ENTOMOPATHOGENIC NEMATODES AS SAFE BIOCONTROL AGENTS USED AGAINST HARD AND SOFT TICKS

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ABSTRACT

The objective of this study is to find safe biocontrol agents as alternatives to chemical acaricides used against ticks. Forty two species and/or isolates of local and foreign entomopathogenic nematodes belong to the genera *Heterorhabditis* and *Steinernema* were tested against the females of both the soft tick, *Argas persicus* and the hard tick *Boophilus annulatus* that are veterinary pests infesting farm animals and poultry. Twenty three of these species or isolates of EPN were *Heterorhabditis* sp. and nineteen of these species or isolates were *Steinernema* sp. All these species and/or isolates were ranked according to their virulence which measured by their mortality percentages within three days following nematode infections Entomopathogenic nematodes are promising biocontrol agents of the soft tick *Argas persicus* and the hard tick *Boophilus annulatus*. They have a great influence on ticks when used in a concentration of 1000 infective nematode juveniles/ml for 5 cm Petri dish. The soft ticks were more sensitive to Heterorhabditidae isolates than Steinernematidae, but the Steinernematidae isolates were more efficient in controlling hard ticks. Tick pH was 3.8 which is acidic and doesn't suit nematode growth and reproduction inside the tick.

INTRODUCTION

Ticks are acarines comprised of two major taxonomic families, the Ixodidae, hard ticks, and Argasidae, soft ticks. The Ixodidae consist of 13 genera and 650 species while the Argasidae is comprised of 5 genera and 170 species (Sonenshine, 1991). The diversity of pathogenic organisms transmitted by ticks exceeds that found in all other arthropods. They serve as vectors of several animal diseases including Anaplasma, Babesia, Cowdria, Escherichia, Theileria (Sonenshine, 1993). They also

transmit important human zoonotic diseases such as; Lyme disease, Rocky Mountain Spotted Fever, relapsing fever, and Q fever (Sonenshine, 1993). In addition to the transmission of pathogens, ticks often provoke animal toxicosis, weight loss, hide damage, and tick paralysis. Annual worldwide losses from ticks and their pathogens have been conservatively estimated at $7 billion (U.S.). Ticks are obligate blood feeding ectoparasites and ingest large amounts of vertebrate blood. They are economically important pests of animals and vectors of dangerous animal and human diseases worldwide. Ticks infestation is currently controlled mainly by spraying or dipping with chemical pesticides, but the effectiveness of acaricides has been decreased because of increasing tick resistance to these chemicals. Tick and tick-borne diseases are considered the greatest animal disease problem all over the world. The conventional method of tick control using chemical acaricides is fraught with several problems e. g. environmental pollution, chemical residues in meat, milk products and in wool, development of tick resistance and the exorbitant costs. Alternative innovative, environmentally friendly and cost-effective methods of tick control are therefore needed (Kaaya, 2004). This phenomenon, as well as problems of contamination of the environment and pollution of food, have stimulated the search for alternative methods to control ticks. Among the reasons for the increased interest in biological control is the wide public awareness of the environmental damage resulting from chemical pesticides. However, biocontrol employing entomopathogenic nematodes has rapidly developed into a sub discipline of insect pathology.

In the present investigation, the susceptibility of soft ticks (*Argas persicus*) and hard ticks (*Boophilus annulatus*) to entomopathogenic nematodes were tested to find out non chemical and safe ways to control these parasites. The pH of the non infected ticks was measured to determine whether it is a limiting factor in the inhibition of nematode development or not.

MATERIL AND METHODS

The present study was carried out at laboratories of the Applied center for Entomonematodes, Department of Agricultural Zoology and Nematology, Faculty of Agriculture, Cairo University.

Tested ticks

Semi and fully engorged adults of the soft tick *Argas persicus* and an other hard tick *Boophilus annulatus* (Arachnida: Ixodidae) used in the present study were collected from the chicken breeding houses at the Faculty of Agriculture, Cairo

University and cattle farms in Giza and Gharbyia Governorates. Collected ticks were identified according to Hoogstraal *et al.*, (1981). Different stages of the *Argas persicus* (males, females and nymphs) and only adults of *Boophilus annulatus* were used in this study (Figs. 1 & 2).

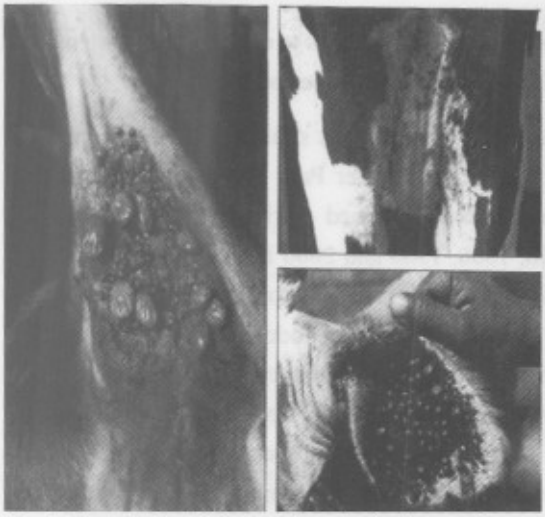

Fig. 1: Hard ticks of *B. annulatus* infesting cattles at the experimental station, Faculty of Agriculture, Cairo University and cattle farms in Giza and experimental station of Agricultural Research Center, Gemmeza, Gharbyia Governorate.

