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Title: Mosquito biological control using fungal products

ABSTRACT

Previously, we demonstrated the possibility of *Metarhizium anisopliae* formulation with Suneem 1% against mosquito born disease (*Anopheles gambiae* sl) which develop in suburbs of Dakar (Sénégal). In this project, we performed molecular technique based on PCR assays after DNA extraction, on *Anopheles gambiae* sl complex samples previously collected from 2009 to 2010 in many areas in the suburbs of Dakar. All insects were demonstrated by PCR methods to be *Anopheles arabiensis* the most species of malaria vector in Senegal. In the department of bioindustries at Gembloux Agro-Bio Tech, several techniques of fungi production were tested. Particularly, wheat bran presented an optimal production of fungal mass production in solid, semiliquid and liquid media, much more than rice grain. These results allowed us to consider it to substitute the other cereals that are more expensive. With conidia produced in solid medium, the lethal doses against *Culex quinquefasciatus* larvae were obtained in 24h for *Aspergillus clavatus* ($DL_{50} = 1,7 \cdot 10^8$ spores/ml; $DL_{90} = 7, 2 \cdot 10^8$ spores/ml) $P < 0,0001$, 48h for *Metarhizium anisopliae* ($DL_{50} = 1,6 \cdot 10^8$ spores/ml, $DL_{90} = 6,5 \cdot 10^8$ spores/ml) $P < 0,0001$ and 72 h for *Metarhizium sp* ($DL_{50} = 1,75 \cdot 10^8$ spores/ml; $DL_{90} = 9,9 \cdot 10^8$ spores/ml) $p = 0, 0046$. With liquid medium, we extrapolated the Erlenmeyer flasks system and developed a new method of spores and secondary metabolites (microtoxins) productions in 20 L Bioreactors using a mould materiel (packing) and wheat bran. Two molecular kinds ($M > 10kD$ and $M < 10 kD$) were separated by ultrafiltration. The metabolites solution favorites in 24hours mortalities between 10% to 93.75% ($M > 10 kD$) and 2% to 55% ($M < 10kD$) against *Culex quinquefasciatus* larvae. With Electronic microscopy, we revealed that, *Aspergillus clavatus* spores secreted microtoxins in gut larvae after conidial application.

Transmission Electronic Microscopy revealed that, larval tissues (cuticular epithelium, gut cells and muscle) were gradually destroyed after exposure. In parallel, increasing conidial concentration was showed in the gastrointestinal tract and were activated and presented beginning germinated tubes, vacuoles and oily globules as well as an increasing cell wall. However, *Aspergillus clavatus* conidia produced in bioreactor and formulated with sunflower oil, allows a regressive survival mosquito adults between 53.3% (day 1) to 10.8% (day 4) after spraying 7.9×10^7 spores/ml dose. These results suggested the possibility of simultaneous mass production of microtoxins and conidia against mosquitoes with less expensive substrate in bioindustries.

In parallel, we have demonstrated that *A. clavatus*, *M. anisopliae* and *M. sp* were very effective against survival and reproductive of an other pest model, namely the *Acyrtosiphon pisum* aphid (Aphididae).