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# MENADIONE INCREASING THE VIRULENCE OF *Metarhizium anisopliae* s.s. TO *Rhipicephalus microplus* LARVAE

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There are several *in vitro* and *in vivo* studies relating the susceptibility of *Rhipicephalus microplus* to *Metarhizium* spp. However, it is known that toxic metabolites, especially those with oxidizing potential, can both interfere on the fungal growth and modulate the interaction between the pathogen and the host. In this context, menadione is a synthetic quinone, commonly used in laboratory to evaluate cell viability, which acts in the production of superoxide radicals by redox cycling. Due to this, first, we aimed to determine the oxidative effects on the germination of *Metarhizium anisopliae* s.s. (ARSEF 2521) after growth on potato-dextrose-agar medium (PDA) plus 0.002% Benomyl and supplied with menadione ranging from 0.01 to 0.13 mM. Twenty-four hours after inoculation, the isolate showed low tolerance to menadione with a median lethal concentration (LC 50) of 0.038 ± 0.0051 mM. Second, we tested the oxidative effects on fungal development and conidia production, growing for 15 days in supplemented PDA (PDA MEN) or not (PDA CTR) with half of the menadione LC 50 (0.019 mM). The colonies of PDA MEN had olive-green color and conidiation restricted to the center, with a white border of cotton-like mycelium. In addition, the presence of menadione reduced the number of conidia quantified per cm² of each colony area by 40%. Finally, for the biological assays to *R. microplus* larvae, we used a control solution (sterile distilled water plus 0.1% Tween 80) and eight aqueous suspensions (four for PDA CTR and four for PDA MEN) in the concentrations of $10^8$, $10^7$, $10^6$ and $10^5$ conidia $mL^{-1}$. The fungal exposure was performed by immersion of the specimens for three minutes, being analyzed 5, 10, 15 and 20 days post-treatment. The PDA MEN conidial suspension at $10^8$ conidia $mL^{-1}$ showed higher larval mortality, killing over 6 times more after 5 days, 3 times after 10 days, and approximately 1.5 times after 15 days, when compared to PDA CTR. In conclusion, our results represent the relevance of studying oxidative stress during media growth and host infection, as well as elucidating that it may act positively on biocontrol efficacy.

**Key-words**: fungal stress; cattle tick; efficacy; arthropod pathogenic fungi; biological assay
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