Nematicidal Effect of Biological Control Agents and Other Chemical Compounds on *Meloidogyne incognita* Infesting Tomato Plants

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ABSTRACT

Greenhouse experimental studies were conducted to evaluate certain biological control agents and other chemical compounds belonging to different groups to suppress the population density of root-knot nematodes (*Meloidogyne incognita*) in the sandy soil on tomato plants cv. Super strain B.

The biological control agents were the antagonistic bacterium *Pseudomonas flouroscence*, antagonistic fungus *Trichoderma harazianum* and their mixture. Meanwhile, the chemical compounds included cadusafos, fosthiazate, carbofuran and oxamyl, as well as the biopesticide abamectin.

The data revealed that carbofuran and *P. flouroscence* proved to be the most effective treatments suppressing the final population of root-knot nematodes (*Meloidogyne incognita*). Both carbofuran and *P. flouroscence* showed the same effect as the mean reduction of population density reached 92.7%, followed by the mixture of *T. harazianum* and *P. flouroscence* (88.9 %), then *T. harazianum* alone which achieved a reduction of 88.1%. Fosthiazate was the least effective treatment on population density giving a reduction of 53.7%.

Cadusafos gave the highest reduction percentage (55.3%) on galls/5g roots, followed by abamectin, oxamyl and fosthiazate which recorded 54, 53.9 and 51.1% reduction, respectively. Meanwhile, *T. harazianum* recorded the least reduction in galls (11.5%).

Abamectin gave the highest reduction (77.2 %) of root-egg masses/5g roots followed by fosthiazate, oxamyl and the mixture of *T. harazianum* and *P. flouroscence* (63.9%, 60.9% and 60.4% reduction), respectively.

All the evaluated treatments proved to be effective in enhancing the plant growth of tomatoes and showed indirect effect on the length and weight of root and shoot systems. Abamectin was the superior treatment in increasing the root system length by 44.2 %. on the other hand, the mixture of *T. harazianum* and *P. flouroscence* decreased the root system length by 4.1 %.

***P. flouroscence* was the most effective treatment achieving an increase of 88.7 % in root system fresh weight, followed by abamectin and cadusafos which gave 87.4% and 81.0% increasing. However, *T. harazianum* showed the least increase in root system fresh weight (20.9%).**

Also, *P. flouroscence* gave the highest increase percentage of the shoot system length followed by abamectin and carbofuran.

In respect to the shoot system weight, abamectin gave the highest increase over all the tested treatments (94.4%), followed by fosthiazate and *P. flouroscence* which recorded increase of 90.9% and 89.4%, respectively. Vice versa, *T. harazianum* recorded the least increase in both shoot system length and weight giving increase of 58.7% and 72.8%, respectively, compared with the untreated check.

INTRODUCTION

Tomato (*Lycopersicon esculentum*, Mill) represents an important vegetable crop in Egypt. Tomato fruits are considered to be one of the important sources for carbohydrates, protein, fats, fiber, minerals and vitamins (Howeedy *et al*., 2003).

Meloidogyne, the root-knot nematodes, contains more than 70 described species, four of them (*M. incognita*, *M. arenaria*, *M. javanica* and *M. hapla*) are responsible for 95% of infestations (Sasser *et al*., 1983). Root-knot nematodes (*Meloidogyne* spp.) cause high levels of economic loss in a multitude of agricultural crops worldwide. They are capable of severely damaging a wide range of crops, in particular vegetables, causing dramatic yield losses mainly in tropical and sub-tropical agriculture (Sikora and Fernandez, 2005).

Among the root-knot nematodes, *Meloidogyne javanica*, *M. incognita*, *M. arenaria*, and *M. hapla* are of major agronomic importance, being responsible for at least 90% of all damage caused by these nematodes (Castagnone-Sereno, 2002). These nematodes can be particular menace in third world countries where most peasant farmers are unaware of these ‘‘hidden enemies’’ and do not take steps to manage them.

A number of methods for the management of the root-knot nematode such as chemical control, organic amendments, resistant varieties, soil solarization and biological control have been tried with different levels of successes for the protection of tomato plants (Randhawa *et al*., 2001& Sakhuja and Jain, 2001). Biopesticides and microbial pathogens are being a new

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line which developed and improved to be an important tool in the IPM programs. A wide variety of soil organisms are known as predators or parasites of plant-parasitic nematodes (Coleman and Crossley, 1996; Dindal, 1990 and Stirling, 1991). Several attempts have been made to use antagonistic fungi to control root-knot nematodes (Sharon *et al*., 2001).

