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Full Length Research Paper

The use of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, as bio-pesticides for tick control

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This paper reports promising results on the use of entomopathogenic fungi for tick control. The aqueous formulations of the entomopathogenic fungi, *Metarhizium anisopliae* isolate RS2 and *Beauveria bassiana* isolate NM2 induced mortalities ranging from 36-64% in adult *Rhipicephalus (Boophilus) appendiculatus* and 40-50% in *Boophilus decoloratus* in the laboratory. In *Rhipicephalus evertsi evertsi*, mortality increased with conidial concentration in all tick stages and oil formulation outperformed (p<0.05) the aqueous formulation in all experiments. In the field potted grass experiment, both aqueous and oil formulations of *M. anisopliae* and *B. bassiana* induced high mortalities in *R (B). appendiculatus* and *Amblyomma variegatum*, especially in larvae, where mortality reached 100% in both tick species. In nymphs of both *R (B). appendiculatus* and *A. variegatum*, mortality was about 100% with oil and 80% with aqueous formulations (p<0.05), whereas in adults, mortality was 20-40% (aqueous) and 65-100% (oil), respectively. In the semi-field experiment, ticks (*R (B). appendiculatus*) feeding on cattle were sprayed with fungi (aqueous formulation) and allowed to continue feeding until they drop off. Half of the dropped off ticks were maintained in the field and the rest in the laboratory and the fungi induced significant mortalities and reduced fecundity and egg hatchability in both categories but the results were not significantly different from each other (p>0.05). In the field paddocks sprayed with *B. bassiana* and *M. anisopliae* tick counts were much lower than those in the control paddock (p<0.05). After monthly spraying for 6 months, the mean numbers of adult *R (B). appendiculatus* on cattle were reduced by 80% in *B. bassiana* and 92% in *M. anisopliae* sprayed paddocks compared to control paddock. These observations show that *M. anisopliae* and *B. bassiana*, have potential as tick mycopesticides.

Keywords: Biological control, *Beauveria bassiana*, formulations, *Metarhizium anisopliae*, Ticks.

INTRODUCTION

Ticks of genus *Amblyomma* and *Rhipicephalus* are economically important pests of livestock in most parts of the world (Kaaya et al., 1996; Correia et al., 1998). *Rhipicephalus* sp. carries causal organisms for Babesiosis, Piroplasmosis, Cattle Tick Fever, Texas Fever, Anaplasmosis, causes tick toxicosis to cattle and its saliva contains toxins that cause paralysis in lambs, adult sheep and calves (Kaaya et al., 1996; Hedimbi et al., 2011). *Amblyomma* sp. carries causal organisms for Rickettisial diseases including Heartwater (Kaaya et al., 1996; Kaaya and Hassan, 2000; Hedimbi et al., 2011). Ticks are mainly controlled with acaricides applied on cattle by means of plunge dips or spray races. Although acaricides have been shown to reduce tick population and their incidences, their main disadvantages have been environmental pollution, development of resistant tick strains, and escalating costs (Norval et al., 1970).

This has prompted searches for alternative methods of tick control. Entomopathogenic fungi have been used successfully to control various agricultural and pasture pests. In Brazil, they have been sprayed with airplanes in large fields to control sugarcane pests (Gillespie and Claydon, 1989), whereas in Indonesia and Malaysia, they have been used to control the rhinoceros beetle, a serious pest of oil palms (Munaan and Wikardi, 1986). In Australia, entomopathogenic fungi have been used to control the subterranean pasture pest, *Adoryphorus couloni* (Rath, 1992; Rath et al., 1995) and field tests

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have produced promising results with other Australian pests such as the plague locust, *Phaulacridium vittatum,* and the wingless grasshopper, *Chortoicetes terminifera* (Milner et al., 1994; Hooper et al., 1995). However, there is no report in the literature on the use of entomopathogenic fungi for control of livestock pests although some promising results have been reported by various researchers (Kaaya et al., 1996; Correia et al., 1998; Gindin et al., 2002; Polar et al., 2005; Leemon et al., 2008; Hedimbi et al., 2011; Kaaya et al., 2011). This paper reports promising results on the use of entomopathogenic fungi for tick control. The paper also reports the use of aqueous and vegetable oil in formulation of fungal conidia to suppress on-host and off-host tick populations under field and laboratory conditions.

