International Journal of Agriculture Sciences
ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 13, Issue 3, 2021, pp.-10684-10688.
Available online at https://bioinfopublication.org/pages/jouarchive.php?id=BPJ0000217

Research Article

IN VITRO EFFICACY OF ENTOMOPATHOGENIC NEMATODES AS A BIOCONTROL OF SHEEP TICKS

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Received: March 01, 2021; Revised: March 25, 2021; Accepted: March 26, 2021; Published: March 30, 2021

Abstract: The present study was conducted to assess the efficacy of two entomopathogenic nematodes (EPNs) *viz, Steinernema abbasi* and *Heterorhabditis indica* as a biocontrol against engorged female's ticks of *Haemaphysalis, Hyalomma* and *Rhipicephalus*. EPN efficacy was assessed at a concentration of 500,1000, 2000, 4000, 6000 and 8000 infective juveniles / petri dish in triplicates. *Heterorhabditis indica* was effective in inducing 100 % mortality within 72hrs at all concentrations whereas *Steinernema abbasi* induced 50 to 100% mortality at 214hrs at 8000 infective juvenile's concentration against *Haemaphysalis, Hyalomma* and *Rhipicephalus*.

Keywords: *Biocontrol, Entomopathogenic nematodes, Steinernema abbasi, Heterorhabditis indica, Ticks*

Citation: Sudha Rani R., *et al*., (2021) *In vitro* Efficacy of Entomopathogenic Nematodes as a Biocontrol of Sheep Ticks. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 13, Issue 3, pp.- 10684-10688.
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Academic Editor / Reviewer: Anindita Paul

Introduction

Ticks are common and widely distributed in all agro-ecological zones of tropical countries like India. Among the livestock, small ruminants are the most affected by ticks of veterinary importance, hindering their productivity. The haemoprotozoan diseases caused by ticks are among the major diseases of sheep causing serious economic loss to small farmers and to the national economy [1]. The desire to minimize chemical pesticides and to offset rising prices of new pesticides is fostering the search for alternatives. As a whole, pest bio control agents are far more environment friendly than chemical pesticides. Biological control of pathogens includes bacteria, fungi and parasitic wasps. Entomopathogenic nematodes (EPN's) are widely distributed throughout the world and have been isolated from many types of natural and managed habitats in a wide variety of soils. In the last two decades EPNs have received much attention due mainly to their potential as biopesticides against insect pests, and agricultural pests. Therefore, the need of biological control of tick's gains importance and EPN's holds a promising future for tick control [2]. EPNs serve as vectors of bacteria, which achieve quick kill of the target pest and thus have high potential capability in pest management. The present study evaluates the in vitro efficacy of the EPN's *Steinernema abbasi, Heterorhabditis indica* on field ticks collected from different places of Karnataka.

Materials and Methods

Collection of ticks

The engorged female ticks were collected from different sheep flocks located in different places of Karnataka viz., Bangalore, Belgaum, Bellary, Chitradurga, Davangere, Mandya and they were identified based on the morphological characters [3,4]. The collected ticks were put into glass vials and labelled with place and date of collection.

The glass vials mouth was wrapped in cotton muslin for oxygen supply and transported to the laboratory for in vitro tests.

Procurement and Preparation of EPNs concentrations

The two species of EPN's *viz*., *Heterorhabditis indica* and *Steinernema abbasi* for the study were procured from the ICAR - National Bureau of Agricultural Insect Resources (NBAIR), Bangalore and were used against the engorged female's ticks collected from naturally infested sheep flocks of Bangalore, Belgaum, Bellary, Chitradurga, Davanagere and Mandya regions.

EPNs dose preparation: was done by calculation of concentration of EPNs by dilution method of counting where the stock solution of each EPN was mixed thoroughly to make a homogenous suspension. A volume of 50 µl from the thoroughly mixed diluted suspension was drawn on to a watch glass and counted under stereo zoom microscope. This process was repeated at least five times to check the accuracy. The average of five values was taken as mean IJs/50 µl sample. Later this was further multiplied by the dilution factor (20) to get the total number of nematode IJs per ml.

