NEMATICIDAL ACTIVITY OF SOME BIOPESTICIDE AGENTS AND MICROORGANISMS AGAINST ROOT-KNOT NEMATODE ON TOMATO PLANTS UNDER GREENHOUSE CONDITIONS

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Abstract: A pot experiment was carried out under greenhouse conditions to study the impact of the evaluated treatments namely abamectin, azadirachtin 0.15%, azadirachtin 0.03%, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Paecilomyces lilacinus* and oxamyl against root-knot nematode (*Meloidogyne incognita*) on the tomato plants cv. Super strain B. The results indicated that the most of the tested treatments obviously reduced root galls and remarkably increase tomato plant growth characters significantly and egg masses on root system, as well as, juvenile's numbers in the soil.

P. lilacinus was the most effective treatment on both galls and egg masses achieving 88.23 and 76.94% reduction, respectively. While, less effective treatment was *P. fluorescens* achieving 57.53% galls reduction. Azadirachtin 0.03% was the least effective treatment giving 40.37% reduction of egg masses.

The superior treatment that suppressed nematode populations was oxamyl recording (88.90%) followed by abamectin (78.69%) reduction. Moreover, azadirachtin 0.15% was the least effective treatment which recorded 60.15% reduction.

On the other hand, plants free nematode recorded the highest plant parameters for shoot system length, fresh shoot weight, dry shoot weight and root system length with values of 24.15, 107.53, 211.59 and 46.17% increase, respectively. Azadirachtin 0.15% was the least effective treatment on shoot system length and fresh and dry shoot weight. While, oxamyl recorded the least increase in root system length estimated by 18.47%.

B. subtilis recorded the highest increase in fresh root weight followed by *P. fluorescens* with value of 125.75 and 86.57%, consecutively. Vise versa, *P. fluorescens* was the superior treatment to increase the dry root weight by 68.14% followed by *B. subtilis* which recorded 35.40%. The least effective treatment in improving fresh root weight was azadirachtin 0.15% which recorded 54.85% increase. Regarding to dry shoot weight *P. lilacinus* and azadirachtin 0.15% were the least effective treatments with values of 8.85 and 2.66% reduction, respectively.

Key words: root-knot nematode, tomato, greenhouse, *Meloidogyne incognita*

INTRODUCTION

Root-knot nematodes, *Meloidogyne* contain more than (70) described species, four species (*M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica*) are responsible for 95% of infestations (Sasser *et al*. 1983). 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).

A number of methods for the management of root-knot nematodes 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).

There is general agreement that the toxic action of organophosphate and carbamate pesticides upon nematodes and insects is caused by their ability to inhibit acetyl cholinesterase (AChE) in various parts of the nervous system, thereby disrupt nervous transmission at that location (Corbett *et al*. 1984).

Rhizosphere microorganisms may provide defense against pathogen attack (Weller 1988). The rhizoplane and rhizosphere are colonized or otherwise occupied by many microorganisms. Plant growth promoting bacteria produce plant growth promoting substances and antibiotics. They are capable of providing substantial protection against nematode diseases (Siddiqui and Mahmood 1999).

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A wide variety of soil organisms are known predators or parasites of plant-parasitic nematodes (Dindal 1990; Stirling 1991; Coleman and Crossley 1996). Several attempts have been made to use antagonistic fungi to control root-knot nematodes (Sharon *et al*. 2001). Nematophagous fungus *P. lilacinus* was discovered by Jatala *et al*. in 1979 at the International Potato Center in Lima, Peru (Oclarit *et al*. 2009).

In India for hundred years, the farmers were used the neem tree (*Azadirachta indica*) for its pesticidal, antifungal and anti feedant properties. Neem is available in simple home-made formulations like seed powder, seed kernel powder, seed cake powder, dry leaf powder and aqueous extracts made from them (Javed *et al*. 2008). The ability of eggs to hatch and mobility of juveniles is reduced by various neem products. It has also been demonstrated that neem products are very effective in reducing the root-knot nematode disease incidence and so ultimately improve plant health.

