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Full Length Research Paper

Pathogenicity of the entomopathogenic fungus *Metarhizium anisopliae* to the red-legged tick, *Rhipicephalus evertsi evertsi*

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Rhipicephalus evertsi evertsi **is an economically-important tick of livestock in Africa mainly due to its ability to transmit *Babesia equi* to horses, *Anaplasma marginale* to cattle and to cause paralysis in lambs, adult sheep and calves. This study investigated the pathogenicity and hence the bio-control potential of *Metarhizium anisopliae* to eggs and all other off-host stages of *R. e. evertsi*. The eggs and larvae were infected by placing them on filter paper wetted with conidial suspension. Tick instars were infected by dipping in *M. anisopliae* suspenssion (1×10 ^{3}–10 ^{8} conidia/ml) and incubated together with the eggs at 25°C and 100% relative humidity (RH). The m ortality of eggs and all tick stages tested increased with increasing conidial concentration and were higher (P>0.05) in conidia formulated in oil than in those formulated in water. For instance, the LC_{50} in unfed larvae was 1×10 ^{4} conidia /ml in oil formulation and 1×10 ^{5} conidia /ml in water formulation. No difference was observed in mortality between fed and unfed tick stages.**

Key words: *Rhipicephalus evertsi evertsi*, *Metarhizium anisopliae*, formulation, mortality, biological control.

INTRODUCTION

Currently, ticks are mainly controlled by chemical acaricides. The main disadvantages of acaricides are their high costs, development of resistance in ticks, environmental and food contamination and their residues (De Castro, 1997, Garcia-Garcia et al., 2000). The use of natural enemies such as entomopathogenic fungi is generally perceived to be ecologically preferable to chemical treatment for controlling pests (Benjamin et al., 2002). Biological control agents are natural, more environmentally-friendly, potentially less expensive than chemical pesticides, and problems with resistance are less likely to occur (Whipps and Lumsden, 2001; Polar et al., 2005, Zimmermann, 2007). The formulation in which the conidia are suspended is known to influence the efficacy of the fungus (Kaaya and Hassan, 2000; Hedimbi et al., 2008, 2011). Conidia formulated in oil have been reported to induce higher tick mortalities than those formulated in water alone (Kaaya and Hassan, 2000; Maranga et al., 2005; Hedimbi et al., 2011).

Rhipicephalus evertsi evertsi is a two-host tick, an economically important pest of livestock throughout most parts of Africa. It is a vector of bovine anaplasmosis, equine babesiosis and causes paralysis in sheep and calves (Walker et al., 2003). Despite being an economically important pest of livestock, the susceptibility of *R. e. evertsi* to potential biological control agents such as *Metarhizium anisopliae* has not yet been invesitigated.

This study aims to investigate the susceptibility of all off host stages of *R. e. evertsi* to infection by *M. anisopliae* in water and oil formulations and hence its potential as a

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biological control agent for this tick species.

MATERIALS AND METHODS

Ticks

Engorged *R. e. evertsi* ticks were collected from Zebu cattle in Northern Namibia in 2007. All tick instars were fed on tick naive rabbits (Kaaya et al., 1996; Hedimbi et al., 2008). The ticks were fed on cloth-covered shaved backs or ears of the rabbits. Ticks not being used immediately after drop were stored at 14° C until use (Samish et al., 1999; Hedimbi et al., 2008).

Fungi

M. anisopliae RS2 (originally isolated from *Amblyomma variegatum* ticks) was cultured in Petri dishes for 3 weeks at 25° C and 100% relative humidity (RH) on Sabourauds Dextrose Agar (Kaaya et al., 1996; Hedimbi et al., 2008; 2011). Conidia were harvested by rinsing agar with sterile distilled water containing 0.05% (v/v) Triton X-100. The Conidia were then washed twice in sterile distilled water by centrifugation at 5000 rpm for 5 min (Hedimbi et al., 2008; 2011).

Inoculants preparation

Water formulations were prepared in sterile distilled water containing 0.05% Triton X-100, while oil based formulations were prepared in sterile distilled water containing 20% olive oil + 0.05% Triton X-100. A hemocytometer was used to determine the concentration of conidia in the initial suspension. Serial dilutions were then made to obtain the desired concentration of conidia (Hedimbi et al., 2008; 2011).

