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# MANAGEMENT OF *Macrophomina phaseolina* BY EXTRACTS OF AN ALLELOPATHIC GRASS *Imperata cylindrica*

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The soil-borne fungal plant pathogen *Macrophomina phaseolina* (Tassi) Goid. causes rot disease in about 500 plant species worldwide. This study was conducted to assess the antifungal potential of an allelopathic grass *Imperata cylindrica* (L.) Beauv. for the management of this devastating plant pathogen. In laboratory bioassays, various concentrations viz. 0, 0.5, 1.0, …, 3.0 g 100 $mL^{-1}$ of methanolic shoot, root and inflorescence extracts of the test allelopathic grass were appraised for their antifungal activity against the pathogen. Generally, higher concentrations of methanolic extracts of all the three parts of the grass exhibited variable antifungal activity. Shoot extract proved to be the most effective. All the concentrations of shoot extract significantly reduced the fungal biomass by 29–76% over control. Methanolic shoot extract was further fractionated using *n*-hexane, chloroform, ethyl acetate and *n*-butanol. In antifungal activity bioassays of these fractions, chloroform fraction was found to be the most effective followed by *n*-hexane and aqueous fractions. All the concentrations of these fractions significantly reduced fungal biomass. Various concentrations of *n*-hexane, chloroform and aqueous fraction reduced fungal biomass by 27–97%, 68–100% and 32–100%, respectively. Present study concludes that chloroform fraction of methanol shoot extract of *I. cylindrica* possesses highly active antifungal constituents for the management of *M. phaseolina.*
**Keywords:** Antifungal activity, *Imperata cylindrica*, *Macrophomina phaseolina*, natural fungicides

## INTRODUCTION

Cogongrass [*Imperata cylindrica* (L.) Beauv.] is a problematic weed of the tropical region that has invaded over 500 million ha worldwide and causes significant losses both in cultivated and non-cultivated areas (MacDonald 2007; Mohamad *et al*., 2011). About 62-90% reduction in yield of different crops have been documented due to this weed (Koch *et al*., 1990; Avav, 2000; Chikoye *et al*., 2001). Once established, it is extremely competitive with crops and neighboring plant communities (Koger and Bryson, 2004). The grass is known to exhibit strong allelopathic activity against many crops, weeds and forest tree species (Hussain and Abidi, 1991; Cerdeira *et al*., 2012. Anjum *et al.* (2005) has reported that *I. cylindrica* can limits the spread of *Parthenium hysterophorus* possibly through its allelopathic exudates. Various secondary metabolites particularly phenolic compounds in foliage, roots and rhizomes of this grass are responsible for strong allelopathic activity and contribute to its extreme invasiveness and competitiveness (Koger and Bryson, 2004). Many recent studies have shown that allelopathy can be used for the management of pests and diseases (Farooq *et al*., 2011; Javaid and Shoaib, 2012). The allelopathic potential of *I. cylindrica* may be exploited as effective tool for the management of phytopathogens. Few earlier studies have shown that aqueous extracts and root exudates of this grass suppress root colonization by mycorrhizal fungi (Afzal *et al*., 2000; Javaid, 2008). In addition, Bajwa *et al*. (2002) and Shafique *et al*. (2004) reported pronounced antifungal activity of shoot and root extracts of this grass against *Fusarium* spp.