Fig. 2: Soft ticks of *Argas persicus* collected from wall cracks in chicken breeding houses at Faculty of Agriculture, Cairo University.

Mass rearing of the Entomopathogenic Nematodes

Cultures of Steinernema and Heterorhabditis were continuously propagated on the greater wax moth, *Galleria mellonella*. The adapted technique was similar to that of Dutky *et al.*, (1964), Tayseer (1980, 1984), (Abdel-Kaway 1981, 1985) and Ahmed, (1992).

Susceptibilty tests

Entomopathogenic nematodes were tested for their ability to kill replete ticks of *Argas persicus*, and *Boophilus annulatus*

Susceptibility of *Argas persicus* and *Boophilus annulatus* ticks as hosts of the nematodes

Five fully engorged females of the hard tick *B. annulatus* and/or of the soft tick *A. persicus* were placed in each of 5 cm diameter Petri dishes padded with filter paper and infested with 1000 IJs of 42 nematodes isolated belong to Heterorhabditea

and Steinarnematedae in 1 ml. of distilled water (Samish and Glazer, 1992). Each treatment was replicated in five dishes. The dishes were incubated at 25 °C. Tick mortality was determined daily for three days. The experiment was repeated three times.

Susceptibility of *B. annulatus* and *Argas persicus* females to different concentrations of entomopathogenic nematodes using the filter paper technique

Suspension of infective juveniles from various strains of Steinernema, and Heterorhabditis were poured into five 9-cm diameter Petri dishes lined with filter paper (Whatman No.1). The suspension contained various concentrations of infective juveniles i.e. 250, 500, 750, 1000, 1500 and 2000 IJs per replicate on hard ticks and 100, 200, 300, 400, 500, and 600 IJs per replicate on soft ticks. The control treatment was done with distilled water without nematodes. Five fully engorged females of *B. annulatus* or *A. persicus* were placed in each dish (replicate). Five replicates of each nematode concentration were done to determine the susceptibility of both hard and soft ticks at 25 + 2 °C. The mortality percentage was determined daily for three days. The experiment was repeated three times. The tick cadavers were collected and placed on white trap dishes (White, 1927).

One on one bioassay

Entomopathogenic nematodes were tested for their ability to kill replete ticks of the species *Argas persicus* and *Boophilus annulatus*. Engorged ticks were placed individually in a multi-well plate of 24 wells. Five grams of sterilized sand were placed at the bottom of each well. Three plates were placed as a treatment. Nematode suspension at different concentrations of 200, 300, 400, 500 and 600 IJs/ml.were prepared. Five hundred µl from the nematode suspension were added with a micropipette to each of the sand-padded wells. A single adult of tick (hard or soft) was placed to each well and the wells were sealed with their lid and incubated at 25 °C. The susceptibility of ticks to nematode infection was tested with 3 nematode species belong to family Heterohabditidae and 3 species belong to family Steinernematidae. Tick mortality was recorded 24 and 48 hours post-infection. Tick mortality is expressed as a percentage of the total number of ticks tested.

In all experiments, effect of nematodes on ticks was judged in comparison with unexposed control ticks by determining the percentage of ticks that died as evidenced by the change of color associated with purification. Treatments were arranged in a complete randomized design.

Statistical analyses

Data were subjected to statistical analyses of variance according to Snedcor and Cochran (1980). Means of treatments and their interactions were compared using least significant difference test at 0.05 and 0.01 probability levels.

All mortality data were corrected for natural mortality using Abbott's formula, (Abbott, 1925).

RESULTS AND DISCUSSION

Susceptibility of fed adult females of both the soft tick, *Argas persicus* and the hard tick, *Boophilus annulatus* exposed to entomopathogenic nematodes

