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MANAGEMENT OF DAMPING OFF DISEASE BY EXTRACTS OF *ALBIZIA LEBBECK* (L.) BENTH

SHAHNAZ HANIF, KHAJISTA JABEEN* AND SUMERA IQBAL

Department of Botany, Lahore College for Women University, Jail Road, Lahore, Pakistan

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Abstract

Antifungal potential of various parts of *Albizia lebbeck* (L.) Benth. was evaluated against the destructive damping off disease causing fungus *Rizoctonia solani* Kuhn. Different concentrations of leaf, fruit, bark and root methanol extracts *viz.,* 1, 2, 3, 4 and 5% were tested against *R. solani*. Leaf extract was found more effective than fruit, bark and root extracts. The methanolic leaf extract of *A. lebbeck* was fractioned between *n*-hexane, chloroform, ethyl acetate and *n*-butanol. The bioactivities of these isolated fractions were tested against *R. solani*. The chloroform fraction was significantly inhibited the test fungus growth. So, this chloroform fraction was further analyzed to separate various constituents through column chromatography. Eleven sub-fractions were isolated from column chromatography of chloroform extract and their minimum inhibitory concentration (MIC) was evaluated against *R. solani*. Synthetic fungicide (Metalaxyl + mancozeb, 72 WP) was used as reference fungicide. After 72 hrs incubation period fraction (7) and the fungicide were effectively suppressed the spore germination of *R. solani* with MIC of 0.00019 mg/ml. The present study can be concluded that *A. lebbeck* possesses active antifungal constituents against *R. solani*.

Introduction

Rhizoctonia solani Kuhn, is a destructive soil borne pathogen responsible for distressing damping off disease. *R. solani* inhabits soil in the form of sclerotia and affects newly germinated seedlings of the host plant, results in wilting, decay and death of succulent tissues. Its common hosts are alfalfa, peanut, soybean, lima bean, potato, tomato, cucumber, papaya, eggplant and corn. This destructive damping off fungus caused up to 50% yield losses worldwide in large number of economically important crops (Wallwork 2000).

Organic soil alteration and crop rotation with non-susceptible crop are eco-friendly method for controlling damping-off disease (Dey 2005). However, these cultural practices are not completely effective, and *Rhizoctonia* disease remains a constant problem. Many commercial synthetic fungicides such as benzimidazoles and mancozeb are used to control *R. solani* (Myresiotis *et al.* 2007). But, the progress of resistance in this pathogenic fungus to common fungicides and increasing harmful effects on human and environment has given a chance to search for new plant derivatives that can slow down the fungal pathogenecity. Use of natural products for the management of fungal plant diseases is considered as a good alternate to synthetic fungicides, due to their less negative impact on the environment (Hanekamp and Kwakman 2004). Botanical compounds (plants allelochemicals) are more environmentally safe than synthetic chemicals (Hashim and Devi 2003).

Albizzia lebbeck (L.) Bennth. belongs to Fabaceae, contains proteins, macrocyclic alkaloids, saponin, phenolic glycosides and flavonoids (Mishara *et al.* 2010, Elazki *et al.* 2012). This plant also possesses analgesic and anti-inflammatory properties (Shah 2009). Due to these properties of *A. lebbeck*, the current study was aimed to study the *A. lebbeck* antifungal potential against *R. solani.*

*Author for correspondence: <khajista_1@hotmail.com>.

Material and Methods

A. lebbeck leaves, fruits, bark and roots were collected from Lahore College for Women University, Lahore, Pakistan. After extensive washing with tap water, the plant material was surface sterilized with 1% sodium hypochlorite solution followed by distilled water. These plant materials were dried at 40°C in an electric oven and grinded to form powder. The test fungal species *R. solani* was isolated from the rhizospheric soil of *Rosa indica* L. on PDA medium. After purification the pure cultures were preserved on 2% PDA (Potao Dextrose Agar) medium and were kept at 4°C in refrigerator.

