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Evaluation of arbuscular mycorrhizal fungus, fluorescent *Pseudomonas* and *Trichoderma harzianum* formulation against *Fusarium oxysporum* f. sp. *lycopersici* for the management of tomato wilt

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ABSTRACT

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Synder and H.N. Hans is the major limiting factor in the production of tomato. An effort was made to develop an eco-friendly approach to control *Fusarium* wilt in tomato using fluorescent *Pseudomonas*, *Trichoderma harzianum* and *Glomus intraradices*, an arbuscular mycorrhizal fungus (AMF). Besides direct interaction with plant pathogens, bioagents have been reported to induce systemic resistance in plants. In the present study, a large number of *Trichoderma* sp. and pseudomonad isolates were evaluated for their efficacy to control *Fusarium* wilt of tomato. *T. harzianum* was multiplied on six different substrates out of which Jhangora, an undertilized grain crop, proved to be the superior substrate. Application of *T. harzianum* and fluorescent *Pseudomonas* by seed bio-priming significantly increased seed germination (22–48%) and reduced the days required for germination (2.0–2.5 days). All bioagents used in this study significantly reduced the incidence of wilt in pot and field trials and combinations of bioagents were more effective than single isolate treatments. The combination of fluorescent *Pseudomonas*, *T. harzianum* and AMF provided significantly better control than uninoculated treatment, reducing disease incidence and severity by 74% and 67% in pots and field, respectively. The combination treatments also increased yield by 20%. Addition of cow dung compost (CDC) further reduced disease and improved yield in all treatments. Comparing to control (-CDC), the combination of all three bioagents with CDC significantly reduced disease by 81 and 74% in pots and field, respectively and enhanced the yield by 33%.

1. Introduction

Vegetables are rich sources of nutrients and vitamins, and play a major role in a balanced diet for human beings. India is the second largest producer of vegetables in the world (next to China) and accounts for about 15% of the world's production of vegetables. Tomato is most important because of its use in all kinds of vegetarian diets. The major disease contributing to the loss in the production of this important crop is *Fusarium* wilt, which is caused by pathogenic formae specialis i.e. *lycopersici* of the soil-inhabiting fungus *Fusarium oxysporum* (Sacc.) W.C. Synder and H.N. Hans. Although the use of resistant cultivars against *Fusarium* wilt is a viable option, the occurrence and development of new pathogenic races is a continuous problem (Jarvis, 1998; Jones et al., 1991). Hence, application of fungicides is a normal practice, which may not be very effective since the disease appears late in the crop growth and the persistence of fungicides throughout the crop growth is always doubtful. Thus, biological control has potential for the management of this disease. A variety of soil microorganisms have demonstrated antagonistic activity in the control of various soil-borne plant pathogens, including *Fusarium* wilt pathogen. *Fusarium* wilt-suppressive soils are known to occur in many regions of the world and suppression has generally been shown to be biological in origin. Antagonists recovered from *Fusarium* wilt-suppressive soils have been used to reduce *Fusarium* wilt diseases of several different crops (Paulitz et al., 1987; Postma and Rattink, 1992; Alabouvette et al., 1993; Minuto et al., 1995; Larkin et al., 1996). It has been suggested that microorganisms isolated from the root or rhizosphere of a specific crop may be better adapted to that crop and may provide better control of diseases than organisms originally isolated from other plant species (Lucy et al., 2004). Such plant-associated microorganisms may prove to be better biocontrol agents because they are adapted to the rhizospheric effect of that particular plant. The use of combinations of multiple antagonistic organisms may also provide improved disease control over

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the use of single organisms. Multiple organisms may enhance the level and consistency of control by providing multiple mechanisms of action, a more stable rhizosphere community and effectiveness over a wide range of environmental conditions (Pandey and Maheshwari, 2006). In particular, combinations of fungi and bacteria may provide protection at different times or under different conditions and occupy different or complementary niches. Such combinations may overcome inconsistencies in the performance of individual isolates. Several researchers have observed improved disease control using various biocontrol organisms such as *Trichoderma* sp. (Roberti et al., 1996; Lewis et al., 1998; Adekunle et al., 2001) and *Pseudomonas* sp. (Lemanceau et al., 1992; Lemanceau and Alabouvette, 1993; Leeman et al., 1996; Duijff et al., 1998). Arbuscular mycorrhizal fungi (AMF) have also been reported in combating the soil-borne diseases by inducing plant defense proteins i.e. PR proteins (Somssich and Hahlbrock, 1998; Agrawal et al., 2002; Bart et al., 2002; Pozo et al., 2002; van Loon et al., 2006) and physical barriers (Sharma et al., 1992). AMF is biotrophic in nature, surviving within the root system until crop maturity, and hence may give mechanical strength to plant roots against soil-borne plant pathogens (Sharma et al., 1992). The potential for controlling *Fusarium* wilt, a major threat in tomato in many parts of the country, was evaluated by using *Trichoderma harzianum*, pseudomonads (microorganisms of saprophytic nature) and *Glomus intraradices,* an arbuscular mycorrhizal fungus. Secondly, the process of using these three microorganisms (*T. harzianum*, fluorescent *Pseudomonas* and *Glomus intraradices*) was adjusted in such a way so that the optimum population of bioagents remains in the rhizosphere to combat the disease.