IJs per ml = Average IJs/50 µl X 20 (dilution factor)

The experiment was carried out as described by Silva et al. (2012)[6]. A total of 180 engorged females were divided into six groups, each containing 24 engorged ticks with statistically similar weights (P≥ 0.05) each carrying out the experiment in four replicates for each dose of EPNs (*Heterrorhabditis indica, Steinernema abbasi*) so that each group consisted of 4 ticks along with a control group of 4 ticks. The experiment was carried out in petri dishes of 9cm in diameter containing 15g of sterilized sand as substrate. Five doses of each EPN at a concentration of 500, 1000, 2000, 4000, 6000, 8000 IJs/1.5ml aqueous suspension were prepared using stock solution of 10000IJs/ml. The petridishes were incubated at temperature 26°C to 30°C and RH≥80% in dark.

Table-1 LC50 values calculated from dose response bio assays conducted with Heterorhabditis indicia and Steinernema abbasi of EPN species against Haemaphysalis bispinosa ticks

Nematode spp	Incubation period (hrs)	LC 50 (IJs)	Fiducial limit (95%)	Nematode Spp	Incubation period	LC50	Fiducial limit (95%)
H.indica	24hrs	1300.81	759.98-226.51	*S.abbasi*	24hrs	3789.61	2360.08-6085.27
	48hrs	575.58	372.19-890.13		48hrs	1835.82	995.83-3384.34
	72hrs	48.34	13.78-169.57		72hrs 96hrs	1255.44 480.64	742.74-2122.06 281.19-846.77
					120hrs	448.02	98.33-672.76

Table-2 LC50 values calculated from dose response bio assays conducted with Heterorhabditis indica and Steinernema abbasi EPN species against Haemaphysalis intermedia ticks

Nematode spp	Incubation period (hrs)	LC 50 (IJs)	Fiducial limit (95%)	Nematode Spp	Incubation period	LC50	Fiducial limit (95%)
H.indica	24hrs	1227.75	715.52-2106.7	*S.abbasi*	24hrs	3596.11	2580.08-6085.27
	48hrs	433.65	212.62-884.48		48hrs	1396.61	772.67-2524.39
	72hrs	205.68	90.33-468.344		72hrs 96hrs	650.18	352.98-1197.60 428.25 219.29-1164.80
					120hrs	193.28	94.58-394.97

Table-3 LC50 values calculated from dose response bio assays conducted with Heterorhabditis indica and Steinernema abbasi of EPN species against Haemaphysalis kutchensis ticks

Nematode spp	Incubation period (hrs)	LC 50 (IJs)	Fiducial limit(95%)	Nematode Spp	Incubation period	LC50	Fiducial limit (95%)
H.indica	24hrs	995.52	509.98-1943.32	*S.abbasi*	24hrs	3088.36	1962.01-4861.31
	48hrs	227.20	91.33-565.19		48hrs	1763.36	1107.52-2807.5
	72hrs	164.40	72.06-375.09		72hrs 96hrs	757.22 338.18	425.16-1348.62 168.84-677.36
					120hrs	48.34	13.78-169.57

Table-4 LC50 values calculated from dose response bio assays conducted with Heterorhabditis indica and Steinernema abbasi of EPN species against Hyalomma anatolicum anatolicum ticks

Nematode spp	Incubation period (hrs)	LC 50 (IJs)	Fiducial limit (95%)	Nematode Spp	Incubation period	LC50	Fiducial limit (95%)
H.indica	24hrs	847.33	493.34-1455.31	*S.abbasi*	24hrs	3789.69	1962.01-4861.31
	48hrs	393.77	183.08-846.93		48hrs	2285.23	1382.82-3776.56
	72hrs	193.28	94.58 – 394.97		72hrs 96hrs	1120.43 710.66	665.35-1886.77 351.52 - 1436.75
					120hrs	383.39	237.71-618.33

Table-5 LC50 values calculated from dose response bio assays conducted with Heterorhabditis indica and Steinernemaabbasi ofEPN species against Hyalomma marginatum isaaci ticks

