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LABORATORY AND FIELD STUDIES ON ENTOMOPATHOGENIC NEMATODES AS A BIOCONTROL AGENT FOR THE CATTLE TICK

**BOOPHILUS ANNULATUS** (ACARI: IXODIDAE)

HANAN, A. EL-SADAWY & SOBHY ABDEL-SHAFY

(Accepted October 2006)

1. Department of Parasitology and Animal Diseases, Veterinary Division, National Research Center, Dokki, Giza, Egypt.


**SUMMARY:** Five Egyptian heterorhabditids and two imported steinernematids isolates were evaluated against engorged females of the cattle tick, *Boophilus annulatus* under laboratory conditions. All tested strains entomopathogenic nematodes (EPN) showed high pathogenicity against *B. annulatus*. The most virulent strains were *Heterorhabditis* sp. S1 and *Heterorhabditis* sp. EG1 recorded 100% mortality for all suspensions on the 4th day post treatment. LC50 values of *Heterorhabditis indica* RM1, *Heterorhabditis* sp. ISK1, *H. bacteriophora* TWF, *Steinernema carpocapsae* All strain and *S. riobravae* SR were 52, 63, 636, 2375, and 5700 IJs/ml, respectively. In field trials, two techniques; soaked-cotton ball and spraying with 2000 IJs/ml nematode suspensions were applied on cows infested by semi-fed females of *B. annulatus*. The treated ticks were dropped from their hosts within 12 h post infection.

**INTRODUCTION**

Ticks are considered the second rank to mosquitoes as vectors of infectious agents because they are the most important arthropods which transmit pathogens to animals (BALASHOVE, 1972). Besides their transmitting pathogens such as virus, bacteria and protozoa, they are also directly responsible for damage to animals and production in livestock. The economic important of ticks and tick borne diseases together with costs of control treatments, has been estimated at 7 billion dollars globally in the livestock sector (MCKOSKER, 1979 and GRAY, 1985). Chemical acaricides are becoming less sustainable due to their resistance among the ticks (NOLAN & SCNITZERLING, 1986), increasing costs and toxicity of their residues in milk and meat (WILLADSEN, 1997).

Entomopathogenic nematodes (EPN) are lethal to many important soil insect pests and safe for plants and animals. Nematodes with their symbiotic bacteria kill insects in 24-48 h. Nematode applications do not require specialized equipments as masks, pressurized, mist, electrostatic fund and aerie sprayers. (GEORGIS & GAUGLER, 1991). Moreover, nematode production is easily accomplished using standard fermentation in tanks up to 150000 liters. The engorged females of *B. annulatus* (Say) and *Hyalomma dromedarii* (Koch) appeared to be the most susceptible stage to penetration and killing by the nematodes (SAMISH & GLAZER, 1991, 1992, GLAZER & SAMISH, 1993, HASSANAIN et al., 1997 and EL-SADAWY & HABEEB, 1998). Also, they showed highly virulent towards mature and immature stages of soft ticks *Argas persicus* (Oken, 1818) under laboratory conditions.
The association between EPN and the Egyptian cattle tick *B. annulatus* under laboratory and field conditions are poorly understood. So, this work aims to shed light on the susceptibility of this tick species to different nematode strains under controlled conditions and trials to apply EPN under field conditions.

**Material and Methods**

1 — **Collected ticks:** The cattle ticks *B. annulatus* were collected from private farm, Qous city, Qena Governorate, Egypt. *B. annulatus* was identified according to Walker et al. (2003). Ticks were collected as engorged females from ground of cows’ pen and incubated at 25 °C and 75 % RH for laboratory experiments.

2 — **Nematode propagation:** Species/strains of nematodes were maintained on the greater wax moth larvae *Galleria mellonella* according to Dvorak et al., (1964). All nematode infective juveniles (IJJs) were used within the first week of harvest. The seven species/strains of nematodes are propagated at Parasitology and Animal Diseases Department, National Research Center, Dokki, Giza, Egypt. The Egyptian and imported nematode locations recorded in Table 1.

3 — **Laboratory application:** Susceptibility of engorged females of *B. annulatus* to EPN was studied under laboratory conditions. Nematode suspended in concentrations of 4000, 2000, 1000, 500 and 250 infective juveniles (IJJs/ml). Each concentration contained 5 replicates and each replicate contained 5 females. These females placed in plastic cups 50 cc full of 10 gm clean sand (one female/cup). Soil moistened with 1.5 ml nematode suspension in each cup. Control moistened with tap water only. All the experiment incubated at 25 °C and 75% RH. Cups were checked every 24 h and mortality was recorded on the 4th day post application. Data analyzed statistically by computing program SPSS, (1999). LC50 values were calculated according to Finny, (1971).

