Metarhizium anisopliae blastospore more virulence than the conidia against *Aedes* larvae

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Introduction

Mosquitoes are major vectors of a wide range of diseases affecting human health (e.g. malaria, dengue, yellow fever). The most important genera are *Anopheles , Culex* and *Aedes,* which are mostly found in tropical areas but are gradually extending their range due to climate change and transport of goods [1].

Chemical pesticides are used to control adult mosquitoes while the bacterium *Bacillus thuringiensis* is used to control the aquatic larval stages. More recently, strains of the entomopathogenic fungus (EPF), *Metarhizium anisopliae,* have been identified that are highly efficacious in controlling mosquito larvae [2]. Most attention has focussed on use of aerial conidia to control mosquito larvae. Both "dry" and "wet" formulations of conidia will kill mosquito larvae following ingestion due to protease induced stress [3].

The current study investigated the use of blastospores for the control of *Aedes aegypti* larvae. Blastospores are far more economical to produce and due to their hydrophilic nature readily suspend in water (Table 1).

Materials & Methods

Dose mortality studies: Ten *Ae. aegypti* larvae (L_{3-4}) in 100 ml water were exposed to different concentrations (10^6, 10^7, 10^8 conidia ml^{-1}) of aerial conidia (wet and dry formulations) and blastospores of *M. anisopliae* ARSEF 4556 (Figs 1, 2).

Dry conidia were dusted onto the water surface while wet formulations were prepared by suspending conidia in 0.03% Aqueous Tween 80. Blastospores were suspended in water only. Mortality was recorded daily for 7 days.

Microscopy: Both light and electron microscope studies were conducted to: (1) better understand the characteristics of conidia and blastospores and (2) determine the mode of pathogenesis. Low temperature scanning electron microscopy (LTSEM) was conducted as described by Butt *et al.*, [3].

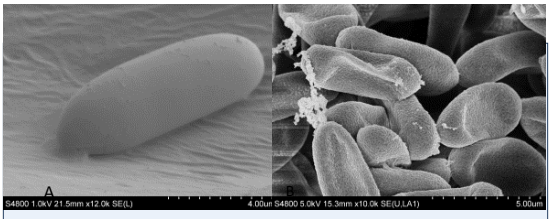

Fig 1. *M. anisopliae* blastospore (A) and aerial conidia (B).

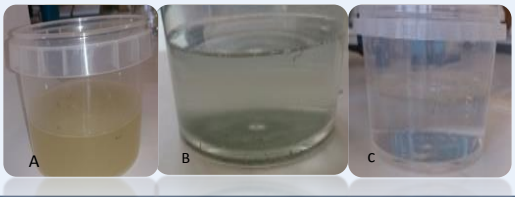

Fig 2. *M. anisopliae* applied to *Aedes* larvae. Formulations tested: blastospores (A), conidia suspension (B), dry conidia (C).

Results & Discussion

Blastospores were clearly more virulent for *Aedes* larvae than conidia at all the doses tested (Fig. 3). Mortality for both conidia and blastospores was dose dependent. The hydrophilic blastospores readily adhered to the larval surface whereas the hydrophobic conidia did not. Blastospores produced copious mucilage which also helped with adhesion to the surface of the mosquito larvae (Fig. 4). Both blastospores and conidia were ingested by healthy larvae. Multiple penetration sites were observed of the gut but also integument which may explain why the blastospores were more aggressive than the conidia (Figs. 4, 5). Key attributes of blastospores and conidia are summarised in Table 1.

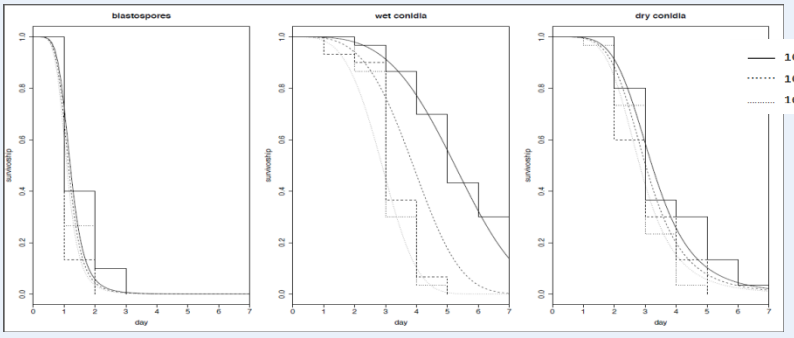

Fig.3 Survival curves of *A. aegypti* larvae exposed to different concentrations and formulations of *Metarhizium anisopliae 4556*

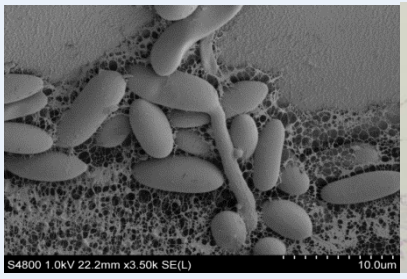

Fig.4 LTSEM. Blastospores adhering to surface of *Aedes* larval cuticle.

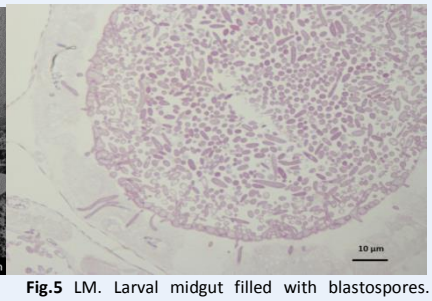

Fig.5 LM. Larval midgut filled with blastospores. Some blastospores have germinated and penetrated the gut wall.

Attributes	Aerial Conidia	Blastospores
Production time	15-17 days	2-3 days
Hydrophobicity	Hydrophobic (need surfactant to suspend in water)	Hydrophilic, readily suspends in water
Death due to	Protease induced stress	Multiple infection sites
Role of *Pr1*	Significant role in pathogenesis	Little or no role in pathogenesis
LT_{50} at 1×10^6 conidia ml^{-1}	3.1 days (wet), 5.1 days (dry)	0.8 day

Table.1 Summary of attributes of *M. anisopliae* blastospores and aerial conidia infecting mosquito larvae.

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