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IMPACT OF SOIL TEXTURE ON THE INFECTIVITY OF DIFFERENT SPECIES OF ENTOMOPATHOGENIC NEMATODES AGAINST GREATER WAX MOTH (*GALLERIA MELLONELLA* L.)

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A B S T R A C T

Entomopathogenic nematodes (EPNs) are effective biological control agents against many soil-inhibiting insect pests. Different soil related factors have influence on the efficacy of these nematodes. The recent study was planned to evaluate the infectivity of four species of EPNs i.e. *Heterorhabditis bacteriophora*, *H. indica*, *Steinernema feltiae* and *S. asiaticum* in different soil textures (loamy sand, sandy loam and clay loam). The *in vitro* assessment of the infectivity was done by exposing last larval instar of *Galleria mellonella* to nematodes. The results revealed that the infectivity was the greatest in sandy loam soil (71.42%) followed by clay loam (54.75%), while it was the lowest in loamy sand (41.63%). Among all species examined, *H. bacteriophora* showed maximum infectivity (69.82%), followed by *H. indica* (52.36%), *S. feltiae* (52.36%) and *S. asiaticum* (49.19%) showing similar trends.

Keywords: *Galleria mellonella*, *Heterorhabditis bacteriophora*, *H. indica*, infectivity, soil texture, *Steinernema asiaticum*, *S. feltiae*.

INTRODUCTION

Entomopathogenic nematodes (EPNs) belonging to families Steinernematidae and Heterorhabditidae control a vast range of economically important insect pest (Shapiro-Ilan *et al*., 2002; Aatif *et al*., 2015). The genus *Heterorhabditis* is mutually associated with *Photorhabdus* bacteria, while *Steinernema* has association with *Xenorhabdus* bacteria (Chen *et al*., 2003). These nematodes are effectively used against different insects in the soil under cryptic habitats. Soil provides the best environment for action and survival of EPNs because it is their natural habitat (Gaugler, 1988). The characteristics of soil can contribute to differences in efficacy of EPNs (Kaya 1990; Shapiro-Ilan *et al*., 2000). The efficacy of EPNs is greatly influenced by soil texture because movement of nematodes, their host finding ability and survival are affected by the soil texture and pore size (Kaya and Gaugler, 1993). Hence, with the increase in clay content, the dispersal and survival of nematodes decreases (Georgis and Poinar 1983; Barbercheck and Kaya 1991; Kung *et al*., 1990a). The dispersal of EPNs whether Horizontal or vertical, as well as their pathogenicity and survival decrease with the increase in the overall proportion of silt and clay (Kung *et al.,* 1990b). There are large pore sizes and low moisture potentials of sandy loam soil and sand, compared to clay and clay loam. These sand properties offer rich aeration environment for nematodes and sufficient space for their free movement (Molyneux and Bedding, 1984). Although some general conclusions have been drawn about the effects of different soils on efficacy of EPNs, various EPNs species may be affected differently by soil types (Molyneux and Bedding 1984; Kung *et al*., 1990a). Moreover, each soil type has a variety of distinctive characters which may have different effects on soil biota (Barbercheck 1992). Consequently, a study on the effects of different soil textures within a particular bio-control program is necessary. Therefore, the objective of current study was to evaluate the effect of different soils on the infectivity of native and exotic species of EPNs.

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MATERIALS AND METHODS

Preparation of soil: Soil samples used in this experiment were collected from different experimental areas of Department of Plant Pathology, University of Agriculture, Faisalabad. The soil was thoroughly mixed and dried by spreading in a thin layer on plastic sheet in sun. After drying the large stones and plant debris were removed by sieving through 3.5 mm pore size sieve. Care was taken that pebbles and crumbs were not removed to avoid the compacting. These collected soil samples were analyzed and characterized for sand, silt, clay and organic matter content from Soil Testing Laboratory, Faisalabad, Pakistan. Only loamy sand, sandy loam and clay loam soils were used. Soil moisture release curves were established using filter paper method for determining soil metric potential (Hamblin, 1981). The soils were prepared at -10kPa soil water potential and filled into plastic vials. During filling, the soil was uniformly compacted by tapping the vials and lightly pressing the substrate surface.

EPNs: *Heterorhabditis bacteriophora, H. indica, Steinernema feltiae* and *S. asiaticum* were used for experimentation.

Galleria mellonella: The larvae of *G. Mellonella* were reared on cereal diet in laboratory (Wiesner, 1993).

EPNs culturing: The culture of all the species of EPNs was maintained on *G. mellonella* larvae. The emerging IJs were harvested from white traps (White, 1927), and were stored at 10°C in shallow clear plastic containers having lids with suspension being no more than 1 cm in depth to ensure sufficient oxygen availability. All stocked nematode cultures were again cultured after every 4 months. For current study fresh culture of EPNs (less than 2 weeks old) was used.

