Original Research Article

LARVICIDAL POTENTIAL OF CONIDIA SUSPENSION OF *ASPERGILLUS FLAVUS* AGAINST *ANOPHELES MOSQUITOES*

ABSTRACT

Aim:This study was aimed at investigating thelarvicidal 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 *Aspergillusflavus* 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 3.3×10^3conidia/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 4.2×10^9conidia/mL at 24-hours; 2.1×10^4 and4.0×10^5 conidia/mL at 48-hours; 1.3×10^3 and 3.2×10^4 conidia/mL at 72-hours.

Conclusion: These results indicated that *Aspergillis flavus* conidia suspension are pathogenic to immature stage of *Anopheles* mosquito and could be suggested as a biological control for mosquito management.

Key words: *Aspergillus flavus; Anopheles* mosquito; Bioassay; Biolarvicide.

1.0 INTRODUCTION

Mosquitoes are known vectors of human andanimal pathogens. Millions of people are killedby mosquito-borne diseases every year such asmalaria, dengue, chikungunya, yellow fever,encephalitis and filariasis [24]. Vector control sanitation, habitat disruption and personalprotection from mosquito bites are the most adopted measures employed to control and protectpeople from infection of these diseases [37]. Over thepast few decades, many countries organizedofficial programs of mosquito vector control.Currently, synthetic chemical insecticides againstadults or larva have been the mainstay and are themost widely used for control of malaria vectors.Mosquito larvae are the attractive targets for theseinsecticides because mosquitoes breed in water andthus, it is easy to deal with them in this habitat [5].The indiscriminate use of chemical insecticides totarget adult mosquitoes results to problemssuch as mosquito resistance, environmentalcontamination, and health risk to humans and non-target organisms. To reduce these problems,there is an urgent need to develop alternatives toconventional chemical insecticides, which aresafe, effective, biodegradable

and highly selective.There has been an increasing awareness in the use biological control agents asalternative to chemical control of mosquitoes.Among the eminent biological control agents areentomopathogenic microorganisms such as fungiand 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. Th

ese 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 themost essential among all the entomopathogenicmicroorganisms due to easy delivery, chances toimprove formulation, vast number of pathogenicstrains known, easy engineering techniques and itsability to control both sap sucking pests such asmosquito and aphids as well as pest with chewingmouth parts. They include severalphylogenetically, morphologically andecologically diverse fungal species which evolveto exploit insects with their main route of entrybeing through the insect's integument, byingestion or via wounds or trachea [35]. Mostentomopathogenic fungi can be grown on artificialmedia; being natural mortalityagents which are environmentally safe, there is aworldwide interest in the use and manipulation ofentomopathogenic fungi for biological control ofinsects and other arthropod pests[31]. They display ahigher degree of effectiveness in infecting theirhost, acting as regulators for numerous harmfulinsects including both domestic and forestinsects.In general mosquitoes have shown susceptibility towardsentomopathogenic fungi and their extracts.They have low toxicity to non-target organismsand using entomopathogenic fungi as larvicidesmay be a promising lead for biologicalcontrol of mosquitoes due to their selectivetoxicity and ready decomposability in theecosystem [6, 32]. Also, unlike the dangerswhich are associated with the process ofproduction of synthetic insecticides, the processfor the manufacture of microbial products is safeand less toxic. Conidia suspensions and extract of different entomopathogenic fungi, notably *Aspergillus fumigatus, Aspergillus terreus, Aspergillus nidulans,*

Lecanillium lecanni, 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'sdox 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^{o}N 8.4753^{o}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^{o}N 8.4753^{o}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 perti-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 conidiosphore was harvested from 10days 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 3.3×10^3conidia/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 27^0C, 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.

2.61 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].

3.0RESULTS

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 conidiosphores as shown in figure 1 and 2 [20, 22, 34].

