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FUNGISTATIC EFFECTS OF *MELIA AZEDARACH* L. AGAINST EARLY BLIGHT OF POTATO

[a]Khajista Jabeen*, [a]Kashifa Noureen, [b]Shagufta Naz, [a]Sumera Iqbal, [a]Shahnaz Hanif
[a] *Department of Botany, Lahore College for Women University, Lahore, Pakistan.*
[b] *Department of Biotechnology, Lahore College for Women University, Lahore, Pakistan.*

A B S T R A C T

The antifungal activity of *Melia azedarach* L. was evaluated against *Alternaria solani* Sorauer. the causal agent of early blight of potato (*Solanum tuberosum* L). Screening bioassays with methanolic leaf and fruit extracts were performed *in vitro* against *A. solani*. Results obtained from this bioassay showed that leaf extract of *M. azedarach*, exhibited strong antifungal activity over the fruit extracts, as 5% concentration retarded the *A. solani* diameter by 90%. Due to high antifungal activity of leaf extract, this part of *M. azaderach* was used for further bioassays. Leaf extract was partitioned between n-hexane, chloroform, ethyl acetate and n-butanol. The minimum inhibitory concentration (MIC) of isolated fractions and a commercial synthetic fungicide Metalaxyl + Mancozeb, 72 WP was investigated against *A. solani*. Different concentrations of fungicide and isolated fractions ranging from (0.19 mg to 100 mg mL^{-1}) were used in MIC bioassay, and data was recorded after 24, 48 & 72 hrs incubation period. n-hexane fraction of the methanolic extract of *M. azaderach* leaves and synthetic fungicide were found highly effective against *A. solani* with MIC value of 0.19 mg mL^{-1}. All tested fractions showed less antifungal activity.

Keywords: *Alternaria solani*, Antifungal, Methanolic extract, MIC.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a tuberous crop of the family Solanaceae (also known as the nightshades). It is the world's fourth largest high yielding food crop having high nutritive value and serves as a major source of carbohydrates and large amount of vitamin C. Pakistan is seventh largest potato producing country with an annual of 2148.26 thousand tons over an area of 127.75 thousand hectares (Anonymous, 2010).

Early blight of potato caused by *Alternaria solani* is a devastating disease of potato crop that causes high yield losses. This disease mainly affects leaves and stems, but under favorable conditions causes significant defoliation and enhanced the possibility for tuber infection. *A. solani* produces small, darkened lesions on the plants that spread into growing black spots of dead tissue and often killing most of the plant in the long run. Preventative measures for managing this disease like ridging, mulching, tillage, crop rotation are often used to reduce tuber contamination. Resistant cultivars are also available having blight resistant genes, but these varieties are short lived as pathogen also changes its genetic makeup and after a short time period resistant variety become susceptible. Blight disease can efficiently be controlled by the application of fungicide like azoxystrobin. However this approach is not safe as chemicals used in crop protection pollute the environment and caused the health hazards (Pritchard, 2004).

Rising public awareness on environmental problems requires alternative disease management strategies which are less pesticide dependent or based on naturally occurring compounds. Use of biologically active natural products to control plant diseases in many fields is extensively studied now a day (Raju, 2001). Different plants from various plant families like Acanthaceacea, Amaranthaceae, Magnoliaceae and Meliaceae have potential antifungal properties. (Mansilla and Palenzuela, 1999). Plant extracts play an important role in the inhibition of seed borne diseases and have strong antifungal activity against various economically important phytopathogenic fungi (Charmaine *et al.,* 2005).

* Corresponding Author:
Email: khajista_1@hotmail.com

Dhraik (*Melia azedarach* L.) is a deciduous tree of family Meliaceae and is widely studied for its pharmacological importance. Various parts of *M. azedarach* like fruit, bark and leaves possess antifungal, antibacterial, nervous system depressant, anti-cancerous and antiviral activity (Khan *et al.,* 2008).

The present study is, therefore, designed to evaluate the antifungal potential of *M. azedarach* against the destructive plant pathogenic fungus *A. solani*, the causal agent of early blight of potato.

