Management of root knot nematode, *Meloidogyne incognita* infecting sugar beet as affected by certain bacterial and fungal suspensions

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Abstract: Three experiments were conducted in a greenhouse to study the nematicidal activity of *Bacillus subtilis*, *B. megaterium, B. pumilus* and *Pseudomonas fluorescens*, *Trichoderma harzianum*, *T.viride* and *T. vierns* against nematode parameters of *Meloidogyne incognita* infecting sugar beet. Experiment I was conducted to test *B. subtilis*, *B. megaterium, B. pumilus* and *P. fluorescens*, *T. harzianum*, *T. viride* and *T. vierns* as single treatment. *T. vierns* highly reduced the galls number by 74%, followed by *B. pumilus* (73%), *T. viride* (73%), *B. subtilis* and *T. harzianum* (71%), *B. megaterium* (71%) and *P. fluorescens* (60%). Also, *T. vierns* caused the highest percentages reduction of egg-masses numbers (80%), followed by *B. pumilus*, *T. harzianum* and *T. viride* that caused 74%, *B. megaterium* (68%), *B. subtilis* (65%) and *P. fluorescens* (61%), respectively. Experiment II was conducted to test *T. vierns* and *B. pumilus* as single treatment at doses of 10, 20 and 30 ml pot^{-1}. Results showed that treatments significantly reduced the number of J_2, galls and egg-masses by 79%-95%; 61%-78% and 64%-87%, compared to 97%, 80% and 88% with Micronema®, respectively. The treatments, also, by *T. vierns* and *B. pumilus* enhanced the length of shoot, fresh and dry weight of shoot, tuber weight and TSS% content of sugar beet. Experiment III revealed that *T. vierns* and *B. pumilus* when applied at both times of treatments reduced the numbers of J_2; galls and egg-masses by 86%-96%, 68%-81% and 69%-89%, compared to 97%. 79% and 87% with Micronema®, respectively. *T. vierns* when applied at one and/or second times was effective in enhancing the growth parameters *viz*., length of shoot, fresh and dry weight of shoot and root (Tuber) weight as well as total soluble sugars (TSS) percentage content than *B. pumilus*.

Keywords: Management, *Meloidogyne incognita*, sugar beet, bacterial and fungal suspensions

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1 Introduction

Root-knot nematodes, such as *Meloidogyne incognita*, cause severe agricultural losses for many crops as well as sugar beet (Tranier et al., 2014). Chemical control is widely used in all countries of the world in 1960s, for controlling the agricultural soil pathogens. But agricultural chemicals became causing environmental pollution; they were capable of inducing pest resistance, development of disease resistance, toxic hazards to human plants and domestic animals, high costs and limited availability in many developing countries or their diminished effectiveness following repeated applications (Dong and Zhang, 2006; Lamovšek et al., 2013; Bhattacharjee and Dey, 2014). Therefore, from the last two decades, scientists applied the biopesticides of environmentally and toxicologically safe characteristics to humans and animals. In the greenhouse, *B. subtilis* highly reduced the numbers of juveniles in soil, galls and egg masses of *M.incognita* and *Rhizoctonia solani* in

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eggplant (El-Nagdi and Abd-El-Khair, 2008). El-Nagdi et al. (2011) reported that the nematicidal effects of certain commercial bio-control products were tested against *M. incognita* on sugar beet, compared with the fenamiphos and cadusaphos. *In vitro*, the mortalities of *M. incognita* J_2 caused by the tested bio-control products. Also, in the greenhouse and open field, the bio-control products reduced the numbers of the tested nematode parameters. All treatments increased growth parameters as well as the percentage of total soluble solids TSS% in sugar beet plants. Abd-El-Fattah et al. (2012) reported that *Trichoderma viride* highly reduced nematode parameters, compared to the untreated control. In glasshouse and in field experiments, after 6 months of application, no significant differences were recorded between liquid and solid formulations of *T. viride* in reducing the tested nematode parameters. Soil treated with *T. viride* increased the plant growth, survival of plants and root yield. Mostafa et al. (2014) reported that the product, Bio-Arc (*Bacillus megaterium*) at the rates of 5, 10, 15 and 20 mL and Nemastrol [containing glycosynolates (12%), chitinase (12×10^5 IU), cytokinins (200 ppm), flavonoids (5%) and β 1-3, Glucanase (2×10^5 IU] at the rate of 0.25 mL have nematicidal activity against *M. incognita* infection and improved growth parameters of sugar-beet with various levels of success. The application of Bio-Arc + Nemastrol increased the activities of related enzymes i.e. Peroxidase (PO) and Polyphenol Oxidase (PPO) in roots of sugar-beet infected with *M. Incognita*, compared to control. El-Nagdi and Youssef (2015) reported that the commercial product of Micronema (containing strains of *Serratia* sp., *Pseudomonas* sp., *Azotobacter* sp., *Bacillus circulans* and *B. thuringiensis*) reduced parameters of root knot nematode, *M. incognita* on sugar beet. Also, the treatment enhanced the plant growth criteria and TSS% at different degrees.

