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THE COMPINED EFFECT OF *Heterorhabditis bacteriophora* AND SOME PLANT RESIDUES ON *Meloidogyne incognita* INFECTING TOMATO PLANTS

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ABSTRACT

The effect of *Heterorhabditis bacteriophora* (Hb) (Poinar, 1975). solely or mixed with some plant residues were evaluated against root knot nematode *Meloidogyne incognita* (Mi) in tomato under greenhouse conditions. One thousand IJs of Hb were applied simultaneously with 1000 Js of Mi to the soil with or without one of the plant residues (clover, sunn hemp, marigold, rice straw, and sawdust) in different three weights (0.5, 1 and 2%). The experiment lasted 37 days, and the variables were evaluated as: number of galls, females, immature stages and eggmasses per plant. Adding of EPNs alone affected Mi variables: (133, 100, 17, and 96 when compare to the control 143, 114, 22, and 99, respectively), but did not show any significant differences in the plant growth. Addition of EPNs and the plant residues improved both of the plant health and EPNs activity at the concentration of 0.5%: The effect of EPNs on numbers of galls and females were increased by the residues of saw dust, clover, and Rice. Marigold residue reported a little effect on the numbers of females and eggmasses. At the concentration of 1%, the effect of EPNs on galls, females, and egg masses improved by adding clover, marigold followed by sunn hemp. At the highest concentration of plant residues (2%), the best significant effects of EPNs were obtained by marigold followed by sunn hemp. The immature stages of Mi did not report any significant reduction all over the treatments.

Keywords: Biological control, root-knot nematode, *Meloidogyne incognita*, entomopathogenic nematode, *Heterorbabditis bacteriophora*, plant organic matters.

INTRODUCTION

Due to environmental concerns and increased regulations on use of chemical fumigants, more strategies for management of *Meloidogyne* spp. are currently being investigated (Nico *et al*., 2004). Treatment with entomopathogenic nematodes (EPNs) is regarded not only as a biopesticidal control agents of insect pests of various crops, but also as an alternative for the management of plant-parasitic nematodes (Grewal *et al.,* 2005). Chevalier and Webster, (2006) said that various direct or indirect effects of EPNs applications may occur within the soil community. They added that the spraying of millions of IJs EPNs for insect biocontrol led to an unnaturally high nematode population density in the soil for the first few days after release and significantly reduced the abundance species richness, maturity, and diversity of the nematode community by reducing the numbers of genera and abundance of plant-parasitic, but not free-living nematodes. The EPNs can also change the species composition of the soil community (Somasekhar *et al*., 2002). The IJs is a difficult target for antagonists because of its capacity to escape parasitism and predation by invading roots quickly as

Meloidogyne juveniles inoculated experimentally in pots or on agar surfaces can be found in roots after 24 hours. Stirling (1991) mentioned that once a crop is planted, plant-parasitic nematodes tend to aggregate near roots and biological control agents must therefore be effective at the root-soil interface. Many workers emphasized the role of *Crotalaria* spp. and *Tagetes* spp. in reducing the populations of *Meloidogyne* spp. (Wang *et al*., 2001; Alston *et al*., 2003; Wang *et al*., 2003a,b, 2004a,b, Sosamma and Jayasree, 2002; Rocha *et al*., 2004a,b; Jourand *et al*., 2004). Moreover, Wang *et al*., (2004a,b) stated that amendment with *Crotalaria juncea* increased the abundance of bacterivorous and fungivorous nematodes and the nematophagous fungus, *Harposporium anguillulae*. Sitaramaiah and Singh (1974) observed that tomato seedlings raised in soil amended with neem, oil-cakes or sawdust showed a reduced level of infection by nematodes when transferred to non-amended soil. Kaplan and keen, (1980) believed that there is no concrete evidence for the involvement of simple, pre-formed phenolic compounds or their oxidation products in the incompatibility of plants to nematodes. It is well known that healthy plants growing under ideal environmental conditions are able to tolerate nematode damage much better than unthrifty plants. And so, in the present work, the suppression of Mi infectivity is the goal of adding EPNs. While the twin goals of adding plant residues as an organic manure are to improve the plant growth in order to tolerate the nematode infection by increasing the soil fertility. The second is to take the advantage of the plant residues as nematicidal substances.

