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Characterization of Midgut and Salivary Gland Proteins of *Hyalomma dromedarii* Females Controlled by Entomopathogenic Nematodes

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Abstract: Biological control of hard ticks, *Hyalomma dromedarii* (Acari: Ixodidae) using entomopathogenic nematode of two families; Heterorhabditidae and Steinernematidae was studied. The protective effect of controlled ticks including haemolymph and haemocytes against these biological agents were also investigated. It was found that heterorhabditid strains cause a higher effect in biological control of engorged female *H. dromedarii* than those of stienernematid strains. It induced mortality rates ranged from 12-92% versus 4-88% for stienernematid strains. It was also found that these entomopathogenic nematodes can not reproduce within the exposed ticks. SDS-PAGE of proteins extracted from midguts and salivary glands infected with 4000 IJs tick^{-1} separated 21 and 25 protein bands versus 13 and 19 protein bands from non-infected ones, respectively. It was concluded that entomopathogenic nematodes of family Heterorhabditidae proved to have a potential acaricidal effect in the control of hard ticks. Moreover, the controlled ticks released unknown proteins in their haemolymph that may promote the haemocytes to phagocyte the nematodes as a type of defense mechanism.

Key words: Biological control, entomopathogenic nematodes, hard ticks, *Hyalomma dromedarii*, immune response

INTRODUCTION

Ticks are important acarines infesting most farm animals and poultry. Its obligatory blood sucker arthropods causing severe economic losses through blood feeding and destructing of skin which led to decrease in milk, meat yields and egg production. Moreover, the ticks are considered an important vector for transmission of many viral, bacterial, rickettesial and parasitical pathogens such as *Anaplasma* sp., *Babesia* sp. (Zayed, 2004). Ticks control has been affected primarily by application of chemicals which often produce undesirable effects. Acaricids require stringent application regimes where ticks develop resistance to them. The residual chemicals often cause environmental pollution, which may cause toxic reactions in domestic animals and the expense of this labor-intensive control method is enormous (Norval *et al*., 1992). Biological control has become a promising alternative for controlling ticks, since it minimizes the problems caused by chemical control and moreover, solves the problem of Acaricids resistance.

Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae have been used for biological control of several important insects of plant (Akhurst, 1983). Their pathogenicity depends in large part on a mutualistic nematode-bacterial complex (With *Xenorhabdus* and *Photorhabdus*, respectively) (Gaugler, 2002). Recent studies have shown that engorged females of some tick species are susceptible to infection with entomopathogenic nematodes (Samish and Glazer, 2001).

The aim of study is to define the nematode's recommendation dose which can be used for ticks biocontrol and characterization of midgut and salivary gland proteins of infected *H. dromedarii* females with entomopathogenic nematodes.

MATERIALS AND METHODS

Collection of ticks: One thousand engorged females of *Hyalomma dromedarii* (Acari: Ixodidae) were collected from sandy soil of resting house of naturally infested camels at Berkash market, Giza province, Egypt during summer of 2005. The ticks were identified according to description of Hoogstraal and Kaiser (1959).

Entomopathogenic nematode culture: Infective third stage juveniles (IJs) of eight different species of entomopathogenic nematode belonging to

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Heterorhabditidae and Steinernematidae families were maintained and at Parasitology and Animal Diseases Department, National Research Center which cultured in the last instar larvae of *Galleria mellonella* (L.) according to the method of Dutky *et al*. (1964). Steinernematid nematodes species were *Steinernema carpocapsae* DD136 (Sc)., *S. agriotes* (Sa), *S. riobrave* (Sr), *S. carpocapsae* All. Heterorhabditid nematodes species were *Heterorhabditis bacteriophora* HP88, *H. bacteriophora* (Eg1), *H. bacteriophora* (TWF) and *H. indicus* (RM1). The strains of Eg1, TWF and RM1 are of Egyptian isolates.

