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EFFECT OF SOIL TEXTURE AND MOISTURE ON THE EFFICACY OF ENTOMOPATHOGENIC NEMATODES, HETERORHABDITIS BACTERIOPHORA AND STEINERNEMA CARPOCAPSAE

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ABSTRACT: *This work aims to study the impact of the soil type and soil moisture on the efficacy of the entomopathogenic nematodes , which consider one of the most important factors that affect the ability of entomopathogenic nematodes (EPNs) to penetrate and kill the target pest. The evaluated EPNs are Heterorhabditis bacteriophora (H88) and Steinernema carpocapsae (All strain) and their influence on the last instar larvae of the greater wax moth, Galleria mellonella. In the first experiment, five levels of soil moisture were applied i.e. 25, 50, 75, 100, and 125 % of field capacity in sandy soil. The obtained results indicated that there were significant differences between the tested levels of soil moisture and the ability of EPNs to penetrate and kill the host insect . Generally, the highest percentage of nematode penetration to G. mellonella larvae as well as their mortality was obtained at 100 % soil moisture , followed by 75,125 and 50 %, whereas the application of both nematodes at 25 % soil moisture showed the lowest mortality percentages. Regarding to the effect of three soil types i.e. clay, loamy and sandy, the obtained results indicated that there were significant differences in the ability of EPNs to penetrate and kill the host insect for both tested EPN species, as well as the tested soil types. The highest reduction percentages of the greater wax larvae were recorded in the sandy soil, followed by loamy soil for the H. bacteriophora and S. carpocapsae, followed by sandy soil, while clay soil recorded the least reduction percentages. Regarding to the effect of soil types on the ability of EPNs penetration to wax larvae ,results indicated that the highest reduction percentages were recorded with were generally higher in the loamy soil, followed by clay and sandy.*

Key words: *Entomopathogenic nematodes, penetration, infectivity, soil texture, soil moisture, Heterorhabditis bacteriophora, Steinernema carpocapsae*

INTRODUCTION

Rhabditid nematodes of the Steinernematidae and Heterorhabditidae families are lethal to a broad range of economically important insect pests (Journey and Ostlie, 2000). Entomopathogenic nematodes (EPN) are often applied to sites and ecosystems that routinely receive other inputs that may interact with nematodes, including behavior, physiology, geographic origin and physical factors of the habitat (Doucet *et al*., 1996 ; Ehlers & Gerwien 1993 and Kaya, 1990).

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; Koppenhofer *et al.*,1995), the most critical factors being soil moisture and soil texture (Kaya and Gaugler 1993 ; Gaugler, 2002, Susurluk, 2006 and Mwaniki, *et al*., 2010).

Several reports indicate that the soil environment affects the efficacy of entomopathogenic nematodes in suppressing pests inhabiting soil (Gaugler, 1988 and Choo & Kaya, 1991). Moreover, the application of nematodes has produced discouraging or inconsistent results in field conditions because of a poor understanding of nematode virulence in different soil types. Therefore, knowledge on the behavior of a specific nematode species in the soil environment is essential to developing an effective biological control program (Kaya,1990). Overall, entomopathogenic nematodes are more infective in sandy and sandy loam soils than in clay soils (Georgis & Poinar, 1983a; 1983b; Molyneux & Bedding, 1984; Kung *et al.*, 1990; Barbercheck & Kaya, 1991 and Lezama-Gutiérrez *et al*., 2006)

Entomopathogenic nematodes also require adequate soil moisture levels for their survival and locomotion (Kaya, 1990; Kung *et al.* , 1991; Glazer 2002 ; Grant and Villani 2003), which may vary among nematode species and isolates and among different soil types. Low soil moisture levels can be lethal to these entomopathogens. However, some species develop survival strategies under water stress conditions, by reducing the body surface area exposed to the air and their cell metabolism. This process, known as anhydrobiosis, allows the nematode to become resistant to desiccation, and it can be reversible when the soil becomes wet again. On the other hand, high soil moisture levels can cause oxygen depletion and restrict the mobility of entomopathogenic nematodes (Koppenhöfer *et al.,* 1995 and Patel *et al.,* 1997).