Data in table 1 showed that, the most potent species and/or isolate belong to the genus *Heterorhabditis* were the local isolates, EBN48, EBN7 and EBN39. They highly affect the soft tick, *A. persicus*, and reached 100% mortality after 24 hours of nematode treatment. While the least virulent species or isolates of *Heterorhabditis* spp. were the local isolates, EGBa1, EBN1, EASAc, and *Heterorhabditis taysearae* since they caused 0 to 20 % mortality after 24 hours post treatment with nematodes. In contrast, the most potent species and/or isolates tested against females of the hard tick, *Boophilus annulatus* were, , EBN48 EBN10k, and USANJ14, these nematodes gave 80-100% mortality after 24 hours post-infection. Moreover, the least virulent species and/or isolates were, EGBa1, *Heterorhabditis taysearae*, and OBma, they gave from 13.33 to 26.66% mortality after 48 hours of treatment with nematodes (Table 1). Data in Table 2 showed that, the best species and/or isolates of *Steinernema* spp. were, USA10 and EGBats. They gave 100% mortality with *Argas persicus* after 48 hours of nematode infections. The lowest mortality rate was recorded with the isolate, PGcycamora which gave 26.66% mortality after 72 hours following the exposure to nematodes. In contrast, the best isolates and/or species of *Steinernema* tested against the hard tick *Boophilus annulatus* were, USA5mon, EGS29, *S. riobravae* and EGBats. They gave from 86.66 - 100% mortality after 48 hours post-infection with nematodes. While, the lowest mortality rate was estimated from isolate and/or species, PGfig2, it gave 20% mortality after 72 hours following the exposure to nematodes. However, mortality increased through time with all nematode isolates. There were high significant differences between all nematodes isolates used to kill ticks and the control at all times of exposure.

Susceptibility of the fed and unfed nymphs, males and females of the soft tick *Argas persicus* exposed to different concentrations of EPN:

The susceptibility of fed and unfed nymphs, males and females of the soft tick *A. persicus* to two nematode isolates one belong to the genus *Heterorhabditis* and the second belong to the genus *Steinernema* were determined. Different stages of *A. persicus* were found susceptible to the infection by the two nematodes (Table 3). Mortality rates differed according to the host stage and the nematode species. Although mortality rates were close to each other in all tick stages treated with the highest nematode concentration of 600 IJs/ml in the two nematode isolates used in this experiment. At a lower concentration of 100 IJs/ml, however, fully engorged female mortality rates were higher than those of males and nymphes in both isolates of *Heterorhabdites* sp. isolate EBN48 and *Steinernema* sp. isolate USA5-mon. Fully engorged females were more susceptible to *Heterorhabditis* sp. at all infection levels than *Steinernema* spp. moreover, the males and nymphs were less susceptible to nematode infection than the engorged females. No mortality was recorded among unfed nymphs at 24 hours post-infection at lower concentrations of 100, and 200 IJs/ml. with both isolates. Fully engorged adult females were generally the most sensitive while, immature stages were the least. Generally, increasing the concentration of nematode IJs in the dish was accompanied by higher mortality rates. There were high significant differences between all concentrations of the two used nematode isolates against all stages and the control (Table 3).

One on one bioassay

Data in Table (4) showed that the replete females of *Boophilus annulatus* and *Argas persicus* were also highly susceptible to the nematode infection with *Steinernema* sp., isolate EGB10b when tested in multiwell plate after 48 hours post-infection.

Infectivity studies under laboratory conditions using multiwell plate, 24 Wells, padded by filter paper, demonstrated that high tick mortality of > 90% can be achieved at a nematode concentration of 240 IJs/tick, it gave 93.33% with *B. annulatus* and 100% with *A. presicus*. While the lowest concentration 60 IJs/tick gave 33.33 with *B. annulatus* and 20% with *A. persicus* after 48 hours post-infection. The mortality percentage with 180 IJs/tick was 73.33% and 86.66% with *B. annulatus* and *A. presicus* respectively. Moreover, the highest mortality rate of 100% was achieved with 300 IJs/tick against *B. annulatus* and 240 IJs/tick against *A. persicus*. These results can be attributed to the size and shape of the body

openings in the tick exposed to nematodes and the thickness of the tick integument which may halt nematode penetration into the tick hemocoel.

Data in table (5) illustrated that sensitivity of fully engorged females of hard tick, *Boophilus annulatus* to different concentrations of *Heterorahbditis* sp., Isolate EBN10k at 24 and 48 hrs using the multiwell plate (24 wells) padded by 5 g. of sterilized sand. Data demonstrated that, the percentage mortality increased with exposure time and nematode concentrations. The mortality percent was 36, 44, 76, 84, and 92 after 24 hrs of treatment with nematodes but it increased to 72, 84, 92, 96, and 100, after 48 hrs of exposure to nematode concentrations of 200, 300, 400, 500 and 600 IJs/ tick, respectively.

Two entomopathogenic nematodes belong to *Heterorhabditis* sp. EBN10k and PGs and two belong to *Steinernema* sp., EGG4 and EGB10b were tested against adults of the soft tick *A. persicus* and the hard tick, *B. annulatus*. No differences between mortality rates due to the exposure to these nematodes after three days post-infection using 750 IJs/dish. The mortality percentages were 78 and 96% with *Steinernema* spp., EGG4 and EGB10b, respectively against the soft tick, while it was 88 and 95 against the hard tick. On the contrary, the *Heterorhabditis* sp., EBN10k and PGs gave from 96 – 100% mortality against soft and hard ticks respectively, three days post-infection. At a lower concentration of 250 IJs/dish, the mortality rate was from 8 – 20% against soft and hard ticks respectively, after 24 hrs post-infection by all nematode isolates. The highest concentration of 2000 IJs/dish has given mortality percent ranged from 82 to 100% after 24 hours post-infection with all isolates. There were high significant differences between all nematode isolates used at all exposure times and the control (Table 6).

