Pathogenicity Evaluation of Eight Egyptian Isolates of Entomopathogenic Nematodes against Two Tick Species; *Argas persicus* and *Boophilus annulatus*

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ABSTRACT

Eight Egyptian isolates of entomopathogenic nematodes (EPN), four belong to the genus *Heterorhabditis* and four belong to *Steinernema* were evaluated against adult females of the soft tick *Argas persicus* and engorged females of the hard tick, *Boophilus annulatus* in the laboratory. Results indicated that two EPN isolates (A4 and 10k) of *Heterorhabditis* were rapidly fatal and have high pathogenicity (100% mortality after 72 hours) against *A. persicus* when treated with 1000 nematode Infective Juveniles (IJs/ml). Meanwhile, only one isolate (5B) of *Steinernema* gave accumulated mortality of 93.33% against *A. persicus*. Furthermore, *Heterorhabditis* isolates (10 K and A4) caused 93.33% and 86% mortality to *B. annulatus*. The most effective EPN isolates of *Steinernema* were 5B and AT4 against *B. annulatus*, they both gave ca 86.7% mortality. The LC_{50} values of *Heterorhabditis* spp. (A4 and 10K isolates) after 72 hrs post infection and LT_{50} were (27.03 and 257.03 IJs/ml) and (11.94 and 19.2 hrs.), respectively against *A. persicus*. While, the LC_{90} was 360.24 and 765.72 IJs/ml for A4 and 10K isolates, respectively. Meanwhile, A4 and 10K isolates exhibited relatively high values of LC_{50}, LC_{90} and LT_{50} against *B. annulatus*, represented by (358.57±0.71 and 812.83 IJs/ ml); (1258.9 and 2485.9 IJs/ml.) and (25.64 and 51.28 hrs.), respectively. The present study cleared that Entomopathogenic nematodes (EPN) isolates of the genus *Heterorhabditis* spp. (A4 and 10K) are potentially useful biological tools for tick control.

Keywords: Biological control, *Argas persicus*, *Boophilus annulatus*, Entomopathogenic nematode, *Heterorhabditis* spp., *Steinernema* spp.

INTRODUCTION

Soft and hard ticks are very serious obligate hematophagous ectoparasites of birds and mammals in all worldwide subtropical countries. Tick problems and diseases transmitted by ticks are one of the main difficulties in these countries for the development of livestock breeding (Guglielmone *et al.* 2003). They cause direct damage to their animal hosts, since they are vectors of many varieties of human and animal pathogens. consider the most important vectors of pathogens (viruses, bacteria, and protozoa) affecting human and cattle worldwide (Peter, 2005; Jongejan and Uilenberg 2005 and De la Fuente *et al.* 2008). Tick control is difficult for a number of reasons; ticks lay huge number of eggs, have more than one developmental stage and parasitize numerous hosts. Chemical control with acaricides considered the best method but development of resistant ticks to these acaricides (Foil *et al.* 2004) remains a big problem. Furthermore, adverse effects of chemical acaricides (acute and chronic toxicity) on the hosts, contamination of animal and plant products, such as milk and meat (George *et al.* 2008) have worsen the problem. So, the development of alternative safe methods for tick controls becomes necessary. Among biocontrol agents tested against soft ticks were entomopathogenic nematodes (EL-Sedawy and Habib 1998, EL-Sadawy and Abdel-Shafy 2007, Basu *et al.*, 2012) and fungi (Samish *et al.* 2004, Ostfeld *et al.* 2006, Samish *et al.* 2008).

Entomopathogenic nematodes exhibited high efficiency against Ixodidae and Argasidae (Kaaya *et al.* 2000, Hussaini 2001) and showed highly virulent towards mature and immature stages of soft tick, *Argas persicus* in the laboratory (EL-Sedawy and Habib 1998) The efficiency of EPN against the Egyptian cattle tick *B. annulatus* in the laboratory and the field is poorly studied. Thus, this work was conducted to shed light on the susceptibility of two tick species to eight indigenous nematode isolates in the laboratory and to determine the potent nematode isolates as well as the most effective concentration required to apply in the field.

