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Dr. Greeshma Rao UB
MVSc Scholar, Department of Veterinary Parasitology, COVAS Parbhani, Maharashtra, India

Dr. BW Narladkar
Associate Professor, Department of Veterinary Parasitology, COVAS Parbhani, Maharashtra, India

Correspondence
Dr. Greeshma Rao UB
MVSc Scholar, Department of Veterinary Parasitology, COVAS Parbhani, Maharashtra, India

Role of entomopathogenic fungi in tick control: A Review

Dr. Greeshma Rao UB and Dr. BW Narladkar

Abstract
Entomopathogenic fungi are known to infect different tick species and the efficacy of these interesting fungi to control ticks is highly strain-specific. They comprise a wide range of morphologically, phylogenetically, and ecologically diverse fungal species fungal bio control agents have been tested under laboratory and to certain extent at field level too against *R. (B.) microplus* ticks. The results are significant in both trials and they can be recommended for field use. The fungi such as *Beauveria bassiana, Verticiliium lecanii and Metarhizium* anisopliae and are well known entomopathogens that have been commercially developed as biopesticides. With increased commercialization, entomopathogenic fungi are poised to become a significant component of integrated pest management. This article reviews these promising microbial agents, their action against against ticks; the scope of applicability in field conditions and potential of these fungi as biological control agent against *R. (B.) microplus* ticks.

Keywords: Entomopathogenic fungi, *Beauveria bassiana, Verticiliium lecanii, Metarhizium anisopliae,* biological control agent, *R. (B.) microplus*

1. Introduction

Ticks are obligate blood-feeders that require an animal host to survive and reproduce. They feed on a wide variety of mammals, birds, reptiles, and even amphibians [1]. Ticks can be a nuisance; their bites can cause irritation and, in the case of some ticks, paralysis. Severe infestations on animals can cause anemia, weight loss, and even death from the consumption of large quantities of blood. Ticks can also transmit many human and animal disease pathogens, which include viruses, bacteria, rickettsiae, and protozoa [1].

The present technology to control ticks is based mainly on the use of chemical products; however, the ability of *B. microplus* to develop resistance to different acaricides, the demands of consumers for chemical free foods and the negative environmental effects of acaricides call for the development of alternative strategies [2]. Although entomogenous fungi have been used widely for the control of agricultural and forest pests, little effort has been made to evaluate the applicability of bio-control potentials of entomogenous fungi against ticks which are vectors of human and animal diseases.

Among these fungi, *Beauveria bassiana* and *Metarhizium anisopliae* received major attention [3]. Different studies on the potential use of *M. anisopliae* and *B. bassiana* as entomopathogenic agents were performed in laboratories; however there is an urgent need for checking the novel strains and even fungal species from different geographic regions to find out potent species. This review has multiple aims. The first is to know the biology of three important entomopathogens which are found effective against egg and adult stages of *R. (B.) microplus* ticks. Secondly, their mechanism of action against target species; applicability and incorporation in integrated pest management approach.

2. Classification of Entomopathogenic Fungus: The major taxa containing the entomopathogenic fungi are depicted in the Table 1

Table 1: Outline classification of fungal entomopathgenic genera and the susceptible insects hosts

Class	Genus	Insects
Phycomycetes	*Coleomycete*	Mosquitoes
	Entomopthora	Diptera, hemiptera
	Massospora	Cicada
Ascomycetes	*Cordiceps*	Cicadidae
Basidiomycetes	*Septobasidium*	Scale
Deuteromycetes	*Beauveria*	Lepidoptera, Coleoptera Homoptera
	Metarhizium	Coleoptera, Lepidoptea, Orthoptera, Hemiptera, Diptera
	Aspergillus	*Heliozella stenella*
	Spicara	Locust
	Hirsutella	Mites
	Paecilomyces	Cerambycids, sawflies, Lepidopterous larvae
	Verticillium	Whiteflies, Aphids, scale insects mealy bug, thrips, mites

3. Biology of the entomopathogenic fungi

The biology of *Metarhizium* and *Beauveria* fungui are highlighted here

Beauveria and *Metarhizium* both these fungi belong to Deuteromycetes [4]. Within the Class Deuteromycetes a morphological group of fungi known as Hyphomycetes exists. These are filamentous fungi that reproduce by conidia generally formed aerially on conidiophores arising from the substrate. The most common route of host invasion is through the external integument, although infection through the digestive tract is possible [5]. Conidia attach to the cuticle, germinate, and penetrate the cuticle. Once in the haemocoel, the mycelium grows throughout the host, forming hyphal bodies called blastospores [5]. Death of the insect is often due to a combination of the action of fungal toxins, physical obstruction of blood circulation, nutrient depletion and/or invasion of organs. After the host has died, hyphae usually emerge from the cadaver and under suitable abiotical conditions; conidia are produced on the exterior of the host. These are then dispersed by wind or water [5].

