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Laboratory evaluation of virulence of Chinese *Beauveria bassiana* and *Metarhizium anisopliae* isolates to engorged female *Rhipicephalus* (*Boophilus*) *microplus* ticks

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HIGHLIGHTS

- We evaluated the virulence of 20 isolates against *Rhipicephalus* (*Boophilus*) *microplus*.
- Four isolates of entomopathogenic fungi were highly virulent against this tick.
- Four isolates have potential for applications to control this tick.

GRAPHICAL ABSTRACT

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ABSTRACT

Rhipicephalus (*Boophilus*) *microplus*, an important ectoparasite that can transmit *Babesia* and *Anaplasma*, has caused inestimable economic losses around the world. Traditionally, acaricides are used for the control of ticks. However, drawbacks of chemical control, such as resistance, environmental pollution, and traces in food promote alternative strategies to pesticides. Microbial control is one option to reduce tick populations. In this study, we investigated the pathogenicity of thirteen *Beauveria bassiana* isolates and seven *Metarhizium anisopliae* isolates to the engorged female *R.* (*B.*) *microplus* ticks using different conidial concentrations of 10^7, 10^8 and 10^9 conidia mL^{-1}. Fourteen days after treatment, three *B. bassiana* isolates (B.bAT01, B.bAT03, B.bAT13) and one *M. anisopliae* isolate (M.aAT04) resulted in 100% mortality of engorged female ticks with conidial concentrations of 10^8 and 10^9 conidia mL^{-1}. Isolates of B.bAT01, B.bAT03, B.bAT13 and M.aAT04 at all conidial concentrations could reduce the reproductive efficiency index (REI) of *R.* (*B.*) *microplus* ticks.

1. Introduction

Ticks, obligatory ectoparasites of terrestrial vertebrates, transmit various pathogenic microorganisms, such as protozoa, rickettsiae, spirochaetes and viruses (Jongejan and Uilenberg, 2004). The cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Acari, Ixodidae) is an obligatory haematophagous ectoparasite and a single host species in almost all tropical and subtropical countries. Apart from physical damage to their host and blood spoliation, *R.* (*B.*) *microplus* is a major vector for bovine babesiosis and anaplasmosis (Monteiro et al., 2010; Jittapalapong et al., 2010). In China, *R.* (*B.*) *microplus* results in severe economic losses (Luo et al., 2003).

Though various techniques have been developed, tick population control still relies on chemical acaricides during their parasitic phase. Presently, chemical acaricides (that include organochlorines, organophosphates, carbamates, amidines and pyrethroids) are most effective reagents in tick control (Tuininga et al., 2009). The unlimited and indiscriminate use of these acaricides has resulted in many problems, such as heritable resistance of ticks, risk to non-target organisms, environmental pollution, trace contamination in food

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products, and increasing cost of tick control (Pourseyed et al., 2010; Angel-Sahagún et al., 2010). Therefore, alternative approaches are needed to reduce or replace the application of acaricides.

During recent years, biological control of ticks with entomopathogenic fungi has received considerable attention (Ment et al., 2010). Because of their wide distribution, low risk for humans and animals, environmental safety, high virulence against ticks and potential low cost, entomopathogenic fungi have been investigated as potential agents for biological control of ticks (Samish and Rehacek, 1999; Pirali-Kheirabadi et al., 2007). Among the entomopathogenic fungi examined for pathogenicity against ticks, two species commonly investigated are *Beauveria bassiana* and *Metarhizium anisopliae* (Polar et al., 2008). *B. bassiana* and *M. anisopliae* show high virulence to various life stages of ticks, and several studies substantiate the effect of these fungi on *R.* (*B.*) *microplus*. Ojeda-Chi et al. (2010) reported that isolates of *M. anisopliae* killed 100% of engorged female *R.* (*B.*) *microplus* ticks at 1×10^6 conidia mL^{-1} dose. Some strains of *M. anisopliae* caused up to 100% mortality in *R.* (*B.*) *microplus in vitro* within 2 days (Leemon and Jonsson, 2008). Fernandes et al. (2003) observed a high percentage of larval mortality and a low percentage of egg hatching in ticks treated with *B. bassiana*.

The entomopathogenic fungi *B. bassiana* and *M. anisopliae* have been used widely for biological control of agricultural and forest pests (Quesada-Moraga et al., 2007; Wraight et al., 2010), but few studies have been reported regarding their virulence against *R.* (*B.*) *microplus* in China. The aim of the present study was to evaluate the susceptibility of engorged female *R.* (*B.*) *microplus* ticks to thirteen strains of *B. bassiana* and seven strains of *M. anisopliae*, to discover potential strains for controlling *R.* (*B.*) *microplus* ticks in China.

2. Materials and methods

2.1. Ticks

R. (*B.*) *microplus* ticks were originally obtained from naturally infested cattle in Sichuan Province in western China. The ticks were maintained in an incubator at 27 ± 1° C and 80 ± 5% RH (relative humidity) in our laboratory. For this study, the larval *R.* (*B.*) *microplus* ticks were put onto two calves, and the engorged female ticks were collected, which were randomly divided into different groups.

