

## Evaluation of antifungal activity of Meliaceae family against *Macrophomina phaseolina*

Hina Ashraf and \*Arshad Javaid

Institute of Mycology & Plant Pathology, University of the Punjab, Lahore, Pakistan.

\*E. mail: arshadjpk@yahoo.com

### Abstract

*Macrophomina phaseolina* (Tassi) Goid is a soil-borne fungal pathogen that causes root rot disease in more than 500 plant species but no registered fungicide is available for its control. The present study was carried out to investigate the antifungal activity of aqueous leaf extracts of *Azadirachta indica* A. Juss., *Melia azedarach* L. and *Toona ciliata* Roxb. (Meliaceae) against *M. phaseolina*. Aqueous extracts of 5-20% concentrations of both *A. indica* and *M. azedarach* significantly reduced biomass of *M. phaseolina* by 34 – 85% and 43 – 78%, respectively. By contrast, aqueous extracts of *T. ciliata* stimulated the growth of the fungus at all the tested concentrations.

**Keywords:** Antifungal, *Azadirachta indica*, *Macrophomina phaseolina*, *Melia azedarach*, Meliaceae, *Toona ciliata*.

### Introduction

*Macrophomina phaseolina* (Tassi) Goid., a soilborne fungus causes charcoal rot, infects the root and lower stem of over 500 plant species and has a wide geographic distribution (Wyllie, 1988; Das *et al.*, 2008). Charcoal rot is an important disease during dry, hot weather or when unfavorable environmental conditions stress the plant. *M. phaseolina* also causes charcoal rot in sunflower, an important oil-seed crop ranking next to soybean. Charcoal rot generally appears after flowering but seedling blights have also been reported. Symptoms on stalks appear as silver-gray lesions near the base, which 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. Seed yield and oil content are reduced. Numerous tiny black fungus bodies called sclerotia are formed on the decayed tissues giving the stalks a charred appearance. There is not any registered fungicide against the charcoal rot pathogen.

Scientists are on their way 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). Recently Masoko *et al.* (2007) studied the antifungal activities of leaf extracts of 24 South African *Combretum* species

and found methanolic extracts of *C. moggii* and *C. petrophilum* very effective against many fungal species. Some members of family Meliaceae are known to exhibit antifungal properties (Ram *et al.*, 2000). The present study was, therefore, undertaken to evaluate the antifungal potential of aqueous extracts of three plant species of Meliaceae viz. *Azadirachta indica*, *Melia azedarach* and *Toona ciliata* against *M. phaseolina*.

### Materials and Methods

*M. phaseolina* was isolated from sunflower plants infected with charcoal rot disease. Infected plant tissue showing charcoal rot characteristics and bearing fungal sclerotia were selected. The tissue was cut into 5 mm long and 2-3 mm thick pieces. These pieces were surface sterilized with 1% NaOCl solution for about 2 minutes followed by thorough washing with sterilized water. These surface sterilized pieces were transferred to malt extract agar (MEA) medium in 9 cm diameter Petri plates. The plates were incubated in dark at 30±2 °C. *M. phaseolina* was also and the pure culture was maintained in refrigerator at 4 °C.

Fresh leaves of the three selected test tree species of Meliaceae were collected from University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan during April 2008. After thorough washing with sterilized water plant materials were blended @ 20 g 100 mL<sup>-1</sup> of sterilized distilled water. Materials were passed through a muslin cloth and then filtered using Whatman No. 1 filter paper. The resultant 20%

(w/w) stock solution was stored at 4 °C in a refrigerator.