Nematode spp	Incubation period (hrs)	LC 50 (IJs)	Fiducial limit(95%)	Nematode Spp	Incubation Period	LC50	Fiducial limit (95%)
H.indica	24hrs	867.53	480.42 – 1566.6	*s.abbasi*	24hrs	3234.69	2136.51-4897.35
	48hrs	187.19	86.59 – 404.66		48hrs	1918.47	1195.50-3078.66
	72hrs	71.99	20.63 – 251.210		72hrs 96hrs	839.02 710.66	498.83-1411.22 351.52 -1436.75
					120hrs	517.79	306.02-876.12

Table-6 LC50 values calculated from dose response bio assays conducted with Heterorhabditis indica and Steinernema abbasi of EPN species against Rhipicephalus haemaphysaloides ticks

Nematode spp	Incubation period (hrs)	LC 50 (IJs)	Fiducial limit (95%)	Nematode Spp	Incubation Period	LC50	Fiducial limit (95%)
H.indica	24hrs	719.52	365.51– 1416.42	*S.abbasi*	24hrs	2969.91	2969.91-8149.80
	48hrs	217.50	92.35 – 512.21		48hrs	1577.22	1968.20-4317.20
	72hrs	48.34	13.78 – 169.57		72hrs 96hrs 120hrs	818.88 566.72 193.28	759.98-2226.51 20.63-251.21 94.58-394.97

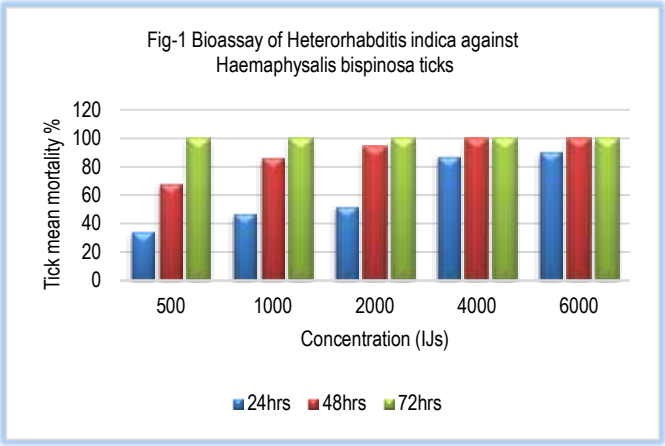
Table-7 LC50 values calculated from dose response bio assays conducted with Heterorhabditis indica and Steinernema abbasi of EPN species against Rhipicephalus sanguineus ticks

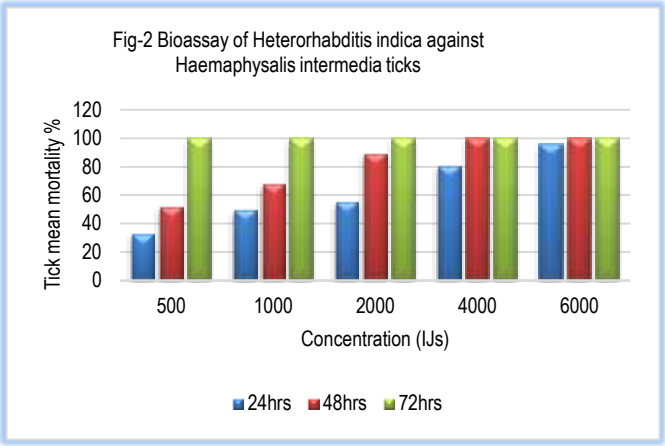
Nematode spp	Incubation period (hrs)	LC 50 (IJs)	Fiducial limit(95%)	Nematode Spp	Incubation Period	LC50	Fiducial limit (95%)
H.indica	24hrs	984.02	527.73 – 1834.85	*S.abbasi*	24hrs	4919.77	2969.91-8149.80
	48hrs	478.95	236.75 – 968.93		48hrs	2914.98	1968.20-4317.20
	72hrs	193.28	94.58 – 394.97		72hrs 96hrs	1300.81 71.99	759.98-2226.51 20.63-251.21

