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Hatchability of eggs of deltamethrin and amitraz resistant cattle tick *Rhipicephalus microplus* of Maharashtra state on treatment with acaropathogenic fungus *metarhizium anisopliae*

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Abstract
Taking into an account the high prevalence of ticks and tick born diseases (TTBDs) in cattle, reflections of indiscriminate use of chemical acaricides in the form of environmental contamination, toxicity and development of acaricide resistance and researcher's inclination towards eco-friendly alternative to chemical acaricides in India, a project was designed with an aim to evaluate non-chemical approach acaropathogenic fungus *Metarhizium anisopliae* for the control of acaricide resistant strain of cattle tick *Rhipicephalus microplus* from north Maharashtra region of the state. Acaropathogenic fungus *M. anisopliae* against resistant *R. microplus* tick revealed significant reduction in hatchability rate upto 51.06 % in untreated eggs of treated females and 70.89 % in treated eggs of untreated females of resistant tick isolate. The results indicated that the direct exposure of eggs to the conidia reduced hatchability to considerable level.

Keywords: Cattle tick, *Rhipicephalus microplus*, *Metarhizium anisopliae*, hatchability, biological control agent

Introduction
India is predominantly an agricultural country with about 70 per cent of its population dependent on income from agriculture. Uncertainty of rains and changing climate and droughts brought the agriculture under great losses leading to suicides of the farmers. In such situation, animal rearing became most reliable allied source of income for farmers. Though the livestock especially cattle is one major resource for the farmers, it has few weaker links too. As like other animals, cattle also suffer from many infectious diseases including parasitic diseases as a major obstacle in the health and performance of animals.

Ticks, among all the ectoparasites, are very important and harmful haematophagus ecto-parasites of mammals, birds and reptiles throughout the world [1] and vectors of several diseases in animals (*viz. Babesia* spp, *Theileria spp.*, *Anaplasma marginale* etc.) which are a major constraint to animal productivity while causing considerable level morbidity and mortality in animals. Ticks have major economic impact through their vector potential and direct losses due to their detrimental effect and the efforts taken for preventive measures directed against them. In India, the damage caused by ticks and tick borne diseases (TTBDs) to livestock is considered very high [2]. A recent estimate of US$ 498.7 million per annum has been calculated as the cost of TTBDs in India [3].

In practice too, ticks are controlled at present mostly by chemicals acaricides. However, the concern because of: 1) increasing concerns about environmental safety and human health (e.g. the gradual increase in use of chemical insecticides in several countries is stimulating the growing market of 'organic' food; 2) the increasing cost of chemical control; and 3) the increasing resistance of ticks to pesticides forced the researchers to find an alternative as bio-control agent. Numerous potential tick bio-control agents including pathogens, parasitoids and predators of ticks has been documented [4, 8].

Thus, present study was planned with an objective to evaluate a biological tool i.e. an acaropathogenic fungus *Metarhizum anisopliae* against acaricide resistant isolate of *R. microplus in vitro* as a novel, non-chemical approach.

Materials and Methods

Collection of Ticks Deltamethrin (RF-19.75) and amitraz (RF-2.06) resistant fully engorged live dropped adult female ticks of R. *Microplus*, [9] were collected from cattle sheds of local farmers at Udgir, Dist. Latur, Maharashtra state along with detail information about the Acaricide used, and frequency of treatment and post application efficacy of acaricides. The ticks were collected in specially designed insect breeding plastic dishes / jars (Himedia Laboratories Pvt. Ltd. India) bearing the mesh window ventilator which allow air and moisture exchange. These insect dishes / jars kept in plastic basket and transported to the Entomology Laboratory, Department of Veterinary Parasitology, Bombay Veterinary College, Mumbai. The collected ticks were cleaned with distilled water, transferred to fresh vials with proper labels and kept at 28 OC and 85 percent relative humidity for ovi position [10].

Procurement of Acaro-pathogenic fungus

The acaro-pathogenic fungus Metarhizium anisopliae was tested against resistant strain of Rhipicephalus micro plus from Maharashtra State. The fungal powder/ culture containing 1x108 conidia per gram of powder, used in the present experiment were procured from Biological control unit, Dept. of Agril. Entomology, Mahatma Phule Krishi Vidyapeeth Rahuri, Dist. Ahemednagar, Maharashtra State.

