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Effect of oil-based formulations of acaripathogenic fungi to control *Rhipicephalus microplus* ticks under laboratory conditions

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ABSTRACT

The formulations of acaripathogenic fungi to control ticks have been widely studied. The present study evaluated the efficacy of oil-based formulations of *Metarhizium anisopliae sensu lato* (s.l.), isolate Ma 959, and *Beauveria bassiana*, isolate Bb 986, on different *Rhipicephalus microplus* stages, comparing the efficacy between aqueous suspensions and 10, 15 and 20% mineral oil formulations. Twelve groups were formed: one aqueous control group; three mineral oil control groups, at 10, 15 or 20%; two aqueous fungal suspensions of *M. anisopliae* s.l. or *B. bassiana*; and three formulations of *M. anisopliae* (s.l.) or *B. bassiana* containing 10, 15, and 20% mineral oil. To prepare aqueous suspensions and oily formulations, fungal isolates were cultivated on rice grains in polypropylene bags. The conidial suspensions and formulations had a concentration of 10^8 conidia/mL. Bioassays were repeated twice. After treatment, the following biological parameters of engorged females were evaluated: hatching percentage, egg production index, nutritional index, and percentage of tick control. The following parameters were evaluated in the bioassays with eggs: period of incubation, period of hatch, and hatching percentage. Mortality was evaluated in bioassays with larvae. *M. anisopliae* s.l. and *B. bassiana* oil-based formulations were more effective than aqueous suspensions against *R. microplus* eggs, larvae and engorged females, however, there was no significant difference between the three oil concentrations used. *M. anisopliae* s.l. and *B. bassiana* formulated in mineral oil reached 93.69% and 21.67% efficacy, respectively, while *M. anisopliae* s.l. and *B. bassiana* aqueous suspensions attained 18.70% and 1.72% efficacy, respectively. *M. anisopliae* s.l. oil-based formulations caused significant effects in all biological parameters of engorged females while *B. bassiana* oil-based formulations modified significantly the nutritional index only. Eggs treated with *M. anisopliae* s.l. and *B. bassiana* oil-based formulations showed hatching rates that decreased 102.5 and 3.65 times, respectively. In the bioassay with larvae, *M. anisopliae* s.l. oil-based formulations caused nearly 100% mortality five days after treatment, while larva treated with *B. bassiana* oil-based formulations reached 100% mortality at day 20 after treatment. Larva from oil-based control groups showed mortality at day 15 after treatment, which indicated a possible toxic effect of the oil for this *R. microplus* stage. The results showed that the fungal mineral oil formulations tested were more effective than the aqueous suspension. Oil-based formulations at 10, 15 and 20% enhanced the activity of *M. anisopliae* s.l. Ma 959, and *B. bassiana* Bb 986, isolates against *R. microplus* eggs, larvae, and engorged females tick. Mineral oil was effective as an adjuvant in formulations of *M. anisopliae* s.l., Ma 959, and *B. bassiana*, Bb 986, for the control of *R. microplus* under laboratory conditions.

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1. Introduction

Rhipicephalus microplus Canestrini, 1888 (Murrel and Barker, 2003), commonly known as the cattle tick, is an ectoparasite of great importance to livestock producers because it causes economic losses in Brazil estimated at two billion dollars per year (Grisi et al., 2002). To control this ectoparasite, stock breeders and dairy farmers use chemical acaricides indiscriminately, which contributes to food and environmental contamination and the development of chemical resistance in some tick populations. In an effort to avoid these problems, microbial control has been attracting increasing attention as a tool for the integrated management of cattle ticks.

Acaripathogenic fungi are potential agents for biological control since all tick stages have been found naturally infected by several species of these organisms. Among acaripathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana* have shown efficacy against various stages of many tick species (Bittencourt et al., 1992; Samish et al., 2001; Fernandes and Bittencourt, 2008). Although the virulence of these acaripathogenic fungi has been demonstrated under laboratory conditions, their efficacy declines considerably under field conditions since fungal action is affected by environmental factors such as temperature, humidity, solar radiation, rainfall, as well as the microclimatic elements in the entomopathogen's habitat (Inglis et al., 2001; Huang and Feng, 2009; Ment et al., 2010).

