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Field Suppression of Fusarium Soil Borne Diseases of Tomato Plants by the Combined Application of Bio Agents and Chitosan

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Two biocontrol agents, *Trichoderma harzianum* and *Bacillus subtilis* proved their high antagonistic effect against wide spectrum of plant pathogens in many previous works, two commercially formulated bio agents products, Plant guard (*Trichoderma harzianum*) and Rhizo–N (*Bacillus subtilis*) and Chitosan at 1.0 g/L were applied as seed bed treatments alone or in combination with chitosan at 0.5 g/L as foliar spray for controlling Fusarium crown and root rot (FCRR) as well as Fusarium wilt (FW) diseases of tomato plants under field conditions. Field evaluation of these treatments in an area of heavy inoculum and using cv. Super Strain indicated that all tested treatments significantly suppressed disease incidence and severity of FCRR and FW of tomato as compared to untreated controls. The most effective treatments were *T. harzianum, B. subtilis* and Chitosan combined with chitosan at 0.5 g/L. as foliar spray which reduced the disease incidence and disease severity of FCRR and FW of tomato. All tested treatments significantly reduced the density of *Fusarium* spp. in the treated soil as compared with control. The

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highest reduction was obtained with *T. harzianum, B. subtilis* and Chitosan combined with chitosan at 0.5 g/L. as foliar spray. The results showed a significant effect of all the treatments on fruit yield of the tomato plants relative to control. These combined treatments could provide sustainable management of Fusarium crown and root rot as well as Fusarium wilt in tomato under field conditions.

Keywords: Tomato plants; Solanum lycopersicum L.;T. harzianum; B. subtilis; chitosan; Fusarium crown and root rot; fusarium wilt.

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) plants are among of most important vegetable crops in Egypt. Fusarium wilt (FW) of tomato plants caused by *Fusarium oxysporum* f.sp. *lycopersici* (FOL) and Fusarium crown and root rot (FCRR) caused by *Fusarium oxysporum* f.sp. *radicis lycopersici* (FORL) are the most damaging soil-borne diseases of tomato and becoming more common in greenhouse tomato production. The disease occurs in both the greenhouse and the field on tomato worldwide and causes significant losses in tomato production [1,2,3]. Biological control agents based on bacterium *Bacillus subtilis* and fungus *Trichoderma harzianum* have been promising in the control of seed and soil borne diseases [4,5,6]. Chitosan (Deacetylated chitin biopolymer), is currently obtained from the outer shell of crustaceans such as crabs, krills and shrimps. Chitosan exhibits a variety of antimicrobial activities [7,8]. On the other hand, chitosan induces host defense responses against several plant diseases [9,10,11]. Chitosan applied as seed or soil treatments was shown to control several diseases of many plant species [12].Chitosan induce host defense responses in both monocotyledons and dicotyledons [9,10,11]. It has been extensively utilized as a foliar treatment to control the growth, spread and development of many diseases involving viruses, bacteria, fungi and pests [7,10]. It has also been used to increase yield and tuber quality of potatoes [13,14,15].

The present study aims are evaluation of two bio-agents as original isolates, *T. harzianum* and *B. subtilis* or formulated in commercial products as Plant guard and Rhizo- N respectively as well as Chitosan at 1.0 g/L applied as seed bed treatments alone or in combination with chitosan at 0.5 g/L as foliar spray for controlling Fusarium crown and root rot as well as Fusarium wilt diseases of tomato plants under field conditions.

2. MATERIALS AND METHODS

2.1 Sources of Bio agents and Commercial Products

Trichoderma harzianum and *Bacillus subtilis* were obtained from the Plant Pathology Department of the National Research Centre, Giza, Egypt, and had proved their high antagonistic ability during previous work [8]. Two commercial biocides *i.e*. Plant guard and Rhizo-N produced by El-Nasser Co., Egypt were used. Plant guard is a suspension containing $3.6x10^7$ viable spores of *T. harzianum*, while Rhizo-N is a powdery formulation of *B. subtilis* containing 3.6 $x10^6$ cells/g. Fungal and bacterial cultures were maintained on potato dextrose agar (PDA) and nutrient agar slant media at 5±1℃ as stock cultures until use. All isolates were refreshed by growing at the optimum growth conditions at the beginning of the present experiments. Chitosan samples from shrimp shells (2-Amino-2-deoxy-(1→4)-*β*-D-glucopyranan and Poly-(1,4-*β*-D-glucopyranosamine) with high molecular weight (HMW~600000 Dalton) produced by Sigma-Aldrich Chemicals Company were used in the present work.