2. Materials and methods

2.1. Isolation and maintenance of pathogens and biocontrol agents

Wilt-infected plants of tomato were collected from disease-prone areas of district Bareilly (Altitude 550 ft AMSL, 28.22N and 79.27E). Isolation of *Fusarium* sp. was made from the infected plants on Komada's *Fusarium*-selective medium (Komada, 1975). The isolated *Fusarium* sp. were preserved at 4 °C in potato dextrose agar (PDA) slants, which contained 200 g peeled potato, 20 g dextrose and 20 g agar. Biological control agents i.e., *Trichoderma* sp. and *Pseudomonas* sp. were isolated from a disease-suppressive soil from the same location by dilution plate method on PDA and modified King's B agar medium containing protease peptone—20.0 g/L; K_2HPO_4—4.0 g/L; $MgSO_4$—0.4 g/L; glycerol—8.0 ml/L; agar—20.0 g/L (pH was adjusted to 7.0 ± 0.2 prior to autoclaving), respectively. Plates were incubated at 27 ± 1 °C. The disease-suppressive soil was detected when soil from this area showed very low disease levels of wilt. Further, when the soil from this and neighboring fields was brought to the laboratory and amended with pathogenic *Fusarium* isolate isolated from infected plants as described above, the suppressive soils showed only 10% mortality of plants, whereas the other soils showed ~90% of tomato plants with wilt symptoms Isolates of *Trichoderma* sp. and pseudomonads were maintained on PDA and modified KB agar slants at 4 °C, respectively. The pseudomonad isolates were also stored as glycerol stocks at −20 °C. Arbuscular mycorrhizal fungus (AMF) was isolated from the same soil. The soil was subjected to multiplication of AMF in trap culture using *Vigna mungo*, *Tagetes* sp., maize (*Zea mays* L.) and sorghum (*Sorghum biocolor* L.) for 3 cycles of 60 days each.

2.2. Characterization of pathogens and biocontrol agents

Based on microscopic studies, the pathogen was identified as *Fusarium oxysporum* on the basis of presence, shape and size of macro- and micro-conidia (Booth, 1971). Isolates of *Trichoderma* sp. were identified by conidia and phialide structures as *T. harzianum*. Bacterial isolates recovered on King's B were identified as fluorescent *Pseudomonas* using various biochemical tests: growth on different carbon sources (acetate, pyruvate, succinate, glucose, lactate and L-alanine) and fluorescence under UV light. Isolates of pseudomonads were screened for functional and also disease-suppressing properties such as P-solubilization on Pikovaskya agar, siderophore production (Schwyn and Neilands, 1987) and rhamnolipid production (Siegmund and Wagner, 1991) in three replications. A total of 37 isolates of fluorescent *Pseudomonas* and 45 isolates of *T. harzianum* were recovered. The AMF spores were identified as *G. intraradices*, *Acaulospora scrobiculata*, *Glomus mossaeae* (Schenck and Perez, 1990). On the basis of population density, *G. intraradices* was found to be the dominant species and was further used in this study.

2.3. Standardization of substrate for mass multiplication of *T. harzianum*

Six different substrates in three replications were used for multiplication of *T. harzianum.* The substrates were jhangora (*Echinochloa frumentacea* (Roxb.) Link), mandua (*Eleusine coracana* (L.) Gaertn.), sorghum (*Sorghum bicolor* L.), polygonum (*Polygonum hydropiper* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) straw. One-fourth volume of 250 ml conical flask was filled with substrates pre-soaked in water for 24 h. To each flask with substrate, 75 ml water and 5% jaggery (concentrate of sugarcane juice) was added before autoclaving at 15 lbs and 121 °C for 60 min. After cooling, five discs of bioagent (5 mm) were inoculated in each flask from 6–7 days old culture plate of *T. harzianum* and incubated at 27 ± 1 °C in BOD incubator for 1 week. Number of conidia per gram of substrate was recorded on PDA and presented as log value/g of substrate. The entire experiment was repeated for reproducibility of the results.

2.4. Inoculum production of Trichoderma and fluorescent *Pseudomonas*

Based on the results, jhangora was further used for the multiplication of *T. harzianum* keeping the same procedure as discussed above. Fully colonized grains were air-dried in shade at room temperature for 4–5 h and were powdered in laboratory blender. The prepared inoculum was mixed in talcum powder so as to obtain 10^6 conidia/g of talcum powder. Log phase culture of fluorescent *Pseudomonas* multiplied on KB broth was inoculated in the talcum powder separately so as to maintain 10^9 cfu/g of talcum powder. The inoculated talc with the respective bioagents was kept at 4 °C until needed. For the co-inoculation treatment, talcum powder having bioagents, *T. harzianum* and fluorescent *Pseudomonas* separately as discussed above, was mixed together in equal amounts at the time of treatment.

2.5. Mass multiplication of pathogen and AMF

Isolates of *Fusarium oxysporum* were grown for 7 days on sorghum grains that were pre-soaked and autoclaved 24 h previously. Fully colonized grains were air-dried for 4 h and powdered in laboratory blender, resulting in an inoculum concentration of ~10^5 conidia/g, and mixed into soil at 5% (w/w) to prepare potting mix.

Glomus intraradices (M) was isolated from the trap culture and was mass propagated through monosporal culture using maize (*Zea mays* L.) as a host. It was further produced in bulk using maize as a test plant by repeated sowing and harvesting of the plants after every 60 days for three cycles in sterilized soil:sand (1:1) mixture. Watering was done with deionized water as and when

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required. The inoculum concentration was assessed by MPN counts (Porter, 1979) and the inoculum was found to contain ~50 infectious propagules (IP)/g of soil.