The aim of this research is to study the nematicdial activity of certain bacterial and fungal suspensions against *M. incognita* on sugar beet.

2 Materials and methods

2.1 Plant species

Sugar beet cv. Gazelle seeds were sown in this work.

2.2 Bio-control agents

Seven bio-control agents, i.e. four bacterial agents *viz*., *Bacillus subtilis*, *B. megaterium, B. pumilus* and *Pseudomonas fluorescens* and three fungal bio-control agents *viz*., *Trichoderma harzianum*, *T. viride* and *T. vierns* were isolated from Egyptian soil and identified according to standard microbiological methods in Plant Pathology Department, National Research Centre (NRC).

2.3 Preparation of bio-control agent inoculums

For the preparation of bacterial biocontrol agent inoculums**,** each bacterium of *B. subtilis*, *B. megaterium, B. pumilus* and *P. fluorescens* was grown for four days on nutrient sucrose (2%) broth medium at 28ºC. Then, the bacterial suspension was adjusted to 3×10^8 colony forming unit CFU ml^{-1}. For preparation of fungal bio-control agents, each fungus of *T. harzianum*, *T. viride* and *T. vierns* were separately grown on PDA broth medium and incubated at 28ºC for one week. The fungal suspension of each fungus was adjusted to 3×10^8 propgules mL^{-1} (Abd-El-Khair and Haggag, 2007).

2.4 Pot experiment design

Three experiments were conducted in a screen house at Plant Pathology Department, NRC for studying the nematicidal activity of the tested bio-control agents against *M. incognita* infecting sugar beet. Pots (30cm diameter), containing 3 kg of a solarized mixture of sandy loam soil (1:1, sand: loam), were arranged in a completely randomized design on a bench and maintained at 20°C±5°C. After seed germination, 21 days from sowing, each pot was thinned to one plant. The pots were inoculated in four holes made around the plant with 1,000 newly hatched J_2 of *M. incognita* per pot. Four replicated pots were used per each treatment as well as the untreated control. The plants were irrigated regularly.

2.4.1 Pot experiment I

This experiment was conducted to study the nematicdal activity of *B. subtilis*, *B. megaterium, B. pumilus, P. fluorescens*, *T. harzianum* and *T. viride* each as single treatment at rate of 20 ml pot^{-1} against *M. incognita* infecting sugar beet. The experiment was divided to 7 treatments as follows; 1) *B. subtilis*; 2) *B. megaterium*; 3) *B. pumilus*; 4) *P. fluorescens*; 5) *T. harzianum*; 6) *T. viride*; 7) *T. vierns* and 8) nematode

only as control. At the same time of nematode inoculation, the sugar beet plants were treated with each bio-control agent inoculum suspension at the tested rates at four holes around the plant.

2.4.2 Pot experiment II

This experiment was conducted to study the nematicdial activity of application of *T. vierns* and *B. pumilus* each as single treatments at different doses of 10, 20 and 30 mL pot^{-1} against *M. incognita*. The experiment was divided to 8 treatments as follows; 1) *T. vierns* (10 mL pot^{-1}); 2) *T. vierns* (20 mL pot^{-1}), 3) *T. vierns* (30 pot^{-1}); 4) *B. pumilus* (10 mL pot^{-1}); 5) *B. pumilus* (20 mL pot^{-1}); 6) *B. pumilus* (30 pot^{-1}); 7) Micronema® and 8) nematode only. At the same time of nematode inoculation, sugar beet plants were treated with each bio-control agent inoculum suspension at the tested rates in four holes around the plant.

