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Role of soil amendment with *Trichoderma harzianum*, benomyl and wilt pathogen on growth and yield of tomato grown in tropical soil

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Chemical fungicides commonly used in modern agriculture to control the pathogenic soil microbes have serious environmental implications. Efficacy of soil amendment with *Trichoderma harzianum* and Benomyl ($C_{14}H_{18}N_4O_3$) against wilt pathogen was evaluated on tomato plants grown in tropical soil. Both *Trichoderma harzianum* and Benomyl inhibited the wilt pathogen and promoted the growth and yield of tomato. Antagonistic activity of *Trichoderma harzianum* against the wilt pathogen and its growth promoting ability on tomato plants was found to increase when it was applied in combination with benomyl in the soil. This fungus may be used as alternative to the hazardous chemicals to combat the wilt diseases and raise the yield of tomato.

Key words: *Trichoderma harzianum*, Benomyl, *Fusarium* wilt, Tomato

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) which is one of the important vegetable crops all over the globe is subjected to attack by several soil borne fungal pathogens that cause serious diseases such as wilt (Morsy *et al.* 2009). *Fusarium* wilt of tomato continues to incur yield losses at different locations where it is endemic (Amni and Sidovich, 2010). Benomyl ($C_{14}H_{18}N_4O_3$) [Methyl [1-[(butylamino) carbonyl] -1H-benzimidazol-2-yl]carbamate] is one of the systemic benzimidazole fungicides which is used to combat this disease (http://en.wikipedia.org). It is selectively toxic to microorganisms and to invertebrates, especially earthworms (http://en.wikipedia.org). Its half life in soil ranges from six months to one year (http://en.wikipedia.org). It binds strongly to the soil and does not dissolve in water to a great extent. Hence, the fungicide has hazardous impact on ecosystem. Therefore, the use of antagonistic soil microbes such as *Trichoderma harzianum* to combat wilt pathogen is quite attractive (Hyakumachi *et al.* 1992). *T. harzianum* is common in agricultural soils and has been reported to stimulate plant growth by the production of phytohormones, degradation of complex substrates (Altmore *et al.* 1999) and /or suppression of pathogenic soil microbes (Hyakumachi *et al.*, 1992, 1994). Altmore *et al.*, (1999) have investigated the capability of *T. harzianum* Rifai 1295-22 (T-22) to solubilize some insoluble or sparingly soluble minerals *in vitro* and reported that T-22 is able to solubilize MnO_2, metallic zinc and rock phosphate (mostly calcium phosphate) in a liquid sucrose-yeast extract medium. This phosphate solubilising activity of *T. harzianum* might be responsible for its plant growth promoting ability. *T. harzianum* has been reported to exhibit antagonistic activity against *Fusarium oxysporum* f.s. *lycopersici* (FOL) that causes the wilt of tomato (Hyakumachi *et al.*, 1992, 1994; Dubey *et al.*, 2007). We have tested the efficacy of benomyl (chemical fungicide) and the antagonistic activity of *T. harzianum* in checking *Fusarium* wilt and raising growth and yield of tomato in tropical soils. The bioagent has exhibited antagonistic activity against the wilt pathogen by competing at the active sites, reduced the intensity of disease development and subsequently, stimulated the growth and yield of plants. Antagonistic activity of *T. harzianum* against the test pathogen and its growth promoting ability increase when it is applied in combination with the fungicide in the soil. This fungus therefore, holds potential for use as alternative to the chemicals for sustainable.

MATERIALS AND METHODS

Isolation of fungi from rhizosphere

Healthy and wilted tomato plants were collected at regular growth intervals i.e. seedling, vegetative, flowering and fruiting stages from a field at Varanasi (Karaudi), India. Care was taken to dig out, as far as possible, the whole root system with a sterilized spatula. The root systems were then brought to the laboratory in separate polyethylene bags. The roots were given gentle taping to loosen-off the lightly adhering soil, in order to have just the rhizosphere soil attached to the root system. Small pieces of roots (2 cm) were cut with sterilized scissors under aseptic condition and 25 such root pieces for each sample were transferred to flasks (one for healthy and the other for diseased roots) containing 100 ml of sterilized distilled water. The flasks were shaken vigorously with the help of a shaker to get a homogenous suspension of the rhizosphere soil. Taking this as the stock solution, conventional soil dilution plate method (Warcup, 1950) was followed for isolation of the rhizosphere fungi. Dilutions of 1:100, 1:1000, and 1:10000 were prepared. Three replicates of sterilized Petri plates were inoculated with one ml aliquots from all the diluted suspensions. To this was added 20 ml melted and cooled (40° C) potato dextrose agar (PDA) medium and the plates were rotated slowly in clock-wise and anti-clock wise directions to disperse the soil solution uniformly in the culture medium. All the inoculated plates were then incubated at 25±2 °C. The plates were examined regularly and the colonies of fungi appearing on the medium were transferred into fresh sterilized Petri plates containing PDA medium to avoid over-running by the fast growing forms. The pure cultures of *Trichoderma harzianum* and *Fusarium oxysporum* f. sp. *lycopersici* thus, isolated were preserved on PDA slants at 4°C.

