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Full Length Research Paper

Effect of single application of *Trichoderma viride* and *Pseudomonas fluorescens* on growth promotion in cotton plants

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The most important and economically cultivated cotton plant was selected to test the growth promotion by *Trichoderma viride* and *Pseudomonas fluroescens* with and without pathogens, *Rhizoctonia solani* and *Macrophomina phaseolina*. Of these, *T. viride* was found to be more effective than *P. fluroescens* on shoot and root length elongation. Seed germination percentage, root length, shoot length, fresh weight, dry weight and vigour index were significantly increased by *T. viride* and *P. fluroescens* . *T. viride* inoculated cotton plants increased 2.7 and 2.4 fold, where as *P. fluorescens* increased 2 and 1.8 fold for shoot and root length respectively. Pre-treated cotton seeds by *T. viride* showed 4 and 3.1 fold and *P. fluorescens* showed 3.1 and 2.8 fold shoot and root length elongation respectively when compared with the control.

Key words: *Trichoderma viride*, *Pseudomonas fluorescens*, cotton plant, plant growth promotion.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is the third largest important economic crop in India produced for cloth and other kind of things of human need serving many other important uses (Hutchinson et al., 1947). The plant is tropical in nature and it grows best in warm temperatures (Table 1). Recently, efficient and exploitive agriculture throughout the world is practiced at great cost to the environment. After decades of warning, the inappropriate usage of pesticides has led to development of more than 500 resistant pathogens (Georghiou, 1990). The increased pressure from public and environmental scientists, on the ill effects of chemical pesticides led to the genesis of bio-control agents (Nakkeeran et al., 2005).

Some bacteria and fungi prevent diseases and enhance plant growth. Beneficial free-living soil bacteria that increase plant growth are generally referred to as plant growth-promoting bacteria and are found in association with the roots of various plants (Kloepper et al., 1991; Sajjad et al., 2001; Shanmugaiah 2005, 2007). Beneficial microbes associate with plants in several ways. Some may inhabit the rhizosphere, taking advantage of root exudates; others may live on root or leaf surfaces and some may colonize intracellular spaces and vascular tissues inside the plant (Preston, 2004). Plant- associated microbial diversity encompasses symbionts, protecting their host against various aggressions and to determine the ecological success of plants. They drastically modify plant communities and to improve the inventory of diversity and functions of *in situ* plant-associated microorganisms (Selosse et al., 2004). Despite, entophytes, which colonize and reside in internal plant habitats, they were proved effective in plant growth promotion and disease control in a wide range of crops (Manjula et al., 2002).

Trichoderma species are effective in the control of soil/seed borne fungal diseases in several crop plants (Kubicek et al., 2001). *Trichoderma* sp. and other beneficial root-colonizing fungi also enhance plant growth and

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Table 1. Cotton cultivation of different verities in different zones of India

Zones	Varieties	States
North	Hirsutum and Arboreum	Punjab, Haryana, northern Rajasthan and part of Uttar Pradesh
Central	Hirsutum, Arboreum, Herbaceum and Hybrid Zones	Gujarat, Madhya Pradesh and Maharashtra.
South	Hirsutum, Arboreum, Herbaceum, Barbadense and Hybrid Zones	Andhra Pradesh, Tamil Nadu and Karnataka

Source: Choudhary and Laroia (2001).

productivity (Balasubramanian, 2003). However, many resistance-inducing fungi and bacteria increase both shoot and root growth, some non-pathogenic root-colonizing fungi also have similar effect (Harman et al., 2004). The increased growth response induced by *Trichoderma* sp. has been reported for many crops such as beans (*Phaseolus vulgaris*) cucumber (*Cucumis sativus*), pepper (*Capsicum annum*), carnation (*Dianthus carophyllus*), maize (*Zea mays*), and wheat (*Tritichum aestivum*) (Lo and Lin, 2002). *Pseudomonas* species are effective root colonizers and biocontrol agents, by their production of antibiotics and other antifungal metabolites including antibiotics, hydrogen cyanide and siderophores (O'Sullivan and O'Gara, 1992). Increased dry weight and plant height were recorded with *Pseudomonas* sp.MML2212 and *Pseudomonas fluorescens* on rice and green gram when compared with the control (Mathivanan et al., 2005, Shanmugaiah et al., 2005, 2008). Most of the strains of plant growth promoting rhizobacteria are from *Pseudomonas* sp. particularly *P. fluorescens* strains. In recent years, more emphasis has been laid on the combined use of biocontrol agents with plant growth promotion.

