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First report on the use of bio-fungal agent *Verticillium lecanii* against tropical cattle tick, *Rhipicephalus microplus* (Acarina: Ixodidae)

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Abstract
The research was conducted to evaluate the pathogenicity and compare the potency of old and new conidia of entomopathogenic fungus *Verticillium lecanii* against the egg and adult stages of the cattle tick, *Rhipicephalus (B.) microplus.* The fungal powder procured in the year 2012 and 2016 was used in the research to compare their efficacy against various developmental stages of *R. microplus* ticks. The ticks and eggs were treated with fungi at concentrations $1g\times10^8$ conidia/litre, 2×10^8 conidia/litre $3g\times10^8$ conidia/litre, $5g\times10^8$ conidia/litre, $6g\times10^8$ conidia/litre. Both fungal treatments (old and new) 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. The highest mortality was recorded at the concentration $5g\times10^8$ conidia/litre. The hatching rates by the treated groups were inversely proportional to the concentration of conidia used. An *in-vivo* trial carried out suggested that fungi had a good tickicidal and ovicidal property might constitute an additional method for integrated tick management.

Keywords: *Verticillium lecanii*, *R. (B.) microplus*, integrated tick management, tickicidal, ovicidal

1. Introduction
Amongst many parasites infesting livestock; ticks are obligate, blood-feeding ectoparasites of vertebrates (particularly mammals and birds) belonging to the class Arachnida, Order Acari [1]. They feed by inserting specialized piercing mouthparts into the skin of the host, avoiding detection by using a salivary pharmacopeia of anti-inflammatories, analgesics, antihistamines and anticoagulants [2]. Though all ticks are considered as important from livestock health and production point of view, *Rhipicephalus microplus* (formerly *Boophilus microplus*) is considered to be the most important tick parasite of livestock in the world.
R. 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 and can be found worldwide in subtropical and tropical regions. The tick *Boophilus microplus* is a bovine ectoparasite that causes economic losses in herds of tropical and subtropical areas, due to the diseases it transmits and to the parasitism itself, which causes reductions in milk yield, calf production and high costs to control the tick [3].
In practice too, ticks are controlled at present mostly by chemicals acaricides. The indiscriminate use of acaricides against *B. microplus* is toxic to the environment as well as causing resistance in the ticks. The search for alternative ways to control this parasite has led to the investigation of several species of entomopathogenic fungi as potential biological control agents against *B. microplus* and other ticks. Taking into consideration the role in *Rhipicephalus (B) microplus* in disease transmission, in lowering down of health, production and reproduction. it becomes a need to control these ticks by involving herbal and biological strategies. Therefore present study was to evaluate the efficicay of the entomopathogenic fungi *Verticillium lecanii* against the egg and adult stages of the cattle tick, *R. (B.) microplus.*

2. Materials and Methods
Study area: The research work was undertaken at the Department of Veterinary Parasitology and College of Veterinary and Animal Sciences (MAFSU) Parbhani, Maharashtra, India.

Fungal Species studied in the experiment: *Verticillium lecanii*

Procurement of the Fungal Powder: The fungal powder was procured from Biological control unit, Dept of Agril Entomology, Mahatma Phule Krishi Vidyapeeth Rahuri Dist Ahemednagar (MS), India. The fungal powder procured in the year 2012 and manufactured in the year 2016 was used in the research to compare their efficacy against various developmental stages of *Rhipicephalus (B.) microplus* ticks.

Collection of Ticks
Requisite numbers of blood engorged female ticks were collected from cattle (cows/bullock) body using forceps at the junction of skin and ticks.

Identification
Ticks were identified under zoom stereoscopic microscope as *Rhipicephalus (B.) microplus* before being introduced in the experiment [4].

Experimental design
For all *in vitro* trials, working concentration were prepared as 1,2,4,5 and 6gm powder+ 5ml jaggery + 1 liter of water. The standard test procedure was followed with little modification [5, 6, 7, 8].

For adult ticks: In each diluted concentration 10 female ticks were dipped for 1 minute, were dried on filter paper and then placed in a test tube as single tick per tube, followed by closure of the tube with muslin cloth and a 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 a piece of muslin cloth tied with rubber bands. These tubes were maintained in desiccators in which humidity levels were maintained @ 75 %. 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 a different fungal concentration solution, dried on filter paper and then transferred in tubes. These tubes were maintained in desiccators in which humidity levels were maintained @ 75 %. The eggs were observed for hatching, till the period hatching process of eggs in the control group were completed.

3. Criteria's for assessment of efficacy of *Metarhizium anisopliae*
a. Mortality of adult ticks and eggs
b. Reduction in egg laying capacity
c. Hatchability of eggs from treated females
d. Hatchability of treated eggs

4. Statistical analysis
The data obtained from various parameters were analyzed by employing two factor Factorial Experiment using computer application, WASP.

