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Phytochemicals of *Melia azedarach* Inhibiting the Growth of *Rhizoctonia solani*

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Black scurf is one of the oldest and common diseases of potato stems and stolons below the soil surface caused by *Rhizoctonia solani*. The present study was carried out to investigate the antifungal potential of *Melia azedarach* against this soil-borne fungal pathogen. Different concentrations (1, 2, …, 5%) of aqueous, methanol, *n*-hexane and chloroform extracts of leaves, stems and fruits of *M. azedarach* were prepared and were evaluated for their *in vitro* antifungal activity. Data were analyzed by Tukey HSD test at 5% level of significance. All the extracts showed variable antifungal activity. In general, leaf extracts exhibited the highest inhibitory effect against growth of the fungal pathogen followed by stem-bark and fruit extracts, respectively. Among the various extracts, leaf chloroform extract, stem-bark methanolic extract and fruit aqueous extract showed the best antifungal activity resulting in 20–89%, 4–85% and 28–70% reduction in fungal biomass over corresponding control treatments, respectively.

Key words: *Rhizoctonia solani*; *Melia azedarch*; Antifungal activity; Fungal biomass.

Soil-borne fungi are considered as major constraint in agro-ecosystem. *Rhizoctonia solani* is among those soil-inhabiting fungi that infect numerous plant species including potato, tomato and wheat [1-4]. It is worldwide in distribution, has physiologic strains and wide host range. It has a tendency to attack young tissues, especially bedding plants grown from seeds are susceptible to pre-emergence damping-off that accounts for a main portion of disease loss due to *R. solani*[5]. *Rhizoctonia* canker commonly called black scurf is one of the oldest and common infections of potato stems and stolons below the soil surface. The pathogen is also involved in early dying syndrome of potato [6].

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Fungicides are the primary means of controlling *R.* solani [7,8]. However, this approach not only uneconomical but also pollutes the environment [9]. There is an increasing awareness about the health hazards involved in the use of chemical pesticides. Therefore, much attention is being focused on alternative methods and new strategies to control plant pathogens[10,11]. Among the alternative methods, plant extraxts and pure constituents have been proved ecofriendly. Several recent studies have shown the antifungal properties of many plant species against common plant pathogens [12-15].

Medicinal plants are not only an important source of new pharmaceuticals but also serves as sources of antimicrobial agents. Plant extracts are known to play important role in suppressing of seed-borne pathogen *Fussarium oxysporum* resulting in improved seed germination [16].Several plant families like Acanthaceae, Anonaceae, Amranthaceae, Magnoliaceae and

Meliaceae are known for their antifungal properties[17]. Many members of *Meliaceae* are being screened worldwide for their medicinal applications, one among them is *Melia azedarach* L. Many tribal use members of family Meliaceae for both fungal and bacterial ailments since ancient times. *M. azedarach* is a native of Tropical Asia but introduced and naturalized in many other countries like Australia, Africa, United States of America and many Arab countries. Screening of three species of *Meliaceae* family namely *Azadirecta indica, Toona ciliata* and *M. azedarach* were done against *Macrophoma phaseolina* and it was found that the aqueous extracts of, these plants exhibit antifungal potential [18]. There are reports that residues of *M. azedarach* contain antifungal acids namely hydroxy coumarin scopoletin, pinoresinol, 4- hydroxyl-3-methycinnamaldehyde and vanillin [19,20]. The objective of present study was to investigate the antifungal activity of aqueous as well as organic solvent extracts of leaf, stem bark and fruits of *Melia azedarach* against *R. solani.*

MATERIALS AND METHODS

Isolation of target fungal species

R. solani was isolated from potato tuber infected with black scruf disease. Infected portions bearing the fungal sclerotia were cut into small pieces and surface sterilized with 1% sodium hypochlorite solution for 2 minutes. Thereafter, thorough washing with sterilized water was done and pieces were placed on potato dextrose agar (PDA) medium in 9-cm diameter Petri plates and incubated at 25±2 °C for one week. Pure culture was stored in the refrigerator at 4 °C.

