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MYCOCIDAL ABILITY OF *TOONA CILIATA* AGAINST *RHIZOCTONIA SOLANI*

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ABSTRACT

Among different diseases of potatoes, black scurf is the oldest and common disease of stems and stolons, below the soil surface caused by *Rhizoctonia solani*. The present study was carried out to investigate the antifungal potential of *Toona ciliata* M. Roem. extracts against this soil-borne fungal pathogen. Different concentrations (1, 2, 3, 4, 5%) of aqueous, methanol, *n*-hexane and chloroform extracts of leaves, stems and fruits of *T. ciliata* were evaluated for their *in vitro* antifungal activity. All the extracts showed variable antifungal activity. In general, stem bark and leaf extracts exhibited the highest inhibitory effect against growth of the fungal pathogen followed by fruit. Among the various extracts, stem bark and leaf methanol extract, followed by stem-bark and leaf chloroform extract showed the best antifungal activity resulting in 0–83% and 0-60% reduction in fungal biomass over corresponding control treatments, respectively. The implications of using the *T. ciliata* extracts in controlling *R. solani* are discussed.

Key words: *Toona ciliata*, *Rhizoctonia solani*, Antifungal ability, Fungal biomass.

INTRODUCTION

Black scurf of potato caused by the fungus *Rhizoctonia solani* Ku¨hn is a common and commercially important disease of potato tubers found in all production areas of the world. This disease affects potato development from emergence to harvest (EL Bakali and Martin, 2006).

Quality of the crop gets deteriorated through the development of black scurf on tubers but also in some instances deformed tubers and cracks (Grosch *et al*., 2005). Due to the stem and stolon infection at the beginning of the season, yield can be reduced and in some cases it can cause complete death of the stem. These losses can go up to 30% due to the disease (Lehtonen, 2009).

At present quick and effective management for most of plant pathogenic fungi is generally achieved by the use of synthetic fungicides. The massive use of synthetic fungicides in crop defense from plant pathogenic fungi had severe environmental impact (Osman and Al-Rehiayam, 2003). The inappropriate use of agrochemicals especially fungicides were found to possess adverse effects on ecosystems and a possible carcinogenic risk than insecticides and herbicides together (Stranger and Scott, 2005).Moreover, resistance by pathogens to fungicides has rendered certain fungicides ineffective (Zhonghua and Michailides, 2005; Dissanayake, and Jayasinghe, 2013).

In recent years, a large number of synthetic pesticides have been banned in the western world because of their undesirable attributes such as high and acute toxicity, long degradation period, accumulation in food chain and an extension of their power to destroy both useful organism and harmful pests (Gatto *et al*., 2011). Aforementioned considerations, necessitate the search for alternative control measures to reduce the dependence on the synthetic fungicides.

Medicinal plants extracts are promising as alternative or complementary control means because of their anti-microbial activity, nonphytotoxicity, systemicity as well as biodegradability (Talibi *et al*., 2012; Ibrahim and Al-Ebady, 2014). Plants produce a great deal of secondary metabolites; many of them exhibit antifungal activity. Well-known examples of these compounds include flavonoids, phenols and phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides glucosinolates and tannins (Singh *et al*., 2010 ;Dissanayake and Jayasinghe, 2013).

Perusal of literatures reports the medicinal properties of most of the plants bearing biological activity. One such species is *Toona ciliata* (Meliaceae), which has been exploited for many traditional uses like construction purpose, dye preparation, furniture, medicines etc. (Negi *et al.* 2011). Another study revealed the presence of carbohydrates, proteins, phytosterols, flavonoids, glycosides, tannins and phenolic compounds in *T. ciliata* (Kavitha and Satish, 2013). *T. ciliata* along with Siderin, a compound isolated from petroleum ether extract showed significant antibacterial activity and also exhibited significant cytotoxicity (Chowdhury *et al*., 2003). The chloroform extract of the leaves of plant *T. ciliata* also showed the inhibitory activity against the bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus* and *Salmonella* (Bibi *et al*., 2011) Natural tetratriterpenoids such as cedrelone isolated from the powdered wood of *T. ciliata* showed the antifungal activity of the plant against the *Puccinia arachidis*, a groundnut rust pathogen (Kumar *et al*., 2012). We

