Mycopath (2013) 11(2): 75-79 **Research Article**

Management of *Macrophamina phaseolina* by methanolic extracts of *Ficus benghalensis* L.

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Abstract

Antifungal potential of *Ficus benghalensis* L. was evaluated against *Macrophamina phaseolina* (Tassi) Goid., causal agent of charcoal rot in many plant species. Leaf extract of *F. benghalensis* exhibited strong antifungal activity. Methanolic leaf extract was portioned among *n*-hexane, chloroform, ethyl acetate and *n*-butanol. Different concentration of fungicide Metalaxyl+Mancozeb and organic fractions (1000-1.95 mg mL^{-1}) were assessed by minimum inhibitory concentration (MIC) assay against *M. phaseolina*. *n*-butanol fraction and fungicide were found the most effective with 1.95 mg mL^{-1} MIC.

Keywords: Antifungal, *Ficus benghalensis*, *Macrophomina phaseolina*, methanolic extract.

Introduction

Macrophomina phaseolina (Tassi) Goid. is one of the most damaging seed and soil-borne pathogen, infecting about 500 plant species in more than 100 families throughout the world. Under favourable conditions the fungus causes many diseases like damping off, seedling blight, collar rot, stem rot, charcoal rot and root rot in various economically important crops (Kunwar *et al.,* 1986; Mihail and Taylor, 1995; Srivastava and Singh, 1990).

Conventional agricultural practices like crop rotation, mulching, green manures and compost, biological pest control, and mechanical control although these measures somehow control the charcoal rot disease but these methods are not so effective. The best method of controlling this disease is the use of resistant varieties (Ilyas *et al.*, 1999). However, the cultivars with resistance not exhibited the desired results due to lack of stability in tolerance because of appearance of new pathotypes of the pathogen (Singh and Reddy, 1991). Charcoal rot is controlled by seed dressing and foliar spray of fungicide but these pesticides are chemical compounds or biological organisms used to kill or inhibit fungus or fungal spores. The residues of pesticides interrupt natural processes and ecosystems and pose menace to human and animal health (Mancini *et al.*, 2008). Increasing awareness towards the ecosystem and environment has made a marked shift from synthetic materials to bio-products. Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails (Okigbo and Ogbonnaya, 2006). Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to the synthetic pesticides (Verma and Dubey, 1999).

Ficus benghalensis (Moraceae, Mulberry family) is commonly known as Banyan tree or Vata or Vada tree in Ayurveda. It is used in Ayurveda for the treatment of diarrhea, dysentery, piles, teeth disorders, rheumatism, skin disorders like sores and to boost immune system, as a hypoglycemic (Mukherjee *et al.,* 1998). *F. benghalensis* has potential antimicrobial and antifungal activities as it contains various glucoside, 20-tetratriaconthene-2-one, 6-heptatriacontene-10-one, pentatriacontan-5-one, beta sitostirol-alpha-D-glucose and meso-inositol have been isolated from the bark (Gayathri *et al*., 2009). The present study was therefore designed to evaluate the antifungal potential of *F. benghalinsis* against *M. phaseolina* the causal agent of charcoal rot of various economically important crops.

Materials and Methods

Selection and collection of test plant material

F. benghalensis was chosen to evaluate the antifungal activity of its different parts leaf and roots against *M. phaseolina* the cause of charcoal rot in various economically important crops. Leaves and roots of *F. benghalensis* were collected from the Defence Housing Authority Lahore. This plant material was dried under the sunlight and stored in polythene bags.

Procurement and culture maintaining of *M. phaseolina*

Pure culture of test fungal species *M. phaseolina* was prepared by transferring the diseased stem of plant on potato dextrose agar *(*PDA) medium. Stem was first sterilized by 1% sodium hypochloride solution and then it was inoculated on 2% PDA medium. This culture was identified by observing its morphological and microscopic features and was sub cultured and maintained on 2% PDA and stored in refrigerator at 4 °C.

