Persistence and virulence of the entomopathogenic fungus *Metarhizium anisopliae* under simulated field conditions to control adult *Aedes aegypti*

Aline T. Carolino [1]*, Adriano R. de Paula[1], Tariq M. Butt[2], Richard I. Samuels[1]

[1]State University of North Fluminense –Darcy Ribeiro (UENF), [2]College of Science, Swansea University, Singleton Park, SA2 8PP,UK.
***[1] carolinoat@gmail.com**

INTRODUCTION

Dengue fever (DF) is the fastest growing mosquito transmitted disease. The principle vector is *Aedes aegypti* but the dengue virus is also transmitted by *Aedes albopictus*, a highly invasive species. Conventional vector control methods are failing to prevent DF epidemics and alternatives are urgently needed [2].

Entomopathogenic fungi, such as *Metarhizium anisopliae* and *Beauveria bassiana*, are potential candidates to use in integrated vector management programmes. Recent studies have identified strains of *M. anisopliae* which are highly pathogenic to *A. aegypti* adults and larvae [1,3].

Here we investigated the virulence and persistence of three formulations of *M. anisopliae* against female *A. aegypti* under simulated field conditions.

MATERIALS & METHODS

- Conidia of *M. anisopliae* strain ESALQ818 formulated in Tween 80 (0.05%, v/v), vegetable oil or vegetable oil + isoparaffin were applied to sheets of black cloth. The black cloth was immersed in a fungal suspension at a concentration of $1x10^9$ conidia/ml^{-1}. Controls were treated with carrier only.

- The five sheets were hung from furniture as shown in Fig 1. Fifty mosquito were released in the room. At the end of the fifth day, a trap (BG-Sentinel™ Biogents Ltd.Germany) was placed for 24h in the rooms and the number of mosquito captured recorded. The procedure was repeated at the following time points: 0–5, 6–11, 12–17, 18–23,24–29 and 30–35 days .

- Conidial viability of the formulant (vegetable oil + isoparaffin) and the control was monitored over a 30 day period. At each time point the cloths containing conidia were re-suspended in Tween 80 and sprayed directly onto mosquitoes using a Potter tower (Burkhart Ltd. UK).

Fig. 1. Fungus impregnated black cloth (Left)
Fig. 2 Adult mosquito infected with *M. anisopliae* (Centre).
Fig. 3 BG-Sentinel Trap (Right).

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RESULTS & DISCUSSION

- The sheet containing conidia exposed to natural conditions (under a veranda) for 2 to 18 days, appeared to be more “virulent” causing a reduction in mosquito survival from 28 to 60%, which was statistically different from the control group (76.6%). Following 24 and 30 days of exposure to natural conditions there was no statistical difference compared to the control (Table 1).
- In the trial where the sheet was hung on furniture, the formulation of the fungus in Tween 80 (0.05%, v/v) and vegetable oil appeared to persist for 6-11 days, showing a significant difference compared to that of the controls (p>0.05). When formulated with vegetable oil + isoparaffin the persistence of conidia increased, within the 18–23 day group (64% survival) and was statistically different to that of the controls (87% survival) (Table. 2).

Table 1 Percentage survival of mosquitoes following spray application of conidia retrieved from cloths that had been left under natural extra-domicile conditions for periods of 2 to 30 days

Period exposed to natural conditions (days)	% end point survival	χ^2 (df = 1)	p
2	28.8	31.01	<0.0001*
6	41.1	16.5	<0.0001*
12	48.8	9.01	0.0027*
18	60	5.26	0.0218*
24	70	0.578	0.4471
30	70	0.0004	0.9894
CONTROL[$]	76.6	-	-

Table 2 Mean survival rates of *Aedes aegypti* released into rooms containing black cloths impregnated with fungal conidia using three different carriers

	Survival (%)		
Time since cloths first placed in rooms (days)	Conidia + T	Conidia + V	Conidia + V + I
0–5	38 ± 1 b	36.6 ± 1.5 b	32.6 ± 2.08 d
6-11	49.3 ± 2.51 b	50 ± 2.64 b	40.6 ± 0.57d
12 -17	81 ± 2.08 a	78.6 ± 2.80 a	60 ± 2.64 c
18-23	82 ± 1 a	81.3 ± 3.21 a	64.6 ± 2.51 bc
24-29	ND	82.6 ± 1.52 a	77.3 ± 0.57 ab
30-35	ND	ND	83.3 ± 2.51 a
Control	83.3 ± 2.51 a	84.6 ± 2.08 a	87.3 ± 4.16 a

CONCLUSIONS

- Black cloth impregnated with *M. anisopliae* formulated in vegetable oil + isoparaffin increased mortality of *A. aegypti* in simulated field conditions for ~20 days.
- The formulation improved persistence and thus offered potential use in the field.
- Attractant semiochemicals could further enhance the efficacy of the fungus impregnated black cloth for adult mosquito control.

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