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Comparative Efficacy of *Metarhizium anisopliae* and Amitraz in Control of *Rhipicephalus decoloratus* on Cattle under Field Conditions in Kenya

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ABSTRACT

Ticks and tick-borne diseases represent a major constraint to cattle production in Kenya. The use of entomopathogenic fungi is an important and promising component of bio-control agents for controlling devastating tick species which include *Rhipicephalus appendiculatus*, *Amblyomma variegatum* and *Rhipicephalus* (*Boophilus*) *decoloratus*. The aim of this study was to evaluate the efficacies of the fungus *Metarhizium anisopliae* ICIPE 7 and the acaricide, amitraz in controlling *R. decoloratus* in naturally infested cattle under field conditions. This study was undertaken during the short rains (October to November 2014) in Narok County, Kenya. Twenty animals (steers and heifers) were randomly allocated to four treatment groups; Control (water + 0.05% Triton X–100 + 15% canola oil); *M. anisopliae* ICIPE 7 isolate alone (1×10^9 conidia ml^{-1}); Amitraz alone at recommended concentration of 0.2% and combination of *M. anisopliae* ICIPE 7 isolate (1×10^8 conidia ml^{-1}) and amitraz (0.1%).

The overall *R. decoloratus* load was high at the start of treatment (day 0) in all the four groups and was highest along the dewlap followed by the back region while the head region had no blue ticks. All the three treatments reduced tick counts significantly on days 7, 14, 21 and 28 relative to the control group with percentage efficacy on day 28 post treatment being 69.2% for ICIPE 7, 67.1% for ICIPE 7 plus amitraz and 94.9% for amitraz alone. Treatments with *M. anisopliae* ICIPE 7 alone and *M. anisopliae* ICIPE 7 isolate plus amitraz attained mortality of 93.5% compared to 2.3% of the control group and all fungal infected ticks developed mycosis. This study has demonstrated that *M. anisopliae* ICIPE 7 isolate was as effective as amitraz and when combined as a spray reduced the tick population significantly. This association could be used as a tool for integrated control of *R. decoloratus*.

1. INTRODUCTION

Ticks are one of the leading vectors of pathogens which cause diseases of economic importance to the livestock industry in Africa (Minjauw and McLeod, 2003; Walker et al., 2003). Tick infestations may cause paralysis or toxicosis (they inject a toxin into the animals while feeding on them) and physical damage (tick worry, loss of blood, weight loss, direct injury to hides) to livestock. In East Africa, *Rhipicephalus* (*Boophilus*) *decoloratus* is among the common devastating tick species and their main host is cattle (Walker et al., 2003). *Rhipicephalus decoloratus* transmits the protozoan *Babesia bigemina*, the cause of babesiosis (red water fever) in cattle, as well as the rickettsia, *Anaplasma marginale* which causes anaplasmosis. These tick-borne diseases (TBD = delete) lead to high production losses and death in cattle (Ocaido et al., 2009) and due to lack of successful vaccination programs against these infections, the only alternatives are to treat the infested animals and to control the tick vectors.

Control of ticks in Kenya relies extensively on chemical acaricides but frequent uses have disadvantages such as tick resistance and ecological pollution (Raynal et al., 2013). Tick resistance may be shared among different acaricides which have the same mode of action (Vudriko et al., 2016).

Amitraz, a formamidine acaricide, plays an important role in the control of ticks that infest cattle (Jonsson and Hope, 2007), dogs (Kumar et al., 2001) and wild animals (Pound et al., 2000), as well as parasitic mites of honey bee (Floris et al., 2001). Effectiveness of amitraz is through its toxic effects on a receptor for a neuromodulator, octopamine (Dudai et al., 1987). Therefore, it leads to overexcitation and consequently paralysis and death in arthropods. Amitraz played a critical role in the control of the southern cattle tick, *R. microplus* in countries where resistance to both organophosphate and pyrethroid pesticides reached unacceptable levels (George et al., 1998). However, tick resistance to amitraz has been detected even though the exact mechanism of resistance is not yet known (Mendes et al., 2013; Muyobela et al., 2015).