Experimental design: One thousand of healthy engorged females were first put individually in plastic pots (25 cm in diameter 2 cm in height) containing 10 g of clean, moistened and sieved sand (Kocan *et al*., 1998). These pots containing ticks were divided into 8 groups, each containing 125 pots. Each group was subdivided into 5 subgroups, each containing 25 pots. Each subgroup was divided into 5 replicates, each with 5 pots. The replicates of group 1 to group 2 were infected with infective juveniles of *S. carpocapsae* DD136 (Sc)., *S. agriotes* (Sa), *S. riobrave* (Sr), *S. carpocapsae* All, *H. bacteriophora* HP88, *H. bacteriophora* (Eg1), *H. bacteriophora* (TWF) and *H. indicus* (RM1), respectively at concentrations of 4000, 2000, 1000, 500 and 250 IJs which previously suspended in 1.5 mL tap water. The infection was applied by introducing the suspended infective juveniles on the ticks and moistened sand. After the infection, the pots were covered tightly with plastic lid and incubated at 25°C and 20% RH. A control replicate was treated with 1.5 mL of tap water only was also incubated at the same conditions. Mortality rates of tick were recorded at 2, 4 and 6 days post infection.

Collection of tick haemolymph: Fifty engorged female of ticks *H. dromedarii* were infected with 4000 IJs tick^{-1} of *H. indicus* RM1. The cuticles of the tested ticks were first cleaned with slightly moistened cotton. The haemolymph of ticks were collected using capillary tube with buffered anticoagulant (pH, 7.2) by made an incision of 2-3 mm long in the dorsum just posterior to the scutum (Roberta, 1970). The collected haemolymph was immediately examined using phase contrast microscope. Haemolymph control sample were collected from 20 non infected semi-engorged females ticks.

Collection of salivary glands and midguts: Salivary glands and midguts were separated from semifed *H. dromedarii* females collected from naturally infected camels as a method described by Purnell and Jouyner (1968). Female tick was first fixed at ventral surface in a wax-filled Petri dish and covered by thin layer of cold normal saline. Dorsal integument of the tick was then carefully removed under dissecting microscope using a fine sharp scalpel and finally the salivary gland and the midguts were separated

Preparation of salivary glands and midguts antigens: Thirty salivary glands and 10 midguts were separately homogenized in 1 mL of PBS (pH 7.2 in an ice bath). Suspensions of both salivary glands and midguts were centrifuged at 3000 rpm for 15 min at 4°C. The supernatant of each suspension was harvested as antigen and stored at -30°C until used.

Sodium dodecyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE): Haemolymph of infected (2 days PI) and non-infected *H. dromedarii* were electrophorsed by SDS-PAGE using a dis continuous gel system of Laemmli (1970). After staking, separating and simultaneously pouring gel, the comb was inserted by a slop way. The haemolymph antigens (50-100 μg lane^{-1}) were treated with the reducing buffer 12% SDS containing 0.7 M 2-mercoptoethanol, 5% glycerol and 0.001% bromophenol bleu) in the ration of 1:2. The treated antigens were immersed in a boiling water bath for 2 min to ensure protein denaturation. After polymerization of the gel (about 2 h) and removing of the comb, unstained protein molecular weight marker (Amersham Company, USA) and the treated antigens were loaded in the wells. A voltage of 100 v. was applied until the bromophenol blue had reached the bottom of the gel. The gel was then stained with 0.025% commassie blue (0.25 g L^{-1}) at room temperature, over night. To visualize the protein bands for each antigen, the gel was washed several times with destaining solution (45% methanol, 5% glacial acetic acid and 50% distilled water) until the back ground become completely clear. Finally, the gel was photographed.

Statistical analysis: The data were subjected to statistical analysis using t-test and F-test (one way classification least significant differences LSD) according to (Snedercor and Cochron, 1967). The significance of the main effects was determined by analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range test ($p<0.05$, 0.01). All analysis was made using a software package Costat, a product of Cohort Software Inc. Berkeley, California.

RESULTS

Effect of entomopathologic nematodes in control of engorged *H. dromedarii* females ticks (Susceptibility test): The results indicated that all heterorhabditis and stienernematid strains were found to be effective in the control of engorged *H. dromedarii* females causing variable mortality rates on the exposed ticks (Table 1). These mortality rates were increased as inoculum level and/or exposure time of applied nematode increased. Heterorhabditis strains showed to cause higher mortality rates than those of stienernematid strains. It induced mortality rates varied from 88-92, 52-92, 36-64, 12-52 and 12-36% versus 76-88, 24-68, 8-20, 8-16 and 4-16% for stienernematid strains at inoculum levels of 4000, 2000, 1000, 500 and 250 IJs tick^{-1}, respectively. Moreover, LD_{50} (460-1053) indicated also that heterorhabditis strains induce the highest effect as biologic agents especially *H. indicus* (RM1) in the control of this species of hard tick.

