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Soil moisture effects on the activity of three entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) isolated from Meghalaya, India

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Abstract Entomopathogenic nematodes (EPNs) are obligate parasites of insects that are widely distributed in soils throughout the world. They have great potential for use as biological control agents for insect pests. It is known that strains of *Steinernema* and *Heterorhabditis* isolated from different geographical regions exhibit differences in their ecological traits, such as infectivity, establishment, survival, reproduction, etc. A precise knowledge of these factors is therefore an essential pre-requisite for devising successful strategies to use these nematodes in biological control programmes. The present study investigated the effect of soil moisture on the activity (as measured by number of nematodes established in hosts) of three entomopathogenic nematode species (*Heterorhabditis indica* Poinar, Karunakar & David; *Steinernema thermophilum* Ganguly & Singh; *Steinernema glaseri* Steiner), isolated from forest soils in Meghalaya, India, under laboratory conditions. The experiments for EPNs were conducted at 25 ± 2°C (30 ± 2°C for *S. thermophilum*) in a sandy loam soil (85% sand, 12% silt and 3% clay, pH 6.54). Last instar larvae of wax moth, *Galleria mellonella* served as the experimental insect host. The soil moistures tested were 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 25% (w/w). The study revealed that soil moisture has marked influences on establishment of infective juveniles of different nematode species in insect host. While, *S. thermophilum* showed establishment at 4% and above soil moistures, *H. indica* and *S. glaseri* showed establishment at 5% and above soil moistures. The optimum soil moisture for different nematode species were noted as: *H. indica* 8–18%, *S. thermophilum* 6–20%, and *S. glaseri* 8–25%. Further, a minimum of 6% soil moisture was noted to be essential for achieving 100% host mortality for all the three nematode species.

Keywords Entomopathogenic nematodes · Soil moisture · *Heterorhabditis indica* · *Steinernema thermophilum* · *Steinernema glaseri* · *Galleria mellonella* · Biological control · Meghalaya

Introduction

Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* are obligate pathogens of insects (Poinar 1979) that have been found in many diverse climates throughout the world (Hominick et al. 1996). Interest in EPNs as biological control agents of insect pests has increased rapidly because they possess many of the attributes of an ideal biological control agent, including broad host range, high virulence, host seeking capability, and ease of mass production (Gaugler and Kaya 1990; Kaya and Gaugler 1993). Applications have been made against a variety of insect pest species with varying degrees of success (Gaugler 1988). The only non-parasitic stage in the life-cycle of an EPN is the third stage infective juvenile (IJ) which is found in the soil (Boemare 2002). The IJs locate the insect hosts, enter their natural body openings and release symbiotic bacteria carried in their intestines (Poinar 1979). The combination of nematode/bacterial virulence factors kills the host within 24-48 h (Hominick et al. 1996). The infective juveniles feed upon the rapidly multiplying bacteria and debris, and subsequently mature, mate, and produce two or more generations within the insect cadaver before emerging into the environment as IJs

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in search of a new host. The IJs proceed to complete their development into the adults, which mate, lay eggs, and produce thousands progeny using the host cadaver as a nutritional base. Upon exhaustion of nutritional base, IJs emerge from the depleted cadaver into the soil where they may persist for months until they locate and infect new hosts (Gaugler and Kaya 1990).

The success of nematode applications for insect control in soil depends on the IJ's ability to disperse and persist until it can locate a host (Kaya 1990; Lewis 2002; Koppenhöfer and Fuzy 2007). Several studies have demonstrated that IJs of different EPNs differ in their ecological and behavioral traits with regard to their persistence and survival in the soil (Kaya 1990; Koppenhöfer et al. 1995). Among these, the soil moisture is considered to be one of the most important variables that affect nematode performance and survival in the soil (Kaya 1990; Glazer 2002; Grant and Villani 2003). It has been found that the low soil moisture can adversely affect nematode activity and survival, but a considerable number of EPNs could survive some degree of dehydration if the drying process is gradual (Womersley 1987). Rapid decline in soil moisture can occur in the upper soil layers, especially in sandy soils with little water retention capacity, and EPNs are very frequently isolated from such soils (Hara et al. 1991; Strong et al. 1996). It thus emerges from the foregoing account that soil moisture range in which IJs can be active may differ among different EPN species (Koppenhöfer et al. 1995; Grant and Villani 2003). It is primarily due to these reasons that if EPNs are to be used as effective and predictable biocontrol agents, a precise knowledge of their moisture requirements is an essential pre-requisite to maximize their success in the fields.

