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Kirubakaran.S.A
PG and Research Department of Biotechnology, Srimad Andavan Arts and Science College (autonomous) T.V.kovil, Trichy

Haripriya.R
PG and Research Department of Biotechnology, Srimad Andavan Arts and Science College (autonomous) T.V.kovil, Trichy

Naveenraj.D
PG and Research Department of Biotechnology, Srimad Andavan Arts and Science College (autonomous) T.V.kovil, Trichy

P.Thirumalaivasan
PG and Research Department of Biotechnology, Srimad Andavan Arts and Science College (autonomous) T.V.kovil, Trichy

Correspondence:
P.Thirumalaivasan
Head, PG and Research Department of Biotechnology,
Srimad Andavan Arts and Science College (autonomous)
T.V.kovil, Trichy

Virulence of *Metarhizium anisopliae* soil and commercial isolates against *Culex quinquefasciatus* Say, a vector of Bancroftian filariasis, and *Aedes aegypti* L., a vector of dengue fever

Kirubakaran.S.A, Haripriya.R, Naveenraj.D, P.Thirumalaivasan

Abstract
Virulence of five soil isolates (WRMa1, WRMa3, WRMa7, MTCC6060 and MTCC 6067) from the Tamil Nadu, India region and two commercial isolates (designated as MMa4, MMa5) of *Metarhizium anisopliae* was tested against third instar larvae and adult female of *Aedes aegypti and Culex quinquefasciatus.* The WRMa3 produced significantly higher mortality than other isolates against third instars, with a lethal threshold level (LT_{50}) of 4.2 (±0.16) days for *Ae. aegypti* and 3.9 (±0.18) days for *C. quinquefasciatus* respectively. The same isolate produced 75% mortality, with LT_{50} of 5.61 days and LC_{50} of 2×10^4 conidia/ml in adult female *Ae. aegypti.* With C. *quinquefasciatus,* 96% mortality was observed with an LT_{50} of 3.2 days and an LC_{50} of 1.8×10^8 conidia/ml. The effective isolate, WRMa3, was tested further against the survival of the both mosquitoes at higher dose of 2×10^8 conidia/ml. The total life span of fungus-treated mosquitoes of *Ae. aegypti* and *C. quinquefasciatus* were significantly reduced when compared to uninfected mosquitoes. The results indicate that the WRMa3 soil isolate of *M. anisopliae* can be effective in the control of *C. quinquefasciatus* and *Ae. Aegypti* larvae and adults.

Keywords: *Metarhizium anisopliae; Aedes aegypti; Culex quinquefasciatus;* virulence; mortality; survival

1. Introduction
Mosquitoes are medically important vectors of parasites and pathogens that infect more than 700 million people annually around the world (Beier 1998; Mackenzie *et al*., 2004;WHO 2007).Prevention of the spread of these diseases depends primarily on vector control (WHO,1999).

Aedes aegypti (L.) (Diptera: Culicidae) is a highly anthropophilic species (Harrington *et al*.,2001) and acts as a vector and transmitter of diseases such as dengue fever, dengue hemorrhagic fever, chikungunya and yellow fever. Dengue and dengue haemorrhagic fever (DHF) are epidemic in tropical and sub-tropical regions around the world, including Africa, the Eastern Mediterranean, the Americas, South- East Asia and the Western Pacific(Mackenzie *et al*., 2004;Scholte *et al*.,2007).*Culex quinquefasciatus* Say (Diptera: Culicidae) is a significant vector of Bancroftian filariasis in tropical and subtropical regions. In India alone, 25 million peoples were affected by microfilaria, and 19 million people suffered from filarial disease manifestations (NCID, 1990; Koroma *et al*., 2012).

Mosquito control is accomplished primarily through destruction of breeding sites with application of the chemical pesticides such as organophosphates, carbamates, and pyrethroids. However, widespread use of chemical pesticides has caused environmental health problem such as toxicity to humans and non-target organisms Many chemical pesticides are persistent in the ecosystem (WHO, 1999; Ariee *et al*., 2001) and in addition, excito-repellent effects of chemical synthetic insecticides (Ariee *et al*., 2001) and the emergence and spread of the insecticide resistance(Senthilnathan *et al*., 2005; Senthilnathan *et al.,* 2006) are beginning to reduce the effectiveness of insecticides.

Biological and pesticide-free control methods such as use of growth regulators, mosquito-eating fishes, botanical insecticides and microbial insecticides have been explored during past decade and have proven to be effective in the regulation of the mosquitoes. Such methods pose little risk to non-target organisms and human health (Senthilnathan, 2007; Kalaivani *et al.,* 2012). Biological control measures also have been successful in diverse ecological and geographical ranges (Kalaivani *et al.,* 2012; Thanigaivel *et al.,* 2012).

Biological control of vectors by the application of the entomopathogenic fungi has been developed in recent years, and several fungal species of *Metarhizium, Beauveria, Lagenidium, Coelomyces, Tolypocladium* and *Culicinonmyces* genera are known to infect mosquitoe species (Harrington et al.,2001;Abdul-Ghaai et al., 2012). Even though the mosquito is not a

natural host for entomopathogenic fungi (Veea *et al*., 1968; Ansari *et al*., 2010) studies from the several authors have shown that *M. anisopliae* has virulence against egg, larvae and adult stages of the mosquito (Scholte *et al.,* 2007;Silva *et al*.,2004;Kanzok *et al*.,2006; Mohanty *et al* 2008). The entomopathogenic fungi have great advantage and differ from other biocontrol agents in that they need not be ingested. The spores of the fungi adhere to and penetrate the cuticle, proliferate internally, and produce toxins to kill mosquitoes (Scholte *et al*.,2007; Luz *et al*., 2012) sustainable control of the mosquito by the fungi may be possible by identifying and selecting effective strains compatible to the environments in which they are applied.