Entomopathologic nematodes infection of haemolymph and the mechanism of tick protection response against it: Phase contrast microscopy revealed that *H. indicus* RM1 larvae were first observed to penetrate *H. dromedarii* at 24 h post-exposure and remained inside it throughout the whole experiment (Fig. 1). The greatest numbers of nematode were observed in ticks haemolymph collected at 4 days post-exposure. Aggregations of nematode larvae were showed engulfed and encapsulated in phagocytic cells of haemolymph as a mean of protection. As a result of encapsulation, the nematodes can not reproduce inside the infected ticks as it occur in other insect larvae. Therefore, they did not emerge from the

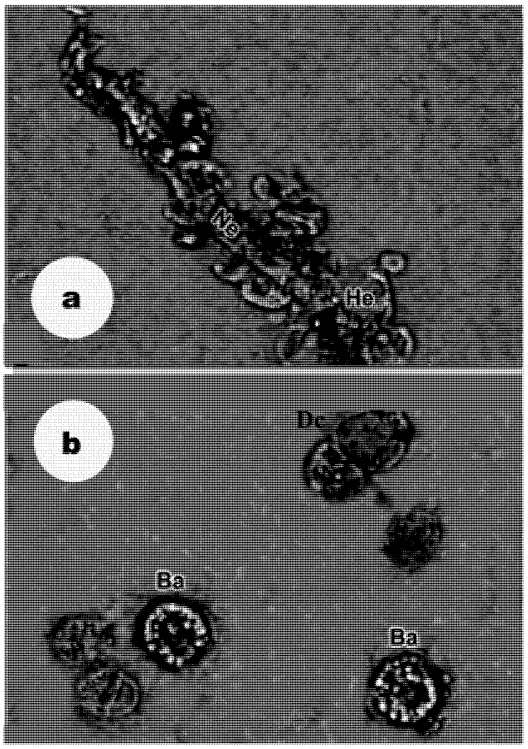

Fig. 1: Phase contrast micrographs of *Hyalomma dromedarii* haemolymph infected with *Heterorhabditis indicus* (IJs): A and B; Non infected haemocytes (Pr = Prohemocyte, Sp = Spherul cell, Pl = Plasmatocyte), C; The Capsule of haemocytes (He) around the nematode (Ne), D; The encapsulated bacteria, *Photorhabdus lummences* (Ba) within haemocytes; Dc, dividing haemocytes

Table 1: Effect of various nematode strains at different concentrations upon the lethal dose of 50 (LD_{50}) of engorged *H. dromedarii* females after 6 days of exposure

	Mortality rates							
	Entomopathologic nematode species							
Infection dose (concentration)	Heterorhabditis strains				Stienernematid strains			
(IJS tick^{-1})	RM1^{A}	HP_{88}^{A}	TWF^{B}	Eg1^{B}	All^{C}	Sa^{D}	Sr^{C}	Sc^{C}
4000	88.0^{a}	92.0^{a}	92.0^{a}	88.0^{a}	88.0^{a}	12.0	84.0^{a}	76.0^{a}
2000	92.0^{a}	80.0^{ab}	80.0^{a}	52.0^{b}	68.0^{a}	24.0	24.0^{b}	24.0^{b}
1000	64.0^{b}	64.0^{b}	56.0^{b}	36.0^{bc}	8.0^{b}	20.0	12.0^{bc}	20.0^{b}
500	52.0^{b}	36.0^{c}	12.0^{c}	40.0^{bc}	8.0^{b}	12.0	8.0^{bc}	16.0^{b}
250	36.0^{c}	36.0^{c}	12.0^{c}	28.0^{c}	4.0^{b}	12.0	8.0^{bc}	16.0^{b}
Control	0.0^{d}	0.0^{d}	0.0^{c}	0.0^{d}	0.0^{b}	0.0	0.0^{c}	0.0^{b}
LD_{50}	460.0	579.0	982.0	1053.0	1675.0	202E+05	2809.0	2809.0
F value	68.46	25.33	44.45	33.43	38.01	2.45	31.44	11.91
p-value	0.000*	0.000*	0.000*	0.000*	0.000*	0.064	0.000*	0.000*