hypothesize that various parts of *T. ciliata* plant and various solvents show differential inhibitory activities against *R. solani*. 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 *T. ciliata* against *R. solani.* As many bioactive compounds have extracted from different parts of *T.ciliata* in organic solvents therefore commercially it can be exploited for the isolation of these bioactive compounds which could be a source for antifungal activities. On the basis of these findings an attempt has been made to control an economically important fungus *R.solani* with the organic extracts of different parts of *T.ciliata*.

MATERIALS AND METHODS

Isolation of Fungal species: Infected potato tuber with *R. solani* was cut into small pieces and surface sterilized with 1% sodium hypochlorite solution for 2 minutes, for the isolation of the pathogen. Sterilized water was used for the thorough washing, 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: The parts of *T. ciliata* tree, like leaves, stem-bark and fruits were collected from University of the Punjab, Lahore, Pakistan. Plant parts 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.

Antifungal activity: Powdered plant material of 20 g was soaked in 100 ml of sterilized distilled water, n-hexane, chloroform and methanol for 24 h and filtered to prepare 20% extract. The desired quantity of 2 ml was achieved by drying then in an oven at 45 ºC. Final volume up to 100 ml was made 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 treatment means were worked out using Tukey HSD test at 5% level of significance using computer software Statistics 8.1.

RESULTS

The present study was designed to see the effect of different concentrations (ranging from 1–5%) of organic solvents extracts of stem-bark, leaves and fruit of *T. ciliata* against *R. solani*. Obtained results showed variability in the fungicidal effect among different parts of *T. ciliata* with reference to its parts and organic solvents. Stem-bark and leaves showed better antifungal activity than fruit extract. Among the different concentrations, 5% concentration of all the solvent extracts displayed the pronounced decrease in the growth of the organism. Overall methanol solvent has proved to be the best one in extracting the compounds from all the parts of the plant and thus reducing the fungal growth and its highest concentration (5%) is the promising one among all the other concentration. In comparison with the other solvents and parts, 5% concentration of methanol extract of stem-bark showed the widest zone of inhibition. The methanol extract at 5% concentration produced the highest effect ($P<0.01$) with the mean reduction of 1 followed by chloroform (2.59) and n-hexane (10.80) (Table 1). At the same concentration, aqueous extract of stem-bark produced maximum diameter with mean reduction of 28±1.53.

The result showed that the leaves extract in different solvents had antifungal activity on the tested *R. solani*. Leaves extract followed the trend of stem extracts. The highest ($P<0.01$) antifungal activity was shown by the methanol extract (4), whereas the least inhibitory effect (48.67) was depicted by the aqueous extract of fruit (Table 1B).Choloroform and n-hexane reduced the growth of the fungus (8.50 and 16.33) respectively.

Fruit extracts of *T. ciliata* exhibited the least effect as compared to other parts of the plant but followed the same trend for the solvents i.e., methanol extract of fruit produced the inhibition zone with a mean reduction of 10 (Table 1C), whereas aqueous extract repeated the trend. Chloroform and n-hexane displayed a mean reduction of 12.87 and 27.00 respectively. The minimum inhibitory effect of the aqueous extract was on an average 43. The zone of inhibition for the fungus was more ($P<0.01$) with methanol, followed by chloroform and *n*-hexane. The results clearly depicted that chloroform and n-hexane produced a similar effect. Overall, a non-significant affect ($P>0.05$) was observed for 1% concentration with all the solvents and parts of the plant.

