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The effect of earthworms on vertical dispersal of entomopathogenic nematode, *Steinernema feltiae* (Rhabditida: Steinernematidae)

Böcek paraziti nematod, Steinernema feltiae *(Rhabditida: Steinernematidae)'nin dikey dağılımı üzerine toprak solucanlarının etkisi*

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ABSTRACT

Vertical dispersal of the entomopathogenic nematode, *Steinernema feltiae* was tested with earthworms in soil columns. One of them is grey worm, *Aporrectodea caliginosa* and the other is reddish-brown worm, *Lumbricus rubellus*. Nematode vertical dispersal was estimated after a 3-week period. S. feltiae reared in the last instar of the greater wax moth, *Galleria mellonella*. Vertical dispersal of *S. feltiae* was increased in the presence of earthworms. For this motion, *L. rubellus* is more effective than A.caliginosa.

Keywords: *Aporrectodea caliginosa, Lumbricus rubellus, Steinernema feltiae,* Nematode dispersal

ÖZET

Böcek paraziti nematod, *Steinernema feltiae*'nin dikey dağılımı toprak kolonlarda, toprak solucanları ile test edilmiştir. Bu toprak solucanlarından birisi gri toprak solucanı *Aporrectodea caliginosa*, diğeri ise kırmızımsı-kahve renkli toprak solucanı *Lumbricus rubellus*'dur. Nematodun dikey dağılımı 3 haftalık bir period sonrası değerlendirilmiştir. *S. feltiae* büyük mum güvesi, *Galleria mellonella*'nın son evresinde yetiştirilmiştir. *S. feltiae*'nin dikey dağılımı toprak solucanlarının mevcudiyetinde artış göstermiştir. Bu hareket için, *L. rubellus*, A.caliginosa'dan daha etkili olmuştur.

Anahtar sözcükler: *Aporrectodea caliginosa, Lumbricus rubellus, Steinernema feltiae,* Nematod dağılımı

1. Introduction

The term entomopathogenic nematode is used in the literature to refer almost exclusively to species of *Steinernema* and *Heterorhabditis*. The dispersal and behaviour of these nematodes were reviewed by some researchers and more recently with emphasis on population biology (1, 2).

The nematode, *Steinernema feltiae* Filipjev, 1934 is an entomopathogenic nematode. It has good potential as a biological control agent of insects, especially in the soil environment (3).

Population of *Steinernema* and *Heterorhabditis* species collected around the world often were found differ in unforeseen ways from these first discovered, in their thermal optima, reproductive behaviour, host finding behaviour and survival strategies (4).

Nematodes spread through the soil very slowly under their own power. The overall distance travelled by a nematode probably does not exceed a meter per season. Nematodes faster in the soil when the pores are lined with a thin film of water than when the soil is water logged (5). In addition to their own movement, however,

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nematodes can be easily spread by anything that moves and can carry particles of soil (6, 7, 8, 9).

Earthworms improve soil by modification of soil structure, by aeration and drainage, and by breaking down and distributing organic matter. The ability of earthworms to change soil structure and move large amounts of soil indicates that nematode movement may increase in the presence of earthworms. Furthermore, entomopathogenic nematodes have been found to have no deleterious effects on earthworms. The present study was conducted to determine the effects of earthworms on vertical dispersal of entomopathogenic nematodes.

