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Biocontrol potential of entomopathogenic nematodes against camel tick, *Hyalomma dromedarii* (Acari: Ixodidae)

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ABSTRACT

Entomopathogenic nematodes (EPNs) are a well-established biocontrol agent for insect pests. Using such a bio-agent to eliminate ticks has only been reported in a few cases. The current study intended to determine how the camel tick, *Hyalomma dromedarii* Koch, 1844 (Acari: Ixodidae), engorged female, was affected by various concentrations and timing of exposure to infected juveniles of five EPN isolates from two species. The six groups of engorged females, each with 25 ticks, were subjected to five concentrations of 50, 100, 150, 300, and 600 infective juveniles (IJs)/female in order to perform the work. Female mortality was observed every day for up to 72 h. The groups' average weights were statistically equivalent. The following biological parameters: initial weight of the female (mg); pre- and oviposition periods (days); survival period (days); initial weight of the egg mass (mg); hatching percentage (%H); nutritional efficacy index (NEI); and the control efficacy (%) of the engorged females as influenced by *Heterorhabditis indica* NEM-23 and *H. bacteriophora* NEM-26 infections were also measured. The results showed a linear increment in female mortality with the increase in EPN concentrations. Three days after being exposed to *H. bacteriophora* at 300 or 600 IJs/Female, all *H. dromedarii* individuals died, while the group containing 50 IJs/Female of the same EPN reached 75 %. All biological parameters were altered by EPNs, and there were extremely noticeable variations between the concentrations and the untreated group. These findings indicate that both *Heterorhabditis* isolates acted negatively on the parameters that were being observed, encouraging one to believe that this EPN was effective in controlling engorged females of *H. dromedarii* in the laboratory.

1. Introduction

Vectors and vector borne diseases could pose a severe hazard to people, animals, and wildlife all over the world (Guglielmone et al., 2015, Dantas-Torres et al., 2012). Ticks are second-most among viral, bacterial, rickettsial, and protozoan agents compared to mosquitoes (Dantas-Torres, 2018). Ticks directly harm cattle through painful bites, blood loss, skin damage, and anorexia, which results in decreased development, in addition to serving as infection vectors (Jabbar et al., 2015). *Hyalomma dromedarii* (Koch, 1844) belongs to family Ixodidae (Barker and Murrell, 2004); was predominantly tick-infected Sinai (95.6 %), Sudan (89 %), and Benha and Belbis in Egypt (57.13 %) camels (van Straten and Jongejan, 1993, Elghali and Hassan, 2009, Ramadan, 1997, Al-Ghamdi et al., 2020, Ashjaran and Sheybani, 2019). It is found in semi-deserts and deserts from Saudi Arabia to northwest India (Hoogstraal, 1979). Camels are the main animals that adult *H. dromedarii* infect. Although cattle are also taken into consideration, camels are the primary feeders of ticks and mites. According to (FAOSTAT, 2019), Riyadh province has the greatest percentage of camels in Saudi Arabia, which is one of the countries with recent significant increase in the camel population (Faye, 2015). The country had a population of about 500,000 in 2017 (FAOSTAT, 2019). The dromedary camel is important to the economy and culture of the Arab world. Numerous illnesses have a major adverse effect on camel production; especially when there are inadequate veterinary services available (Megersa, 2010). Ticks are one of many endo- and ectoparasites that negatively impact camels' health, performance, and productivity (Megersa, 2010). Camels are infested by more than 20 species of ixodid (Hoogstraal et al., 1981, Banaja and Ghandour, 1994), and it is vital that they are actively controlled. Ticks of the genus *Hyalomma* are the most prominent species among them (Elghali and Hassan, 2009, Fard et al., 2012), and they may act as transmitters for *Babesia* spp., and *Theileria* spp. (El Kady, 1998, Fard et al., 2012, Al-Deeb et al., 2015, Barghash and Hafez, 2016, Alanazi et al., 2018), and *Anaplasma* spp. (Fard et al., 2012). However, it's still unclear whether *Hyalomma* spp. ticks have the ability of serving as these infections' vectors. Many factors make it difficult to control tick