MATERIALS AND METHODS

1- Multiplication and maintenance of the entomopathogenic nematodes:

IJ3s of *Heterorhabditis bacteriophora* (Hb) were obtained from the nematodes laboratory in National Research Center in Dokky El Giza . These nematodes were propagated on the fifth instar larvae of the greater wax moth (*Galleria mellonella* L.), Insect larvae were placed in a 9-cm-diam Petri dish lined with a moistened filter paper and exposed to about 100 IJ3s at 25 ºC. in the lab. After 2 days, dead larvae (cadavers) were removed, rinsed thoroughly in tap water then transferred to spongy trap dishes. After 10-12 days, huge number of IJ3s was collected. These IJ3s were used for experiments during 2-week period after emergence.

2-Root-knot nematode culture : Culture of *Meloidogyne incognita* (Kofoid & White) (Mi) was maintained in the screenhouse on tomato plants (*Lycopersicon esculentum* L. var. *Castel Rock*) grown in 15-cm pots in sterile sandy loam soil (750g/each). The pots were watered daily with tap water as needed and nutrient solution was added once a week. When nematode inoculum as a second-stage juveniles (J2s) was needed, galled tomato roots were washed thoroughly with tap water, cut into pieces and placed in the mist chamber for egg hatching. The first catch was discarded, then the following hatched J2s were collected daily and refrigerated at ٦ ^{o}C for the experimental use.

3-Plant materials incorporated in soil: The vegetative parts of the 4th cutting of clover, *Trifolium alexandrinum*, those of sunn hemp, *Crotalaria juncia*, marigold, *Tagetes erecta*, the harvest, rice straw, *Oryza sativa* and sawdust were used as soil amendments. The air-dried vegetative parts and rice straw were chopped to small pieces. The five plant materials were singly mixed with soil (0.5, 1, and 2% w/w). The following table indicates the percentage content of organic carbon (C) and total nitrogen (N), and C:N ratio for each plant material (Entsar 2009):

English name	**Scientific name**	**C%**	**N%**	**C/N ratio**
Clover	*Trifolium alexandrinum* (FamilyFabaceae)	48.46	2.40	20.2:1
Sunn hemp	*Crotalaria juncia* (FamilyFabaceae)	48.88	1.44	33.9:1
Marigold	*Tagetes erecta* (Family Asteraceae)	49.70	1.26	39.4:1
Rice hay	*Oryza sativa* (Family Graminaceae)	47.60	0.88	54.1:1
Sawdust	Unknown	57.50	0.47	122.3:1

4-The greenhouse experiment: To evaluate each plant residue, 10 pots (15-cm-dim) were filed with sandy clay soil and mixed with 0.5% (W.W) of the plant residue and then 3 weeks-tomato-seedling was transplanted in each pot. After one week, 1000 IJs of Mi were inoculated in each plant/pot. Half of the pots were inoculated with 1000 3IJs of Hb simultaneously. The suspension was poured into holes in the soil around the plant stem. The experiment was repeated with 1% and 2% in the same way. The plants were watered when needed. The experiment was ended after 37 days from the nematode inoculation.

5-Determination of plant growth and nematode parameters: At the end of the experiment, roots were washed and fresh weight of roots and shoots were measured. Thereafter, roots were stained with acid fuchsine in cold lacto-phenol (McBeth *et al*., 1941) and stored in it for not less than 24 hr. Stained roots were rinsed in water and cut into pieces to facilitate counting of galls, females, egg masses, and immature stages.

6-Statistical analysis: The data of all experiments were statistically analyzed using analysis of variance procedure proposed by Snedecor and Cochran (1969). The differences between means were compared using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS

Results of the present investigation showed that: 1- Adding of EPNs alone affected Mi parameters e.g. galls, females, immature stages, and egg masses variables evaluated (133, 100, 17, and 96 when compared to the control, 143, 114, 22, and 99, respectively). This findings agree with those of Riegel *et al*., 1998 Fallon *et al*., 2002; Molina *et al*., 2007, Pérez and Lewis, 2004, and Shapiro-Ilan *et al*, 2006b. 2- Adding EPNs with some plant residues, reduced Mi infection. For examples at the concentration of 0.5% (Table 1, Fig. 1) the effect of EPNs on galls and females increased with saw dust, clover, and Rice hay when compared to the control (for galls: 79, 82,

and 95 while the females recorded : 72, 57, and 77, respectively). On the other hand, marigold reported a little effect on females and eggmasses (86 and 78, respictively).

