Research Note

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Effects of destruxin A on *Rhipicephalus* (*Boophilus*) *microplus* ticks (Acari: Ixodidae)

Efeitos da destruxina A no carrapato *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae)

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Abstract

This study evaluated the effects of destruxin A on *Rhipicephalus* (*Boophilus*) *microplus* females, since this toxin is one of the likely causes of high mortality induced by the entomopathogenic fungus *Metarhizium anisopliae* in arthropods. Ticks were immersed or inoculated with different concentrations of destruxin A. Despite the doses applied, there were no deaths or significant alterations in oviposition between the groups treated with destruxin A and the control groups. No other external effect caused by destruxin, such as tetanic paralysis, was observed in these engorged female ticks after the treatment.

Keywords: *Metarhizium anisopliae*, fungal metabolites, biological control.

Resumo

Este estudo avaliou os efeitos da destruxina A em fêmeas de *Rhipicephalus* (*Boophilus*) *microplus*, uma vez que essa toxina é uma das prováveis causas da alta mortalidade induzida pelo fungo entomopatogênico *Metarhizium anisopliae* em artrópodes. Os carrapatos foram imersos ou inoculados com diferentes concentrações de destruxina A. Apesar das doses aplicadas, não houve mortes ou alterações significativas de postura entre os grupos tratados com destruxinas A e os grupos controle. Nenhum outro efeito externo provocado pela destruxina A, tal como paralisia tetânica, foi observado nas fêmeas ingurgitadas de carrapato após o tratamento.

Palavras-chave: *Metarhizium anisopliae*, metabólitos fúngicos, controle biológico.

Introduction

The cattle tick *Rhipicephalus* (*Boophilus*) *microplus* causes significant economic losses to cattle farmers due to reduced milk and meat production, slower growth of infested animals and disease transmission. In Brazil, these economic losses have been estimated as two billion dollars a year (GRISI et al., 2002). Tick control is generally based on the use of chemical acaricides, but their continual application and improper use have many negative side effects, including the development of chemical resistance in tick populations and food and environmental contamination. Biological control is an alternative to the use of chemical acaricides. Of all the entomopathogenic fungal genera and species that have been tested, *Metarhizium anisopliae* is among the most often investigated, because of its potential for controlling tick species worldwide (FERNANDES; BITTENCOURT, 2008).

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The infection pathway of *M. anisopliae* consists of attachment of the spore to the cuticle, germination and formation of appressoria, thereby leading to penetration through the cuticle. After overcoming the host's response and immune defense reactions, this fungus spreads within the host by forming hyphal bodies, outgrowing the dead host and producing new conidia (ZIMMERMANN, 2007). While spreading within the host, the fungus produces secondary metabolites, which have a toxic effect on the host. Thus, the efficiency of this fungus is due not only to its physical proliferation, but also to its chemical action. Studies on insects have shown that there is a direct relationship between *M. anisopliae* virulence and destruxin production (PAL et al., 2007). However, the mechanisms for this effectiveness against ticks are unclear.

Destruxins are toxic secondary metabolites produced by entomopathogenic fungi, including *M. anisopliae*, and are considered to have insecticidal properties. This group of cyclic depsipeptides is composed of five amino acids and one α-hydroxy acid. Thirty-eight destruxin analogues have been reported to date (SCHRANK;

VAINSTEIN, 2010). They differ in the R-group of the hydroxyl acid residue and appear to have overlapping but different biological effects. These effects include insecticidal, herbicidal and antiviral ones (HU; REN, 2004). However, the mechanism through which destruxin production increases fungal pathogenicity is not totally understood. Studies have reported that destruxins may be involved with insect-cuticle dissolution, immune system suppression and interference in host ion channels (causing tetanic paralysis), as well as other cell dysfunctions (DUMAS et al., 1996; VILCINSKAS et al., 1997). The target organs of these cyclic peptides in insects generally include the gut, Malpighian tubules and circulating hemocytes (DUMAS et al., 1996). The reports on insects also include different modes of action, such as contact, antifeedant and growth regulation modes. Nevertheless, there are no reports on the effects of destruxin on ticks. Moreover, clarification of the mechanisms involved in the process of tick infection by fungi is a crucial step towards developing new biological agents that can be used against these ectoparasites.