Twenty grams dried powder of each plant part was soaked in 100 ml of methanol for three days at room temperature. After three days these extracts were dried at room temperature and various concentrations (1 - 5%) of methanolic extract leaf, bark, root and fruit were made.

Mycelial discs (5 mm) was prepared using sterilized cork borer from the tip of 7 days old culture of *R. solani* and was placed in the center of each Petri plate after solidification of PDA medium. Three replicates were made for each treatment. All these plates were incubated at 25ºC for one week. After 7 days, fungal growth diameter 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 formula:

$$\text{Growth Inhibition (\%)} = \frac{\text{Growth in Treatment} - \text{Growth in Control}}{\text{Growth in Control}} \times 100$$

A. lebbeck leaves (1000 g) were soaked in 2.5 liters of methanol for one week. After one week this methanolic extract was evaporated under vacuum on rotary evaporator at 40°C, yield 64 g of gummy mass. Adequate quantity of distilled water was added in the gummy mass and successively partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol at room temperature (Jabeen *et al.* 2013). This partitioning was resulted as gummy mass of *n*-hexane (7 g), chloroform (20 g), ethyl acetate (7 g), *n*-butanol (8 g) and remaining water fraction.

In vitro antifungal activity of these four isolated fractions was studied against *R. solani* through agar serial dilution method given by Jabeen *et al.* (2014). Experiment was done by applying appropriate amount of all isolated fractions into 5 ml of PDA medium and 0.01 - 2.00 mg/ml final concentrations were made. Control Petri plates were without any extract.

The chloroform soluble fraction (13 g) was subjected to column chromatography for the separation of different chemical constituents. The column was filled with silica gel (E. Merck, 230 - 400 mesh) and eluted with *n*-hexane, *n*-hexane - chloroform, chloroform-methanol and methanol. This separation gave 11 sub-fractions.

These 11 sub-fractions isolated from chloroform fraction of methanolic extract of *A. lebbeck* leaves were evaluated through minimum inhibitory concentration assay. The MIC of these fractions along with a commercial synthetic fungicide (Metalaxyl + mancozeb, 72 WP) was tested against the test fungus *R. solani*. All the fractions and fungicide were diluted by serial dilution method and the MIC assay was conducted in test tubes (Jabeen *et al.* 2011). Maximum 0.1 mg of each fraction was dissolved in 1 ml of DMSO (dimethyl sulphoxide) and 1 ml of distilled water and this concentration was further serially diluted and the minimum tested concentration was 0.00019 mg/l. Conidial concentration of 1×10^5 was prepared from seven days old culture of *R. solani* and 100 µl of this was added to test tubes of 1.6 cm diameter and 15 cm length. Test tubes containing DMSO and distilled water was served as control. These test tubes were incubated at 25 - 30°C after 24, 48 and 72 hrs, MIC of these isolated organic fractions and fungicide was observed visually by using inverted microscope to study the fungal mycelial growth.

The data was analyzed statistically by applying ANOVA followed by Duncan's Multiple Range Test (Steel *et al.* 1997).

Results and Discussion

In present study *Albizia lebbeck* (leaves, fruits, bark and root) methanolic extract was evaluated against causal agent of damping off disease *R. solani*. In all concentrations, *A. lebbeck* methanolic extract significantly suppressed the test fungus growth. In case of leaf extract maximum 33% reduction in the test fungus radial diameter was observed in 5% concentration (Fig. 1A). Methanolic fruit extract of *A. lebbeck* also significantly suppressed the test fungal growth. The 5% concentration of this extract was also found highly effective against the *R. solani* with 19% reduction compared to control (Fig. 1B). Bark and root extracts also possess antifungal potential against the target pathogen (Fig. 1C. D). Earlier Bobby *et al.* (2012) reported that methanolic extract of *A. labbeck* has inhibitory potential against a number of plant pathogens. Recently Ascencion *et al.* (2015) stated that the leaf residues of *Brassica napus*, *B. rapa* and *B. juncea* significantly retarded the biomass of *R. solani*.