Abamectin is a mixture of macro cyclic lactone metabolites produced by the fungus *Streptomyces avermitilis,* which used as a seed treatment to control plant-parasitic nematodes on cotton and some vegetable crops. Abamectin was effective on both *M. incognita* and *R. reniformis* in tomato plants (Faske and Starr 2006). Also, abamectin has a nematicidal effect against *M. incognita* and *R. reniformis* on cotton plants as seed treatment (Faske and Starr 2007). Furthermore, abamectin proved highly activity against lesion nematodes (*Pratylenchus* spp.) as a seed treatment on corn with reduction evaluated by 25–72% (Cochran *et al*. 2007).

This investigation aimed to study the positive performance of biological agents and azadirachtin against root-knot nematodes, (*M. incognita*), which considered among the most difficult crop pests to control. Furthermore, to evaluate the effect of antagonistic microorganisms as alternative and safety method in Integrated Pest Management (IPM) programs to management the root-knot nematodes. Moreover, to study the impact of used treatments on plant growth.

MATERIALS AND METHODS

Compounds used

Chemical nematicide

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

Achook® 0.15% EC (Azadirachtin), dimethyl (3*S*,3a*R*,4*S*,5*S*,5a*R*,5a^{1}*R*,7a*S*,8*R*,10*S*,10a*S*)-8- acetoxy- 3,3a,4,5,5a,5a^{1},7a,8,9,10-decahydro-3,5- dihydroxy-4- {(1*S*,3*S*,7*S*,8*R*,9*S*,11*R*)-7-hydroxy-9-methyl-2,4,10-trioxatetracyclo [6.3.1.0^{3,7}.0^{9,11}] dodec-5-en-11-yl}- 4- methyl-10[(*E*)-2-methylbut-2- enoyloxy]-1*H*,7*H*-naphtho[1,8a,8-*bc*:4,4a-*c′*]difuran-3,7a- dicarboxylate.

Nimbecidine® 0.03% EC(Azadirachtin), dimethyl (3*S*,3a*R*,4*S*,5*S*,5a*R*,5a^{1}*R*,7a*S*,8*R*,10*S*,10a*S*) -8- acetoxy- 3,3a,4,5,5a,5a^{1},7a,8,9,10-decahydro-3,5- dihydroxy-4-{(1*S*,3*S*,7*S*,8*R*,9*S*,11*R*)-7-hydroxy-9-methyl-2,4,10-trioxatetracyclo [6.3.1.0^{3,7}.0^{9,11}]dodec-5-en-11-yl}- 4- methyl-10[(*E*)-2-methylbut-2- enoyloxy]-1*H*,7*H*-naphtho[1,8a,8-*bc*:4,4a-*c′*]difuran-3,7a- dicarboxylate.

Tested antagonistic microorganisms

The evaluated microorganisms in this experiment were commercial compounds as follow:

1 – Bio cure-B® contain 1*10^{9} cell/ml of bacterium (*Pseudomonas fluorescens*),

2 – Stanes sting® contains 1*10^{9} cell/ml of bacterium (*Bacillus subtilis*),

3 – Bio-Nematon® contains 1*10^{9} cfu/ml of fungus (*Paecilomyces lilacinus*).

Pots experiment was carried out under greenhouse conditions using tomato plants (*Lycopersicon esculentum,* Mill) cv. Super strain B as host plant for *M. incognita* (Kofoid and White) Chitwood. The Pots (15 cm in diameter and 20 cm in depth) were filled with 1kg mixture of clay and sand (2:1 v/v), and the nematode eggs were applied at the rate of 5,000 eggs/pot, three days after seedling. Each treatment was replicated four times and each replicate contains two seedlings. The fresh and dry weight of shoot and root were determinated in addition to shoot and root length. Also, egg masses and galls number per root system and number of juveniles per 250 g soil were evaluated. The roots were stained for 15 minutes in an aqueous solution of Phloxine B stain (0.15 g/l water) then washed with running tap water to remove residual stain and to detect the presence of nematode egg masses (Holbrook *et al*. 1983).