The purpose of this study was to evaluate the effects of destruxin A from *M. anisopliae* on engorged *R.* (*B.*) *microplus* females, using immersion and inoculation methods for infection. This communication reports the first comprehensive bioassays on destruxins acting against ticks.

Material and Methods

Rhipicephalus (*Boophilus*) *microplus* colony was maintained at the Federal Rural University of Rio de Janeiro, which is located in Seropédica, RJ, Brazil. To prepare the destruxin solution, one milligram of destruxin A from *M. anisopliae* (Sigma®; D4921 Destruxin A) was diluted in 1 mL of sterile distilled water. Firstly, 200 µL of acetone PA (Merck®) was added to optimize the dilution, followed by 500 µL of sterile distilled water. After stirring, the solution was placed in a water bath for acetone evaporation. The final volume was then made up to 1 mL by adding 500 µL of water.

For the immersion bioassays on engorged females, four groups containing six engorged female ticks of similar weights were tested: a control group and three treatment groups using destruxin A at concentrations of 5, 10 or 20 parts per million (ppm). The destruxin solutions were diluted, according to each concentration, in sterile distilled water plus 0.05% Tween 80. In the control group, the engorged females were immersed only in sterile distilled water with 0.05% Tween 80. Each engorged female was immersed in the solution for five minutes, as described by Drummond et al. (1973), with modifications.

In the inoculation bioassay, a perforation was made at the body insertion of the fourth leg, using a hypodermic needle. The inoculum was individually calculated based on the weight of each female, as 0.075, 0.15 and 0.3 mg of destruxin per gram of tick. Two control groups were established for the inoculation bioassay: one composed of engorged females injured by a needle, with no inoculation, and the other composed of females inoculated with 5 µL of physiological solution. Each inoculated group was composed of six engorged female ticks, of similar weights. The immersion and inoculation bioassays were performed twice.

After treatment, the engorged females were placed in Petri dishes, labeled and incubated at 27 ± 1 °C and RH ≥ 80%. Some biological parameters of the females were analyzed to determine the effects of destruxin A on *R.* (*B.*) *microplus*. The egg mass laid by each female was weighed daily and placed into individual test tubes. The eggs were then incubated at the same temperature and RH, to allow the larvae to hatch.

The main parameters studied were: oviposition period (OP), hatching percentage (HP), egg production index (EPI) and nutrient index (NI). The EPI was calculated as the ratio between the total weight of eggs and the initial weight of engorged females and the NI was calculated as the ratio between the total weight of eggs and the subtraction between the initial and residual female weights (BENNETT, 1974). The parametric data (OP) were assessed using analysis of variance followed by the Student-Newman-Keuls test. The nonparametric data (NI, EPI and HP) were assessed using the Kruskal-Wallis test followed by Student's *t* test. P values less than 0.05 were considered to be significant.

To detect whether there was any tetanic paralysis, the movement of the engorged females' legs was observed.

To test the viability and effectiveness of the destruxin A used in these bioassays, larvae of *Galleria mellonella*, a species known to be sensitive to this toxin (DUMAS et al., 1996; ROBERTS, 1966; VILCINSKAS et al., 1997), were also subjected to treatment. The larvae were provided by the Brazilian Agriculture and Livestock Research Company (Empresa Brasileira de Pesquisa Agropecuária; Embrapa), from the Embrapa Dairy Cattle Research Unit, Juiz de Fora, MG, Brazil. Twelve final-instar *G. mellonella* larvae (around 250 mg) were distributed into two groups. In the first group, 50 µL of sterile distilled water was applied to the body surface of each larva and the same volume of destruxin solution, at 10 ppm, was applied to each larva in the second group. The bioassays were performed twice.

