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Compatibility of *Trichoderma* spp. with fungicides and phytoextracts for integrated management of Alternaria Leaf Blight of *Ocimum sanctum* L.

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Chemical hazards are now becoming very alarming and as such scientists are in search of different alternative methods of plant disease control. In present communication an attempt has been made to control the fungal pathogen, *Alternaria* spp. causing leaf blight of *Ocimum sanctum* following non-chemical method. The biocontrol fungus *Trichoderma viz, T. viride, T. lignorum, T. harzianum, T. hammatum and T. recei* were being challenged against *in vitro* growth of the pathogen and was found that *T. viride was* the most potent antagonist to control the pathogen with 84.04% efficiency. Some plant extracts like, *Azadirachta indica, Clerodendrum inermme, Datura metel, Cassia occidentalis, Cassia alata and Cymbopogon flexuosa* (Lemon grass) were tested against the pathogen and it was recorded that *Azadirachta indica* (Neem) inhibited the growth of the pathogen with 78% efficiency. The most potent antagonist *T. viride* as screened was subjected to varying concentrations of some selective fungicides in order to determine the fungicidal sensitivity of the said antagonist and was noted that *T. viride* had the highest tolerance limit to the systemic fungicide carbendazim up to a concentration of 1%. An integrated combination was being formulated using *T. viride* (1 ml cell-free culture filtrate), *Azadirachta indica* (1 ml of the ethanolic extract of leaf) and the screened systemic fungicide carbendazime (1 ml of 0.01%) which offered cent per cent growth inhibition of the pathogen under *in vitro* condition. Field trial experiment was conducted by applying this integrated formulation which exhibited over 75% control of the disease.

Key words: Leaf blight, *Ocimum sanctum, T. viride, T. lignorum, T. harzianum, T. hammatum, T. recei,* integrated control, *Azadirachta indica, Clerodendnlun inermme, Datura metel, Cassia occidentalis, Cassia alata* and *Cymbopogon flexuosa.*

INTRODUCTION

Traditional herbal medicines are increasingly being used not only by "TRTdeveloping countries but also by the developed countries in their primary health care system. A bulk of our rural population relies on the drug resources of plant origin and for this reason research efforts on medicinal plants are increasing day by day to maintain a steady supply and to meet their ever increasing demand. *Ocimum sanctum* L. ('Tulsi') is an important medicinal plant belonging to the family Lamiaceae with plethora of uses as expectorant and as tonic. The extract of leaves and inflorescences are used in the treatment of cough, bronchitis, asthma, hopping cough and bronchial congestion, respiratory troubles etc. Not only that, due to its tremendous medicinal value, the plant is regarded as a religious species to the Hindus and it is supposed to be the most effective means of conservation of this plant and also to save the plant from anthropogenic threat.

Leaf blight of 'Tulsi' caused by *Alternaria* alternata is very prevalent disease which occurs in severe form every year all over India. The disease causes shrinkage of the leaves with concomitant development of brownish blade lesions over the whole surface of the leaves. Lesions appear first on

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the margins of the leaf and then rapidly spread throughout the whole surface of the leaf which ultimately causes an enormous loss of the photosynthetic area of the leaf and thereby decreases the productivity. So, the control of the disease is an urgent need and a thrust area of research. In recent decades chemical hazards are becoming very alarming and that's why today's scientists are trying their best for developing different alternative methods of plant disease management other than the conventional chemical method. Several countries including India has already banned the use of a number of highly toxic and hazardous chemicals and imposed restrictions on the use of many others to prevent environmental pollution. Considering the facts, research work has been carried out for successful control of the disease following different non-chemical methods. Search for effective biocontrol agents for the management of plant diseases has been intensified in recent years to reduce the dependence on ecologically hazardous chemicals. The soil borne plant growth promoting fungus (PGPR) *Trichoderma* spp. has gained considerable importance for management of soil borne plant pathogens either alone or in integrated combination with some selective fungicides in extremely lower dosage (below minimum lethal dose) and/or with some phytoextracts. Possibility of the use of different *Trichoderma* spp. and phytoextracts for successful biological control of the pathogen is being investigated in the present communication. An attempt has been made for integrated management of Alternaria blight of 'Tulsi' using the antagonistic fungus *Trichoderma viride,* phytoextract of neem *(Azadirachta indica)* and a systemic fungicide carbendazim(0.01%) which offer cent per cent growth inhibition of the pathogen under *in vitro* condition and over 75% control of the disease under pot culture experiment.

