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Survival of Entomopathogenic Nematodes in Sterilized vs Non-Sterilized Soil

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ABSTRACT

This study was conducted to determine the survival of entomopathogenic nematodes (EPN) in soil. Entomopathogenic nematodes [(*Steinernema asiaticum* (Anis), *S. glaseri* (Steiner), *Heterorhabditis indica* (Poinar) and *H. bacteriophora* (Poinar)] were applied to non-sterilized and sterilized soil either with or without tomato plants and roots. Formalin was used for the sterilization of sandy loam soil (72% sand, 17% silt and 8% clay). Nematodes were recovered from the soil immediately after application and 7 days after application using sieving method. There was no significant difference seen in all the treatments immediately after application. Nematode recovery ranged from 43.22 to 45.42%. The percentage of the entomopathogenic nematodes recovered after seven days of application ranged from 1.87 to 7.83%. Number of live *S. asiaticum, S. glaseri, H. indica* and *H. bacteriophora* were severely reduced in non-sterilized soil with or without tomato roots after 7 days. There was a significant difference in survival rate of *S. asiaticum, S. glaseri, H. indica* and *H. bacteriophora* in sterilized soil.

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SAK performed experimental work. IUH and KN helped in experimental work and data analysis. ZU and AS wrote the article. NJ and SAA supervised the work.

Key words
Entomopathogenic nematodes , Persistence , Sterilization, Soil, Attract, Female tail extract

INTRODUCTION

Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* symbiotically associated with bacteria of the genera *Xenorhabdus* and *Photorhabdus*, respectively, are safe antagonists as a commercial bioinsecticide for many economically important soil pests including root- knot nematodes in ornamentals, vegetables, fruit and turf (Klein, 1990; Georgis and Manweiler, 1994; Ehlers and Peters 1995; Georgis *et al*., 2006). The specialized third stage juvenile, called the infective juvenile, is the only free living stage which upon finding suitable host enters through natural openings (anus, spiracles, and mouth), penetrate into the hemocoel and discharge the symbiotic bacteria that multiply and kill the hosts within 48 h by septicemia (Burnell and Stock, 2000). EPIV survive naturally in the soil and are effective as inundative biological control agents of many soil insects (Klein, 1990; Kaya and Gaugler, 1993). *Steinernema carpocapsae* (Weiser) and *Steinernema glaseri* in sand, sandy loam, clay loam and clay over 16 weeks (Kung *et al*., 1990). Survival of both nematodes was different in all soil types. EPN survival and population depend directly upon the abiotic characteristics of soil and the biology, behavior, and interspecific interactions of coexisting species (Puza and Mracek, 2005; Gruner *et al*., 2007). Soil parameters such as soil temperature, texture, bulk density, pH, organic content and soil water potential can affect juvenile behavior, survival, and infectivity to hosts (Brown and Gaugler, 1997; Aguilar *et al*., 1999; Koppenhöfer and Fuzy, 2006). Temperatures above 40°C and below 8°C are lethal for most EPNs (Griffin, 1993; Grewal *et al*., 1994). *Steinernema feltiae* was more efficient than *H. bacteriophora* at different temperatures (12, 18, and 24°C), especially at 12°C. These nematodes have been used against *Meloidogyne incognita* (Khan *et al*., 2016) and this successful use of nematodes as biological tool is critically dependent upon many other factors such as soil moisture, air and soil temperatures, soil texture, application time and crop variability and features (Kaya, 1993; Gaugler, 2002). Therefore, this investigation was undertaken to determine the effect of sterilized soil versus non-sterilized soil in combination with presence and absence of tomato shoots and roots separately on the survival of EPN (*Steinernema asiaticum, S. glaseri, Heterorhabditis indica* and *H. bacteriophora*).

MATERIALS AND METHODS

EPN were applied to non-sterilized or sterilized soil either with or without tomato plants and roots. The sterilization of sandy loam soil (72% sand, 17% silt and

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8% clay) was accomplished by applying formalin. Diluted formalin (1:320) was poured in the small heap of soil and covered with polythene sheet to stop the fumes completely. This process continued for a week. After a week, the soil was spread out to get rid of the residual formalin, was mixed thoroughly and then filled in the pot again.