Results

There was no statistically significant difference ($P \geq 0.05$) between the groups treated with destruxin A and the control groups. In other words, not only was there no contact virulence, but also the females' biological parameters that were analyzed did not differ from those of the control groups, even when the toxin was injected. The data are shown in Tables 1 and 2. The treated engorged females did not present any paralysis just after infection.

Galleria mellonella larvae treated with destruxin A presented immediate tetanic paralysis, while the water-treated group did not suffer any paralysis. This result indicates that the destruxin A solutions were viable.

Discussion

Although in this study the doses applied to the female ticks were above the LD_{50} for insects (AMIRI et al., 1999; THOMSEN; EILENBERG, 2000; SREE et al., 2008), the females also remained alive and did not show any change during the oviposition process. Similarly, Hu et al. (2009) reported there was no significant difference in oviposition shown by the whitefly *Bemisia tabaci*

Table 1. Biological parameters of engorged *Rhipicephalus* (*Boophilus*) *microplus* females immersed in destruxin A and incubated at 27 ± 1 °C and RH ≥ 80%.

Group	OP (days)	EPI (%)	HP (%)	NI (%)
control	17.5 ± 2.72[a]	62.84 ± 8.79[a]	98.63 ± 0.74[a]	81.14 ± 9.69[a]
5 ppm	16.88 ± 2.53[a]	61.94 ± 7.09[a]	98.25 ± 0.71[a]	79.9 ± 10.32[a]
10 ppm	17.63 ± 2.06[a]	62.84 ± 8.21[a]	96.25 ± 6.6[a]	81.93 ± 9.83[a]
20 ppm	15.88 ± 4.36[a]	49.82 ± 20.44[a]	85.25 ± 29.08[a]	79.23 ± 10.24[a]

OP: oviposition period; HP: hatching percentage; EPI: egg production index; NI: nutrient index; ppm: parts per million. The data are expressed as mean ± standard deviation. Means followed by the same letter in the same column do not differ statistically (P ≥ 0.05).

Table 2. Biological parameters of engorged *Rhipicephalus* (*Boophilus*) *microplus* females inoculated with destruxin A (mg per gram of tick) and incubated at 27 ± 1 °C and RH ≥ 80%.

Group	OP (days)	EPI (%)	HP (%)	NI (%)
IJ	17 ± 1.77[a]	61.98 ± 6.27[a]	96.0 ± 3.93[a]	79.92 ± 7.35[a]
INps	16.5 ± 2.88[a]	54.84 ± 6.82[a]	96.0 ± 3.93[a]	75.28 ± 5.32[a]
INd 0.075	16.25 ± 1.28[a]	59.37 ± 8[a]	96.88 ± 1.55[a]	79.22 ± 10.33[a]
INd 0.15	16.13 ± 1.81[a]	57.24 ± 10.98[a]	97.25 ± 3.45[a]	77.55 ± 11.85[a]
INd 0.3	16.38 ± 1.69[a]	56.91 ± 12.21[a]	98.0 ± 1.31[a]	77.86 ± 11.81[a]

OP: oviposition period; HP: hatching percentage; EPI: egg production index; NI: nutrient index; IJ: females only injured by the needle; INd: females inoculated with destruxin; INps: females inoculated with physiological solution. The data are expressed as mean ± standard deviation. Means followed by the same letter in the same column do not differ statistically (P ≥ 0.05).

between a group treated with destruxins and the control group. Since the toxicity of molecules is usually attributed to the interaction between the substance and its target protein (HU et al., 2009), the results suggest that target proteins were absent from the organs or tissues relating to the tick oviposition process. Furthermore, based on these results, destruxin A production was not a determining factor for entomopathogenic fungal virulence towards *R.* (*B.*) *microplus* females, since the hosts' biological parameters did not change. This is an important observation with regard to clarifying the process of fungal infection in tick biological control.

Bioassays using other destruxin analogues are required in order to determine the real role of production of fungal secondary metabolites and their effects on ticks.

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