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populations, including ticks' large egg production, multiple developmental stages, and ability to parasitize a variety of hosts (EL Roby et al., 2018). Using repellents and acaricides is a common and effective way to mitigate the threat that ticks cause to humans, animals, and livestock. Nevertheless, repeated usage and inadequate dosage of chemicals resulted in ticks developing resistance, and the cost of traditional ectoparasitic medications was exorbitant (Foil et al., 2004, Klafke et al., 2017, Monteiro et al., 2020). According to (George et al., 2008), the use of repellents and synthetic pesticides to control target pests of veterinary and medical importance became increasingly difficult. This led to adverse effects of these chemicals (acute and chronic toxicity) on the hosts and contamination of animal and plant products, including milk and meat. In Saudi Arabia, attempts had been previously made to assemble some key information on the important ectoparasites that infest camels (*Camelus dromedarius*). Additionally, there is a growing need for the creation of novel, chemical-free tick management techniques with a lower risk of polluting the environment, food, or animals, as well as causing human and animal intoxication (Samish, 2000). Entomopathogenic nematodes (EPNs) were one type of bio-agent explored for tick control (EL-Sadawy and Abdel-Shafy, 2007, Samish et al., 2008, Basu et al., 2012, Monteiro et al., 2014, Monteiro and Prata, 2015, EL Roby et al., 2018). With the help of mutualistic bacteria, EPNs eliminate ticks and other arthropods; *Xenorhabdus* and *Photorhabdus* species of bacteria are symbiotically linked to *Steinernema* and *Heterorhabditis* species, respectively (Gaugler and Kaya, 1990). As the only free-living stage, infective juvenile nematodes infect hosts by natural openings such as the mouth, anus, and spiracles, or sometimes through the cuticle. Once inside the host, they release their bacterial symbionts into the hemocoel, where they kill the host (Koppenhöfer et al., 2007). Engorged *Rhipicephalus microplus* females were vulnerable to infection by various EPN species, according to laboratory testing (Singh et al., 2018). It has been suggested in new research to use EPNs directly in pastures (Monteiro et al., 2020). Engorged females look for moist areas shielded from sunlight to oviposit in after reaching engorgement, separating from their animal host, and dropping to the ground (Chagas et al., 2001). A favorable soil microclimate is created by these circumstances, allowing EPN to survive and spread infection on engorged females. The survival and virulence of EPNs can be affected by different abiotic factors, particularly temperature, with optimal conditions varying at the species level, and even among isolates of the same species (Rohde et al., 2010). The temperature threshold at which a nematode species can survive, infect, and reproduce is generally close to the climatic conditions of its geographic origin (Molyneux and Bedding, 1984). In the same context, the isolation of native EPN as biological control agents against mosquitoes that may spread serious human diseases like yellow fever, Chikungunya, Zika, and dengue has been the subject of numerous investigations (El-Sadawy et al., 2018, Kovendan et al., 2018, Yüksel et al., 2023). The biocontrol ability of EPNs *S. siamkayai*, *H. indica*, *S. glaseri*, and *S. abbasi* against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* larvae was assessed by (Dilipkumar et al., 2019). In the same way, (Thanwisai et al., 2022) concluded that EPN, *H. indica* and its symbiotic bacteria *Photorhabdus* are larvicidal against *A. aegypti* and *C. quinquefasciatus* mosquitoes and may serve as effective biocontrol agents. In Saudi Arabia, research on the effectiveness of EPNs against the camel tick *H. dromedarii* is missing or inadequate. Therefore, the objective of the current study is to assess the laboratory acaricidal activity of five isolated native EPNs against *H. dromedarii*.

2. Materials and methods

2.1. Tick

On the same trial day, the engorged females of tick, *Hyalomma dromedarii* (about 10 not parasitized ticks, homogenized in size, age, and shape per camel) were manually gathered from naturally infested camels without previous treatment with acaricides on a farm located in the city of Taif, Saudi Arabia. To verify that the gnathosoma was intact and would allow the use of surviving ticks, engorged females were examined under a stereo microscope (Fig. 1).

2.2. EPNs

The EPNs used in this study were provided by Prof. Dr. Ahmed Noureldeen, Department of Biology, College of Sciences, Taif University, Taif, Saudi Arabia. According to (Kaya and Stock, 1997), five isolates of the acquired EPNs, i.e., *Steinernema feltiae* NEM-29 (OP578208), *Heterorhabditis indica* NEM-18 (OP578197), *H. indica* NEM-19 (OP578198), *H. indica* NEM-23 (OP578199), and *H. bacteriophora* NEM-26 (OP578205) were reproduced in last instar larvae of the greater wax moth, *Galleria mellonella* L. (Pyralidae, Lepidoptera). EPNs' emerging infective juveniles (IJs) were collected daily from nematode traps and kept (20 mL aliquots) in 40 mL cell culture bottles at 15 °C in an incubator. All IJs were stored at 8 °C in a refrigerator and used up to two weeks post-emerged in the bioassays. For preparation of suspensions, ten aliquots (10 µL) each of the stored IJs were quantified and the average number of IJs/sample was then estimated, and the suspensions were adjusted to the concentrations required based on this mean.