Improvements in the biological control of ticks must include research on formulations to maintain fungal viability and pathogenicity given the negative interference of environmental conditions on the action of acaripathogenic fungi in the field. Many studies have shown the efficacy of acaripathogenic fungal formulations in controlling ticks (Kaaya and Hassan, 2000; Maranga et al., 2005; Polar et al., 2005; Leemon and Jonsson, 2008; Ángel-Sahagún et al., 2010; Angelo et al., 2010; Kaaya et al., 2011; Peng and Xia, 2011). When added to fungal suspensions, mineral and vegetable oils increase adhesion of the conidia to arthropod surfaces, which protects fungi from unfavorable environmental conditions (Alves, 1998). Here, we report on studies where the efficacy against different cattle tick stages was compared between aqueous suspensions and formulations of *M. anisopliae sensu lato* (s.l.) and *B. bassiana* containing 10, 15, and 20% mineral oil.

2. Materials and methods

2.1. Ticks

Engorged *R. microplus* females were collected from the floor of cattle pens holding naturally infested calves at the W. O. Neitz Parasitological Research Station that is part of the Department of Animal Parasitology, Veterinary Institute, Rio de Janeiro Federal Rural University (UFRRJ), Brazil. The calves had no recent contact with any chemical acaricides. Female ticks were taken to the laboratory and washed in a 1% sodium hypochlorite solution for cuticle asepsis, after which they were rinsed in sterile distilled water and dried with sterile paper towels. Then, these females were submitted to the treatment with fungal suspensions.

2.2. Fungal isolates

The isolates Ma 959 of *M. anisopliae* s.l. and Bb 986 of *B. bassiana* were obtained from the Entomology Department of Luiz de Queiroz School of Agriculture, of the University of São Paulo (USP), Brazil. Fungal isolates were maintained on potato dextrose agar (PDA) (Merck) at $25 \pm 1\,^{\circ}C$ and RH $\geq$80% for 15 days. Thereafter, the fungi were kept at $4\,^{\circ}C$.

2.3. Preparation of fungal suspensions and formulations

2.3.1. Aqueous suspension

Fungi were cultivated on rice grains in polypropylene bags (Alves, 1998). The bags were inoculated with *M. anisopliae* s.l. or *B. bassiana* maintained as described above. After fungal growth, a portion of the rice was placed in a beaker (100 mL) and the conidia were suspended in a sterile aqueous Tween 80 solution (0.1%). After homogenization for conidial release, the suspensions were filtered through sterile gauze. The conidial concentration was quantified using a hemacytometer according to Alves (1998). The conidia aqueous 0.1% Tween 80 suspension was adjusted to 10^8 conidia/mL.

2.3.2. Oil formulations

The mineral oil proportions used to prepare the formulations were adapted from Angelo et al. (2010). The formulations contained 10, 15, or 20% sterile mineral oil (Vetec Química Fina Ltda., Rio de Janeiro, Brazil) and were prepared with the following proportions: (i) 89% of the aqueous suspension, 10% mineral oil and 1% Tween 80; (ii) 84% of the aqueous suspension, 15% mineral oil and 1% Tween 80; and (iii) 79% of the aqueous suspension, 20% of mineral oil and 1% Tween 80.

2.3.3. Conidial viability test

Conidial viability was determined by plating an aliquot of the aqueous suspension and each oil formulation on PDA medium plus 0.05% chloramphenicol followed by incubation at $25 \pm 1\,^{\circ}C$. Conidial germination was observed after 24 h and 48 h (Alves, 1998).

2.4. Bioassays

Three groups were formed in the bioassays with aqueous suspensions: a control group treated with sterile distilled water and 0.1% Tween 80, and two groups treated with *M. anisopliae* s.l. or *B. bassiana* suspensions. In the oil formulation bioassays, three groups were formed for each oil concentration (10, 15 or 20%): a control group, treated with sterile distilled water, 1% Tween 80 and the respective mineral oil concentration, and the two other oil based formulations of *M. anisopliae* s.l. or *B. bassiana*, with the appropriate proportions of water, mineral oil, and Tween 80. All bioassays were repeated twice.

