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Effect of soil amendment with plant growth promoting fungi on growth and yield of tomato

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Trichoderma harzianum, T. koningii, T. virens, T. viride and a white sterile fungus (which did not fructify) were isolated from the rhizosphere of tomato. The growth promoting ability of these fungi was tested on tomato plants grown in soil amended with their inocula. All these rhizosphere fungi were found to enhance the growth as well as yield of tomato significantly. This might be attributed to the ability of such fungal genera to produce plant growth promoting substances such as hormones, degrade complex substrates and/or suppress the deleterious soil microorganisms. These fungi may be of agricultural importance for raising yield of tomato.

Key words: Plant Growth Promoting fungi (PGPF), *Trichoderma* spp., tomato growth and yield

INTRODUCTION

Many rhizosphere fungi have been reported to promote the growth and yield of crop plants (Hyakumachi, 1994). Lindsey and Baker (1967) have reported that rhizosphere fungi stimulate shoot growth in plants. *Aspergillus flavus, Penicillium corylophilum, Penicillium cyclopium, Penicillium funiculosum, and Rhizopus stolonifer* have been reported to enhance seed germination and seedling growth of fababean, melochia, sesame and soybean (Hasan, 2002). Egamberdieva (2008) has observed that rhizobacteria isolated from wheat and pea produced indole-3 acetic acid (IAA) which might account for the overall synergistic effect of these microorganisms on growth of the plants. *Burkholderia cepacia* MCI 7 has been reported as a promising plant growth promoting inoculant for maize in the soil infested with a pathogenic strain of *Fusarium moniliforme* (Bevivino *et al.*, 2000). Plant growth promoting effect of *Cladosporium cladosprioides*, *Trichoderma harzianum* and *Trichoderma virens,* isolated from the rhizosphere of *Pisum sativum and Cicer arietinum,* has also been established *in vitro* and *in vivo* (Singh *et al,* 1995). In the researches conducted by Baker (1988), it has been reported that when *T. harzianum* has been added to greenhouse soil or growth medium at a rate higher than 10^5 CFU/g soil, the fungus promotes height, flowering and branching in *Petunia sp.* and *Chrysanthemum* sp. The indigenous soil Plant Growth Promoting Fungi (PGPF) have been reported to suppress pathogenic *Pythium* spp. and stimulate the growth as well as yield of cucumber (Hyakumachi, 1994). The metabolites of *T. harzianum, T. virens* and *T. viride* have been reported to enhance seed germination and increase plumule as well as radicle length of chickpea (Dubey *et al*, 2007). *T. harzianum* (strains T22, T39 and A6) and *T. atroviride* (strain PI) enhance the seed germination as well as seedling growth of tomato, canola and pea (Vinale *et al*, 2008).

MATERIALS AND METHODS

Isolation of fungi from rhizosphere of tomato

Tomato plants were collected at regular growth intervals i.e. seedling, vegetative, flowering and fruiting stages from a field of Agricultural Farm, Banaras Hindu University (BHU), Varanasi (India) and a farmer's field of Karaudi, situated adjacent to the BHU campus, in sterilized polyethylene bags and brought to the laboratory of Microbial Ecology and Plant Pathology, Department of Botany, BHU. 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 in length) of different diameters were cut with sterilized scissors under aseptic condition and twenty five such root pieces for each one were transferred into two separate 250 ml Erlenmeyer flasks (one for healthy and the other for diseased root) 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 was followed for isolation of the rhizosphere fungi. Dilutions of 1:100, 1:1000, and 1:10000 were prepared in separate sterilized conical flasks for the study. 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 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 the fungi thus isolated were preserved on Potato Dextrose Agar slants at 4°C.