This study was conducted to compare the virulence of seven commercially and locally obtained isolates of *M. anisopliae* against the larvae and adult stages of *Ae. aegypti and C. quinquefasciatus*.

2. Materials and Methods

2.1. Fungal isolates

Seven isolates of *M. anisopliae* were used in for the studies. Two isolates (MTCC 6060 and MTCC 6067) were purchased from the microbial type culture collection (MTCC), Institute of Microbial Technology (IMTECH) Chandigarh, India. Three isolates WRMa1, WRMa3, WRMa7 (Table. 1) were isolated from the soil of nearby rice fields using "Galleria bait method (Zimmermann 1986) The isolated *M. anisopliae* was identified with aid of taxonomic keys (Humber 1997) under microscopic observation (Optika- Fluo series B-600TiFL, Italy) at 100X magnification. Bio- Magic (T. Stanes, Coimbatore, India) and Sun Agro Meta-L (Sun Agro Biosystem Pvt. Limited, Chennai, India) provided two commercial isolates designated as WRMa4 andWRMa5. After isolation, *M. anisopliae* was subcultured by three times in Sabouraud dextrose agar (SDA) media to obtain the pure culture and to avoid the effect of the commercial formulation on fungi. All the collected isolates were cultured in SDA (Hi-media, India) amended with 0.1% yeast extract incubated at 27±1^{0}C at 95% humidity in a biological oxygen demand (BOD) incubator.

2.2. Mosquito culture

Mosquito cultures of *Ae. aegypti* and *C. quinquefasciatus* were obtained from a colony that been maintained at the Biopesticides and Environmental Toxicology Laboratory (BET Lab), SPK Centre for Excellence in Environmental Sciences since 2007 without exposure to any pesticides. They were maintained at 27±2°C and 75–85% RH under a 14:10 L/D photoperiod. Larvae were fed a diet of Brewer's yeast, dog biscuits and algae in a ratio of 3:1:1, respectively. Pupae were transferred from the trays to a cup containing tap water and placed in screened cages (23×23×32 cm) where adults emerged. Adults were maintained in 30×30×30-cm glass cages. Adults were continuously provided with 10% sucrose solution in a jar with a cotton wick. On day 5 post-emergence, adults were deprived of sugar for 12 h, then provided with a mouse placed in resting cages overnight for blood feeding.

2.3. Conidial production and viability

The *M. anisopliae* isolates were grown in SDA (Hi-media, India) amended with 0.1% yeast extract and the conidia were harvested from 15-day-old culture of the isolates and passed through sieves to avoid mycelia and conidia clumping. The harvested conidia were refrigerated at 4^{0}C in screw cap tubes. Viability of the collected conidia of the all seven isolates was determined by the direct plating method(Daoust and Robert ,1982).The spores were suspended in the water solution containing 0.001% Triton-X-100 and water- spore suspensions were placed in the centre of the SDA plates and evenly spread using sterile glass L-rods and incubated at 27^{0}C for 24 hour. Viability was determined by observing 200-300 conidia germinations under a microscope (Optika-Fluo series B-600TiFL, Italy) at 100X. Conidia were classified as germinated when the length of the germ tube matched at least the width of the conidium.

2.4. Identification of effective strain

Seven *M. anisopliae* isolates were initially tested with concentrations of 2×10^8 conidia/ml against the larval stage of the mosquito by surface application to identify an effective isolate. The effective isolates were tested against the larvae and adult stages of the mosquitoes.

2.5. Bioassay with the mosquito larvae

The bioassay was carried out using third-instar larvae and adult female of *Ae. aegypti* and *C. quinquefasciatus* by application of conidia to the water surface of the culture dish. Concentrations from 1×10^2 to 2.5×10^8 were used for identification of lethal concentration (LC_{50}). Ten mg of unformulated conidia were weighed and added directly on the surface of each dish containing 20ml of distilled water. Formulations were spread evenly over the surface with a brush and 20 larvae were used per concentration. Five replications were carried out for each concentration and the control larvae were placed in the distilled water. The treated larvae were fed with a diet of Brewer's yeast, dog biscuits and algae in a ratio of 3:1:1 during the experimental period and the bioassay chamber were placed at 27±2°C and 90-95% RH under a 14:10 L/D photoperiod. Observation was carried from the second day of the treatment to eighth day. The dead larvae were removed from the bioassay dishes and placed in the moist chamber for the sporulation of the conidia.