The tested compounds were applied to evaluate their efficacy on the root-knot nematode *(M. incognita)*. The compounds were utilized according to the recommended dose such as oxamyl and all of the antagonistic microorganisms. While, Vertemic® used at the rate of 11.11 ml/l, Achook® and Nimbecidine® were used at the rate of 5 ml/l. All treatments were applied for one time, three days after nematode inoculation as a soil drench. The experimental total time was sixty two (62) days then the plants were up-rooted and determined root galls and egg masses.

Statistical analysis

Data of the present study were subjected to the analysis of variance test (ANOVA) as complete randomized design (CRD), for greenhouse experiment. The least significant difference (LSD) at the 5% level of probability was determined using Costat program (1988).

RESULTS AND DISCUSION

Data in table 1 indicated that *P. lilacinus* was the most effective treatment which recorded 88.23% galls reduction followed by abamectin, oxamyl and azadirachtin 0.15% giving 86.87, 78.09 and 69.31% reduction, respectively. While, the least effective treatment was *P. fluorescens* that gave 57.53% galls reduction per plant root.

Furthermore, the performance of the used treatments on egg masses was also evaluated. The superior treatment was *P. lilacinus* followed by *B. subtilius* and abamectin giving 76.94, 71.69 and 68.94% reduction, consecutively. Azadirachtin 0.03% was the least effective treatment which recorded 40.37% reduction.

Data represented in table 1 showed the influence of the evaluated treatments on root-knot nematode population densities which considered an important indicator to the efficacy of the used compounds which recorded reduction ranged from (60.15 and 88.90%). The collection data in table 1 showed that the evaluated treatments suppressed the nematode population densities. Oxamyl was the superior treatment that reduced juveniles in the soil giving 88.90% followed by abamectin, *P. lilacinus* and *P. fluorescens* which recorded 78.60, 76.24 and 76.16% reduction, respectively. While, azadirachtin 0.03%, *B. subtilis* and azadirachtin 0.15% recorded 63.91, 60.37 and 60.15% reduction, respectively.

The present results are in agreement with those reported by Oclarit *et al*. (2009) who found that *P. lilacinus* strain UP1 was effective against *M. incognita* which attacking tomato under screen house condition in pot experiments and significantly reduced the number of galls, nematodes and egg masses compared with Nemacur.

Korayem *et al*. (2008) found that abamectin at the tested concentrations significantly reduced most nematode parameters and enhanced plant growth parameters.

Monfort *et al.* (2006) and Faske and Starr (2007) indicated that abamectin has a nematicidal effect against *M. incognita* and *R. reniformis* on cotton plants. Also, Kavitha *et al.* (2007) indicated that *P. fluorescens, B. subtilis* and *T. viride* significantly decreased the nematode population. Sharma *et al.* (2008) found that *P. fluorescens* decreased nematode penetration and galling by 54 and 70%, respectively.

There were multitude investigations interpreted the actions of *P. lilacinus* on plant parasitic nematodes as follow: Jatala *et al*. (1985) mentioned that *P. lilacinus* caused substantial egg deformation in *M. incognita*, these deformed eggs never matured or hatched.

In addition to killing juveniles and females of *M. incognita* and *Globodera pallida*. In the laboratory test this fungus infects eggs of *M. incognita* and destroys the embryos within 5 days because of simple penetration of the egg cuticle by individual hypha aided by mechanical and/or enzymatic activities (Jatala 1986). Also, *P. lilacinus* suppressed root knot infections which resulted in fewer galls developing in the root system. (Linderman 1992; Siddiqui *et al*. 2001; Prakob *et al*. 2007). The serine protease produced by *P. lilacinus* might play a role in penetration of the fungus through the eggshell of the nematodes (Bonants *et al*. 1995). On the other hand, early developed eggs were more susceptible than the eggs containing fully developed juveniles. As observed by transmission electron microscopy, fungal hypha penetrated the *M. javanica* female cuticle directly (Khan *et al*. 2006).