2.6. Pathogenicity test

Isolates of *F. oxysporum* (F) recovered from diseased tomato plants were tested for their pathogenicity on tomato variety Pant Tomato-3. Tomato seedlings after 21 days were transplanted in 2 kg pots filled with steam-sterilized soil mixed with pathogen as described above. Control pots were without any inoculation of pathogen. Five seedlings were transplanted in each pot and replicated three times. To ensure the pathogenicity, the experiment was repeated. Wilted plants were recorded over time for disease occurrence.

Soil used in the present study for pot and field trials was a sandy loam having pH 6.81, EC—0.3 dS/m, organic matter 0.81%, total P—12 kg/ha, Total *N*—95 kg/ha, Zn—0.545 ppm, Mn—23.1 ppm, Fe—16.81 ppm, Cu—1.01 ppm, and S—9.5 ppm.

2.7. Inhibition of F. oxysporum f. sp. lycopersici by antagonists

Pathogenic *F. oxysporum* f. sp. *lycopersici* (FOL) isolate was further used to assess the antagonistic effect of 45 isolates of *T. harzianum* on PDA by dual plate assay by placing 5 mm disc of pathogen (8–10 days old culture) and antagonist (6–7 days old culture) on the two opposite side of one Petriplate. Efficacy of 37 isolates of fluorescent *Pseudomonas* was tested against FOL by dual plate assay on Petriplates containing Kings' B media and PDA (1:1v/v) using bangle method where the bangle (70 mm dia) was dipped for 2 min in the culture of bacterial antagonist 'fluorescent *Pseudomonas* multiplied in KB broth' and placed on the solidified medium (modified KB and PDA 1:1v/v) in a Petriplate. The 5 mm disc of pathogen taken from 8- to 10-day-old culture was kept in the middle of bangle. Control plates had only FOL. Petriplates were sealed with parafilm and incubated at 27 ± 2 °C in BOD incubator for 6 days. Radial growth of FOL was recorded and percent inhibition was calculated. The entire experiment was repeated for the confirmation of results.

The percent growth inhibition was calculated by formula $I = C - T/C \times 100$, where I = percent growth inhibition, C = radial growth of pathogen without antagonist, and T = radial growth of pathogen with antagonist.

2.8. Compatibility test amongst bioagents

Compatibility test between selected *T. harzianum* and fluorescent *Pseudomonas* isolates was performed on modified Kings' B media and PDA (1:1v/v) using bangle method as described above. Growth of *T. harzianum* was recorded after four days. AMF being biotrophic in nature, the compatibility amongst the three bioagents was assessed in a chamber system of dimension 7" × 1.5" × 5.5" (L × W × D) in a glass house. Compatible isolates of *T. harzianum* (T_{35}) and fluorescent *Pseudomonas* (P_{16}) were assayed for its competitiveness with *G. intraradices* in sterilized soil on tomato variety Pant Tomato-3 and maize variety Pragati with four replications. The chamber was divided equally in three compartments using 45 μm mesh (Fig. 1). Pre-germinated seeds of tomato and maize were used for sowing in either side of the chamber. *T. harzianum* and fluorescent *Pseudomonas* were either not applied or applied in the middle chamber either singly or in combination through talcum powder based carrier so as to maintain a conidial count of 10^6 and 10^9 cfu/g of soil, respectively. AMF was inoculated at 50 IP/seed. The inoculum was provided in holes and seeds of maize were sown in these holes. Control plants received washings of AMF inoculum. Percent AMF infection was observed in tomato plants using method proposed by Biermann and Linderman (1981) after staining of roots (Sharma et al., 1988) of 1-month-old plants. The experiment was repeated and average of the two was recorded.

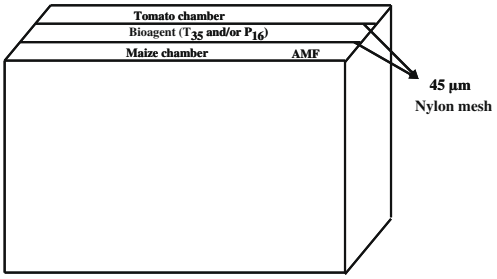

Fig. 1. Sketch of chamber used in this study.

2.9. Seed bio-priming

Tomato seeds variety Pant T-3 were bio-primed with inoculum (T_{35} and P_{16} either singly or in combination) produced using talcum powder as carrier. Ten gram of formulation of the bioagents in talc along with 0.1 g gum arabic used as adhesive was mixed in 25 ml of water. Tomato seeds were soaked in this slurry for 24 h at room temperature and then transferred on moist filter paper in Petriplates. Ten seeds were placed in each plate and replicated five times. Control seeds received only talcum powder with adhesive and were processed in the same manner as mentioned with the treatments.