2. Materials and Methods

Vertical dispersal of nematodes in the presence or absence of earthworms was tested in soil columns over a 3-week period. *Steinernema feltiae* Filipjev, 1934 was obtained from Buglogical Control Systems, Tucson and reared in the last instar of the greater wax moth, *Galleria mellonella* L. (Figure 1). Earthworms, grey worm, *Aporrectodea caliginosa* Savigny, 1826 was obtained from the field soil of Osmaniye-Turkey, (37° 03,0' N; 36° 13,8'E) (Figure 2A) and reddish-brown worm, *Lumbricus rubellus* Hoffmeister, 1843 was obtained from the forest soil of Karabük-Turkey, (41° 12,0' N; 32° 37,2' E) (Figure 2B). For identification of earthworms, Mısırlıoğlu (10) were used. Both species were reared on partly decomposed sheep manure. Soil columns were constructed by joining six 4 cm long sections of 5 cm diameter polyvinyl chloride (PVC) pipe and filling them with 39 % sand, 19 % silt and 42 % clay to a bulk density of approximately 1.4 g /cm3. Soil moisture was standardized to field capacity in each column. The soil columns received one of three treatments: the addition of either two *A. caliginosa* or *L. rubellus*, or no earthworms. All treatments were applied to soil columns testing both upward and downward dispersal with three replications for each treatment direction combination. Earthworms were allowed to tunnel for 10 days before nematodes were added to each column. Soil columns were incubated at 20-23 °C and approximately 95 % relative humidity.

Downward movement: Approximately 5.000 nematodes were applied in 100 µl water to the surface of each soil column. *Steinernema feltiae* tends to move upward; therefore, to encourage downward migration of nematodes, two wax moth larvae (hereafter referred to as "forage larvae") were enclosed in cloth mesh and placed on the bottom end of each column. After two weeks the soil columns were dismantled. The wax moth larvae in each column were checked for mortality, and the soil from each 4 cm long section of PVC pipe was removed

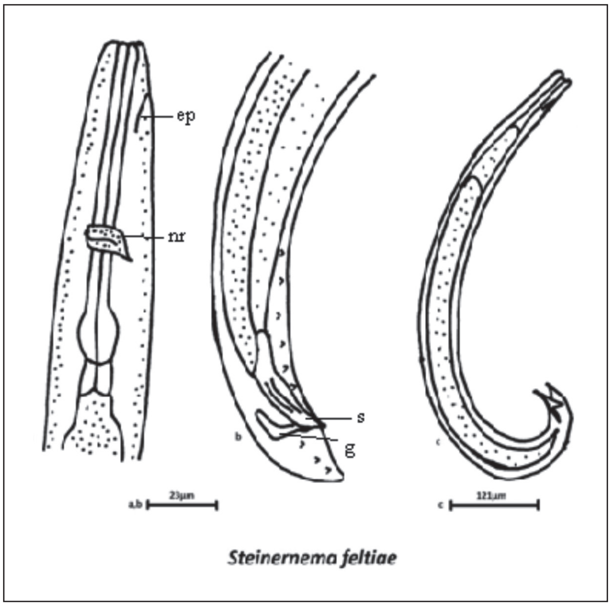

Figure 1: Steinernema feltiae. a. Anterior region - excretory pore (ep), nerve ring (nr) b. Posterior region –spicules (s), gubernaculum (g) c. Entire body.

Figure 2: A) Aporrectodea caliginosa, **B)** Lımbricus rubellus.

and placed in a plastic Petri dish (9 cm diameter) with 12 fresh *G. mellonella* larvae (hereafter referred to as "test larvae"). The Petri dishes were placed at 25 °C and approximately 70 % relative humidity for 3 days, after which mortality of test larvae was recorded. Differences in mortality of test larvae were used to indicate differences in numbers of nematodes.

Upward movement: Approximately 5.000 nematodes in 100 µl water were applied to soil on the bottom end of the column and allowed to migrate for 2 weeks, after which the columns were dismantled and soil from each section was exposed to test larvae as described previously. Because *S. feltiae* tend to disperse upward, forage larvae were not required in the columns testing upward dispersal of nematodes.

Statistical differences in nematode dispersal among treatments were determined by doing an analysis of variance on the numbers of dead larvae infected from soil in the bottom three sections of columns testing downward dispersal, and in the top three sections of columns testing upward dispersal. The numbers of dead larvae, caused by nematodes that dispersed to the most distant three sections of the columns, were than compared among the treatments using contrast.

