Development of formulations and delivery systems to control economically important ticks with entomopathogenic fungi

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Declaration

The experimental material and results described in this thesis is my original work (except where the input of others is acknowledged), conducted in the Programme for Phytomedicine, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, and at the International Centre for Insect Physiology and Ecology, Nairobi, Kenya. This work has not been submitted in any other form to any other University or academic institution. I declare the above statement to be true.

Signed: ……………………………………

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Date: …………………………………….

Dedication

To my family

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List of Abbreviations used

AAAP Attraction-Aggregation-Attachment Pheromone

CO_2 Carbon dioxide

DCM Dichloromethane

DRC Democratic Republic of Congo

ICIPE International Centre of Insect Physiology and Ecology

LC_{50} Lethal concentration needed to kill fifty percent (50%) of ticks

SP Direct inoculation and indirect substrate contamination of ticks for duration of 0-5 minutes

SS Direct inoculation of ticks and indirect substrate contamination for duration of 12 hours (SS)

SW Indirect inoculation by substrate contamination for a 12-hour duration

ULV Ultra low volume

UV Ultraviolet

Publications from this thesis

Published paper

F. Nchu, N.K. Maniania, A. Touré, A. Hassanali, J.N. Eloff

The use of a semiochemical bait to enhance exposure of *Amblyomma variegatum* (Acari: Ixodidae) to *Metarhizium anisopliae* (Ascomycota: Hypocreales). Veterinary Parasitology 160: 279-284

Manuscript in preparation

F. Nchu, N.K. Maniania, A. Touré, A. Hassanali, J.N. Eloff

Performance of *Metarhizium anisopliae*-treated semiochemical-baited trap in reducing *Amblyomma variegatum* population in the field. Submitted to Veterinary Parasitology.

F. Nchu, N.K. Maniania, A. Hassanali, J.N. Eloff

Laboratory screening of entomopathogenic fungi against *Rhipicephalus pulchellus* Gerstäcker (Acari: Ixodidae).

F. Nchu, N.K. Maniania, A. Touré, A. Hassanali, J.N. Eloff

Optimizing modes of infection of *Rhipicephalus* ticks (Acari: Ixodidae) with mitosporic entomopathogenic fungus in the laboratory. Submitted to Journal of Experimental and Applied Acarology

Published book chapter

Maniania N.K., Nchu, F. and Ekesi, S. 2007. Fungal pathogen for biocontrol of ticks. In Ekesi S. and Maniania N. K. (eds). Use of entomopathogenic fungi in biological pest management. Research SignPost, Kerala, India. ISBN 978-81-308-0192-6.

Conference presentations

International Congress on Entomology, held in Durban South Africa from 6-12 June 2008.
Nguya Maniania, Felix Nchu, Susan Dimbi, Sunday Ekesi. 2008. Entomopathogenic fungi vs semiochemicals: Mutualistic affair against pests

World Conference on Medicinal and Aromatic Plants (WOCMAP IV), held in Cape Town from 9-14 November 2008.
Felix Nchu, Maniania Nguya, Alione Touré, Ahmed Hassanali and Kobus Eloff. The use of a semiochemical bait to enhance exposure of *Amblyomma variegatum* (Acari: Ixodidae) to *Metarhizium anisopliae* (Ascomycota: Hypocreales)

SUMMARY

Due to the rapid development of tick resistance to synthetic chemical acaricides and the potential risk posed by these chemicals to non-target species, efforts are intensifying towards the development of entomopathogenic fungi as an environmentally benign alternative tick control strategy. The main objective of this study is the development of formulations and delivery systems for control of ticks with entomopathogenic fungi.

Screening of entomopathogenic fungal isolates is the first step towards the development of mycoacaricides. Twelve isolates of *Metarhizium anisopliae* (Metschnik.) Sorok. and three isolates of *Beauveria bassiana* (Bals.) Vuill. (Ascomycota: Hypocreales) were evaluated for pathogenicity against unfed *Rhipicephalus pulchellus* Gerstäcker (Acari: Ixodidae) adults under laboratory conditions. Out of the 15 isolates screened, 4 isolates were virulent against *R. pulchellus*, inducing significantly (F value = 11.86; DF = 14, 75; $P < 0.0001$) higher tick mortalities; i.e. ICIPE 78 (76.1 ± 5.9%), ICIPE 69 (62.6 ± 5%), ICIPE 62 (49.8 ± 5.8%) and ICIPE 60 (49.6 ± 7.7%), four weeks post-treatment compared to the others. The results of this study demonstrate that *R. pulchellus* ticks were susceptible to some *M. anisopliae* isolates.

