

Full Length Research Paper

Antifungal activity of leaf extracts of some medicinal trees against *Macrophomina phaseolina*

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Present study was carried out to investigate the antifungal activity of leaf extracts of four medicinal trees namely *Syzygium cumini* (L.) Skeels, *Eucalyptus citriodora* Roxb. (family Myrtaceae) *Azadirachta indica* L. and *Melia azedarach* L. (family Meliaceae), against *Macrophomina phaseolina* (Tassi) Goid, the cause of charcoal rot disease in more than 500 plant species. Three organic solvents viz. methanol, chloroform and ethyl acetate were used for extraction from the leaves. Antifungal bioassays were carried out by using leaf extracts of 0.3, 0.6, ..., 1.5% w/v concentrations in 100 ml conical flasks containing 15 ml of malt extract broth. In general, all the extracts significantly reduced the biomass of the target fungal species. However, there was significant difference among the plant species and extracting solvents for their antifungal activity. Highest antifungal activity was exhibited by ethyl acetate extract of *A. indica* followed by chloroform extract of the same tree species where different concentrations of these extracts reduced the fungal biomass by 81 to 90% and 78 to 84%, respectively as compared to control. Present study concludes that the leaf extracts of allelopathic trees especially ethyl acetate and chloroform extracts of *A. indica* contain natural fungicides which can be used for the management of *M. phaseolina*.

Key words: Allelopathic trees, charcoal rot, leaf extracts, *Macrophomina phaseolina*, natural fungicides.

INTRODUCTION

Macrophomina phaseolina is a soil-borne fungal plant pathogen that causes charcoal rot disease in more than 500 different monocotyledonous and dicotyledonous plant species including such important crops as sorghum, soybean, alfalfa, maize etc (Wyllie, 1993; Ma et al., 2010). It exists in soil as sclerotia, a compact mass of hardened mycelial structures, which can remain dormant for many years and produces hyphae under appropriate conditions which infect the roots of host plants (Ammon et al., 1974). High variation in pathogenicity or genetic diversity or both has been reported in *M. phaseolina* that confirms the ability of the pathogen to survive and adapt to the various environmental conditions (Vandemark et al., 2000; Mayek-Pérez et al., 2001; Baird et al., 2010). Charcoal rot disease can also be transmitted through seeds (Abawi and Pastorcorrales, 1990). This disease can result in severe loss in hot and dry years and drastic weather changes due to global warming pose a serious

threat to crops that are susceptible to *M. phaseolina* (Gaige et al., 2010). So far, there is not any registered fungicide against the charcoal rot pathogen.

Scientists are engaged to achieve some plant derived compounds to control diseases. Natural plants products are biodegradable, exhibit structural diversity and complexity and rarely contain halogenated atoms. These can act directly as pesticides or may provide structure lead for pesticidal discovery (Duke et al., 2000). Several plant families like Acanthaceae, Amranthaceae, and Magnoliaceae are known for their antifungal properties (Neerman, 2003). Masoko et al. (2007) reported that methanolic extracts of *Combretum moggii* and *Combretum petrophilum* were very effective against many fungal species. Many recent studies have shown that both crude extracts and purified isolated compounds from plants can effectively be used as natural fungicides for the management of plant diseases (Javaid and Amin, 2009; Jabeen and Javaid, 2010; Kanwal et al., 2010; Riaz et al., 2010). The present study was, therefore, undertaken to investigate the antifungal potential of organic solvent extracts of four medicinal tree species of family Meliaceae and Myrtaceae to control *M. phaseolina*.

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EXPERIMENTALS

Isolation of *M. phaseolina*

Mungbean plants infected with root rot were collected. The diseased portions of roots were cut into 0.5 cm pieces and surface sterilized by 1% sodium hypochlorite solution for 1 min and then thoroughly rinsed with sterilized water. These pieces were placed on malt extract agar plates aseptically and incubated at 25±2°C for 7 days. After 7 days, the fungal colonies appeared on root pieces were purified on fresh malt extract agar plates. The colour of fungal colony was grey that darkens with age. Characteristic black coloured oblong microsclerotia were observed. On the basis of these characteristics, the isolated fungus was identified as *M. phaseolina* (Wyllie, 1993; Watanabe, 2002).

