Suppression of the Root-Knot Nematode, *Meloidogyne incognita* in Tomato Plants by Application of Certain Entomopathogenic Nematode Species Under Greenhouse Conditions

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Abstract

The suppressive effects of six species of entomopathogenic nematodes (EPNs) against *Meloidogyne incognita* infected tomato were assessed in two separate trials under greenhouse conditions. The tested EPN species were *Heterorhabditis bacteriophora*, *H. megidis, H. zealandica, Steinernema feltiae, S. glaseri* and *S. riobravae*. In the first trial, EPNs were applied as a liquid suspension of alive or dead infective juveniles (IJs) at rate of 5000 IJs/plant in sandy and clay soils, while in the second trial, two *Galleria mellonella* infected cadavers of six-day old per plant were used in sandy soil. In the two experiments, application of EPNs was accomplished simultaneously with inoculation of *M. incognita*. Carbofuran as a standard synthetic nematicide was used as comparison treatment at recommended rate of 0.2 g/plant. Control treatments received only water and *M. incognita* at rate of1000 IJs/plant.

Two months after inoculation, galling (as indicated by number of galls/plant) and reproduction (as indicated by number of egg masses /plant) as well as damage (as indicated by fresh and dry weight of areal parts) were assessed. Data showed that, treatment of carbofuran surpassed all other treatments in minifying galling and reproduction of *M. incognita* in sandy and clay soils. On the other hand, curative applications of alive or heat-killed IJs significantly (P≤ 0.05) diminished gall formation and egg mass production in tomato roots with slightly amelioration in fresh and dry weight of tomato shoot. Steinernematid species were more comparatively effective than heterorhabditid ones. General means for number of galls and egg masses for steinernematid species were 52.50 and 25.33 with percent reduction of 55.45 and 64.49 %, respectively. Whereas, the parallel values for heterorhabditid species were 69.67 and 36.33 with percent reduction of 40.88 and 49.07%, respectively compared to treatment of *M. incognita* alone. Treatments of alive IJs overwhelmed those of dead IJs in decreasing number of galls (with percent reduction of 56.53 and 39.79% respectively) and egg masses (with percent reduction of 63.16 and 50.39%, successively). Moreover, utilization of two *G. mellonella* infected cadavers markedly lowered number of galls and egg masses

and insignificantly (P≤ 0.05) improved plant growth parameters to certain extent. General means of percentage reduction in galls and egg masses were 58.46 and 54.74%, consecutively.

Key words: Entomopathogenic nematodes, *Heterorhabditis* spp., *Steinernema* spp., *Meloidogyne incognita*, tomato, biological control, carbofuran.

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable plants in the world. The crop is now by far the largest vegetable crop in Egypt. In 2014, the country ranked 5th in the world with total cultivated area reached 214,016 hectares produced 8,288,043 tons **(FAO, 2014).** Between various obstacles including fungi, bacteria and viruses in cultivating this crop, root knot nematodes (RKNs) are recognized as a major pathogen of tomato **(Kamran *et al*., 2010).** On the other hand, *Meloildogyne* spp. (Goeldi) are more widely distributed throughout the world as a major depressive factor in food production especially in tropical, subtropical and Mediterranean climates. Plant parasitic nematodes (PPNs) including *Meloidogyne* spp. cause an estimated annual loss of $125 billion globally **(Chitwood, 2003).** Many studies have been carried to asses damage of RKNs on tomato.The yield loss ranged between 25 to 100 % **(Kaur *et al*., 2011 and Ebrahim *et al*., 2015).**

In Egypt, RKNs *M. incognita* (Kofoid and White) Chitwood and *M. javanica* (Treub) Chitwood, are considered one of the main problems in tomato production particularly in the newly reclaimed sandy areas **(Ibrahim, 1985).** On the other hand, chemical control have several problems, such as high cost, pollution of environment, toxicity to man and animals as well as disturbance of the natural balance. Therefore, finding safer alternatives to chemical nematicides is one of the top priorities for future nematology. Moreover, due to environmental concerns and increased regulations on use of nematicides, more strategies for management of RKNs were currently investigated **(Nico *et al*., 2004).** Biological control using EPNs is one potential alternative to chemical nematicides.

Entomopathogenic nematodes (EPNs) are currently marketed worldwide for biological control of insect pests. Several greenhouse studies have demonstrated suppression of PPNs by application of EPNs **(Grewal *et al*., 1999; Ishibashi & Kondo, 1986; Perry *et al*., 1998 and Jagdale *et al*., 2009).** Likewise, field trials also indicate potential of EPNs to control PPNs **(Grewal *et al*., 1997; Jagdale & Grewal, 2008 and Caccia *et al*., 2013).** 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 **(Bird &Bird, 1986; Fallon *et al.,* 2002 and Perez & Lewis, 2004).** Most experiments evaluating the suppressive effects of EPNs have focused on the use of living IJs **(Grewal *et al*.,**

1997; Smitley *et al*., 1992 and Somasekhar *et al*., 2000). Whereas some authors tested the effect of heat-killed IJs **(Grewal *et al*., 1999 and Jagdale *et al*., 2002)** or infected insect cadavers **(De Valle *et al*., 2013 and Shapiro-Ilan *et al*., 2003)**. Therefore, the objective of this study was to assess the impact of six species of EPNs applied as live or dead IJs as well as *G. mellonella* infected cadavers compared to the nematicide carbofuran in suppressing *M.incognita* infecting tomato in sandy and clay soils under greenhouse conditions.

