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Effect of some biological nematicides combined with oxamyl in the control of citrus nematode, *Tylenchulus semipenetrans*

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ABSTRACT

This experiment was carried out to explore the role of two bio-nematicides namely : Mycorrhizae (Arbuscular mycorrhiza) and Nemafree (*Serratia marcescens*) with oxamyl singly and in combination to control citrus nematode, *Tylenchulus semipenetrans* under greenhouse conditions. Several methods of control were evaluated, using a chemical pesticide or biological agents (fungi and bacteria), or conducting chemical control, then biological control and vice versa, to determine their efficacy against citrus nematode infecting Valencia orange seedlings. Statistical analysis of the obtained data indicated that the single treatment of the oxamyl nematicide recorded the highest reduction % of citrus nematode larvae reaching (63.8 %), followed by Mycorrhizae (39.5%) and Nemafree (23.1%). The treatment of (oxamyl then Mycorrhizae) recorded the greatest effect in reducing nematode population, where larvae number after 4 weeks of application was 671.9 larvae per 100 cm^3 of soil, as the lowest number of larvae compared to other treatments with a percentage of 48.3% of the total number of larvae, compared with 1300 larvae per 100 cm^3 of soil in control. The treated seedlings with (oxamyl then Mycorrhizae) recorded the lowest number of females in citrus roots , reaching 8.0 female per gram root, with a reduction percentage of 71.4 % compared with other treatments.

Key words: Citrus nematode, biological control, Nemafree, Mycorrhizae, citrus orchards.

INTRODUCTION

Citrus is one of the most important fruit crops through the worldwide. In Egypt, citrus plantation is nearly 420.678 feddan which represents about 33.08% of the total area of fruit trees. Citrus fruits occupied the first rank as the most important crop for production and exportation to other countries. Citrus-parasitic nematode, *Tylenchulus semipenetrans* Cobb., consider one of the most serious pest of citrus trees is capable of damaging mature trees and

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down grading fruit quality. It is a parasite of several woody plant species, and aptly named because it is ubiquitous in the citrus producing regions of the world (Sorribas *et al*., 2008). *Tylenchulus semipenetrans* nematode is well-adapted to citrus, with very high numbers required to significantly affect the growth and health of its host (Maafi and Damadzadeh, 2008). Despite the long history of biological control in plant pathology, recent advances in biotechnology have sparked renewed interest and vigor in the pursuit of biocontrol as an emerging technology. Although chemical nematicides such as organophosphate and carbamate nematicides are highly effective in controlling nematodes, their usage has been restricted in recent years because of increasing concerns about their effects on the environment and food (Anastasiadis *et al*., 2008; Kiewnick and Sikora, 2006).

Vesicular-arbuscular mycorrhizal (VAM) fungi (*Glomus mosseae*) are obligate symbionts that increase nutrient uptake by plants, especially phosphorus and other minor elements (Gerdemann, 1968; Smith,1987). The mycorrhizal fungi contribute to the control of plant disease, and the mechanisms by which they do so have been well documented (Ahmed *et al*., 2009). This mycorrhizal working on increasing nutrient uptake, growth rates and hormonal activity in roots (Linderman, 1992). The biological control of soil-borne plant pathogens and nematodes by antagonistic microorganisms is a potential non-chemical means of plant disease control. A wide range of bacteria (Moussa and Abo-Korah, 2016) used to reduce a range of plant parasitic nematodes.

Therefore, the objective of the present study aimed to evaluate the effect of two bio pesticides in comparison with a chemical nematicide, singly or in combination on the development of citrus nematode, T. semipenetrans infecting Valencia citrus seedlings.

MATERIALS AND METHODS

The experiment was carried out under greenhouse conditions of the Faculty of Agriculture, Menoufia University, Shebin El-Kom.

One seedling of the Valencia orange (seven months old) was planted in plastic pot 25 cm in diameter filled with 4 kg sterilized clay-sand mixed soil (1:1, v/v). Fifteen days later, 4000 J2 of T. semipenetrans were pipetted into three holes around the rhizosphere of each seedling.

Nemafree® bio nematicide (a commercial suspension of bacterium, *S. marcescens* Bizio, having 1 × 109 cells/ml water) produced by the Egyptian Ministry of Agriculture and Land Reclamation, mixed thoroughly with Talcum powder at 50% (w/w) and applied at the rate of 10 g/ plant, 15 days after nematode inoculation.

