Evaluation of Certain Agrochemical and Biological Agents Against *Meloidogyne incognita* on Tomatoes

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ABSTRACT

An experiment was conducted in a greenhouse to evaluate the biological performance of some agrochemical and biological agents against root-knot nematode on tomatoes planted in clay soil. The antagonistic bacterium *Pseudomonas flouroscence* was proved to be the most effective tool suppressing the infection of root-knot nematodes on tomatoes during the period of inspections. The mean of population reduction of *P. flouroscence* was (79.4%), followed by the mixture of antagonistic fungus and bacterium *Trichoderma harazianum* plus *Pseudomonas flouroscence* (73.0%), then fosthiazate (60.3%), while the least effective one was abamectin (22.5%).

On the other hand, abamectin applied as soil drench gave the highest reduction percentage (81.6 % of root galls / 5g roots) followed by oxamyl (62.4%), then fosthiazate (61.5%). Carbofuran recorded the least reduction percentage of 33.1 % of root galls /5g roots.

Meanwhile, abamectin gave the highest reduction percentage on egg masses / 5g roots (79.1 %), followed by oxamyl and *P. flouroscence* giving 61.4 and 39.9 reduction percentages. The least effective treatment was *Trichoderma harazianum* which increased the egg masses by 18.8 %.

Some treatments showed an indirect effect on the root and shoot system length and weight. The antagonistic fungus *Trichoderma harazianum* was the superior treatment which increase the root system length by 39.1 %, whilst abamectin didn't show any increase.

***Trichoderma harazianum* as well as fosthiazate proved to be the most effective treatment on the root system fresh weight as they gave 86.8 and 85.5% increase, respectively. Abamectin was the least effective treatment giving 5.3% increase.**

On the other hand, *T. harazianum* was effective and gave the highest increase percentage of shoot system length followed by oxamyl (54.9 and 46.8%, respectively). Meanwhile, the same trend was obtained in case of shoot system weight giving increases of 87.8 and 81.9%, respectively. Abamectin treatment was the least efficient on its effecton both root system length and weight increase.

INTRODUCTION

Tomato fruits are considered to be one of the important nutritive sources for carbohydrates, minerals and vitamins. Tomato plants (*Lycopersicon esculentum* Mill) represent an important vegetable crop in Egypt, showed that [every 100 g contains: 4.7gcarbohydrates, 1.1g protein, 0.2g fats, 0.5g fiber, 13mg calcium, 27mg phosphor, 244mg potassium, 3mg sodium, 0.5mg iron, 23mg ascorbic acid, 0.06mg thiamin, 0.04mg riboflavin, 0.7mg niacin and 900 iu vitamin A] (Howeedy *et al*., 2003).

The production of tomato plants is affected by several factors mainly, rain fall, temperature, soil fertility, time of planting, plant density, infestations of destructive pests and diseases infection that cause great loss and damage. The root-knot nematodes, *Meloidogyne* spp., have been recognized as one of the serious pests causing remarkable losses of vegetables production especially tomato crop (Ibrahim, 1985).

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 a particular menace in third world countries where most peasant farmers are unaware of these ‘‘hidden enemies’’ and do not take steps to manage them.

Fumigant and non-fumigant nematicides have been used against plant–parasitic nematodes with good results since 1950s. They have been used extensively for controlling root-knot nematode that threat the production of many fruits, vegetables and nursery crops (Noling and Becker, 1994).

There is a general agreement that the toxic action of organophosphate and carbamate pesticides upon nematodes and insects is caused by their ability to inhibit acetylcholinesterase (AChE) in various parts of the nervous system, thereby, disrupt nervous transmission at that location (Corbett *et al*., 1984). Using biopesticides being a new line developed and improved to be an important tool in the IPM programs.

The aim of the present study is to investigate the effectiveness of some agrochemical and biological agents against the root-knot nematodes, *Meloidogyne*

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incognita, and to through a light on the antagonistic microorganism as safety methods to reduce effectively the population density of plant-parasitic nematodes in the soil.

