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In-vitro Studies on Efficacy of Native Entomopathogenic Nematodes (*Steinernema carpocapsae*) on Cattle Ticks

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Abstract: Entomopathogenic nematodes (EPNs) have been successfully used as biological control agents for insects of economically important crops. In the present study, bio-efficacy of two different strains of entomopathogenic nematodes, *Steinernema carpocapsae* STSLU and *S. carpocapsae* STUDR against two different cattle hard ticks, *Rhipicephalus microplus* and *Hyalomma savignyi* was evaluated based on percentage mortality under laboratory conditions. The adult female cattle ticks (of both species) were treated with infective juveniles (IJs) of both the strains of *S. carpocapsae* at different inoculum levels. All the treatments were replicated four times at 20°C. Percentage mortality of the cattle ticks was determined every 24 hours up to 120 hours from the time of inoculation. The experimental results showed the cattle tick *R. microplus* was more susceptible to both strains of *Steinernema carpocapsae* than that of *H. savignyi.* Further, *S. carpocapsae* STSLU was more efficient than *S. carpocapsae* STUDR and caused 100 and 97.5% mortality of *R. microplus* and *H. savignyi*, respectively at a concentration of 250 IJs Petri dish^{-1} after 120 hours of inoculation. The entomopathogenic nematodes can be cultured easily in an artificial medium and have high reproductive efficiency, broad host range, long storage ability, ease of application and being safe for the host make them promising bio-control agent against *R. microplus* and *H. savignyi.* This may be evaluated further in field conditions in different seasons and temperatures. Future research may be directed towards emerging technologies of ticks control without acaricide uses.

Keywords: Ticks, biological control, entomopathogenic nematodes, *Steinernema carpocapsae, Rhipicephalus microplus, Hyalomma savignyi*

Ticks can be found on many hosts, including cattle, buffalo, horses, donkeys, goats, sheep, deer, pigs, dogs and wild animals. Ticks are one of the leading monetary menaces to the cattle industry worldwide, affecting productivity, health and welfare. They are obligate blood-feeding ectoparasites that infest 80% of the cattle worldwide (Grisi *et al.*, 2014). Livestock

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are the major source of livelihood but due to unhygienic condition in the shed and in open grazing system, the chances of infestation with ectoparasite in livestock are very high and cause heavy blood losses, irritation, hide damage and weight losses resulting in lower productivity (Kaur *et al*. 2016). Loss of appetite in heavily tick-infested cattle was found responsible for 65% of the bodyweight reduction (Seebeck *et al.,* 1971). These ectoparasites are among the most critical health problems like babesiosis, theileriosis, anaplasmosis and anemia (Kocan *et al*., 1998). Ticks are highly responsible for economic losses worldwide, putting food safety at risk (Fernanedz-salas *et al*., 2017). In India, almost all the livestock species suffer from tick infestations. Cost of ticks and ticks born diseases (TTBDs) in animals, in India has been estimated as a direct loss of more than 2000 crore per annum (Ghosh *et al*., 2007). According to the FAO (2004), 80% of the world's cattle population is exposed to ticks infestation and has estimated the impact of 7.3 US $ head^{-1} year^{-1}. In addition to directly affecting their hosts, ticks are also the most important group of parasitic arthropods as vectors of pathogens that affect domestic animals and wildlife (Perez de Leon *et al*., 2020). Tick-borne pathogens are the foremost reason for transboundary livestock diseases, listed as notifiable by the World Organization for Animal Health (Esteve- Gasent *et al*., 2020). The TTBDs have been recognized as a major cause of production loss predominantly in tropical and subtropical countries of the world (Kumar and Nagarajan, 2013 and Mondal *et al*., 2013). Since the beginning of 20thcentuary investigators have documented numerous potential tick bio-control agents including pathogens and parasitoids (Samish *et al*., 2001). Entomopathogenic nematodes (EPNs) are parasites of insects. These are characterized by carrying specific symbiotic bacteria of the genus *Xenorhabdus* or *Photorhabdus* in their intestine (Boemare *et al*., 1993). Symbiotic bacteria play an important role in the pathogenicity of the nematodes bacteria complex to insect host and the subsequent reproduction of the nematodes in the host (Akhurst and Boemare 1990). EPNs are currently used as biopesticides to control several important insect pests worldwide (Shapiro Ilan *et al*., 2002).