2.4.3 Experiment III

This experiment was conducted to study effect of the number of times of the treatment (one and two times) of *T. vierns* and *B. pumilus* each as single treatment against *M. incognita* infecting sugar beet. All bio-control agents were tested two times with a 15-day interval between each treatment(time) at the rate of 20 mL pot^{-1}. The experiment was divided to six treatments as follows; 1) *T. vierns* (one time pot^{-1}); 2) *T. vierns* (two times pot^{-1}); 3) *B. pumilus* (one time pot^{-1}); 4) *B. pumilus* (two times pot^{-1}); 5) Micronima®; and 6) nematode only. At the same time of inoculation, sugar beet plants were treated with 20 mL pot^{-1} of each bacterial suspension around the plant. After 15 of the first treatment, the treatments were repeated with 20 mL pot^{-1} of each bio-control agent inoculum separately.

After 6 months of nematode inoculation, effects bio-control agents against the nematode parameters of *M. incognita* such as numbers of juveniles (J_2) in soil; galls and egg-masses on the entire root system of sugar beet plants were recorded. For J_2 in soil, the sugar beet plants were carefully uprooted and number of J_2 in the soil was extracted using a sieving and decanting technique and were counted under a light microscope. Numbers of galls and egg-masses on the entire root system of sugar beet were also recorded (Barker, 1985).

Effects of bio-control agents on the plant growth and yield parameters such as shoot length in cm, shoot weight (fresh and dry) in g, fresh root (Tuber) weight in g per plant were recorded after 6 months of nematode inoculation. Total soluble solids percent (TSS%) was measured in fresh weight of roots by using hand refractometer.

2.5 Statistical analysis

Statistical analysis of the obtained data was performed through Computer Statistical Package (COSTAT) User Manual Version 3.03, Barkley Co., a computer-based program. Analyses were including analysis of variance (ANOVA) procedures which reported by Snedecor and Cochran (1977). Duncan's Multiple Range Test (DMRT) was applied to compare the means for each treatment at 5% level of probability.

3 Results

3.1 Experiment I

Results in Table 1 revealed that the tested bio-control agents *viz*., *B. subtilis*, *B. megaterium, B. pumilus, P. fluorescens, T. harzianum*, *T. viride* and *T. vierns* reduced the number of galls in the range of 13 to 19, equaling to 60%-74% reduction ,compared to 48 galls in nematode only treatment ,respectively. *T. vierns* highly reduced the galls number by 74%, followed by *B. pumilus* (73%), *T. viride* (73%), *B. subtilis* (71%), *T. harzianum* (71%), *B. megaterium* (69%) and *P. fluorescens* (60%), respectively (Table 1).

Table 1 Effect of some bio-control agents on nematode parameters of *M. incognita* on sugar beet cv. Gazelle

Bio-control agents	Averages of *M. incognita* parameters					
	Galls			Egg-masses		
	Count		Red. %	Count		Red. %
	No.	Log_{10}		No.	Log_{10}	
Bacillus subtilis	14.0	1.15**d**	71	11.0	1.04**b**	65
Bacillus megaterium	15.0	1.18**c**	69	10.0	1.00**bc**	68
Bacillus pumilus	13.0	1.11**e**	73	8.0	0.90**c**	74
Pseudomonas fluorescens	19.0	1.28**b**	60	12.0	1.08**b**	61
Trichoderma harzianum	14.0	1.15**d**	71	8.0	0.90**c**	74
Trichoderma viride	13.0	1.11**e**	73	8.0	0.90**c**	74
Trichoderma vierns	12.3	1.10e	74	6.3	0.80e	80
Nematode only	48.0	1.68**a**	-	31	1.49**a**	-

Note: Means are averages of four replicates. Means followed by different letter (s) are significantly different according to Duncan's Multiple Range Rest at $p \leq 0.05$.