2.10. Assessment of effectiveness of bioagents against wilt

2.10.1. Pot trial

Tomato seedlings were raised using bio-primed seeds (var. Pant Tomato-3) with either T_{35} or P_{16} or in combination or without any inoculation. Control seeds received only the talcum powder with adhesive. Sterilized soil was filled in plastic tray of dimension 60 × 45 × 10 cm (L × W × D). AMF was applied in furrows at 50 IP/seed. The control treatment received only AMF inoculum washing in furrows. Watering was done as and when needed. After 21 days, four seedlings of tomato were transplanted in pots of 2 kg capacity filled with same sterilized soil. An extra dose of T_{35} and P_{16} was given in tomato seedlings by root dipping in formulation discussed above either singly or in combination depending on the treatment. Fully colonized sorghum grains with FOL was mixed in soil at 5% (10^6 conidia/g sorghum powder) in all of the treatments except absolute control before transplanting the seedlings. Pot trial was laid down in two sets, one in which cow dung compost (CDC) was added to the potting mix at 5 g/pot (10t/ha) and other which was not amended with CDC. CDC was obtained from Livestock Research Centre of the University, which is prepared by amending wheat straw in the pits for 3 months to obtain the matured compost. CDC was analyzed for its nutrient content which was: N—0.29%, P_2O_5—0.18%, Cu—2.85 ppm, Zn—14.61 ppm and Fe—420.3 ppm. There were nine treatments: absolute control (without pathogen), FOL, T_{35} + FOL, P_{16} + FOL, M + FOL, T_{35} + P_{16} + FOL, T_{35} + M + FOL, P_{16} + M + FOL and T_{35} + P_{16} + M +FOL, each with four replications. Plant height, disease incidence using 1–5 scale and mycorrhizal infection was recorded after 1 month of transplantation. The entire experiment was repeated and mean data is presented.

2.10.2. Field test

A field naturally infested with the pathogen.and having a history of wilt occurring every year was chosen for the field trials.

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Bio-primed tomato seeds (var. Pant Tomato–3) with T_{35} and P_{16} either alone or in combination, respective to the treatments in field, were placed in furrows made in 1 sq. m area to raise seedlings of tomato. AMF inoculum was provided at 50 IP/seed in furrows. Watering was done as and when it was needed. Twenty-one-day-old seedlings of tomato were transplanted at 30 cm plant to plant and 60 cm row-to-row distance. Tomato seedlings were further inoculated with T_{35} and P_{16} prepared on talc-based carrier as discussed above by root dipping either singly or in combination depending on treatment. Experimental trial was laid out in two sets: with and without CDC, added at 5t/ha in randomized block design. There were eight treatments: control, T_{35}, P_{16}, M, $T_{35} + P_{16}$, $T_{35} + M$, $P_{16} + M$, and $T_{35} + P_{16} + M$ and each treatment was replicated four times. Plot size was 25 sq. m. for each replication. Agronomic parameters taken were plant height and number of branches from 9 sq. m. area of each plot. Measurements were taken every 15 days after transplanting till the harvest of crop, however the data is provided only for two harvests. Wilt incidence was recorded at periodical intervals till maturity of crop and total wilted plants per plot were presented. At the end of trial, yield was recorded in all the treatments. The experiment was conducted in two consecutive years: 2006 and 2007.

2.11. Data analysis

The observations recorded in percent were angularly transformed prior to statistical analyses and all data were subjected to analysis of variance using a completely randomized design (factorial) for glass house experiment and in randomized block design for field experiment (Gomez and Gomez, 1984).

3. Results

3.1. Standardization of substrate for mass multiplication of *T. harzianum*

Out of the six substrates used, significantly higher numbers of conidia of *T. harzianum* were found in jhangora followed by mandua, sorghum and polygonum. Rice and wheat straw were least hydrolysed and colonized by *T. harzianum* (data not shown).

3.2. Pathogenicity test

Amongst ten isolates of *Fusarium oxysporum*, isolate number five was found to be significantly more pathogenic on tomato variety Pant T-3 as all five plants from each replicate were dead and hence it was proven as *Fusarium oxysporum* f. sp. *lycopersici*. However, only F7 was a weak pathogen and showed significantly lower pathogenecity compared to all other isolates, whereas F6, F1, F8 and F9 showed comparable pathogenecity (data not shown).

3.3. Functional properties of fluorescent *Pseudomonas*

Amongst 37 isolates of fluorescent *Pseudomonas*, 17 were found to be phosphate solubilizers which formed a clear zone around the colony on specific media. Production of an orange halo zone against dark blue background suggested that 18 isolates were siderophore producers. Dark blue coloured zone around the colonies of 23 isolates were recorded as positive for rhamnolipid production. Isolate number 5, 8, 16, 24 were found to be positive for all the three properties. Amongst these four, isolate No. 16 was proven best on the basis of various functional tests i.e. P-solubilization, siderophore production and rhamnolipid production (data not shown).

3.4. Inhibition of *F. oxysporum f. sp. lycopersici* by antagonists

The maximum percent inhibition of growth of *F. oxysporum* f. sp. *lycopersici* (FOL) was observed by *T. harzianum* isolate 35 (48%) followed by isolate numbers 23, 19, 38, 31, 7, 26, 13 and 14 (ranging from 30% to 45%). The evaluation of antagonistic activity of 37 isolates of fluorescent *Pseudomonas* against FOL showed that four isolates inhibited more than 45% growth. However, isolate number 16 gave maximum inhibition i.e. 56%.

3.5. Compatibility test amongst different isolates of *T. harzianum* and fluorescent *Pseudomonas*

Based on the inhibition data, six isolates, each of *T. harzianum* and fluorescent *Pseudomonas* were selected. Selected isolates of fluorescent *Pseudomonas* were also found to be functionally superior based on their P-solubilization, siderophore production and rhamnolipid production activity. On the basis of compatibility test, isolate number 35 of *T. harzianum* (T_{35}) having highest growth (61 mm) in presence of isolate number 16 of fluorescent *Pseudomonas* (P_{16}), these (T_{35} and P_{16}) were selected for further studies (Table 1).

