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Natural Occurrence of Entomopathogenic Nematodes (Rhabditida: Steinernematidae And Heterorhabditidae) in Syrian Soils

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Abstract: Two-hundred-eleven soil samples from five ecologically diverse habitats in 14 various locations in Syria were assessed to detect natural populations of entomopathogenic nematodes by using *Galleria* baiting technique. Five soil samples (2.37%) in three sites contained entomopathogenic nematodes. All samples were positive for *Heterorhabditis*. These five heterorhabditids were identified as *Heterorhabditis bacteriophora*. The soil texture of the entomopathogenic nematode positive soils were loamy sand, sandy loam, and silt with weakly basic (pH 7.9) to medium basic (pH 8.7) and little organic content (0.56%) to medium (2.20%). Electrical conductivity for the nematode positive soils varied from 0.30 (non-saline) to 4.65 mS/cm (moderately saline).

KEY WORDS: Entomopathogenic nematodes, *Heterorhabditis*, natural occurrence, Syria

INTRODUCTION

Entomopathogenic nematodes (EPNs) have been used for insect control since the 1930s[33]. These nematodes have been applied successfully against soil-inhabiting insects[13,15,22] as well as above-ground insects in cryptic habitats[21,6]. They have many ideal properties to be biological control agent; wide host spectrum, killing the host within 48 h, easy commercial production in vivo or in vitro, active host seeking, long-term efficacy, easy application, compatibility with most chemicals, and environmental safety. EPNs are also variable in pathogenisity, host searching behavior, and survivability, making them suitable in biological control programs.

After Bedding and Akhurst[5] developed the *Galleria*-bait method, a number of surveys have been carried out around the world, including Spain[16], Egypt[32], Sri Lanka[3], Norway[19], Canada[26,7], Tennessee[31], Scotland[9], Germany[12], Hawaii[18], Israel[14], Ireland[8,17], Great Britain[20], Finland[35], Hungary[27], Australia[2], Sweden[10], North Carolina[1], Florida[4], Puerto Rica[29], Czechoslovakia[25] and Portugal[30]. The habitat and soil characteristic where these nematodes were isolated vary: pastures, forests, field crops, orchards, beaches, and in calcareous, sandy loam, loam, humus and sandy, and humus and organo-mineral soils[1,18].

There has been no information on the natural distribution of EPNs in Syria. Our objective was to conduct a survey on these nematodes to document their occurrence in different habitats and locations, and develop local isolates for possible use in biological control programs.

MATERIALS AND METHODS

A total of 211 soil samples from five different habitats at 14 sites in Syria were collected at various times in 2002 and 2003 (Table 1 and Figure 1). The habitats were forest, pasture, field crops (wheat, faba bean, cotton), vegetable, and fruit orchards/vineyards. At the each site, six to eight random samples were taken in a 100 m^2 area at one to three m intervals to a depth of 10-15 cm, using a hand shovel/shovel or soil corer with a volume of 1000-1500 ml. These samples were put in a plastic bag (43 cm x 46 cm x 20 mm) and mixed well. Approximately 750 ml of soil was taken and placed in a 1000-ml plastic container with a cover containing nine 1mm-diameter holes. Associated vegetation, date, and site location were recorded on container. The samples were kept in a cooler at 12-15 ^oC during transportation.

Late instar larvae of Mediterranean flour moth, *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralididae) used in this study were obtained from Department of

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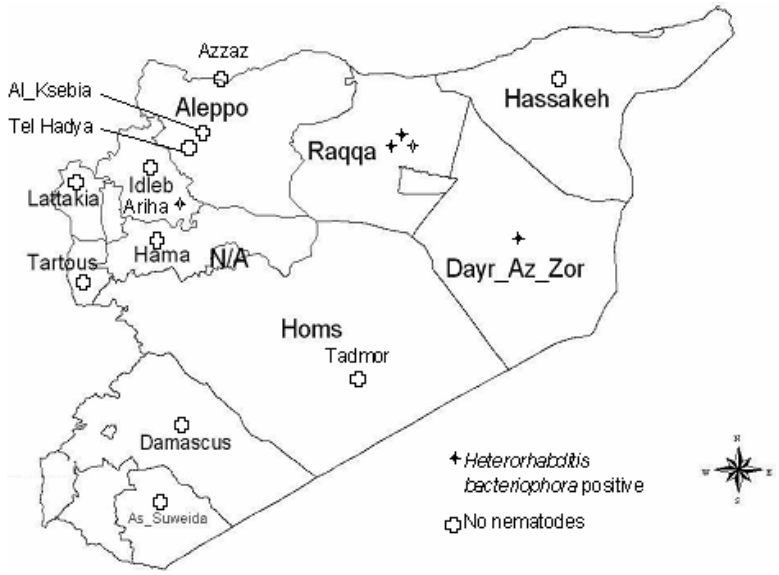

