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RESEARCH ARTICLE

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Larvicidal Efficacy of Entomopathogenic Fungus, Metarhizium anisopliae against Culex quinquefasciatus (Say) (Diptera: Culicidae)

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ABSTRACT

Biological control potential of Metarhizium anisopliae against Culex quinquefasciatus was evaluated. M. anisopliae was isolated from soil using soil suspension method with selective isolation media. Bioassay was made to determine its efficacy against 4th instar larvae of C. quinquefasciatus. Three different concentrations; 1 × 10^6, 1 × 10^7 and 1 × 10^8 conidia/ml were made and tested. Results showed that, mortality increased as the period of exposure increased and also increased as the conidia concentration increased. The mortality recorded in lowest dose of 10^6 conidia/ml was 60%, and again 80% mortality was recorded at dose of 10^7 conidia/ml. whereas highest dose level of 10^8 conidia/ml caused high mortality of up to 90%. The lethal concentration causing 50% mortality (LC_{50}) of 4th instars larvae of C. quinquefasciatus was also varied according to concentration of spores and duration of exposure. The result showed that LC_{50} values of M. anisopliae isolate were 3.8 × 10^8, 2.5 × 10^6, 2.0 × 10^5 and 1.7 × 10^7 conidia/ml after 24, 48, 72 and 96 Hours exposure respectively. These results indicated that M. anisopliae isolated is pathogenic to immature stage of C. quinquefasciatu and could be suggested for development as a biological control for mosquitos' management.

Keywords: Culex quinquefasciatus, Entomopathogenic fungi, Metarhizium anisopliae, Biocontrol

INTRODUCTION

Lymphatic filariasis, commonly known as elephantiasis, is one of the most serious neglected tropical diseases. Infection occurs when filarial parasites are transmitted to humans through mosquitoes [1]. Mosquito control, sanitation, habitat disruption or personal protection from mosquito bites are the most widely measures employed to control and protect people from infection of these diseases [2]. Over the past few decades, many countries organized official programs of mosquito vector control [3].

Currently, synthetic chemical pesticides against adults or larvae have been the mainstay and are the most widely used for control of Mosquitoes [4]. Mosquitos' larvae are the attractive targets for these pesticides because mosquitoes breed in water and thus, it is easy to deal with them in this habitat [5,6]. The indiscriminate use of these chemical has given rise a problems such as Mosquito resistance, environmental contamination and health risk to humans and non-target organisms [7-9]. As a result, there is an urgent need to develop alternatives to conventional chemical insecticides, which are safe, effective, biodegradable and highly selective. In recent years, there has been an increasing interest in the possibility of using biological control agents as alternative to chemical control of mosquitoes. Among the eminent biological control agents, plants extract [5,10,11] and Entomopathogenic microorganisms such as bacteria and fungi [7-9,12-15] are the most widely used for mosquito control, and they are used throughout the world with great advantage and success. Fungal biocontrol agents are the most important 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 [16]. They include the genera of *Metarhizium, Beuvaria, Paecilomyces* and many more [17,18], and are in use to manage various Mosquitoes species [19].

Most Entomopathogenic Fungi can be grown on artificial media [20,21]. Base on some literatures [22,23], many fungal species have been isolated from soil samples and identified. Despite the benefits associated with Entomopathogenic

fungi, there has been little information on the use of indigenous fungal pathogens of insects for the control of Mosquitoes in Nigeria. But a number of studies have found that entomopathogenic fungi (Hypomycetes) significantly control species of mosquitoes.

The objective of this study was therefore to isolate different fungal isolate of entomopathogenic fungi, for selection of virulent isolate *M. anisopliae* specie and evaluate in laboratory, it efficacy in the control of Larvae of Culex mosquito, *C. quinquefasciatus.*

MATERIAL AND METHODS

Study area and sampling site

The study was conducted at Umaru Musa Yar'adua University Katsina at latitude 12º 53' N and longitude 7º 35' E. Soil sample were collected from insect hibernation site including fields characterized by soil with a lot of leaf litters that typically covers the ground and grasses, shrubs and shade of trees.

Collection of soil sample

Soil sample about (1000 g. each) were taken from different depth of (0-20 cm) with a trowel after removing litter or weeds and placed in appropriately labeled plastic bags and the global position of the site using Global Positioning System (GPS) was recorded.