MATERIALS AND METHODS

Ticks rearing

Rhipicephalus (Boophilus) appendiculatus, *Amblyomma variegatum, Rhipicephalus evertsi evertsi,* and *Boophilus decoloratus*, obtained from cattle, were used. Ticks were maintained in the laboratory at 25 °C at 100% relative humidity (RH) and were fed on rabbits as explained by Kaaya et al., (1996).

Fungi culturing

Metarhizium anisopliae isolate RS2 and *Beauveria bassiana* isolate NM2 (both strains were originally isolated from *Amblyomma variegatum*) were cultured in petri dishes for 3 weeks at 25°C and 100% RH on Sabouraud's Dextrose Agar (SDA). 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 haemocytometer was used to determine the concentration of conidia in the initial suspension. Serial dilutions were then made to obtain the desired concentration of conidia.

Laboratory infection experiments

Engorged adult female ticks (male ticks naturally die after mating) or engorged nymphs were infected by dipping in *M. anisopliae* or *B. bassiana* conidia suspensions (1×10^8 conidia/ml) formulated in water (0.05% Triton X-100) or oil emulsion (20% sunflower oil + 0.05% Triton X-100 in water). No fungi were added to the formulation in the control groups. They were then placed on filter papers in disposable petri-dishes (70 mm diameter). Adult *R (B). appendiculatus* and adult *B. decoloratus* were infected with *M. anisopliae* and *B. bassiana*; whereas engorged nymphs and engorged adult female *R. e. evertsi* were infected only with *M. anisopliae* (at concentrations of 1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia/ml). Each petri dish contained 30 adults or nymphs and the experiment was done in triplicates. The dishes containing ticks were incubated at 25 °C and 100% RH. LC_{50} was calculated by determining the minimum concentration of fungal suspension that was required to cause 50% mortality in tick instars.

Semi-field experiment

M. anisopliae and *B. bassiana* were tested on *R (B). appendiculatus* feeding on zebu cattle. Aqueous formulations containing 1×10^8 conidia/ml were prepared and sprayed on the ticks engorging on cattle in the field. After engorgement and dropping, the ticks were placed individually in nylon tetrapacks, sealed and left in the grass under a tree for 6 weeks, after which mortalities, fecundity and egg hatchability were recorded. Non-infected ticks were also collected and incubated in the laboratory at 25 °C at 100% RH for comparison. Mortalities, fecundity and egg hatchability were also recorded for 6 weeks. The experiment was done in triplicates.

Determination of pathogenicity of *B. bassiana* and *M. anisopliae* against *R. appendiculatus* and *A. variegatum* in vegetation

Fungal concentrations of 1×10^8 conidia/ml were prepared in water or peanut oil emulsions and each life stages of *R (B). appendiculatus* and *A. variegatum* sealed in nylon tetrapacks containing 50 adult ticks per tetrapack, dipped in the fungal suspensions, and placed in potted grass and left in the field for 6 weeks, after which tick mortalities were recorded for the 2 formulations. The experiment was done in triplicates.

Determination of impact of fungi on on-host tick populations in the field

Conidia of *M. anisopliae* and *B. bassiana* were produced on a substrate of ground maize (*M. anisopliae*) or rice (*B. bassiana*). These substrates were chosen because they are readily available and relatively cheaper compared to artificial growth media. The substrates were autoclaved for 1 hour at 121°C and 15 lb/sq inch pressure, transferred to plastic buckets and inoculated with a 3 day-old fungal culture (50 ml). They were then incubated for 21 days at 25°C and 70% RH and then allowed to dry for 5 days at room temperature before conidia were harvested by sieving. The conidia were stored in the refrigerator (4-6°C) until required for field spraying. Viability tests were conducted prior to each field experiment by culturing the conidia on SDA