Metarhizium: Metarhizium is one of the most common entomopathogenic fungi, with a worldwide distribution [4]. The species is soil-borne and infects predominantly soil-dwelling insects. *Metarhizium anisopliae* has a large host-range, including arachnids and five orders of insects comprising over 200 species [5]. On terrestrial insects, the life cycle begins with a conidium attaching to the host cuticle, forming an appressorium, followed by a penetration peg to enter the cuticle. After entering the haemocoel, hyphae are formed that produce and release toxins, killing the host 4-16 days (depending mainly on the host species) after contamination. These toxins include Destruxins, Swainsinone, and Cytochalasin C [6]. Histopathological studies of elaterid tissues infected by *Metarhizium anisopliae* suggest that toxins (destruxins) kill the host by inciting degeneration of the host tissues due to loss of the structural integrity of membranes and then dehydration of cells by fluid loss. Many laboratory studies have shown the potential of *Metarhizium anisopliae* as a mosquito control agent [5].

Conidia normally require a relative humidity of at least 92% to germinate [7]. Conidia that are stored under dry conditions show higher germination rates (initially 96%, dropping to 80% after 60 days) than conidia formulated in paraffin oil (from 93% to 73) [8]. Conidia are found to survive longest at a combination of moderate temperatures and high RH (26 ^{0}C and 97% RH or 19 ^{0}C and 97% RH) or low temperature and low RH (40 ^{0}C and 0% RH). The fungus can easily be grown *in vitro* and storage conditions are more critical to spore survival and virulence than the substrate upon which conidia are produced. Its failure to germinate in the mosquito environment until actual exposure to a host and its resulting persistence in the environment, as well as the fact that its effect is not limited to periods of host molting (as for *Beauveria bassiana*), make this fungus a very promising control agent.

Beauveria: Beauveria is one of the most frequently isolated entomogenous fungal genera and has a cosmopolitan distribution. Conidia of *Beauveria bassiana* are effective in killing mosquito larvae when applied as a conidial dust to the water surface of breeding sites. Conidia are hydrophobic, thus floating on the water surface, and contact mosquito larvae that feed below the surface mainly at the tip of the siphon [5]. Humidity is considered as one of the critical factors affecting the outcome of both laboratory and field-tests with *Beauveria bassiana* [9]. Infection does not appear to be dependent on temperature. For conidia however, high temperatures may be harmful, especially in combination with high humidity conditions [7]. The effective stages of the fungus against larvae are conidia and blastoconidia, the latter stage being far more pathogenic. A problem associated with using conidia is that they have no residual effect. Although growing blastoconidia is relatively easy, production has been abandoned because of the difficulties of storing this type of conidium [7].

Verticillium lecanii: Fungi in the *genus Lecanicillium* (formerly classified as the single species *Verticillium lecanii*) are important pathogens of insects and some have been developed as commercial bio pesticides [10]. Some isolates are also active against phytoparasitic nematodes or fungi. *Lecanicillium* spp. use both mechanical forces and hydrolytic enzymes to directly penetrate the insect integument and the cell wall of the fungal plant pathogen. In addition to mycoparasitism of the plant pathogen, the mode of action is linked to colonization of host plant tissues, triggering an induced systemic resistance of pests [10]. Until recently, the form genus *Verticillium* contained a wide variety of species with diverse host ranges including arthropods, nematodes, plants and fungi [11]. Species of *Lecanicillium* have a wide host range and have been isolated from a variety of insect orders. *Lecanicillium* spp. generally follow the typical pathway of pathogenesis of entomopathogenic mitosporic fungi: adherence of conidia to the host cuticle; germination of conidia; penetration of the cuticle; production of blastospores within the hemocoel; ramification of the mycelia and invasion of tissues causing death of the host; and finally, production of conidia on the surface of the cadaver. *Lecanicillium muscarium* has a broader host range and has been isolated from a range of substrates, mainly insects and fungi and has been commercialized as the bio-pesticides [12].

