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1	Biological Control of Cattle Ticks through Native
2	Entomopathogenic Nematodes (Steinernema Carpocapsae)
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6	

7 Abstract

 $_{\ensuremath{\scriptscriptstyle 8}}$ Entomopath
ogenic nematodes have been successfully used as biological control agents for

⁹ insects of economically important crops. In the present study, the bioefficacy of two different

¹⁰ strains of entomopathogenic nematodes, Steinernema carpocapsae STSLU and S. carpocapsae

¹¹ STUDR against two different cattle hard ticks, Rhipicephalus microplus and Hyalomma

¹² savignyi was evaluated based on percentage mortality under laboratory conditions. The adult

¹³ female of cattle ticks were inoculated with infective juveniles (IJs) of the strains S.

¹⁴ carpocapsae at different inoculum levels. All the treatments were replicated four times at 20°

¹⁵ C in a B.O.D. incubator. The percentage mortality of the cattle ticks was determined every 24

- ¹⁶ hours up to 120 hours from the time of inoculation.
- 17

18 Index terms—ticks, biological control, epns, entomopathogenic nematodes.

¹⁹ 1 Introduction

icks can be found on many hosts, including cattle, buffalo, horses, donkeys, goats, sheep, deer, pigs, dogs, and wild 20 animals. Ticks are one of the leading monetary menaces to the cattle industry worldwide, affecting productivity, 21 health and welfare. They are obligate blood-feeding ectoparasites that infest 80 percent of the cattle worldwide 22 (Grisi et al., 2014). Livestock are the major source of livelihood but due to unhygienic in a herd and open grazing 23 the chances of ectoparasite in livestock will be more common and causing heavy blood losses, irritation, hide 24 25 damage and weight losses resulting in lower productivity (Kaur et al., 2016). Loss of appetite in heavily tick-26 infested cattle was found responsible for 65 % of the bodyweight reduction ??Seebeck, 1971). These ectoparasites are among the most critical health problems like babesiosis, theileriosis, anaplasmosis and anemia. Ticks are 27 highly responsible for economic losses worldwide, putting food safety at risk (Fernanedz-salas et al., 2017). In 28 29 India, almost all the livestock species suffer from tick infestations India alone the cost of ticks and ticks born diseases (TTBDs) in animals has been estimated direct loss of more than 2000 crore per annum (Ghosh et al., 30 2007). According to the FAO (2004), 80 % of the world's cattle population is exposed to ticks infestation and has 31 estimated the impact of 7.3 US S/head/year. In addition to directly affecting their hosts, ticks are also the most 32 important group of parasitic arthropods as vectors of pathogens that affect domestic animals and wildlife (Perez 33 de Leon et al., 2020). Tick-borne pathogens are the foremost reason for transboundary livestock diseases, listed 34 as notifiable by the World Organization for animal health (Esteve-Gasent et al., 2020). The TTBDs have been 35 36 recognized as a major cause of production loss predominantly in tropical and subtropical countries of the world 37 (De Castro, 1997; ??arthiban et al., 2010;Lurthu et al., 2012;Arunkumar and Nagarajan, 2013;Mondal et al., 38 2013). Since the beginning of 20 th centuary investigators have documented numerous potential tick bio-control 39 agents including pathogens, parasitoids and predictors of ticks (Samish & Alexseev, 2001). Entomopathogenic nematodes (EPNs) are parasites of insects. These are characterized by carrying specific symbiotic bacteria of 40 the genus Xenorhabdus or Photorhabdus in their intestine (Boemare et al. 1993). Symbiotic bacteria play 41 an important role in the pathogenicity of the nematodes bacteria complex to insect host and the subsequent 42 reproduction of the nematodes in the host (Akhurst and Boemare 1990). EPNs are currently used as biopesticides 43 to control several important insect pests worldwide ??Shapiro Ilan et al., 2002). 44

