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Effect of Different Bioagents on the Population Density of *Meloidogyne incognita* Infected Tomato Plants

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ABSTRACT

The effect of two isolates of fungi (*Glomus mosseae* - *Trichoderma harzianum*); two isolates of bacteria (*Azotobacter chroococcum* - *Pseudomonas fluorescens*), and two nematodes (*Steinernema feltiae- Diplogaster lheritieri*) was evaluated against root knot nematode, *Meloidogyne incognita* infecting tomato plants under greenhouse conditions. The experiment was conducted in plastic pots (25 cm) at the Research Station of the Faculty of Agriculture in Shebin El-kom, El-Menoufia Governorate. Statistical analysis of the obtained results recorded that predatory nematode, *D. lheritieri* gave the highest reduction percentage (84.0%) in *M. incognita* juveniles, as well as it reduced root gall index (60.0%) and mature females (72.3%), compared to the nematicide oxamyl that gave 84.2 & 80.0 %, respectively. Triple treatment of *D. lheritieri* + *A. chroococcum* + *G. mosseae* was superior recording only 184.7 J_2 / 100 cm^3 soil with a reduction percentage of 88.5 % compared to the rest of bio-agents. From the obtained results, it can be recommended to use such treatment which achieved the highest decrease percentages of soil nematodes, number of females and root knot galls compared to oxamyl.

***Keywords*:** Root knot nematode, biological control, bacteria, mycorrhizae, predator, entomopathogenic nematode

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) belongs to Family Solanaceae, which is grown in temperate and warm regions. Tomatoes contain several antioxidant vitamins (vitamins C, B and A) and contain many sugars, minerals, vegetable acids, iron and a quantity of proteins. It is considered one of the vegetables of great economic importance (Bashir et al., 2018). *Meloidogyne incognita* is a sedentary endoparasitic species of the root knot nematodes and is considered one of the most important pests that infect tomatoes and cause great economic losses by attacking the roots of tomatoes and cause root knot disease (Manju and Sankari, 2015). Scientists conducted many researches to control root knot nematodes by safe microorganisms to avoid the harmful effects of chemical nematicides. Vesicular-arbuscular mycorrhizal (VAM) fungi (*Glomus mosseae*) penetrates the roots of the host and enters into the cells and multiplies with the presence of some outside the roots extending in the soil and works to facilitate the absorption of water, salts and nutrients to the roots (Ahmed et al., 2009). *Trichoderma harzianum* considered an effective bio-agent against root-knot nematodes, and studies have shown that it reduces root-knot nematode eggs and is used in integrated control programs (El-Deeb et al., 2018). *Azotobacter chroococcum* and *Pseudomonas fluorescens* strains affect root-knot nematodes, directly by excreting toxic substances that lead to the death of nematode juvenile, or indirectly by changing the rhizosphere environment (Youssef and Eissa, 2014). Entomopathogenic nematode, *Steinernema feltiae* is used in biological control of insect pests, but many studies have proven their efficacy against plant-parasitic nematodes (Jagdale et al., 2009). Studies have shown that *S. feltiae*

reduced root knot nematode, *M. incognita* juvenile infected tomatoes (El-Ashry et al., 2018). *Diplogaster lheritieri* has proved to be successful in biological control programs for plant parasitic nematode. It has a high ability to attack plant-parasitic nematodes, as it is equipped with teeth that cut its prey and feed on it (Anwar et al., 2005; Sanchez-Moreno et al., 2007). The harmful effects of chemical control on human and animal health, as well as the plants, have promoted scientists to think about alternative& safe and harmless methods for the environment,

Hence the idea of this research under study was to use safe agents and microorganisms on humans and plants to control root knot nematode, *M.–incognita* infecting tomato plants.

