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Research Article

In vitro efficacy of three species of entomopathogenic nematodes on biocontrol of cattle tick *Rhipicephalus haemaphysaloides* Neumann (Acarina: Ixodidae)

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ABSTRACT: A study was conducted to assess three entomopathogenic nematodes (EPNs) *viz, Steinernema carpocapsae, Steinernema thermophilum* and *Heterorhabditis indica* on engorged females of cattle tick *Rhipicephalus haemaphysaloides.* Three concentrations of 1250, 2500 and 5000 infective juveniles (IJs) / Petri dish in triplicates were evaluated. *Heterorhabditis indica* induced 100% mortality within 48 -72 h at all three concentrations. There was a significant reduction in the egg production i.e. 0.1-14.1mg in *R. haemaphysaloides* at all three concentrations whereas 255.25 mg of eggs were produced by untreated control ticks. The egg mass weight was reduced by EPNs and infertile eggs were produced (no hatchability) *S. carpocapsae* induced 100% mortality in *R. haemaphysaloides,* within 72-96 h. There was no significant difference in the egg production but infertile eggs (1% hatchability) were produced. *S. thermophilum* induced 100% mortality within 72-144 h. There was a significant reduction in the egg production i.e. 12-98.4 mg in *R. haemaphysaloides,* at all three concentrations in contrast to 255.25 mg of eggs produced by untreated control ticks. 41% hatchability was found in the control group. Among the three EPNs *H. indica* was highly pathogenic at a concentration of 1250 IJs/ petri dish, inducing 100% mortality within 48-72 h when compared to *S. carpocapsae* and *S. thermophilum.*

KEY WORDS: Biocontrol, entomopathogenic nematodes, *Heterorhabditis indica, Rhipicephalus haemaphysaloides, Steinernema caprocapsae* and *S. thermophilum*

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INTRODUCTION

Ticks are commonly occurring ectoparasites and act as vectors for important protozoan diseases *viz*., Babesiosis, Theileriosis and Ehrlichiosis. Among the different species of ticks, *Rhipicephalus haemaphysaloides* Neumann is a three host tick of cattle, sheep and goat. It has been estimated that 80 percent of the world's cattle population is exposed to tick infestation. Ticks cause great economic losses to livestock amounting to 498.7 million US dollars in India (Minjauw and Mc Leod, 2003). Tick infestation is a major threat to livestock particularly during summer and rainy seasons. It is therefore, imperative that ticks should be controlled.

Control of ticks continually relies on the use of chemical acaricides with the drawback of chemical residues in the food chain as well as in the environment in addition to the threat of emergence of acaricide resistant tick population. Therefore, the development of alternative control strategies like biological control which is more environment friendly is worth exploring. Biological control of ticks with entomopathogenic nematodes (EPN) was found to be promising in the previous studies (Samish *et al*., 2004). EPNs belonging to the families Steinernematidae and Heterorhabditidae have been found to be useful for the biological control of ticks (Samish *et al*., 1999). Therefore, a study was undertaken to observe the bio efficacy of three entomopathogenic nematodes for the control of the cattle tick *R. haemaphysaloides*.

MATERIALS AND METHODS

The engorged female cattle ticks were collected from naturally infested HF cross breed cows of Halasur and Ravuthnahalli in Bangalore and were identified based on the morphological characters as described by Walker (1994). A total of 150 engorged females were divided into three groups of 50 ticks each with statistically similar weights ($P > 0.05$) for treating each group with each EPNs (*Heterorhabditis indica, Steinernema caprocapsae* and *S. thermophilum*) separately. They are obtained from Biocontrol

Research Laboratory, Yelahanka, Bengaluru. Each group was subdivided into three subgroups containing 15 ticks each for carrying out the experiment in triplicate for each dose of EPNs as described by Vasconcelos *et al*., (2004) so that each group consisted of 5 ticks. The control group of 5 ticks were maintained for each group and the experiment was carried out in Petri dishes of 5 cm diameter.

Each of the treatment group containing 5 ticks was treated with 1.5ml of aqueous solution containing EPNs at concentrations of 1250, 2500 and 5000 IJs per Petridish. The experiment was carried out in triplicates for each dose of all the three EPNs. The control consisted of 1.5ml distilled water without any nematodes. These Petridishes were kept at room temperature (26°C - 30°C) in dark. The female ticks were observed daily to check for mortality and egg-laying.

Tick mortality

Tick mortality was recorded at every 24 h interval till the complete mortality occurred in treatment groups based on the visual observations such as absence of leg reflex and changes in coloration of the external surface of the tick.

