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Assessment of Insecticidal Activity of *Beauveria* spp against *Anopheles* Mosquitoes in Katsina State, Nigeria

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Abstract
This study investigates the biological activity of EPF, *Beauveria* spp against *Anopheles* mosquitoes, a key malaria vector, in Katsina State. Collection of mosquito larvae was carried in three local government areas (LGAs) of Katsina State namely Rimi, Kaita and Mani. The sampling of wild *Anopheles* mosquito larvae was carried from identified breeding sites such as permanent water bodies, rice fields, small water pools or impoundments. The larvae were placed in plastic container and reared under laboratory conditions for adult emergence in the insectary of Umaru Musa Yar'adua University, Katsina (UMYUK). Naturally infected arthropod cadavers (cockroaches, grasshoppers, spiders, scorpions, beetles, ants and mosquitoes) were gathered from different habitats like tires, pots and garden and fungi were isolated using modified techniques. Morphological and microscopic analyses identified the fungal species and selected *Beauveria* spp for this study. Pathogenicity of the isolated fungi was evaluated through larvicidal and adulticidal mosquito bioassays. Three concentrations (10^6, 10^7 and 10^8 conidia/mL) were tested against *Anopheles* mosquito within 72-hours. Conidial suspensions of *Beauveria* spp resulted in significant larval adult mortality ranging from 36.0% to 96.5% and 80.5% to 90.0%, respectively. This research highlights the potential of these EPF as eco-friendly biocontrol agents for pest and malaria vector management. The study suggests the need for further investigations into their long-term effectiveness, environmental impact, and field applications.

Keywords: *Anopheles* mosquito, *Beauveria* spp, Entomopathogenic fungi, Mortality, Pathogenesis

1. Introduction
Malaria, a deadly disease spread by mosquitoes, has a major effect on people's health all over the world. [1]. In 2020, the World Health Organization (WHO) recorded 241 million cases of malaria and 627,000 deaths from the disease globally [2]. The WHO African region accounts for 95% (228, millions) of all reported malaria cases. Malaria remains the leading cause of mortality and morbidity in sub-Saharan Africa, where *Plasmodium falciparum* is the main malaria parasite. Either the parasite (*Plasmodium*) or the vector (mosquitoes) can be controlled to prevent malaria. However, due to its resistance, *Plasmodium* is less effective against the variety of malaria medications currently on the market [3].
On the other hand, the majority of the genus Anopheles's main malaria vector species have demonstrated insecticide resistance. In most parts of the world, case management and vector control using indoor residual spraying (IRS) and long-lasting insecticide-treated nets (LLITNs) have long been the mainstays of malaria control [4]. The effectiveness of these control methods against vector mosquitoes is threatened by insecticide resistance in mosquito populations, behavioral shifts such as avoiding treated nets or buildings, and operational constraints including inadequate funding and coverage [5].
Entomopathogenic fungi (EPF) appear to be a promising alternative method for controlling malaria vectors. The development of EPF as biopesticides against vector mosquitoes is a good fit [6]. EPF have been reported as either insect pathogens or they injure insects to some degree [7]. Among the more than 750 species and 85 genera of EPF, two genera—*Beauveria* and *Metarhizium*—have been utilized as components in "myco-insecticides" [8]. EPF species from

the *Beauveria* are well-researched biocontrol agents that manage a variety of insects [9]. Selection pressure in the insects is unlikely because it takes a long time for the EPF to infect and kill the insects. In both lab and field settings, *B. bassiana* and *M. anisopliae* were used to control vector mosquitoes. Thus, in Katsina State, Nigeria, the purpose of this study was to assess the pathogenicity of *Beauveria* against adult and immature *Anopheles* mosquitoes using various fungal isolates.

2. Materials and Methods

2.1 Study Area and Sampling Sites

Collection of mosquito larvae was carried out in three local government areas (LGAs) of Katsina State, Nigeria, while the experiments took place in Umaru Musa Yar'adua University, Katsina (UMYUK). The selected LGAs are Rimi, Kaita and Mani

2.2 Mosquito Larval Collection and Rearing of Adults

A sampling of wild *Anopheles* mosquito larvae was carried out from breeding sites at various locations of the selected LGAs. Different larval stages were collected and transported promptly to the microbiology laboratory of UMYUK. The larvae were maintained under laboratory condition of 27°C, 70-75% r.h. and 12:12h photoperiod. The larvae were fed with yeast powder mixed with cabin biscuits twice in every 24 hours until adult emergence. The emerged adult mosquitoes were maintained at the same ambient environmental conditions as that of larvae in mosquito cages.

