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Field application of *Trichoderma harzianum* and *Bacillus subtilis* combined with *Rhizobium* for controlling *Fusarium* root rot in Faba bean in organic farming

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ABSTRACT

The antagonistic potential effects of both *Trichoderma harzianum* (Plantgard) and *Bacillus subtilis* (Rhizo-N) were tested separately or each combined with *Rhizobium* against *F. solani,* the causal of root rot in Faba bean plants. Treatments evaluated in field application in organic farming on two faba bean cultivars (Giza$_3$ and Sakha$_{11}$)**.** Field experiments were carried out in private organic farm cultivated in El-Sharkiya Governorate. This field is well known as naturally heavily infection with *F. solani*. Ecah *T. harzianum* and *B. subtilis* combined with *Rhizobium* reduced the diseases incidence of *F. solani* as pre- emergence & post- emergence damping off as well as root rot, compared to each treatment only and control. Application of the same treatments increased the percentages of survival faba bean plants. The treatments increased the growth parameters per plant *viz*., shoot length, both fresh & dry shoot weight, branches number, root length and both fresh & dry root weight. Yield parameters has been also increased as pods number and fresh & dry weight of pod per plant.

Key words: *Bacillus subtilis,* Biological control, *Fusarium solani,* Faba bean, Field application, organic farming, *Rhizobium and Trichoderma harzianum.*

Introduction

Faba bean (*Vicia faba* L.) is one of the most common legumes in Egypt, where it is an excellent source of protein and complex carbohydrates (Rabey *et al*., 1992). *Fusarium solani* as soil borne pathogen was found to be associated with root rot symptoms of faba bean plants in organic farming (Abdel-Monaim, 2013), where causes economic losses in yield (Golpayegani *et al*., 2010; Habtegebriel &Boydom, 2015 and Shafique *et al*., 2016). The biological management is considered an environmentally acceptable alternative to chemicals. The organic farmers can used this approach for controlling root rot plant pathogens (Van Bruggen and Termorshuizen, 2003 and Van Bruggen *et al*., 2016). *Trichoderma* is a very effective biological mean for soil borne plant disease management. *Bacillus subtilis* also is an organism that can be used for disease management at organic vegetables, because it is widespread in nature and it has been recovered from soil, water and decomposing plant materials.

B. subtilis and *Trichoderma harzianum* showed antagonistic activity against *F. solani* in greenhouse pot experiments, where the treatments protected significantly the bean seedlings against pathogen. *Trichoderma harzianum* was highly protected the treated plants than *B. subtilis* (Abeysinghe, 2007). As soil treatments, *Bacillus pumilus* and *Trichoderma viride* reduced significantly the pre- and post-emergence damping off incidence caused by *F. solani* under artificial infection in greenhouse experiments. The best protection of damping off disease was obtained by *T. viride*, followed by *B. pumilus.* All treatments improved plant survival and growth parameters (Kamel and El-Khteeb, 2012). *Trichoderma viride* and *Bacillus megaterium* individually or in combination controlled damping-off and root rot/wilt diseases of Faba bean plants in pots and field. These treatments also increased the survival plants as well as the fresh and dry weights in pots. In field, these treatments significantly increased growth parameters and yield components (Abdel-Monaim, 2013). *Trichoderma hamatum* significantly controlled the Faba bean root rot incidence caused by *F. solani* (Habtegebriel and Boydom,

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2015). Coating seeds of Faba bean by *T. harzianum* and *T. viride*, before sowing in the soil, were strongly suppressed the disease incidence of *Fusarium oxysporum* (Mahmoud, 2016).

The objective of this research was to evaluate the antagonistic potential effect of both *T. harzianum* (Plantgard) and *B. subtilis* (Rhizo-N) alone or combined with *Rhizobium* bacteria against *F. solani,* the causal organism of root rot Faba bean plants grown under organic farming .The role of the applied treatments in reducing *Fusarium* root rot disease incidence and enhancing the growth and yield parameters in plant were studied.

Materials and Methods

1. Isolation and identification of *F. solani* pathogen:

Naturally infected Faba bean plants showing root rot symptoms were collected from organic farm of El-Sarkiya Governorate, Egypt. Infected parts of plant roots were washed with tap water and then surface sterilized with 3% (W/W) NaOCl for 2 min. Sterilized parts were washed twice with sterile water and then cut into small pieces (1cm length) .The sterilized root pieces were placed on surface of antibiotic amended potato dextrose agar medium (PDA) in Petri dishes (9 cm diameter). Plates were incubated at 30±2°C for one week. A single hyphae tip was cultured on new PDA plates. *F. solani* was identified based on the characteristics described by Barnett and Hunter (1972) and Booth (1985) after Koch's postulates that demonstrated the pathogen is the causal agent of root rot of Faba bean plants.