3.6. Compatibility amongst three bioagents

It was observed that consortial inoculum of bioagents enhanced shoot length, shoot fresh and dry shoot weight of tomato plants significantly in comparison to control (Fig. 2). Data of maize are not shown as we were trying to see the impact of *T. harzianum* and fluorescent *Pseudomonas* on the AMF hyphae, which was to pass through middle chamber inoculated with these two bioagents to give the infection in tomato. Mycorrhizal infection recorded was 85%, 46%, 51% and 63% in treatments inoculated with AMF (no inoculation in middle chamber), T_{35} (inoculated in middle chamber), P_{16} (inoculated in middle chamber) and $T_{35} + P_{16}$ (inoculated in middle chamber), respectively. No infection either in maize or tomato was recorded in control treatment.

Table 1
Growth of different isolates of *T. harzianum* against fluorescent *Pseudomonas*.

Fluorescent *Pseudomonas* isolates	Growth of *T. harzianum* isolates (mm)						LSD ($P = 0.05$)
	T7	T19	T23	T31	T35	T38	
P14	55.46	57.36	46.40	51.00	59.90	57.00	0.66
P16	55.00	57.00	54.00	59.00	61.00*	59.00	0.38
P18	54.80	35.44	48.60	58.65	54.43	53.30	0.41
P24	53.77	48.57	33.22	58.17	47.30	46.24	0.26
P29	48.57	40.71	50.94	51.33	53.55	46.35	0.38
P30	49.31	44.29	51.33	46.35	45.86	52.14	0.31

* Depicts the maximum growth of *T. harzianum* with fluorescent *Pseudomonas*.

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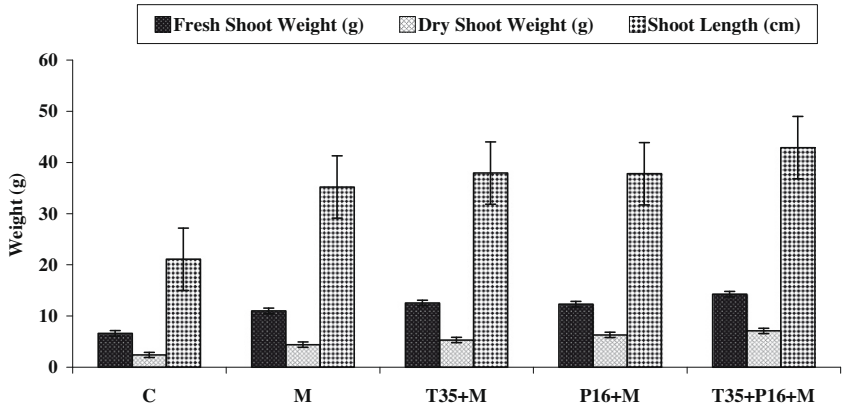

Fig. 2. Compatibility and plant growth promotory activities of bioagents on tomato plants. Bars on the pillars represents LSD ($P = 0.05$).

3.7. Seed bio-priming

Bio-priming of seeds enhanced seed germination by 48% on treatment with the combination of bioagents ($T_{35} + P_{16}$) (Fig. 3A). Duration taken by the seeds to germinate was reduced significantly by 2–2.5 days when seeds were bio-primed with the bioagents either individually or in combination (Fig. 3B).

3.8. Assessment of efficacy of bioagents in controlling Fusarium wilt of tomato in pot trial

Fusarium wilt was significantly suppressed in all treatments compared to the untreated control. Single inoculation of T_{35}, P_{16} and mycorrhiza reduced disease severity by almost 47%, 37% and 47% respectively, whereas combination of $T_{35} + P_{16}$, $T_{35} + M$ and $P_{16} + M$ reduced disease by 63, 58 and 58%, respectively. Addition of CDC further reduced the disease severity by 5–25% with single and dual inoculation of either of the bioagents. A combined inoculation of all three bioagents significantly reduced disease severity by 74%, which was further reduced by 25% with the addition of CDC. The combined treatment of all bioaogents with CDC reduced disease incidence by 81% relative to the without CDC control (Table 3). Shoot dry weight was increased in all the treatments compared to control ranging from 40% to 237%. Maximum increase was recorded in the combined treatment of all the bioagents. There was further increase in shoot dry weight with the amendment of CDC ranging from 27% to 79%. The combined treatment of all the bioagents with CDC increased shoot dry weight by 283% relative to the without CDC control (Table 2).

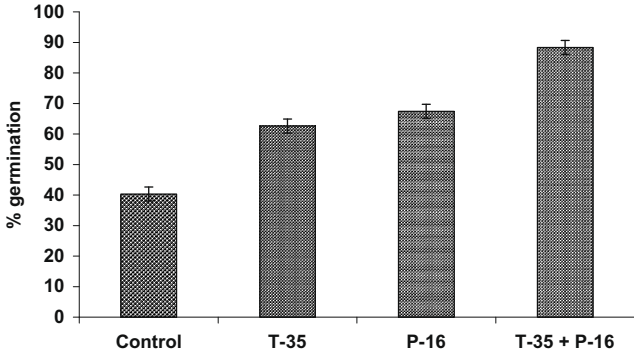

Fig. 3A. Effect of seed bio-priming with bioagents on seed germination. Bars on the pillars represents LSD ($P = 0.05$).

