Research Article

Antifungal activity of *Syzygium cumini* L. against *Rhizoctonia solani*

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Abstract

Antifungal activity of *Syzygium cumini* (L.) Skeels bark and leaves were evaluated against *Rhizoctonia solani* Kuhn, the causal agent of damping-off. Extracts of bark and leaves of *S. cumini* were prepared in methanol at a concentrations viz. 1%, 2%, 3%, 4% and 5% were tested against the target pathogen. Methanolic bark extract of *S. cumini* was found more effective and showed high antifungal potential as compared to leaf extract. The methanolic bark extract was subjected to bioassay guided fractionation and different organic fractions like ethyl acetate, chloroform, *n*-hexane and *n*-butanol were isolated. The isolated fractions were further serially diluted to check their Minimum Inhibitory Concentration along with a synthetic fungicide Puslan (72% WP). The MIC of various concentrations from (1.56-200 mg mL⁻¹) was recorded for each fraction at the intervals of 24, 48 and 72 hours. *n*-butanol and Puslan were highly effective in inhibiting the mycelium growth of *R. solani* with MIC of 1.56 mg/ml.

Key words: Antifungal activity; Damping off; Jaman; MIC; Plant extract

Introduction

Rhizoctonia solani Kuhn is a soil born fungal pathogen that causes damping off disease in members of different plant families such as Leguminosae, Graminae, Solanaceae, Brassicaceae, Cucurbitaceae, Malvaceae, Chenopodiaceae, Cyperaceae and Caryophyllaceae [1, 2]. The pathogen inhabits in the soil as thick walled sclerotia and brown colour hyphae. Due to heavy yield losses in economical important crops by *R. solani* have been reported worldwide [3, 4].

Since, the pathogen causes large economic losses therefore, a number of management techniques have been developed. Cultural practices such as soil mulching, planting date adjustment, deep ploughing, irrigation timing and crop rotation can help to control

the disease [5]. But these practices are not effective enough to control the disease. Resistant varieties can also be used in disease control but it has been found that genetic resistance cannot be much effective measure [6]. Although fungicides help to reduce this fungal disease, but the chemical residues of fungicides can persist inside plant tissue and such residues persisting in fruit or vegetables may cause hazardous effects on consumers health [7-10].

The problems raised by using synthetic pesticides increased the need of alternative biodegradable pesticides [11]. The plant world can provide nontoxic and biodegradable natural products which can be effective in controlling many phytopathogens. These bioactive natural compounds are secondary metabolites

including terpenoids, quinines and tannins [12].

Syzygium cumini (L.) Skeels is an evergreen tree belongs to family Myrtaceae native to sub-continent region. *S. cumini* is rich in compounds containing anthocyanins, acylated flavonol glycosides, glucosides, ellagic acid, isoquercetin, kaemferol and myrecetin, myricetin, 3-O-4-acetyl-L-rhamnopyranoside, quercetin, triterpenoids, esterase, galloyl carboxylase, tanninsbetulinic acid, friedelin, epi-friedelanol, beta-sitosterol, eugenin and fatty acid ester of epi-friedelanol [13-15]. So, the present study is designed to evaluate the antifungal activity of *S. cumini* against *R. solani*.

Materials and methods

The present study was conducted at Plant Pathology Lab at Botany Department, Lahore College for Women University, Lahore, Pakistan, in 2013. Leaves and bark of *S. cumini* were collected from the Government Employees Co-operative Housing Society Town Ship, Lahore (Longitude: 74°20' 36" E, Altitude: 688 ft). The plant material was dried under sunlight and stored in polythene bags. Culture of test fungus *R. solani* was prepared by sprinkling contaminated soil on PDA medium. This culture was sub-cultured and maintained on 2% PDA (Potato Dextrose Agar) and stored in refrigerator at 4°C for future use.

Antifungal bioassays

Twenty grams of dried material of each plant part of *S. cumini* was soaked in 100 ml methanol and left for three days at room temperature. After three days the soaked material was filtered through an autoclaved muslin cloth. This methanolic extract was evaporated to reduce their volume up to 2 ml under dried oven at 35°C and then diluted by adding appropriate amount of distilled water to make the final volume of 100 ml. The stock solvent extracts thus, made were

stored in refrigerator at 4°C and used within four days.

