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Laboratory evaluation of *Beauveria bassiana* and *Metarhizium anisopliae* in the control of *Haemaphysalis qinghaiensis* in China

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Abstract *Haemaphysalis qinghaiensis*, a prevalent tick species in China, is an ectoparasite that preferentially infests small ruminants and can transmit *Theileria* sp. and *Babesia* sp. In this study, we evaluated the pathogenicity of individual and mixed infections of the fungi *Beauveria bassiana* and *Metarhizium anisopliae* to *H. qinghaiensis* nymphs. The estimated LC_{50} for ticks immersed in solutions of *B. bassiana*, *M. anisopliae* and a mixture thereof were: 5.88056×10^4, 2.65×10^4, and 2.85×10^4 conidia mL^{-1} respectively, and the nymphal mortality ranged from 52 to 100 %. Thus, these results suggest a potential approach for the biocontrol of *H. qinghaiensis*.

Keywords *Haemaphysalis qinghaiensis* · *Beauveria bassiana* · *Metarhizium anisopliae* · Biological control · Entomopathogenic fungi

Introduction

Ticks are important hematophagous arthropods that are distributed almost worldwide, but particularly in tropical and subtropical areas. They can have direct deleterious effects on their hosts, including damage to skin, blood loss, decreased milk yield, and loss of weight. In addition, and perhaps more seriously, ticks can also transmit various pathogens to their

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host animals and also to humans. They can also result in economic losses, particularly in the tanning industry, given their effects on the hides of their hosts. Ticks are considered to be second only to mosquitoes as vectors of human disease (Zhou et al. 2009). The annual worldwide cost of the control of ticks and tickborne diseases (TTBDs) has been estimated to be between US$13.9 and $18.7 billion (Angel-Sahagun et al. 2010).

The three-host tick, *Haemaphysalis qinghaiensis*, is a species endemic to China, where it is widely distributed on the western plateau. It preferentially infests small ruminants and can transmit *Theileria* sp. and *Babesia* sp. (Yin et al. 2002). *Haemaphysalis qinghaiensis*, and the protozoal diseases that it transmits, result in significant economic losses to livestock production in China.

Chemical acaricides, which are the primary and most commonly used methods to control tick populations, have many disadvantages, including environmental pollution, food contamination, appearance of acaricide-resistant ticks, increasing costs, and impacts on nontarget organisms (You and Fujisaki 2009; Pourseyed et al. 2010). These drawbacks are driving research into alternative, sustainable strategies for more efficient tick control (Bharadwaj and Stafford 2010).

Entomopathogenic fungi infect arachnids by direct penetration of the cuticle and, thus, can be used to control sucking arthropod pests (Shang et al. 2012). Given their global dispersal, relatively low risk to humans, animals and ecosystems, high virulence against ticks, and the fact that they are easy to produce commercially, entomopathogenic fungi are considered to be acceptable biological control agents (Leemon and Jonsson 2008). Recently, interesting results (Camargo et al. 2014; Golo et al. 2015) have been published relating to the use of the fungi *Beauveria bassiana* and *Metarhizium anisopliae* to control ticks. *B. bassiana* and *M. anisopliae* are the predominant fungal species infecting ticks; they have a broad host range and the ability to penetrate the arthropod cuticle. Different strains of *B. bassiana* and *M. anisopliae* are pathogenic to several kinds of tick.

Although there have been various studies of the individual effects of *B. bassiana* and *M. anisopliae* on ticks, few studies have considered the pathogenicity of their synergistic effects. Thus, with the aim of developing a new tick control method, we evaluated the virulence of *B. bassiana*, *M. anisopliae*, and the synergism thereof, on *H. qinghaiensis* nymphs.

Materials and methods

Ticks

For laboratory experiments, unfed *H. qinghaiensis* nymphs were collected from naturally infested sheep. After collection, the ticks were maintained in an incubator at 28 ± 2 °C and 80 ± 5 % relative humidity (RH) in glass tubes sealed with hydrophilic cotton. Two to three-week-old unfed nymphs were randomly divided into five groups of ten ticks for each treatment.

Fungal growth and preparation of conidial suspensions

Isolates of *B. bassiana* and *M. anisopliae* were originally obtained from soil samples collected in China. The fungi were maintained on potato dextrose agar (PDA) slopes and kept at 4 °C. Fungal isolates were cultured with fresh PDA Petri plates in an incubator at

26–28 °C and 75–85 % RH. Spores were harvested into sterilize aqueous 0.05 % Tween 80 solution by scraping the surface of the plate after 12 days; the suspension was then homogenized on a vortex mixer. Conidia concentrations were determined by direct count using a hemocytometer.