MATERIALS AND METHODS

Selection and collection of test plant: *Melia azedarach* was chosen to appraise the antifungal activity against *Alternaria solani,* the cause of early blight of potato. Leaves and fruits of the *M. azedarach* were collected from the Lahore College for Women University, Lahore. This plant material was dried at 40°C in an electric oven and stored in polythene bags.

Procurement and culture maintaining of target fungus specie *A. solani:* Culture of *A. solani* was procured from Ayub Agriculture Research Centre, Jhang Road, Faisalabad. This culture was sub cultured and maintained on 2% PDA (Potato Dextrose Agar) and stored in refrigerator at 4 °C.

Preparation of organic solvent extract: Twenty grams of each plant part dried materials were weighed on Shimadzu A X 120 and soaked in 100 mL organic solvent (methanol) and left for three days at room temperature. After three days materials were filtered through an autoclaved muslin cloth. These methanolic extracts were evaporated to reduce its volume to 2 mL in dried oven at 35 ˚C and then diluted by adding appropriate amount of distilled water to make a final volume of 100 mL. The stock solutions were stored in refrigerator at 4 °C and used within four days.

Antifungal bioassays: PDA (Potato Dextrose Agar) 2% was prepared by adding 2 g PDA in 100 mL of distilled water in 250 mL conical flask; this solution was autoclaved at 121ºC for 30 minutes. To 75 mL of PDA medium, 25 mL of stock solutions were added to prepare 5% w/v concentration of the extracts in the medium. The lesser concentrations of 4, 3, 2 and 1% were prepared by adding 20, 15, 10 and 5mL of the stock solutions to 80 mL of PDA. Control treatments were without any plant extracts. Each concentration was supplemented with Chloromycetin @50 mg 100 mL^{-1} of the medium to avoid bacterial contamination.

In vitro antifungal bioassay was conducted with organic solvent extract. 20 mL of each applied concentration was poured in sterilized 9 cm Petri plates. Mycelial discs of 5 mm was prepared by using a cork borer from the pure culture of *A. solani* and placed in the center of each Petri plate after solidification of the PDA medium. These plates were incubated for 7 days at 25±2°C**.** After 7 days fungal radial growth was measured by taking average of three diameters taken at right angles for each colony. Percentage growth inhibition of the fungal colonies was measured by using the following formula:

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

Bioassays guided fractionation: Leaf extracts of *M. azedarach* were exhibited strong antifungal potential in retarding the *in vitro* growth of *A. solani.* This extracts was selected for further investigation.

Partitioning of plant material: Leaves of *M. azedarach* were partitioned with n-hexane, chloroform ($CHCl_3$) followed by ethyl acetate (EtOAc) and n-butanol at room temperature. 310 g leaves of *M. azedarach* were fully extracted with methanol (MeOH) (1 L 300 mL) at room temperature. The extract was evaporated under vacuum on rotary evaporator (Buchi Switzerland R-210) at 40 ^{0}C; give up 33.67 g of gummy mass. This methanolic extract (33.67 g) was partitioned between n-hexane and water. The aqueous fraction was successively partitioned with chloroform, ethyl acetate and n-butanol (Ahmed *et al.,* 2007) according to increasing polarity order. This partitioning was yielded as gummy mass of n- hexane (1.09 g), chloroform (4.20 g), ethyl acetate (1.11 g), n-butanol (3.48 g) and remaining water fraction. This partitioning was made by use of separating funnel.

Assessment of minimum inhibitory concentration (MIC) of the isolated fractions: The MIC values of the isolated fractions and a synthetic fungicide (Metalaxyl+Mancozeb 72 WP) were tested in test tubes by serial dilution micro dilution assay (Jabeen *et al.,* 2013) with small alterations. The four isolated fractions were dissolved in DMSO (Dimethyl Sulfoxide) and were serially diluted with water in test tubes. Maximum 100 mg mL^{-1} concentration was prepared by adding 2 mL of DMSO and 2 mL of distilled water, this concentration was further serially diluted and the minimum applied concentration was 0.19 mg mL^{-1}. Freshly prepared PD medium was added to seven days old fungal culture of *A. solani* to reach a final conidial concentration of 1×10^5,

100 µL of this was added to test tubes having a diameter of 1.6 cm and 15 cm long. Test tubes containing DMSO and distilled water was used as control. These test tubes were incubated at 25-30 °C. The MIC of the fractions was observed visually after 24, 48 and 72 hours by using inverted microscope to study the fungal mycelial growth.