MATERIALS AND METHODS

The experiments were carried out at the laboratory of the Applied Center for Entomonematodes (ACE), Department of Agricultural Zoology and Nematology, Faculty of Agriculture, Cairo University and repeated in Plant Protection Dept., Faculty of Agriculture, Minia University at the same laboratory environment.

1. Animal sources:
Ticks;

The ticks, *Boophilus annulatus* adults were collected as engorged females from the ground below the cows' in their cattle farms in Giza Governorate. The collected ticks were incubated at 25 °C and 75 % RH for laboratory experiments. Semi and fully engorged adult of *Argas persicus* were collected from the chicken breeding houses at Shosha region, Minia governorate. Ticks were identified according to (Hoogstraal *et al.* 1981).

Nematode propagation:

The eight nematode isolates used in this work were propagated and reared on last instar larvae of *Gallaria mellonella* L. (Lepidoptera: Pyralidade) (Ehlers 2001; EL Roby 2011 and 2018). Last instar larvae of *G. mellonella* were reared on old bee wax at 28±2 °C and relative humidity 65±5 % in the insect rearing laboratory. The emerging infective juveniles (IJs) were harvested from nematode traps and stored in sterilized water at 10°C (Yadav and Lalramliana 2012). All emerged nematode infective juveniles (IJs) were stored at 8°C and used within a range of two weeks of nematode harvest.

1. Evaluation the virulence of EPN isolates against tested ticks:

To evaluate the virulence of all tested nematode isolates against engorged females of *B. annulatus* and adults of *A. persicus*, ten females placed in each of 5 cm diameter Petri dishes with filter paper and infected with 1000 IJs of nematodes isolated in 1 ml. of distilled water (Samish and Glazer 1992) under laboratory conditions. Each treatment was replicated three times (three Petri dishes/ treatment). The experiment was repeated twice. All the treatments were incubated at 25 °C and 75% RH. Petri dishes were checked after 24, 48 and 72 hrs post application and mortality was recorded on each interval. Mortality percentage was corrected using Schneider-Orellis formula (Puntener 1981) at each intervals.

$$\text{Corrected Mortality \%} = \frac{(\text{Mortality \% in treatment} - \text{Mortality \% in control})}{100 - \text{control mortality}} \times 100$$

The virulence of all tested nematode isolates against engorged females of *B. annulatus* and adults of *Argas persicus* was assessed as corrected mortality %. Completely randomized design was used. ANOVA test was applied and means of mortality were differentiated with the Least Significant Differences (LSD) test.

2. Susceptibility of *B. annulatus* and *Argas persicus* females to A4 and 10K isolates.

Based on the corrected mortality % obtained in the first experiment, the lethal concentration values (LC_{50}, LC_{90} and LC_{99}) of the most virulent strains (A4 and 10K) as well as the time required to kill 50% of the tick population (LT_{50}) were determined.

Estimation the lethal concentration values (LC50, LC90 and LC99):

Suspension of infective juveniles from two nematode isolates of *Heterorhabditis* spp. (A4 and 10K), were poured into three 5-cm diameter Petri dishes with filter paper. The suspension contained various concentrations of infective juveniles i.e. 100, 200, 300, 400, 500, and 1000 IJs per replicate on soft ticks and 250, 500, 750, 1000, 1500 and 2000 IJs per replicate on hard ticks and the control treatment was done with distilled water without nematodes. Ten fully engorged females of *B. annulatus* or *A. persicus* were placed in each Petri dish (replicate). Each treatment was replicated three times (three Petri dishes/ treatment). All the treatments were incubated at 25 °C and 75% RH. The mortality percentage was determined daily for three successive days. The experiment was repeated two times. Corrected mortality means were subjected to the Probit analysis to calculate the lethal concentrations (Finney 1971) after 72 hrs.

Estimation the LT50 value:

To evaluate the time required to kill 50% of the tick population (LT_{50}) due to the impact of the selected nematode isolates as the most potent nematodes. The same technique was adopted. Results were taken after 2, 6, 12, 16, 24, 36 and 72 hours with a volume of 1.0 mL. of aqueous suspension per Petri dish with a concentration of LC_{99} for each strain. Three replicates were used for each treatment. The experiment was replicated twice. Corrected mortality means and log time were subjected to the Probit analysis to calculate (LT_{50}) (Finney 1971).