2.4.1. Bioassay with engorged females

Twelve groups with 10 females of similar weight were formed. Each female was weighed, identified and submerged for 3 min in 1 mL of the test materials. Afterwards, the females were labeled, attached to Petri dishes and incubated at 27 ± 1 °C and RH ≥80%. The egg mass laid by each female was weighed daily and placed into individual test tubes. The eggs were then incubated at the same temperature and RH to allow the larvae to hatch. The following parameters were evaluated: hatching percentage; egg production index (EPI) (EPI = weight of egg mass/initial weigh of engorged female × 100) (Bennett, 1974); nutritional index (NI) (NI = weight of egg mass/initial weigh of engorged female − residual weight of engorged females × 100) (Bennett, 1974); and percentage of tick control (CP). The reproductive efficiency (RE) (RE = weight of egg mass/initial weigh of engorged female × hatching percentage) was used to calculate the CP (CP = mean RE of control group − mean RE of treated group/mean of control group × 100) (Drummond et al., 1971).

2.4.2. Bioassay with eggs and larvae

Engorged females were held in Petri dishes and incubated at 27 ± 1 °C and RH ≥80% for oviposition. The eggs laid until the tenth day of oviposition were used in the bioassay with eggs and larvae. Egg aliquots of 50 mg were placed in test tubes sealed with cotton plugs. Each group was formed by eight test tubes. In the egg bioassay, the eggs were immersed in 1 mL of conidial suspension or formulation for 3 min. The test tubes were then turned upside down to remove the excess conidial suspension/formulation through absorption by the cotton plug. The eggs were held at 27 ± 1 °C and RH ≥80%. The biological parameters evaluated were: incubation period; hatching period; and hatching percentage. The methodology used in the bioassay with larvae was similar to that used in the egg bioassay. Larval treatment was performed on the tenth day after total larval hatching. The tubes with hatching percentage below 95% were discarded. Mortality was evaluated every five days up to day 20 after treatment.

2.5. Fungi growth on host cuticle

Dead engorged females, eggs, and larvae from all treatment groups were incubated at 25 ± 1 °C and RH ≥80% to allow fungal growth and further evaluations of their characteristics (Samson and Evans, 1982).

2.6. Statistical analysis

The periods of egg incubation and hatching were assessed using analysis of variance (ANOVA) followed by the Student–Newman–Keuls test (SNK) with a significance level of 5% ($p \leq 0.05$). The hatching percentage, NI, EPI, and mortality percentage of larvae were assessed by the Kruskal–Wallis test followed by the Student's *t*-test with a significance level of 5% ($p \leq 0.05$) (Sampaio, 2002).

3. Results

3.1. Conidial viability

Aqueous conidial suspensions of *M. anisopliae* s.l. and *B. bassiana* were 100% viable within 24 h at 25 ± 1 °C, and RH ≥80% while oil-based conidial formulations were 100% viable after 48 h of incubation under the same conditions.

3.2. Fungi growth on host cuticle

R. microplus engorged females treated with *M. anisopliae* s.l. oil-based formulations including 15 and 20% mineral oil started showing fungal growth on the cuticle three days after treatment while fungal growth on the cuticle of females treated with the oil-based formulations at 10% commenced four days after treatment. Conspicuous fungal growth was noted initially on the cuticle of engorged females treated with *M. anisopliae* s.l. aqueous suspensions at six days post-treatment. Finally, engorged females treated with the aqueous suspension and oil-based formulations of *B. bassiana* showed fungal growth on their cuticle until 14 days after treatment.

3.3. Bioassays with engorged females

M. anisopliae s.l. oil-based formulations reduced 14 and 12 times the percentage of larval hatching as compared to the control groups and the group treated with the aqueous fungal suspension, respectively (Table 1). The NI and EPI of females treated with *M. anisopliae* s.l. oil-based formulations declined significantly ($p < 0.01$; degree of freedom [df] = 7) in comparison with the control groups. A significant reduction ($p < 0.05$; df = 7) of these biological parameters was also observed when the formulations with 15 and 20% oil were compared with the *M. anisopliae* s.l. aqueous suspension. However, no significant difference ($p < 0.05$; df = 7) was observed between the group treated with the *M. anisopliae* s.l. 10% oil formulation and the same aqueous fungal suspension (Table 1).