Potato Dextrose Agar (2%) was prepared by adding 2 g PDA in 100 ml distilled water in 250 ml conical flask and this solution was autoclaved at 121°C for 30 minutes. Different concentrations 1, 2, 3, 4 and 5% were prepared for experimental treatment. To make 1% w/v concentration 2 g of PDA was added in 95 ml distilled water, to this solution 5 ml of stock solution was added. Similarly, the remaining concentrations (that are 2, 3, 4 and 5%) were prepared by adding 10 ml, 15 ml, 20 ml and 25 ml of stock solution respectively. Each concentration was supplemented with Chloromycetin capsule @ 50 mg/100 ml to avoid bacterial contamination.

In vitro antifungal bioassay was conducted with the organic solvent extract. Three replicates of each applied concentration were prepared by pouring media into sterilized plates. Mycelial discs of 5 mm was prepared by using a cork borer from the pure culture of *R. solani* and placed in the center of each petri plate after solidification of PDA medium. These plates were incubated for 7 days at 25 ± 2 °C. 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:

Growth inhibition (%) =

$$\frac{\text{Growth intreatment} - \text{Growth in control}}{\text{Growth in control}} \times 100$$

Bioassay guided fractionation

Bark extract of *S. cumini* found more effective in screening bioassay so, the methanolic bark extract of *S. cumini* was partitioned with *n*-hexane, chloroform followed by ethyl acetate and *n*-butanol at room temperature. 300 g dried bark of *S. cumini* was fully extracted with 500 mL methanol at room temperature. The obtained extract was evaporated under vacuum on rotary evaporator (Buchi Switzerland R-

210) at 40°C, this gave up turbid methanolic extract. The methanolic extract was then partitioned by using separating funnel between *n*-hexane and water. The obtained aqueous fraction was then partitioned successively with chloroform, ethyl acetate and *n*-butanol [16] according to increasing order of polarity. This partitioning yielded 2.2 g *n*-hexane fraction, 2.1 g chloroform fraction, 0.2 g ethyl acetate fraction, 1.45 g *n*-butanol fraction and the remaining was water fraction.

The MIC values of the isolated fractions and synthetic fungicide Puslan 72 WP (Metalaxyl+Mancozeb) were determined in test tubes by serial dilution micro assay [17]. The four isolated fractions and synthetic fungicide were dissolved in DMSO (Dimethyl Sulfoxide). Maximum 200 mg/ml 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 1.56 mg/ml. Freshly prepared malt extract medium was added to seven days old fungal culture of *R. solani* and 1×10^5 100 µl mycelial suspension was prepared and added in each concentration of all the four fractions, fungicide and control set. All the test tubes were incubated at 25-30°C. The MIC of the fractions was observed after 24, 48 and 72 hours with the help of inverted microscope and the fungal mycelia growths were measured. .

The data was statistically analyzed by applying ANOVA followed by Duncan's Multiple Range Test at significant level $P \leq 0.05$ by using co-stat software [18].

Results and discussion

In the present study the methanolic extract bark and leaf extract of *S. cumini* were evaluated for their antifungal potential against *R. solani*. All the applied concentrations of the methanolic bark extract of *S. cumini* (1- 5%) significantly retarded the *in vitro* growth of the test

fungus. However, concentration 5% was found to be highly effective and significantly surpassed all other treatments by reducing 43% of the fungal growth when compared to the control treatment. Followed by 3 and 4% which suppressed the colony diameter of *R. solani* by 35% and 40% respectively. Concentration 1% explored low significant effect and resulted in reduction of 25% of the fungal growth (Fig. 1 & 2). Data regarding the methanolic extract of *S. cumini* leaves on *in vitro* growth of *R. solani* is presented in (Fig. 3 & 4). All the applied concentrations significantly retarded *R. solani* growth but the reduction amounts were less when compared to the observed bark antifungal activity. The maximum significant inhibition of the fungal growth observed at 5% concentration, which caused 28% reduction in the test fungal growth diameter when compared to control treatment. This concentration significantly surpassed all other treatment effects except 4% concentration. 1% concentration explored low significant effect and resulted in reduction of 25% of the fungal growth. Earlier Jabeen and Javaid [19] studied the antifungal potential of leaves, stem and root bark of *S. cumini* against *Ascochyta rabiei*. Aqueous, *n*-hexane and ethanol extracts of *S. cumini* significantly retarded the growth of the test fungus. Fifty two plants of different plant families were studied for their antifungal activity by Satish *et al.* [20]. Among all the tested plant aqueous extract of *S. cumini* alongwith *Datura stramonium*, *Acacia nilotica*, *Lawsonia inermis*, *Peltophorum pterocarpum*, *Punica granatum*, *Eucalyptus globules*, *Polyalthia longifolia*, *Mimusops elengi*, *Prosopis juliflora* and *Peltophorum pterocarpum*, showed strong antifungal activity against the tested *Aspergillus* species. Kuate *et al.* [21] stated that antifungal activity *S. jambosis* due to the presence of friedeloactone and friedelintriterpenes.

