EFFECTS OF ISOLATE 986 OF THE FUNGI *BEAUVERIA BASSIANA* (BALS.) VUILL., ON EGGS OF THE TICK *ANOCENTOR NITENS* (NEUMANN, 1897) (ACARI:IXODIDAE).

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SUMMARY: Laboratory tests were conducted under controlled conditions (27 °C and 80% relative humidity - R.H.) with the entomopathogenic fungi *Beauveria bassiana* (isolate 986) to verify its patogenicity on eggs of the tick *Anocentor nitens*. Ten 50 mg egg batches were immersed in each conidia suspension tested (10^5, 10^6, 10^7 and 10^8 conidia/ml) plus a control group, of distilled water and spreading adhesive. The following parameters were assessed: egg incubation period, hatching period, hatching percentage and larval mortality rates ten days after the end of hatching period. The results obtained according to statistical analysis showed a significant difference for the incubation period, which was smaller in the treated groups (average of 22 days) when compared to the control group (average of 25 days); Ten days after hatching, larval mortality rates were higher than 80% in the treated groups, while in the control group such rate was smaller than 3%. The larval hatching period showed a significant difference at the 10^8 conidia/ml concentration when compared to the control group (averages of 4.1 and 6.6 days, respectively). Larval hatching rates did not show significant differences when several treatments were compared to controls. These results suggest that isolate 986 of the fungi *B. bassiana* interferes in the development of stages of the biological cycle of this tick representing an interesting alternative for its use in biological control.

KEY WORDS: Biological control, *Anocentor nitens*, fungi, *Beauveria bassiana*.

INTRODUCTION

The tick *Anocentor nitens* belongs to the Ixodidae family and it is responsible for transmitting several agents of equine diseases. DENNING (1988) mentions this species as a vector of equine piroplasmosis; and points out also, that the lesion caused by tick puncture can facilitate bacterial infection and myiasis, mutilating the ears.

Beauveria bassiana is a deuteromycete fungi commonly found in soil. It is considered by several authors (KAAYA *et alii*, 1996, MWANGI, 1990, BITTENCOURT *et alii* 1992) as pathogenic to some tick species, being widely used on the biological control of agricultural pests (ANDERSON, 1982). A great advantage of this fungi over conventional pesticides is its persistence on the host population, and its ability to reduce longevity, increasing mortality rates of larval and adult insect populations (ROBERTS & CASTILLO, 1980). MONTEIRO (1997) evaluated the effect of four concentrations of the fungi *B. bassiana* (isolate 986 and 747) on eggs of *Rhipicephalus sanguineus* and observed that larval hatching of treated eggs at different concentrations was lower than that of controls. The entomopathogenic fungus *Metarhizium anisopliae* is highly pathogenic to the black-legged tick, *Ixodes scapularis* (ZHIOUA *et alii*, 1997) and shows considerable potential as a microbial control agent for the management of this tick.

The objective of this work is to evaluate the *in vitro* effect of isolate 986 of the fungi *B. bassiana* on eggs of the tick *A. nitens* under laboratory conditions.

MATERIALS AND METHODS

A. nitens engorged females were collected from naturally infested horses. The ticks were washed with distilled water, dried, and then placed individually in Petri dishes and kept under controlled environmental conditions (27°C and 80% R. H.) to lay eggs. After the beginning of egg-laying, the egg masses were weighed and separated in aliquots of 50mg. They were then placed in test tubes with cotton plugs; and returned to the previously described controlled conditions.

The fungi *B. bassiana* (isolate 986) was chopped previously in rice medium (ALVES, 1986) in order to collect large amounts of conidia. Later on, four suspensions with different concentrations (10^8, 10^7, 10^6, 10^5 conidia/ml) were prepared using distilled water and spreading adhesive at 1%. The count were made with the help of a Neubauer chamber.

Ten replicates were used for each concentration tested. Each one placed in a test tube with 50 mg of eggs. The test tubes were immersed in three ml of each suspension for three minutes (treated groups); after what they were turned up side down to discard the suspension surplus. The control group was also composed by ten test tubes with 50 mg of eggs each. They were immersed in three ml of water plus spreading adhesive (1%), for the same time used in the treated groups.

The parameters assessed were: egg incubation period, hatching period, hatching percentage and larval mortality rates ten days after the end of hatching period. The statistical procedures used were analysis of variance (ANOVA) followed by the Tukey-Kramer and Kruskall Wallis tests.

The non hatched material was washed in sodium hypoclorite for one minute, rinsed in de-ionized water, dried in filter paper and placed under controlled environmental conditions (27°C, 80% R.H.) for fungi isolation.

RESULTS AND DISCUSSION

The results obtained are presented in Table 1.

The incubation period was significantly smaller for all treatments when compared to controls. The biological data observed in controls (mean incubation period of 25.1 days) is in agreement with SANAVRIA *et alii* (1996), who used the same environmental conditions in his study of the biology of this tick. BORGES' *et alii* (1993) observed an average of 23 days and DESPINS (1992) using the temperature of 25°C and 75% R.H. found 28.6 days for that parameter. Such results demonstrate the reliability of our data. BITTENCOURT *et alii* (1996) tested the fungi *B. bassiana* on eggs of *Boophilus microplus* kept under 27°C and 80% R.H., observing that the incubation period was larger on the treated group in the controls, with an average of 28 to 30.67 days for the 10^8 conidia/ml concentration and 21.33 to 22 days for the control group. BITTENCOURT *et alii* (1994a) tested the fungi *Metarhizium anisopliae* on eggs of *B. microplus* kept under the same conditions mentioned above and also registered an increase in the incubation period on treated groups when compared to controls. The difference found between the two works was due to the methodology used, which quantified all groups that started hatching until the last day of evaluation and not until the last day of hatching. MONTEIRO (1997) tested the fungi *B. bassiana* under laboratory conditions (27°C, 80% R.H.) on eggs of *Rhipicephalus sanguineus* and verified that the incubation period for treated groups did not present any significant difference when compared to controls after the statistical analysis he. Thus, it was demonstrated that the fungi can act in a different way depending upon tick species.

