International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:6, No:9, 2012

Evaluation of Antifungal Potential of *Cenchrus pennisetiformis* for the Management of *Macrophomina phaseolina*

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Abstract**—***Macrophomina phaseolina* is a devastating soil-borne fungal plant pathogen that causes charcoal rot disease in many economically important crops worldwide. So far, no registered fungicide is available against this plant pathogen. This study was planned to examine the antifungal activity of an allelopathic grass *Cenchrus pennisetiformis* (Hochst. & Steud.) Wipff. for the management of *M. phaseolina* isolated from cowpea [*Vigna unguiculata* (L.) Walp.] plants suffering from charcoal rot disease. Different parts of the plants viz. inflorescence, shoot and root were extracted in methanol. Laboratory bioassays were carried out using different concentrations (0, 0.5, 1.0, …, 3.0 g mL^{-1}) of methanolic extracts of the test allelopathic grass species to assess the antifungal activity against the pathogen. In general, extracts of all parts of the grass exhibited antifungal activity. All the concentrations of methanolic extracts of shoot and root significantly reduced fungal biomass by 20–73% and 40–80%, respectively. Methanolic shoot extract was fractionated using *n*-hexane, chloroform, ethyl acetate and *n*-butanol. Different concentrations of these fractions (3.125, 6.25, …, 200 mg mL^{-1}) were analyzed for their antifungal activity. All the concentrations of *n*-hexane fraction significantly reduced fungal biomass by 15–96% over corresponding control treatments. Higher concentrations (12.5–200 mg mL^{-1}) of chloroform, ethyl acetate and *n*-butanol also reduced the fungal biomass significantly by 29–100%, 46–100% and 24–100%, respectively.

Keywords**—**Antifungal activity, *Cenchrus pennisetiformis*, *Macrophomina phaseolina*, natural fungicides

I. Introduction

MACROPHOMINA PHASEOLINA is an important fungal pathogen, infecting more than 500 plant species and also has the ability to survive as a saprophyte for up to 15 years in the soil [1]. It has a wide host range including crop plants namely mungbean, sesame, maize, chickpea, cowpea, sunflower, sorghum, cotton, peanut [2]–[4], and forest trees including *Pinus*, *Abies*, *Cassia*, *Pseudotsuga* [5],[6]. It causes dry root rot, charcoal rot, dry weather wilt, seedling blight disease and ashy stem blight in susceptible hosts [7]. It is a soil-borne fungus that survives mainly as microsclerotia that act as primary inoculum and repeatedly germinate during the whole season of the crop. These microsclerotia are produced in root as well as stem tissues of host plants However, in many crops such soybean, this fungus is also seed-borne [8].

Cloncurry buffel grass *Cenchrus pennisetiformis*) is a summer growing perennial grass of family Poaceae. It is palatable plant species with good forage quality and used for cattle grazing.

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In Punjab, Pakistan it generally grows along the road sides. The weed is known to have antifungal and herbicidal potential [9],[10]. The present study was therefore carried out to assess the antifungal activity of this grass for the management of *M. phaseolina*

II. Materials and Methods

A. Isolation and Identification of Fungal Pathogen

Stem portions of the cowpea plants suffering from charcoal rot disease were surface sterilized with 1% sodium hypochlorite, thoroughly rinsed sterilized water, dried plated on malt extract agar medium under aseptic conditions. The plates were incubated at 28 ^{o}C in the dark for one week. The isolated fungal pathogen was purified and sub-cultured. The isolated fungus was identified as *M. phaseolina* on the basis of characteristic black-coloured oblong microsclerotia [11].

B. Screening Bioassays

Dried shoot (leaves+stem), root and inflorescence of *C. pennisetiformis* were thoroughly grinded to fine powders. These powdered plant samples were soaked at 150 g L^{-1} of the methanol in air tight jars separately for 7 days room temperature. Afterwards extracts were obtained from soaked materials by filtering through an autoclaved muslin cloth followed by filter papers and preserved in plastic bottles. The leftover plant materials were again soaked in 500 mL methanol, filtered and preserved in plastic bottles. Filtrates were combined and evaporated in a rotary evaporator under vacuum.

