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Research paper

Laboratory and field evaluation of entomopathogenic fungi for the control of amitraz-resistant and susceptible strains of *Rhipicephalus decoloratus*

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ABSTRACT

Rhipicephalus decoloratus causes serious economic losses in cattle industry every year in East Africa. Biological control using entomopathogenic fungi is seen as a promising alternative to chemical acaricides being used for their control. The pathogenicity of *Metarhizium anisopliae* and of *Beauveria bassiana* isolates was tested in the laboratory against amitraz-resistant and amitraz-susceptible strains of *R. decoloratus*. Unfed larvae were sprayed with conidial suspensions of 1×10^9 conidia ml^{-1}. Fungal isolates were pathogenic to *R. decoloratus* larvae, causing mortality of between 10.0 and 100% and between 12.1 and 100% of amitraz-susceptible and amitraz-resistant strains, respectively. The LT_{50} values of selected fungal isolates varied between 2.6–4.2 days in amitraz-susceptible strain and between 2.8–3.9 days in amitraz-resistant strain. The LC_{50} values varied between 0.4 ± 0.1 and $200.0 \pm 60 \times 10^3$ conidia ml^{-1} and between 0.1 ± 0.1 and $200.0 \pm 31.0 \times 10^3$ conidia ml^{-1} in amitraz-susceptible and amitraz-resistant strains, respectively. *Metarhizium anisopliae* isolate ICIPE 7 outperformed the other isolates and was selected for compatibility study with amitraz and field trial. ICIPE 7 was compatible with amitraz. In the field, four treatments including control, ICIPE 7 alone, amitraz alone and ICIPE 7/amitraz were applied on cattle. All the treatments significantly reduced the number of ticks on all the sampling dates: 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 ($F_{3,8} = 8.170$; $P = 0.0016$) compared to the control. Results of the present study indicate that ICIPE 7 can be used for the management of amitraz-resistant strain of *R. decoloratus*.

1. Introduction

Ticks and tick-borne diseases (TBDs) represent a major constrain to livestock production in tropical and subtropical regions (Walker et al., 2003). The most devastating species in East Africa region include *Rhipicephalus appendiculatus*, *Amblyomma variegatum* and *Rhipicephalus decoloratus*. The latter affects mainly cattle and can occasionally feed on horses and sheep but they do not complete their life-cycle on them (Walker et al., 2003). *R. decoloratus* are vectors of anaplasmosis caused by *Anaplasma marginale* and babesiosis caused by *Babesia bigemina* in cattle. Both diseases cause high production losses and frequently lead to death (Melendez, 2000; Stuen et al., 2003). In the absence of effective vaccines against anaplasmosis and babesiosis, control of the vector remains the best option.

Tick control is mainly based on the use of chemical acaricides such as amitraz, pyrethroids and organophosphates (Pound et al., 2009). However, chemical control has resulted in toxicological and environmental hazards as well as unselective killing including non-target organisms (Ducornez et al., 2005; Schulze et al., 2005). More importantly, ticks have developed resistance to various classes of compounds (Castro-Janer et al., 2010). This has prompted the search for alternative tick control measures. The use of entomopathogenic fungi (EPF) is among the strategies being considered (Maniania et al., 2007). Three mycoacaricides based on *Metarhizium anisopliae* have been developed and commercialized for the control of Ixodid ticks (Faria and Wraight, 2007). An integrated tick management strategy using EPF as core component could address

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Table 1
Identity of the fungal isolates used in the study and their germination on Sabouraud Dextrose Agar at 25 ± 2 °C.

Fungal species	Isolate	Locality (Country)	Source	Year of isolation	Germination (%)
Metarhizium anisopliae	ICIPE 41	Lemba (Democratic Republic of Congo)	Soil	1990	98.8 ± 0.8
	ICIPE 74	Mtwapa (Kenya)	Soil	1990	96.6 ± 2.7
	ICIPE 68	Matete (Democratic Republic of Congo)	Soil	1990	96.6 ± 2.7
	ICIPE 719	Machakos (Kenya)	Soil	2013	97.3 ± 5.2
	ICIPE 9	Matete (Democratic Republic of Congo)	Galleria	1990	100.0 ± 0.0
	ICIPE 91	Senegal	Locust	2003	98.5 ± 1.0
	ICIPE 7	Rusinga Island (Kenya)	*Amblyomma variegatum*	1996	100.0 ± 0.0
Beauveria bassiana	ICIPE 279	Kericho (Kenya)	Soil	2005	97.2 ± 2.2
	ICIPE 609	Meru (Kenya)	Soil	2008	97.6 ± 1.7
	ICIPE 676	Kenya	Soil	2008	97.2 ± 2.2
	ICIPE 644	Mauritius	Unknown	2007	96.6 ± 5.4
	ICIPE 718	Mbita (Kenya)	*Amblyomma variegatum*	2013	100.0 ± 0.0

the problem of acaricide resistance in ticks. Amitraz was recently reported to inhibit the development of *Beauveria bassiana* (Alizadeh et al., 2007) and as a component of integrated tick management, compatibility between EPF and an acaricide must be evaluated before any integration.

