevented, making the soil free of the accumulation of allelochemicals that retard the growth of agricultural crops. Belz *et al.* (2007) reported that the sesquiterpene lactone parthenin which is biosynthesized by *Parthenium* species is thought to play a role in its allelopathic interference with surrounding plants. However, despite the fact that parthenin is released from various plant parts into the soil, little is known about its relative contribution to overall allelopathic effects. They further added that species differed considerably in their sensitivity to leaf extracts: whereby *A. conyzoides* was most sensitive with ED50-values for root length of 24.8 mg FM/ml and 53.6 mg FM/ml for inhibition of germination. The effects of parthenin on germination were on average more than three times lower. Stronger effects of parthenin on root growth than on germination were previously reported by Batish *et al.* (2002).

Although Belz *et al*. (2007) reported a stimulatory effect of parthenium weed on other plant species, it remains speculative what caused these observed stimulatory effects, although root length in the present experiments was not affected. Belz *et al*. (2007) further added that the observed stimulatory effects of leaf extracts of *P. hysterophorus* were probably mostly attributable to parthenin. At present, it remains speculative as to what masked the hormetic effect of parthenin in leaf extracts. Synergistic effects among extract constituents could have enhanced the phytotoxicity of parthenin, or other extract constituents with greater phytotoxicity could have interfered with parthenin hormesis.

Table 6 Effect of parthenium root extracts on root length (cm) of barley.

Concentration ($g\ L^{-1}$)	Time Run I	Time Run II	Concentration means
0	5.02 a	4.90 a	4.96 a
10	1.94 b	4.28 a	3.11 b
50	0.00 c	2.13 b	1.06 c
100	0.00 c	1.23 b	0.61 c
Time means	1.74 b	3.13 a	

LSD value for concentration means = 0.715
LSD value for time means = 1.013
Values followed by different letters are significantly different at P≤ 0.05 level according to LSD test.

CONCLUSIONS

Parthenium hysterophorus has the potential to adversely affect crops, resulting in low yields due to the release of allelochemicals into the soil. Dense stands of *P. hysterophorus* can release these chemicals and thus can affect the germination and growth of subsequent crops. These allelochemicals could be used as a resource i.e. bioherbicide for weed control. The negative effect of this species is concentration dependent. At higher concentrations, this species will cause more losses to crop plants than lower concentrations. A pure stand of parthenium weed may be due to its strong allolopathic effect on neighbouring plants and thus its infestation should be discouraged in crop and rangelands to avoid the accumulation of allelochemicals in the soil. Further intensive studies are needed to explore the full knowledge of allelopathy of such invasive weeds. Overall the results indicated that barley was more sensitive to the parthenium weed extracts than maize. This might be due to species response to allelochemicals or different inherent response. Several researchers have claimed that the response of different species to allelochemicals is different. Amin *et al.* (2007) reported that *Lepidium* sp. was more sensitive than wheat. These are encouraging results and could lead to a better understanding of crop/weed competition because the exploration of the field of alleopathy provides unlimited opportunities for agricultural scientists since allelochemicals can be used both for weed and pest control. The harmful impact of allelopathy can be exploited for pest and weed control (Narwal 1994; Kohli *et al.* 1998). The effects of parthenin on germination were on average more than three times lower. Stronger effects of parthenin on root growth than on germination were previously reported by Batish *et al.* (2002).

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