Mycorrhizae as bio nematicide contain 300 propagules/gram (Arbuscular mycorrhiza) were inoculated in soil 15 days after nematode inoculation. Each pot received endo-mycorrhiza inoculated with (3g) infested soil and (0.5 g) of the onion roots colonized by *Glomus mosseae*. Plants were thinned to one plant per pot after 5 days (Amin and Mostafa, 2000).

Oxamyl 24% L is a chemical nematicide (methyl 2-(dimethylamino)-N-(methylcarbamoyloxy)-2-oxoethanim-idothioate) C7H13N3O3S, it is commonly sold under the trade name Vydate, and was applied at the rate of 2ml/seedling, 15 days after nematode inoculation. All the treatments were singly applied, also binary treatments were done where the first treatment is applied, and two weeks later, the second treatment was added in the same pot. The mean average of air temperature was ranged from 22-26 ºC. and the relative humidity was ranged between 60-70%. Treatments were replicated three times and arranged as randomized complete block design (RCBD).

Nematode Extraction and Enumeration:

Each soil sample was carefully mixed, and an aliquot of 100 cm^3 was processed for nematode extraction according to methods described by Christie and Perry (1951) and Southey (1970). About 300-400 ml of water were added to the soil in a glass beaker (1000 ml) and the mixture was agitated by fingers, after few seconds the suspension was poured onto a 60 mesh-sieve and passing suspension was collected in another clean glass beaker. Materials caught on the 60 mesh-sieve were discarded, while the collected suspension was then poured onto a 200 mesh-sieve. Materials remain on the sieve were thoroughly washed by a gentle streamed of water into a 200 ml beaker. The resulting suspension containing nematodes was then transferred to a Modified Baermann pan fitted with soft tissue paper for the separation of active nematodes from debris and fine soil particles. After 72 hrs. nematode water suspension was collected and concentrated to 20 ml in a vial by using a 350 mesh-sieve. An aliquant of 1 ml each of nematode suspensions were pipetted off, placed in a Hawksley counting slide and examined using a stereomicroscope.

Nematode counts were made after 4,8,12,16,20 and 24 weeks of application. At the end of the experiment, the roots are washed and placed in Lactophenol acid fuchsine for 24 hours, then the females in the roots are counted using a microscope (Daykin and Hussey 1985).

Statistical analysis:

All obtained data were subjected to ANOVA test using a computer program (CoStat, 2008) to determine Duncan's multiple range test and the LSD 5% (least significant difference). In addition, Abbott's formula was used to determine the increase percentages of vegetative characters.

Reduction percentages were counted according to Abbott's (1925):

$$\text{Reduction \%} = \left(1 - \frac{no\ in\ Treatment\ after\ treatment}{no\ in\ Control\ after\ treatment}\right) x100$$

RESULTS:

The obtained results in Tables (1, 2) indicated that the chemical pesticide oxamyl gave the highest results in reducing nematode population, as the number of larvae after 4 weeks of application was 715.5 juveniles in 100 cm^3 soil, recorded the lowest number of larvae compared to other treatments by a percentage of 47.3% of the total number of juveniles, compared with 1357.6 juveniles per 100 cm^3 of soil at control. In addition, the chemical pesticide gave the highest results in reducing the number of juveniles in soil where it decreased to 101 larvae in 100 cm^3 soil after 24 weeks of application, as the lowest number of juveniles compared to other treatments, with a percentage of 95.3% of the total number of larvae, compared with 2149.3 juveniles per 100 cm^3 soil in control.

The bio-nematicide of bacterial origin Nemafree gave the lowest results in reducing nematode population, as the number of juveniles after 4 weeks of application reached 931 larvae in 100 cm^3 soil, as the largest number of larvae compared to other pesticides with a percentage of 31.4% of the total number of larvae, compared with 1357.6 juveniles per 100 cm^3 of soil in control. Also, Nemafree application gave the lowest results in reducing nematode population, as the number of larvae, after 24 weeks of application, where it was 397 larvae in 100 cm^3 soil, as the largest number of larvae compared to other treatments, with a percentage of 81.5% of the total number of larvae, compared with 2149.3 juveniles / 100 cm^3 soil in control.

Table 1: Effect of bio-nematicides and oxamyl on the population density of citrus nematode, *T. semipenetrans* infected citrus seedlings under green house conditions.