Materials and Methods

1. Source and culturing of entomopathogenic nematodes:

Infective juveniles (IJs) of the tested nematode species were friendly obtained from Department of Entomology and Nematology, University of Florida, USA by Dr. Fahiem Elborai. The nematode species were *Heterorhabditis bacteriophora*, *H.megidis*, *H. zealandica*, *Steinernema feltiae*, *S. glaseri* and *S. riobravae*. They were cultured separately in last instar larvae of the greater wax moth *Galleria mellonella* L. according to the technique of **Dutkey *et al*., (1964).** IJs emerged from cadavers were stored in distilled water at 12°C for 1 week until applied in pots **(Woodring & Kaya, 1988).** Dead infective juveniles used in this study were gained by heating 250 ml nematode suspensions in water path for 3 minutes at 60°C.

2. Culturing of the root knot nematode, *M. incognita:*

Pure culture of *M. incognita*, was maintained in the greenhouse on the tomato susceptible cultivar Super Strain B for using as source of inoculum. Species identification was based on juvenile measurements and examination of perineal pattern system of adult females according to **Eisenback *et al*., (1981)** and **Jepson, (1987).** Infected tomato roots were cut into pieces of 2-cm long and placed in a 600 –ml flask with 200 ml of 0.5% sodium hypochlorite (180 ml water + 20 ml Clorox). The tightly capped flack was shaken for 3 minutes. The shaking partially dissolved the gelatinous matrix thus freeing eggs from egg masses **(Hussey & Barker 1973).** The liquid suspension of eggs was poured through a 200-mesh sieve nested upon a 500-mesh sieve. Eggs collected on the 500-mesh sieve were immediately washed free of residual sodium hypochlorite solution under a slow stream of tap water and incubated in Petri dishes at 25±1°C until hatching. Newly hatched juveniles were collected by using a micropipette.

3. Impact of alive and dead infective juveniles of EPNs on galling and reproduction of *M. incognita* infecting tomato under greenhouse conditions:

Tomato plant was chosen because it is severely attacked by the root-knot nematode *M. incognita* as well as it's regional economic importance. Seeds of the susceptible tomato cv. Super Strain B were soaked in sterile distilled water in Petri dishes and kept in an incubator at 26±°C. After 48 hours seeds were germinated in

clay pots of 20-cm diameter containing steam sterilized sandy soil. At the two leaf stage,seedlings were singly transplanted to formalin sterilized 20-cm diameter plastic pots filled with steam sterilized soil. This experiment was carried out in two soil textures i. e., clay soil (40.7% clay), (49.5% silt) (9.8% sand) and sandy soil (95.7% sand), (1.2% silt) and (3.1% clay). One week after transplanting, when seedlings were approximately 10 cm in height, they were inoculated with 1000 newly hatched IJs of *M.incognita* per plant. Inocula were obtained from available pure culture formerly prepared and propagated in the greenhouse. IJs were added by pipetting 2 ml of the inoculum suspension into three holes around the root system. Immediately after inoculation the holes were covered with moist soil. EPN treatments (*Heterorhabditis bacteriophora, H. megidis*, *H. zealandica*, *Steinernema feltiae*, *S. glaseri* and *S.riobravae*) were applied at the same time to tomato seedlings at concentration of 5000 IJs per seedling. Alive or heat- killed IJs were placed on the soil surface in 2 ml water with a pipette.The amount of water used to add the nematodes was the same for all plants within a block. The nematicide, carbofuran (Furadan 10% G), at 0.2 g per pot was applied instantly after *M.incognita* inoculation according to the recommended rate based on formulated form by incorporating the exact amount in the upper 3 cm of soil pot. Control treatments included inoculation of *M. incognita* IJs alone as well as healthy plants without nematode inocula. Each treatment was replicated three times. All treatments were arranged in a complete randomized block design in the greenhouse at 27±4°C., and all received similar horticultural treatments.

Two months after inoculation, plants were removed carefully from pots and data on plant growth (fresh and dry weight of shoot) were recorded. Roots and surrounding soil in the pots were soaked in tap water for two hours to facility removing adhering soil and keep egg masses on root surface. Roots were wrapped in tissue paper to prevent drying out during the steps of evaluation. Moreover, numbers of galls and egg masses were counted per root system under a dissecting microscope.

4. Effect of two cadavers of *G. mellonella* infected with EPNs on galling and reproduction of *M. incognita* infecting tomato under greenhouse conditions:

To obtain insect cadavers, last instar *G. mellonella* larvae were exposed to 1000 IJs of each species in 9-cm diameter Petri dishes lined with one wet filter paper. The Petri dishes were incubated at 25±1°C. for four days, and dead larvae were subsequently transferred to new Petri dishes lined with dry filter paper for a further two days till the typical signs of EPN infection were noticed **(Woodring & Kaya, 1988 and Del Valle *et al*., 2013).**

Experiment to test the effect of EPNs-infected cadavers on *M. incognita* infected tomato was conducted using the same protocol mentioned before in the

previous experiment with the following difference. Instead of using live or dead IJs, EPNs-infected cadavers were applied. The infected cadavers were added simultaneously with *M. incognita* inoculation. In treatments involving the use of infected cadavers, two cadavers were added per pot. The cadavers were buried 2 cm below the soil surface and 2.5 cm from the stem, diametrically opposite each other. The insect cadavers used were infected 6 days before application **(Del Valle *et al*., 2008).** During the experimental period, the mean temperature was at 22± 4ºC. Sixty days after inoculation with *M. incognita*, the following variable were recorded for each plant: number of galls and egg masses per root system as well as fresh and dry weight of aerial parts.