The NI was the only biological parameter statistically affected ($p < 0.05$; df = 7) by both the *B. bassiana* aqueous suspension and *B. bassiana* oil-based formulations (Table 1).

The formulations of *M. anisopliae* s.l. containing 10, 15, or 20% mineral oil showed control percentages of 58.1, 93.7 and 87.5%, respectively while the aqueous suspension produced 18.7% control (Table 2). From all the groups treated with *B. bassiana*, the 10 and 20% oil formulations showed the highest efficacy achieving 18.1 and 21.6% control, respectively (Table 2).

3.4. Bioassays with eggs

The *M. anisopliae* oil-based formulations significantly reduced ($p < 0.01$; df = 7) the incubation period, hatching period, and hatching percentage when compared to the other treatments (Table 3). The hatching percentage in the groups treated with the *M. anisopliae* s.l. oil-based formulations was reduced as much as 102.5 times. In contrast, the *M. anisopliae* s.l. aqueous suspension significantly reduced

Table 1
Effect on biological parameters of *Rhipicephalus microplus* engorged females immersed in formulations of *Metarhizium anisopliae* s.l., Ma 959 isolate or *Beauveria bassiana*, Bb 986 isolate containing water or various concentrations of mineral oil.

	Hatching percentage (%)	Egg production index	Nutrient index
Aqueous control group[a]	96.9 ± 9.46 a	61.62 ± 3.61 a	75.32 ± 5.67 a
Control with 10% mineral oil	92.9 ± 16.32 a	57.66 ± 9.41 ab	69.25 ± 7.84 a
Control with 15% mineral oil	97.0 ± 3.50 a	57.49 ± 8.10 ab	67.29 ± 7.73 ab
Control with 20% mineral oil	97.8 ± 4.13 a	58.73 ± 5.67 ab	67.57 ± 6.47 ab
Ma 959 aqueous suspension	88.4 ± 31.23 a	49.36 ± 18.05 bc	56.16 ± 20.20 bc
Ma 959 formulation with 10% mineral oil	51.0 ± 49.17 b	24.10 ± 24.22 cd	28.93 ± 28.41 cd
Ma 959 formulation with 15% mineral oil	7.0 ± 22.14 b	5.39 ± 17.05 d	6.23 ± 19.71 d
Ma 959 formulation with 20% mineral oil	17.0 ± 36.53 b	9.14 ± 18.02 d	11.36 ± 21.73 d
Aqueous control group	96.00 ± 7.04 A	54.73 ± 5.43 A	71.02 ± 7.63 A
Control with 10% mineral oil	92.38 ± 11.17 A	52.44 ± 12.51 A	67.58 ± 14.44 A
Control with 15% mineral oil	96.86 ± 7.16 A	55.22 ± 4.62 A	70.67 ± 5.17 A
Control with 20% mineral oil	91.43 ± 12.24 A	55.29 ± 4.35 A	71.78 ± 1.34 A
Bb 986 aqueous suspension	93.69 ± 10.33 A	54.82 ± 4.94 A	64.44 ± 7.89 B
Bb 986 formulation with 10% mineral oil	87.64 ± 15.97 A	47.67 ± 11.09 A	59.03 ± 10.76 B
Bb 986 formulation with 15% mineral oil	97.69 ± 3.28 A	56.26 ± 5.61 A	64.54 ± 6.60 B
Bb 986 formulation with 20% mineral oil	92.67 ± 7.44 A	51.61 ± 8.51 A	63.63 ± 8.65 B

[a] Refer to the text for details on the treatments tested. Experiments were conducted at 27 ± 1 °C and RH ≥80%. Means followed by the same lower letter in the same column do not differ statistically ($p \geq 0.05$), and means followed by the same capital letter in the same column do not differ statistically ($p \geq 0.05$). Means are followed by standard deviation of 10 replicates per bioassay. The bioassay was repeated twice.

