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Management of *Meloidogyne incognita* Race 1 Through the Use of Entomopathogenic Nematodes in Tomato

Sajid Aleem Khan,[1] Nazir Javed,[1] Muhammad Kamran,[2]* Huma Abbas,[1] Asma Safdar[2] and Imran ul Haq[1]

[1]*Department of Plant Pathology, University of Agriculture, Faisalabad*
[2]*Department of Plant Pathology, University College of Agriculture, University of Sargodha, Sargodha, Pakistan*

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Authors' Contribution
NJ designed and supervised the work. SAK executed the experimental work and also wrote the article. MK analyzed the data. HA and AS cultured RKN, while IUH reared EPN.

Key words
Entomopathogenic nematode, root-knot nematodes, Tomato.

ABSTRACT

Entomopathogenic nematodes; *Steinernema asiaticum* (Anis), *Steinernema glaseri* (Steiner), *Heterorhabditis indica* (Poinar) and *Heterorhabditis bacteriophora* (Poinar) were investigated for their effect on *Meloidogyne incognita* (Kofoid and White) in tomato. They were applied at different levels before and at the same time with the application of root-knot nematodes. Suppression of *M. incognita* varied with application rates of *S. asiaticum, S. glaseri, H. indica* and *H. bacteriophora* in tomato. The high application rates of *S. asiaticum, S. glaseri, H. indica* and *H. bacteriophora* applied both at the same time and before reduced *M. incognita* egg production. The low rates of *S. asiaticum, S. glaseri, H. indica* and *H. bacteriophora* were not as effective as high application rates. The findings of this study suggested that entomopathogenic nematodes could be used for the management of root-knot nematodes.

INTRODUCTION

The cultivated tomato (*Lycopersicon esculentum* Mill.) belongs to *Solanaceae* family and grown successfully throughout the world including Pakistan as well. Within the past century, it has become one of the most popular and widely consumed vegetable crop, growing in outdoor fields, greenhouses and net houses. Tomato is one of the important vegetable crops of Pakistan and is cultivated over 46,230 ha with the annual production of about 468,140 t (Anonymous, 2007). Among various obstacles including fungi, bacteria and viruses in cultivating this crop, root knot nematodes *Meloidogyne incognita* is widespread and recognized as a major pathogen of tomato (Fourie and McDonald, 2000; Kamran *et al*., 2013).

Continuing environmental problems associated with the use of nematicides have resulted in a sense of urgency regarding the search for alternative nematode management strategies (Veremis and Roberts, 1996). Entomopathogenic nematodes (EPN) are effective for the management of root knot nematodes (RKN) Grossman, 1997) and used as bioinsecticides against soil pests (Klein 1990, Georgis and Manweiler, 1994). EPN can provide better management for plant-parasitic nematodes due to environmentally safe and their non target effect without affecting the free living nematodes that play an important role in nutrient cycling (Raichon *et al*., 1994; Bonning and Hammock, 1996; Somasekhar *et al.*, 2002). There was little information available on the application rate of EPN against root knot nematodes. Objective of study was to assess the application of EPN at different levels against RKN as control measure.

MATERIALS AND METHODS

Rearing of EPN

The greater wax moth *Galleria mellonella* (L.) were obtained from bee hives infected with *G. mellonella.* Last instar larvae of *G. mellonella* were separated for nematode culture, leaving small sized larvae for moth emergence and egg laying. Fresh laid eggs were transferred to modified artificial diet prepared by mixing oat, wheat, rice and maize porridge (20 g), yeast granules (50 g) in solution of 80 ml warm honey and 100 g glycerol (Alrubei and Al-Izzim, 1986). This diet with *Galleria* eggs was incubated at 27ºC for hatching. The last instars developed were taken out and used for storage and nematode isolation/multiplication. The already available EPN *Steinernema glaseri, S. asiaticum, Heterorhabditis indica* and *H. bacteriophora* (these species were previously obtained from University of Reading, UK) were evaluated against *Meloidogyne incognita* race 1. The EPN were reared on the late instars larvae of *G. mellonella,* the greater wax moth. The *in vivo* production of EPN was slightly modified from the basic methods described by Poinar (1979) and summarized by Woodring and Kaya (1988). Larvae were kept at 15°C. EPN were collected from dead *G. mellonella* larvae by

* Corresponding author: mkamran.uaf.pk@gmail.com
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modified White trap (White, 1927) and then stored at about 10-15ºC. Live infective juveniles were used within fifteen days of emergence from the cadavers of insect host.