Extract preparation

Leaf, stem-bark and fruit materials of *M. azedarach* were collected. Plant materials were thoroughly washed in running tap water and surface sterilized with 1% sodium hypochlorite solution, thoroughly washed with sterilized water, dried at 40 °C in an electric oven and grinded to form powder. This powder was then stored in polyethylene bags and used according to the need of experimental work to make extracts with water, methanol, *n*-hexane and chloroform.

Bioassays with aqueous and organic solvent extracts

Twenty grams of powdered materials of each plant part were soaked in 100 mL of sterilized distilled water, *n*-hexane, chloroform and methanol for 24 hours and filtered to prepare 20% extracts. The extracts were dried in an oven at 45 °C until the desired quantity (2 mL) was received. Then the final volume was made up to 100 mL by adding autoclaved sterilized distilled water. Potato dextrose broth was autoclaved and cooled to 40 °C. Eighty milliliters of medium were poured in 250 mL flasks. Appropriate quantities of stock solutions and distilled water were added to make 1, 2, …, 5% (v/v) concentrations and to make a final volume of 100 mL in each flask. Control treatments were without plant extracts and only received distilled water or methanol, *n*-hexane or chloroform (2 mL in 100 mL water). Five millimeter diameter actively growing mycelial disks of *R. solani* were transferred to the flasks aseptically. Flasks were incubated at 25 °C ± 2 for one week in an incubator. Each treatment was replicated three times. Fungal biomass in each flask was filtered, dried to constant weight and weighed after one week.

Statistical analysis

Two factor Completely Randomized Design (CRD) was applied. All the data were analyzed by analysis of variance (ANOVA). The comparisons among means were worked out using Tukey HSD test at 5% level of significance.

RESULTS

Leaf extracts were the most effective in reducing fungal growth as compared to stem-bark and fruit extracts. Among all the extracts screened with different solvents, chloroform extracts showed promising results with maximum inhibition in fungal biomass over control. It was also observed that from 1–5 % concentrations of different aqueous and organic solvent extracts from all the parts of plant, 5% concentration showed maximum reduction in fungal biomass (Table 1-3).

Aqueous extracts of fruit of *M. azedarach* at all concentration reduced biomass of *R. solani* by 28–70%. Similarly, all the concentrations of metanolic and *n*-hexane extracts of fruit significantly declined the biomass of target fungal pathogen by 25–55% and 21–40%, respectively. Chloroform extract, however, not proved much inhibitory against the growth of fungal pathogen. Antifungal effect of lower concentrations of 1%

and 2% of fruit chloroform extract was insignificant. By contrast, higher chloroform extract concentrations significantly lowered the fungal biomass by 24–81% over corresponding control treatment (Table 1).

In general, various types of stem extracts of *M. azedarach* exhibited variable antifungal activity. Methanol stem extract showed the best antifungal activity resulting in 4–85% reduction in fungal biomass at different concentrations.

Table 1. Effect of aqueous and organic solvent extracts of *M. azedarach* fruit on biomass of *Rhizoctonia solani*

Conc.	Fungal biomass (mg)				Mean
(%)	Water	Methanol	*n*-hexane	Chloroform	
0%	216.67±8.82a	210.00±5.77a	123.33±3.33d	103.33±3.33ef	163.33±15.4A
1%	156.67±3.33b	156.67±3.33b	93.33±3.33fgh	96.00±3.06fgh	125.67±9.45B
2%	143.33±3.33bc	146.67±3.33bc	97.00±0.58fg	89.00±0.58f-i	119.00±7.96B
3%	116.67±3.33de	130.00±5.77cd	94.00±0.58fgh	77.67±1.45hij	104.58±6.26C
4%	96.67±3.33fg	116.67±3.33de	84.00±0.58ghi	48.33±0.88k	86.42±7.57D
5%	65.00±2.89jk	93.33±3.33fgh	74.00±0.58ij	19.00±0.58l	62.83±8.29E
Mean	132.50±11.8B	142.22±8.99A	94.28±3.73C	72.22±7.21D	

Means sharing different letters in a row or column are significantly different (P≤0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 2. Effect of aqueous and organic solvent extracts of *M. azedarach* stem-bark on biomass of *Rhizoctonia solani*