Tick resistance to acaricides of different groups, food safety concerns and environmental awareness have led to exploration of other ways of controlling ticks such as use of natural methods (Chandler et al., 2000; Hussain et al., 2014).

Biological control of ticks using naturally occurring entomopathogenic fungi is being considered as one of the alternatives (Maniania et al., 2007; Murigu et al., 2016). Entomopathogenic fungi have broad host spectrum and can penetrate their host cuticle directly unlike other entomopathogens which require to be ingested first (Maniania et al., 2007). Most entomopathogenic fungi belong to the orders Entomophthorales and Hypocreales and currently, approximately 51 genera have been recognized with more than 900 fungal species (Maniania et al., 2007; Hussain et al., 2014). Although they have shown good potential in the laboratory, results from the field have been generally inconsistent (Alonso-Diaz et al., 2007; Kaaya et al., 2011).

A number of mycoinsecticides have been developed and commercialized in different parts of the world. Most of these products were developed based on *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosoroseus* propagules. They are currently available in North and South American countries, Europe and Asia, with few in Middle East and Africa (Hussain et al., 2014; Maina et al., 2018). Biopesticides in combination with *M. anisopliae* have been seen as a viable option for commercial purposes in tick control (Faria and Wraight, 2007). An incorporated approach in managing tick resistance to acaricides using a combination of

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acaricides and entomopathogenic fungi could tackle these setbacks but this should be explored first before they are combined. For example, amitraz was previously reported to hinder the growth of *B. bassiana* (Alizadeh et al., 2007). However, other studies have demonstrated that amitraz in 1.6 ppm and 40 ppm enhanced vegetative growth of *M. anisopliae* but 200 ppm concentration reduced sporulation by 50% (Schumacher and Poehling, 2012). In a recent study in Kenya, *M. anisopliae* ICIPE 7 isolate was found to be effective against *R. decoloratus* under laboratory and field conditions (Murigu et al., 2016). The aim of the current study was to determine the comparative efficacy of *M. anisopliae* ICIPE 7 and amitraz in controlling *R. decoloratus* in naturally infested cattle under field conditions.

2. MATERIALS AND METHODS

2.1 Study area

The study was carried out in Transmara West Sub-county of Narok County, Kenya. The county lies within the Great Rift Valley and climatic conditions are strongly influenced by altitude and physical features. Temperatures range from 20^{o}C (January-March) to 10^{o}C (June-September) with an average of 18^{o}C. Rainfalls are bi-modal with long rains being experienced between the months of February and June while the short rains are experienced between August and November. Rainfall ranges from 2,500 mm in wet season to 500 mm during the dry season. The two dominant vegetation types in the county include forest land in the Mau area and grasslands and shrubs in the lowland areas of Suswa, Osupuko**,** Loita as well as the Mara sections in Transmara (Anonymous, 2018).

The trial was carried out at a private farm situated about 12 km from Kilgoris town: 1^{o}00’S, 34^{o}53’E, 1716 m above sea level (GPS reading). Approximately 0.81 hectares paddock was the study site although animals grazed anywhere freely in the 52.6 hectares that constituted the farm. The red oat grass, *Themeda triandra* was the predominant vegetation. The grass was between 5 cm and 25 cm in height. The trial was conducted from October to November 2014 during the rainy season. High abundance of *R. decoloratus, R. appendiculatus* and *A. variegatum* characterized this period (Walker et al 2003). During the trial period, the prevailing climatic conditions of the area were recorded. Ethical clearance was obtained from the Directorate of Veterinary Services **(DVS = delete)**, Nairobi, for this study.