MATERIALS AND METHODS

Nematode culture:

Meloidogyne incognita second juveniles (J_2) were collected from pure culture reared on cotton *Gossypium barbadense* (Gallini) var. Delta Pin 61. Galled roots of cotton were washed in the water to clean them from the soil sticking to the roots, then the roots were cut to pieces of 1 cm long and stirred in sodium hypochlorite NaOCl (0.5%) for 3 minutes and shacked well in sterile water to free eggs from egg masses (Kerry and Bourne, 2002). Eggs were incubated in modified Baermann funnel at 25 ± 1°C for 4 days to obtain second stage juveniles (J_2) according to the method of Gray (1984) to use it in the experimental infection procedure (Chuixu et al., 2013).

Preparation of treatments:

1- Fungi:

Glomus mosseae and *Trichoderma harzianum* fungi isolates were obtained from Cairo Mercin, Ain Shams University, Egypt. Mycorrhizae inocula were applied 15 days before nematode infection. Pots receiving endo-mychorrhiza was each inoculated with 3g infested soil and 0.5 g of the onion roots colonized by *G. mosseae* (Amin and Mostafa, 2000). *T. harzianum* ($1x10^{10}$ vital spores), was applied at the rate of 0.2 g/plant (El-Deeb et al., 2018).

2- Bacteria:

Efficient local strains of *Azotobacter chroococcum* and *Pseudomonas fluorescens* were obtained kindly from Pharmaceutical Department, Agricultural Research Center, Giza, Egypt. The bacterial strains were prepared for application according to the method described by Mahfouz (2003). The prepared culture from each bacterial strain contained 10^7cell/ml, and was applied at the rate of 3 ml /plant, at the same time of nematode inoculation.

3- Nematode:

Pure culture of predatory nematode, *Diplogaster lheritieri* was obtained from Nematology and Biotechnology Laboratory, Faculty of Agriculture, Fayoum University and reared under laboratory conditions.

Steinernema feltiae was obtained kindly from a pure culture by Dr. Ahmed Azaze from Entomology and Nematology, Agricultural Research Center, Giza, Egypt. It was separately cultured in last instar larvae of the greater wax moth *Galleria mellonella* L. this technique according to El-Ashry et al. (2018). At the same time of adding *M. incognita* infection, the predatory nematode, *D. lheritieri* was added at a rate of 100 individual per pot, as well as entomopathogenic nematode, *Steinernema feltiae* was

added at the same time of adding root knot nematode at the rate of 5000 IJs per pot, for single or combined treatments (El-Ashry et al., 2018).

4- Oxamyl :
Vydate 24% L is a chemical insecticide and nematicide: (methyl 2-(dimethylamino)-N-(methylcarbamoyloxy)-2-oxoethanimidothioate) $C_7H_{13}N_3O_3S$. It is commonly sold under the trade name Vydate. It was applied at the rate 2 ml /pot and added at the time of nematode inoculation.

Experimental preparation and design:
Experiment layout was randomized complete block design with ten replicates. This experiment was carried out in the experimental station of the Faculty of Agriculture at Menoufia University, Egypt. The experiment was done under greenhouse conditions and each treatment consisted of 10 replicates. One seedling 30 day-old of tomato, *Solanum lycopersicom* cv. Login was planted in plastic pots 25 cm containing 3kg of sterilized clay-sand mixed soil (1:3 v/v). After seven days of seedlings adaptation, 3000 J_2 /3 kg soil of *M. incognita* were added by pipette into three holes around each seedling.

Sampling Procedures:
Soil samples were collected from soil around tomato plants after 30,60 and 90 days of nematode inoculation, using a hand trowel where the dried surfaces of soil was removed and samples were taken from the wetted rhizosphere region of the soil and transferred to the laboratory to extract nematodes and determine the population of root knot nematode.

Nematode Extraction and Numeration:
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.

