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Studies on the impact of soil amendment with fungal consortium, chemical fungicide and wilt pathogen on growth and yield of Tomato in vegetated western Himalayan agro ecosystems

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It is well proven fact that the chemical fertilizers and fungicides that are of common use in agriculture have negative effect on environmental health. Many beneficial fungi and bacteria that are known to flourish in the rhizosphere of crop plants are a better alternative to the harmful chemicals. These microbes can be isolated, mass cultured and used for plant growth promotion and pathogen suppression. In the present study we studied the effect of soil amendment with fungal consortium consisting of *Trichoderma viride*, *Trichoderma asperellum*, *Hypocrea lixii / Trichoderma harzianum*, *Trichoderma atrobrunneum*, *Penicillium ortum, Hypocera lixii* and *Penicillium oxalicum* in presence of wilt pathogen and chemical namely, Benomyl ($C_{14}H_{18}N_4O_3$) on growth and yield of tomato crop cultivated in vegetated western Himalayan agroecosystems. Antagonistic activity of the consortium 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 fungal consortium may be used as alternative to the hazardous chemicals to combat the wilt diseases and raise the yield of tomato.

Key words: Fungal consortium, *Trichoderma*, Benomyl, wilt pathogen

INTRODUCTION

Crop plants such as tomato, maize, wheat, rice and potato are attacked by the soil pathogens (Morsy *et al*, 2009). Diseases like *Fusarium* wilt of crop plants including tomato, being endemic, causes losses in terms of yield at different places in agricultural fields in the country (Amini and Sidovich, 2010). The chemicals including Benomyl ($C_{14}H_{18}N_4O_3$) [Methyl [1-[(butylamino)carbonyl]-1H-benzimidazol-2-yl]carbamate] which is one of the systemic benzimidazole fungicides, are used in agriculture to suppress the pathogens and enhance plant growth and yield. However, the chemicals are not eco-friendly. For instance, benomyl (half life ranging from six months to one year in the soil), binds strongly to the soil and does not dissolve in water to a great extent, is selectively toxic to microbes and earthworms (Indu *et al*, 2015). The fungicides are therefore, hazardous to the ecosystem and ecology (Indu *et al*, 2015). A better and well known alternative to the chemicals are the microbial consortia consisting of beneficial soil fungi such as *Trichoderma viride*, *Trichoderma asperellum, Hypocrea lixii / Trichoderma harzianum, Trichoderma atrobrunneum*, *Penicillium ortum, Hypocera lixii* and *Penicillium oxalicum* (Hyakumachi *et al.* 1992; Indu *et al*. 2015). The species of *Trichoderma* are common in agricultural soils and are known to enhance plant growth through the suppression of pathogenic soil microbes (Hyakumachi *et al.* 1992, 1994), degradation of complex substrates (Altmore *et al.* 1999), production of phytohormones. The solubilizing ability of *Trichoderma harzianum* Rifai 1295-22 (T-22) for minerals *in vitro* was investigated (Altmore *et al,* 1999). It has been found that T-22 was able to solubilize metallic zinc, MnO_2 and rock phosphate (mostly calcium phosphate) in a liquid sucrose-yeast extract medium (Altmore *et al.* 1999). The ability of *T. harzianum* to solubilize phosphates might be responsible for its plant growth promoting activity (Altmore *et al.* 1999). Investigations on *Trichoderma* strains showed that these fungi exhibit antagonistic activity against the soil pathogens including *Fusarium oxysporum* f.sp.

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lycopersici that causes the wilt of tomato (Hyakumachi *et al,* 1992, 1994; Dubey *et al,* 2007). In the present investigation, studied the effect of soil amendment with fungal consortium consisting of *Trichoderma viride*, *Trichoderma asperellum*, *Hypocrea lixii / Trichoderma harzianum*, *Trichoderma atrobrunneum, Penicillium ortum, Hypocera lixii* and *Penicillium oxalicum* in presence of wilt pathogen and chemical namely, Benomyl ($C_{14}H_{18}N_4O_3$) on growth and yield of tomato crop cultivated in vegetated western Himalayan agroecosystems. Antagonistic activity of the consortium 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. The consortium reduced the intensity of disease, exhibited antagonistic activity against the wilt pathogen by competing at the active sites, and subsequently, promoted the growth and yield of plants. This fungal consortium may be used as alternative to the hazardous chemicals to combat the wilt diseases and raise the yield of tomato.