*Macrophomina phaseolina* is an important soil-borne phytopathogen, has a wide geographic distribution, causes diseases in about 500 plant species and can survive for up to 15 years in the soil as a saprophyte (Indera *et al.,* 1986; Kaur *et al*., 2012). It survives in the soil mainly as microsclerotia that germinate repeatedly during the crop-growing season (Gupta *et al*., 2012). In Pakistan, 67 economic hosts of *M. phaseolina* including sunflower, cotton, rice, maize, cucurbits, mungbean, okra, and wheat have been reported (Shehzad *et al*., 1988; Javaid and Amin, 2009; Javaid and Saddique, 2011). Diseases caused by *M. phaseolina* are responsible for massive yield losses in crops (Kaur *et al*., 2012), which demand necessary economic friendly control measures. Earlier studies have shown that crude extracts of some allelopathic plants such as *Datura metel, Chenopodium album* and *C. murale,* as well as purified compounds from mango leaves can effectively control growth of *M. phaseolina* (Javaid and Amin, 2009; Kanwal *et al.,* 2010; Javaid and Saddique, 2012). However, studies regarding the use of extracts of *I. cylindrica* for management of *M. phaseolina* are entirely lacking. The present research work was, therefore, carried out to assess the antifungal potential of methanolic extracts of different parts of *I. cylindrica* against *M. phaseolina*.

## MATERIALS AND METHODS

***Preparation of methanolic extracts:*** Dried shoot, root and inflorescence of *I. cylindica* were thoroughly grinded to a fine powder and 150 g of each plant material was soaked in 1.0 L methanol in air tight jars for 7 days. 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 evaporated in rotary evaporator under vacuum to reduce the volume up to 20 mL. Then 20 mL of each extract was poured in small beakers and put in the oven at 45°C to completely evaporate the methanol.

***Bioassays with methanolic extracts*:** Crude methanolic extracts (8.4 g) of various parts of the *I. cylindrica* grass were dissolved in sterilized distilled water to prepare 14 mL of stock solution. Seventy six milliliter malt extract (ME) medium was autoclaved in 250 mL conical flasks and cooled at room temperature. To avoid bacterial contamination, chloromycetin at 50 mg 100 $mL^{-1}$ of the medium was added. Six concentrations viz. 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, 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 of distilled autoclaved water to 76 mL of ME broth.

*M. phaseolina* was isolated from cowpea plants showing the symptoms of charcoal rot disease. Mycelial discs of 5 mm diameter were removed from the edges of 6-7 days old actively growing culture of *M. phaseolina* and put in each conical flask. Flaks 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.

***Fractionation of methanolic extracts*:** Three kilograms of dried crushed material of shoots of *I. cylindrica* were exhaustively extracted with methanol (7 L × 2) for one week. After filtration, the extracts were evaporated under vacuum on a rotary evaporator at 45°C to yield 150 g of extract. The crude methanolic shoot extract was mixed in 300 mL distilled water and the mixture was partitioned with 400 mL of *n*-hexane in a separating funnel. The upper layer *n*-hexane was collected and evaporated in a rotary evaporator till dryness to obtain 6.4 g of this fraction. The aqueous phase was subjected to further partition by successive solvents including 400 mL of each of chloroform, ethyl acetate and *n*-butanol to yield 4.7 g chloroform fraction, 2.7 g ethyl acetate fraction, 4.1 g *n*-butanol fraction and 3.7 g aqueous fraction.

***Bioassays with different fractions of methanolic shoot extract*:** The bioactivities of the four organic solvents and the aqueous fraction were evaluated against the target fungal species by liquid culture method in 10 mL test tubes according to Chuang *et al.* (2007). Weighed amount (1.2 g) of each of the five fractions of methanolic shoot extract of *I. cylindrica* 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, 25, 12.5, 6.25 and 3.125 mg $mL^{-1}$. For control, 0.5 mL of DMSO was dissolved in 5.5 mL malt extract broth and serially double diluted to prepare control treatments to various corresponding extract concentrations.

Bioassays were conducted in 10 mL volume glass test tubes each containing 1 mL of medium. Test tubes were inoculated with one drop of mycelial and conidial suspension of *M. phaseolina* aseptically. There were three replicates of each treatment. Test tubes were incubated at room temperature for 7 days. Thereafter, fungal biomass from each test tube was collected by filtration and died in an electric oven at 60°C.

