



## EVALUATION OF SOME ORGANIC FORMULATIONS AND THEIR COMBINATIONS AS INTEGRATED MANAGEMENT PROGRAMS FOR ROOT KNOT NEMATODE *MELOIDOGYNE SPP.* ON CUCUMBER PLANTS UNDER GREENHOUSE CONDITION

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### Abstract

A greenhouse trial was conducted in Erbil Governorate/Iraq with natural infected soil by *Meloidogyne spp* to evaluate some organic formulations namely; Tervigo 20SC by two doses 8 L/ha and 10 L/ha, Nematron, *Paecilomyces lilacinus* and their combinations compared to Oncal 5% G as a reference nematicide to manage root-knot nematode *Meloidogyne spp.* on cucumber plants. The results showed good efficacy of tested treatments in reducing nematode numerical density in the soil as well as the number of galls on cucumber roots. *Paecilomyces lilacinus* was the superior treatment in reducing nematode population and galls index by 44.72, 88.92% respectively, while Tervigo at 8 L/ha was the least by 37.89, 61.67% respectively compared to control treatment. All involved treatments showed a positive effect on plant shoot system growth studied parameters; Tervigo at 8 L/ha + Nematron recorded the highest significant increase in plant length by 236.00 cm. followed by Nematron + *Paecilomyces lilacinus* while, Nematron + *Paecilomyces lilacinus* had more effect on fresh weight of plant shoot system recording a significant increasing of 880 g followed by *Paecilomyces lilacinus* alone 836.67g. For dry weight parameter, Nematron was the highest treatment that recorded 121.33 g followed by treatment of Nematron + *Paecilomyces lilacinus* which gave 118.00 g compared with control treatment 99.33g. Tervigo @8 L/ha + Nematron was the superior in realizing highest yield weight of 2862.50 g compared to control treatment 2186.11 g. As for root system growth parameters, highest root length of 38 cm was recorded by Nematron + *Paecilomyces lilacinus*. All treatments were recorded less weight than control treatment due to its efficacy in reducing number of galls on the root, *Paecilomyces lilacinus* recorded the least root fresh and dry weight of 296.67, 2.97 g compared to inoculated control treatment 438.33, 4.67 g respectively.

**Keywords:** Tervigo, Nematron, Cucumber, IPM, Root Knot Nematode

### Introduction

Root knot nematode *Meloidogyne spp* is one of many pathological causes for cucumber plants (Michereff *et al.*, 2005). It is causing economic losses at various rates for agricultural crops, it has reached up to 25% on the bean plant and 43-78% on flax plant and ranged between 30-40% on tomato, while was reaching to 37-55% on potato (Charchar *et al.*, 2003). As most of nematode pesticides were banned or restricted due to their high toxicity to humans and the environment, recently new and less toxic natural and modern nematode pesticides for humans and animals have been developed that have the same effect against nematodes that infect various agricultural crops (Adegbite and Adesiyani, 2005). Using of biomaterials and plant organic compounds is one of good alternatives to resist root-knot nematode and obtain a satisfactory management to reduce nematode density and improve plant growth, as well as to create an environment that helps to increase the population of nematode's natural enemies (Hassoun, 2011). Abamectin is one of the biocides that are being re-evaluated for using against root-knot nematodes *Meloidogyne spp.* and it has been successfully used by a number of researchers to combat nematode root-knot on tomato and cotton through seed treatment which has proved high efficiency in controlling nematode of *M. incognita* (Debeer, 2010). It was also found that cucumber seed treatment with abamectin is reduced the rate of root penetration by the juveniles *M. incognita* (Beeker *et al.*, 2006). In Iraq, Abamectin was tested with two plant extract formulations named; Garland, Resyst on the tomato plants by watering and gave positive and significant results in reducing the juvenile's population and nodes index of *Meloidogyne spp.* (Mater, 2016). Ali and Zewain (2018)

Found that introducing of abamectin in IPM program along with *Trichoderma harazianum*, *Paecilomyces lilacinus*, plant organic formulations and its combinations gave better results than chemical nematicide (Oncal 5% G) against *Meloidogyne spp* on cucumber plants.

