PATHOGENICITY UNDER LABORATORY CONDITIONS OF THE FUNGI *BEAUVERIA BASSIANA* AND *METARHIZIUM ANISOPLIAE* ON LARVAE OF THE TICK *RHIPICEPHALUS SANGUINEUS* (ACARI: IXODIDAE).

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SUMMARY: The in vitro pathogenicity of three isolates of fungi *Metharizium anisopliae* (Ma 959, Ma 319 e Ma E9) and two isolates of *Beauveria bassiana* (Bb 986 e Bb 747) was evaluated on the tick larvae of *Rhipicephalus sanguineus* kept under controlled conditions (27°C and 80% R.H.), or under room temperature. Four concentrations of each isolate (10^5, 10^6, 10^7 e 10^8 conidia/ml) were tested to evaluate larval mortality rates. The lethal doses to 50% (LD_{50}) and to 90% (LD_{90}) of the population were assessed. The mean larval mortality in treated groups ranged from 13.3 to 100%, depending upon the used isolate and its concentration. Larval mortality in the control group ranged from 10 to 46.6%. The most pathogenic concentration was Ma 319 kept at 27°C, and 80% R.H. (LD_{90} = 3.5 x 10^5 conidia/ml).

KEY WORDS: *Rhipicephalus sanguineus*, *Beauveria bassiana*, *Metharizium anisopliae*, entomopathogenic fungi, microbial control.

INTRODUCTION

The tick *Rhipicephalus sanguineus* (Latreille, 1806) is an ectoparasite that can be a vector for several pathogenic agents, mainly for the Canidae family, such as *Babesia canis*, *Hepatozoon canis* and *Ehrlichia canis* (CHRISTOPHERS, 1907; DAVOUST & PARZY, 1989; CONCEIÇÃO-SILVA *et alii*, 1988). Furthermore, its saliva toxins cause host reactions: a remarkable irritation, and intense itching. Because of scratching, skin lesions appear, being susceptible to bacterial infection and myiasis.

The fungi *Metharizium anisopliae* (Metscnikof) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin, both from the Hyphomycetes Class, are the species widely studied for pest control purposes, probably because of their wide geographical distribution and variety of hosts (ALVES, 1986). The majority of research conducted with these fungi had the control of agricultural pests as a target although some authors already showed good results using *B. bassiana* and *M. anisopliae* for the control of livestock pests.

BITTENCOURT *et alii* (1994) tested the efficacy of *M. anisopliae* against eggs and larvae of *Boophilus microplus*. They observed a reduction of larval hatching and an increase of tick mortality, when compared to controls.

The use of *B. bassiana* conidia suspensions on engorged larvae of *R. sanguineus* reduced the ecdisis rate, in proportion to the increase of concentration (BARBOSA *et alii*, 1996).

The purpose of this work was to verify the in vitro pathogenicity of three isolates of *Metharizium anisopliae* (Ma 959, Ma 319 e Ma E9) and two isolates of *Beauveria bassiana* (Bb 986 e Bb 747) on larvae of the tick *Rhipicephalus sanguineus* kept under different temperatures.

MATERIALS AND METHODS

The experiment was conducted at the Station for Parasitological Research W. O NEITZ, from the Departamento de Parasitologia Animal, Instituto de Biologia, Universidade Federal Rural do Rio de Janeiro.

Rhipicephalus sanguineus engorged females were collected from naturally infested dogs in Campo Grande, Rio de Janeiro, and colonized under laboratory conditions. Larvae obtained from the colony were used in the experiments with the fungi *B. bassiana* and *M. anisopliae*.

The eggs of *R. sanguineus* were separated in 150 batches of 25 grams each and placed in 30 ml test tubes. Seventy-five

of these tubes were kept under controlled conditions of temperature and relative humidity (27°C and ± 80% R.H.). The remainder 75 tubes were kept at room temperature. After hatching, the larvae were used in the tests.

The three isolates of *M. anisopliae* used were named as follows: MaE9 (standard isolate), Ma319 (ant isolate) and Ma959 (tick isolate). Two isolates of *B. bassiana* were used: Bb986 (tick isolate) and Bb747 (ant isolate). The suspensions of each isolate were made with conidia grown in rice medium, using deionized water. Three drops of the surfactant Tween 80 were added to each 100 ml of deionized water. The suspensions were titrated in four different dilutions (10^8, 10^7, 10^6 e 10^5 conidia/ml). The control group was treated in a suspension of deionized water plus Tween 80 prepared as previously described. Each treatment was made on three larval replicates hatching from 25 mg of eggs.

The test tubes with larvae were sealed with cotton plugs. Ten ml of each suspension under test were injected into the tubes by means of a disposable syringe with needle. After 2 minutes shaking the tubes were turned up side down to discard the excess of suspension, and then returned to their previous environment of temperature and relative humidity. After 10 days of exposition to the fungi, the mortality rate was assessed under a stereoscopic microscope. Mortality was calculated in relation to the controls (ABBOTT, 1925).