4. Mode of action

On adult ticks

a) The conidial germination and formation of appressoria are the important events in the interaction between entomopathogenic fungi and the arthropod hosts. Lipid composition of tick epicuticle selectively affects germination of conidia of entomopathogenic fungi; thus results in mortality of ticks. Adhesion, germination and production of conidia are recognized as the main virulence factors against arthropods. This type of the mode of action is observed against all stages i.e. egg, larva, nymph and adult; thus fungus appears to be the potent anti-tick agent [13].

b) After penetration of arthropod cuticle by the germ tubes, fungi rapidly invade the internal organs and thus killing the host arthropod. These fungi also release mycotoxins which may also be responsible partly for mortality (Table 2) [14].

c) Different strains of *Beauveria bassiana* are able to produce several cuticle degrading hydrolytic extracellular enzymes such as chitinases and B-1, 3 glucanases, all of which are considered to be determinants of fungal pathogenicity. These fungi secrete toxic metabolites during the infection process that contribute to the establishment and progression of the disease. Also, *B. bassiana* and *B. amorpha* produce subtilisin-like proteases and chitinases in the presence of *R. (B.) microplus* tick [15].

d) Mechanical pressure, due to apressorium formation and degradation by the synergistic action of hydrolytic enzymes: *M. anisopliae* is a broad host range deuteromycete first recognized as a potential candidate for biological control of agricultural pets in 1880. The fungus actively invades the hosts through the cuticle by mechanical pressure, due to apressorium formation and degradation by the synergistic action of hydrolytic enzymes such as proteases, chitinases and lipases [16].

Table 2: Toxins produced by the various entomopathogenic fungi

Sl No.	Name of fungus	Toxins produced	Remarks
1	*Entomophthora*	Caronata and a protinacirus mycotoxins	It is natural excretory product.
2	*Aspergillus flavous*	Afflatoxin	Caused histological changes in organic even at very small doses
	A. parasiticus		*Helothis* is more susceptible to afflatoxin than *spotoptera*. Afflatoxin 2 types- (a) and (b) (b) is more toxic than (a)
	A. niger		
	A. ochraceus	Aspochracin	
3	*Metarhizium anisopliae*	Distruxin (a) Distruxin (b) (Distruxin a & b	Paralysis when injected in wax and silk moth Distructor (a) is more toxic than (b) chemically it is cyclic depsi peptide.
4	*Beauvaria bassiana (White muscurdine fungus)*	*Beauvaricin*	It is depsi peptide
5	*Verticillium lecanii*	*Dipicolinic acid and Didepsi peptide (protein) Bassianolide*	It causes atony. Interfere, the process of chitin formation and exposure the insect easily to the attack of natural enemy.

On eggs of ticks

1. **Role of Pentane and DCM:** The surface of ticks, lipids namely pentane and DCM has relevance with germination of conidia and formation of appressoria of the fungal strains[10]. Therefore the effect of fungus on eggs of particular tick species is nothing but interaction between conidial density and lipids present on the surface of tick egg. Lipids present on the egg of *R. sanguineus,* stimulated germination of more conidia within 12 hrs of contact than the other two species of ticks which indicate the difference in susceptibility of tick eggs to the different fungal strains [17].
2. **Egg wax and age of the tick eggs:** Susceptibility of tick eggs to the *M. anisopliae* was also observed on the basis of one factor i.e. age of the egg. In the literature it is mentioned that reduction in egg resistance to the fungus could be caused by the changes in the egg cuticular compounds as a result of oxidation [16]. As the eggs are laid by the female tick, Gene's organ secretes a waxy coating onto each egg [16]. This Coating both renders the eggs-water proof and contains fungicidal components. The wax becomes harder after secretion, raising its melting point and making it stickier and better able to hold the egg mass. This process would thus improve the waterproofing properties of the wax and enhance the protection against various environmental hazards. However at the same time it seems to reduce the egg's resistance to *Metarhizium,* as the age of the egg advances, oxidation of their surface wax proceeds and the amount of unsaturated fatty acid in their tegument declines resulting in weakening of anti–fungal properties. As a result tick eggs appear to be more susceptible to the fungus compared to adult ticks [18].

Fungal infection of engorged female ticks often resulted in longer periods of pre-oviposition, oviposition time, egg incubation, and egg hatching of the egg mass, as well as in lowered egg production [19, 20]. Thus, amalgamation of these observations, it can be said that there is a relatively long-lasting sub-lethal influence of the fungi on their tick hosts.

5. 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 [21]. The efficacy with satisfactory results of *M. anisopliae* and *B. bassiana* against the cattle tick in the drought prone area i.e. Marathwada region of Maharashtra, which is categorized under tropical arid zone where humidity levels are always low except during monsoon season [21]. 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 and these two factors vary from geographic area to area [18].