Global environmental changes and continued increase in degradation of the natural ecosystem shows the importance of using IPM as a good management technique for soil-borne pests (Peachey *et al.*, 2001). Aromatic and medicinal plants could be considered as an important source for many chemical compounds and oils that can be used as organic nematicides with safe effect on humans and environment (Chitwood *et al.*, 2002). Due to the importance of these factors in reducing the use of chemical nematicides in order to find suitable alternatives that can work by same efficiency, in managing *Meloidogyne spp.* pest. For this purpose, this study came as an attempt to evaluate an integrated management program and its effectiveness in influencing *Meloidogyne spp.* population on cucumber plants by using following organic factors and its combinations:

Tervigo 20 SC @10 L/ha, Tervigo 20 SC @ 8 L/ha, *Paecilomyces lilacinus* and Nematron comparing with conventional method by chemical nematicides (Oncal 5% G) that currently practiced by Iraqi farmers.

### Materials and Methods

This field trial was carried out in a greenhouse at Qushtappa district south of Erbil governorate in February 2017. Cucumber seeds (Karol) were planted in plastic trays and then transferred to the greenhouse and planted at the age of 2-3 leaves. The soil in greenhouse was naturally infected with root-knot nematode by estimated density of 500

juveniles per 100 g of soil. Cucumber plants also were inoculated by 500 juveniles and eggs after seven days of planting to enhance the density of nematodes *Meloidogyne* spp. in the soil. Nematode eggs were extracted by a sieve of 200 mesh and 500 mesh then collected in Baker and counted under dissection stereo- microscope by placing 1 ml of egg extract and counting the number of eggs in this volume and then estimated the total number of eggs. 2 mL of egg extract were added to the soil by pipette at a depth of 2 cm by making three pits with equal distances 3 cm around the plant.

The treatments involved in the experiment were used as follows:

1. Tervigo 20 SC 10 L/ha (Abametin + FE- chlate) Producing by (Syngenta / Spain) tested at rate 0.42 ml with 50 ml water per plant.
2. Tervigo 20 SC 8 L/ha tested 0.33 ml with 50 ml water per plant tested.
3. *Paecilomyces lilacinus*

Pure culture of bio-control agent was used after its developing on Potato dextrose agar in a petri dish 9 cm and then were loaded onto millet medium as follows: The total required quantity of millet grains was sterilized in the autoclave for 20 minutes at 1.5 kg / cm<sup>2</sup> pressure and 121 °C and then left the flasks at room temperature until their temperature got dropped. 250 g of millet grains was placed in each 500 ml/l flasks with 100 ml distilled water, then millet grains were inoculated by 5 tablets of fungus culture growing on the PDA medium at 7 days old. The flasks were placed in the incubator at temperature of 26 ± 1 °C and left for 14 days with shaking continuously to ensure the homogeneous diffusion of the fungal mycelium on the millet medium (19). Millet medium loaded with bio-control fungus was added to the soil at a rate of 3 g of millet per plant by making a longitudinal incision around the plant with a depth of 5-7 cm of soil.

4. Nematron (Garlic based liquid plant extract formulation), produced by (Cosmocell Company/ Mexico), used as an organic fertilizer and anti-nematode, it contains natural garlic oil and vegetal extracts and some nutrients such as phosphorus and potassium, added at the rate of 4 ml / liter of water with 50 ml of solution per plant. This treatment is repeated five times during the season with interval of 10 days between each two applications.
5. Chosen combinations among above-said formulations were applied as well by same doses as follow:

Tervigo 20 SC @ 8L/ha + Nematron.

Tervigo 20 SC @ 8L/ha + *Paecilomyces lilacinus*.

Nematron + *Paecilomyces lilacinus*.

Tervigo 20 SC @ 8L/ha + Nematron + *Paecilomyces lilacinus*.

