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Evaluation of antifungal activity of aqueous extracts of two asteraceous plant species

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Abstract

The antifungal activities of shoot and root extracts of two Asteraceous plants species viz. *Parthenium hysterophorus* L. and *Ageratum conyzoides* were determined against *Macrophomina phaseolina* (Tassi) Goid., the cause of charcoal rot disease of sunflower (*Helianthus annus* L.). A measured reduction in *M. phaseolina* biomass was observed due to aqueous extracts of different concentrations. Lowest concentration of 2% of both root and shoot extract of *P. hysterophorus* markedly suppressed the biomass. Whereas in case of *A. conyzoides* 4% of both root and shoot extract was proved most effective.

Key words: *Parthenium hysterophorus, Ageratum conyzoides,* antifungal activity, *Macrophomina phaseolina*

Introduction

Macrophomina phaseolina, a soil-borne fungus causes charcoal rot and other root rot infections. The fungus can infect the root and lower stem of over 500 plant crops as sunflower, soybeans, corn, and sorghum (Dhingra and Sinclair, 1977, White, 1999, Das *et al.*, 2007). Charcoal rot is one of the destructive diseases of sunflower. It generally appears after flowering but seedling blights have also been reported. Symptoms on stalks appear as silver-gray lesions near the base and eventually decay the stem and tap root, leaving a shredded appearance. Stems become hollow, rotted, and may lodge easily. Plants show poor seed fill, premature ripening, and undersized heads. Yield and oil content of seeds become reduced. Sclerotia develope on decayed tissues giving the stalks a charred appearance.

To avoid the implication of yield losses due to plant diseases, variety of control measures presently are in use. Chemical control of *M. phaseolina* is not much effective and economical because the pathogen is seed borne and difficult to eradicate. This situation demands an alternative approach for management of seed-borne and other saprophytic and parasitic fungi. Scientists are on their way to achieve some plant derived compounds to control diseases (Singh *et al.,* 2004**)**. Natural products or plant derived compounds contribute to a great extent in fighting against pathogenic microorganisms (Vyvyan, 2002). Aqueous extract of many allelopathic plants are known to exhibit antifungal properties. Plant extracts or plant-derived compounds are likely to provide a valuable source of new medicinal agents (Carvalho and Ferreira, 2001; Kayser and Kiderlen, 2001) and the urgent need for alternative treatment has led to screen natural products for potential use in the therapy of leishmaniasis and fungal infections. Bajwa *et al*. (2001) found inhibitory potential in aqueous extracts of three asteraceous allelopathic species against growth of *Aspergillus niger*. Recently Shafique *et al.* (2005) have reported that the aqueous extracts of allelopathic plants have considerable potential to control seed borne mycoflora. With the objective to contribute to these studies, the antifungal activity of crude extracts obtained from *P. hysterophorus* and *A. conyzoides* was investigated against *M. phaseolina*.

Materials and Methods

Fresh samples of *P. hysterophorus* and *A. conyzoides* were collected from Experimental Station, Mycology & Plant Pathology Department, University of the Punjab, Lahore, Pakistan and washed thoroughly under running tap water, dried with blotting paper and cut into small pieces. The soluble ingredients of the plant material were then extracted by solubilization in water. Aqueous extraction of water soluble ingredients of plant material was carried out according to Bajwa *et al.* (2004). A 50% w/v stock solution of plant extract was obtained by soaking the crushed plant materials in sterilized