At the end of the experiment, tomato roots were washed carefully under running tap water in order to remove soil particles. A sample of one gram for each composite root sample (Shafiee and Mendez, 1975) was immediately stained in Lactophenol acid fuchsine for 24 hours, then the females were counted using a stereomicroscope (Daykin and Hussey, 1985) as well as root gall index was computed according to Taylor and Sasser (1978) (Table 1).

Table 1: Rating scale levels of gall numbers

Number of galls/ root system	Gall index
0	0
1-2	1
3-10	2
11-30	3
31-100	4
>100	5

Statistical analysis:

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

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

$$\text{Reduction \%} = \left(1 - \frac{\text{No of nematodes after treatment}}{\text{No of nematodes in Control}}\right) * 100$$

RESULTS

The obtained results in Tables (2&3) revealed that the combined treatment of *D. lheritieri + A. chroococcum + G. mosseae* was superior recording only 184.7 individual nematodes/ 100 cm^3 soil with a reduction rate of 88.5 % compared to the rest of the materials, followed by the binary treatment *D. lheritieri + A. chroococcum* and the binary treatment *D. lheritieri + G. mosseae*, where 228.4 and 248.0 nematodes/100 cm^3 soil were found, with a reduction of 86.0 and 84.8%, respectively. While the binary treatment of *G. mosseae + P. fluorescens* gave 702.6 nematodes/100 cm^3 soil with a reduction rate of 59.8%, and *T. harzianum + P. fluorescens* also gave 698.7 nematodes/100 cm^3 soil with a reduction rate of 59.5%, recording the lowest reduction percentages.

Table 2: Effect of bio-treatments on the population density of *Meloidogyne incognita* infected tomato plants.

Treatments	Aver. No. of *M. incognita* juveniles/ 100 cm^3 soil			
	Days post-treatments			
	30 Days	60 Days	90 Days	Overall mean
T. harzianum + P. fluorescens	781.9 c	713.3 b	601.0 b	698.7 b
G. mosseae + P. fluorescens	815.6 b	701.9 c	590.3 c	702.6 b
T. harzianum + A. chroococcum	694.9 e	487.0 g	428.0 e	536.6 d
G. mosseae + A. chroococcum	627.0 g	471.9 h	317.3 g	472.1 f
S. feltiae + G. mosseae	739.0 d	567.0 d	497.9 d	601.3 c
S. feltiae + A. chroococcum	701.0 e	549.3 e	334.9 f	528.4 d
D. lheritieri + G. mosseae	449.0 i	203.9 i	91.0 h	248.0 g
D. lheritieri + A. chroococcum	407.9 j	192.0 j	85.3 i	228.4 h
S. feltiae + A. chroococcum + G. mosseae	649.9 f	516.0 f	297.0 i	487.6 e
D. lheritieri + A. chroococcum + G.mosseae	374.9 k	118.3 k	61.0 j	184.7 i
Vydate 24% L	497.0 h	187.0 j	84.9 i	256.3 g
Control	1497.9 a	1796.9 a	2101.3 a	1798.7 a

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

Table 3: Reduction percentage of *Meloidogyne incognita* infected tomato in the soil after application of different bio-agents.

Treatments	Reduction %			
	30 Days	60 Days	90 Days	Overall mean
T. harzianum + P. fluorescens	47.8	60.3	71.4	59.8
G. mosseae + P. fluorescens	45.6	60.9	71.9	59.5
T. harzianum + A. chroococcum	53.6	72.9	79.6	68.7
G. mosseae + A. chroococcum	58.1	73.7	84.9	72.2
S. feltiae + G. mosseae	50.7	68.5	76.3	65.2
S. feltiae + A. chroococcum	53.2	69.4	84.1	68.9
D. lheritieri + G. mosseae	70.0	88.7	95.7	84.8
D. lheritieri + A. chroococcum	72.8	89.3	95.9	86.0
S. feltiae + A. chroococcum+ G. mosseae	56.6	71.3	85.9	71.3
D. lheritieri + A. chroococcum + G. mosseae	75.0	93.4	97.1	88.5
Oxamyl (Vydate24 % L)	66.8	89.6	96.0	84.1