Preparation of mass culture

The mass culture of the rhizosphere fungi was prepared on barley grains (Shivanna *et al.*, 1994). Clean and intact barley grains were taken for this purpose. The grains were pre-wetted by boiling them in water for 20-30 minutes so as to raise the moisture content of the grains up to 40-50% and to make them soft enough for the profuse growth of the fungus. After boiling, the grains were spread on wire mesh so as to drain the excess of water. The grains were then mixed with gypsum (calcium sulphate 2%) and chalk powder (calcium carbonate 0.5%) on dry weight basis to check pH of the medium and prevent grains from sticking with each other. Clean glucose bottles were filled with such barley grains (100g each) which were then steam sterilized for 1-2 hour. The bottles were then allowed to cool at room temperature and inoculated with five agar blocks (5 mm diameter each) cut from the margin of actively growing culture of each fungus. The bottles were incubated at 25 ± 2°C for 10 days. The bottles were shaken once or twice daily for rapid and uniform colonization of the fungi. Barley grains colonized by the rhizosphere fungi were air dried and aseptically stored at 4°C for further use.

Preparation of pots

The soil sample was collected from tropical agricultural field at Varanasi, India. The soil was air dried at room temperature and ground to fine powder form with the help of pestle and mortar. The pure inoculum of *T. harzianum* , which was prepared on barley grains, was mixed separately with sterilized natural soil (1% w/w). Chemical fungicide namely benomyl ($C_{14}H_{18}N_4O_3$) was mixed separately with sterilized natural soil samples at the rate of 0.24 kgh^{-1} (w/w) (Hoeven and Bollen, 1980). The pure inoculum of the test pathogen *F. oxysporum*. f.s. *lycopersici* (FOL) prepared separately on barley grains was mixed with each sample of sterilized natural soil inoculated with the chemicals and pure inoculum of *T. harzianum* (1% w/w). The soil samples so prepared were separately filled in clay pots (15 x 25 cm). The pots were kept at room temp. for a week during which *T. harzianum* and the test pathogen developed and colonized the soil particles. Soil supplemented with barley grains without inocula was used as control. The moisture level of the soil (25-30%) was maintained by watering the pots from time to time. Twenty surface sterilized seeds of variety H-24 of tomato were sown in each pot 8days after combined soil amendment with *T. harzianum* , benomyl and wilt pathogen. The experiments were set in replicates of three pots in a greenhouse. The observations for the combined effect of the bioagent, fungicide and wilt pathogen on growth and yield of tomato plants were made on plant height, branches/ plant, fruits/plant and weight of 100 dry seeds at 45, 65, 95 and 115 days after sowing (DAS). Ten plants

were uprooted randomly from each treatment and the plant length above the ground was measured in cm and average height per plant was calculated. Branches and/or fruits on all the tomato plants when formed and developed under each treatment were counted and average number of branches and/or fruits per plant was calculated. The seeds harvested from the ripened fruits separately in each treatment were air dried. One hundred dry seeds were randomly selected from triplicate sets from individual treatments and were weighed.

RESULTS AND DISCUSSION

The results clearly indicated that *Trichoderma harzianum* and the fungicide promoted the growth of tomato in presence of wilt pathogen FOL (Table 1). Out of all the treatments, *T. harzianum* + benomyl was found to be the maximum growth promoter at 45 DAS; this was followed by *T. harzianum* and benomyl sulphate in that order. Plant growth promotion by the bioagent and the fungicide was significantly higher than the control ($P < 0.05$). In most of the treatments growth promotion of tomato increased with time up to 115 DAS. Plant growth at 45 DAS was significantly lower in the FOL inoculated compared to un-inoculated plants. Antagonistic activity of *T. harzianum* against the test pathogen and its growth promoting ability increased when it was used in combination with the fungicide.Maximum number of branches/plant was recorded at 115 DAS with *T. harzianum* + benomyl treatment that was followed by *T. harzianum* and benomyl which were significantly higher than control ($P < 0.05$). In FOL inoculated plants, no branching was observed.Maximum number of fruits/plant were recorded at 115 DAS with *T. harzianum* + benomyl treatment that was followed by *T. harzianum* and benomyl which were significantly higher than control ($P < 0.05$). In FOL inoculated plants, no fruit setting was observed. Weight of 100 dry seeds observed at 115 DAS, was maximum in *T. harzianum* + benomyl treatment which was followed *T. harzianum* and benomyl which were significantly higher than control ($P < 0.05$).

Among all the treatments, *T. harzianum* + benomyl was found to be the best (at 115 DAS) as plant growth promoter in terms of all the four parameters viz, plant height, no. of branches/plant, no. of fruits/plant and weight of 100 dry seeds. An overall 2-folds increased yield in terms of fruit set was observed in bioagent and fungicide treatments which were significantly higher than the control ($P < 0.05$). In FOL inoculated plants, intensity of wilt was high, plant growth poor without producing any yield.