2.3. Bioassays

2.3.1. Virulence of EPNs

Five engorged females of *H. dromedarii* with the same statistically significant weights ($P > 0.05$) were placed in Petri dishes (9-cm-diameter) filled with 20 g autoclaved sand to assess the potency of all EPN isolates against tick. Each Petri dish received 2 mL of distilled water containing 250, 500, 750, 1,500, and 3,000 IJs of each nematode isolate, at rates of 50, 100, 150, 300, and 600 IJs/female, with five repetitions, yielding a total of 25 engorged females/treatment. Two mL of distilled water free of nematodes was added to the Petri dish as the control treatment. The plates were parafilm-sealed and maintained in a controlled growth room at a temperature of 26 ± 1 °C, a relative humidity 80 ± 5 %, and a light–dark cycle of 12:12. During the first three days following the beginning of the experiment, the females were observed daily to check for mortality by examinations of leg movements as well as by smell and color changes. At each exposure period, the following mortality percentages were adjusted utilizing the Schneider-Orellis formula (Puntener, 1981):

$$\text{Corrected Mortality } \% = \left(\frac{\%\ \text{MT} - \%\ \text{MC}}{100 - \%\ \text{MC}}\right) \times 100$$

Where MC: Mortality in Control, MT: Mortality in Treatment.

The lethal concentration values (LC_{50} and LC_{90}) and time (LT_{50}) of

Fig. 1. *Hyalomma dromedarii* engorged female.

the EPNs needed to eliminate 50 % of the tick population were calculated using Probit analysis based on the corrected mortality % (Finney, 1971).

2.3.2. Biological parameters of tick

The engorged females that were still alive after each of the treatments with the most virulent EPN strains (*H. indica* NEM-23 and *H. bacteriophora* NEM-26) were taken out of the dishes and examined under a stereoscopic microscope in case the IJs on the tegument could be seen. After being observed, these females got clean with distilled water, dried with paper towels to remove any excess moisture, and then weighed to determine their initial weight (IW). They were then put in equal-sized dishes without nematodes or sand for oviposition. Until the last tick in each treatment died, the egg masses were daily collected. Each female's egg mass was weighed independently (EW), put into labelled 10-ml modified syringes with the distal end cut off, sealed with hydrophilic cotton, and then deposited once more in the controlled growth chamber (26 ± 1 °C and 80 ± 5 % RH). The females' residual weight (RW) was determined after the oviposition period by weighing them once again. Periods of females survival (SP), oviposition (OP), and preoviposition (POP) as well as nutritional efficacy index (NEI), larval hatching percentage (H%), and the efficacy of treatments (Egg-laying inhibition index, ELII) were additional biological factors estimated as follows (Drummond et al., 1973, Gonçalves et al., 2007, Monteiro et al., 2020).

$$\text{Egg laying index (ELI)} = \frac{\text{EW (mg)}}{\text{IW (mg)}}$$

$$\text{Egg} - \text{laying inhibition index (ELII)} = \frac{\text{ELI control group} - \text{ELI treated group}}{\text{ELI control group}} \times 100$$

$$\text{Nutritional efficacy index (NEI)} = \frac{\text{EW (mg)}}{\text{IW (mg)} - \text{RW (mg)}} \times 100$$

2.4. Statistical analysis

Mean ± standard error (M ± SE) was employed to express the results. A two-way analysis of variance (ANOVA) followed by Duncan's multiple range test was performed to examine the tick mortality rate. The COSTAT program (Version 6.400) was used to conduct all of the analyses. Using SPSS Version 23, t-tests were undertaken on the LC_{50} and LC_{90} values, the 95 % confidence limits of the lower and upper values, the slope and intercept, and the χ^2 values of the tested EPNs ($P < 0.05$), with *P*-values less than 0.05 indicating significant. At significance level of 5 % ($P < 0.05$), the nonparametric data were analyzed by the Kruskal–Wallis test followed by the Student-Newman-Keuls test to compare the means due to the non-normal distribution of data.

3. Results

3.1. Acaricidal activity of EPNs

Table 1 display the average corrected mortality percentages of engorged females *H. dromedarii*, which ranged from 0.0 to 100 % following three exposure periods of five EPNs application at five different concentrations. The findings showed that concentration and exposure duration had a direct impact on the EPNs' efficiency (Table 1). According to the results, IJs from all EPNs generated a range of mortality compared to control treatment ($P < 0.05$), indicating that they had a significant effect on *H. dromedarii* mortality to a certain point. Except *S. feltiae* at 50 IJs/Tick, at every studied concentration and exposure time, the *H. dromedarii* engorged females were sensitive ($P < 0.05$) to every EPN. Mortality rate average of 74.3 % was significantly recorded for *H. bacteriophora* followed by *H. indica* NEM-23 that recorded 53.9 % mortality, whereas, *S. feltiae* showed the least virulence against camel tick engorged females with mortality percentage of 26.6 % (Table 1). One hundred percent mortality rate from *H. dromedarii* was the highest and recorded when the individuals were exposed to 300 and 600 IJs/Tick of *H. bacteriophora* for 72 h. Additionally, a direct correlation between exposure duration and mortality percentage ($P < 0.05$) was noticed. Thus, as the exposure increased, the mortality percent

Table 1
Acaricidal activity of five EPNs on the camel tick, *H. dromedarii* under laboratory condition.