Table (1): Effect of *Heterorhabditis bacteriophora* (Hb) and some plant residues (0.5%) on the developmental stage of *Meloidogyne incognita* (Mi) :

	Galls	Femals	Immat.	egg mas.
C + Mi	96 ^{ab}	89 ^{ab}	32 ^{abc}	87 ^{bcd}
C + Mi+Hb	82 ^{a}	57 ^{a}	32 ^{abc}	74 ^{bc}
R + Mi	97 ^{ab}	78 ^{ab}	45 ^{bcd}	75 ^{bc}
R + Mi+Hb	95 ^{ab}	77 ^{ab}	35 ^{abc}	69 ^{ab}
Cj + Mi	162 ^{c}	170 ^{e}	60 ^{d}	119 ^{d}
Cj + Mi +Hb	151 ^{c}	144 ^{cde}	50 ^{cd}	115 ^{cd}
S + Mi	95 ^{ab}	109 ^{abc}	22 ^{ab}	80 ^{bcd}
S + Mi +Hb	79 ^{a}	72 ^{ab}	15 ^{a}	35 ^{a}
T + Mi	145 ^{c}	157 ^{de}	29 ^{abc}	108 ^{bcd}
T + Mi +Hb	132 ^{bc}	86 ^{ab}	26 ^{abc}	78 ^{bcd}
Mi	143 ^{c}	114 ^{bcd}	22 ^{ab}	99 ^{bcd}
Mi+Hb	133 ^{bc}	100 ^{abc}	17 ^{a}	96 ^{bcd}

Plant materials: clover (C), sunn hemp (Cj) and marigold vegetative parts (T), Rice straw (R) and sawdust (Sd). In each column, means not followed by the same letter differ significantly from one to another at the 0.05 level of significance (Duncan, 1955).

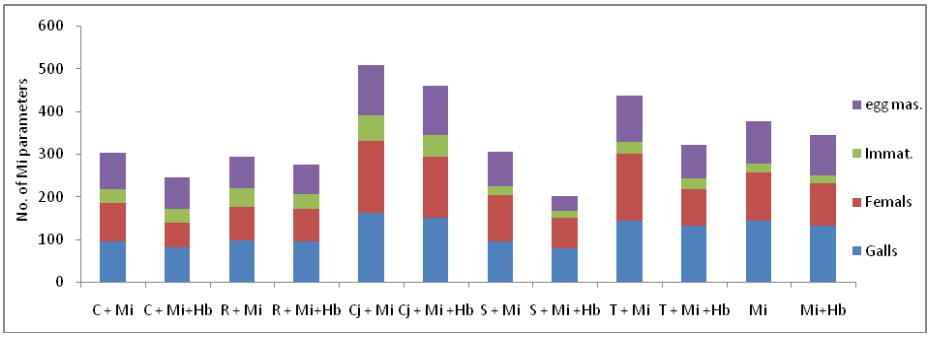

Fig. (1): No. of *Meloidogyne incognita* (Mi) parameters in tomato plants treated with *Heterorhabditis bacteriophora* (Hb) and plant residues (0.5%).

At the concentration of 1% (Table 2, Fig. 2), the effect of EPNs on galls, females, and egg masses improved with adding clover, sun hemp followed by marigold (52, 45, and 33 for clover, 83, 75, and 32 for marigold, while it was 93, 82, and 32 for sunn hemp, respictively).