Fig. 1: Locations in Syria where soil samples were taken and distribution of naturally occurring *Heterorhabditis bacteriophora*

Plant Protection of Aleppo University. Bioassays of the soil samples were performed using the methods described by Bedding and Akhurst[5] within 24 h after sampling. Five to seven larvae buried in the each sample and the containers were held at 25 ± 2 ºC. Checks for mortality were made six to eight d after larvae were buried by removing the larvae from soil. Dead larvae from each soil sample, after rinsing in sterile distilled water, were individually put on a modified White trap where infective juveniles (IJs) were collected[36]. The trap consisted of a folded 11 cm filter paper (three mm in depth after folding) in a Petri dish (100 x 15 mm). The Petri dish was filled with 15-20 ml of distilled water and the dead larva was kept on the filter paper in the petri dish 10-12 d to collect IJs. The IJs that migrated into water were exposed to five to eight *E. kuehniella* larvae on a filter paper in a Petri dish (100 x 15 mm) to verify pathogenicity and complete Koch's postulates. The IJs of pathogenic isolates were cultured in the laboratory and stored at eight to ten ºC in tissue culture flasks for 20-30 d.

The nematodes were identified morphologically by examining morphometrics for IJs and first-generation males reared in late instar larvae of *Galleria mellonella* L. The key for the genera *Steinernema*, *Neosteinernema*, and *Heterorhabditis* by Nguyen and Smart[28] was used for identification.

Positive soil samples for nematodes were analyzed for pH, electrical conductivity, organic matter, and for soil texture in the Soil Laboratory, ICARDA.

RESULTS AND DISCUSSIONS

Five soil samples (2.37%) out of 211 in three sites contained EPNs. All samples contained heterorhabditid nematodes. Five isolates of *Heterorhabditis* were identified as *Heterorhabditis bacteriophora*; one from field crops (faba bean) in Dayr_Az_Zor, one from forest in Ariha, and two from field crops (sugar beet and wheat) and one from orchard/vine yard (olive) in Raqqa (Table 1). We found EPNs occurred more in the field crops with 5.08% of the samples positive for *Heterorhabditis* followed by orchard/vine yard with 2.27% and forest with 2.04% (Table 1).

Frequencies of EPNs vary. We found only heterorhabditids in Syria soils (2.37%). Hara et al. (18) obtained 6.3% *Heterorhabditis* and only 0.06% *Steinernema*. There were sand grains in 95.5% of soil samples positive for heterorhabditid species. This was 40% for heterorhabditids in our study. Akhurst and Brooks[1] isolated heterorhabditids (*H. heliothis*), with 16.9% and steinernematids (*S. glaseri*, *S. feltiae*, and *S. sp.*), with 2.2% in North Carolina. On the other hand, in a survey conducted in Tennessee nursery soils, half of positive soil samples contained heterorhabditid nematodes. *H. bacteriophora* was the only heterorhabditid species as in our study[31]. Homonick and Briscoe[20] recovered *Steinernema* from 48.6 % of the 403 sites from different parts of Britain but only one site yielded *Heterorhabditis*. While steinernematids occurred in 33 of 35 sites, heterorhabditid nematodes were isolated from only two sites in Spanish soils[16]. Canhilal and Carner[11] obtained 12.3% heterorhabditids and 4.6% steinernematids in 8 sites over 130 samples in South Carolina. These differences in the distribution of EPNs are probably due to the availability of susceptible hosts and environmental factors such as soil texture, soil moisture, temperature, and cultural practices.

The incidence of EPNs in different habitats also varies in different regions of the World. Mracek[25] isolated nematodes more from forest than cropland in

Table 1: Locations, habitats, and percent nematode incidence of sites containing *Heterorhabditis bacteriophora* in 2002 and 2003, Syria.

