

# EXAMINING THE POTENTIAL OF ENTOMOPATHOGENIC FUNGI AS AN ALTERNATIVE TO CHEMICAL INSECTICIDES FOR CULEX QUINQUEFASCIATUS CONTROL

<sup>1</sup>Suleiman Sani

## Article Info

**Keywords:** Biological control; Entomopathogenic Fungi; *M.anisopliae*; *Culex quinquefasciatus*

## Abstract

Mosquito species that transmit parasitic diseases are typically controlled using chemical insecticides, which can harm the environment and public health. This study assesses the potential of the entomopathogenic fungus *Metarhizium anisopliae* as an eco-friendly alternative for controlling *Culex quinquefasciatus* larvae in the lab. After treating fourth instar larvae with various concentrations of the fungus, mortality rates were monitored over 120 hours. The results indicate that *Metarhizium anisopliae* could be a viable bio-control agent for *Culex quinquefasciatus* and merits further investigation. The experiment was allowed to run for a period of 120 hours. Mortality was recorded at an interval of 24 hours. Probit analysis was used to determine LC<sub>50</sub>, LC<sub>90</sub>, LC<sub>95</sub> and LC<sub>99</sub>. For all the selected concentration, highest mortality of 42(70.0%), 48(80.0%), 51(85.0%) and 57(95.0%) were recorded after 120 hour.  $1.79 \times 10^3$ ,  $3.96 \times 10^4$ ,  $4.97 \times 10^5$ ,  $7.58 \times 10^8$  conidia/ml were the LC<sub>50</sub>, LC<sub>90</sub>, LC<sub>95</sub> and LC<sub>99</sub> recorded respectively. *Metarhizium anisopliae* has the potential to be used as bio control agent for *Culex quinquefasciatus* and is suitable candidate for further research

## Introduction

Several mosquito species are known to serve as vectors for several parasitic diseases such as Malaria, Filariasis, Yellow fever, Japanese encephalitis, Dengue, and Zika virus [1]. Malaria and Filariasis are among the most common prevalent parasitic disease globally, as of 2011, 1.3 billion were at risk of the filariasis and also about one hundred and twenty million people were infected with disease globally (Singh & Prakash, 2011) [2]. Presently conventional control measures of several mosquitos borne infection like malaria and Lymphatic filariasis heavily rely on vector control which in turn depends heavily on the use of chemical insecticide like parathyroid and personal preventives which involves the use of Insecticide Treated Nets (ITN) and Indoor Residual Spraying (IRS) [4,5].

The adverse consequences of synthetic chemical insecticides in the environment are a serious and major public health problem. Over the last fifty years, many problems have been resulted due to the misuse of synthetic insecticides in Agriculture and Public Health Programs, such as insecticide resistance, environmental pollution, toxic hazards to human and other non-target organisms [6] coupled with accumulation of these

<sup>1</sup> Abubakar Tafawa Balewa University Bauchi, Nigeria

chemicals in the food chain and environmental pollution [7], these necessitates exploring eco-friendly and biological control methods [8]. Therefore in past few years' interest in mosquito control using entomopathogenic fungi is evident, mainly due to continuous and increasing levels of insecticide resistance and increase in global risk of mosquito-borne diseases. The use of enteropathogenic fungi has so many advantages over conventional insecticide and pesticide, in that, having target selectivity, environmental compatibility, economic variability, and novel mode of action, safer to environment and beneficial organisms as well as rational approach at a long run [9].

Entomopathogenic fungi which are normally found in soil and are widespread in temperate agro-ecosystems and semi-natural habitats [10] are a very heterogeneous group of insect pathogens, there nearly 700 species belonging to approximately 100 orders.

Entomopathogenic fungi have the ability to target the larval stage of the pest and vector right from the breeding site which is more advantageous and attractive targets for the fact that insects like mosquitoes usually breed in stagnant water and thus, it is easy to deal with them in such habitat (breeding site)[11]. The genus *Metarhizium* is pathogenic to a large number of insect species thus, are among the natural enemies of pests and vectors. It causes a disease known as 'green muscardine' in insect hosts because of the green colour of its conidial cells [12]. Entomopathogenic fungal metabolites could be an alternative source for mosquito larvicides because they constitute a potential source of bioactive compounds and generally free from harmful effects [13]. For example, *M. anisopliae* has a large hostrange, including Arachnids and five orders of insects, comprising over 200 species. Despite the fact that, mosquitoes are not listed as natural hosts for *M. anisopliae* some strains have shown to be virulent against mosquito larvae. Spores (conidia) of *M. anisopliae* have been known for some time to be infectious to adults and emerging pupae of some insects. Therefore, the aim of the present study was to isolate and determine the efficacy of *Metarhizium anisopliae* in the control of larval stage of *Culex quinquefasciatus*.