EPNs	Concentrations (IJs/Tick)	[a] Mortality ± standard error (%)			EPN Means
		24 h	48 h	72 h	
S. feltiae	**50**	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	**26.6 e**
	100	8.0 ± 4.9	20.0 ± 11.0	33.3 ± 7.8	
	150	20.0 ± 0.0	36.0 ± 4.0	41.7 ± 4.2	
	300	20.0 ± 0.0	40.0 ± 0.0	50.0 ± 5.1	
	600	36.0 ± 4.0	40.0 ± 0.0	54.2 ± 4.2	
***H. indica* NEM-18**	**50**	20.0 ± 0.0	24.0 ± 4.0	33.3 ± 4.2	**36.9 d**
	100	28.0 ± 4.9	28.0 ± 4.9	29.2 ± 8.3	
	150	28.0 ± 4.9	40.0 ± 0.0	54.2 ± 4.2	
	300	36.0 ± 4.0	40.0 ± 0.0	50.0 ± 5.1	
	600	40.0 ± 0.0	44.0 ± 4.0	58.3 ± 0.0	
***H. indica* NEM-19**	**50**	20.0 ± 0.0	28.0 ± 4.9	41.7 ± 7.8	**48.5c**
	100	28.0 ± 4.9	48.0 ± 4.9	54.2 ± 7.8	
	150	36.0 ± 4.0	56.0 ± 4.0	62.5 ± 4.2	
	300	40.0 ± 0.0	60.0 ± 0.0	70.8 ± 5.1	
	600	52.0 ± 4.9	64.0 ± 4.0	66.7 ± 5.1	
***H. indica* NEM-23**	**50**	32.0 ± 4.9	36.0 ± 4.0	41.7 ± 4.2	**53.9b**
	100	32.0 ± 4.9	40.0 ± 0.0	54.2 ± 7.8	
	150	40.0 ± 6.3	60.0 ± 6.3	75.0 ± 4.2	
	300	44.0 ± 4.0	68.0 ± 4.9	79.2 ± 0.0	
	600	56.0 ± 4.0	72.0 ± 4.9	79.2 ± 0.0	
H. bacteriophora	**50**	36.0 ± 4.0	56.0 ± 7.5	75.0 ± 4.2	**74.3 a**
	100	48.0 ± 4.9	68.0 ± 8.0	88.0 ± 4.9	
	150	52.0 ± 4.9	87.5 ± 5.1	91.7 ± 5.1	
	300	60.0 ± 6.3	92.0 ± 4.9	100.0 ± 0.0	
	600	68.0 ± 4.9	92.0 ± 4.9	100.0 ± 0.0	
Exposure Time Means		**35.2c**	**49.6b**	**59.3 a**	

[a] Each replicate had five engorged females, and there were five replicates in each treatment. [b] Each column's numbers represented mortality ± standard error. According to Duncan's Multiple Range Test ($P < 0.05$), means in the column or row with different letters differ significantly.

significantly increased, and a female mortality rate of 59.3 % was observed 72 h post-exposition. At all test periods, high concentrations of *H. bacteriophora* and *H. indica* NEM-23 were related to high mortality percentages, whereas *S. feltiae* was linked to low mortality. In contrast to the IJs of *H. bacteriophora*, which significantly increased tick mortality at all times tested at low concentration (50 IJs/Tick), *S. feltiae*'s mortality rate at the three exposure times (0.0 %) was not significantly different than the untreated ticks.

Similarly, the IJs of *H. bacteriophora* and *H. indica* NEM-23 were the most effective against *H. dromedarii* engorged females 72 h after treatment, with LC_{50} values of 25.7 and 63.9 IJs/Tick, and 123.3 and 754.3 IJs/Tick for LC_{90}, respectively (Table 2). The IJs of *H. indica* NEM-19 were the third most virulent and that *H. indica* NEM-18 and *S. feltiae* were the least effective after different exposure times against *H. dromedarii* with LC_{50} values of 76.2, 251.4 and 306.1 IJs/Tick, respectively after 72 h exposition. The highest degree of homogeneity for the *H. dromedarii* population was observed for *H. bacteriophora* and *H. indica* NEM-23 with slope values of 1.36, 1.44, and 2.07, and 1.04, 1.09, and 1.29, respectively at 24, 48, and 72 h-exposure, whereas, the tick response to the other three EPN species was heterogeneous, as evidenced by their low slope values (Table 2).

As displayed in Table 3, the similar pattern was observed in terms of the lethal time required to kill 50 % of tick-engorged females. The *H. bacteriophora* and *H. indica* NEM-23 IJs were more potent against *H. dromedarii*, with lower LT_{50} values of 21.2 and 36.6 h, respectively; followed by *H. indica* NEM-19 (46.8 h), then *H. indica* NEM-18 (89.9 h), while the *S. feltiae* IJs had an LT_{50} of 99.9 h.

