



Larvicidal Activity of Entomopathogenic Fungus, *Paecilomyces* Specie Against Fourth Instar Larvae of the Mosquito *Culex quinquefasciatus* (Culicidae: Diptera)

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Abstract

Biological control potential of *Paecilomyces* spp against *Culex quinquefasciatus* was evaluated. *Paecilomyces* spp was isolated from soil using soil suspension method with selective isolation media. Bioassay was made to determine its efficacy against 4th instar larvae of *Culex 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 50%, and again 70% 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 80%. The lethal concentration causing 50% mortality (LC₅₀) of 4th instars larvae of *C. quinquefasciatus* was also varied according to concentration of spores and duration of exposure. The result showed that LC₅₀ values of *Paecilomyces* spp isolate were 5.3×10^8 , 3.8×10^7 , 2.0×10^6 and 2.3×10^7 conidia/ml after 24, 48, 72 and 96 Hours exposure respectively. These results indicated that *Paecilomyces* spp isolated was pathogenic to immature stage of *C. quinquefasciatus* and could be suggested for development as a biological control for mosquitos' management.

Keywords: *Culex quinquefasciatus*, Entomopathogenic fungi, *Paecilomyces* spp, Biocontrol

INTRODUCTION

Mosquitoes are among the most serious insect pests affecting the health of millions of people by transmitting the disease-causing agent (pathogen) of several diseases, including encephalitis, dengue, yellow fever, malaria and filariasis (Jackman and Olson 2002). Vector 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 (WHO, 2013). Over the past few decades, many countries organized official programmes of mosquito vector control (Wilke, 2009).

Currently, synthetic chemical pesticides against adults or larvae have been the mainstay and are the most widely used for control of Mosquitoes (Arivoli *et al.*, 2012; Al-Hussaini and Hergian, 2014). Mosquito larvae are the attractive targets for these pesticides because mosquitoes breeds in water and thus, it is easy to deal with them in this habitat (Arivoli *et al.*, 2012). 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 (Seye *et al.*, 2012; Bhan *et al.*, 2013; Benserradj and Mihoubi, 2014). 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, plant extract (Rajkumar and Jebanesan, 2005; Remia and Logaswamy, 2009; Arivoli *et al.*, 2012) and entomopathogenic microorganisms such as bacteria (Mulla, 1991; Ramirez-Lape and Ramirez -Suero, 2012) and fungi (Scholte *et al.*, 2008; Seye *et al.*, 2012; Bhan *et al.*, 2013; Butt *et al.*, 2013; Benserradj and Mihoubi, 2014) 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 (Khan *et al.*, 2012). They include the genera of *Metarhizium*, *Beuveria*, *Paecilomyces* and many more (Goettel and Inglis, 1997; Shahid *et al.*, 2012), and are in use to manage various Mosquitoes species (Singh and Prakash, 2014).

Most entomopathogenic Fungi can be grown on artificial media (Shin *et al.*, 2009; Posades *et al.*, 2012). Base on some literatures (Richard, 2005 and Watarabe 2010), many fungal species have been isolated from soil samples and identified. The main route of entrance of the entomopathogenic fungi is through the insect's integument and by ingestion method or through the wounds or trachea. The virulence of fungal entomopathogens involves four steps: adhesion, germination, differentiation and penetration. Each step is influenced by a range of integrated intrinsic and external factors, which ultimately determine the pathogenicity (Shahid *et al.*, 2012). Of all the 31 species of *Paecilomyces* spp, 14 species are known pathogens of arthropods (Gayathri *et al.*, 2010). *Paecilomyces* species is a geographically widespread group of many entomopathogens that can infect different orders of insects in all stages of development and can be frequently isolated from soil (Alessandro *et al.*, 2013).

Like most entomopathogenic fungi, *Paecilomyces* spp., infects its host by breaching the cuticle. Various metabolites allow the pathogen to physically penetrate the host as well as inhibit its regulatory system (Hussein *et al.*, 2016).

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.

The objective of this study was therefore to isolates different fungal isolates of entomopathogenic fungi, for selection of virulent isolates *Paecilomyces* species and evaluate in laboratory, it efficacy in the control of Larvae of *Culex* mosquito, *Culex quinquefasciatus*.

Material and Methods

Study Area and Sampling Site

The study was conducted at Umaru Musa Yar'adua University, Katsina at latitude 12° 53' N and longitude 7° 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 (1000g. each) were taken from different depth of (0-20cm) 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 0.4mm mesh sieve for breaking soil lumps and separating litter remains (Abdullah *et al.*, 2015).

Isolation of *Paecilomyces* specie from soil by selective culture media

A selective entomopathogenic fungal isolation media (Posades *et al.*, 2012), with cetyltrimethyl ammonium bromide (Sigma Aldrich Co., USA) were used in the isolation of the fungal isolate, *Paecilomyces* spp from soil. Isolation media plates were prepared with a 20 g/l of rolled oatmeal, 20 g/l of agar 0.6g/l of Cetyltrimethyl ammonium bromide (CTAB) and 0.1 g/l of streptomycin to retard bacterial growth.

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 (100µl) 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 putative 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 (Richard, 2005 and Watarabe, 2010).