After homogenization, the concentration of conidia was determined with a hemocytometer and adjusted to concentrations of 10^5, 10^6, 10^7, 10^8, and 10^9 conidia mL^{-1} with 0.05 % Tween 80 in distilled water. In preparing the suspension of the fungal cocktails, co-formulations of 10^9 conidia mL^{-1} of *B. bassiana* and *M. anisopliae* were mixed at ratios of 1:1. The adjusted final concentrations were 10^5, 10^6, 10^7, 10^8, and 10^9 conidia mL^{-1}.

Laboratory bioassays

Suspensions ranging from 10^5 to 10^9 conidia mL^{-1} of *B. bassiana*, *M. anisopliae*, and *B. bassiana* + *M. anisopliae* (Bb + Ma) were prepared. Ten *H. qinghaiensis* nymphs per treatment were immersed for 30 s in each conidial suspension and eliminated the excess suspension. As controls, ticks were immerged in the same volume of sterile water containing 0.05 % Tween 80. Each trial was conducted with five repetitions. After the treatment, the treatment ticks were placed individually in a Petri dish and kept in an incubator at 28 ± 2 °C and 80 ± 5 % RH. The ticks were observed every 72 h to check for mortality.

Data analysis

To evaluate the mortality and LC_{50} of *H. qinghaiensis* nymphs for each treatment, mortality data were analysed using a one-way analysis of variance (ANOVA) via SPSS. The data collected were based on the percentage mortality 21 days after treatment. The median lethal concentrations (LC_{50}), respective confidence limits, regression equation, and resistance ratios were determined using the probit-analysis method.

Results

The mortality of the *H. qinghaiensis* nymphs immersed in the different fungal strains are shown in Table 1. The mortalities of *B. bassiana*, *M. anisopliae*, and Bb + Ma were not significantly different from each other, whereas any group treated with any fungal strain showed significant differences compared with the control treatments. All these three

Table 1 Mean percent mortality of *Haemaphysalis qinghaiensis* nymphs obtained after treatment with *Beauveria bassiana*, *Metarhizium anisopliae* and their associations at different concentrations

	10^5	10^6	10^7	10^8	10^9	Control
B. b	0.52 C d	0.68 C c	0.76 C b	0.86 C a	0.92 B a	0.00 e
M. a	0.62 A d	0.84 A c	0.88 A b	0.98 A a	1.00 A a	0.00 e
B. + M.	0.58 B d	0.72 B c	0.84 B b	0.90 B a	0.96 B a	0.00 e
Control	0.00 D	0.00 D	0.00 D	0.00 D	0.00 C	0.00

Means followed by the same uppercase letter in the same line and by the same lowercase letter in the same column did not differ significantly ($p < 0.05$)

treatment groups were pathogenic to *H. qinghaiensis* at concentration of 10^5, 10^6, 10^7, 10^8, and 10^9 conidia mL^{-1} in the laboratory, but the highest mortality (92–100 %) was obtained at the concentration of 10^9 conidia mL^{-1}, mortalities were lower at the other concentrations. The mixture of strains (Bb + Ma) showed 96 % efficacy compared with control unfed nymphs at the same concentration, and no dead ticks were observed in the control groups. The mortality of the ticks in the mixed-infection treatments were consistently the intermediate of the two single infection treatments.

The LC_{50} of *B. bassiana*, *M. anisopliae*, and Bb + Ma on *H. qinghaiensis* nymphs are shown in Table 2. The estimated LC_{50} for ticks immersed in *B. bassiana*, *M. anisopliae* and Bb + Ma were 5.88×10^4, 2.65×10^4, and 2.85×10^4 conidia mL^{-1}, respectively. This indicates a lower LC_{50} for *M. anisopliae* and the mixture of both than for *B. bassiana*.

The temporal mortality of unfed *H. qinghaiensis* nymphs infected with fungal conidia is presented in Fig. 1. All three treatments resulted in >50 % mortality at 9 days post-inoculation (DPI) at a concentration of 10^8 conidia mL^{-1}. The fungal cocktail (Bb + Ma) resulted in 96 % mortality at 18 DPI for 10^9 conidia mL^{-1}.

Discussion

Entomopathogenic fungi are major pathogens of ticks. Given their ability to penetrate the external cuticle of arthropods, their wide distribution, wide host range, low risk to humans and animals, ease of production, and environmental safety, entomopathogenic fungi are frequently evaluated as biocontrol agents (Hussain et al. 2014). Studies showed that entomopathogenic fungi are potential pathogens to use for the control of ticks (Ojeda-Chi et al. 2010). Among the entomopathogenic fungi examined for pathogenicity against ticks, *M. anisopliae* and *B. bassiana* are the most commonly studied species (Fernandes et al. 2012).

