Original Research

Laboratory Evaluation of the Entomopathogenic Fungus *Beauveria bassiana* against the Cattle Tick *Rhipicephalus (B.) microplus* (Acarina: Ixodidiae)

Greeshma Rao, U. B. and Babasaheb Wamanrao Narladkar

COVAS, Parbhani, Maharashtra, INDIA

***Corresponding author:** greeshmarao12@gmail.com

Rec. Date:	Jan 11, 2018 09:51
Accept Date:	Mar 03, 2018 03:40
DOI	10.5455/ijlr.20180111095133

Abstract

The research was conducted to evaluate the pathogenicity of fungus namely Beauveria bassiana on the egg and adult stages of the cattle tick Rhipicephalus (B.) microplus. The two fungal strains of Beauveria bassiana were used to compare their efficacy against egg and adult stages of Rhipicephalus (B.) microplus ticks. The susceptibility of the tick to the species of entomopathogenic fungi was evaluated by treating engorged female ticks and eggs with fungi at concentrations viz. $1g\times10^8$ conidia/litre, 2×10^8 conidia/litre $3g\times10^8$ conidia/litre, $5g\times10^8$ conidia/litre6g$\times10^8$ conidia/litre. The treatment of ticks and egg was followed as per standard procedure and were observed for further fungal action. The both fungal treatments showed at par results in terms of mortality of adult ticks, reduction in egg laying capacity and hatchability of the treated eggs in comparison with the control and found to be concentration dependent. The hatching rates by the treated groups were inversely proportional to the concentration of conidia used. The results showed that use of Beauveria bassiana fungus might constitute an additional method to an integrated tick management.

Key words: Integrated Tick Management, *Beauveria bassiana, Rhipicephalus (B.) microplus*

How to cite: Greeshma, B., & Narladkar, B. (2018). Laboratory Evaluation of the Entomopathogenic Fungus Beauveria bassiana Against the Cattle Tick Rhipicephalus (B.) microplus (Acarina: Ixodidiae). International Journal of Livestock Research, 8(7), 101-108. doi: 10.5455/ijlr.20180111095133

Introduction

Rhipicephalus (B.) microplus is a hard tick that can be found on many hosts including cattle, buffalo, horses, donkeys, goats, sheep, deer, pigs, dogs and some wild animals *Rhipicephalus (B.) microplus* can be found worldwide in subtropical and tropical regions. Because of its one-host life cycle all stages of development occur on the host at the same time and the presence on a single host of approximately 70 engorged female can represent a total parasitic population exceeding 10,000 ticks (Greeshma, 2017). *Rhipicephalus (B.) microplus* has been proved as an important tick in the tropical areas like India. Its infestation results in retarded development of affected animals causing reduced milk and meat production (Sutherst *et al*., 1983). Tick control throughout the world is based mainly on the repeated use of chemical acaricides. Unlimited use of this tick controlling measures has resulted in problems related to environmental pollution, milk contamination and resistance development in the target species (Onofre *et al.*, 2001). Biological control is becoming an increasingly attractive approach to tick management because of: Increasing trend of people towards pesticide free food, meat, milk, pork etc. with increasing concern about environmental safety and human health. Biological method of tick control involves the use of entopathogenic fungi. It is known to infect different tick species and the efficacy of these interesting fungi to control ticks is highly strain-specific. An efficient biological control agent for of *Rhipicephalus (B.) microplus* must be able to reduce the reproductive biological parameters of engorged females (Perinotto, 2012). Therefore present study was planned with the objective of evaluating the efficacy of two strains of *Beauveria bassiana* against egg and adult stages of *Rhipicephalus (B.) microplus* ticks.

Materials and Methods

The research work was undertaken at Department of Veterinary Parasitology and College of Veterinary and Animal Sciences, (MAFSU) Parbhani, Maharashtra, India.

Fungal Species studied in the Experiment: *Beauveria bassiana*

Procurement of the Fungal Powder

The fungal powders were procured from two different colleges of Maharashtra state.

a. Department of Plant Pathology, College of Agriculture, Vasantrao Naik Marathwada Agricultural University, Parbhani was used in the research to compare their efficacy against various developmental stages of *Rhipicephalus (B.) microplus* ticks (BP).
b. Biological control unit, Department of Agril Entomology, Mahatma Phule Krishi Vidyapeeth Rahuri Dist Ahemednagar (MS)- procured during the year (BR).

Collection of Ticks and Identification

Requisite number of blood engorged female ticks was collected from cattle body using forceps at the junction of skin and ticks. Ticks were identified as *Rhipicephalus (B) microplus*under zoom stereoscopic microscope before being used for the experiment based on the keys (Walker, 2003).