distilled water for 48 h at 30±2 °C. It was then passed through muslin cloth and finally through Whatman Filter Paper No.1 under aseptic conditions. The extracts were stored at 4 °C in sterilized flasks. To avoid contamination and prospective chemical alterations, the extracts were used within 3 to 4 days. Aqueous extract bioassays were carried out in liquid medium. The basal medium employed to grow the test fungus was 2% malt extract (ME) medium in 250 mL conical flasks. To avoid bacterial contamination, antibacterial Chloromycetin capsules @ 1 capsule 100 mL^{-1} of medium were used. The lower concentrations of 2, 4 and 6% aqueous extracts were prepared by adding appropriate quantity of sterilized distilled water. To 80 mL of ME, 20 mL of each of 2–6% extract of each plant sample was added. Control received the same quantity of distilled water. Inoculum discs of 5 mm diameter, obtained from 7-days old actively growing fungal cultures of *M. phaseolina* was transferred to flasks aseptically and incubated at 25±2 °C. The mycelial biomass from triplicate samples for each treatment was collected on pre-weighed filter papers after 10 days. Their dry weight yield was determined after 24 h oven drying at 60 °C (Bajwa *et al.*, 2006).

Standard errors of means of three replicates of each treatment were computed using computer software Microsoft Excel. All the data were analyzed by analysis of variance (ANOVA) using computer software SPSS. Following the ANOVA, Duncan's Multiple Range Test (Steel and Torrie, 1980) was applied to separate the treatment means using Computer software COSTAT.

Results and Discussion

The data tested through ANOVA revealed that the effect of plant species (S), plant part (P) and extract concentration (C) were highly significant ($P \leq 0.001$) for dry fungal biomass production. The interactive effect of S×C was also significant for the studied parameter. However, the interactive effects of S×P, P×C and S×P×C were insignificant (Table 1).

Effect of aqueous extract of *P. hysterophorus* on fungal biomass:

The data on dry biomass production in the growth of 10 days after incubation revealed an excessive interference of aqueous extract with the growth of the test fungal species. The statistically significant arrest in growth was evidenced at all concentrations (2-6%) of shoot extract of *P. hysterophorus* in comparison to control, however, 2% concentration proved most effective than other concentrations. (Fig. 1 A). The maximum allelopathic stress was induced by 2% concentration of shoot extract causing a decline of about 72% (Fig. 2A) in the biomass production of *M. phaseolina*.

Investigative studies of root extract of *P. hysterophorus* revealed that statistically significant reduction was obtained in biomass of fungus in all the treatments as compared to control. Whereas the response of change in concentration was insignificant as 68, 65.32 and 69.34 % reduction was exhibited by 2, 4 and 6 % concentrations of root extract of *P. hysterophorus*, respectively (Fig. 1 A & 2 A).

Effect of aqueous extract of *A. conyzoides* on fungal biomass:

In case of aqueous shoot extract of *A. conyzoides*, the mycelial yield of the test fungus species was found to be significantly depressed in experimental treatments. The relative intensity of this effect, however, was found to vary with the concentration of the extract employed and maximum reduction in biomass production was evidenced at 4% concentration (Fig. 1B). There was 48, 69 and 59% reduction in biomass of *M. phaseolina* due to various concentrations (2–6%) of aqueous shoot extract of *A. conyzoides* (Fig. 2 B).

The antifungal activity of root extract of *A. conyzoides* also depicted similar pattern of growth inhibition as that of shoot extract. The lowest concentration of 2 % of aqueous root extract exhibited a persistent negative effect on the fungal biomass production. In contrast to lower concentrations, where the allelopathic stress was very obvious in terms of arrested dry biomass increments, 6% depicted less negative effect on biomass production but significantly lower than control (Fig. 1B).

In the present study, aqueous shoot and root extracts of *P. hysterophorus* and *A. conyzoides* were used against *M. phaseolina*, a pathogenic fungus. The results of this conceptual study clearly reflect that the test weeds have inherent ability to induce allelopathic effects on mycelial growth rate of fungi. In a similar study, recently Amin and Javaid (2007) evaluated antifungal activity of aqueous leaf, stem, root and inflorescence extracts of three *Chenopodium* spp. viz. *C. album* L., *C. murale* L. and *C. ambrosioides* L. against *M. phaseolina* and reported a significant reduction in the biomass of target fungal species.