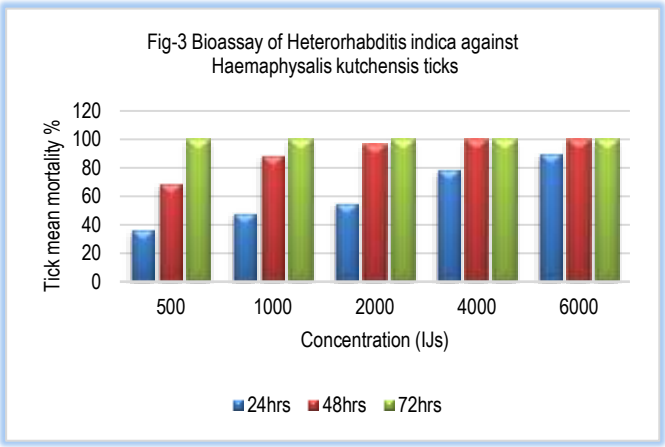
Tick mortality, effect on egg laying and hatchability: The engorged ticks were observed for mortality for every 24hrs interval, till the complete mortality in treatment groups based on visual observation such as absence of leg reflex and changes in coloration of external surface of the tick. When complete mortality of ticks in treatment group is observed, the egg masses were removed, weighed and transferred to sterilized test tubes. Every day egg counting was done till the start of tick mortality in control group.

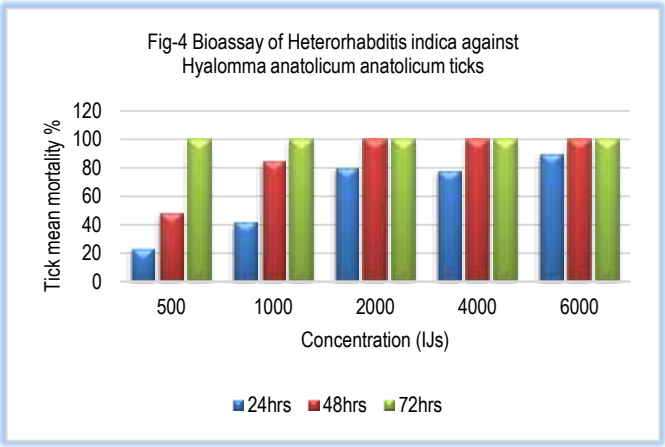
Results

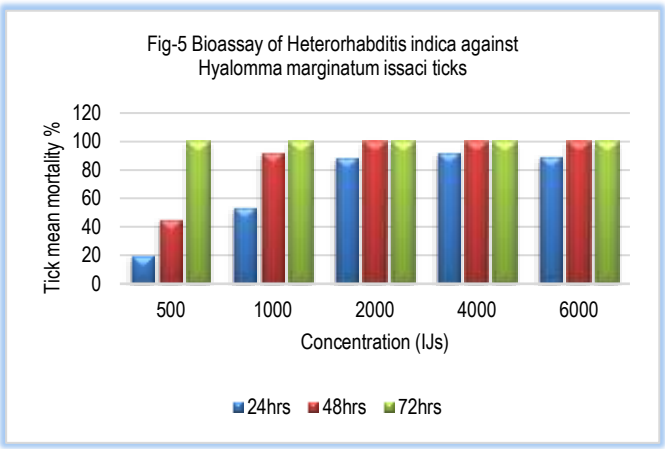
The efficacy of entomopathogenic nematodes (EPNs) *viz*., *Heterorhabditis indica, Steinernema abbasi* in biocontrol of ticks was undertaken. EPN efficacy was assessed at a concentration of 500, 1000, 2000,4000, 6000 and 8000 infective juveniles(IJs) / petri dish in triplicates on *Haemaphysalis bispinosa, Haemaphysalis kutchensis, Haemaphysalis intermedia, Rhipicephalus haemaphysaloides, Rhipicephalus sanguineus, Hyalomma anatolicum anatolicum, Hyalomma marginatum issaci* species. In the present bio assay study, *H.indica* induced 100 per cent mortality against all species of ticks *i.e*., *Haemaphysalis bispinosa , Haemaphysalis kutchensis, Haemaphysalis intermedia, Rhipicephalus sanguineus, Rhipicephalus haemaphysaloides, Hyalomma anatolicum anatolicum* and *Hyalomma marginatum issaci,* at 500IJs concentration / petridish at 72hrs and the increasing concentration (2000IJs) of *H.indica* resulted in inducing 100 per cent mortality within 48hrs and thereby causing a reduction of egg mass weight, hatching per cent, egg production index per cent with significant reduction difference ($p<0.05$) between the treated and control group [Fig-1 to 7]. Whereas *Steinernema abbasi* resulted in inducing 100 per cent mortality in about 192 hrs to 216 hrs against all species of ticks thereby causing a reduction of egg mass weight, hatching per cent, egg production index per cent with significant reduction difference ($p<0.05$) between the treated and control group [Fig-8-14]. The LC50 and LC90 values indicated that *H.indica* was more virulent against all species of engorged ticks when compared to *S.abbasi*.