2.2 Preparation of Antagonistic *T. harzianum* Inoculums

Inoculum of *T. harzianum* was prepared by growing isolate in 50.0 mL potato dextrose broth (PDB) medium in 250 mL Erlenmeyer flasks for 15 days at 25±2℃, and the growing upper solid layers were washed and blended in sterilized water. Colonies forming units (cfu) were adjusted to 10^6 cfu/mL using haemocytometer slide [16] A few drops of the emulsifier Tween 80 (Sigma Co.) and sticker were added.

2.3 Preparation of *B. subtilis* Inoculum

The antagonistic bacterium *B. subtilis* was grown in nutrient broth medium and incubated in a rotary shaker at 200 rpm for 72 h at 28 ±2℃. The bacterial cells were harvested by centrifugation at 6,000 rpm for 10 min, washed twice with sterilized water and re-suspended in sterilized distilled water. The concentrations of bacterial cells in the suspensions were adjusted to 10^8 cells/mL (cfu/mL) [17].

2.4 Field Experiment

2.4.1 Management of fusarium crown and root rot of tomato plants under field conditions

Two bioagents as original isolates of *T. harzianum* or *B. subtilis or* formulated in commercial products as Plant guard and Rhizo–N respectively as well as Chitosan at 1.0 g/L were applied as single treatments or in combination with chitosan at 0.5 g/L as foliar spray were applied for controlling Fusarium crown and root rot as well as Fusarium wilt diseases of tomato plants under field conditions in private farm with history of infested soil by root rot and wilt pathogens of tomato. Field experiment was carried out, in the private farm of Fayoum Governorate, Egypt. Tomato cv. Super Strain B was sown in the transplanting tray containing peat–moss soil which was mixed individually with each tested treatment as described earlier. Tomato seedlings were transplanted in field after 30 days of sowing. A field experiments consisted of plots (7x10 m) each comprised of 12 rows and 50 transplants/row. Treatments were conducted in randomly complete block design with three replicates (plots) for each particular treatment as well as control. This experiment was conducted following completely randomized block design in two successive growing seasons (2013/2014) in a field naturally infected with the causal organisms of Fusarium diseases of tomato. The following 12 treatments were evaluated under field conditions.

2.4.1.1 Treatments

Two bio agents as original isolates of *T. harzianum* and *B. subtilis* or formulated in commercial products as Plant guard and Rhizo -N respectively as well as Chitosan at 1.0 g/L were applied as seed bed treatments singly or in combination with chitosan at 0.5 g/L as foliar spray as follows (Table 1).

All biological treatments or Topsin were applied as seed bed treatment (tomato seeds were sown in transplants production foam trays containing peat-moss soil mixed individually with each tested treatment). Half of treatments divided into two groups one, sprayed with chitosan at 0.5 g/L after 20 and 40 days of transplanting and the other sprayed with water. Tomato seeds (cv. Super Strain B) were sown in treated soil for 30 days. Two seedlings/hill were transplanted with 50 cm apart between hills. Untreated seedlings were used as control.

2.5 Disease Assessments

2.5.1 Disease incidence

Fusarium crown and root rot as well as Fusarium wilt disease incidence were assessed on the basis of field symptoms 60 days after transplanting. All infected plants were picked up and examined for the causal organism for specific root disease. Root samples were also subjected to isolation for the pathogens in the laboratory.

2.5.2 Disease severity

Disease severity were estimated 60 days after transplanting according to Rowe [18] using a rating scale of (0 – 5) based on root discoloration or leaf yellowing grading, *viz*., 0 = neither root discoloration nor leaf yellowing, 1= 1-25% root discoloration or one leaf yellowing 2= 26-50% root discoloration or more than one leaf yellowing, 3= 51-75% root discoloration with one wilted leaf, 4= up to 76% root discoloration or more than one leaf wilted, and 5= completely dead plants.