Samples were subjected for fungal isolation within one week of collection. Before use, samples were thoroughly mixed and passed through a 0.4 mm mesh sieve for breaking soil lumps and separating litter remains [24].

Isolation of m. anisopliae from soil by selective culture media

A selective entomopathogenic fungal isolation media [25-27], with cetyltrimethyl ammonium bromide (Sigma Aldrich Co., USA) were used in the isolation of the fungal isolate; *M. anisopliae* from soil. The media is often called isolation medium. Isolation media plates were prepared with a 20 g/l of rolled oatmeal, 20 g/l of agar 0.6 g/l of cetyl trimethyl ammonium bromide (CTAB) and 0.1 g/l of streptomycin to retard bacterial growth.

About 0.1 g of soil sample was diluted in 10 ml of prepared 0.05% of tween 80 in a test tube. One hundred microlitres (100 µl) of soil suspension were then placed aseptically above solidified media in petridishes. The suspensions were then spread using an L-shape glass rod. The plates were prepared in triplicates and incubated at room temperature until growth of fungi is observed.

After 3 to 5 days, fungal growths of different colonies were observed in the isolation medium. A strand of mycelium is aseptically transferred onto Potato Dextrose Agar (PDA) medium (Micromedia Trading house, Ltd. Hungary) and incubated in the dark at about 24°C, to obtain pure colonies of different fungi species. After 3 to 5 days pure fungal growth was observed in each culture.

Identification of fungi

To identify entomopathogenic fungi isolated from soil, pure colonies of the observed fungi were prepared on microscope slides. A sterile needle was used to collect a strand of mycelia and placed on a glass slide to study for micro-morphological features using a microscope (Olympus CX31 series, England). (X10 X20 X40 and X100) The macroscopic and microscopic characteristics of the isolated fungi were examined using reference text [22,23].

Production of conidial suspension (concentration) and counting of spores

Spore suspensions were prepared from 21 day-old surface culture of entomopathogenic fungi on PDA medium. A mixture of conidia and hyphae was harvested by flooding the Petri dishes with 20 ml of sterile distilled water in 0.05% Tween 80. The resultant suspension was then placed in universal bottles containing 3 mm glass beads. The conidial suspension was then vortexed for 10 min to produce a homogeneous suspension.

To establish the concentration of the conidia in the solution, a hemocytometer (France) was used to count the number of conidia under a compound microscope (Olympus CX31 series, England). The conidial suspension was further diluted with 0.5% Tween 80 solution, until it reaches a concentration with a countable number of spores. After having the established concentration of conidia, suspensions were diluted with distilled water to the concentrations of 1×10^6, 1×10^7 and 1×10^8 conidia/ml

Mosquito larvae rearing

C. quinquefasciatus larvae collected from stagnant water within the study area were brought and maintained in the laboratory at a temperature of 27°C, relative humidity of about 70% and a photoperiod of 12:12 h.

Different instars of mosquitoes were maintained in separate containers at a density of 50 larvae per container in distilled water at pH 7.0. Larvae were provided with yeast powder as food media in every 24 hours. To counteract evaporation water was added daily.

Larvicidal bioassay

Laboratory bioassay was done following methods of with some modifications. Conidia of *M. anisopliae* were tested against the 4th instar larvae of *C. quinquefasciatus* by adding fungal suspension to a beaker containing 20 ml of distilled water with 10 larvae of the 4th instar. Each beaker was inoculated with 1 ml of fungal suspensions (10^6, 10^7 and 10^8 conidia/ml). Control treatments were carried out by addition of 20 ml of distilled water only. Each assay was replicated three times. Larvae were fed with yeast powder and their mortality was observed in a 24 h interval for 7 days.

Mycosis test

A mycosis test was made to see how many of the dead larvae actually died from fungal infestation. Three Petri dishes were prepared, two with distilled water and one with 70% ethanol. The dead larvae were dipped one by one, first in distilled water, then in ethanol and in distilled water again in order to kill the fungus on the surface of the larvae. Each Petri dish contained larvae from the three concentrations and the different fungal isolate were kept separately. The process was repeated for all the replicates. If fungal subsequently started to grow on the larvae, the fungal isolate had penetrated the larvae cuticle, meaning that the larvae had died from the fungal infection.

