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Full Length Research Paper

Evaluation of antifungal activity of methanolic extracts of *Dicanthium annulatum* for the management of *Macrophomina phaseolina*

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The present study was carried out to assess the antifungal potential of an allelopathic grass *Dicanthium annulatum* (Forssk.) Stapf. against *Macrophomina phaseolina* (Tassi) Goid. isolated from cowpea [*Vigna unguiculata* (L.) Walp.] plants suffering from charcoal rot disease. Different parts of this grass namely shoot, root and inflorescence were extracted in methanol. After evaporation of methanol, different concentrations (0, 0.5, 1.0, …, 3.0 g ml^{-1}) of the extracts were prepared and their antifungal activity was studied. In general, extracts of all the three parts exhibited antifungal activity. However, a marked variation in antifungal activity among the extracts of different parts of the test grass was observed. There was 7 to 51%, 29 to 71% and 33 to 81% reduction in fungal biomass due to different concentrations of shoot, root and inflorescence extracts of *D. annulatum*, respectively.

Key words: Antifungal activity, charcoal rot, *Dicanthium annulatum*, *Macrophomina phaseolina,* methanolic extracts.

INTRODUCTION

Allelopathic interactions have been widely reported both in wild (Bajwa et al., 1997; Sarah et al., 2011), as well as in cultivated members of family Poaceae (Cheema and Khaliq, 2000; Xu et al., 2012). Allelopathy is considered to be one of the promising options for sustainable pest management (Khanh et al., 2007; Zahid et al., 2012). Several published reports have confirmed the allelopathic activity of family Poaceae owing to occurrence of phenolic compounds, hydroxamic acids and flavonoids (Sanchez-Moreiras et al., 2003; Adams et al., 2010; Scrivanti et al., 2011). It has been reported that water soluble ability of such allelotoxins facilitates to reach the immediate habitat by various mechanisms (Samreen et al., 2009; Hisashi et al., 2009).

Dicanthium annulatum (Forsk.) Stapf, commonly known as marvel grass is an important perennial grass species of tropical and subtropical regions (Dabadghao and Shankarnarayan, 1973). It is famous as a range grass in the moist low land and plains of Pakistan exhibiting allelopathic activity against the susceptible plant species (Dirvi and Hussain, 1979). The grass is utilized as forage due to its easy and cheaper development from seed (Kumar et al., 2008). There are reports of exploiting allelopathic potential of this grass for the management of weeds (Javaid and Anjum, 2005), and some plant pathogenic fungi namely *Fusarium oxysporum* and *Fusarium solani* (Bajwa et al., 2001; Shafique et al., 2004). However, there is no report regarding antifungal activity of this grass against devastating soil- and seed-borne plant pathogenic fungus *Macrophomina phaseolina.* This fungus is responsible for charcoal rot disease in about 500 plant species belonging to more than 100 plant families (Babu, 2007). Amongst its 500 host, 67 have been reported from Pakistan (Shehzad et al., 1988). Yield losses due to *M. phaseolina* may reach up to 100% depending on plant species (Iqbal et al., 2010). Keeping in view the allopathic potential of *D. annulatum*, current investigation was designed to asses the antifungal potential of methanolic extracts of root,

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shoot and inflorescence of this weed against most notorious plant pathogen *M. phaseolina*.

MATERIALS AND METHODS

Isolation and culturing of *Macrophomina phaseolina*

Charcoal rot infected cowpea [*Vigna unguiculata* (L.) Walp.] plants were obtained from National Agricultural Research Centre (NARC), Islamabad, Pakistan. Infected stem portions of the plants were surface sterilized with 1% sodium hypochlorite solution followed by thorough washing with autoclaved water. These surface sterilized pieces were placed on malt extract agar (MEA) medium under aseptic conditions and the plates were incubated at 28°C in the dark for 1 week. The isolated fungal pathogen was sub-cultured on MEA in 9 cm diameter Petri plates for culture purification. Identification was done on the bases of colony colour and characteristics microsclerotia (Wyllie, 1993; Watanabe, 2002). The pure culture was stored in refrigerator at 4°C.