The severity of Fusarium wilt was assessed 60 days after transplanting using the following scale.0= no symptoms, 1= slight yellowing or wilting of foliage, 2= moderate yellowing or wilting of foliage, 3= complete yellowing or wilting of foliage, 4= plant dead

$$\text{Disease severity \%} = \frac{\sum (\text{Disease grade} \times \text{Number of plants in each grade})}{\text{Total number of plants} \times \text{Highest disease grade}} \times 100$$

Table 1. Treatments

Single treatments	Combined treatments
1- T. harzianum at 50 ml/kg soil	6- *T. harzianum* + Chitosan at 0.5 g/L as foliar spray
2- *B. subtilis* at 50 ml/kg soil	7- *B. subtilis* + Chitosan at 0.5 g/L as foliar spray
3- Chitosan at 1.0 g/kg/ soil	8- Chitosan + Chitosan at 0.5 g/L as foliar spray
4- Plant guard at 3.0 mL/kg soil	9-Plant guard + Chitosan at 0.5 g/L as foliar spray
5- Rhizo - N at 3.0 g/kg soil	10- Rhizo - N+ Chitosan at 0.5 g/L as foliar spray
11- Topsin – M 70% at 3g/kg soil (As comparison)	
12- Control (un treated plants)	

2.6 Effect of Bio agents and Chitosan Treatments on Fusarium Population Density

The population densities of *Fusarium* spp. were determined by assaying soil samples in the laboratory, using serial dilutions on modified peptone – PCNB agar medium, as described by Ioannou [19]. From each treatment, 10 samples were taken and bulked into a composite sample. Soil sampling was done before treatments and after 30 and 60 days of transplanting tomato seedlings.

2.7 Effect of Bio Agents and Chitosan Treatments on Fruit Yield of Tomato Plants

After 55 days of tomato transplanting up to the end of the experiment, tomato fruits were collected periodically (one time per week). Tomato yield per treatment was recorded and the average of the fruit yield in tons per feddan (4200 m^{-2}) was calculated for each treatment.

2.8 Statistical Analysis

All experiments were set up according to a randomized complete-block design. One-way ANOVA was used to analyze differences between treatments. A general linear model option of the analysis system SAS (48) was used to perform the ANOVA. Tukey test for multiple comparisons among means was utilized [20].

3. RESULTS

In the present study, we investigated the suppressive effect of isolates of *T. harzianum* and *B. subtilis* or formulated in commercial products as Plant guard and Rhizo-N respectively as well as Chitosan at 1.0 g/L when applied alone as seed bed treatments or in combination with chitosan at 0.5 g/L as foliar spray in addition to the Fungicides (Topsin –M 70% at 3g/kg soil) on the occurrence of Fusarium crown and root rot as well as Fusarium wilt diseases of tomato plants caused by FCRR, and FOL, respectively under field conditions.

3.1 Suppressive Effects against Fusarium Crown and Root Rot Disease by *Bio* agents and Chitosan Treatments under Field Conditions

Results in Table (2) show that all tested treatments significantly reduced disease incidence and severity of FCRR of tomato plants as compared with control. The most effective treatments were *T. harzianum, B. subtilis,* and Chitosan 1.0 g/L combined with chitosan at 0.5 g /L. as foliar spray which reduced the disease incidence of FCRR by 64.8, 65.4 and 64.0%, respectively In addition to its reduced the disease severity by 75.0%. Meanwhile, single treatments showed moderate effect.

3.2 Suppressive Effects against Fusarium Wilt Disease by Bio agents and Chitosan Treatments under Field Conditions

Results in Table 3 indicate that all tested treatments significantly reduced disease incidence and severity of Fusarium wilt of tomato plants as compared with untreated plants (control). The highest reduction was obtained with *T. harzianum, B. subtilis,* chitosan 1.0 g/L combined with chitosan at 0.5 g/L. as foliar spray which reduced the Fusarium wilt incidence by 65.7, 69.5 and 63.8% respectively. In addition to *T. harzianum* and *B. subtilis* combined with chitosan at 0.5 g/L. as foliar spray reduced the disease severity more than 71.4%. Meanwhile, single treatments showed moderate effect.