2. Bio- control agents:

Trichoderma harzianum (Plantgard, $30x10^6$ cells per ml) was applied at rate of 250 cm^3/ 100 liter of water for soil treatment and at rate of $4cm^3$/kg for seed treatment. *Bacillus subtilis* (Rhizo-N, 30 milion cells per gram) was applied at rate of 250 g/ 100 liter of water for soil treatment and at rate of 4g/kg for seed treatment.

3. Field experiment:

Field experiment was carried out in private farm cultivated under organic farming system in El-Sarkiya Governorate. The field trial (32 plots) was designed in complete randomized block with four plots as replicates for each treatment as well as untreated control. Each experimental unit area was $12m^2$ (4 × 3m). Each plot included 5 rows and each row was 4m in length and 60 cm width .Each bio-control agent inoculum was incorporated with the 20 cm of the soil surface. Then, the soil was irrigated before 7 days before sowing (Abd El- Khair *et al*., 2004). The field treatments were as follows;

1. *T. harzianium* as soil treatment + *Rhizobium* as seed treatment (TSo+RSe).
2. *B. subtilis* as soil treatment + *Rhizobium* as seed treatment (BSo+RSe).
3. *T. harzianium* as soil treatment (TSo).
4. *B. subtilis* as soil treatment (BSo).
5. *T. harzianium* as seed treatment (TSe).
6. *B. subtilis* as seed treatment (BSe).
7. *Rhizobium* as seed treatment (RSe).
8. Untreated control (UC).

Faba bean seeds (cv. $Giza_3$ and $Sakha_{11}$, obtained from the Legumes Crops Research Department, Agricultural Research Centre, Giza, Egypt**)** were separately planted in hole 25 cm at rate of 2 seeds / hole at 20cm space in all treatments . All plots received the agricultural practices according to farming system.

3.1. Disease assessment:

Effects of the tested *T. harzianium* and *B. subtilis* in reducing the damping off disease incidence at pre- and post-emergence stages were recorded after 15 and 30 days after sowing. The percentages of root rot incidence and survival healthy plants were recorded after 45 days after sowing (Abd-El-Khair *et al*., 2010 and Kamel &El-Khateeb, 2012).

3.2. Vegetative growth parameters:

After one 30 days , random samples of 20 faba bean plants for each treatment as well as the control were removed from the soil and the roots were washed with water. Then, the plant shoot growth parameters *viz*., shoot length , shoot fresh and dry weight and branches number as well as root parameters *viz*. root length and root fresh & dry weight per plant were estimated.

After four months of sowing, random samples of 20 faba bean plants for each treatment as well as the control have been collected. The plant growth parmeters were measured as mentioned before.

3.3. Yield parameters:

The yield parmeters was determined as pods number and fresh & dry weight of pod per plant for each particular treatment as well as the control at the end of growing season.

4. Statistical analysis:

Statistical analysis of the obtained data was performed through Computer Statistical Package (CO-STATE) originated by Anonymous (1989). Duncan's Multiple Range Test was applied to compare the means for each treatment at 5% level of probability.

Results

The field application of *T. harzianum* and *B. subtilis* alone or combined with *Rhizobium* reduced the diseases incidence of *F. solani viz*. pre- emergence of damping off , post- emergence of damping off and root rot, compared to RSe only or untreated control (Table 1).

Table 1: Effects of *Trichoderma harzianium* and *Bacillus subtilis* separately or each combined with *Rhizobium* bacteria on diseases parameters of faba bean (Cvs. Giza$_3$ and Sakha$_{11}$) in organic farming.