## **Materials and Methods**

### **Study area**

The study was conducted at Abubakar Tafawa Balewa University Bauchi (ATBU), Yelwa Campus (Longitude 9.792°East and Latitude 10.279°North) Bauchi Local Government Area, Bauchi state, Nigeria.

### **Mosquito (*Culex quinquefasciatus*) collection and breeding**

One hundred and ninety two blood fed mosquitoes were collected from their resting site in some selected houses of Gombe Metropolis using Aspirators. The collected mosquito species were placed in collecting cubs and transported to the insectary unit of Biological Sciences Laboratory of Gombe state university for confirmation and identification. 109 (56.77%) were identified to be *Culex quinquefasciatus* and released in to two separate breeding cages for rearing and breeding, while in the cages they were fed with 10% sugar solution. Five egg cups each containing 500 ml dechlorinated water was placed in each for eggs laying. Pieces of filter paper were placed on to the water and allowed to float for easy recovery of the eggs laid. The eggs were collected from the filter paper the following morning and transferred in to containers (45 × 20 cm) containing 500 ml of unchlorinated water. No any food was provided to the containers until the first instars appear, then they were fed with 10% yeast and filtering was conducted once the water was dirty. The larvae were monitored until third and fourth instar larvae emerged, and were ready for the experimentation.

### **Fungal culture and conidial production**

An inoculum of *Metarhizium anisopliae* was obtained from the Biological Science Department Abubakar Tafawa Balewa University, Bauchi. Method of [8] was adopted, but slight modification in culturing of *Metarhizium anisopliae*. In this, briefly the *Metarhizium anisopliae* were cultured on Potato Dextrose Agar (PDA) medium in an aseptic cupboard fumes and incubated at 28°C for 3 days. The conidia were harvested by scraping the surface of 3 days old culture suspended in distilled water. The mixture was stirred with a magnetic stirrer for 10 minute. The conidial concentration was determined using a hemocytometer which was

used to count the number of conidia under a compound microscope. The conidial suspension was further diluted with 0.05% Tween 80 solution, until it reaches a desired concentration with a countable number of spores. After having the established concentration of conidia, suspensions were diluted with distilled water to the concentrations of  $1 \times 10^7$ ,  $10^8$ ,  $10^9$ , and  $10^{10}$  conidia/ml. Only distilled water and 0.01% of poly sorbent added in the control treatment.

### Bioassays

Laboratory bioassay was done following the methods of World Health Organisation (WHO, 2015) with some modifications. Conidia of *M. anisopliae* were tested larvae of *C. quinquefasciatus* by adding fungal suspension to plastic cups containing water with 20 larvae of *C. quinquefasciatus*. Each plastic cup was inoculated with 1 ml of fungal suspensions of varying concentration ( $10^7$ ,  $10^8$ ,  $10^9$ , and  $10^{10}$  conidia/ ml). Control treatments were prepared by adding of 20 ml of distilled water and 0.01% poly sorbent only. Each assay was replicated three times. Larvae were fed with yeast powder and their mortality was observed in a 24 h interval for 5 days.

### Mortality determination

A glass rod was used to determine whether the larvae are dead or not. The rod was dipped into the container and bring very close to suspected dead larvae (which usually lie flat on the water surface). For the larvae that was still alive responded rapidly by either bending or moving away from the rods. On the other hand, for the dead larvae no matter how close the rod was brought, there was no any respond.