2.2 Evaluation of efficacy of *Metarhizium anisopliae* ICIPE 7 and amitraz against *Rhipicephalus decoloratus*

2.2.1 Test acaricide

The synthetic acaricide, used in this trial was amitraz commercialized under the brand name Triatix®, Cooper K-Brands Ltd formulated in 12.5% emulsion concentration and 87.5% inert ingredients and was obtained from Coopers K-Brands Limited, Nairobi. The acaricide was used at the recommended field usage rate of 250 ppm. In the study area, *R. decoloratus* resistance to amitraz was confirmed through molecular techniques by Hatta et al (2013).

2.2.2 Fungal isolate

The *M. anisopliae* ICIPE 7 isolate was obtained from the ICIPE's Arthropod Germplasm Centre and was mass produced as detailed by Nana et al (2012). This isolate was recently found to be compatible with amitraz under *in-vitro* conditions (Murigu et al., 2016).

2.2.3 Test animals

A mixture of 25 steers and heifers were selected based on their weight and age (approximately 150-175 kg; 1.5 years old) from the main herd of cattle of Maasai Zebu and Sahiwal crosses. These animals were dewormed and provided with mineral supplements as well as being provided with diagnostic and clinical services during the pre-trial, trial and one month post-trial periods. To become infested with ticks, they were allowed to graze for two weeks on pasture and were free from chemical interventions of any kind to prevent tick infestation. Total whole body tick counts that included *R. appendiculatus, R. decoloratus* and *A. variegatum* for the pre-trial animals were done on the first day of the study. The counts were used to rank the animals from highest to lowest number of ticks. A homogenous sample of 20 cattle was selected from the initial group of twenty five cattle having attained the mandatory 150-250 ticks per animal as per the trial protocol. Five animals were eliminated because three had very low tick counts and the other two, one was pregnant and the other was very aggressive.

A stratified randomized complete block design method (Olsson 2018) was used to allocate the twenty cattle to the four treatment groups; Control (water + 0.05% Triton X-100 + 15% canola oil; the former is used to break the surface tension of water and ensure that spores are evenly distributed in water as a suspension while, the latter is used to maintain spore viability on the animal skin); *M. anisopliae* ICIPE 7 isolate alone (1×10^9 conidia ml^{-1}); Amitraz alone at recommended concentration of 0.2% and combination of *M. anisopliae* ICIPE 7 isolate (1×10^8 conidia ml^{-1}) and amitraz (0.1%). The *M. anisopliae* conidia were enumerated as described by Goettel and Inglis (1997). Only *R. decoloratus* were counted during the trial period.

2.2.4 Application of treatments

The four treatment groups comprising five animals per group were brought to the crush and sprayed with their respective treatments using a knapsack sprayer. Treatments were applied once a week for four weeks. Animals were sprayed starting with the head including the ears, lower parts moving to the flank, back and belly then front legs and axillae for both sides before finishing with tail switch. By the end of the spray treatment all cattle were thoroughly wetted.

2.2.5 Assessment of efficacy of treatments

After each spray application, whole body tick counts of naturally infested cattle were conducted on days 0, 3, 5 and 7. Ticks were counted on the 3 regions on each animal. Head (head, ear, neck, the dewlap to the point of the sternum), shoulder (outer and inner foreleg from point of the sternum back to the start of the fore belly) and the back (ribs, tail and tail switch, udder and scrotum and hind legs) with much attention on blue ticks predilection sites like back, upper legs, neck, shoulder, dewlap and belly (Walker et al., 2003). The treatment groups were grazed separately to avoid rub off with the other groups and from the neighboring animals throughout the trial period.

The proportion of ticks infected with the fungus was determined by, collecting 8-10 ticks from each animal in fungus-treated groups and control. They were placed in sterile a Petri dish and maintained in room temperature (25–27 ◦C) for 10 days. Mortality was recorded and dead ticks removed and put in a petri dish which had damp filter paper for the fungus to growth on the surface of the dead ticks.

The effectiveness of the different treatments was established as the percentage reduction of the population of ticks by comparing with the negative control population (Bittencourt et al., 2003).