Statistical analysis: Data were analyzed statistically by applying ANOVA followed by Duncan's multiple range test (Steel *et al.,* 1997).

RESULTS AND DISCUSSION

Potato is the world's fourth-largest food crop having high nutritive values. Early blight of potato is a devastating disease of potato crop that causes high yield losses caused by *A. solani*. Biological control is the way to avoid environmental pollution to minimize the intensive use of chemical fungicides. Use of biologically active natural products to control plant diseases in many fields is extensively studied. There are many plants like Euclyptus, Alstonia, Melia, Azadirachta spp. showed antifungal activities (Charmaine *et al.,* 2005).

The present study was designed to investigate the methanolic extract of leaves and fruits of *M. azedarach* for their antifungal activity against the test fungus *A. solani*. The analysis of variance (ANOVA) showed significant effect of different plant parts of *M. azaderach* solvent extracts and their different concentrations on growth of fungus. All the data was analyzed by using Co-stat software followed by Duncan' Multiple Range test at 0.05 level of significance (DMR) (Steel *et al.,* 1997).

Methanolic leaf extracts of *Melia azedarach* significantly inhibited the *in vitro* growth of test fungus *A. solani*. Higher concentration (5%) was found highly significant, resulted in reduction of fungal growth up to 90%. 4% concentration also effectively retarded the fungal colony diameter (81%) when compared with control treatment. Lower concentrations 1, 2 & 3% of *M. azedarach* also significantly reduced the test fungus diameter viz. 69%, 76% & 71% respectively against *A. solani* (Fig. 1 & 2). Earlier Carpinella *et al.* (2003) studied the ethanolic and n-hexane extracts from fruit, seed kernels and senescent leaves exhibited fungistatic activity against *Aspergillus flavus*, *Diaporthe phaseolorum* var. *meridionales*, *Fusarium oxysporum*, *F. solani, F. verticillioides*, and *Sclerotinia sclerotiorum.*

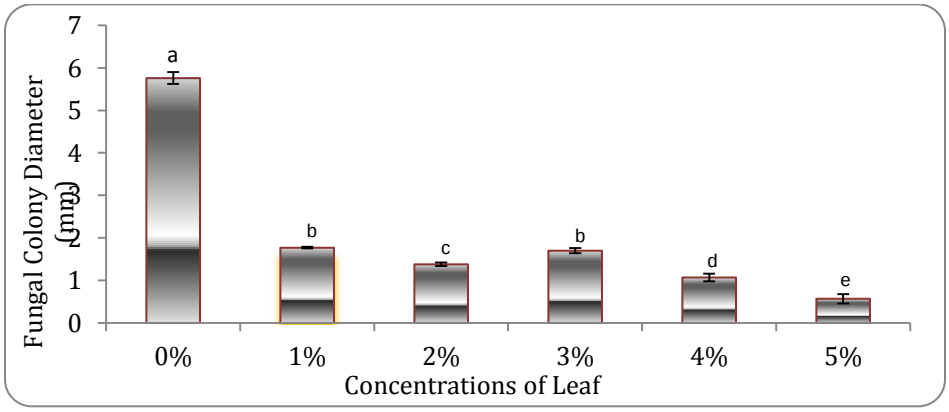

Figure 1. Effect of *M. azedarach,* methanolic leaf extracts on *in vitro* growth of *A. solani.* Vertical bars show standard error of means of three replicates. Values with different letters show significant difference as determined by DMR Test.