The number of egg-masses as the percentages reduction was in the range of 61.3% to 74.2%, compared to nematode only treatment, respectively. *T. vierns* caused the highest percentages reduction of egg-masses numbers (80%), followed by *B. pumilus*, *T. harzianum* and *T. viride* that caused by 74%, *B. megaterium* (69%) and *B. subtilis* (65%) with significant ($p \leq 0.05$) differences were recorded among treatments (Table 1).

The tested bio-control agents enhanced the length of shoots of sugar beet in the range of 39 to 48 cm, compared to 39 cm in nematode only treatment. *B. subtilis* achieved the highest shoot length (48 cm), followed by *B. megaterium* (44.5 cm), *T. vierns* (44.5 cm), *B. pumilus* and *T. harzianum* (43.3 cm), *T. viride* (41.8 cm) and *P. fluorescens* (40.5 cm), respectively (Table 2).

Table 2 Effects of some bio-control agents on growth parameters of sugar beet cv. Gazelle infected by *M. incognita*

Bio-control agent	Averages of growth and yield parameters						Total soluble solids (TSS %)
	Length of shoots, cm		Weight of shoots, g		Weight of root (Tuber), g		
	Length, cm	Inc., %	Weight, g	Inc., %	Weight, g	Inc., %	
Bacillus subtilis	48.0**a**	23	153.0*a*	112	195.5**a**	136	17.8**a**
Bacillus megaterium	44.5**b**	14	113.6**b**	58	102.7**e**	24	16.8**bc**
Bacillus pumilus	43.8**bc**	12	98.3**e**	36	109.7**b**	32	16.8**bc**
Pseudomonas fluorescens	40.5**e**	4	90.3**f**	25	85.9**f**	4	16.0**cd**
Trichoderma harzianum	43.3**c**	11	103.2**d**	43	106.4c	28	17.0**bc**
Trichoderma viride	41.8**d**	7	102.5**e**	42	105.8**e**	28	16.5**cd**
Trichoderma vierns	44.5**d**	14	104.0c	44	107.2**d**	29	17.0**bc**
Nematode only	39.0**f**	-	72.1**g**	-	82.9**g**	-	15.5**d**

Note: Means are averages of four replicates.Means followed by different letter(s) are significantly different according to Duncan's Multiple Range Test at $p \leq 0.05$.

The treatments increased the weight of shoots in the range of 90.3 to 153.0 g, compared to 72.1 g in nematode only treatment. *B. subtilis* achieved the highest shoot weight (153.0 g), followed by *B. megaterium* (113.6 g), *T. vierns* (104.0 g), *T. harzianum* (103.2 g), *T. viride* (102.5 g), *B. pumilus* (98.3 g) and *P. fluorescens* (90.3 g), respectively (Table 2).

The weights of tuber were in the range of 85.9 to 195.5 g, compared to 82.9 g in nematode only, where *B. subtilis* caused the highest root weight (195.5 g), followed by *B. pumilus* (109.7 g), *T. vierns* (107.2 g), *T. harzianum* (106.4 g), *T. viride* (105.8 g), *B. megaterium* (102.7 g), and *P. fluorescens* (85.9 g), respectively (Table 2).

The treatments also increased the percentages of total soluble solids (TSS%) in the range of 16.0% to 17.8%, compared to 15.5% in nematode only treatment, where *B. subtilis* achieved the highest percentages TSS (17.8%), followed by *T. vierns* (17.0%), *T. harzianum* (17.0%), *B. pumilus* (16.8%), *B. megaterium* (16.8%), *T. viride* (16.5%) and *P. fluorescens* (16.0%), respectivily (Table 2).

3.2 Experiment II

It is clear that the nematicidal effect of *B. pumilus* or *T. vierns* against J_2 numbers increased with the rate increase. Results showed that the averages of J_2 number in soil of sugar beet were in the range of 86.7 to 343.3 J_2/200 soil with *B. pumilus* and *T. vierns* when treated at the rates of 10, 20 and 30 mL pot^{-1}, respectively compared to averages of 53.3 and 1615 J_2/200 soil with Micronema® and nematode only. In other words, the two bio-control agents significantly reduced the J_2 number by 79%-95%, compared to 97% with Micronema®. *B. pumilus* has highly reduction to J_2 reaching to 81%-95%, where significant differences were recorded among applied rates, compared to the reduction of 79%-86% with *T. vierns* with no significant differences (Table 3).