***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

***Antifungal activity of methanolic extracts*:** Among the three plant parts assayed, methanolic shoot extract exhibited the highest antifungal activity against the target fungal pathogen. All the concentrations of shoot extract showed significant antifungal effect against *M. phaseolina*. There was gradual decrease in fungal biomass with the increasing concentration of the plant extract. There was 29–78% suppression in biomass of the fungus due to different concentrations of the shoot extract (Fig. 1A). Root extract showed comparatively lower activity than the shoot extract. The adverse effect of all except the lowermost concentration (0.5%) was significant. Various concentrations of the extract declined fungal biomass by 12–65% (Fig. 1B). Inflorescence extract showed the lowest antifungal activity where only concentrations higher than 1% exhibited significant negative effect on fungal growth. Various concentrations of this extract decreased fungal biomass by 6–59% (Fig. 1C). Earlier, some workers reported that aqueous extracts of *I. cylindrica* suppressed the growth of mycorrhizal as well as pathogenic fungi (Bajwa *et al.*, 1996; Afzal *et al.,* 2000; Shafique *et al*., 2004). Hussain and Abidi (1991) identified different phenolic compounds viz. gallic acid, caffeic, ferrulic, p-hydroxybenzoic, pcoumaric, vanillic, chlorogenic and syringic acids as the allelopathic agents. Phenolic acids are powerful antioxidants and have been reported to

demonstrate antibacterial, antifungal and antiviral actions (Galeotti *et al.,* 2008; Mattila and Hellstrom, 2007). Gallic acid and ferrulic acid are the active phenolic compounds known to possess great antifungal potential (Shalini and Srivastava, 2009). These compounds are known to retard the growth of different fungi including the species of *Fusarium*, *Trichoderma*, *Aspergillus* and *Rhizopus* (Hussain *et al.,* 2009). Recently, Cerdeira *et al.* (2012) reported 4-(2-butenylidene)-3,5,5-trimethyl-2-cyclohexen-1-one (also called tabanone) in *I. cylindrica* that might be responsible for antifungal activity.

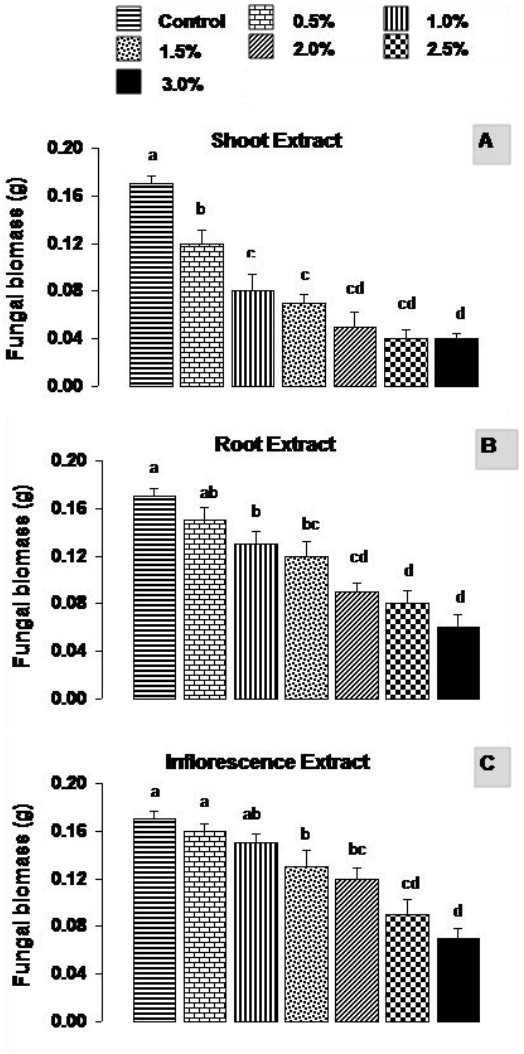


**Figure 1. Effect of different concentrations of methanol extract of shoot, inflorescence and root of *Imperata cylindrica* on biomass of *Macrophomina phaseolina*.** Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by DMR test.