Crude methanolic extracts (8.4 g) of each of the three different parts of the grass were dissolved in 2 mL of dimethyl sulphoxide (DMSO) and raised the volume to 14 mL stock solution by adding sterilized distilled water. Separately a mixture of DMSO in water (2 mL DMSO + 12 mL H_2O) was prepared to keep the quantity of DMSO constant in different treatments. Seventy six milliliters malt extract broth was autoclaved at 121 ^{o}C for 30 minutes in 250 mL conical flasks and cooled at room temperature. In order to avoid bacterial contamination, chloromycetin at 50 mg 100 mL^{-1} of the medium was also added. Six concentrations (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 g 100 mL^{-1}) were prepared 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 mixture of DMSO in water , respectively, to each flask containing 76 mL autoclaved malt extract broth. 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 addition of 4 mL DMSO + distilled water mixture to 76 mL of the growth medium.

Mycelial discs (5 mm) were cut from actively growing *M. phaseolina* culture with the help of a sterilized cork borer and put in each conical flask. Flaks were incubated in an incubator at 28 °C for 10 days. Thereafter, fungal biomass was filtered through pre weighed filter papers and oven dried at 70 °C.

C. Bioassays with Different Fractions of Methanolic Shoot Extract

Three kilograms of dried crushed shoot material of *C. pennisetiformis* was thoroughly extracted with 7 L methanol twice at room temperature for one week each. Extracts were combined, filtered and evaporated at 45 °C on a rotary evaporator under vacuum to get 100 g crude methanolic extract. The crude extract was dissolved in 500 mL water and the solution was partitioned with 500 mL of *n*-hexane in a separating funnel several times till all the *n*-hexane soluble constituents were separated. The *n*-hexane phase was collected and evaporated in a rotary evaporator to get 4.0 g of this fraction. The aqueous phase was further partitioned by successive solvents viz. chloroform, ethylacetate and *n*-butanol to yield 4.0 g chloroform, 2.0 g ethyl acetate and 3.8 g of *n*-butanol fraction. Lastly, the remaining aqueous fraction was evaporated to give 2.9 g gummy mass of this fraction.

Antifungal activities of various fractions of methanolic shoot extract were assessed against *M. phaseolina* by liquid culture method in 10 mL test tubes following Javaid and Saddique [12]. Equal amount (1.2 g) of each of the five fractions of methanolic shoot extract of *C. pennisetiformis* was dissolved in 0.5 mL of DMSO and added to 5.5 mL of malt extract broth. This stock solution (200 mg mL^{-1}) was serially double diluted by adding malt extract broth to prepare lower concentrations of 100, 50, …, 3.125 mg mL^{-1}. A series of control treatments was prepared so that both control and experimental treatments have the same concentrations of DMSO. For this purpose, 0.5 mL of DMSO was dissolved in 5.5 mL malt extract broth and serially double diluted to prepare control treatments corresponding to various extract concentrations. One milliliter of medium was poured in each 10-mL test tube. Tubes were inoculated with one drop of mycelial plus sclerotial suspension of *M. phaseolina* aseptically. Tubes were incubated at room temperature for 7 days. Fungal mass in each test tube was filtered, dried weighed [12].

D. Statistical Analysis

All the data were analyzed by ANOVA followed by Duncan's Multiple Range Test to delineate the treatment means [13].

III. Results and Discussion

A. Screening Bioassays

ANOVA revealed that the effect of different plant parts of the test grass species (P) and concentration of the methanolic extracts (C) was highly significant ($P \leq 0.001$) for biomass of *M. phaseolina.* Similarly, the interactive effect of P×C was also significant for fungal biomass production (Table I).