5. Results and Discussion
The average mortality, egg laying capacity, hatchability of eggs from treated female ticks and hatchability of the treated eggs count observed after the treatment with fungi showed variation in the mortality count at different concentrations and their average count at different concentrations are depicted in the Table 1,2,3,4 respectively.

Mortality of engorged adult female ticks
If you look into numerical values in the Table 1 it appears that amongst 10 ticks treated for each fungus at different concentrations; maximum average mortality of 1(in each tube 1 out of 1 tick died meaning 100%) was recorded for only *Verticillium lecanii* new suggesting fungus has effect in causing mortality of adult ticks *in-vitro*, however mortality rate appears to be concentration dependent and with increasing concentration of fungal conidia mortality count was also increased. Therefore as an adulticide both the tested fungal strains exerted much expected effect (Table 1). These values indicates that three species of entomopathogenic fungi established as BCA are having good amount of activity and can exert as cidal (killing) effect on adult ticks.

Table 1: Showing mean mortality of *Rhipicephalus (B) microplus* female ticks after treatment with fungi at different concentrations

Sl. 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
l	*Verticillium lecanii* Rahuri Old	$^{p}0.00^{a}$ ±0.00 0-0	$^{p}0.29^{b}$ ±0.18 0-1	$^{p}0.43^{c}$ ±0.20 0-1	$^{p}0.57^{d}$ ±0.20 0-1	$^{p}0.71^{e}$ ±0.18 0-1	(5%) 0.120
II	*Verticillium lecanii* Rahuri New	$^{p}0.00^{a}$ ±0.00 0-0	$^{pq}0.15^{b}$ ±0.15 0-1	$^{p}0.29^{c}$ ±0.18 0-1	$^{p}0.71^{d}$ ±0.18 0-1	$^{q}1.00^{e}$ ±0.00 1-1	
III	Control	$^{p}0.00$ ±0.00 0-0	$^{q}0.00$ ±0.00 0-0	$^{q}0.00$ ±0.00 0-0	$^{q}0.00$ ±0.00 0-0	$^{r}0.00$ ±0.00 0-0	(1%) 0.162
	Critical Difference(CD)	(5%)0.167			(1%)0.224		

Superscripts a, b, c, d, e indicates significant differences between concentrations (amongst the rows)
Superscripts p, q, r indicates significant differences between different fungi (amongst the columns)

Reduction in egg laying capacity of the treated female ticks
The treatment with both fungi, in terms of reduction in egg laying capacity showed significant variation at different concentrations and was significantly reduced as compared to control group.
The average numbers of eggs laid were 1.00 and 4.29 by the ticks treated with VRN and VRO respectively (Table 2). From Table 2, it can be assessed that *Verticillium lecanii* New @ 6g/l fungal treatments proved as best as compared to old one.

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

Sl. 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
l	*Verticillium lecanii* Rahuri Old	^{q}36.43±27.58 0-200	^{p}31.43±20.87 0-150	^{q}17.15±13.93 0-100	^{p}10.71±3.85 0-30	^{p}4.29±2.02 0-10	(5%) 80.218
II	*Verticillium lecanii* Rahuri New	^{q}53.15±41.94 0-300	^{p}39.29±35.20 0-250	^{p}39.29±26.97 0-200	^{p}10.71±6.93 0-50	^{p}1.00±0.73 0-5	
III	Control	^{r}1578.55±261.32 350-2315	^{r}1578.55±261.32 350-2315	^{r}1578.55±261.32 350-2315	^{q}1578.55±261.32 350-2315	^{q}1578.55±261.32 350-2315	(1%) 105.42
	Critical Difference(CD)	(5%) 107.621			(1%)141.448		

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

Hatchability of eggs laid by treated female ticks

Both fungal treatments for female ticks, though not resulted in causing the mortality of adult female ticks at all five different concentrations tried, but have showed the effect in terms of reduction in hatchability of eggs laid by such treated females. Even 10% eggs could not be hatched as against hatchability of 97.7 in control tick eggs. At the concentration of 6g/l, all the eight fungal treatments showed the effect to their maximum and hatching of eggs could be noted to the lowest. The hatchability recorded to the tune of 0.29 and 0.71 for VRN and VRO respectively (Table 3).

