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Beauveria bassiana: Synergistic effect with acaricides against the tick *Hyalomma anatolicum anatolicum* (Acari: Ixodidae)

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ABSTRACT

Owing to the need to combat the spread of chemical acaricide resistance in ticks, we evaluated the efficacy of a mixture of the entomopathogenic fungus *Beauveria bassiana* AT17 and acaricides for the control of *Hyalomma anatolicum anatolicum* in China. A mixture of *B. bassiana* AT17 at the concentration of 10^8 conidia/mL and the synthetic pyrethroid deltamethrin at concentrations of 2500, 250, 25, 5, 2.5, 0.5 and 0.25 ppm was tested *in vitro*. The germination capability, vegetative growth, conidia production, and viability of *B. bassiana* AT17 were assessed and the efficacy of the mixture in killing engorged *H. anatolicum anatolicum* females was measured. High mortality rates were achieved when the entomopathogen was combined with different concentrations of deltamethrin. Neither *B. bassiana* AT17 nor deltamethrin alone at the same concentrations could cause the higher mortality rates seen with the combination. In addition the combination killed the ticks more rapidly than did either agent alone (3–5 days more rapidly). Our results indicate that the mixture of *B. bassiana* AT17 and deltamethrin has potential as a new type of reagent for integrated control of *H. anatolicum anatolicum*.

1. Introduction

Hyalomma anatolicum anatolicum (Acari: Ixodidae) is a three-host tick that not only causes weakness of animals by blood sucking, but also transmits various pathogens to man and other animals, including viruses, bacteria and parasites (Guan et al., 2009; Li et al., 2010; Luo et al., 2003a; Sun et al., 2010). It is one of the most widely distributed tick species infesting cattle, sheep and goats in Xinjiang Province, China. The economic losses caused by ticks are difficult to calculate, due to its direct or indirect transmission of pathogenic agents, and control of ticks would greatly improve the control of tick-borne diseases.

Numerous studies are currently under way to investigate effective control strategies for minimizing the damage caused by ticks. Chemical acaricides such as synthetic pyrethroids, organophosphates and amitraz have a pivotal role in the control of *Rhipicephalus* (*Boophilus*) *microplus* and *Rhipicephalus sanguineus*, especially permethrin acaricide (Roma et al., 2010) and synthetic pyrethroid (Mencke et al., 2003) that act in the oocytes and nervous system of the ticks, respectively. Control of ticks through use of acaricides is an important approach in disease management. However, insufficient concentrations, improper application methods and other inappropriate uses of chemical products to treat ticks have led to acaricide-resistant populations, creating future problems in controlling the parasite (Furlong, 1993). In Mexico, tick resistance to acaricide is recognized in several states (Rodriguez-Vivas et al., 2007). Currently, due to the issues concerning resistance, environmental pollution and public sanitation, acaricide use has been limited. Therefore, there has been an increased focus on immune prophylaxis and biocontrol. In Brazil, the Lamiacea family of plants has been used to develop new technologies to be used in the strategic control of ixodidae ticks (Oliveira et al., 2009, 2010).

With the aim of establishing more rational and efficient strategies for controlling ticks, several studies have used fungi as biological controllers (Fernandes and Bittencourt, 2008; Kaaya and Hassan, 2000; Samish et al., 2004). Entomopathogenic fungi are important natural predators of arthropods and have been widely used in the control of agricultural insect pests in China (Sun et al., 2010). To find new reagents for efficient control of ticks in china, we assessed the efficacy of a mixture of *Beauveria bassiana* AT17 and different concentrations of acaricides in controlling *H. anatolicum anatolicum* ticks. We aimed to evaluate the synergistic effect of entomopathogenic fungi and different concentrations of deltamethrin on the biological parameters of engorged females of *H. anatolicum anatolicum*.

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2. Materials and methods

2.1. Ticks

H. anatolicum anatolicum engorged females were collected from naturally infected cattle and sheep in Xinjiang Province in the northwest of China (Luo et al., 2003b). All off-host stages were maintained in an incubator at 27 °C with nearly 80% relative humidity (RH), and the on-host stages were fed on either sheep or rabbits. A total of 240 engorged female ticks were used for this study and the initial weight of the females was homogeneous. The ticks were divided into 16 groups, each containing 15 engorged females; one group was used as the control, seven were immersed for 5 min in different concentrations of deltamethrin, seven were immersed in the mixture of fungus and deltamethrin and one was immersed in fungus (Table 2).