Table (2): Effect of *Heterorhabditis bacteriophora* (Hb) and some plant residues (1%) on *Meloidogyne incognita* (Mi):

	Galls/plant	Femals/plant	Immature stages/plant	egg mas.
C + Mi	59 ^{a}	56 ^{ab}	46 ^{c}	45 ^{a}
C + Mi+Hb	52 ^{a}	45 ^{a}	32 ^{abc}	33 ^{a}
R + Mi	111 ^{bcd}	105 ^{bcd}	25 ^{ab}	108 ^{b}
R + Mi+Hb	106 ^{bcd}	103 ^{bcd}	21 ^{a}	106 ^{b}
Cj + Mi	93 ^{abc}	86 ^{abc}	43 ^{bc}	40 ^{a}
Cj + Mi +Hb	93 ^{abc}	82 ^{abc}	42 ^{bc}	32 ^{a}
S + Mi	134 ^{cd}	151 ^{d}	24 ^{ab}	107 ^{b}
S + Mi +Hb	118 ^{bcd}	99 ^{bc}	22 ^{a}	107 ^{b}
T + Mi	112 ^{bcd}	81 ^{abc}	26 ^{abc}	32 ^{a}
T + Mi +Hb	83 ^{ab}	75 ^{abc}	19 ^{a}	32 ^{a}
Mi	143 ^{d}	114 ^{cd}	22 ^{a}	99 ^{b}
Mi+Hb	133 ^{cd}	100 ^{bc}	17 ^{a}	96 ^{b}

Plant materials: clover (C), sunn hemp (Cj) and marigold vegetative parts (T), Rice straw (R) and sawdust (Sd).
In each column, means not followed by the same letter differ significantly from one to another at the 0.05 level of significance.

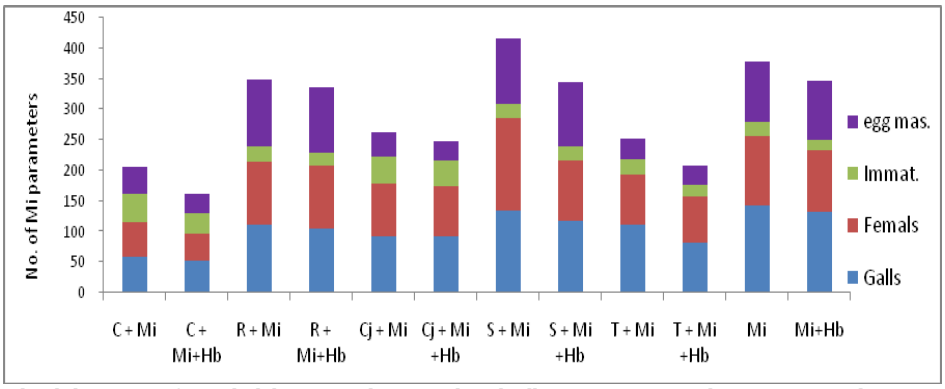

Fig.(2): No. of *Meloidogyne incognita* (Mi) parameters in tomato plants treated with *Heterorhabditis bacteriophora* (Hb) and plant residues (1%).

At the highest concentration of plant residues, 2% (Table 3, Fig. 3) a significant effect was obtained when marigold was added followed by sun hemp (53, 29, and 17 for marigold while it was 66, 48, and 39 for sunn hemp for galls, females, egg masses, respictively), and rice hay showed a non significant effect (88, 79, and 79 for the same parameters).

Immature stages of Mi did not affected with the different plant residues.

Moreover, adding EPNs didn't report any effect on the plant growth, while the plant residues improved plant growth in some cases, e.i, rice hay

reported an effect at the different concentration. Sun hemp and marigold showed more effectivness at the concentration of 1, 2% than the low concentration (0.5%).

Table (3): Effect of *Heterorhabditis bacteriophora* (Hb) and some plant residues (2%) on *Meloidogyne incognita* (Mi):