5. Statistical analysis:

The experiments were carried out in a completely randomized block design with 3 replications for each treatment and each replicate consisting of one plant. Data were subjected to analysis of variance (ANOVA). Moreover, student t-test was used to compare number of galls and egg masses in the two groups i.e. heterorhabditid species vs steinernematid species and alive IJs vs dead IJs in the experiment where IJs of EPNs were used as an aqueous suspensions. Means were compared by Duncan's multiple range test at P ≤ 0.05 level of probability **(MSTAT, 1987).**

Results

1. Suppression of the root- knot nematode, *M. incognita* by application of alive and dead infective juveniles of EPNs:

Data in table (1) indicated that application of EPNs reduced galls and egg masses of *M. incognita* in sandy soil under greenhouse conditions. Plants received *M. incognita* alone showed a relatively higher values of galls (116.00) and egg masses (75.67) indicating the susceptibility of tomato cv Super Strain B to this nematode. Treatment of carbofuran surpassed all other treatments in reducing galling and reproduction of *M. incognita*. Since, number of galls and egg masses per root system were 21.33 and 14.33, respectively, with remarkable reduction values reached 81.61 and 81.06 %, respectively.

Utilization of alive or dead IJs of EPN species significantly (P ≤ 0.05) reduced gall formation and egg mass production on the treated tomato plants. Treatments of steinernematid species gave best results in relation to treatments of heterorhabditid species. Number of galls and egg masses in plants treated with *S. feltiae*, *S. glaseri* and *S. riobravae* were 47.67 (24.67), 37.67 (20.00) and 39.67 (23.00), respectively, while the parallel values for *H. bacteriophora*, *H.megidis* and *H. zealandica* were 51.00(31.67), 55.67 (33.33) and 66.67 (35.67), respectively. Likewise, ranges of percent reduction in galls and egg masses for *Steinernema* spp. were 58.90 to 67.52% and 67.39 to 73.56%, respectively, whereas the respective values for

Heterorhabditis spp. were 42.52 to 56.03 % and 52.86 to 58.14 %, consecutively.

The same results were obtained when dead IJs were added simultaneously with *M. incognita* IJs (table 1). Comparing number of galls and egg masses in plants treated with alive and dead IJs, showed that alive IJs markedly reduced galling and reproduction of *M. incognita* compared to dead ones. Number of galls in alive and dead, *S. glaseri*, *S. feltiae* and *S. riobravae* were 37.67 (70.67), 47.67 (66.33) and 39.67 (53.33), respectively. The parallel values for egg masses were 20.00 (38.33), 24.67 (34.00) and 23.00 (25.67), respectively. The same trend was found with alive and dead *H. bacteriophora*, *H. megidis* and *H. zealandica*. Their respective values for galls were 51.00 (76.33), 55.67 (91.00) and 66.67 (89.67), successively while the parallel values for egg masses were 31.67 (40.33), 33.33 (47.00) and 35.67 (46.67), respectively. In general, it could be concluded that curative application of carbofuran and IJs of EPNs significantly reduced gall formation and egg mass production of *M. incognita* in tomato plants. Steinernematid species were more effective than heterorhabditid ones. Moreover, alive IJs of all tested six EPN species gave good results compared to dead treatments.

Table (1): Effect of simultanoeusly application of *M. incognita* (1000 IJs/plant) and alive or dead six species of EPNs (5000 IJs/plant) on suppressing galling and reproduction on tomato plants in sandy soil under greenhouse conditions.

Treatments	Alive Infective Juveniles (IJs)		Dead Infective Juveniles (IJs)	
	Number of galls/Plant (Reduction %)	Number of egg masses/Plant (Reduction %)	Number of galls /Plant (Reduction %)	Number of egg masses/Plant Reduction %)
Control (*M.incognita* alone)	116.00 a	75.67 a	116.00 a	75.67 a
M.incognita + carbofuran	21.33 d (81.61)	14.33 e (81.06)	21.33 e (81.61)	14.33 e (81.06)
M.incognita + *H.bacteriophora*	51.00 bc (56.03)	31.67 bcd (58.14)	76.33 c (34.19)	40.33 bc (46.70)
M.incognita + *H.megidis*	55.67 bc (52.08)	33.33 bc (55.95)	91.00 b (21.55)	47.00 b (37.88)
M.incognita + *H. zealandica*	66.67 b (42.52)	35.67 b (52.86)	89.67 b (22.69)	46.67 b (38.32)
M.incognita + *S. feltiae*	47.67 bc (58.90)	24.67 bcde (67.39)	66.33 c (42.81)	34.00 cd (55.06)
M.incognita + *S. glaseri*	37.67 cd (67.52)	20.00 de (73.56)	70.67 c (39.07)	38.33 bc (49.34)
M.incognita + *S. riobravae*	39.67 cd (65.80)	23.00 cde (69.60)	53.33 d (54.02)	25.67 d (66.07)

