LARVICIDAL ACTIVITY OF *BEAUVERIA BASSIANA* AND *PAECILOMYCES* SPECIE ON *CULEX QUINQUEFASCIATUS* (SAY) (DIPTERA: CULICIDAE)

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Abstract

Biological control potential *of Beauveria bassiana and Paecilomyces* species against *Culex quinquefasciatus* was investigated. The two fungi have been reported to exhibit insecticidal activity against mosquito and other insects. *Beauveria bassiana and Paecilomyces* spp was isolated from soil using soil suspension method with selective isolation media procedures. Bioassay was made to determine their efficacy against 4th instar larvae of *C. quinquefasciatus.* Three different concentrations; $1x10^7$, $1x10^6$ and $1x10^5$ conidia/ml were made and tested. Results showed that mortality increased with increase in conidia concentration and exposure time. The mortality recorded in lowest dose of 10^5 conidia/ml was 70% and 50% in *Beauveria bassiana* and *Paecilomyces* spp respectively, and again 80% and 60% mortality were recorded at dose of 10^6 conidia/ml. The highest dose level of 10^7 conidia/ml of *Beauveria bassiana* and *Paecilomyces* spp isolate caused high mortality up to 90% and 80% respectively. 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 *Beauveria bassiana* isolate after 24-, 48-, 72- and 96-Hours exposure time were 3.9×10^8, 2.6×10^6, 2.0×10^4 and $1.6x10^6$ conidia/ml. Similarly, LC_{50} values *Paecilomyces* spp isolate after 24,46,72,96-Hours exposure were 6.3×10^8, 4.8×10^7, 3.0×10^4 and 2.3×10^6 conidia/ml respectively. These results indicated that *B. bassiana* and *Paecilomyces* spp isolated is pathogenic to immature stage of *C. quinquefasciatu*s and although not as efficient as chemical larvicides and could be suggested for development as a biological control for mosquito management so as to avoid concerns associated with chemical larvicides

Keywords: *Beauveria bassiana*, Biocontrol, *Culex quinquefasciatus*, Entomopathogenic fungi, *Paecilomyces* spp.

Introduction

Malaria is transmitted by female mosquitoes of the genus *Anopheles*, and it is a major public health challenge in developing countries. Malaria is Africa's major cause of mortality in those younger than 5 years of age and constitutes 10% of the continent's overall disease burden (Akande and Musa, 2005; WHO, 2017). Malaria is arguably the most serious vector borne disease worldwide. The already alarming mortality rate caused by malaria is increasing, caused in part by the increase in mosquito resistance to chemical insecticides. Nearly half of the world population is at risk of contracting malaria, and over one million people, mostly African children, die of the disease every year (Gimba and Idris, 2014; Thu *et al*., 2017). The disease is widespread in the tropical and subtropical regions that exist in a broad band around the equator. This includes much of Sub-Saharan Africa, Asia, and Latin America (WHO, 2014).

Entomopathogenic fungi (EPF) cause lethal infections in their host, and they are the natural regulator of many insect pests including those living in soil by epizootics (Goettel *et al*., 2005). These microorganisms have attracted remarkable attention for their usage in biological control programs of insect pests in both agriculture and forestry as environmentally safe agents (Lacey *et al*., 2015; Strasser *et al*., 2010). Among these fungi, *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschn) Sorokin are the most studied fungi in terms of commercial production (Goettel *et al*., 2005; Meyling and Eilenberg, 2007). Majority of the entomopathogenic fungi infects their host with the attachment of conidial spores on the external body surface (cuticle). Soil is an important reservoir for many entomopathogen and they have a great importance in terms of biological control. Many entomopathogenic fungi such as *B. bassiana*, *M. anisopliae*, and *Paecilomyces* spp. spend a significant period of their life cycle in soil (Jackson *et al*., 2000).

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

Materials and Methods

Soil sample collection and storage

Soil sample was collected from three different locations around Biochemistry department, Bayero University Kano (11.9836ºN 8.4753ºE). The soil surface was first cleared of leaves and other litters and hole 10cm below the ground was made using hand-trowel. About 200g of soil was then collected in a clean polyethene bag. The soil collected was air-dried and then stored at room temperature (Dong *et al*., 2016).