2.3 Isolation of EPF from Arthropod Cadavers

Cadavers of naturally infected arthropods were gathered from various locations, including tires, pots, fields, botanical gardens, and homes. Cockroach, spider, grasshopper, scorpion, ant, beetle, and mosquito cadavers exhibiting outward manifestations of mycosis were gathered and promptly delivered to the Microbiology lab at UMYUK. The EPF were isolated using the procedure of Thakur and Sandhu [10]. Direct fungal isolation was followed by external conidia from dead insects being placed in 1.5% water agar containing 0.05 mg/l chloramphenicol. It was cultivated for five days at 25±2 °C. Every culture was inspected using a stereomicroscope. The slide culture procedure was performed using fungal mycelium hyphae for pure culture isolation. The morphological taxonomic characteristics of conidia-forming mycelia and conidia structure were microscopically identified [11].

2.4 Morphological Characterization and Fungal Identification

EPF isolates were examined using traits like colony shape, colour, texture, and growth pattern on potato dextrose agar (PDA). The slide culture technique was used to identify isolated fungal colonies under a microscope [12]. A sterile petri dish with a glass slide was filled with a 1 cm block of PDA. After inoculating the agar block with a seven-day-old fungal culture, a cover slide was placed over it. The culture was incubated at 28 °C for five days. After carefully removing the cover slide and observing the fungal structure, a drop of lactophenol cotton blue with lactic acid was added.

The morphological features were used to identify the fungus isolates as described by Humber [13]. Using a light microscope (Olympus CX31 series, England), the morphological characteristics of the colony, conidial size and form, and mycelia growth rate were examined. Images were captured with an OPTIKA 4083, and microscopic identification was carried out using objectives X40 and X100.The microscope is equipped with a B2 digital camera.

2.5 Production of Conidial Suspension and Counting of Spores

A hemocytometer was used to count the conidia under a compound microscope (Olympus CX31 series, England) in order to determine the concentration of the conidia in the solution. To get a concentration with a countable number of spores, the conidial suspension was further diluted with 0.5% Tween 80 solution. Suspensions were diluted with distilled water to achieve concentrations of $1x10^6$, $1x10^7$, and $1x10^8$ conidia/ml once the conidia concentration was determined.

2.6 Determination of Larvicidal Effect of *Beauveria* spp against *Anopheles* Mosquito

Laboratory bioassay was done following methods of Benserradj and Mihoubi [14]. Conidia of *Beauveria* spp were tested against the *Anopheles* larvae by adding fungal suspension to a beaker containing 20 ml of distilled water with ten 4th instar larvae. Each beaker was inoculated with 1 ml of fungal suspension (10^6, 10^7 and 10^8 conidia/ml). Control treatment was carried out by adding 20 ml of distilled water only. Each assay was replicated three times. Larval mortality was observed in a 24hrs interval for 3 days.

2.7 Determination of Adulticidal Effect of *Beauveria* spp against *Anopheles* Mosquitoes

In order to study the pathogenesis of the isolated fungi and its filtrate 10 adult mosquitoes were placed in each of the CDC bottles and replicated three times. The adult mosquitoes were sprayed with spore suspension and the fungal filtrate separately. Another set of 10 adult mosquitoes was sprayed with distilled water as a control treatment. Adult mortality was recorded after 30, 60 and 90 minutes; the most pathogenic fungi were selected through the highest mortality rates of fungal spore suspension and fungal filtrate.

2.8 Data Analyses

Data were analyzed using simple percent for mortality and distribution of EPF using Excel 2010. Also ANOVA was employed to determine if there were significance differences in mortalities of mosquito larvae and adults among various concentrations across time interval.

3. Results

3.1 Morphological Characteristics of *Beauveria* spp Isolated from Arthropod Cadavers in Katsina State, Nigeria

Results show the morphological characteristics of *Beauveria* spp isolated from arthropod cadavers (Table 1).

Table 1: Morphological characteristics of *Beauveria* spp recovered from arthropod cadavers in Katsina State, Nigeria

Macroscopic	Microscopic	Fungi Detected
Colonies are usually white to pale-coloured, and may have a powdery or cottony texture	Produce hyaline conidiophores and conidia	*Beauveria* spp

3.2 Distribution of *Beauveria* spp according to Host Arthropods

Percentage distribution showed that EPF occurred most on spider (32.35%) followed by cockroaches (19.12%), grasshoppers (17.65%), scorpions and ants (13.24%), beetles (2.94%) and mosquitoes (1.47%) (Table 2).

Table 2: Prevalence of *Beauveria* spp according to host arthropods

Host Arthropod	Number	Prevalence (%)
Spider	22	32.35
Cockroaches	13	19.12
Grasshopper	12	17.65
Scorpion	09	13.24
Ants	09	13.24
Beetle	02	2.94
Mosquito	01	1.47
Total	68	100.00

3.3 Larvicidal Activity *Beauveria* spp against *Anopheles* Mosquito

Results show that larvicidal effects of *Beauveria* spp resulted in *Anopheles* larval mortality which ranged from 36.0 to 96.5 at the various cornidial concentrations within 24 to 72 hours (Table 3).