Treatments	Disease assessments							
	Damping off %				Root rot incidence %		Survival plants %	
	Pre-emergence %		Post-emergence %					
	Giza$_3$	**Sakha$_{11}$**	Giza$_3$	**Sakha$_{11}$**	Giza$_3$	**Sakha$_{11}$**	Giza$_3$	**Sakha$_{11}$**
T. harzianium (soil) + *Rhizobium* (seed)	9.3h*	**6.7h**	2.2e	**2.1d**	1.5e	**2.1e**	96.3a	**95.8a**
B. subtilis (soil) + *Rhizobium* (seed)	12.0g	**8.0g**	2.3e	**2.2d**	1.5e	**2.2e**	96.2a	**95.6b**
T. harzianium (soil)	18.7f	**12.0f**	3.3d	**3.0cd**	3.3d	**3.8d**	93.4b	**93.2c**
B. subtilis (soil)	20.0e	**13.4e**	3.3d	**3.2bcd**	3.3d	**3.9d**	93.4b	**92.9d**
T. harzianium (seed)	21.3d	**20.0d**	3.4cd	**3.3bcd**	5.1c	**5.0c**	91.5c	**91.6e**
B. subtilis (seed)	22.7c	**21.3c**	3.5c	**3.4bc**	5.2c	**5.1c**	91.3c	**91.5f**
Rhizobium (seed)	40.0b	**36.7b**	5.6b	**5.3b**	6.7b	**7.4b**	87.7d	**87.3g**
Untreated control (UC)	42.7a	**40.0a**	10.5a	**10.0a**	12.8a	**13.3a**	76.7e	**76.7h**

*Means are averages of four replicates followed by same small letter(s) are not significant according to Duncan's Multiple Range Test at $p \leq 0.05$

TSo+RSe, BSo+RSe, TSo, BSo ,TSe and TSe treatments highly reduced the incidence of pre-emergence of damping off in the ranges of 6.7 -22.7% (9.3-22.7 for cv. Giza$_3$ and 6.7-21.3% for cv. Sakha$_{11}$) compared to disease incidence of 40.0 & 36.7% with RSe only and 42.7 & 40.0% in the untreated control in the two cultivars, respectively. The incidence of post-emergence of damping off with the same applied treatments ranged from 2.1-3.5% (2.2-3.5% and 2.1-3.4% in cvs Giza$_3$ and Sakha$_{11}$) , compared to disease incidence of 5.6 & 5.3% and 10.5 & 10.0% in the two cultivars with RSe only and the untreated control , respectively . Results also showed that the treatments highly reduced the root rot incidence in the ranges of 1.5-5.2% (1.5-5.1% for cv. $Giza_3$ and 2.1-5.1% for cv. $Sakha_{11}$), compared to 6.7 & 7.4% (RSe only) and 12.8& 13.3% (Untreated control), respectively. Details of the ability of different applied treatments for reducing the incidence of disease assessments caused by *F. solani* are shown in Table (1). Results cleared that TSo+RSe treatment significantly reduced the incidence of pre- & post- emergence damping off as well as root rot ,followed by BSo+RSe, TSo , BSo ,TSe and TSe . Application of tested treatments increased the percentages of faba bean plants survival in the ranges of 91.3-96.3% (91.3 – 96.3% for cv. $Giza_3$ and 91.5 - 95.8% for cv. $Sakha_{11}$), compared to 87.7 & 87.3% with rhizobium only and 76.7 & 76.7 % in untreated control, respectively (Table 1).

After one month of sowing; application the treatments improved the tested shoot, compared to RSe only or untreated control. The shoot length of faba bean with application of TSo+RSe , BSo+RSe, TSo , BSo ,TSe and BSe treatments ranged from 27.0-40.7cm (27.0 -40.7 cm for cv. $Giza_3$ and 28.3 - 38.7cm for cv. $Sakha_{11}$) , comparing with lengths of 30.3 7 26.3 cm and 16.3 & 15.0cm with RSe only and in untreated control . In cv. $Giza_3$, BSo+RSe significantly the shoot height, followed by TSo+RSe, BSo, BSe, TSo and TSe, while in cv. $Sakha_{11}$ BSo+RSe also significantly the shoot length, followed by TSo+RSe, BSo, TSo, BSe, and TSe, respectively .The fresh weight of shoot ranged from 19.6-27.2g (21.2-27.2g and 19.6-21.1g), while the fresh weights were 19.3 & 16.3g and 17.4 & 14.8g with RSe only and untreated control for cvs $Giza_3$ and $Sakha_{11}$, respectively. The TSo+Rse and TSo+Rse significantly increased the fresh weight of plants cvs $Giza_3$ and $Sakha_{11}$, respectively .The dry weight of shoot ranged from 2.54-4.16g (2.74-4.16 g for cv. $Giza_3$ and 2.54-3.99g for cv. $Sakha_{11}$), compared to 2.50 & 2.33g and 1.97 & 1.97 g with RSE only and untreated control. TSo+RSe was significantly increased the shoot dry weight in two faba bean cultivars , followed by , BSo+RSe, TSo , BSo ,TSe and BSe . BSo+RSe was significantly increased the shoot dry weight two faba bean cultivars , followed by , TSo+RSe , TSo , BSo ,TSe and BSe . The leaves numbers, in cultivars of $Giza_3$ and $Sakha_{11}$, ranged from 10.3-18.0 leave (11.0-18.0 leave for cv. $Giza_3$ and 10.3 -14.0 leaves for cv. $Sakha_{11}$), compared to 10.7 & 10.3 and 9.7 & 9.0 leave with RSe only and untreated control. BSo+RSe was significantly increased the leave numbers in two faba bean cultivars , followed by , TSo+RSe , TSo , BSo , BSe and TSe (Table 2).