Preparation of mass culture of the rhizosphere fungi

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 2% Gypsum (calcium sulphate) and 0.5% chalk powder (calcium carbonate) on dry weight basis. These would help to check the pH of the medium and prevent them from sticking with each other. Clean glucose bottles were filled with 100 g (in each case) of such barley grains and the mouth of such bottles were plugged with non-absorbent cotton, which were then steam sterilized in autoclave at 126.5°C for 1-2 hrs. The bottles were then allowed to cool at room temperature and were inoculated with five agar blocks (5 mm diameter each) cut from the margin of the actively growing culture of each fungus. The bottles were incubated at 25 ± 2°C for ten days. The bottles were shaken once or twice daily for rapid and uniform colonization of the fungi. The 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 the agricultural field, Banaras Hindu University and brought to the laboratory. 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 each rhizosphere fungus, which was prepared on barley grains (method described earlier), was mixed separately with sterilized natural soil at the rate of 1% (w/w). The soil samples so prepared were separately filled in clay pots (15 x 25 cm size) and were kept at room temperature for one week during which each rhizosphere fungus developed and colonized the soil particles. Soil supplemented with barley grains without inoculum 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 H-24 variety of tomato were sown in each pot on eighth day after the soil amendment with the rhizosphere fungi. The experiments were set in replicates of three pots put under greenhouse condition. The observation for the effect of rhizosphere fungi on growth and yield of tomato plants was made in terms of plant height, dry weight of the plant, number of fruits per plant, and weight of 100 dry seeds at 30, 50, 80, and 100 days after sowing (DAS) as described below.

(a) Plant height : 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.

(b) Dry weight of plant : The same plants used for

plant height were oven dried for 48 hrs and average dry weight per plant was calculated.

(c) Number of fruits per plant : Fruits on all the tomato plants when formed and developed under each treatment, were counted and average number of fruits per plant was calculated.

(d) Weight of 100 dry seeds : The seeds separated out 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. Their average weight (in g.) was recorded.

RESULTS AND DISCUSSION

Effect of soil amendment with the PGPF on growth and yield of tomato

Rhizosphere fungi namely *T. harzianum, T. koningii, T. virens, T. viride* and the white sterile fungus were tested *in vivo* for their growth promoting ability. The growth promoting effect of these fungi was investigated on tomato plants grown in soil amended with their inocula. The experiments were conducted in pots kept under greenhouse condition. The growth and yield of tomato was recorded in terms of plant height, dry weight of the plant, number of fruits/plant and weight of 100 dry seeds. Observations were made at 30, 50, 80 and 110 days after sowing (DAS). All these fungi showed stimulatory effect on growth and yield of tomato.

Maximum height of tomato plants was recorded with *T. virens* treatment at 10 DAS (10.23 cm). This was followed by *T. harzianum* (10.5 cm), *T. koningii* (9.36 cm), the white sterile fungus (9.1 cm) and *T.* viride (9.06 cm) treatments which was significantly higher than the control (6.7 cm). The plant height, when recorded at 50 DAS, was found to have increased approximately 1.5 times in almost all the treatments, maximum being in case of *T. virens* treatment (16.13 cm)

Interestingly, at 80 DAS, a two-fold increase in plant height was recorded in almost all the treatments, which clearly indicated that the plant height increased at a faster rate between 50 and 80 DAS. Maximum plant height was recorded in case of *T. harzianum* treatment at 80 DAS (30.9 cm). (Fig.2 and 3)

When final observation for plant height was made at 110 DAS, maximum plant height was recorded in case of *T. harzianum* treatment (40.06 cm). This was followed by the treatment with *T. virens* (39.06 cm), *T. viride* (36.23 cm), *T. koningii* (34.86cm) and the white sterile fungus (34.1 cm) (Fig.4)

Dry weight of the plants increased gradually when recorded at 30, 50, 80 and 110 DAS. At 30 DAS, maximum dry weight of tomato plant was recorded in case of *T. harzianum* and *T. virens* treatments (0.96 g). This was followed by *T. koningii* (0.68 g), the white sterile fungus (0.67 g) and T. *viride* (0.66 g) treatments as compared to control (0.54g). Dry weight of the plant was found to have increased approximately 2 times in almost all the treatments at 50 DAS, maximum being in case of white sterile fungus treatment (1.25g). Almost a two-fold increase was recorded in dry weight of the plant between 30 to 50 DAS. During 50 to 80 DAS, the dry weight of the plant increased gradually. At 80 DAS, maximum dry weight was observed in *T. virens* treatment (1.77 g). At 110 DAS, maximum dry weight of the plant was recorded in *T. koningii* treatment (1.97 g). At this stage (110 DAS), this was followed by *T. virens* (1.92g), *T. harzianum* (1.89 g), *T. viride* (1.85g), the white sterile fungus (1.75 g) and control (1.50 g) (Table 1b)

