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Effect of Metarhizium robertsii in Rhipicephalus microplus hemocytes

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Abstract : The bovine tick, Rhipicephalus microplus, is an arthropod of great importance in veterinary medicine leading to anemia, weight loss, animals' leather depreciation and also acting as a vector of many pathogens. In this way, the parasitism causes a loss of 3.24 billion dollars per year in Brazil. Knowingly, entomopathogenic fungi act as natural controller of some arthropods, acting mainly by active penetration through the cuticle. However, it can also act on the hemolymph and through the production of mycotoxins. Hemocytes are responsible for the cellular immune response and participate in the processes of phagocytosis, nodulation and encapsulation and may undergo changes when challenged by pathogens. The aim of the present study was to evaluate changes in R. microplus hemocytes after inoculation of Metarhizium robertsii using transmission electron microscopy. The isolate ARSEF 2575 and 200 engorged R. microplus females were used. The groups were divided into control, in which the females were inoculated with 5 µL of sterile distilled water solution and 0.1% Tween 80, and a group inoculated with 5 µL of fungal suspension at the concentration of 10^7 conidia mL^{-1}. The experiment was performed in duplicate and each group contained 50 females. Twenty-four hours after fungal inoculation, hemolymph was collected through the cuticle dorsal surface perforation of the tick females. After collection, the hemolymph samples were centrifuged at 500 x g for 3 minutes at 4 °C, the plasma was discarded and the hemocyte pellet was resuspended in 50 µl PBS. The suspension material was fixed in 2% glutaraldehyde in Millonig buffer for three hours. After fixation, the material was centrifuged at 500 x g for 3 minutes, the supernatant was discarded and the cells were resuspended in a wash solution. Subsequently, the cells were post-fixed with 1% osmium tetroxide in phosphate buffer for one hour at room temperature and dehydrated in increasing concentrations of ethanol, and then embedded in Epon resin. The ultrathin sections were examined under the LEO EM 906E transmission electron microscopy at 80kV. The ultrastructural results revealed that.in control group, the cells were considered intact, in which the granulocytes were observed with granules of different electrodensities, intact mitochondria and cytoplasm without vacuolization. In addition, granulocytes showed plasma membrane projections similar to pseudopodia. Plasmatocytes presented as irregularly shaped cells, with the eccentric nucleus, agranular cytoplasm and some cells presented pseudopodia. Nevertheless, in the group exposed to the fungus, most of the cells presented in degeneration. The granulocytes found had fewer granules in the cytoplasm and more vacuoles. Plasmatocytes, after treatment, presented many vacuoles also in the cytoplasm and the lysosomes presented great amount of electrodense material in their interior. Thus, the results suggest that the fungus has a depressant action in the immune system of the tick, not only by the cell degranulation, but also suggesting that this leads to morphological changes in the hemocytes and may even trigger processes such as phagocytosis.

Keywords : bovine tick, cellular defense, entomopathogenic fungi, immune response
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