From these results, the lethal doses (LD_{50} and LD_{90}) were assessed by probit analysis (FINNEY, 1971; LITCHFIELD & WILCOXON, 1949).

RESULTS AND DISCUSSION

The per cent mortality of each treatment and the infective means are presented in Tables 1 and 2. The lethal dose values for 50 and 90% of the population are shown in Figures 1 and 2.

The mean per cent mortality in groups treated with different isolates of *B. bassiana* e *M. anisopliae* was greater than in the control groups. The single exception was the isolate Ma 319 kept at room temperature, in which it 13.3% mortality occurred in the suspensions with 1.24×10^5 and 10^6 conidia/ ml, against 30% for the controls (Table 1). BITTENCOURT *et alii* (1994) tested two isolates of *M. anisopliae* on *B. microplus* larvae and found mortality rates ranging from 29.3% up to 97.3% according to the suspension used. This was also high when compared to controls (6.7% up to 7.3%). The high mortality rate found among the control groups disagrees with the results found by BITTENCOURT *et alii* (1994). This result might be caused by the presence of other contaminant microorganisms in the culture medium by the time of isolation procedures.

Table 1 - Mortality rates of *Rhipicephalus sanguineus* larvae treated with different isolates and concentrations of *Metarhizium anisopliae* (Ma) ten days after inoculation.

	DL_{50}	DL_{90}
Ma E9 27C	140000	4000000
Ma E9 r.t.	77000	1.60×10^9
Ma 959 27C	78000	4400000
Ma 959 r.t.	3000000	1.20×10^8
Ma 319 27C	13300	350000
Ma 319 r.t.	4300000	4.00×10^9

r.t. = room temperature

Table 2 - Mortality rates of *Rhipicephalus sanguineus* larvae treated with different isolates and concentrations of *Beauveria bassiana* (Bb) ten days after inoculation.

	DL_{50}	DL_{90}
Bb 747 27C	1200000	2.10×10^7
Bb 747 r.t.	2150000	5.30×10^8
Bb 986 27C	475000	1.20×10^7
Bb 986 r.t.	220000	1.10×10^9

r.t. = room temperature

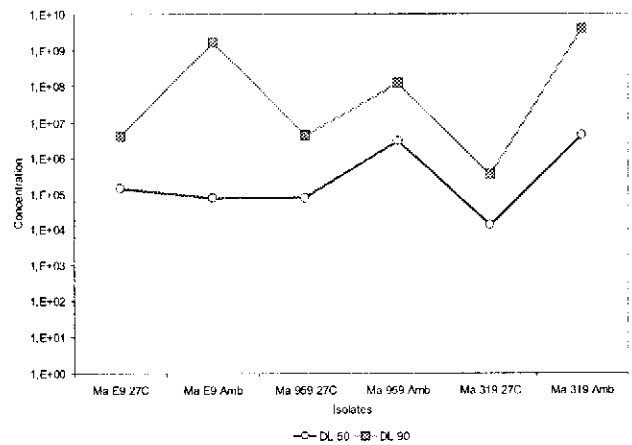

Fig. 1 - Lethal doses to 50% and 90% of *Rhipicephalus sanguineus* larvae treated with different isolates of *Metarhizium anisopliae* (Ma) under different temperatures.

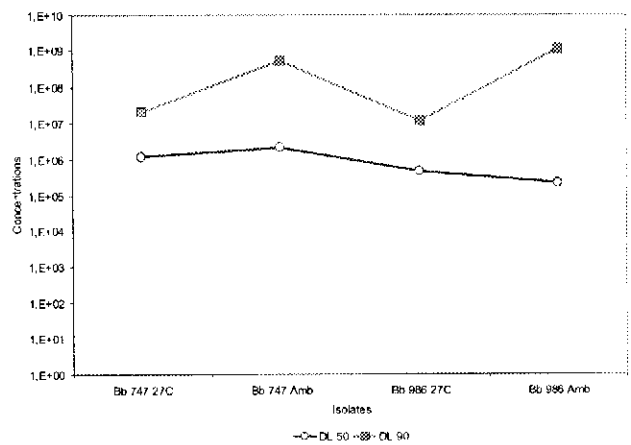

Fig. 2 - Lethal doses to 50% and 90% of *Rhipicephalus sanguineus* larvae treated with different isolates of *Beauveria bassiana* (Bb) under different temperatures.

BITTENCOURT *et alii* (1996) evaluated the action of *B. bassiana* on larvae of *B. microplus* kept under controlled environment (27°C and + 80% R.H.) and found out a mean per cent mortality ranging from 29.33% to 66% for the suspensions with 10^5 conidia/ ml. In the present experiment the mortality rate was from 13.3% to 96% for *M. anisopliae* (Table 1) and from 60% to 90% for *B. bassiana* (Table2). These same authors also observed a maximum mortality of 82% for the suspension with 10^8 conidia/ ml, contrasting with a 100% mortality occurring in the present work. These finding may suggest that such variation was due to the higher susceptibility of *R. sanguineus* larvae to the effect of the used microorganisms, than *B. microplus* larvae.