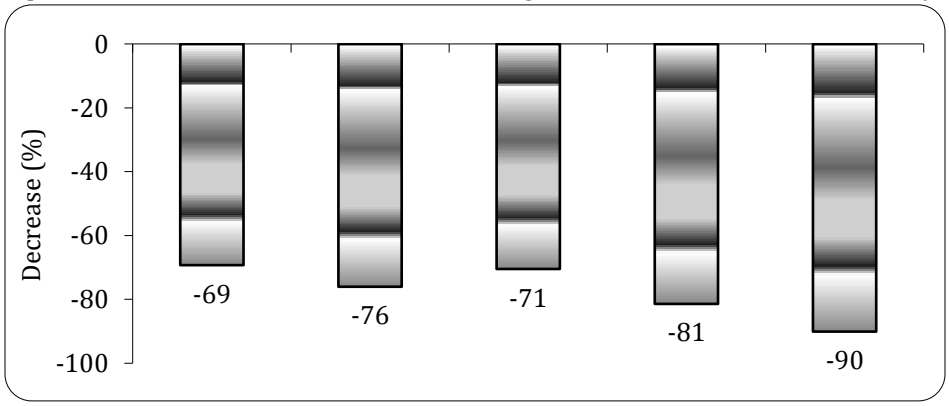

Figure 2. Percentage increase/decrease in diameter of *A. solani* due to diff. concentrations of methanolic leaf extract of *M. azedarach.*

In vitro activity of methanolic fruit extract of *M. azedarach* was also tested against *A. sloani* (Fig. 3 & 4). All the applied concentrations of *M. azedarach* fruit extract significantly inhibited the growth of test fungus. 3% and 4% concentration most effectively retarded the fungal colony diameter to 87% & 82% respectively as compared to the control. Other applied concentrations of fruit extract (1% & 2%) also significantly suppressed the radial growth of *A. solani* upto 60% & 56%. *M. azedarach* is rich in scopoletin, vanillin, hydrocoumarin derivatives and the antifungal activity of *M. azedarach* might be due to the presence of these alkaloids & phenolics compounds (Carpenilla *et al.,* 2005).

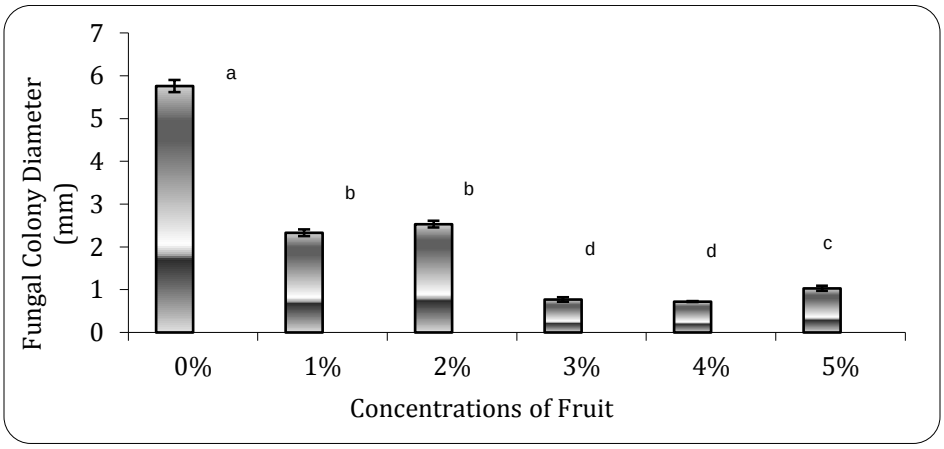

Figure 3. Effect of *M. azedarach,* methanolic fruit extracts on *in vitro* growth of *A. solani* Vertical bars show standard error of means of three replicates. Values with different letters show significant difference as determined by DMR Test.

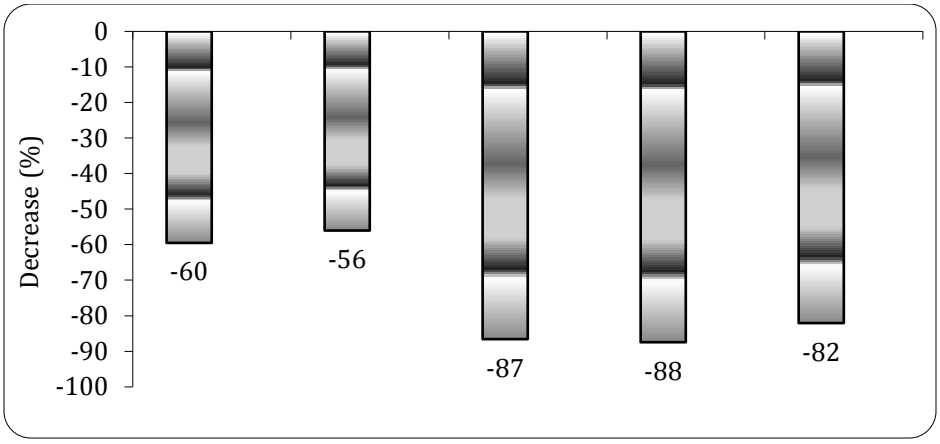

Figure 4. Percentage increase/decrease in diameter of *A. solani* due to different concentrations of methanolic fruit extract of *M. azedarach.*