Culturing of M. incognita

Culture of *M. incognita* was maintained on roots of susceptible tomato variety 'Moneymaker' in greenhouse. Juveniles were isolated from infested roots by modified Whitehead and Hemming tray method (White-head and Hemming, 1965). Only freshly hatched second stage juveniles (24-48 h old) were used. Three weeks old tomato nursery 'Moneymaker' was planted in small pots containing 240 ml formaline sterilized soil (72% sand, 17% silt and 8% clay). The sterilization of soil was accomplished by applying formalin. Diluted formalin (1:320) was poured in the small heap of soil, mixed thoroughly and covered with polythene sheet to avoid the evaporation completely. This process continued for a week. After a week sheet was removed and the soil was spread uniformly to get rid from residual formalin and then filled in the pots.

Experimental Procedure

After two weeks when tomato plants established their root system, second stage juveniles of *M. incognita* at 750/plant and EPN at the rate of 1,000, 2,500 and 5,000 were applied at same time in 240 ml modules (pot) in the rhizosphere by making 3-4 holes near the base of plant with sharp wooden needle (Campos and Campos, 2005) and filled with soil to prevent drying. Plants inoculated with *M. incognita* only were kept as control. Each application level of EPN had its respective control. These plants were randomized in a glass house at 22-38ºC and each treatment was replicated fifteen times. Another experiment was conducted by applying the EPN before RKN. After thirty days, they were removed from pots and the root balls were shaken until most of the soil had been dislodged from the root. After washing and taking root and shoot weight roots were placed in a phloxine B solution (0.15 g/litre tap water) for about 15-20 min. The stain was absorbed by the gelatinous matrix, which took a pink to red color while roots remained either unstained or very lightly stained, whereas eggs remained viable. Excess stain was removed by three consecutive rinses in one-liter beaker filled with water. After staining, roots were wrapped in tissue paper to prevent drying out during the steps of the procedure of evaluation. Stained egg masses were counted. Then total number of females was counted by staining in acid fuchsin (Bridge *et al.*, 1982). Number of egg masses and females were counted on the whole root system (Quesenberry *et al.*, 1989; Anwar *et al.*, 2007) under a stereomicroscope (Olympus SZ 61) at 3.5X magnification. Results were subjected to statistical analysis and analysis of variance was done using DMR test at 1% probability level by using M Stat version 2.3.

RESULTS

Effect of EPN at various densities and time of application of M. incognita *(applied at same time with RKN)*

Table I shows the effect of EPN applied at the same time with RKN. Root weight was significantly higher in the treatment where only RKN applied. It indicated the maximum invasion of root-knot nematodes. Root weight varied significantly in all the treatments when the EPN and root-knot nematodes applied at the same time. Minimum root weight was recorded in untreated control plants. There was not any significant difference in the treatment level 2500 and 5000 except for *S. glaseri* at 500 level that was significantly different ($p<0.01$). Though the shoot weight was lower than the root-knot nematodes treatment but was non significant ($P<0.01$). Shoot weight was also non significant in all the treatments (Table I). Shoot weight in the treatment where RKN were applied was lower as compared to the other treatments but it was non significant ($p<0.01$). All the EPN when they were applied significantly reduced the invasion of RKN in tomato root. Maximum invasion of RKN was observed where it was applied alone. All the treatments proved effective in controlling invasion of RKN to tomato roots. All the EPN at their concentration level 5000 proved effective followed by 2500 and 1000. *Steinernema asiaticum* and *H. bacteriophora* at 5,000 gave good results and invasion was lower significantly. Minimum egg masses recorded in *S. asiaticum. H. bacteriophora* and *S. glaseri* and these were statistically similar (Table II). It was very low as compared to the treatment containing only root-knot nematodes that were significantly higher.