Number of fruits per plant was recorded only at 80 and 110 DAS. At 80 DAS, maximum number of fruits per plant was recorded in case of *T. harzianum* treatment (16). This was followed by the treatments of *T. virens* (15), *T. koningii* (12), *T. viride* (10), the white sterile fungus (4) (Table 1c)

In control it was only one. At 110 DAS, the maximum number of fruits per plant was recorded in *T. virens* treatment (26) (Fig.5).This was followed by the treatments with *T. harzianum* (20), *T. koningii* (18) (Fig.4), *T. viride* (13) (Fig.6) and the white sterile fungus (8) (Fig.7). In control the number of fruits per plant was only 4. Weight of 100 dry seeds was recorded to be maximum (0.91 g) at 110 DAS in *T. harzianum* treatment. This was followed by the treatments with *T. koningii (0.85 g),* T. virens as well as *T. viride* (0.78 g) and the white sterile fungus

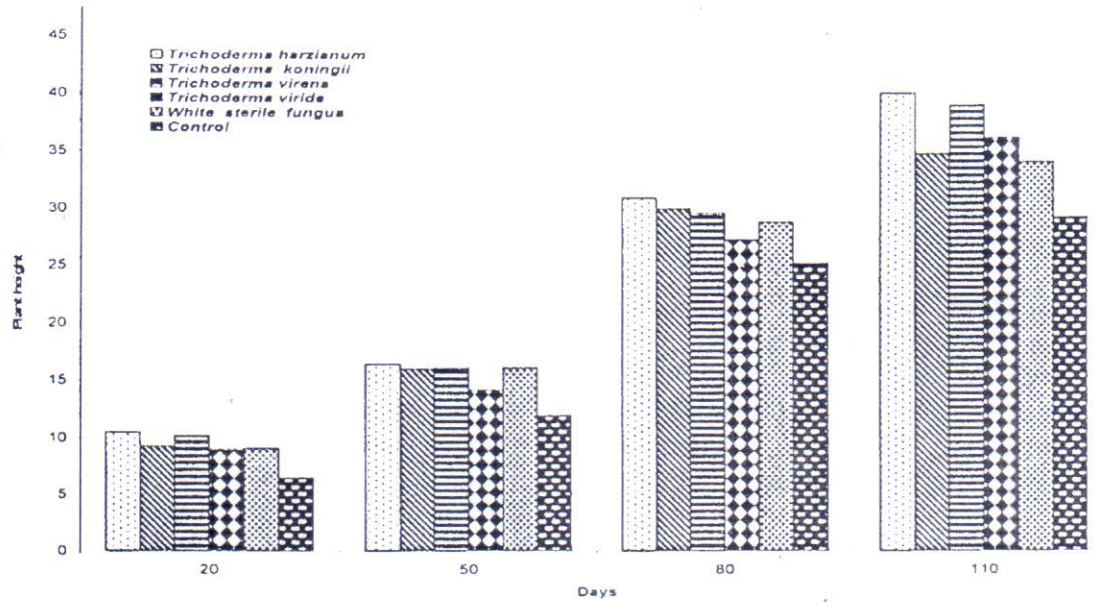

Fig. 1 (a): Effect of soil amendment with the inoculum of the potent PGPF on the height of tomato plants (In pots)

