Impact of microbial agents on *Meloidogyne incognita* management and morphogenesis of tomato

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ABSTRACT

A greenhouse experiment was conducted to evaluate the efficacy of certain microbial agents against *Meloidogyne incognita* infesting tomato plants (cv. super strain B). The treatments were the antagonistic bacteria *Bacillus subtilis* and *Bacillus thuringiensis*, the antagonistic fungus *Paecilomyces lilacinus* and mycorrhizal fungi *Glomus intraradices* and *Glomus macrocarpium* which were compared with the synthesis nematicides Oxamyl and Cadusafos. The *Paecilomyces lilacinus* product was the best treatment in suppressing the root-knot populations in the soil with (85.2%), followed by those with *B. subtilis* and *B. thuringiensis* with 82.6 and 80.5% reduction, respectively. Also, *P. lilacinus* increased the shoot length and fresh weight of the root system by 229.0% and 476.46%, respectively. The most effective treatment in reducing root galls and egg masses of the nematode was Oxamyl. *Bacillus thuringiensis* increased shoot weight and root length and was the most effective treatment. *Glomus macrocarpium* was the least effective treatment as galls and egg masses. *Glomus macrocarpium* produced the lowest increase in root length and *B. subtilis* the lowest increase in root fresh and dry weights.

Key words: *Bacillus subtilis*, *Bacillus thuringiensis*, *Glomus* sp., *Meloidogyne incognita*, *Paecilomyces lilacinus*, *Solanum lycopersicum*

INTRODUCTION

Tomato *Solanum lycopersicum* Mill. is an important vegetable crop in Egypt; it is attacked by several destructive pests and diseases that cause severe damage. One of the important pests of tomatoes is the plant-parasitic nematodes, especially root-knot nematodes. Among the root-knot nematodes, *Meloidogyne javanica* (Treub) Chitw., *M. incognita* (Kofoid and White) Chitw., *M. arenaria* (Neal) Chitw., and *M. hapla* Chitw. are of major agronomic importance, being responsible for at least 90% of all damage caused by nematodes (Castagnone-Sereno, 2002), as well as nematodes cause an estimated $118b annual losses to world crops (Atkinson *et al*., 2012). In a survey the root-knot nematodes have been found the most predominant group targeted by 48% of global nematicides use across crops (Haydock *et al*., 2006) .A wide variety of soil organisms are known as predators or parasites of plant-parasitic nematodes (Coleman and Crossley, 1996) and several attempts have been made to use antagonistic fungi to control root-knot nematodes (Sharon *et al*., 2001). Arbuscular mycorrhizal fungi (AMF) are widely used in nurseries as they enhance nutrient and water availability. They have also been to improve the growth of tomato plants (Saad *et al*., 2011; Saad *et al*., 2012).

Paecilomyces lilacinus (Thom) Samson is a soil-inhabiting fungus that is capable of parasitizing nematode eggs, juveniles and females thus reducing soil population densities of plant parasitic nematodes. Strains of this fungus have been formulated for use in controlling nematodes in several countries (EPA, 2005; Kiewnick and Sikora, 2003, 2006). *Bacillus thuringiensis* Berliner produces parasporal crystalline proteinaceous inclusions. Most of these crystal proteins or δ-endotoxins are toxic to larvae of lepidopteran, dipteran or coleopteran insects (Knowles and Dow, 1993). Strains exhibiting toxicity against pathogenic protozoa, mites and nematodes have also been reported (Fettelson *et al*., 1992). Pioneering studies of *B. thuringiensis* (Bt) crystal

(*Cry*) proteins that intoxicate free-living nematodes have raised the hypothesis that nematicidal Bt *Cry* proteins might be able to intoxicate plant parasitic nematodes and provide biocontrol against these pests (Wei *et al*., 2003). Among the plant growth-promoting rhizobacteria (PGPR), Pseudomonas and Bacillus are the genera most commonly described as having PGPR but many other taxa also contain PGPR (Barea *et al*., 2005). Some strains of *Bacillus subtilis* have exhibited enormous potential as biocontrol agents in the management of root-knot nematodes (Karanja *et al.*, 2007; Khalil *et al*., 2012).

