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Effect of *Steinernema glaseri* and *Heterorhabditis indica* on the Plant Vigour and Root Knot Nematodes in Tomato Roots at Different Densities and Time of Applications

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Abstract.- *Steinernema glaseri* and *Heterorhabditis indica* applied at different inoculum levels before and simultaneously with root knot nematodes were investigated for invasion of *Meloidogyne* spp. in tomato. Suppression of *M. incognita* varied with application rate of *S. glaseri,* and *H. indica* The high application rates of, *S. glaseri* and *H. indica* applied both same and before reduced invasion of *M. incognita*. The low rate of *S. glaseri* and *H. indica* were not as effective as high rates. Both entomopathogenic nematodes when applied at 1250 and 2500/pot 24 h before or at the same time reduced the invasion of root knot nematodes in tomato root. Whereas *S. glaseri* applied at 500 /pot at the same time with the root-knot nematodes reduced the invasion. Both the entomopathogenic nematodes (EPN) applied 24 h before or at the same time with root knot nematodes at 2500/pot reduced the invasion as compared with 500/pot. Only *H. indica* significantly reduced invasion when it was applied at 1250/pot 24 h before the root knot nematodes. Whereas *S. glaseri* applied at the same time and *H. indica* 24 h before at 2500/pot significantly reduced the invasion as compared with 1250/pot. *S. glaseri* was more effective in reducing the invasion as compared to *H. indica.* Recovery of both the entomopathogenic nematodes when they were applied alone was comparatively more than when applied with the root knot nematodes. But in case of *H. indica* its recovery was more when it was applied at the same time with the *M. javanica.*

Key words. *Steinernema glaseri, Heterorhabditis indica,*root knot nematodes, entomopathogenic nematodes.

INTRODUCTION

Root-knot nematodes (RKN) are notoriously difficult to manage because of their high reproductive potential. Economic damage on tomato can occur with root-nematode densities of 0.1–1.0 nematodes per cm^3 soil at planting (Sikora and Fernandez, 2005). Whitehead (1998) suggested that 99.9% control is required in order to prevent the subsequent build-up of damaging population because of the reproductive potential of *Meloidogyne* spp.

On tomato these nematodes can cause 24-38% loss, where sequential cropping of one susceptible crop after another is practiced with up to four per year. In the absence of effective control it would lead to total crop failure. Plant parasitic nematodes cause global losses to crop plants with an estimated loss of $ 125 billion per year in the tropics (Chitwood, 2003). The nematode infected plants show poor growth and become less productive because the damaged root systems are less efficient in absorbing water and nutrients. Current dissatisfaction with chemicals nematicides, due to safety issues, environmental concern and limited use of many products (*e.g.*, methyle bromide) has stimulated interest in control strategies that are ecologically compatible with current production system. In fact developing alternative to hazardous chemical nematicides is one of the top priorities for the future of nematology. Several control strategies, such as host plant resistance, rotation with non-hosts, sanitation and avoidance, destruction of residual crop roots, and judicious use of nematicides have been reported to effectively control RKNs (Whitehead, 1998).

Entomopathogenic nematodes (EPN) of Steinernematidae and Heterorhabditidae families have been used as biological control agents against different insect pests. These nematodes are soil-dwelling organisms, and are obligate parasites of insects. The only stage which lives freely on the soil is the infective juvenile or J3 and to complete their life cycles, those J3s must find a suitable host. When they locate the host, penetrate into them through the body natural openings (Poinar, 1979;

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Burnell and Stock, 2000) or also through the cuticle in Heterorhabditidae (Bedding and Molyneux, 1982), reaching the hemocoel and releasing a symbiotic bacterium (*Xenorhabdus* in *Steinernema* and *Photorhabdus* in *Heterorhabditis*) which starts reproducing and finally kills the insect by septicaemia between 24 to 72 h. Some species of EPN such *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.