RESULTS AND DISCUSSION

Eight isolates of local entomopathogenic nematodes belong to the genera *Heterorhabditis* and *Steinernema* were tested against the females of the soft tick, *A. persicus* and the hard tick, *B. annulatus* four of these EPN isolates belong to *Heterorhabditis* spp. (A4, SNAB, 10K and S4) and the other four belong to *Steinernema* spp. (5B, AT4, B32 and 5S). All these isolates were ranked according to their virulence which measured by their accumulated mortality percentages within three days following nematode infections (Tables 1 and 2). Data in Table 1 showed that, the most potent isolates belong to the genus *Heterorhabditis* were A4 and 10K. They highly affected the soft tick, *A. persicus*, and caused 100% mortality after 72 hours post nematode treatments, followed by S4 which gave accumulated mortality of 93.33 % after 72 hrs with no significant differences between them. In addition, isolates A4 and 10K showed rapid pathogenicity against *A. persicus* (86.7 and 83.3 respectively) after 24 hours of infection. While the lowest virulent isolate of *Heterorhabditis* spp. was, S4 since it caused 26.7% and 43.77% mortality after 24 hrs and 48 hrs post treatment (Table1). Two strains belong to genus *Steinernema* (5B and 5S) both recorded 93.33 % mortality after 72 hrs post treatment of *A. persicus.*

Table 1. Virulence of entomopathogenic nematode isolates at 1000 IJs/ml against the soft tick *A. persicus* throughout 3 days post nematode infections.

Nematode genera	Strain	Mortality% post treatment intervals (hrs.)		
		24	48	72
Heterorhabditis	A4	86.7±11.5	93.3±11.5	100.0±0.0
	SNAB	53.3±11.5	80.0±10.0	93.3±11.5
	10K	83.3±15.3	93.3±11.5	100.0±11.5
	S4	26.7±11.5	43.7±05.8	93.33±5.7
Steinernema	5B	40.0±10.0	80.7±11.5	93.33±11.5
	AT4	30.0±10.0	66.7±11.5	80.0±0.0
	B32	26.7±11.5	46.66±8.5	60.0±0.0
	5S	40.0±00.0	86.7±11.5	93.3±11.5
LSD (.01)***		16.27	23.51	21.54

*Differences between means are highly significant according to the LSD test.

As shown in Table (2), the most potent isolates tested belong to *Heterorhabditis* spp. against *B. annulatus* were 10K and A4 isolates, they showed accumulated mortality of 93.33 and 86 % after 72 hours post nematode exposure, respectively. While the most effective isolates from *Steinernema* against *B. annulatus* were (5B and AT4), both gave accumulated mortality of (86.7 %.). Moreover, the least virulent species from *Heterorhabditis* was S4 it gave mortality of 10, 33.3 and 76.7% after 24, 48 and 72 hrs, respectively. The lowest mortality rate was recorded with isolate (5S) against *B. annulatus* which gave 3.33, 6.7 and 23.33% mortality after 24, 48 and 72 hrs following the exposure to

nematodes. In contrast, the best isolates of *Steinernema* tested against the hard tick *Boophilus annulatus* were (5B and AT4). They gave 86.7% accumulated mortality after 72 hrs post nematode infection. Highly significant differences were recorded between all nematodes isolates used to kill ticks and the control at all times of exposure. Data in Table 1 and 2 demonstrate that, the mortality percentages caused by *Heterorhabditis* isolates increased with exposure time to reach 100,100, 93.33 and 93.33% after 72 hrs of exposure to A4, 10K, SNAB and S4 nematode isolates, respectively. While mortality rate using nematode isolates of *Steinernema* spp. never reached 100% through the three days of nematode exposure to the ticks.

The LC_{50} and LC_{90} values of the tested isolates A4 and 10K against *A. persicus* and hard tick, *B. annulatus* females are given in Table (3) together with their Slope (b) of LDP Line.