Root-knot nematodes are among the most difficult crop-pests to be controlled. Several methods are known to manage the root-knot nematode. They include the use of nematicides, organic amendments, resistant cultivars, soil solarization and biological control, which have been used with different levels of success on tomatoes (Randhawa *et al*., 2001; Sakhuja and Jain, 2001). Due to the adverse effects of pesticides on the environment and human health, this investigation aimed to evaluate the performance of certain biological-based products, in comparison with synthetic nematicides to control *M. incognita* in tomato.

MATERIALS AND METHODS

The biological-based products tested were: i) Stanes sting®, containing 1×10^9 cell/mL of the bacterium *Bacillus subtilis* (Ehrenberg) Cohn (Stanes company, India); ii) Bio-Nematon®, containing 1×10^9 cfu/mL of the fungus *Paecilomyces lilacinus* (Stanes company, India); iii) Dipel 2x® containing 32,000 iu/mg of bacterium *B. thuringiensis* var. *kurstaki* (Valent company, Canada). Stanes sting® and Bio-Nematon® were diluted at a rate of 10 mL/litre water and every plant received 50 mL of the suspensions. Dipel 2x® was used at a rate of 5 kg/feddan equivalent to 1.3 g/m^2 (Radwan, 1999). Two species of arbuscular mycorrhizal fungi (AMF) were also tested: a strain of *Glomus intraradices* from the Hanover University, Germany obtained from the Agricultural Botany Department, Faculty of Agriculture, Saba Basha, Alexandria University, and a strain of *Glomus macrocarpum* from the Gottingen University, Germany obtained from the Soil and Agriculture Chemistry Department, Faculty of Agriculture, Saba Basha, Alexandria University. The obtained culture media of both AMF were inoculated to tomato at the rate of 10 g/plant. The performance of these products and AMFs was compared with that of the synthetic nematicides *i*) Rugby® 10% G (a.i. cadusafos, *S*, *S*-di-sec-butyl *O*-ethyl phosphorodithioate) at a rate of 0.6 g a.i./m^2 and *ii*) Vydate®10% G (a.i. Oxamyl,*N*,*N*-dimethyl-2-methyl carbamoyloxyimino-2-(methylthio) acetamide] at a rate of 0.5 g a.i./m^2. Non treated plots served as a control.

The experiment was conducted in a sandy soil greenhouse infested by *M. incognita* and cropped to tomato cv. Super strain B. Identification of the species of the root-knot nematode (*Meloidogyne incognita*) was done by using the perineal patterns method according to Taylor and Nelscher (1974). The greenhouse contains a completely separated cement basins which was divided into 24 plots and every plot area was 3 m^2, each having three rows of five plants each spaced 30 cm within the row. The plots were arranged according to a randomized complete block design (RCBD) and each treatment was replicated three times.

Four weeks old tomato seedlings were transplanted. During the course of the experiment, irrigation and fertilization were made via a drip system. Soil samples were collected before transplanting and at the termination of the experiment (3 months after transplanting) according to Barker (1985). Five sub-samples were collected from 5 to 20 cm depth of each plot to form a composite sample of approximately 1 kg, which was then thoroughly mixed. Second stage juveniles (J2) of *M. incognita* were extracted from a 250g sub-sample soil of each plot, using the sieving and Baermann plates' technique (Ayoub, 1980), and counted under a stereomicroscope. At termination of the experiment, the plants were uprooted and shoot length, shoot weight; root length, root weight, galls number and egg masses per 5 g root system were also estimated. To count the egg masses, it were stained by dipping the roots for 15 minutes in an aqueous solution of phloxine B (0.15 gm /L water) and then washed with running tap water to remove excess stain

(Holbrook *et al*., 1983). The reduction in the nematode population density expressed as a percentage was calculated at the end of the experiment according to Henderson and Tilton's (1955) equation. The data were subjected to the analysis of variance and means compared according to the least significant difference (LSD) at the 5% level of probability, using Costat program (1988).

