



Larvicidal Potential of Conidia Suspension of *Aspergillus flavus* against *Anopheles* Mosquitoes

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ABSTRACT: *Aim:* This study was aimed at investigating the larvicidal potential of the conidia suspension of *Aspergillus flavus* against *Anopheles* mosquitoes. *Method:* *Aspergillus flavus* was isolated from soil using soil suspension procedures and was identified using morphological characteristics. Bioassay was performed to determine the efficacy of *Aspergillus flavus* conidial suspension against early 4th instar larvae of *Anopheles* mosquito using WHO-2005 protocol with slight modifications. *Results:* Four different concentration of conidial suspension; 3.3×10^6 , 3.3×10^5 , 3.3×10^4 and 3.3×10^3 conidia/ml were tested, and the results show that; mortality increases with increase in conidial concentration and exposure time. *Conclusion:* These results indicated that *Aspergillus flavus* conidia suspension are pathogenic to immature stage of *Anopheles* mosquito and could be suggested as a biological control for mosquito management.

RESEARCH PAPER

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1.0 INTRODUCTION

Mosquitoes are known vectors of human and animal pathogens. Millions of people are killed by mosquito-borne diseases every year such as malaria, dengue, chikungunya, yellow fever, encephalitis and filariasis [24]. Vector control sanitation, habitat disruption and personal protection from mosquito bites are the most adopted measures employed to control and protect people from infection of these diseases [37]. Over the past few decades, many countries organized official programs of mosquito vector control. Currently, synthetic chemical insecticides against adults or larva have been the mainstay and are the most widely used for control of malaria vectors. Mosquito larvae are the attractive targets for these insecticides because mosquitoes breed in water and thus, it is easy to deal with them in this habitat [5]. The indiscriminate use of chemical insecticides to target adult mosquitoes results to problems such as mosquito resistance, environmental

contamination, and health risk to humans and non-target organisms. To reduce these problems, there is an urgent need to develop alternatives to conventional chemical insecticides, which are safe, effective, biodegradable and highly selective. There has been an increasing awareness in the use biological control agents as alternative to chemical control of mosquitoes. Among the eminent biological control agents are entomopathogenic microorganisms such as fungi and bacteria [8].

Entomopathogens are microorganisms capable of infecting and invading live insects at various developmental stages (larvae, pupae and adult) and ultimately killing their host through feeding on their body nutrients and secreting biochemical toxins. This brings about reduction in the population of pest and vector to a level that does not cause economic or health impact [4]. Bacterial and fungal entomopathogens are

widely employed as bio-control agents of mosquito worldwide, where fungi are most preferred because they are relatively easier to deliver. These entomopathogens also have higher chances of improving formulations, vast number of pathogenic strains, and wider range of host and are easily subjected to molecular transgenesis [17].

In recent years, interest on mosquito-killing fungi is reviving, mainly due to continuous and increasing levels of insecticide resistance and increasing global risk of mosquito-borne diseases. Historically, both environmental and biological controls of mosquitoes were exclusively aimed at larval stages and as such have been successful in a variety of geographical and ecological settings within the class of Dueteromycetes, especially Ascomycetes that have entomopathogenic fungi such as *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces formosus* species [16].

Fungal biocontrol agents are the most essential among all the entomopathogenic microorganisms due to easy delivery, chances to improve formulation, vast number of pathogenic strains known, easy engineering techniques and its ability to control both sap sucking pests such as mosquito and aphids as well as pest with chewing mouth parts. They include several phylogenetically, morphologically and ecologically diverse fungal species which evolve to exploit insects with their main route of entry being through the insect's integument, by ingestion or via wounds or trachea [35]. Most entomopathogenic fungi can be grown on artificial media; being natural mortality agents who are environmentally safe, there is a worldwide interest in the use and manipulation of entomopathogenic fungi for biological control of insects and other arthropod pests [31]. They display a higher degree of effectiveness in infecting their host, acting as regulators for numerous harmful insects including both domestic and forest insects. In general mosquitoes have shown susceptibility towards entomopathogenic fungi and their extracts. They have low toxicity to non-target organisms and using entomopathogenic fungi as larvicides may be a promising lead for biological control of mosquitoes due to their selective toxicity and ready decomposability in the ecosystem [6, 32]. Also, unlike the dangers which are associated with the process of production of synthetic insecticides, the process for the manufacture of microbial products is safe and less toxic. Conidia suspensions and extract of different entomopathogenic fungi, notably *Aspergillus fumigatus*, *Aspergillus terreus*, *Aspergillus nidulans*, *Lecanillium lecanii*, *Hirsutella thompsoni*, *Paecilomyces formosus*, *Meterhizium anisopliae*, *Beaveria bassiana*, *Lagenidium giganteum*, among others have been reported to exhibit promising larvicidal activity against mosquito larvae [10, 13, 21, 30]. In view of this, this research focuses on evaluating