Table 3
LT_{50} of five EPNs against the engorged females of *H. dromedarii*.

EPNs	LT_{50} (h) (95 % LCL–UCL)	Slope ± SE	Intercept	X^2	*P*-value
S. feltiae	99.9 (78.1–192.6)	0.78 ± 0.38	−2.70	0.11	0.002
***H. indica* NEM-18**	89.9 (65.9–155.2)	1.25 ± 0.41	−1.62	0.49	0.040
***H. indica* NEM-19**	46.8 (35.1–66.1)	1.29 ± 0.37	−2.16	0.45	0.001
***H. indica* NEM-23**	36.6 (24.4–47.1)	1.33 ± 0.37	−2.07	0.07	0.000
H. bacteriophora	21.2 (14.6–26.1)	2.42 ± 0.42	−3.21	0.19	0.000

LT_{50}—time required to kill 50% of ticks, lower confidence limit (LCL), upper confidence limit (UCL), Chi-square (X^2), standard error (SE), and probability (*P*).

3.2. Biological parameters of tick

All biological parameters for engorged *H. dromedarii* females were affected by *H. indica* NEM-23 and *H. bacteriophora* exposition (Tables 4, 5). Although there was no difference between the treated groups, there were significant differences ($P < 0.05$) among the control and treated groups in all biological parameters examined. Prior to egg laying, the average weight of engorged females was between 171.6 and 249.1 mg, and when exposed to different levels of *H. indica* NEM-23 IJs except those treated with 600 IJs, no variations ($P < 0.05$) were observed among treated groups (Table 4). Regarding the pre-oviposition and oviposition duration of *H. dromedarii* engorged females; there were significant variations in results between the untreated and treated groups ($H = 48.299$, $P < 0.001$; $H = 58.574$, $P < 0.001$). Except concentration of 50 IJs, there was a statistical difference among treated groups in the pre-oviposition period, ranging from 1.6 to 3 days. The period of oviposition in the treated groups was significantly short ($P < 0.05$), with a range of 4.6 to 7.3 days (Table 4), whereas the control group had a period of 13.5 days. There were no statistical differences noted among IJs levels, however, there was a statistically significant difference in nutritional efficiency index between all groups. For the NEI, the value obtained was of 77.33 % in the control group, which is higher number than those found for *H. indica* NEM-23-treated groups. Concerning egg mass weight, the infection by *H. indica* NEM-23 IJs at different rates delayed the *H. dromedarii* females' oviposition. This was substantially lower in the treated group compared to the untreated one for engorged females ($H = 57.958$, $P < 0.001$). The treatments with 50, 100, 150, 300, and 600 IJs/Female result in decreases ($P < 0.05$) in weight of egg mass, averaging 137.8, 129.5, 124.3, 113.8, and 97.2 mg, respectively (Table 4), but it was 284.4 mg in the control. Regarding the larval hatching percentages, there were statistical differences between all IJs levels and between the treated and the control groups ($H = 59.933$, $P < 0.001$), with values ranged from 42.0 to 69.6 % in treated groups and value of 99.4 % in control. The survival period showed significant differences for various IJ concentrations of *H. indica* NEM-23 and varied in the treated groups from 7.8 to 11.1 days, while, it recorded 18.2 days in the untreated. The best control efficacy (Inhibition of egg laying) was of 42.2 %, obtained from the tick exposed to 600 IJs/Female, whereas, the least control efficacy (23.8 %) was recorded in 50 IJs/Female group (Table 4).

The results of the eight biological parameters for engorged *H. dromedarii* females influenced by *H. bacteriophora* infection are presented in Table 5. In groups containing *H. bacteriophora*, all of the tested biological parameter values, differed significantly with the control group's values ($P < 0.05$). With reference to the parameters initial weight of females, pre-oviposition period, NEI, weight of egg masses,

Table 2
Pathogenicity of five EPNs against *H. dromedarii*.