The objective of this study was to evaluate the influence of soil texture and moisture on the penetration and infectivity of entomopathogenic nematodes , *H. bacteriophora* (H88) and *S. carpocapsae* (All Strain) to last instar larvae of the greater wax moth , *G. mellonella*.

MATERIALS AND METHODS

1-Tested Soils:

Three soil samples were collected from Egypt and described in the field according to the FAO Guidelines (2006), where each soil was dried at room temperature, crushed and sieved through a 2 mm-pore sieve. Physical and chemical properties, in addition to some geographical and morphological properties (Tables 1, 2 and 3) were estimated according to Piper (1950) and Jackson (1973).

Table (1): Physical analyses of the tested soil types.

Soil types	Depth cm	Sp	% CaCo3	% Gravels	% Sand	% Silt	% Clay	Texture class
Clay	0-40	20.0	0.70	--	28.25	23.93	47.82	clay
Loam	0-45	18.0	0.70	--	48.24	34.05	17.71	loam
Sand	0-30	31.0	0.25	1.8	94.30	3.20	2.00	sand

Table (2): Chemical properties of the tested soil types.

Soil types	Depth cm	pH 1 : 2.5	EC dS/m In soil extract	Anions meq/L (soil extract)			Cations meq/L (soil extract)			
				HCO_3^-	Cl^-	SO_4^-	Na^+	K^+	Ca^{++}	Mg^{++}
Clay	0-40	8.12	0.99	1.40	6.50	3.10	6.47	0.13	3.00	1.40
Loam	0-45	8.39	0.24	0.80	1.10	0.59	1.12	0.27	0.70	0.40
Sand	0-30	8.54	0.87	1.20	5.30	2.46	6.74	0.22	1.60	0.40

Table (3): Geographical and some morphological properties of the tested soils

Soil Type and Charachtiristics	Clay	Loam	Sand
Date of collection	5/2015	5/2015	6/2015
Area name	Al-Beheira	Al-Beheira	Cairo Naser city
Longitude	30° 55' 02.15"E	30° 57' 49.35"E	30° 55' 02.15"E
Latitude	: 30° 44' 51.74"N	30° 45' 48.80"N	30° 44' 51.74"N
Elevation	7 m a.s.l	4 m a.s.l.	85 m a.s.l
Topography	flat	flat	flat
Physiography	delta	delta	sand deposits
Parent material	Delta deposits	Delta deposits	Delta deposits
Climate	Semi-arid	Semi-arid	Semi-arid
Land use	cultivated	cultivated	Non cultivated
Description	Dark brown (10 YR 4/3 dry) to very dark grayish brown (10YR 3/3 moist); clay; sub angular blocky; very sticky; very plastic; very firm; hard, very weak effervescence with HCL.	Yellow (10 YR 7/6 dry); to brownish yellow (10 YR 6/8 moist) sand; single grains; non sticky; non plastic; loose, weak effervescence with HCL.	Dark brown (10 YR 3/3 dry) to very dark grayish brown (10YR 3/2 moist); loam; massive; sticky; plastic; firm very weak effervescence with HCL.

2- Rearing of insects and nematodes:

The greater wax moth, *Galleria mellonella* L. larvae were reared in the Biological laboratory of Zoology Dept. of Alazhar University , on artificial diet at a constant temperature of 27±1°C. Entomopathogenic nematodes, *Heterorhabditis bacteriophora* (H88) and *Steinernema carpocapsae* (All strain) were used in bioassays. Nematodes were routinely cultured in the larvae of the greater wax moth, *Galleria mellonella* L. (Woodring and Kaya , 1988) , and stored at 15 ^{o}C as aqueous suspensions in tissue culture flasks 250 ml, no longer than one week before use.

