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Native *Trichoderma* isolates and its impact on growth, fruit yield and disease management of Chilli Wilt in Manipur

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Ten different *Trichoderma* isolates (ThrC1, TvC2, TvC3, ThmC 4, ThrC5, ThrC6, TvC7, TvC8, ThmC9 and TvC10) were isolated from the root rhozosphere of healthy chilli plants from 10 different locations in four different districts of Manipur. According to their growth behaviour five were *Trichoderma viride*, three were *Trichoderma harzianum* and two were *Trichoderma hamatum.* It was observed that *Trichoderma viride* was light greenish with fluffy growth and concentric rings with dense growth at the margin of the colony. One isolate produced coconut like aroma in all the three media tested *viz*. PDA (Potato dextrose agar), OMA (Oat meal agar) and TSM (Trichoderma specific media). Growth of *Trichoderma harzianum* was fast to very fast with whitish greenish to green colour, concentric rings and hyaline hyphae without any significant aroma. Growth of *Trichoderma hamatum* was fast with whitish green to greenish colour, highly fluffy and compact colony, hyaline hyphae without any significant aroma in all the three media. Conidiophores were highly branched nearly at right angle in *Trichoderma harzianum* and *Trichoderma hamatum* and at acute angle in *Trichoderma viride.* These isolates were evaluated for their *in vitro* antagonistic potential against *Fusarium oxysporum* f.sp. *capsici* causing wilt of chilli in Manipur by dual culture technique. It was observed that TvC10 was best followed by ThrC1, ThmC9, TvC3, ThrC6, TvC8, ThrC5, TvC2, TvC7,, ThmC4 resulting to 86.00, 84.22, 83.11, 81.56, 80.67, 79.33, 79.11, 78.44, 76.67 and 76.22 % inhibition in colony growth of *Fusarium oxysporum* f.sp. *capsici*. These isolates were applied as seed treatment as well as soil application and observed that seed treatment as well as soil application with ThrC1 produced maximum shoot length (101.37 cm), root length (13.37 cm), number of fruits (21.23) and weight of fresh fruit (89.27 g/plant) with significant disease reduction. So, native isolates of ThrC1 seed treatment as well as soil application gave most promising and synergistic effect on vegetative growth, yield as well as reduction of wilt of chilli in Manipur.

Key words: Biocontrol, *Capsicum annuum* L.,*Fusarium oxysporum* f.sp. *capsici, Trichoderma* spp.

INTRODUCTION

Chilli (*Capsicum annuum* L.) belongs to the family Solanaceae. It is an important vegetable and spice crop world wide produced and consumed as fresh or processed. In India, it is commercially grown in an area of 9.65 lakhs ha. producing 10.75 lakhs tonnes. In Manipur, Chilli is cultivated in an area of 10,140 ha. producing 6,080 tonnes. Chilli is known to suffer from various diseases of which wilt disease caused by *Fusarium oxysporum* f.sp. *capsici* is a serious one which results in total or partial killing of the standing crops. Several fungicides are recommended for the control of *Fusarium* wilt of chilli, however indiscriminate use of fungicide is harmful to human beings, animals and other beneficial microorganisms present in the ecosystem. Use of environment friendly biological agents can more effectively control many soil borne pathogens *Trichoderma*, a filamentous soil borne saprophytic fungus is known to be one of the best candidates of biocontrol agents for the management of soil borne plant pathogens. Mode of action of this fungus include mycoparasitism, antibiosis, competi-

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tion for nutrients and space, tolerance to stress through enhanced root and plant development, solubilization and sequestration of inorganic nutrients and induced resistance. Biological control of pathogens has proven not only ecologically but also economically sound as seen in the benefit/cost ratio (Salami *et al*, 2005).

Hence, the present study has been carried out to biologically control *Fusarium* wilt of chilli (*Capsicum annuum* L.) by *Trichoderma* spp. and to study their impact on growth and yield of the crop.

MATERIALS AND METHODS

Diseased plant was collected from the field and isolation of the causal pathogen from the infected root of chilli plants was done on potato dextrose agar (PDA) medium. The fungus was identified in the laboratory, Department of Plant Pathology, CAU, Imphal, Manipur and pure culture was maintained by regular subculturing for further studies. Koch's postulate was used to prove the pathogenicity test. Different *Trichoderma* species were isolated by soil dilution plate technique using *Trichoderma* specific medium (TSM) from the soil samples collected from the different locations of chilli growing areas of Manipur *viz*., Imphal West, Imphal East, Bishnupur and Thoubal Districts. Soil was taken from the rhizosphere of healthy chilli plants from 5-6 cm depth of soil. The cultural characteristics of different isolates of *Trichoderma* were studied in three different media, *viz*., potato dextrose agar (PDA), *Trichoderma* specific medium (TSM) and oat meal agar (OMA) to observe the colony colour, growth rate, growth pattern and production of any distinguishing odour. The antagonistic behavior of different isolates of *Trichoderma* against *Fusarium oxysporum* f. sp. *capsici* were done on dual culture plate technique. The pathogen, *Fusarium oxysporum* f. sp. *capsici* was multiplied on growing in rice seed. *Trichoderma* isolates were also mass multiplied in rice bran (80 per cent) + mustard cake (20 per cent) substrate (Pan and Bhagat, 2007).