In respect to LC_{50} and LC_{90} values: The obtained data revealed that A4 isolate had the highest virulence on both *A. persicus* and *B. annulatus* females, presenting LC_{50} equal to 27.03 (18.69- 39.04) and LC_{90} equal to 103.18 IJs/ml. (for *A. persicus*), while, it was significantly less virulent against *B. annulatus* with LC_{50} value of 360.24 (311- 413.2) IJs/ml. and LC_{90} equal to 1258.9 IJs/ml. On contrary, 10K isolate was significantly less virulent against the soft and hard ticks (LC_{50} was 257.03 (213.7-322.8) and LC_{90} of 765.72 (for *A. persicus*) and LC_{50} 812.83 IJs/ml (657.8-1079.2) (for *B. annulatus*). Also, 10K isolate was the least virulent, with LC_{50} value of 812.83 (657.8- 1079.2) IJs/dish against *B. annulatus* females. Based on these results, the (A4) isolate of *Heterorhabditis* sp. considers a

promising candidate as a biological control agent against both soft and hard ticks.

The third assay confirmed the quick pathogenicity of the isolates (A4 and 10K). LT_{50} and LT_{90} values of A4 and 10K isolates against *A. persicus* and *B. annulatus* were estimated and presented in Table (4). The obtained data cleared that the two tested isolates are promising ones for controlling both soft and hard ticks, especially A4 isolate. It was the fastest and potent one against ticks, which gave rapid pathogenicity of LT_{50} (11.94 hrs.) and LT_{90} (54.59 hrs.) in case of *A. persicus* and 25.64 and 104.48 hrs. in case of *B. annulatus*. These values were 19.2 and 104.48 and 51.28 and 626.05 hrs. for the other (10K) isolate in the case of *A. persicus* and *B. annulatus,* respectively.

Table 2. Virulence of Entomopathogenic nematode isolates at 1000 IJs/ml against the hard tick *B. annulatus* throughout 3 days post nematode infections

Nematode genera	Strain	Mortality% post treatment intervals (hrs.)		
		24	48	72
Heterorhabditis	A4	80.0±10.0	80.0±10	86.7±5.8
	SNAB	40.0±10.0	40.0±17.3	83.3±11.5
	10K	33.3±17.3	66.7±11.5	93.33±5.8
	S4	10.0±00.0	33.3±5.8	86.7±20.5
Steinernema	5B	30.9±17.0	53.3±10.0	86.7±11.5
	AT4	36.7±11.5	60.0±05.8	86.7±20.5
	B32	13.3±5.9	60.0±11.5	80.0±10.0
	5S	3.33±5.8	06.7±05.7	23.33±5.7
LSD (.01)***		16.17	29.68	22.54

***Differences between means are highly significant according to LSD test.**

Table 3. LC50 and LC90 of *Heterorhabditis* isolates (A4 and 10K), when treated with LC99 against the non-fed and engorged females of the soft tick, *Argas persicus* and the hard tick, *Boophilus annulatus*.

Tested tick species	Nematode isolates	Slope (b) of LDP Line	LC50 (IJs/female)	LC90 (IJs/female)
Argas persicus	A4	2.12	27.03 (18.69 - 39.04)	103.18
	10K	2.7	257.03 (213.7-322.8)	765.72
Boophilus annulatus	A4	2.12	360.24 (311.1-413.2)	1258.9
	10K	2.68	812.83 (657.8-1079.2)	2485.9

Table 4. LT_{50} and LT_{90} of *Heterorhabditis* isolates (A4 and 10K), when treated with LC99 against the non-fed and engorged females of the soft tick, *Argas persicus* and the hard tick, *Boophilus annulatus*.

Tested tick species	Nematode isolates	Slope (b) of LDP Line	LT50 (hours)	LT90 (hours)
Argas persicus	A4	1.94	11.94 (9.22 - 15.2)	54.59
	10K	2.12	19.2 (16.39- 22.9)	73.77
Boophilus annulatus	A4	2.1	25.64 (19.66 - 39.92)	104.48
	10K	1.18	51.28 (44.27 - 83.72)	626.05