3- Influence of soil moisture on entomopathogenic nematodes:

3-1 Penetration assay:

The efficiency of *H. bacteriophora* and *S. carpocapsae* IJs (Infective juveniles) against *G. mellonella* larvae was investigated in sandy soil at five levels of soil moisture (25, 50, 75, 100, and 125 % of soil field capacity). Experiments were conducted in cylindrical plastic cups (4.5 cm long x 2.5 cm diameter) filled with sandy soil. Approximately 50 IJs of tested nematode species were applied to each cup. Three replicates for each treatment were incubated at 25°C. After 24 hours, one *G. mellonella* larvae was added to each cup, cadavers were dissected and the number of penetrating nematode were counted for 3 – 4 days with the aid of dissecting stereomicroscope and counting slide.

3-2 Infectivity assay:

Cylindrical plastic cups (7.5 long x 6 cm diam.) filled with sandy soil at five levels of soil moisture (25, 50, 75, 100, and 125 % of soil field capacity). Approximately 100 IJs of tested nematode species were applied to each cup. Three replicates per each treatment were incubated at 25°C. After 24 hours, ten *G. mellonella* larvae were added to each cup, insect mortality was determined three days after infection.

4- Influence of soil texture on entomopathogenic nematodes:

4-1 Penetration assay:

This assay was conducted in clay, loam and sand soils at two levels of soil moisture (100 and 50 % of soil field capacity). Experiments were conducted in cylindrical plastic cups (4.5 long x 2.5 cm diameter) filled with tested soil. Approximately 50 IJs of tested nematode species were applied to each cup. Three replicates per each treatment were incubated at 25°C. After 24 hours, one *G. mellonella* larvae was added to each cup, cadavers were dissected and the number of penetrating nematode were counted after 3 – 4 more days.

4-2 Infectivity assay:

Cylindrical plastic cups (7.5 x 6 cm diam.) filled with clay, loam and sand soils three levels of soil moisture (50, 75, and 100 % of soil field capacity). Approximately 100 IJs of tested nematode species were applied to each cup. Three replicates per each treatment were incubated at 25°C. After 24 hours, ten *G. mellonella* larvae were added to each cup, insect mortality was determined three days after infection.

5- Statistical analyses:

The obtained data was statistically analyzed using analysis of variance (ANOVA) at 5 % probability. The measurements were separated using Duncan's Multiple Range Test (DMRT) through CoStat software program (Version 6.400). Copyright © 1998-2008 Cohort Software. 798 Lighthouse Ave. PMB 320, Monterey, CA, 93940, USA.

RESULTS AND DISCUSSION

1): Effect of soil moisture on entomopathogenic nematodes:

Establishment bioassay:

The effects of different soil moistures on *H. bacteriophora* and *S. carpocapsae* nematodes establishment and host mortality, were assayed using *G. mellonella* as the experimental insect host in sand soil. The results showed that, different soil moistures had a marked effect on the penetration of nematodes in insect larvae (Table 4). The soil moistures(of soil field capacity) had significant effects on the establishment of tested nematode species ($P < 0.05$), the highest percentage of *H. bacteriophora* penetration to *G. mellonella* larvae was obtained with 100 % soil moisture (24.66 %) ,followed by 75,125 and 50 % (20.66, 16.66 and 15.33 % ,respectively) at 25 % soil moisture showed the lowest penetration percentage (4.00 %). However, *S. carpocapsae* revealed an increase in their establishment in host only up to 75 % soil moisture, and the same declined thereafter (40.66 %), followed by 100,125 and 50 % (26.00,24.00 and 22.66 %,respectively),the lowest reduction percentage result was recorded at 25 % soil moisture (6.00 %).

Table (4): Soil moisture effects on establishment (Average) of EPNs to *G. mellonella* larvae.