Table 1: (A) Dry Biomass (g) of *Rhizoctonia solani* affected by the aqueous and organic extracts of *T. ciliata* stem

Conc.	Treatment				Mean
	Water	n-hexane	Chloroform	Methanol	
0%	88.67 ± 0.88a	58.67 ± 0.88bc	8.50 ± 0.29fg	8.87 ± 0.09efg	41.18 ± 10.3A
1%	66.33 ± 3.18b	36.33 ± 3.18d	4.60 ± 0.35g	2.60 ± 0.21g	27.47 ± 7.94B
2%	56.33 ± 3.18c	28.67 ± 0.88d	2.80 ± 0.15g	1.80 ± 0.15g	22.40 ± 6.78C
3%	32.67 ± 3.18d	19.67 ± 2.33ef	1.80 ± 0.15g	2.48 ± 0.02g	12.65 ± 4.01D
4%	28.67 ± 5.24e	15.67 ± 0.33efg	2.90 ± 0.06g	1.36 ± 0.04g	7.40 ± 2.52E
5%	28.00 ± 1.53fg	10.80 ± 0.15fg	2.59 ± 0.01g	1.18 ± 0.02g	4.39 ± 1.26E
Mean	45.11 ± 6.88A	36.30 ± 4.30B	3.20 ± 0.66C	2.38 ± 0.74C	

(B) Dry Biomass of *Rhizoctonia solani* affected by the aqueous and organic extracts of *T. ciliata* leaves

Conc.	Treatment				Mean
	Water	n-hexane	Chloroform	Methanol	
0%	106.67 ± 3.33a	103.33 ± 3.33a	73.00 ± 3.51def	78.00 ± 1.53cde	90.25 ± 4.68A
1%	96.67 ± 3.33ab	79.00 ± 0.58cd	58.67 ± 0.88gh	66.00 ± 3.51efg	75.08 ± 4.48B
2%	86.33 ± 3.18bc	64.67 ± 2.60fg	43.33 ± 4.41ij	32.33 ± 1.45jk	56.67 ± 6.38C
3%	79.00 ± 0.58cd	44.00 ± 2.52ij	28.67 ± 0.88kl	12.33 ± 1.45mn	41.00 ± 7.46D
4%	68.00 ± 1.53d-g	34.67 ± 2.60jk	17.67 ± 1.45lm	7.47 ± 0.32mn	31.95 ± 6.96E
5%	48.67 ± 0.88hi	16.33 ± 0.88m	8.50 ± 0.29mn	4.00 ± 0.06n	19.38 ± 5.28F
Mean	80.89 ± 4.67A	57.00 ± 7.05B	38.31 ± 5.53C	33.36 ± 7.05D	

(C) Dry Biomass of *Rhizoctonia solani* affected by the aqueous and organic extracts of *T. ciliata* fruit

Conc.	Treatment				Mean
	Water	n-hexane	Chloroform	Methanol	
0%	139.00 ± 0.58a	88.67 ± 0.88bc	89.67 ± 0.33bc	38.00 ± 1.53hi	88.83 ± 10.8A
1%	96.67 ± 3.33b	79.00 ± 0.58d	76.33 ± 3.18de	28.67 ± 0.88j	70.17 ± 7.67B
2%	88.67 ± 0.88bc	69.00 ± 0.58e	46.00 ± 3.51gh	28.00 ± 1.53j	57.92 ± 6.97C
3%	85.00 ± 2.89cd	58.67 ± 0.88f	28.00 ± 1.53j	19.00 ± 0.58k	47.67 ± 7.90D
4%	48.67 ± 0.88g	58.67 ± 0.88f	16.83 ± 2.68l	19.00 ± 0.58l	30.79 ± 7.01E
5%	43.67 ± 0.88ij	27.00 ± 1.15jk	12.87 ± 0.09l	10.90 ± 0.06l	19.36 ± 3.40F
Mean	81.94 ± 8.29A	63.50 ± 4.74B	42.62 ± 7.71C	21.76 ± 2.67D	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