Means in each column followed by same letter(s) are not significantly different at 5% level of probability according to Duncan's multiple range test.

$$\text{Reduction } \% = \frac{\text{Control - treated}}{\text{Control}} \times 100$$

Effect of *M. incognita* alone or combined with carbofuran or IJs of six EPN species on growth of tomato plants as indicated by fresh and dry weight of shoot system is illustrated in table (2). It was found that, *M. incognita* caused remarkable reduction in tomato growth response in terms of shoot fresh weight (33.37%) and shoot dry weight (30.27%) as compared to healthy plants. On the other hand, all tested treatments ameliorated shoot fresh and dry weight of tomato plants to a certain extent. Alive IJs and carbofuran significantly improved shoot fresh weight of tomato plants. However, insignificant variations in shoot dry weight were detected between treatments. The same result was obtained in fresh and dry weight in treatments of dead IJs. Generally, application of live IJs overwhelmed dead ones in improving plant growth of tomato with all tested EPN species. For instance, percent increase in fresh and dry weight of shoot system in treatment of alive *H. bacteriophora* IJs were 41.16% and 35.64%, while the parallel values for dead IJs were 6.75% and 3.04%, respectively.

Table (2): Fresh and dry weight of tomato shoot system as influenced by application of *M. incognita* alone (1000 IJs/ plant) or combined simultaneously with alive or dead six species of EPNs (5000 IJs/plant) in sandy soil under greenhouse conditions.

Treatments	Alive Infective Juveniles (IJs)		Dead Infective Juveniles (IJs)	
	Fresh weight (g) (Increase %)	Dry weight (g) (Increase %)	Fresh weight (g) (Increase %)	Dry weight (g) (Increase %)
Healthy plants	22.68 a	8.49 a	22.68 a	8.49 a
Control (*M.incognita* alone)	15.11 g	5.92 d	15.11 d	5.92 b
M.incognita + carbofuran	17.70 f (17.14)	6.77 cd (14.35)	17.70 bcd (17.14)	6.77 ab (14.35)
M.incognita + *H.bacteriophora*	21.33 ab (41.16)	8.03 ab (35.64)	16.13 bcd (6.75)	6.10 b (3.04)
M.incognita + *H.megidis*	20.80 bc (37.65)	8.07 ab (36.31)	15.60 d (3.24)	6.52 b (10.13)
M.incognita + *H. zealandica*	18.27 def (20.91)	6.67 cd (12.66)	16.20 bcd (7.21)	6.19 b (4.56)
M.incognita + *S. feltiae*	18.11 ef (19.85)	6.36 cd (7.43)	15.74 cd (4.16)	6.01 b (1.52)
M.incognita + *S. glaseri*	18.70 de (23.75)	7.00 c (18.24)	18.23 b (20.64)	6.95 ab (17.39)
M.incognita + *S. riobravae*	19.60 cd (29.71)	7.30 bc (23.31)	17.95 bc (18.79)	6.25 b (5.57)

Means in each column followed by same letter(s) are not significantly different at 5% level of probability according to Duncan's multiple range test.

$$\text{Increase \%} = \frac{\text{Treated - Control}}{\text{Control}} \times 100$$

Results in table (3) revealed that, in clay soil minimum number of galls and egg masses was detected in carbofuran treatment 18 (12.33) with reduction values of 84.95% (81.59%), respectively, compared to non-treated plants which gained

119.67 galls and 67.00 egg masses. Also, application of alive and dead IJs of EPNs significantly (P≤ 0.05) diminished number of galls and egg masses. In almost cases, IJs of steinernematid species were more effective than IJs of heterorhabditid species in reducing galling and reproduction of *M. incognita.* The only exception was found in the case of *H. zealandica* which exceeded *S. feltiae*, and *S. riobravae* in reducing number of galls in dead IJs treatments, with values of 52.00,57,00 and 67.67, respectively. On the other hand, application of alive IJs provided good results in decreasing number of galls and egg masses in comparison with dead ones. For examples, counts of galls and egg masses in treatment of alive *H. bacteriophora* IJs were 63.33 and 27.33, respectively, while those for dead ones were 75.00 and 39.67, respectively. Also, number of galls and egg masses for alive *S. feltiae* IJs were 55.67 and 20.33, respectively, while those for dead ones were 57.00 and 29.67, respectively.

Table (3): Number of galls and egg masses on tomato plants simultaneously inoculated with *M. incognita* (1000 IJs/plant) and alive or dead six species of EPNs (5000 IJs/plant) in clay soil under greenhouse conditions.