### Mycosis test

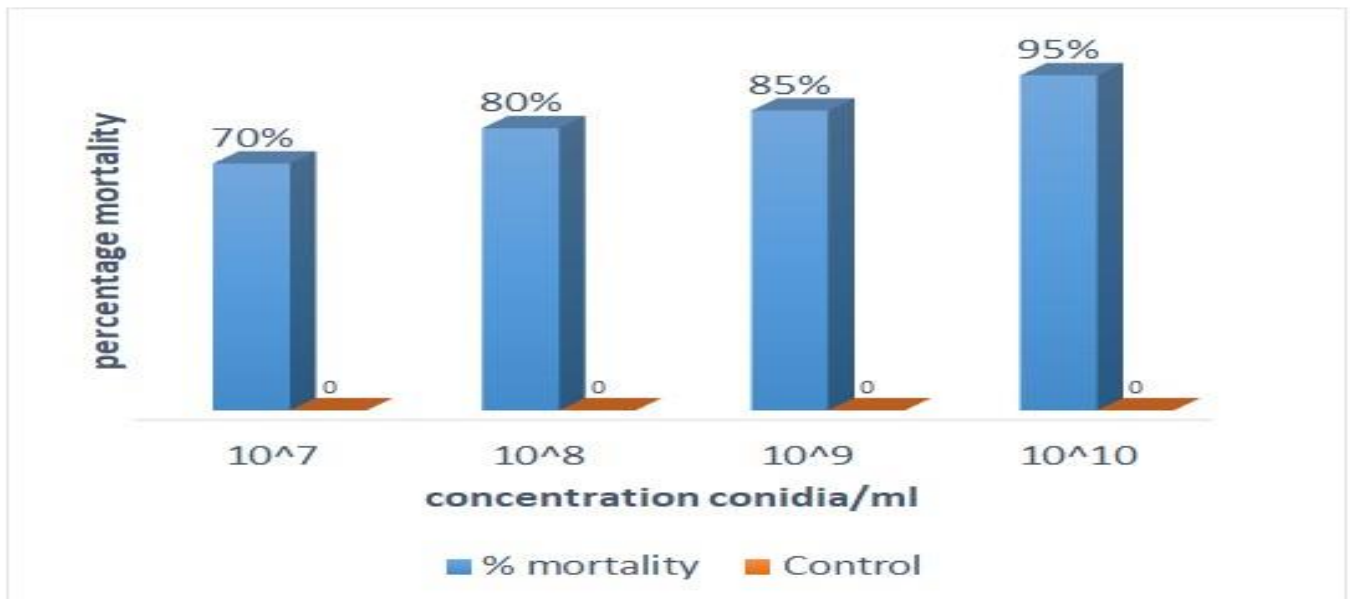
For this test, Three Petri dishes were set up; two containing distilled water and the other one with 70% alcohol. Firstly all dead *Culex quinquefasciatus* larvae were placed in to the distilled water one by one, later in to ethanol and then into the distilled water again in order to kill the fungus on the surface of the larvae. After which microscopic examination of treated *Culex quinquefasciatus* larvae was conducted. Any sign of fungal growth on the body surface the larvae indicated the actual number of insect that died from fungal infestation.

### Statistical analysis

All data generated were entered in to an excel software, and later transferred into a Minitab software for the actual analysis. Probit regression analysis was used to determine the relationship between concentration and mortality of *Culex quinquefasciatus* in given treatment. The  $LC_{50}$ ,  $LC_{90}$ , and  $LC_{99}$  values were calculated with 95% confidence limit

### Results

Result from this study shows that *M. anisopliae* isolate tested against fourth instars larvae of *C. quinquefasciatus* has pathogenic effect in all the treatment, but there was no mortality recorded in all the controls. However, pathogenicity varied according to concentration of spores and period of exposure. For the four concentrations;  $10^7$ ,  $10^8$ ,  $10^9$ , and  $10^{10}$  conidia/ml of the fungal isolate tested, it was observed that, mortality increased with increased in the time of exposure and also increased as the conidia concentration increased (Figure. 1). Highest mortality of 57(95%) was recorded at the highest concentration ( $10^{10}$ ) of conidia/ml applied. Consequently, other lower concentrations of  $10^7$ ,  $10^8$  and  $10^9$  conidia/ml recorded a *Culex* larval mortality of 42(70%), 48(80%) and 51(85%) respectively.



**Figure 1:** Total percentage mortality of *Culex* larvae exposed at different concentration of *Metarhizium anisopliae* isolate.

