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Full Length Research Paper

Mortalities induced by entomopathogenic fungus *Metarhizium anisopliae* to different ticks of economic importance using two formulations

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The objective of this study was to compare the infectivity of *Metarhizium anisopliae* at a concentration of 1 × 10^8 conidia/ml to developmental stages of *Amblyomma variegatum, Rhipicephalus appendiculatus* and *Rhipicephalus evertsi evertsi* in oil and water formulations. The fungus, *M. anisopliae,* induced 50-100% mortalities in all developmental stages of the tick species tested. *R. e. evertsi* was more susceptible to *M. anisopliae* than the other two tick species. However, there was no clear difference in mortality between fed and unfed instars of different tick species. Furthermore, engorged adults were more susceptible than unfed adult ticks. Oil formulated conidia induced significantly higher mortalities ($P<0.05$) than water formulated conidia. The results indicate that the type of conidia formulation is critical to the performance of fungi as biological control agents for ticks.

Keywords: *Amblyomma variegatum,* Formulation, *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi evertsi*

INTRODUCTION

Ticks transmit a variety of pathogenic agents to animals and parasitize a wide range of vertebrate hosts thereby causing great economic loses to livestock (Rajput et al., 2006). The use of natural enemies such as entomopathogenic fungi to manage ticks is generally perceived to be ecologically preferable to chemical treatment (Benjamin et al., 2002). Biological pesticides based on entomopathogenic fungi have several advantages in comparison to chemicals for the control of pests. They are natural, more environmentally friendly, potentially less expensive and the problems with resistance are less likely to occur (Polar et al., 2005; Whipps and Lumsden, 2001). Another advantage of fungi is their ability to grow and spread in the environment through infected ticks present in the soil and also its capacity to cause epizootics due to its wide spectrum (Bittencourt et al., 1999).

Entomopathogenic fungi, especially *Metarhizium anisopliae* and *Beauveria bassiana* are major pathogens of ticks. *M. anisopliae* and *B. bassiana* have high virulence, wide dispersal and ability to penetrate the host via the cuticle (Samish and Rehacek, 1999). They are used against terrestrial insects because of their wide geographical distribution and host range as well as their exceptional ability to germinate even at a relatively low humidity (Samish and Rehacek, 1999). *Amblyomma variegatum* and *Rhipicephalus appendiculatus* have been reported to be susceptible to *M. anisopliae* and *B. bassiana,* inducing high mortalities, causing reduction in fecundity and egg viability (Kaaya et al., 1996).

Ticks of genus *Amblyomma* and *Rhipicephalus* are economically important pests of livestock in most parts of the world. *Rhipicephalus* sp. carries causal organisms for Babesiosis, Piroplasmosis, Cattle Tick Fever, Texas Fever, Anaplasmosis, causes tick toxicosis to cattle and

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its saliva contains toxins that cause paralysis in lambs, adult sheep and calves (Hedimbi et al., 2008; Walker et al., 2003). *Amblyomma* sp. carries causal organisms for Rickettisial diseases including Heartwater (Walker et al., 2003).

The ability of entomopathogenic fungi to induce mortality in ticks in the laboratory and field makes them promising candidates for tick control. The formulation in which the conidia are applied is crucial for the effectiveness of the fungus. This study aims to investigate and compare the susceptibility of different developmental stages (eggs and instars) of various tick species (*A. variegatum, R. appendiculatus* and *R. e. evertsi*) to infection by the entomopathogenic fungus *M. anisopliae* in water and oil formulations.

MATERIALS AND METHODS

Tick rearing

Eggs and instars of ticks used in this study were obtained from the existing tick colonies in the Department of Biological Sciences at the University of Namibia. All the ticks were maintained in a humidity chamber at 25 °C and 100% relative humidity (RH) (Hedimbi et al., 2008; Kaaya et al., 1996).

Inoculant and formulation preparation

M. anisopliae RS2 strain, originally isolated from *A. variegatum* was cultured in petri dishes for 3 weeks in a humidity chamber at 25 °C and 100% RH on Sabouraud Dextrose Agar (Hedimbi et al., 2008; Kaaya et al., 1996). Conidia were harvested by rinsing agar with sterile distilled water containing 0.05% (v/v) Triton X-100. Conidia were then washed twice in sterile distilled water by centrifugation at 5000 revolutions/minute for 5 minutes. A hemocytometer was used to determine the concentration of conidia in the initial suspension. Serial dilutions were then made to get the desired concentration of 1×10^8 conidia ml^{-1} (Hedimbi et al., 2008). Water formulations were prepared in sterile distilled water containing 0.05% Triton X-100 and oil based formulations were prepared in sterile distilled water containing 20% olive oil + 0.05% Triton X-100.