In a comparison between the 4 tested isolates of *Heterorhabditis* against the soft tick *A. persicus* at different nematode concentrations after three days of infection using a one on one assay, data in Table (7) showed that the most effective isolate of *Heterorhabditis* spp. was EBN39, it gave 80% mortality after 72 hours post-infection against the soft tick *A. persicus* when it was infected by 200 IJs/tick. While the mortality percentage was 63% with isolate EBN48 at the same time. The results also revealed that, the mortality percentages were 72 and 95% when the nematode concentration was 300 IJs/tick with isolates EBN48 and EBN39, respectively at three days post-infection, but no different changes in the mortality rates were recorded with both EBN7 and OB5 isolates. The highest mortality rate of 100% was recorded when the ticks were infected with 400 IJs/tick at 72 hrs post-infection with isolates EBN7, EBN39, and OB5 while isolate EBN48 gave only 85%

mortality at the same concentration. Moreover, in the lower concentration of 100 IJs/tick, no significant changes in the mortality rate between the four nematode isolates were recorded. On the contrary, the isolate EBN48 was less effective with the higher concentration of 600 IJs/tick with a mortality rate of 45% after 24 hrs from nematode infection while it reached 100% after three days post-infection. However, when the concentration of nematode IJs/tick increased, mortality rate elevated throughout time.

TABLE (I)

Virulence of heterorhabditid nematode isolates at 1000 IJs/ml against the hard tick *B. annulatus* and the soft tick *A. persicus* throughout 3 days from the initial nematode infections.

Isolate[b]	Mortality %					
	Argas persicus[a]			*Boophilus annulatus*[a]		
	24[d]	48[d]	72[d]	24[d]	48[d]	72[d]
EBN48	100	-	-	100	-	-
USA7mon	46.66	93.33	100	16	73.33	100
EBN5anb	60	100	-	33.33	100	-
PGs	86.66	100	-	0	80	100
EBN1	20	86.66	93.33	24	76.66	100
EGBa1	0	80	93.33	0	26.66	33.33
EGB6b2	60	93.33	100	33	93.33	100
EASac	20	100	-	20	100	-
EBN10k	80	100	-	80	100	-
HP88	33.33	93.33	100	0	46.66	100
Heterorhabditis taysearae	26.66	73.33	100	0	13.33	13.33
EBN9	73.33	100	-	43.33	86.66	100
EBN7	100	-	-	46.66	66.66	93.33
OB5o	93.33	100	-	53	73.33	96.66
EBN	33.33	100	-	6.66	53.33	86.66
EBN39	100	-	-	60	100	-
Obma	60	100	-	0	26.66	73.33
EBN4m	46.66	80	100	28	66.66	80
EBN16m	73.33	100	-	20	46.66	88
Hb strain	53	86.66	100	26.66	80	96.66
EGB12	73.33	86.66	100	26.66	73.33	86.66
UAEs	66.66	100	-	40	60	93.33
USANJ14	46.66	80	100	80	100	-
LSD*	1.611					

*The LSD based on original data at 0.05 and 0.01 probability levels.

*a=NS b (0.83, 1.097); ab (1.178, 1.551); c (0.30, 0.39) and ac (0.425, 0.56).

Nematodes are known to enter their host body cavity mainly via natural orifices. In engorged females of *B. annulatus*, entomopathogenic nematodes were

attracted towards the natural apertures of the ticks or penetrate between mouthparts. On the first day after the death of the tick >30% of the recovered IJs were dead, but at later dates we found over 80% and up to 100% dead nematodes inside the tick body cavity.

The 42 nematode strains that have been tested for their anti-tick activity have shown varying degrees of virulence. In laboratory tested, heterorhabditids were generally more virulent to ticks than steinernematids. The same results were also shown by Kocan *et al.*, (1998) when they have found that nematodes are virulent against engorged *Amblyomma americanum* females and they were attracted towards natural openings in the ticks' body or attempted to penetrate between mouthparts. Oyoun and El-Bishlawy, (1990). They used *Heterorhabdities heliothidis* to control *Argas persicus* and found that a concentration of 600 Infective Juveniles (IJs)/ml caused 73.3% and 100% mortality to *Argas persicus* males and females respectively. Also, El-Sadawy (1994) revealed, the susceptibility of nymphs, males and females of the soft ticks *Argas persicus* to three entomopathogenic nematodes, *Steinernema carpocapsae*, *Heterorhabditis heliothidis*, and *H. bacteriophora*. Different stages of *A. persicus* were susceptible to the infection with the three nematodes. Rate of mortalities differed according to the host stage and to the tested nematode species. She also found that, mortality rates of all stages at higher nematode inoculums of 12000 IJs in the 3 tested nematode species were very close to each other. At a lower concentration of 3000 nematode IJs per tick, meanwhile mortality rates in tick females were higher than those of males and nymphs with both *S. carpocapsae* and *H. bacteriophora*. In case of *H. heliothidis*, mortality rates of females and nymph were almost the same, while that of males was much higher at high concentrations and dropped sharply at lower concentrations. Females were much more affected by *H. bacteriophora* at all inoculums levels than the two other nematode species. The possibility of infecting *Argas persicus* using two species of entomopathogenic nematodes *S. carpocapsae* and *Heterorhabditis bacteriophora*, studied by Hassanain *et al.* (1997) they found that, nymph, males and females of soft ticks were susceptible to the two tested nematode species. They also showed that, mortality rates differed according to the host stage, the nematodes species and concentrations of nematode juveniles. Although nematodes could invade and kill the ticks, they usually fail to reproduce inside the dead ticks.