Treatments	Alive Infective Juveniles (IJs)		Dead Infective Juveniles (IJs)	
	Number of galls/Plant (Reduction %)	Number of egg masses/Plant (Reduction %)	Number of galls /Plant (Reduction %)	Number of egg masses/Plant Reduction %)
Control (*M.incognita* alone)	119.67 a	67.00 a	119.67 a	67.00 a
M.incognita + carbofuran	18.00 d (84.95)	12.33 d (81.59)	18.00 d (84.95)	12.33 d (81.59)
M.incognita + *H.bacteriophora*	63.33 bc (47.07)	27.33 bc (59.20)	75.00 b (37.32)	39.67 b (40.79)
M.incognita + *H.megidis*	77.33 b (35.38)	29.33 bc (56.32)	77.67 b (35.09)	37.33 b (44.28)
M.incognita + *H. zealandica*	60.33 bc (49.58)	31.67 b (52.73)	52.00 c (56.54)	22.00 c (67.71)
M.incognita + *S. feltiae*	55.67 c (53.48)	20.33 bcd (69.65)	57.00 bc (52.36)	29.67 bc (55.71)
M.incognita + *S. glaseri*	17.67 d (85.23)	18.67 cd (72.13)	74.67 b (37.60)	36.00 b (46.26)
M.incognita + *S. riobravae*	42.00 c (64.90)	19.67 bcd (70.64)	67.67 bc (43.45)	28.00 bc (58.20)

Means in each column followed by same letter(s) are not significantly different at 5% level of probability according to Duncan's multiple range test.

$$\text{Reduction } \% = \frac{\text{Control - treated}}{\text{Control}} \times 100$$

Table (4) showed that in clay soil, *M. incognita* infection significantly diminished fresh and dry weight of shoos by 30.08 and 48.08% respectively. On the other hand, carbofuran as well as EPN treatments significantly improved plant growth of tomato plants compared to treatment of *M. incognita* alone. However, variations in fresh and dry weight of aerial parts among carbofuran and EPN

treatments were insignificant (P ≤ 0.05). In dead IJs treatments, maximum fresh and dry weight of shoots were detected in carbofuran treatment with 30.75 and 35.86% increase compared to *M. incognita* alone. Amelioration in tomato growth was relatively higher in treatments of alive IJs than dead ones.

Table (4): Influence of *M. incognita* alone (1000 IJs/ plant) applied at the same time with alive or dead six species of EPNs (5000 IJs/plant) on fresh and dry weight of tomato shoot in clay soil under greenhouse conditions.

Treatments	Alive Infective Juveniles (IJs)		Dead Infective Juveniles (IJs)	
	Fresh weight (g) (Increase %)	Dry weight (g) (Increase %)	Fresh weight (g) (Increase %)	Dry weight (g) (Increase %)
Healthy plants	22.97 a	9.88a	22.97 a	9.88 a
Control (*M.incognita* alone)	16.06 e	5.13 c	16.06 e	5.13 c
M.incognita + carbofuran	21.00 ab (30.75)	6.97 b (35.86)	21.00 ab (30.75)	6.97 b (35.86)
M.incognita + *H.bacteriophora*	20.49 b (27.58)	7.04 b (37.23)	17.13 cde (6.66)	6.41 bc (24.95)
M.incognita + *H.megidis*	19.73 bc (22.85)	7.43 b (44.83)	16.60 e (3.36)	5.56 bc (8.38)
M.incognita + *H. zealandica*	18.26 cd (13.69)	6.67 b (30.01)	17.20 cde (7.09)	6.47 bc (26.12)
M.incognita + *S. feltiae*	18.12 d (12.82)	6.33 bc (23.39)	16.73 de (4.17)	5.72 bc (11.50)
M.incognita + *S. glaseri*	19.71 bc (22.72)	7.53 b (46.78)	19.23 bc (19.73)	7.25 b (41.32)
M.incognita + *S. riobravae*	20.64 b (28.51)	7.48 b (45.80)	18.95 bcd (17.99)	6.56 bc (27.87)

Means in each column followed by same letter(s) are not significantly different at 5% level of probability according to Duncan's multiple range test.

$$\text{Increase \%} = \frac{\text{Treated - Control}}{\text{Control}} \times 100$$

T-test was used to compare number of galls and egg masses in the two groups i.e. heterorhabditid species vs steinernematid species and alive IJs vs dead IJs in the experiment where IJs of EPNs were used as an aqueous suspensions. Results in fig. (1) revealed that general means for number of galls and egg masses were significantly (P ≤ 0.05) lower in steinernematid treatments (52.50 and 25.33, respectively) compared to heterorhabditid treatments (69.67 and 36.33, respectively). Percentages reduction in galls and egg masses for steinernematids were 55.45 and 64.49 % respectively, while the parallel values for heterorhabditids were 40.88 and 49.07 %, respectively. Moreover, the same criteria were significantly lower (P ≤ 0.05) with alive IJs (51.22 and 26.28, respectively) compared to dead IJs (70.95 and 35.39, respectively). Percentages reduction for alive IJs were 56.53 and 63.16%, respectively, while the respective values for dead ones were 39.79 and 50.39%, respectively.