Table 2: Effects of *Trichoderma harzianium* and *Bacillus subtilis* separately or each combined with *Rhizobium* bacteria on shoot growth parameters of faba bean (Cvs. $Giza_3$ and $Sakha_{11}$) in organic farming after one month of sowing.

Treatments	Shoot growth parameters							
	Length (cm)		Fresh weight(g)		Dry weight (g)		Leavesno.	
	$Giza_3$	**$Sakha_{11}$**	$Giza_3$	**$Sakha_{11}$**	$Giza_3$	**$Sakha_{11}$**	$Giza_3$	**$Sakha_{11}$**
T. harzianium (soil) + *Rhizobium* (seed)	39.7a*	**36.0b**	**27.2a**	**26.0**b	4.16a	**3.99a**	15.0b	**12.3b**
B. subtilis (soil) + *Rhizobium* (seed)	40.7a	**38.7a**	25.6b	**27.1a**	3.89b	**3.90b**	18.0a	**14.0a**
T. harzianium (soil)	29.0d	**31.0c**	23.7c	**20.9d**	2.92c	**2.64c**	11.7cd	**12.0b**
B. subtilis (soil)	32.0b	**31.0c**	22.0d	**21.8c**	2.91c	**2.63c**	12.7c	**12.0b**
T. harzianium (seed)	27.0e	**28.3d**	21.4e	**20.2e**	2.74e	**2.54d**	11.3d	**11.7b**
B. subtilis (seed)	31.0bc	**28.7d**	21.2e	**19.6e**	2.79d	**2.55d**	11.0d	**10.3c**
Rhizobium (seed)	30.3c	**26.3e**	19.3f	**16.3f**	2.50f	**2.33e**	10.7de	**10.3c**
Untreated control (UC)	16.3f	**15.0f**	17.4g	**14.8g**	1.97g	**1.97f**	9.7e	**9.0d**

*Means are averages of four replicates followed by same small letter(s) are not significant according to Duncan's Multiple Range Test at $p \leq 0.05$

Field application of the same treatments also increased the root parameters of faba bean *viz.* length; fresh weight and dry weight in the ranges of 13.3-15.7cm (13.3 - 15.0cm for cv. $Giza_3$ and 14.0 – 15.7 cm for $Sakha_{11}$), 2.0-2.8g (2.30- 2.8 for cv. $Giza_3$ and 2.0-2.6g for $Sakha_{11}$) and 0.30-0.45g (0.30 – 0.42g for cv. $Giza_3$ and 0.31 - 0.45g for $Sakha_{11}$), respectively. The root parameters of 10.0cm, 1.9g & 0.29g for cv. $Giza_3$ and 9.3 cm, 1.9 g & 0.26g for $Sakha_{11}$with RSe only compared to 10.0cm, 1.7g and 0.21g for cv. $Giza_3$ and 8.7cm, 1.8g & 0.18g for $Sakha_{11}$in untreated control, respectively. BSo+RSe significantly increased the root parameters, followed by Tso + RSe in two tested cultivars (Table 3).

Table 3: Effects of *Trichoderma harzianium* and *Bacillus subtilis* separately or each combined with *Rhizobium* bacteria on the root growth parameters of faba bean (Cvs. $Giza_3$ and $Sakha_{11}$) in organic farming after one month of sowing.