MATERIALS AND METHODS

Agrochemical and biological agents:-

● Nematicides Used:

A: Organophosphorus

1) Nemathorin® 10% G (fosthiazate), [*RS*-*S*-*sec*-butyl *O*-ethyl2-oxo-1,3-thiazolidin-ylphosphonothioate;(*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

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].

● Biopesticide agents:

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-□-L-*arabino*-hexopyranosyl)-3-*O*-methyl-□-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-□-L-*arabino*- hexopyranosyl)-3-*O*-methyl-□-L-*arabino*-hexopyranoside (ii) (4:1) .

● Antagonistic Fungus (*Trichoderma harazianum*).

The fungus *Trichoderma harazianum* was obtained from the biofertilizer center, Ain Shams University. The suspension was counted under the microscope. It is found that each ml contains 1x10^5 spore. The treated plants received 50 ml of the suspension (5x10^6 spore / plant).

●Antagonistic Bacterium (*Pseudomonas flouroscence*).

The bacterium *Pseudomonas flouroscence* was also obtained from the biofertilizer center, Ain Shams University. The suspension was counted through the spectrophotometer at the wave length of 550 nm. The optical densities were measured and compared with the standard curve and found that each ml contains 3.8 x 10^4 CFU. The treated plants received 50 ml of the suspension (1.9 x10^6 CFU/ plant). mixture of the antagonistic fungus and the antagonistic bacterium (half dose of both (2.5 x 10^6 spore) + (95 x 10^4 CFU) / plant) was also evaluated.

The Greenhouse Experiment.

Greenhouse experiment was carried out on tomato plants cv. Super strain B as a host plant for plant parasitic nematodes (*Meloidogyne incognita*), in the Faculty of Agriculture Saba-Basha greenhouse. The greenhouse contained clay soil (89.5% clay, 0.5% sand and 10% silt). There were 9 different treatments and each treatment consisted of ten replicates (a plant / replicate).

The Soil samples were took according to Barker (1985) for three successive months after treatment to determine the efficacy of the tested compounds on the nematode populations in the soil. The shoot length, shoot weight, root length, root weight, galls number / 5g root, 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 the residual of the stain. To detect the presence of nematode egg masses, the mothod of Holbrook *et al*.(1983) was followed.

The reduction percentage of infection was calculated after 1, 2 and 3 months post-treatment according to the formula of Henderson and Tilton's (1955) as follows:

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

Where:

a = Population in treatment after treatment

b = Population in treatment before treatment

c=Population in check untreated (control) before treatment

d = Population in check untreated after treatment

The fertilizationof plants was carried out daily through the drip irrigation lines (fertigation). The irrigation was performed through the drip irrigation lines two times / day.

Data of the present study 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 computer program Costat and Duncan's Multiple Range (Duncan, 1955).

RESULTS AND DISCUSSIONS

1) The effect of the tested agrochemical and biological agents against the nematodes populations:

The data in Table (1) show the calculated reduction percentages of the tested agrochemical and biological agents on the second stage Juvenile number (J_2) at 250 g clay soil in the greenhouse experiment. The efficacy of the used agrochemical and biological agents [nematicides, biopesticide and antagonistic (fungus/bacterium)] were monthly recorded for three months post-treatment, and the efficacy of the agrochemical and biological agents were varied.

It is obvious that the efficacy of the tested agents and chemical was increased in the second month except cadusafos. On the third month, it was noticed that the efficacy of all treatments decreased except the antagonistic bacterium *Pseudomonas flouroscence* and the mixture of antagonistic bacterium and fungus *Pseudomonas flouroscence* plus *Trichoderma harazianum*. Generally, the most effective treatment was *Pseudomonas flouroscence* against nematode population which recorded a reduction of nematode population calculated by 79.4%. Afterwards, the treatments of *Trichoderma harazianum* plus *Pseudomonas flouroscence*, fosthiazate, oxamyl, carbofuran, *Trichoderma harazianum*, cadusafos and abamectin showed the following reduction percentages of the nematodes population 73.0, 60.3, 60.0, 43.8, 43.2, 41.8 and 22.5%, respectively.