Infection of ticks

Eggs and unfed larvae of each tick species were infected by placing them on a filter paper in disposable petri dishes (65 mm diameter) previously wetted with 1 ml of conidial suspension in each formulation, respectively.
Each petri dish contained between 30-40 eggs or unfed larvae of either tick species. Fed larvae, nymphs and adults of each tick species were infected by dipping them in conidial suspension in each formulation and then placed on filter papers in disposable petri dishes (65 mm diameter). Each petri dish contained between 15-20 instars of either tick species. Three replicates were maintained in each treatment. Controls of either oil or water formulation contained no fungal conidia. All petri dishes were sealed with parafilm and incubated at 25 °C and 100% RH. Fungal infections were viewed under a dissecting microscope. Mortalities (or infection for eggs) were recorded daily up to 21 days post-infection and compared between species as well as water and oil formulations.

Data analysis

Normality of the data were tested using Kolomogorov-Smirnov test and the normally distributed data were analyzed by ANOVA. Means were compared using a post-hoc Scheffé multiple comparison test, using SPSS™ for Windows® version 18 (SPSS, 2010). Three replicates were used in all tests. All analyses were done at confidence interval = 95%, $\alpha = 0.05$.

RESULTS

Eggs: Infection was 50-77% in water and 65-87% in oil formulation. In water formulation, egg infections and mortality were significantly higher ($P<0.05$) in *R. e. evertsi* than in *R. appendiculatus* and *A. variegatum*. In oil formulation, egg infections and mortality were significantly higher ($P<0.05$) in *R. e. evertsi* and *A. variegatum* than an in *R. appendiculatus*. However, there was no significant difference ($P>0.05$) between *R. e. evertsi* and *A. variegatum*. In all tick species, egg infections and mortality were significantly higher ($P<0.05$) in oil than in water formulation (Figures 1-3).

Unfed larvae: Mortality was 55-95% in water and 73-100% in oil formulation. The water formulation induced significantly higher ($P<0.05$) mortalities in *R. appendiculatus* and *R. e. evertsi* than in *A. variegatum*. Mortality was also significantly higher ($P<0.05$) in *R. e. evertsi* than in *R. appendiculatus* with oil formulation. Furthermore, mortalities in the oil formulation were significantly higher ($P<0.05$) than in water formulation in all cases (Figures 1-3).

Fed larvae: Mortality was 51-84% in water and 59-90% in oil formulation. The water formulation induced significantly higher ($P<0.05$) mortality in *R. appendiculatus* than in *A. variegatum*. The oil formulation induced significantly higher ($P<0.05$) mortality than water formulation in *A. variegatum* than in *R. appendiculatus* (Figures 1-3). In both tick species, oil formulation

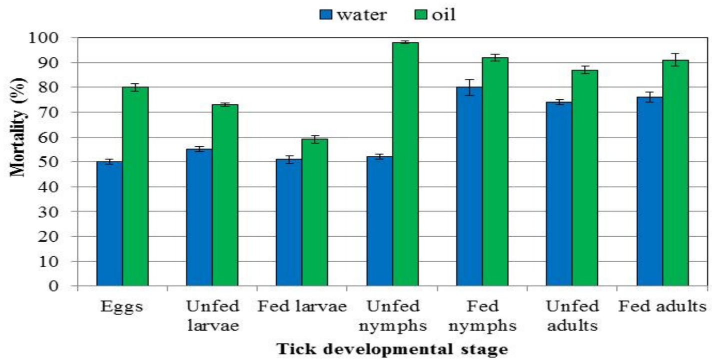

Figure 1. Mortality in various instars of *A. variegatum* induced by *M. anisopliae* ($1x10^8$ conidia/ml) in water and oil formulation. Means (±SE) of three replicates are presented.

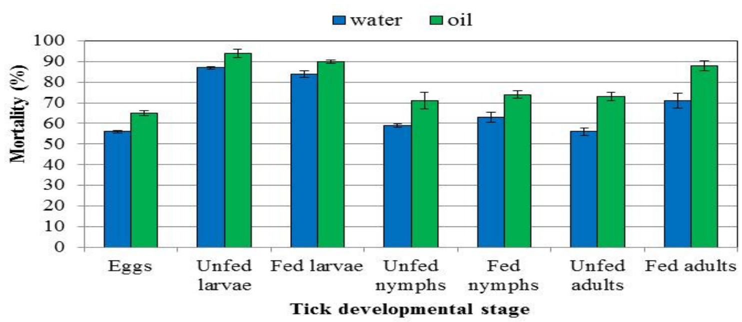

Figure 2. Mortality in various instars of *R. appendiculatus* induced by *M. anisopliae* ($1x10^8$ conidia/ml) in water and oil formulation. Means (±SE) of three replicates are presented.