Galls numbers in roots of sugar beet ranged from 10.0 to 17.3 galls for *B. pumilus* and *T. vierns* when applied at doses of 10, 20 and 30 ml pot^{-1}, compared to 9.0 and 44.7 galls with Micronema® and nematode only treatment, respectively. This means that the treatments reduced the galls numbers by 61%-78%, compared to 80% with Micronema®. *B. pumilus* treatment also highly reduced the gall numbers by 69%-78% with no significant differences among rates were recorded, compared to 61% to 73% reduction with *T. vierns*, where significant differences were recorded between rate of 10ml and Micronema and nematode alone (Table 3).

Table 3 Nematicidal effect of *Trichoderma vierns* and *Bacillus pumilus*, each as single treatment at different rates, against nematode parameters of *M. incognita* on sugar beet cv. Gazelle

Bio-control agent	Rate	Averages of *M. incognita* parameters								
		J_2 in soil			Galls			Egg-masses		
		Count		Red. %	Count		Red. %	Count		Red. %
		No.	Log_{10}		No.	Log_{10}		No.	Log_{10}	
T. virens	10	343.3	2.53**b**	79	17.3	1.24**b**	61	12.0	1.05**b**	64
	20	236.7	2.37**b**	87	14.0	1.14**bc**	69	10.3	1.00**bc**	69
	30	226.7	2.35**b**	86	12.3	1.09**bc**	73	6.3	0.79**cd**	81
B. pumilus	10	306.7	2.48**b**	81	13.7	1.13**bc**	69	6.7	0.82**cd**	80
	20	146.7	2.14**c**	91	12.7	1.09**bc**	72	6.7	0.82**cd**	80
	30	86.7	1.92**d**	95	10.0	1.00**bc**	78	4.3	0.62**d**	87
Micronema®		53.3	1.72**e**	97	9.0	0.94**c**	80	4.0	0.59**d**	88
Nematode only		1615	3.21**a**	-	44.7	1.65**a**	-	33.3	1.52**a**	-

Note: Means are averages of four replicates. Means followed by different letter (s) are significantly different according to Duncan's Multiple Range Test at $p \leq 0.05$.

The nematicidal activity of the tested bio-control agents increased with rate increase (Table 3). The averages of egg-mass numbers ranged from 4.3 to 12.0 egg-masses with *T. vierns* and *B. pumilus* when tested at the tested rates, compared to averages of 4.0 and 33.3 with Micronema® and nematode only, respectively. This means that the treatments reduced the egg-masses by 64 to 87%, while by 97% with Micronema®. *B. pumilus* treatment highly reduced the egg-masses number by 80%-87%, compared to reduction percentages ranged from 64% to 81% with *T. vierns*. Significant differences were recorded among bio-control agents and nematode only, while no significant differences were recorded among bio-control agents.

It is revealed that the shoot length of sugar beet increased with increasing the tested rates of bio-control agents (Table 4). The treatments by *T. vierns* and *B. pumilus* enhanced the length of shoot of sugar beet in the ranges of 42.7-60.7 cm, compared to length of 57.7 and 39.0 cm with Micronema® and nematode only, respectively. This means that the percentages shoot length increase ranged from 10% to 56% with *T. vierns* and *B. pumilus*, compared to 48% with Micronema®. *T. vierns* significantly increased the averages of shoot length by 31%-56%, followed by *B. pumilus* which increased the plant length by 10%-42%.