Due to environmental concerns and increased regulations on use of chemical fumigants, more management strategies for management of RKNs (*Meloidogyne* spp.) are currently being investigated (Nico *et al.,* 2004). Biological control using EPN is one potential alternative to chemical nematicides. Recently it has been demonstrated that EPNs can affect the number of RNK infecting plants, or the number of eggs produced when they are applied near the root system (Fallon *et al.,* 2002; Perez and Lewis, 2002; 2004). The explanation of this effect has been theorized as a result of an allelopathic response produced by the symbiotic bacteria of EPNs which is repellent to RKNs (Grewal *et al.*, 1999), however the application of the bacterium has not shown a consistent suppression of RKN in some studies (Fallon *et al.*, 2004). The objective of the recent study is to determine the effect of different inoculums levels and time of application for 2 species of EPNs (Sg and Hi) on the invasion of RKN to tomato roots and their effect on plant vigour improvement.

MATERIALS AND METHODS

Culture of EPNs

The greater wax moth *Gallaria mellonella (L.)* were obtained from bee hives infected with *G. mellonella.* Last instars larvae of *G. mellonela* 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). Diet with Gallaria was then kept at 27ºC in an incubator. After reaching last instars, they were taken out from the diet and used for storage and nematode isolation/multiplication. *Steinernema glaseri* and *Heterorhabditis indica* were multiplied and harvested from greater wax moth larvae (Lepidoptera: Pyralidae) (Woodring and Kaya, 1988; White, 1927) and later stored at 10°C. Infective juveniles of these nematodes were harvested two weeks after incubation using white traps and washed in three changes of distilled water (Dutky *et al.,* 1964). These juveniles were stored at 10°C and before use they were left over night at 20±3°C.

Culture of RKN M. javanica

The RKN *M. javanica* was maintained on tomato (*Lycopersicon esculentum*) plants cv., Tiny Tim in a glass house at 28±4±C. Eggs of *M. javanica* were collected using a modification of the technique described by McClure (1977). Galled roots with egg masses were washed free of soil, cut into 2-cm long pieces and after placing in 0.26% sodium hypochlorite (commercial bleach) were triturated at 30 s intervals at maximum speed in a two-speed blender. To separate the organic debris from eggs this suspension was poured through a series of sieves. The eggs were collected on 38µm-pore sieve and washed carefully with tap water. The egg suspension was poured on to cotton-wool filter paper (modified Baermann) and incubated at 28°C. The hatched second stage juveniles (J2) were collected daily. Only freshly hatched J2 collected within 48 h were used for experiments.

Effect of S. glaseri *and* H. indica *on the plant vigour and development of* M. javanica *in tomato roots*

One month old tomato plants c.v, Tiny Tim was maintained in 80 ml modules. *S. glaseri* and *H. indica* were applied separately at 500, 1250, 2500 individuals per pot (80 ml) at various time intervals (24 h before, at the same time with or without root-knot). Plants receiving only RKNs were kept as control. Each time and application dose of entomopathogenic nematodes had its respective control. These plants were completely randomised in a glass house in controlled conditions where

temperature was 22-38°C and each treatment was replicated five times. Plants were kept in green house for one week after exposure of plants to root-knot nematodes. Plants were not watered one day before harvesting. Then they were removed from pots and the root balls were shaken until most of the soil had been dislodged from the root. From this soil the EPNs were recovered by plating the soil for 48 h using Baermans funnel method. After washing and taking root and shoot weight these roots were stained in acid fuchsin (Byrd *et al.*, 1983) and macerated. Then total number of nematodes was counted.

In one set of treatment where *S. glaseri* and *H. indica* were applied at the same time with root knot nematodes, the experiment run for 31 days. The number of egg masses and females were counted on the whole root system (Holbrook *et al.*, 1983). Experiment was repeated twice to confirm the data.

Data was analyzed using ANOVA by using SAS statistical software (SAS Institute, Cary, NC, USA). The significance of differences within treatments was separated by using Least Significant Difference test at 5%.