MATERIALS AND METHODS

Isolation of rhizosphere soil fungi

Healthy and wilted tomato plants were collected at regular growth intervals i.e. seedling, vegetative, flowering and fruiting stages from tomato plants cultivated in vegetated western Himalayan agro ecosystems in Kangra region of Himachal Pradesh. Whole rhizosphere system was carefully digged out with the help of spatula. The samples were placed in separate polyethylene bags and shifted to the laboratory. In order to have just the rhizosphere soil attached to the root system, gentle tapping was given to the roots so as to loosen-off the lightly adhering soil. With sterilized scissors under aseptic conditions, small pieces of roots (2 cm) were cut 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. With the help of shaker, flasks were vigorously shaken to get a homogenous suspension of the rhizosphere soil. Taking this as the stock solution, for the isolation of the rhizosphere fungi, conventional soil dilution plate method was followed. Serial dilutions of 1:100, 1:1000, and 1:10000 were prepared. From all the diluted suspensions, three replicates of sterilized Petri plates were inoculated with one ml aliquots. 20 ml melted and cooled (40° C) potato dextrose agar (PDA) medium was added to it. To disperse the soil solution uniformly in the culture medium, the plates were rotated slowly in clock-wise and anti-clock wise directions. The inoculated plates were then incubated at 25±2 °C. To avoid over-running by the fast growing forms, the colonies of fungi appearing on the medium were transferred into fresh sterilized Petri plates containing PDA medium. The pure cultures of fungi thus, isolated were preserved on PDA slants at 4°C.

Preparation of mass culture of fungi

Barley grains were used for preparation of mass culture of isolated fungi (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 fungi. 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 (100 g 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 individual rhizosphere fungi were mixed to prepare the consortium of all the isolated fungi. The grains colonized by the fungal consortium were air dried and aseptically stored at 4°C for further use. The mass culture of the test pathogen (FOL) was similarly prepared separately on barley grains.

Preparation of pots for experiments

The soil sample was collected from agricultural field in Shahpur region of District Kangra in Himachal Pradesh, India. The soil was air dried at room temperature and ground to fine powder form with the help of pestle and mortar. The consortium of fungal isolates namely, *Trichoderma* *viride*, *Trichoderma asperellum*, *Hypocrea lixii / Trichoderma harzianum*, *Trichoderma atrobrunneum* , *Penicillium ortum, Hypocera lixii* and *Penicillium oxalicum* which was prepared on barley grains, was mixed with sterilized natural soil (1% w/w). Chemical fungicide namely benomyl ($C_{14}H_{18}N_4O_3$) was mixed with sterilized natural soil samples at the rate of 0.24 kg h^{-1} (w/w).The pure inoculum of the test pathogen (FOL) prepared separately on barley grains was mixed with the sample of sterilized natural soil inoculated with the chemicals and the consortium (1% w/w). The soil samples so prepared were separately filled in clay pots (15 ´ 25 cm). The pots were kept at room temperature for a week during which consortium 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 8 days after the combined soil amendment with consortium, benomyl and wilt pathogen. The experiments were set in replicates of three pots in a greenhouse. The observations for the combined effect of the consortium, 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 50, 70, 100 and 120 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 are shown in Table 1. As is apparent from the results, the combined soil amendment with consortium and the fungicide stimulated the growth of tomato in presence of wilt pathogen FOL. Out of all the treatments, consortium + benomyl was found to be the maximum growth promoter at 50 DAS; this was followed by consortium alone in that order. Plant growth promotion by the consortium 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 120 DAS. Plant growth at 50 DAS was significantly lower in the FOL inoculated soil compared to uninoculated plants. Antagonistic activity of consortium 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 120 DAS with consortium + benomyl treatment that was followed by consortium alone 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 120 DAS with consortium + benomyl treatment that was followed by consortium alone 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 120 DAS, was maximum in consortium + benomyl treatment which was followed consortium alone which were significantly higher than control ($P < 0.05$). Among all the treatments, consortium + benomyl was found to be the best (at 120 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 combined treatment with consortium and fungicide 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.