<table>
<thead>
<tr>
<th>Species Code No.</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Heterorhabditis indica</em> RM1</td>
<td>Soil sample under a lemon tree, in Alexandria</td>
</tr>
<tr>
<td><em>H. bacteriophora</em> TWF</td>
<td>Soil sample under an apple tree in Salheya, Sharkia province</td>
</tr>
<tr>
<td><em>Heterorhabditis sp.</em> EG1</td>
<td>Soil sample under chinaberry tree in western desert</td>
</tr>
<tr>
<td><em>Heterorhabditis sp.</em> ISK1</td>
<td>Soil sample under mango tree in El-Kassaseen, Esmalia</td>
</tr>
<tr>
<td><em>Heterorhabditis sp.</em> S1</td>
<td>Soil sample from Ras Sidr, South Sinai</td>
</tr>
<tr>
<td><em>Steinernema carpocapsae</em> All</td>
<td>Biosys, Palo Alto, California, USA</td>
</tr>
<tr>
<td><em>Steinernema riobravae</em> SR</td>
<td>Department of Entomology, Rutgers University, NJ</td>
</tr>
</tbody>
</table>

**Table 1:** Source of EPN species/strains.

<table>
<thead>
<tr>
<th>Nematode strain*</th>
<th>Mortality (%)</th>
<th>LC50</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentrations (IJJs/ml)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4000</td>
<td>2000</td>
</tr>
<tr>
<td><em>Heterorhabditis indica</em> RM1</td>
<td>100 a</td>
<td>96 a</td>
</tr>
<tr>
<td><em>Heterorhabditis sp.</em> ISK1</td>
<td>100 a</td>
<td>100 a</td>
</tr>
<tr>
<td><em>Heterorhabditis bacteriophora</em> TWF</td>
<td>88 a</td>
<td>80 a</td>
</tr>
<tr>
<td><em>Steinernema carpocapsae</em> All</td>
<td>60 b</td>
<td>52 b</td>
</tr>
<tr>
<td><em>Steinernema riobravae</em> SR</td>
<td>40 b</td>
<td>36 b</td>
</tr>
</tbody>
</table>

* *Heterorhabditis* sp. S1 and EG1: 100% mortality for each concentration within 48 h post infection.
  a,b,c: different letters in the same column means differences are significant (LSD = 24.98 at P<0.05).

**Table 2:** Susceptibility of engorged females of *B. annulatus* to different EPN strains on the 4th day post treatment.
Fig. 1: Soaked-cotton ball with EPN suspensions on different parts of body’s cows infested by semi fed females of *B. annulatus*. (A. — Neck. B. — Fore leg. C. — Hind leg. D. — Front tail)

Fig. 2: The effect of soaked-cotton ball and spraying with EPN suspensions on semi fed females of *B. annulata*. A. — Tick before treatment with soaked-cotton ball. B. — Tick dropped after 12 h post treatment with soaked-cotton ball. C. — Ticks before spraying. D. — Ticks have been picked by chicken from ground of pens after dropping.
4 — Field application: Location (I): Two nematode strains, *Heterorhabditis* sp. S1 and *Steinernema carpocapsae* All (the most virulent heterorhabditids and steinernematids in susceptibility test) were applied on Frezian cows infested by semi fed females of *B. annulatus*. Nematode suspensions (2000 IJs/ml) were prepared 24 hrs before treatment in the laboratory and kept in icebox. Two techniques were applied in El-Makhzan village, Qous City, Qena Governorate after sunset at 38 °C as follow:

— Sixteen semi fed females of *B. annulatus* were marked on a mature cow in four groups; G1: 1 on neck, G2: 5 on fore leg, G3: 7 on hind leg and G4: 3 on front tail (Table 4 and Fig. 1 A-D). A small piece of cotton ball was immersed in *Heterorhabditis* sp. S1 suspension and put on semi fed females for each group. This procedure was repeated on front tail of a calf infested by 2 semi fed females (G5) using the same suspension. This application was also repeated on 2 semi fed female for neck of a mature cow using *S. carpocapsae* All suspension (G6). In control treatment (G7), cotton ball was immersed in water and put on front tail of a mature cow infested by 5 semi fed females. All soaked-cotton balls were covered with blasters and banded around the treated parts to keep suitable moisture condition which prevents nematodes from desiccation. Cows were roped from its head to prevent cows from removing the plasters. Blasters were removed in the morning, 12 h post treatment and recorded the dropping ticks (Fig. 2B).