Bio assay

Infection of eggs and larvae was achieved by placing 1 ml conidia suspensions (1×10^{3} to 1×10^{8} conidia/ml) on filter papers covering the bottom of 65 mm diameter disposable plastic Petri dishes and placing 40 to 50 tick eggs or larvae into each dish. Nymphs and adults were infected by dipping them in various concentrations of conidial suspenssions (1×10^{3} to 1×10^{8} conidia/ml). The dipped ticks were placed in 65 mm disposable Petri dishes, and incubated at 25°C and 100% RH. Three replicates were set up in eac h experi-ment. The control groups went set up using the same procedure but without fungal conidia. Fungal infections were viewed under a dissecting microscope and mortality recorded 21 days post infection (Hedimbi et al., 2008; 2011). Eggs were regarded as dead when they failed to hatch 21 days post-infection.

Data analyses

Normality of the data was tested using Kolomogorov-Smirnov test and normally distributed data were analyzed by ANOVA and means were compared with a post-hoc Scheffé multiple comparison test, using SPSS™ for Windows® Version 18 (SPSS, 2010). A ll analyses were done at 95% confidence interval (CI), and 0.05 α-value (Hedimbi et al., 2011).

RESULTS

Eggs

Conidia of *M. anisopliae* infected and induced mortality in eggs of *R. e. evertsi*. A significant corelation ($P<0.05$) between concentrations of *M. anisopliae* conidia and egg mortality was observed. Egg infections, in all conidia concentrations tested were significantly lower ($P<0.05$) in the water than in the oil formulation (Figure 1). Furthermore, all infected eggs failed to hatch.

Unfed larvae and fed nymphs

The mortality of unfed larvae was significantly lower ($P<0.05$) in water than in the oil formulation at a concentration of 1×10^{3} to 10^{7} conidia/ml (Figure 2). The LC_{50} in unfed larvae was 1×10^{4} conidia/ml in oil, and 1×10^{5} conidia/ml in the water formulation (Figure 2). In engorged nymphs, mortality was significantly higher ($P<0.05$) in oil than in the water formulation at con-centrations of 1×10^{3}, 10^{5} and 10^{6} conidia/ml. However, no significant difference between oil and water formu-lations was observed at 1×10^{4}, 10^{7}, and 10^{8} conidia/ml (Figure 2).

Unfed and fed adults

Mortality increased with increasing conidial concentra-tions in unfed and fed adults, in both oil and water formulations. In both unfed and fed adults, mortality was significantly higher ($P>0.05$ in oil than in water formu-lation at all concentrations, except at 1×10^{4} conidia/ml. Furthermore, no significant difference was observed in mortality between fed and unfed adult ticks (Figure 3), 21 days post-infection.

DISCUSSION

In this investigation, the mortality of different develop-mental stages of *R. e. evertsi* exposed to increasing concentration of *M. anisopliae* conidia was studied. The concentration of 1×10^{3} conidia/ml induced in the 2 types of formulations studied, low tick mortality; whereas exposure to 1×10^{5} of oil formulation or 1×10^{6} conidia/ml of water formulation induced about 50% mortality in most developmental stages tested. A concentration of $1\times1\ 0^{8}$ conidia/ml in the 2 formulations induced very high mor-tality (over 80%) to all *R. e. evertsi* developmental stages. Furthermore, oil formulation induced significantly higher tick mortality ($P>0.05$) than water formulation at all concentrations.

The ability to improve the performance of myco-pesticides by adding oil was also demonstrated when using the conidia of *M. anisopliae or B. bassiana* under laboratory and field conditions against *Rhipicephalus appendiculatus* and *Amblyomma variegatum* ticks (Kaaya and Hassan, 2000; Maranga et al., 2005). Jenkins and Thomas (1996) also reported that aerial conidia of *M. anisopliae* var. *accridum* in oil formulation were more effective against grasshoppers than conidia suspended