Experimental Design

For all *in vitro* trials, working concentration were prepared as 1,2,4,5 and 6gm powder+ 5gm jaggery+ 1 liter of water which was allowed to soak for 2hours. Test procedure described by Srivastava *et al.* (2008), Bagherwal *et al.* (1994), Maske *et al.* (2000), Kaaya and Hassan, (2000) and Narladkar *et al.* (2015) were followed with little modifications.

Hosted@www.ijlr.org DOI 10.5455/ijlr.20180111095133

For Adult Ticks

In each diluted concentration 10 female ticks were dipped for 1 minute, were dried on filter paper and then placed in test tube as single tick per tube, followed by closure of tube with muslin cloth and rubber band. The mortality of ticks was observed at 24 hrs till 96 hrs. The mortality data were tabulated and efficacy was worked out in terms of per cent mortality. If the ticks were not dead, they were judged for their egg laying capacity; observed for number of eggs laid and compared with control ticks which were only treated with water.

Action on Eggs of Treated Females

The treated female *Rhipicephalus (Boophilus) microplus* ticks not dead, were separately maintained for eggs collection. Eggs collected from such treated female ticks were counted in petri dishes in the batches of 100 numbers, were transferred in tubes which were closed with piece of muslin cloth tied with rubber band. The eggs were observed for hatching, till the period hatching process of eggs in the control group were completed.

On Treated Eggs

The eggs laid by *Rhipicephalus (B.) microplus* ticks in the tubes separately maintained for egg collection, were drawn from tubes in petri dishes. By counting the eggs in 100 numbers were treated with different fungal concentration solution, dried on filter paper and then transferred in tubes. The eggs were observed for hatching till the period all eggs from control tubes were hatched.

Criteria's for Assessment of Efficacy of *Beauveria bassiana*

a. Mortality of adult ticks and eggs
b. Reduction in egg laying capacity
c. Hatchability of eggs from treated females
c. Hatchability of treated eggs

Result and Discussion

The efficacy was judged by using various concentrations of the different fungi on the basis of above mentioned criteria.

Mortality of Engorged Adult Female Ticks

The mortality count, egg laying capacity, hatchability of eggs laid by treated female ticks and hatchability of treated eggs of adult *Rhipicephalus (B.) microplus* female ticks observed after the treatment of various fungal agents showed variation in the mortality count at different concentrations and their average count are depicted in the Table 1,2,3,4 respectively. In case of mortality, at the

 DOI 10.5455/ijlr.20180111095133

concentration of 6g/l, the fungus BP showed significantly higher mortality of 1.00 in comparison with BR which showed 0.71(Table 1).From the results it was assessed that *Beauveria bassiana* established as BCA(Biological Control Agent) are having good amount of activity and can exert as cidal (killing) effect on adult ticks and the concentration required for producing desired effect is largely depend on species of fungi and species of ticks against which it is to be used.

Table 1: Mortality of *Rhipicephalus (B.) microplus* female ticks after treatment with fungi at different concentrations

S. No.	Treatments	1g/l Mean±SE Range	2g/l Mean±SE Range	4g/l Mean±SE Range	5g/l Mean±SE Range	6g/l Mean±SE Range	CD
I	*Beauveria bassiana* Rahuri	$^{p}0.00^{a}\pm0.00$ (0-0)	$^{p}0.00^{a}\pm0.00$ (0-0)	$^{p}0.29^{b}\pm0.18$ (0-1)	$^{p}0.43^{c}\pm0.20$ (0-1)	$^{p}0.71^{d}\pm0.18$ (0-1)	(5%) 0.120)
II	*Beauveria bassiana* Parbhani	$^{p}0.00^{a}\pm0.00$ (0-0)	$^{p}0.00^{a}\pm0.00$ (0-0)	$^{p}0.15^{b}\pm0.15$ (0-1)	$^{p}0.29^{c}\pm0.18$ (0-1)	$^{q}1.00^{d}\pm0.00$ (01-Jan)	(1%) 0.162
III	Control	$^{p}0.00^{a}\pm0.00$ (0-0)	$^{p}0.00^{a}\pm0.00$ (0-0)	$^{r}0.00^{a}\pm0.00$ (0-0)	$^{q}0.00^{a}\pm0.00$ (0-0)	$^{r}0.00^{a}\pm0.00$ (0-0)	
	Critical Difference(CD)	(5%)0.167			(1%)0.224		

Superscripts p, q, r indicates significant differences between different fungi (amongst the column)

While determining the egg laying capacity it was found that treatment with both fungi resulted in significant reduction in egg laying capacity of the female adult ticks and was significantly reduced as compared to control group. It was found that *Beauveria bassiana* Parbhani strain @ concentration of 6g/l) proved as best in comparison to Rahuri strain (Table 2).

