Antifungal potentiality of leaves of some higher plants against *Rhizoctonia solani* causing damping - off disease of Brinjal.

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A screening of leaves of 21 taxa of angiosperms was made for their volatile toxicity against *Rhizoctonia solani*. The volatile substances from *Callistemone lanceolatus* and *Citrus medica* were toxic against *R. solani*. Soil amendment with the leaves of *C. laceolatus* and *C. medica* and the mixture of both plants leaves controlled damping - off disease of brinjal upto 61.11, 72.25 and 83.33% respectively in soil infested with *R. soluni*. Soil amendments with leaves and mixture of leaves of these fungitoxic plants increase the saprophytic fungal community.

Key words: Volatile toxicity, *Rhizoctonia solani*, soil amendment, damping - off disease, fungicides.

INTRODUCTION

Synthetic chemicals with various degree of persistence are employed as fungicides in crop protection. The use of many such fungicides has now been cautioned due to their carcinogenicity, teratogenicity, and other residual toxicities (Bajaj and Ghosh, 1975; Sax, 1987 Arya, 1988; Lingk, 1991). Several of the synthetic fungicides are reported to cause adverse effect on treated soil ecosystem because of their non-biodegradable nature (Shashikant et.al., 1989). Scientists are now looking for some alternatives for the control of plants diseases. in search of better alternatives natural products are considered to be environmentally safe for control of plant diseases (Bye, 1978). Higher plants in the tropics are reservoir of different secondary metabolites and provides an almost limitless source of useful chemicals with different biological properties (Sbragia, 1975). Several higher plants have been found to possess outstanding fugitoxicity against myceiial growth or spore germination of different phytopathogenic fung *in vitro* (Pandey and Dubey, 1991, 1992, 1994; Lee, 2006). Therefore in present piece of work the leaves extract of some plants have been screened for their volatile toxicity and soil amendment with potent fungitoxic plants against *Rhizoctonia solani* causing damping-off of brinjal have been evaluated.

MATERIALS AND METHODS

Fresh leaves of 21 angiosperms were screened for their volatiles against the test fungus *R. solani.* The leaves of (20 g) of each plant were thoroughly washed with 70% ethanol and finally with sterile distilled water in pestle and mortar (1:1 w/v) and filtered through double layered sterilized cheese cloth. The clear extract (aquous extract) thus obtained was assayed for its antifungal activity by the inverted Petriplate Technique (Peach and Tracy, 1955). Potato dextrose agar medium was aseptically poured in Petriplates (10 ml / plate) and was inoculated with discs cut from 7 days old culture of the test fungus. The inoculated plates were inverted upside down. Then 5ml of the

prepared extract was aseptically pipetted to the lid of the Petri plate. Control sets were prepared similarly using 5ml sterilized distilled water. The plates were kept at 25±2°C for 7 days. The experiment was run triplicate. The fungitoxicity was calculated as per formula:

$$\% \text{ mycelial growth inhibition} = 100 \left(\frac{dc - dt}{dc} \right)$$

where,

dc = mean colony diameter of control

dt = mean colony diameter of treatment

Amendment of soil with leaves of *C. lanceolatus* and *C. medica* was done (Pandey and Dubey, 1997) to find out the potentiality of leaves in control of damping-off disease of *Solanum melongena* caused by *R. solani*. Thirty kg of garden soil was collected for setting treatments and controls. Six kg of the soil was filled in 3 earthen pots (2 kg/pot) which served as uninoculated control. Twenty four kg of the soil was inoculated with 120g inoculum of *R. solani* maintained on oat sand. The soil inoculated with *R. solani* was filled (2kg/pot) in to 12 pots separately and kept for one week to establish the infection. Three of infested pot served as inoculated controls. The remaining pots, were amended with leaves pieces (0.25 cm^2) of *C. laceolalus, C. medica* and mixture of leaves pieces (1:1 w/w) of these plants @ 20g/pot (3 pots for each) separately. Twenty days after the amendment, the seeds of brinjal soaked for 6 hrs in sterilized water, were sown (2cm deep) equidistantly in all 15 pots @ 20 seeds/pot. Experiments were repeated thrice. After 14 days of sowing at two leaf stage the percent seedling mortality and percent disease control were calculated by the following formulae (Kataria and Grover, 1976):

$$\% \text{ seedling mortality} = 100 - \frac{\text{Seedling stand in inoculated soil} \times 100}{\text{Seedling stand in uninoculated soil}}$$

$$\% \text{ disease control} = 100 - \frac{\% \text{ disease in treatment set} \times 100}{\% \text{ disease in treatment control}}$$