One last instar *G. mellonella* larvae was released into vial on substrate and vials were sealed with a petri dish bottom to retard moisture loss while allowing gas exchange. After 24 h, EPNs species (*S. feltiae, S. asiaticum, H. bacteriophora* and *H. indica*) were applied in water suspension at the rate of 200 IJs on the substrate surface. At 6 days after treatment, larvae were observed and dead larvae were dissected and nematodes established were counted. For one soil texture four EPN species were tested. There were 28 vials per replication for one type of soil and for three types of soils total 84 vials were used. EPNs were applied in separate 1 ml water suspensions by making 1 cm holes with sharp pointed wood. For determination of nematode infectivity each larva was considered a replicate, surviving larvae were counted as 0's while larvae with clear sign of infection but without nematodes found in them were counted as 1's. For determination of nematode virulence (% larval mortality), groups of 7 larvae were considered as replicate. After application vials were arranged in completely randomized design.

Table 1. Characteristics of various soils used in this study.

Soil texture	%Age sand/clay/silt	Organic matter percentage	pH
Clay loam	34/31/35	2.1	7.2
Sandy loam	69/9/22	1.3	7.5
Loamy sand	81/6/13	0.6	6.9

Statistical analysis: The data were subjected to Analysis of variance (ANOVA) using Statistix 8.1 for windows and the mean values were separated by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) for survival of EPNs at various soil textures showed that effect of treatments and species was significant ($p>$ 0.05). Although interaction effect of treatments and species was not significant, the infectivity of *H. bacteriophora* was higher in sandy loam soil (80.94 %) followed by *H. indica* (76.183%) in the same soil while *S. asiaticum* showed least infectivity (33.283 %) in loamy sand (Table 2).

Table 2. Effect of soil texture on the infectivity of four entomopathogenic nematode (EPN) species under laboratory conditions.

	Percent infectivity of EPN species			
Soil texture	*H. bacteriophora*	*H. indica*	*S. feltiae*	*S. asiaticum*
Sandy loam	80.947 A	76.183 AB	66.660 BC	61.900 CD
Clay loam	66.647 BC	47.613 EF	52.350 DE	52.377 DE
Loamy sand	61.873 CD	33.283 G	38.067 FG	33.283 G

Means followed by different letters are significantly different from each other at p<0.05. Data is mean of three replications.

The infectivity of EPNs species was best in Sandy loam soil (71.42%), followed by clay loam (54.75%), while minimum infectivity was recorded in loamy sand (41.63%) (Table 3). *Heterorhabditis bacteriophora* was the only EPN species showing an overall best infectivity (69.82%) whereas there was no significant difference in infectivity among three other species (Table 4).

Table 3 Main effect of soil texture on the infectivity of entomopathogenic nematodes.

Soil texture	Percent infectivity
Sandy loam	71.42 A
Clay loam	54.75 B
Loamy sand	41.63 C

Means followed by different letters are significantly different from each other at $p<0.05$. Data is mean of three replications.

Table 4. Main effect of infectivity of four entomopathogenic nematode (EPN) species.

EPN species	Percent infectivity
H. bacteriophora	69.82 A
H. indica	52.36 B
S. feltiae	52.36 B
S. asiaticum	49.19 B

Means followed by different letters are significantly different from each other at $p<0.05$. Data is mean of three replications.

Previous work by different scientists have exhibited that infectivity is declined in case of *H. bacteriophora* (Choo and Kaya, 1991; Molyneux and Bedding, 1984), *S. carpocapsae* (Kung *et al*., 1990b; Georgis and Poinar, 1983) and *S. glaseri* (Kung *et al*., 1990b; Georgis and Poinar, 1983b) in soils with finer texture. This decreased infectivity may have some relation with reducing pore space among smaller particles of soil which increasingly obstructs the movement of nematode (Wallace, 1958). The nematodes with large size like *S. glaseri* should, therefore, be more powerfully influenced by reducing pore spaces. Certainly, Molyneux and Bedding (1984) noticed a much stronger fall in infectivity of *S. glaseri* than in the smaller *H. bacteriophora*. However, our study along with work of Shapiro *et al*., (2000) and Georgis and Gaugler (1991) revealed changed trends in the effect of soil texture on infectivity of nematodes. In our study, infectivity of both intermediate sized or small sized EPN species (*H. bacteriophora, S. feltiae*, *S. asiaticum* and *H. indica*) was affected by soil texture.

It is also obvious that the fine textured soil used in our study was a clay loam (sand/silt/clay: 27/37/36%), but the finest soil used in Molyneux and Bedding (1984) was an even finer textured clay (sand/silt/clay: 29/19/52%; called clay loam).

Other researches even detected greater infectivity in fine soils than in soils having coarser texture e.g. Shapiro *et al*., (2000) noticed greater infectivity of *H. bacteriophora* and *S. riobrave* in a silt loam soil as compared to two sandy soils and Georgis and Gaugler (1991) in a summary of many field tests, detected greater effectiveness of *H. bacteriophora* against larvae of *P. japonica* in fine soils. However, in that study the effect of soil-texture was confounded but other factors. Georgis and Gaugler (1991) reported that the movement of nematode was more limited in soil with finer texture and therefore, *H. bacteriophora* was more intense in the top layers of soil where the host larvae usually stay. In addition, finer textured soils keep moisture longer and therefore, deliver better soil moisture conditions for activity of nematode in turf grass which is not frequently irrigated.

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