Isolation and identification of entomopathogenic fungi

Beauveria bassiana and *Paecilomyces* spp were isolated from soil samples around Biochemistry department, Bayero University Kano premises. Before use, samples were thoroughly mixed and passed through 0.4mm mesh sieve for breaking soil lumps. Serial dilutions were made up to three dilutions. From each dilution was plated on DOA (dodine oatmeal agar) selective medium for screening entomopathogenic fungi (containing 200 µg /ml dodine (Beilharz *et al*.,1982) and 50 µg/ml streptomycine (Chase *et al*., 1986, Liu *et al*., 2007; Du *et al*., 2008). The plates were incubated for 14 days at 28°C. After 14 days resulting colonies were purified on SDAY (Sabouraud dextrose agar) and identified using

standard mycological keys. Fungus culture Isolates *Beauveria bassiana* and *Paecilomyces* spp were cultured on PDA (potato dextrose agar) medium and incubated at 28°C for 14 days after inoculation. the conidia were harvested by scraping the surface of 14 days old culture and were suspended in solution of 0.01% Tween80 in distilled water. The mixture was stirred with a magnetic stirrer for 10 min. The conidial concentration of the final suspension was determined by direct count using a hemocytometer (Hazrat *et al*., 2012). Dilutions were made using 0.01% Tween80 to obtain conidia concentrations of 10^7, 10^6, 10^5 conidia/ml.

Mosquito larvae rearing

Culex quinquefasciatus larvae were maintained in the laboratory at a temperature of 27 + 2°C, relative humidity of 70 + 5% and a photoperiod of 14:10h. Different instars of mosquitos were maintained in separate enamel container at a density of 200 larvae per container. Larvae were provided a mixture of yeast powder and biscuit as food every 24 hours. Larvae were reared in distilled water at pH 7.0. To counteract evaporation, water was added daily (WHO, 2014).

Larvicidal bioassays

Conidia of *Beauveria bassiana* and *Paecilomyces* species were tested against mosquito larvae by adding fungal suspension to plastic cups containing 50 ml of distilled water with 25 larvae of the 4th instar. Each cup was inoculated with 1ml of fungal suspensions (10^7, 10^6, 10^5 conidia/ml). Control treatments were carried out by addition of 10 ml of distilled water. Each assay was conducted three times. Larvae were fed and they were observed daily, larvae mortality was evaluated on a daily basis of 10 days (WHO, 2014).

Statistical analysis

Lethal concentration causing 50% mortality (LC50) were estimated by fitting mortality data to probit analysis by using statistical computer programmed, Statistical Package of Social Sciences (SPSS) – 20.

Results and Discussion

Table 1: Larvicidal efficiency of conidial suspension of *Beauveria bassiana* and *Paecilomyces* spp against Culex *quinquefasciatus*

Fungal Species	Concentration (Conidia/ml)	Exposure Time			
		24Hours	48Hours	72Hours	96Hours
Beaveria basssiana	10^5	10	20	40	70
	10^6	30	50	60	80
	10^7	40	60	80	90
Paecilomyces spp	10^5	15	30	40	50
	10^6	30	45	50	60
	10^7	40	50	70	80
Control	0	0	0	0	0

Control group treated by addition of 10 ml of distilled water recorded no mortality for, 24, 48, 72, 96-hours respectively.

Table 2: The LC50 value of *B. bassiana* and *Paecilomyces* spp against 4th mosquito larvae of *Culex quinquefasciatus* after 24, 48, 72, and 96hours exposure time

	Fungal Specie	Exposure Time (Hours)	Probit Equations	LC_{50}(Conidia/ml)
1.	***Beauveria bassiana***	24	0.534x + 3.245	3.9×10^8
		48	0.262x + 2.345	2.6×10^6
		72	0.384x + 2.267	2.6×10^4
		96	0.534x + 3.255	1.6×10^7
	***2. Paecilomyces* spp**	24	0.434x + 2.611	6.3×10^8
		48	0.534x + 3.765	4.8×10^7
		72	0.480x + 3.453	3.0×10^4
		96	0.533x + 2.288	2.3×10^6

Result from this research show that *B. bassiana* and *Paecilomyces spp* isolates tested against 4th instars larvae of *C. quinquefasciatus* have larvicidal activity. Mortality in the control was recorded zero percentage. However, pathogenicity varied due to the concentration of conidia and exposure time. For the three concentrations; $1x10^7$, $1x10^6$ and $1x10^5$ conidia/ml of the fungal isolate tested, it was observed that, mortality increased with increase in conidia concentration and exposure time (Table 1.).