— *Spraying with nematode suspensions*: Fourteen semi fed females of *B. annulatus* were marked on a mature cow; G1: 2, G2: 4, G3: 4, G4: 3. while, G5 on a calf, G6 on a mature cow and G7 on a mature cow included 5, 12 and 5 semi fed females, respectively. G1 to G5 were sprayed with *Heterorhabditis* sp. S1 suspension, while G6 was sprayed with *S. carpocapsae* All suspension. Control group (G7) was sprayed with fresh water (Table 4). Sprayed ticks were checked in the morning, 12 h post treatment to record the dropping ticks.

Location (II): Two nematode strains, *Heterorhabditis* sp. Eg1 and *Steinernema carpocapsae* All (the most virulent heterorhabditids and steinernematids in susceptibility test) were applied on local cows infested by semi fed females of *B. annulatus*. Nematode suspensions (2000 IJs/ml) were prepared 24 hrs before treatment in the laboratory and kept in icebox. Spraying technique was only carried out in El-Saff society, Kebabet, Atfi, Giza Governorate after sunset at 32 °C. G1 and G2 were sprayed with *Heterorhabditis* sp. Eg1 suspension but G3 and G4 were sprayed with *Steinernema carpocapsae* All suspension and fresh water (control) (Table 4). Spraying treatments were randomly applied on the different parts of animal’s bodies because they were highly infestation. In the morning, ground of pens was checked to observe dropping ticks specially semi fed females.

**RESULTS**

1 — Laboratory application.

All tested EPN strains showed various pathogenicity against engorged females of *B. annulatus*. The most virulent strains were *Heterorhabditis* sp. S1 and *Heterorhabditis* sp. EG1. Wherever, they recorded 100% mortality for all suspensions after 48 h post infection. The LC50 values were 52, 63, 636, 2375, and 5700 IJs of the nematode species/strains; *H. indica* RM1, *Heterorhabditis* sp. ISK1, *H. bacteriophorae* TWF, *S. carpocapsae* All and *S. riobravae* SR, respectively. It was obviously that the first two strains were the most virulent ones whereas SR was the lowest. In general, *Heterorhabditis* strains were considered highly effect comparing with *Steinernematid* species/strains especially in low concentrations. Mortality percentages were increased as the increase of exposure time in all applied nematodes (Table 2).

*H. indica* RM1 and *Heterorhabditis* sp. ISK1 caused 100 % mortality for all suspensions on the 7th day post infection. On the other hand, *H. bacteriophorae* TWF, *S. carpocapsae* All and *S. riobravae* SR showed 100 % mortality for only at 1000 to 4000 IJs. In case of *H. bacteriophorae* TWF, the low concentrations 250 and 500 IJs caused mortality 92 and 96 %, respectively. However, *S. carpocapsae* All and *S. riobravae* SR had a weak pathogenicity, (52 to 68 %) and (32 to 64%), respectively. They also were significantly difference than other strains (Table 3).
### Table 3: Susceptibility of engorged females of *B. annulatus* to different EPN strains on the 7th day post treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soaked-cotton ball</th>
<th>Spraying</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated ticks</td>
<td>Dropped ticks*</td>
<td>Treated ticks</td>
</tr>
<tr>
<td>Soaked-cotton ball</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spraying</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: Efficacy of EPN suspensions on semi fed females of *B. annulatus* under field conditions.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Group</th>
<th>Nematode strain</th>
<th>Cow</th>
<th>Position</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>G1</td>
<td><em>Heterorhabditis</em> sp. S1</td>
<td>Mature</td>
<td>Neck</td>
<td>1</td>
</tr>
<tr>
<td>I</td>
<td>G2</td>
<td><em>Heterorhabditis</em> sp. S1</td>
<td>Calf</td>
<td>Tail</td>
<td>2</td>
</tr>
<tr>
<td>I</td>
<td>G3</td>
<td><em>S. carpocapsae</em> All</td>
<td>Mature</td>
<td>Neck</td>
<td>2</td>
</tr>
<tr>
<td>I</td>
<td>G4</td>
<td><em>S. carpocapsae</em> All</td>
<td>Calf</td>
<td>All body</td>
<td>—</td>
</tr>
<tr>
<td>I</td>
<td>G5</td>
<td><em>S. carpocapsae</em> All</td>
<td>Calf</td>
<td>All body</td>
<td>—</td>
</tr>
</tbody>
</table>

* 12 h post treatment
— Treatment did not applied
+ Ticks were sprayed randomly without counting because of heavy infestation.
+ + Semi fed females were observed on ground of cow pens.