Plant-associated microorganisms fulfil important functions for plant growth and health (Gabriele Berg, 2009) such as enhancement of plant growth and protection of plants from various plant pathogens in several crops such as cucumber, radish, tomato, sugar cane, and rice as reported by Viswanathan and Samiyappan (1999), Ongena et al. (2000) and Ramamoorthy et al. (2001). In this study, the effect of talc powder formulation with *P. fluorescens* and *T. viride* applications alone on cotton growth was investigated. The aim of the present study was to evaluate plant growth promotion using *P. fluorescens* and *T. viride* for their ability to promote growth of cotton plants.

MATERIALS AND METHODS

Isolation and maintenance of the pathogens

The present study was carried out with *Rhizoctonia solani* and *Macrophomina phaseolina* isolated from the infected roots of cotton, collected from Tamil Nadu Agriculture College, Madurai, Tamil Nadu, India. 20 ml of sterilized and warmed PDA media were poured into sterilized Petri plates and allowed to solidify. The fungal cultures were inoculated at the centre of the Petri plates by placing a 9 mm disc of 5 days old PDA culture. The plates were incubated at room temperature (28 ± 2°C) for four days.

Multiplication of the pathogens

The inoculums of *R. solani* and *M. phaseolina* were developed by inoculating the pathogens on a sand-maize medium (sand and ground maize grains mixed at the ratio 19:1 and sterilized in polythene bags at 121°C for 30 min and incubated at 28 ± 2°C for 20 days. The pathogens were thoroughly mixed at regular intervals.

Mass production of *T. viride* and *P. fluorescens*

T. viride was obtained from the Botany Department of V. H. N. S. N. College, Virudhunagar, Tamil Nadu, India. Molasses-yeast medium was prepared in a conical flask and sterilized. *T. viride* culture was inoculated by taking a fungal disc from a 10 day old culture and incubated for 10 days. This culture was served as the mother culture. 190 g of soil was taken in a polythene bag with 10 g of corn powder and sterilized. The moisture content of the soil and corn was set at 50%. Fungal discs (5) were collected from the mother culture plate and they were inoculated into the polythene bags containing the growth medium. The culture was incubated at room temperature (28 ± 2°C) for 20 days. After 20 days, the mixture was air dried and they were used for the experiment. *P. fluorescens* was obtained from T. Stans and company Hd, Coimbatore, Tamil Nadu, India. *P. fluorescens* was grown in a nutrient broth and was used for the seed treatment experiments.

Effect of *R. solani, M. phaseolina, T. viride* and *P. fluorescens* on cotton

Cotton seeds were surface sterilized with 1% sodium hypochlorite solution for 20 min and then the seeds were washed in sterile distilled water. Ten seeds per pot containing 25% sand: 75% red soil (5 L pot) was sown and three replicates were maintained for each treatment. Pathogen inoculated and un-inoculated controls were maintained in separate pots in the green house with the temperature ranging from 28 to 32°C and the plants were irrigated every two days. The soil moisture content was maintained at about 70%. After 10 days of growth, germination percentage, root length, shoot length, fresh weight and dry weight were measured and vigour index was calculated. The control pot set without the inoculation of neither pathogens nor antagonists was maintained. 100 g of fungal pathogens such as *R. solani* and *M. phaseolina* per pot and 100 g of fungal antagonist *T. viride* were used in this experiment. 10 g of *P. fluorescens* with talc powder was used per pot in this experiment. The following treatment was carried out for cotton plants:

For *R. solani*: Control, T1; *R. solani*, T2; *T. viride*, T3; *P.*

Table 2. Effect of *R. solani P. fluorescens* and *T. viride* on the growth of cotton

Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	Vigour index
Control	80 ± 1.07*	5.23 ± 2.76**	8 .98 ± 2.08**	3.180 ± 2.98**	0.320 ± 2.12**	1136.8 ± 2.85**
T1	30 ± 1.32**	5.05 ± 1.98**	6.86 ± 2.12**	2.950 ± 1.58**	0.220 ± 2.85**	357.3 ± 2.56**
T2	100 ± 0.0*	12.42 ± 2.36**	20.28 ± 1.83**	11.750 ± 2.30**	3.867 ±1.67**	3270 ± 1.98**
T3	90 ± 2.34**	10.83 ± 0.94*	15.79 ± 2.35**	9.520 ± 1.98**	2.647 ± 2.21**	2395.8 ± 2.32**
T4	80 ± 1.89**	12.02 ± 1.12**	17.87 ± 1.09*	10.050 ± 0.67*	3.184 ± 1.75**	2391.2 ± 1.15**
T5	76 ± 0.98*	10.33 ± 1.89*	12.94 ± 2.25**	8.810 ± 1.12**	2.160 ± 2.52**	1861.6 ± 1.35**

Control: Untreated, T1: *R. solani* treated, T2: *T. viride* treated, T3: *P. fluorescens* treated, T4: *R. solani* with *T. viride* treated, T5: *R. solani* with *P. fluorescens* treated. Values are mean of three replicates with ± standard deviation at $P < 0.05$ and $P < 0.01$ respectively.

fluorescens; T4; *R. solani* + *T. viride*, T5; *R. solani* + *P. Flurorescens.*
For *M. phaseolina*: Control, T1; *M. Phaseolina*, T2; *T. viride*, T3; *P. Fluorescens*; T4; *M. Phaseolina* + *T. viride,* T5; *M. phaseolina* + *P. flurorescens.*

Effect of *T. viride* and *P. fluorescens* on cotton seeds

The conidia of *T. viride* (4×10^6) were collected by sterile distilled water. Cotton seeds were separately soaked with *T. viride* conidia for 2 h and the seeds soaked in distilled water were used as control. Cotton seeds were soaked in a liquid culture of *P. fluorescens* (1×10^8) for 2 h and then these seeds were used for the experiment. The following treatments were carried out for this experiment.

Untreated control, T1; Treated control (*P. fluorescens* separately) T3; *P. fluorescens* + *R. solani* and T4; *P. fluorescens* + *M. phaseolina*.
Untreated Control, T1; Treated control (*T. viride* separately) T3; *T. viride* + *R. solani* and T4; *T. viride* + *M. Phaseolina*.

Measurement of shoot and root length of cotton

The shoot and root length of cotton plants in different treatments were measured 30 days after treatment with standard scaling method.

Fresh, dry weight and vigour index of cotton

Seed growth from the date of sowing the plants (treated and control) were washed thoroughly in running tap water after 30 days. The fresh weights of cotton plants were measured. Uprooted plants were placed in an oven at 60°C for 24 h. Then the dry weights of cotton plants were measured.
Normal seedlings were evaluated for vigour index. The root and shoot lengths of the normal seedlings were measured and vigour index was calculated using the formula by Abdul Baki and Anderson (1973).

VI = (root length + shoot length) × percentage of germination

RESULTS AND DISCUSSION

Effect of *R. solani, T. viride* and *P. fluorescens* on the growth of cotton

The shoot and root length, fresh and dry weight of plants grown in pathogen with antagonist were 12.0 and 17.8 cm, 10.0 and 3.18 g, 2391.2 and 10.3 and 12.9cm, 8.81 and 2.16 g, 1861.6 for T4 and T5 respectively, with plants grown in pathogen alone-inoculated soil. Root length of cotton is known to increase up to the flowering stage (80 – 90 days-old plant), however, no significant increase in root length was observed after this growth stage (Nayakekorala and Taylor, 1990). The root length in cotton is low when compared to other crop species, which leads to a low exploitation of soil nutrients (Brouder and Cassman, 1990). Combination of the pathogen and antagonist was superior to the pathogen alone treatment recorded in cotton. These findings were in accordance with dual inoculation of *Bacillus* sp. and *Glomus manihotis* which significantly improved banana growth parameters (Rodriguez-Romero et al., 2005) and *P. fluorescens* and *Glomus mosseae* BEG 12 increased mycorrhizal colonization of tomato root and improved plant growth (Gamalero et al., 2004).