Effect on egg laying and hatchability

After complete mortality of ticks in treatment group, the eggs masses were removed, weighed and transferred to sterilized test tubes.

Egg counting was done every day till the start of tick mortality in control group. 100 eggs in each EPN group and control group were transferred to sterilized test tubes for egg hatch assay. Test tubes containing eggs were tied with muslin cloth at the mouth and placed in the desiccator containing saturated potassium chloride to maintain the temperature of 27 ±1 °C and relative humidity of >80 % to favour egg hatching.

Haemolymph examination for symbiotic bacteria

In order to assess the cause of mortality of the ticks haemolymph examination was carried out and the procedure was as follows; after the fourth day of treatment, blood smear was prepared with a drop of blood taken from the haemocoel of the tick and fixed with methanol for one minute. Then the smears were stained with Geimsa stain (1: 10 dilution) for 30 minutes. After the staining, the blood smear was examined under the oil immersion objective of the microscope for the presence of rod shaped symbiotic bacteria.

The data of the bioassay experiments were analysed by using minitab-16.50 version, univariate chi square analysis. All the parameters were tested at 1% level of significance (Two tailed test). The Probit analysis with LC50 – 33.91548 16.28951 44.03631 and LT- 50. 435.90039 322.31993 590.70833 was done.

RESULTS AND DISCUSSION

The results are depicted in Table 1. All the three EPNs were pathogenic to *R. haemaphysaloides.* However, *H. indica* was more pathogenic leading to 100% mortality of ticks within 48- 72 h at all three concentrations. Tick mortality of 100%, 80% and 40% was observed at 48, 144 and 168 h for EPN concentration of 1250 IJs / Petri dish of *H. indica, S. thermophilum* and *S. carpocapsae,* respectively. When the concentration of EPNs was increased to 2500 IJs/ Petri dish, tick mortality of 100% was observed at 72, 96 and 96 h for *H. indica, S. thermophilum* and *S. carpocapsae,* respectively. As the concentration of EPNs was increased to two fold than the previous concentration (to 5000IJs/ Petri dish), tick mortality of 100% was observed after 72, 72 and 120 h for *H. indica, S. thermophilum* and *S. carpocapsae,* respectively (figure. 1). In all these experiments there was no mortality in the control group.

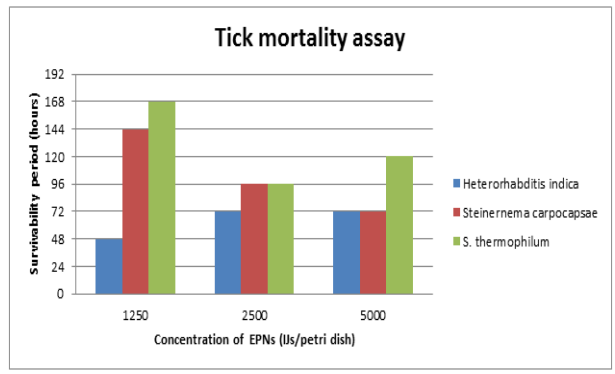

Fig. 1. Tick mortality assay showing the survivability of *Rhipicephalus haemaphysaloides* at different concentrations of three EPNs

Table 1. Comparison of effect of three EPNs on cattle tick *Rhipicephalus hemaphysaloides* (5cm Petridish assay)

EPNs used in the study	Egg mass weight (mg)	Mortality (%)	Survivability(hours)	Hatchability (%)
Heterorhabditis indica	0.1-14.1	100	48-72 hrs	0
Steinernema caprocapsae	No effect	100	72-144 hrs	1
S. thermophilum	12-98.4	100	96-168hrs	0

In egg mass assay, *H. indica* caused a reduction in the egg mass weight with significant differences ($P > 0.01$) between the treated groups (egg mass weight ranged from 0.1-14.1 mg) and the control group (egg mass weight was 255.25 mg). In case of *S. carpocapsae* treated groups, there was no effect on the egg mass weight compared to the control group where as in *S. thermophilum* treated group, reduction in egg mass weight was appreciable (12-98.4 in treated group compared to 255.25 mg in control group).

In addition to the reduction in the egg mass of female ticks in the treated groups, the exposure to the EPNs led to the production of infertile eggs, since the hatching percentage in all the treated groups ranged from 0 to 1 % with significant differences ($p > 0.01$) compared to the untreated control group which showed 41% hatchability.