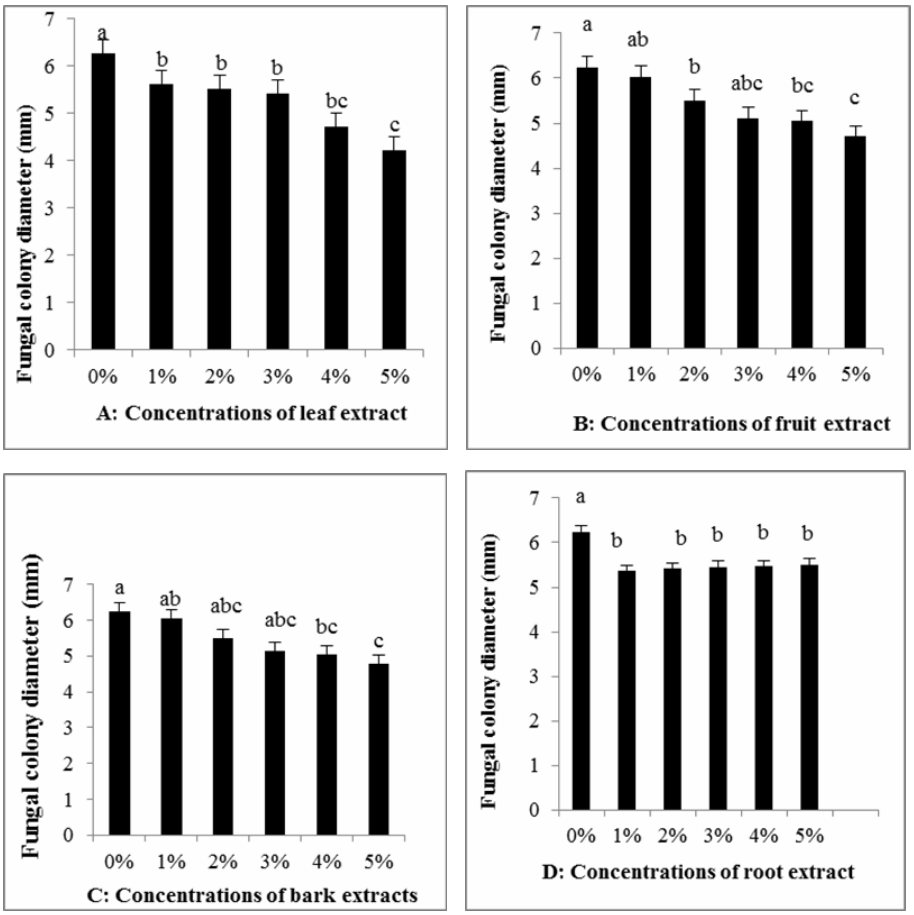

Fig. 1. Changes in radial diameter of *R. solani* by the effects of *A. lebbeck*, (A) leaf extract, (B) fruit extract, (C) bark extract and (D) root extract.

Different organic fractions *viz.*, *n*-hexane, chloroform, ethyl acetate and *n*-butanol were isolated from the crude methanol extract of *A. lebbeck* leaves (Fig. 2). *In vitro* experiments with these isolated organic fractions showed that chloroform fraction was more antifungal as compared to other fractions, as 38% inhibition in test fungal diameter was observed in this fraction. Earlier, Chaddah *et al.* (2011) reported that the presence of alkaloids, glycosides, steroids, flavanoids, saponins, tannins, carbohydrates and reducing sugar might be responsible for the antifungal potential of *A. lebbeck*.