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 and/or penetrate between mouthparts. The eight tested nematode isolates for their anti-tick activity have shown varying degrees of virulence. In our laboratory tests, heterorhabditid isolates were generally more virulent to ticks than steinernematids. Similar conclusion was obtained by (Kaya *et al.* 2000) that nematodes are virulent against engorged females of the tested African tick species and they attracted towards natural openings in the ticks' body or attempted to penetrate between mouthparts. However, the nematodes mode of invasion has not yet been established. In contrast, a study on *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 entomopathogenic nematodes (EL-Sadawy and Abdel-Shafy 2007) (EL- Sedawy and Habib 1998, Hassanain *et al*. 1999, Glazer *et al*. 2001). This differences may be attributed to the ability of heterorhabditid infective juveniles to penetrate through soft cuticle and thin membranes with the help of a the nematode terminal tooth in their head region (Georgis and Gaugler 1991). The differences in the ability of nematode strains to kill ticks in Petri dishes may be attributed to several factors, including the host finding capability of the nematodes where *B. annulatus* females release volatile attracting compounds and also secrete a water soluble nematode repellent. Ticks are also known to secrete allomones, including squalene, that repel predators (Yoder *et al*. 1993, Samish *et al*. 2008). The fact that nematode Infective Juveniles (IJs) may at time survive for up to 4-6 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 of some nematodes against lethal factors by their invasion into more protective organs inside the tick. (Poinar 1989; and Samish *et al*. 2004) indicated that *S. carpocasae* and *H. bacteriophora* are infective against aerial and ground spiders (Arachnida). Whereas thousands of Steinernematidis and Heterorhabditidis infective juveniles were needed to kill these spiders, the data show that as few as 100 infective juveniles per female in petri dish produce 100% mortality of soft ticks and ca 85% of hard ticks. The results demonstrate that A. *persicus* and *B. annulatus* females are highly susceptible to infection by heterorahbditid isolates of (A4 and 10K) and less susceptible to steinernematid isolates (5B and 5S) compared with other non-insect hosts from the same class. Our current data are similar to the results obtained with some nematode susceptible insects (Gaugler 2017)

Although ticks are highly susceptible to nematode infection, they do not seem to be satisfactory hosts for the reproduction of those tested entomopathogenic nematodes. 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 (Samish and Glazer 1992). As observed 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 (Benjamin *et al*. 2002).

Testing tick–nematode interactions, EPN penetrate engorged females of *B. annulatus* tick almost solely via the anus or genital pore (unpublished observations). We also observed that heterorhabditid nematodes killed engorged *A. persicus* and *B. annulatus* females in Petri dishes after less than 12 hrs of exposure, whereas Steinernematidis nematodes needed more than 12 hrs to penetrate into ticks (Glazer *et al*. 2001). The injection of a single heterorhabditid nematode into a tick can cause mortality (Glazer *et al*. 2001). Whereas, the doses of EPN needed to kill 50 or 90% of ticks are comparable to that used commercially in the control of plant insect pests, but the time required to kill ticks is often relatively long (Table 4). Tick mortality caused by EPN seems to be due to the rapid proliferation of the nematode associated symbiotic bacteria within the ticks body, since the nematodes do not go through their natural cycle inside ticks, and most infective juveniles die shortly after entry (Hassanain *et al*. 1999; Glazer *et al*. 2001; Samish *et al*. 2004; Freitas-Ribeiro *et al*. 2005; and Gaugler 2017). *In vitro* experiments demonstrated that tick hemolymph hinders the growth of EPN (Zhioua *et al*. 1995), but the reason(s) for nematode mortality within ticks is not yet fully understood. Interestingly, when the cuticle of *I. scapularis* was physically slit before nematode infection, the nematodes *S. carpocapsae* and *S. glaseri* reproduced successfully (Zhioua *et al*. 1995).