Data shown in table 2 revealed that the most of used treatments recorded an increase of shoot system length. Control without nematode shows the highest increase evaluated by 24.15% followed by *B. subtilis*, *P. fluorescens*, azadirachtin 0.03%, abamectin and oxamyl with values of 20.69, 16.55, 16.55, 12.42 and 11.03% increase, respectively. While, *P. lilacinus* didn't show any effect on plant shoot length. Moreover, azadirachtin 0.15% was the least effective treatment which decreased the shoot length by (2.75%).

Table 1. The effect of biological agents and azadirachtin on galls, egg masses in root system and nematode population for tomato plants infected with *M. incognita*

Treatments	The No. per root system weight				Nematode population [250 g soil]	Reduction [%]
	galls No.	reduction [%]	egg masses	reduction [%]		
Abamectin	45.33 f	86.87	79.00 ef	68.94	725.00 c	78.60
Azadirachtin 0.15%	106.00 d	69.31	96.00 d	62.25	1,350 b	60.15
Azadirachtin 0.03%	122.66 c	64.48	151.66 b	40.37	1,222.66 b	63.91
Bacillus subtilis	129.00 c	62.64	72.00 f	71.69	1,342.33 b	60.37
Pseudomonas fluorescens	146.66 b	57.53	109.33 c	57.01	807.66 c	76.16
Paecilomyces lilacinus	40.66 f	88.23	58.66 g	76.94	805.00 c	76.24
Oxamyl	75.66 e	78.09	85.33 e	66.45	376.00 d	88.90
Untreated check	345.33 a	–	245.33 a	–	3,387.33 a	–

Within a column, numbers followed by different letter(s) are significantly different using LSD at p = 0.05

Table 2. The effects of the evaluated treatments on shoot system length and weight in tomato plants infected with *M. incognita*

Treatments	Shoot system length [cm]	Increase [%]	Shoot system weight [g]	Increase [%]	Dry shoot weight [g]	Increase [%]
Abamectin	54.33 b	12.42	24.00 abcd	54.84	2.96 ab	114.49
Azadirachtin 0.15%	47.00 c	–2.75	19.00 cd	22.58	2.18 bc	57.97
Azadirachtin 0.03%	56.33 ab	16.55	25.50 abcd	64.52	2.88 ab	108.70
Bacillus subtilis	58.33 a	20.69	29.33 ab	89.23	2.96 abc	114.49
Pseudomonas flouroscence	56.33 ab	16.55	25.50 abcd	64.52	3.06 ab	121.74
Paecilomyces lilacinus	48.33 c	0.00	19.50 bcd	25.81	2.39 bc	73.19
Oxamyl	53.66 b	11.03	29.00 abc	87.10	3.48 ab	152.17
Control without nematodes	60.00 a	24.15	32.17 a	107.55	4.30 a	211.59
Untreated check	48.33 c	–	15.50 d	–	1.38 c	–

Within a column, numbers followed by different letter(s) are significantly different using LSD at p = 0.05

On the other hand, *B. subtilis*, oxamyl, *P. fluorescens* and azadirachtin 0.03% were the most effective treatments on the shoot weight with values of 89.32, 87.1, 64.52 and 64.52% increase, respectively. Whilst, azadirachtin 0.15% showed the least value of shoot weight increase (22.58%).

In the case of dry shoot weight there was no significant differences between the all used treatments. Oxamyl treatment was the superior that recorded 152.17% increase in dry shoot followed by *P. fluorescens*, *B. subtilis* and abamectin with values of 121.74, 114.49 and 114.49% increase, consecutively. Whereas, azadirachtin 0.15% was the least treatment which recorded 57.97% increase in dry shoot weight.