Table 2
Control percentage of *Rhipicephalus microplus* engorged females immersed in formulations of *Metarhizium anisopliae* s.l., Ma 959 isolate or *Beauveria bassiana*, Bb 986 isolate containing water or various concentrations of mineral oil.

	Aqueous suspension[a]	Oil-based formulation		
		10%	15%	20%
Metarhizium anisopliae s.l., Ma 959 isolate	18.70	58.12	93.69	87.54
Beauveria bassiana, Bb 986 isolate	1.72	18.07	0.71	21.67

[a] Refer to the text for details on the formulations tested. Experiments were conducted at 27 ± 1 °C and RH ≥80%. The bioassay was repeated twice.

($p < 0.05$; df = 7) the hatching period and hatching percentage as compared to the aqueous control group (Table 3). Neither the *B. bassiana* aqueous suspension nor the oil-based formulations significantly affected ($p < 0.05$; df = 7) the incubation period when *R. microplus* eggs were exposed to that fungus (Table 3). A significant reduction ($p < 0.05$; df = 7) in the hatching period appeared to be related with exposure to the *B. bassiana* aqueous suspension and *B. bassiana* oil-based formulations when compared with the results for the aqueous control group; however, the effect was not observed when results from the same treatments were compared to data obtained for the oil-based control groups (Table 3). The *B. bassiana* aqueous suspension caused no change in the percentage of larvae hatching. By contrast, the oil-based formulations significantly reduced

Table 3
Effect on biological parameters of *Rhipicephalus microplus* eggs immersed in formulations of *Metarhizium anisopliae* s.l., Ma 959 isolate or *Beauveria bassiana*, Bb 986 isolate containing water or various concentrations of mineral oil.

	Incubation period (days)	Hatching period (days)	Hatching percentage (%)
Aqueous control group[a]	21.88 ± 0.34 a	6.88 ± 0.96 a	96.38 ± 1.86 a
Control with 10% mineral oil	19.38 ± 6.79 a	6.63 ± 0.89 ab	96.00 ± 2.88 a
Control with 15% mineral oil	21.86 ± 0.36 a	6.71 ± 0.91 ab	93.57 ± 2.34 ab
Control with 20% mineral oil	21.88 ± 0.34 a	6.38 ± 0.72 ab	93.38 ± 9.32 a
Ma 959 aqueous suspension	21.81 ± 0.40 a	5.63 ± 1.20 b	63.06 ± 31.64 b
Ma 959 formulation with 10% mineral oil	13.81 ± 11.05 b	2.13 ± 2.06 c	2.19 ± 2.32 c
Ma 959 formulation with 15% mineral oil	13.94 ± 11.16 b	1.50 ± 1.71 c	1.38 ± 1.86 c
Ma 959 formulation with 20% mineral oil	14.00 ± 11.21 b	1.19 ± 1.28 c	0.94 ± 1.24 c
Aqueous control group	23.00 ± 0.00 A	9.00 ± 1.03 A	97.75 ± 2.24 A
Control with 10% mineral oil	23.00 ± 0.00 A	8.25 ± 0.68 AB	98.38 ± 2.06 A
Control with 15% mineral oil	23.00 ± 0.00 A	8.50 ± 0.89 AB	93.50 ± 13.24 A
Control with 20% mineral oil	23.00 ± 0.00 A	8.50 ± 0.89 AB	95.00 ± 4.47 A
Bb 986 aqueous suspension	22.94 ± 0.25 A	7.25 ± 1.53 B	91.69 ± 8.93 A
Bb 986 formulation with 10% mineral oil	23.13 ± 0.34 A	7.31 ± 2.50 B	27.00 ± 21.43 B
Bb 986 formulation with 15% mineral oil	23.00 ± 0.00 A	7.44 ± 1.21 B	39.69 ± 20.93 B
Bb 986 formulation with 20% mineral oil	22.88 ± 0.50 A	7.31 ± 2.09 B	47.50 ± 23.87 B

[a] Refer to the text for details on the treatments tested. Experiments were conducted at 27 ± 1 °C and RH ≥80%. Means followed by the same lower letter in the same column do not differ statistically ($p \geq 0.05$), and means followed by the same capital letter in the same column do not differ statistically ($p \geq 0.05$). Means are followed by standard deviation of 10 replicates per bioassay. The bioassay was repeated twice.