Effect of EPN at various densities and time of application of M. incognita *(applied 24h before RKN)*

Root weight at all application rates of EPN were lower than the *M. incognita* alone and were non-significant at 1% probability level (Table I). Root weight and shoot weight where *S. glaseri* (alone) applied was lower but it was not significant at 1% probability level. Shoot weight in the treatment where RKN were applied was lower as compared to the other treatments but it was non significant at 1% probability level. All the EPN when they were applied significantly reduced the invasion of *M. incognita* in tomato root ($P<0.01$). Maximum invasion of *M. incognita* was observed where it was alone. All the treatments proved effective in controlling invasion of

Table I.- Effect of EPN at various densities and time of application of *M. incognita* on tomato growth.

Treatment EPN	RKN level	Applied at same time with RKN		Applied 24 h before RKN	
		Root weight (g)	Shoot weight (g)	Root weight (g)	Shoot weight (g)
S. glaseri alone	1,000	1.89 efgh	5.18 a	1.92 a	5.30 a
	2,500	1.89 efgh	5.27 a	1.98 a	5.22 a
	5,000	1.93 efgh	5.28 a	1.91 a	5.22 a
S .glaseri +RKN	1,000	2.06 cdef	5.57 a	2.22 a	4.99 a
	2,500	1.85 efgh	5.44a	2.14 a	4.99 a
	5,000	1.74 hi	5.47 a	2.19 a	4.98 a
S. asiaticum alone	1,000	1.89 efgh	5.33a	1.92 a	5.22 a
	2,500	1.91 efgh	5.3 a	1.92 a	5.25 a
	5,000	1.87 efgh	5.44 a	1.89 a	5.24 a
S. asiaticum +RKN	1,000	1.97 defgh	5.31a	2.09 a	5.15 a
	2,500	1.91 efgh	5.41 a	2.07 a	5.13 a
	5,000	1.88 efgh	5.41 a	1.99 a	5.32 a
H. indica alone	1,000	1.88 efgh	5.29 a	1.94 a	5.19 a
	2,500	1.92 efgh	5.35 a	1.91 a	5.23 a
	5,000	1.91 efgh	5.38 a	1.92 a	5.27 a
H. indica +RKN	1,000	2.18 cd	5.54 a	2.12 a	5.07 a
	2,500	1.88 efgh	5.62 a	2.12 a	5.07 a
	5,000	1.76 ghi	5.72 a	2.11a	5.14 a
H. bacteriophora alone	1,000	1.89 efgh	5.37 a	1.93a	5.29 a
	2,500	1.88 efgh	5.38 a	1.92 a	5.16 a
	5,000	1.90 efgh	5.3 a	1.95a	5.27 a
H. bacteriophora +RKN	1,000	2.08 cde	5.24 a	2.11a	5.03 a
	2,500	2.01 cdefg	5.29 a	2.07a	5.16 a
	5,000	1.92 efgh	5.34 a	1.99 a	5.24 a
RKN only		2.44 a	4.78 a	2.31 a	4.81 a
Healthy		1.69 c	5.92 a	1.93 a	5.26 a

Numbers followed by different letters in the same columns are significantly different from each other at 1% probability level. Data are mean of fifteen replications.

M. incognita to tomato roots. All the EPN at their concentration level of 5,000 proved effective followed by 2,500 and 1,000. *Steinernema asiaticum* at its 5,000 concentration gave good results and invasion was significantly lower as compared to other treatments (Table II). Minimum egg masses were recorded in *S. asiaticum* and it was lower as compared to the treatment containing only *M. incognita* which were significantly higher ($p<0.01$).