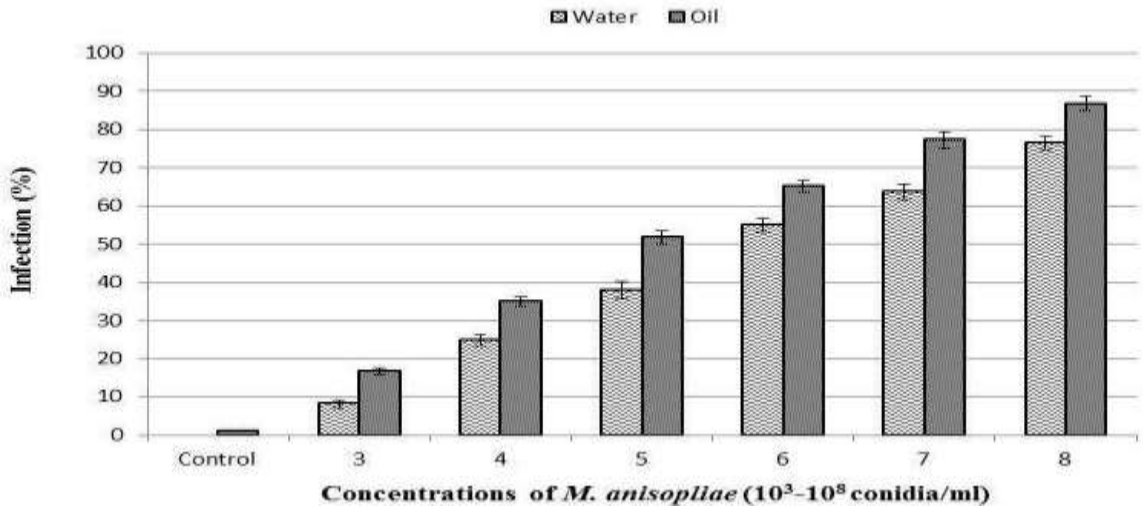

Figure 1. Infection (%) of *R. e. evertsi* eggs induced by *M. anisopliae* conidia in oil and water formulations. Controls contained no conidia. Means (±SD) of three replicates are presented.

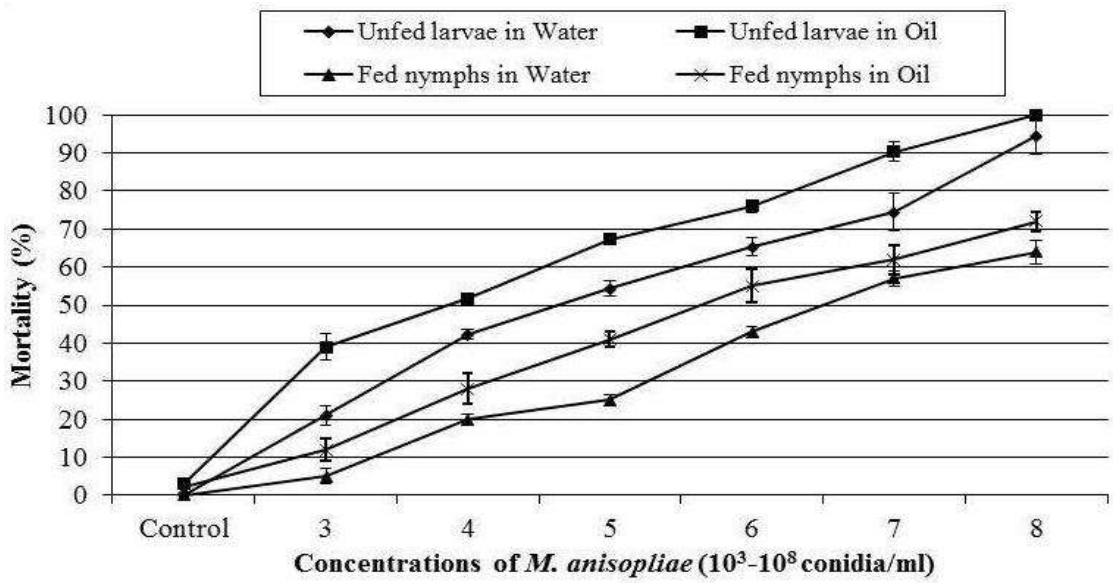

Figure 2. Mortalities (%) of *R. e. evertsi* unfed larvae and fed nymphs induced by *M. anisopliae* conidia in oil and water formulations. Controls contained no conidia. Means (±SD) of three replicates are here presented.

in water. Oil formulations have also been reported to induce higher mortality in desert locusts than water based formulations (Bateman et al., 1993).