Table 4 Effects of *Trichoderma vierns* and *Bacillus pumilus*, each as single treatment at different rates on growth parameters of sugar beet cv. Gazelle

Bio-control agent	Rate	Averages of growth and yield parameters								
		Length of shoots		Weight of shoots				Root (Tuber) weight		Total soluble solids (TSS %)
				Fresh		Dry				
		Length, cm	Inc., %	Weight, g	Inc., %	Weight, g	Inc., %	Weight, g	Inc., %	
T. virens	10	51.0**b**	31	199.3**b**	39	19.8**abc**	64	158.0**b**	34	15.7**ab**
	20	60.3**a**	55	291.7**a**	104	24.6**ab**	103	300.0**b**	154	16.0**ab**
	30	60.7**a**	56	306.0a	114	25.0**a**	107	315.7**a**	168	15.3**ab**
B. pumilus	10	42.7**c**	10	178.3b**c**	25	17.1**c**	41	161.7**b**	37	15.7**ab**
	20	52.7**ab**	35	181.7b**c**	27	18.7**c**	55	170.3**b**	44	17.0**ab**
	30	55.3**ab**	42	183.3**b**	28	19.4**bc**	60	172.3**b**	46	17.3**a**
Micronema®		57.7**ab**	48	269.3**a**	88	20.8**ab**	72	300.0**a**	154	17.3**a**
Nematode only		39.0**c**	-	143.0**c**	-	12.1**d**	-	118.0**b**		14.7**b**

Note: Means are averages of four replicates. Means followed by different letter(s) are significantly different according to Duncan's Multiple Range Test at $p \leq 0.05$.

Results also revealed that the average of shoot fresh weight was increased with increasing the tested rates. *T. vierns* and *B. pumilus* treatments also enhanced the average of shoot fresh weight of sugar beet in the ranges of 178.0-306.0 g, compared to 269.3 and 143.0 g with Micronema® and nematode only treatment, respectively.

In other words, the percentages weight increase ranged from 25%-114%, compared to 88% in Micronema®. *T. vierns* significantly increased the average of shoot fresh weight by 39% to 114%, while *B. pumilus* increased the weight by 25% to 28%.

The dry weight of shoot increased by increasing the tested rate (Table 4). The dry weight of sugar beet shoot ranged from 17.1-25.0 g with *T. vierns* and *B. pumilus*, compared to 20.8 and 12.1g with Micronema® and nematode only, respectively. The treatments increased the shoot dry weight by 41% to 107%, compared to 72% with Micronema® and nematode only treatment. *T. vierns* highly increased the shoot dry weight in the ranges of 64% to 107%, while the increase was ranged from 41% to 60% with treatment of *B. pumilus*.

The tuber weight increased by increasing the applied dose (Table 4). Results revealed that *T. vierns* and *B. pumilus* enhanced the weight of sugar beet tuber in the ranges of 158.0 to 315.7 g, compared to 300.0 and 118.0 g with Micronema® and nematode only, respectively. The percentages tuber weight increase ranged from 34% to 168%, compared to 154% with Micronema®. *T. vierns* highly increased the tuber weight in the ranges of 34% to 168%, compared to range of 37% to 46% with *B. pumilus*.

T. vierns and *B. pumilus* increased the percentages of total soluble solids (TSS %) in treated sugar beet plants in the range of 15.7 to 17.3%, compared to 17.3% and 14.7% in Micronema® and nematode only treatment, respectively. *T. vierns* increased the TSS% in the ranges of 15.3% to 15.7%, while it was in the range of 15.7% to 17.3% with *B. pumilus* (Table 4).

3.3 Experiment III

Results revealed that the averages of J_2 number in soil were ranged from 65.0 to 238.0 J_2/200 soil with *T. vierns* and *B. pumilus* when applied at both times of treatment, compared to averages of 53.7 and 1617 J_2/200 soil with Micronema® and nematode only, respectively. The tested bio-control agents significantly reduced the J_2 number by 86%-96%, compared to 97% with Micronema®. *B. pumilus* has highly reduction to J_2 ranging from 91% and 96%, compared to 86% and 96% reduction with *T. vierns* at one and second time of application, respectively (Table 5).

Table 5 Nematicidal effects of *Trichoderma vierns* and *Bacillus pumilus*, each as single treatment as influenced by the no. of times of treatments, against nematode parameters of *M. incognita* on sugar beet cv. Gazelle.