RM1 = *Heterorhababditis indicus* (native nematode), HP_{88} = *H. bacteriophora* (imported nematode), TWF = *H. bacteriophora* (native nematode), Eg1 = *H. bacteriophora* (native nematode), All = *Steinernema carpocapsae* All (imported nematode), Sa = *S. agriotes* (imported nematode), Sc = *S. carpocapsae* DD136 (imported nematode), Sr = *S. riobravae* (imported nematode), A, B, C, D: Means significant between different doses for eight strains, a,b,c,d: Means significant between different doses for each strain, *: Highly significant p>0.05 non significant

Table 2: Electrophoretic salivary gland profiles of female *H. dromedarii* infected with *H. bacteriophora* HP88 by using Gel-Pro-Analyzer

	Normal		4th day post infection	
Band No.	MW (kDa)	Conc. (%)	MW (kDa)	Conc.(%)
1	241.16*	5.95	241.16*	5.410
2	95.98	2.93	153.73	11.580
3	78.30*	3.36	93.52	1.570
4	61.89*	2.08	84.29	3.030
5	55.16*	1.46	78.30*	1.726
6	52.02*	2.95	69.91	2.050
7	46.68*	3.37	61.89*	2.380
8	37.44	0.12	58.10	0.015
9	35.35*	0.28	55.90*	0.023
10	32.95*	3.80	52.20*	1.610
11	31.40	5.13	46.30*	1.840
12	27.46	15.40	42.90	3.270
13	17.82*	0.24	35.90*	1.320
14	14.12	0.76	33.45	1.030
15	11.68	2.01	32.14*	1.150
16	9.17	5.42	28.56	8.320
17	5.90*	1.94	23.30	6.101
18	4.48*	4.49	19.20	0.230
19	3.72*	18.98	17.30*	0.330
20	-	-	10.60	3.900
21	-	-	8.50	2.320
22	-	-	6.70	2.830
23	-	-	5.60*	1.560
24	-	-	4.40*	4.370
25	-	-	3.70*	15.300

MW: Molecular weight, kDa: Kilo Dalton Conc.: Concentration, *: Shared protein bands

nematode exposed ticks. Moreover, Symbiotic bacteria of nematodes were observed in tick haemolymph at 24 h post-exposure. The bacteria were first observed in the haemolymph and later in the degenerated tissues of all nematode exposed ticks. Cell phagocytosis and encapsulation were also observed around the symbiotic bacteria in tick haemolymphs. A clear space was often shown separating the nematodes from the surrounding tissues and bacteria. Nematode guts were filled with these bacteria that presumably served as a source of food for the nematodes.

Characterization of midgut and salivary gland proteins of *H. dromedarii* females

Salivary glands: SDS-PAGE of proteins extracted from salivary gland homogenates infected with 4000 IJs tick^{-1} of *H. dromedarii* females (Fig. 2) detected 25 protein bands (Table 2). The molecular weights of these bands ranged from 3-241 kDa. However, it was detected only 19 protein bands in non-infected salivary glands with molecular weights ranged also from 3-241 kDa. Twelve shared common protein bands were demonstrated between infected and non-infected salivary glands. The molecular weights of these shared bands were of 241, 78, 61, 55, 52, 46, 35, 32, 17, 5, 4 and 3 kDa (Table 2).

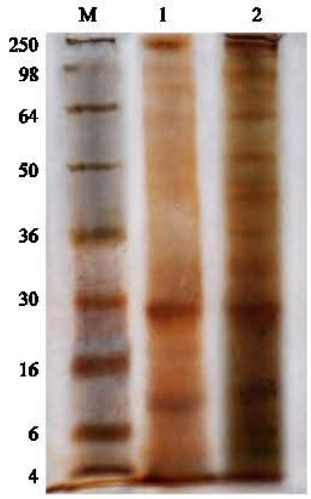

Fig. 2: SDS-PAGE electrophoretic profiles of female *H. dromedarii* salivary glands proteins; Lane M: Molecular weight markers, Lane 1: Non infected salivary glands, Lane 2: Infected salivary glands with entomopathogenic nematodes

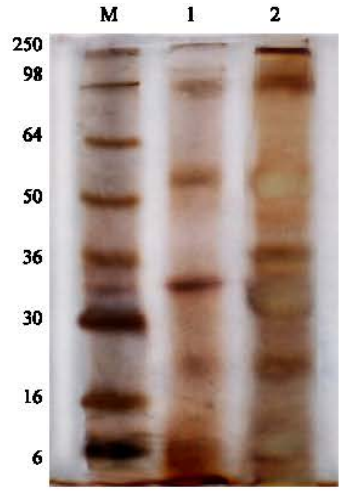

Fig. 3: SDS-PAGE electrophoretic profiles of female *H. dromedarii* midguts proteins; Lane M: Molecular weight markers, Lane 1: Non infected midguts, Lane 2: Infected midguts with entomopathogenic nematodes