Percentage efficacy (%) =

$$\frac{(\text{Average tick population in negative group}) - (\text{Average tick population in treatment group})}{\text{Average tick population in negative group}} \times 100$$

2.3 Data analysis

Percentage mortality in controls was corrected using Abbott's formula (Abbott, 1925) and standardized using arcsine transformation. Analysis of variance (ANOVA) was done at significance level of 95% using PROC GLM. Means were separated as a post-ANOVA procedure (p<0.05) using Student-Newman-Keuls analysis. The test values of lethal concentration and lethal time were calculated using the probit analysis for data correlation (Throne et al., 1995) and compared using ANOVA ($p<0.05$) and separating their means using Student-Newman-Keuls test.

3. RESULTS

3.1 Climatic data

During the study period, the mean maximum temperature of the trial site was 22.7°C with mean minimum temperature being 13.8°C. Relative humidity (RH) was 86.5% 06.00 am relative to 57.3% RH at noon and mean rainfall recorded was 37.2 mm.

3.2 Mean total tick counts per treatment

The mean total tick (*R. decoloratus*) counts after weekly application of treatments from the predilection sites are shown in Fig. 1. The overall tick counts were high in dewlap region followed by the back region. The head region had no *R. decoloratus* ticks.

3.3 Mean tick counts from three body regions

The mean tick counts for the three body regions (head, dewlap, back) per treatment are as shown in Fig. 2. Counts were high on day zero (0) in all the four treatment groups and decreased on day 7 with the dewlap having high tick loads in the control followed by ICIPE 7 alone and ICIPE 7 plus amitraz. Tick counts increased on day 14 with the same trend in treatments but the counts decreased up to day 28 except in control which maintained high tick counts. Amitraz group maintained low tick counts throughout the study period (Fig. 2).

3.4 Overall mean tick counts on animal body after weekly application of different treatments

Before application of treatments, the number of ticks was considerably high in all the treatment groups and varied between 71.8 and 125.6 and was not significantly different ($F_{3, 8}=0.4986$; $P=0.6885$).
Application of treatments significantly reduced the number of ticks on all the sampling days: day 7 ($F_{3, 8}=3.917$; $P=0.0284$), day 14 ($F_{3, 8}=9.090$; $P=0.0275$), day 21 ($F_{3, 8}=37.971$; $P=0.0001$) and day 28 ($F_{3, 8}=8.170$; $P=0.0016$) as compared to the control group. No significant differences were observed between the treatments ($F_{3, 8}=3.917$; $P=0.0284$), except on day 14 when tick reduction was highest in amitraz alone treatment (Table 1). Percentage efficacy of different treatments is as shown in Fig. 3. On day 28 post treatment, tick reduction was 69.2% for ICIPE 7, 67.1% for ICIPE 7 plus amitraz and 94.9% for amitraz alone (Fig. 3).

Table (1). Mean and standard error of means (SEM) *Rhipicephalus decoloratus* counts of treatment groups from day zero to day twenty eight of study

Treatment group	**Day of sampling**				
	1	7	14	21	28
Control	105±32.9	36.2±23.4	57.2±13.3	63.6±4.8	47.4±7.9
ICIPE 7	71.8±19	15.4±3.2	40.4±21.9	18.4±3.3	8.2±7
ICIPE 7 + Amitraz	125.6±59.6	14±8.2	37.2±9.1	21±4.8	14.6±8.1
Amitraz	75.4±15.7	9.4±4.8	12.6±3.8	15.6±2.4	2.4±1.6
P value	0.6889	0.4742	0.1886	<0.0001	0.001
F value	0.4979	0.8758	1.795	32.975	9.023