Preparation of working solution of acaro-pathogenic fungal powder

For all in vitro trials, the working concentration was prepared by mixing 5 g of Fungal powder (108 conidia /gm of powder) in 5 ml of Raw Cow Milk and finally total volume 1 liter by adding 990 ml distilled water. The solution was prepared at least 6 hours prior to the experiment to facilitate the proper soaking of fungal conidia and ensure their germination

Egg Hatch Assay (EHA)

The hatchability of two types of eggs on the basis the source i.e. untreated eggs of treated females (UETF) and treated eggs of untreated females (TEUF) was assessed according to the method of Ribeiro *et al*. [11] with minor modifications. In UETF, 200 eggs were collected from the female ticks which were already treated with M. anisopliae conidia with a control group having eggs of untreated females. While in TEUF, 200 hundred embryonated eggs of untreated females of both groups viz., Group-I (M. anisopliae) and Group-II (Control-milk water) and immersed for 2 min in 1 ml of the test solution. Subsequently, the solution was decanted and after evaporation of the solvent, all vials were covered with a muslin cloth. Eggs were incubated at 28 ± 1 OC and 85 ± 5% relative humidity for 15 days, until hatching was completed. In UETF, eggs of both groups were kept for hatching without any treatment. The hatched larvae and unhatched eggs were counted after the 14th days of incubation period. Each treatment of both the experiments replicated thrice and the following parameters were compared:

1. Hatchability Rate % (H): Determined by counting the number of hatched larvae divided by the total number of incubated eggs with respect to remaining unhatched eggs in a representative sample from the dishes, as described by Amaral [12], with some modifications.

2. The hatchability reduction rate % (HR): Determined by comparing the number of hatched larvae in treated groups (HT) in relation to the control group (HC).

Hatching rate reduction / Inhibition of Hatchability:

$$\% HR = \frac{HC - HT}{HC} \times 100$$

Statistical analysis

The data obtained from various experiments and parameters of the present study was analyzed by employing Chi square test, Completely Randomized Design [13, 14].

Results and discussion

The viability of the fungal conidia was tested in the laboratory using SDA agar culture, examination of fungal colonies and conidia using lacto-phenol cotton blue stain.

Hatchability reduction (HR) (Table 1)

a. HR of untreated eggs of treated females

The per cent hatchability of eggs from ticks of M. anisopliae treated group was 46.00 while it was 94.00 per cent in control groups. The HR reduction of 51.06 per cent was statistically significant at both 1 and 5 per cent level of significance.

b. HR of treated eggs of untreated females (Plate 1)

The eggs of ticks from M. anisopliae treatment group showed only 27.17 per cent hatchability while 93.33 eggs from control group. The hatchability rate reduction of 70.89 per cent in treatment group was highly significant as compared that of control group.

The results indicated that the direct exposure of eggs to the conidia reduced the hatchability to a considerable level as rate of hatchability reduction is significantly more in treated eggs than the untreated eggs of treated females (UETF).

Fig 1: A. Tick eggs treated with conidial suspension kept for hatching, B. Haypha spreading to another egg, C. Dead eggs.

Table. 1: Hatchability response of untreated eggs of treated females and treated eggs of untreated females of Rhipicephalus *Microplus* (resistant isolate) to M. anisopliae.

Acaropathogenic fungus used	No of eggs / Replicate (r=3)	Untreated eggs of treated females			Treated eggs of untreated females		
		No. of eggs hatched	% Hatched	% Hatchability Reduction	No. of eggs hatched	% Hatched	% Hatchability Reduction
M. anisopliae	200	276	46.00±2.46^{y}	51.06	163	27.17±2.13^{a}	70.89
Control	200	564	94.00±1.04^{x}		560	93.33±0.44^{b}	
Statistical analysis	CRD	SE±43.33, CV= 4.684, CD(0.01) = 24.650 CD(0.05) = 14.863 Treatments found Significant at 1% and 5% level of significance			SE±28.33, CV= 4.417, CD(0.01) = 20.010 CD(0.05) = 12.065 Treatments found Significant at 1% and 5% level of significance		

SE-Standard error. Similar superscripts indicate the values statistically at par. r-no of replicates. Different superscripts shows significant difference among the column (between treatments).