Results of the MIC of four isolated fractions in reference with the synthetic fungicide Puslan (Metalaxyl + Mancozeb, 72 WP) against *R. solani* growth revealed that *n*-butanol and synthetic fungicide were found most effective in inhibiting the mycelium production in *R. solani* even at lowest concentration of 1.56 mg, 72 h of incubation period. Other fractions were found ineffective in inhibiting germination of the test fungus. The results of MIC

determination are supported by the literature as Shihabudeen *et al.* [22] found that methanolic extract of *Eugenia jambolana* exhibited the highest antibacterial efficacy against *Staphylococcus aureus* and *Pseudomonas aeruginosa* at lower concentrations.

The present study concludes that different part of *S. cumini* contain antifungal constituents against the test fungus *R. solani*.

Figure 1. Antifungal activity of the methanolic bark extracts of *S. cumini*, on *in vitro* growth of *R. solani*

Figure 2. Percentage inhibition in diameter of *R. solani* due to different concentrations of methanolic bark extract of *S. cumini*.

Figure 3. Antifungal activity of the methanolic leaf extracts of *S. cumini*, on *in vitro* growth of *R. solani*

Figure 4. Percentage inhibition in diameter of *R. solani* due to different concentrations of methanolic leaf extract of *S. cumini*

Table 1. MIC values of different organic fractions isolated from *S. cumini* and the synthetic fungicide Puslan (Metalaxyl+Mancozeb, 72 WP) against *R. solani*, growth after 24, 48 and 72 h incubation period

Fractions	Concentration (mg/ml)							
	200mg	100mg	50mg	25mg	12.5mg	6.25mg	3.13mg	1.56mg
24 hours after of incubation								
Control (H ₂ O)	+	+	-	+	+	+	+	-
Control (DMSO)	-	+	-	+	-	-	+	-
<i>n</i> -Hexane	-	-	-	-	+	+	+	+
Chloroform	-	-	-	+	+	+	-	+
Ethyl acetate	-	-	+	+	+	+	+	+
<i>n</i> -Butanol	-	-	-	-	-	-	-	-
Puslan 72% WP	-	-	-	-	-	-	-	-
48 hours after incubation								
Control (H ₂ O)	+	+	+	+	+	+	+	+
Control (DMSO)	+	+	+	+	+	+	+	-
<i>n</i> -Hexane	-	-	-	+	+	+	+	+
Chloroform	-	-	+	+	+	+	+	+
Ethyl acetate	-	-	+	+	+	+	+	+
<i>n</i> -Butanol	-	-	-	-	-	-	-	-
Puslan 72% WP	-	-	-	-	-	-	-	-
72 hours after incubation								
Control (H ₂ O)	+	+	+	+	+	+	+	+
Control (DMSO)	+	+	+	+	+	+	+	-
<i>n</i> -Hexane	+	+	+	+	+	+	+	+
Chloroform	+	+	+	+	+	+	+	+
Ethyl acetate	+	+	+	+	+	+	+	+
<i>n</i> -Butanol	-	-	-	-	-	-	-	-
Puslan 72% WP	-	-	-	-	-	-	-	-

Mycelium present: (+), Mycelium absent: (-)

Authors' contributions

Conceived and designed the experiments: K Jabeen, Performed the experiments: A Khan, Analyzed the data: K Jabeen, A Khan & S Iqbal, Contributed reagents/ materials/ analysis tools: K Jabeen & S Iqbal, Wrote the paper: A Khan, K Jabeen & S Iqbal.

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