The larval hatching period was inversely proportional to the concentration used. The values found were 4.1 and 5.4 days for 10^8 and 10^5 conidia/ml; and 6.6 days for the control group. Significant variation occurred only between the 10^8 conidia/ml concentration and controls. BITTENCOURT *et alii* (1994a), working with the entomopathogenic fungi *M. anisopliae* on eggs of *B. microplus*, did not found such lack of statistical significance, and observed a larger hatching period in all groups treated with different concentrations when compared to controls. They found a hatching period of 6.8 days for controls and of 13.83 days for the concentration with 10^8 conidia/ml. BITTENCOURT *et alii* (1996) conducted a study with the tick *B. microplus* infected with *Beauveria bassiana* under the same temperature and humidity, and found also a larger hatching period for treated groups than for controls (an average of 8.6 to 9.6 days for the concentration of 10^8 conidia/ml and 5.3 to 5.6 days for the control group). Once again was stated that the pathogenic effect of these fungi depends upon the susceptibility of treated species.

The larval hatching percent rates were lower in the treated groups when compared to controls, but did not present significant differences between treatments and/or with controls after statistical analysis. A mean hatching rate of 82.1% was observed for the control group, similar to the values found by BORGES *et alii* (1993) and DESPINS (1992). Other works that used fungi on tick egg presented different results. At the present work, the treatments showed a hatching rate of 66.44% at the highest concentration used (10^8), and diminished when the concentration was reduced (82,5% for 10^5 conidia/ml). BITTENCOURT *et alii* (1994b and 1996) tested the fungi *M. anisopliae* and *B. bassiana* on eggs of *B. microplus* kept at 27°C and 80% R.H., and found a hatching rate for treated groups ranging from 3.3 to 66% for *M. anisopliae* and from 20 to 86,6% for *B. bassiana*, and rates of 91.3 (*M. anisopliae*) and 93.3% (*B. bassiana*) for controls. Such findings show that this fungi has a greater impact on hatchability of *B. microplus* larvae when compared to *A. nitens* larvae.

The larval mortality rate among treated groups for all treatments varied significantly when compared to controls, since that ten days before the end of hatching, the mortality rate among treated groups was higher than 85%, against a control mortality of only 3%. MONTEIRO (1997) tested the fungi *B. bassiana* in larvae of *R. sanguineus* kept under the same environmental conditions of this work and found mortality rates ranging from 30 to 40% for the controls and from 66.6 to 100% among treated groups. These results demonstrate the susceptibility of larvae to this fungi, both regarding to the development conditions inside the egg as well as after hatching. It was possible to conclude that *A nitens* larvae has a higher susceptibility to the fungi *B. bassiana* than its eggs, showing a high mortality rate ten days after hatching, when the contact with conidia occurs.

The material isolated in humid chamber confirmed the presence of fungi used in the infestations.

The results obtained after evaluation of the entomopathogenical potential of *B. bassiana* (isolate 986) for eggs of *A. nitens* is promising and encourages further research on biological control as a viable and reliable alternative.

SUMÁRIO

Testes em laboratório realizados em condições controladas (27°C e 80% U. R.) com o fungo entomopatogênico *Beauveria bassiana* (isolado 986) foram feitos para verificar a sua patogenicidade para ovos do carrapato *Anocentor nitens*. Para tal, realizou-se a imersão de dez lotes de 50 mg de ovos para cada suspensão de conídios testada (10^5, 10^6, 10^7 e 10^8 conídios/ml) e um grupo controle onde utilizou-se apenas água destilada mais espalhante adesivo. Os parâmetros analisados foram: período de incubação dos ovos, período de eclosão, porcentagem de eclosão e porcentagem de mortalidade das larvas após dez dias do final da eclosão. Os resultados obtidos após a realização dos testes estatísticos evidenciaram uma diferença significativa no período de incubação, o qual foi menor nos grupos tratados (média de 22 dias) quando comparado ao grupo controle (média de 25 dias); a porcentagem de mortalidade das larvas foi superior a 80% nos grupos tratados após 10 dias do final da eclosão, enquanto no grupo controle esse índice foi menor que 3%. O período de eclosão das larvas teve diferença significativa apenas na comparação da concentração 10^8 conídios/ml com o grupo controle, que foi em média de 4,1 e 6,6 dias respectivamente, e o parâmetro de porcentagem de eclosão de larvas não demonstrou diferença significativa quando comparados os diferentes tratamentos com o grupo controle. Esses resultados sugerem que isolado 986 do fungo *B. bassiana* interfere no desenvolvimento de etapas do ciclo biológico deste carrapato representando uma alternativa interessante para a sua utilização em controle biológico.

PALAVRAS-CHAVE: Controle biológico, *Anocentor nitens*, fungo, *Beauveria bassiana*.

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(Received 5 March 1998, Accepted 17 May 1999)