Green house experiment was conducted to know the efficiency of *in vivo Trichoderma* antagonists against *Fusarium oxysporum* f. sp. *capsici* causing wilt of chilli. Three methods of application of *Trichoderma* as seed treatment with *Trichoderma* @ 5g (rice bran + mustard cake) (1×10^8 cfu/g)/kg seed; soil treatment with *Trichoderma* @ 25g (rice bran + mustard cake) (1×10^8 cfu/g)/kg soil and both seed treatment with *Trichoderma* @ 5g (rice bran + mustard cake) (1×10^8 cfu/g)/kg seed as well as soil treatment with *Trichoderma* @ 25g (rice bran + mustard cake) (1×10^8 cfu/g)/kg soil. Plastic pots without *Trichoderma* served as control. Observations on disease incidence was recorded from the germination to the fruiting stage. Growth parameters like plant height, root length was taken at 90 days after planting and yield parameters of chilli like number of green fruits per plant, weight of green fruits, size of fruit (length and diameter) were recorded at harvest.

RESULTS AND DISCUSSION

The isolated fungus was purified and identified as *F. oxysporum* f. sp. *capsici* on the basis of morphological characteristics and taxonomic keys available in the literature. When the fungus isolated from the diseased samples was artificially inoculated in soil and chilli was grown to cause disease. The wilt symptom was developed after 12-15 days of inoculation and it confirmed that wilt disease was due to the pathogen *F. oxysporum* f. sp. *capsici*.

Isolation of Trichoderma isolates and their morphological characters

Three species of *Trichoderma viz*., *Trichoderma harzianum* (3 isolates), *Trichoderma viride* (5 isolates) and *Trichoderma hamatum (*2 isolates) were isolated and identified according to the cultural and morphological characters as follows. The growth of *T. viride* was light greenish with fluffy growth and concentric ring with dense growth at the margin of the colony and one isolate produced coconut like aroma in all the three media. The growth of *T. harzianum* was fast to very fast with whitish greenish to green color, concentric ring, and hyaline hyphae without any significant aroma. The growth of *T. hamatum* was fast with whitish green to greenish colour, highly fluffy and compact colony, hyaline hyphae without any significant aroma in all the three media. *T. harzianum* was fast growing colonies, white to greyish or sometimes yellowish exudates colourless to amber or greenish yellow, odour indistinct or faintly earthy, and hyphae hyaline. Similarly, it was also found that *T. viride* as rapidly growing fungus, aerial mycelium usually limited, side of the growth was colourless to dull yellowish, some isolates with distinctive aromatic odour resembling

Table 1 : Effect of different *Trichoderma* isolates on the growth of *Fusarium oxysporum* f. sp. *capsici*

Trichoderma isolates	Per cent inhibition over control
ThrC1	84.22
TvC2	78.44
TvC3	81.56
ThmC4	76.22
ThrC5	79.11
ThrC6	80.67
TvC7	76.67
TvC8	79.33
ThmC9	83.11
TvC10	86.00
S.E. (d) ±	2.49
C.D. at 5%	5.23

of *T. hamatum* was adapted to excessive soil moisture and *T. viride* was restricted to areas where low temperature prevails, whereas *T. harzianum* is most commonly found in warm climates. The possible reason of presence of *T. harzianum* in comparatively less warm climate (Manipur) may be due to the diverse climatic conditions with high rainfall and comparatively high organic matter content than normal soil with high soil microbial diversity and complex interaction among the soil microorganisms. Indo-Burma region is also considered a hot spot region of biodiversity and Manipur is likely to harbor useful *Trichoderma* isolates.

Table 2 : Effect of different *Trichoderma* isolates on the Wilt incidence and growth of Chilli under net house condition

Treatments	% Disease incidence	Length (cm)		No. of fruits per plant	Size of fruits (cm^2)	Wt. of fruits per plant (g)
		Shoot	Root			
ThrC1 Seed	43.33* (41.15)**	80.97	11.17	17.13	22.07	78.20
ThrC1 Seed + Soil	30.00 (33.21)	101.37	13.37	21.23	32.17	89.27
ThrC1 Soil	36.66 (37.22)	95.23	12.87	20.40	26.63	79.70
ThmC9 Seed	50.00 (45.00)	70.70	8.97	16.23	22.30	67.93
ThmC9 Seed + Soil	33.33 (35.22)	80.17	11.10	19.60	28.53	78.50
ThmC9 Soil	36.66 (37.22)	76.40	9.37	16.87	27.27	69.33
TvC10 Seed	40.00 (39.15)	82.30	10.93	16.73	24.67	74.60
TvC10 Seed + Soil	23.33 (28.78)	97.23	13.03	19.90	32.77	84.17
TvC10 Soil	26.66 (31.00)	88.70	12.73	17.07	26.17	75.37
Control	100.00 (89.19)	68.20	8.63	10.93	21.30	47.87
S.E. (d) ±	2.95	1.72	0.32	1.32	1.84	4.27
C.D. at (5%)	6.19	3.61	0.67	2.77	3.86	8.98