($p < 0.05$; df = 7) this parameter (27–47.5%) when compared to the control groups (93.5–98.4%) (Table 3).

3.5. Bioassays with larvae

The oil-based formulations of *M. anisopliae* s.l. and *B. bassiana* were more efficient in controlling *R. microplus* larvae as compared to the aqueous suspensions (Fig. 1). The mean mortality rate for larvae treated with *M. anisopliae* s.l. oil-based formulations was close to nearly 100% on the fifth day after treatment while the aqueous fungal suspension caused 2.0% larval mortality (Fig. 1A). *B. bassiana* treatments started to cause noticeable larval mortality the tenth day after treatment (Fig. 1D). Mean larval mortality with the *B. bassiana* oil-based formulations was close to 100% at 20 days after treatment while the aqueous suspension caused only 27.4% larval mortality (Fig. 1F).

The control group receiving the control treatment containing 20% mineral oil showed average mortality rates of 28.1, 40.9, and 41.3% on the 15, 20, and 25th days after treatment, respectively (Fig. 1E, F and G). A significant larval mortality rate was observed in the control formulation with 10 or 15% oil on the 20 and 25th days after treatment (Fig. 1F and G). No larval mortality was observed in the control group treated with water.

From the results obtained in the bioassays with engorged females, eggs and larvae, it was possible to evaluate the cumulative effect of the treatments with conidia aqueous suspension or 20% mineral oil based formulation of *M. anisopliae* s.l. Ma 959 isolate and *B. bassiana* Bb 986 isolate on *R. microplus* population (Table 4). It is important to note that the treatments of each tick developmental stage was carried out separately, accordingly the cumulative effect is hypothetical, assuming that the combined treatments would be equivalent.

4. Discussion

The present study showed that the addition of mineral oil to formulations of *M. anisopliae*, isolate Ma 959, and *B. bassiana*, isolate Bb 986, enhanced the pathogenic effects of these fungi against different stages of *R. microplus.* Mineral and vegetable oils are used in formulations as adjuvants to protect conidia and to maximize fungal performance (Alves, 1998). In the present study, the pathogenic activity of *M. anisopliae* s.l. against developmental stages of *R. microplus* was enhanced by formulating the fungus with mineral oil. Our findings are in agreement with the data reported by Angelo et al. (2010). They evaluated a 15% oil-based formulation of *Lecanicillium lecanii* and found significant differences on all *R. microplus* tick stages. The cuticle of most arthropods has hydrophobic characteristics as do some entomopathogenic conidia isolates (Prior et al., 1988). Thus, conidia suspended in water tend to show hampered adherence to the host cuticle. In the present study, the oil-based formulations of *M. anisopliae* s.l. and *B. bassiana* were more effective on *R. microplus* than the aqueous suspensions. These results can be explained by the increased adherence of conidia on the cuticle provided by the oil adjuvant present in the formulations, which has a chitinophilic property. This property of oil increases the affinity of hydrophobic conidia for the tick's cuticle, which enhances the infectivity and consequently the pathogenicity of fungal isolates (Prior et al., 1988). The chitinophilic characteristic of oil likely accelerated the infection process of the fungi used in the present study since the oil formulation caused faster damage to engorged females, eggs, and larvae than the water based fungi suspensions.

Fernandes et al. (2011) evaluated the virulence of 60 *Beauveria* spp. isolates on the immature stages of *R. microplus* and noted that larvae from different populations showed different susceptibility to the isolates tested. Moreover, differences in the susceptibility of two distinct *R. microplus* populations infected with *B. bassiana* and *M. anisopliae* was observed (unpublished data). Thus, the difference between results presented here using *R. microplus* engorged females infected with *B. bassiana* isolate 986 and those reported in the literature may be related to variations in susceptibility of distinct tick populations to the same fungal isolate.