Bioassays with methanolic extracts

Shoots (leaves and stems), inflorescence and roots of *D. annulatum* were collected from University of the Punjab, Quaid-e-Azam Campus Lahore, Pakistan. Fresh plant materials were thoroughly rinsed with tap water and dried in an electrical oven at 45°C. These dried materials were crushed and grinded to fine powder. Two hundred g of each of the three powdered plant parts were soaked in 2 L methanol in air tight glass jars separately for 7 days at room temperature. Afterwards, extracts were obtained from soaked materials by filtering through muslin cloth followed by filter papers and preserved in plastic bottles. Filtrates were evaporated in rotary evaporator under vacuum to reduce the volume to 20 ml and poured in open wide mouth pots and placed in an oven at 40°C to completely evaporate the methanol. Clean sterilized and pre-weighed small glass bottles were used to store the extracts. Bottles were weighed again to get the weight of extracts by subtracting the weight of empty bottles.

In vitro bioassays were carried out with methanolic extracts of shoots, roots and inflorescence. Crude methanolic extracts (8.4 g) of each of the three different parts of the test grass species were dissolved in 2 ml dimethyl sulphoxide (DMSO) and added sterilized distilled water to prepare 14 ml of stock solution. Seventy six ml malt extract (ME) broth was autoclaved at 121°C for 30 min in 250 ml conical flasks and cooled at room temperature. Chloromycetin at 50 mg 100 ml^{-1} of the medium was added to avoid bacterial contamination. Six concentrations (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 g) 100 ml^{-1} were made by adding 0.67, 1.332, 1.998, 2.664, 3.33 and 3.99 ml stock solution and 3.33, 2.668, 2.002, 1.336, 0.67 and 0.01 ml solution of distilled autoclaved water and DMSO (2 ml DMSO + 12 ml H_2O), respectively, to each flask to make total volume of the medium 80 ml. The 80 ml of each treatment was divided into four equal portions in 100 ml conical flasks to serve as replicates. Control treatment was prepared by adding 4 ml solution of DMSO + distilled water to 76 ml of ME broth.

Mycelial discs of 5 mm diameter were removed from the edge of 1 week old actively growing culture of *M. phaseolina* using a sterilized cork borer and put in each conical flask. Flasks were incubated for 10 days in an incubator at 20 ± 2°C. Fungal harvest was taken by filtering the fungal mat through pre weighed Whatman No. 1 filter papers followed by oven drying to gain dry biomass from each flask (Figure 1B), and then filter papers with biomass were weighed again. Fungal growth was measured by subtracting the weight of filter paper from the weight of both fungal mass plus filter paper (Javaid et al., 2012).

Percentage growth inhibition of the fungal biomass was calculated by applying the following formula:

$$\text{Growth inhibition}(\%) = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

Statistical analysis

All the data were analyzed by analysis of variance (ANOVA) followed by Duncan's multiple range test to delineate the treatment means (Steel and Torrie, 1980), using computer software SPSS.

RESULTS AND DISCUSSION

ANOVA reveals that the effect of different parts of the test grass species (P), extract concentrations (C) as well as their interaction (P×C) was significant ($p \leq 0.001$) for fungal biomass (Table 1).

Effect of shoot extract

The effect of methanolic shoot extract of *D. annulatum* on biomass of *M. phaseolina* is shown in Figures 1A and 2. In general, all the concentrations of the extract suppressed fungal biomass. There was a gradual reduction in fungal biomass with the increase in the extract concentration. Lower concentrations of the extract namely 0.5, 1.0 and 1.5% exhibited non-significant effect which decreased fungal biomass by 7, 15 and 19% over control, respectively.

In contrast, the effect of higher concentrations of 2.0 to 3.0% was significant where 29 to 51% reduction in fungal biomass was recorded as compared to control. Earlier, Shafique et al. (2004) have reported up to 15% reduction in biomass of *F. solani* due to aqueous extracts of shoots of *D. annulatum*. Similarly, Bajwa et al. (2001) reported antifungal activity of aqueous extracts of shoots of *D. annulatum* against *F. oxysporum* and *Fusarium moniliforme.*

Effect of root extract

The effect of methanolic root extract of *D. annulatum* on biomass of *M. phaseolina* is shown in Figures 1B and 2. All the concentrations of the methanolic root extract showed significant adverse effect on the biomass of *M. phaseolina.* The lowest concentration of 0.5% exhibited the highest adverse effect and decreased fungal biomass by 71% as compared to control. In general, fungal biomass was increased by increasing extract concentration. There was 29 to 59% reduction in fungal biomass due to 1.0 to 3.0% extract concentrations over control. Javaid et al. (1996) studied the effects of root exudates of this grass on colonization of mycorrhizal fungi in 11 annual and 3 perennial weed species. All the