EPNs are associated with symbiotic bacteria therefore they are extraordinary lethal to many important G soil 45 insect pests. Biological control of insect pests using EPNs has gained importance in current years. Because they 46 are highly virulent and killing their host within 24 to 48 hrs. They can be cultured easily in vivo as well as in 47 48 vitro (on artificial diet), longer 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 49 are safe for plant and animal health. Recently, it has been demonstrated that the entomopathogenic nematode, 50 Steinernema carpocapsae has the potential to use as a biological control agent against cattle tick, Rhipicephalus 51 microplus and Hyalomma savignyi, which is considered to be the most important tick parasite of livestock in 52 the world (Monteiro et al., 2010). The major objective of the present investigation was to determine the effects 53 of Steinernema carpocapsae on mortality of R. microplus and H. Savignyi at different levels of inoculums under 54 laboratory conditions for effective bio-control of cattle ticks. 55

56 **2** II.

57 3 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 plats 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. All the treatments were replicated four times and placed at 20° 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.

65 **4** III.

66 5 Results

The experiment was conducted for evaluating the potential of the entomopathogenic nematodes (EPNs) 67 68 indigenous strains S. carpocapsae against cattle ticks at different inoculum levels under laboratory conditions. The bio-efficacy was tested based on percent mortality of the cattle ticks R. microplus and H. savignyi were found 69 susceptible against both the strains of S. carpocapsae STUDP-1 and STSLU under laboratory conditions. The 70 71 maximum mortality of R. microplus was recorded 100 per cent with S. Carpocapsae STSLU followed by 97.5 with S. carpocapsae STUDP-1 @ 250 IJs per tick after 120 hrs (Table 1). Whereas the maximum per cent mortality 72 of H. Savignyi was 97.5 per cent with S. Carpocapsae STSLU followed by 92.5 with S. carpocapsae STUDP-1 @ 73 74 250 IJs per tick after 120 hrs (Table 2). 75 IV.

76 6 Discussion

Tick mortality caused by EPNs seems to be due to the rapid 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 (Hassanain et al. 1999).

In vitro experiments demonstrated that tick hemolymph hinders the growth of EPNs ??Zangi, 2003). Similar 80 studies in this regard were made by who also reported that infective juveniles (IJs) of different EPNs strains 81 (Steinernema glaseri, S. riobravus, S. carpocapsae, S. feltiae and Heterorhabiditis bacteriophora) appeared to be 82 the most effective in killing ticks and invaded and killed 30 to 100% of replete females. Samish et al. ??2000) 83 reported that the mortality of Rhipicephalus bursa, and Rhipicephalus sanguineus adult ticks were recorded after 84 0.3 to 8.0 days of their exposure in Petri dishes to 5 entomopathogenic nematode strains. Maru et al. (2011) 85 also recorded a cent per cent mortality of R. microplus was observed at 500 S. carpocapsae IJs/Petri plate after 86 the fourth day of inoculation. Similar studies were made by Samish et al. 87

(1999) that the Mexican strain of Steinernema carpocapsae was most efficient, inducing 100% tick mortality
 at a concentration of 50 nematodes per square centimeter to our study 97.5 % mortality of ticks through EPN.
 V.

91 7 Conclusion

92 The development of anti-tick biological control agents is still in its babyhood. Furthermore, the various steps 93 required for commercialization of these products (production, storage and delivery) and education of consumers 94 (storage, application and evaluation of results) are still in the future. Ticks infestation is a significant cause of 95 economic losses to the dairy industry all over the world. At present, acaricides are mostly used for tick's control. To the extent possible, dairy farmers and veterinarians should make use of an integrated tick control strategy based 96 on the utilization of biological control methods, breeding for tick resistance breeds etc. Nematodes are potentially 97 used tools for ticks control because engorged ticks are susceptible to EPNs. However, the use of nematodes may 98 be limited to defined ecological niches because their pathogenicity is reduced by low humidity or temperature and 99