2.6. Adulticidal assay

M. anisopliae isolates were tested against the female *Ae. aegypti* and *C. quinquefasciatus* using methods described by Scholte *et al*., 2007. The adults of both species were passively treated with conidia of the *M. anisopliae* individually. Ten mg of the unformulated conidia were suspended in 0.05% Triton-X-100 solution to prepare conidial suspension. The conidial suspensions for the experiment were prepared one hour before the bioassay. The filter papers were plated with 5 ml of conidia suspension, placed in a plastic box, and covered with muslin cloth to avoid the escape of the adults. Twenty two-d-old females were placed in the plastic box containing the fungus contaminated filter paper and provided with 10% sucrose solution. The fungi infected the mosquito by attachment of the conidia to the mosquito's tarsae. After 24h the mosquitoes were transferred to clean plastic boxes where they had access to 10% sucrose solution. Mosquito mortality was checked from the second day of the treatment up to eight days. The dead mosquitoes were removed from the cages and placed in the moist chamber, where they were incubated at 27^{0}C for the sporulation of the conidia on the cadavers to confirm whether the mosquito mortality was due to fungal infection. The effective *M. ansiopliae* strain conidial suspensions were prepared to study the survival of the both mosquito species. The 10ml of conidial suspensions at the concentration of 2×10^8 conidia/ml were prepared using sterile solution of

0.05% of Triton- X- 100. The concentration of conidia was estimated by direct counting by using a Neubauer improved haemocytometer chamber under microscope (Optika- Fluo series B-600TiFL, Italy) at 40 X magnification.

2.7. Statistical analysis

Percentages of mortality were transformed by arcsine square root to normalize the mean percentages **(**Gomez and Gomez 1984) after correcting for natural mortality (Abbott ,1925)The transformed percentages were subjected to analysis of variance (ANOVA). Differences between the treatments were determined by Tukeys multiple range test ($P<0.05$) (Snedecor and Cochran 1989) Lethal time (LT) to 50% mortality and the lethal concentration (LC) causing 50% mortality were estimated by fitting mortality data to linear regression (Minitab® 16 software package, State College, PA). The longevity of both mosquitoes after treatment was analyzed using a log-rank χ^2 test of equality over strata (PROC LIFE Table) with the Minitab® 16 statistical software package.

3. Results

Seven *M. anisopliae* isolates were tested for comparative virulence analysis study against the two mosquito species. Before the biological assay, the viability studies on the seven isolates were tested and conidial viability range between 90-93%. All seven isolates showed mortality against the larval stages of *Ae. aegypti and C. quinquefasciatus*

The percentage mortality of *C. quinquefasciatus* and *Ae. ageypti* larvae treated with the different fungal strains varied from 59 to 96 % and 51 to 88% respectively. Conidia were found at the tip of the siphon, on anal papillae, and in the oral brushes of dead larvae treated with the fungal conidia. The infected larvae showed initial outgrowth of the *M. anisopliae* mycelia followed by conidial production. Among the tested isolates, VRMa3 showed maximum mortality of 96% and 88% in the larval stage of *C. quinquefasciatus* and *Ae. aegypti*. The probit mortality is compared at different conidial concentrations in Fig. 1. Treated larvae exhibited a sluggish behaviour within few hours of exposure to conidia and either sank or floated. This isolate causes high mortality rates with a shorter LT_{50} of 3.10±0.18 and 3.60±0.33 days for *C. quinquefasciatus* and *Ae. aegypti* respectively than other isolates (Table 2). However, differences in fungal isolate activity against closely related species are a common observation. Adulticidal assay were carried with the all the fungal isolates with the female adults of the both the mosquitoes species. Ten mg of conidia were suspended in the 10 ml 0.05% Triton-X-100 solution and impregnated in the filter paper and exposed to mosquitoes for 48 hours. Adult longevity of the female *Ae. aegypti* and *C. quinquefasciatus* mosquitoes treated with the conidia was significantly lower than the untreated (control). The VRMa3 isolates infected the female adult of mosquitoes more effectively than other isolate used in the study. The VRMa3 isolate showed mortality of 75% with LT50 of 5.61 days in female *Ae. aegypti.* The LT50 of fungi affected *C. quinquefasciatus* was about 3.2 days with a total of 96% mortality. The data related to mortality of the larvae and adults were recorded and statistical data regarding LC_{50} of WRMa3 were calculated (Table 3 and Fig. 2).

Initially, fungal colonies were pale white, and then became pale greenish brown with

a powdery form. Terminal and lateral conidia developed at the same time and were sessile. The mortality rates of the third instar (r^2=0.97) and adult female (r^2=0.99) of *C. quinquefasciatus* were highly correlated with the concentration of the *M. anisopliae* conidia inoculation. A comparison the LC_{50} and LT_{50} values revealed that third instar larvae of *C. quinquefasciatus* were more susceptible than the other instar and *Ae. aegypti*.

At the standard concentration (10 mg unformulated conidia), all fungal strains tested were pathogenic to both mosquito strains *C. quinquefasciatus* and *Ae. aegypti*. However, pathogenicity varied according to fungal strains isolated from the soil (Figs. 3-6). For example, mortalities caused by the strain WRMa3 ranged from 85 to 90% to third instar *C. quinquefasciatus* and it was significantly different from other tested fungal strains ($F6,28 =23.41$; $P<0.0001$) (Fig. 3).

Mortalities caused by *M. anisopliae* strain WRMa3 ranged from 80 to 87%. Further, WRMa1 caused mortalities ranging from 65 to 70% and mortality caused by MTCC6060 was 60% to third instar *Ae. aegypti* ($F6,28 =15.28$; $P< 0.0001$). With regards to dose mortality relationships, WRMa3 strain had lower LT_{50} values for the third instar of both *C. quinquefasciatus* and *Ae. aegypti* (Table 3).