The root-knot nematodes, (*Meloidogyne incognita*) are considered to be the most difficult crop-pests to be controlled and due to the adverse effects of pesticides on the environment and human health, this investigation aimed to: (a) study the positive performance of certain biological control agents and other chemical compounds belonging to different chemical groups against root-knot nematodes, (*Meloidogyne incognita*). (b) determine the effect of microorganisms and biopesticide as safety and alternative control methods. (c) study the impact of the evaluated treatments on plant growth parameters.

MATERIALS AND METHODS

Biological control agents and chemicals compounds:

1) Biological control agents:

A) Antagonistic fungus.

The fungus (*Trichoderma harazianum*) was obtained from the biofertilizer center, Ain Shams University. The suspension was counted by a microscope. Each ml contains $1x10^5$ spores. Every plant received 50 ml of the suspension ($5x10^6$ spore / plant).

B) Antagonistic bacterium.

The bacterium (*Pseudomonas flouroscence*) was obtained from the biofertilizer center, Ain Shams University. The suspension was counted through the spectrophotometer at the wave length of 550 nm. Measuring the optical densities on the standard curve showed that every ml contains 3.8×10^4 CFU. Every plant received 50 ml of the suspension (1.9×10^6 CFU/ plant).

C) Antagonistic fungus and bacterium mixture.

A mixture of the antagonistic fungus and the antagonistic bacterium was employed by mixing the half dose of both {(2.5×10^6 spore) + (95×10^4 CFU)} / plant.

D) Biopesticide agent:

Vertemic® 1.8 % EC (Abamectin), (10*E*,14*E*,16*E*,22*Z*)-(1*R*,4*S*,5'*S*,6*S*,6'*R*,8*R*,12*S*,13*S*,20*R*,21*R*,24*S*)- 6'- [(*S*)-*sec*- butyl] -21,24-dihydroxy-5',11,13,22-tetramethyl-2-oxo-3,7,19-trioxatetracyclo[15.6.1.$1^{4,8}$.$0^{20,24}$]pentacosa-10,14,16,22- tetraene-6-spiro-2'-(5',6'-dihydro-2'*H*-pyran)-12-yl 2,6-dideoxy-4- *O*-,6-dideoxy-3-*O*-methyl-α-L-*arabino*- hexopyranosyl)-3-*O*-methyl-α- L-*arabino*-hexopyranoside (i) mixture with (10*E*,14*E*,16*E*,22*Z*)- (1*R*,4*S*,5'*S*,6*S*,6'*R*, 8*R*,12*S*,13*S*,20*R*,21*R*,24*S*)-21,24- dihydroxy-6'-isopropyl- 5',11,13,22-tetramethyl-2-oxo-3,7,19-trioxatetracyclo[15.6.1.$1^{4,8}$.$0^{20,24}$] pentacosa-10,14,16,22- tetraene-6- spiro-2'-(5',6'-dihydro-2'*H*-pyran)-12-yl 2,6- dideoxy-4-*O*-(2,6- dideoxy-3-*5* -methyl-α-L-*arabino*- hexopyranosyl)-3-*O*-methyl-α-L-*arabino*-hexopyranoside (ii) (4:1) .

2) Chemical compounds:

A) Organophosphorus compounds.

1) Nemathorin® 10% G (fosthiazate), [*RS*-*S*-*sec*-butyl *O*-ethyl 2-oxo-1,3-thiazolidin-3-yl phosphonothioate;(*RS*)-3-[*sec*-butylthio(ethoxy)phosphinoyl]-1,3-thiazolidin-2-one].
2) Rugby® 10% G (cadusafos), [*S*, *S*-di-sec-butyl *O*-ethyl phosphorodithioate].

B) Carbamates compounds.

1) Cartan®10% G (carbofuran),[2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate].
2) Vydate®10% G (oxamyl),[*N*, *N*-dimethyl-2-methylcarbamoyloxyimino-2-(methylthio) acetamide].

The Greenhouse Experiment.

Greenhouse experiment was carried out on a susceptible tomato plants cv. Super strain B to *Meloidogyne incognita*. 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). In the end of the experiment, second stage juveniles (J2) were extracted from 250g soil using sieving and baermann plates' technique (Ayoub, 1980). The greenhouse contained eight different evaluated treatments in sandy soil beside the untreated check and each treatment was replicated ten times.

The Soil samples were monthly collected according to Barker (1985) for three months after treatment to determine the efficacy of the tested nematicides on the nematode population densities in the soil which utilized according to the recommended dose of MAC (Ministry of Agriculture). Meanwhile, the biopesticide abamectin was applied at the rate of 11.11ml / l. The evaluated biological control agent and chemical compounds were applied to the soil for one time.