The aim of this study was to investigate the soil moisture effects on the activity of three EPN strains, viz. *H. indica* Poinar, Karunakar & David, *S. thermophilum* Ganguly & Singh and *S. glaseri* (Steiner), isolated recently from the forest soils in Meghalaya, India. These EPN isolates have shown good potentials for the control of some serious insect pests of vegetable crops in this area (Lalramliana 2007). We report herein our findings related to the effects of different soil moistures on nematode establishment and host mortality in *G. mellonella*, under the laboratory conditions, simulating the ambient moisture conditions that occur in the area of EPNs origin i.e., Meghalaya, India.

Materials and methods

The three EPNs, *H. indica* Poinar, Karunakar & David, *S. thermophilum* Ganguly & Singh and *S. glaseri* (Steiner), used in this study were originally isolated by baiting techniques from forest soils in Meghalaya, India. These nematodes have been characterized in previous studies, using the late instar larvae of the greater wax moth, *G. mellonella* Linnaeus (Lepidoptera: Pyralidae) as a host (Lalramliana 2007). The EPNs were reared in the laboratory on late instar larvae of *Galleria* (0.19–0.25 gm) at 25°C, using the methods of Woodring and Kaya (1988). The IJs that emerged from wax moth larvae cadavers were collected using modified White traps (White 1929), and stored in darkness at 15°C in deionized water. All the experiments were conducted at 25 ± 2°C (30 ± 2°C for *S. thermophilum*) and a sandy loam soil (85% sand, 12% silt and 3% clay, pH 6.54) was used which had been autoclaved at least 2 weeks before use.

Methods of Koppenhöfer et al. (1995) were followed with slight modifications. The experiments were conducted in 24-well tissue culture plates (3 ml arena). The soil moistures tested were 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 25% (w/w). For each treatment, 100 IJs in 25 µl of distilled water were placed on the bottom of each well, which was then filled with pre-wetted soil so as to give the desired soil moisture. A tiny depression was made on top of the soil to accommodate one *Galleria* larva in each well. The plate was covered, sealed with a cellophane tape and turned upside down. During the exposure period, the plates were flipped upside down every 8 h to check the upward movement of *Galleria* larva and to achieve a uniform distribution of IJs. After 72 h exposure, insects were recovered from individual wells and the numbers of dead insects were recorded. They were rinsed in distilled water, dissected in a 0.5% pepsin solution, incubated for 2 h at 37°C to digest the insects' tissues (Mauleon et al. 1993) and the number of IJs established per insect host were recorded. Wells without IJs served as the control to check the mortality, if any, of insect larva. There were 8 replicates of *G. mellonella* per soil moisture for each nematode isolate.

Statistical analysis

Data were subjected to ANOVA. Mean numbers of IJs establishment close to 0 were not included in ANOVA because the data were not normally distributed.

Results and discussion

Entomopathogenic nematodes are lethal parasites of soil-dwelling insects that are being used for the biological control of several agricultural and ornamental crops in many countries (Hominick 2002; Grewal et al. 2005). The success of nematode applications for insect control in soil depends on the IJ's ability to disperse and persist in the soil

until it can locate a host (Kung et al. 1991; Koppenhöfer et al. 1995). Their dispersal and persistence in the soil, in turn, depend upon many abiotic environmental factors, such as soil moisture, temperature, and soil texture (Ames 1990; Kung et al. 1991; Koppenhöfer et al. 1995). Of these, the moisture conditions have been recognized as one of the most important factors in the soil environment affecting survival, virulence and persistence of nematodes (Kaya 1990; Klein 1990, Curran 1993). For instance, nematodes may become dormant at very low soil moisture; on the other hand they may not be able to move freely at very high soil moistures (Grant and Villani 2003). In several studies, variability in efficacy of EPNs has often been found to be associated with soil moistures (Gaugler et al. 1994; Koppenhöfer et al. 1995). Prior to their applications in the fields, it is therefore always advisable to characterize the nematode in terms of its moisture requirements so as to maximize its success in the fields.