RESULTS

P. lilacinus was the most effective treatment to suppress the soil population density of the nematode with 85.2% reduction followed by *B. subtilis, B. thuringiensis* and *G. macrocarpum*, respectively (Table 1). Cadusafos and Oxamyl were the least effective as they recorded 67.6 and 76.8% reduction, respectively. Data in table 2 indicated the activity of tested treatments on galls and egg masses / 5g roots while oxamyl and *P. lilacinus* were the highest effective treatments on root galls achieving 72.73 and 66.67% reduction, respectively, without any significant differences.

Table 1. Initial and final population densities of *M. incognita* juveniles on tomato and percent reduction after the application of treatments plants under greenhouse conditions.

Treatment	Initial Population /250 g soil	Final Population /250g soil	Reduction %
Oxamyl	5830^{e}	2700^{d}	76.84
Cadusafos	7930^{bc}	5120^{b}	67.65
G. macrocarpum	6838.67^{d}	2716.67^{d}	80.14
G. intraradices	9360^{a}	3844.67^{c}	79.42
B. thuringiensis	5380^{f}	2102.67^{f}	80.46
B. subtilis	7773.33^{c}	2697.67^{d}	82.65
P. lilacinus	8153.33^{b}	2405^{e}	85.22
Untreated check	3383.33^{g}	6750^{a}	-

Means followed by different letter(s) within a column are significantly different using LSD at P = 0.05

While, the least effective treatment was *G. macrocarpum* which provided only 42.86% reduction. Respecting, the egg masses parameter oxamyl, *P. lilacinus, B. thuringiensis* and *B. subtilis* were the superior treatments which suppressed egg masses with values of 77.41, 75.97, 74.97 and 71.76 reduction percent without any differ from each other in significance, consecutively. *G. macrocarpum* and Cadusafos showed the less performance with values of 42.86 and 40.1% reduction and without significant, respectively.

Table 2. The efficacy of evaluated treatments on nematode galls and egg masses on tomato plants.

Treatment	Galls/ 5 g roots	Reduction %	Egg mass/5 g roots	Reduction %
Oxamyl	60^{e}	72.73	68^{d}	77.41
Cadusafos	93^{cd}	57.73	172^{b}	42.86
G. macrocarpum	125.7^{b}	42.86	180.33^{b}	40.1
G. intraradices	96^{cd}	56.36	116^{c}	61.46
B. thuringiensis	87.67^{d}	60.15	75.33^{d}	74.97
B. subtilis	102^{c}	53.64	85^{d}	71.76
P. lilacinus	72.33^{e}	66.67	72.33^{d}	75.97
Untreated check	220^{a}	-	301^{a}	-

Values are mean of three replicated plots. Means followed by different letter(s) within a column are significantly different using LSD at P = 0.05

On the other hand, *P. lilacinus* and *B. subtilis* gave the greatest shoot length increase with same significant achieving values 229.03 and 217.19%, respectively (Table 3). Meanwhile, *B. subtilis* did not differ from *G. intraradices* . The bacterium *B. thuringiensis* was the supreme treatment on both shoot fresh and dry weight with 1487.3 and 1391.7% increase, respectively. While, oxamyl recorded the least values of both fresh and dry shoot system weights 381.66 & 382.50% increase, respectively. *Bacillus thuringiensis, G. intraradices* and *B. subtilis* increased root length with the same significant and with values of 79.23, 69.78 and 56.59%, respectively (Table 4). Furthermore, *P. lilacinus, B. thuringiensis*, cadusafos, *G. macrocarpum* and *G. intraradices* recorded the greatest increase of root fresh weight without significant differences. Beyond, *Bacillus thuringiensis*, cadusafos, *G. macrocarpum, G. intraradices* and *P. lilacinus* were the most effective treatments to increase root dry weight giving 732.13, 697.96, 659.86, 589.12 and 557.82%, respectively, with the same significance.