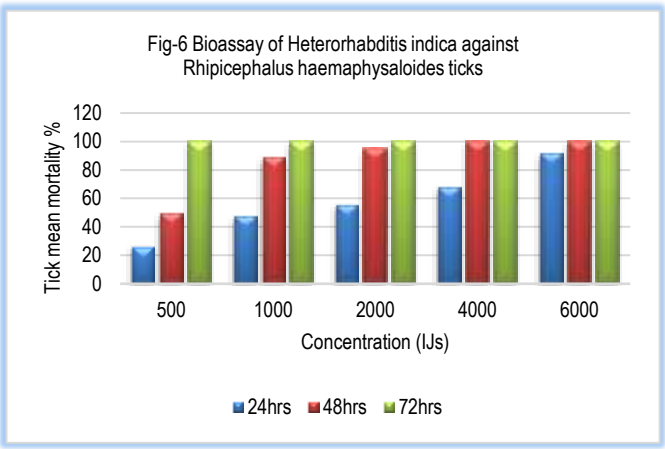


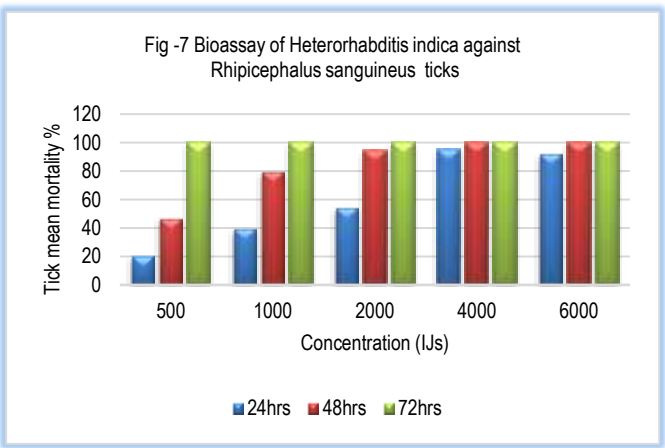


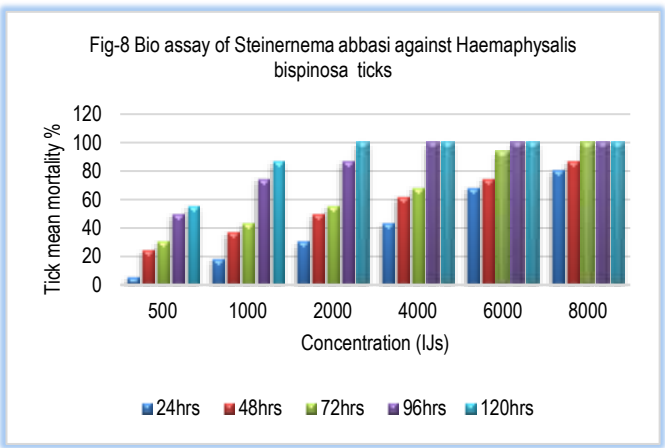






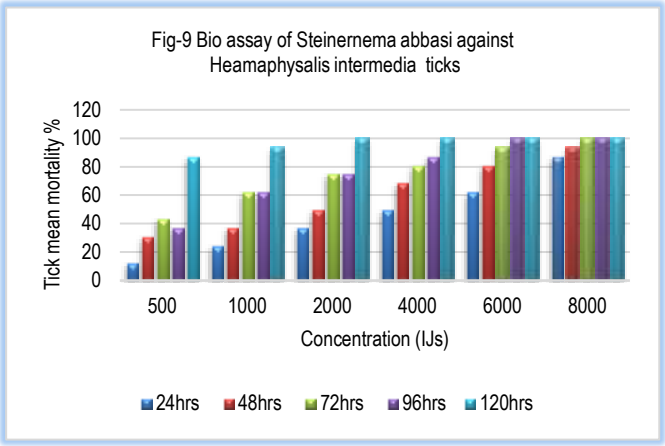


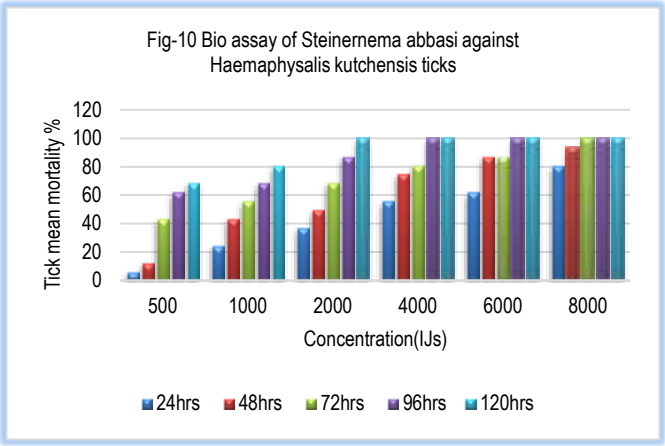


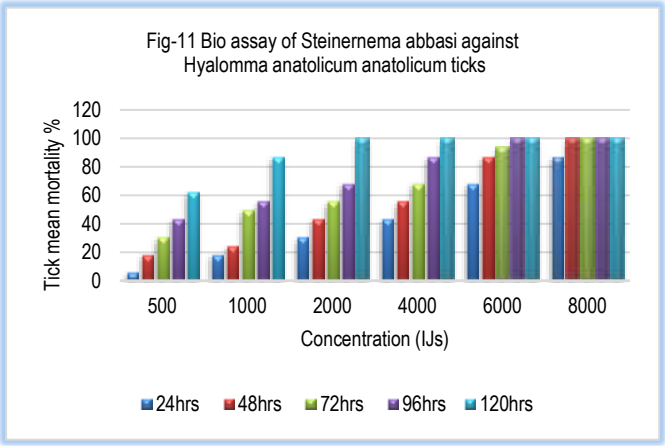


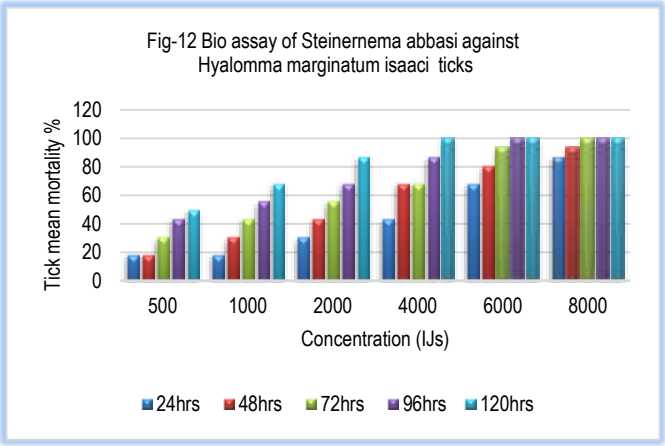
Per cent insect mortality data were analysed by multifactor ANOVA followed by Duncan's multiple range test ($p>0.05$) for separation of means [Table-1 to 7]. LC50 values were calculated according to Finny, (1971)[7].