Bio-control agent	No.of times	Averages of *M. incognita* parameters								
		J_2 in soil			Galls			Egg-masses		
		Count		Red., %	Count		Red., %	Count		Red., %
		No.	Log_{10}		No.	Log_{10}		No.	Log_{10}	
T. virens	1 time	238.0	2.37**b**	86	14.3	1.15**b**	68	10.3	1.00**b**	69
	2 time	65.0	1.81**d**	96	8.7	0.90**c**	81	3.7	0.55**d**	89
B. pumilus	1 time	147.0	2.15**c**	91	12.7	1.09**bc**	72		0.83b**c**	79
	2 time	73.3	1.85**d**	96	11.0	1.00**bc**	75	6.3	0.76c**d**	81
Micronema®		53.7	1.72**d**	97	9.3	0.96**bc**	79	4.1	0.63**cd**	87
Nematode only		1617	3.22**a**	-	44.7	1.65**a**	-	33.3	1.52**a**	-

Note: Means are averages of four replicates. Means followed by different letter(s) are significantly different according to Duncan's Multiple Range Test at $p \leq 0.05$.

The galls numbers in roots of sugar beet were in the range of 8.7 to 14.3 galls with *T. vierns* and *B. pumilus* when applied at both times, compared to 9.3 and 44.7 galls with Micronema® and nematode only, respectively. The tested bio-control agents significantly reduced the galls number by 68%-81%, compared to 79% with Micronema®. *B. pumilus* highly reduced the galls number by 72% and 75%, while *T. vierns* reduced the gall numbers by 68% and 81% at the first and second time of application, respectively (Table 5). The averages of egg-masses number ranged from 3.7 to 10.3 egg-masses with *T. vierns* and *B. pumilus* when at both times, compared to averages of 4.1 and 33.3 with Micronema® and nematode only treatment, respectively. The tested bio-control agents significantly reduced the egg-masses number by 69% to 89%, while it was 87% with Micronema®. *B. pumilus* treatment highly reduced the egg-masses number by 79% to 81%, compared to the

percentages of reduction ranging from 69% to 89% with *T. vierns* (Table 5).

T. vierns and *B. pumilus* enhanced the length of shoot in the ranges of 52.7 to 81.0 cm when applied at both times, compared to 58.0 and 39.3 cm in Micronema® and nematode only treatment, respectively. The percentages of shoot length increase ranged from 34% and 106%, compared to 48% with Micronema®. *T. vierns* highly increased the average of shoot length by 55% to 106%, while *B. pumilus* increased the length percentages by 34% at the first and second times of application, respectively (Table 6).

Table 6 Effects of *Trichoderma vierns* and *Bacillus pumilus*, each as single treatment as influence by no. of times of treatment, on growth parameters of sugar beet cv. Gazelle

Bio-control agent	No.of times	Averages of growth and yield parameters								
		Length of shoots		Weight of shoots				Root (Tuber) weight		Total soluble solids (TSS %)
				Fresh		Dry				
		Length, cm	Inc., %	Weight, g	Inc., %	Weight, g	Inc., %	Weight, g	Inc., %	
T. virens	1 time	60.7**b**	55	292.0**a**	103	24.7**a**	102	301.0**ab**	152	16.0**ab**
	2 time	81.0**a**	106	300.0**a**	109	25.2**a**	107	342.0**a**	187	16.0**ab**
B. pumilus	1 time	52.7**c**	34	180.0**bc**	25	18.7**b**	53	170.3**cd**	43	17.0**ab**
	2 time	52.7**c**	34	206.3**b**	44	22.9**ab**	88	223.3**bc**	88	17.7**a**
Micronema®		58.0b**c**	48	270.7**a**	88	21.0**ab**	72	301.0**ab**	152	17.3**ab**
Nematode only		39.3**d**	-	143.7**c**	-	12.2**c**	-	119.0**d**	-	14.7**b**

Note: Means are averages of four replicates. Means followed by different letter (s) are significantly different according to Duncan's Multiple Range Test at $p \leq 0.05$.