In *B. bassiana* isolate, the mortality of the 4th instar larvae ranged from 10 to 90% after 96 hours post treatment. From Table 1, maximum mortality of 90% was recorded at the highest dose of $1x\ 10^7$ conidia/ml applied. Consequently, the mortality recorded in lowest dose of 10^5 conidia/ml and 10^6conidia/ml as 70%, and 80% respectively.

Similarly, the mortality of the 4th instar larvae due to *Paecilomyces* spp, ranged from 15 to 80% after 96 hours post treatment. From Table 1, maximum mortality of 80% was recorded at the highest dose of 10^7conidia/ml applied. Accordingly, the mortality was recorded in lowest dose of 10^5 conidia/ml and 10^6 conidia/ml as 50%, and 60% respectively.

In this study, the efficacy of entomopathogenic fungi, *B. bassiana and Paecilomyces* spp have been investigated against 4th instars larvae of *C. quinquefasciatus* with maximum mortality reaching 90% and 80% after 96hous exposure time. This study produced results which corroborate the findings of Sani *et al*. (2016) reporting the percentage mortality of *Paecilomyces spp* against *Culex* mosquito larvae to be up to 80% after 96 hours post treatment. Thomas *et al*. (2016) also in his findings reported the percentage mortality of *Aspergillus fumigatus* against *Culex* mosquito reaching up to 96% after 72 hours post treatment. In the same manner, Gayathri *et al*. (2010) reported the pathogenicity of *Paecilomyces fumosoroseus* against *Culex quinquefasciatus* showed 97.73% mortality on 8th day after treatment with 10^8 conidia/ml which is similar to this research with mortality reaching 90% at concentration of 1×10^7 conidia/ml after 96 hours exposure time strengthening the larvicidal effect of the isolate. Ji Hee *et al.* (2014) also revealed that *Paecilomyces fumosoroseus* FG340 caused 100% mortality of *Spodoptera exigua* larvae (Lepidoptera: Noctuidae) 6 days after treatment at a concentration 1×10^4 conidia/ml and Belabid *et al*. (2000) reported that *Metarhizium anisopliae* caused mortality of 93.3% larval of *G. deserticola* at a concentration of 40 x 10^4 conidia/ml, in their work *P. fumosoroseus* appear more virulent than *M. anisopliae*, where as the this study showed the larvicidal superiority of *Beauveria bassiana* over *Paecilomyces* spp.

Conclusion

In this research, entomopathogenic fungi isolate were isolated from soil suspension by the use of DOA (dodine oatmeal agar) selective medium for screening entomopathogenic fungi. Immature mosquito of *C. quinquefasciatus* was exposed to the two isolates of the entomopathogen: *B. bassiana* and *Paecilomyces* spp. Results indicate that, efficacy and LC_{50} varied according to concentration of conidia and exposure tissme. Among the two isolate tested *B. bassiana* isolate recorded lower LC_{50} value and highly pathogenic to 4th instars larvae of *C. quinquefasciatus;* an increase in conidia concentration and exposure time generally increases the mortality and produces faster result.

Recommendation

The result obtained shows great larvicidal efficacy of *Beauveria bassiana* and *Paecilomyces* spp conidia suspension against *Culex quinquefasciatus*, Further investigation on larvicidal potential of conidial suspension against other mosquito larvae as well as the ovicidal, pupicidal and adulticidal activity against other mosquito geniuses should be carried-out. Toxicological study of conidia and secondary metabolites of *Beauveria bassiana* and *Paecilomyces* spp on non-target organisms such as fish, phytoplankton and vertebrates including mammals should be carried-out to determine host range and safety of the fungal conidia and extract.

Conflict of Interest

The authors have no conflict of interest

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