The statistical analysis of the data in Table (3) indicated that the highest reduction percentages treatment that caused the death rate of root-knot nematode, *M. incognita* juvenile was the predatory nematode *D. lheritieri* with a percentage of 84.1%, followed by the treatment with *A. chroococcum* bacteria with a percentage of 68.3% compared to the nematicide Vydate that caused a death rate of 84.0%.
Whereas, the treatment with *D. lheritieri* reduced the percentage of mature females and root gall index to 72.3 & 60.0%, compared to the nematicide Vydate, which reduced by 84.2 & 80.0%, respectively.

Table 4: Influence of the binary and triple effect of bio-treatments on mature female and root gall index of *Meloidogyne incognita* infecting tomato roots.

Treatments	Root gall index	Reduction %	Mature females per1g/root	Reduction %
T. harzianum + P. fluorescens	4.0 b	20.0	18.9 b	31.3
G. mosseae + P. fluorescens	4.0 b	20.0	17.0 c	38.2
T. harzianum + A. chroococcum	3.0 c	40.0	15.0 d	45.5
G. mosseae +A. chroococcum	3.0 c	40.0	14.0 de	49.1
S. feltiae + G. mosseae	3.0 c	40.0	13.9 de	49.5
S. feltiae + A. chroococcum	3.0 c	40.0	12.0 e	56.4
D. lheritieri +G. mosseae	2.0 d	60.0	7.2 f	73.8
D. lheritieri + A. chroococcum	2.0 d	60.0	7.0 f	74.5
S. feltiae + A. chroococcum + G. mosseae	2.0 d	60.0	8.3 f	69.8
D. lheritieri + A. chroococcum + G. mosseae	1.0 e	80.0	3.0 h	89.1
Vydate 24% L	1.0 e	80.0	5.0 g	81.8
Control	5.0 a	-	27.5 a	-

Means in each column followed by the same letter (s) are not significantly different at 5% level.

In addition the obtained results in Table (4) cleared that the triple treatments also reduced the numbers of female nematodes, and the most successful treatment in this direction was the multiple treatment of *D. lheritieri + A. chroococcum + G. mosseae*, which reduced the number of females by 89.1%, followed by the treatment of *D. lheritieri + A.chroococcum* with a decrease of 74.5%. These treatments also reduced the number of root knot galls. The highest treatments in reducing the number of nematode galls were the combination treatment of *D. lheritieri + A. chroococcum + G. mosseae*, giving an 80% decrease in the average number of root knot galls, while the binary treatments of *G. mosseae + P. fluorescens*, or *T. harzianum + P. fluorescens* gave only 20% decreases in both nematode galls and mature females.

From the previous results, it can be recommended to use the triple treatment of *D. lheritieri + A. chroococcum + G. mosseae*, which achieved the highest reduction percentages of nematodes and reduced the number of females and nematode galls more than the percentage achieved by the pesticide Vydate.

DISCUSSION

The obtained results agree with those conducted by Linderman (1992), Ahmed et al., (2009) and Abokorah (2017) who recorded that, vesicular-arbuscular mycorrhizal (VAM) fungus (*Glomus mosseae*) lives with the roots of the plant in a symbiotic life, as it facilitates the absorption of nutrients to the roots of the plant, which leads to an increase in the plant's immunity in resistance to root-knot nematodes, as it competes with the parasitic nematodes on the plant for housing within the root tissues.