EPNs	Exposure Time (h)	LC_{50} IJs/Female (95 % LCL–UCL)	LC_{90} IJs/Female (95 % LCL–UCL)	Slope ± SE	Intercept	X^2	*P*-value
S. feltiae	24	731.4 (538.3–1424.7)	1332.5 (929.9–1932.7)	0.52 ± 0.16	−4.29	2.47	0.001
	48	649.3 (437.9–1134.1)	1212.9 (884.9–1620.7)	0.53 ± 0.32	−3.06	4.07	0.002
	72	306.1 (201.4–653.3)	1142.6 (849.2–1409.5)	0.59 ± 0.29	−3.27	5.28	0.002
***H. indica* NEM-18**	24	680.3 (450.1–912.7)	1249.3 (891.6–1644.1)	0.70 ± 0.31	−1.71	0.56	0.095
	48	630.1 (429.3–889.5)	1143.2 (864.8–1543.1)	0.74 ± 0.27	−1.55	2.23	0.001
	72	251.4 (178.6–409.1)	1080.6 (822.1–1305.4)	0.75 ± 0.31	−1.81	3.29	0.014
***H. indica* NEM-19**	24	532.2 (258.3–908.1)	1059.9 (889.5–1414.2)	0.79 ± 0.32	−2.16	0.90	0.012
	48	134.6 (107.8–214.7)	978.5 (845.4–1364.3)	0.81 ± 0.31	−1.77	1.28	0.009
	72	76.2 (28.3–160.3)	801.6 (653.7–1104.8)	0.97 ± 0.31	−1.31	1.74	0.025
***H. indica* NEM-23**	24	426.7 (251.4–807.9)	958.7 (776.1–1264.9)	1.04 ± 0.33	−1.55	0.36	0.054
	48	120.8 (46.1–211.8)	933.3 (621.6–1236.7)	1.09 ± 0.35	−2.02	1.12	0.002
	72	63.9 (12.9–110.9)	754.3 (499.7–1086.5)	1.29 ± 0.34	−1.88	2.09	0.001
H. bacteriophora	24	134.2 (97.4–322.5)	871.8 (713.9–1133.7)	1.36 ± 0.39	−1.58	0.95	0.016
	48	35.7 (6.3–64.2)	156.9 (119.4–311.5)	1.44 ± 0.42	−2.12	2.03	0.004
	72	25.7 (2.4–45.7)	123.3 (79.2–212.7)	2.07 ± 0.69	−2.92	0.87	0.003

LC_{50}— 50% tick-killing concentration, LC_{90}— 90% tick-killing concentration, SE—standard error, X^2—Chi-square value, LCL—lower confidence limit, UCL—upper confidence limit, and *P*-value—probability.

Table 4
Biological parameters of *H. dromedarii* engorged females exposed to *H. indica* NEM-23 at five rates under laboratory conditions.

IJs/Female [a] (n)	[b] Biological Parameters							
	IW (mg)	POP (days)	OP (days)	NEI (%)	EW (mg)	H (%)	SP (days)	Control efficacy (%)
50 (14)	$249.1^{b} \pm 5.3$	$3.0^{b} \pm 0.0$	$7.3^{b} \pm 0.1$	$59.3^{b} \pm 1.3$	$137.8^{b} \pm 1.4$	$69.6^{b} \pm 0.6$	$11.1^{b} \pm 0.1$	$23.8^{e} \pm 0.4$
100 (11)	$232.7^{bc} \pm 3.4$	$2.3^{c} \pm 0.1$	$6.3^{c} \pm 0.1$	$59.1^{b} \pm 1.2$	$129.5^{b} \pm 1.1$	$62.3^{c} \pm 0.7$	$9.5^{c} \pm 0.3$	$26.2^{d} \pm 0.4$
150 (6)	$210.8^{bc} \pm 2.7$	$2.2^{c} \pm 0.2$	$5.8^{cd} \pm 0.2$	$62.0^{b} \pm 1.1$	$124.3^{bc} \pm 1.9$	$54.8^{d} \pm 1.9$	$9.0^{cd} \pm 0.0$	$30.5^{c} \pm 0.3$
300 (5)	$200.4^{bc} \pm 0.4$	$2.0^{c} \pm 0.0$	$5.4^{d} \pm 0.2$	$58.9^{b} \pm 1.7$	$113.8^{bc} \pm 3.5$	$49.4^{e} \pm 1.7$	$8.2^{cd} \pm 0.2$	$35.2^{b} \pm 0.7$
600 (5)	$171.6^{c} \pm 1.2$	$1.6^{c} \pm 0.2$	$4.6^{e} \pm 0.2$	$58.7^{b} \pm 1.8$	$97.2^{c} \pm 2.6$	$42.0^{f} \pm 0.7$	$7.8^{d} \pm 0.2$	$42.2^{a} \pm 1.7$
Control (24)	$408.3^{a} \pm 14.3$	$3.7^{a} \pm 0.2$	$13.5^{a} \pm 0.2$	$77.3^{a} \pm 2.7$	$284.4^{a} \pm 7.2$	$99.4^{a} \pm 0.2$	$18.2^{a} \pm 0.3$	–

[a] Numbers in the brackets represented surviving engorged females for whom biological characteristics were measured. [b] In each column, numbers denoted the mean ± standard error. Student-Newman-Keuls and Kruskal-Wallis tests show that means with same letters in each column not significantly differ ($P < 0.05$). Initial weight (IW), preoviposition (POP), oviposition (OP), nutritional efficacy index (NEI), eggs weight (EW), larval hatching percentage (H%), and females survival period (SP).

Table 5
Biological parameters of *H. dromedarii* engorged females exposed to *H. bacteriophora* NEM-26 at five rates under laboratory conditions.