Midgut: SDS-PAGE of proteins extracted from midgut homogenates infected with 4000 Ijs tick^{-1} of *H. dromedarii* females (Fig. 3) detected 21 protein bands versus 13 protein bands in the non-infected guts (Table 3). These protein bands of infected midguts exhibited great variations in their molecular weights which ranged from 4-233 kDa versus 3-250 kDa in non-infected

Table 3: Electrophoretic midgut protein profiles of female *H. dromedarii* infected with *H. bacteriophora* HP88 (4000 IJs tick^{-1}) by using Gel-Pro-Analyzer

	Normal		4th day post infection	
Band No.	MW (kDa)	Conc. (%)	MW (kDa)	Conc.(%)
1	250.000	6.8727	233.820	9.460
2	73.062	3.9180	104.780	10.470
3	48.917*	7.7869	91.967	8.130
4	41.659*	1.6233	74.870	0.530
5	35.552*	3.4632	66.437	0.130
6	33.576*	2.4010	61.464	0.033
7	31.766	10.3290	57.052	0.966
8	29.568	2.4750	48.820*	0.292
9	26.842*	1.3971	44.903	1.794
10	22.229	0.6985	41.549*	0.366
11	19.603	1.5482	35.900*	5.490
12	8.8206	7.6592	33.390*	2.520
13	3.7628	33.3590	32.070	4.090
14	-	-	30.370	5.610
15	-	-	26.560*	2.010
16	-	-	20.650	8.857
17	-	-	17.140	0.780
18	-	-	14.390	1.440
19	-	-	11.103	6.680
20	-	-	6.6056	7.210
21	-	-	4.405	3.690

kDa: Kilo Dalton, MW: Molecular weight, *: Shared Protein bands, Conc.: Concentration

ones. Five shared common protein bands with molecular weights of 48, 41, 35, 33 and 26 kDa were detected between the infected and non-infected guts (Table 3).

DISCUSSION

This study found that the engorged females of *H. dromedarii* were highly susceptible to infection by heterorhabditid nematodes than those belonging to steinernematid. Mortality rates of ticks were proportional to either the dose or exposure time of applied nematodes. The four tested heterorhabditis strains; *H. bacteriophora* HP88, *H. indicus* RM1, *H. bacteriophora* Eg1 and *H. bacteriophora* TWF were more virulent than Steinernematid strains; *S. carpocapsae* DD136, *S. riobravae*, *S. carpocapsae* all and *S. agriotes*. These results were evident in the higher mortality rates recorded after increasing exposure times and dosages of nematodes. These findings were in agreement with other studies reported by Hassanin *et al.* (1997), Kocan *et al.* (1998), El Sadawy and Habeeb (1998) and Glazer *et al.* (2001). The death of ticks was attributed to the fact that infective juveniles of nematodes carry a specific bacteria in their intestine. After the invasion of these juveniles into the tick body, they release its bacteria (*Photorhabdus* sp. for heterorhabditid and *Xenorhabdus* sp. for steinernematids) which multiply rapidly releasing large amount of protolytic enzymes that hydrolyses the tick protein and subsequently decreases the resistance of the tick to the bacteria causing death (Kocan *et al.*, 1998; Samish *et al.*, 2000; Vasconcelos *et al.*, 2004).

As reported by Samish and Glazer (1992), Glazer *et al.* (1991), Hassanin *et al.* (1997), Kocan *et al.* (1998) and present study found that the applied nematodes did not multiply in ticks and consequently not complete their life cycle inside the infected ticks. Therefore, there were no subsequent generations of infective juveniles releasing from the cadaver searching to infect new hosts. The most rapid mortality of engorged females *H. dromedarii* was obtained with infective juveniles of *H. bacteriophora* HP88, *H. indicus* RM1, *H. bacteriophora* TWF and *H. bacteriophora* Eg1. This finding might be attributed to the ability of heterorhabditid infective juveniles to penetrate through soft cuticles and thin membranes with the help of a cuticular tooth in their head region. These results were in accordance with the previous study of different tick models by Georgis (1990), Samish and Glazer (1992) and El Sadawy and Habeeb (1998).