larvicidal efficacy of conidial suspension of *Aspergillus flavus* on *Anopheles* mosquito.

2.0 MATERIALS AND METHODS

Fungal growth medias and selective proteins such as Potato dextrose agar, Czapek'sdcox agar/ broth, Cetyl-trimthyl ammonium bromide (CTAB), Chloramphenicol, synthetic chemical larvicides/ insecticides such as Malathion (781.25 mg/L), Temephos (156.25 mg/L) as well as chemicals used for fungal identification procedures such as Tween-20, Lacto-phenol cotton blue were supplied by the department of biochemistry, Bayero University, Kano. DNA extraction kit and PCR reagents were purchased from Sigma-Aldrich Inc., USA while laboratory apparatus and machineries used in this research were obtained from Biochemistry department laboratories and Microbiology department laboratory complex, Bayero University, Kano, Nigeria.

2.1 Collection of soil sample

Soil sample about (200 g) was collected from insect hibernation site including fields characterized by soil with a lot of leaf litters that typically cover the ground and grasses, shrubs and shades of trees at a depth of 0-20 cm using trowel after removing litter or weeds and placed in appropriately labelled plastic bags within Bayero University Kano premises (11.9836°N 8.4753°E). Before use, samples were thoroughly mixed and passed through 0.4 mm mesh sieve for breaking of soil lumps [1].

2.2 Isolation of entomopathogenic fungi from soil

Soil sample about (200 g) was collected from insect hibernation site including fields characterized by soil with a lot of leaf litters that typically cover the ground and grasses, shrubs and shades of trees at a depth of 20 cm using trowel after removing litter or weeds and placed in appropriately labelled plastic bags within Bayero University Kano premises (11.9836°N 8.4753°E). Before use, samples were thoroughly mixed and passed through 0.4 mm mesh sieve for breaking of soil lumps [25].

2.3 Isolation and identification of *Aspergillus flavus*

The fungus was isolated from soil using soil suspension procedures [14]. Soil suspension was prepared by weighing 0.1g of soil into 10mL 0.05% Tween-20. 100µL of the soil suspension was inoculated into a petri-dishes of solidified Czapek's media (3g NaNO₃, 0.5g MgSO₄.7H₂O, 1g K₂HPO₄, 0.5g KCl and FeSO₄.7H₂O), supplemented with 0.6 g/L of CTAB and 0.1 g/L of streptomycin. The plates were incubated at room temperature in the dark for 3-5days. Micro and macro morphological characteristics of the isolate was used for identification of fungal genus [27, 35].

2.4 Formulation of conidial suspension

Fungal conidiospore was harvested from 10 days old culture in 0.05% Tween-20 (used as negative control), its concentration was determined using hemocytometer, after which, four concentrations (3.3×10^6 , 3.3×10^5 , 3.3×10^4 and 3.3×10^3 conidia/ml) were formulated by serial dilution [11, 37].

2.5 Mosquito Larvae Collection, Identification and Maintenance

Mosquito larvae collected from stagnant water from Auyo Local Government Area of Jigawa State were brought and maintained in the insectaria laboratory at a temperature of 27°C , relative humidity of about 70% and a photoperiod of 12L: 12D h. Anopheles larvae were identified using morphological and behavioral characteristics as described by Gilles and Coetzee (1987)[15]. Fourth instars of Anopheles larva were transferred into separate containers and were maintained according to WHO-2005 protocol [37].

2.6 Bioassay

Bioassay was conducted according to WHO-2005 protocol with slight modification.