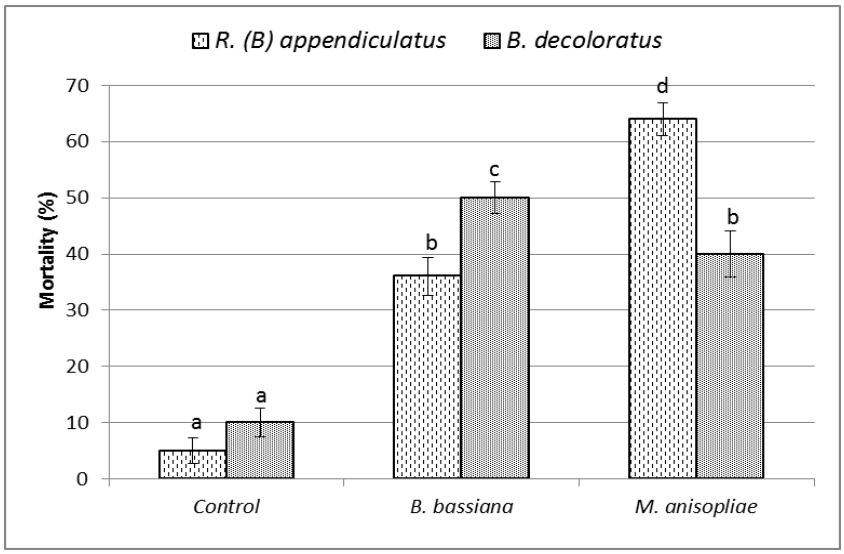

Figure 1: Mortality (%) in adult *R (B). appendiculatus* and *B. decoloratus* induced by *B. bassiana* and *M. anisopliae* in aqueous formulation in the laboratory. Means ±SE of three replicates are presented. Means followed by the same are not significantly different from each other (Schefé multiple comparison test, P>0.05).

for 24 hours and determining the percent germination. The conidia were mixed thoroughly with water and 1% Tween-80 in 240 liter metal drums to obtain a final concentration of 1×10^8 conidia per ml of water. These suspensions were then sprayed on grass once per month on 5 acre paddocks previously seeded with approximately 2×10^5 larvae of *R (B). appendiculatus*. The first paddock was sprayed with *B. bassiana*, the second with *M. anisopliae* and the third acted as control. Spraying started 3 months after seeding paddocks with larvae and continued for 6 months. Each paddock contained 5 zebu cattle. The control paddock was sprayed with 1% Tween-80 in water only. On-host tick populations were counted on all cattle (whole body count) throughout the experimental period (9 months).

Data analysis

Normality of data was tested using the Kolomogorov–Smirnov test and normally distributed data were analysed by ANOVA and means were compared using a post-hoc Scheffé multiple comparison test, using SPSS for Windows® version 18 (2010).

RESULTS

The aqueous formulations of *M. anisopliae* and *B. bassiana* induced mortalities ranging from 36 to 64% in adult *R (B). appendiculatus*, and 40 to 50% in *B. decoloratus* in the laboratory (Fig. 1). In the *R. e. evertsi* in which concentration-mortality relationship was investigated, it was observed that mortality increased with conidial concentration in all tick stages and that oil formulation outperformed the aqueous formulation in all experiments ($p<0.05$). Figure 2 show mortalities in engorged nymphs and adults of *R. e. evertsi*. The LC_{50} in engorged nymphs and engorged adults was 1×10^5 conidia/ml in oil and 1×10^6 conidia/ml in water formulation (Fig. 2).

In the field potted grass experiment, both aqueous and oil formulations of *M. anisopliae* and *B. bassiana* induced high mortalities ($p<0.05$) in *R. appendiculatus* and *A. variegatum*, especially in larvae, where mortality was 100% in both tick species. The oil formulation, however, induced a higher mortality in both tick species in nymphs and adults than the aqueous formulation ($p<0.05$). In nymphs of both *R (B). appendiculatus* and *A. variegatum*, mortality was about 100% with oil and 80% with aqueous formulations, whereas in adults, mortality was 20-40% (aqueous) and 65 to 100% (oil), respectively ($p<0.05$) (Figures 3 and 4).