The VRMa3 soil isolates from rice field were encountered as an effective virulence strain against the larval and adult stages of the *C. quinquefasciatus* and *Ae. aegypti* mosquito species and were classified as highly virulent in the initial screening assay. The VRMa3 show virulence greater than the commercial isolate used in the study. The isolates virulence to *C. quinquefasciatus* were in the order of VRMa3>VRMa1> MMa4> VRMa7> MTCC6060> MMa5> MTCC6067 and virulence were in the order of VRMa3>MMa5> VRMa1> MMa4> MTCC6067> VRMa7>MTCC6060 for *Ae. aegypti*. From the larvicidal and adulticidal assay it has been shown that tested effective strain show higher virulence against the *C. quinquefasciatus* than *Ae. aegypti* (Figs.3-5)

The longevity of *C. quinquefasciatus* that had been treated with conidia was significantly lower than those of the control groups (χ^2 = 31.9260; df=1, P=0.0001, Fig. 7). Further, total *Ae. aegypti* survivorship was also significantly reduced on WRMa3 treatment at

2×10^8 concentration. (χ^2 =13.6975; df=1, P=0.0001, Fig. 8). Approximately 60-65% of both pupae on untreated carrier control reached the adult stage.

4. Discussion

M. anisopliae is a soil-borne cosmopolitan entomopathogenic fungus known to infect a wide range of insect orders. Comparative virulence of seven isolates of five soil isolates (WRMa1, WRMa3, WRMa7, MTCC6060, and MTCC 6067) from various geographical region and two commercial isolates (MMa4, MMa5) of *M. anisopliae* were tested against the third-instar larvae and female adult of the *Ae. aegypti and C. quinquefasciatus.* The potential of entomopathogenic fungi for the control of vectors has long been recognized (Scholte *et al*., 2007).

Unlike other biocontrol agents, the entomopathogenic fungi are contact insecticides and, unique in infecting the host insect, they need not to be ingested by the specific host. The fungi penetrate the cuticle and establish in the host. The advancement of entomopathogenic fungi has been made in recent years in development of spores and mycelium-based biocontrol agent for the mosquito population regulation which reduced inputs of chemical pesticide in the environment (Strasser *et al.,* 2000).Large numbers of fungal conidia for

mosquito control programmes have been produced in simple and relatively inexpensive artificial media (Goettel *et al*., 1984; Lacey and Undeen ,1986). Thus insect pathogenic fungi show more advantage than many other biocontrol agents in the management of the mosquito. A number of entomopathogenic fungi have been so for used effectively to control *Aedes spp.* and *Culex spp* mosquito vector for the last few decades (Riba *et al*., 1986; Lacey *et al*., 1988; Ravallec *et al*., 1989; Alves *et al.*, 2002; Silva *et al*., 2004; Scholte *et al*., 2007). However, the success in the use of entomopathogenic fungi as mycoinsecticides largely depends on strain selection of highly virulent strains, since natural variation found to exist between strains (Soper and Ward 1981)

M. anisopliae strains found to be highly effective in reducing larval survival in laboratory condition were reported by the several authors (Scholte *et al*.,2004; Scholte *et al*.,2007; Silva *et al*.,2004; Kanzok *et al* .,2006). Significant differences were found to exist between the isolate. Such differences in virulence are due to genetic variability of the entomopathogenic fungi complexes (Hajek and Leger1994). In addition to genetically based virulence, activity of *M. anisopliae* in the mosquito larvae has been related to susceptibility of the mosquito species (Ravallec *et al*., 1989) moulting period of the larvae (Goettel *et al*., 1984) dose and viability of fungi conidia, the pathogen invasion, recent- host passage, formulation, and application techniques (Daoust and Robert 1982;Riba *et al*., 1986;Miranpuri and Khachatourians 1990). In this study, the *M. anisopliae* was applied to the water surface as dry unformulated conidia at a concentration of 10 mg, which cause mortality on the larvae by germinating and growing on the siphon perispiracular valves on the tip of the siphon. This process causes blocking of the respiratory siphon. As a result of perispiracular infection, larvae succumbed due to asphyxiation after obstruction of the tip of the respiration siphon, followed by development of mycelium down the respiratory tract, which resulted in impairment in the breathing mechanism (Roberts 1970).Larvae can also ingest dry conidia where they, apparently without germination, release lethal substances such as destruxin mixture into the gut (Crisan 1971). The application of dry conidia to the water surface is known to have prolonged activity against mosquito larvae (Roberts 1970).

Treating the habitat with dry spores is practical for bio-control. Dry conidia will float longer and have a better chance of coming into contact with larvae during air intake (Lacey *et al*.,1988). Similarly Scholte *et al* 2003 reported the pathogenicity of the *M. anisopliae* on adult stages of the C. *quinquefasciatus* and *An. gambiae.* Both the mosquito species were found to susceptible to infection with unformulated and oil-formulated conidia of the fungus.

The results from the larvicidal and adulticidal screening assay indicate that all the tested isolates show significant mortality on the third instar larvae and adult females of both mosquito species. The finding of *in vitro* biological assay of the larvae and adult of the both species show that the *M. anisopliae* infected the mosquito based on dose and time-dependent mortality relationships. *M. anisopliae* strain WRMa3 provided maximum mortality. Also it is more effective than the commercial isolates used in the study. Similarly, selection of effective strains of *M. anisopliae* against the mosquito species carried out by several authors. Ramoska (Ramoska 1982) shown that the selected effective strain used in his experiment suppressed *C. quinquefasciatus* larval populations for a month. Daoust and Roberts (1982) tested 52 strains from a variety of hosts from nine countries on of *C. pipiens* larvae and selected effective isolates and further tested the virulence isolates to larvae of *Ae. aegypti* and *Anopheles stephensi* and shown that the strains most virulent to *C. pipiens* proved to be highly pathogenic to larvae of *Ae. aegypti* and *An. stephensi* . Blanford *et al* (2005) selected an isolate of moderate virulence, which is currently used in a commercially available biological product for agricultural pests, 324 as a candidate for *Anopheles* control.

