Title: USING *Tenebrio molitor* AS AN ALTERNATIVE MODEL HOST TO STUDY *Candida albicans* AND *Cryptococcus neoformans* INFECTIONS

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Abstract:

The use of invertebrates in research has is increasingly growing, as it has significant advantages ethics, logistics and economic on mammalian models and is capable of high performance testing on a large scale and at low cost.Our objective in this study was to evaluate the potential alternative host larvae of *Tenebrio molitor* (Coleoptera order) infected with *Candida albicans* (causes of superficial and invasive infections) and *Cryptococcusneoformans*(causing meningitis). Two standard strains were used in the experiment, *C. neoformans* ATCC 28957 and *C. albicans* SC5314.Larvae weighing around 200 mg were chosen and they were inoculated with a Hamilton syringe in the membranous region, above the legs of the larva. Each larva received 5 µL of a suspension containing $1x10^4$,$1x10^5$, $2x10^5$, $3x10^5$ or$1x10^6$colony forming unit (CFU) of *C. albicans* and$1x10^4$,$1x10^5$, $1x10^6$or $1x10^7$ CFU of *C. neoformans*. There were two control groups, one that was inoculated with sterile PBS and the other with 10^6heat-inactivated cells. Then, the larvae were incubated at 37°C and the number of dead larvae was scored on intervals of 12 hours during 10 days. The results were analyzed by GraphPad Prism 5 program and were arranged in a survival curve using the Kaplan-Meier method. To evaluate the tissue damage, histopathology was performed,infecting the larvae with 10^6CFU/larvae and then incubating for 12 h at 37°C. After this period, the internal structures were collected and fixed in 10% formalin. After 12 hours fixation, the tissue were stainedwith Periodic Acid-Schiff(PAS) for *C. albicans* infected larvae and Gomori'sMethenamine Silver (GMS) for *C. neoformans* infected larvae.The concentration of $1x10^6$ of *C. albicans* was able to kill all larvae in 24 hours while $3x10^5$ CFU killed within 72 hours, other concentrations were not able to kill all larvae, but the result was significantly different compared to controls. For*C. neoformans*, the concentration of $1x10^7$ was capable of killing the larvae in 72 hours and $1x10^6$ in 192 hours.In the photomicrograph,*C. albicans*presented hyphae invading the host tissue and *C. neoformans*presented as yeast containing capsule. Those morphologies are also seem in histological preparations of mammalian infected tissue.It was concluded that *Tenebrio molitor* is a good choice to study the infection of these two fungal species, investigating their virulence traits.

Key-words:*Tenebrio molitor*, *Candida albicans*, alternative model host, *Cryptococcus neoformans.*

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