Supporting this hypothesis is also the result of probit analysis conducted with data concerning the mortality rates of larvae treated with *M. anisopliae* isolates, in which the lethal doses (LD_{50}) ranged from 1.3×10^4 up to 4.3×10^6 conidia/ ml (Fig. 1). For the *B. bassiana* isolates this value ranged from 2.2×10^5 to 1.2×10^6 conidia/ ml (Fig. 2). In the paper by BITTENCOURT *et alii* (1996) the LD50 found for *B. bassiana* isolates ranged from 6.83×10^6 and 1.01×10^7 conidia/ ml.

Larvae treated with the Ma E9 isolate and kept under controlled temperature (±27 °C) showed a mortality higher than 80% in all suspensions (Table 1); and the probit analysis showed a mean LD_{50} of 1.4×10^5 conidia/ ml (Fig. 1). Such result was different from the one found among larvae treated with the same isolate but kept under room temperature, in which the mean mortality was 70% (Table 1), although without variation in the LD_{50}.

The Ma 319 isolate produced 100% mortality in suspensions with 1.24×10^8 e 1.24×10^7 conidia/ ml when kept at 27 °C. For larvae kept under room temperature the per cent mortality was from 63.3% to 66.6% (Table 1). The LD_{90} was 4.0×10^9 conidia/ ml in larvae kept under room temperature and 3.5×10^5 for those kept at 27 °C (Fig. 1). The low mortality observed for the 10^5 and 10^6 suspensions reflected high LD_{50} and LD_{90} values.

B. bassiana produced the highest larval mortality index (100%) with the suspension containing 10^8 conidia/ ml kept at 27 °C (for both isolates). The lowest larval mortality index (60%) was found in the suspensions with 10^5 and 10^6 conidia/ ml of the following isolates: Bb 747 kept at controlled temperature and Bb 986 kept under room temperature (Table 2). The LD_{50} and LD_{90} observed for these isolates were respectively 1.2×10^7 and 1.1×10^9 conidia/ ml (Fig. 2). It was also observed that a huge increase in the number of conidia is required in the suspension to reach the LD_{90} (considering LD_{50} as the initial dose) (Figs. 1 and 2). After perusing these figures, it was evidenced that for all evaluated isolates the LD_{90} was higher in samples kept underat room temperature, when compared to those kept under controlled environmental conditions. Such data suggested that field results may be different from those observed in the laboratory. BITTENCOURT *et alii* (1994) and CASTRO *et alii* (1996), using the same 959 isolate of *M. anisopliae* for control of *B. microplus* larvae, also observed different results comparing laboratory and field data.

A few hours after immersion in the fungi, larval motility slows down, equilibrium is affected, and hyphas make their appearance on the idiosomas. These hyphas were identified as coming from *M. anisopliae* and *B. bassiana*, according to the suspension used for the infection. Such findings corroborate the action of these fungi on the biology of the ixodid.

After these experiments with *M. anisopliae* and *B. bassiana* on larvae of *R. sanguineus* we conclude that those fungi are pathogenic for the larvae. We also found, through analysis of LD_{90}, that the most effective isolate was the Ma 319 kept at 27 °C (LD_{90} was 3.5×10^5 conidia/ ml). For the isolate Ma E9 kept under the same conditions the LD_{90} was 4.0×10^6 conidia/ ml (Figs. 1 and 2). It was also verified that using controlled environments we get more uniform data, specially on control groups.

Although more research is needed to evaluate the effect of these fungi under field conditions, the results presented in this paper indicate a high potential for using these fungi in the integrated control of *R. sanguineus*.

SUMÁRIO

A patogenicidade in vitro de três isolados do fungo *Metharizium anisopliae* (Ma 959, Ma 319 e Ma E9) e dois isolados de *Beauveria bassiana* (Bb 986 e Bb 747) foi avaliada em larvas do carrapato *Rhipicephalus sanguineus* mantidas em temperatura controlada (27°C e 80% U.R.) e ambiente. Quatro concentrações (10^5, 10^6, 10^7 e 10^8 conídios/ml) de cada isolado foram testadas para avaliar o percentual de mortalidade das larvas, calculando-se as doses letais para 50% (DL_{50}) e 90% (DL_{90}) da população. A média de mortalidade nos grupos tratados variou entre 13,3 e 100% dependendo do isolado e da concentração utilizada e a mortalidade de larvas no grupo controle variou entre 10 e 46,6%. A concentração mais patogênica foi o Ma 319 mantido em 27°C, e 80% U.R. (DL_{90} = $3,5 \times 10^5$ conídios/ml).

PALAVRAS-CHAVE: *Rhipicephalus sanguineus*, *Beauveria bassiana*, *Metharizium anisopliae*, fungos entomopatogênicios, controle microbiano.

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