RESULTS

There was no significant effect of any treatment on the root and shoot weight of tomatoes. The root weigh where *S. glaseri* applied at 2500 with root knot nematodes at the same times was low and had a significant effect, but this effect could not be repeated again as the experiment was repeated twice (Table I). Similarly shoot weight where *S. glaseri* were applied at 1250 with root knot 24 h before was comparatively lower than other treatments (Table I).

Recovery of *S. glaseri* from soil after one week of its application was significantly different ($P<0.05$) from *H. indica*. *S. glaseri* and *H. indica* recovered from soil were significantly different from each other at different densities and time of application (24 h before or after application). Similarly it was insignificant ($P = 0.54$) at its method of application (with or without RKN). Recovery of *S. glaseri* was more when it was applied without root-knot nematodes in contrast to *H. indica*. Recovery of both EPNs when they were applied alone were comparatively more than when they applied with the RKNs. In case of *H. Indica*, the recovery was more when it was applied at the same time with RKN (Table II).

Both the EPNs when applied at 1250 and 2500/pot 24 h before or at the same time significantly ($P<0.05$) reduced the invasion of RKNs in tomato root. Whereas only *S. glaseri* applied at 500 /pot at the same time with the RKNs significantly reduced the invasion (Table III). Both the EPN applied 24 h before or at the same time with RKNs at 2500/pot significantly ($P<0.05$) reduced the invasion as compared with 500/pot. Only *H. indica* significantly reduced invasion when it was applied at 1250/pot 24 h before the RKNs as compared with 500/pot.Whereas *S. glaseri* applied at the same time and *H. indica* 24 h before at 2500/pot significantly reduced the invasion as compared with 1250/pot (Table IV). *S. glaseri* was more effective in reducing the invasion as compared to *H. indica*.

Table IV indicating that both the EPN differed significantly ($P<0.05$) with control in reducing the number of egg masses at all levels of their application (500, 1250 and 2500).There was no significant difference between the EPN at various doses expect *S. glaseri* at 500/pot which differed significantly with *H. indica* at same and 2500/pot level of application. Both the EPN at 2500/pot differed significantly ($P<0.05$) with other densities in reducing the number of egg masses. They also significantly ($P<0.05$) reduced the number of females as compared with control.

DISCUSSION

The effect of S. glaseri *and* H. indica *was investigated on* M. javanica *in tomato roots*

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 from the application of nematode biomass to the soil (Ishibashi and Kondo, 1986), behavioral response

Table I.- Effect of EPN on root weights and shot of tomato plant.

Treatments	Time of EPN application	Densities of EPNs (IJ/pot)					
		500		1250		2500	
		Alone	With RKN	Alone	With RKN	Alone	With RKN
Root weight							
S. glaseri	24 h before	1.57±1.26	1.90±0.21	1.72±0.06	1.64±0.13	1.49±0.15	1.51±0.07
	Simultaneous	1.56±0.075	1.47±0.19	1.97±0.15	1.93±0.16	1.78±0.14	1.43±0.15
H. indica	24 h before	1.55±1.16	1.76±0.17	1.54±0.10	1.73±0.11	1.48±0.13	1.77±0.07
	Simultaneous	1.76±0.05	1.59±0.14	1.82±0.11	1.71±0.22	1.53±0.09	1.71±0.22
Control (Root-knot)				1.782±0.089			
Healthy plants				1.786±0.188			
Shoot weight							
S. glaseri	24 h before	4.39±0.06	4.62±0.35	4.49±0.17	3.98±0.26	4.43±0.05	4.28±0.21
	Simultaneous	4.93±0.12	4.11±0.20	4.97±0.20	4.55±0.17	4.83±0.31	4.65±0.19
H. indica	24 h before	4.83±0.23	4.53±0.29	4.64±0.35	4.99±0.42	4.52±0.16	4.92±0.56
	Simultaneous	4.75±0.21	4.60±0.27	5.37±0.23	4.86±0.21	4.98±0.27	5.04±0.34
Control (Root-knot)				4.21±0.34			
Healthy plants				4.87±0.39			