Malt extract broth was sterilized by autoclaving at 121 °C. In 250 mL flasks, 80 mL of malt extract medium was poured and cooled to room temperature. Appropriate quantities of stock solutions and distilled water were added to make 1, 2, 3 and 4% (w/v) media with final volume of 100 ml in each flask. Control received 20 ml of distilled water to make the final volume of the growth medium to 100 ml. Actively growing mycelial discs of *M. phaseolina* were prepared using a cork borer of 5 mm diameter and transferred to the flasks aseptically. Each treatment was replicated three times. Flasks were incubated at 25 °C for 7 days on an electric shaker. After 10 days, fungal biomass in each flask was filtered, dried to constant weight and weighed. Percentage reduction in fungal biomass due to various employed concentrations of the extracts over control were calculated by applying the following formula:

$$\text{Biomass reduction (\%)} = \frac{\text{Biomass in control} - \text{biomass in extract treatment}}{\text{Biomass in control}} \times 100$$

All the data were subjected to one-way analysis of variance followed by mean separation through Duncan's Multiple Range Test (Steel and Torrie, 1980).

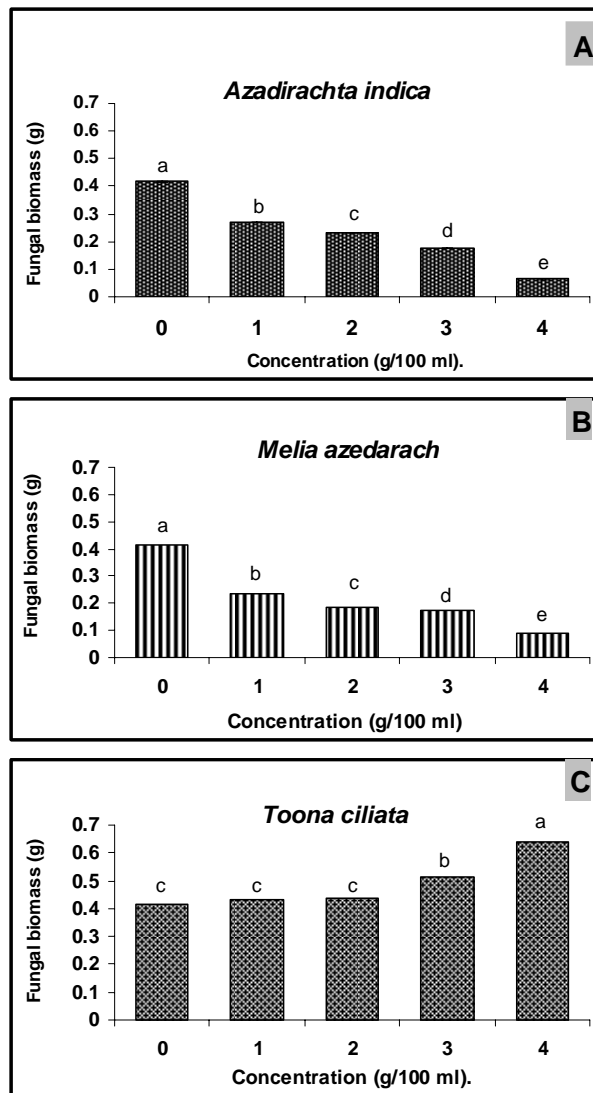
Aqueous extracts of *A. indica* significantly suppressed the biomass of *M. phaseolina*. There was a gradual decrease in fungal biomass with the increase in extract concentration (Fig. 1A). Aqueous extracts of 5–20% concentrations reduced the fungal biomass by 34 – 85% (Fig. 2). Earlier, Ramos *et al* (2007) have reported 35% growth reduction of mycelia of *Phytophthora* on neem leaf extract media. Saha *Tet 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*. Govindachari *et al.* (1999) reported that n-hexane fractions of leaf extracts of *A. indica* showed highly antifungal activity against spore germination of *Fusarium oxysporum* and *Colletotrichum Lindemuthianum*. 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.