MATERIALS AND METHODS

Study of the disease symptoms : The disease was manifested by visible symptoms like dark brown to black spots on the leaves, specially on the marginal areas which latter spread towards the centre and ultimately the total leaf became wrinkled, dark brown to blackish brown and dried within a period of 15-20 days and causes an enormous loss in the photosynthetic area of the leaf.

Isolation and identification of the pathogen : *Alternaria alternata* was isolated from the diseased leaf of 'tulsi' and maintained in potato dextrose agar PDA) medium at 4°C. The pathogen was identified by LARA, New Delhi.

Screening of the antagonistic fungi : Different antagonistic fungi like *Trichoderma viride, T. lignorum, T. harzianum, T. hammatum and T. recei* were tested against *in vitro* growth of the pathogen following 'dual culture plate technique' (Royse and Ries, 1978) and 'food poisoning technique' (Mondal *et al.,* 1995).

Dual culture plate technique (Royse and Ries, 1978) : In this technique antagonistic activity of the *Trichoderma* spp. were tested against *in vitro* growth of the pathogen on PDA plate. 20 ml of sterilized melted PDA medium was poured in sterilized plates, allowed to solidify and 5 mm inoculum disc of each of the pathogen and *Trichoderma* spp. were placed on the plates approximately 6 cm. away from each other and incubated at 28±1°C. Five replicates were maintained for each treatment. Observations of antagonistic activity of *Trichoderma* spp. were recorded after 24 h for 7 days.

Food poisoning technique *(Mondal et al l995)* : In this technique 20 ml of PDA medium was poured in each of the stemizea fetridish and allowed to solidify. Just before solidification 2 ml of the cell-free culture filtrate of 7 days old culture of each of the antagonists grown in liquid broth were added to the medium and mixed thoroughly. After solidification each of the Petridishes were inoculated with 5 mm inoculum disc of the test pathogen and incubated at 28±1°C for 7 days. At the end of incubation, the radial growth of the pathogen was measured and percentage growth inhibition of the pathogen was calculated.

Screening of the phytoextracts : *In vitro* effect of different phytoextracts like leal extracts of *Azadirachta indica, Cassia alata, Cassia occidentalis,* Lemon grass *(Cymbopogon flexuosa), Datura metel, Clerodendrum inermme* against the test pathogen was studied following the same 'food poisoning technique'. 15 ml potato dextrose broth was taken in lOO ml conical flask, sterilized, mixed

thoroughly with different volumes (ml) of respective plant extracts and inoculated with the pathogen and incubated for 7 days. Total biomass production was measured and the spore germination percentage was calculated and these two parameters were taken as an index of the inverse growth of the pathogen.

Determination of fungicidal sensitivity of Trichoderma viride : Fungicidal sensitivity of me antagonistic strain *Trichoderma viride* was determined by using some selective fungicides like carbendazim, tridemorph, calixin, captan and mancozeb following food poisoning technique (Mondal *et al.,* 1995) in order to combine them with the antagonist for effective integrated management of the disease.

Integrated control of the disease : Various integrated combinations of *T. viride,* Neem extract *(Azadirachta indica)* and a systemic fungicide-carbendazim (cell-free culture filtrate of *T viride* + neem leaf extract + 0.01% Carbendazim) were prepared and trial has been conducted to control the pathogen following food poisoning technique (Mondal *et al.,* 1995). Bioefficacy of various integrated combinations was tested against spore germination of the pathogen in broth culture and per cent reducuonjn spore germination per microscopic field was calculated against the control after 48 hrs of incubation at 28 ± 1°C temp.

Pot culture experiment : Pot culture experiment was conducted to test the efficacy of different integrated combinations using culture filtrate of *T. viride*, ethanolic extract of Neem leaves and Carbendazim (0.01% concentration) under field condition when each of the pots were inoculated simultaneously with the inoculum of the test pathogen and one of the integrated combinations formulated. The symptoms of the disease if appeared were recorded at 7 days interval for one month.