Effect of *M. phaseolina T. viride* and *P. fluorescens* on the growth of cotton

When *M. phaseolina* was present in the soil, it reduced the germination of cotton seeds up to 40%. There was a reduction of about 40% in the growth characters of the plants in control and T1. In T2 and T3 treatments, there was 20% increased shoot and root length, fresh and dry weight and vigour index of the plant, when compared to control and T1 (Table 3). Significant level of germination was observed in all the other treatments. The reason for the increase in growth parameters and vigour index may

Table 3. Effect of *M. phaseolina, P. fluorescens* and *T. viride* on the growth of cotton.

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	Vigour index
Control	80 ± 1.90**	5.23 ± 2.35**	8 .98 ± 1.98**	3.180 ± 1.00*	0.320 ± 0.96*	1136.8 ± 1.05*
T1	40 ± 1.25**	6.28 ± 2.21**	7.68 ± 1.75**	3.150 ± 1.90**	0.420 ± 1.10**	558.4 ± 1.88**
T2	100 ± 0.0*	15.82 ± 1.96**	23.40 ± 2.31**	12.75 ± 2.05**	4.780 ± 1.43**	3922 ± 1.73**
T3	80 ± 1.53**	10.90 ± 2.02**	14.49 ± 1.66**	7.880 ± 1.95**	2.436 ± 1.95**	2031.2 ± 2.02**
T4	100 ± 0.85*	12.22 ± 1.98**	18.95 ± 2.32**	11.150 ± 2.54**	3.526 ± 1.57**	3117 ± 2.41**
T5	80 ± 1.12**	11.93 ± 1.65**	14.28 ± 1.36**	9.120 ± 2.66**	1.860 ± 2.08**	2096.8 ± 2.53**

Control: Untreated, T1: *M. phaseolina* treated, T2: *T. viride* treated, T3: *P. fluorescens* treated, T4: *M. phaseolina* with *T. viride* treated, T5: *M. phaseolina* with *P. fluorescens* treated. Values are mean of three replicates with ± standard deviation at *P < 0.05 and **P < 0.01 respectively.

Table 4. Effect of *Pseudomonas fluorescens* with pathogens on the growth of cotton pre-treated seeds**.**

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	Vigour index
Control	80 ± 1.188**	4.30 ± 2.20**	7 .90 ± 1.18**	1.680 ± 2.15**	0.120 ± 1.98**	976 ± 2.08**
T1	100 ± 1.86**	13.65 ± 1.98**	22.76 ± 2.15**	12.995 ± 2.08**	4.008 ± 1.82**	3641 ± 1.96**
T2	70 ± 2.25**	10.72 ± 2.13**	14.57 ± 1.87**	8.864 ± 1.87**	2.290 ± 2.30**	1770.3 ± 2.16**
T3	80 ± 1.86**	11.93 ± 1.86**	15.88 ± 1.90**	7.225 ± 1.66**	2.030 ± 2.18**	2224.8 ± 1.34**

Control: Untreated, T1: bacteria treated, T2: *R. solani* with *P. fluorescens* treated, T3: *M. phaseolina* with *P. fluorescens* treated. Values are mean of three replicates with ± standard deviation at *P < 0.05 and **P < 0.01 respectively.

be due to certain plant growth hormones and secondary metabolites produced by *P. fluorescens* and *Trichoderma harzianum* which are known to have increased growth rate as reported by Lynch and Hobbie (1991). A similar type of higher field emergence was also observed in field conditions using different cultivars of cotton (Kimura et al., 1992).