IJs/Female [a] (n)	[b] Biological Parameters							
	IW (mg)	POP (days)	OP (days)	NEI (%)	EW (mg)	H (%)	SP (days)	Control efficacy (%)
50 (6)	$187.0^{b} \pm 4.8$	$2.3^{b} \pm 0.2$	$5.2^{b} \pm 0.2$	$45.3^{b} \pm 2.7$	$79.8^{b} \pm 3.1$	$29.7^{b} \pm 1.1$	$8.2^{b} \pm 0.3$	$46.3^{c} \pm 1.9$
100 (3)	$128.3^{b} \pm 3.8$	$1.7^{b} \pm 0.3$	$4.7^{b} \pm 0.3$	$43.8^{b} \pm 1.8$	$52.7^{b} \pm 3.9$	$21.3^{c} \pm 1.9$	$7.3^{b} \pm 0.3$	$55.4^{b} \pm 0.6$
150 (2)	$91.5^{b} \pm 1.5$	$1.5^{b} \pm 0.5$	$2.5^{c} \pm 0.5$	$40.7^{b} \pm 0.2$	$35.0^{b} \pm 1.0$	$0.0^{d} \pm 0.0$	$5.5^{b} \pm 0.5$	$61.3^{a} \pm 0.7$
300 (0)	–	–	–	–	–	–	–	–
600 (0)	–	–	–	–	–	–	–	–
Control (24)	$408.3^{a} \pm 14.3$	$3.7^{a} \pm 0.2$	$13.5^{a} \pm 0.2$	$77.3^{a} \pm 2.7$	$284.4^{a} \pm 7.2$	$99.4^{a} \pm 0.2$	$18.2^{a} \pm 0.3$	–

[a] Numbers in the brackets represented engorged females which survived and whose biological measurements could be examined. [b] Each column's numbers proved mean ± standard error. Means with different letters within the same column differ significantly ($P < 0.05$) according to Kruskal-Wallis and Student-Newman-Keuls Tests. Initial weight (IW), preoviposition (POP), oviposition (OP), nutritional efficacy index (NEI), eggs weight (EW), larval hatching percentage (H%), and females survival period (SP).

and survival period, results for IJ concentrations were not differ significantly, with values ranged from 91.5 to 187.0 mg, 1.5 to 2.33 days, 40.69 to 45.31 %, 35.0 to 79.8 mg, and 5.5 to 8.2 days, respectively (Table 5). For the oviposition period, the value obtained for the rate 150 IJs/female was of 2.5 days, which is less than those observed for the control and other treated groups, indicating significant variations among treatments and between the treatments and control group ($H = 23.879$, $P < 0.001$). Similarly, high significant differences ($H = 24.901$, $P < 0.001$) between treated and untreated groups in relation to egg hatching percentage were also recorded. No larvae were hatched from the eggs treated with level of 150 IJs/Female, while 21.3 and 29.7 % eggs were hatched when exposed to 100 and 50 IJs/Female of *H. bacteriophora*. The control percentage (61.3 %) of the 150 IJs/Female level was considerably high ($P < 0.05$), while in the 100 and 50 IJs/Female levels, the control efficacies were of 55.4 and 46.3 %, respectively (Table 5).

4. Discussion

The current study's findings proved that the five isolates of EPNs at different levels and exposure times were virulent to *H. dromedarii* engorged females to some extent with average mortality percentage values ranged from 26.6 to 74.3 % (Table 1). A linear increase in mortality occurred with increasing IJs concentration and exposition. It was clear from the results that the *Heterorhabditis* spp. was more effective than *S. feltiae* against the engorged females under laboratory conditions, which indicates low virulence. Low concentration of *S. feltiae* 50 IJs/Female failed to kill any of the engorged females at all of exposure periods. When ticks were treated with high concentrations of *H. bacteriophora*, the optimum engorged female's mortality was 100 % after 3 days; nevertheless, when exposed to low concentration of this nematode, more than 70 % of females died within the same duration. These findings are consistent with those of (Samish et al., 1999), who discovered that a high mortality index applying low nematode rates indicates a specific tick species' susceptibility to a nematode species. The presence of a tooth on the front tip of the *H. bacteriophora* IJs, which promotes penetration and infection, may explain the increased vulnerability of *Boophilus microplus* engorged females. These data were also confirmed by those of (Mauleon et al., 1993), who mentioned that all of the *B. microplus* individuals survived after being exposed to a suspension of 1,000 *S. carpocapsae* IJs from nine different strains. Based on these findings, it is believed that *B. microplus* must release nematode-killing agents in the hemocel that prevent nematode reproduction. Moreover, the methods employed to collect infectious juveniles and the acquisition period, which is closely related to their infectivity in lab experiments, may be responsible for *S. carpocapsae*'s lack of pathogenicity. The present results investigated that the time needed to kill 50 % of engorged females and the lethal concentration of nematodes which killed 50 % and 90 % of the individuals up to the third day of exposure were both lower in *H. bacteriophora* than that of *S. feltiae* and all other *Heterorhabditis* species (Tables 2,3). The results matched with (Vasconcelos et al., 2004) discovered, who observed that when compared to the results for *S. glaseri* IJs (i.e., 1,250 and 4,160 IJs/Petri dish, respectively), *H. bacteriophora* IJs' LC_{50} and LC_{90} values of 874 and 1,930, respectively, were low. According to earlier research (Singh et al., 2018); a number of EPN species are lethal to engorged females of *R. microplus*. In semi-field trials, the use of EPNs to soil containing engorged females had excellent results (Alekseev et al., 2006, Monteiro et al., 2020); however, the same result did not occur when applying EPNs to infested cattle (Goolsby et al., 2018). Similar findings were also attained by (EL Roby et al., 2018), who tested eight Egyptian isolates of EPNs in a laboratory (four belonged to the genus *Steinernema* and four to *Heterorhabditis*) against engorged females of the hard tick, *B. annulatus* and adult females of the soft tick *Argas persicus*. They reported that *Heterorhabditis* isolates (10 K and A4) at 1000 IJs/ml have high potency which accomplished 100 % mortality three days post-infection against *A. persicus,* whereas, only one *Steinernema* isolate (5B) produced an overall mortality rate of 93.33 %. Meanwhile, mortality rates for 10 K and A4 isolates of