DISCUSSION

The effect of *S. asiaticum, S. glaseri, H. indica* and *H. bacteriophora* was investigated on *M. incognita* in tomato roots. Harvesting of treatments was done after 28 days. Reduction of RKN was recorded in EPN treatments along with reduced number of females in EPN treated roots. It can be concluded that it was due to a delayed development/maturation effect of EPN on the maturity of root-knot nematodes.

Different factors are responsible for the suppressive effects of EPN 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 CO_2 and other root exudates (Robinson, 1995) increased density of predators resulting

Table II.- Effect of EPN at various densities and time of application of *M. incognita* on nematode reproduction.

Treatment EPN	RKN level	Applied at same time with RKN		Applied 24 h before RKN	
		Females	Egg mass	Females	Egg mass
S. glaseri alone	1,000	0g	0h	0g	0h
	2,500	0g	0h	0g	0h
	5,000	0g	0h	0g	0h
S .glaseri +RKN	1,000	181cd	143.4de	206c	174.2b
	2,500	170.6d	127.6e	164d	126.8d
	5,000	109ef	72.6g	123.2e	87.8ef
S. asiaticum alone	1,000	0g	0h	0g	0h
	2,500	0g	0h	0g	0h
	5,000	0g	0h	0g	0h
S. asiaticum +RKN	1,000	198c	129e	196.6c	129.4d
	2,500	131.8e	93.6f	148.2d	93.20e
	5,000	104.4f	68g	101f	67g
H. indica alone	1,000	0g	0h	0g	0h
	2,500	0g	0h	0g	0h
	5,000	0g	0h	0g	0h
H. indica +RKN	1,000	227b	165bc	228.4b	164.6b
	2,500	205bc	149cd	188c	140.6cd
	5,000	114.4ef	82fg	120.8e	88ef
H. bacteriophora alone	1,000	0g	0h	0g	0h
	2,500	0g	0h	0g	0h
	5,000	0g	0h	0g	0h
H. bacteriophora +RKN	1,000	276.8a	180.4b	239.6b	176b
	2,500	204.4bc	147cde	194.8c	149.2c
	5,000	98.2f	66g	116.4ef	74.4fg
RKN only		285.8a	212.4a	282a	211.2a
Healthy		0g	0h	0	0h

Numbers followed by different letters in the same columns are significantly different from each other at 1% probability level. Data are mean of fifteen replications.

from the application of nematode biomass to the soil (Ishibashi and Kondo, 1986), behavioral response and increased natural enemies (Grewal *et al.*, 1999) and production of allelochemicals by the entomopathogenic nematode symbiotic bacteria complex (Grewal *et al.*, 1999; Hu *et al.*, 1999; Samaliev *et al.*, 2000; Lewis *et al.*, 2001; Jagdale *et al.*, 2002). Nematicidal properties of metabolites of symbiotic bacteria *Xenorhabdus* spp. associated with *Steinernema* spp. (Grewal *et al.*, 1999; Hu *et al.*, 1999; Samaliev *et al.*, 2000) and *P. temperate* and *P. luminescens* with *H. megidis* and *H. bacteriophora* (Boemare, 2002) might be responsible for the suppressive effect of EPN on RKN. The difference in the suppressive effect might be due to the difference of the associated bacteria and its toxic metabolites. 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). EPN belonging to Steinernematids were found in tomato roots. *Steinernema* spp. has ability to enter in roots by following infecting root-knot nematodes (Fallon *et al.*, 2002). *M. incognita* suppression using Heterorhabditis was less consistent than steinernematids. It can be concluded that the *Steinernema* spp. were more efficient in suppressing *M. incognita* due to their ability to enter the roots and release associated bacteria inside the roots. The bacteria inside the root

tissue release allelochemicals those are toxic and repellent to RKN (Grewal *et al*., 1999; Fallon *et al.*, 2002).

Entompathogenic nematodes can be successfully applied for the management of root-knot nematodes, as they are environmentally safe and could be successfully used in integrated disease management programme.

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