Treatments	Root growth parameters					
	Length (cm)		Fresh weight (g)		Dry weight (g)	
	$Giza_3$	**$Sakha_{11}$**	$Giza_3$	**$Sakha_{11}$**	$Giza_3$	**$Sakha_{11}$**
T. harzianium (soil) + *Rhizobium* (seed)	15.0a*	**15.3a**	2.3b	**2.4b**	0.41a	**0.39b**
B. subtilis (soil) + *Rhizobium* (seed)	15.0a	**15.7a**	2.8a	**2.6a**	0.42a	**0.45a**
T. harzianium (soil)	14.0ab	**15.7a**	2.2bc	**2.1c**	0.39b	**0.31d**
B. subtilis (soil)	14.7ab	**15.0ab**	2.0de	**2.0cd**	0.35c	**0.35c**
T. harzianium (seed)	13.3b	**14.0b**	2.1cd	**2.0cd**	0.30d	**0.31d**
B. subtilis (seed)	14.0ab	**14.0b**	2.0de	**2.0cd**	0.36c	**0.36c**
Rhizobium (seed)	10.0c	**9.3c**	1.9e	**1.9de**	0.29d	**0.26e**
Untreated control (UC)	10.0c	**8.7c**	1.7f	**1. 8e**	0.21e	**0.18f**

*Means are averages of four replicates followed by same small letter(s) are not significant according to Duncan's Multiple Range Test at $p \leq 0.05$

After four months of sowing; treatments of field application increased the shoot parameters of faba bean *viz*. length; fresh weight; dry weight and leaves number , compared to RSe only or untreated control . The shoot length were the ranges of 71.0-97.7 cm (86.3-91.7cm for cv. $Giza_3$ and 71.0-97.7cm for cv. $Sakha_{11}$), compared to 83.0 & 68.0 cm and 78.0 & 57.3cm in RSe only and untreated control. BSo+Rse and Tso+Rse were highly increased the shoot length of cvs $Giza_3$ and $Sakha_{11}$, respectively. The fresh weight of shoot ranged from 84.1 to 142.0g (86.0-142.0g for cv. $Giza_3$ and 84.1-136.9g for cv.$Sakha_{11}$) , compared to 75.5 & 83.8 g and 44.2 & 5758.5g in RSe only and untreated control. The dry weight of shoot were 37.9-97.6g (56.7-91.7cm for cv. $Giza_3$ and 37.9 – 94.1f for $Sakha_{11}$), compared to 45.1 & 29.3 g and 22.8 & 27.5g in RSe only and untreated control. The dry weight of shoot were at the range of 37.9-97.6g (56.7-91.7cm for cv. $Giza_3$ and 37.9 – 94.1f for $Sakha_{11}$), compared to 45.1 & 29.3 g and 22.8 & 27.5g in RSe only and untreated control. The number of shoot branches were at the range of 1.7 – 2.3 branch (1.7-2.3 branch for cv. $Giza_3$ and 2.3 – 3.7 branch for cv. $Sakha_{11}$), compared to 1.3& 2.0 branch and 1.3 & 1.7 branch in RSe only and untreated control. TSo+RSe followed by BSo+RSe were significantly increased the shoot parameters viz. fresh & dry weight and number of branches (Table 4).

Table 4: Effects of *Trichoderma harzianium* and *Bacillus subtilis* separately or each combined with *Rhizobium* bacteria on shoot growth parameters of faba bean (Cvs. $Giza_3$ and $Sakha_{11}$) in organic farming after four month of sowing.

Treatments	Shoot growth parameters							
	Length (cm)		Fresh weight (g)		Dry weight (g)		Branches no.	
	$Giza_3$	**$Sakha_{11}$**	$Giza_3$	**$Sakha_{11}$**	$Giza_3$	**$Sakha_{11}$**	$Giza_3$	**$Sakha_{11}$**
T. harzianium (soil) + *Rhizobium* (seed)	91.3a*	**97.7a**	142.0a	136.9a	97.6a	**94.1a**	2.3ab	**3.7a**
B. subtilis (soil) + *Rhizobium* (seed)	91.7a	**82.0b**	102.8ab	**124.7ab**	87.9a	**75.5b**	2.7a	**3.0ab**
T. harzianium (soil)	87.0ab	**74.0bc**	102.0ab	**100.4abc**	65.8b	**41.2c**	1.7ab	**2.7abc**
B. subtilis (soil)	87.3ab	**73.3bc**	86.5bc	**88.5bc**	57.5bc	**40.7c**	2.0ab	**2.7abc**
T. harzianium (seed)	86.3ab	**72.0bc**	100.0ab	**98.6abc**	64.3bc	**40.0c**	1.7ab	**2.3bc**
B. subtilis (seed)	87.0ab	**71.0bc**	99.7ab	**84.1bc**	56.7bc	**37.9cd**	1.7ab	**2.3bc**
Rhizobium (seed)	83.0bc	**68.0cd**	75.5bc	**83.8bc**	45.1c	**29.3d**	1.3b	**2.0bc**
Untreated control (UC)	78.0c	**57.3d**	44.2c	**58.5c**	22.8d	**27.5d**	1.3b	**1.7c**