Conc.	Fungal biomass (mg)				Mean
(%)	Water	Methanol	*n*-hexane	Chloroform	
0%	126.67±3.33ab	139.67±0.33a	98.00±1.15cd	90.33±0.33de	113.67±6.14A
1%	113.33±6.67bc	135.67±2.96a	96.33±1.86d	90.00±0.00de	108.83±5.57A
2%	96.67±3.33d	86.67±3.33def	86.00±0.58def	69.00±0.58gh	84.58±3.17B
3%	96.67±3.33d	70.00±5.77gh	87.33±0.88def	69.00±0.58gh	80.75±3.82B
4%	73.33±3.33fgh	65.00±2.89gh	78.00±0.58efg	59.00±0.58hi	52.67±7.96C
5%	68.67±0.33i	20.00±5.77j	74.00±0.58fgh	49.00±0.58i	54.08±6.82C
Mean	85.89±9.37A	86.17±10.19A	86.61±2.15A	71.06±3.67B	

Means sharing different letters in a row or column are significantly different (P≤0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

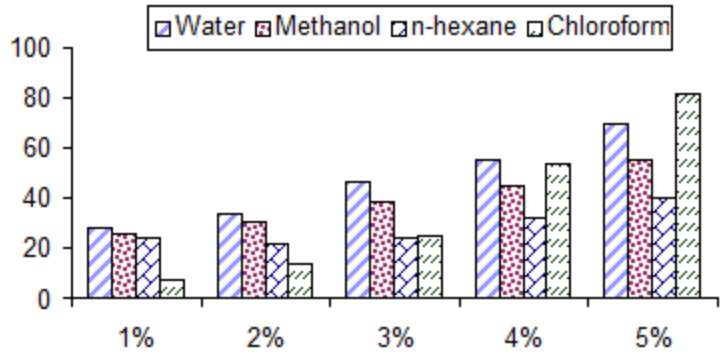
Table 3. Effect of aqueous and organic solvent extracts of *M. azedarach* leaves on biomass of *Rhizoctonia solani*

Conc.	Fungal biomass (mg)				Mean
(%)	Water	Methanol	*n*-hexane	Chloroform	
0%	116.7±3.3 a	106.7±3.3 a	89.7±3.3 b	49.3±0.7 gh	90.6±7.8 A
1%	76.7±3.3 b-e	86.7±3.3 bc	80.0±5.8 bcd	39.3±0.7 hi	70.7±5.8 B
2%	76.7±3.3 b-e	73.3±3.3 cde	78.0±0.6 b-e	29.0±0.6 ij	64.3±6.2 C
3%	58.3±4.4 fg	65.0±2.9 ef	68.0±0.6 def	19.0±0.6 jk	52.6±6.0 D
4%	48.3±1.7 gh	49.3±0.7 gh	58.0±0.6 fg	8.3±0.3 kl	41.0±5.8 E
5%	49.3±0.7 gh	13.3±3.3 kl	48.3±0.3 gh	5.0±0.6 l	29.0±6.1 F
Mean	71.0±5.8 A	65.7±7.2 B	70.3±3.5 A	25.0±3.9 C	

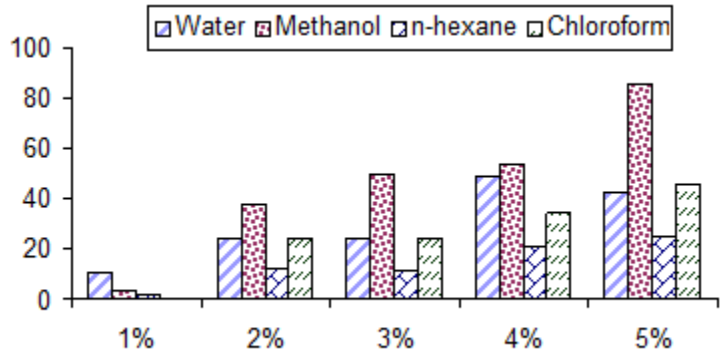
Means sharing different letters in a row or column are significantly different (P≤0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Aqueous and chloroform extract also showed pronounced antifungal activity resulting in 11–45% and 0.4–46%, respectively. *n*-hexane extract showed the least antifungal activity where only the highest concentrations of 4% and 5% significantly reduced fungal biomass by 20% and 24%, respectively (Table 2, Fig. 1).