3. Results

Steinernema feltiae dispersed significantly farther downward when either *A. caliginosa* or *L. rubellus* were present than when no earthworms were present. The numbers of test larvae killed when exposed to soil from column sections from the lower half of the columns were significantly greater when earthworms were present than when they were not (Table 1). Indicating that the nematodes dispersed farther downward with earthworms present than earthworms absent. Forage larvae were always infected with nematodes after 2 weeks when earthworms were present but never infected when earthworms were absent.

Nematodes moved further upward when earthworms were present than when no earthworms were present. The number of test larvae killed by nematodes when exposed to soil from column sections in the upper half of the columns was significantly greater when *L. rubellus* was present than when either *A. caliginosa* was present or when no earthworms were present (Table 1). Nematodes were detected in the opposite most section of the columns when earthworms were present but when earthworms were not present. This method is similar to Shapiro's method (12).

4. Discussion

Entomopathogenic nematodes in the genus *Steinernema* have many attributes as biological control agents, including wide host ranges, safety to nontarget organisms, and vectoring a bacterium, *Xenorhabdus* sp., which rapidly kills insect hosts (3). Despite their great potential as biological control agents, results of nematode applications have been inconsistent. Biotic and abiotic factors that influence the efficacy of nematode applications must be investigated (11).

Interactions with soil invertebrates may increase nematode dispersal. A phoretic relationship was observed between nematophagous mites and *Steinernema carpocapsae*. Shapiro et al. (12) reported increased dispersal of *S. carpocapsae* in the presence of earthworms. Earthworms are not adversely affected by entomopathogenic nematodes (13). Therefore, it may be possible to exploit the association between *S. carpocapsae*

Table 1: Number of dead assay larvae in soil columns.

	Downward movement			Upward movement		
Depth (cm)	**0**	**Ac**	**Lr**	**0**	**Ac**	**Lr**
0-5	12.7	12.0	8.9	0	2.3	4.4
5-9	14.5	14.7	13.1	1.5	6.8	10.2
9-12	15.1	14.9	14.6	8.3	10.9	13.6
12-15	7.4	15.1	14.7	12.5	15.8	16.6
15-18	3.3	13.6	10.5	12.8	12.2	18.1
18-24	0.3	2.2	1.3	18.9	19.3	19.1
a.m.	3.6 a	10.3 b	8.8 b	3.2 a	6.6 a	9.4 b

0: no earthworms, Ac: Aporrectodea caliginosa, Lr: Lumbricus rubellus

a.m. average motality of assay larvae in the bottom three sections in downward movement and top three sections in upward movement experiment. Means followed by the same latter are not significantly different. Numbers represent means of three replications.

and earthworms in the soil to increase the efficacy of the nematode as a biological control agent.

More of the nematodes associated with earthworms were dispersed upward compared with those associated with each other. The differences in nematode distributions in the soil columns may be caused by differences in earthworm behaviour.

Channels produced by *L. rubellus* are vertically oriented, whereas *A. caliginosa* generally produces horizontally oriented burrows (14). It may be expected that *A. caliginosa* would be most suitable in enhancing nematode dispersal horizontally if earthworms were incorporated into biological control applications to enhance nematode dispersal to species of earthworm used would need to be considered.

Increased dispersal of *S. feltiae* may have resulted from direct or indirect effects of earthworms. Dispersal may have been increased directly by transportation of nematodes on the surface of the earthworms, or in the digestive tract of the earthworm. Two *L. rubellus* and four *A. caliginosa* were examined after exposure to S. feltiae in soil columns. Live nematodes were found in dissected earthworms, in earthworm casts, and in the debris washed from the surface of the earthworms. Mortality was observed in *G. mellonella* larvae exposed to nematodes isolated from dissected earthworms, but nematodes were not observed to reproduce and emerge. Nematodes found in the casts and debris of earthworms were able to infect and reproduce in larvae of the *G. mellonella* (4). Therefore, these observations suggest that earthworms may serve as phoretic hosts of *S. feltiae*. Phoresy of entomopathogenic nematodes has been observed on nematophagous mites. Phoresy may have adaptive value for entomopathogenic nematodes by allowing them to disperse further than they could on their own energy (15).