Table.1: Effect of soil amendment with *Trichoderma harzianum*,benomyl and wilt pathogen on growth and yield of tomato grown in tropical soil

Treatment	Days	Plant ht. (cm)	Branch/ Plant	Fruit/ plant*	100 dry seeds (g)**
Trichoderma	45	11.99 ± 0.00	3.00 ± 0.00	-	-
harzianum	65	17.00 ± 0.00	4.00 ± 0.00	-	-
	95	34.00 ± 0.00	7.00 ± 0.00	13 ± 0.0	-
	115	39.00 ± 0.00	7.00 ± 0.00	22 ± 0.0	0.86 ± 0.0
Benomyl	45	11.35 ± 0.00	2.00 ± 0.00	-	-
	65	17.00 ± 0.02	3.00 ± 0.00	-	-
	95	33.05 ± 0.00	6.00 ± 0.00	12 ± 0.0	-
	115	38.75 ± 0.00	6.00 ± 0.00	20 ± 0.0	0.85 ± 0.0
T.harzianum	45	13.60 ± 0.00	3.00 ± 0.00	-	-
+ Benomyl	65	18.50 ± 0.00	4.00 ± 0.00	-	-
	95	34.70 ± 0.00	6.00 ± 0.00	15 ± 0.0	-
	115	40.00 ± 0.00	8.00 ± 0.00	24 ± 0.0	0.89 ± 0.0
FOL	45	5.75 ± 0.00	0.00 ± 0.00	-	-
	65	8.00 ± 0.00	0.00 ± 0.00	-	-
	95	12.00 ± 0.00	0.00 ± 0.00	-	-
	115	12.00 ± 0.00	0.00 ± 0.00	-	-
Control (without treatment)	45	9.50 ± 0.00	1.00 ± 0.00	-	-
	65	13.00 ± 0.00	3.00 ± 0.00	-	-
	95	22.10 ± 0.00	3.00 ± 0.00	8 ± 0.0	-
	115	27.00 ± 0.00	4.00 ± 0.00	13 ± 0.0	0.40 ± 0.0

±, Standard error of mean of three replicates (SEM); -, Not recorded; *Yield in terms of number of fruits only; **Weight taken when seeds were ready for harvesting; Data were statistically analyzed which were found to be significant ($P < 0.05$)

Trichoderma spp. are known for their antagonistic activity against the pathogenic soil microbes (Howell and Stipanovic, 1983). Inhibition of mycelial growth of *Fusarium oxysporum*, *Heterobasidion annosum* and *Phytophthora* spp. by non-volatile metabolites of *Trichoderma* spp. has been reported (Etebarian *et al.*, 2000) and the reasons have been attributed to the production of substances such as antibiotics, toxins, etc. in the culture filtrates of the test microorganisms (Skidmore, 1976). It has been reported that *Trichoderma* spp. produce non-volatile substances such as Trichodermin which could be the cause of inhibition of the growth of FOL in the present study (Dennis and Webster, 1971). Vinale *et al.* (2006) reported that *T. harzianum* produced a metabolite identified as T22azaphilone(83) that

inhibited the growth of *Rhizoctonia solani*, *Pythium ultimum* and *Gaeumannomyces graminis var. tritici*. *T. aggressivum* has been reported to produce an antifungal metabolite (3, 4-dihydro-8-hydroxy-3-methylisocoumarin) that inhibited the growth of *Agaricus bisporus* and other fungi (Krupke *et al.*, 2003). John *et al.* (2004) studied the interaction between *T. harzianum* and *Eutypa lata*, the pathogen which causes dieback disease of grapevine and reported that the metabolites produced by *T. harzianum* reduced the growth of this test pathogen *in vitro*. Eziashi *et al.* (2006) tested the metabolites of *Trichoderma* spp. against *Ceratocystis paradoxa* and found them to be growth inhibitory. Narisawa *et al.* (2002) reported that *Verticillium dahliae* causing wilt disease of eggplant was suppressed by *Heterconium chaetospira, Phialocephala fortinii, Penicillium* sp. and *Trichoderma* sp. *T. harzianum, T. viride* and *T. virens* have been found to suppress the mycelial growth of *Fusarium oxysporum* f. sp. *ciceris* and enhance the growth and yield of this crop plant (Dubey *et al.*, 2007).

In the present study, antagonistic activity of *T. harzianum* against the test pathogen and its growth promoting ability increased when it was applied in combination with the fungicide. This might be due to combined effect of the treatments (Dubey *et al*, 2007). Adhilakshmi *et al.* (2008) studied the combined effect of bioagents and chemicals on alfalfa wilt pathogen *Fusarium oxysporum* f. sp. *medicaginis* and found that the application of chemicals in combination with bioagent in the soil significantly reduced the wilt disease incidence accompanied by improved plant growth as well as yield.

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