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

Sl. 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
l	*Verticillium lecanii* Rahuri Old	$^{p}6.15^{a}$±1.89 0-12	$^{p}3.15^{b}$±0.40 2-5	$^{p}2.15^{bc}$±1.37 0-10	$^{p}1.85^{c}$±1.39 0-10	$^{p}0.71^{c}$±0.36 0-2	(5%) 1.290
II	*Verticillium lecanii* Rahuri New	$^{q}9.15^{a}$±2.55 0-17	$^{q}5.85^{b}$±2.84 0-20	$^{p}1.43^{c}$±1.43 0-10	$^{p}1.00^{c}$±0.38 0-2	$^{p}0.29^{c}$±0.29 0-2	
III	Control	^{r}97.57±0.71 95-100	^{r}97.57±0.71 95-100	^{q}97.57±0.71 95-100	^{q}97.57±0.71 95-100	^{q}97.57±0.71 95-100	(1%) 1.703
	Critical Difference(CD)	(5%)1.748			(1%)2.295		

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

Hatchability of treated eggs

Eggs harvested from the female ticks were directly exposed to the treatment of various concentrations of fungi. The both fungi had shown significant effect in reducing the hatching percentage (hatchability/ eclosion) of eggs of *R. (B) microplus*.

Both fungi resulted either in killing the embryo (ovicidal) or has not allowed to hatch and failed to yield next generation larvae/seed ticks. The hatchability recorded to the tune of 0.00, and 9.80 for VRN and VRO respectively (Table 4).

Table 4: Showing mean hatchability of the *Rhipicephalus (B.) microplus* tick eggs after treatment with various fungi at different concentrations

Sl. 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
l	*Verticillium lecanii* Rahuri Old	$^{p}73.60^{a}$±8.20 41-85	$^{p}64.00^{b}$±4.05 50-72	$^{p}33.00^{c}$±6.63 10-50	$^{p}11.00^{d}$±1.88 5-15	$^{p}9.80^{d}$±2.43 4-20	(5%) 4.521
II	*Verticillium lecanii* Rahuri New	$^{q}39.40^{a}$±8.27 11-60	$^{q}8.20^{b}$±5.21 0-25	$^{q}3.60^{c}$±1.86 0-10	$^{q}1.60^{c}$±1.02 0-5	$^{q}0.00^{c}$±0.00 0-0	
III	Control	^{r}96.80±0.86 95-100	^{r}96.80±0.86 95-100	^{r}96.80±0.86 95-100	^{r}96.80±0.86 95-100	^{r}96.80±0.86 95-100	(1%) 5.940
	Critical Difference(CD)	(5%) 6.067			(1%)7.974		

Superscripts a, b, c, d, e indicates significant differences between concentrations (amongst the rows)
Superscripts p, q, r indicates significant differences between different fungi (amongst the columns.

Present study results proved that the fungi tested are found to be the promising biological control agents against an important tick pest of cattle. Hence present research has two important components, one as tick and other as fungi tested as BCAs.

Susceptibility of tick species to fungal infection

Potency and activity of the fungus is largely dependent on climatic/environmental factors such as temperature and humidity. At a high temperature, low humidity fungus will not work. However *M. anisopliae* found to work at 60-70% humidity where the temperature ranges between 20-30ºC [7], [8]. In conclusion, testing of fungi at different geographical locations are necessary because population growth of entomopathogenic fungi in an environment depends among many other factors, a) Climatic condition b) type of grass utilized [9], and these two factors vary from geographic area to area.

Why fungi are more efficacious against eggs than adults?

The entomopathogenic fungi are the promising one as ovicidal compared to adulticide and therefore entomopathogenic fungi can be considered as a potential means of reducing tick population by killing egg masses. This fact has been proved through research of several workers including the present one. Tick eggs in contrast to many insect eggs are highly susceptible and upto 100% of eggs exposed to fungi under laboratory conditions did not hatch [10, 11].

Probable reasons for higher effect of fungi on tick eggs as compared to adult are

a) Most tick belongs to Ixodidae family; lay the eggs in large masses on the upper layer of the ground, in niches with high relative humidity which is favorable for the growth of fungus and for making contact of conidia to the ticks eggs.
b) Conidia of entomopathogenic fungi spread on the ground which land in natural environment in which they persist for many months and wait for new tick egg masses [12].

Advantages of application of fungus as anti-tick agent

a) Use of entomopathogenic fungi against ticks has advantage that the fungus penetrates into the integument [13] and not necessary that fungus shall be ingested by the ticks. This type of penetration makes the application of fungus easier and it facilitates quick and easy host-fungi contact.
b) Second advantage of fungi against the tick is that fungi are more specific to target organisms under field condition and this specific nature reduces the level of hazards to non target organisms [14].

Owing to these advantages fungal BCAs are gaining more and more popularity in tick control programmes and therefore research conducted in the present study also gains due importance.

Efficacy of newly harvested and stored fungi

In the present study fungus in 2 forms as old and new were evaluated. New means the fungal conidia which are recently harvested from the growing culture media and old means conidia harvested before 4 years from the growing culture media and stored in dark place away from the sunlight at room temperature.

a) To test the potency of old and new conidia
b) To judge the longevity of conidia

After study it was observed that, whether the fungus is new or old, *at par* efficacy was recorded, though numerically slight differences were in evidence. Similarly after four years of storage, fungi hold the good potency.