In the semi-field experiment where ticks (*R (B). appendiculatus*) feeding on cattle were sprayed with fungi (aqueous formulation) and half maintained in the field and the other half in the laboratory, the fungi induced significant mortalities and reduced egg production and hatchability ($p<0.05$). Depending on whether ticks were maintained in the field or in the laboratory, mortalities in the control group ranged from 10-20%, 65-75% with *B. bassiana* and 65-78% with *M. anisopliae*. The corresponding egg hatchability was 85-90%, 30-45% and 30-60% (Fig. 5).

In the field pastures sprayed with *B. bassiana* and *M. anisopliae* once per month, (1×10^8 conidia/ml), tick populations were much lower ($p<0.05$) than in the

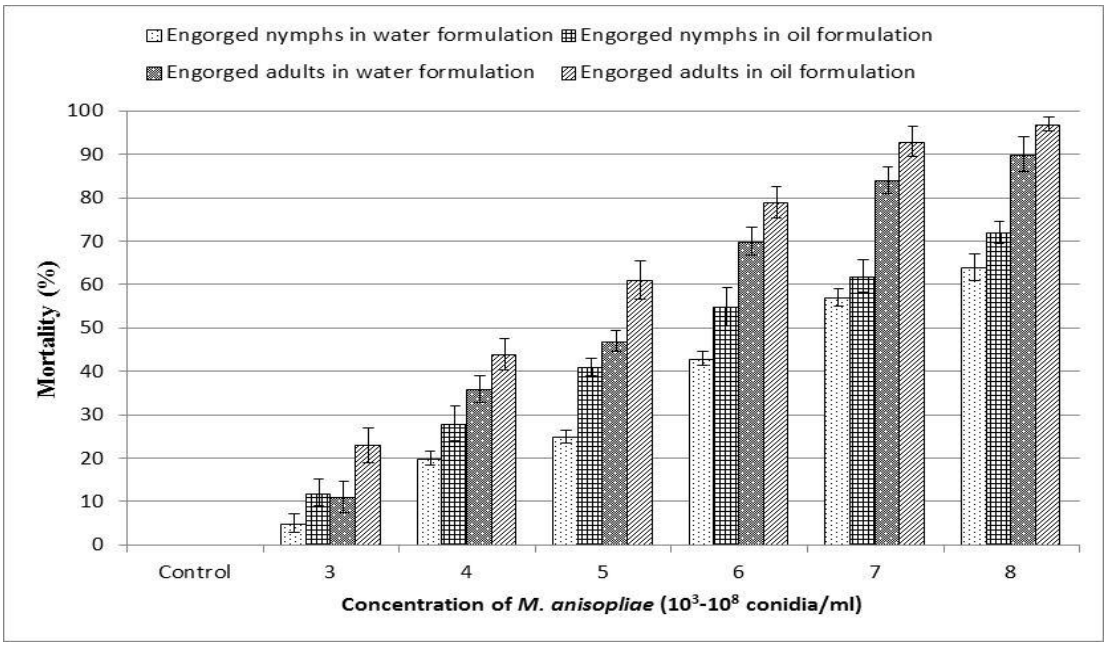

Figure 2: Mortality (%) in *R. e. evertsi* engorged nymphs and engorged adults induced by various concentrations of *M. anisopliae* conidia in aqueous and oil formulations. Means ± standard errors of 3 replicates are presented.