Tomato plants (cv. Money maker), grown in soil, were washed out and repotted in the sterilized or non-sterilized soil 24 h before nematode application. Holes in the bottom of pots were sealed with 20 m mesh to allow water drainage but prevent nematodes from being washed out. Four species of EPNs were used *i.e. Steinernema glaseri, S. asiaticum, Heterorhabditis bacteriophora* and *H. indica*. The concentration of EPN was 5000 IUS per pot. Each treatment was replicated five times. Nematodes were recovered from the soil immediately after application or 7 days after application using a sieving method. Roots were washed carefully to avoid damage. Recovering the nematodes immediately after nematode application gave a percentage nematode recovery for the sieving method (Barker, 1985). The sieving method consisted of first collecting the soil in 200 ml water in a 1000 ml beaker. The suspension was stirred vigorously and was allowed to settle for 5 to 10 seconds. The supernatant, leaving a stone/clay suspension behind, was then passed over a coarse (2 mm mesh size) sieve (10 cm diameter), sieving out smaller stones and debris, and was collected in another 1000 ml beaker. The suspension collected was passed over a 38 µm sieve (10 cm diameter) placed over a third 1000 ml beaker. Nematodes were collected on the 38 µm sieve and were rinsed off with little water and collected in a 250 ml beaker. The stone/clay suspension left in the first beaker was again diluted with water and the whole stirring/settling/ decanting/sieving process was repeated four times. The number of EPN per sterilized or non-sterilized soil pot was estimated. Only live nematodes were counted, non-motile nematodes were checked with a dissecting needle for viability. They were counted under stereomicroscope.

$$\text{Percent recovery} \frac{\text{Recovered juveniles}}{\text{Added juveniles}} \text{ X } 100$$

RESULTS

Nematodes were retrieved from the soil by using the sieving method. There was no significant difference seen in all the treatments (Table I). Nematode recovery ranged from 43.22 to 45.42%. This was done to assess the actual recovery from soil EPN were recovered after seven days from sterilized and non-sterilized soil from all the pots with or without tomato roots and tomato. Number and percentage of the EPN that survived in the soil were calculated on the basis of the recovery method (Sieving method). The percentage of the EPN recovered seven days after nematode application ranged from 1.87 to 7.83% (Table II). Based on the recovery method it meant that 4.32 to 16.68% of the total EPN survived. All the entomopathogenic varied significantly in their survival. *S. glaseri* was highest surviving after seven days and it was significantly higher ($p<0.01$) from the other treatments while *H. indica* and *H. bacteriophora* did not differ significantly. Maximum survival of all the EPN was in the sterilized soil. Percentage survival was lowest in the non-sterilized soil. Survival percentage in the sterilized soil ranged from 8.06 to 40.97% while in non-sterilized it ranged from 4.57 to 15.29% (Table III). There was also low number of surviving EPN in non-sterilized treatments of tomato as compared to the sterilized soil treatments. There was not any significant difference between *H. indica* and *H. bacteriophora* survival after seven days.

DISCUSSION

The present study demonstrated that soil sterilization had a direct and strong effect on the efficiency of EPNs. The use of tomato shoots and roots, on the other hand, was of minor significance. Numbers of live *S. asiaticum, S. glaseri, H. indica* and *H. bacteriophora* were severely reduced in non-sterilized soil of with or without tomato plants after 7 days. There was a significant difference in survival rate of *S. asiaticum, S. glaseri, H. indica* and *H. bacteriophora* in sterilized soil. Low survival of EPN in non-sterilized soil might be due to predation of nematode by soil predators. The sterilized soil + tomato roots treatment also showed a significantly reduced number of viable EPN. Tomato roots were only rinsed in tap water before planting in the sterilized soil might had few microorganisms, predators and antagonists which moved from rhizosphere and could account for the low survival rate of EPN in the sterilized soil + tomato roots as compared to sterilized soil treatment. Our results are in conformity with Susurluk (2006) who concluded that efficiency of the two nematodes (*Steinernema feltiae* and *Heterorhabditis bacteriophora*) was greater in sterile than in nonsterile conditions, and was greater in sandy soils than in clay soils. Soil biotic factors can play important role in the survival of EPN. These factors can have different interactions with EPN like antagonism, additivity and synergism depending upon nematode species and relative timing or rate of application (Barbercheck and Kaya, 1990; Thurston *et al.*, 1994; Koppenhofer *et al*., 1997). Different soil medium and temperature influenced the

Table I.- Number and percentage of EPN recovery immediately after application in non-sterilized and sterilized soil.