In the present study, we characterized three locally isolated EPN strains from Meghalaya, India (*H. indica*, *S. thermophilum* and *S. glaseri*) in terms of effects of different soil moistures on nematode establishment and host mortality, using *G. mellonella* as the experimental insect host. As it is evident from the results, different soil moistures had a marked effect on the establishment of nematodes in insect larvae (Fig. 1a–c; Table 1). The soil moistures had significant effects on the establishment of *H. indica* ($F = 19.951$; $df = 10, 51$; $P < 0.05$), except for 8–18% moisture, where differences were insignificant ($P > 0.05$) (Fig 1a). Similarly, significant effects of soil moistures were also observed on the establishment of *S. thermophilum* ($F = 8.871$; $df = 10, 51$; $P < 0.05$) (Fig. 1b) and *S. glaseri* ($F = 8.849$; $df = 10, 51$; $P < 0.05$) (Fig 1c). However, *S. thermophilum* did not show any significant difference in its establishment from 6 to 20% soil moisture, and *S. glaseri* from 14 to 25% moisture ($P > 0.05$). Though, establishment of *S. thermophillum* in host was recorded at 4% and above soil moistures, establishment of *H. indica* and *S. glaseri* could take place only at 5% and above moistures. Interestingly, *S. glaseri* showed an increase in its establishment as the soil moisture increased, with the highest establishment observed at 25% soil moisture. On the other hand, *H. indica* and *S. thermophilum* revealed an increase in their establishment in host only up to 18% soil moisture, and the same declined drastically thereafter, reaching to a minimum at 25% soil moisture. With regard to the mortality of insect larvae at 72 h exposure time, only *S. thermophilum* was able to cause insect mortality (16.67) at 4% soil moisture. But at 5% moisture, highest mortality of insect larva was observed for *S. thermophilum* (40.50%), followed by *H. indica* (33.33%) and *S. glaseri* (21.88%) (Table 1). The soil moistures 6% and above caused a 100% larval mortality for all the nematodes studied. A careful scrutiny of data regarding IJs establishment reveals that insect mortality does not directly depend upon nematode establishment in host, as there could be other factors such as the virulence of the nematodes' symbiotic bacteria, how fast these bacteria are released and the survival rate of IJs penetrated which may come into interplay in determining insect mortality (Koppenhöfer et al. 2007).