However, the nematodes mode of invasion has not yet been established. In contrast, a study on two *Boophilus* spp. has shown that, no obvious relationship was observed between the size of spiracles, size of genital openings or cuticle thickness

and relative susceptibility to two strains of entomopathogenic nematodes (Maulean *et al.*, 1993, Hill 1998, El-Sadawy *et al.*, 1998, Hassanain *et al.*, 1997 and Glazer *et al.*, 2001). This finding might be attributed to the ability of heterorhabditid infective juveniles to penetrate through soft cuticle and thin membranes with the help of a terminal tooth in their head region (Poinar and Georgis, 1990). In most cases, strains virulent to one tick species and one stage were also highly virulent to other tick species and certain stages (Samish *et al.*, 1999). The mortality increased linearly up to 100% when the exposure time was increased.

TABLE (II)

Virulence of steinernematid nematode isolates at 1000 IJs/ml against the hard tick *B. annulatus* and the soft tick *A. persicus* throughout 3 days from the initial nematode infections.

Isolate[b]	Mortality %					
	Argas persicus[a]			*Boophlius annulatus*[a]		
	24[d]	48[d]	72[d]	24[d]	48[d]	72[d]
EGG4	33.33	33.33	80	0	66.66	93.33
PGfig1	6.66	73.33	100	20	66.66	100
PGfig2	53.33	73.33	100	20	20	20
EBN-Gauava	26.66	46.66	73.33	6.66	80	80
S. feltiae (sfn27)	26.66	80	80	33.33	73.33	96.66
Egs29	66.66	86.66	100	40	86.66	100
USA5-mon	46.66	66.66	93.33	60	100	-
BBNn3	53.33	73.33	100	0	20	60
EGB10b	26.66	80	80	20	60	100
EGB2	40	73.33	93.33	46.66	66.66	100
EGBa	60	86.66	100	40	73.33	86.66
S. riobravae	6.66	66.66	93.33	53.33	93.33	100
OBefc	0	46.66	80	0	33.33	86.66
EGS32b	26.66	60	60	26.66	53.33	76.66
PG cycamora	20	26.66	26.66	20	60	80
USA10	53.33	100	-	44	73.33	100
EGBats	46.66	100	-	53.33	86.66	100
I13m	0	40	60	40	73.33	93.33
LSD*	0.474					

*The LSD based on original data at 0.05 and 0.01 probability levels.

*a- NS, b (0.470, 0.596), ab (0.639, 0.843) c (0.639, 0.843), ac= NS, bc (0.783, 1.033) and abc (1.108, 1.48).

The differences in the ability of nematode strains to kill ticks in Petri dishes or in soil may be attributed to several factors, including the host finding capability of the nematodes in the soil where *B. annulatus* females release volatile attracting compound(s) and also secrete a water soluble nematode repellent. Ticks are also known to secrete allomones, including squalene, that repel predators (Yoder *et al.*, 1993). The fact that IJs

may at time survive for up to 6 days within the tick can possibly be explained by variability in the efficiency of some anti nematode factor(s) among individual ticks. It could also be attributed to variation in susceptibility of individual nematodes within the population or to the protection devices of some nematodes against lethal factors by their invasion into more protective organs in the tick.

TABLE (III)

Comparative test of two potent isolates of entomopathogenic nematodes against fed and unfed adults and nymphs of soft tick *Argas persicus* at different nematode concentrations after 48 hours from the initial nematode infection.

IJs Conc.	% Mortality											
	Heterorhabditis sp. (EBN48)						*Steinernema* sp. (USA5mon)					
	Unfed			Fed			Unfed			Fed		
	N[@]	M[#]	F[*]	N	M	F	N	M	F	N	M	F
100	0	8	52	8	36	72	0	4	16	4	16	44
200	0	24	68	24	48	88	0	16	28	12	28	56
300	4	48	80	48	60	96	4	36	44	28	48	68
400	12	56	88	56	76	100	8	44	62	44	56	76
500	20	68	96	68	80	100	24	52	76	52	72	80
600	48	80	100	76	96	100	32	76	80	72	92	100
LSD[**]	0.408											

[@] N = Nymphs, [#]M = Males and [*]F = Females.