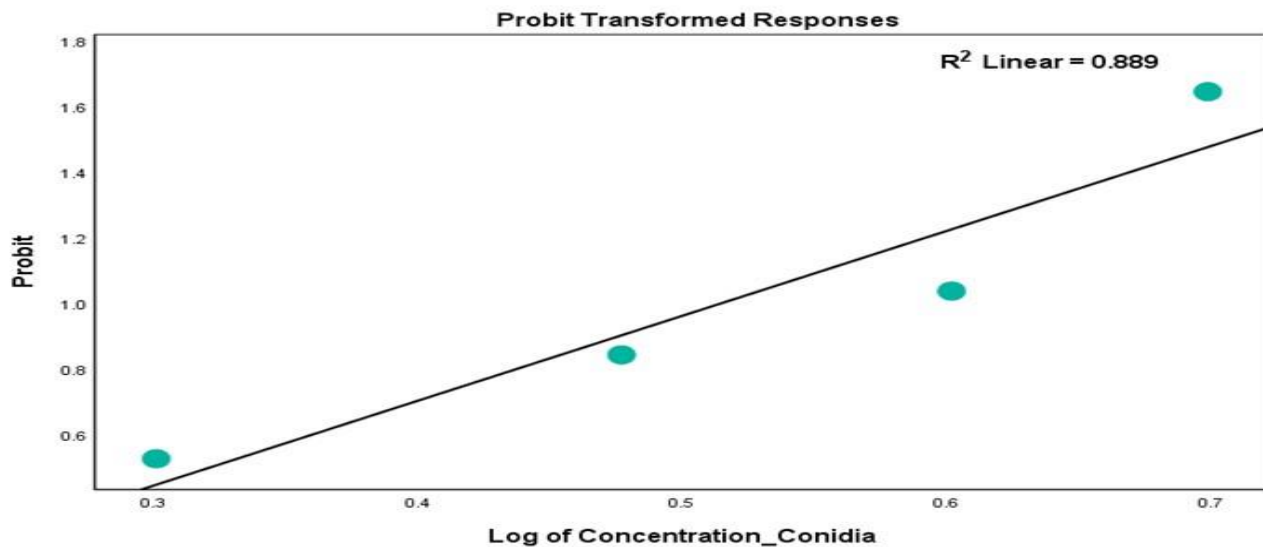
Mortality for the efficacy of *Metarhizium anisopliae* was assessed against *Culex quinquefasciatus* larvae based on the exposure period at various conidia concentration ( $10^7$ ,  $10^8$  and  $10^9$  conidia/ml). The result revealed that, mortality increases as the exposure time also increases as shown in Table 1 below. At the concentration of  $10^7$ ,  $10^8$ ,  $10^9$ , in 24 hours of exposure time there was no any mortality but at  $10^{10}$  concentration 3(5%) larval mortality was recorded. After 48 hours of post exposure, mortality increases to 3(5%), 6(10), 9(15) and 12(20%) at  $10^7$ ,  $10^8$ ,  $10^9$ ,  $10^{10}$  Conidia/ml respectively. 15(20), 17(28.3), 18(30) and 26(43.3%) mortality were observed after 72 hours of exposure time using  $10^7$ ,  $10^8$ ,  $10^9$ ,  $10^{10}$  Conidia/ml. 26(43.3), 30(50), 32(53.3) and 41(68.33%) after 96 hours of exposure to the concentration of  $10^7$ ,  $10^8$ ,  $10^9$ ,  $10^{10}$  Conidia/ml while at 120 hours of exposure to the same concentration 42(70), 48(80), 51(85) and , 57(95%) mortality were respectively recorded.

Concent ration (Conidia g/ml)	Time/hour				
	24 h	48 h	72 h	96 h	120 h
	Mortality (%)	Mortality (%)	Mortality (%)	Mortality (%)	Mortality (%)
107	0	3(5.0)	15(20)	26(43.3)	42(70.0)
108	0	6(10.0)	17(28.3)	30(50.0)	48(80.0)
109	0	09(15.0)	18(30.0)	32(53.3)	51(85.0)
1010	3(5.0)	12(20.0)	26(43.3)	41(68.33 )	57(95.0)
Control	0	0	0	0	0

**Table 1:** Percentage mortality (%) of mosquito larvae *Culex quinquefasciatus* exposed at different time and concentrations of *Metarhizium anisopliae*.