Table II.- EPN (actual values and log values) recovered from soil at the time of harvesting (one week after application)

Treatments	Time of EPN application	Densities of EPNs (IJ/pot)					
		500		1250		2500	
		Alone	Pathogen	Alone	Pathogen	Alone	Pathogen
EPN (actual values)							
S. glaseri	24 h before	152.40±24.80	172.2±46.2	325.6±102.1	298.8±90.5	715.2±73.5	557.0±131.4
	Simultaneous	148.60±28.58	116.2±34.75	268.6±58.0	215.6±38.8	684.2±101.4	600.8±128.2
H. indica	24 h before	49.0±14.44	55.8±23.99	137.2±29.66	142.0±20.73	236.4±58.1	243.0±45.3
	Simultaneous	44.6±9.45	71.8±27.89	118.4±35.15	198.8±58.1	335.0±69.4	362.8±63.9
EPN (log values)							
S. glaseri	24 h before	2.15±0.07	2.17±0.12	2.42±0.13	2.40±0.12	2.84±0.04	2.69±0.10
	Simultaneous	2.13±0.09	2.0±0.10	2.38±0.09	2.30±0.08	2.81±0.07	2.73±0.10
H. indica	24 h before	1.59±0.15	1.62±0.15	2.10±0.08	2.13±0.06	2.30±0.12	2.35±0.08
	Simultaneous	1.60±0.10	1.72±0.17	2.01±0.11	2.22±0.12	2.47±0.11	2.52±0.08

Densities of EPN; P <0.05; SED; 0.05 Treatments; P< 0.05; SED; 0.045; Time; P =0.08; SED; 0.045; Method (alone or with pathogen); P = 0.54; SED; 0.045; Treatments x Densities x Time x Method); P = 1.0; SED; 0.15

Table III.- Effect of *H. indica* and *S. glaseri* applied 24 h before or at the same time with root-knot nematodes on the invasion of root-knot nematodes within tomato root.

Treatments	Densities of EPNs (IJ/pot)					
	500		1250		2500	
	24 h before	Simultaneous	24 h before	Simultaneous	24 h before	Simultaneous
S. glaseri	110.0±17.40	93.0±8.58	84.4±9.26	86.8±5.70	67.8±11.21	54.2±9.59
H. indica	129.0±5.73	110.4±13.12	90.0±9.34	81.0±16.95	62.4±10.78	61.6±13.03
Control (RKN)			131.20±15.05			

Treatments; P< 0.05; SED; 12.73; Densities of EPN; P <0.05; SED; 13.17; Time: P=0.35; SED; 12.73; Treatments x Densities x Time; P = 0.76; SED; 16.66

Table IV.- Effect of EPN on number of females and egg masses.

Treatment	Densities of Entomopathogenic nematodes (IJ/pot)					
	500		1250		2500	
	Egg masses	Females	Egg masses	Females	Egg masses	Females
S. glaseri	43	94	72	97	15	80
H. indica	78	147	73	108	9	33
Control (root-knot nematodes	127.0	225.7	127.0	225.7	127.0	225.7

Egg masses:	Females;
Treatments; P<0.05 SED: 16.41	P<0.05; SED; 16.81
Densities; P<0.05 LSD; 16.41	P<0.05; SED; 16.81
Treatments x Densities P= 0.36; SED; 28.42	P=0.09; SED; 29.12

and increased natural enemies (Grewal *et al*., 1999) 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). 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 EPNs on root knot nematodes. 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). EPNs 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 Heterorhabditids was less consistent than steinernematids. Our results are in confirmitty with the work done by different workers (Grewal *et al.,* 1999; Fallon *et al*., 2002, 2006; Jagdale and Grewal, 2008). 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 RKNs (Grewal *et al.,* 1999; Fallon *et al*., 2002). EPNs can be successfully applied as the environment safe management approach for RKNs.

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