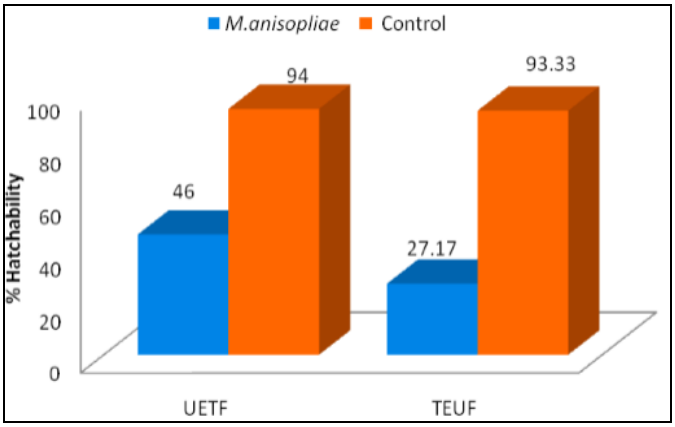

Fig. 1: Hatchability response of untreated eggs of treated females and treated eggs of untreated females of Rhipicephalus *Microplus* (resistant isolate) to M. anisopliae

The observations on hatchability reduction (51.06 %) of the eggs laid by female ticks treated with M. anisopliae during the present study is in agreement with report of few studies undertaken by Camargo *et al*, [15] and Narladkar *et al*, [16] who reported 57.30 and 75.29 per cent hatchability. However, some of the studies [17, 18] recorded 70-100 per cent reduction in HR.

Some researchers [19, 21, and 15] reported significant reduction in hatchability of treated eggs of untreated females which supports the findings of the present study with HR of 70.89 per cent. However, Rao [22] reported 99.15 per cent reduction in HR which higher than the finding of the present study. The variation the hatchability rates on various parameters may be attributed to the source of M. anisopliae, its strain, viability, the form of conidia, the diluents used for preparation of suspension. The studies were conducted at different locations, though the species of tick was same, there may be minor variations in the Cuticular wax, susceptibility of the respective ticks strain to the acaropathogenic fungi M. anisopliae. Thus, the literature reveals different incarnation combinations of formulations and concentrations on different types of hard ticks

The performance of the bio control agent can be improved further by undertaking more studies on different species and strains of the fungus and promising strains can be subsequently selected. The integration of this agent with semio chemicals, the interaction between the bio control agent and the target ticks can be enhanced to achieve minimum desired results.

If the use of biological control agents like acaropathogenic fungi Metarhizium anisopilae implemented in judicious and scientific way, it may replace the harmful chemical acaricides partially and definitely help in minimizing the risk of its persistence and accumulation in the environment leading to pollution, poisoning, food residues and resistance development in ticks. For control of ticks on livestock, the possibility of using fungal bio-pesticide is very limited and its application on animal body as topical application needs to be validated through lab animal toxico-pathological studies. However, it can be of great value for the purpose in the animals sheds. Though, the fungal powder or liquid formulations are available in the market at a very cheaper rate, there are few reports of side effects viz. keratitis, and respiratory infections in some animals and human beings especially children [23, 26]. Hence, it very much essential to take personal safety precautions while its application. It should be kept out of children's reach. The application of the powder formulation in premises too close to the residential areas should be avoided.

Rhipicephalus misfire plus being a one host tick, completes its life cycle in two phases' viz. on-host and off-host on the basis of environmental conditions. Egg is the only life cycle stage which occurs off host. Taking the advantage of off-host period, their hidden breeding places like below the objects on the floor and mangers, cracks and crevices in walls of the shed, grazing land to be targeted [27]. For this part of life cycle, bio-control agents like M. anisopliae evaluated during the present study can be used to target the off host stages of tick.

Conclusion

The results of the present study are unique as it was conducted on tick species which is resistant to deltamethrin and amitraz. It was revealed that a state of moderate to high effectiveness of acaropathogenic fungus M. anisopliae against the egg stage of resistant isolate of R. micro plus and suggests need of further study by using and shows a ray of hope as an non-chemical option for the effective control of off host egg stages of acaricide resistant ticks at their breeding places in the animal sheds. The data generated may be useful in effective control of deltamethrin and amitraz resistant isolate of R. micro plus off the host stages and may hence lead to lesser number of infective stages available in the environment and thus may contribute largely to control of resistant isolates of ticks and slow down acaricide resistance process.

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