EPNs are associated with symbiotic bacteria therefore they are extraordinary lethal to many important soil insect pests. Biological control of insect pests using EPNs has gained importance in current years. Because they are highly virulent and kill their host within 24 to 48 hrs. They can be cultured easily *in vivo* as well as *in vitro* (on artificial diet), possess long storage ability, have a high reproductive potential, broad host range and can easily be applied in soil and foliage without adverse effects on non-target organisms (Georgis *et al*., 1991). They are safe for plant and animal health. Recently, it has been demonstrated that the entomopathogenic nematode, *Steinernema carpocapsae* has the potential to be used as a biological control agent against cattle tick, *Rhipicephalus microplus* and *Hyalomma savignyi*, which are considered to be the most important tick parasite of livestock in the world (Monteiro *et al*., 2010). The major objective of the present investigation was to determine the effects of *Steinernema carpocapsae* on mortality of *R. microplus* and *H. Savignyi* at different levels of inoculums under laboratory conditions for effective bio-control of cattle ticks.

Materials and methods

The bio-efficacy test of indigenous EPNs strains of *Steinernema carpocapsae* STSLU and *S. carpocapsae* STUDR were conducted on important cattle tick, *Rhipicephalus microplus* and *Hyalomma savignyi* under laboratory conditions. Total sterilized 24 Petri plates were used for this experiment. The 25 cattle ticks were placed on Whatman filter paper no. 1 in each Petri plate and inoculated infective juveniles (IJs) from both the strains of *S. carpocapsae* at different inoculum levels *viz*., 50, 100, 150, 200 and 250 IJs Petri plate^{-1}. All the treatments were replicated four times and placed at 20^{o} C under B.O.D. incubator condition. The observations were taken on per cent mortality of cattle ticks after every day up to 5 days from the time of inoculation.

Results and Discussion

The maximum mortality of *R. microplus* was recorded 100% with *S. carpocapsae* STSLU followed by 97.5 with *S. carpocapsae* STUDR @ 250 IJs per tick after 120 hrs (Table 1). Whereas the maximum per cent mortality of *H. savignyi* was 97.5% with *S. carpocapsae* STSLU followed by 92.5% with *S. carpocapsae* STUDR @ 250 IJs per tick after 120 hrs (Table 2). Tick mortality caused by EPNs seems to be due to the rapid

Table 1. Bioefficacy of S. carpocapsae STUDR and S. carpocapsae STSLU against R. microplus

No. of IJs/ insect	EPNs	Percent mortality at different time intervals (hrs.)				
		24	48	78	96	120
50	*S. carpocapsae* STUDR	10.0 (18.44)	25.0 (30.00)	37.5 (37.76)	60.0 (50.77)	72.5 (58.37)
	S. carpocapsae STSLU	12.5 (20.70)	27.5 (31.63)	47.5 (43.57)	65.0 (53.73)	75.0 (60.00)
100	*S. carpocapsae* STUDR	22.5 (28.32)	40.0 (39.23)	52.5 (46.43)	70.0 (56.79)	85.0 (67.21)
	S. carpocapsae STSLU	25.0 (30.00)	45.0 (42.10)	67.5 (55.24)	75.0 (60.00)	85.0 (67.21)
150	*S. carpocapsae* STUDR	35.0 (36.27)	50.0 (45.00)	67.5 (55.24)	82.5 (65.27)	92.5 (74.11)
	S. carpocapsae STSLU	42.5 (40.69)	55.0 (47.87)	75.0 (60.00)	85.0 (67.21)	92.5 (74.11)
200	*S. carpocapsae* STUDR	52.5 (46.43)	65.0 (53.73)	75.0 (60.00)	92.5 (74.11)	95.0 (77.08)
	S. carpocapsae STSLU	55.0 (47.87)	75.0 (60.00)	85.0 (67.21)	92.5 (74.11)	97.5 (80.90)
250	*S. carpocapsae* STUDR	67.5 (55.24)	77.5 (61.68)	85.0 (67.21)	95.0 (77.08)	97.5 (80.90)
	S. carpocapsae STSLU	65.0 (53.73)	82.5 (65.27)	90.0 (71.56)	97.5 (80.90)	100.0 (90.00)
Control	Water	0.0	0.0	0.0	0.0	0.0
SEm±		0.637	1.302	2.709	2.806	2.443
CD (0.05%)		1.920	3.924	8.166	8.457	7.363
CV (%)		16.98	9.41	10.53	8.44	6.37