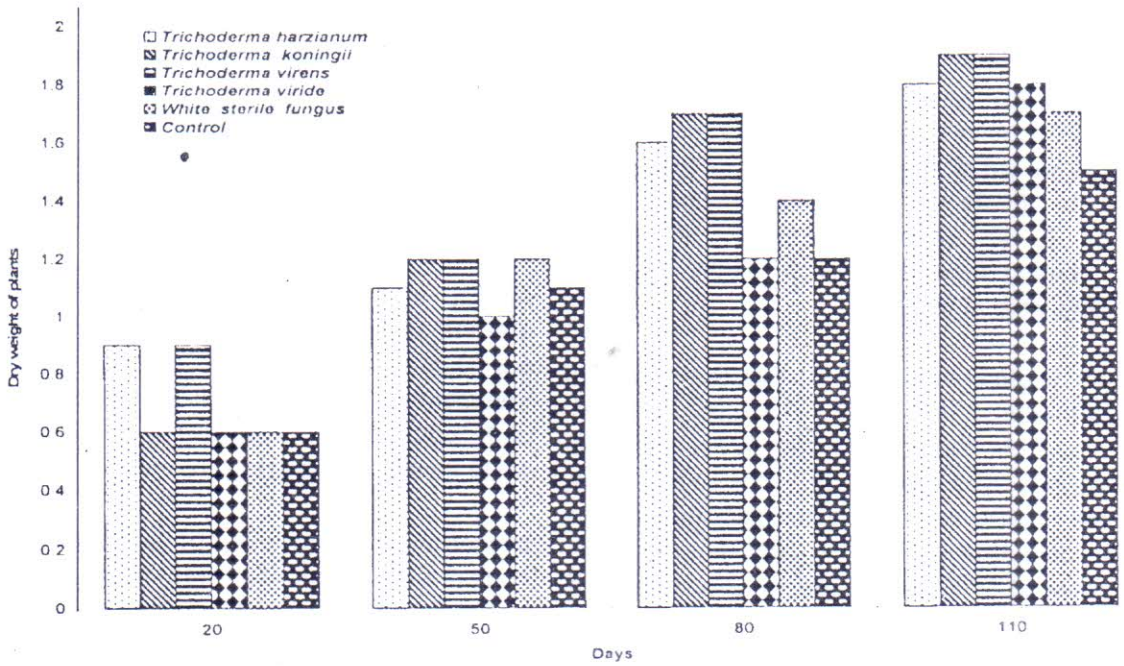

Fig. 1 (b): Effect of soil amendment with the inoculum of the potent PGPF on the dry weight of tomato plants (in pots)