Further the effective strain WRMa3, was studied on the long-term survival of the both mosquitoes species. Reduction in the mosquito longevity reduces the average number of blood feeding, reduction in the number of blood meals taken reduces the probability of the vector acquiring a disease agent and inhibit the normal development of parasites (Blanford et al., 2005) and reduced fecundity was already been reported .The results from the current study show that the longevity of *M. anisopliae*-infected *Ae. Aegypti* and *C. quinquefasciatus* is significantly lower than control. Similarly total mortality and mean survival times (expressed as LT_{50}) of the both species were observed by Scholte *et al* (2007) and it was found that the fungus infected mosquitoes have shorter survival times than control. The virulence of isolates selected for biological control programs of mosquito may be important as any small alteration in kill time could have consequences for the transmission of the disease. Therefore application of the fungi provides alternative technology for regulating large population of mosquito larvae and adults. Both the mosquito species were susceptible to the WRMa3 isolate but *C. quinquefasciatus* third instar larvae were more prone to mycosis than *Ae. aegypti.* Biological control of mosquito by the *M. anisopliae* soil isolates found to be promising and WRMa3 from the Tirunelveli district of the rice field habitat were selected as effective strain and good candidate against both mosquito species. There is potential to use this strain in the sustainable management of mosquitoes in the field and to develop it as a commercial mycoinsecticide.

Figures and tables

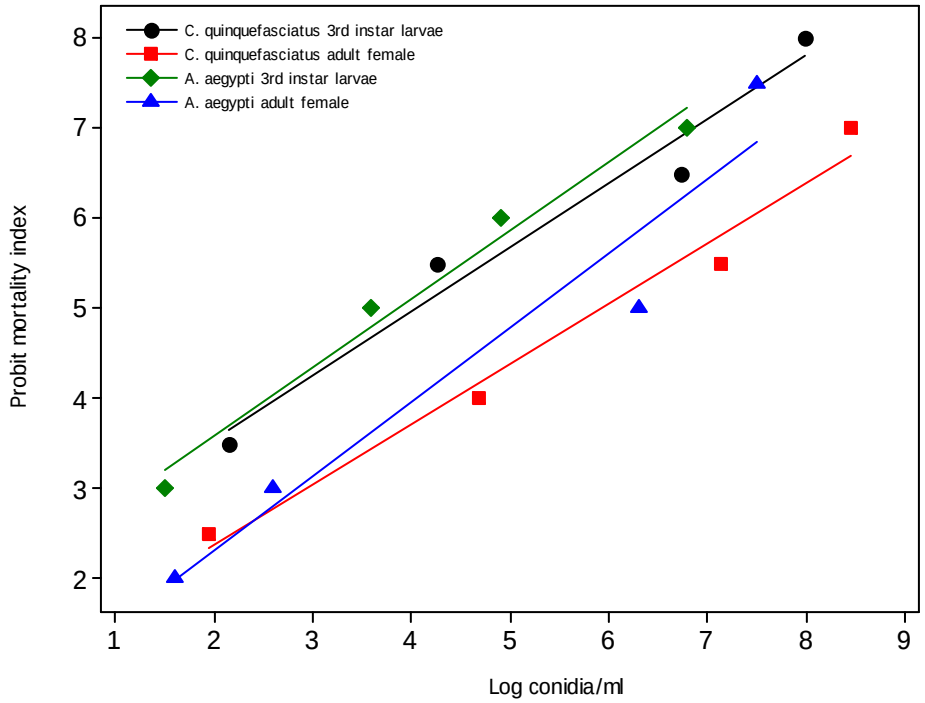

Fig 1: Mortality of *C. quinquefasciatus* and *A. aegypti* exposed to different concentrations of *M. anisopliae* isolate (WRMa3).

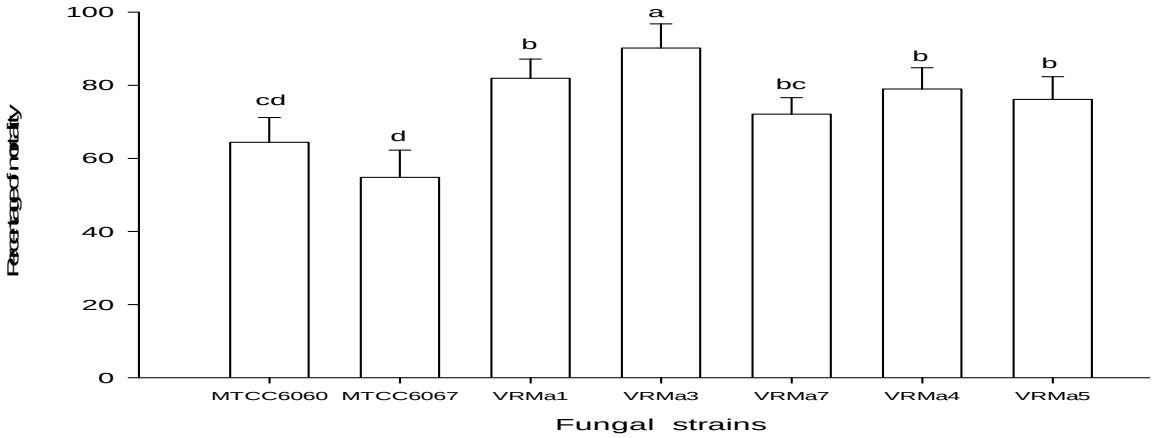

Fig 2: Percentage of mortality of 3rd instar *C. quinquefasciatus* treated with 10 mg of unformulated conidia of fungal isolates. Means (SEM±) followed by the same letters above bars indicate no significant difference ($P < 0.05$) according to a Tukey test.