TABLE I
ANALYSIS OF VARIANCE FOR THE EFFECT OF DIFFERENT CONCENTRATIONS OF METHANOL SHOOT, INFLORESCENCE AND ROOT EXTRACTS OF *SORGHUM HELEPENSE* ON *IN VITRO* GROWTH OF *MACROPHOMINA PHASEOLINA*

Sources of variation	df	SS	MS	F values
Treatments	20	0.103	0.005	23.3**
Plant parts (P)	2	0.008	0.004	18.13**
Conc. (C)	6	0.089	0.015	67.42**
P × C	12	0.005	0.000	2.07*
Error	63	0.014	0.000	
Total	83	0.117		

*, **Significant at $P \leq 0.05$ and $P \leq 0.001$, respectively

All the concentrations of both methanolic shoot and root extracts significantly reduced fungal biomass by 20–73% and 40–80%, respectively. There was a gradual decrease in fungal biomass with the increase in extract concentration. Inflorescence extract was found comparatively less antifungal as compared to other plant parts. There was 7–67% reduction in fungal biomass due to different concentrations of inflorescence extract. Only the effect of 1.5% and higher concentrations was significant as compared to control (Fig. 1).

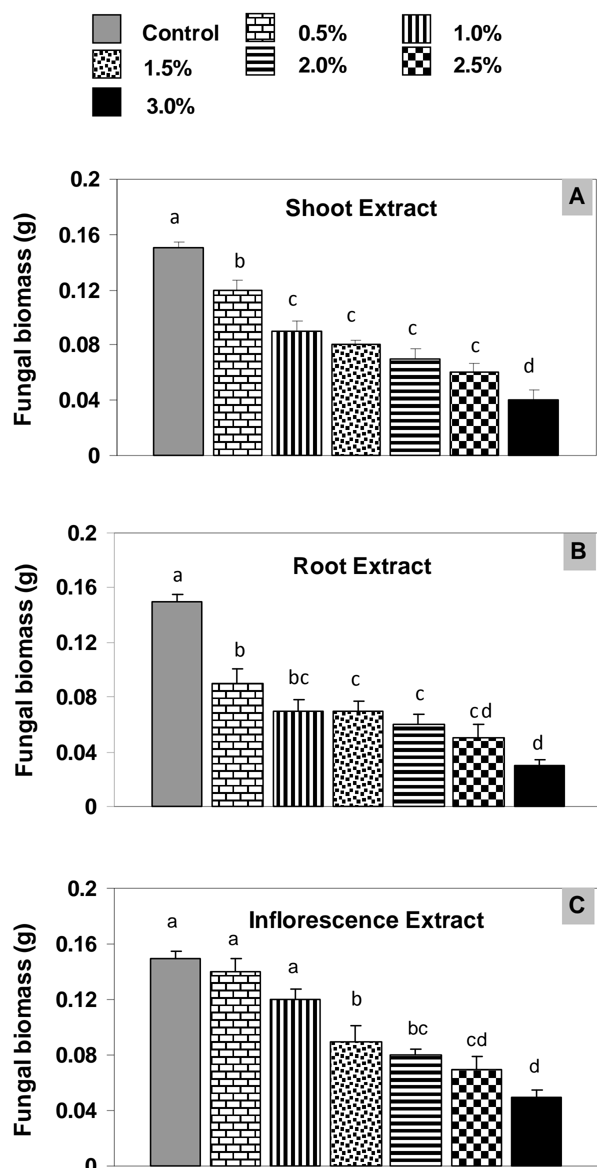

Fig. 1 Effect of different concentrations of methanol extract of shoot, inflorescence and root of *Cenchrus pennisetiformis* on biomass of *Macrofomina phaseolina*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test

Earlier, Shafique *et al.* [9] evaluated the antifungal potential of *C. pennisetiformis* and reported a significant reduction in the biomass of *Fusarium solani*.

B. Antifungal Activity of Different Fractions of Methanolic Shoot Extract

All the concentrations of *n*-hexane fraction significantly inhibited the fungal biomass as compared to control. The higher concentrations viz. 200, 100 and 50 mg mL^{-1} were highly effective and inhibited the fungal growth by 96, 76 and 61%, respectively, over control. Lower concentrations were comparatively less effective and reduced the fungal biomass by 15–27% (Table II).