6. Strain/concentration of the fungus to be used for producing knock down effect

To have a desired effect on tick's mortality (knock down effect), particular concentration of conidia of fungal BCAs appears as must. Concentration of the conidia has direct relationship with mortality of ticks. As the dose increased mortality count was also increased [18].

While testing *Metarhizium anisopliae* (three strains), *Beauveria bassiana* (six strains) and *Lecanicillium psalliotae* (two strains) on different developmental stages of *Rhipicephalus (Boophilus) annulatus* virulence varied according to the species and strain of the fungus [22]. Similarly they also observed that different stages suffer or susceptible to the different fungal strain also varies).

7. 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. 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 [23].

The eggs are more susceptible to the fungal infection as compared to adult female and unfed larvae [23]. The efficacy of *L. lecanii* species was equal to *M. anisoplaie* and *B. bassiana* on the eggs and larval stages of *R. annulatus*. Probable reasons for higher effect of fungi on tick eggs as compared to adult are

a) Most tick belongs to Ixodidae family; lays 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 egg.
b) The 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 [24].

8. Application on field (grazing land x cattle body surface)

There appears to be controversy amongst scientists, many scientists advocated the application of fungal BCAs on grazing land while many are of the opinion that fungal BCAs shall be applied on cattle body.

Taking into consideration both the opinions detailed in the above paragraphs following conclusions can be drawn,

a) It indicated the capabilities of these two fungi in the field conditions. Even not a single biological control agent has been reported to cause this much of mortality.
b) Host factors which are contributing in decreasing the efficiency of entomopathogenic fungi, when applied on the body surface of the cattle, are UV-A and UV-B radiation and heat from sunlight. Owing to this fact, present study is of the opinion that, application of fungus shall be limited to the application in the cattle shed, where maximum numbers of eggs are laid by the ticks like *R. (B.) microplus*, rather than using fungi on cattle body.
c) *M. anisopliae* shows limited performance when applied on skin surface because high temperature at the skin surface reduces the efficacy of conidia [25].
d) The fungal efficacy can be poor in the environments where water availability is reduced[16]. It suggests that drought prone area where environment is considered as dry may not be suitable for fungus. However, even with less humidity levels in the geographical area of the present study, we observed the results of all three fungi. The results are more satisfactory against the egg stage, when applied in the cattle shed. The cattle shed can be more suitable for better fungal efficacy on tick eggs, the reasons could be a) no sunlight UV-A and UV-B radiation and heat from sunlight, b) amount of humidity in the dark corners and under mangers, and c) amount of humidity created through evaporation of water from dung and urine of the animals.

9. Conclusion

In nutshell it can be said that susceptibility of ticks to a particular fungus largely depend on tick genera and species; fungus species, strain, conidial concentration; temperature, humidity and geographic area. The detailed understanding of biology of the fungus would provide better knowledge of its appropriate utilization in field conditions. The better proving of ovicidal by the fungi would be helpful in controlling emergence of seed ticks thus inhibit tick population.

Owing to these advantages these fungi are gaining more and more popularity in tick control programmes and therefore research conducted in the present study also gains due importance. Further studies that include the selection and testing of many isolates in the laboratory and under field conditions can provide essential information on the anti-tick efficacy and of various fungal species as potential biological control agents. Finding additional studies on fungi and other biological control agents against ticks and their strategic use can be a magic wand and may be a boon for Indian farmers.

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11. References

1. Kirby CS. Tick management handbook. Stafford KC. Tick Management Handbook: An Integrated Guide for Homeowners, Pest Control Operators, and Public Health Officials for the Prevention of Tick-Associated Disease. Connecticut Agricultural Experiment Station. 2007; 1-78.
2. Kay BH, Kemp DH. Vaccines against arthropods. Am. J. Trop. Med. Hyg. 1994; (50):87-96.
3. Kalsbeek V, Frandsen F, Steenberg T. Entomopathogenic fungi associated with Ixodes ricinus ticks. Exp. Appl. Acarol. 1995; 19(1):45-51.
4. Scholte EJ, Knols BGJ, Samson RA, Takken W. Entomopathogenic fungi for mosquito control: A review. 24pp. Journal of Insect Science. 2004*;* 19(4):1-24.
5. Goettel MS, Inglis GD. Fungi: Hyphomycetes. In: Lacey LA editor. Manual of Techniques in Insect Pathology. 5-3. 1997; 46(1):213-248.
6. Strasser H, Vey A, Butt TM. Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of *Metarhizium, Tolypocladium* and *Beauveria* species? Biocontrol Science and Technology. 2000; 10(6):717-735.
7. Ferron P. Pest control by the fungi *Beauveria* and *Metarhizium*. In: Burgess HD, editor. Microbial Control of Pests and Plant Diseases 1970-1980. 1981; 24(1):465-482.
8. Morley DJ, Moore D, Prior C. Screening of *Metarhizium* and *Beauveria* spp. conidia with exposure to simulated sunlight and a range of temperatures. Mycological Research. 1995; 100(1):31-38.
9. Boucias DR, Pendland JC. Entomopathogenic fungi; Fungi Imperfecti. In: Boucias DR, Pendland JC, editors. Principles of Insect Pathology. 1998; 10(1):321-359.
10. Goettel MS, Koike M, Kim JJ, Aiuchi D, Shinya R, Brodeur J. Potential of *Lecanicillium* spp. for management of insects, nematodes and plant diseases. J Invertebr Pathol. 2008; 98(3):256-261.