In this study, a concentration of 1×10^{8} conidia/ml of *M. anisopliae* induced mortalities of 95% in water and 100% in oil formulation to *R. e. evertsi* unfed larvae. Monteiro et al. (1998) reported mortality of 95% in unfed larvae of *R. sanguineus* exposed to fungi formulated in oil 20 days post infection. Although the reason for higher mortality induced by oil formulations is not known, it is believed to be due to the fact that oil blends better with insect's lipophilic cuticle than water, and that oil spreads rapidly, presumably carrying fungal conidia to areas of the cuticle that are normally protected from unfavorable environmental conditions (Wraight et al., 2001).

A concentration of 1×10^{8} conidia/ml in oil formulation induced a mortality of 91% in unfed adults and 97% in fed adults of *R. e. evertsi* ($P>0.05$). Benjamin et al. (2002) reported that a high conidial concentration (4×10^{9} conidia/ml) was required to induce almost 100% mortality among unfed adult *Ixodes scapularis* ticks. In another study, a concentration of 1×10^{7} conidia/ml induced 100%

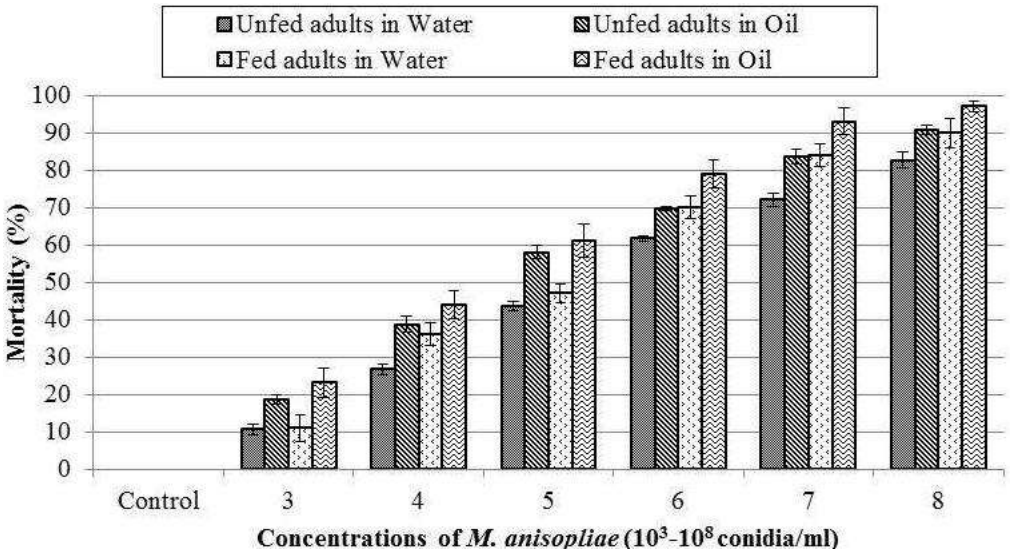

Figure 3. Mortalities (%) of *R. e.evertsi* unfed and fed adults induced by *M. anisopliae* conidia in oil and water formulations. Controls contained no fungal conidia. Means (±SD) of three replicates are presented.

mortality in engorged adult *I. scapularis* (Zhioua et al., 1997; Benjamin et al., 2002). Mwangi et al. (1995) reported mortalities of 35 and 81%, respectively in unfed and fed adult *R. appendiculatus* Neumann exposed to 1×10^{6} conidia/ml of *M. anisopliae.* The pathogenicity of the fungus *M. anisopliae* on engorged females of *R. e. evertsi* was evaluated and a high mortality rate was observed, with 90 and 97% in water and oil formulation, respectively at 1×10^{8} conidia/ml. *M. anisopliae* has been reported to have caused 100% mortality in engorged adult female *Boophilus microplus* (Polar et al., 2005). This observation suggests that blood in the engorged ticks does not influence fungal pathogenicity to ticks.

No report on pathogenicity and biological control potential of *M. anisopliae* on *R. e. evertsi*, a tick of great economic importance in Africa could be found in the literature. This study has shown that *M. anisopliae* may have a potential as a mycopesticide for control of different developmental stages of *R. e. evertsi* ticks.

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