Previous studies on ticks were also demonstrated that there were marked differences in the virulence of various applied nematode species when used as a biological mean against *A. americanum*, *I. scapularis*, *B. annulatus*, *H. excavatum* and *R. bursa* (Samish and Glazer, 1992; Glazer and Samish, 1993; Mauleon *et al.*, 1993; Zhioua *et al.*, 1995; Kocan *et al.*, 1998; Samish *et al.*, 1999, 2000). It was found that the strains of nematodes species were also varied greatly in their effectiveness against insects (Coroli *et al.*, 1996; Ricci *et al.*, 1996). Therefore, the factors influencing the mortality rates of ticks were probably included; dose and penetration rate of nematodes in the host haemocoel (Glazer, 1992; Coroli *et al.*, 1996; Ricci *et al.*, 1996), proliferation rate of nematodes within the host (Samish *et al.*, 2000) and finally efficiency of the ticks to protect itself against the nematode symbiotic bacteria and their deleterious secretions (Wang and Bedding, 1996).

The difference in nematode virulence is often attributed to the forgoing strategy of Lewis *et al.* (1992) and Campbell and Gauglar (1993). However, in the present study, both nematodes and ticks were placed in a close proximity so that the need to seek the target host was eliminated. Therefore, it may be speculated that penetration rate of nematodes and perhaps both rate of development and virulence of the symbiotic bacteria within the ticks played a major role in the nematode-tick interaction. These observations similar to that reported for Egyptian cotton leaf worm by Glazer *et al.* (1991).

The present study found that a high tick mortality at comparatively low concentration of heterorhabditid strains indicate high susceptibility to *H. dromedarii* and their developmental stages to a particular nematode strain. These findings further imply that the high mortality rates may be related not only to the concentration of nematodes, but also to other factors. Therefore, it was noteworthy that a complete mortality was recorded in the susceptible insects with 24-48 h of exposure at the same concentrations used in this study (Coroli *et al*., 1996). Moreover, our results revealed complete mortality of *H. dromadarii* females after 6 days. This delay may be attributed to that the ticks exhibited suboptimal conditions for the nematodes or symbiotic bacteria or both. This conclusion may similar to the fact that all studied tick species have not supported a full nematode life cycle (Glazer and Samish, 1993; Zhioua *et al*., 1995; Samish *et al*., 2000).

In general, the susceptibility of the different tick species studied here was far less affected by their particular species than by their developmental stages. However, very pronounced differences were demonstrated between engorged females of several other tick species in terms of susceptibility to nematodes (Mauleon *et al*., 1993; Samish *et al*., 1996; Kocan *et al*., 1998). The preimaginal stages of ticks are evidently far less susceptible to nematodes than are adults. *Ixodes scapularis* larvae and nymphs are totally resistant to nematode strains which killed the former's engorged females (Zhioua *et al*., 1995; Hill, 1998). These differences may be attributed to anatomical differences. Nematodes are known to enter insects via natural openings; anus, spiracles, mouth and genital apertures (Peters and Ehlers, 1994). Moreover, nematodes also are able to penetrate the ticks via thin areas of insect cuticle (Bedding and Molyneux, 1982). The relation between the penetration sites of the various nematodes and their efficiency in killing insects has been demonstrated (Glazer *et al*., 1991; Glazer, 1992). It was found that when the nematodes placed near ticks, it concentrate in the vicinity around their mouth, spiracles, anus and genital apertures (Kocan *et al*., 1998; Samish *et al*., 1999).

Results obtained by phase contrast microscopy clearly demonstrated that the nematodes penetrated engorged female *H. dromedarii*. Moreover, the released bacteria, *Photorhabdus* or *Xenorhabdus* were proliferated within the tick cadaver which showed to be the cause of tick mortality. Similar observations confirming these results were obtained by Samish and Glazer (1991), Kocan *et al*. (1998) and Samish *et al*. (2000). Therefore, it should be noted that the symbiotic bacterium that was released upon invasion into the haemolymph had a vital role in the pathogenic process.