	Galls/plant	Femals/plant	Immature stages/plant	egg mas.
C + Mi	110 ^{cde}	86 ^{cde}	37 ^{ab}	111 ^{d}
C + Mi+Hb	104 ^{bcde}	84 ^{cde}	28 ^{ab}	109 ^{d}
R + Mi	107 ^{bcde}	87 ^{cde}	22 ^{a}	78 ^{bcd}
R + Mi+Hb	88 ^{abc}	79 ^{bcde}	22 ^{a}	79 ^{bcd}
Cj + Mi	102 ^{bcde}	51 ^{abc}	73 ^{c}	43 ^{ab}
Cj + Mi +Hb	66 ^{ab}	48 ^{abc}	46 ^{b}	39 ^{ab}
S + Mi	100 ^{bcd}	82 ^{bcde}	27 ^{ab}	98 ^{cd}
S + Mi +Hb	86 ^{abc}	64 ^{abcd}	16 ^{a}	61 ^{abc}
T + Mi	66 ^{ab}	40 ^{ab}	33 ^{ab}	20 ^{a}
T + Mi +Hb	53 ^{a}	29 ^{a}	28 ^{ab}	17 ^{a}
Mi	143 ^{e}	114 ^{e}	22 ^{a}	99 ^{cd}
Mi+Hb	133 ^{de}	100 ^{de}	17 ^{a}	96 ^{cd}

Plant materials: clover (C), sunn hemp (Cj) and marigold vegetative parts(T), Rice straw (R) and sawdust (Sd). In each column, means not followed by the same letter differ significantly from one another at the 0.05 level.

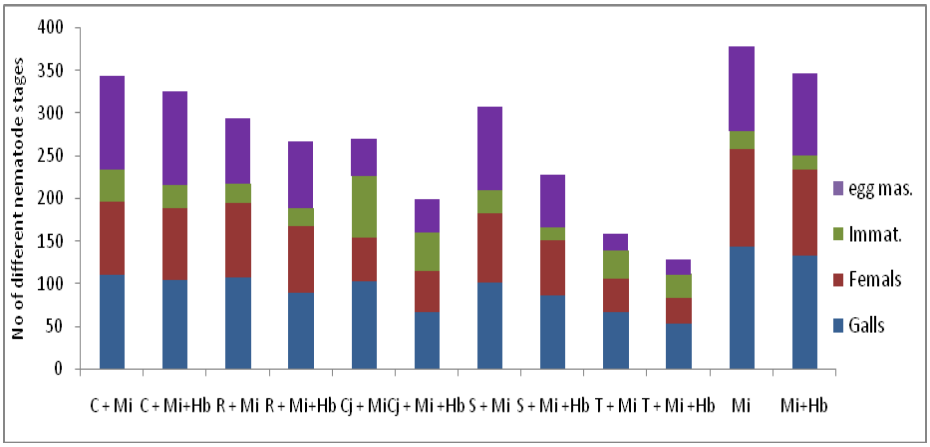

Fig. (3): No. of *Meloidogyne incognita* (Mi) parameters in tomato plants treated with *Heterorhabditis bacteriophora* (Hb) and plant residues (2%).

DISCUSSION

Different factors are responsible for the suppressive effects of EPNs on plant-parasitic nematodes as competition between the nematode groups for space in rhizosphere (Bird and Bird, 1986; Tsai and Yeh, 1995), attraction towards the CO2 and other root exudates (Robinson, 1995), some species of

EPNs such as *Steinernema glaseri*, *S. carpocapsae and Heterorhabditis megidis* have been reported following root plants (Bird and Bird, 1986; Kanagy and Kaya, 1996) or their exudates when insect consume them (Rasmann *et al.,* 2005), probably as a result of a defensive strategy used by plants to protect themselves from insect attacks. (Nazir *et al*., 2012), and production of allelochemicals by the EPNs symbiotic bacteria complex (Grewal *et al*., 1999; Hu *et al.,* 1999; Samaliev *et al.,* 2000; Lewis *et al*., 2001). The difference in the suppressive effect might be due to the difference of the associated bacteria (*Xenorhabdus* spp*. a*ssociated with *Steinernema* spp*.* and *Photorhabdus temperate* and *P. luminescens* with *H. megidis* and *H. bacteriophora*) and its Nematicidal toxic metabolites (Grewal *et al.,* 1999; Hu *et al*., 1999; Samaliev *et al.,* 2000 and Boemare, 2002). Moreover The secondary metabolites 3,5-dihydroxy-4-isopropylstilbene (DST) and indole, which are obtained from filtrates of *P. luminescens*, exhibit nematicidal properties on egg hatching (Hu *et al.,* 1999). In addition, the cell-free extracts of *Xenorhabdus* spp. were found to be toxic and repellent to *M. incognita* juveniles and inhibited its egg hatching (Grewal *et al*., 1999). However, Pérez and Lewis (2004) suggested that *Steinernema* species might be more effective in the suppression of root-knot nematodes than *H. bacteriophora* because of their greater capacity to penetrate roots and to release symbiotic bacteria inside them within roots, the bacteria would release allelochemicals that are toxic and repellent to *Meloidogyne* spp. (Grewal *et al*., 1999). In addition, Kajak *et al*., (1991) reported that manure applications did not protect *Steinernema feltiae* populations from decline when the applications were accompanied by inorganic fertilizer treatments. The effects of inorganic fertilizer may be more important in the long term when nematodes are used for inoculative biological control. By contrast, organic manure used as fertilizer may encourage the nematode establishment and recycling, and might be a useful tool for conservation biological control composted manure was not harmful. These authors hypothesized that this detrimental effect may have been attributed to decomposition of fresh manure, leading to reduced oxygen availability. Rodriguez-Kabana (1986) indicated that decreases in phytoparasitic nematode numbers following organic fertilizer applications tended to be associated with the growth of antagonistic organisms, especially nematophagous fungi. Finally, further studies are required to emphasis this efficacy in field applications.