Increased dispersal of *S. feltiae* may have resulted from indirect effects of earthworms. Nematode dispersal is affected by soil moisture, structure and texture. For example, the dispersal of *S. feltiae* is greater in soils with coarser textures than in soils with high clay contents. Soil texture, moisture and structure are altered by earthworm burrowing. Therefore, nematode dispersal may be different in earthworm burrows than in soil void of earthworms.

References

1. N. Ishibashi, E. Kondo. Behavior of infective juveniles Pp. 139-150 in R. Gaugler and H. K. Kaya eds. Entomopathogenic Nematodes in Biological Control. Boca Raton, FL: CRC Press (1990).
2. M.J. Downes, C.T. Griffin. Dispersal behaviour and transmission strategies of the entomopathogenic nematodes Heterorhabditidae and Steinernematidae. Biocontrol 6: 347-356 (1991).
3. H.K. Kaya, R. Gaugler. Entomopathogenic nematodes. Annual Review of Entomology 38: 181-206 (1993).
4. R.F. Denno, D.S. Gruner, I. Kaplan. Potential for entomopathogenic nematodes in biological control: a meta-analytical synthesis and insights from trophic caucade theory. Journal of Nematology 40: 61-72 (2008).
5. N.D. Epsky, D.E. Walter, J.L. Capinera. Potential role of nematophagous microarthropods as biotic mortality factors of entomogenous nematodes (Rhabditida: Steinernematidae, Heterorhabditidae). Journal of Economic Entomology 81: 821-825 (1988).
6. G. Georgis, R. Gaugler. Predictability in biological control using entomopathogenic nematodes. Journal of Economic Entomology 84: 713-720 (1991).
7. H.K. Kaya. Soil ecology. Pp. 93-115 in R. Gaugler and H.K. Kaya, eds. Entomopathogenic nematodes in biological control. Boca Raton, FL: CRC Press (1990).
8. Q.L. Wang, J.Y. Cui, Z.X. Li, C.Q. Liu. Effect of soil moisture on the motion of *Steinernema glaseri* (Nematoda: Steinernematidae). Chinese Journal of Biological Control. 11: 150-152 (1995).
9. K.E. Lee. Earthworms: Their ecology and relationship with soils and land use. Sydney, Australia: Academic Press (1985).
10. M. Mısırlıoğlu. Toprak solucanları biyolojileri, ekolojileri ve Türkiye türleri. Nobel Yayın Dağıtım 104s. (2011).
11. A.S. Molyneux, R.A. Bedding. Influence of soil texture and moisture on the infectivity of *Heterorhabditis* sp. D1 and *Steinernema glaseri* for larvae of the sheep blowfly *Lucilia cuprina*. Nematologica 30: 358-365 (1984).
12. D.I. Shapiro, E.C. Berry, L.C. Lewis. Interaction between nematodes and earthworms: Enhanced dispersal of *Steinernema carpocapsae*. Journal of Nematology 25: 189-192 (1993).
13. P. Timper, H.K. Kaya, R. Gaugler. Dispersal of the entomogenous nematode *Steinernema feltiae* (Rhabditida: Steinernematidae) by infected adult insects. Environmental Entomology 17: 546-550 (1988).
14. T.J. Walker. Mole crickets in Florida. Florida Agricultural Experimental Bulletin, No. 845. 54 pp. (1984).
15. R.J. Zimmerman, R.J, W.S. Cranshaw. Short-term movement of *Neoaplectana* spp. (Rhabditida: Steinernematidae) and *Heterorhabditis* "HP 88 strain (Rhabditida: Heterorhabditidae) through turfgrass thatch. Journal of Economic Entomology 84: 875-878 (1991).