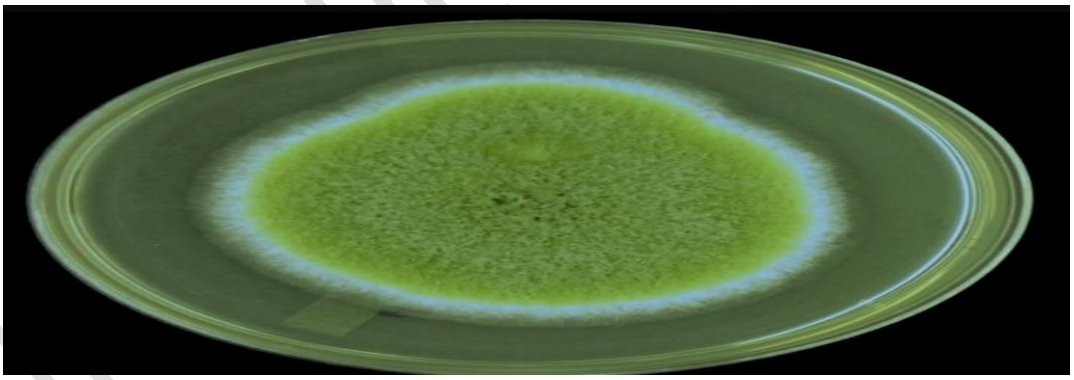

Figure 1: Two weeks old mono-cultured plates of fungal isolate

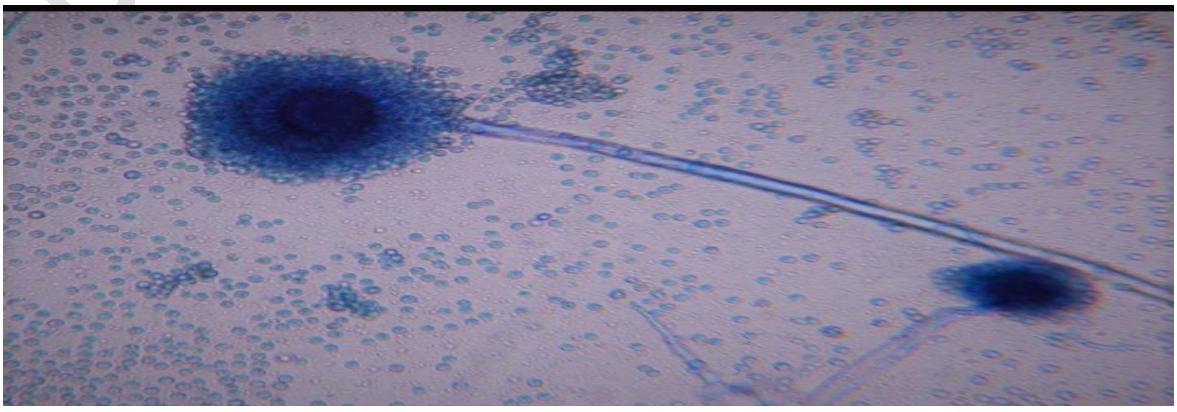

Figure 2: Micro Slide Image of the Fungal Isolate viewed under X100 magnification.

This study reveals the larvicidal potential ofconidia 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 4.2×10^9conidia/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			
	3.3×10^6	14			
72- Hours				1.3×10^3	3.2×10^4
			y = 0.80x + 3.00		
	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 ofBogus *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 8^{th} day after treatment with 10^8 conidia/mLwhich 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 periodsincreased.

Furthermore, a research conductedin East Africa to determine the pathogenicity ofentomopathogenic fungi against several strains ofadult *Anopheles gambiae* revealed a high infectionrates ranging from 46 to 88% with *Metarhiziumanisopliae* being the most pathogenic strain [14]. Inrecent years, there is a considerable amount ofattention focusing on identifying potentialmosquitocidal fungus from natural sources foreffective control of mosquitoes as a key measureto curtailing the vector borne diseases in human.A study was also conducted in Asia researching the larvicidalpotential of *Lagenidium giganteum*, a water weed,leading to its efficacies in killing the tested vectorswith appreciable safety to non-target organismsand good biological stability [27]. According to alarge-scale field trial conducted in the UnitedStates, mycelium extract of *Lagenidium giganteum*caused 40-90% infection rates in *Culex tarsalis*and *Anopheles freeborn sentinel* larvae [3]. The potentials of many fungi have beenestablished for mosquito control, nevertheless,only a few have received commercial attentionand are marketed for use in vector controlprograms 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.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

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