On examination of blood smears prepared out of the haemocoel of dead ticks, rod shaped *Photorhabdus* spp. symbiotic bacteria were observed.

Entomopathogenic nematodes (EPNs) were used successfully to control different insect pests (Grewal *et al*., 2001) and the use of EPNs to control ticks was also reported (Samish *et al.,* 2008). Thirteen ixodid tick species and two argasid species were susceptible to nematodes, adults being the target stage (Samish *et al*., 2000). The control of *R.* (*B.*) *microplus* (Vasconcelos *et al.,* 2004; Carvalho *et al*., 2010; Monteiro *et al*., 2012) and *R. annulatus* (Samish and Glazer, 1992; Samish *et al*., 2000; Glazer *et al*., 2001) with EPN was widely reported.

The present study was conducted to assess the bio efficacy of three EPNs, *S. carpocapsae, S. thermophilum* and *H. indica* on engorged female cattle tick of *R. haemaphysaloides*. Under laboratory conditions, *H. indica* caused significant deleterious effects on the biology of *R. haemaphysaloides* compared to the other two EPNs. It was also reported by Silva *et al.,* (2012) that *H. indica* affected the reproductive biology of *Rhipicephalus microplus* (Canestrini). Monteiro *et al*., (2014) reported these two entomopathogenic nematodes, *Heterorhabditidis bacteriophora* and *H. indica* pathogenic to partially engorged females of *Dermacentor nitens* Neumann.

The tick mortality in the present study with *H. indica* (100% within 48-72 h) and *S. carpocapsae* (96-168 h) was in accordance with Glazer *et al*. (2001) who had also found higher mortality in heterorhabditid strains than steinernematids strains to engorged females of *B. annulatus* ticks.

The results of the present study also demonstrated that exposure to the infective juveniles (IJs) for 48 hours was sufficient for *H. indica* to penetrate into the haemocoel causing changes in the evaluated parameters often killing the females before the onset of oviposition. Silva *et al*. 2012 also demonstrated that *H. indica* LPP1 is one of the most virulent species of EPN under Laboratory conditions. They evaluated the virulence of three Heterorhabditis and six Steinernematids on engorged female ticks of *Boophilus annulatus* Say, they found that six hour exposure time of ticks to Heterorhabitids can result in > 80% mortality but only 20 65 % resulted against Steinernematids However in our study we used *H. indica* which was also found to be pathogenic when compared to Steinernematids . Heterorhabitids were generally more virulent to ticks than Steinernematids (Glazer *et al*., 2001). Heterorhabditid and Steinernematid nematodes differ in their mode of reproduction in heterorhabditid nematodes, the first generation individuals are produced by self-fertile hermaphrodites (hermaphroditic) but subsequent generation individuals are produced by cross fertilization involving males and females (amphimictic). In Steinernematid nematodes with an exception of one species, all generations are produced by cross fertilization involving males and females (amphimictic).

The tick mortality in the present study (100%) was also in accordance with Kaaya *et al*., (2000) who had also found 92-100% mortality with *S. carpocapsae,* but found very low percent mortality (13-50%) at higher concentration of 5000 IJs/ petridish, whereas in the present study 100% mortality was observed after 72 h at the concentration of 5000 IJs/ petridish. This variation could be attributed to the variation in the susceptibility of tick species.

The three EPNs used in the present study not only caused a significant reduction in the egg mass weight, but led to the production of infertile eggs and the hatching percentage declined significantly in all the treated groups compared to the control groups.

The findings of the present study with regard to 100% tick mortality, significant reduction in the egg production and no hatchability were in accordance with Silva *et al*. (2012) who had reported 99.7-100% mortality of *R. microplus*, significant reduction in egg mass weight (0.1-2.2 mg) and hatching percentage 0-5% with *H. indica.* Monteiro *et al.* (2014) found 77.1-95.9% mortality of *Dermacentor nitens* Neumann, significant reduction in egg mass weight (3.2-14.4 mg) and 47.5-56.7%, hatchability with *H. indica.*

The lower mortality in *D. nitens* could be attributed to resistance of *D. nitens* to *H. indica* (Monteiro *et al.,* 2014).

The findings of the present study with *S. thermophilum* revealed that it was more pathogenic to cattle ticks (100% in *in vitro* studies) than agricultural crop pests (40.5-46% in *in vivo* studies). This happened to be the first report of this species of EPN on the cattle ticks and since it is available for the agricultural pest control in India its potential as a bio control agent for cattle ticks was explored.

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