However, few studies have examined the virulence of *B. bassiana* and *M. anisopliae*, either individually or in combination, to ticks. Thus, in this study, we examined the virulence of the individual strains and a mix of *B. bassiana* and *M. anisopliae* to *H. qinghaiensis* nymphs. The data demonstrated the susceptibility of *H. qinghaiensis* to isolates of *B. bassiana* and *M. anisopliae* and that this susceptibility was similar to that of a combination of the two species. This result contrasts with a previous investigation of the mortality of the tick *Amblyomma variegatum* resulting from fungal suspensions of *B. bassiana* and *M. anisopliae,* which indicated that the fungal cocktails induced higher mortalities than each fungi alone, although the differences were mostly not statistically significant (Maranga et al. 2005).

Table 2 Values of LC_{50} of *Beauveria bassiana*, *Metarhizium anisopliae* and a cocktail of the two fungi on *Haemaphysalis qinghaiensis* nymphs and the respective confidence limits, regression equation, and resistance ratio

Treatment	Regression equation	LC_{50}	Confidence limits (95 %)	Resistance ratio
B.b	−1.587 + 0.333x	5.88056×10^4	2.33452×10^3–2.98534×10^5	0.067
M.a	−2.442 + 0.548x	2.64973×10^4	2.15226×10^3–9.77589×10^4	0.103
B.b + *M.a*	−1.682 + 0.377x	2.84857×10^4	1.08134×10^3–1.47422×10^5	0.074

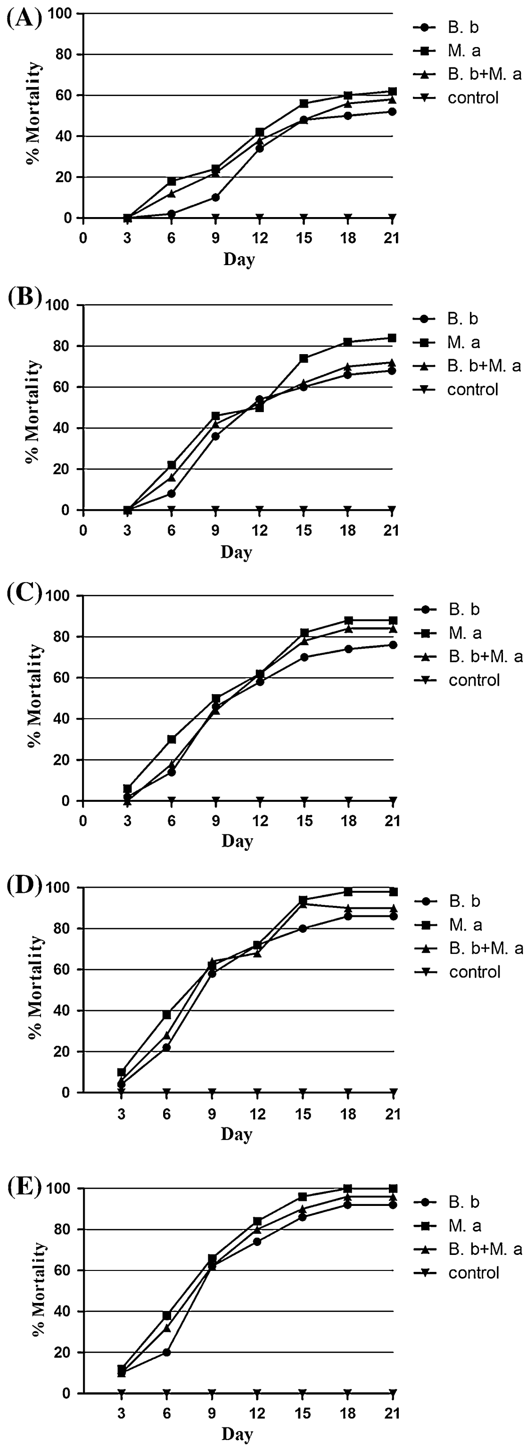

Fig. 1 The temporal mortality (%) of unfed nymphal *Haemaphysalis qinghaiensis* infected with fungal conidia: **a** 10^5, **b** 10^6, **c** 10^7, **d** 10^8, **e** 10^9 conidia mL^{-1}. B. b = *Beauveria bassiana*, M. a = *Metarhizium anisopliae*

In conclusion, this study suggests a new approach for the biocontrol of *H. qinghaiensis* ticks using fungal strains. However, subsequent studies are needed to improve the synergistic effects of *B. bassiana* and *M. anisopliae* against these ticks. The pathogenicity of *B. bassiana* and *M. anisopliae* should then be followed up by field studies, under the various climatic conditions of the geographical distribution area of the tick.

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