	Habitats (No. positive samples / no. samples tested)					
Location	Forest	Pasture	Orchard/vineyard	Field crops	Vegetable	[a]% inc. of H in locations
Azzaz	5	4	5	3	-	-
Raqqa	4	4	1(H)/3	2(H)/6	1	16.67
Hassakeh	5	8	3	7		-
Idleb	4	3	4	3	3	-
Ariha	1(H)/5	3	3	1	2	7.14
Tel Hadya	8	3	3	2	-	-
Al_Ksebia	2	3	2	4	-	-
Hama	2	2	2	2	1	-
As-Suwayda	1	4	4	7	-	-
Damascus	3	4	4	5	1	-
Dayr_Az_zor	4	3	4	1(H)/8	1	5.00
Tadmor	2	2	2	3	-	-
Tartous	2	3	2	4	-	-
Lattakia	2	2	3	4	2	-
Total	1 (H) / 49	- / 48	1 (H) / 44	3 (H) / 59	- / 11	
[a]% inc. of H[a] in habitats	2.04	-	2.27	5.08	-	

[a]% incidence of *Heterorhabditis bacteriophora*

Table 2: Mean (± SE) of characterizations of soil containing *Heterorhabditis bacteriophora* and % of positive samples in 2002 and 2003, Syria.

Soil texture	% Positive samples	% Organic content	Electrical conductivity (mS/cm)	pH
Silt	20.00	2.20	2.71	8.1
Loamy sand	20.00	0.56	0.63	8.7
Sandy loam	20.00	1.74	0.30	8.1
Not estimated-1	20.00	2.06	3.14	7.9
Not estimated-2	20.00	1.25	4.65	8.1
Mean ± SE		1.56±0.30	2.28±0.81	8.17±0.13

Czechoslovakia, while Gracia and Palomo[16] found no difference for the incidence of nematodes among cultivated fields, woodlands, or pastures in Spain. Akhurst and Brooks[1] recovered more nematodes in cropland than forest, orchard/vineyard, or pasture. We also obtained more nematodes in cropland than orchard and forest (5.08% vs. 2.27% and 2.04%, respectively) similar to Akhurst and Brooks's[1].

The proportions of clay, sand and silt could not be estimated for two positive soil samples taken from Raqqa. Two positive soil samples contained sand and one did not contained sand. Soil texture for heterorhabditid positive soil samples was loamy sand, silt, and sandy loam (Table 2).

Electrical conductivity averaged 2.28 mS/cm for nematode positive soils. It varied from non-saline (0.30 mS/cm) to moderately saline (4.65 mS/cm) (Table 2). Tolerance of soil salinity differs with crop. For instance, while carrot yields are reduced in soils with electrical conductivity as low as 1.0 mS/cm, cotton yields are not adversely affected until 7.7 mS/cm electrical conductivity[24]. Our results showed that EPNs are also well adapted to different soil salinity as indicated by Thurston *et al.*[34].

Soil pH ranged from weakly basic (7.9) to medium basic (8.7) for *Heterorhabditis*-positive soils (Table 2). Kung *et al*.[23] found that survival and pathogenicity of steinernematids were reduced only slightly as the tested soil pH decreased from pH 8 to pH 4, but survival and pathogenicity drastically decreased at pH 10. Canhilal and Carner[11] stated that they have got steinernematids and heterorhabditids in various soil pH from strongly acidic (4.3) to neutral (7.0) in a survey conducted in South Carolina. These findings show that EPNs tolerate a wide range of soil pH.

Organic matter of nematode positive soils averaged 1.56% varying little organic content (0.56%) to medium (2.20%). It ranged for *Heterorhabditis*-positive soils from little organic content (0.7%) to high (7.8%) in Canhilal and Carner's[11] study. These results indicate that EPNs are well adapted to different soil organic content as to different soil textures, salinity, and pH.

Conclusion: Natural distribution of EPNs in Syrian soils was put forth and documented first time. Some characteristics such as soil texture, organic content and electrical conductivity of nematode positive soils were also determined. These local isolates can be used against economic insect pests especially the ones in soil and cryptic habitats in biological control programs in Syria. Future studies should be conducted for this purpose.

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