Table 2: Mean reduction in egg laying capacity of *Rhipicephalus (B) microplus* female ticks after treatment with fungi at different concentrations

S. No.	Treatments	1g/l Mean±SE Range	2g/l Mean±SE Range	4g/l Mean±SE Range	5g/l Mean±SE Range	6g/l Mean±SE Range	CD
I	*Beauveria bassiana* Rahuri	$^{p}68.00\pm40.96$ (0-300)	$^{p}66.29\pm24.55$ (11-150)	$^{p}53.15\pm27.34$ (10-200)	$^{p}47.57\pm18.61$ (0-100)	$^{p}25.00\pm13.04$ (0-100)	(5%) 80.218
II	*Beauveria bassiana* Parbhani	$^{p}12.00\pm1.84$ (5-20)	$^{p}9.43\pm2.47$ (0-20)	$^{p}8.57\pm1.14$ (5-13)	$^{p}8.85\pm2.64$ (0-20)	$^{p}0.00\pm0.00$ (0-0)	(1%)105.425
III	Control	$^{q}1578.55\pm261.32$ (350-2315)	$^{q}1578.55\pm261.32$ (350-2315)	$^{q}1578.55\pm261.32$ (350-2315)	$^{q}1578.55\pm261.32$ (350-2315)	$^{q}1578.55\pm261.32$ (350-2315)	
	Critical Difference(CD)	(5%) 107.621			(1%)141.448		

Superscripts p, q indicates significant differences between different fungi (amongst the column)

In case of hatchability of eggs laid by treated female ticks it was noted that both the fungi resulted in significant reduction in hatchability of the eggs, which were harvested from the treated female ticks (eggs

  DOI 10.5455/ijlr.20180111095133

were not directly exposed to the fungal treatment). From this it was evident that both fungi tried in the present study have shown the effect not only as cidal of female engorged ticks but also exerted ovicidal activity. In reference to hatchability of treated eggs both fungi had shown significant effect in reducing the hatching percentage of eggs of *Rhipicephalus (B.) microplus*. The both fungi applied to tick eggs which resulted in reducing the hatchability of treated eggs and therefore if used at the sites where tick deposits the eggs, can prove as best biological control agent. In nutshell it can be said that susceptibility of ticks to a particular fungus largely depend on-

1) Tick genera and species(genetics), their physiology
2) Fungus species, strain and conidial concentration
3) Temperature, humidity and geographic area

Therefore present study also recommends that, before using any fungal BCA in a particular geographic area, location based trial is must.

The Reasons behind Using the Different Isolates of the Same Fungi

In the present study *Beauveria bassiana* were used as isolates from MPKV Rahuri and Parbhani. The reason behind using isolates from two different places is to evaluate whether there exist any isolate/ strain difference and can it affect the potential of fungus in terms of cidal effect against adult and egg stages of *Rhipicephalus (B.) microplus* and in terms of hatchability. After study it was found that no significant difference were noted and both the isolates of both the fungi showed *at par* activity, though there are numerical differences (Tables 1, 2, 3 ,4).

Table 3: Showing mean hatchability of eggs harvested from *Rhipicephalus (B.) microplus* female ticks treated with various fungi at different concentrations

S. No.	Treatments	1g/l Mean±SE Range	2g/l Mean±SE Range	4g/l Mean±SE Range	5g/l Mean±SE Range	6g/l Mean±SE Range	CD
I	*Beauveria bassiana* Rahuri	$^{p}4.85^{a}\pm2.18$ (0-14)	$^{p}4.57^{a}\pm1.92$ (0-12)	$^{p}1.29^{b}\pm0.48$ (0-3)	$^{p}0.43^{b}\pm0.20$ (0-1)	$^{p}0.15^{b}\pm0.15$ (0-1)	(5%) 1.290
II	*Beauveria bassiana* Parbhani	$^{p}3.57^{a}\pm1.57$ (0-10)	$^{p}3.15^{a}\pm2.81$ (0-20)	$^{p}2.43^{ab}\pm1.60$ (0-10)	$^{p}1.85^{b}\pm0.76$ (0-5)	$^{p}1.57^{b}\pm0.71$ (0-5)	(1%) 1.703
III	Control	$^{q}97.57^{a}\pm0.71$ (95-100)	$^{q}97.57^{a}\pm0.7$ (95-100)	$^{q}97.57^{a}\pm0.71$ (95-100)	$^{q}97.57^{a}\pm0.71$ (95-100)	$^{q}97.57^{a}\pm0.71$ (95-100)	
	Critical Difference(CD)	(5%)1.748			(1%)2.295		