2.2. B. bassiana strain and conidia suspensions

The *B. bassiana* AT17 strain was isolated in China and was cultured on PDA (potato dextrose agar) in Petri plates for 2 weeks at 26 °C and 80% RH. The conidia suspensions were harvested by washing the plates with 0.05% Tween-80 sterilization solution. A hemocytometer and microscope were used to determine the conidia concentration and to adjust it to 10^8 conidia/mL.

2.3. Synthetic pyrethroid solutions

Solutions were prepared using the commercial product Bayer® (CAS No.: xk13-003-00459, Chinese), which had shown potential biological activity against *R.* (*Boophilus*) *microplus* ticks in our laboratory (Sun et al., 2004). Seven solutions were prepared at the concentrations of 2500, 250, 25, 5, 2.5, 0.5 and 0.25 ppm, through successive dilutions in sterile distilled 0.05% Tween-80 solution. The control group was exposed only to the sterile distilled 0.05% Tween-80 solution.

2.4. Compatibility of B. bassiana AT17 with deltamethrin

2.4.1. Bioassay of conidia germination at different deltamethrin concentrations

Spore powders of the *B. bassiana* AT17 were suspended in 0.05% Tween-80 sterilization solution and sterile quartz sand to give high concentrations of conidia suspensions. The suspensions of conidia with concentration of 1×10^6 conidia/mL were prepared by dilution of the high concentrations of conidia suspensions in the germination solution (40 g/L cane sugar and 10 g/L peptone in distilled water), in which different concentrations of deltamethrin were contained, as indicated in Table 1. Those suspensions of conidia were cultured by oscillation at 26 °C for 24 h, germination and non-germination spores number (>100) were counted using a microscope and hemacytometer to calculate germination rate. Each experiment was performed in triplicate and the germination rate with 0.05% Tween-80 sterilization solution was measured as a control.

2.4.2. Bioassay of colony at different dilution of deltamethrin

The PDA culture medium was autoclaved for sterilization. When its temperature reached about 45 °C, different concentrations of deltamethrin were added and mixed, then the medium was put onto Petri dishes aseptically. A 4 mm diameter sterilization puncher was used to make a hole in the plates of different concentration. Spores powders were suspended with 0.05% Tween-80 sterilization solution and sterile quartz sand to give high concentrations of conidia suspensions, 1×10^6 conidia/mL were prepared as described above and approximately 50 μL samples of conidia suspensions were injected into the holes. The plates were cultured at 26 °C and 80% RH, and the diameter of the colony was measured and the changes in the color of spores were observed every day. Each experiment was performed in triplicate and PDA culture medium with 0.05% Tween-80 sterilization solution was used as a control.

2.5. Bioassay of tick immersion tests (AIT)

Deltamethrin was diluted with the 0.05% Tween-80 sterilization solution as indicated in Table 1 and the solutions were homogenized by shaking. The efficacy of each group against engorged *H. anatolicum anatolicum* females was tested by immersing 15 engorged females in the deltamethrin solution for 5 min according to the method previously described by Roma et al. (2009). The ticks in the control group were exposed only to 0.05% Tween-80 sterilization solution.

At the same time, the efficacy of *B. bassiana* AT17 in combination with deltamethrin was assessed. *B. bassiana* AT17 were cultivated in PDA, and its spores were suspended in 0.05% Tween-80 sterilization solution, and adjusted to 1×10^8 conidia/mL with different dilutions of deltamethrin (Table 1). A total of 120 engorged female ticks were divided into eight groups; the ticks in seven groups were considered as experimental groups, which were submerged in conidia and deltamethrin suspensions for 5 min, and the eighth group considered as a control group and immersed in the 1×10^8 conidia/mL conidia suspensions under the same conditions.

After treatment, ticks were placed individually in Petri dishes (50 × 15 mm) and incubated in a controlled environment (26 °C and RH ⩾ 80 ± 10%) for 21 days. Mortality of ticks was recorded daily until the end of the experiment (21 days) (Hedimbi et al., 2008). A corrected mortality rate was calculated using the following formula recommended by Food and Agriculture Organization of the United Nations (FAO, 2004)

$$\text{Corrected mortality} = \frac{\%\text{treated mortality} - \%\text{controls mortality}}{100 - \%\text{controls mortality}} \times 100$$

Table 1
Inhibitory effect of different concentrations of deltamethrin.