Aqueous extracts of different concentrations of *M. azedarach* also significantly suppressed the

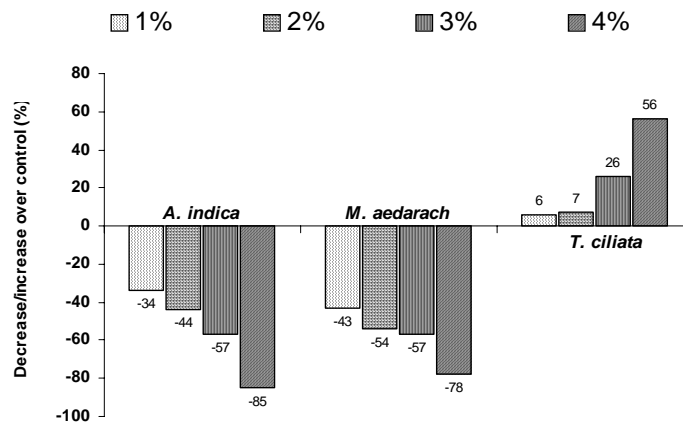
biomass of *M. phaseolina*. Similar to that *A. indica*, extracts of different concentrations of *M. azedarach* exhibited gradual increase in antifungal activity with the increase in extract concentration (Fig. 1B). There was 43–78% decline in fungal biomass due to different concentrations of aqueous extracts of *M. azedarach* (Fig. 2). Earlier Carpinella *et al.* (2003) reported that extracts from fruit, seed kernels and senescent leaves of *M. azedarach* exhibited fungistatic activity against *Aspergillus flavus*, *Diaporthe phaseolorum* var. *meridionales*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium verticillioides*, and *Sclerotinia sclerotiorum*. They attributed the antifungal activity of *M. azedarach* due to the presence of compounds namely hydroxycoumarin scopoletin, vanillin, 4-hydroxy-3-methoxycinnamaldehyde and (±) pinoselinol (Carpinella *et al.*, 2003, 2005). Recently Jabeen *et al.* (2008) have reported that leaf extract of *M. azedarach* suppressed the *in vitro* growth of *Ascochyta rabiei*, the cause of chickpea blight. Jabeen (2008) isolated four antifungal constituents namely β- amyrin, ursolic acid, benzoic acid and 3-5 dimethoxy benzoic acid from leaves of *M. azedarach*.

In contrast to that of other two species of Meliaceae, aqueous extracts of *T. ciliata* promoted the fungal growth. Extracts of lower concentrations of 5 and 10% showed an insignificant effect on biomass of *M. phaseolina*. However, higher concentrations of 15 and 20% significantly enhanced fungal biomass by 28 and 58%, respectively (Fig. 1C & 2). In contrast to the present study, earlier Govindachari *et al.* (2000) reported antifungal activity of *T. ciliata* against *Puccinia arachidis*, a groundnut rust pathogen. This difference in activity could be due to specificity of the plant extracts towards different fungal species. Similar specificity has also been reported for the extracts of other plant species against fungal growth (Bajwa *et al.*, 2007, 2008; Braga *et al.*, 2007).

The present study concludes that the aqueous extracts of *A. indica* and *M. azedarach* contain antifungal constituents. Since no registered fungicide is available against *M. phaseolina*, this preliminary study would be helpful to isolate and identify the antifungal compounds from these plant species for the management of charcoal rot of sunflower.



**Fig.1:** Effect of different concentrations of aqueous extracts of *Azadirachta indica*, *Melia azedarach* and *Toona ciliata* on biomass of *Macrophomina phaseolina* in liquid culture. Values with different letters show significant difference as determined by Duncan's Multiple Range Test at  $P \leq 0.05$ .



**Fig. 2:** Percentage increase/decrease in biomass of *Macrophomina phaseolina* due to different concentrations of aqueous extracts of *Azadirachta indica*, *Melia azedarach* and *Toona ciliata* as compared to control.