Collection of plant materials

Fresh leaves of four tree species viz. *Syzygium cumini*, *Eucalyptus citriodora*, *Azadirachta indica* and *Melia azedarach* were collected from University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan. After thorough washing with tap water, leaves were sun-dried, crushed and stored in polythene bags.

Preparation of organic solvent extracts

One hundred grams of thoroughly crushed leaf materials of the four test plant species were soaked in 1000 ml of each of the three organic solvents viz. methanol, chloroform and ethyl acetate. Materials were left for 7 days at room temperature. After that extracts were filtered through muslin cloth followed by filtration by Whatman filter paper No. 1. Organic solvents were evaporated under vacuum in a rotary evaporator. Weighed amount of crude extracts (1.8 g) of each of the four tree species in different solvents was mixed in sterilized distilled water to prepare 6 ml of stock solution. Fifty seven milliliters malt extract broth was autoclaved in 250 ml conical flasks and cooled at room temperature. Five concentrations viz. 1.5, 1.2, 0.9, 0.6 and 0.3 g 100 ml⁻¹ were prepared by adding 3, 2.4, 1.8, 1.2, and 0.6 ml stock solutions, and 0, 0.6, 1.2, 1.8 and 2.4 ml sterilized distilled water, respectively, to each flask to make the total volume of the medium 60 ml. This 60 ml medium of each treatment was divided into three equal portions in 100 ml conical flasks. For control treatment, 3 ml of sterilized distilled water was added to 57 ml of malt extract medium.

Antifungal bioassays

Mycelial discs of *M. phaseolina* were prepared using a sterilized 5 mm diameter cork borer from the tips of 7 days old fungal culture and transferred to each flask. Each treatment was replicated three times. Flasks were incubated at room temperature for 7 days. After 7 days the fungal biomass in each flask was filtered and dried to constant weight in an electric oven and weighed.

Statistical analysis

All the data were analyzed by analysis of variance (ANOVA) followed by Duncan's Multiple Range Test to separate the treatment means at $P \leq 0.05$, using computer software SPSS.

RESULTS AND DISCUSSION

Analysis of variance revealed that the effect of test plant

species (P), extracting solvents (S) as well as concentrations (C) was significant for biomass of *M. phaseolina*. Similarly, the interactive effects of P x S, P x C and S x C were also significant for fungal biomass. In contrast, the effect of P x S x C was insignificant for this studied parameter (Table 1).

Antifungal activity of *A. indica*

Among the four test plant species, extracts of *A. indica* were found most effective against *M. phaseolina*. Ethyl acetate extracts exhibited highest antifungal activity resulting in 81 to 90% reduction in the target fungal biomass. Similarly, different concentrations of chloroform extract reduced the fungal biomass by 78 to 84%. Methanol extract was found comparatively less antifungal exhibiting 65 to 76% suppression in fungal biomass over control (Figure 1A). Earlier, Ashraf and Javaid (2007) reported up to 85% reduction in biomass of *M. phaseolina* by aqueous extracts of *A. indica*. Similarly, Ramos et al. (2007) have reported 35% growth reduction of mycelia of *Phytophthora* on neem (*A. indica*) leaf extract media. Saha et al. (2005) recorded 100% inhibition of spore germination of *Pestalotiopsis theae* (Saw.) Stey., *Colletotrichum camelliae* Mess., *Curvularia eragrostidis* (P. Hennings) Meyer, and *Botryodiplodia theobromae* Patouillard due to extracts of *A. indica*. Various compounds including diterpenoids, triterpenoids, polyphenolics, sulphurous compounds, and polyacetate derivatives have so far been isolated from neem (Kumar and Dev, 1993) which may be responsible for antifungal activity. NIM 76, a spermicidal fraction from neem oil, possessed antifungal activity (Sairam et al., 2000).