Moisture% (of field capacity)	*H. bacteriophora*			*S. carpocapsae*		
125	8.33	bc	(A)	12.00	b	(A)
100	12.33	a	(A)	13.00	b	(A)
75	10.33	ab	(B)	20.33	a	(A)
50	7.66	c	(B)	11.33	b	(A)
25	2.00	d	(A)	3.00	c	(A)

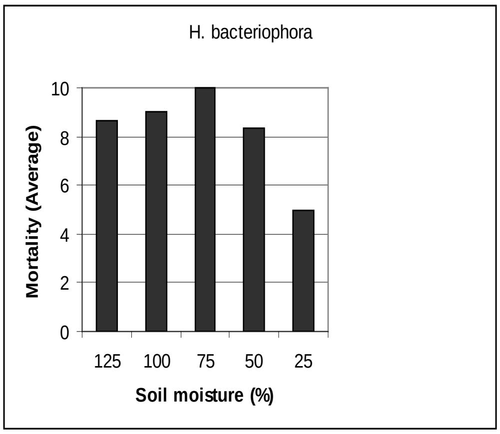
Means followed by the same letter within a column and or (row) are not significantly different by (P=0.05) according to Duncan's multiple –range test .

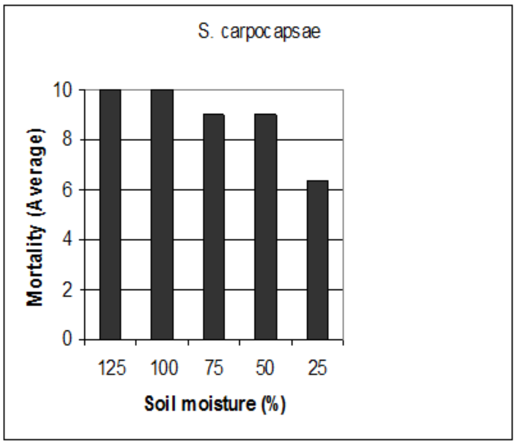
The dispersal and persistence of entomopathogenic nematodes in the soil, in turn, depend upon many abiotic environmental factors, such as soil moisture, temperature, and soil texture (Ames, 1990; Kung *et al*., 1991; Koppenhöfer *et al*., 1995). Of these, the moisture conditions have been recognized 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). 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.

Efficacy bioassay:

Regarding to the mortality of *G. mellonella* larvae at 72 h exposure time, *H. bacteriophora* was more effective in the treatments at 75% of field capacity, causing 100% mortality. Followed by 100,125 and 50 % (90.00, 86.66 and 83.33 %, respectively), the low mortality was found at 25 % soil moisture (50.00 %). On the other hand, S. *carpocapsae* induced greater host mortality when soil moisture was at 100 and 125 % of field capacity, causing 100 % mortality, with low efficiency in the other treatments (Fig. 1). Generally, at all tested soil moisture levels, the highest mortality of insect larva was observed for S. *carpocapsae*, except for 75 % soil moisture, where H. *bacteriophora* was more efficient than S. *carpocapsae*, with 100 % and 90 % mortality, respectively.

Several studies indicate that soil moisture influence infectivity of entomopathogenic nematodes, demonstrating, in general, a decrease in infectivity as soil moisture decreases (El- Sadawy, 2001; Grant & Villani , 2003 and Alekseev *et al.*, 2006). Similar studies conducted with *H*. *bacteriophora* DI and *S*. *glaseri* KG (Molyneux & Bedding, 1984) and with *S*. *glaseri* NC and *S*. *carpocapsae* ALL (Koppenhöfer *et al.*, 1995) also resulted in lower nematode infectivity in extreme, low and high (near the saturation point) soil moistures. The low infectivity of both nematodes at the lowest moisture content is probably related to the lack of water between the pores, which is also limiting for nematode locomotion. Another possibility for the low infectivity at the lowest moisture content is that these nematodes have developed physiological and behavioral adaptations that allow them to reduce their metabolism, entering a state of anhydrobiosis (Grewal, 2000 and Glazer, 2002). Studies have been demonstrated that some species of the *Steinernema* have the ability to enter a state of anhydrobiosis when exposed to low moisture contents (Koppenhöfer *et al.*, 1995), but nothing is clear on this issue regarding to *Heterorhabditis* sp.