Superscripts a,b indicates significant differences between concentrations (amongst the rows); Superscripts p,q indicates significant differences between different fungi (amongst the column

Humidity is considered as one of the critical factors affecting the outcome of both laboratory and field-tests with *Beauveria bassiana*. The author Clark *et al.* (1968) mention a study by Hart and MacLeod, (1955), who found optimal germination of *Beauveria* conidia occurring at relative humidities above 94%. Infection does not appear to be dependent on temperature (Schaerffenberg, 1964; Ferron, 1981). For

DOI 10.5455/ijlr.20180111095133

conidia however, high temperatures may be harmful, especially in combination with high humidity conditions. The effective stages of the fungus against larvae are conidia and blastoconidia, the latter stage being far more pathogenic (Miranpuri and. Khachatourians, 1991). Although growing blastoconidia is relatively easy, production has been abandoned because of the difficulties of storing this type of conidium (Ferron, 1981).

Table 4: Showing mean hatchability of the adult Rhipicephalus (B) microplusfemaletick eggs after treatment with various fungi at different concentrations

S. No.	Treatments	1g/l Mean±SE Range	2g/l Mean±SE Range	4g/l Mean±SE Range	5g/l Mean±SE Range	6g/l Mean±SE Range	CD
I	*Beauveria bassiana* Rahuri	$^{pt}26.80^{a}\pm3.36$ (15-35)	$^{p}14.80^{b}\pm2.95$ (10-25)	$^{p}10.20^{b}\pm1.50$ (5-14)	$^{p}4.39^{c}\pm1.96$ (1-12)	$^{p}3.80^{c}\pm1.12$ (2-8)	(5%) 4.521
II	*Beauveria bassiana* Parbhani	$^{q}84.80^{a}\pm1.99$ (11-79)	$^{q}78.40^{b}\pm3.14$ (68-85)	$^{p}9.47^{c}\pm4.24$ (11-36)	$^{q}8.60^{c}\pm1.50$ (5-12)	$^{p}6.60^{c}\pm1.64$ (1-10)	(1%) 5.940
III	Control	$^{r}96.80^{a}\pm0.86$ (95-100)	$^{r}96.80^{a}\pm0.86$ (95-100)	$^{q}96.80^{a}\pm0.86$ (95-100)	$^{r}96.80^{a}\pm0.86$ (95-100)	$^{q}96.80^{a}\pm0.86$ (95-100)	
	Critical Difference(CD)	(5%)6.067			(1%)7.974		

Superscripts a, b, c indicates significant differences between concentrations (amongst the rows); Superscripts p, q, r indicates significant differences between

Paiao *et al.* (2001) observed that, *B. bassiana* fungus had no significant effect on ovipostioning by *B. microplus* tick while it had high effect on hatchability of eggs. Disagreeing to this, in the present study, we observed different isolates of same fungi with different concentration showed significant effect on ovipositioning and hatchability of *B. microplus* ticks.

LC_{50} Values in Comparison with LD_{50} Values in Rats for Different Fungi and Herbal Acaricide

LC_{50} values for *Beauveria bassiana* was calculated by Bioassay method given by Finney in 1952 of LC_{50} (by Formula methods and Graph method) by using software available on the web probitanalysis.wordpress.com (Annonymous, 2017a)(Table 5).

From the comparison between LC_{50} values and their corresponding LD_{50} values in rats, it can be assessed that LC_{50} values are quite less than LD_{50} values indicating these fungi are safe for use and not harmful to domestic animals and human being.

 DOI 10.5455/ijlr.20180111095133

Table 5: Indicating LC_{50} values and its corresponding LD_{50} values in rats *Beauveria bassiana* against adult female *R. (B.) microplus* tick eggs

Fungus	LC_{50} values for eggs from treated female	LC_{50} values for treated eggs	LD_{50} values in rats	References
BR	1g/l	2.094g/l	*Beauveriabassiana*HF23 at a dose of 4.05 x10^9cfu per animal	BeauveriabassianaHF23 Final Draft of EPA United States Environmental protection Agency
BP	1g/l	1.633g/l		Biopesticide Registration Action Document December 22, 2006 (www.epa.gov/pesticides), (Anonymous 2017b)

Conclusion

Both the strain of *Beauveria bassiana* tested showed significant activity in terms of mortality of adult ticks, reducing egg laying capacity, less hatchability and less hatchability of treated eggs. The activity was optimum at 5g/l and hence it can be recommended for tick control programmes, subject to the evaluation under field conditions. Both the fungi showed higher activity when applied to the egg stage compared to the adult ticks. Thus present study concludes that fungal treatments are better choice as ovicidal as compared to tickicidal in the tick control programmes.