Treatments	Aver. no. of *T. semipenetrans* juveniles/ 100 cm^3 soil — weeks post-treatments						Overall mean	Female population per 1 g root
	4 weeks	8 weeks	12 weeks	16 weeks	20 weeks	24 weeks		
Nemafree	931.0 b	814.5 b	706.3 b	597.0 b	507.0 b	397.0 b	658.8 b	20.0 ab
Mycorrhizae	901.0 c	794.5 c	613.0 c	490.0 c	364.5 c	199.3 c	560.4 c	16.0 b
Oxamyl	715.5 d	638.6 d	491.0 d	302.5 d	187.0 d	101.0 d	405.9 d	12.0 b
Control	1357.6 a	1600.2 a	1759.5 a	1915.5 a	2037.0 a	2149.3 a	1803.2 a	26.0 a
LSD 5%	9.4	9.4	9.4	9.4	9.4	9.4	9.4	8.1

values in each column followed by the same letter are not significantly different at 5% level.

Table 2: Reduction percentages of *T. semipenetrans* infected citrus seedlings treated with bio pesticides under green house conditions.

Treatments	Reduction % by Abbott formula						Overall mean	Females reduction %
	4 weeks	8 weeks	12 weeks	16 weeks	20 weeks	24 weeks		
Nemafree	31.4	49.1	59.9	68.8	75.1	81.5	60.9	23.1
Mycorrhizae	33.6	50.4	65.1	74.4	82.1	90.7	66.1	39.5
Oxamyl	47.3	60.0	72.0	84.2	90.8	95.3	74.9	63.8

Roots of seedlings treated with chemical pesticides oxamyl contain only 12 citrus nematode female per gram of roots with a percentage of 53.8% compared to 26 females/g roots of untreated seedlings, followed by the treatment of Mycorrhizae with 16 females / g roots as 39.5 reduction percentages. Meanwhile, the roots of seedlings treated with bio-nematicide of bacterial origin Nemafree contain 20 female per gram of roots with a reduction percentage of 23.1%

compared to 26 females / g roots of untreated seedlings.

As shown in Tables (3, 4) the obtained results indicated that the treatment of (Oxamyl then Mycorrhizae) recorded the greatest effect in reducing citrus nematode population, as the number of larvae after 4 weeks of application reached 671.9 juveniles in 100 cm3 soil, recording the lowest number of larvae compared to other treatments with a percentage of 48.3% of the total number of juveniles, compared with 1299.7 juveniles per 100 cm3 of soil of control treatment. Moreover, after 24 weeks of the application of the treatment of Oxamyl then Mycorrhizae recorded the greatest effect in reducing nematode population, as the number of larvae was only 53.3 juveniles per 100 cm^3 of soil, as the lowest number of juveniles compared to other treatments, with a percentage of 97.6% of the total number of larvae, compared to 2209 juveniles per 100 cm^3 of soil in control treatment. The treatment of Nemafree then Mycorrhizae recorded the least effect in reducing citrus nematode population, as the number of larvae, after 4 weeks of application, was 924 larvae per 100 cm^3 of soil, representing the highest number of larvae compared to other treatments with a percentage of 28.9% of the total number of larvae, compared to 1299.7 juveniles per 100 cm^3 of soil in control treatment.

In addition, after 24 weeks of application, the treatment of Nemafree then Mycorrhizae recorded the least effect in reducing citrus nematode population, as the number of larvae was 232 larvae per 100 cm^3 of soil, which is the lowest number of larvae compared to other treatments with a reduction percentage of 89.5% of the total number of larvae, compared with 2209 juveniles per 100 cm^3 of soil in control treatment. The lowest number of females on citrus roots was recorded at the treatment of Oxamyl then Mycorrhizae recording 8 female /g roots, with the highest reduction percentage as 71.4%, followed by the treatment of Mycorrhizae then Oxamyl recorded 10 female /g roots, with a reduction percentage a 64.6%. Meanwhile, the treatment of Nemafree then Mycorrhizae recorded the highest number of females, as 15 female per gram occupied the least reduction percentages of females as 46.4% compared to control treatment.

Table 3: Effect of bio-nematicides on juvenile numbers of citrus nematode, *T. semipenetrans* infected citrus seedlings under greenhouse conditions.