2.2. Fungal growth and preparation of conidial suspensions

Thirteen isolates of *B. bassiana* and seven isolates of *M. anisopliae* were originally obtained from soil and a tick collected from 14 provinces of China (Table 1). These fungal isolates were maintained on potato dextrose agar (PDA) and kept at 4° C. All isolates were cultured with PDA on petri plates in an incubator at 26–28° C and RH > 80% for 12 days. Conidia were harvested by scraping the surface of the plate and placed into sterilized aqueous solution containing 0.05% Tween 80. After homogenization, concentrations of conidia were determined with a haemocytometer and adjusted to final concentrations of 10^7, 10^8 and 10^9 conidia mL^{-1} with 0.05% Tween 80 in distilled water.

2.3. Laboratory bioassays

Evaluation of fungal virulence was carried out according to the method described in Frazzon et al. (2000). Bioassays of *B. bassiana* and *M. anisopliae* suspensions (10^7, 10^8 and 10^9 conidia mL^{-1} of each isolate) were conducted for pathogenicity by immersing 10 engorged *R.* (*B.*) *microplus* females in each of the suspensions for approximately 30 s and then placing them on paper towel to soak up the excess. Control groups (with 10 engorged *R.* (*B.*) *microplus* females per group) were treated with sterile water containing 0.05% Tween 80 instead of conidial suspension. Each trial was repeated three times. After treatment, ticks were individually placed in glass tubes sealed with hydrophilic cotton and kept in an incubator at 27 ± 1° C and 80 ± 5% RH for oviposition. Ticks were observed daily to evaluate biological parameters.

Table 1
Details of fungal strains used in this study.

Strain	Fungus	Original host	Geographical origin
B.bAT01	*B. bassiana*	Soil	Anhui, China
B.bAT02	*B. bassiana*	Soil	Anhui, China
B.bAT03	*B. bassiana*	Soil	Guizhou, China
B.bAT04	*B. bassiana*	Soil	Zhejiang, China
B.bAT05	*B. bassiana*	Soil	Zhejiang, China
B.bAT06	*B. bassiana*	Soil	Jiangsu, China
B.bAT07	*B. bassiana*	Soil	Fujian, China
B.bAT08	*B. bassiana*	Soil	Hunan, China
B.bAT09	*B. bassiana*	Soil	Heilongjiang, China
B.bAT10	*B. bassiana*	Soil	Guizhou, China
B.bAT11	*B. bassiana*	Soil	Guangxi, China
B.bAT12	*B. bassiana*	Soil	Hubei, China
B.bAT13	*B. bassiana*	*B. microplus*	Guizhou, China
M.aAT01	*M. anisopliae*	Soil	Sichuan, China
M.aAT02	*M. anisopliae*	Soil	Shanxi, China
M.aAT03	*M. anisopliae*	Soil	Anhui, China
M.aAT04	*M. anisopliae*	Soil	Henan, China
M.aAT05	*M. anisopliae*	Soil	Gansu, China
M.aAT06	*M. anisopliae*	Soil	Guangdong, China
M.aAT07	*M. anisopliae*	Soil	Guizhou, China

2.4. Data analysis

Treatment data were evaluated using a formula described by Ojeda-Chi et al. (2010). The reproductive efficiency index (REI = egg mass weight/engorged female weight) was determined for each female tick. Dead ticks were counted and mortality rates were calculated. Analyses of variance (ANOVA) were used to evaluate levels of significance of the virulence of *B. bassiana* and *M. anisopliae* against *R.* (*B.*) *microplus* female ticks.

3. Results

All isolates of *B. bassiana* and *M. anisopliae* were pathogenic to *R.* (*B.*) *microplus* at concentration of 10^7, 10^8 and 10^9 conidia mL^{-1} in the laboratory. The lethal activity of all treatment and control groups to engorged females are presented in Fig. 1. Fourteen days

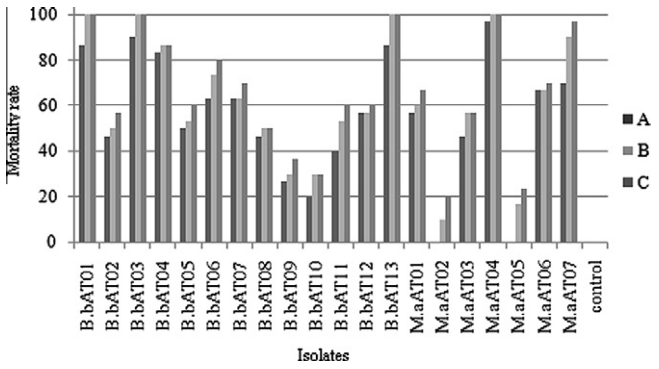

Fig. 1. Mortality of *R.* (*B.*) *microplus* female caused by *M. anisopliae* and *B. bassiana* (concentrations of fungal strains: (*A*) 10^7 conidia mL^{-1}, (*B*) 10^8 conidia mL^{-1}, (*C*) 10^9 conidia mL^{-1}).