TABLE II
EFFECT OF DIFFERENT FRACTIONS OF METHANOL SHOOT EXTRACT OF *CENCHRUS PENNISETIFORMIS* ON BIOMASS OF *MACROPHOMINA PHASEOLINA*

Methanolic fraction	Conc. of DMSO (mL mL^{-1})	Extract conc. (mg mL^{-1})	Fungal biomass (mg)
Control	0.1666	0	2.53 j-l
	0.0833	0	2.96 i-k
	0.0416	0	3.66 e-h
	0.0208	0	4.07 d-f
	0.0104	0	4.20 c-e
	0.0052	0	4.40 b-d
	0.0025	0	4.93 ab
n-hexane	0.1666	200	0.13 n
	0.0833	100	0.90 m
	0.0416	50	1.35 m
	0.0208	25	2.60 j-l
	0.0104	12.5	3.43 g-i
	0.0052	6.25	3.90 d-g
	0.0025	3.125	4.23 c-e
Chloroform	0.1666	200	0.00 n
	0.0833	100	0.00 n
	0.0416	50	0.90 m
	0.0208	25	1.24 m
	0.0104	12.5	2.93 i-k
	0.0052	6.25	3.46 g-i
	0.0025	3.125	4.83 ab
Ethyl acetate	0.1666	200	0.00 n
	0.0833	100	0.00 n
	0.0416	50	0.90 m
	0.0208	25	1.40 m
	0.0104	12.5	2.33 l
	0.0052	6.25	4.80 ab
	0.0025	3.125	5.10 a
n-butanol	0.1666	200	0.00 n
	0.0833	100	0.9 m
	0.0416	50	2.63 j-l
	0.0208	25	2.99 ij
	0.0104	12.5	3.23 hi
	0.0052	6.25	3.83 d-g
	0.0025	3.125	4.66 a-c
Aqueous	0.1666	200	0.00 n
	0.0833	100	0.00 n
	0.0416	50	2.40 kl
	0.0208	25	2.60 j-l
	0.0104	12.5	3.59 f-h
	0.0052	6.25	4.0 d-g
	0.0025	3.125	4.23 c-e

Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test.

Chloroform and ethyl acetate fractions were equally effective in suppressing the growth of the target fungal pathogen. Higher concentrations (100 and 200 mg mL^{-1}) of both the fractions completely inhibited the fungal growth. The adverse effect of all except the lower most concentration 3.125 mg mL^{-1} of chloroform fraction was significant. Similarly, all the concentrations of ethyl acetate fraction except 6.25 and 3.125 mg mL^{-1} significantly reduced the fungal biomass as compared to control (Table II). *n*-butanol fraction also showed marked inhibitory effect on growth of the target fungal species. The highest concentration (200 mg mL^{-1}) concentration of this fraction completely arrested the fungal growth. Other concentrations of this fraction reduced fungal biomass by 10–76% (Table II). The 200 and 100 mg mL^{-1} concentrations of aqueous fraction were highly effective and reduced the growth by 100%.

Lower concentrations of aqueous fraction showed variable antifungal activity against *M. phaseolina*. There was 15–100% reduction in fungal biomass due to various concentrations of aqueous fraction (Table II).

The variation in antifungal activity of various organic fractions may be attributed to different chemical nature of the four organic solvents, especially the difference in the polarity of these compounds. Possibly the low molecular weight compounds were dissolved in *n*-hexane fraction, as this is a non-polar solvent.

The moderately polar compounds were dissolved in the chloroform and ethyl acetate fraction and highly polar compounds in *n*-butanol fraction [14].

The present concludes that shoot of *C. pennisetiformis* contains potent antifungal compounds for the management of highly problematic soil-borne plant pathogenic fungus *M. phaseolina.* Antifungal bioassays with different fractions of methanolic shoot extract further reveals that these antifungal compounds are of diverse polarity nature as all the fractions showed pronounced antifungal activity. Further studies are needed to identify these antifungal constituents for their use in the formulation of nature product based fungicides for the management of *M. phaseolina.*

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