(Fig. 1): Effect of soil moisture on the mortality of *G. mellonella* larvae by entomopathogenic nematodes

2): Effect of soil texture on entomopathogenic nematodes:

Establishment bioassay:

In this study, the entomopathogenic nematodes *H. bacteriophora* and *S. carpocapsae* were able to penetrate the last instar larvae of *G. mellonella* in the tested soil types. The analysis of variance of the obtained data showed highly significant differences (P < 0.05) in the reduction percentages of wax larvae for soil type, soil moisture and nematode species. At the tested three soil textures, the highest penetration percentage of both nematode species for insect larva was observed at 100 % soil moisture, except in clay soil, where S. *carpocapsae* at 50 % soil moisture was more efficient than that at 100 % moisture (Table 5). Also, at 50 % soil moisture in all tested soil textures, *S. carpocapsae* showed an increase in its establishment (34.66, 40.66 and 22.66 % , respectively). On the other hand, the maximum penetration percentage under 100 % soil moisture was occurred by *H. bacteriophora* (32.66 and 62.66 %) in clay and loam soil ,respectively , while in sandy soil, *S. carpocapsae* was more efficient (26 %) than *H. bacteriophora* (24.66 %). Overall, EPNs in loam soil, showed an increase in its establishment as compared with clay and sandy soils.

Efficacy bioassay:

In this part of the work, there was a significant difference between the soil textures and nematode species (P < 0.05) (Fig., 2) in larvae reduction. Comparing the effect of the tested soil textures, results indicated that soil type had a significant effect on the ability of the nematodes to kill *G. mellonella* larvae. Comparing the IJ species, *S. carpocapsae* caused higher rates of mortality in all different soil textures tested than *H. bacteriophora* at the three soil moistures , except in sand soil, where *H. bacteriophora* at 75 % soil moisture was greater efficient (100 %) than S. *carpocapsae* (90 %).

Finally, mortality caused by both IJs of EPN species was the greatest in the loam soil followed by the sand and clay soi.

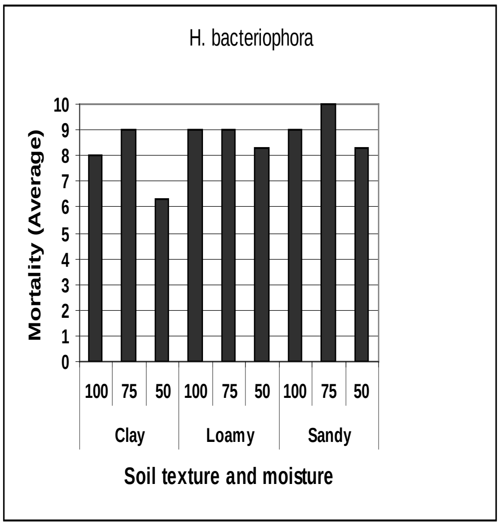
The obtained results confirmed this hypothesis and corroborate the reports of Georgis & Poinar (1983 a& b), Gaugler (1988), Choo & Kaya (1991), Shapiro *et al.*, 2000 and Lezama-Gutiérrez *et al*. (2006). The number and size of soil pores also could play a diminishing role on the reduction of cumulative percent mortality because the space availability and water films are diminished in the clay type, affecting the movement of the nematodes in the soil

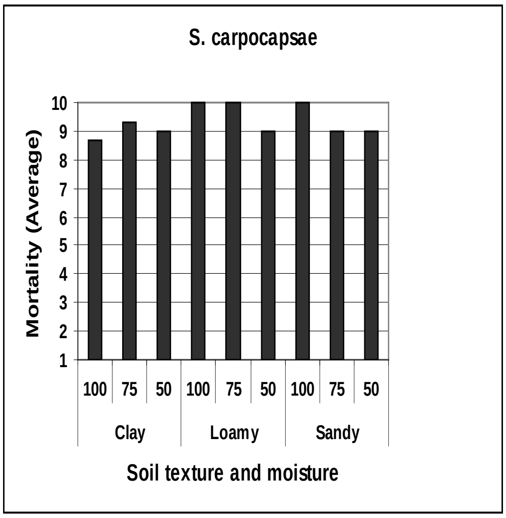
(Barbercheck & Kaya 1991and Gouge *et al.* 2000). Eben & Barbercheck (1997) observed that *S. carpocapsae* was more efficient in sandy loam soil than sandy one. Some species are ambushers (e.g., *S. carpocapsae*) that tend to remain near the soil surface and attach to and infect mobile hosts at the soil-litter interface (Campbell & Gaugler 1993). Other species (i.e., *Heterorhabditis bacteriophora*) are cruisers that have an active searching strategy and are more effective against less mobile insects in the soil (Lewis *et al.* 1992 and Campbell & Gaugler, 1997). Depending on the nematode species, length of exposure, and concentration, various soil nutrients may enhance or hinder entomopathogenic nematode infectivity and virulence (Shapiro *et al.* 1996, Bednarek and Gaugler 1997, Jaworska *et al.*, 1999). For example, Jaworska *et al.* (1999) reported an increase in virulence of *S. carpocapsae* and *H. bacteriophora* upon treatment with Mg or Mn ions, Moreover , Ishibashi and Kondo (1986) found that the addition of organic matter to soil (i.e., compost) increased the efficacy of the entomopathogenic nematodes.