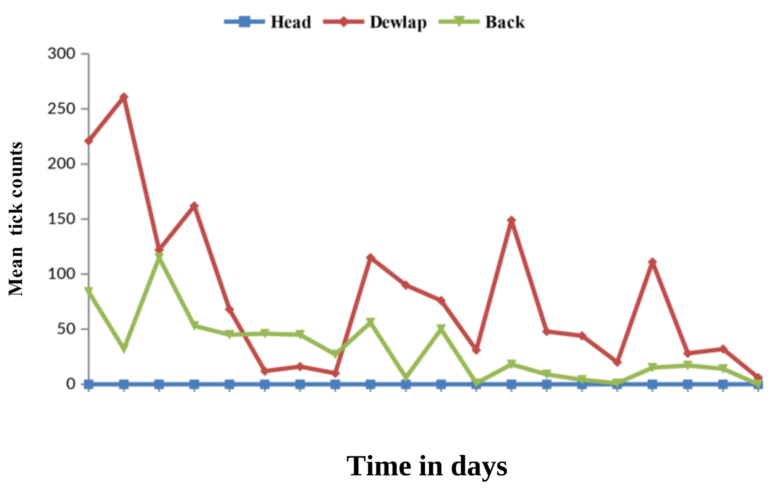

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Fig. 1: Mean total tick counts per treatment

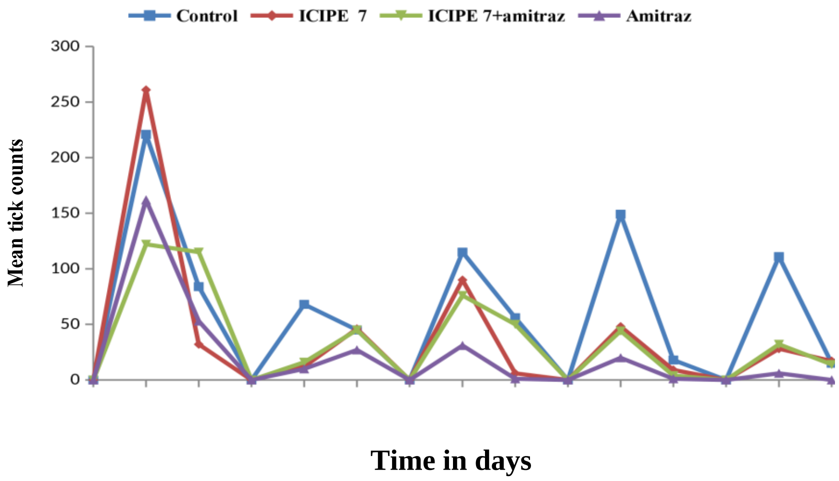

Fig. 2: Mean tick counts in three body regions per treatment

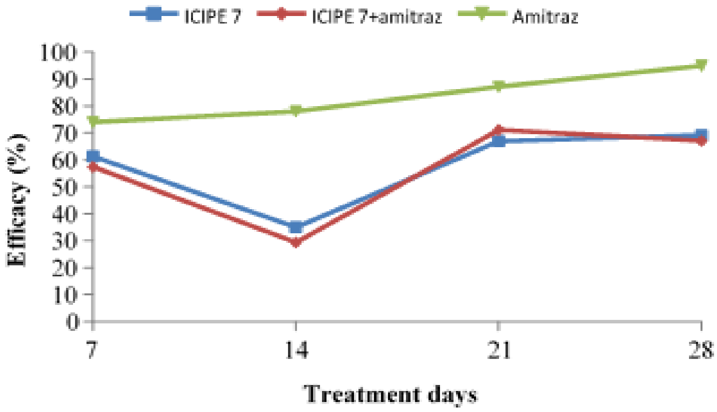

Fig. 3: Percentage efficacy of different treatments

3.5 Mean percentage mortality of *Rhipicephalus decoloratus* following treatment with *Metarhizium anisopliae* ICIPE 7 and amitraz

Mortality varied between 89% (ICIPE 7) and 98% (ICIPE 7 + Amitraz) in fungus treatment groups compared to 2.3% in the control group (Table 2). All dead blue ticks from fungus alone and combination fungus-amitraz treatments developed mycosis.