*Means are averages of four replicates followed by same small letter(s) are not significant according to Duncan's Multiple Range Test at $p \leq 0.05$

Field application of the same treatments also increased the root parameters of faba bean *viz.* length; fresh weight and dry weight in the ranges of 13.3-15.7cm (13.3 - 15.0cm for cv. $Giza_3$ and 14.0 – 15.7 cm for $Sakha_{11}$), 2.0-2.8g (2.30- 2.8 for cv. $Giza_3$ and 2.0-2.6g for $Sakha_{11}$) and 0.30-0.45g (0.30 – 0.42g for cv. $Giza_3$ and 0.31 - 0.45g for $Sakha_{11}$), respectively. The root parameters of 10.0cm, 1.9g

& 0.29g for cv. $Giza_3$ and 9.3 cm, 1.9 g & 0.26g for $Sakha_{11}$with RSe only compared to 10.0cm, 1.7g and 0.21g for cv. $Giza_3$ and 8.7cm, 1.8g & 0.18g for $Sakha_{11}$in untreated control, respectively. BSo+RSe significantly increased the root parameters, followed by TSo+RSe in the two tested cultivars (Table 5).

Table 5: Effects of *Trichoderma harzianium* and *Bacillus subtilis* separately or each combined with *Rhizobium* bacteria on root growth parameters of faba bean (Cvs. $Giza_3$ and $Sakha_{11}$) in organic farming after four month of sowing.

Treatments	Root growth parameters					
	Length (cm)		Fresh weight (g)		Dry weight (g)	
	$Giza_3$	**$Sakha_{11}$**	$Giza_3$	**$Sakha_{11}$**	$Giza_3$	**$Sakha_{11}$**
T. harzianium (soil) + *Rhizobium* (seed)	20.7a*	**21.7a**	18.4ab	**15.2a**	9.0a	**7.7a**
B. subtilis (soil) + Rhizobium (seed)	20.3a	**20.7a**	21.0a	**14.4ab**	9.6a	**7.5a**
T. harzianium (soil)	17.0ab	**20.0a**	16.0ab	**12.6abc**	8.4ab	**6.0b**
B. subtilis (soil)	18.3ab	**20.0a**	18.9ab	**12.0bc**	9.4a	**6.2b**
T. harzianium (seed)	16.0ab	**19.5a**	15.3b	**11.3bc**	7.1b	**5.9b**
B. subtilis (seed)	17.0ab	**19.4a**	17.7ab	**11.0bc**	8.7ab	**6.0b**
Rhizobium (seed)	16.7ab	**18.0ab**	15.5b	**10.1c**	7.1b	**4.4c**
Untreated control (UC)	14.0b	**15.0b**	7.9c	**9.1c**	3.7c	**2.9d**

*Means are averages of four replicates followed by same small letter(s) are not significant according to Duncan's Multiple Range Test at $p \leq 0.05$

Field application of the applied treatments also increased the root parameters of faba bean *viz*. length; fresh weight and dry weight in the ranges of 16.0-21.7cm (16.0-20.7cm for cv. $Giza_3$ and 19.4 – 21.7 cm for $Sakha_{11}$), 11.0-15.2g (15.3- 21.0g for cv. $Giza_3$ and 11.0-15.2g for $Sakha_{11}$) and 5.9 – 9.6g (7.1 – 9.6g for cv. $Giza_3$ and 5.9 – 7.5g for $Sakha_{11}$), respectively. The root parameters of 16.7cm, 7.9g & 3.7g for cv. $Giza_3$ and 15.0 cm, 9.1 g & 4.4g for $Sakha_{11}$with RSe only compared to 14.0cm, 7.9g and 0.213.7g for cv. $Giza_3$ and 15.0cm, 9.1g & 2.9g for $Sakha_{11}$in untreated control, respectively. TSo+RSe and BSo+RSe significantly increased the root length in two cultivars. BSo+RSe significantly increased the root fresh and dry weight in cv. $Giza_3$, while TSo+RSe in $Sakha_{11}$.