Methanolic leaf extract was partitioned with various organic solvent viz. n-hexane, chloroform, ethyl acetate and n-butanol (Ahmed *et al.,* 2007). The MIC values of the isolated fractions were recorded by making different concentrations (0.19 mg – 100 mg mL^{-1}) by serial dilution method against *A. solani* (Table.1). n-hexane and synthetic fungicide were found to be most antifungal as its highest and lowest (0.19 mg -100 mg mL^{-1}) concentration totally inhibits the conidial germination of *A. solani* even after 72 hrs incubation period. Other fractions were comparatively less antifungal and the chloroform fraction was least effectual. Several compounds have been isolated from *M. azedarach* including bakayanin, quercitrin, rutin, backalactone 6 β-hydroxy-4-stigmastem-3-one and 6 β-hydroxy-4-campesten-3-one,4, 5-dihydroxy-7-0-α-L-rhamnopyranosyl-(1-4)-β-glucopyranoside, cystine, serine, arginine, glycine, glutamic acid, threonine, methionine, leucine, lycine and praline (Khan *et al.,* 2008). Some of them like β-sitosterol, β-amyrin, ursolic acid , benzoic acid , 3,5 dimethoxybenzoic acid and maesol are used as antifungal agents against chickpea blight (Jabeen *et al.,* 2011).

Table 1. MIC values of different organic fractions of methanolic leaf extract of *M. azedarach* and synthetic fungicide Metalaxyl+Mancozeb against *A. solani*, after 24, 48 and 72 hours incubation periods.

Fractions	Concentration (mg mL^{-1})									
	100	50	25	12.5	6.25	3.125	1.56	0.78	0.39	0.19
24 hours after incubation										
Control (H_2O)	+	+	+	+	+	+	+	+	+	+
Control (DMSO)	-	-	-	-	-	+	+	+	+	+
n-Hexane	-	-	-	-	-	-	-	-	-	-
Chloroform	-	-	-	+	+	+	+	+	+	+
Ethyl acetate	-	-	-	+	+	+	+	+	+	+
n-Butanol	-	-	-	+	+	+	+	+	+	+
Metalayl+Mancozeb, 72WP	-	-	-	-	-	-	-	-	-	-
48 hours after incubation										
Control (H_2O)	+	+	+	+	+	+	+	+	+	+
Control (DMSO)	-	-	-	-	-	-	+	+	+	+
n-Hexane	-	-	-	-	-	-	-	-	-	-
Chloroform	-	-	-	+	+	+	+	+	+	+
Ethyl acetate	-	-	+	+	+	+	+	+	+	+
n-Butanol	-	-	-	+	+	+	+	+	+	+
Metalayl+Mancozeb, 72WP	-	-	-	-	-	-	-	-	-	-
72 hours after incubation										
Control (H_2O)	+	+	+	+	+	+	+	+	+	+
Control (DMSO)	+	+	+	+	+	+	+	+	+	+
n-Hexane	-	-	-	-	-	-	-	-	-	-
Chloroform	+	+	+	+	+	+	+	+	+	+
Ethyl acetate	+	+	+	+	+	+	+	+	+	+
n-Butanol	+	+	+	+	+	+	+	+	+	+
Metalayl+Mancozeb, 72WP	-	-	-	-	-	-	-	-	-	-

Mycelium Present: +; Mycelium Absent: -

CONCLUSION

Present study concludes that *M. azedarach* leaves possess substantial antifungal potential against *A. solani* and n-hexane fraction is much effective against the target fungus with 0.19 mg mL^{-1} MIC.

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