Data in parenthesis are angular transformed values

Table 2. Bioefficacy of S. carpocapsae STUDR and S. carpocapsae STSLU against H. savignyi

No. of IJs/ insect	EPNs	Percent mortality at different time intervals (hrs.)				
		24	48	78	96	120
50	*S. carpocapsae* STUDR	5.0 (4.05)	12.5 (20.70)	17.5 (24.73)	32.5 (34.76)	57.5 (49.31)
	S. carpocapsae STSLU	5.0 (4.05)	12.5 (20.70)	27.5 (31.63)	47.5 (43.57)	67.5 (55.24)
100	*S. carpocapsae* STUDR	12.5 (20.70)	25.0 (30.00)	32.5 (34.76)	52.5 (46.43)	70.0 (56.79)
	S. carpocapsae STSLU	15.0 (22.79)	25.0 (30.00)	47.5 (43.57)	65.0 (53.73)	75.0 (60.00)
150	*S. carpocapsae* STUDR	25.0 (30.00)	42.5 (40.69)	55.0 (47.87)	67.5 (55.24)	80.0 (63.44)
	S. carpocapsae STSLU	30.0 (33.21)	47.5 (43.57)	57.5 (49.31)	75.0 (60.00)	85.0 (67.21)
200	*S. carpocapsae* STUDR	37.5 (37.76)	55.0 (47.87)	65.0 (53.73)	80.0 (63.44)	87.5 (69.30)
	S. carpocapsae STSLU	42.5 (40.69)	65.0 (53.73)	75.0 (60.00)	85.0 (67.21)	92.5 (74.11)
250	*S. carpocapsae* STUDR	45.0 (42.13)	62.5 (52.24)	77.5 (61.66)	90.0 (71.56)	92.5 (74.11)
	S. carpocapsae STSLU	57.5 (49.31)	72.5 (58.37)	82.5 (65.27)	90.0 (71.56)	97.5 (80.90)
Control	Water	0.0	0.0	0.0	0.0	0.0
SEm±		0.636	1.311	2.739	2.856	2.453
CD (0.05%)		1.909	3.933	8.217	8.567	7.359
CV (%)		16.87	9.29	10.57	8.47	6.36

Data in parenthesis are angular transformed values.

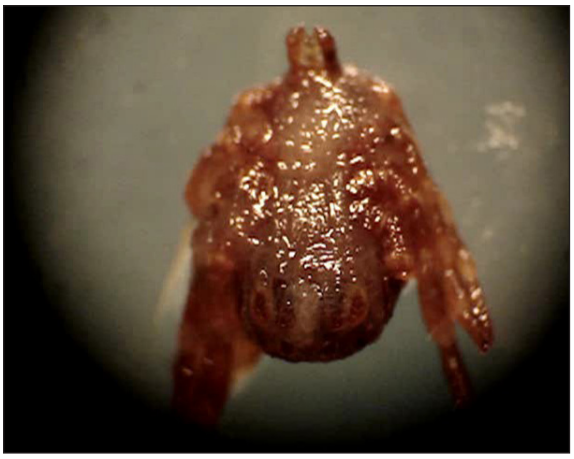

Fig. 1. R. microplus infected with S. carpocapsae.