Treatments	Aver. no. of *T. semipenetrans* juveniles/ 100 cm^3 soil						Overall mean	Female per 1 g root
	weeks post-treatments							
	4 weeks	8 weeks	12 weeks	16 weeks	20 weeks	24 weeks		
Oxamyl + Nemafree	703.0 e	612.0 f	474.3 f	291.6 d	168.3 f	83.3 f	388.6 f	10.3 b
Nemafree + Oxamyl	876.3 d	768.9 c	657.3 c	514.0 b	391.4 c	203.0 c	568.5 c	13.0 b
Oxamyl + Mycorrhizae	671.9 f	594.3 g	402.3 g	231.9 c	107.0 g	53.3 g	343.5 g	8.0 b
Mycorrhizae+ Oxamyl	881.3 d	694.4 e	511.3 e	370.9 d	209.0 e	94.9 e	460.3 e	9.9 b
Nemafree + Mycorrhizae	924.0 b	801.3 b	677.9 b	517.6 b	460.1 b	232.0 b	602.2 b	15.0 b
Mycorrhizae+ Nemafree	891.9 c	708.9 d	576.3 d	428.0 c	297.3 d	117.9 d	503.2 d	14.1 b
Control	1299.7 a	1559.9 a	1841.0 a	1979.9 a	2109.3 a	2209.0 a	1833.1 a	28.0 a
LSD 5%	8.8	14.4	8.8	39.2	8.8	8.8	8.8	8.8

+ mean that the second treatment was applied 15 days after the first
values in each column followed by the same letter are not significantly different at 5% level

Table 4: Reduction percentages of *T. semipenetrans* infected citrus seedlings treated with bio pesticides under greenhouse conditions.

Treatments	Reduction %of citrus nematode juveniles in soil by Abbott formula						Overall mean	Reduction %of citrus nematode females
	4 weeks	8 weeks	12 weeks	16 weeks	20 weeks	24 weeks		
Oxamyl + Nemafree	45.9	60.8	74.2	85.3	92.0	96.2	75.7	63.2
Nemafree + Oxamyl	32.6	50.7	64.3	74.0	81.4	90.8	65.6	53.6
Oxamyl + Mycorrhizae	48.3	61.9	78.1	88.3	94.9	97.6	78.2	71.4
Mycorrhizae + Oxamyl	32.1	55.5	72.2	81.3	90.1	95.7	71.2	64.6
Nemafree + Mycorrhizae	28.9	48.6	63.1	73.9	78.2	89.5	63.7	46.4
Mycorrhizae + Nemafree	31.4	54.5	68.7	78.4	85.9	94.7	68.9	49.6

+ means the second treatment was applied 15 days after the first

DISCUSSION

The control of plant parasitic nematodes has been a difficult task for decades. Remarkable reduction of nematode population has been achieved by application of nematicide of chemical origin. However, due to the hazardous effects of chemical on the environment, great interest has focused on biological control methods. Several strategies, including resistance cultivars, organic soil amendments, and biological control have been developed for the management of plant parasitic nematodes (Kimenju *et al*., 2008).

Oxamyl is one of the effective systemic nematicide against plant-parasitic nematodes, which succeeded in eliminating nematodes in good proportions. In spite of our finding that Oxamyl along with NPK overwhelmed all the treatments in controlling nematode and increasing tomato yield, the authors recommend further studies about agrochemicals integrated regimes (Osman, *et al*., 2015).

Research on the use of antagonistic microorganisms to control plant parasitic nematodes is receiving increasingly greater attention (Hallman *et al*., 2009). The results are in agreement with those reported for the efficacy of *Bacillus thuringiensis* and *Serratia* sp on nematodes :*H. glycine*, *T. semipenetrans* and *M. javanica* (Banna and Horani, 2007). Similarly, the bacterial preparations caused inhibitory effect on multiplication of cyst nematode, *Globodera rostochiensis* (Trifonova, 2010).

The obtained results are in agreements with Abo-Korah (2017) who reported that the application of mycorrhizae might play an important role in enhancing parasitic activity of nematophagous fungi in the soil and stimulating plant growth. It also increase plant immunity and its resistance to parasitic nematodes.

REFERENCES

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal Econ. Entomology*, 18: 265-267.

Abo-Korah, M.S.(2017). Biological control of root-knot nematode, *Meloidogyne javanica* infected Ground Cherry using two nematophagous and mycorrhizal Fungi. *Egyptian Journal of Biological Pest Control*, 27(1): 111-115.

Ahmed, S.H., Abdelgani, M.E. and Yassin, A.M. (2009). Effects of interaction between vesicular-arbuscular mycorrhizal (VAM) fungi and root knot nematodes on Dolichos bean (*Lablab niger* Medik.)

plants. *American-Eurasian Journal of Sustainable Agriculture,* 3:678–683.