Table 2. Field evaluation of combined applications of bio-agents and chitosan for the suppression of Fusarium crown and root rot in tomato plants cv. Super Strain B

Treatment	Foliar application	Fusarium crown and root rot disease incidence*			
		Disease incidence %	Reduction %**	Severity %	Reductio n%
T. harzianum	Chitosan 0.0 g/L	16.0 c	53.9	0.4 c	50.0
B. subtilis		15.0 c	56.8	0.4 c	50.0
Plant guard		22.3 b	35.7	0.6 b	25.0
Rhizo N		21.0 b	39.5	0.6 b	25.0
Chitosan 1.0 g/L		16.0 c	53.9	0.4c	50.0
T. harzianum	Chitosan 0.5 g/L	12.2 d	64.8	0.2 d	75.0
B. subtilis		12.0 d	65.4	0.2d	75.0
Plant guard		15.8 c	54.5	0.4c	50.0
Rhizo N		16.4 c	52.7	0.4c	50.0
Chitosan 1.0 g/L		12.5 d	64.0	0.2 d	75.0
Topsin –M 70% at 3g/kg soil		22.3 b	35.7	0.6 b	25.0
Control (un treated plants)		34.7a	0.0	0.8 a	0.0

**Average percentages of diseases incidence for the two successive seasons 2013-2014.*
***Reduction as compared to the untreated control. Means followed by the same letters are not significantly different according (P= 0.05)*

Table 3. Field evaluation of combined applications of bio-agents and chitosan for the suppression of Fusarium wilt in tomato plants cv. Super Strain B

Treatment	Foliar application	Fusarium wilt disease incidence*			
		Disease incidence %	Reduction %**	Severity %	Reduction%
T. harzianum	Chitosan 0.0 g/L	10.0 d	52.4	0.4 cd	42.9
B. subtilis		10.5 d	50.0	0.4 cd	42.9
Plant guard		17.4 b	17.1	0.5 b	28.6
Rhizo N		16.6 b	21.9	0.6 b	14.3
Chitosan 1.0 g/L		11.7 d	44.3	0.5 b	28.6
T. harzianum	Chitosan 0.5 g/L	7.2 e	65.7	0.2 e	71.4
B. subtilis		6.4 e	69.5	0.2 e	71.4
Plant guard		13.5 c	35.7	0.4 cd	42.9
Rhizo N		13.1 c	37.6	0.3 de	57.1
Chitosan 1.0 g/L		7.6 e	63.8	0.3 de	57.1
Topsin –M 70% at 3g/kg soil		16.6 b	21.9	0.6 b	14.3
Control (un treated plants)		21.0 a	0.0	0.7 a	0.0

**Average percentages of diseases incidence for the two successive seasons 2013-2014.*
***Reduction as compared to the untreated control. Means followed by the same letters are not significantly different (P= 0.05)*

3.3 Effect of Bio-agents and Chitosan Treatments on Fusarium Density in Soil

Effect of bio agents and chitosan as soil treatments alone or in combination with chitosan as foliar spray on population density of *Fusarium* spp. 30 and 60 days after transplanting was tested. Results in Table 4 show that all tested treatments significantly reduced Fusarium density in the treated soil as compared with control. The highest reduction was obtained with *T. harzianum, B. subtilis,* chitosan 1.0 g/L combined with chitosan at 0.5 g/L. as foliar spray which reduced the Fusarium density by 75.0% 60 days after transplanting. Meanwhile, single treatments showed moderate effect.

3.4 Effect of Bio-agents and Chitosan Treatments on Fruit Yield of Tomato Plants

Results in Table 5 indicate that all tested treatments significantly increased the fruit yield of tomato plants compared with untreated plants (control). The highest increase in fruit yield was

Table 4. Effect of bio-agents and chitosan alone or in combination on population density of *Fusarium* spp. in naturally infested soil

Treatment	Foliar application	Average population densities of Fusarium spp. cfu x10 ^{3} /g dry soil			
		Days after planting			
		30 days	Reduction % *	60 days	Reduction %
T. harzianum	Chitosan	2.0b	68.8	1.8 c	65.4
B. subtilis	0.0 g/L	2.1 b	67.1	1.7 c	67.3
Plant guard		2.2 b	65.6	2.0 b	61.5
Rhizo N		2.5 b	60.9	2.4 b	61.5
Chitosan 1.0 g/L		2.4 b	62.5	2.3 b	55.8
T. harzianum	Chitosan	1.5 d	76.6	1.3 d	75.0
B. subtilis	0.5 g/L	1.4 d	78.1	1.3 d	75.0
Plant guard		2.0 b	68.8	1.7 c	67.3
Rhizo N		2.0 b	68.8	1.8 c	65.4
Chitosan 1.0 g/L		2.4 b	62.5	1.3 d	75.0
Topsin –M 70% at 3g/kg soil		2.2 b	65.6	2.0 b	61.5
Control (un treated plants)		4.6 a	0.0	5.2 a	0.0