### Discussion

Our results agree with Samish & Glazer (1991) found that engorged females of *B. annulatus* ticks are highly susceptible to infection by the entomopatho-
genic steiernematids and heterorhabditids. In laboratory application, results indicated that 5 heterorhabditids and 2 steiernematids showed high pathogenicity against engorged females of *B. annulatus*. Moreover, heterorhabditidis were significantly more virulent to ticks than steiernematides in agreement with other reports (Mauleon et al., 1993, Hassanain et al., 1997, El-Sadawy & Habeeb, 1998, Hill, 1998 and Glazer et al., 2001). As well as, *Heterorhabditis* sp. S1 and Eg1 recorded 100% mortality for all concentration after 48 h in comparing with steiernematidis species, *S. carpocapsae* All and *S. riobravae* SR which recorded lower virulent for all concentrations.

Ticks were killed when placed on the soil surface which inoculated with the nematode species (Mauleon et al., 1993, Hassanain et al., 1997 and El-Sadawy & Habeeb, 1998). This finding agrees with our results where all the seven used nematodes killed *B. annulatus* with high mortality percentage when it placed on the inoculated sandy soil. Therefore, laboratory test can supply with the most virulent nematode strains which can be used in field especially in wet ground of pens, where the engorged females live. This wet environment is suitable for both nematodes and ticks. El-Sadawy & Habeeb, (1998) succeeded in applying EPN in control of engorged females of *H. dromedarii* in sandy soil under laboratory conditions. However, field application of EPN on ticks that live on the body of animals did not apply before. So, we have tried to use two techniques (soaked-cotton ball and sprayer) on semi fed females of *B. annulatus* during its feeding on cows.

While feeding on a host, ticks are resistant to nematodes except on moist feeding sites (Samish et al., 1999 and Kaaya et al., 2000). However, these nematodes are highly sensitive to ultraviolet light and desiccation and EPN have been found to be most efficacious in soil or other protected environments (Kaaya & Gaugler 1993). Therefore, some precautions had been considered in field application to save suitable conditions for nematode survival, such as the application had been carried out after sunset to avoid UV harmful effect and save a high humidity to prevent nematode from desiccation whereas, animal houses were in cultivated land which was semi saturated was water vapor comes from plant ooze.

In this investigation, three strains of EPN had been applied successfully in the field against semi fed females of *B. annulatus; Heterorhabditis* sp. S1, *Heterorhabditis* sp. EG1 and *S. carpocapsae* All. Soaked-cotton ball or spray techniques succeeded to constrain semi fed female of *B. annulatus* to leave the host body within 12 h post application. In both soaked-cotton ball and spray techniques, we can not record the dead time because the semi fed females dropped on the ground and the birds picked them in the morning (12 hrs post application). Additionally field trials carried out at 38°C in location (I) and 32°C in location (II). This result indicated that EPN were thermotolerance strains and it can be virulent at high temperature in agreement with high mortality percentage which had been recorded on *Heterorhabditis* sp. S1 to *H. dromedarii* at 33 °C (El-Sadawy & Habeeb, 1998).

EPN can reduce the tick infestation. The tested nematodes led to dropping semi fed females without complete their feeding. This result means that dropped semi fed or fed females either dead or picked up by birds before complete their preoviposition period. However, the preoviposition period of *B. annulatus* is 6-7 days (Jagannath et al., 1982). An addition, if we repeat the same procedures on infesting cows, then another semi fed or fed stages will be dropped. Finally, more ticks will be reducing from cows and will be killed without laying eggs because the nematodes caused 100% mortality on the 2nd day post application in case of *Heterorhabditis* sp. S1 and *Heterorhabditis* sp. EG1 and 7th day pos application in case of *S. carpocapsae* All.

All tested EPN strains had various pathogenic effects on *B. annulatus* under laboratory condition. *Heterorhabditis* sp. S1, *Heterorhabditis* sp. EG1 and *S. carpocapsae* All showed strongly effect on semi fed females of *B. annulatus* infesting cows under field condition. These investigations consider the first step towards large scale field application of nematodes as agents for tick control in future.

**Acknowledgement**

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