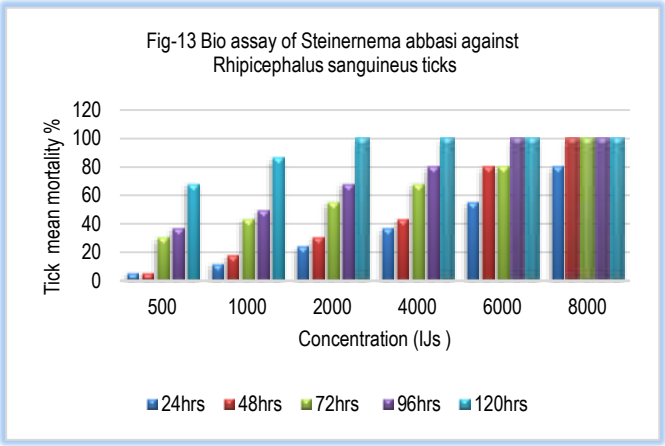
Entomopathogenic nematodes are used to control different insect pests successfully in various locations around the world [8], and more recently research work is being conducted on the use of EPNs to control ticks (Samish et al., 2008).

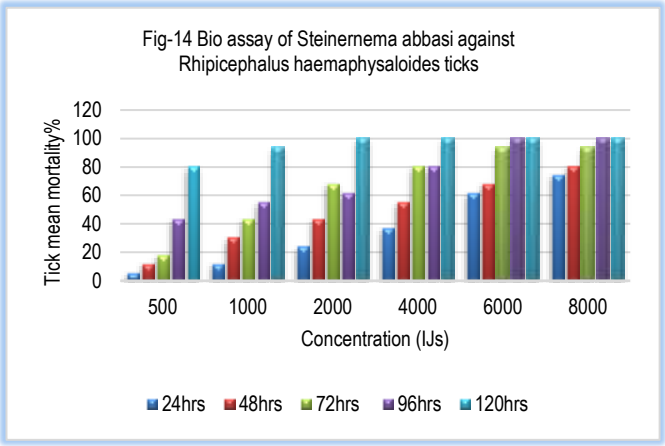












Most of the research studies have been directed toward the control of *R. microplus* [9-13] and *Rhipicephalus annulatus* [14-19] and also against *R.sanguineus, R.haemaphysaloides* by Hussain *et al*. (2016) [20]. In this study two EPN species i.e., *Heterorhabditis indica* and *Steinernema abbasi* were tested against sheep ticks i.e., *Haemaphysalis bispinosa, Haemaphysalis intermedia, Haemaphysalis kutchensis, Hyalomma anatolicum anatolicum, Hyalomma marginatum isaaci, Rhipicephalus haemaphysaloides* and *Rhipicephalus sanguineus* by bioassay test. It was found that among two EPNs used, *Heterorhabditidis indica* were more effective against all species of ticks than *Steinernema abbasi* suggesting that EPN efficiency is greatly influenced by its dose and is in agreement with other reports [21-24].

Conclusion

In the present bio assay study increasing concentration of *H.indica* resulted in inducing 100 per cent mortality within 48 to 76 hrs against all species of ticks *i.e., Haemaphysalis bispinosa, Haemaphysalis kutchensis, Haemaphysalis intermedia, Rhipicephalus sanguineus, Rhipicephalus haemaphysaloides, Hyalomma anatolicum anatolicum* and *Hyalomma marginatum isaaci*. Whereas *Steinernema abbasi* induced 100 per cent mortality against all species of ticks by 120 hrs. Among the two EPN species tested, *Heterorhabditis indica* was most virulent with minimum values of LC50 at 48 hrs of incubation and induced 100% mortality Whereas *S.abbasi* showed higher LC50 values at 48 hrs of incubation. Many researchers have used these bio assays to test the efficacy of EPN against various insect pests [25-28].

Application of Research

To combat the chemical acaricides resistance problem, alternate method was explored to control ticks by bio control agents.

Research Category: Bio control agent (entomopathogenic nematodes).

Abbreviations: cm: centimeter, mg/ml: milligram/ml, °C: degree Celsius, % RH: per centage relative humidity, LC: lethal concentration, FAO: Food and Agriculture Organization.

Acknowledge / Funding: Author thankful to Department of Veterinary Parasitology, CAFT, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bengaluru, 560024, Karnataka, India

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Research project name or number: PhD Thesis

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Bangalore, Belgaum, Bellary, Chitradurga, Davangere, Mandya

Nematodes Names: *Steinernema abbasi, Heterorhabditis indica*

Conflict of Interest: No Conflict of Interest

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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