Table (5): Effect of soil texture on the penetration of EPNs to *G. mellonella* larvae.

Soil types & Moisture %		*H. bacteriophora*			*S. carpocapsae*		
Clay	100	16.33	b	(A)	12.33	d	(B)
	50	10.00	c	(B)	17.33	c	(A)
Loamy	100	31.33	a	(A)	27.00	a	(A)
	50	12.33	c	(B)	20.33	b	(A)
Sandy	100	12.33	c	(A)	13.00	d	(A)
	50	7.66	d	(B)	11.33	d	(A)

Means followed by the same litters within a column and or (row) are not significantly different by (P = 0.05) according to Duncan's multiple –range test .





(Fig. 2): Effect of soil type on the mortality of *G. mellonella* larvae by entomopathogenic nematodes

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تأثير قوام ورطوبة التربة على كفاءة النيماتودا الممرضة للحشرات

*Steinernema carpocapsae*و*Heterorhabditis bacteriophora*

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الملخص العربى

يهدف هذا البحث الى تقييم تاثير كلا من نوع التربة ورطوبة التربة والتى تعتبر من اهم العوامل التى تؤثر فى قدرة النيماتودا المتطفلة على الحشرات على اختراق وقتل العائل الحشرى . تم استخدام نوعين من هذة النيماتودا وهما النوع (H88) *Heterorhabditis bacteriophora* والنوع (All strain) *Steinernema carpocapsae* وتأثيرهم على العمر الاخير من دودة الشمع الكبيرة *Galleria mellonella*. فى التجربة الاولى ، تم اختبار خمسة مستويات رطوبة وهى ١٠٠،١٢٥،٢٥،٥٠،٧٥ ٪ من السعة الحقلية للتربة الرملية ، ودلت النتائج على وجود فروق معنوية بين المستويات المختبرة من رطوبة التربة فى قدرة يرقات النيماتودا على اختراق وقتل العائل الحشرى لكلا من نوعى النيماتودا المختبرة. وأشارت النتائج ان اعلى النسب المئوية للاختراق وموت العائل الحشرى مع معاملة رطوبة تربة ١٠٠ ٪ تلاها ٧٥ ،١٢٥، ٥٠ ٪ وكانت اقل النتائج تحت رطوبة تربة ٢٥ ٪. وفى تجربة نوع التربة تم اختبار ثلاثة انواع من التربة وهى الطينية والطميية والرملية ودلت النتائج على وجود فروق معنوية فى قدرة النيماتودا المتطفلة على الحشرات على اختراق وقتل العائل الحشرى لكلا من نوعى النيماتودا المختبرة وكذلك انواع التربة المختبرة ، وكانت اعلى النتائج فى موت اليرقات قد سجلت فى الارض الرمليه يليها الطميية بالنسبة للنوع *H. bacteriophora* أما *S. carpocapsae* فكانت أعلى النتائج فى الارض الطميية يليها الرملية وكانت اقل النتائج لكلا النوعين قد سجلت فى الارض الطينية ، وبالنسبة لقدرة نيماتودا الحشرات على الاختراق، كانت اعلى النتائج فى التربة الطميية يليها الطينية ثم الرملية .