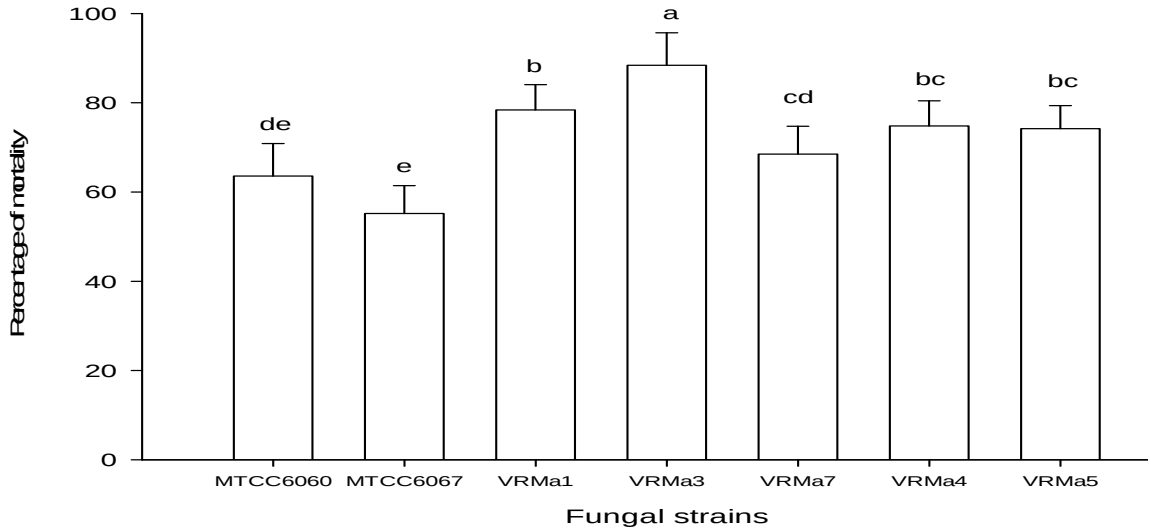

Fig 3: Percentage of mortality of adult female *C. quinquefasciatus* treated with 10 mg of unformulated conidia of fungal isolates. Means (SEM±) followed by the same letters above bars indicate no significant difference ($P < 0.05$) according to a Tukey test.

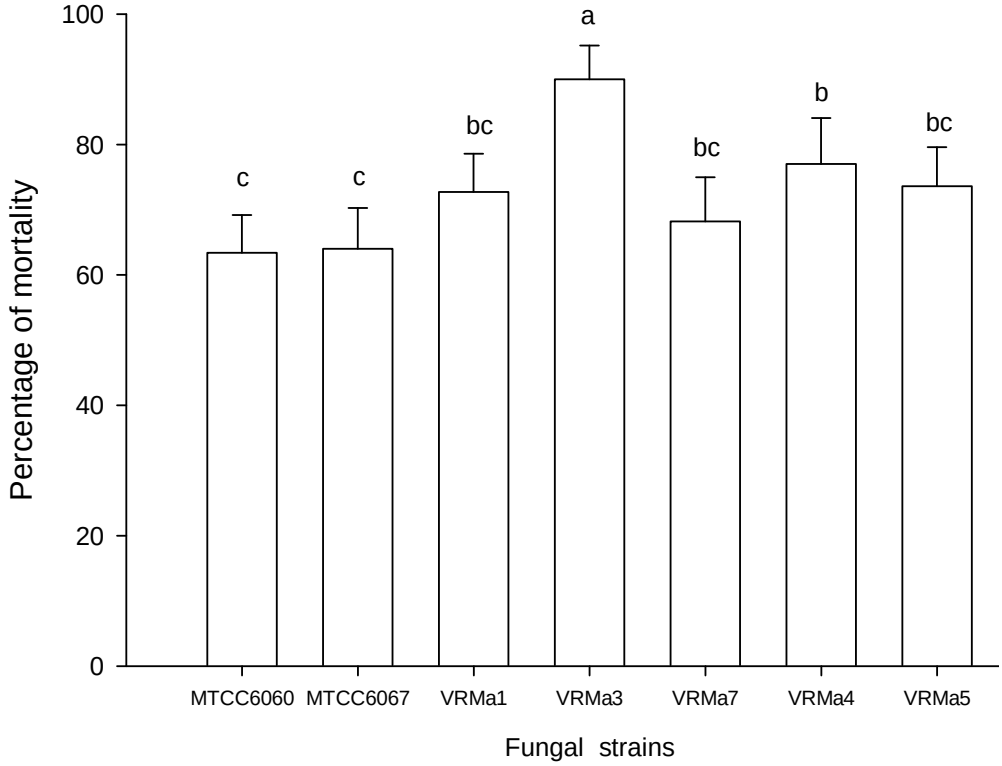

Fig 4: Percentage of mortality of 3rd instar *A. aegypti* treated with 10 mg of unformulated conidia of fungal isolates. Means (SEM±) followed by the same letters above bars indicate no significant difference (P < 0.05) according to a Tukey test.