These findings are in agreement with those reported by Sharma (1999) and Krishnaveni and Subramanian (2004) indicated that *Trichoderma harzianum*, *Trichoderma viride* and *P. fluorescens* were effective in controlling the plant parasitic nematodes. On the other hand Stephan (1995) and Badawi and Abu-Gharbieh (2000) showed that the treatments of following nematicides**:** Cadusafos liquid, carbofuran and oxamil were effective in controlling *M. javanica*. Monfort *et al.*(2006) and Faske and Starr (2007) indicated that abamectin has a nematicidal effect against *M. incognita* and *Rotylenchulus reniformis* on cotton plants.

The possible mode of action of the antagonistic bacterium *Pseudomonas fluorescens* strain that it produces hydrogen cyanide (HCN), as a secondary metabolite (Imran *et al*., 2006) and that gas has its effect on nematode. The suppression of nematode multiplication by *Pseudomonas fluorescens* could be due to its capability of altering root exudates which alter nematode behavior and suppress nematode population in root system (Oostendrop and Sikora, 1989).

Furthermore, the action of the antagonistic bacterium against plant parasitic nematode could be explained due to the antibiotic production and competition with pathogens for essential nutrients such as iron, and more indirectly through plant growth promotion (Gamlie and Katan, 1993 and Siddiqui and Mahmood, 1998).

2) The biological performance of agrochemical and biological agents on root galls and egg masses

Data represented in Table (2) indicated the influence of the agrochemicals and biological agents on the galls and egg masses / 5 g root in the clay soil**.** Abamectin was the most effective treatment which recorded 81.6% reduction of root galls, followed by oxamyl, fosthiazate, cadusafos, *Trichoderma harazianum, Pseudomonas flouroscence*, *Trichoderma harazianum* plus *Pseudomonas flouroscence* and carbofuran which gave 62.4, 61.5, 56.2, 51.9, 46.8, 34.5 and 33.1% reduction, respectively.

Table 1. The effectiveness of the tested agrochemical and biological agents expressed as mean reduction% of nematode populations

Treatments	The mean reduction (%) of nematodes populations at different intervals post treatment (months)		
	One month after treatment	Two months after treatment	Three months after treatment
Fosthiazate	24.4	80.3	60.3
Carbofuran	46.1	71.0	43.8
Cadusafos	69.4	42.4	41.8
Oxamyl	43.7	73.6	60.0
Abamectin D*	31.5	58.9	22.5
Trichoderma harazianum	52.2	60.2	43.2
Pseudomonas flouroscence	30.7	72.0	79.4
*Trichoderma h. + Pseudomonas f.***	38.9	46.5	73.0

*(D): Soil drench application at the rate of 11.11 ml/l.

** The mixture was composed by the half dose of each ($2.5*10^6$ spores+ $95*10^4$ CFU) / plant.

Table 2. The effectiveness of the tested agrochemical and biological agents expressed as reduction% of galls and egg masses

Treatments	Egg masses / 5g roots		Galls / 5g roots	
	Average (NO.)	Reduction (%)	Average (NO.)	Reduction (%)
Fosthiazate	328.3 bc	61.5	450.0 abc	22.8
Carbofuran	570.0 b	33.1	396.7 bc	31.9
Cadusafos	373.3 bc	56.2	353.3 bcd	39.4
Oxamyl	320.0 bc	62.4	225.0 cd	61.4
Abamectin D*	156.7 c	81.6	121.7 d	79.1
Trichoderma harazianum	410.0 bc	51.9	691.7 a	-18.8
Pseudomonas flouroscence	453.3 b	46.8	350.0 bcd	39.9
*Trichoderma h. + Pseudomonas f.***	558.3 b	34.5	476.7 abc	18.2
Untreated check	851.7 a	−−	582.5 ab	−−
L.S.D$_{0.05}$	260.99	−−	243.64	−−

Numbers followed by different letter(s) within a column are significantly different using LSD at P = 0.05.
*(D): Soil drench application at the rate of 11.11 ml/l.
** The mixture was composed by the half dose of each ($2.5*10^6$ spores+ $95*10^4$ CFU) / plant.