100 differences in the susceptibility among the various tick stage and species. Ticks have numerous natural enemies

but Entomopathogenic have only a limited pragmatic role in tick's control. At present TTBDs control is mainly 101 affected by the widespread use of acaricides like organophosphates, carbamates, pyrethroids, BHC/cyclodines, 102 amidines, macrocyclic lactones and benzoyl phenyl ureas leading to various problems such as resistance, residues, 103 environment pollution and high cost. These factors reinforce the need for alternative approaches to control 104 ticks infestations. Several plants and herbs have been shown to possess anti-tick insecticidal, growth-inhibiting, 105 antimolting and repellent activities. A number of reports are available on the use of vaccines for tick control on 106 the horizon effect of different extracts of plant material on tick species. Due to severe problems associated with 107 the continuous use of acaricides on animals, integrated ticks management is recommended. Increasing public 108 health concern over tick-born diseases demands the strategic control of ticks on animals that transmit diseases 109 to human beings. The development of improved formulations is also important. Finally, in-depth studies are 110 needed to elucidate the interaction between nematodes and ticks under field conditions.



Figure 1: Fig. 1 : Fig. 2 :

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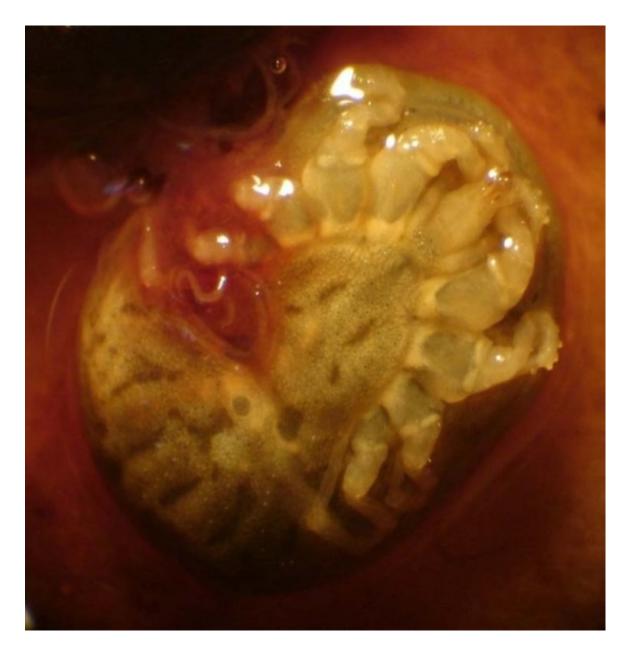


Figure 2:

KH (2021). Advances in int research for area-wide mitig disease burden. In Book: A Management: Development Pereira R, Vreysen MJB, Ed Raton, FL, USA, p 251-274	 R, Mahesh M & Raman M e in cattle of Tamil Nadu adu, J Vet AnimSci, 6: chell RD, Miller RJ, Lohmeyer egrated tick management ation of tick-borne rea-Wide Integrated Pest and Field Application, ds, CRC Press: Boca . & Glazer I (1999). Efficacy of e strains against sus females (Acari: ield conditions. Kimron agan, Israel, Journal Med & Glazer I(2000). Mortality 	 entomopathogenic nematodes, Kimron Veterina Institute, Bet Dagan, Israel, Journal of Parasito 86 (4): 679-84. 23. Samish M, Gindin G, Alekseev E & Glazer Pathogenicity of entomopathogenic fungi to different developmental stages of Rhipicephalus sanguineus, J Parasitol, 87: 1355-1359. 24. Seebeck RM, Springell PH & O'Kelly JC(19 Alterations in the host metabolism by the speci and anorectic effects of the cattle tick (Boophilusmicroplus) in food intake and body weight growth, Aust J Biol Sci, 24: 373-380. 25. Shapiro Ilan, Gouge DH & Koppeenhfer AM Factors affecting commercial success: case study in cotton, turf and citrus. In Book: Gaugler R. Entomopathogenic Nematology. CABI, New Yo 333-356. 26. Zangi G (2003). Tick Control by Means of Entomopathogenic Nematodes and Fungi, In M thesis, The Hebrew University of Jerusalem, Isr 				
No. of IJs/ insect	EPNs 24 Percent mort	ality at different time intervals	(hrs.) 48 78 96 1			
50	S.carpoca psa e	25.0	37.5 60.0 7			
	STUDP-					
	1					
	(18.44)	(30.00)	(37.76)(50.77) (
	S.carpocaþ£afe	27.5	47.5 65.0 7			
	STSLU (20 70)	(21.62)				
100	(20.70)	(31.63)	(43.57)(53.73) (
100	S.carpoca ² 28a ⁵ e	40.0	$52.5 \ 70.0 \ 8$			
	STUDP-					
	1 (20, 22)	(20.22)	$(A6 \ A2)(56 \ 70)$ (
	(28.32) S.carpoca 25:a le	(39.23) 45.0	(46.43)(56.79) (67.5 75.0 8			
	STSLU	45.0	01.5 15.0 8			
	(30.00)	(42.10)	(55.24)(60.00) (
150	S.carpoca p 5ale	50.0	(05.24)(00.00) (67.5 82.5 9			
100	STUDP-	00.0	01.0 02.0 0			
	1					
	(36.27)	(45.00)	(55.24)(65.27) (
	S.carpoca	55.0	75.0 85.0 9			
	STSLU					
	(40.69)	(47.87)	(60.00)(67.21) (
200	S.carpoca țis ăe	65.0	75.0 92.5 9			
	STUDP-					
	1					
	(46.43)	(53.73)	(60.00)(74.11) (
	S.carpoca písa le	75.0	85.0 92.5 9			
	STSLU					
	(47.87)	(60.00)	(67.21)(74.11) (
250	S.carpocatosáe	77.5	85.0 95.0 9			
	STUDP-					
	1	(61.60)				
	(55.24)	(61.68)	(67.21)(77.08) (
	S.carpocatisale	82.5	90.0 97.5 1			