2. Suppression of *M. incognita* infected tomato by application of two cadavers of *G. mellonella* infected with six species of EPNs under greenhouse conditions:

Efficacy of two *G. mellonella* cadavers separately infected with six species of EPNs compared to carbofuran in controlling *M. incognita* infecting tomato was studied in sandy soil under greenhouse conditions (table 5). Cadavers of six- day after death or carbofuran were applied at the same time with *M. incognita* IJs. It was clear that, all the tested treatments significantly (P ≤ 0.05) minified galls and egg masses in relation to plants infected with *M. incognita* alone which gained the maximum values of galls (122.00) and egg masses (72.67). However, insignificant variations (P ≤ 0.05) were detected between carbofuran and most of the tested EPN species in reducing galls and egg masses of *M. incognita*. Amongst EPN species, *S. glaseri* followed by *S. riobrave* gave the highest percent reduction in galls (71.58 & 71.04%) and egg masses (67.43 & 66.97%) respectively. Whereas, *H.megidis* proceeded by *H. zealandica* showed the lowest percent reduction in galls (40.43 & 53.01%) and egg masses (38.07 & 44.50%) respectively. However, *H. bacteriophora* and *S. feltiae* were found with intermediate effect. General means of percent reduction in galls and egg masses for all EPN species were 58.46 and 54.74% respectively.

Table (5): Effect of curative application of *G. mellonella* cadavers infected with six species of EPNs (two/plant) on *M. incognita* (1000 IJs/ plant) infecting tomato in sandy soil under greenhouse conditions.

Treatments	Number of galls/plant (Reduction %)	Number of egg masses /plant (Reduction %)	Fresh weight (g)/ plant (Increase %)	Dry weight (g) / plant (Increase %)
Healthy plants	-	-	22.68 a	8.49 a
Control (*M.incognita* alone)	122.0 a	72.67 a	17.36 b	5.24 b
M.incognita +carbofuran	25.67 c (78.96)	14.33 c (80.28)	20.18 ab (16.24)	7.11 ab (35.68)
M.incognita+ *H.bacteriophora*	47.33 bc (61.20)	26.00 bc (64.22)	19.88 ab (14.51)	5.88 b (12.21)
M.incognita + *H.megidis*	72.67 b (40.43)	45.00 b (38.07)	19.37 ab (11.57)	6.03 b (15.07)
M.incognita + *H. zealandica*	57.33 bc (53.00)	40.33 bc (44.50)	18.70 ab (7.71)	5.33 b (11.71)
M.incognita + *S. feltiae*	56.67 bc (53.55)	38.33 b (47.25)	19.20 ab (10.59)	5.87 b (12.02)
M.incognita + *S. glaseri*	34.67 c (71.58)	23.67 bc (67.43)	21.44 ab (23.50)	6.90 ab (31.67)
M.incognita + *S. riobravae*	35.33 c (71.04)	24.00 bc (66.97)	20.58 ab (18.54)	6.41 ab (22.32)

Means in each column followed by same letter(s) are not significantly different at 5% level of probability according to Duncan's multiple range test.

$$\text{Reduction } \% = \frac{\text{Control - treated}}{\text{Control}} \times 100$$

$$\text{Increase } \% = \frac{\text{Treated - Control}}{\text{Control}} \times 100$$