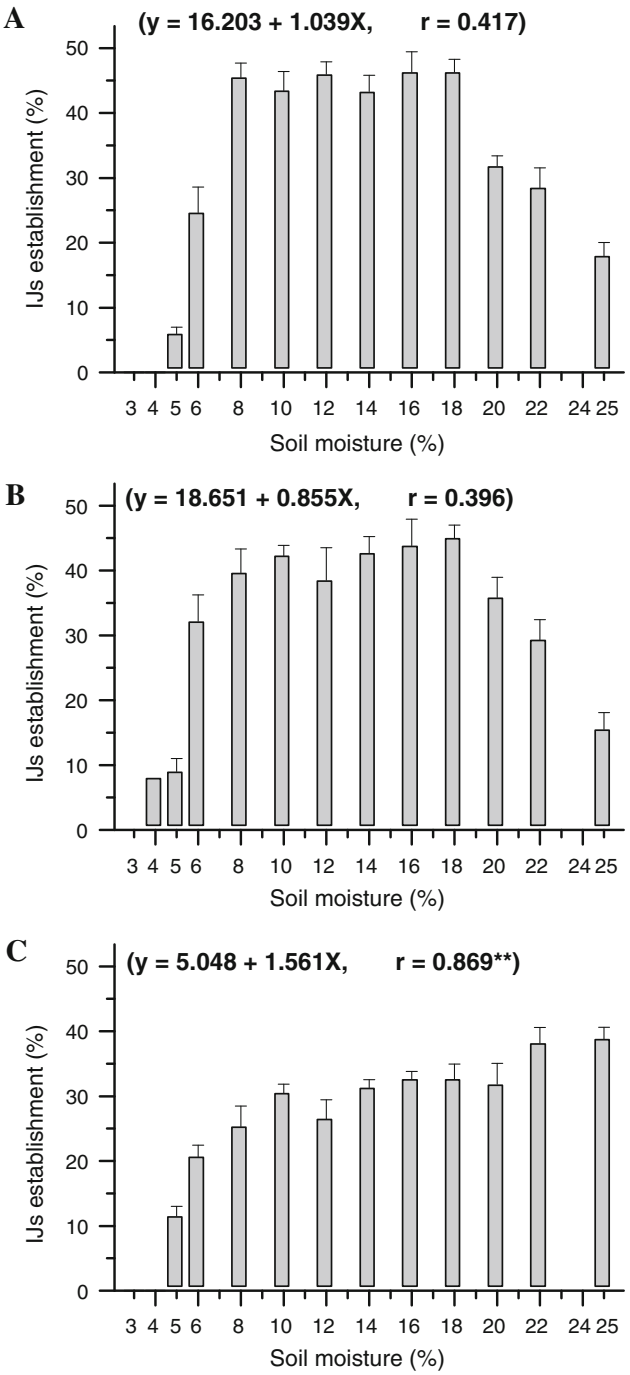

Fig. 1 Soil moisture effects on establishment of entomopathogenic nematodes. **a** *H. indica.* **b** *S. thermophilum.* **c** *S. glaseri*

Table 1 Soil moisture effects on mortality of *Gailleria mellonella* larvae following three days post-exposure to entomopathogenic nematodes

	Mortality rate (%)			
Soil moisture (%) →	3	4	5	6–25
Heterorhabditis indica	–	–	33.33	100
Steinernema thermophilum	–	16.67	40.5	100
Steinernema glaseri	–	–	21.88	100

It is, therefore, evident from this study that of all the EPNs studied, *S. thermophilum* established in the host at rather low soil moisture as compared to *H. indica* and *S. glaseri*. However, all nematodes could establish in host at 5% and above soil moistures, though their establishment rate varied. At 5% soil moisture, insect mortality was minimum for *S. glaseri* and maximum for *S. thermophilum*. However, at 6% and above soil moisture, 100% mortality of insect larvae was recorded for all the nematode species studied. Taken as a whole, our study clearly indicates that establishment of different EPNs vary markedly with different soil moistures. The results of present study are in agreement with Ganguly and Singh (2001) who observed that *S. thermophilum* can infect and multiply well on several pests of crops at a wide range of soil moisture (3–16% w/w) conditions. In a similar manner, our findings on the effect of soil moisture on establishment of *S. glaseri* are also in agreement with Grant and Villani (2003) who reported that, in general, the virulence of *S. glaseri* increases with soil moisture content in sandy loam soil. Also, Koppenhöfer et al. (1995) in their study on the effect of different soil moistures on pathogenicity of *S. carpocapsae* and *S. glaseri* observed that considerable establishment of IJs of *S. carpocapsae* occurred at 4–5% moistures, however, nematodes establishment declined at the highest soil moisture studied (19%). In the present study, *S. glaseri* could not establish at 3% soil moisture and its highest establishment was observed at 19% soil moisture. In another related study, Koppenhöfer et al. (2000) reported that IJs of *S. monticolum* did not infect wax moth larvae at 2 and 3% soil moistures. In this case, the establishment started at 3.5% and reached to its peak at 6% moisture, with very low establishment at 19%. We believe that variability in the establishment of *S. monticolum* could be associated with specific strain of nematode or due to a different host species.

Womersley (1990) reported that infective stages of EPNs are exclusively associated with the microenvironment provided by interstitial spaces of the soil, the water dynamics of which vary depending on moisture availability. It is therefore likely that differences in infective juvenile's size and/or their behavior of nematode species could also be among other important factors that determine their establishment in host at different soil moistures (Koppenhöfer et al. 1995). In the present study, IJs of *S. glaseri* being the largest (length >800 μm) than the rest two nematode species, established more at higher soil moisture levels. Whereas, the smaller sized species, like *H. indica* and *S. thermophilum* could establish more at comparatively lower soil moisture levels and their establishment declined at higher soil moisture levels (i.e., above 18% soil moisture). Koppenhöfer et al. (1995) in their study on influence of soil moisture on entomopathogenic nematode species opined that large-sized IJs of *S. glaseri* need a thicker water film (i.e., higher soil moisture) for optimal movement than the small-sized IJs of other EPN species. This may have contributed to the observed differences in the establishment of different nematode species.

In conclusion, this study suggests that soil moisture greatly influence the establishment of EPNs in insect host. In the present study, the optimum soil moisture for different nematode species were noted as: *H. indica* 8–18%, *S. thermophilum* 6–20% and *S. glaseri* 8–25%. Further, a minimum of 6% soil moisture seems congenial for achieving 100% host mortality.

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