Field application of the treatments increased the yield parameters of faba bean *viz*. pods number; pod fresh and dry weight at harvest. The pods number was at the range of 7.0-11.3 pod (7.0-11.3 pod in cv. $Giza_3$ and 7.0-11.3 pod in cv. $Sakha_{11}$), compared to 6.0 & 6.7 pod (RSe) and 5.7 & 6.0 pod in untreated control. The pod fresh weight was at the range of 5.9-8.3g (5.9-7.8 g in cv. $Giza_3$ and 6.0-8.3 pod in cv. $Sakha_{11}$), compared to 3.1 & 5.6g (RSe) and 1.7 & 2.9 g in untreated control. The pod dry weight was at the range of 2.0 – 3.6g (2.0-3.6 g in cv. $Giza_3$ and 2.0-3.7 g in cv. $Sakha_{11}$), compared to 1.8 & 1.9g (RSe) and 0.5 & 1.7 g in untreated control (Table 6).

Table 6: Effects of *Trichoderma harzianium* and *Bacillus subtilis* separately or each combined with *Rhizobium* bacteria on fruits parameters of faba bean (Cvs. $Giza_3$ and $Sakha_{11}$) in organic farming at harvest.

Treatments	Fruit parameters					
	Fruit no.		Fresh weight(g)		Dry weight (g)	
	$Giza_3$	**$Sakha_{11}$**	$Giza_3$	**$Sakha_{11}$**	$Giza_3$	**$Sakha_{11}$**
T. harzianium (soil) +*Rhizobium* (seed)	11.3a*	**10.3a**	7.8a	**8.3a**	3.6a	**3.7a**
B. subtilis (soil) + *Rhizobium* (seed)	10.3ab	**10.0a**	7.7a	**7.9b**	3.4b	**3.0b**
T. harzianium (soil)	9.7abc	**9.7a**	6.6b	**6.4c**	3.0c	**2.3c**
B. subtilis (soil)	7.7abc	**9.3ab**	6.2c	**6.1d**	3.1c	**2.1d**
T. harzianium (seed)	9.3abc	**7.3bc**	6.0d	**6.0d**	2.1d	**2.0de**
B. subtilis (seed)	7.0bc	**7.0c**	5.9d	**6.0d**	2.0d	**2.0de**
Rhizobium (seed)	6.0c	**6.7c**	3.1e	**5.6e**	1.8e	**1.9e**
Untreated control (UC)	5.7c	**6.0c**	1.7f	**2.9e**	0.5f	**1.7f**

*Means are averages of four replicates followed by same small letter(s) are not significant according to Duncan's Multiple Range Test at $p \leq 0.05$

Discussion

With the rising awareness of the adverse effects of chemical pesticides, people are looking for organically grown vegetables because organic foods are healthier than those conventionally grown. Therefore, organic agriculture has expanded worldwide, but the soil borne fungi pathogens play an important role in disease development causing low yields by killing the plants or damaging the product. The bio-control agents that accumulate of specific compounds that may be antifungal can be applied in organic farming for disease control as alternative pesticides (Ahanger *et al*., 2013, Shafique *et al*., 2016 and Van Bruggen & Finckh, 2016). Pathogen suppression under organic farming depend upon quality of residues, stage of their decomposition, microbial activity, microbial population dynamics and nutrient concentrations (Ahanger *et al*. 2013). In this work, both *T. harzianum* and *B. subtilis* were separately applied (as soil treatment) combined with *Rhizobium* bacteria (as seed treatment) for controlling of *F. solani* in faba bean plants grown under organic farming systems.