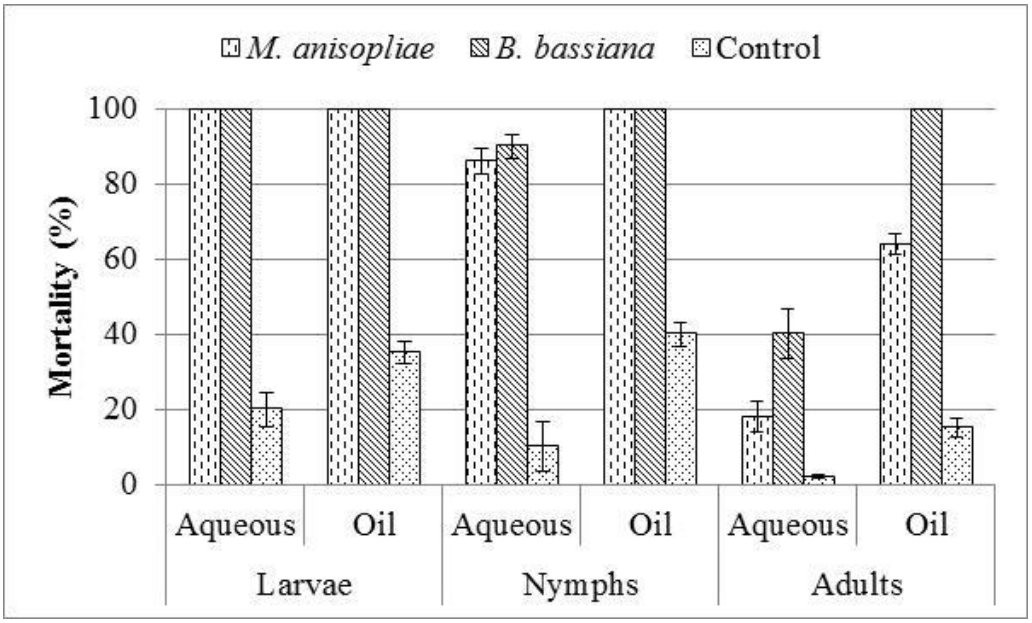

Figure 3: Mortality (%) induced by aqueous and oil formulations of *B. bassiana* and *M. anisopliae* conidia maintained in the field to: larvae, nymphs and adults of *R (B). appendiculatus*.

control paddocks. After 6 months of spraying, the mean numbers of adult *R (B). appendiculatus* on cattle had been reduced by 80% in *B. bassiana* and 92% in *M. anisopliae* sprayed paddocks compared to controls (Fig. 6). Heavy rainfall in January to March flooded the pastures killing most of the ticks and washing away the fungi from the grass, hence low tick counts and no clear effect of fungal treatment till later after rainfall

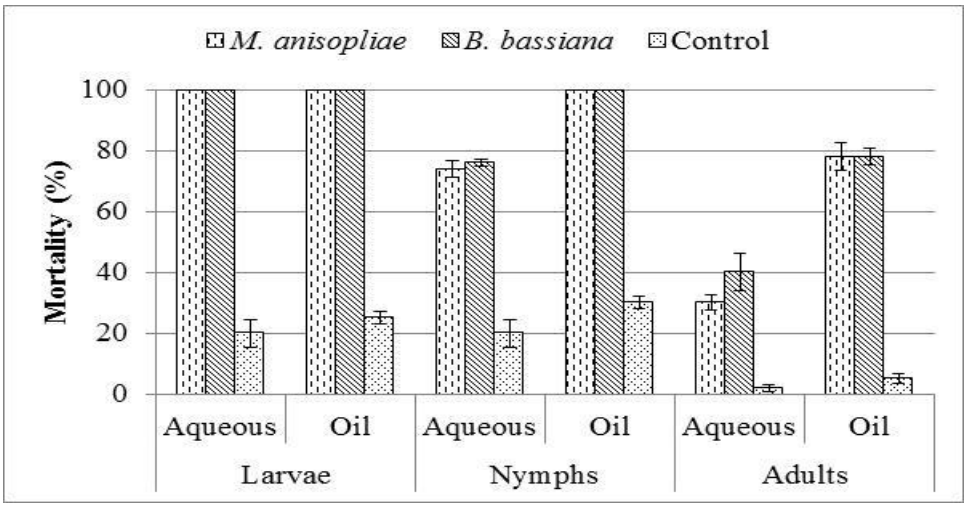

Figure 4: Mortality (%) induced by aqueous and oil formulations of *B. bassiana* and *M. anisopliae* conidia maintained in the field in larvae, nymphs and adults of *A. variegatum*.