[**]The LSD is based on original data at 0.05 and 0.01 probability levels.

a and b (SN), c (0.229, 0.303) d (0.162, 0.214), ad (0.229, 0.303), bd (0.229, 0.303), cd (0.398 , 0.525) and acd (0.563, 0.742).

TABLE (IV)

Sensitivity of fully engorged females of the hard tick, *B. annulatus* and the soft tick, *A. persicus* to different concentrations of *Steinernema*, EGB10b isolate after 48 hrs using multiwell plates (24 wells each) filled with filter paper.

Nematode IJs/tick	% Mortality	
	B. annulatus	*A. persicus*
60	33.33	20
120	46.66	53.33
180	73.33	86.66
240	93.33	100
300	100	100

Poiner and Thomas (1985) have shown in laboratory trials that *S. carpocasae* and *Heterorhabditis heliothidis* (= *H. bacteriophora)* are infective against aerial and ground spiders (Arachnida). Whereas thousands of steinernematid and heterorhabditid infective juveniles were needed to kill these spiders, the data show that as few as 1000 infective juveniles per female in petri dish produce >90% mortality of *B. annulatus* ticks. The results demonstrate that engorged *B. annulatus* females are highly susceptible to infection by steinernematid and heterorahbditid

nematodes compared with other non-insect hosts from the same class and similar to results obtained with susceptible insects (Poinar 1986).

TABLE (V)

Sensitivity of fully engorged females of the hard tick *B. annulatus* to different concentrations of EBN10k, a heterorhabditid isolate at two different times in a multi-well plate (24 wells) filled with sterilized sand.

Nematode IJs/tick	% Mortality	
	24	48
200	36	72
300	44	84
400	76	92
500	84	96
600	92	100

TABLE (VI)

Comparison between four strains of entomopathogenic nematodes against adults of the soft tick *A. persicus* and the hard tick *B. annulatus* at different nematode concentrations and time intervals.

IJs Conc.	Time elapsed	Corrected percent mortality							
		Steinernema spp.				*Heterorhabditis* spp.			
		EGG4		EGB10b		EGN10k		PGs	
		A*	B**	A	B	A	B	A	B
250	24	8	16	20	20	12	20	20	16
	48	24	36	32	36	28	40	36	36
	72	36	72	68	60	68	75	60	64
500	24	13	24	32	32	32	32	28	18
	48	39	48	56	44	60	60	56	48
	72	53	80	72	80	88	88	80	80
750	24	35	36	40	48	48	44	48	44
	48	65	64	72	64	76	80	80	64
	72	78	88	96	95	100	100	96	96
1000	24	67	56	74	65	68	68	64	56
	48	73	84	88	87	92	96	96	84
	72	85	100	100	100	100	100	100	100
1500	24	84	68	84	80	80	80	72	88
	48	92	100	100	100	100	100	100	100
	72	100	-	-	-	-	-	-	-
2000	24	90	82	100	100	85	100	87	95
	48	100	100	-	-	100	-	100	100
	72	-	-	-	-	-	-	-	-
LSD***		0.283							

*A = Argas, **B = Boophilus.

***The LSD is based on original data at 0.05 and 0.01 probability levels.

A (0.095, 0.125), c (0.11, 0.145), d (0.13, 0.177), ad (0.233, 0.307), bd (0.191 , 0.251), cd (0.269 , 0.355) and acd (0.467 , 0.61).

Although ticks are highly susceptible to nematode infection, they do not seem to be satisfactory hosts for the reproduction of those entomopathogenic nematodes studied. The reproduction and infective juvenile formation in non-insect hosts are rarely completed even when the host has been killed (Poinar, 1989). Furthermore, nematode development was inhibited by a host defense reaction of arthropods with low susceptibility such as millipedes (Poinar and Thomas, 1985).

A few days after juvenile nematodes penetrate or injected into ticks, all or most of them die inside their tick host. Although in rare cases they have survived as IJs or even started to develop within the tick but they never completed their life cycle (Hill, 1998). However, when the cuticle of ticks was slit artificially before their infection, the nematodes were able to complete their life cycle (Samish, personal communications).

TABLE (VII)

Comparison between four isolates of *Heterorhabditis* spp. against the soft tick, *A. persicus* with different nematode concentrations at three time intervals using one on one assay.

IJs/tick	Exposure time	Corrected percent mortality			
		EBN48	EBN7	EBN39	OB5o
100	24	10	15	12	18
	48	27	40	35	32
	72	52	58	60	52
200	24	14	22	27	27
	48	35	55	48	42
	72	63	70	80	72
300	24	22	34	42	38
	48	48	63	76	56
	72	72	85	95	88
400	24	26	45	58	54
	48	56	72	85	75
	72	85	100	100	100
500	24	34	57	73	67
	48	68	85	100	86
	72	93	100	-	100
600	24	45	65	85	82
	48	80	100	100	97
	72	100	-	-	100
LSD*	0.371				

*The LSD is based on original data at 0.05 and 0.01 probability levels.
a (0.155, 0.204), b (0.219, 0.288), ab (0.379, 0,5) c (0.179, 0.236) and ac (0.309, 0.409).