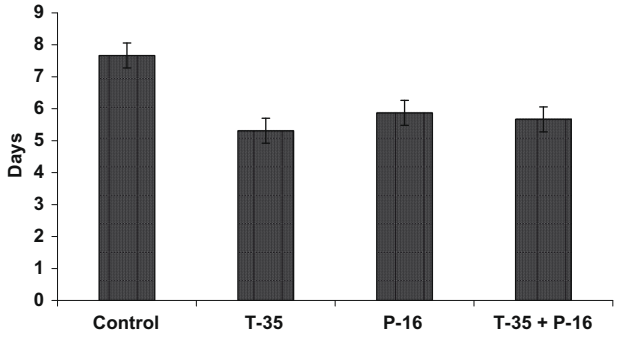

Fig. 3B. Effect of seed bio-priming with bioagents on days taken for seed germination. Bars on the pillars represents LSD ($P = 0.05$).

3.9. Field trial

Single inoculation of mycorrhizal fungi suppressed the disease severity by 30–31%, however its combined inoculation with either T_{35} or P_{16} reduced the disease by 38–49% in 2006 and 40–53% in 2007 and the combination of all three bioagents reduced disease severity by 67% and 69% in 2006 and 2007, respectively. Amendment of CDC further reduced disease severity in conjunction with all other treatments ranging from 23% to 44% with an average of ~35%. The combined treatment of all the bioagents reduced

Table 2
Influence of bioagents on dry shoot weight of tomato plants in pots under glass house condition.

Treatments	Mean dry shoot weight (g)		
	CDC+	CDC−	Mean
C	0.79	0.58	0.69
FOL	0.25	0.14	0.20
T_{35} + FOL	0.89	0.79	0.84
P_{16} + FOL	0.81	0.65	0.73
M + FOL	1.21	1.04	1.12
$T_{35} + P_{16}$ + FOL	1.54	1.34	1.44
T_{35} + M+FOL	1.06	0.85	0.96
P_{16} + M + FOL	1.04	0.84	0.94
$T_{35} + M + P_{16}$ + FOL	2.22	1.96	2.09
Mean	1.09	0.91	
LSD ($P = 0.05$) for CDC = 0.034, Treatments = 0.072 and Treatments X CDC = 0.10			

CDC = cow dung compost, CDC+ = with CDC; CDC− = without CDC; C = control; FOL = *Fusarium oxysporum* f. sp. *lycopersici*; T = *Trichoderma harzianum*; P = Fluorescent *Pseudomonas*; M = *Glomus intraradices*.

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Table 3
Influence of bioagents on wilt incidence caused by *F. oxysporum* f. sp. *lycopersici* on tomato in pots under glass house condition.

Treatments	Mean wilt incidence (%)		
	CDC+	CDC−	Mean
C	0 (00.0)	0 (00.0)	0 (00.0)
FOL	90.1 (71.7)	95.4 (77.7)	92.7 (74.7)
T_{35} + FOL	40.1 (39.3)	50.5 (45.3)	45.3 (42.3)
P_{16} + FOL	54.8 (47.8)	60.0 (50.8)	57.4 (49.3)
M + FOL	40.2 (39.3)	50.4 (45.2)	45.3 (42.3)
T_{35} + P_{16} + FOL	28.1 (32.0)	35.4 (36.5)	31.8 (34.3)
T_{35} + M+FOL	29.8 (33.1)	40.0 (39.2)	34.9 (36.2)
P_{16} + M + FOL	35.0 (36.3)	40.4 (39.4)	37.7 (37.9)
T_{35} + M + P_{16} + FOL	18.1 (25.2)	24.4 (29.6)	21.3 (27.4)
Mean	37.4 (36.1)	44.0 (40.4)	
LSD (P = 0.05) for CDC = (0.5), Treatments = (1.1) and Treatments X CDC = (1.5)			

Cdc = Cow dung compost, CDC+ = with CDC; CDC− = without CDC; C = control; FOL = *Fusarium oxysporum* f. sp. *lycopersici*; T = *Trichoderma harzianum*; P = fluorescent *Pseudomonas*; M = *Glomus intraradices*. Figures in parentheses are transformed angular values.

the disease severity with CDC by 63% compared to its control. Similar results were observed in the second year also, with the disease severity reduced by 84% with all the three bioagents and CDC compared to without CDC control (Table 6). Individual or combination of bioagents increased plant height by 63–81%, which was further improved with the addition of CDC by 3–19% in different treatments except the combination of P_{16} and M where the difference was not significant. Plant height was significantly higher with the combination of all three bioagents (Table 4). The number of branches increased by 40–60% in different treatments, however, significantly maximum number of branches were recorded in the plants provided the combination of all three bioagents (Table 5). The yield was increased ranging form 4% to 20% in different treatments in 2006 and 6–25% in 2007. The maximum yield (20% higher than control) was recorded in the plots where a combined inoculation of all the three bioagents was used which was increased to 33% greater than the control when combined with CDC. The yield increase in the same treatment was in the order of 43% in second year (Table 6).