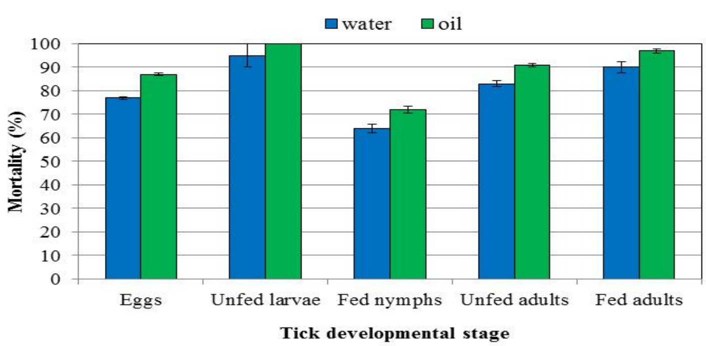

Figure 3. Mortality in various instars of *R. evertsi evertsi* induced by *M. anisopliae* ($1x10^8$ conidia/ml) in water and oil formulation. Means (±SE) of three replicates are presented.

outperformed the water formulation.

Unfed nymphs: Mortality was 52-59% and 71-98% in water and oil formulations, respectively. With both water and oil formulations, there was no significant difference in mortality between *R. appendiculatus* and *A. variegatum* (Figures 1-3).

Fed nymphs: Mortalities were 63-80% in water and 72-92% in oil formulation. The water formulation induced significantly higher (P<0.05) mortality in *A. variegatum* than in *R. appendiculatus* and *R. e. evertsi* whereas oil formulation induced significantly higher (P<0.05) mortality than water formulation in all cases (Figures 1-3).

Unfed adults: Mortality was 56-83% in water and 73-91% in oil formulation. With water formulation, mortality in *R. e. evertsi* was significantly higher (P<0.05) than in *A. variegatum* which was also significantly higher (P<0.05) than in *R. appendiculatus*. Like in all other cases, oil formulation outperformed water formulation (Figures 1-3).

Fed adults: Mortality was 71-90% in water and 88-97% in oil formulation. The pattern was similar to that observed in unfed adults with *R. e. evertsi* having the highest mortality compared to the other 2 tick species (Figures 1-3). The oil formulation outperformed the water formulation.

DISCUSSION

In this study, *M. anisopliae* was found to be capable of causing mortalities to various developmental stages of, *A. variegatum, R. apendiculatus* and *R. e. evertsi* in both water and oil formulations. Gindin et al. (2003) and Kaaya et al. (1996) also observed that *M. anisopliae* was pathogenic to various developmental stages of various tick species particularly *R. appendiculatus* and *R. sanguineus* under laboratory and field conditions. Although some differences in susceptibility of different instars and tick species were observed in this study, mortalities varied from 50-100%. This suggests that *M. anisopliae* has the potential to control mixed tick infestations which occurs commonly in African grazing pastures especially because the fungus was pathogenic to all instars of all the 3 tick species tested.

Conidia formulated in oil induced higher mortalities to all developmental stages of ticks tested than those formulated in water, indicating that formulation to which conidia are suspended is critical to the performance of a mycoacaricide. In a similar study, conidia of *M. anisopliae* and of *B. bassiana* in oil formulation were reported to induce higher mortalities in *R. appendiculatus* and *A. variegatum* than conidia formulated in water, under laboratory and field conditions (Kaaya and Hassan, 2000). Jenkins and Thomas (1996) also reported that, conidia of *M. anisopliae* var. *accridum* in oil formulation were more effective against grasshoppers than conidia in water suspension. Oil formulations of *M. flavoviridae* conidia have also been reported to induce higher mortalities than water formulations in the desert locust (Bateman et al., 1993).

The reason for the better performance of oil formulation may be due to the fact that, oil blends better with the tick cuticle since the cuticle is lipophilic and hydrophobic (Bateman et al., 1993). Furthermore, upon contacting the cuticle, oil spreads rapidly and presumably carries conidia to areas of the body that are protected from unfavorable ambient environmental conditions. The structure of the integument of the tick makes it highly impermeable, restricting water loss from the body (Polar et al., 2005), thus water for germination of conidia may not be readily available hence the use of oil formulation may provide the needed moisture. High mortality in ticks should be interpreted with caution. Placing ticks in the same container may have caused cross infection between infected and non-infected ticks. This may be true especially in cases of unfed stages which are highly mobile and their movement may have caused a transfer of inoculum from one tick to another.

Although there was no clear difference in mortality between fed and unfed stages in larval and nymphal stages, fed adult ticks appeared to be more susceptible than unfed adult ticks. Engorged adult ticks consume a large volume of blood which stretches the cuticle, probably making the germ tubes of the fungus penetrate more easily. This overstretching of the cuticle may also expose the spiracles through which the fungus may penetrate more easily.

CONCLUSION

From the current study, it can be concluded that all instars of the tick species tested were highly susceptible to *M. anisopliae.* The oil formulation induced higher mortalities than water formulations and adult *R. e. evertsi* appeared to be more susceptible, followed by *A. variegatum* and *R. appendiculatus*. Furthermore, fed adult ticks were more susceptible than unfed adult ticks. The results of this study suggest that, due to its acaricidal activity, *M. anisopliae* could be used in bio-control programs to control the populations of different tick species and/or different stages of ticks. Therefore further investigation is required to identify broad spectrum isolates, which are pathogenic to all developmental stages of several tick species yet not pathogenic to non-targets under field conditions.

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