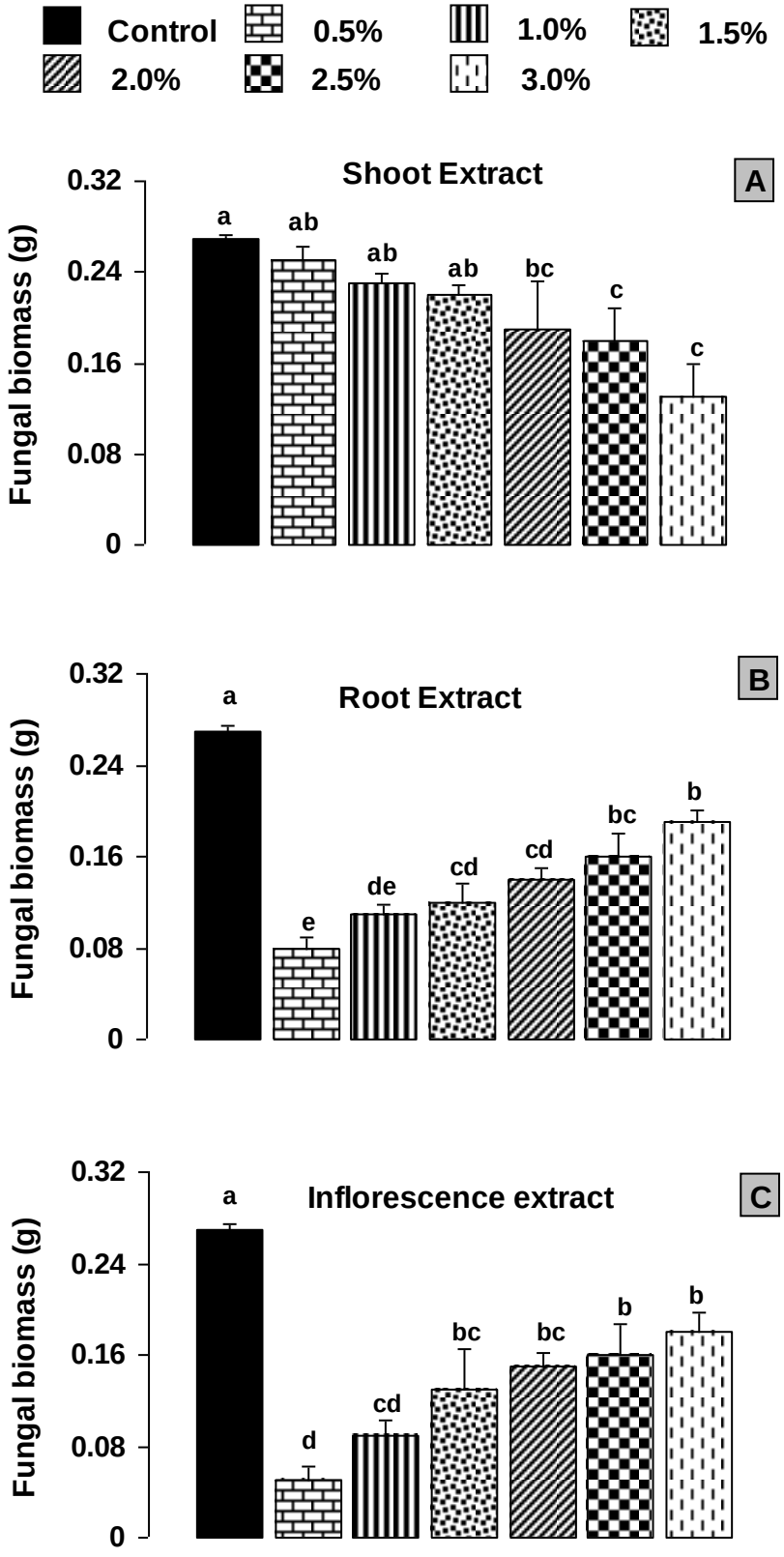

Figure 1. Effect of different concentrations of methanol extract of shoot, inflorescence and root of *D. annulatum* on biomass of *M. phaseolina*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Duncan's multiple range test.