Heterorhabditis were 93.33 % and 86 %, however, both *Steinernema* isolates (5B and AT4) caused 86.7 % mortality. It was evident from the present investigation that the two most virulent EPNs, *H. indica* NEM-23 and *H. bacteriophora* NEM-26, had a further effect on the biological characteristics of engorged females of *H. dromedarii*. All of the engorged females exposed to 300 or 600 IJs/Tick *H. bacteriophora* in the initial experiments of current study were killed within 3 days post-infection, thus only three concentrations of this nematode were evaluated for biological parameters. Longer exposure times and higher rates of the two EPNs appear to strengthen their impacts on the biological parameters of *H. dromedarii* engorged females analyzed in this study by allowing a larger amount of nematodes to find and enter the host. The biological parameters of the females in the treated and control groups had statistically different means, highlighting the heterogeneity in several groups and establishing that the variations in these measurements were caused by the two tested nematodes (Tables 4,5). With reference to the parameters survival, oviposition, and pre-oviposition periods of groups treated with *H. bacteriophora*, being shorter than those of *H. indica*-treated ones, whereas, NEI, EW, and H % parameters were higher in *H. indica* treatments than those of *H. bacteriophora* ones, confirming the potency of *H. bacteriophora* over *H. indica*. This suggests that the metabolic conversion process or the synthesis of the nutrients required for egg formation within the pre-oviposition stage were unaffected by the EPN infections. Thus, the best control efficacy (61.3 %) was obtained from *H. bacteriophora* at 150 IJs/Female, and in the same rate of *H. indica*, efficacy was lower with value of 30.5 % only. Results that were better than those observed in the current study were reported by (Silva et al., 2012, Monteiro et al., 2020), who noted that using a similar concentration (150 IJ/engorged female) of *H. bacteriophora* HP88 and *H. indica* LPP1 lead in an almost 90 % efficacy, causing a noticeably decreased in the number and viability of the laying eggs by *R. microplus* females. One year later, (Monteiro et al., 2021) concluded that the acaricides chlorfenvinphos, amitraz, and deltamethrin as well as the essential oil of *Lippia triplinervis* were in compatibility with EPNs against *R. microplus* engorged females with an efficiency recorded over 95 % (except for HP88 + deltamethrin), and it reached 100 % in LPP1 + amitraz treatment. Likewise, the present data are in line with those previously recorded by (Monteiro and Prata, 2015), who mentioned that these two strains outperform the results obtained when utilizing EPNs from the genus *Steinernema* and are among the most active against *R. microplus*.

5. Conclusion

The current investigation concluded that a 24-h in vitro exposure was sufficient to allow the EPNs to infect and kill the engorged *H. dromedarii* females. However, in order to achieve efficient results, considering the high lethality and deficiency of the tick biological parameters, a 48-h in vitro treatment with *H. bacteriophora* NEM-26 was required. The findings provide a strong basis for prospective biocontrol agents that could be applied to camel tick management. More laboratory studies to evaluate the influence of computability with other bio-agents or acaricides on the action of EPNs against camel tick are required to supply critical information that will help conducting field studies.

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CRediT authorship contribution statement

Bander Albogami: Conceptualization, Funding acquisition, Data curation, Writing – original draft, Writing – review & editing, Visualization, Investigation, Validation, Formal analysis, Methodology, Supervision, Resources, Project administration, Software.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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