These results were in agreement with those obtained by Sharma *et al*. (1997) and Pathan *et al*. (2005) **who** 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. Also, Sharma *et al.* (2008) found that *Pseudomonas fluorescens* decreased nematode penetration and galling by 54 and 70%, respectively, compered with the untreated check.

In the case of egg masses, abamectin was the most effective treatment that gave 79.1% reduction, followed by oxamyl, *P. flouroscence*, cadusafos, carbofuran, fosthiazate, *T. harazianum* plus *P. flouroscence* achieving reduction percentages of 61.4, 39.4, 39.4, 31.9, 22.8 and 18.2, respectively. Vice versa, *T. harazianum* was the least effective treatment in egg masses which gave an increase as low as 18.8%.

3) Indirect effects of certain agrochemical and biological agents on the length and weight of the root system.

Data in Table(3) showed that *Trichoderma harazianum* was the most effective treatment to increase the root system length in the clay soil giving an increase of 39.1% over untreated check followed by cadusafos, fosthiazate, carbofuran, oxamyl, *P. flouroscence* and *T. harazianum* plus *P. fluorescens* which achieved 19.6, 17.2, 17.2,15, 6.8 and 3.5 % increase, respectively. It could be observed that there were no any significant differences between untreated check and all the running treatments except *T. harazianum*. Abamectin didn't showed any side effect on the root system length.

On the other hand, the highest increase of the root system fresh weight was induced by *T. harazianum* and fosthiazate, followed by carbofuran, *T. harazianum* plus *P. flouroscence*, *P. flouroscence*, oxamyl, cadusafos and abamectin which gave 86.8, 85.5, 73.9, 62.8, 58.7, 54.8, 19.3 and 5.3 % increase, respectively.

These findings are in agreement with those recorded by Alam *et al*.(1995) and Asawalam and Adesiyan (2001) who indicated that carbofuran improved the plant growth and increased mean fruit number and fruit weight. Also, Hafez and Sundararaj (2006) found that fosthiazate increased the total yield and the marketable yield of potato**.**

Tripathi and Singh (2006) found that the length and weight of root and shoot system significantly increased when tomato plants treated with *Paecilomyces lilacinus* and *Trichoderma viride* in combinations with mustard cake and Furadan.

4) Indirect effects of certain agrochemical and biological agents on the length and weight of the shoot system.

Data in Table (4) explain that the highest increase of the shoot system length was attaine by treatment of *Trichoderma harazianum* that gave 54.9% increase, followed by oxamyl, fosthiazate, *P. flouroscence*, cadusafos, carbofuran, abamectin and *T. harazianum* plus *P. fluorescens* that gave 46.8, 43.4, 42.7, 42.2, 40.4, 36.7 and 35.1 % increase, respectively.

Moreover, *Trichoderma harazianum* recorded the highest increase of 87.8% in the shoot system fresh weight followed by oxamyl, fosthiazate, *P. flouroscence*, *T. harazianum* plus *P. fluorescens*, carbofuran, abamectin and cadusafos that gave descending percent increase amounted to 81.9, 78.1, 75.9, 72.2, 70.6, 69.5 and 67.9, respectively.

Stephan (1995) and Rather *et al*. (2007) indicated that carbofuran treatments showed maximum plant growth, also Kavitha *et al.* (2007) found that *Pseudomonas fluorescens*, *Bacillus subtilis* and *Trichoderma viride* gave a significant increase in the plant growth parameters.

Trichoderma harzianum showed antagonistic effects against the root-knot (*Meloidogyne incognita*), and the possible mode of actions of this fungus were:

- The ability to colonize plant roots and penetrate into the epidermis by mechanisms similar to those of mycorrhizal fungi and to produce compounds that stimulate growth and plant defense mechanisms (Harman *et* al., 2004).
- *Trichoderma* strain may produce or release compounds that induce localized or systemic plant resistance responses (Harman *et* al., 2004).
- *Trichoderma* strains produce volatile and non volatile toxic metabolites such as, arzianic acid, alamethicins, tricholin, peptaibols antibiotics, 6-penthyl-α-pyrone, massoilactone, viridin, gliovirin, glisoprenins, heptelidic acid and others (Vey *et* al., 2001).
- The chitinolytic system of *Trichoderma* comprises many enzymes such proteases that together with chitinases are able to degrade nematode egg-shell thus helping penetration (Tikhonov *et* al., 2002).