The objectives of the present study were to screen fungal isolates of *B. bassiana* and *M. anisopliae* for their virulence against amitraz-resistant and amitraz-susceptible strains of *R. decoloratus,* to evaluate the compatibility of selected fungal isolate with acaricide and to evaluate the field efficacy on-host ticks.

2. Materials and methods

2.1. Ethical considerations

Laboratory bioassays were carried out at the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi and field trial at a private farm in Transmara District. The fungal isolates were obtained from the ICIPE's Arthropod Germplasm Centre and no permission was required since the Centre operates under a Headquarters' agreement with the Kenyan Government. Ethical clearance was obtained from the Division of Veterinary Services (DVS), Nairobi, for field trial.

2.2. Ticks

The larvae of amitraz-resistant strain of *R. decoloratus* were obtained from Acarology Laboratory, Veterinary Department, Ministry of Agriculture, Livestock and Fisheries, Nairobi, Kenya, while the amitraz-susceptible strain was obtained from International Livestock Research Institute (ILRI), Nairobi, Kenya.

2.3. Fungal isolates

Seven isolates of *M. anisopliae* and 6 of *B. bassiana* were used in the present study. They were obtained from the International Centre of Insect Physiology and Ecology (ICIPE) Arthropod Germplasm Centre; their origin and place of isolation are presented in Table 1. Fungal isolates were cultured on Sabouraud Dextrose Agar (SDA) in Petri dishes (90-mm) and incubated (SANYO INCUBATOR®, Wood Dale, IL) at 25 ± 2 °C in complete darkness. The viability of conidia was determined before any bioassay by the method described by Goettel and Inglis (1997). Conidia were harvested from 3-week-old cultures with a sterile spatula. Conidial suspensions (0.1 ml) titrated to 1×10^6 conidia ml^{-1} were spread-plated on Petri dishes containing SDA medium. A sterile microscope cover slip (2 × 2 cm) was placed on top of the agar in each plate. Plates were incubated in complete darkness at 25 ± 2 °C and examined after 20 h. Percentage germination of conidia was determined by counting the number of germinated conidia (a germ tube two times the diameter of the propagule) from 100 spores counted randomly on the surface area covered by each cover slip under the light microscope (400 ×). Four replicate plates per isolate were used.

2.4. Mass production

For field experiment, conidia were produced on long white rice as substrate in polyethylene bags. The rice was autoclaved for 40 min at 121 °C and was inoculated after cooling with 50 ml 3 days-old broth and maintained in an incubation room (23–27 °C, 35–60% Relative Humidity) for three weeks. The substrate was allowed to dry for 5 days at room temperature before the conidia were harvested by sieving. The conidia were stored for two weeks in the refrigerator (4–6 °C) before being used for the field trial.

2.5. Preparation of conidial suspension

Conidia of each isolate were harvested from a 3-week-old culture by scrapping the surface of sporulating culture. Conidia were suspended in sterile distilled water containing 0.05% Triton X-100 in universal bottles with 3 mm diameter glass beads. The conidial suspension was vortexed for 5 min for homogenization. Conidial concentration was determined using an improved Neubauer haemocytometer (Celeromics, UK). Different concentrations were obtained through serial dilutions with distilled water containing 0.05% Triton X-100.

2.6. Acaricide

A synthetic acaricide, amitraz (Triatix®, Cooper K-Brands Ltd), was used in the experiments. In field trial, amitraz was used at the recommended concentration of 0.2% that corresponds to 250 ppm.

2.7. Laboratory bioassays

2.7.1. Efficacy of amitraz against larvae of amitraz-resistant and amitraz-susceptible strains of R. decoloratus

Two concentrations of acaricide (62.5 ppm and 125 ppm) were used. Ten ml of each concentration were sprayed on larvae using a hand sprayer. Controls were sprayed with sterile distilled water only. The treated larvae were transferred into 90 mm Petri dish containing filter paper at the base and sealed with Parafilm to prevent larvae from escaping. Larvae were then maintained in an incubator at 25 ± 2 °C and 75% RH. Mortality was recorded daily for 7 days. Ten larvae were used per replicate and the experiment was replicated four times.

2.7.2. Pathogenicity of isolates of anisopliae and bassiana against larvae of amitraz-resistant and amitraz-susceptible strains of R. decoloratus

Larvae of *R. decoloratus* were sprayed with 10 ml of fungal suspension titrated at 1×10^9 conidia ml^{-1} using Burgerjon's spray