The survival data of biological vector, *Culex quinquefasciatus*, closely fitted the Probit distribution model which showed a significant increase in the mortality rate of *C. quinquefasciatus* following exposure to different doses of conidia. It can be indicated from this result that survival rates were inversely related to the

exposure of dose. That is, the higher the dose of conidia to the test organism, the increase in the mortality rate, While the lowest mortality rate was observed in the lowest concentration of conidia ( $10^7$ ) and the higher mortality rate was found in the higher concentration of conidia ( $10^{10}$ ), as shown in figure 3



**Figure 3:** Probit survival curve for larva of *Culex quinquefasciatus* infected with different doses of conidia of the entomopathogenic fungus *Metarhizium anisopliae*.

LC	Estimate	Lower bound	Upper bound
LC <sub>50</sub>	1.79	0.23	2.54
LC <sub>90</sub>	3.96	2.8	25.51
LC <sub>95</sub>	4.97	3.37	82.92
LC <sub>99</sub>	7.58	4.46	810.92

**Table 2:** Estimation of Lethal Concentrations (LC) of *Culex quinquefasciatus* exposed to different concentration of conidia

The result of the Probit modelling analysis (Table 1) revealed the lethal concentrations that cause 50% (LC<sub>50</sub>), 90% (LC<sub>90</sub>), 95% (LC<sub>95</sub>) and 99% (LC<sub>99</sub>) mortality of *Culex quinquefasciatus* exposed to different doses of conidia of the entomopathogenic fungus *Metarhizium anisopliae*.

As shown in Table 3,  $1.79 \times 10^3$  conidia/ml (95% CI was 0.23 to 2.54),  $3.96 \times 10^4$  conidia/ml (95% CI was 2.80 to 25.51),  $4.97 \times 10^5$  conidia/ml (95% CI was 3.37 to 82.92),  $7.58 \times 10^8$  conidia/ml (95% CI was 4.46 to 810.92) were reported as the LC<sub>50</sub>, LC<sub>90</sub>, LC<sub>95</sub> and LC<sub>99</sub> respectively.

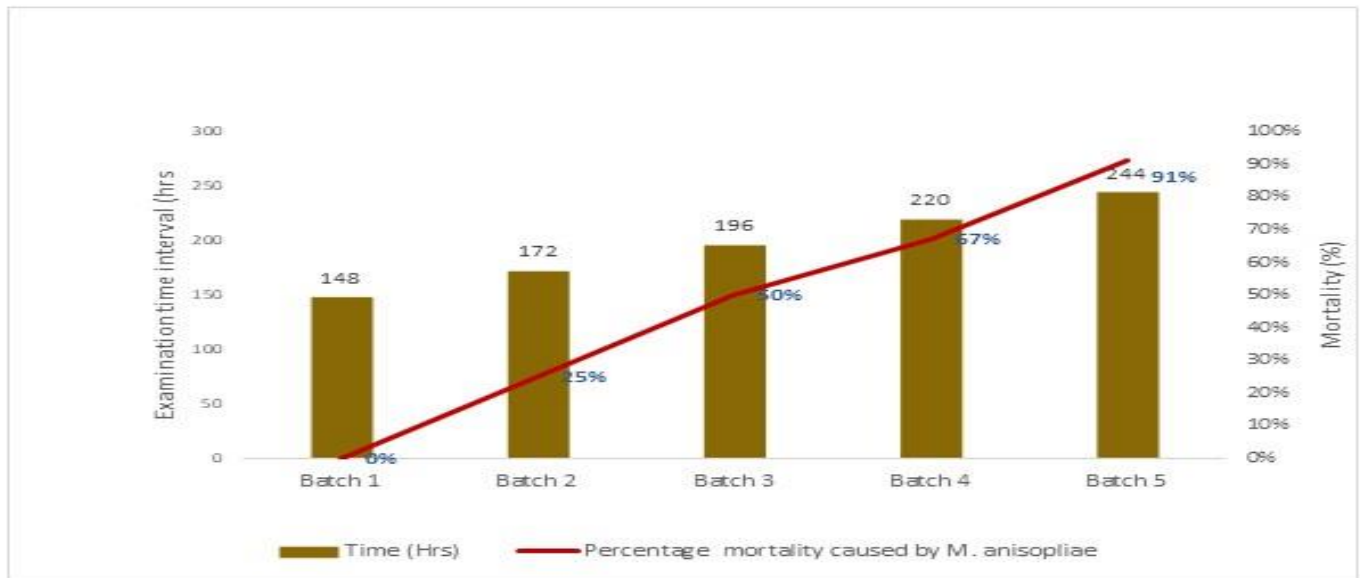
Specie	Respo nse	R2	Pears on Goodn ess of fit Chi square	Sig. level	95% confidence interval	
					Lower bound	Upper bound
<i>Culex quinqu efascia tus</i>	$y = 3.71 + 0.52x$	0.89	10.17	0.02*	2.69	4.72

\*represent significant ( $P < 0.05$ ). Since the significance level is less than 0.05, there is significance between the observed and the expected value.