Table (2). Mean percentage mortality (± SD) of *Rhipicephalus decoloratus* following treatment with *Metarhizium anisopliae* ICIPE 7 and amitraz

Treatment group	Mortality (%) (± SD)
Control	2.3±0.4^{a*}
ICIPE 7	89±3.6^{b}
ICIPE 7 + Amitraz	98±2.4^{b}

*Same letters (a, b) indicates no significance

4. DISCUSSION

In the present field study, the overall *R. decoloratus* load was high at the start of treatment (day 0) in all the four study groups and was highest along the sides of the body, shoulders, neck and dewlap which are preferred sites of attachment for this tick (Huruma et al., 2015). weekly (for four weeks) treatment application significantly reduced one-host *R. decoloratus* population by 69.2% for *M. anisopliae* ICIPE 7 isolate alone, by 67.1% for ICIPE 7 plus amitraz, and by 94.9% for amitraz alone relative to the control. In earlier studies, Correia et al. (1998) reported no effect of *M. anisopliae* on *R. microplus* on cattle contrary to present findings while Castro et al. (1997) reported more than 50% tick reduction of *R. microplus* after spraying the animals once. On the other hand, 90% reduction was demonstrated in *R. microplus* after applying *B. bassiana* and *M. anisopliae* for five weeks (Rijo-Camacho, 1996). *Rhipicephalus microplus* reduction was also demonstrated on cattle treated with two isolates of *M. anisopliae* after treatment for 3 weeks (Polar et al., 2005) while a reduction of 40 to 91.2% was achieved by weekly application of *M. anisopliae* by Alonso-Diaz et al. (2007). Kaaya et al. (2011) on the other hand demonstrated 83% reduction of *R. decoloratus* when *M. anisopliae* (1×10^8 conidia/ml) was in oil formulation.

The field trial was done in the middle of the day and this could have affected the conidial germination due to the UV-A, UV-B radiation and heat from the sun (Francisco et al., 2008). Higher percentage efficacies were attained when similar experiment was performed in late afternoon (Alonso-Díaz et al 2007; Anelise et al., 2015). The present study was done with temperatures of 22.7^0C and the RH was 57.3% which was within the normal range (RH: 55% 75% and temperature: 25^0C - 27^0C) for *Metarhizium* spp. growth (Michalak et al., 2007).

Combination of *M. anisopliae* and amitraz had lower efficacy (67.1%) as compared to amitraz alone (94.9%) as the amitraz concentration added to the *M. anisopliae* was lower than the manufacturer's recommended concentration. Other field studies registered high efficacies with the same combination but amitraz concentration was equivalent to the manufacturers' recommendation and was combined with *M. anisopliae* at 1×10^8 conidia ml^{-1} (Anelise et al., 2015). During the treatment period, animals were re-infested as the grazing fields were heavily infested with ticks. Three to five days after application of treatments, the tick population went down but on day seven, more new ticks were observed on cattle. Bahiense et al. (2006) demonstrated that treatment of *R. microplus in-vitro* with combination of the entomopathogenic fungus (EPF) and acaricide (deltamethrin) at recommended doses improved the efficacy of the entomopathogen. Thus, integrating a compatible repellent with an EPF is indicated.

The mean percentage mortality of *R. decoloratus* after treatment with ICIPE 7 and amitraz was 93.5% as compared to the control which attained 2.3% mortality with all dead ticks developing mycosis. In previous studies, ticks were shown to die after application of fungi with mycosis being observed indicating the viability of conidia in spite of the effects of the environment on the host (Polar *et al.*, 2008). Mycosis was also observed on surfaces of treated ticks with the fungus recovered from infected tick cadavers (Murigu et al., 2016).

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5. CONCLUSIONS

In conclusion, the present study has demonstrated that *M. anisopliae* ICIPE 7 isolate was as effective as amitraz and could be used for the management of amitraz-susceptible and resistant strains of *R. decoloratus.* This isolate when combined with amitraz as a spray reduced the tick population significantly and this association can be used as a tool for integrated control of *R. decoloratus* and other tick species on cattle. Further studies should be undertaken to improve this isolate as a mycoacaricide.

6. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

7. ACKNOWLEDGEMENTS

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