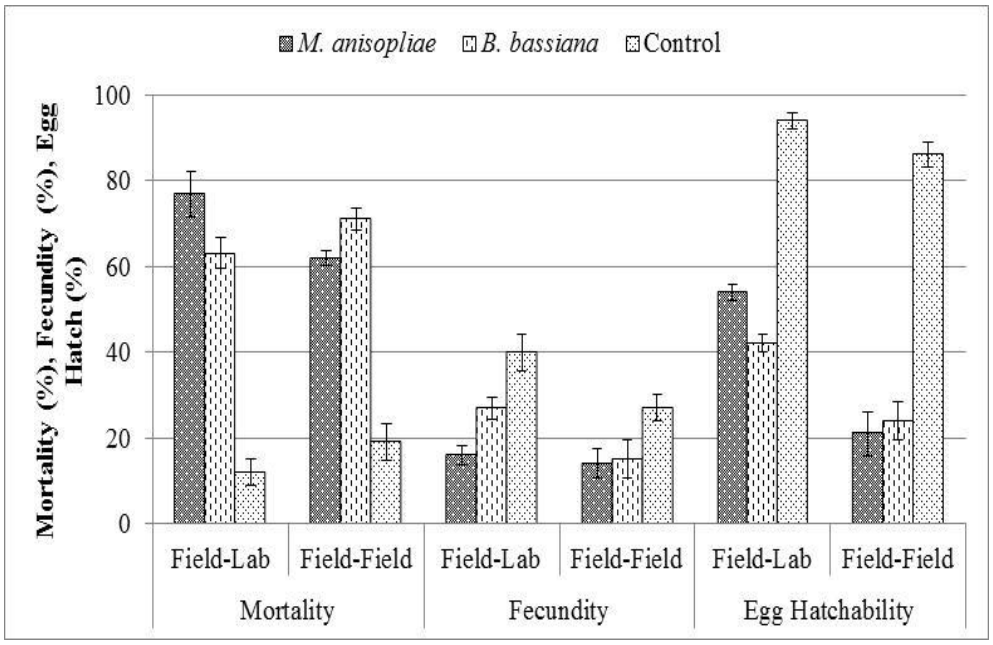

Figure 5: Mortality, fecundity and egg hatch (%) in *R (B). appendiculatus* infected with conidia of *B. bassiana* and *M. anisopliae*. Control contained no fungal conidia.

diminished (Fig. 6).

DISCUSSION AND CONCLUSIONS

The results of the laboratory, semi-field and field studies reported in this paper clearly show that *B. bassiana* and *M. anisolpiae* are pathogenic to the economically important African tick species and therefore have potential as biological control agents for these ticks. Since fungi can be mass-produced on local substrates, this method of tick management is likely to be cheaper and will save foreign currencies used to import acaricides (Kaaya and Hassan, 2000). Furthermore, resistance in ticks is not likely to develop as fast as in the case of chemical acaricides since fungi are capable of producing mycotoxins that weaken arthropod immune systems.

In all our experiments, the oil-based formulation was observed to be more effective than the water formulation. This might be due to the fact that oil blends better with the lipophilic tick cuticle as compared to