Determination of tick pH

Tick homogenate pH was estimated using phosphate buffer saline PBS buffer (pH 7.0). The estimated pH was 3.8; this degree of acidity is not suitable for the nematode to live in tick cadevar. The effects of soil pH on the survival and pathogenicity of both plant-parasitic nematodes and/or insect-pathogenic nematodes have been scarcely studied. Banage and Visser (1965) observed that *Dorylaimus* sp. survived a few minutes at a pH of 1-2.5, a few hours at pH 3.0-6.0, and several days at pH 6.5-10. Morgan and Maclean (1968) reported that *pratylenchus penetrans* survived over the pH spectrum of most agricultural soils from 5.1 to 6.5, and survived best at a pH of 5.5-5.8. No information is available on the effect of soil pH on survival and pathogenicity of entomopathogenic nematodes. In the study of Kung *et al.* (1990), steinernematid nematodes survival and pathogenicity decreased only slightly as the tested soil pH decreased from pH 8 to pH 4, but their survival drastically declined and they showed no ability to kill *G. mellonella* larvae at a pH of 10. Moreover, no marked nematicidal effect on the persistence of these two nematode species were observed when they were exposed to soils with a pH rang of 4-8. These findings are in agreement with previous studies on other plant-parasitic nematodes. They also suggested that steinernematid nematode persistence and efficacy is unlikely to be adversely affected at the pH spectrum of most agricultural soils (>pH 10). However, a nematicidal effect can be expected. In our study, steinernematid and heterorhabditid nematode survival decreased when tested with the tick homogenate. Our study suggested that the pH of tick homogenate which is 3.8 was not suitable for the living of the entomopathogenic nematodes. This result is in agreement with Banage and visser (1965). Engorged ticks contain concentrated vertebrate blood. As most nematodes were dead when the ticks were dissected and mixed with much blood, our visual technique to recover nematodes from the tick cadaver probably did not reveal all the existed nematodes.

REFERENCES

ABBOTT, W. S. (1925): A method of computing the effectiveness of an insecticide. *(J. Econ. Entomol. 18: 265 -267).*

ABDEL-KAWY, A. M. (1981): Physiological effects of entomophagous nematodes on their insect hosts. *(Unpublished M. Sc. Thesis, Fac. Agric., Cairo Univ).*

ABDEL-KAWY, A. M. (1985): Biological control of some lepidopterous pests by the nematode Neoaplactana carpocapsa and the physiological changes associated with it. *(Unpublished Ph.D. Thesis, Fac. Agric., Cairo Univ).*

AHMAD, N. M. (1992): Comparative studies on the effect of pesticides and the entomogenous nematodes in controlling some economic insects. *(Unpublished Ph.D. Thesis, Fac. Agric., Cairo Univ).*

AYAAD,T. H.; N. A.RASHDAN, F. K. ADHAM, R. M.GABRE and K. M. EL-KAMMAH. (2000): The immune defense system of the cattle tick *Boophilus annulatus* (Say) (Acari: Ixodidae): *I. microscopical* and ultrastructural characterization of hemocytes. *(Interat. J. Acarol. 26 (1): 3-9).*

BANAGE, W. B. and S. A. VISSER (1965): The effect of some fatty acids and pH on a soil nematode. *(Nematologica 11: 255-262).*

DUTKY, S.R.; J. V. THOMSON, and G. E. CANTWELL (1964): A technique for the mass propagation of the DD-136 nematode. *(J. Insect Pathol., 6 (4): 417-422).*

EL-SADAWY, H. A. and S. M. HABEEB (1998): Testing same entomopathogenic Nematodes for the biocontrol of *Hyalomma dromedrii* Koch (Acarina: Ixodidae). *(J. Union Arab Biol, Cairo 10A Zoology 1-11).*

EL-SADAWY, H. A.(1994): The use of nematodes in the biological control of some pests and parasites of farm animals. *(Unpublished Ph .D. Thesis, Faculty of Agriculture, Cairo university pp.186).*

EL-SADAWY, H. A. and R. M. ABDEL-RAHMAN (2001): Effect of different hosts on dispersal and host finding ability of Heterorhabditis nematode strain from Egypt. *(Int. J. Nematol.11: 63-68).*

GLAZER, I.; E. ALEKSEEV, and M. SAMISH (2001): Factors affecting the virulence of entomopathogenic nematode to engorged female *Boophilus annulatus* ticks. *(J. Parasitol. 87: 808-812).*

HASSANAIN, M. A.; A.A. DERBALA ; N.A. ABDEL-BARRY; M.A. EL-SHERIF and H.A. EL-SADAWY (1997): Biological control of ticks (Argasidae) by entomopathogenic nematodes.*(Egyptian J. Biological Pest Control. 7: 41-46).*