Preparation of methanolic extract

Twenty grams of each plant part dried materials were weighed on Shimadzu AX 120 was soaked in 100 mL methanol and left for three days at room temperature . After three days material were filtered through an autoclaved muslin cloth. Organic solvent extracts was evaporated to reduce it volume up to 2 mL under dried oven at 35 °C and then diluted by adding appropriate amount of distilled water to make final volume of 100 mL. These stock solvent extract were stored in refrigerator at 4 °C and used within four days.

Antifungal bioassays

PDA was prepared by adding 2 g PDA in 100 mL of distilled water in 250 mL conical flask this solution was autoclaved at 121 °C for 30 minutes. To 80 mL of PDA and adequate amount of distillated water was added in each flask of all applied concentrations of leaf and roots of *F. benghaensis*. Various v/v applied concentrations viz. 4, 5, 3, 2 and 1% were prepared by adding appropriate amount of stock solution to PDA medium. Control treatments were without any plant extracts. Each concentration was supplemented with Chloromycetin capsule @ 50 mg 100 mL 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 and each concentration was replicated thrice. Mycelial discs of 5 mm was prepared by using a cork borer from the pure culture of *M. phasolina* and placed in the center of each Petri plate after solidification of PDA medium .These plates were incubated for 7 days at 25±2 ^{o}C After 7 days fungal growth diameter was measured by taking average of three diameters taken at right angles for each colony.

Fractionation guided bioassays

Leaf extract of *F. benghalensis* exhibited strong antifungal potential in retarding the *in vitro* growth of *M. phaseolina*. This extracts was selected for further investigation. Leaves of *F. bengalensis* were portioned with *n*-hexane, chloroform followed by ethyl acetate and *n*-butanol at room temperature. Dried leaves (250 g) of *F. bengalensis* were fully extracted with 750 mL methanol at room temperature. The extract was evaporated under vacuum on rotary evaporator (Buchi Switzerland R-210) at 40 °C give up 16 g gummy mass. This methanolic extact (16 g) was portioned between n-hexane and water. The aqueous fraction was successively portioned with chloroform, ethyl acetate and n-butanol (Jabeen *et al.,* 2013) according to increasing polarity order. This portioning was yielded as gummy mass of *n*-hexane (2 g) chloroform (1 g) ethyl acetate (3 g) and *n*-butanol (2 g) and remaining was water fraction. This portioning was made by use of separating funnel

MIC values of the isolated fractions and synthetic fungicide (Metalaxyl+Mancozeb 72 WP) were tested in test tubes by serial dilution micro assay (Jabeen *et al.,* 2011) with small alterations. The four isolated fractions were dissolved in DMSO (dimethyl sulfoxide) and were serially diluted with water in test tubes. Maximum 1000 mg mL^{-1} concentration was prepared by adding 1 mL of DMSO and 1 mL of distilled water, this concentration was further serially diluted and the minimum applied concentration was 1.95 mg mL^{-1}. Freshly prepared PDA medium was added to seven days old fungul culture of *M. phesolina* to reach a final conidial concentration 1×10^{5}, 100 uL of this was added to test tubes having a diameter of 1.6 cm and 15 cm long. Test tube containing DMSO and distilled water was used as control. These test tubes were incubated at 25-30 ^{o}C. The MIC of the fractions was observed visually after 24, 48 and 72 hours by using inverted microscope to study the fungal mycelia growth.

Statistical analysis

Data were analyzed statistically by applying ANOVA followed by Duncan's Multiple Range test (Steel and Torrie, 1980)

Results and Discussion

In present study, the methanolic leaf and root extract of *F. benghalensis* were investigated against the target fungus *M. phaseolina*. The analysis of variance (ANOVA) showed significant effect of different plant parts of *F. bengalensis* solvent extracts and their different concentrations on growth of test fungus *M. phaseolina*.