The results indicated that the presence of either *T. harzianum* or *B. subtilis* combined with *Rhizobium* significantly reduced the damping off and root rot incidence in rhizosphere of faba bean plants. The protection exerted by the *T. harzianum* combined with *Rhizobium* against *F. solani* was pronounced than *B. subtilis* combined with *Rhizobium,* comparing to each treatment separately .The reduction may be related to the decline of the population density of *F. solani* in soil. These results are agreement with those recorded by Abeysinghe (2007), Kamel & El-Khateeb (2012), Błaszczyk *et al*.(2014) and Woo *et al*. (2014). *Bacillus subtilis* and *T. harzianum* effectively antagonized against *F. solani* f. sp. *phaseoli* in dual Petri plate assays and significantly protected bean seedlings in greenhouse pot experiments compared to the untreated control plants. *Trchoderma harzianum* enhanced the root growth (Abeysinghe, 2007). *In vitro* tests, *B. pumilus* and *T. viride* significantly reduced the mycelial growth of *F. solani*. In greenhouse experiment, *B. pumilus* and *T. viride* significantly reduced the pre- and post-emergence damping off disease incidence of *F. solani* under artificial infection. The best protection of damping off disease was obtained by *T. viride*, followed by *B. pumilus.* All treatments improved the survival plant and growth parameters (Kamel and El-Khateeb, 2012). Błaszczyk *et al*.(2014) showed that *Trichoderma* spp. and *B. subtilis* are a very large group of microorganisms that play a significant role in protecting plants against fungal pathogens and have positively affect on plants by stimulating plant growth . Woo *et al*. (2014) indicated that the *B. subtilis* and *T. harzianum* can be used as biological control agents approach as alternatives to synthetic agro-chemicals for controlling *Fusarium* root rot disease in organic farming , where *Trichoderma* spp. are presently marketed as bio-pesticides, biofertilizers, growth enhancers and stimulants of natural resistance .The bio-control agents reduced the pathogens infections by different mechanisms like competition for space and nutrients, antibiosis, mycoparasitism, hyphal interactions, enzyme secretion, plant growth promotion, degradation of the toxins produced by the pathogen and induction of the defense responses in plants (Howell , 2003 and Delete *et al*., 2015).

The results revealed that *T. harzianum* or *B. subtilis* combined with *Rhizobium* significantly increased the growth parameters viz. shoot length, shot fresh weight, shoot dry weight, leave number and branches per plant as well as root parmeters viz. root length, root fresh weight and root dry weight the damping off and root rot incidence caused by *F. solani* in rhizosphere of faba bean plants*,* comparing to each treatment separately. The treatments also increased the yield parameters *viz*. pods no. per plant, pod fresh and dry weight. *Trichoderma* spp. positively affects plants by stimulating plant growth (Błaszczyk *et al*., 2014). *Trichoderma harzianum* enhanced growth of root system as evidenced by increased biomass may be positively acted in this respect (Vinale *et al*., 2006 and Błaszczyk *et al*., 2014). The plant growth promoting rhizobacteria (PGPR) are rhizospheric microbes produced bioactive substances and promote plant growth and/or protect them against pathogens. Root colonizing bacteria also improved the plant growth through direct stimulation by producing growth regulators or by suppression of pathogens PGPR have to be highly competitive to successfully colonize the root zone (Raaijmakers *et al.*, 2002).

Our results also are inagreement with those recorded by Shaban and El-Bramawy (2011). They reported that the combined treatments of *T. harzianum* with *R. leguminosarum* significantly increased the plant growth parameters viz. branches /plant, pods /plant, seeds /pod, mean seed weight and then increase seed yield of the broad bean, chickpea and lupine plants. The results suggested that *Trichoderma* sp. and *Rhizobium* spp. can be used as biological control of damping off and root rot diseases which causing significant yield losses in legumes field crops (Hassan *et al*., 2015). The symbiotic rhizobia nodulating -Faba bean plants produced statistically equal shoot dry weight, shoot length and plant total nitrogen (Argaw, 2012). *Rhizobium* significantly increased the plant dry weights with *F. solani* infection (Golpayegani *et al*., 2010).*Rhizobium* strains improved the plant growth and N_2 fixation *in Vicia faba* plants (Othman and Tamimi, 2016).

Results in this work cleared that disease management in organic farming is largely based on the maintenance of biological diversity and soil health including nitrogen-fixing (Van Bruggen *et al*., 2016). Positive effects of seed factorization with *Rhizobium* in greenhouse were evident in terms of emergence and plant growth and sustainability in crop production of faba bean, Colonization by *R. leguminosarum* was stimulated by fluorescent *Pseudomonas* sp. (Shirin, 2014). It is obvious that root diseases in organic agriculture are generally less severe owing to greater soil health. Therefore, the soil microbial community and nitrogen availability play an important role in disease development and yield (Van Bruggen and Finckh, 2016).

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