**Reduction as compared to the untreated control. Means followed by the same letters are not significantly different according (P= 0.05)*

Table 5. Effect of bio-agents and chitosan alone or in combination on fruit yield of tomato plants (cv. Super Strain B) under filed conditions

Treatment	Foliar application		Total fruit yield*	
			Ton/Feddan	Increase %**
T. harzianum	Chitosan	0.0 g/L	16.4 c	46.4
B. subtilis			16.5 c	47.3
Plant guard			15.2 d	35.7
Rhizo N			14.8 d	32.1
Chitosan 1.0 g/L			14.8 d	32.1
T. harzianum	Chitosan	0.5 g/L	18.0 a	60.7
B. subtilis			18.4 a	60.7
Plant guard			16.1 c	43.8
Rhizo N			15.6 c	39.3
Chitosan 1.0 g/L			17.5 b	56.3
Topsin –M 70% at 3g/kg soil			16.1 c	43.8
Control (un treated plants)			11.2 e	0.0

**Average fruit yield of tomato plants for the two successive seasons 2013-2014.*
***Increase as compared to the control. For each column, means followed by the same letter are not significantly different (P= 0.05)*

obtained with *T. harzianum, B. subtilis* combined with chitosan at 0.5 g/L. as foliar spray by 60.7%. Moderate effect was obtained with combined treatments between chitosan at 1.0 g/L. were applied as seed bed treatments and chitosan at 0.5 g/L as foliar spray which increased the tuber yield more than 56.3%. Meanwhile, both treatments, when used single, were less effect.

4. DISCUSSION

Fusarium wilt of tomato plants caused by *Fusarium oxysporum* f.sp. *lycopersici* (FOL) and Fusarium crown and root rot caused by *Fusarium oxysporum* f.sp. *radicis lycopersici* (FORL) are the most damaging soil-borne diseases of tomato and becoming more common in greenhouse tomato production. The disease occurs in both the greenhouse and the field worldwide and causes significant losses in tomato production [21,22,23,24]. Biological control agents based on the biocontrol bacterium *Bacillus subtilis* and the biocontrol fungus *Trichoderma harzianum* have been promising in the control of seed and soil borne diseases [4,5,6]. This effect was also observed in study reported by Bakeer [25] who found that seed dip treatment and soil drench treatment with *B. subtilis* and *T. viride* as bioagent, Plant Garud and Rizo-N as commercial biocide products reduced the incidence of bean infection with root rot under green house and field condition.

Chitosan applied as seed or soil treatments was shown to control several diseases in many plant species [12]. Chitosan induces host defense responses in both monocotyledons and dicotyledons [9,10,11]. In the present study results indicated that the most effective treatments were *T. harzianum*, *B. subtilis* and Chitosan 1.0 g/L combined with chitosan at 0.5 g /L. as foliar spray which reduced the disease incidence of FCRR by 64.8, 65.4 and 64.0% respectively in addition to the reduced the disease severity by 75.0%. Moreover, results indicated that the most effective treatments were *T. harzianum*, *B. subtilis* and Chitosan 1.0 g/L combined with chitosan at 0.5 g/L. as foliar spray which reduced the Fusarium wilt incidence by 65.7, 69.5 and 63.8%, respectively. In addition they reduced the disease severity more than 57.1%. These findings agree with those of studies showing that chitosan treatment can cause induced resistance and increase enzyme activities in many plants [26]. HilaL [27] who found that chitosan was able to enhance the growth of many crops. The underlying mechanisms for this plant growth promoting action may be attributed to effects on plant physiological processes such as nutrient uptake, cell elongation, cell division, enzymatic activation and protein synthesis [28]. In addition to, Benhamou [29] found that the increased resistance of bacterized tomato roots to *Fusarium* infection can be triggered by specific alterations in the physiology of the host plant due to the effect exerted by chitosan.

All tested treatments significantly reduced density of *Fusarium* spp. in the treated soil as compared with control. The highest reduction was obtained with *T. harzianum*, *B. subtilis* and *Chitosan* 1.0 g/L combined with chitosan at 0.5 g /L. as foliar spray which reduced the Fusarium density by 75.0% after 60 days of transplanting. As for tomato yield, the highest increase in yield was obtained with *T. harzianum*, *B. subtilis* and Chitosan 1.0 g/L combined with chitosan at 0.5 g /L. as foliar spray which increased the tomato yield by 60.7, 60.7 and 56.3%.