Different dilution proportion	Active constituent (ppm)	Conidia germination (%)	Diameter of colony (mm)	Speed of growth (mm/d)
10^{-1}	2500.00	0.00 ± 0.00^{e}	0.00 ± 0.00^{g}	0.00 ± 0.00^{f}
10^{-2}	250.00	0.00 ± 0.00^{e}	0.00 ± 0.00^{g}	0.00 ± 0.00^{f}
10^{-3}	25.00	0.00 ± 0.00^{e}	38.67 ± 0.64^{f}	2.74 ± 0.08^{e}
2^{-3}	5.00	38.00 ± 1.00^{d}	55.61 ± 0.99^{e}	3.91 ± 0.12^{d}
10^{-4}	2.50	66.92 ± 1.61^{c}	58.55 ± 0.39^{d}	4.10 ± 0.17^{cd}
2^{-4}	0.50	77.00 ± 1.73^{b}	63.11 ± 0.23^{c}	4.40 ± 0.20^{bc}
10^{-5}	0.25	93.22 ± 0.75^{a}	67.29 ± 0.78^{b}	4.72 ± 0.14^{b}
CK (*B. bassiana* AT17)	0.00	94.76 ± 3.00^{a}	70.34 ± 0.48^{a}	5.21 ± 0.25^{a}

Note: The difference between means with different small letters in a column is significant ($p < 0.05$).

Table 2
Mortality rates of *H. atolicum anatolicum* treated with deltamethrin and *B. bassiana*.

Groups	Active constituent (ppm)	Fungi (1×10^8 conidia/mL)	Mortality (%)											Corrected mortality (%)
			1st	3rd	5th	7th	9th	11th	13th	15th	17th	19th	21st	
Chemicals	2500.00	–	46.67	60.00	66.67	80.00	86.67	93.33	93.33	93.33	93.33	93.33	93.33	92.85
	250.00	–	20.00	40.00	53.33	60.00	60.00	66.67	66.67	66.67	66.67	66.67	66.67	64.29
	25.00	–	6.67	26.67	33.33	46.67	53.33	60.00	60.00	60.00	60.00	60.00	60.00	57.14
	5.00	–	0.00	0.00	26.67	53.33	53.33	53.33	53.33	53.33	53.33	53.33	53.33	50.00
	2.50	–	6.67	6.67	6.67	13.33	20.00	20.00	40.00	40.00	40.00	40.00	40.00	35.71
	0.50	–	0.00	6.67	20.00	20.00	20.00	20.00	20.00	26.67	26.67	26.67	26.67	21.43
	0.25	–	0.00	0.00	0.00	6.67	6.67	13.33	13.33	13.33	13.33	13.33	13.33	7.14
Mixture	2500.00	+	66.67	66.67	93.33	93.33	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	250.00	+	33.33	53.33	73.33	86.67	93.33	93.33	100.00	100.00	100.00	100.00	100.00	100.00
	25.00	+	53.33	93.33	93.33	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	5.00	+	0.00	53.33	80.00	86.67	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	2.50	+	20.00	53.33	80.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	0.50	+	6.67	53.33	93.33	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	0.25	+	20.00	26.67	86.67	93.33	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Fungi	0.00	+	0.00	0.00	0.00	13.33	20.00	53.33	93.33	100.00	100.00	100.00	100.00	100.00
Control	0.00	–	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.67	6.67	6.67	6.67	0.00

2.6. Statistical analysis

Statistical analysis was performed using DPS (Data Processing System) software for Windows. The mean values of conidia germination, colony diameter and speed of growth were analyzed by Tukey's test, with *p*-values of less than 5% indicating significance ($p < 0.05$).

3. Results

3.1. Deltamethrin effect on conidia germination and growth of B. bassiana AT17

The biological efficacy of *B. bassiana* AT17 conidia and different concentrations of deltamethrin was evaluated by measuring conidial germination (Table 1). Deltamethrin at a concentration of 0.25 ppm showed high compatibility with *B. bassiana* AT17, and the germination rate exceeded 93%; however, this was not significantly different from the rate seen with the control. Our results also showed that deltamethrin at concentrations of 25–2500 ppm could completely inhibit conidia germination; in contrast, concentrations of 0.25–0.5 ppm had little inhibitory effect. At the same time, the effect of different concentrations of deltamethrin on the vitality of conidium was studied (Table 1). The results showed that the colony diameter and speed of growth of *B. bassiana* AT17 were deltamethrin-dosage dependent. The germination capability of conidia was inhibited with deltamethrin concentrations of 250–2500 ppm; however, little, if any, inhibition was seen with concentrations of 0.25–0.5 ppm. In particular, deltamethrin at a concentration of 0.25 ppm had minimal inhibitory effects on *B. bassiana* conidia as the colony diameter exceeded 67 mm.