**Table 4:** Probit transformed response for *Culex quinquefasciatus* following exposure to varying doses of conidia of *Metarhizium anisopliae*.



Mycosis results showed that the hyphae of the fungal conidia start to appear after 172 hours post treatment with 25 % *quinquefasciatus*. Highest (91%) conidial appearance was observed at 244 hours of post treatment. Conidial appearance on the surface of 67% and 50% for mosquito larvae after 220 hours and 196 hours of post treatment respectively. There was no conidial growth on the surface of *C. quinquefasciatus* after 148 hours of post treatment as shown in figure 5 below.



**Figure 5:** percentage mortality of *Culex quinquefasciatus* caused by *Metarhizium anisopliae* during five batches examination of cadavers under microscope.

### Discussion

The present study evaluates the bio-efficacy of the fungus, *M. anisopliae* against larval stage of *C. quinquefasciatus* under laboratory conditions. The findings from the study revealed a very promising outcome, where *M. anisopliae* demonstrated high level of pathogenic activity against *C. quinquefasciatus* larva. This is not surprising, as previous studies revealed that Spores and metabolites of entomopathogenic fungi have been reported as larvicides/biocontrol agents against larval stage of different mosquitoes species [14], coupled with the fact that *M. anisopliae* is among the best natural enemy of several biological vector [15]. Therefore *M. anisopliae* could serve as a better replacement present chemical insecticide and pesticide in a future. Findings from this study are in agreement to the findings of [16] who reported that, *M. anisopliae* causes significant mortality in different mosquito including *C. quinquefasciatus*. The larvicidal activity of *M. anisopliae* is dose dependent, this findings is in concordance with findings [17] who equally reported positive correlation between larval mortality and concentration of the entomopathogenic fungi (*M. anisopliae*), for that high level of larval mortality of 95% was reported from  $1 \times 10^{10}$  conidia/ml which was the highest concentration. This result is the very similar to the findings of [5] who equally reported high larval mortality of 91% at a conidial concentration of  $1 \times 10^8$  Conidia/ml. this clearly demonstrate the impact of concentration on larvicidal activity of the entomopathogenic fungi. In addition, larvicidal activity of *M. anisopliae* against *C. quinquefasciatus* is directly proportional to the time of exposure that is larval mortality increases as sharply from 24 hours up to 120 hours, this finding is in agreement with findings of [18] who also reported similar condition. Therefore the longer the exposure time of larvae to the *M. anisopliae* isolate more the metabolite and other active component and toxins such as Destruxin, Bavericin, and Efraeptins [18] are secreted by *M. anisopliae* thereby penetrate deeply and establish themselves and eventually kill the larvae.

Irrespective of time, the lethal concentration that will kill at least 50% ( $LC_{50}$ ) recorded in this study was  $1.79 \times 10^3$  conidia/ml with the range of 0.23-2.54 conidia/ml. Considering this low value of  $LC_{50}$  and a very lower boundary range of 0.23 confirmed the effectiveness of *M. anisopliae* as good biological control agent. This result is by far lower than  $3.9 \times 10^8$  reported as  $LC_{50}$  conidia /ml of *Metarhizium anisopliae* against mosquito

larvae after 24 by [20]. Mycosis result of this study confirmed the actual effectiveness of *M. anisopliae* as more 90% of the larval mortality in was attributed to *M. anisopliae* isolate due to the development hypae of the fungal conidia on the dead *C. quinquefasciatus* larvae 244 hours after treatment.

### Conclusion

*M. anisopliae* significantly demonstrate high level of efficacy in the control of larval stage of *C. quinquefasciatus*, as 95% of larval mortality was reported at higher conidia/ml concentration of  $1 \times 10^{10}$ . Likewise other lower concentrations demonstrate relatively good larvicidal activity even at 24 hour of larval exposure. Therefore in a near future entomopathogenic can favourably compete and even replace conventional synthetic insecticide.

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