Antifungal activity of *M. azedarach*

All the leaf extracts of *M. azedarach* significantly reduced biomass of *M. phaseolina*. Chloroform extract was found more effective than the other two types of extracts. There were 38 to 60, 57 to 78 and 56 to 75% reduction in fungal biomass due to different concentrations of methanol, chloroform and ethyl acetate leaf extracts of *M. azedarach* as compared to control (Figure 1B). These results in line with the findings of some earlier workers. Carpinella et al. (2003) reported that ethanolic extracts senescent leaves of *M. azedarach* exhibited fungistatic activity against *Aspergillus flavus*, *Diaporthe phaseolorum* var. *meridionales*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium verticillioides*, and *Sclerotinia sclerotiorum*. Similarly, Jabeen et al. (2008) reported antifungal activity of ethanol and chloroform leaf extracts of this tree species against *Ascochyta rabiei*. The antifungal activity of *M. azedarach* could be attributed to the presence of antifungal compounds namely hydroxycoumarin scopoletin, vanillin, 4-hydroxy-3-methoxycinnamaldehyde and (±) pinoresinol (Carpinella

Table 1. Analysis of variance for the effect of different concentrations of leaf extracts of four allelopathic trees on *in vitro* growth of *M. phaseolina*.

Sources of variation	df	SS	MS	F value
Treatments	71	0.663	0.009	42.6**
Plant species (P)	3	0.062	0.021	94.2**
Extracting solvents (S)	2	0.022	0.010	49.5**
Concentration (C)	5	0.535	0.107	488.1**
P x S	6	0.013	0.002	10.3**
P x C	15	0.016	0.001	4.9**
S x C	10	0.006	0.0006	2.7*
P x S x C	30	0.008	0.0003	1.3 ^{NS}
Error	144	0.031	0.0002	
Total	216	2.86		

*, **, Significant at $P \leq 0.01$ and 0.001 , respectively, NS: Nonsignificant.