Table 3. The influence of tested agrochemical and biological agents on length and weight of the root system of tomato plants

Treatments	Root system			
	Length (cm)		Fresh weight (g)	
	Average	Increase (%)	Average	Increase (%)
Fosthiazate	23.3 b	17.2	49 a	85.5
Carbofuran	23.3 b	17.2	27.2 b	73.9
Cadusafos	24.0 b	19.6	8.8 b	19.3
Oxamyl	22.7 b	15.0	15.7 b	54.8
Abamectin D*	19.3 b	0.0	7.5 b	5.3
Trichoderma harazianum	31.7 a	39.1	53.9 a	86.8
Pseudomonas flouroscence	20.7 b	6.8	16.8 b	58.7
*Trichoderma h. + Pseudomonas f.***	20.0 b	3.5	19.1 b	62.8
Untreated check	19.3 b	--	7.1 b	--
L.S.D$_{0.05}$	5.73	--	19.93	--

Numbers followed by different letter(s) within a column are significantly different using LSD at P = 0.05.
***(D)**: Soil drench application at the rate of 11.11 ml/l.
** The mixture was composed by the half dose of each ($2.5*10^6$ spores+ $95*10^4$ CFU) / plant.

Table 4. The influence of tested agrochemical and biological agents on length and weight of the shoot system of tomato plants

Treatments	Shoot system			
	Length (cm)		Fresh weight (g)	
	Average	Increase (%)	Average	Increase (%)
Fosthiazate	78.3 b	43.4	190.0 bc	78.1
Carbofuran	74.3 b	40.4	141.7 bc	70.6
Cadusafos	76.7 b	42.2	130.0 c	67.9
Oxamyl	83.3 ab	46.8	230.0 b	81.9
Abamectin D*	70.0 b	36.7	136.7 bc	69.5
Trichoderma harazianum	98.3 a	54.9	341.7 a	87.8
Pseudomonas flouroscence	77.3 b	42.7	173.3 bc	75.9
*Trichoderma h. + Pseudomonas f.***	68.3 b	35.1	150.0 bc	72.2
Untreated check	44.3 c	-----	41.7 d	-----
L.S.D$_{0.05}$	17.57	-----	86.09	-----

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

*(D): Soil drench application at the rate of 11.11 ml/l.

** The mixture was composed by the half dose of each ($2.5*10^6$ spores+ $95*10^4$ CFU) / plant.

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الملخص العربي

تقييم بعض المبيدات النيماتودية والكائنات المضادة على نيماتودا تعقد الجذورالمتطفلة على نباتات الطماطم

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تم تقييم بعض المبيدات النيماتودية (الفوزثيازيت, الأوكساميل, الكاربوفيوران, الكادوسافوس) وأحد المركبات الحيوية (الأبامكتين) وكذلك أحد الفطريات المضادة (*Trichoderma harazianum*) وبكتريا مضادة (*Pseudomonas flouroscence*) لدراسة تأثيرها على نيماتودا تعقد الجذور *Meloidogyne incognita* التى تصيب نباتات الطماطم فى التربة الطينية تحت ظروف الصوب البلاستيكية.

وقد بينت الدراسة أن البكتريا المضادة *Pseudomonas flouroscence* منفردة أعطت أفضل النتائج فى خفض تعداد اليرقات بالتربة بمقدار 79.3% وقد تبعها فى ذلك التأثير خليط الفطر والبكتريا المضادة بمقدار خفض وصل الى 73%.بينما كان مركب الأبامكتين أفضل المعاملات المختبرة فى خفض العقد النيماتودية وكتل البيض/ 5جم جذوربمقدار81.6% و79.1% على التوالى.

ولقد أوضحت النتائج أيضا فاعلية الفطر المضاد *Trichoderma harazianum* وتحقيقه أفضل النتائج من حيث زيادة طول ووزن المجموع الجذرى والخضرى لنباتات الطماطم مقارنة بالمعاملات الأخرى.