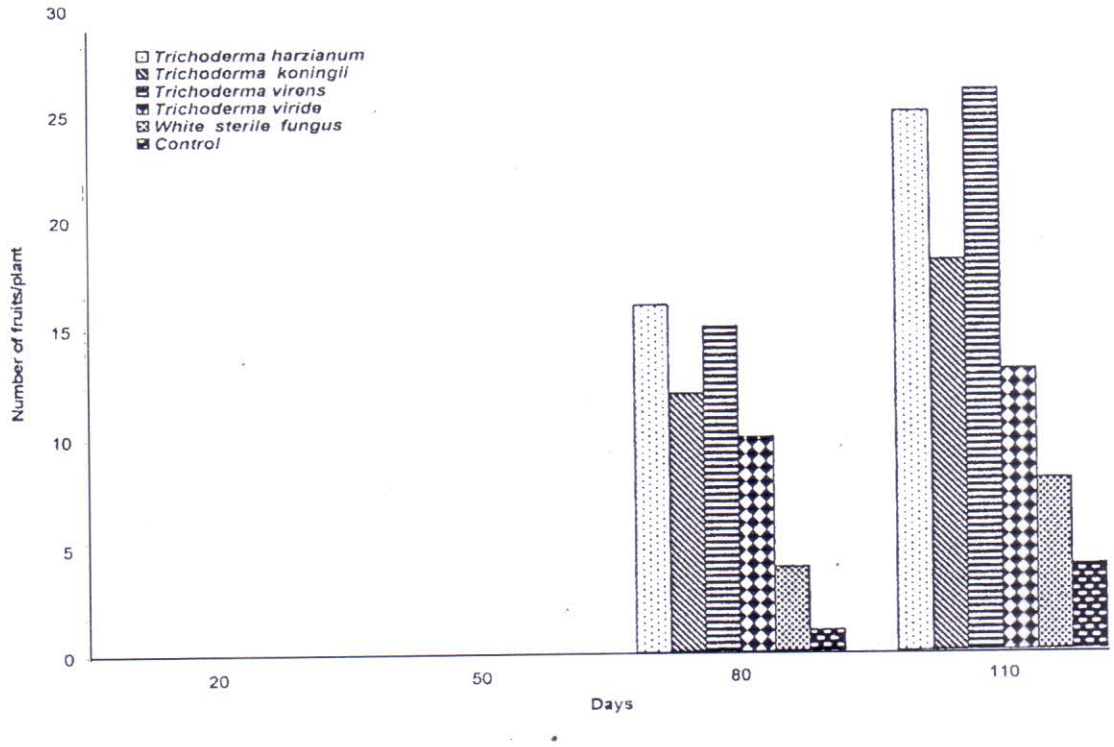

Fig. 1 (c): Effect of soil amendment with the inoculum of the potent PGPF on fruiting of tomato plants (in pots)

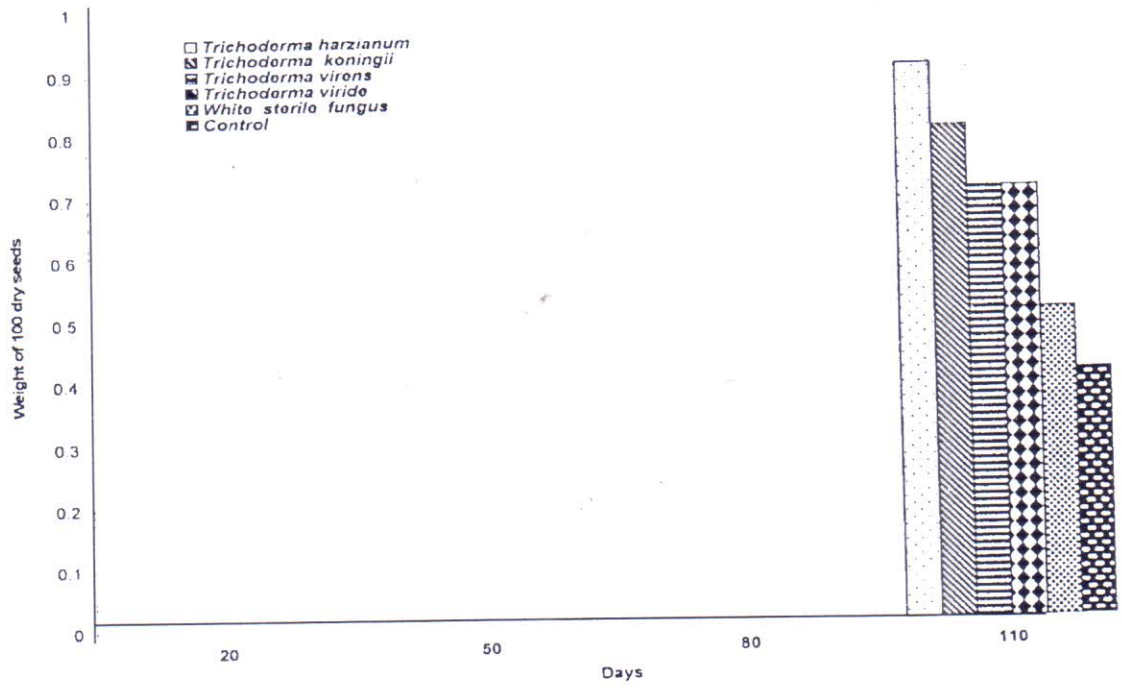

Fig. 1(d): Effect of soil amendment with the inoculum of the potent PGPF on the weight of dry seeds of tomato (in pots)

Fig. 2 : Control (for all the following photo-plates) in pots

Fig. 3 : Effect of soil amendment with the inoculum of *T. harzianum* on growth and yield of tomato

(0.59 g). In control the dry weight of 100 seeds was only 0.40 g. (Table 1d)

Plant growth promotion by the rhizosphere fungi has been reported by some earlier workers also (Hyakumachi, 1994; Al-Azouni, 2008). The plant growth promotion by the rhizosphere fungi may be because of the production of growth promoting substances such as hormones, complex substrate degradation and/or suppression of deleterious microorganisms by these fungi.