4. Discussion

Fusarium oxysporum f. sp. *lycopersici* is one of the yield limiting factors of tomato (*Lycopersicon esculentum* L.) across the world. Due to the soil-borne nature of the disease, use of chemicals in controlling the wilt is hardly successful. Inconsistencies in biocontrol under varying environmental conditions have been a common limitation of many biocontrol agents. A number of isolates of *T. harzianum* and fluorescent *Pseudomonas* recovered from suppressive soils were screened against *F. oxysporum* and selected isolates were found to be highly effective and also compatible with each other: T_{35} and P_{16}, were used to develop a sound technique for combating wilt disease under pot and field conditions. Mycorrhizal fungus, *Glomus intraradices*, was also found to be compatible with these bioagents. Pseudomonads are active against many of the diseases caused by several soil-borne fungal pathogens (Pierson and Weller, 1994; Van Peer et al., 1991; Bangera and Thomashow, 1996; Buysens et al., 1996; Duffy et al., 1996; Handelsman and Stabb, 1996). Root-colonizing plant-beneficial fungi like AMF are important in protecting plants from root pathogens (Sharma et al., 1992). Many of the studies have focused on use of the integrated management of soil-borne plant pathogens using pseudomonads and *Trichoderma* spp. and *Trichoderma* spp. and AMF, however, use of these bioagents together has not been documented

Table 4
Effect of bioagents on plant height in cm at 45 and 60 days interval after transplantation of tomato.

Treatments	45 Days						60 Days					
	2006			2007			2006			2007		
	CDC+	CDC−	Mean	CDC+	CDC−	Mean	CDC+	CDC−	Mean	CDC+	CDC−	Mean
Control	33.83	28.41	31.12	34.67	29.97	32.32	34.09	30.63	32.36	35.67	32.54	34.11
M	54.49	49.56	52.03	56.34	51.09	53.72	56.33	53.61	54.97	57.28	54.56	55.92
T_{35} + M	52.78	47.39	50.09	54.48	48.87	51.68	50.48	45.27	47.87	52.78	47.34	50.06
P_{16} + M	49.71	50.64	50.18	51.23	52.48	51.85	47.52	48.39	47.95	49.43	50.12	49.78
T_{35} + P_{16} + M	53.39	51.42	52.41	54.76	52.65	53.70	54.80	52.64	54.31	56.34	53.78	55.06
Mean	48.84	45.48		50.30	47.01		48.64	46.34		50.30	47.67	
LSD (P = 0.05) CDC	0.40			0.38			0.95			0.47		
Treatments	0.63			0.60			1.51			0.74		
CDC X Treatments	0.89			0.85			2.13			1.05		

CDC+ = with CDC; CDC− = without CDC; C = control; M = *Glomus intraradices*; T = *Trichoderma harzianum*; P = fluorescent *Pseudomonas*.

Table 5
Effect of bioagents on branches of tomato at 45 and 60 days interval after transplantation.

Treatments	45 Days						60 Days					
	2006			2007			2006			2007		
	CDC+	CDC−	Mean	CDC+	CDC−	Mean	CDC+	CDC−	Mean	CDC+	CDC−	Mean
Control	12.04	10.74	11.39	13.75	11.79	12.77	13.39	11.92	12.66	14.28	12.87	13.58
M	16.81	15.60	16.21	18.03	16.98	17.51	19.04	18.96	19.00	21.45	20.78	21.12
T_{35} + M	17.42	17.41	17.42	18.99	18.76	18.88	18.82	18.04	18.43	20.37	20.08	20.22
P_{16} + M	16.37	15.78	16.08	17.87	17.23	17.55	17.70	17.09	17.40	19.65	19.34	19.50
T_{35} + P_{16} + M	17.85	16.70	17.28	19.24	17.45	18.34	19.28	18.64	18.96	21.99	20.19	21.09
Mean	16.10	15.24		17.58	16.44		17.65	16.93		19.55	18.65	
LSD (P = 0.05)CDC	0.24			0.18			0.08			0.59		
Treatments	0.38			0.29			0.12			0.93		
CDC X Treatments	0.54			0.41			0.17			1.31		

CDC+ = with CDC; CDC− = without CDC; C = control; M = *Glomus intraradices*; T = *Trichoderma harzianum*; P = fluorescent *Pseudomonas*.

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Table 6
Effect of bioagents on wilt incidence and yield of tomato.

Treatment	Wilt incidence (%)						Yield (q/h)					
	2006			2007			2006			2007		
	CDC+	CDC−	Mean	CDC+	CDC−	Mean	CDC+	CDC−	Mean	CDC+	CDC−	Mean
Control	53.5 (47.0)	77.7 (35.1)	65.6 (41.0)	47.1 (43.4)	75.6 (33.3)	61.3 (38.3)	116.80	98.53	107.66	125.67	101.78	113.72
M	33.0 (29.6)	53.9 (31.1)	43.5 (30.3)	30.1 (26.7)	52.8 (28.4)	41.5 (27.6)	122.65	108.42	115.53	130.78	111.67	121.23
T_{35} + M	24.4 (26.3)	39.5 (61.9)	31.9 (44.1)	20.3 (20.0)	35.6 (60.4)	27.91 (40.18)	128.67	111.36	120.01	135.87	120.89	128.38
P_{16} + M	26.7 (47.2)	47.6 (38.9)	37.1 (43.1)	22.6 (46.6)	44.7 (36.6)	33.62 (41.60)	120.84	102.44	111.64	127.59	108.48	118.03
T_{35} + P_{16} + M	19.7 (43.6)	25.7 (30.4)	22.7 (37.0)	11.7 (41.9)	23.6 (29.0)	17.6 (35.5)	131.54	118.54	125.04	145.89	127.37	136.63
Mean	31.4 (38.7)	48.9 (39.5)		26.4 (35.7)	46.4 (37.5)		124.10	107.86		133.16	114.04	
LSD (P = 0.05) CDC	(0.5)			(0.9)			0.92			1.16		
Treatments	(0.3)			(0.6)			1.46			1.84		
CDC X Treatments	(0.8)			(1.3)			2.06			2.60		

CDC+ = with CDC; CDC− = without CDC; C = control; M = *Glomus intraradices*; T = *Trichoderma harzianum*; P = fluorescent *Pseudomonas*. Figures in parentheses are transformed angular values.

to control *Fusarium* wilt in tomato. The present study is demonstrating the way of using antagonists together in the integrated management practice of controlling *Fusarium* wilt disease of tomato. The most important aspect of this study was the use of a combination of three bioagents: fluorescent *Pseudomonas*, *T. harzianum* and *G. intraradices*, which has potential not only to control the disease but also to enhance plant growth.