Kaaya and Hassan (2000) observed significant changes in all developmental stages of *Amblyomma variegatum* and *Rhipicephalus appendiculatus* treated with *M. anisopliae* and *B. bassiana* formulations containing 15% peanut oil; however, they documented that larvae were most susceptible by showing nearly 100% mortality against that stage. In the present study, *R. microplus* eggs and larvae were more susceptible than engorged females when treated with *B. bassiana* oily formulations. Additionally, *B. bassiana* oil formulations were clearly better at controlling eggs and larvae as compared to the aqueous fungal suspension, since only the groups treated with the oil-based formulations showed significant changes.

It is worth noting the toxic effect of mineral oil alone on *R. microplus* larvae that increased with time. However, mineral oil showed no toxic effect on *R. microplus* eggs and engorged females. We hypothesize that there are differences in susceptibility to mineral oil between life stages of *R. microplus*. Abdel-Shafy and Soliman (2004) reported similar observations while studying the toxic effect of five essential oils against different life stages of *Rhipicephalus annulatus.* These authors found that *R. annulatus* larvae were most susceptible followed in decreasing susceptibility by the egg stage, and engorged females.

Conidia formulations have the potential to be an important biological tool for tick control. Features required to develop a useful formulation include the duration of the viability, virulence, and efficacy of acaripathogenic fungi under field, and the incorporation of ingredients that promote adherence of conidia on the tick surface and elements providing protection against adverse environmental conditions. Entomopathogenic fungi formulations have been studied intensely and some have been used to control agricultural pests effectively (Prior et al., 1988; Bateman et al., 1993; Batista Filho et al., 1994; Alves et al., 1996). Experience in the development of entomopathogenic fungi used to control agricultural pest can be applied to prepare fungal formulations for tick control. However, considerable research is required to develop formulations that are effective for tick control (Samish et al., 2004). The experiments reported here documented that mineral oil was effective as an adjuvant in formulations of *M. anisopliae*, Ma 959, and *B.*