Methanaolic leaf extracts of *F. bengalensis* was significantly inhibited the *in vitro* growth of test fungus *M. phaseolina.* Higher concentrations 5% and 4% were effectualy retarded the fungal growth up to 88% and 85% respectively. Other applied concentrations viz. 1%, 2% and 3% of *F. bengalensis* were also suppressed the test fungus diameter up to 82%, 79% and 84% respectively (Fig. 1 & 2). Methanolic root extract of *F. benghalensis* also significantly inhibited the growth of test fungus. All the applied concentrations of root extract showed pronounced antifungal activity but 4% and 5% concentration most effectively retard fungal colony diameter 86% and 89% respectively as compared to control. Other concentrations of root extract (1%, 2% and 3%) were also significantly retarded the diameter of *M. phaseolina* up to 82%, 80% and 84% (Fig. 1 & 4). Earlier Wang *et al.* (2011) studied methanol extract of *F. sarmentosa* to detect potent inhibitory activities against *Fusarium graminearum, Pumpkin fusarium*, *Curvularia lunata*, *Septoria zeicola*, *Botrytis cinerea*, and *Rhizoctonia solani*. Flavonoids isolated from *F. sarmentosa* displayed excellent inhibitory activity against *F. graminearum* and *S. zeicola*. Similar studies showed that phenolics, flavanoids, terpenoids and saponins contents appear to be responsible for antifungal activity of *F. benghalensis* (Aswar *et al.,* 2008; Shi *et al*., 2011).

Leaf extract was selected for further treatment and different organic fractions viz. *n*-hexane, chloroform, ethyl acetate and *n*-butanol were portioned from methanolic extract of *F. benghalensis* leaves. MIC of four isolated fractions along with a reference synthetic fungicide Metalaxyl+Mancozeb, 72 WP was observed against the test fungus (Table 1). *n*-butanol and synthetic fungicide were found to be most antifungal as highest and lowest concentrations totally inhibits the conidial germination of *M. phaseolina* even after 72 hrs incubation period. *n*-hexane and chloroform fractions were comparatively less antifungal and ethyl acetate fraction was least effectual. Both the control treatments aqueous and dimethyl sulphoxide stimulated the spore germination of *M. phaseolina*. Similar results were reported by Jabeen *et al.* (2013) as *n*-hexane fraction of *Calotropis procera* leaves and synthetic fungicide retarded *M. phaseolina* spore germination after 48 hrs incubation. This variable activity of organic fractions may be due to the fact that different compounds were soluble in different solvents according to their polarity. The results of the MIC determinations are supported by the literature as Keute *et al.* (2009) studied the MIC of the various fractions of methanol extracts from the stem bark of *F. ovata* against *Streptococcus faecalis, Candida albicans* and *Microsporum audouinii*. This study suggested that *F. ovata* is more fungicidal due to the presence of 3-friedelanone, taraxeryl acetate, betulinic acid, oleanoïc acid, 2-hydroxyisoprunetin, 6,7-(2-isopropenyl furo)-5,2,4-trihydroxyisoflavone, cajanin and protocatechuic acid. Similarly Sardari *et al.* (2009) investigated the antifungal activity of herbal extracts of *Rheum ribes*, *F. bengalensis*, *Morus alba*, *Musa sapientum*, *Arnebia decumbens*, *Citrus limon*, *Fraxinus excelsior*, *Rumex acetosella* and *Arnebia echioides.* These herbal extracts showed antifungal activity at 250 µg mL^{-1}. The present study concludes that different parts of *F. benghalensis* contain antifungal substances that showed variable antifungal activity.