The rhizosphere is a region populated by several beneficial microorganisms and is thought to be a region of first line of defense for roots against attack by pathogenic fungi. In this regard, Chakraborty et al. [30] found that *T. harzianum* strains produce chitinase protein which showed clear hyphal lysis *in vitro*. Also, *B. subtilis* showed inhibition zones against *Rhizoctonia. solani, Colletotrichum truncatum, Sclerotina sclerotium, Macrophomina phaseolina, Phomopsis* spp., *Pythium aphanidermatum*, *F. verticilloides, F. equiseti, F. solani, F. oxysporum* and *F. oxysporum* f.sp. *lycopersici* under *in vitro* conditions [31,32]. The inhibitory effect of biocontrol agents might be related mainly to the antagonistic properties, which involve parasitism and lysis of pathogenic fungi and/or competition for limiting elements in the rhizosphere, mainly iron and carbon [33,34]. However, another possible mechanism has been suggested by Baraka et al. [35] namely; induced resistance in plants to soilborne fungal attack. Singh et al. [36] reported that *Bacillus subtilis* isolates exhibited strong antagonistic activity against *M. phaseolina* and other phytopathogens including *F. solani* and *R. solani*.

Chitosan or deacetylated chitin, is currently obtained from the outer shell of crustaceans such as crabs, krills and shrimps. Chitosan exhibits a variety of antimicrobial activities [7,8]. On the other hand, chitosan induces host defense responses against several plant diseases [9,10,11]. In the present study results indicated that bio agents combined with chitosan as foliar spray significantly reduce both diseases and increased the tomato yield. The increase of yield obtained in this study, could be attributed to the effect of biocontrol agents as plant growth promoters [37]. In this respect, Kulikov et al. [38] reported that the antimicrobial activity increased with the increase in chitosan molecular weight and seems to be faster on fungi and algae than on bacteria. Fungicidal activity of chitosan has been documented against various species of fungi and oomycetes [8,39]. Some of the derivatives also repressed spore formation at rather high concentrations [7]. Recently, Palma-Guerrero et al. [40] demonstrated that chitosan is able to permeabilize the plasma membrane of *Neurospora crassa* and kills the cells. In general, chitosan is able to reduce the *in vitro* growth of a number of fungi and oomycetes [41]. For instance, chitosan was reported to exert an inhibitory action on the hyphal growth of numerous pathogenic fungi, including root and necrotrophic pathogens, such as *Fusarium oxysporum*, *Botrytis cinerea*, *Monilina laxa*, *Alternaria alternata* and *Pythium aphanidermatum* [42].

On the other hand, chitosan induces host defense responses against several plant diseases [9,10,11]. Moreover, chitosan has been extensively utilized as a foliar treatment to control the growth, spread and development of many diseases involving viruses, bacteria, fungi and pests [7,10,43]. It has also been used to increase yield and tuber quality of potatoes [13]. Chitosan had different properties *i.e.* inhibitory effect against pathogenic fungus and had the ability to be potent elicitors of plant defense resistance. In addition, chitosan was reported to induce callose formation and proteinase inhibitors [44]. In the present study, the role of chitosan could be acting as antifungal activity and induced resistance. It could be suggested that combined treatments between bio agents and chitosan as foliar spray might be used for controlling tomato root disease under field condition. On the other hand, the antagonistic isolate *T. harzianum*, was most active than the commercial bio agent plant guard in reducing disease and increase of tomato yield in field conditions. However, their efficacy may vary depending on isolate and or formulation of the respective product in addition to the nature of the strain used in the preparation of bio agent product and its stability or no change in their effectiveness for a long time. Successful colonization of host rhizospheres by *T. harzianum* is very important for effective control of soil borne pathogens.

5. CONCLUSION

Under field conditions, two bio agents, *T. harzianum* and *B. subtilis* or formulated in commercial products as Plant guard and Rhizo-N, respectively as well as Chitosan showed the great capacity for disease suppression and increased the fruit yield of tomato plants. Combined treatments between bio agents and chitosan showed the greatest capacity for disease suppression and highest increase in fruit yield of tomato plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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