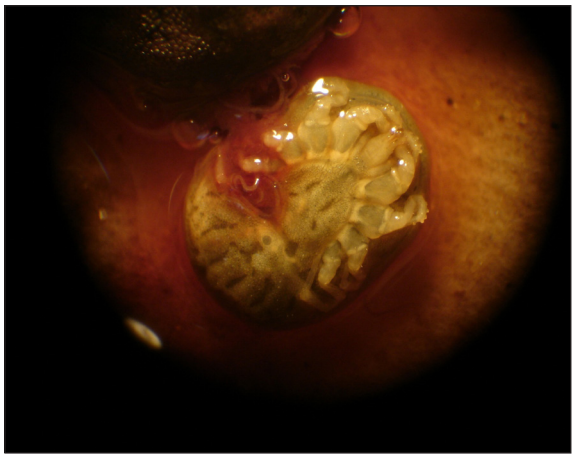

Fig. 2. H. savignyi infected with S. carpocapsae.

proliferation of the nematode symbiotic bacteria within the ticks, since the nematodes do not go through their natural cycle within ticks and most infective juveniles die shortly after entry into ticks (Hassanain *et al.,* 1999). *In vitro* experiments demonstrated that tick hemolymph hinders the growth of EPNs (Zangi, 2003). Similar studies in this regard were carried out by Kocan *et al.,* (1998) who also reported that infective juveniles (IJs) of different EPNs strains (*Steinernema glaseri*, *S. riobravus*, *S. carpocapsae*, *S. feltiae* and *Heterorhabiditis bacteriophora*) appeared to be the most effective in killing ticks and invaded and killed 30 to 100% of replete females. Samish *et al.,* (2000) reported that the mortality of *Rhipicephalus bursa*, and *Rhipicephalus sanguineus* adult ticks were recorded after 0.3 to 8.0 days of their exposure in Petri dishes to 5 entomopathogenic nematode strains. Maru *et al.,* (2011) also recorded a cent per cent mortality of *R. microplus* was observed with 500 *S. carpocapsae* IJs Petri plate^{-1} after the fourth day of inoculation. Similar studies were made by Samish *et al.,* (1999) that the Mexican strain of *Steinernema carpocapsae* was most efficient, inducing 100% tick mortality at a concentration of 50 nematodes per square centimeter in comparison to our study of 97.5 % mortality of ticks through EPNs.

Conclusion

Ticks infestation is a significant cause of economic losses to the dairy industry all over the world. At present, acaricides are mostly used for ticks control. Nematodes are potentially used tools for ticks control because engorged ticks are susceptible to EPNs. Our results have shown that EPNS can control ticks. However,

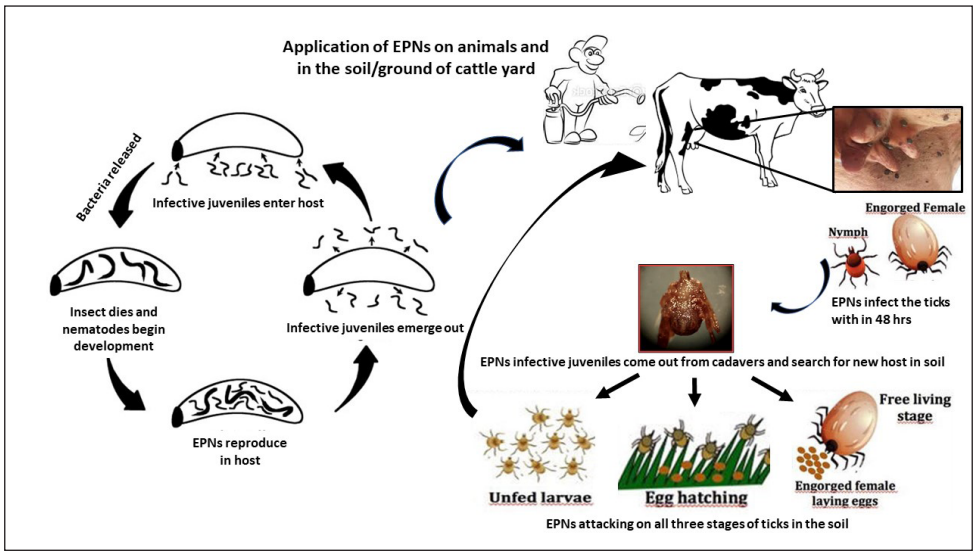

Fig. 3. Diagrammatic representation of using entomopathogenic nematodes for controlling cattle ticks.

the use of nematodes may be limited to defined ecological niches because their pathogenicity is reduced by low humidity or temperature and differences in the susceptibility among the various tick stage and species

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Competing Interests

The author declares that they have no competing interests.

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