RESULTS AND DISCUSSION

It is evident from the results (Table 1) that among the *Trichoderma* species tested; *T. viride* was the best to inhibit in *vitro* growth of the pathogen with 84.04% efficiency followed by *T. harzianum* which offered 77.78% growth inhibition of the pathogen in 'dual culture plate technique assay'. In contrast, the same antagonists offered 76.67% and 72.22% inhibition of the pathogen in 'food poisoning technique' (Table 2). The phytoextract of neem (ethanolic extract of leaves) exhibited 72.22% inhibition of the diametric growth of the pathogen when 3ml of this phytoextract was added with 15 ml of potato dextrose broth (Table 3). It had been inferred from the experiment regarding determination of fungicidal sensitivity of the most suitable antagonist- *T. viride* that this antagonist was less sensitive or insensitive to the systemic fungicide carbendazim even up to a dose of (1%) but in this concentration sporulation was very minimum and insignificant (Table 4). However, in the concentrations higher than this level *T. viride* exhibited some degree of sensitivity. Possibility of

Table 1 : Effect of antagonistic fungi on growth of *Alternaria* spp. following 'dual culture plate technique'

Antagonist	Colony diameter (mm)			Growth inhibition of the pathogen (%)*
	Pathogen	Antagonist	Inhibition zone	
Trichoderma viride	12	70	6	84.04 ± 0.477
Trichoderma lignorum	30	55	4	65.56 ±0.814
Trichoderma harzianum	20	66	4	77.78 ± 1.100
Trichoderma hammatum	55	30	5	38.89 ±0.331
Trichoderma recei	48	40	2	46.67 ± 0.885
Control	90	-	-	-

* Data are the mean values of five replicates

SEm ± 0.03 ; CD at 5% 1.82

Table 2 : Efficacy of the cell-free culture filtrates of microbial antagonists on mycelial growth of *Alternaria alternata* following 'food poisoning technique'

Antagonist	Colony diameter of the pathogen (mm)	Growth inhibition of the pathogen* (%)
Trichoderma viride	21	76.67 ± 0.524
Trichoderma lignorum	29	67.78 ± 0.670
Trichoderma harzianum	25	72.22 ± 0.751
Trichoderma hammatum	64	28.89 ± 0.921
Trichoderma recei	60	33.33 ± 1.100
Control	90	-

* Data are the mean values of five replicates

SEm ± 0.039 ; CD at 5% 0.118

Table 3 : Effect of phytoextracts on growth *of Alternaria alternata* following 'food poisoning technique'

Phytoextract	Quantity of phytoextract (ml)	Radial growth of the pathogen (mm)	Growth inhibition of the pathogen (%)
Cassia alata	1	68	24.44 ± 0.03
	2	36	60.00 ± 0.07
	3	27	70.00 ± 0.01
Cassia occidentalis	1	61	41.11 ± 0.61
	2	49	45.55 ± 0.04
	3	35	50.00 ± 0.07
Azadirachta indica	1	66	25.66 ± 0.01
	2	33	63.33 ± 0.02
	3	25	72.22 ± 0.05
Datura metel	1	77	14.44 ± 0.03
	2	56	37.77 ± 0.09
	3	37	58.89 ± 0.04
Clrodendrum inermme	1	85	5.55 ± 0.06
	2	67	25.55 ± 0.04
	3	44	51.12 ± 0.02
Cymbopogon flexuosa	1	72	20.00 ± 0.09
	2	53	41.11 ± 0.05
	3	33	63.33 ± 0 01
Control	-	90	-

* Data are the mean values of five replicates

SEm ± 2.09 ; CD at 5% 8.025

Table 4 : Fungicidal sensitivity of *Trichoderma viride*

Fungicide	Dose of fungicide (%)	Growth of *Trichoderma viride*
Carbendazim	0.001	++
	0.010	++
	0.100	++
	1.000	+
	1.500	–
	2.000	–
Tridemorph	0.001	++
	0.010	++
	0.100	–
	1.000	–
	1.500	–
	2.000	–
Benomyl	0.001	++
	0.010	++
	0.100	–
	1.000	–
	1.500	–
	2.000	–
Captan	0.001	++
	0.010	+
	0.100	–
	1.000	–
	1.500	–
	2.000	–
Mancozeb	0.001	++
	0.010	+
	0.100	–
	1.000	–
	1.500	–
	2.000	–

+ = Growth only
++ = Growth and sporulation
– = No growth

the combination of most potent antagonist (*T. viride*) and the most active phytoextract (*Azadirachta indica*) with the systemic fungicide carbendazim at a concentration bellow minimum lethal dose (0.01%) had been explored which successfully inhibited the growth of the pathogen cent per cent (Table 5) and proved its excellencies by controlling the disease over 75% under pot culture experiment (Table 6).