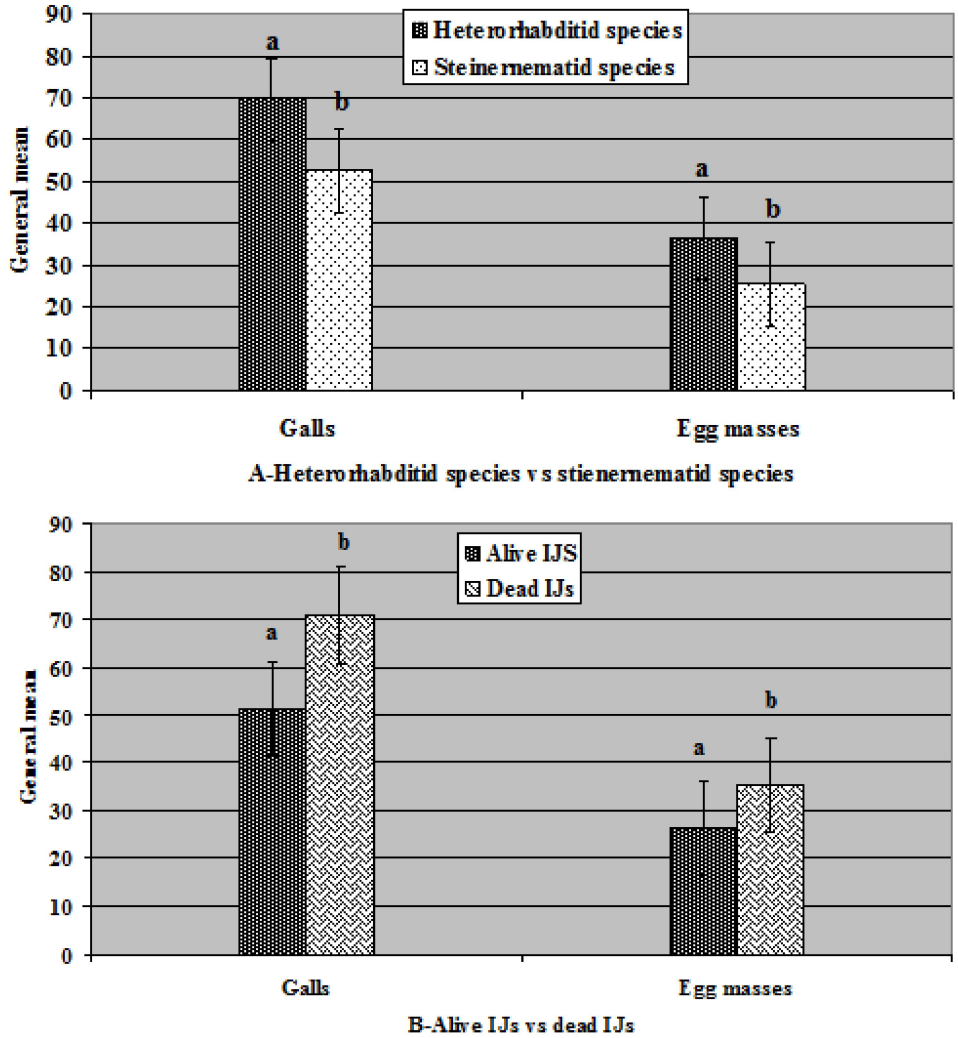

Fig. (1): General means for number of galls and egg masses as influenced by two groups i.e heterorhabditid species vs steinernematid species (A) and alive IJs vs dead IJs (B). Different litter above bars indicate statistical significance ($P \leq 0.05$).

Application of carbofuran as well as two cadavers of heterorhabditid or steinernematid nematodes insignificantly ($P \leq 0.05$) enhanced fresh and dry weight of tomato shoot when compared to plants inoculated with *M. incognita* alone. Ranges of percent increase in fresh and dry weight of aerial parts were 7.71-23.50% and 1.71-35.68%, respectively (table 5).

Discussion

Obtained results in our study confirmed with those reported by many authors who observed suppressive effects of EPNs against RKNs under laboratory and greenhouse conditions **(Bird & Bird, 1986; Grewal *et al*., 1999; Perez & Lewis, 2004; Aatif *et al*., 2016 and Khan *et al*., 2016).** Most of these studies were conducted on *M. incognita* infected tomato plants. Likewise, suppressive effects of EPNs have been demonstrated on other PPNs under field conditions like *Tylenchorhynchus* spp. and *Pratylenchus penetrans* **(Smitley *et al.*, 1992),** *Belonolaimus longicadatus* and *Criconemoides* spp**. (Grewal *et al*., 1997),** *Globodera rostochiensis* **(Perry *et al*., 1998),** *Aphelenchoides fragariae* **(Jagdale & Grewal, 2008)** and *Nacobbus aberrans* **(Caccia *et al.,* 2013).** On the other hand, negative effects of EPN application in reducing RKNs were reported in other studies **(Fallon *et al*., 2002; Molina *et al*., 2007 and Shapiro-Ilan *et al*., 2006).** Lewis & **Grewal (2005)** reported that in some cases the use of EPNs does not always reduce PPN populations and the effects of their interaction vary with EPN and PPN species, the host crop and the impact on PPNs.

The suppressive effects of EPNs on PPNs may attribute to many factors. Fore instances, attraction of *S.glaseri* to tomato roots and suppression may be due to competition between the two nematode groups for space **(Bird &Bird, 1986);** increase density of predators resulting from the application of nematode biomass to the soil **(Ishibashi & Kond, 1986)**, production of allelochemicals of EPN symbiotic bacteria complex **(Grewal *et al*.,1999; Hu *et al*., 1999 and Lewis *et al*., 2001)** and application of *S.carpocapsae* IJs and its symbiotic bacteria (*X. nematophilus*) stimulated the activity of P-peroxidase, G- peroxidase and catalase enzymes which responsible for inducing systemic resistance in plants **(Jagdale *et al*., 2009).**

Our results showed that, in most cases EPNs belonging to sterinernematids were more effective than EPNs belonging to heterorhabditids in controlling *M. incognita* infecting tomato. It may be due to the ability of *Steinernema* spp. to enter tomato roots and release their symbiotic bacteria which produce allelochemicals that are toxic or repellent to *M. incognita* **(Grewal *et al*.,1999).** Also**, Fallon *et al*. (2002)** reported that *S.feltiae* and *S. riobravae* but not *H.indica* were found intercellular in the root cortex of soybean plants that had been infected with RKN.

Treatments of heat-killed EPNs significantly diminished galling and reproduction of *M. incognita* nearly as alive ones. This results is in accordance with results of **Grewal *et al*., (1999)** who showed that application of heat-killed EPNs

suppressed root penetration by PPNs. Moreover, **Jagdale *et al*., (2002)** published the first report concerning using of dead *S.carpocapsae* IJs which were as effective as live nematodes in controlling PPNs under field conditions. In contrast to PPNs, population of free living nematodes remained unaffected. These results were of practical importance because the use of dead EPNs may help in overcoming difficulties in formulation, storage and transportation associated with living EPNs.