The shoot length, shoot weight, root length, root weight, galls number / 5g roots, egg masses / 5 g root system and number of juveniles / 250 g soil were determined. The roots were stained for 15 minutes in an aqueous solution of phloxine B stain (0.15 g /l water),

then they have been washed with running tap water to remove residual stain and to emphasize nematode egg masses (Holbrook *et al*., 1983).

The reduction percentage of infection was calculated after one, two and three months from treatment according to Hende nd Tilton' (1975) as follows:

$$\text{Reduction \%} = \{1 - \frac{a}{b} \times \frac{c}{d}\} \times 100$$

Where:

a = Population density in treatment after application

b = Population density in treatment before application

c = Population density in check untreated (control) before application

d= Population density in check untreated after application

Fertilization and irrigation

Fertilization was carried out through the drip irrigation lines (fertigation). On the other side, the irrigation was carried out through the drip irrigation lines two times / day.

Statistical analysis

The data were subjected to the analysis of variance test (ANOVA) as complete randomized design for greenhouse experiment. The least significant difference (LSD) at the 5% level of probability was determined using a Costat program and Multiple Range (Duncan, 1955).

RESULTS AND DISCUSSION

The influence of biological control agents and other chemical compounds on the nematode population:

The data presented in Table (1) indicated the reduction percentage of the evaluated biological control agents and other chemical compounds on the numbers of second Juvenile stage (J_2) at 250 g sandy soil. The efficacy was monthly recorded for three successive months after treatment.

It is obvious that the efficacy of the biological control agents and other chemical compounds was varied. It was noticed that most of the treatments increased the reduction percentage in the second month. While the results showed decreasing in the efficacy of carbofuran and cadusafos. On the other hand, the biological performance of carbofuran, cadusafos, *T. harazianum* and *P. flouroscence* were increased at the third month. Moreover, the effectiveness of fosthiazate, oxamyl, abamectin and the mixture of *T. harazianum* plus *P. flouroscence* were decreased in the third month.

The results of the effectiveness of the tested compounds against the root-knot nematode populations revealed that both carbofuran and *P. flouroscence* gave 92.7% reduction, followed by the mixture of *T. harazianum* and *P. flouroscence* and *T. harazianum* alone which recorded reductions of 88.9% and 88.1%, respectively.

These results are in agreement with those obtained by Sharma and Sharma (1995); Enokpa *et al.* (1996); Indira *et al*. (2001) and Kumari and Sivakumar (2005) who confirmed that carbofuran gave reduction of galls and nematode population of *Meloidogyne incognita*.

Also, Rich *et al*. (1994) and Lawrence and McLean (1995) reported that fosthiazate reduced the nematodes galls and *Meloidogyne incognita* numbers. Ibrahim *et al*. (2010) found that *Pseudomonas fluorescens* was superior treatment in reducing of nematodes population by 79.4%.

The possible action of the antagonistic bacterium *Pseudomonas fluorescens* strain was due to the capability of altering root exudates which could alter nematode behavior and suppress nematode population in root system (Oostendrop and Sikora, 1989). Also, the antibiotic production and competition with pathogens for essential nutrients such as iron, and more indirectly through plant growth promotion (Gamliel and Katan, 1993 and Siddiqui and Mahmood, 1998). Moreover, the production of hydrogen cyanide (HCN) as a secondary metabolite (Imran *et al*., 2006).

Table 1. The reduction percentage of the nematode population due to the application of biological control agents and other chemical compounds in tomato plants

Treatments	The reduction percent age of nematodes at three intervals after application		
	First month	Second month	Third month
Abamectin	86.5	86.6	74.0
Carbofuran	87.6	80.8	92.7
Cadusafos	72.0	71.6	83.1
Fosthiazate	59.5	87.2	53.7
Oxamyl	79.3	91.9	56.4
Pseudomonas flouroscence	81.1	84.6	92.7
Trichoderma harazianum	80.5	86.6	88.1

P. flouroscence + T. harazianum	85.9	92.3	88.9

The influence of biological agents and other chemical compounds on the rate of counted root galls and egg masses:

Data in Table (2) indicated that all treatments were effective against root galls and egg masses / 5 g roots compared with the untreated check. Cadusafos was the superior treatment which recorded 55.3 % reduction of root galls followed by abamectin, oxamyl and fosthiazate which recorded 54%, 53.9%, and 51.1% reduction, respectively.These results are in agreement with those of Sharma *et al.* (2008) who found that *Pseudomonas fluorescens* decreased nematode penetration and galling by 54% and 70%, successively. Also, Bhat *et al*. (2005); Pathan *et al*. (2005) and Singh (2006) indicated that carbofuran was the most effective treatment in reducing the larval population and the gall number. Kalaiarasan *et al*. (2006) exhibited that chitinolytic biological control agents *Pseudomonas fluorescens* and *Trichoderma viride* decreased the galls number / plant.