3.2. Synergistic effect of B. bassiana AT17 with acaricides against Hyalomma anatolicum anatolicum

The mortality rate of the engorged female ticks increased with increasing concentrations of deltamethrin. The lowest mortality rate (7.14%) was observed in the group treated with deltamethrin at a concentration of 0.25 ppm and the highest mortality rate (92.85%) was observed in the group treated with deltamethrin at a concentration of 2500 ppm.

The fungal suspension was able to kill *H. anatolicum anatolicum* engorged females with a mortality rate of up to 100% at the concentration of 10^8 conidia/mL. The first dead tick was observed on the 7th day after treatment and all the ticks had died by the 15th day, five to seven days after tick death, fungal mycelium of *B. bassiana* was observed on the surface of dead ticks in this experiment.

The susceptibility of engorged *H. anatolicum anatolicum* females to deltamethrin and *B. bassiana* AT17 was shown in this study. As shown in Table 2, all mixtures of *B. bassiana* AT17 and deltamethrin were associated with higher mortality rates than seen with deltamethrin alone. All groups treated with the combination had 100% mortality. In the combination-treated ticks, dead ticks appeared slightly swollen and their cuticle and legs were stained red. Tick death was observed at 3–11 days after treatment. The combination of the fungus at a concentration of 10^8 conidia/mL and deltamethrin at 0.25 ppm was associated with significantly higher mortality compared with the corresponding concentrations of either agent alone; in addition, tick death was reported about 6 days earlier than with either agent alone.

4. Discussion

Globally, tick control is still based almost exclusively on chemical acaricides. However, use of chemical acaricides for the control of arthropods often results in the development of tick resistance. Madzimure et al. (2010) suggested that, although the efficacy was less than that seen with the amitraz-based acaricide Tick buster®, aqueous *Lippia javanica* was effective at controlling cattle ticks and could provide an effective tick control option when synthetic products were unavailable or unaffordable, particularly in remote parts of southern Africa. Here, we described that the synergistic activity of *B. bassiana* AT17 with chemical acaricides in controlling *H. anatolicum anatolicum* is one of the models for controlling ticks in China, so as to settle the issues in terms of tick resistance, environment and product quality.

In a previous study, Sun et al. (2004) showed that deltamethrin could eradicate *R.* (*Boophilus*) *microplus* at its four developmental stages, and the concentrations of deltamethrin were 125, 11.5, 1.98, 0.105 and 0.01 ppm in their study, resulting in the mortality rates of 45.0–95.6% in engorged females. In our study, high dose of the deltamethrin was required to reach high mortality as shown in Table 2. This may be due to the susceptibility of different ticks. However, *B. bassiana* AT17 and deltamethrin at a concentration of 0.25 ppm were shown to be the optimal combination for control of *H. anatolicum anatolicum* (Table 2); the dead fastigium of ticks was found after about 3–7 days. Indeed, the combination showed significantly greater efficacy than seen with either the fungus or acaricide alone.

The use of *B. bassiana* with chemical acaricides may be suitable for tick control because chemical acaricides can rapidly numb ticks, while fungi contain toxins that can kill the ticks. Gindin et al. (2002) showed that *R.* (*Boophilus*) *annulatus* females were infected with strains of *M. anisopliae* and *B. bassiana* before egg lying. They reported that the highest mortality rate was 90% at 7 days. Our results showed the highest mortality rate was 100% at 11 days before egg laying. It is interesting to note that in contrast to the use of fungi alone, the mixture had better efficacy in killing ticks before egg laying.

In conclusion, the data presented here show that deltamethrin and *B. bassiana* AT17 act synergistically and more effectively eliminate ticks than does either agent alone. This is the first report on the synergistic effects of *B. bassiana* and deltamethrin in China. Use of the combination is likely to allow use of reduced concentrations of the two agents, reduced costs, optimization of benefits and increased safety and efficacy. However, an in-depth study of the interaction between *B. bassiana* and acaricides under field conditions is needed to prove the usefulness of the mixture for tick management in practice.

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