Penetration route of entomopathogenic nematodes into ticks is still controversial. Samish and Glazer (1991) reported that nematodes entered *B. annulatus* via the mouth parts. However, for the same tick species, *B. annulatus*, genital opening was also suggested as another route of penetration (Mauleon *et al*., 1993). Similarly in insects, stienernematids IJs used oral and anal openings as penetration routes (Poinar, 1979). Furthermore, spiracles were considered as an alternative routes using dorsal labial tooth (Triggiani and Poinar, 1976). Heterorhabditis nematodes were suggested to penetrate the insect hemocoel through soft intersegmental areas of the cuticle (Bedding and Molyneux, 1982).

Present results demonstrated that the integumental thickness of the ticks were suggested to be another factor affecting the entrance of applied nematodes. The integument is thicker in late instars (adult males and females) than early ones (larvae and nymphs) where it increases after each molt. Therefore, size of natural openings was subsequently affected. It becomes narrower in late instars which hinders the entrance of applied nematodes. Moreover, the mechanical stretch of the cuticle in engorged ticks may affect the size of some natural openings. Therefore, it should be taken into account that large meals cause dramatic internal changes, integument thickness and cuticle plasticity (Triggiani and Poinar, 1976; Bedding and Molyneux, 1982; Kocan *et al*., 1998).

The present study focused on the time elapsed between application of nematodes and death of ticks as an important factor not only in terms of host-parasite interaction but also it indicates whether, or/and how long engorged female tick still could lay eggs after they drop off onto nematode infested soil. Moreover, it could also determine if, or how long, or both, various unfed ticks could serve as a vector of medical disease agents in nematode infested soil.

SDS-PAGE results of midguts and salivary glands of applied ticks showed a remarkable increase in protein bands if compared with non-infected control ticks. It reached 25 and 21 bands in infected salivary glands and midguts versus 19 and 13 bands in non-infected ones, respectively. These findings agreed with those reported by Saad and Kamal (1994), Hernandez *et al*. (1995) and Abdel Wahab *et al*. (1999). They showed that the gut and salivary gland SDS- dissociated proteins of *B. annulatus* and *R. sanguineus* were separated into 15 and 16 bands respectively. The protein bands post infection with nematode showed presence of additional bands. This may be attributed to the capability of the ticks to release a protein as a type of defense against applied nematodes. Similar changes were described post-injection of *Parasarcophage surcoufi* with *H. bacteriophora* HP88.

All of the isolated protein bands were replaced by new ones referred as an immune protein (Ayaad *et al*., 2001; El-Kady *et al*., 2005).

The obtained results demonstrated also absence of some bands in infected midguts and salivary glands if compared with non-infected ticks. This disappearance of some bands may be interpreted to that the applied nematodes secrete toxic materials and proteolytic enzymes which hydrolyse protein of the tick. This run in full agreement with Poinar (1979), El Sadawy (1994) and Boemare (2002). They reported that the infective juvenile penetrates insect host through natural body openings such as the mouth and spiracles. Once inside the host, species-specific, symbiotic bacteria (*Xenorhabdus* sp. for *Steinernema* sp. and *Photorhabdus* sp. for *Heterorhabditis* sp.) are released from the gut into the insect body cavity causing a bacterial septicemia. In response, the combination of nematode/bacterial virulence factors kills the host within 24-48 h.

Furthermore, the findings of the present study demonstrated the presence of 5 common shared protein bands between infected and non-infected midgut. Also, there were 10 common shared protein bands between infected and non-infected salivary glands. These shared bands can be considered as the specific protein bands of midgut and salivary gland. This is in agreement with that reported by Hernandez *et al*. (1995) who studied SDS-PAGE proteins of eggs, larvae, nymphs, male and female salivary glands and midgut extracts of *R. sanguineus*. A common band of molecular weight higher than 250 KDa was observed, although with different intensity (Hernandez *et al*., 1995).

In conclusion, entomopathogenic nematodes, belonging to the families Steinernematidae and Heterorhabditidae, are complex nematode-bacteria with a broad insect-host range. The effectiveness of these biological agents in killing the parasites was mainly ascribed to the septicemia induced by massive release of symbiotic bacteria, in the latter stage of the infection (Samish and Glazer, 2001; Brivio *et al*., 2004). The insect-nematode system studied by Brivio *et al*. (2004) provided unique opportunities for studying the interactions of insect immunity with immune-suppressive nematodes. In particular, the immunoevasion mechanisms involving parasite body surface through which they are able to escape host immune recognition (You, 2005; Imamura *et al*., 2005; Dinglasan *et al*., 2005).

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