Table 3. Effect of used treatments on both tomatoe shoot system length (cm) and weight (g).

Treatment	Shoot system					
	Length (cm)	Increase %	Fresh weight (g)	Increase %	Dry weight (g)	Increase %
Oxamyl	84.33^{cd}	172.03	88^{e}	381.66	17.37^{d}	382.5
Cadusafos	77.33^{d}	149.45	118.33^{de}	547.67	39^{b}	983.33
G. macrocarpum	86.67^{cd}	179.58	140^{cd}	666.28	30.3^{c}	741.67
G. intraradices	90^{bc}	190.32	190^{b}	939.96	35.8^{bc}	894.44
B. thuringiensis	78.33^{d}	152.68	290^{a}	1487.30	53.7^{a}	1391.67
B. subtilis	98.33^{ab}	217.19	170^{bc}	830.49	34.17^{bc}	849.17
P. lilacinus	102^{a}	229.03	165^{bc}	590.03	34.3^{bc}	852.78
Untreated check	31^{e}	--------	18.27^{f}	--------	3.6^{e}	--------

Values are mean of three replicated plots. Means followed by different letter(s) within a column are significantly different using LSD at P = 0.05

However, *B. subtilis* recorded the least activity towards both root fresh and dry weight with values of 206.46 and 246.94% increase, respectively.

DISCUSSION

Our results indicate that using microbial agents suppressed the root-knot nematodes and resulted in positive changes in plant growth. *Paecilomyces lilacinus* was the best treatment which reduced root galls and egg masses in tomato plants under greenhouse conditions (Khalil *et al.*, 2012). Moreover, the activity of *P. lilacinus* attributed to ability to infect eggs, juveniles and females of *M. javanica* by direct hyphal penetration (Khan *et al*., 2006). Moreover, *P. lilacinus* contains protease and chitinase which play an important role in the degradation of the egg shell (Khan *et al*., 2004). Meanwhile, Khan *et al*. (2012) recorded an enhancement in growth and yield of eggplants with biocontrol agents *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Trichoderma harzianum* as a result to suppress galls formation and egg masses. Also, the using of bioproducts showed decreasing in the second stage juveniles and root galls on tomatoes. (Radwan *et al.*, 2012).

Bacillus subtilis and *Bacillus thuringiensis* are considered the most used bacteria against plant parasitic nematodes and this agreement with those obtained by Dawar *et al.* (2008) who recorded that *B. subtilis*, *B. thuringiensis* and *B. cereus* significantly reduced eggs hatching of *M. javanica* *in vitro* whereas mortality of larvae was significantly increased with an increase in exposure time. Prakob *et al*. (2009) found that *B. subtilis*, *Pseudomonas aeruginosa* (Schröter) Migula and *P. lilacinus* decreased nematode population densities, suppressed nematode infection and galls on lettuce plants roots.

Also, Siddiqui (2000) suggested that rhizobacteria and *B. subtilis* not only enhance plant growth but also suppress root-knot infection and nematode density in the soil. The reduction of plant parasitic nematodes associated with *B. subtilis* may be attributed to diverse mechanisms which involve phytohormones production, mineral solubilisation, reduction of the activity of egg hatching factors, alteration of root exudates and inhibition of nematode penetration into the roots as well as reducing galling (Karanja *et al.*, 2007).

Furthermore, several reports clarified that the basic mechanisms of *B. subtilis* included direct parasitism, production of extracellular antibiotics or other substances, stimulation of host defenses, enhance plant growth, induce systemic resistance in plants, control the plant diseases and secreting volatile nematicidal products (Ji *et al.*, 2006; Kloepper and Ryu, 2006 and Huang *et al*.,2009). Also, Prakob *et al.* (2009) recorded that the weight of lettuce plants increased in soil treated with the three biocontrol agents *B. subtilis*, *P. aeruginosa* and *P. lilacinus*. On the other hand, Mena *et al.* (1996) recorded that the *B. thuringiensis* var. *kurstaki* controlled

Table 4. Effect of used treatments on both tomatoes root system length (cm) and weight (g).