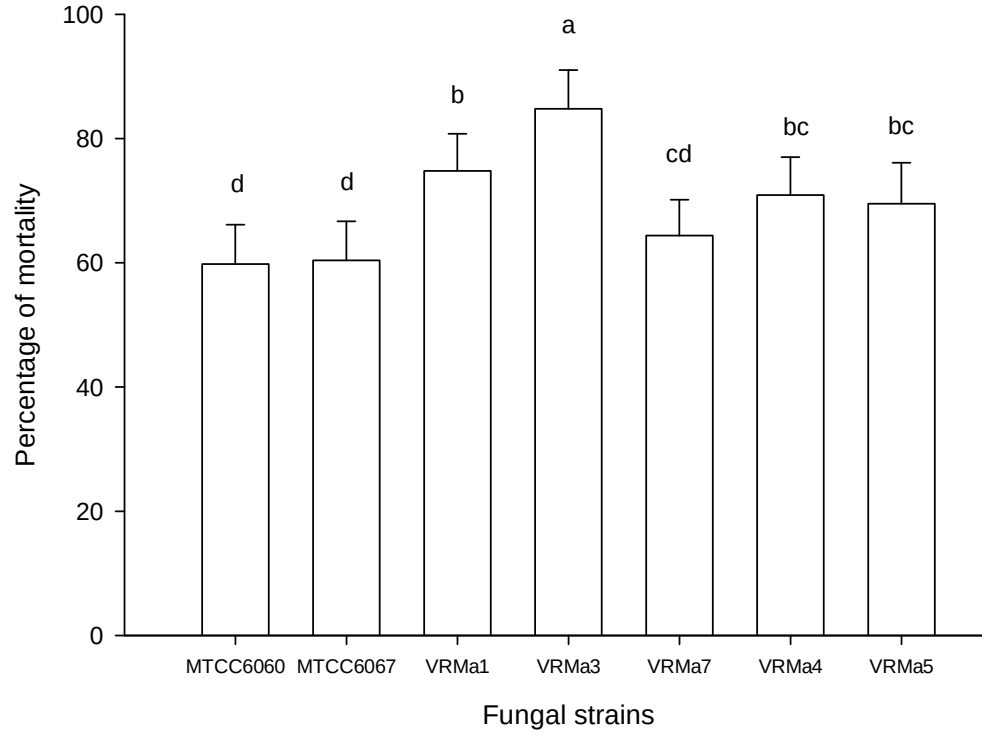

Fig 5: Percentage of mortality of adult female *A. aegypti* treated with 10 mg of unformulated conidia of fungal isolates. Means (SEM±) followed by the same letters above bars indicate no significant difference (P < 0.05) according to a Tukey test.

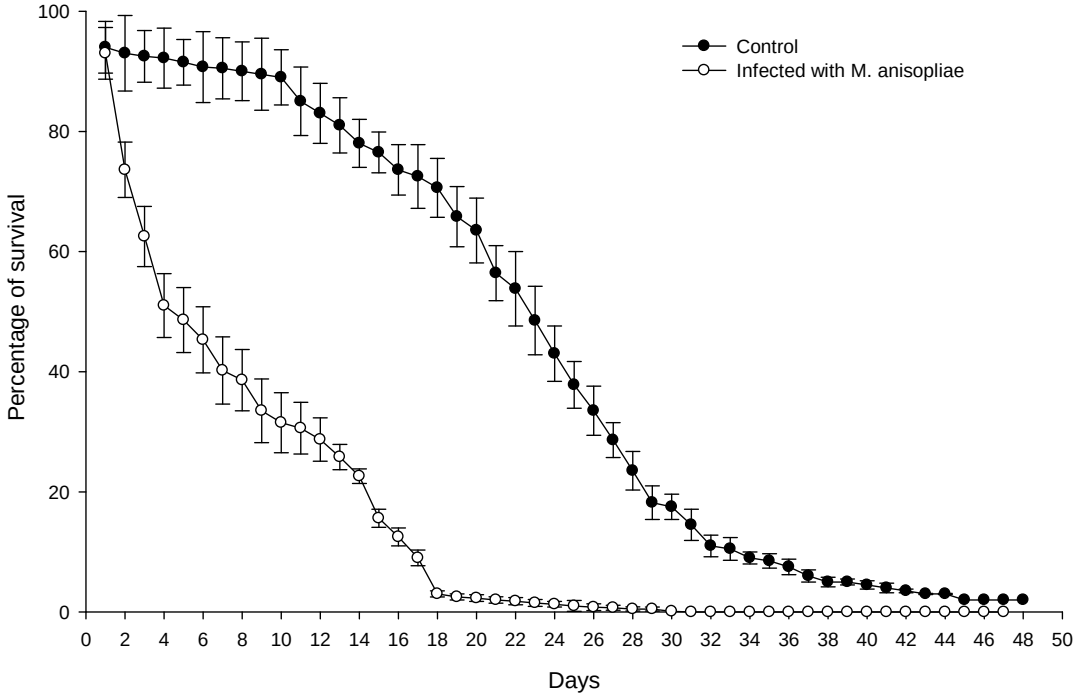

Fig 6: Daily mean percentage (±SEM) of survival of *C. quinquefasciatus* after treatment with *M. anisopliae* (WRMa3) at 2×10^8 conidia/ml concentration. Survivorship curves differ at the $\alpha = 0.05$ confidence interval according to log-rank statistics.