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

Table 1 : Effect of soil amendment with fungal consortium, in presence of wilt pathogen and chemical namely, Benomyl ($C_{14}H_{18}N_4O_3$) on growth and yield of tomato crop cultivated in vegetated western Himalayan agro ecosystems

Treatment	Days	Plant ht. (cm)	Branch/Plant	Fruit/plant*	100 dry seeds (g)**
Fungal consortium	50	13.00 ± 0.00	4.00 ± 0.00	-	-
	70	20.00 ± 0.00	6.00 ± 0.00	-	-
	100	32.00 ± 0.00	9.00 ± 0.00	15 ± 0.0	-
	120	33.00 ± 0.00	9.00 ± 0.00	20 ± 0.0	0.80 ± 0.0
Chemical (Benomyl)	50	12.00 ± 0.00	3.00 ± 0.00	-	-
	70	18.00 ± 0.00	5.00 ± 0.00	-	-
	100	25.00 ± 0 00	6.00 ± 0.00	14 ± 0.0	-
	120	32.00 ± 0.00	6.00 ± 0.00	16 ± 0.0	0.79 ± 0.0
Consortium + Benomyl	50	14.00 ± 0.00	5.00 ± 0.00	-	-
	70	21.00 ± 0.00	7.00 ± 0.00	-	-
	100	33.70 ± 0.00	10.00 ± 0.00	16 ± 0.0	-
	120	34.50 ± 0.00	10.00 ± 0.00	22 ± 0.0	0.82 ± 0.0
Pathogen	50	4.00 ± 0.00	0.00 ± 0.00	-	-
	70	7.00 ± 0.00	0.00 ± 0.00	-	-
	100	9.00 ± 0.00	0.00 ± 0.00	-	-
	120	9.00 ± 0.00	0.00 ± 0.00	-	-
Control (without treatment)	50	10.00 ± 0.00	3.00 ± 0.00	-	-
	70	15.00 ± 0.00	5.00 ± 0.00	-	-
	100	24.10 ± 0.00	6.00 ± 0.00	10 ± 0.0	-
	120	28.00 ± 0.00	6.00 ± 0.00	15 ± 0.0	0.60 ± 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 ($P < 0.05$)

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).

Suppression of deleterious microorganisms by the fungicides and their stimulatory effect on the growth and yield of plants has been reported (Trybom and Jeschke, 2008). Nasir (2003) evaluated the effect of five systematic fungicides namely Captan, Vitavax, Dithane M-45, Thiram and Benomyl on the growth of seed-borne fungi of soybean and found that either of these fungicides was able to reduce the growth of *Alternaria alternata, Cladosporium cladosporioides, Macrophomina phaseolina, Drechslera specifera, Fusarium oxysporum* and *Rhizoctonia solani*. Bharath *et al*. (2005) studied the effect of Topsin and Dithane M-45 on the growth and yield of watermelon (*Citrullus lanatus*) and reported that these fungicides suppressed the fungal pathogens and significantly enhanced the growth and yield of watermelon.

Raghavendra *et al.* (2005) investigated the compatibility of phyton (a phytotonic) with Captan and GLSTIN and their effect on the yield of safed musli (*Chlorophytum borivilianum*) and found that GLSTIN either alone or in combination with phyton had the ability to suppress *Fusarium solani* causing tuber rot of safed musli and enhanced the yield of this plant. Azoxystrobin has been reported to enhance the growth and yield of rice (Groth, 2008).

In the present study, antagonistic activity of consortium comprising of *Trichoderma viride*, *Trichoderma asperellum*, *Hypocrea lixii / Trichoderma harzianum*, *Trichoderma atrobrun- neum*, *Penicillium ortum, Hypocera lixii* and *Penicillium oxalicum* 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 as reported earlier (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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