Moreover, in the case of egg masses, abamectin was the effective treatment that gave 77.2% reduction, followed by fosthiazate, oxamyl and the mixture of *P. flouroscence* plus *T. harazianum* achieving 63.9%, 60.9% and 60.4% reduction, in respect. *T. harazianum* showed the least reduction in both root galls and egg masses / 5 g roots which gave 11.5% and 38.3%, consecutively.

The finding results are similar to those reported by Khalil (2009) and Ibrahim *et al*. (2010) who found that abamectin, oxamyl and fosthiazate were the most effective treatments against egg masses of the root-knot nematode on tomato plants in clay soil cultivated under greenhouse conditions.

Also, Sharma *et al*. (1997) and Pathan *et al*. (2005) found that *P. lilacinus* along with furadan significantly reduced the number of galls / plant, egg-masses / root and eggs / egg-mass, the number of larvae / 200 g soil and females / 5 g root.

The action of the antagonistic fungus *Trichoderma* spp in multitude investigations, were interpreted as a producer of volatile and non volatile toxic metabolites such as arzianic acid, alamethicins, tricholin, peptaibols antibiotics, viridian and others (Vey *et al*., 2001). Tikhonov *et al.* (2002) found that the chitinolytic system of *Trichoderma* comprises many enzymes such proteases that together with chitinases are able to degrade nematode egg-shell. Also, the competition for nutrients specially iron which essential for viability decreased the available nutrients for the nematode (Eisendle *et al*., 2004).

Moreover, there are a general agreement that the toxic action of organophosphate and carbamate pesticides on nematodes and insects is due to their ability to inhibit acetylcholinesterase (AChE) in various parts of the nervous system thereby disrupt nervous transmission at that location (Corbett *et al*., 1984).

Table 2. The influence of biological control agents and other chemical compounds on the root galls and egg masses in tomato plants

Treatments	NO. / 5g roots			
	Galls		Egg masses	
	Average (NO.)	Reduction (%)	Average (NO.)	Reduction (%)
Abamectin	478.3 bc *	54.0	338.3 ef	77.2
Carbofuran	611.7 b	41.2	710.0 bc	52.2
Cadusafos	465.0 bc	55.3	860.0 b	42.1
Fosthiazate	508.3 bc	51.1	536.7 cdef	63.9
Oxamyl	480.0 bc	53.9	580.0 cdef	60.9
Pseudomonas flouroscence	633.3 b	39.1	613.3 cd	58.7
Trichoderma harazianum	920.0 a	11.5	916.7 b	38.3
P. flouroscence + T. harazianum	631.7 b	39.3	588.3 cde	60.4
Untreated check	1040.0 a	--	1485.0 a	--

*Within a column, values followed by different letter (s) are significantly different using LSD at P = 0.05

Table 3. The influence of biological control agents and other chemical compounds on length and weight of both of root and shoot system in tomato plants

Treatments	Root system				Shoot system			
	Length		Fresh weight		Length		Fresh weight	
	Average (cm)	Increase (%)	Average (g)	Increase (%)	Average (cm)	Increase (%)	Average (g)	Increase (%)
Abamectin	31.7 a *	44.2	69.2 ab	87.4	93.7 ^{ab}	66.9	280.0 ^{a}	94.4
Carbofuran	20.3 bcd	12.8	18.0 de	51.7	93.0 ^{abc}	66.7	100.0 ^{defg}	84.2
Cadusafos	22.7 abcd	22.0	45.8 bcd	81.0	77.3 ^{cd}	59.9	118.3 ^{cdefg}	86.6
Fosthiazate	27 abc	34.3	38.1 cd	78	82.3 ^{bcd}	63.3	173.3 ^{bc}	90.9
Oxamyl	24.3 abcd	27.2	41.0 cd	78.8	84.3 ^{abcd}	63.2	85.3 ^{efg}	81.5
Pseudomonas flouroscence	24.3 abcd	27.2	76.8 a	88.7	100.0 ^{a}	69.0	148.3 ^{bcde}	89.4
Trichoderma harazianum	20.0 bcd	11.5	11.0 c	20.9	75.0 ^{d}	58.7	58.0 ^{gh}	72.8
P. flouroscence + T. harazianum	17.0 d	-4.1	24.2 cde	64.1	80.7 ^{bcd}	61.6	76.7 ^{fgh}	79.4
Untreated check	17.7 cd	--	8.7 e	--	31.0 ^{e}	--	15.8 ^{h}	--

*Within a column, values followed by different letter (s) are significantly different using LSD at P = 0.05.