Moreover, the article results are in agreement with several attempts have been made to use *T. harzianum* to control plant-parasitic nematodes. Al-Hazmi and Javeed (2016) reported decrease in egg production of the root-knot nematode, *M. javanica* on tomato plants; soil juveniles (J_2); and increased host growth when treated with *T. harzianum* and it is important that biocontrol isolates are able to compete and persist in the environment rapidly colonize and efficiently proliferate on newly formed roots. In addition, Weller et al. (2002) and Youssef and Eissa (2014) recorded that, *A. chroococcum* inhibit the activity of parasitic nematodes by releasing ammonia gas that suffocates nematodes and leads to the death of the juvenile of parasitic nematodes on the plant and it also works to raise soil fertility. Both *Azotobacter chroococcum* and *P. fluorescens* reduce the numbers of parasitic nematodes and improved the vegetative characteristics of hibiscus plants (Moussa and Abo-Korah, 2016).

Diplogaster sp. is a very important predatory nematode caused significantly reduction in nematode population in the roots of tomato. *Diplogaster* sp. as a predator of second stage juveniles of *M. javanica* by prey on parasitic nematodes with their teeth were studied by Khan and Kim (2005); McSorley et al. (2006); Sanchez-Moreno et al. (2007).

Entomopathogenic nematodes (EPNs) are currently marketed worldwide for biological control of insect pests. Several greenhouse studies have demonstrated suppression of plant parasitic nematodes (PPNs) by application of EPNs (Jagdale et al., 2009). These findings suggest the possibility of exploiting the antagonistic potential of EPNs for biological control of PPNs. Moreover, it has been shown that EPNs can affect the populations of RNKs infecting plants, when they are applied near the root system (El-Ashry et al., 2018).

Chemical nematicides control has disadvantages like high expensive and hazard to human health and the environment. Thus, there is urgently needed to replace chemical control. Now, several alternative techniques are available, including biocontrol agents

and soil amendments (El-Deeb et al., 2018). Today, chemical nematicides are losing their popularity among farmers for protecting their crops from nematode infestations. Some safe procedures for nematode control have been developed passed on biological control agents and organic amendments; however, there is still a need for alternative, friendly methods or compounds for effective nematode control to be developed (El-Ashry et al., 2018).

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الملخص العربى

تأثير بعض المواد البيولوجية على نيماتودا تعقد الجذور التى تصيب الطماطم

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قسم الحشرات الاقتصادية والحيوان الزراعى - كلية الزراعة - جامعه المنوفية - شبين الكوم - مصر

تم اجراء هذة التجربة بمحطة البحوث بكلية الزراعة بشبين الكوم محافظة المنوفية وذلك لدراسة تأثير بعض المواد البيولوجية والحيوية فى مكافحة نيماتودا تعقد الجذور التى تصيب نباتات الطماطم حيث تم زراعة شتلات الطماطم صنف لوجين فى اصص بلاستيكية سعة ٣ كجم وعمل عدوي بنيماتودا تعقد الجذور وتطبيق بعض المواد البيولوجية من فطر – بكتريا – نيماتودا ومقارنه هذه المواد بالمبيد الكارباماتى اوكساميل ٢٤٪ .

أظهر التحليل الإحصائي للنتائج المتحصل عليها أن المعاملة المنفردة باستخدام النيماتودا المفترسة *D. lheritieri* أعطت أعلى نسبة انخفاض في تعداد النيماتودا بنسبة ٨٤,٠٪ ، كما أدت إلى انخفاض معدل العقد الجذرية والإناث الناضجة بنسبة ٦٠,٠٪ ، ٧٢,٣٪ ، مقارنة بمبيد النيماتودا أوكساميل الذي أعطى ٨٤,٢٪ و ٨٠,٠٪. أعطت المعاملة الثلاثية لـ *D. lheritieri + A. chroococcum + G. mosseae* اعلى النتائج بنسبة خفض للنيماتودا فى التربة بنسبة ٨٨,٥ ٪ مقارنة ببقية المواد. من النتائج التي تم الحصول عليها ، يمكن التوصية باستخدام هذه المعاملة الثلاثية والتي حققت أعلى نسب انخفاض في النيماتودا وتقليل عدد الإناث وتكوين العقد الجذرية متفوقة على المبيد اوكساميل.