Treatment	Non sterilized soil	%	Non sterilized soil+tomato	%	Non sterilized soil+tomato roots	%
Non-sterilized soil						
S. glaseri	2271.33a	45.42	2234.33a	44.69	2217a	44.34
S. asiaticum	2115.66a	42.31	2174a	43.47	2198.66a	43.64
H. indica	216a	43.38	2144.33a	42.89	2148a	42.96
H. bacteriophora	2161.33a	43.22	2123.33a	42.47	2186.33a	43.72
Sterilized soil						
S. glaseri	2245.33a	44.90	2263.66a	45.3	2257a	45.14
S. asiaticum	2177.66a	43.55	2196a	43.9	2178.33a	43.57
H. indica	2164a	43.28	2180.66a	43.6	2169.66a	43.39
H. bacteriophora	2149.66a	42.99	2140.33a	42.8	2168.33a	43.37

Table II.- Number and percentage of EPN recovery after seven days in non-sterilized and sterilized soil.

Treatment	Non sterilized soil	%	Sterilized soil+tomato	%	Sterilized soil+ tomato roots	%
Non-sterilized soil						
S. glaseri	375.0a*	7.38a	339.0a	6.78a	315.0a	6.30a
S. asiaticum	253.7b	5.07b	206.7b	4.13b	193.3b	3.86b
H. indica	145.0c	2.90c	99.00c	1.98c	93.67c	1.87c
H. bacteriophora	145.0c	2.90c	115.7c	2.31c	103.7c	2.07c
Sterilized soil						
S. glaseri	898.3a*	17.97a	355.0a	7.10a	344.7a	6.89a
S. asiaticum	314.0b	6.280b	245.0b	4.90b	239.0b	4.78b
H. indica	186.0c	3.720c	129.7c	2.59c	106.0c	2.12c
H. bacteriophora	173.7c	3.473c	132.3c	2.64c	122.3c	2.44c

*Means sharing the same letter within the column do not differ significantly at 1% probability level. Data is mean of five replications.

Table III.- Number and percentage of EPN survival after seven days and non-sterilized and sterilized soil.

Treatment	Non sterilized soil	%	Sterilized soil+tomato	%	Sterilized soil+ tomato roots	%
Non- sterilized soil						
S. glaseri	820.0a*	16.68a	764.5a	15.29a	718.2a	14.36a
S. asiaticum	586.0b	11.72b	477.4b	9.54b	446.6b	8.927b
H. indica	335.0c	7.1c	228.7c	4.57c	216.4c	4.327c
H. bacteriophora	336.4c	6.73 c	268.c	5.36c	240.5c	4.81c
Sterilized soil						
S. glaseri	2048a*	40.97a	809.4a	16.19a	785.8a	15.71a
S. asiaticum	725.3b	14.51b	566.0b	11.32b	552.1b	11.04b
H. indica	429.7c	8.593c	299.5c	5.987 c	244.9c	4.893c
H. bacteriophora	402.9c	8.06c	307.0c	6.177c	283.8c	5.67c

*Means sharing the same letter within the column do not differ significantly at 1% probability level. Data is mean of five replications.

survival of EPN (Salma and Shahina, 2013). Survival of EPN is adversely affected by infection or predation by certain phages, bacteria, protozoans, nematophagous fungi, predacious mites and nematodes etc. (Kaya, 2002). Similarly, Ishibashi and Kondo (1986, 1987) also proved that EPN were affected by natural enemies; therefore, when they placed infective juveniles in sterilized and non-sterilized soils, the infective juveniles survived

longer in sterile soils than in non-sterile soils. Competitive abilities of particular EPN species for control programs can impact the establishment, persistence, and population dynamics of introduced EPNs and induction and expression of stress response genes (Rodriguez *et al.*, 2004; Somvanshi *et al.*, 2008).

Statement of conflict of interest

Authors have declared no conflict of interest.

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