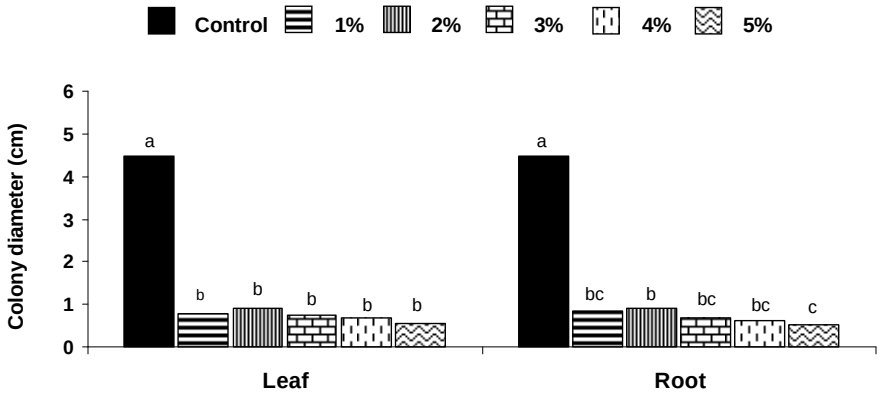

Fig. 1: Effect of methanolic leaf and root extracts of *Ficus benghalensis,* on growth of *Macrophamina phaseolina.* Vertical bars show standard error of means of three replicates. Values with different letters show significant difference as determined by DMR Test.

Table 1: MIC values of different fractions isolated from *F. bengalensis* and synthetic fungicide Metalaxyl+Mancozeb against *M. phaseolina* after 24, 48 and 72 hrs incubation period.

Fractions	Concentration (mg mL^{-1})									
	1000	**500**	**250**	**125**	**62.5**	**31.25**	**15.62**	**7.81**	**3.90**	**1.95**
24 hours after incubation										
Control (H_2O)	+	+	+	+	+	+	+	+	+	+
Control (DMSO)	+	+	+	+	+	+	+	+	+	+
n-Hexane	-	-	-	-	-	-	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-	-	-
Ethyl acetate	-	-	-	-	-	-		-	-	-
n-Butanol	-	-	-	-	-	-	-	-	-	-
Metalayl+Mancozeb	-	-	-	-	-	-	-	-	-	-
48 hours after incubation										
Control (H_2O)	+	+	+	+	+	+	+	+	+	+
Control (DMSO)	+	+	+	+	+	+	+	+	+	+
n-Hexane	-	-	-	-	-	-	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-	-	-
Ethyl acetate	+	+	+	+	+	+	+	+	+	+
n-Butanol	-	-	-	-	-	-	-	-	-	-
Metalayl+Mancozeb	-	-	-	-	-	-	-	-	-	-
72 hours after incubation										
Control (H_2O)	+	+	+	+	+	+	+	+	+	+
Control (DMSO)	+	+	+	+	+	+	+	+	+	+
n-Hexane	-	-	-	-	-	-	-	-	-	-
Chloroform	+	+	+	+	+	+	+	+	+	+
Ethyl acetate	+	+	+	+	+	+	+	+	+	+
n-Butanol	-	-	-	-	-	-	-	-	-	-
Metalayl+Mancozeb	-	-	-	-	-	-	-	-	-	-

\+ Mycelium appeared.
\- Mycelium did not appear.

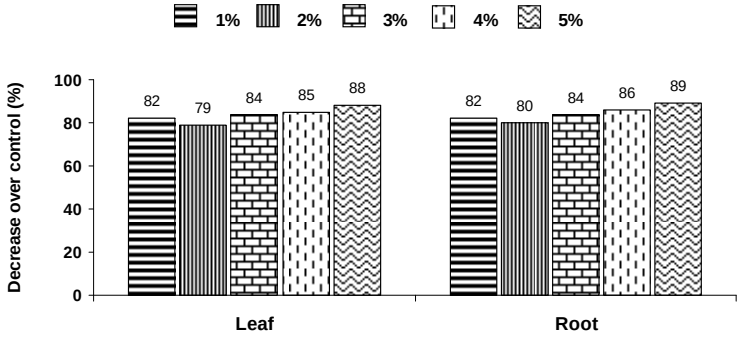

Fig. 2: Percentage increase/decrease in diameter of *Macrophamina phaseolina* due to different concentrations of methanolic root extract of *Ficus bengalensis.*

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