Effect of pre-treated seeds with *P. fluorescens* on the growth of cotton

Three inoculated strains of *P. fluorescens* produced several plant growth promoting phyto-hormones, including indole-3-acetic acid (Jeon et al., 2003). The production of plant growth regulators by the microorganisms is another important mechanism often associated with growth stimulation (Vessey, 2003). In the present study, cotton seeds were pre-treated with *P. fluroescens* (T1); there was an increased 20% in germination over the control. Similarly, morphological characters also increased over the control, when compared to T2 and T3 and there was a 10 to 20% increase because bacteria was inoculated in the soil (Table 4). Cotton seeds were pre-treated with *P. fluroescens*; T2 treatment showed marginal loss over the untreated control, but root length showed remarkable increase than control. Furthermore, with pathogen inoculated in the soil, only 20 to 30% loss was observed in germination as against the control. Pathogenecity might cause little damage and this was also recorded in the case of root and shoot length, fresh and dry weight and vigour index in T2 and T3 (Table 4). Five bacteria (*P. fluorescens*, *P. fluorescens* subgroup G strain 2, *Pseudomonas marginalis*, *Pseudomonas putida* subgroup B strain 1 and *Pseudomonas syringaestrain* 1) were evaluated to determine their promoting effect on the growth of mature healthy tomato plants grown under hydroponic conditions (Gravel et al., 2007).

Effect of pre-treated seeds with *T. viride* on the growth of cotton

Cotton seeds pre-treated with *T. viride* (T1) showed 100% germination. The root and shoot length, fresh and dry weight and vigour index also showed significant improvement compared to control (Table 5). IAA production plays a crucial role in plant growth promotion. The increased dry matter production was correlated with the increased production of IAA (Indole acetic acid) MG6, UV10 and MNT7 and it was also found that *Trichoderma harzianum* stimulated plant growth, including floricultural and horticultural plants (Baker et al., 1984). A mixture of three different plant growth-promoting rhizobacteria applied as seed treatment, showed intensive plant growth promotion (Raupach and Kloepper, 1998). These responses have occurred with higher population densities, applied as conidial suspension or as mycelium and spore in a peat bran medium. Increased growth by *Trichoderma* sp. was also induced by a diffusible growth-regulating factor (Windham et al., 1986). Similarly, *T. harzianum* and *T. viride* and their fusants enhanced rice and tomato

Table 5. Effect of *Trichoderma viride* with pathogens on the growth of cotton pre-treated seeds**.**

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Fresh weight(g)	Dry weight (g)	Vigour index
Control	80 ± 1.93**	4.30 ± 2.32**	7 .90 ± 2.35**	1.680 ± 1.55**	0.120 ± 2.21**	976 ± 2.21**
T1	100 ± 0.0*	16.87 ± 1.65**	24.96 ± 1.95**	15.128 ± 1.97**	4.102 ± 1.65**	4183 ± 1.65**
T2	90 ± 1.73**	14.96 ± 1.08*	19.10 ± 1.65**	10.275 ± 2.05**	3.126 ± 1.92**	3065.4 ± 1.72*
T3	100 ± 0.0*	15.90 ± 1.23**	21.20 ± 2.06**	9.960 ± 1.85**	3.880 ± 2.05**	3710 ± 1.07*

Control: Untreated, T1: *T. viride* treated, T2: *R. solani* with *T. viride* treated, T3: *M. phaseolina* with *T. viride* treated. Values are mean of three replicates with ± standard deviation at *P < 0.05 and **P < 0.01 respectively.

shoot and root lengths (Balasubramanian, 2003) .

Increased effect of pathogens on the growth of cotton pre-treated with *T. viride* remarkably increased over the control. In T2, when *R. solani* was present in the treatment, only 10% reduction was recorded. Similarly in T3 where *M. phaseolina* was present in the treatment, it showed 100% germination (Table 5). When seeds were pre-treated with *T. viride,* seed germination was observed over the control and similar results were also observed in morphological characters. In *R. solani* with *T. viride* inoculated soil only, 90% germination was observed, but 100% germination of seeds was observed in *M. phaseolina* with *T. viride* inoculated soil (Table 5).

The introduction of *Trichoderma* strains with or without pathogens did not affect existing beneficial populations. The effect of *T. viride* in the presence of *M. Phaseolina* and *R. solani* was significant than the *P. fluorescens* strain which resulted in an increased plant biomass because bacteria and fungal population also increased (data not shown). This might be as a result of slightly deleterious effect of this strain causing increased root leakage/damage, which allows a greater population of aggressive rhizosphere and root colonizers such as *P. fluorescens* and *T. viride.* It could be concluded that a clear demonstration has showed promotion in cotton growth, however, the plant growth promotion would be confirmed by field experiments.

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