2.6.1 Conidial Bioassay

A set of 5 disposable cups each containing 15 fourth instars larvae was prepared. 4 cups were treated with one concentration of conidial suspension prepared as stated above, while the remaining cup was treated with 0.05% Tween-20 as negative control. The whole experimental set-up was prepared in triplicate and the result was reported as average of the three replicates [30].

3.0 RESULTS

Aspergillus flavus was identified using macro and micro-morphological characteristics of the cultured fungal isolate. Based on the observed characteristics; colonies have distinct margin and are covered with fluffy well developed aerial plane mycelium on the surface and culture appears yellow green when young and turns jade green as the culture ages. Spores were spherical in shape and colonies consisting of dense conidiospores as shown in figure 1 and 2 [20, 22, 34].

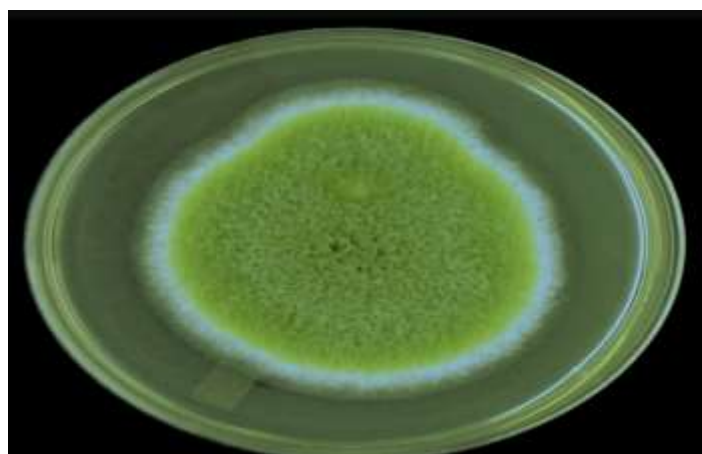


Fig-1: Two weeks old mono-cultured plates of fungal isolate

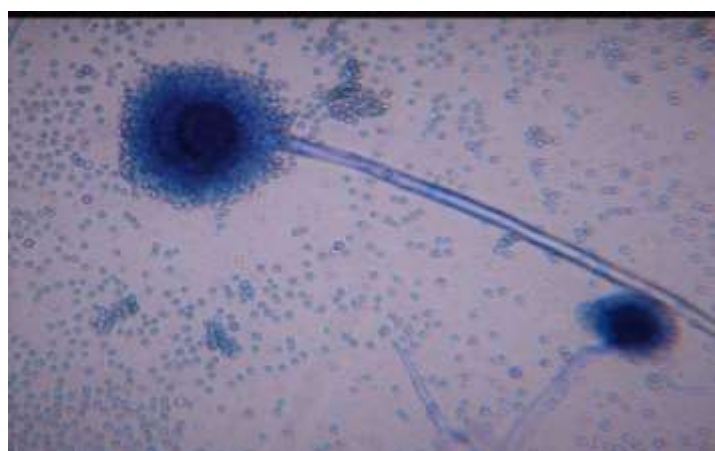


Fig-2: Micro Slide Image of the Fungal Isolate viewed under X100 magnification

This study reveals the larvicidal potential of conidia suspension sourced from the entomopathogenic fungus (*Aspergillus flavus*). Four different concentration of conidial suspension; (3.3×10^6 , 3.3×10^5 , 3.3×10^4 and 3.3×10^3 conidia/ml) were tested, and the results show that; mortality increases with increase in conidial concentration and exposure time. The lowest mean percentage mortality (12%) was recorded at 3.3×10^3 conidia/mL and the highest mean percentage mortality (78%) was recorded at 3.3×10^6 conidia/mL at 72-hours post exposure. The lethal concentration of

conidial suspension causing 50% and 90% mortality of the larvae was found to be 1.6×10^8 and 4.2×10^9 conidia/mL at 24-hours; 2.1×10^4 and 4.0×10^5 conidia/mL at 48-hours; 1.3×10^3 and 3.2×10^4 conidia/mL at 72-hours as shown in Table 1. During the current study, the conidial suspensions were stored under constant temperature (26°C - 27°C) and relative humidity (85-95%), thus, it is unknown if fluctuating environmental conditions would affect the length of residual activity.