## References

- Bajwa R, Shafique S, Shafique S, 2007. Appraisal of antifungal activity of *Aloe vera*. *Mycopath*, **5**: 5-9.
- Bajwa R, Javaid A, Shafique S, Javaid A, Jabeen K, Shafique S, 2008. Fungistatic activity of aqueous and organic solvent extracts of three rice varieties against phytopathogenic fungi. *Allelopathy J.* **22(2)**: 363-370.
- Braga FG, Maria LM, Bouzada RL, Matos MO, Moreira FO, Scio E, Coimbra ES, 2007. Antileishmanial and antifungal activity of plants used in traditional medicine in Brazil. *J. Ethnopharmacol.*, **111**: 396-402.
- Carpinella MC, Giorda LM, Ferrayoli CG, Ferrayoli CG, 2003. Antifungal effects of different organic extracts from *Melia azedarach* L. on phytopathogenic fungi and their isolated active components. *J. Agric. Food Chem.*, **51**: 2506-2511.
- Carpinella MC, Ferrayoli CG, Ferrayoli CG, 2005. Antifungal synergistic effect of scopoletin, a hydroxycoumarin isolated from *Melia azedarach* L. fruits. *J. Agric. Food Chem.*, **53**: 2922-2927.
- Das IK, Fakrudin B, Arora DK, 2008. RAPD cluster analysis and chlorate sensitivity of some Indian isolates of *Macrophomina phaseolina* from sorghum and their relationships with pathogenicity. *Microbiol. Res.*, **163**: 215-224.
- Duke SO, Dayan FR, Romaine JG, Rimando AM, 2000. Natural products as sources of herbicides: status and future trends. *Weed Res.*, **40**: 99-111.
- Govindachariu TR, Suresh G, Masilamani S, 1999. Antifungal activity of *Azadirachta indica* leaf hexane extract. *Fitoterapia* **70**: 417-420.
- Govindachariu TR, Suresh G, Gopalakrishnan G, Masilamani S, Banumathi B, 2000. Antifungal activity of some tetranortriterpenoids. *Fitoterapia*, **71**: 317-320.
- Jabeen K, Javaid A, Athar M, 2008. Fungistatic activity of aqueous and organic solvent extracts of *Melia azedarach* against *Ascochyta rabiei*. *Pak. J. Phytopathol.*, **20**: 143-149.
- Jabeen K, 2008. Natural compounds from allelopathic trees as antifungal agents against *Ascochyta rabiei*. Ph. D. Thesis, University of the Punjab, Lahore, Pakistan.
- Kumar DC, Dev S, 1993. *Neem Research and Development*. In: Randhawa NS, Parmar BS, editors. New Delhi, India: Society of Pesticide Science, pp.63-96.
- Masoko P, Picard J, Eloff JN, 2007. The antifungal activity of twenty-four southern African *Combretum* species (Combretaceae). *South African J. Bot.*, **73**:173-183.
- Neerman MF, 2003. Sesquiterpenes lactones a diverse class of compounds found in essential oils possessing antibacterial and antifungal properties. *Int. J. Aromath*, **13**:114-120.
- Ram MS, Ilavazhagan G, Sharma SK, Dhanraj SA, Suresh B, Parida MM, Jana AM, Devendra K, Selvamurthy W, 2000. Antimicrobial activity of a new vaginal contraceptive NIM-76 from neem oil (*Azadirachta indica*). *J. Ethnopharmacol.*, **71**: 377-382
- Ramos AR., Falcão LL, Barbosa GS, Marcellino LH, Gander ES, 2007. Neem (*Azadirachta indica* A. Juss) components: Candidates for the control of *Crinipellis pernicioso* and *Phytophthora* ssp. *Microbiol. Res.*, **162**: 238-243.
- Saha D, Dasgupta S, Saha A, 2005. Antifungal activity of some plant extracts against fungal pathogens of tea (*Camellia sinensis*). *Pharmac. Biol.*, **43**: 87-91.
- Steel RGD, Torrie JH, 1980. *Principles and Procedures of Statistics*. McGraw Hill Book Co., Inc, New York, USA.
- Wyllie TD, 1988. Charcoal rot of soybean-current status. In Soybean diseases of the north central region. Wyllie TD. and Scott DH. (eds.) APS Press, St. Paul, MN.