$\mathbf{2}$

No. of IJs/ in-	EPNs Percent mortality at different time intervals (hrs.) 24 48 78 96					8 78 96	120
$ \frac{\text{sect}}{50} $	S. carpocapsae STUDP-1	5.0	12.5	17.5	32.5		57.5
		(4.05)	(20.70)	(24.73)	(34.76)	(49.31)	
	S. carpocapsae STSLU	5.0	12.5	27.5	47.5	()	67.5
		(4.05)	(20.70)	(31.63)	(43.57)	(55.24)	
100	S. carpocapsae STUDP-1	12.5	25.0	32.5	52.5	. ,	70.0
		(20.70)	(30.00)	(34.76)	(46.43)	(56.79)	
	S. carpocapsae STSLU	15.0	25.0	47.5	65.0		75.0
		(22.79)	(30.00)	(43.57)	(53.73)	(60.00)	
150	S. carpocapsae STUDP-1	25.0	42.5	55.0	67.5		80.0
		(30.00)	(40.69)	(47.87)	(55.24)	(63.44)	
	S. carpocapsae STSLU	30.0	47.5	57.5	75.0		85.0
		(33.21)	(43.57)	(49.31)	(60.00)	(67.21)	
200	S. carpocapsae STUDP-1	37.5	55.0	65.0	80.0	. ,	87.5
		(37.76)	(47.87)	(53.73)	(63.44)	(69.30)	
	S. carpocapsae STSLU	42.5	65.0	75.0	85.0	· · · ·	92.5
		(40.69)	(53.73)	(60.00)	(67.21)	(74.11)	
250	S. carpocapsae STUDP-1	45.0	62.5	77.5	90.0	()	92.5
		(42.13)	(52.24)	(61.66)	(71.56)	(74.11)	
	S. carpocapsae STSLU		72.5	82.5	90.0	()	97.5
		(49.31)	(58.37)	(65.27)	(71.56)	(80.90)	
$\begin{array}{c} {\rm ControlWater} \\ {\rm SEm} \pm \end{array}$		0.0	0.0	0.0	0.0	、 /	0.0
		0.636	1.311	2.739	2.856	2.453	
CD		1.909	3.933	8.217	8.567	7.359	
(0.05%)							
CV		16.87	9.29	10.57	8.47		6.36
(%)							

 $[Note:\ Data\ in\ parenthesis\ are\ angular\ transformed\ values]$

Figure 4: Table 2 :

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¹¹⁴ .2 Competing Interests

- 115 The author declares that he has no competing interests.
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