Unyayar *et al.* (2000) reported the production of auxin and abscisic acid by the fungus *Phanerochaete chrysosporium* ME446 immobilized on polyurethane foam. *Aspergillus niger, A. flavus, Penicillium corylophilum, P. cyclopium, P. funiculosum* and *Rhizopus stolonifer* have been reported to produce gibberellin (Hasan, 2002). Cristescu *et al.* (2002) studied ethylene production by phytopathogenic fungus *Botrytis cinerea* causing post harvest rot of perishable plant products including tomato and found that this fungus has the ability to produce ethylene *in vitro*. Ethylene (C_2H_2) is known as fruit ripening hormone in higher plants. Ankit *et al.* (2008) observed auxin-like activity of the extract from hypertrophied tissue of *Acacia eburnea* infected with the rust pathogen *Ravenelia esculenta* and reported that the hypertrophy of the host plant tissue might be due to indole-3-acetic acid (IAA) produced by this pathogen.

Plant Growth Promoting Fungi (PGPF) have been reported to mineralize the organic substrates and may, therefore, provide the plants with necessary mineral nutrients in an easily assimilating form (Hyakumachi, 2000a,b). Altmore *et al.* (1999) investigated the capability of *Trichoderma harzianum* Rifai 1295-22 (T-22) to solubilize some insoluble or sparingly soluble minerals *in vitro*.and reported that T-22 was 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.*

Fig. 4 : Effect of soil amendment with the inoculum of *T. koningii* on growth and yield of tomato

Fig. 5 : Effect of soil amendment with the inoculum of *T. virens* on growth and yield of tomato

Fig. 6 : Effect of soil amendment with the inoculum of *T. viride* on growth and yield of tomato

Fig. 7 : Effect of soil amendment with the inoculum of the White sterile fungus on growth and yield of tomato

harzianum might be responsible for its plant growth promoting ability. Kang *et al.* (2002) reported the ability of *Fomitopsis* to solubilize tri-calcium phosphate. Gibson and Mitchell (2004) studied the nutritional influences on solubilization of metal phosphates by ericoid mycorrhizal fungi and found that *Hymenoscyphus ericae* (an ericoid mycorrhizal mycobiont) has the ability to solubilize zinc and calcium phosphates ($Ca_3(PO_4)_2$ on solid agar plates. Richa *et al.* (2007) tested the efficacy of *Aspergillus tubingensis* and *A. niger* to solubilize rock phosphate and found that both these fungi have the ability to solubilize rock phosphate and also enhanced the growth and yield of maize in rock phosphate amended soil. El-Azouni (2008) tested the efficacy of *Aspergillus niger* and *Penicillium italicum* to solubilize tri-calcium phosphate (TCP) in vitro as well as their effect on the growth of soybean (Glycine max) *in vivo* and reported that both these fungi showed high TCP solubilising ability on agar plates and their dual inoculation in pot experiments significantly increased the yield and dry matter of soybean plants.

Hyakumachi (1994) observed plant growth promotion effect in cucumber which was due to the suppression of *Pythium* spp. by PGPF which were indigenous in the soil. Elad (2000) studied the biological control of foliar pathogens of cucumber by means of *T. harzianum* and found that 4 foliar pathogens namely *Botrytis cinerea, Pseuperonospora cubensis, Sclerotinia sclerotiorum* and *Sphaerotheca fusca* causing grey mould, downy mildew, white mould and powdery mildew diseases of cucumber, respectively, were suppressed by *T. harzianum* under greenhouse conditions. 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* were found to be suppressed, by the mycelial growth of *Fusarium oxysporum* f. sp. *ciceris* and enhanced the growth and yield of this crop plant (Dubey *et al*, 2007).

In the present investigation, a significant increase in the growth and yield of tomato with *T. harzianum* and *T. virens* in comparison to other rhizosphere fungi might be due to the compatibility of these fungi to their surrounding environment. The capacity of rhizosphere PGPF to colonize and inhabit certain components of root zone suggests that they have great potential for beneficially altering the composition of the rhizosphere leading to improved plant health.

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