The effects of soil amendment with leaves of *C. lanceolatus*, *C. medica* and mixture of leaves of these plants on soil mycoflora were investigated by the method of Khalis and Manoharachari, (1985). Sterilized earthen pots were filled with 1 kg of garden soil. The soil was amended with 5, 10 and 15% of leaf pieces of young leaves (0.5 cm^2) of *C. lanceolatus*, *C. medica* and mixture of both plant pieces (1: 1 w/v) separately. Controls sets contained unamended soil. The pots were watered regularly with equal amount of sterilized water. After 30 days, all the sets were subject to mycoflora analysis separately by the Waksman□s dilution plate method (Wakman, 1952) as well as warcup□s soil plate method (Dhingra and Sinclair, 1986) using martin agar medium. The soil from the top 1 cm was removed in a glass container. The container was kept in a water bath for 30 minutes at 60°C and mycoflora analysis was made.

RESULTS AND DISCUSSION

During screening of leaves of angiospermic taxa, most plant species showed either poor (below 50%) or moderate (above 50% and below 100%) fungitoxicity. However leaf extracts of *C. lanceolatus* and *C. medica* inhibited the growth of test fungus completely (Table - 1). In pot experiments soil amended with leaves of *C. lanceolatus*, *C. medica* and mixture of both plant leaves showed control of damping - off by 61.11, 72.25 and 83.33% respectively in soil infested with *R. solan*i (Table - 2) . The number of fungal types greatly increased as a result of soil amendment with the leaves and the mixture of leaves of the test plants. The soil amended with 5, 10 and 15% of *Callistemone* leaves harboured 12, 11 and 11 saprophytic species respectively. By amendment with 5, 10 and 15% *Citrus* leaves isolated 10, 9 and 9 species respectively.

While the mixture of both plant leaves harboured 10 saprophytic species in each set. Increase the percentage occurrence of fungi such as *Trichoderma hargianum* and *T.viride* observed in amended soils (Table - 3). In unamended set 24 fungal species were isolated. Moreover in amended soils the fungus namely *Botrytis cineria* completely disappeared Some fungi namely *Alternaria brassicae, A. raphani, Cercospora cajani, C. capsici, Drechslera graminea, Fusarium semtectum, Pythium debaryanum, P. proliferum and Rhizoctonia solani* respectively disappeared in leaves mixture of both the plants. These findings indicate that for the management of disease caused by *R. solani* the leaves of *C. lanceolatus* and *C. medica* may be used. The increase in number of some saprophytic fungi in soil amended with leaves and leaves mixture of the test plants may be an additional merit in soil disease control. These saprophytic fungi may provide disease control through antagonism.

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Table 1. Fugitoxicity of volatile compounds of leaves of some higher plants against *Rhizoctonia solani* (± S.E. of the Mean)

Plant tested	% inhibition of growth of *Rhizoctonia solani*
Acacia arabica	45.2±0.04
Alizia labbeck	46.3±0.03
Annona squamosa	43.4±0.05
Antirrhinum of orontium	42.3±0.03
Artocarpus heterophyllus	23.3±0.03
Cllistemone lanceolatus	100
Cannabis sativus	75.5±0.04
Cassia nodasa	35.5±0.03
Cirus medica	100
Daucas carota	24.3±0.06
Gomphrena globosa	19.5±0.07
Ipomea fistulosa	32.2±0.05
Morus indica	54.3±0.04
Phyllanthus emblica	65.2±0.02
Polyalthia longifolia	44.1±0.03
Psidium guajava	43.3±0.04
Punica granatum	63.3±0.05
Raphanus sativus	63.6±0.06
Ranunculus scleratus	73.8±0.07
Saraca indica	26.2±0.07
Ziziphus mauritiana	33.3±0.06

Table-2. Soil amendment with leaves of *Callistemone lanceolatus*, *Citrus medica* and mixture of leaves of these plants (1:1 w/w) for control of damping-off disease of brinjal (*Solanum melongena)* caused by *Rhizoctonia solani*.

