

Using micro-injection technique to assess fungal toxicity in mosquito control

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Introduction

- Topical application of insecticidal compounds allows directly exposing these substances on insect tissues and measuring their toxicity while ignoring many factors.
- But application on mosquito larvae remains difficult considering their aquatic lifestyle.
- Micro-injection could be used for the direct deposition of toxic compounds in the larvae.
- This technique has already been used to highlight the pathogenicity of bacteria and viruses to mosquito larvae.

Objective

- Micro-injection of spores was evaluated for the estimation of the toxicity of entomopathogenic fungi in mosquito control.

Materials & Methods

- Capillaries exhibiting an injection tip with an external diameter of 0.5mm have been designed from silica tubes.
- For each treatment, a capillary is mounted on a pump connected to a flow rate regulator.
- Spores from entomopathogenic fungi (*Aspergillus clavatus*, *Metarhizium anisopliae*, and *Metarhizium* sp) were suspended in Ringer's solution (10^7 spores per ml).
- Culex quinquefasciatus* larvae were injected with 500nl of spore solution.
- Batches were incubated at a 16L/8D photoperiod, $25 \pm 2^\circ\text{C}$ and 75-80% RH.
- The distribution of spores stained with methylene blue and injected into the body of larvae was also observed according to the system described.

Data analysis

- Mortalities were recorded daily for 72h and corrected with Abbott formula. Comparisons were performed by ANOVA-1.

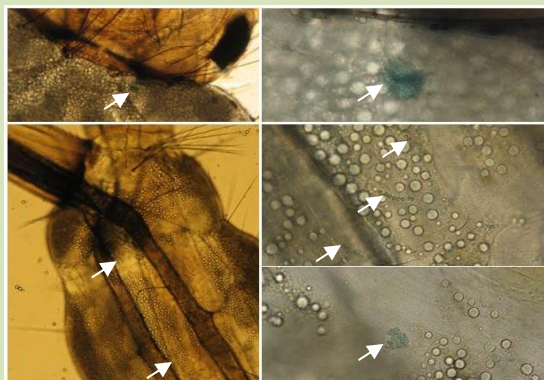


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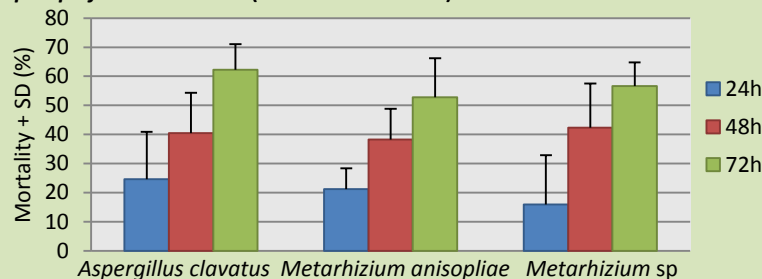


Results

(1) *Culex quinquefasciatus* larvae injected (500nl) with *Aspergillus clavatus* spores (10^7 per ml) stained with methylene blue. Spores spread over the whole body (arrows).



(2) Corrected mortality (and standard deviation) induced by injection (500nl) of entomopathogenic fungi at the dose of 10^7 spores per ml in *Culex quinquefasciatus* larvae (3rd and 4th instars).



Mortalities differed statistically from control groups since 48h ($p < 0.001$). However, no significant differences were observed between the strains.

(3) Post-mortem emergences of filaments from dead larvae were observed in the case of the three fungal strains confirming spore viability.



Aspergillus clavatus



Metarhizium anisopliae



Metarhizium sp

Conclusions

- Culex quinquefasciatus* larvae mortality could be related to the toxic effect of entomopathogenic spores after injection but additional experiments are still needed.

Perspectives

- Injection of inactivated spores (or inert bodies of similar size) could help to prove the toxic effect for each fungal strain, and reject the hypothesis of a response due to the presence of foreign bodies.