***Antifungal activity of different fractions of methanolic shoot extracts*:** Data regarding the antifungal effect of different factions of methanolic shoot extract of *I. cylindrica* is presented in Figure 2 A-E. Chloroform fraction exhibited the highest antifungal activity where all the concentrations significantly suppressed the fungal growth. Higher concentrations of 50–200 mg $mL^{-1}$ of this fraction completely retarded the growth of *M. phaseolina.*

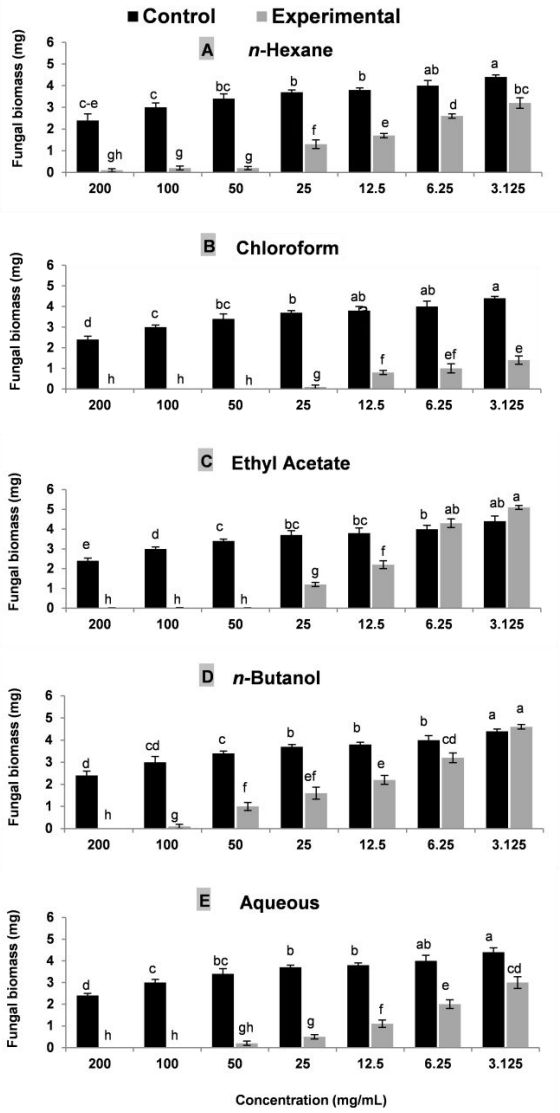


**Figure 2. Effect of different fractions of methanol shoot extract of *Imperata cylindrica* on biomass of *Macrophomina phaseolina*.** Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by DMR test.

The decrease in fungal biomass due to lower concentrations (3.125–25 mg $mL^{-1}$) of chloroform fraction was 68–97% (Fig. 2B). Similar to that of chloroform fraction, all the concentrations of *n*-hexane fraction significantly reduced the biomass of pathogen. Various concentrations of this fraction decreased fungal biomass by 27–97%. However, none of the concentration completely checkered the fungal growth (Fig. 2A). Higher concentrations of 50–200 mg $mL^{-1}$ exhibited very high activity resulting in 99% reduction in fungal biomass. The reduction in fungal biomass due to 25 and 12.5 mg $mL^{-1}$ concentrations of this fraction was 68% and 42%, respectively. Conversely, the lower most concentrations of 6.25 and 3.125 mg $mL^{-1}$ increased fungal biomass by 8% and 12% as compared to control (Fig. 2C). Similarly, higher concentrations of 50–200 mg $mL^{-1}$ of *n*-butanol fraction proved highly antifungal resulting in 70–100% reduction in fungal biomass while the effect of lower most concentration was insignificant (Fig. 2D). Aqueous fraction of methanolic shoot extract showed remarkable antifungal activity. All the concentrations of this fraction significantly decreased the biomass of the fungus by 32–100% (Fig. 2E).

***Conclusion*:** The present study concludes that *I. cylindrica* shoot possesses the remarkable antifungal potential. Especially the chloroform fraction of methanolic shoot extract of this grass exhibited highly antifungal activity against *M. phaseolina.* Further studies are needed to isolate and identify the active antifungal constituents.

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