The antagonistic property of *Trichoderma* spp. may well be due to their capacity for production of different antifungal antibiotics or other antifungal organic compounds which are volatile and non-volatile in nature (Bruce *et. al,* 1984).These organic compounds contain phenylalanine, ethanol, 2,3-dimethyl butane, acetaldehyde, 3-methyl -2-hexanol, isopropyl alcohol, 2-methyl pentane,etc.(Bruce *et. al,* 2000). Some antibiotics isolated from different species of *Trichoderma* are Trichodermin' (Krisosherekova, and Mishchenk, 1990); Alimethicin (Meyer and Reusser,1967); Dermadin (Pyke and Dietz, 1996); Suzukacillin (Ooka *et al, 1966)* etc. which have been proved to be highly effective against several phytopathogenic fungi.In addition to the production of different antibiotics *Trichoderma* spp. are reported to produce several hydrolytic enzymes like cellulase, chitinase. laminarinase etc. which impart their mycoparasitic property (Haran *et al,* 1993; Bruce *et al., 1995;* Dutta and Chatterjee, 2004, 2005; Chakraborty *et al.* 2004). The intensified pectolytic enzyme activity of plant pathogens during their pathogenesis had been reported to be reduced in presence of a strain of *Trichoderma* (Urbanek *et al.*,1989; Dutta and Chatterjee, 2005). Antifungal activity of'Neem'' *(Azadirachta indica)* extract has already been well documented by the works of several scientists. Such antifungal activity of neem extract may be due to the presence of some active compounds like azadirachtin, nimbidin, nimbicin, nimbin etc.

Successful biological control of pest and diseases can be achieved through the integrated way of disease management by applying antagonists along with some compatible form of fungicides and/or biocides (Wilson *et al,* 1991) Thus, the screening of suitable antagonists and determination of their compatibility with fungicides and plant extracts is newly emerging area of modern research and may certainly be a positive approach towards minimization of environmental hazards Compatibility of *Trichoderma* spp. with the systemic fungicide 'Carbendazim' is well studied by Ahmed and Baker (1988), Kay and Stewart (1994) and Dutta and Chatterjee (2004).

Table 5: Integrated control of the pathogen *Alternaria alternate* under *in vitro* condition

Integrated combination	Colony diameter of the pathogen (mm)	Percent Spore Germination of the pathogen per microscopic field	Reduction in spore germination over control (%)	Growth inhibition of the pathogen (%) *
T. viride (cell-free culture filtrate 1 ml) + Carbendazim (I ml of 0.01% concentration)	14	4	63.64	84.44 ± 1.447
T. viride (cell-free culture filtrate 1 ml) + Neem extract (1 ml ethanolic extract of leaves)	10	2	81.81	88.89 ± 1.000
T. viride (cell-free culture filtrate 1 ml) + Carbendazim (1 ml) of 0.01% concentration) + Neem extract (1 ml ethanolic extract of leaves)	0	0	100	100 ± 0.955
Control	90	11	0	0

* Data are the mean values of five replicates
SEm ± 0.60 ; CD at 5% 1.554

Table 6 : Integrated management of the disease under pot culture

Integrated combination	Total no. of leaves	No. of infected leaves	Leaf infection (%)	Reduction of leaf infection over control* (%)
T. viride + Carbendazim (0.01% concentration)	42	12	28.57	47.52
T. viride + Neem extract	31	7	22.58	57.40
T. viride + Carbendazim (0.01% concentration) + Neem extract	45	6	13.33	75.92
Control	49	26	54.16	

* Data are the mean values of five replicates
SEm ± 4.27 CD at 5% 12.25

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