HILL, D. E. (1998): Entomopathoenic nematodes as control agents of deveopmental stages of the). Black-legged tick, *Ixodes scapularis. (J. Parasitology 84: (6) 1124 – 1127).*

HOOGSTRAAL, H.; H. Y. WASSEF and W. BUTTIKER (1981): Ticks (Acarina) of saudi Arabia- Fam. Argasidae, Ixodidae, *(Fauna of Soudi Arabia 3: 25-110).*

KAAYA G.P. (2004): Biocontrol of ticks using Entomogenous fungi; development of cheap fungal culture methods. *(Third African acarology symposium 11-15 January, Faculty of Agriculture Cairo Unive. Egypt).*

KOCAN, K.M.; M.S. PIDHERNEY, E.F. BLOUN, P.L.CLAYPOOL, M. SAMISH, and I. GLAZER (1998): Interaction of entomopahtogenic nematodes (Steinernematidae) with selected species of Ixodid ticks (Acari: xodidae). *(J. Med. Entomol. 3: 514-519).*

KUNG, S.; R.GAUGLER and H. K. KAYA (1990): Influence of soil pH and oxygen on Persistence of Steinernema spp. *[J. Nematology 22 (4): 440-445].*

MAULEON H., N. BARRE and S. PANOMA (1993): Pathogenicity of 17 isolates of entomopathogous nematodes (Steinernematidae and Heterorhabditidae) for the ticks *Amblyomma variegatum* (Fabricius), *Boophilus microplus* (Conestrini) and *Boophilus annulatus* (Say). *(Experimental and Applied Acaology 17: (11) 831-838).*

MORGAN, G.T. and A.A. MACLEAN (1968): Influence of soil pH on an introduced population of *Pratylenchus penetrans. (Nematologica. 14: 311-315).*

OYOUN, L.M.I. and S.M. EL-BISHLAWY (1990): Parasitic effects of *Heterorhabiditis heltiothidis* (Nematoda) on the mortality rate of certain acarines. Egypt. *(J. Wildflile and National Resources, 8 (19): 19 – 25).*

POINAR, G.O. Jr. (1986): Entomophagous nematodes. *[In Biological Plant and Health Protection (ed. J.M. Franz), pp. 95-121. G. Fischer Verlag, Stuttgart].*

POINAR, G.O. JR. (1989): Non-insect hosts for the entomogenous rhabditoid nematodes *Neoaplectana* (Steinernematidae) and *Heterorhabditidis* (Heterorhabditidae). *(Rev. Nematol. 12: 423-428).*

POINAR, G. O., JR., and G. M. THOMAS (1985): Laboratory nfection of spiders and harvestmebt (Arachnidae: Archaneae and Opiliones) with Neoaplectana and Heterorhabditis nematodes (Rhabditoidae). *(J. Archanology. 13: 297-302).*

POINAR, G. O. JR., and R. GEORGIS (1990): Charactarization and field application of *Heterorhabditis bacteriophora* strain HP88 (Heterorhabditidae: Rhabditida). *(Rev. Nematol. 13: 387-393).*

SAMISH M.; E. ALEKSEEV and I. GLAZER (1999a): Efficacy of entomopathogenic nematode strain against engorged *Boophilus annulatus* female (Acar: Ixodidae) under simulated field conditions. *(J. Med. Entomol. 36: 727-732).*

SAMISH, M. and I. GLAZER (1992b): Infectivity of entomopathogenic nematodes (*Steinernematides* and *Heterorhabditidae*) to females ticks of *Boophilus annulatus* (Arachnida: Ixodidae). *(J. Medical Entomology. 29: (4) 614-618).*

SNEDECOR, G.W. and W.G. COCHRAN (1980): Statistical Methods 7th ed. Iowa state Univ. *(Press Ames, Iowa, USA).*

SONENSHINE, D. E. (1991): The biology of ticks vol. *(1. Oxford University Press, New York).*

SONENSHINE, D. E. (1993): Biology of ticks, Vol. *(2. Oxford universtity Pres, New York).*

TAYSEER, M.A.H. (1980): Utilization of the nematode *Neoaplactana carpocapsae* in the biological control of the cotton lesfworm *Spodotera littoralis. (Unpublished M. Sc. Thesis, Fac. Agric., Cairo Univ).*

TAYSEER, M.A.H. (1984): Factors affecting potentialities of entomogenous nematdoesin the biological control of insect pest. *(Unpublished Ph. D. Thesis, Fac. Agric., Cairo Univ).*

WHITE, C.F. (1927): A method for obtaining infective larvae from culture. *(Science 66: 302-303).*

YODER, J.A., R.J. POLLACK and A. SPIELMAN (1993): An ant-diversionary secretion of ticks: first demonstration of an acarine allomone. *(J. Insect Physiol. 39: 429-435).*