6. Oncal 5% G (with active ingredient -Aminoforacarb produced by OAT Company/Japan) used as a reference chemical nematicide used by Iraqi farmers for the purpose applied by rate of 3.5 g per plant after 7 days of planting.

All mentioned applied doses for formulations involved in this trial are recommended by their producers.

Results were taken after 70 days from planting date, plants were up-rooted and root galls and plant targeted characteristics were determined.

Root nodes index is used according to Bridge and Page (1980) as follows:

0 = No knots, 1 = Few small knots difficult to find. 2 = small knots only but clearly visible. 3 = Some larger knots visible, main roots clean .4 = larger knots predominate but main Roots clean. 5 = 50 % of the roots effected, knotting on some main roots. 6 = nodes spread over 60 % of the root, 7 = nodes spread over 70 % of the root, 8 = nodes spread over 80 % of the root, 9 = nodes spread over 91 % of the root, 10 = nodes spread over 100 % of the root.

The changes in the values of the studied characteristics were estimated as a percentage by applying of following formula:

$$\% \text{ of reduction} = \frac{\text{Average of treatment} - \text{Average of check treatment}}{\text{Average of check treatment}} \times 100$$

### Statistical analysis

Data of the present study were subjected to the analysis of variance test (ANOVA) as (RCBD)

The averages of studied traits were tested according to the Duncan multiple test.

### Results and discussion

#### The effect of tested treatments on galls and nematode population of cucumber infected with *Meloidogyne* spp.

Results in table-1 indicated that all evaluated treatments had a positive and significantly effect in galls reducing of *Meloidogyne* spp nematodes and *Paecilomyces* was the most effective treatment which recorded 44.72% reduction followed by Tervigo @10 L/ha and Nematron by 41.41% galls reduction for each of both treatments while, Tervigo @8 L/ha + *Paecilomyces* + Nematron treatment was the least effective as recorded only 34.37% gall reduction.

On the other hand, data shown in table -1 revealed also that tested treatments had a good and significantly effect in reducing the nematode population densities in the soil. *Paecilomyces* was the superior treatment that reduced juveniles' number in the soil with a significant effect giving 88.93 % followed by dual treatment of Nematron + *Paecilomyces* and Oncal nematicide which recorded 86.80, 79.72% population reduction, respectively. while, Tervigo 8 L/ha was the least effective with recorded value of 61.67% reduction only.

The present results are coming in agreement with result reported by (Oclarit *et al.* (2009) who found that *P. lilacinus* was effective against *M. incognita* nematode on tomato plants under greenhouse condition with significant reduction of galls number and nematodes egg masses compared with Nematicur nematicide. Another study had shown the significant effect of Tervigo @10 L/ha and *P. lilacinus* and Nematron treatments in reducing the symptoms of root-knot nematode on cucumber plants and nematode population in the soil compared with Vydate10/L nematicide under greenhouse condition (Ali and Zewain, 2018). Kalil *et al.* (2012) indicated that abamectin and *P. lilacinus* were superior treatment in reducing galls and juveniles population densities on tomato.

The difference effect of treatments may due to the type of active substances that contain or due to the mechanism of their influence on the pathogen, The effect of abamectin may be due to the presence of bacteria *Streptomyces avermitilis* or its produced antibiotics which have an important role in the formation of certain compounds like (Extracellular protease) or chitins that affect the pathogen cells and this effect can be similar to or even superior to the nematicides. Several previous studies have also indicated the ability of *P. lilacinus* to parasitize nematode eggs and subsequently suspend its development to juveniles as well as nematode susceptibility to secretion of antibiotics by the fungus that impede the movement of nematodes in the soil which explains the superiority of this treatment in reducing the number of nematodes in the soil and reducing the root nodes as a result. Also, all of dual and triple treatments had an integrated compatible performance, which participated in realizing a significant effect on nematodes compared with inoculated check treatment.