Treatment	Root system					
	Length (cm)	Increase %	Fresh weight (g)	Increase %	Dry weight (g)	Increase %
Oxamyl	24.33^{bcd}	37.69	41 ^{b}	372.90	7.33^{bc}	398.64
Cadusafos	22.67^{cd}	28.30	45.83^{ab}	428.61	11.73^{a}	697.96
G. macrocarpum	22^{cd}	24.51	43.60^{ab}	402.88	11.17^{a}	659.86
G. intraradices	30.33^{ab}	69.78	42.5^{ab}	390.20	9.8^{ab}	589.12
B. thuringiensis	31.67^{a}	79.23	48.6^{ab}	460.55	12.10^{a}	723.13
B. subtilis	27.67^{abc}	56.59	26.57^{c}	206.46	5.10^{c}	246.94
P. lilacinus	24.33^{bcd}	37.69	49.97^{a}	476.36	9.67^{ab}	557.82
Untreated check	17.67^{d}	--------	8.67^{d}	--------	1.47^{d}	--------

Values are mean of three replicated plots. Means followed by different letter(s) within a column are significantly different using LSD at P = 0.05

M. incognita and *Radopholus similis* Cobb on *Cucurbita pepo* L., Radwan (1999) observed that the shoot and root length and fresh weight of tomato plants were increased in the presence of *B. thuringiensis* var. *kurstaki* and Oxamyl. Also, Radwan (2007) recorded that *Bt* products such as Dipel® and Turex® have been shown to reduce damage caused by root-knot nematodes.

The plant growth were augmented with all evaluated bioproducts and there are certain reports were abolished this action for direct and indirect mechanisms, the direct growth promoting mechanism is depending on substances which released from bacteria or facilitation of uptake nutrients (Glick *et al*., 1999), and/or facilitation the absorption of iron and inducing of phytohormones production such as auxins, cytokinins, gibberellins (Patten and Glick, 2002; Vessey, 2003). Otherwise, the indirect mechanism through production of inhibitory substance or by increasing of natural resistance of the host (Cartieaux *et al*., 2003)

On the other hand, Jothi and Sundarababu (2002b) and Shreenivasa *et al*. (2007) found that AMFs reduced root infection by many parasitic nematodes. Also, Jothi and Sundarababu (2001) recorded that *Glomus* spp. reduced the nematode populations of *M. incognita* as well as the numbers of galls. Besides, arbuscular mycorrhizal fungi specially *Glomus* spp. increased the shoot and root systems of tomato plants (Khalil, 2009; Saad *et al.*, 2011; Saad *et al.*, 2012), as well as improved the plant growth in various crops (Jothi and Sundarababu, 2001; Krishnaveni and Subramanian, 2004).

The suggested mechanisms of Arbuscular mycorrhizal fungi against plant parasitic nematodes are summarized as follow: *i*) improved plant vigor and growth to offset yield loss normally caused by nematodes, *ii*) physiologically alteration or reduction of root exudates that are responsible for chemotactic attraction of nematodes, *iii*) directly retarding nematode development or reproduction within the root tissue, and *iv*) enhance and encourage the endophytes and endoparasitic-nematodes to compete for the same site in the root. Also, higher chitinase activity and ß-1, 3-glucana in roots was recorded (Jothi and Sundarababu, 2002a; Pozo *et al*., 2002), as well as enhance the host tolerance and augmenting resistance through the increasing of root growth and slowing down nematodes development, changes in root exudates which decrease attraction of nematode and attracted the plant growth promoting bacteria and an increase in phenols in roots (Sood, 2003; Hol and Cook, 2005).

It could be concluded that the microbial agents *B. thuringiensis* var. *kurstaki*, *P. lilacinus*, *B. subtilis* and the arbuscular mycorrhizal fungi (*Glomus* spp.) reduced *M. incognita* and improved the plant growth and, therefore, appear promising for

inclusion in an environmentally friendly integrated management of the root-knot nematode.

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