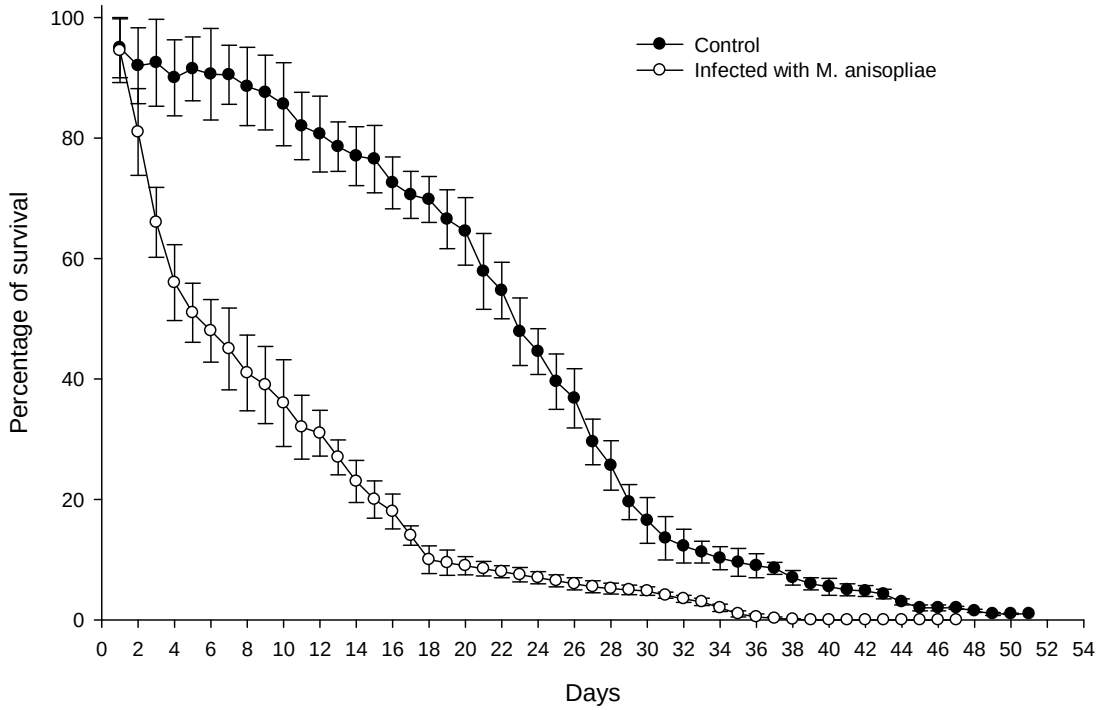

Fig 7: Daily mean percentage (±SEM) of survival of larval *A. aegypti* after treatment with *M. anisopliae* (WRMa3) at 2×10^8 conidia/ml concentration. Survivorship curves differ at the $\alpha = 0.05$ confidence interval according to log-rank statistics.

Table 1: Detail of the fungal isolates used in the study

M. anisopliae isolates	Substrate / source	Geographical Location
MTCC 6067	Soil	Mundoomuzhy, Pathinamthitta, Kerala
MTCC 6060	Soil	Nilambur, Malapuram, Kerala
WRMa1	Soil[a]	Alwarkurichi, Tirnelveli (dt), Tamil-Nadu
WRMa3	Soil[a]	Kalakadu, Tirneleveli (dt), Tamil-Nadu
WRMa7	Soil[a]	Ambasamduram, Tirneleveli(dt), Tamil-Nadu
MMa4	Bio- Magic[b]	N/A
MMa5	Sun Agro Meta-L[c]	N/A

MTCC- Microbial type culture collection; [a]-Soil from the rice field habitat; N/A- not available; b-Product from T. Stanes, Coimbatore, India; [C]-Product from the Sun Agro Biosystem Pvt. Limited, Chennai, India.

Table 2: LT_{50} in d for 3rd-instars of the mosquito species treated with 10 mg of unformulated conidia of fungal isolates.

Mosquito species	Fungal isolates						
	MTCC 6060	MTCC 6067	WRMa1	WRMa3	WRMa7	MMa4	MMa5
C. quinquefasciatus	5.7±0.28	6.7±0.17	4.2±0.16	3.9±0.18	5.1±0.11	4.6±0.13	5.3±0.09
A. aegypti	6.4± 0.31	6.1±0.35	4.8±0.37	4.2±0.33	5.8±0.15	4.9±0.30	5.6±0.19

Table 3: Probit equations and susceptibilities of conidia of *M. anisopliae* (WRMa3) strain against different instars of mosquito species (Fiducial limits in parenthesis; '*x*' is the log concentration of conidia of *M. anisopliae* in conidia/ml)

	C. quinquefasciatus		*A. aegypti*	
	3rd instar	Adult female	3rd instar	Adult female
Probit equation	2.095+0.715 *x*	1.03 + 0.670 *x*	0.703+0.915 *x*	0.545 + 0.742 *x*
LC_{50} (conidia/ml)	1.8×10^{8}	2×10^{5}	1.9×10^{9}	2.2×10^{4}
Fiducial limit	(1.65×10^{8}–1.98×10^{8})	(1.85×10^{5}–2.15×10^{5})	(1.75×10^{9}–2.05×10^{5})	(1.95×10^{4}–2.35×10^{4})

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