The treatments of *T. vierns* and *B. pumilus* enhanced the averages of shoot fresh weight in the range of 180.0 to 300.0 g when tested at both times, compared to 270.7 and 143.7 g with Micronema® and nematode only treatment, respectively. The treatments increased the shoot fresh weight by 25% to 109%, compared to 88% in Micronema®. *T. vierns* highly increased the average of shoot fresh weight by 103% and 109%, while *B. pumilus* increased the weight percentages in the range by 25% and 44% at the first and second time of application, respectively (Table 6).

The dry weight of sugar beet shoot ranged from 18.7 to 25.2 g with *T.vierns* and *B. pumilus* when applied at both times, compared to 21.0 and 12.2g with Micronema® and nematode only treatment, respectively. The treatments increased the shoot dry weight by 88% to 107%, compared to 72% with Micronema®, compared to control. *T. vierns* highly increased the average of shoot dry weight by 102% and 107%, while *B. pumilus* increased the weight percentages in the range by 53% and 88% at the first and second time of application, respectively (Table 6).

Results revealed that *T. vierns* and *B. pumilus* enhanced the tuber weight in the range of 170.0 to 342.0 g when applied at both times, compared to 301.0 and 119.0 g with Micronema® and nematode only treatment, respectively. The percentages tuber weight increases were in the range 43% to 187%, compared to 152% with Micronema® (Table 6). *T. vierns* and *B. pumilus* also increased the percentages total soluble solids (TSS %) in the range of 16.0% to 17.7% when treated at both times, compared to 17.3% and 14.7% in Micronema® and nematode only treatment, respectively. *T. vierns* increased the TSS% by 16.0%, while it was in the range of 17.0% to 17.7% with *B. pumilus* (Table 6).

4 Discussion

Survey results in Egypt revealed that the *Meloidogyne incognita* was the common plant parasitic nematodes in the rhizospheres of sugar beet (Massih, 1985; El-Sherif and El-Deen, 2010). Our results in experiment I indicated that showed *B. subtilis*, *B. megaterium*, *B. pumilus*, *P. fluorescens*, *T. harzianum* and *T. viride* had nematicidal activity against *M. incognita* as well as enhancement the growth parameters of sugar beet. Results of experiment II showed that *T. vierns* and *B. pumilus* had nematicidal activity against nematode parameters of *M. incognita*, when applied at doses of 10, 20 and 30 mL pot^{-1}, but less

than Micronema. Results also showed that the nematicidal effects of *T. vierns* and *B. pumilus* increased with increasing the applied rates. *B. pumilus* has highly nematicidal activity against *M. incognita* than *T. vierns*. The two-tested bio-control agents were more effective in reducing J_2 in soil and egg-masses and galls on roots, respectively. Results, also, revealed that the *T. vierns* and *B. pumilus* enhanced the growth parameters of sugar beet as well as TSS% content. *T. vierns* was highly effective in enhancement of growth parameters of sugar beet more than *B. pumilus*. Results of experiment III revealed that *T. vierns* and *B. pumilus* when used at two times of treatment were more effective in reducing the nematode parameters of *M. incognita* than at one time. The differences in nematode reduction were clear when used twice than one.

These results are in agreement with those recorded by Viterbo et al. (2002). They reported that the use of specific mycolytic soil micro-organisms to control plant pathogens is an ecological approach to overcome the problems caused by standard chemical methods of plant protection. The ability to produce lytic enzymes is a widely distributed property of rhizosphere-competent fungi and bacteria. Due to higher activity of *Trichoderma*, as bio-control agent due to the lytic enzymes production, compared to other soil microorganism that produce the same class of enzymes, therefore, *Trichoderma* has been widely used as antagonistic fungal agents against several pests as well as plant-growth enhancers. Myco-parasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolies and induction of plant defence are typical biocontrol actions of these fungi (Dababat and Sikora, 2007; Verma et al., 2007; Gajera et al., 2013). Mohammed et al. (2008) reported that *T. viride* application significantly increased the growth components of potato. Shaban and El-Bramawy (2011) also suggested that the *Rhizobium* spp. and *Trichoderma* spp. can be used as biological control of some soil-borne fungal diseases causing significant yield losses in legumes. This suggests that the microbial counts play an important role in increasing antagonistic effects of the microfauna against soil-borne diseases (Panneerselvan and Saravanamutha, 1996; Harman, 2006).

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