Female ticks were killed up to six times quicker (the LT_{50} was one day for *R. bursa*) than engorged ticks (6 days for *R. bursa*) (Samish *et al*. 2004). This may be connected to the strong anti-bacterial activity of the tick haemolymph (Caroli *et al*. 1996). The eight nematode isolates tested for anti-tick activity showed varying degrees of virulence, however, Heterorhabditidis nematodes were generally more virulent to ticks than Steinernematidis. This fact was proven in the current results and corroborate in several publications by (Hill 1998, Glazer *et al*. 2001, Freitas-Ribeiro *et al*. 2005, El-Borai *et al*. 2007, George *et al*. 2008, Morton and Del Pino 2008, El-Borai *et al*. 2012, Gassmann *et al*. 2012, Laznik and Trdan 2016, Memari *et al*. 2016, Gaugler 2017). Nematode isolates virulent to one tick stage of one certain tick species were found, in most cases, to be also highly virulent to other tick species and stages (Kaya *et al*. 2000, Glazer *et al*. 2001, Samish *et al*. 2004). Our data confirmed the potentially of local EPN as useful biological tools for tick control because: engorged ticks are susceptible to some EPN and also reside in locations that are preferred by many EPN isolates; in addition immobile ticks attract mobile nematodes. However, the use of nematodes may be limited to defined ecological niches because their pathogenicity is reduced by low humidity and/or temperature, high concentrations of animal manure or silt, and by differences in the susceptibility among the various tick stages and species. The wide genetic variation found among many nematode isolates, and presumably in nematode isolates yet to be found in the future, means that genetic manipulation of nematodes could increase the range of ecological conditions in which they could be successfully applied against ticks. The development of improved commercial formulations is also important. Finally, in-depth studies are needed to clarify the interactions between nematodes and their tick hosts in the field.

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التقييم المعملي للقدرة المرضية لثمان عزلات محلية من النيماتودا الممرضة Entomopathogenic nematodes لنوعين من القراد المصري Argas persicus والقراد الجامد Boophilus annulatus

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تم تقييم ثماني عزلات مصرية محلية من النيماتود الممرضة (EPN) (أربعة تنتمي إلى جنس Heterorhabditis وأربعة إلى Steinernematids من القراد اللين Argas persicus والقراد الصلب ، Boophilus annulatus تحت ظروف المعمل. وأظهرت النتائج أن عزلتين A4 و 10K، لهما كفاءة ممرضية عالية وسريعة ضد الإناث البالغة للقراد اللين A. persicus والذي أعطى نسبة موت تراكمي 100% بعد 72 ساعة عند المعاملة بـ 1000 طوريرقي معدي /مل في حين أن عزلة واحدة فقط من عائلة (5B) Steinernematidae سجلت نسبة موت متراكمة (93.33 %) ضد A. persicus. وأظهرت العزلة 10K والمنتمية للجنس Heterorhabditis اعلي نسبة موت تراكمي بعد 72 ساعة ضد الأناث من القراد الجامد B. annulatus (93.33 %) تليها العزلة A4 ايضا من جنس Heterorhabditis ضد B. annulatus (موت تراكمي 86%) واوضحت النتائج أن السلالات الأكثر فعالية من steinernematids ضد B. annulatus القراد الجامد والتي أظهرت نسبة موت تراكمي خلال 72 ساعة 86.7 % العزلتان (5B , AT4) . وبلغت قيمة الجرعة النصف مميتة LC50 لعزلتين من Heterorhabditis spp (A4 و 10K) والتي تم تقديرها بعد 72 ساعة (27.03 و 257.03 / IJs ml علي التوالي) والزمن اللازم لقتل 50% من الأفراد LT50 ضد القراد اللين A. persicus (11.94 و 19.2 ساعة) على التوالي لكلا السلالتين.بينما كانت قيمة الجرعة القاتلة لـ90 % من الأفراد المعرضة (LC90) 360.24 و 765.72 /IJs مل، على التوالي. بينما أظهرت نتائج التقييم الحيوي علي القراد الجامد لكلا السلالتين أن قيم Lc50 بلغت 358.57 و ml/IJs 812.83 و LC90 كانت 1258.9 و ml/IJs. 2485.9 وقيم LT50 كانت 25.64 و 51.28 ساعة. للسلالتين على التوالي ضد B. annulatus. أشارت النتائج إلى أن النيماتودا الممرضة للحشرات (العزلات) Heterorhabditis spp (EPN) A4و K 0 1) تعتبر عناصر بيولوجية ناجحة لمكافحة القراد.

الكلمات المفتاحية: المكافحة البيولوجية ، النيماتودا الممرضة للحشرات ، Heterorhabditis ، steinernematids ,القراد اللين Argas persicus والقراد الجامد Boophilus annulatus