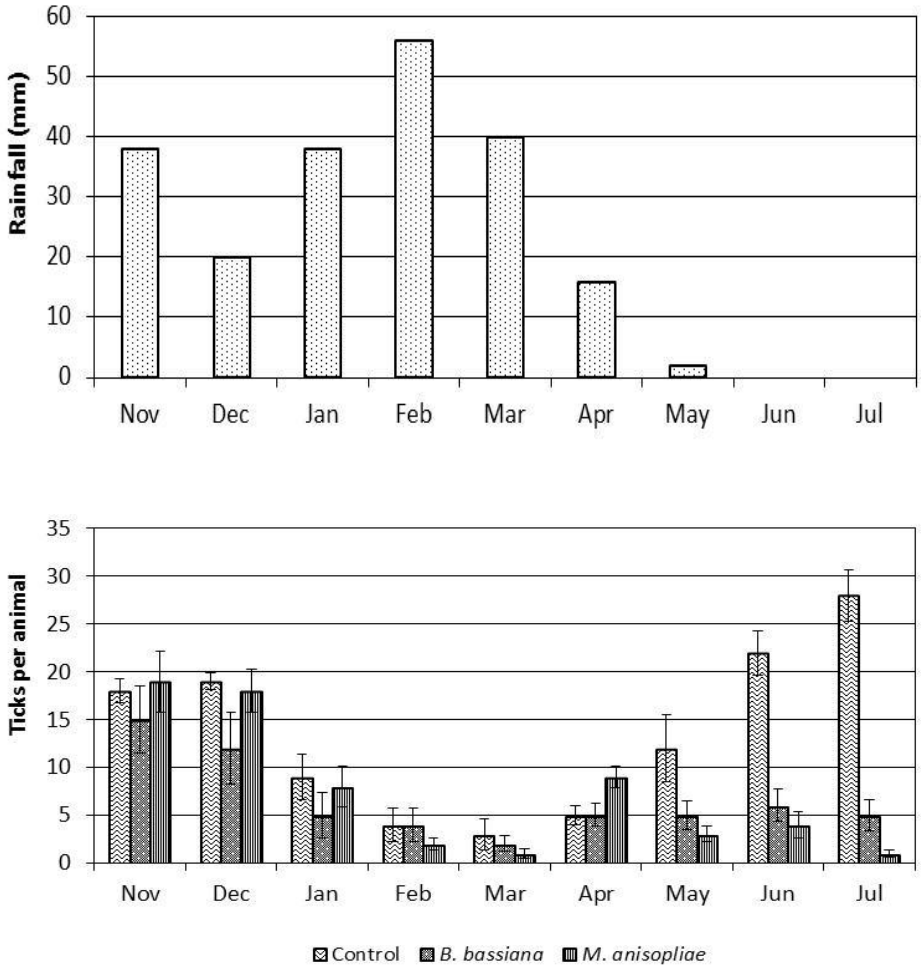

Figure 6: *R (B). appendiculatus* tick counts on cattle grazing on pastures sprayed with entomopathogenic fungi. Top graph shows mean monthly rainfall in mm.

water since the cuticle is hydrophobic (Hedimbi et al., 2011; Kaaya et al., 2011). Furthermore, oil provides moisture for the fungus to germinate for a longer period than water which evaporates faster (Kaaya et al., 2011). The experiment in Fig. 5 was conducted to compare the mortality, fecundity and egg hatchability from ticks maintained in the field and those maintained in the laboratory. Although there were some differences, the results were not significantly different suggesting that good results obtained in the laboratory with selected fungal isolates may be a rough prediction of good field results although this may not always be the case.

Horizontal transmission of infection from fungus-infected to non-infected arthropods has been reported (Baker et al., 1994). This may lead to fungal epizootics (Kaaya and Hassan, 2000), especially in moist environments. Heavy rainfall in January to March (Figure 6) flooded the pastures killing most of the ticks and washing away the fungi from the grass, hence low tick counts and no clear effect of fungal treatment till later after rainfall diminished. The amount of ticks in the control group were slightly high during heavy rainfall (Jan-Feb) compared to the treatments due to the fact that tick numbers in control group were already high prior to heavy rainfall period compared to the treatments. Non-target organisms within the environment may also serve as secondary hosts on which fungi are maintained and propagated, thus promoting later infections in the target host populations (Goettel and Johnson, 1992). This epizootic phenomenon may reduce the frequency of field applications, probably to only a few times in a year thus reducing the cost of tick control and incidences of tick-borne diseases.

Vegetation acts as a reservoir for ticks that attach on cattle. Hence, spraying ticks in vegetation will have an advantage of killing more ticks than spraying directly on cattle, thus reducing the numbers of ticks that would have attached on cattle and hence the frequency of acaricide application (Kaaya and Hassan, 2000).

However, spraying fungus directly on cattle to kill on-host ticks will be much cheaper and the fungi will come into contact with fewer non-target organisms (Kaaya et al., 2011).

Earlier observation that acaricides do not severely affect the viability of the fungal conidia (Kaaya et al., 1996) strengthens the prospects of developing an integrated approach for tick control consisting of low levels of acaricides in combination with entomopathogenic fungi. Since in addition to causing direct mortality to ticks, the fungi also significantly reduce tick fecundity and egg viability, the impact of fungi on tick populations is likely to be significant. Rath et al., (1995) observed that application of *M. anisopliae* to control the subterranean scarab, *Adoryphorus couloni* (Burmeister) in pasture had no adverse effects on non-target invertebrates, hence mycopesticides are likely to be more environmentally friendly than the conventional acaricides.

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