Indirect effect of studied biological agents and other chemical compounds on the length and weight of the root system:

The results in Table (3) showed the side effect of the different evaluated treatments on length and weight of the root system. It is clear that abamectin was the most effective treatment on the root system length which gave 44.2% increase, followed by fosthiazate, oxamyl and *P. flouroscence* that recorded 34.3, 27.2 and 27.2% increase, in sequence. While, the least effective treatment was the mixture of *P. flouroscence* plus *T. harazianum* giving a reduction of 4.1%. Therefore, it could be said that the mixture of *P. flouroscence + T. harazianum* was the only treatment that decreased the root length compared with the other running treatments.

Pseudomonas flouroscence alone was the superior treatment in increasing the fresh weight of root system by 88.7 %, followed by abamectin, cadusafos and oxamyl achieving 87.4, 81.0 and 78.8% increase, respectively, while, *T. harazianum* exhibited the least root system weight (11 g) and recorded an increase of 20.9% .

Indirect effect of tested biological agents and other chemical compounds on the length and weight of the tomatoes shoot system.

Data shown in Table (3) indicated the effect of the evaluated treatments on the length and weight of the shoot system in the sandy soil. *P. flouroscence* exhibited the highest increase in shoot system length (69.0 %), followed by abamectin and carbofuran achieving increase of 66.9 and 66.7%, consecutively.

Moreover, abamectin recorded the highest significant increase in shoot system weight which estimated by 94.4%, followed by fosthiazate and *P. flouroscence* that gave 90.9% and 89.4%, respectively. The fungus *T. harazianum* showed the least increase in both length and weigh of the shoot system performing 58.7% and 72.8%, respectively.

These findings are in agreement with those reported by Krishnaveni and Subramanian (2004) and Shanthi and Sivakumar (2005) who indicated that the yield of those plants treated with *Pseudomonas fluoroscence* was increased. Also, Kavitha *et al.* (2007) found that *Pseudomonas fluorescens*, *Bacillus subtilis* and *Trichoderma viride* showed a significant increase in the plant growth parameters. Also, Ibrahim *et al*. (2010) found that *Trichoderma harazianum*, oxamyl and fosthiazate increased the length and weight of the shoot system significantly.

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الملخص العربي

التأثير النيماتودى لعوامل المكافحة الحيوية وبعض المركبات الكيماوية على نيماتودا تعقد الجذور المتطفلة على نباتات الطماطم

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تم دراسة تأثير بعض عوامل المكافحة الحيوية وبعض المركبات من مجاميع كيماوية مختلفة على نيماتودا تعقد الجذور المتطفلة على نباتات الطماطم وكذلك دراسة تأثيرها الفعال على نمو نباتات الطماطم فى التربة الرملية تحت ظروف الصوب البلاستيكية.

هذا وقد تضمنت عوامل المكافحة الحيوية الفطر المضاد (*Trichoderma harazianum*) والبكتريا المضادة (*Pseudomonas flouroscence*) وخليطهما وكذلك أحد المركبات الحيوية (الأبامكتين)، بينما ضمت المركبات الكيماوية المختبرة مركبى الفوزثيازيت والكادوسافوس من مجموعة المبيدات الفسفورية العضوية ومركبى الأوكساميل والكاربوفيوران من مجموعة المبيدات الكارباماتية.

وقد أوضحت الدراسة أن استخدام الكاربوفيوران والبكتريا المضادة *Pseudomonas flouroscence* أدى الى تسجيل أعلى نسبة خفض فى تعداد اليرقات بالتربة قدر بـ ٩٢,٧%، بينما سجل مركب الكادوسافوس أعلى نسبة خفض للتورمات الجذرية بمعدل ٥٥,٣% وكذلك سجل مركب الأبامكتين أعلى نسبة خفض فى كتل البيض قدرت بـ ٧٧,٢%.

وقد بينت الدراسة أيضا أن البكتريا المضادة *Pseudomonas flouroscence* أعطت أفضل النتائج فى زيادة وزن المجموع الجذرى وكذلك طول المجموع الخضرى بمعدل ٨٨,٧% و٦٩% على التوالى، بينما سجل مركب الأبامكتين أفضل النتائج من حيث زيادة طول المجموع الجذرى وزيادة وزن المجموع الخضرى بمعدل ٤٤,٢% و ٩٤,٤% على التوالى بالمقارنة مع النباتات الغير معاملة.