THE USE OF ENTOMOPATHOGENIC FUNGI AGAINST *ANOPHELES FUNESTUS* Giles (Diptera: Culicidae)

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ABSTRACT

Malaria vector control relies primarily on the application of chemical insecticides. The increasing incidence of insecticide resistance in target vector populations coupled with the threat of environmental contamination are of major concern in terms of this approach. The use of biological agents to complement existing insecticide based control strategies has been proposed, e.g. *Metarhizium anisopliae* and *Beauveria bassiana.* The efficacy of *M. anisopliae* (Metschnikoff) Sorokin strain ICIPE-30 and *Beauveria bassiana* (Balsamo) Vuillemin isolate I93-825 alone or in combination was assessed against laboratory strains of the major malaria vector *Anopheles funestus.*

Samples of adult females from three laboratory strains of *An. funestus* were exposed to dry conidia of *M. anisopliae* and *B. bassiana* for 3, 6 or 24 hours. Of these *An. funestus* strains, Fang was fully susceptible to all insecticides, Fumoz was partly resistant to pyrethroid insecticides and Fumoz-R has been intensively selected for pyrethroid resistance. Following inoculations, the rate of mortality in all strains was approximately 6-fold higher in fungus infected cohorts compared to their corresponding uninfected control cohorts. Susceptibility to fungal infection in the colonies appeared to follow their pattern of susceptibility to insecticide whereby Fang showed higher rates of mortality following fungus infection than Fumoz and Fumoz-R. Further, Mosquitoes placed in forced proximity of fungal spores for only three hours showed significantly lower rates of mortality than those placed in similar circumstances for 24 hours, showing that the probability of acquiring an infection is a function of time and that a longer potential exposure time leads to the acquisition of greater numbers of infective spores. Approximately 99% of all fungus infected mosquitoes (infection confirmed by followup sporulation tests on cadavers) died within 14 days of acquiring their infection. Fourteen days is the maximum time required by malarial parasites to reach the infective sporozoite stage.

Experiments were designed to quantify a possible interaction between susceptibility to the pyrethroid insecticide permethrin and susceptibility to *B. bassiana* or *M. anisopliae* infection, based on the hypothesis that intoxication or infection with one of these agents exerts a synergistic effect on susceptibility to the other. Further, the effect of a non insecticidal substance that inhibits the activity of monooxygenases - piperonyl butoxide (PBO) – on subsequent susceptibility to fungus infection in pyrethroid resistant *An. funestus* was tested. Fumoz-R infected with fungus proved significantly more susceptible to pyrethroid intoxication post fungus infection than uninfected samples from the same cohort. Pre-exposure to PBO did not affect subsequent susceptibility to fungus infection, suggesting that monooxygenases play a negligible role in protection against fungus infection.

Experiments were designed to test for variation in fungus induced mortality rates between blood fed and unfed cohorts of female *An. funestus* as well as to test for differences in fecundity in response to fungal infection. Females blood fed post exposure to fungus showed a slightly higher rate of mortality compared to unfed fungus infected females. Females blood fed prior to fungus infection showed comparable rates of mortality with those of unfed fungus infected cohorts. These results suggest that blood-feeding may affect susceptibility to fungus infection, although the effect is slight at best and of no concern in terms of fungal

pathogenicity. Fungus infected females produced significantly fewer eggs than uninfected females. The proportion of progeny from fungus infected females surviving to adulthood was also significantly reduced by comparison to the progeny of uninfected females.

In order to test the effectiveness of entomopathogenic fungi in semi-field conditions it is important to first survey the local malaria vector species composition including their insecticide susceptibility status. Baseline mosquito surveillance was conducted in the Mamfene region of northern KwaZulu-Natal, South Africa, in order to assess the feasibility of using clay pots treated with dry conidia of entomopathogenic fungi as a delivery/infection system in a field environment. *Anopheles arabiensis*, *An. parensis*, *An. funestus*, *An. merus* and *An. quadriannulatus* were collected inside houses between February and September, 2005. A sample of *An. parensis*, a non-vector, was shown to present false positives for the presence of *P. falciparum* circumsporozoites using the standard ELISA method. This result highlighted the importance of accurate species identification and vector incrimination, and showed how over-reliance on standard methodologies without suitable quality assurance can lead to inaccurate information about malaria transmission dynamics in a given area. Resistance to permethrin (pyrethroid) was detected in *An. arabiensis* and *An. parensis.* Biochemical analysis and insecticide-synergist assays showed monooxygenase elevation leading to monooxygenase based permethrin detoxification in *An. arabiensis.* Preliminary laboratory tests revealed that clay pots treated with dry conidia of *B. bassiana* or *M. anisopliae* are suitable for spore delivery to anopheline mosquitoes resting inside them. However, the efficacy of treated pots, measured in terms of relative infectivity, decreased with increasing time lapse since treatment, so that by 3 months post treatment their efficacy was negligible under standard laboratory conditions.

I conclude that dry conidia of *B. bassiana* and *M. anisopliae* are effective pathogens against *An. funestus*. Infective spores can be delivered using either an attractant such as sucrose or by treating the surfaces of preferred resting sites such as clay pots. Their pathogenicity is not significantly affected by monooxygenase based insecticide resistance, and in almost all cases fungus infected females die within 14 days of acquiring an infection, regardless of bloodfeeding status. Further, fungal infection significantly attenuates the expression of insecticide resistance, and also significantly reduces fecundity and fertility in infected *An. funestus* females. These factors enhance the potential of entomopathogenic fungi as biocontrol agents in areas where resistance to insecticide occurs in target vector populations.