6. *In vivo* trials

An *in vivo* (off- host) trial was undertaken to judge the suitability of the fungus on the *R. (B.) microplus* ticks and eggs at field level. During the trial @ concentration of 5g/l were sprayed in the cattle shed at the breeding places where large number of eggs were laid and adult ticks were found lodged. After treatment on day 5, 15 and 21, PI observation was recorded. It was observed that both fungi caused of adult ticks, and resulted in approximately >80% prevention of hatching of treated eggs. The eggs which have come in contact with fungal treatment showed blackish discoloration; change their shape, caused shrinkage indicating death of the embryo.

7. Conclusions

The results obtained in this study, demonstrate the efficacy of *Verticillium lecanii* fungus to control *Rhipicephalus microplus* ticks. When the old stock of the fungus was evaluated, it had given at par activity with their respective newly harvested conidia, indicating that, if fungal powder is stored away from the sunlight at room temperature in dry conditions, it can hold activity for more than 4 years. The efficacy of the fungal treatments was found to be concentration dependent. Hence it is concluded that a minimum concentration of 5×10^8 conidia/litre shall be used to achieve desired effect. The field trial has suggested *Verticillium lecanii* can work better when applied on the egg stage of tick. Spraying rate should be 30 ml / m^2 area of the solution containing 5×10^8 conidia /litre. As this fungus is capable of inducing high mortalities, decreased fecundity, and egg hatchability, they have a great potential for tick control. Their ability to reduce egg hatchability will certainly have a greater impact on tick population than the direct mortality on engorging ticks, which may destroy only a few dozen ticks.

Thus the use of this fungus in a biological control programme could reduce the use of chemical acaricides and could result not only in lower costs to the farmer but also in a lower environmental impact and less damage to nature.

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9. References

1. Wall RL, Shearer D. Veterinary ectoparasites: biology, pathology and control. John Wiley & Sons, 2008.
2. Sonenshine DE. Biology of Ticks, New York: Oxford University Press. 1993, 2.
3. De Castro JJ, Newson RM. Host resistance in cattle tick control. Parasitology Today. 1993; 9(1):13-17.
4. Walker AR, Bouattour A, Camicas JL, Estrand-Perna A, Horak IJ, Latif AA *et al*. Ticks of Domestic Animals in Africa: A guide to identification of species. 1st Ed, Bioscience Reports Publication, Scotland, and Edinburg, UK. 2003; 44(1):149-209.
5. Srivastava R, Ghosh S, Mandal DB, Azhahianambi P, Singhal PS, Pandey NN *et al*. Efficacy of *Azadirachta indica* extracts against *Boophilus microplus*. Parasitol Res 2008; 104(1):149-153.
6. Maske DK, Kolte SW, Jangde CR. Efficacy of neem based based compound "Nimbitor"against ectoparasites of cattle. Indian Veterinary Journal. 2000; 77(1):103-106.
7. Kaaya GP, Hassan S. Entomogenous fungi as promising biopesticides for tick control Entomogenous fungi as promising biopesticides for tick control. Experimental & applied acarology. 2000; 24(12):913-926.
8. Narladkar BW, Shivpuje PR, Harke PC. Fungal Bio-Control Agents for Integrated Management of *Rhipicephalus (Boophilus) microplus* Ticks. Indian Veterinary Journal. 2015; 92(5):34-37.
9. Gauss CLB, Furlong J. Comportamento de larvas infestantes de Boophilus microplus em pastagem de Brachiaria decumbens. Ciência Rural. 2002; 32(3):467-472.
10. Paiao JCV, Monteiro AC, Kronka SN. Susceptibility of the cattle tick *Boophilus microplus* (Acari: Ixodidae) to isolates of the fungus *Beauveria bassiana*. World Journal of Microbiology and Biochemistry. 2001; 17(3):245-251.
11. Gindin G, Samish M, Zangi G, Mishoutchenko A, Glazer I. The susceptibility of different species and stages of ticks to entomopathogenic fungi. Experimental and Applied Acarology. 2003; 28(1):283-288.
12. Fargues J, Robert PH. Persistence of conidia of 4 entomopathogenic hyphomycetes in soil *Beauveria bassiana* (Bals.) Vuill. *Metarhizium anisopliae* (Metsch) Sor., *Nomuraea rileyi* (F.) Samson and *Paecilomyces fumosoroseus* Wize, in controlled conditions. Agron. Sci.

Prod. Veg. Env. 1985; 5(1):73-79.
13. Madelin MF, Robinson RK, Williams RJ. Apressorium like structures in insects parasiting deuteromycetes. Journal of Invertebrate Patholology. 1967; 9(3):404-412.
14. Goettel MS, Johnson DL. Environmental impact and safety of fungal biocontrol agents. Food and Agricultural Organization. 1992, 356-361.