Table-1: Larvicidal efficiency of *Aspergillus flavus* conidial suspension against *Anopheles* mosquitoes

Exposure time	Concentration (conidia/ml)	Percentage Mortality	Probit equation	LC ₅₀ (conidia/ml)	LC ₉₀ (conidia/ml)
24_Hours		43	$y = 0.90x + 3.30$	1.6×10^8	4.2×10^9
	3.3×10^6	26			
	3.3×10^5	18			
	3.3×10^4	12			
48_Hours	3.3×10^3		$y = 0.85x + 3.10$	2.0×10^4	4.0×10^5
	3.3×10^6	63			
	3.3×10^5	50			
	3.3×10^4	27			
72- Hours	3.3×10^6	14	$y = 0.80x + 3.00$	1.3×10^3	3.2×10^4
	3.3×10^6	78			
	3.3×10^5	64			
	3.3×10^4	33			
	3.3×10^3	25			

Positive control group (treated with 1ml 0.05% tween-20 and distilled water) records no mortality.

4.0 DISCUSSION

The findings in this study support the findings of Bogus *et al.* [9] who found that, an increase in the concentration of conidial suspension and time generally increase percentage mortality. The study also produced results which corroborate the findings of Sani *et al.* [30] reporting the percentage mortality of *Paecilomyces* spp against *Culex* mosquito larvae to be up to 80% after 96 h post treatment. Thomas *et al.* [38] also in his findings reported the percentage mortality of *Aspergillus fumigatus* against *Culex* mosquito reaching up to 96% after 72 h post treatment. Gayathri *et al.* [14] reported the pathogenicity of *Paecilomyces fumosoroseus* against *Culex quinquefasciatus* with 97.73% mortality on 8th day after treatment with 10^8 conidia/mL which is similar to this research with mortality reaching 98% at concentration of 6.6×10^7 conidia/mL after 72 h exposure time. In this study, mortality in the control was recorded zero percentage, pathogenicity varied according to concentration of conidial suspension and period of exposure. For the four concentrations of the conidial suspension isolate tested (Table 1). These findings further support the idea of Al-Hussaini and Hergian, [2] and Benserradj and Mihoubi [7] who reveal that larval mortality percent and LC50 of *Culex*

quinquefasciatus increased as exposure periods increased.

Furthermore, a research conducted in East Africa to determine the pathogenicity of entomopathogenic fungi against several strains of adult *Anopheles gambiae* revealed a high infection rates ranging from 46 to 88% with *Metarhizium anisopliae* being the most pathogenic strain [14]. In recent years, there is a considerable amount of attention focusing on identifying potential mosquitocidal fungus from natural sources for effective control of mosquitoes as a key measure to curtailing the vector borne diseases in human. A study was also conducted in Asia researching the larvicidal potential of *Lagenidium giganteum*, a water weed, leading to its efficacies in killing the tested vectors with appreciable safety to non-target organisms and good biological stability [27]. According to a large-scale field trial conducted in the United States, mycelium extract of *Lagenidium giganteum* caused 40-90% infection rates in *Culex tarsalis* and *Anopheles freeborni* sentinel larvae [3]. The potentials of many fungi have been established for mosquito control, nevertheless, only a few have received commercial attention and are marketed for use in vector control programmes globally [35].

The basic mechanism of pathogenesis behind is entrance through the external integument. Besides, infection through digestive tract is also possible [3].

Conidia attach to the cuticle, germinate and penetrate the cuticle. Once in the hemocoel, the mycelium grows and spreads throughout the host, forming hyphae and producing blastospores. Humidity is a key factor for high and rapid killing of insects by entomopathogenic fungi, and further development on cadavers [18, 29].

5.0 CONCLUSION

Conidia suspension of *Aspergillus flavus* have promising larvicidal activity against *Anopheles* mosquito larvae, the vector of *Plasmodium* parasite that causes malaria which is widely distributed in the Northern guinea savannah vegetation of Nigeria. The mortality was observed based on concentration and exposure time for conidial bioassay irrespective of the concentration.

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Conflict of interest

The authors declared no conflict of interests regarding the publication of this manuscript.

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