In experiment of using EPN –infected insect cadavers, it was found that adding two *G. mellonella* infected cadavers of six-day old simultaneously with *M. incognita* IJs, obviously lowered number of galls and egg masses in tomato roots like that caused by the systemic nematicide carbofuran applied at the recommended dose. Similar results were obtained under laboratory and greenhouse conditions **(Shapiro –Ilan *et al*., 2003; and Del Valle *et al*., 2013).** The use of such application method improve survival and dispersal compared to aqueous suspension application method **(Shapiro & Lewis, 1999). Hu *et al*., (1999)** revealed that the secondary metabolite 3,5 dihydroxy-4- isopropylstilbene from nematode killed insects inhibited egg hatch of *M. incognita* and caused significant mortality of *Aphelenchoides rhytium* and *Bursaphelenchus* sp. Furthermore, EPN- killed insects filled with both symbiotic bacteria and different life cycle stages of nematodes produce high concentration of ammonia which can be toxic to PPNs **(Grewal *et al*., 1999 and Shapiro *et al*., 2000).**

Application of IJs of EPNs in aqueous solution has some disadvantages such as IJs formulation decrease infectivity, survival during storage, transportation difficulties and the need for adequate irrigation equipment (**Grewal, 2002).** Therefore, application of EPNs-infected insect cadavers provides an option for the control of PPNs because the lack of some disadvantages of aqueous suspension **(Shapiro–Ilan *et al*., 2012). Dolinski *et al*., (2015)** concluded that pest control using EPNs formulated as insect cadavers is an attractive approach for many reasons. Since IJs emerged from cadavers are more infectious and have a higher dispersal capacity as well as prolonged longevity compared to IJs applied in aqueous suspension. Moreover, the cadavers itself appears to serve as protection against harmful environmental extremes such as freezing and desiccation.

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تثبيط نيماتودا تعقد الجذور *Meloidogyne incognita* التي تصيب نباتات الطماطم باستخدام بعض أنواع النيماتودا الممرضة للحشرات تحت ظروف الصوبة

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الملخص العربي

تم دراسة تأثير ستة أنواع من النيماتودا الممرضة للحشرات وهي:

Heterorhabditis bacteriophora; *H. megidis*; *H. zealandica*; *Steinernema feltiae*; *S. Glaseri*; *S. riobravae*

على نيماتودا تعقد الجذور *Meloidogyne incognita* التي تصيب نباتات الطماطم في تجربتين منفصلتين تحت ظروف الصوبة، وفي التجربة الأولى، تم استخدام نيماتودا الحشرات في صورة معلق مائي يحتوى على يرقات الطور المعدي في صورة حية وأخرى ميتة بمعدل ٥٠٠٠ طور يرقى/ نبات، بينما استخدمت في التجربة الثانية اثنين من جثث يرقات دودة الشمع الكبيرة *Galleria mellonella* لكل نبات بعد مرور ستة أيام من إصابتها بالأنواع محل الاختبار من النيماتودا وفي كلتا التجربتين استخدمت نيماتودا الحشرات في نفس التوقيت مع إجراء العدوى بنيماتودا تعقد الجذور، كما استخدم المبيد النيماتودي الكاربوفيوران (الفيورادان ١٠% مجبب) بالتركيز الموصى به (٠.٢ جرام/نبات) للمقارنة وأجريت العدوى لنباتات المعاملة الضابطة الموجبة بمعدل ١٠٠٠ يرقة/ نبات في حين تركت نباتات المعاملة الضابطة السالبة بدون أي إضافات، وبعد شهرين من المعاملة تم تقييم درجة التعقد (المشار إليها بعدد العقد / نبات) والتكاثر (المشار إليه بعدد كتل البيض/ نبات) بالإضافة إلى الضرر (المشار إليه بالوزن الطازج والجاف لأجزاء النبات الخضرية).

أوضحت النتائج المتحصل عليها إلى أن المعاملة بالكاربوفيوران كانت أقل المعاملات في عدد العقد وكتل البيض في التربة الرملية والطينية، ومن ناحية أخرى اتضح أن المعاملات العلاجية باستخدام يرقات الطور المعدي سواء كانت حية أو ميتة قللت وبصورة معنوية من تكون العقد وكتل البيض على جذور نباتات الطماطم مع حدوث زيادة نسبية في الوزن الرطب والوزن الجاف للأجزاء الخضرية لنباتات الطماطم، وكانت الأنواع التابعة لنيماتودا *Steinernema* أكثر فعالية نسبيًّا مقارنة بالأنواع التابعة لنيماتودا *Heterorhabditis* فقد وجد أن المتوسط العام لعدد العقد وكتل البيض في الأنواع التابعة لنيماتودا *Steinernema* هو ٥٢.٥٠ و٢٥.٣٣ مع نسبة خفض مقدارها ٥٥.٤٥ و٦٤.٦٩% على التوالي مقارنة بالنباتات المعاملة بنيماتودا تعقد الجذور فقط، في حين كانت القيم الخاصة بنيماتودا *Heterorhabditis* هي ٦٩.٦٧ و٣٦.٣٣ مع نسبة خفض مقدارها ٤٠.٨٨ و٤٩.٠٧% على التوالي، وكانت أفضل المعاملات هي التي استخدمت فيها اليرقات الحية والتي تفوقت على المعاملة باليرقات الميتة في إحداث نقص في عدد العقد وصل إلى ٥٦.٥٣ و٣٩.٧٩% على التوالي وكذلك خفض في عدد كتل البيض بنسبة بلغت ٦٣.١٦ و٥٠.٣٩% على التوالي، كما أدى استخدام اثنين من

جنث يرقات دودة الشمع الكبيرة إلى خفض عدد العقد وكتل البيض بدرجة واضحة مع تحسن القياسات الخاصة بنمو النبات بدرجة غير معنوية وكان المتوسط العام للخفض في عدد العقد وكتل البيض ٥٨.٤٦ و٥٤.٧٤% على التوالي.