Aqueous extracts of both parts of *P. hysterophorus* and *A. conyzoides* significantly

reduced the fungal biomass. However, variability among the aqueous extracts of different parts was evident. This reduction in dry fungal biomass in the presence of allelochemicals could probably be attributed to the rate of mitosis (Cornman, 1946) and enhanced cellular respiration (Singh and Kohli, 1999). Different concentrations of both shoot and root extracts of *P. hysterophorus* declined fungal biomass by 50-72% and 65-69%, respectively whereas in case of shoot and root extracts of *A. conyzoides* 48-69% and 48-71% reduction in biomass production was evidenced, respectively. These findings are in line with the work conducted by Shafique *et al.* (2006) who reported 60% reduction in incidence of *Alternaria alternata* on wheat due to aqueous leaf extract of *C. album.* Earlier Bajwa *et al.* (2004) reported the allelopathic potential of *P. hysterophorus*, against three pathogenic fungal species viz. *Drechslera hawaiiensis, Alternaria alternata* and *Fusarium monilifrome*. The significant growth inhibition was exhibited at lower concentrations.

It is concluded from the present study that aqueous extracts have fungitoxic potential and are the best and economical way to control *M. phaseolina* which is a very destructive pathogen of sunflower.

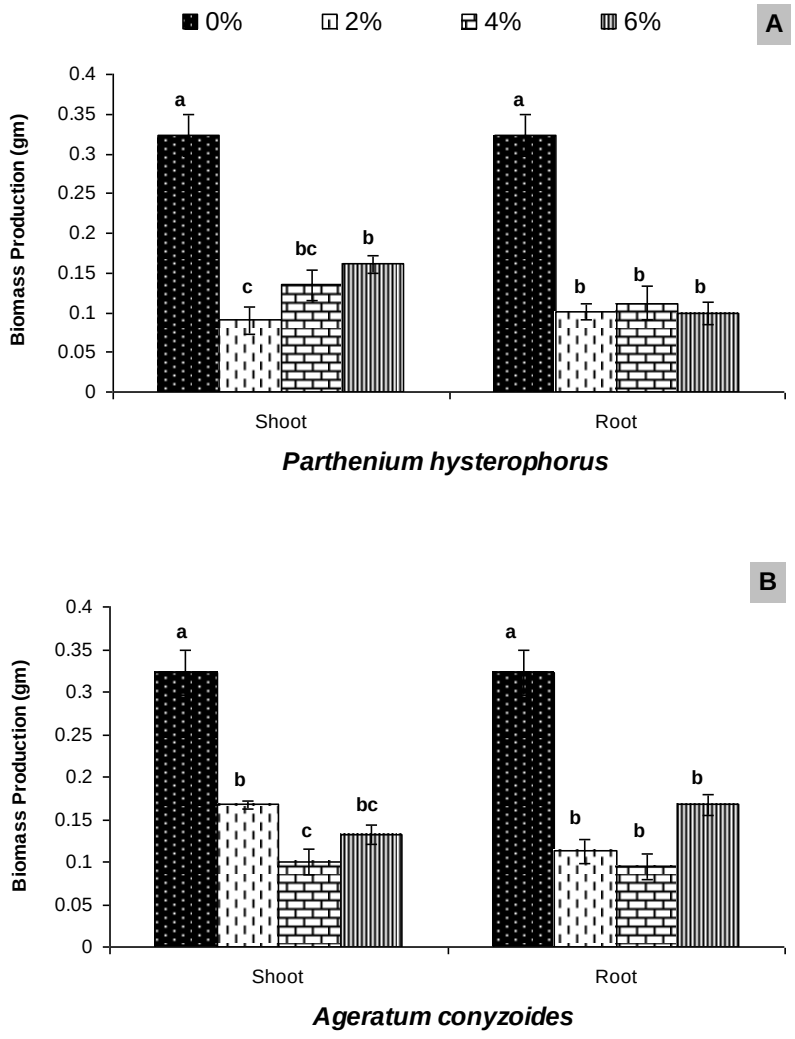

Fig. 1: Effect of different concentrations of aqueous extract of *Parthenium hysterophorus* and *Ageratum conyzoides* on dry biomass production of *Macrophomina phaseolina* after 10 days of incubation.
Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference (P≤0.05) as determined by DMR test.