References

1. Anonymous. 2017a. probitanalysis.wordpress.com.Designed by Dr Alpha Raj. This calculator is based on the method of Finney (1952). [D. J. Finney (1952) Probit Analysis (2nd Ed), Journal of the Institute of Actuaries, 78 (3): 388-390]. Last seen 15-06-2017.
2. Anonymous. 2017b.*Beauveria bassiana* HF23 Final Draft of EPA United States Environmental protection Agency Bio-pesticide Registration Action Document December 22, 2006.www.epa.gov/pesticides, Last seen 15-06-2017.
3. Bhagerwal RK, Sharma A and Dhanotiya RS. 1994. Studies on efficacy of deltamethrin against different stages of *Hyalomma anatolicum anatolicum*. Indian Vet J 71: 1073-1076 on efficacy of some latest acaricide against different stages of ticks.
4. Clark TB, Kellen W, Fukuda T, Lindegren JE. 1968. Field and laboratory studies on the pathogenicity of the fungus *Beauveria bassiana* to three genera of mosquitoes. *Journal of Invertebrate Pathology* 11: 1-7.
5. Ferron P. 1981. Pest control by the fungi *Beauveria* and *Metarhizium*. In: Burgess HD, editor. *MicrobialControl ofPests and Plant Diseases* 1970-1980, 24:465-482. London, Academic Press.
6. Greeshma Rao U B. 2017. In-vitro evaluation of fungal bio-control agents and herbal bio-pesticide against cattle tick, *Rhipicephalus microplus* (Acarina: Ixodidae), MVSC thesis, Approved by the Maharashtra Animal and Fishery Sciences University,Nagpur.
7. Hart MP and MacLeod DM. 1955. An apparatus for determining the effects of temperature and humidity on germination of fungus spores. Canadian Journal of Botany33: 289-292.
8. Maske DK, Kolte SW and Jangde CR. 2000. Efficacy of neem based based compound "Nimbitor"againstectoparasites of cattle. Indian Vet J 77: 103-106.

  DOI 10.5455/ijlr.20180111095133

9. Miranpuri GS and Khachatourians GG. 1991. Infection sites of the entomopathogenic fungus *Beauveriabassiana*in the larvae of the mosquito *Aedesaegypti*. EntomologiaExperimentalisetAplicata59: 19-27.
10. Narladkar BW, Shivpuje PR and Harke PC. 2015. Fungal Bio-Control Agents for Integrated Management of *Rhipicephalus (Boophilus) microplus*Ticks. Indian Vet. J., May 2015, 92 (5): 34 – 37.
11. Onofre SB, Cindia MM., Neiva M. de B and Joao LA. 2001. Pathogenicity of four strains of entomopathogenic fungi against the bovine tick *Boophilus microplus*. American Journal of Veterinary Research 62(9):1478-80 DOI:10.2460/ajvr.2001.62.1478.
12. Paiao JCV, Monteiro AC and Kronka SN. 2001. Susceptibility of the cattle tick *Boophilus microplus* (Acari: Ixodidae) to isolates of the fungus *Beauveria bassiana*. World Journal of Microbiology and Biochemistry 17, 245–251.
13. Perinotto WMS, Angelo IC, Golo PS, Quinelato S, Camargo MG, Sá FA and Bittencourt VR. 2012. Susceptibility of different populations of ticks to entomopathogenic fungi, Exp Parasitol, Mar; 130(3): 257-60. doi: 10.1016/j.exppara.2011.12.003. Epub 2011 Dec 26.
14. Srivastava R, Ghosh S, Mandal DB, Azhahianambi P, Singhal PS, Pandey NN and Swarup D. 2008. Efficacy of *Azadirachta indica* extracts against *Boophilusmicroplus*. Parasitol Res 104:149–153, DOI 10.1007/s00436-008-1173-3.
15. Sutherst RW, MaywaldGF, Kerr JD and Siegeman DA. 1983. The effect of the cattle tick (*Boophilus microplus*) on the growth of *Bos indicus* × *Bos taurus* steers. Aust. Vet. J. Agric. Res., 34, 317–327.
16. Schaerffenberg B. 1964. Biological and environmental conditions for the development of mycoses caused by *Beauveria* and *Metarrhizium.* Journal of Insect Pathology 6: 8-20.