Pathogen	Average No. of healthy seedlings					% seedling mortality				% disease control		
	Control Unino.	Ino.	C.L.	C.M.	C.L.±C.M.	Control	Treatment C.L.	C.M.	C.L.±C.M.	C.L.	C.M.	C.L.±C.M.
Rhizoctonia solani	25±0.03	07±0.02	18±0.01	20±0.01	22±0.04	72±0.03	28±0.01	20±0.01	12±0.01	61.11±0.03	72.25±0.02	83.33±0.01

Table-3. Percent occurrence of fungi in soil amended with leaves of *Callistemone lanceolatus* and *Citrus medica* and combination of both plant leaves.

Fungi	% Occurrence Leaves amended (w/w)									
	C. lanceolatus				*C. medica*			C. lanceolatus + *C. medica*		
	Control	5%	10%	15%	5%	10%	15%	5%	10%	15%
Fungi stimulated										
Aspergillus flavus	14±0.05	14±0.02	20±0.02	25±0.01	15±0.03	22±0.01	30±0.03	16±0.01	25±0.0	35±0.04
A. fumigatus	07±0.02	06±0.01	08±0.03	10±0.02	07±0.02	10±0.03	12±0.01	09±0.01	12±0.01	15±0.01
A. luchuensis	04±0.03	06±0.02	06±0.03	08±0.04	08±0.01	10±0.01	15±0.01	08±0.03	14±0.01	16±0.02
A. parasiticus	03±0.02	01±0.01	04±0.02	06±0.03	ND	ND	ND	06±0.03	08±0.01	12±0.01
Botrytis cineria	02±0.03	ND	ND	ND	ND	ND	ND	ND	ND	ND
Penicillium citrinum	10±0.05	12±0.01	14±0.01	16±0.02	13±0.01	16±0.01	18±0.01	14±0.01	16±0.03	18±0.03
P. digitatum	09±0.02	10±0.02	12±0.01	14±0.01	12±0.03	13±0.02	15±0.01	16±0.01	18±0.01	20±0.01
P. italicum	07±0.01	09±0.01	10±0.03	11±0.02	09±0.03	12±0.01	14±0.02	12±0.01	18±0.03	20±0.01
P. vermiculatum	04±0.03	04±0.01	05±0.02	05±0.01	06±0.01	08±0.01	10±0.02	07±0.01	10±0.02	15±0.01
Trichoderma viride	09±0.01	11±0.03	14±0.03	18±0.01	13±0.01	16±0.01	20±0.01	15±0.01	20±0.03	25±0.01
T. hargianum	07±0.02	09±0.01	11±0.02	15±0.01	10±0.02	11±0.02	15±0.02	12±0.02	16±0.02	20±0.03
Fungi affected										
Aspergillus niger	10±0.03	08±0.01	06±0.02	02±0.01	03±0.01	ND	ND	ND	ND	ND
A. sulphureus	03±0.02	01±0.01	ND	ND	ND	ND	ND	ND	ND	ND
Alternaria brassicae	04±0.03	03±0.01	02±0.01	ND	02±0.02	01±0.01	ND	ND	ND	ND
A. raphani	04±0.02	04±0.02	03±0.02	ND	03±0.01	01±0.01	ND	ND	ND	ND
Cercospora cajani	05±0.01	03±0.01	02±0.03	01±0.01	03±0.02	02±0.02	ND	ND	ND	ND
C. capsici	07±0.02	05±0.03	04±0.02	02±0.02	04±0.02	02±0.03	ND	ND	ND	ND
Cladosporium capsici	10±0.03	09±0.01	08±0.02	03±0.01	07±0.01	05±0.01	03±0.01	08±0.01	05±0.02	02±0.03
Colletotricum capsici	08±0.02	08±0.01	07±0.02	05±0.01	07±0.01	05±0.01	03±0.01	04±0.01	03±0.01	01±0.02
Drchslera							ND	ND	ND	ND

graminea	07±0.02	06±0.02	03±0.01	01±0.01	-06±0.02	02±0.01	ND	ND	ND	ND
Fusarium										
semitectum	06±0.02	04±0.01	-2±0.01	ND	ND	ND	ND	ND	ND	ND
Pythium										
debaryanum	07±0.01	07±0.01	05±0.02	03±0.01	05±0.01	03±0.01	ND	ND	ND	ND
P. proliferum	05±0.02	03±0.02	ND	ND	ND	ND	ND	ND	ND	ND
Rhizoctonia										
solani	06±0.01	03±0.01	01	01±0.02	03±0.02	ND	ND	ND	ND	ND

N.D. = Not detected.

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