DISCUSSION

The present study revealed that stem-bark of *T. ciliata* possessed more antifungal properties than leaves and fruit. Singh *et al.* (2010) found that stem bark of medicinally important plants possess antibacterial and antifungal activity. Maikai *et al.* (2009) also found that stem-bark extracts of *Ximenia americana* had antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, Bacillus subtilis, Proteus vulgaris* and *Candida albicans*. Joshi *et al.* (2003) reported the fungicidal activity of *Alianthus excelsa* against *Aspergillus niger, A. fumigatus, Penicillium frequentence, P. notatum* and *Botrytis cinerea*. The stem-barks are rich sources of tannins and other phenolic compounds. Tannins inhibited the growth of various fungi, yeast, bacteria and virus. Tannins are water-soluble polyphenols and their presence in plants mostly located in dead or dying cells. They exert an inhibitory effect on many enzymes due to protein precipitation and hence they may contribute a protective function in barks and heartwood (Singh *et al*., 2010). Chung *et al.* (1998) documented the antimicrobial activities of tannins against many fungi, yeast, bacteria, and viruses. Kumar *et al.* (2012) reported the presence of tetranortriterpenoids in the stem bark and heartwood of *T. ciliata*. Govindachari *et al.* (2000) further investigated that tetranortriterpenoids such as cedrelone found in the stem bark of *T. ciliata* was very effective in reducing rust pustule emergence caused by *Puccinia arachidis*, a groundnut rust pathogen.

Methanolic and chloroform extracts showed considerable antifungal activity than *n*-hexane in this study, which was in line with the findings of Mondali *et*

al. (2009) and Vadlapudi and Kaladhar (2012), respectively. This is suggestive of the fact that some compounds are more active in methanolic and chloroform extracts in their antifungal activity than n-hexane. Similar results were again reported by Vadlapudi (2012) with *Avicennia alba* against some bacteria and fungi. Methanol extract shown better antifungal activity than chloroform extract. These findings are in agreement with the findings of Satish *et al.* (2007). Shirzadian *et al*. (2009) compared antifungal properties of ethanol, petroleum ether and water extracts of some plants against some pathogenic fungal pathogens including *Alternaria alternata*, and found highest antifungal activity among ethanolic extracts. Similarly Rizwana *et al.* (2012) reported that alcoholic extracts possess more antibacterial activity than chloroform This shows that some compounds are more effective and extraction is more efficient in polar solvents rather then nonpolar (Zaker and Mosallanejad, 2010; Mahmoud *et al*., 2011; Bassey *et al*., 2013; Rajendran *et al*., 2014).

Aqueous extracts of the plants also showed considerable antifungal effects by reducing the fungal biomass (Masih *et al*., 2014). Satish *et al*., 2007 screened aqueous extracts of 52 plants from different families against 8 important species of Aspergillus. Among 52 plant species tested, aqueous extract of *Acacia nilotica*, *Achras zapota*, *Datura stramonium*, *Emblica officinalis*, *Eucalyptus globules*, *Lawsonia inermis*, *Mimusops elengi*, *Peltophorum pterocarpum*, *Polyalthia longifolia*, *Prosopis juliflora*, *Punica granatum*, *Sygigium cumini* revealed significant antifungal activity against one or the other *Aspergillus* species tested.

The highest concentration of all the extracts showed maximum inhibition of *R. solani.* It has also been reported that as the concentration increases antifungal activity also increases (Aslam *et al*., 2010; Ibrahim *et al*., 2014). Recently, Ibrahim *et al*. (2014) evaluated that methanolic extracts of leaves and stem of *Mimosa pudica* plant against *Trichophyton verrocusum, T. mentagrophytes, Microsporumvnanum, Aspergillus niger and A. flavus* and found that the highest concentration possessed the highest antifungal property.

Conclusion: The present study illustrates that all parts of *T. ciliata* viz. stem, leaf and fruit displayed reduction in the growth of the fungus. Simultaneously all the concentration of the extracts poured some affect on the colony of the fungus; however the highest concentrations of the extracts were more pronouncing in decreasing the growth of *R. solani*. Commercial exploitation of the compounds found in the extracts of the plant could be used for the reduction of the fungi causing fatal diseases of plants and animals. The results have revealed that economically important fungus *R.solani* can be successfully controlled by the bioactive compound present in different parts of *T.ciliata* which were expressed in organic solvents. The extracted compounds can be used as an alternate of synthetic fungicides in future

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