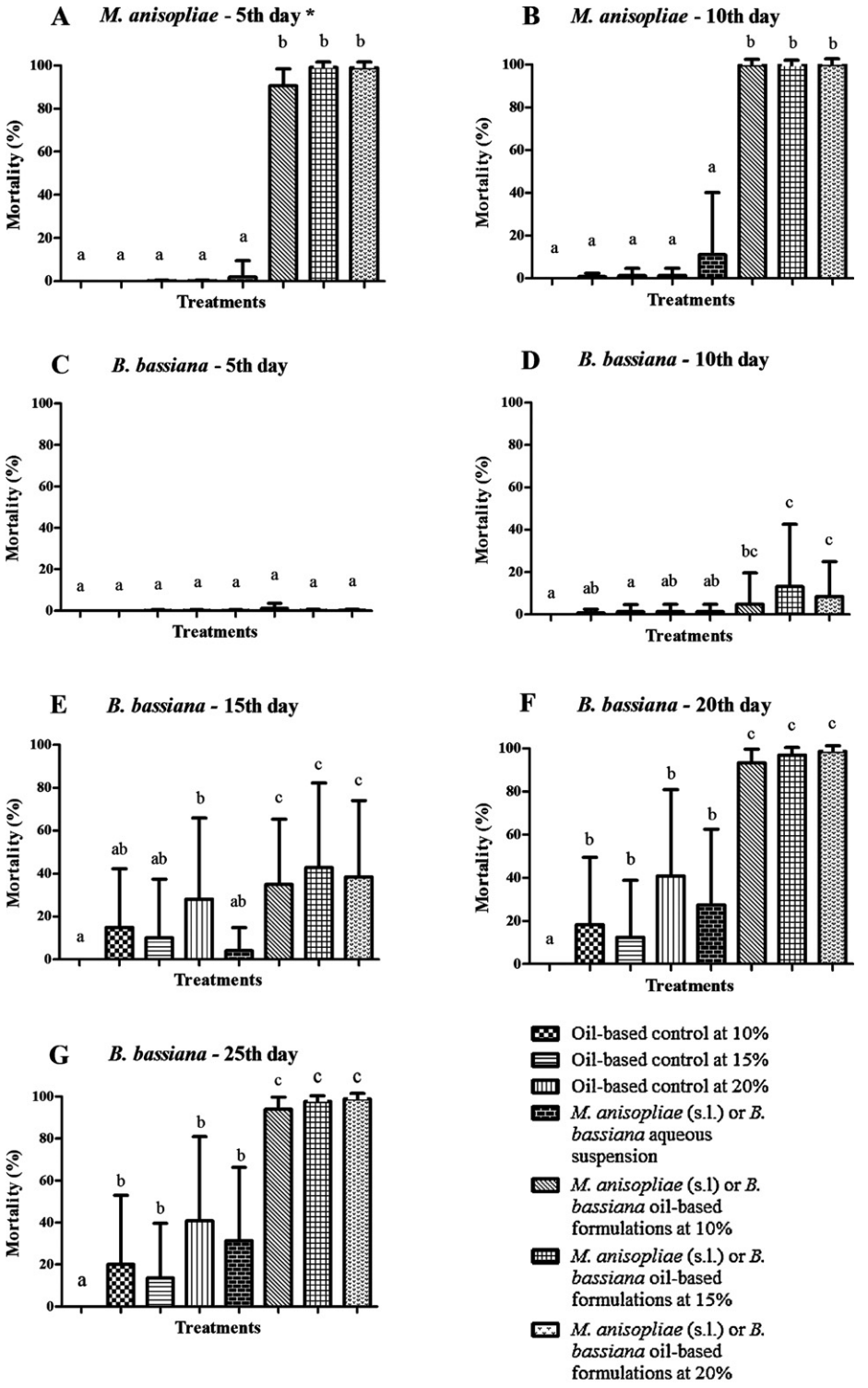

Fig. 1. Mortality of *Rhipicephalus microplus* larvae treated with aqueous conidial suspensions or mineral oil-based formulations of *Metarhizium anisopliae* s.l. Ma 959 isolate or *Beauveria bassiana* Bb 986 isolate. Mortality was observed at 5-day intervals. (A and B) 5th and 10th days, respectively, after treatment with *Metarhizium anisopliae* s.l., Ma 959 isolate; (C–G) 5th, 10th, 15th, 20th and 25th days, respectively, after treatment with *Beauveria bassiana*, Bb 986 isolate. The experiments were conducted at $27 \pm 1\,^{\circ}C$ and RH $\geq$80%. Means are followed by standard deviation of 10 replicates per bioassay. The bioassay was repeated twice. No bar for the water control is shown because it caused insignificant larval mortality.

Table 4
Estimated cumulative effect on engorged females, eggs and larvae of *Rhipicephalus microplus* after immersion in formulations of *Metarhizium anisopliae* s.l., Ma 959 isolate or *Beauveria bassiana* Bb 986 isolate containing water or 20% mineral oil.

Treatments		1st treatment	2nd treatment	3rd treatment
		Females	Eggs	Larvae with 10 days
Ma 959 aqueous suspension[a]	Total number of individuals	100[b]	243,000[c]	153,235
	Percentage of control (%)	18.70[d]	36.94[e]	11.06[f]
	Number of survivors[g]	81	153,235	136,287
Ma 959 formulation with 20% mineral oil	Total number of individuals	100	36,000	338
	Percentage of control (%)	87.54	99.06	100
	Number of survivors	12	338	0
Bb 986 aqueous suspension	Total number of individuals	100	294,840	270,338
	Percentage of control (%)	1.72	8.31	2.75
	Number of survivors	98	270,338	262,903
Bb 986 formulation with 20% mineral oil	Total number of individuals	100	234,990	111,620
	Percentage of control (%)	21.67	52.50	17.00
	Number of survivors	78	111,620	92,640

[a] Refer to the text for details on the treatments tested.
[b] Hypothetical number.
[c] Estimated number of eggs, assuming that each *Rhipicephalus microplus* female has an oviposition average of 3000 eggs (Furlong et al., 2004).
[d] Refer to the text (Section 2.4.1) for details on how the calculation was performed.
[e] Percentage of unviable eggs in the bioassay with eggs.
[f] Mortality percentage of larvae in the bioassay with larvae.
[g] Difference between the number of individuals and the control percentage.

bassiana, Bb 986, for the control of *R. microplus* under laboratory conditions. Field testing of oil-based formulations is required to assess the practical utility of entomopathogenic fungi for the integrated control of *R. microplus*.

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