Amin, A.W. and F.A. Mostafa (2000). Management of *Meloidogyne incognita* infecting Sunflower by integration of *Glomus mosseae* with *Trichoderma viride*, *T. harzianum* and *Arthrobotrys oligospora*. *Egyptian Journal of Agronematology,* 4(2): 102-113.

Anastasiadis, I.A., Giannakou, I.O, Prophetou-Athanasiadou, D.A. and Gowen, S.R. (2008). The combined effect of the application of a biocontrol agent *Paecilomyces lilacinus*, with various practices for the control of root-knot nematodes. *Crop Protection*., 27:352–361.

Banna L.A. and Horani H.K. (2007). Efficacy of *Bacillus thuringiensi* integrated with other non-chemical materials to control *Meloidogyne incognita* in tomato. Nematologia Mediterranea 35:69-73.

Christie, J. R. and Perry, V. G. (1951). Removing nematodes from soil. Proc.Helm. Soc. Wash., 18:106-108.

CoStat 6.400 (2008). Statistical CoHort Software program, Copyright © 1998- 2008 CoHort Software 798 Lighthouse Ave. Monterey CA, USA.

Daykin, M. E., and Hussey, R.S. (1985). Staining and histo pathological techniques in nematology. In: Barker, K. R.; C. C. Carter and J. N. Sasser (eds), An advanced treatise on Meloidogyne, V. II Methodology,pp. 39-48. N. CarolinaStateUniv.Graphics,Raleigh.

Gerdemann, J. W. (1968). Vesicular-arbuscular mycorrhiza and plant growth. *Annull Review. of Phytopathology*, 6: 396-418.

Hallman J., Davies K.G. and Sikora R. A.(2009). Biological control using microbial pathogens, endophytes and antagonists. In: Root-knot Nematodes. Perry R.N., Moens M., Starr J.L (Eds.). Wallingford, UK, CAB International: 380-411.

Kiewnick, S. and Sikora, R.A. (2006). Biological control of the root-knot nematode, *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. *Biological Control*,38:179–187.

Kimenju, J. W., A. M. Kagundu, J. H. Nderitu, F. Mambala, G. K. Mutua and Kariuki, G. M. (2008). Incorporation of green manure plants into bean cropping systems contribute to root- knot nematode suppression. *Asian Journal of plant Sciences* 7: 404- 408.

Linderman, R. G. (1992). Vesicular-arbuscular mycorrhizae and soil microbial interactions. In: Mycorrhizae in Sustainable Agriculture. Eds. G. J. Bethlenfalvay and R. G. Linderman. pp 45–70.

Maafi, Z. T. and Damadzadeh, M. (2008). Incidence and control of the citrus nematode, *Tylenchulus semipenetrans* Cobb, in the north of Iran. *Nematology*, 10(1): 113-122.

Moussa, M.M. and Abo-Korah, M.S. (2016). New successful strategy by application of antibiotic and foliar fertilizer for controlling of *Meloidogyne javanica* infected Gladiolus plants and its connectedness of plant characteristics. *Life Science Journal*,13(1):142-151.

Osman Hamida, A.; Hoda, H. Ameen; Mohamed M. M. and Alkelany U.S.(2015). Effect of Integrating Inorganic Fertilizer with either Micronema, Compost, or Oxamyl on Suppressing Plant Parasitic Nematode *Meloidogyne incognita* Infecting Tomato Plants under Field Conditions. *Middle East Journal of Agriculture*, 4: 707-711.

Smith, G. S. (1987). Interactions of nematodes with mycorrhizal fungi. In Vistas on Nematology. Eds. J. Veech and D. Dickson. pp 292-300.

Sorribas, F. J., Verdejo-Lucas S. and Pastor, J. (2008). Population densities of *Tylenchulus semipenetrans* related to physicochemical properties of soil and yield of clementine mandarin in Spain. *Plant Disease*, 92: 445-450.

Southey, J. F. (1970). Principles of sampling for nematodes, pp-1-4 in Laboratory methods for work with plant & soil nematodes. Min. Agric., Fish & Food Tech. Bull. 2. Her Hajesty's Stationery Office, London.

Trifonova Z.T. (2010). Studies on the efficacy of some bacteria and fungi for control of *Globodera restochiensis*. *Journal of Agricultural Sciences, Belgrade*,*55*(1), 37-44.

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