

# Biofilms from entomopathogenic fungi in mosquito control

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## Introduction

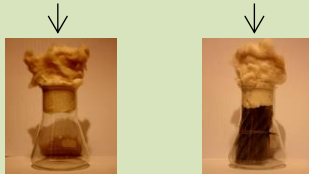
- Mosquitoes (Diptera: Culicidae) are zoonotic vectors responsible for numerous infectious diseases of medical and veterinary importance such as filariasis, malaria and encephalities.
- As part of an integrated vector control, metabolites secreted by entomopathogenic fungi could be developed as biopesticides.
- In this context, filamentous microorganisms growing on a support as a biofilm in a liquid medium would offer several advantages in bioreactor regarding performances and metabolites recovery.

## Objective

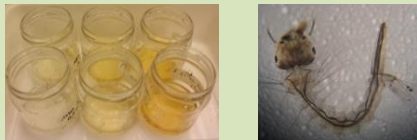
- The production of insecticidal metabolites by the fungus *Aspergillus flavus* growing as a biofilm on a solid support was assessed.

## Materials & Methods

- Three inoculum levels were cultured in shake flask with or without a stainless steel support.  
10<sup>1</sup> - 10<sup>3</sup> and 10<sup>6</sup> spores per ml



- Liquid cultures were filtered to eliminate spores and filaments.
- Toxicity tests were performed on *Culex quinquefasciatus* larvae using dilutions of 1, 2, 4, 6, 8 and 10% of culture filtrates.



- For each treatment, batches of 20 larvae (3rd and 4th instars) were incubated in laboratory conditions (16L/8D photoperiod, 25±2°C and 75-80% RH) in 4 replicates.

## Data analysis

- Mortalities were recorded daily for 72h and corrected with Abbott formula in order to determine LC<sub>50</sub> and LC<sub>90</sub> by regression.

## Results

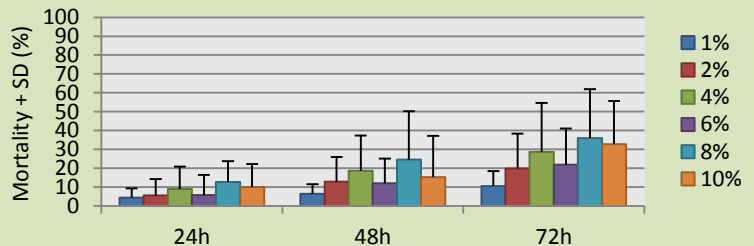
(1) The results indicated that *Aspergillus flavus* tended to form pellets in submerged culture. The size and the amount of pellets were affected by the initial inoculum level of spores.



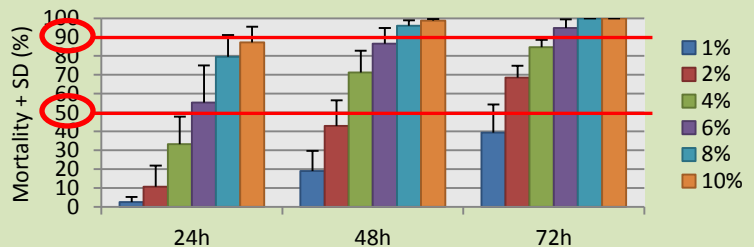
Under similar conditions, the filaments fixed on the stainless steel support and didn't appear in free form in the liquid culture.



(2) Toxicity tests revealed differences between both free and fixed forms. Interestingly, culture filtrates from initial inoculum level of 10<sup>6</sup> spores per ml grown in free form lead to low mortality of *Culex quinquefasciatus* larvae.



By contrast, culture filtrates from equivalent spore level grown on a support lead to high mortality with LC<sub>50</sub> = 2.2% and LC<sub>90</sub> = 7.2% after 48h (p<0,001).



## Conclusions

- Growth of *Aspergillus flavus* as a biofilm on a solid support favored the secretion of insecticidal metabolites in the liquid medium.

## Perspectives

- Secretomes could be compared between these culture conditions by proteomic and metabolomic approaches.