These findings are in agreement with those given by Krishnaveni and Subramanian (2004) and Shanthi and Sivakumar (2005) who found that yield of plants treated with *P. fluorescens* was increased. Also, Kavitha *et al.* (2007) found that *P. fluorescens, B. subtilis* and *Trichoderma viride* showed a significant increase in the plant growth parameters.

There are several possible mechanisms involved in prevention of nematode development by neem products were mentioned as follows: 1) Alam *et al*. (1980) suggested that the involvement of phenolic compounds absorbed systemically by the roots of tomato plant exposed to neem formulations, might have induced tolerance against nematodes. 2) Khan *et al*. (1974) reported that the narcotic effect of neem formulations could be due to by-products (ammonia, formaldehyde, phenols and fatty acids) released during their decomposition. 3) Khan *et al*. (1974) and Devakumar *et al*. (1985) claimed direct toxicity of neem formulations due to nimbin, salanine, thionemone, Aza (azadirechtin) and nimbidine. 4) The nematicidal action of neem formulations is not only due to the compounds present within the neem product namely nimbidin and thionimone but also due to other by-products such as ammonia, formaldehyde, phenols, and fatty acids produced during decomposition of neem formulations (Khan *et al*. 1974).

Data represented in table 3 indicated the positive performance of the evaluated treatments on the root length and weight. It's obvious that there were no significant differences between *P. fluorescens*, abamectin, *B. subtilis*, azadirachtin 0.15%, azadirachtin 0.03%, *P. lilacinus* and oxamyl in their effect on root system length.

Also, *B. subtilis* recorded the highest increase of root weight with value of 125.75%, followed by *P. fluorescens*, oxamyl, abamectin, *P. lilacinus*, azadirachtin 0.03% and azadirachtin 0.15% which recorded 86.57, 79.66, 70.90, 68.47, 67.16 and 54.85% increase, respectively, without any significant differences.

On the other hand, *P. fluorescens* was the superior treatment that gave 68.14% increase in dry root weight followed by *B. subtilis*, oxamyl, abamectin and azadirachtin 0.03% with values of 35.40, 20.35, 8.85 and 8.85% increase, respectively. *P. lilacinus* and azadirachtin 0.15% were the least effective treatments which had indirect effect on the dry root weight estimated by 8.85 and 2.66% decrease, consecutively.

These findings are in agreement with those obtained by Basu and Karuppagnaniar (2009) who found that Inoculation with fluorescent Pseudomonas or *G. fasciculatum* or both increase the root and shoot length, plant vigour index, dry weight and total N and P content significantly.

Finally, it could be concluded that the results from this study indicated that using of both antagonistic microorganisms and biopesticides achieved a highly activity against the root-knot nematode, in addition gave increasing in plant growth. Therefore, the results imply that it should focus on using biological agents as a safety method for human and environment to management the root-knot nematode in Egypt.

Table 3. The effects of the evaluated treatments on root system length and weight in tomato plants infected with *M. incognita*

Treatments	Root system length [cm]	Increase [%]	Root system weight [g]	Increase [%]	Dry root weight [g]	Increase [%]
Abamectin	30.66 ab	41.55	9.16 bc	70.90	1.23 cde	8.85
Azadirachtin 0.15%	29.00 ab	33.89	8.30 b	54.85	1.10 fg	–2.66
Azadirachtin 0.03%	27.66 ab	27.70	8.96 b	67.16	1.23 def	8.85
Bacillus subtilis	29.17 ab	34.67	12.10 a	125.75	1.53 b	35.40
Pseudomonas fluorescens	26.00 bc	20.04	10.00 b	86.57	1.90 a	86.14
Paecilomyces lilacinus	31.00 ab	43.12	9.03 b	68.47	1.03 g	-8.85
Oxamyl	25.66 bc	18.47	9.63 bc	79.66	1.36 bcd	20.35
Control without nematodes	31.66 a	46.17	8.70 b	62.31	1.43 bc	26.55
Untreated check	21.66 c	–	5.36 c	–	1.13 efg	–

Within a column, numbers followed by different letter(s) are significantly different using LSD at p = 0.05

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