Table 1. Analysis of variance for the effect of different concentrations of methanol shoot, inflorescence and root extracts of *D. annulatum* on *in vitro* growth of *M. phaseolina*.

Sources of variation	df	SS	MS	F values
Treatments	20	0.320	0.0159	10.8*
Plant parts (P)	2	0.046	0.0234	15.8*
Extract concentration (C)	6	0.156	0.0260	17.6*
P × C	12	0.116	0.0097	6.5*
Error	63	0.093	0.0014	
Total	84	2.820		

*, Significant at $P \leq 0.001$.

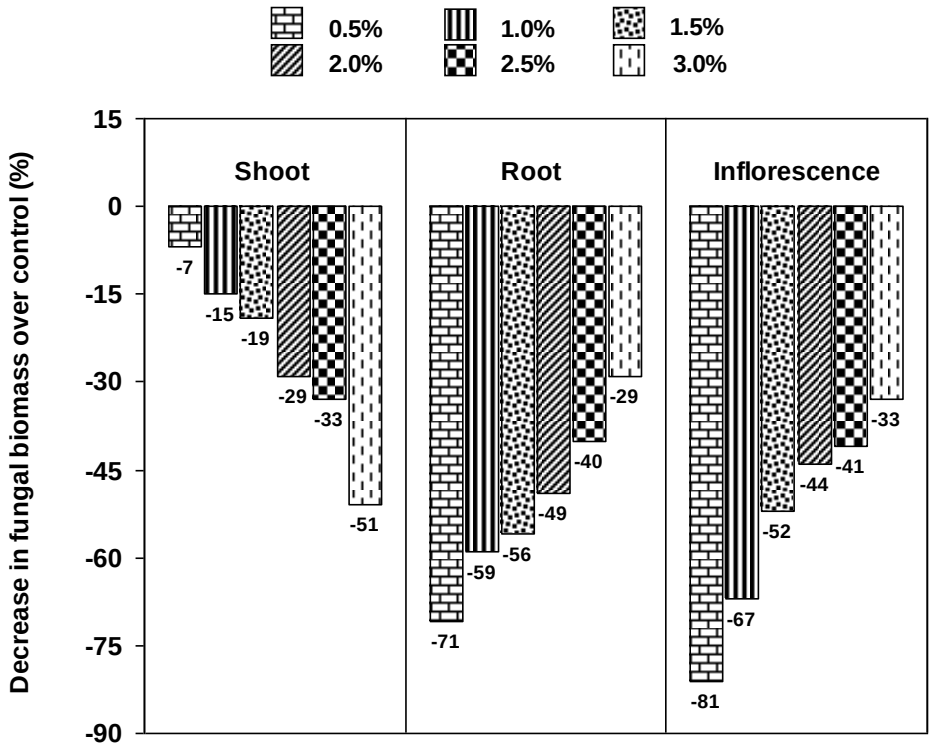

Figure 2. Percentage increase/decrease in biomass of *M. phaseolina* due to different concentrations of methanolic shoot, root and inflorescence extracts of *D. annulatum* over control.

annual test species showed low mycorrhizal colonization when growing in *D. annulatum* dominating localities indicating the antifungal nature of root exudates of this grass under natural environmental conditions.

Effect of inflorescence extract

The effect of methanolic inflorescence extract on biomass of *M. phaseolina* is shown in Figures 1C and 2. All the inflorescence extract concentrations showed significant adverse effect on the biomass of *M. phaseolina*. Similar to that of root extract, the amount of fungal biomass increased with increasing concentration of the extract. Lowest concentration of 0.5% showed highest decrease of 81% in fungal biomass as compared to control, while higher concentrations of 1.0 to 3.0% resulted in 33 to 67% decrease in fungal biomass as compared to control. Earlier, Ashraf and Javaid (2009) reported increased growth of *M. phaseolina* by increasing the concentration of aqueous leaf extract of *Toona ciliata*. Similarly, Riaz et al. (2008) found that the lower concentrations of aqueous extracts of wheat (*Triticum aestivum* L.) and sunflower (*Helianthus annus* L.) were more effective than the

higher concentrations against *F. oxysporum* f.sp. *gladioli*.

The present study concludes that methanolic extracts of various parts of *D. annulatum* posses antifungal constituents for the management of *M. phaseolina*. Further studies are required to isolate and identify the active antifungal ingredients from various parts of this weed.

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