*Mean of 3 replications; **Figure in parenthesis indicates angular transformation

coconut and *T. hamatum* was characterized as moderately rapid growing fungus, white to grayish mycelium, indistinct odour, hyaline hyphae. Similar observations were made (Pan and Bhagat, 2008) where they comprehensively reported that the *T. harzianum* and *T. viride* were fast growing green coloured mycoparasitic fungi with distinct coconut or faintly earthy aroma, whereas *T. hamatum* has relatively slow growth. Conidiophores were highly branched nearly at right angle in *T. harzianum* and *T. hamatum* and nearly at acute angle in *T. viride.* Prolonged dry condition of soil reduced the population of *Trichoderma* and *Gliocladium* and also concluded that certain strains

Antagonism against Fusarium oxysporum f. sp. capsici on different Trichoderma spp.

Antagonistic efficiency of *Trichoderma* isolates against *F. oxysporum* f. sp. *capsici* were evaluated *in vitro* by dual culture technique and the results indicated that all the tested *Trichoderma* isolates were found to have statistically significant effect on the growth of the pathogenic fungus *F.oxysporum* f. sp. *capsici*. Among the *Trichoderma* isolates, TvC10 showed the highest inhibition on the growth of *F.oxysporum* f. sp. *capsici* where the inhibition percentage was 86 per cent. Rest of the nine *Trichoderma* isolates *viz*., ThrC1, ThmC9, TvC3,

ThrC6, TvC8, ThrC5, TvC2, TvC7, ThmC4 inhibited the colony growth of *F. oxysporum* by 84.22, 83.11, 81.56, 80.67, 79.33, 79.11, 78.44, 76.67, and 76.22 per cent respectively (Table 1). *T. viride* showed the best performance in *in vitro* biological control of *F.oxysporum* followed by *T. harzianum, T. aureoviride, T. koningii and T. pseudokoningii*, respectively, resulting in 62, 36, 24, 18 and 6 per cent reduction in colony growth of *F. oxysporum* respectively (Sahi *et al*, 2007). The pathogens like *R. solani*, *Pythium* spp., *S. rolfsii*, *Macrophomina phaseolina* and *Fusarium oxysporum* were significantly inhibited by *Trichoderma* spp. *in vitro* condition(Chaudhary *et al*, 2006; Kumar and Hooda, 2007).

Effect of different Trichoderma isolates on the management of Chilli Wilt

In vivo efficacy of three potent *Trichoderma* isolates *viz*., ThrC1, ThmC9 and TvC10 on the wilt incidence, growth parameters like plant height, root length and yield parameters of chilli like number of green fruits per plant, weight of green fruits, size of fruit (length and diameter) were studied and showed that all the treatments significantly decrease disease incidence rather than the control(100%). The minimum disease was observed in seed treatment as well as soil application of TvC10 isolate (23.33%) statistically at par with the soil application of TvC10 isolate (26.66%). Whereas maximum disease incidence was noticed in the seed treatment with ThmC9 isolate (50.00%) followed by seed treatment with ThrC1 isolate (43.33%) statistically at par with seed treatment with TvC10 isolate (40.00%). It was also observed that seed treatment as well as soil application of ThrC1 isolate produced less disease (30.00%) statistically at par with seed treatment as well as soil application of ThmC9 isolate (33.33%). Seed treatment as well as soil application with ThrC1 produced maximum shoot length (101.37 cm), root length (13.37 cm), number of fruits (21.23) and weight of fresh fruit (89.27 g/plant) with significant disease reduction (Table 2). Studies confirmed that *T. harzianum* and *T. viride* enhanced seed germination, root and shoot length (Dubey *et al*, 2007) as well as increased the frequency of healthy plants, and boosting yield (Rojoa *et al*, 2007). Crop productivity in fields increased up to 30 per cent after the addition of *T. hamatum* or *T. koningii*.

The result in the present study showed that in *in vitro* experiment among the *Trichoderma* isolates TvC10 gave the best performance where the per cent inhibition of *Fusarium oxysporum* f. sp*. capsici* was 84.22 %. It also gave the least disease incidence 26.66% in net house condition. The *Trichoderma* isolates showed diversity in their colony colour and linear growth on PDA, OMA and TSM. Seed treatment as well as soil application of ThrC1 (*Trichoderma harzianum* C1) gave maximum biometric data *i.e.* shoot and root length, no. of fruits per plant, size of fruits and weight of fresh fruits per plant. Our results indicate the possibility of using native *Trichoderma* isolates for the management of wilt disease in chilli.

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