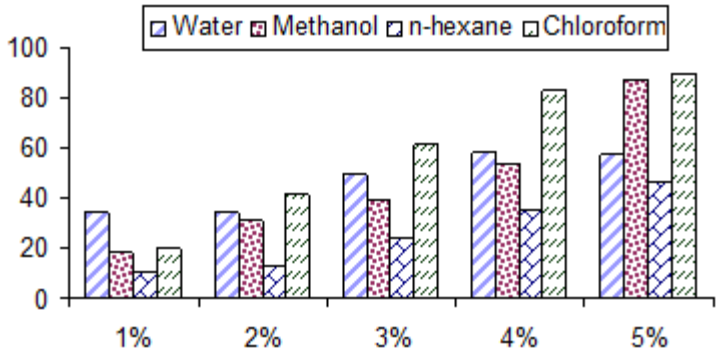
Different concentrations of leaf extracts of *M. azedarach* inhibited the fungal growth to variable extents Chloroform extract was found to be the most effective among the other extracts used in this experiment, followed by methanol aqueous and *n*-hexane extract (Table 3). There was 20–89%, 12–87%, 34–58% and 10–21% reduction in fungal biomass by different concentrations of chloroform, methanol, aqueous and *n*-hexane extract, respectively (Fig. 1).



a) Fruit



b) Steam-bark



c) Leave

Fig. 1. Percentage decrease in fungal biomass due to various extract treatments over control

DISCUSSION

Present study reveals that leaves of *M. azedarach* posses more antifungal properties than stem-bark and root, similarly leaves of *Jatropha curcas* extract possess better antifungal properties when compared to stem and root extracts [21]. Leaf is one of the major accumulatory parts of plants for bioactive compounds and people have generally preferred it for its therapeutic purposes [22]. It has been reported that ethanolic extracts of senescent leaves of *M. azedarach* exhibited fungistatic activity against *Diaporthe phaseolorum* var. *meridionales*, *Aspergillus flavus*, *Fusarium solani*, *F. oxysporum*, *F. verticillioides*, and *Sclerotinia sclerotiorum* [19]. Similarly, fungicidal activity of ethanol and chloroform leaf extracts of this tree species against *Ascochyta rabiei* , was reported[23]. The antifungal activity of *M. azedarach* could be attributed to the presence of antifungal compounds namely vanillin, hydroxycoumarin scopoletin, (±) pinoresinol and 4-hydroxy-3-methoxycinnamaldehyde [19, 20]. Recently, five compounds namely â-sitosterol, â-amyrin, ursolic acid, 3-5 dimethoxy benzoic acid and benzoic acid have been isolated from the leaves of *M. azedarach*, which showed antifungal activity against *A. rabiei* [24]. Overall methanolic and chloroform extracts showed considerable antifungal activity over *n*-hexane. However, in the stem-bark extracts, methanol extracts showed more pronounced antifungal effect than chloroform extract. Similarly, it was reported that alcoholic extracts possess more antibacterial activity than chloroform extracts[15]. This shows that some compounds are more effective and extraction is more efficient in more polar rather than in less polar solvents [25]. Similar results have been reported by previous researchers [26-28].

Aqueous extracts of fruit also showed considerable antifungal effects by reducing the fungal biomass. Our findings are in line with the findings of other scientists [18].They screened aqueous extracts of three plants of Meliaceae and found that both *Azadirachta indica* and *M. azedarach* significantly reduced biomass of *M. phaseolina* by 34–85% and 43–78%, respectively. It has been reported by earlier workers,that as the concentration increases antifungal activity also increases [29, 30]. Recently, methanolic extracts of three parts of *Sorghum halepense* Pers. against *M. phaseolinaand* were evaluated and found that the highest concentration possessed the highest antifungal property [31].

In conclusion, generally all types of extracts from *M. azedarch* leaf, stem and fruit showed different levels of antifungal activity against *R. solani*. The best antifungal activity was exhibited by chloroform extract of leaf, methanolic extract of stem-bark and aqueous extract of fruit resulting in 20–89%, 4–85% and 28–70% reduction in fungal biomass over control, respectively.

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