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 20ml of sterile distilled water in 0.05% Tween 80. The resultant suspension was then placed in universal bottles containing 3mm 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 haemocytometer (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

Culex 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 12:12h.

Different instars of mosquitoes were maintained in separate containers at a density of 50 larvae per container in distilled water of 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 Benserradj and Mihoubi, (2014) with some modifications; conidia of *Paecilomyces* spp. were tested against the mosquito larvae, *C. quinquefasciatus* by adding fungal suspension to a beaker containing 20ml of distilled water with 10 larvae of the 4th instar. Each beaker was inoculated with 1ml of fungal suspensions (10^6 , 10^7 and 10^8 conidia/ml). Control treatments were carried out by addition of 20ml of distilled water only. Each assay was replicated three times. Larvae were fed with yeast and their mortality was observed in a 24 hrs 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 isolates 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 IBM SPSS statistics-20.

Results and Discussion

The Results obtained from this study showed that *Paecilomyces* specie isolate tested against 4th instars larvae of *Culex quinquefasciatus* has some 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 of time period, and also increased as the conidia concentration increased (Table 1.). The mortality of the 4th instar larvae, for instant, ranged from 10 to 80% after 96 hours post treatment. As can be seen from the Table 1 maximum of mortality was recorded at the highest dose of 10^8 conidia/ml applied. Accordingly, the mortality was recorded in lowest dose of 10^6 conidia/ml and 10^7 conidia/ml as 50%, and 70% respectively. Whereas highest dose level of 10^8 conidia/ml spp caused the highest 80%.

Table1: Percentage mortality (%) of *Culex quinquefasciatus* larvae exposed to different concentrations of *Paecilomyces* spp isolate

Fungal Specie	Concentration (Conidia/ml)	Time			
		24 Hours	48Hours	72Hours	96Hours
<i>Paecilomyces</i> spp	10^6	10	30	40	50
	10^7	20	40	50	70
	10^8	40	50	70	80
Control	0	0	0	0	0

The mortality percentage and duration of exposure were expressed using a bar chat column in Figure 1; below

The lethal concentration causing 50% mortality (LC₅₀) was also varied according to concentration of spores and duration of

exposure. The LC₅₀ values were 5.3×10⁸, 3.8 × 10⁷, 2.0 × 10⁶ and 2.3×10⁷ conidia/ml after 24, 48, 72 and 96 Hours exposure, respectively.

Table 2. The LC₅₀ value of *Paecilomyces* Spp against 4th mosquito larvae of *Culex quinquefasciatus* after 24, 48, 72, and 96 hours

Fungal Specie	Time of exposure	Probit equation	LC ₅₀
<i>Paecilomyces</i> spp	24Hours	0.413x + 3.255	5.3 × 10 ⁸
	48Hours	0.524x + 3.671	3.8 × 10 ⁷
	72Hours	0.426x + 2.523	2.0 × 10 ⁶
	96Hours	0.522x + 2.860	2.3 × 10 ⁷

In this study, the efficacy of Entomopathogenic fungi, *Paecilomyces* Species has been demonstrated against 4th instars larvae of *Culex quinquefasciatus*. Very little information exists on the isolation of entomopathogenic fungi for the control of insect pests in Nigeria, but the use of hypomecete entomopathogenic Fungi for the control of adult and immature mosquitoes species has already been recognized from different part of the world (Scholte, *et al.*, 2008; Gayathri *et al.*, 2010; Bilal *et al.*, 2012; Butt *et al.*, 2013; Benserradj and Mihoubi, 2014 Rashed *et al.*, 2014). The result of this study indicate that, Entomopathogenic fungi, *Paecilomyces* species were the fungal pathogen isolated from the soil and were previously detected along with some of the most widely used groups of hypomecete entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium* spp (Sooker *et al.*, 2008; Hasan *et al.*, 2012; Esparza Mora *et al.*, 2016). Furthermore, this fungal specie isolated had been previously reported to have pathogenic effect in some insect species such as *Bactrocera cucurbitae*, *B. zonata* and *Lipaphis Erysimi* (Sooker *et al.*, 2008; Ujjanand Shahzad, 2012).

This study produced results which seemed to be consistent with that of Gayathri *et al.* (2010)

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who found that, *Paecilomyces fumosoroseus* were effective against 4th instars larvae of *Culex quinquefasciatus* inducing at least 74% mortality. These findings further support the idea of Al-Hussaini and Hergian(2014) and Benserradj and Mihoubi, (2014) who reveal that larval mortality percent and LC₅₀ of *C. quinquefasciatus* increased as exposure periods increased.

Conclusion and Recommendation

In this study, entomopathogenic fungi isolate *Paecilomyces* spp were obtained from soil suspension by the use of novel dodine free-selective medium with the Cetyltrimethyl ammonium bromide (CTAB). Immature mosquito of *Culex quinquefasciatus* were exposed to one isolate of hypomecetes entomopathogenic fungi isolate, *Paecilomyces* spp. Results indicate that, efficacy and LC₅₀ varied according to concentration of spores and period of exposure. An increase in the concentration of spores and time generally increases the mortality and might generate faster result.

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

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