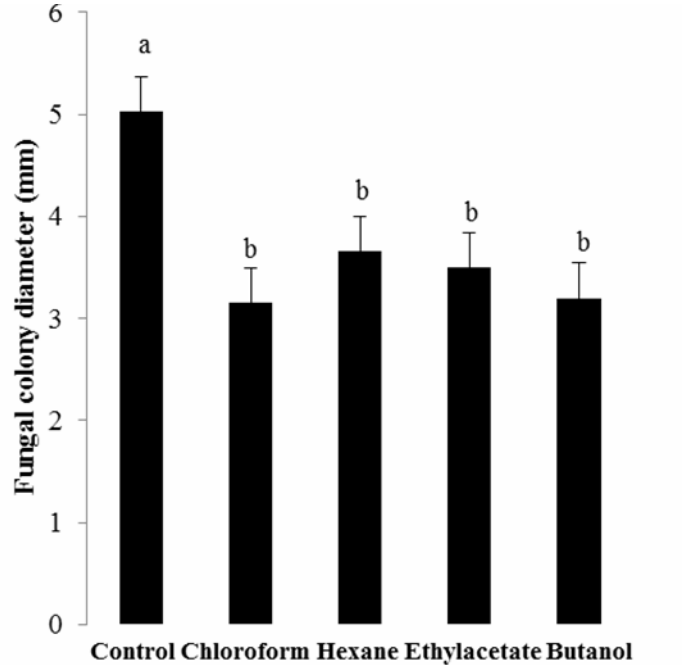

Fig. 2. Effect of various organic fractions of *A. lebbeck* leaf extract on *in vitro* growth of *R. solani*.

Column chromatography was performed with chloroform fraction to separate various antifungal fractions. For this purpose column was eluted with *n*-hexane; *n*-hexane-chloroform: chloroform-methanol and methanol. Eleven fractions were separated from column chromatography and the MIC (Minimum inhibitory concentration) for these fractions (0.1 mg/ml to 0.00019 mg/ml) and synthetic fungicide was tested (Table. 1). Fraction 7 and the synthetic fungicide were found most effectual as their lowest concentration 0.00019 mg/ml completely inhibited the spore germination of *R. solani.* Other fractions 5, 8 and 9 were also effective but their order of effectiveness was little bit low. Earlier Shahid and Firdous (2012) studied the antimicrobial potential of *A. lebbeck* and *Acacia leucophloea* (Roxb.) Wild. by minimum inhibitory concentration assay. They suggested that both the species have promising antimicrobial potential. Previously, Ueda *et al.* (2003) and Jangwan *et al.* (2010) also suggested that bioactivity of *A. lebbeck* leaf might be due to the presence of saponin.

On the basis of these findings, the present study concluded that *A. lebbeck* has significant antifungal potential against *R. solani.* Future researches on the effective fractions with strong fungitoxic potential would be exploited to develop cost effective natural fungicide against *R. solani.*

Table 1. MIC values of different organic fractions isolated from *A. lebbeck* and fungicide against *R. solani* after 24, 48 and 72 hrs incubation periods.

Fractions	mg/ml									
	0.1	0.05	0.025	0.0125	0.0062	0.0031	0.0015	0.0007	0.0003	0.0019
After 24 hrs incubation										
H_2O	+	+	+	+	+	+	+	+	+	+
(DMSO)	+	+	+	+	+	+	+	+	+	+
1	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-
Fungicide	-	-	-	-	-	-	-	-	-	-
After 48 hrs incubation										
H_2O	+	+	+	+	+	+	+	+	+	+
(DMSO)	+	+	+	+	+	+	+	+	+	+
1	-	-	-	-	-	-	-	-	+	+
2	-	-	-	-	-	-	-	-	+	+
3	-	-	-	-	-	-	-	-	+	+
4	-	-	-	-	-	-	-	-	+	+
5	-	-	-	-	-	-	-	-	+	+
6	-	-	-	-	-	-	-	-	-	+
7	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	+
9	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	+
11	-	-	-	-	-	-	-	-	-	+
Fungicide	-	-	-	-	-	-	-	-	-	-
After 72 hrs incubation										
H_2O	+	+	+	+	+	+	+	+	+	+
(DMSO)	+	+	+	+	+	+	+	+	+	+
1	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+
5	-	-	-	-	-	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+
7	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	+	+	+
9	-	-	-	-	-	-	-	-	+	+
10	+	+	+	+	+	+	+	+	+	+
11	+	+	+	+	+	+	+	+	+	+
Fungicide	-	-	-	-	-	-	-	-	-	-

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