#### **The effect of tested treatments in shoot system growth and weight yield for cucumber infected with *Meloidogyne* spp.**

Data represented in table 2 revealed that all tested treatments on cucumber plants recorded a significant increase of shoot system growth compared with inoculated check treatment. The results showed that dual treatment of Tervigo@ 8 L/ha with Nematron were recorded the highest length of plant by 236 cm followed by Nematron + *Paecilomyces* and Tervigo 10 L/ha which recorded 232.67, 231.033 cm. while, Oncal Nematicide recorded the lowest length increase of plant by 225.67 cm compared with inoculated check treatment recorded 220.67 cm.

In the case of shoot weight, the tested treatments recorded an increase in wet and dry shoot weight Nematron + *Paecilomyces* were the superior treatments by value 880.00 g followed by Nematron with value of 836.67 g. while, Nematron was the superior treatment as it increased the dry shoot weight by value 121.33 g followed by Nematron + *Paecilomyces* by value 116.00 g. Tervigo@ 8 L/ha + *Paecilomyces* were least recorded 107.00 g dry shoot weight.

On the other hand, Tervigo @ 8 L/ha + Nematron was the significantly superior treatment that gave 2862.50 g weight yield with significant difference followed by Tervigo @8 L/ha + *Paecilomyces* and Nematron + *Paecilomyces* with values of 2692.00, 2673.61 g respectively.

These findings are coming in agreement with those results by Mater (2016) and Kalil *et al.* (2012) (who found that Abamectin and *P. lilacinus*, showed an increase in the plant growth parameters. Also, Ali (2018) found that yield of plants treated with Nematron and Tervigo 10 L/ha were increased. Al-Shibani (2005) reported that the fungus produces some growth regulators that increase some plant growth criteria and increases the efficiency of absorption of micronutrients Mn, Fe, Cu, Zn and K by plants.

These results may attribute to the active constituents of the substances tested which stimulated plant growth because they contain essential nutrient elements for cucumber plants, these substances vary in their effectiveness according to their different types and chemical structures as well as being negatively affect the nematodes, which reduces the incidence of the disease, this explains the difference between all applied treatments and inoculated control treatment.

#### **The effect of evaluated treatments on root system growth for cucumber infected with *Meloidogyne* spp.**

Data shown in table 3 revealed that most of tested treatments recorded an increase in shoot system length, the dual treatments showed more increase in length of root system and Nematron + *Paecilomyces* was the significant superior treatment as recorded 38.00 cm followed by Tervigo 8 L/ha + *Pacilomyces* + Nematron by 37.33 cm with no significant difference between them. while, Oncal Nematicide was recorded the least length of 34.33 cm with no significant difference with inoculated control treatment 33.33 cm.

Data represented in table 3 showed also the effective role of all evaluated treatments in protecting root system of cucumber against nematode attack where fresh root weights have become less than inoculated control treatment which recorded 438.33 g, *Paecilomyces* treatment was the least in root system fresh weight by 296.67 g. while, triple treatment recorded the highest fresh weight of 376.67g but still is significantly differ from inoculated control treatment 438.33 g.

In case of dry root weight, Tervigo 10 L/ha treatment recorded an increase with value 4.30 g comparing with inoculated control treatment, *Pacilomyces* and Oncal Nematicide were the two treatments that recorded highest decrease in dry weight of 2.97, 3.07 g respectively with no significant difference between them.

These findings are in agreement with many studies; Hannawi (2014) found that using of *P. lilacinus* improved the growth properties of total root system and have an important role in providing plant nutrients as well as competition for nodes caused by the infection of these worms, these compounds also have the ability to deprive nematodes from some important compounds and promote systemic resistance in the plant. Ali and Zewain (2018) reported that Abamectin had a high ability to reduce the numerical density of nematodes and improve the growth of the root system, the presence of Abamectin helps to provide iron element into the soil through (Siderophor) compounds and presence of chelated iron within new formulation of Abamectin used in this study which participated in increasing the size of host roots through improving their growth and reducing penetration by nematode juveniles.

**Table 1 :** The effect of tested treatments on galls and nematode population of cucumber infected with *Meloidogyne* spp.