Regardless of the organism used, an important criterion for a successful implementation of bioagent is the preparation of microbial biomass of high efficacy with a high level of viability and vigour. Several isolates of *Trichoderma* spp. have been developed in large amounts of biomass containing conidia and chlamydospores on substrates having inexpensive ingredients (Lewis and Papavizas, 1984). Vargas-García et al. (2005) also reported that agricultural wastes could be used as substrates for the preservation and growing of ligno-cellulolytic fungi. We used the cheaper substrate jhangora which is an underutilized crop in the different regions of Himalaya and is easily available. Also, inoculum grown on this substrate gets ready in just 1 week, which is appreciably less than with other substrates like sorghum, another inexpensive substrate. Use of talcum powder, an inert material, as a carrier in this study also reduces the chances of growth of any other contaminants. Thus, the results indicated that it was possible to use inexpensive substrates to produce viable inoculum of *T. harzianum*, which could be utilized in the field to manage soil-borne plant pathogens. Bio-priming of seeds with individual isolates or combinations of bioagents enhances germination, as well as shortens the time to germination suggesting the benefits of the tested organisms.

Many types of composted material has been shown to suppress diseases, including hardwood bark (Chef et al., 1983; Trillas-Gay et al., 1986), pine bark (Cebolla and Pera, 1983; Orlikowski, 1983; Pera and Calvet, 1989), poplar bark (Garibaldi, 1988), wood shavings (Cebolla and Pera, 1983), cork and grape marc (Trillas et al., 2002), olive pumice (Pera and Calvet, 1989), cattle manure (Reuveni et al., 2002), sewage sludges (Cotxarrera et al., 2002) and vermicompost (Garibaldi, 1988; Szczech, 1999; Szczech et al., 1993). Several of these have been used successfully for consistent biological control, particularly for containerized crops. In the present study conducted under glasshouse condition, it was observed that the combination of the bioagents in soil amended with CDC were more efficient in controlling disease. On its own, organic matter content is necessary, but it is not sufficient alone for suppressiveness of disease. Organic matter provided by CDC is of high quality because of its cellulosic content and level of available energy and thus supports the growth of suppressive microbes. Our results clearly demonstrated the possibility of using CDC for better management of wilt in tomato. This could also be because of low carbon content of soil under tropical situation and hence use of CDC might support the growth of biocontrol agents like *Trichoderma harzianum*..

Under field conditions, the combination of any two bioagents was found to be effective in controlling disease. The seed treatment with the combination of *T. harzianum*, fluorescent *Pseudomonas* and further inoculation of infective propagules of *G. intraradices* to raise seedlings in conjunction with root dipping in the mixture of *T. harzianum* and fluorescent *Pseudomonas* at the time of transplanting consistently showed the best performance in enhancing the plant growth along with an increase in the yield of tomato and suppression of disease. The effect was more pronounced in CDC inoculated plots. Presence of carbon and nitrogen in CDC helps the inoculated bioagents to grow, persist and sustain itself for longer duration (unpublished data). The stages of growth, decline, or persistence of a population of *Fusarium* in soil depend on the ecological balance and nutrient availability (Woltz and Jones, 1981). The most critical limitation is chemical energy and competition for energy sources present in soil (Lockwood, 1988). Other microorganisms (bacteria, actinomycetes and fungi) compete according to the magnitude of their genetically controlled reproductive capacity. Suppression of *F. oxysporum* by competitive microorganisms seems to be mainly due to depletion of available carbon sources and antibiotic production (Marshall and Alexander, 1960). These results are in agreement with the biological control of diseases in growth media formulated with stabilized organic components being dependent upon the concentration of slow release sources of nutrients for growth and activity such as carbohydrates (hemicellulose, cellulose, and so on) (Hoitink et al., 1993, 1996; Borrero et al., 2004). Thus, use of the consortia of bioagents (*T. harzianum,* fluorescent *Pseudomonas* and *G. intraradices*) against *Fusarium* wilt not only suppressed the disease incidence but also helps in sustenance and growth promotion of crop through their different plant growth promotory and nutrient uptake properties.

5. Conclusion

The present study thus promotes the integrated management approach of disease suppression through usage of various effective bioagents that inhabit in the rhizosphere. Their use probably does not alter the rhizospheric community as they were isolated from the suppressive soils and were highly populated and share same ecological niche in rhizosphere of plants. The method of inoculation of bioagents might ensure the availability of biocontrol agents to combat the disease. Also the use of CDC helps in growth and sustenance of these bioagents by providing them the necessary nutrients required for their growth so as to attain a certain population

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and compete with the pathogen for nutrients and indirectly helping in disease control.

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