Statistical analysis

Lethal concentration causing 50% mortality (LC_{50}) were estimated by fitting mortality data to probit analysis by using statistical computer programmed, Statistical Package of Social Sciences (SPSS)-20.

RESULTS AND DISCUSSION

Result from these study show that *M. anisopliae* isolate tested against 4th instars larvae of *C. quinquefasciatus* has pathogenic effect. Mortality in the control was recorded zero percentage. However, pathogenicity varied according to concentration of spores and period of exposure. For the three concentrations; 1×10^6, 1×10^7 and 1×10^8 conidia/ml of the fungal isolate tested, it was observed that, mortality increased with increased in the time period and also increased as the conidia concentration increased (Table 1). The mortality of the 4th instar larvae, for instant, ranged from 20 to 90% after 96 h post treatment. As can be seen from the Table 2, maximum mortality was recorded at the highest dose of 10^8 conidia/ml applied. Consequently, the mortality was recorded in lowest dose of 10^6 conidia/ml and 10^7 conidia/ml as 60%, and 80% respectively. Whereas highest dose level of 10^8 conidia/ml caused the highest mortality 90%.

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

Fungal Specie	Concentration (Conidia/ml)	Time			
		24 h	48 h	72 h	96 h
Metarhizium anisopliae	10^6	20	30	50	60
	10^7	40	50	70	80
	10^8	50	70	80	90
Control	0	0	0	0	0

Table 2: The LC_{50} value of *Metarhizium anisopliae* against 4th mosquito larvae of *Culex quinquefasciatus* after 24, 48, 72, and 96 h

Fungal Specie	Time of exposure	Probit equation	LC_{50} (Conidia/ml)
Metarhizium anisopliae	24 h	0.522x + 4.448	3.8×10^8
	48 h	0.262x + 2.094	2.5×10^6
	72 h	0.3887x + 2.622	2.0×10^5
	96 h	0.426x + 2.523	1.7×10^7

The lethal concentration causing 50% mortality (LC_{50}) of 4th instars larvae of *C. quinquefasciatus* was also varied

according to concentration of spores and duration of exposure. The LC_{50} values of *M. anisopliae* isolate tested is 3.8×10^8, 2.5×10^6, 2.0×10^5 and 1.7×10^7 conidia/ml after 24, 48, 72 and 96 Hours exposure period respectively.

In this study, the efficacy of Entomopathogenic fungi, *M. anisopliae* has been demonstrated against 4th instars larvae of *C. quinquefasciatus*. Very little information exists on the isolation of entomopathogenic fungi for the control of insect pests in Nigeria, but the use of entomopathogenic Fungi for the control of Mosquito has long been recognized from different part of the world [6,9,15,25]. Moreover, this fungus specie isolated from soil had been previously detected in the soil from different part of the world along with some of the most widely used groups of fungal pathogens such as *Beauveria bassiana* and *Paecilomyces* species [26,27]. The results of this study indicate that, Entomopathogenic fungi; *M. anisopliae* was pathogenic to 4th instars larvae of *C. quinquefasciatus* and percentage mortality reaches 90%. This study produced results which corroborate the findings of who found the percentage mortality of *M. anisopliae* against culex mosquito larvae to be up to 96% after 96 hours post treatment [9]. The efficacy of *M. anisopliae* has been reported to others immature Mosquitoes species, such as *Aedes aegypti* [15] *Anophele gambiae* [14], *Aedes albopictus* [25]. These findings further support the idea [9], who found that, an increase of the concentration of spores and time generally increases percentage mortality.

CONCLUSION AND RECOMMENDATION

In this study, entomopathogenic fungi isolate were obtained from soil suspension by the use of novel dodine free-selective medium with the Cetyl trimethyl ammonium bromide (CTAB). Immature mosquito of *C. quinquefasciatus* was exposed to one isolate of hypomecetes entomopathogenic fungi isolate: *M. anisopliae*. Results indicate that, efficacy and LC_{50} varied according to concentration of spores and period of exposure. *M. anisopliae* isolate had lower LC_{50} value and is highly pathogenic to 4th instars larvae of *C. quinquefasciatus*; an increase in the concentration of spores and time generally also increases the mortality and might generate a faster result.

The fungal species isolated should be confirmed through advance techniques and also should be used as mycoinsecticides in the combat against Mosquitoes and various insect pests.

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