Table 3: Larval Mortality of *Anopheles* Mosquitoes Treated with *Beauveria* spp

Fungicidal Concentrations	Mean Mortality (% ± SEM)		
	Exposure Period (Hrs)		
	24	48	72
B1×10^6	36.0±0.0^c	57.5±0.5^b	87.5±1.5^a
B1×10^7	41.5±0.0^c	69.5±0.5^b	93.0±0.0^a
B1×10^8	45.5±0.0^b	83.5±0.5^a	96.5±0.0^a

Key: B1 = *Beauveria* spp

Values with different superscripts across the same row shows significant difference at $p<0.05$

Adulticidal effect of *Beauveria* spp against *Anopheles* Mosquitoes

Table 4 shows varying degrees of adult mortality of *Anopheles* mosquitoes treated with varying concentrations of *Beauveria* spp. The mortality varied from 20.0 to 22.0%, 47.5 to 61.5% and 80.5 to 90.0% at 30, 60 and 90 minutes, respectively.

Table 4: Mortality of Adult *Anopheles* Mosquitoes Treated with *Beauveria* spp

Fungicidal Concentrations	Mean Mortality (%± SEM)		
	Exposure Period (Mins.)		
	30	60	90
B1×10^6	20.5±0.5^c	47.5±1.5^b	80.5±0.5^a
B1×10^7	20.0±0.0^c	62.5±0.5^b	82.5±0.5^a
B1×10^8	22.0±1.0^c	61.5±0.5^b	90.0±0.0^a

Key: B1 = *Beauveria* spp

Values with different superscripts across the same row shows significant difference at $p<0.05$

4. Discussion

The present study isolated, characterized and identified EPF, *Beauveria* spp, from arthropod cadavers in Katsina State, Nigeria. Similar findings by Shang *et al.* [15] and Cokola *et al.* [16] revealed high prevalence of *Beauveria* species in insect cadavers across different ecosystems.

The observed mortality trend was consistent with previous reports that EPF such as *Beauveria* spp exhibited time and dose-dependent pathogenicity against mosquito larvae [17]. It is observed that as conidial concentrations increases, the likelihood of spore attachment, germination, and penetration into the larval cuticle is enhanced, resulting in mortality [18].

In the present study, the observed mortality supports Shang *et al.* [[15], who recorded mortality ranging from 40 to 95% in a similar experimental setup. The concentration of *Beauveria* spp used in this research falls within the array tested by previous researchers, indicating consistency in the effectiveness of *Beauveria* spp as a larvicidal agent.

Beauveria spp is a well-known EPF with a wide range of hosts. It infects insects through direct contact, penetrating the host cuticle and proliferating within the host body, ultimately leading to the insect's death. Studies have demonstrated its effectiveness against numerous pests, including aphids, whiteflies, and various beetles. It was reported that *B. bassiana* was effective in managing populations of the Colorado potato beetle (*Leptinotarsa decemlineata*) and the silver leaf whitefly (*Bemisia argentifolii*) [19].

The study evaluated adult survival of *Anopheles* mosquitoes treated with *Beauveria* spp and found that the fungi exhibited promising effect against *Anopheles* mosquitoes. The time and dose-dependent pattern observed is consistent with the infection biology of the *Beauveria* spp, in which fungal propagules adhere to the insect cuticle, germinate, penetrate and cause systematic infection leading to mortality as observed by Wang *et al.* [20] and Agullera-Samarantino *et al.* [21]. Furthermore, previous studies reported that adult *Anopheles* were highly susceptible to *Beauveria* spp exposure, with mortality increasing with dose and exposure duration, similar to what was recorded at 90 minutes in the current study [22]. The concentration-dependent response of these fungi against *Anopheles* mosquitoes is evident, as higher concentrations lead to increased mortality within a shorter period.

5. Conclusions

Our study confirmed the morphological characteristics of *Beauveria* spp. The results of this study confirm the potent larvicidal effects of *Beauveria* spp against *Anopheles* mosquitoes. The data clearly show that *Beauveria* spp are effective in inducing larval mortality in *Anopheles* in a concentration and time dependent manner, with statistical significant differences observed across exposure periods. Overall, the findings demonstrate that *Beauveria* spp is highly pathogenic to adult *Anopheles*, producing 80-90% mortality within 90 minutes at higher concentrations, confirming its potential as a biocontrol agent. These findings support the use of *Beauveria* spp as a promising bio-control agent for managing insect pests. It is therefore recommended that further investigation on the impact of environmental factors such as temperature and humidity on the efficacy of *Beauveria* spp should be carried out to provide valuable insights for optimizing its application in pest management strategies.

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