Since the process of strain selection is an important step in the development of insect pathogens for biological control, an appropriate bioassay technique for infecting different life stages of a host is of paramount importance. Bioassays were conducted in the laboratory to evaluate the efficacy of different methods of inoculation using *R. pulchellus* as a model. In a preliminary experiment, an oil-based formulation of *M. anisopliae* titred at 10^9 conidia ml^{-1} was applied to *R. pulchellus* adults using a Burgerjon spray tower or a microapplicator. Inoculation by microapplicator yielded poor results (25.0% tick mortality) compared to Burgerjon's spray tower (52.3% tick mortality) , although the mean number of fungal conidia on *R. pulchellus* adults was lower ($1.5 \times 10^4 \pm 1.1 \times 10^3$ conidia ml^{-1}) after spraying by Burgerjon's spray tower compared to 1×10^6 conidia ml^{-1} obtained with the microapplicator. Thus, inoculation by Burgerjon's spray tower was selected for further investigations. Different modes of inoculation were tested and included direct spray of inoculum on the tick and substrate (SS), direct spray on the substrate and tick followed by transfer of the tick to clean uncontaminated Petri dish (SP) or indirect inoculation of ticks through substrate (SW). The LC_{50} values following contamination of nymphs ($LC_{50} = 1.4 \times 10^7$ conidia ml^{-1}) and adults ($LC_{50} = 6.7 \times 10^7$ conidia ml^{-1}) in SS were significantly lower compared to SP; nymphs

(LC_{50} = 5.7 x 10^8 conidia ml^{-1}) and adults (LC_{50} = 5.3 x 10^9 conidia ml^{-1}) and SW; nymphs (LC_{50} = 5 x 10^8 conidia ml^{-1}) and adults ($LC_{50} > 10^{10}$ conidia ml^{-1}). Although the LC_{50} value in SS was the lowest, it recorded the highest tick mortality among control ticks (24.2% at two weeks post-treatment) and (23.3% at three weeks post-treatment) in nymphs and adults respectively compared to SP (2.5 and 5.8% respectively) and SW (0.0 and 0.0). Results show that among the modes of contamination tested, SP was the most appropriate for contaminating *R. pulchellus* adults. SW and SP were identified as appropriate techniques for infecting the *R. pulchellus* nymphs with conidia formulated in oil.

The integrated use of semiochemicals and entomopathogenic fungus (hypocreales) may optimise the performance of a fungus as a biocontrol agent against ticks. Initially, experiments were conducted to evaluate the attraction of the hunter tick *Amblyomma variegatum* Fabriscius (Acari: Ixodidae) to semiochemicals. In one of the experiments, the simultaneous release of 1-octen-3-ol and AAAP together with CO_2 from a trap in simulated field plots attracted up to 94 ± 6% of adult ticks from a distance of 6 m, and up to 24.0 ± 5.1% from 8 m. Formulations of *M. anisopliae* (dry powder, oil, and emulsifiable) applied within the trap baited with AAAP and 1-octen-3-ol resulted in high levels of contamination of the ticks attracted to the traps. However, 48 hr after autoinoculation, 89.1 and 33.3% of conidia were lost in dry powder and oil formulations, respectively. Emulsifiable formulation lost the lowest number of propagules (17.1%). Samples of ticks attracted to the baited traps were transferred to plastic basins containing grass and maintained for 5 weeks. The experiment was conducted in the rainy and dry seasons. The emulsifiable formulation gave the highest relative reduction in tick numbers in both seasons: 54.7 and 46.5% in the rainy and dry seasons, respectively, followed by the oil formulation (32 and 23.8%) and the powder formulation (38.0 and 24.4%).

Following the high attraction and contamination of *A. variegatum* under semi-field conditions, experiments were done to evaluate the efficacy of *M. anisopliae*-treated semiochemical-baited traps for control of *A. variegatum* under field conditions. Unfed *A. variegatum* adults (118) were seeded in each 100-m plot. An emulsifiable formulation of *M. anisopliae* (consisting of 49.5% sterile distilled water, fungal conidia, 49.5% corn oil [CHEF cooking oil, Premier Oil Mills LTD] and 1% Tween 80) titrated at 10^9 conidia ml^{-1} was applied in a semiochemical-baited traps (900 cm^2) which were placed at 5 spots within the plot. The control and fungal treatments were repeated after 14 and 28 days soon after rotating the traps clockwise (45^{o}) in order to cover different sections of the plot. In the control plots, traps baited with semiochemicals only were used. Six weeks after the initiation of the experiments, five semiochemical-baited traps (untreated) were deployed in each plot for 3 successive days to trap ticks in the treated and control plots. The percentage of ticks recovered in the fungus-treated plots were significantly lower (31.1 ± 5.2%) than in the control plots (85.6% ± 3%) ($P < 0.001$), which represented a relative tick reduction of 63.7%. Mortality of 93.8 ± 2.3% was observed among the ticks that were recovered from the field and maintained in the laboratory for two weeks; while only 3.3 ± 0.9% died from the control plots.

The results of this study open up the possibility of developing an environmentally friendly, low cost product to control these economically important ticks.

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