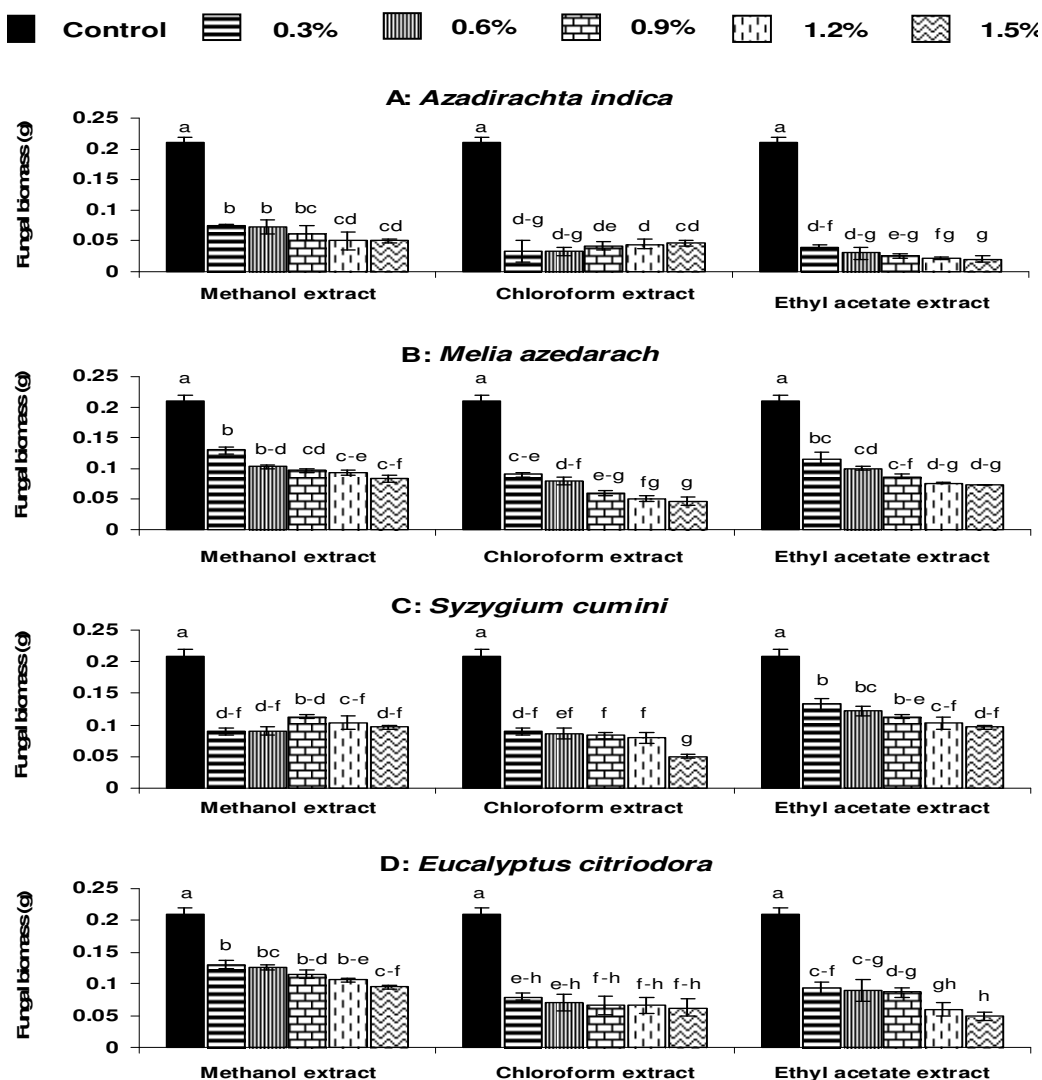


Figure 1. Effect of different concentrations of methanol, chloroform and ethyl acetate extracts of leaves of four tree species on biomass of *M. phaseolina*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test.

et al., 2003; Carpinella and Ferrayoli, 2005). Recently, Jabeen et al. (2011) have isolated five compounds namely β -sitosterol, β - amyryn, ursolic acid, benzoic acid, 3-5 dimethoxy benzoic acid from leaves of *M. azedarach*, which showed antifungal activity against *A. rabiei*.

Antifungal activity of *S. cumini*

All the concentrations of leaf extracts of *S. cumini* in the three organic solvent extracts significantly declined the fungal biomass with chloroform extract being the most effective one. Different concentrations of methanol, chloroform and ethyl acetate extracts suppressed the biomass of *M. phaseolina* by 48 to 57, 57 to 76 and 37 to 54%, respectively, over control (Figure 1C). The antifungal activity of the *S. cumini* leaves extract may be due to tannins and other phenolic constituents. *S. cumini* is known to be very rich in gallic and ellagic acid polyphenol derivatives (Chattopadhyay et al., 1998). Also, acylated flavonol glycosides, kaempferol, myricetin and other polyphenols have been isolated from *S. cumini* leaves (Mahmoud et al., 2001; Timbola et al., 2002), which may be responsible for antifungal activity.

Antifungal activity of *E. citriodora*

A significant reduction in target fungal biomass was recorded due to different concentrations of the leaf extracts of *E. citriodora* in the three different organic solvents. Chloroform extract exhibited the highest antifungal activity resulting in 62 to 81% reduction in fungal biomass. There were 38 to 57 and 52 to 74% decline in fungal biomass due to different concentrations of methanol and ethyl acetate leaf extracts over control (Figure 1D). Earlier, Fiori et al. (2000) reported that crude extracts of *E. citriodora* were very effective in suppressing the growth of fungus *Didymella bryoniae*. Recently, Jabeen and Javaid (2008) reported the antifungal activity of alcoholic and chloroform extracts of leaves of *E. citriodora* against *A. rabiei*.

Present study concludes that organic solvent extracts of allelopathic trees especially chloroform extracts contain antifungal constituents and can effectively be used for the management of *M. phaseolina*. Further studies are required to isolate and identify these effective antifungal ingredients from these extracts. There is possibility that once identified, the structures of these natural fungicides may be used as lead for the preparation of new synthetic fungicides effective against an economically important fungal species for which there is no known registered fungicide available at present.

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