Treatments	Galls index/ Plant	Reduction (%)	Nematode population (100 g soil)	Reduction (%)
Tervigo 10 L/ha	2.83 de	- 41.41	278.67 c	- 67.30
Tervigo 8 L/ha	3.00 cd	- 37.89	326.67 b	- 61.67
Oncal Nematicide	3.00 cd	- 37.89	172.83 f	- 79.72
Nematron	2.83 de	- 41.41	290.00 c	- 65.97
<i>Paecilomyces</i>	2.67 e	- 44.72	94.33 h	- 88.93
Terv. 8 L/ha + Nematron.	3.00 cd	- 37.89	207.50 e	- 75.65
Terv. 8 L/ha + <i>Pacilomyces</i>	3.00 cd	- 37.89	316.67 b	- 62.84
Nematron + <i>Paecilomyces</i>	2.83 ed	- 41.41	112.50 g	- 86.80
Terv. 8 L/ha + <i>Pacilomyces</i> + Nematron	3.17 cb	- 34.37	253.33 d	- 70.27
Non-inoculated control	3.33 b		288.33 c	
Inoculated control	4.83 a		852.17 a	

Each value represents an average of three values. Averages that share the same letter are not significantly different at the probability level of 0.05. (-) The impairment than control treatment.

**Table 2 :** The effect of tested treatments in shoot system growth and weight yield for cucumber infected with *Meloidogyne* spp

Treatments	Shoot system length (cm)	Fresh weight of shoot system (g)	Dry weight of shoot system (g)	Weight yield (g)
Tervigo @10 L/ha	231.33 ab	753.33 e	113.00 bc	2577.78 d
Tervigo @8 L/ha	228.00 bc	709.00 g	108.00 de	2520.72 f
Oncal Nematicide	224.33 cd	650.00 i	107.00 de	2519.94 f
Nematron	227.00 bcd	766.67 d	121.33 a	2569.44 d
<i>Paecilomyces</i>	227.33 bcd	836.67 b	110.00 cd	2566.67 d
Tervigo @ 8 L/ha + Nematron	236.00 a	780.00 c	115.67 b	2862.50 a
Tervigo @ 8 L/ha + <i>Pacilomyces</i>	226.33 bcd	690.00 h	105.67 e	2692.00 b
Nematron + <i>Paecilomyces</i>	232.67 ab	880.00 a	116.00 b	2673.61 c
Terv. 8 L/ha + <i>Pacilomyces</i> + Nemat.	230.00 abc	766.67 d	108.00 de	2548.61 e
Non-inoculated control	235.00 a	730.00 f	106.33 de	2516.67 f
Inoculated control	220.67 d	620.00 j	99.33 f	2186.11 g

Each value represents an average of three values. Averages that share the same letter are not significantly different at the probability level of 0.05.

**Table 3 :** The effect of tested treatments on root system growth for cucumber infected with *Meloidogyne* spp.

Treatments	Root system length (cm)	fresh weight of Root system (g)	Dry weight of Root system (g)
Tervigo @10 L/ha	36.00abc	366.33 c	4.30 ab
Tervigo @8 L/ha	36.00 abc	370.00 c	3.53 de
Oncal Nematicide	34.33 bc	306.67 fg	3.07 ef
Nematron	35.67 abc	373.33 c	3.93 bcd
<i>Paecilomyces</i>	35.33 abc	296.67 g	2.97 f
Tervigo @ 8 L/ha + Nematron	35.00 abc	316.67 ef	3.77 bcd
Tervigo @ 8 L/ha + <i>Pacilomyces</i>	34.33 bc	333.33 d	3.60 cde
Nematron + <i>Paecilomyces</i>	38.00 a	326.67 de	3.77 bcd
Tervigo@ 8 L/ha + <i>Pacilomyces</i> + Nematron	37.33 ab	376.67 c	3.60 cde
Non-inoculated control	35.33 abc	396.67 b	4.20 abc
Inoculated control	33.33 c	438.33 a	4.67 a

Each value represents an average of three values. Averages that share the same letter are not significantly different at the probability level of 0.05.

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