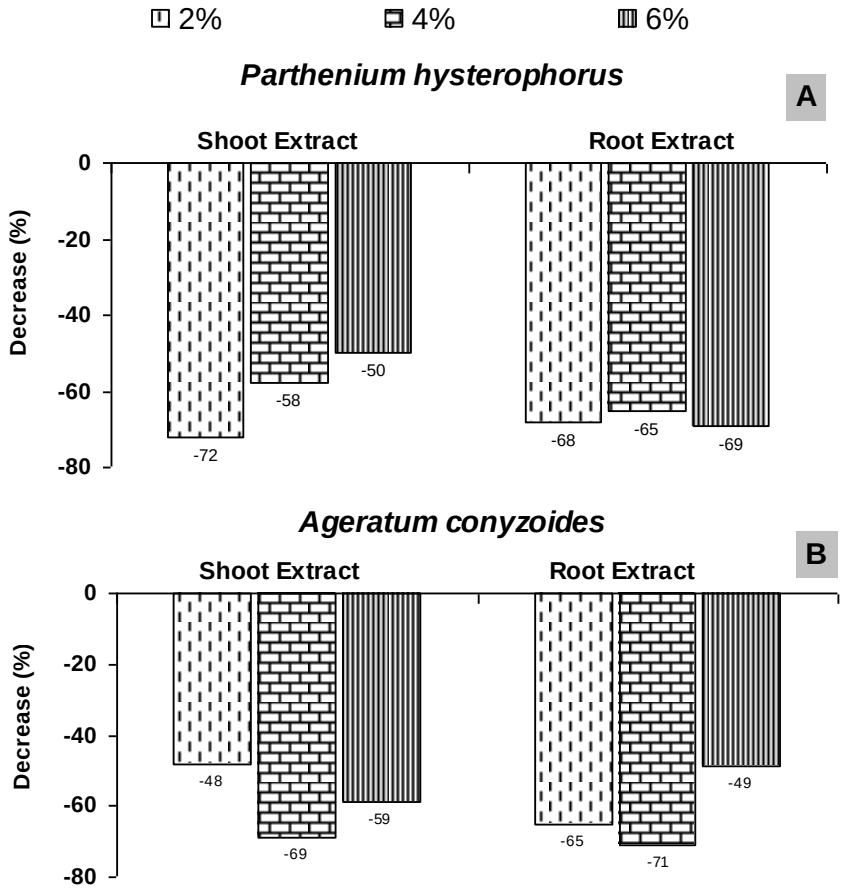

Fig. 2: Percentage decrease in biomass of *Macrophomina phaseolina* due to different concentrations of aqueous root and shoot extracts of *Parthenium hysterophorus* and *Ageratum conyzoides*.

Table 1: Analysis of variance (ANOVA) for the effect of different concentrations of aqueous root and shoot extracts of *Parthenium hysterophorus* and *Ageratum conyzoides.*

Source of variance	df	Sum of Squares	Mean Square	F values
Treatments	15	4.07×10^{-2}	2.71×10^{-2}	26.7^{**}
Plant species (S)	15	8.33×10^{-6}	8.33×10^{-6}	0.008^{**}
Plant part (P)	1	5.2×10^{-3}	5.2×10^{-3}	5.131^{*}
Extract conc. (C)	3	3.85×10^{-1}	1.28×10^{-1}	126^{**}
S × P	1	5.63×10^{-5}	5.63×10^{-5}	0.055^{ns}
S × C	3	8.7×10^{-3}	2.9×10^{-3}	2.856^{*}
P × C	3	3.56×10^{-3}	1.2×10^{-3}	1.17^{ns}
S × P × C	3	4.02×10^{-3}	1.34×10^{-3}	1.32^{ns}
Error	32	3.25×10^{-2}	1.01×10^{-3}	
Total	48	1.806		

*,**, significant at P≤0.05 and 0.001, respectively
ns: non-significant

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