11. Zare R, Gams W. A revision of *Verticillium* section Prostrata. IV. The genera *Lecanicillium* and *Simplicillium*. Nova Hedwigia. 2001; 73(1, 2):1-50.
12. Faria MR, Wraight SP. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biol. Control. 2007; 43(3):237-256.
13. Cafarchia C, Davide I, Roberta I, Rafael ANR, Riccardo PL, Daniele P. *et al*. Native strains of *Beauveria bassiana* for the control of *Rhipicephalus sanguineus* sensu lato. Parasites and Vectors. 2015; 8 (1):80.
14. Kaaya GP, Esther NM, Elizabeth AO. Prospects for Biological Control of Livestock Ticks, *Rhipicephalus appendiculatus* and *Amblyomma variegatum* using the Entomogenous Fungi *Beauveria bassiana* and *Metarhizium anisopliae*. Journal of Invertebrate Pathology. 1996; 67(1):15-20.
15. Campos RA, Arruda W, Boldo JT, da Silva MV, Neiva M, Joao L *et al*. *Boophilus microplus* infection by *Beauveria amorpha* and *Beauveria bassiana*: SEM analysis and regulation of subtilisin-like proteases and chitinases. Curr. Microbiol. 2005; 50(5):257-261.
16. Gindin G, Dana M, Asael R, Itamer G, Michael S. Pathogenicity of *Metarhizium anisopliae* (Hypocreales:Clavicipitaceae) to Tick Eggs and the effect of egg Cuticular Lipids on Conidia. Journal of Medical Entomology. 2009; 46(3):531-538.
17. Ment D, Gindin G, Soroker V, Glazer I, Rot A, Samish M. *Metarhizium anisopliae* conidial responses to lipids from tick cuticle and tick mammalian host surface. J. Invertebr. Pathol. 2010; 103(2):132-139.
18. Greeshma Rao. In-vitro evaluation of fungal bio control agents and herbal acaricide against cattle tick *R. microplus*, MVSc thesis, Maharashtra Veterinary and Fishery Sciences University, Nagpur. 2017.
19. Bittencourt VREP, Mascaranhas AG, Faccini JLH. Mecanismo de infecção do fungo *Metarhizium anisopliae* no carrapato *Boophilus microplus* em condições experimentais. Ciência Rural. 1999; 29(2):351-354.
20. Gindin G, Samish M, Alekseev E, Glazer I. The Susceptibility of *Boophilus annulatus* (Ixodidae) Ticks to Entomopathogenic Fungi. Journal of Biocontrol Science and Technology. 2001; 11(1):111-118.
21. Narladkar BW, Shivpuje PR, Harke PC. Fungal Bio-Control Agents for Integrated Management of *Rhipicephalus (Boophilus) microplus* Ticks. Indian Vet. J. 2015; 92(5):34-37.
22. Pirali K, Hamidreza H, Mehdi RA, Saeed B, Rasoul Z, Mehran G *et al*. Biological control of *Rhipicephalus (Boophilus) annulatus* by different *strains of Metarhizium anisopliae, Beauveria bassiana* and *Lecanicillium psalliotae* fungi. Parasitol Res. 2007; 100(6):1297-1302
23. 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.
24. 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.
25. Leemon DM, Turner LB, Jonsson NN. Pen studies on the control of cattle tick (*Rhipicephalus* (*Boophilus*) *microplus*) with *Metarhizium anisopliae* (Sorokin). Veterinary Parasitology. 2008; 156(3):248-260.