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التـأثير المشـترك لنيمـاتودا الحشـرات هيتيرورابـديتيس بـاكتريوفورا وبعـض المخلفـات النباتية على "ميلويدوجينى انكوجنيتا" فى نباتات الطماطم

انتصار حلمى طه
قسم وقاية النبات- كلية الزراعة-جامعة عين شمس

تناول البحث دراسة تأثير نيماتودا الحشرات من جنس هيتيرورابديتيس باكتريوفورا على اصابة نيماتودا الميلودوجينى انكوجنيتا للطماطم فى الصوبة وذلك من خلال اضافة نيماتودا الحشرات (١٠٠٠ يرقة) مع نيماتودا النبات (١٠٠٠ يرقة) فى نفس الوقت لشتلات طماطم عمر ثلاثة اسابيع بعد اسبوع من الشتل فى أصص محتوية على ٧٥٠ جم تربة رملية طمبية (١:١) مع الاحتفاظ بعدد من المكررات بدون نيماتودا الحشرات (للمقارنة). فى محاولة لتحسين فعل نيماتودا الحشرات وتحسين نمو النباتات لزيادة مقاومة الاصابة بنيماتودا النبات أو تحملها ، تم خلط التربة قبل الزراعة فى بعض المكررات ببعض المخلفات النباتية (البرسيم، قش الارز، الكروتالاريا، نشارة الخشب والقطيفة) بتركيزات مختلفة(٠.٥%، ١%و٢%) وكانت النتائج كالتالى:

عند المعاملة بنيماتودا النبات و اضافة نيماتودا الحشرات كان هناك تأثير لنيماتودا الحشرات على اعداد العقد النيماتودية والاناث والاطوار الغير كاملة وكتل البيض (١٣٣، ١٠٠، ١٧ و٩٦) فى حين كانت المقارنة (١٤٣، ١١٤، ٢٢ و٩٩) ولم يسجل تأثير على نمو النباتات. اضافة بعض المخلفات النباتية ادى الى تحسن فى تأثير نيماتودا الحشرات كالتالى:

عند تركيز ٠.٥% ادت اضافة نيماتودا الحشرات مع نشارة الخشب ثم البرسيم وأخيرا قش الارز الى خفض فى اعداد العقد النيماتودية وكذلك أعداد الاناث وكتل البيض كما ادت اضافة القطيفة الى خفض قليل فى اعداد العقد والاناث.

عند تركيز ١% كان التأثير الافضل عند اضافة نيماتودا الحشرات مع مخلفات البرسيم، القطيفة يليها الكروتالاريا.

عند التركيز الاعلى ٢% كانت النتائج أفضل عند اضافة نيماتودا الحشرات مع مخلفات القطيفة ثم الكروتالاريا.

فى حين لم يتم تسجيل انخفاض معنوى لاعداد الاطوار الغير كاملة لنيماتودا النبات مع جميع المخلفات النباتية.

قام بتحكيم البحث

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