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Identification and Biocontrol Potentials of Entomopathogenic Fungi, *Isaria fumosorosea* Isolated from the *Anophelines* Mosquito in Katsina, Northwest, Nigeria

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Abstract
Malaria vector control primarily relies on insecticides to kill vectors. However, the rising concern of insecticide resistance in vector populations and the environmental health risk pose significant challenges to this approach. Therefore, this study is determined to identify indigenous isolates of entomopathogenic fungi (EPF) found naturally infecting malaria vectors and to test their biological potentials as part of integrated vector management (IVM). Isaria fumosorosea was identified by morphological characterization (macroscopic and microscopic observation) and tested its virulence against larval and adult Anopheles mosquitoes using three different I. fumosorosea conidia concentrations (1×10^6, 1×10^7, and 1×10^8 conidia/mL). The Isaria fumosorosea isolate reduced the population of larvae and adult Anopheles *mosquitoes compared to the control treatment. Findings from this study recommend that locally isolated EPF have the potential to be developed as biopesticides in the future to control adult* Anopheles *mosquitoes.*

Keywords: Entomopathogenic fungi, biocontrol, malaria, *Isaria fumosorosea*

INTRODUCTION
Mosquitoes are among the most serious insect pests, affecting millions of people's health by transmitting the pathogens that cause various diseases, such as dengue, encephalitis, yellow fever, malaria, and filariasis (Raghavendra *et al.*, 2011; Accoti *et al.,* 2021; Eba *et al.*, 2021). *Anopheles* mosquito is one of the most serious mosquito species, threatening the health and life of millions of people by transmitting the pathogens that cause malaria and filariasis, which represent Nigeria's major public health diseases (Mahe *et al.*, 2022).

Vector control with insecticides or breeding habitat disruption is the most common method used to manage and protect people from these diseases. However, recent vector control programs using insecticides have led to the emergence and widespread of insecticide resistance in *Anopheles* and many other mosquito species (Accoti *et al.,* 2021; Bichi *et al.*, 2024)

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Therefore, developing safe, highly selective, and biodegradable alternatives to current chemical insecticides is important. Biological control agents, such as entomopathogenic fungi (EPF), have recently been identified as a potential alternative to synthetic chemicals in controlling mosquitoes. Local isolates of EPF offer a unique and sustainable approach to controlling indigenous insect pests because they are naturally adapted to the local environment (Sani *et al.,* 2023). However, very little information exists on discovering indigenous EPF isolates as biological control agents to suppress mosquitoes. Identification of new isolates and species of EPF from various hosts or localities is one of the good steps towards attaining the sustainable use of biopesticides under the framework of integrated vector management. Therefore, this study determined to identify and evaluate the potential of EPF-infecting *Anopheles* mosquitoes as a contribution to integrated vector management (IVM) strategies for mosquito control.

MATERIALS AND METHODS

Sampling site and mosquito sample collection

A survey for the collection of *Anopheles* mosquitoes was conducted in a breeding site at different locations (Kaita and Rimi Local Government Areas) in Katsina state, Nigeria. Adult mosquitoes were collected using different methods such as indoor resting catches, beating methods, and window exit methods. The samples were placed in an appropriately labelled white vial container and transported to the insectary for identification of EPF.

Isolation of EPF from the samples of dead mosquito

Dead mosquito samples were examined on a stereomicroscope to detect the potential presence of EPF on mosquito cadavers. The sample was immersed in 70% ethanol for 5 min to surface sterilize it, followed by 5 times water washes with distilled water in a biosafety cabinet.
The samples of mosquito cadavers treated were transferred into a surface-sterilized Petri dish and used surface sterile forceps to pick and place them into the PDA media plates supplemented with antibiotic (100 mg/ml chloramphenicol). Two samples on each plate were replicated 8 times and incubated at room temperature for 10 days. The presence of EPF growth on the plates inoculated with mosquito cadavers was observed daily until the EPF growth was observed.

Morphological Identification of EPF

Upon being isolated, the fungal grown on plates were sub-cultured on PDA plates and incubated at room temperature for seven days. Pure plates of the fungal growth were microscopically prepared for observation under a microscope. A surface sterilized needle was used to pick fungal mycelia and placed in a clean glass slide having a single drop of lacto phenol blue mounted or distilled water. The fungal isolates was identify based on the reference text of morphological characteristics described by Watanabe, (2010). Morphological characteristics of the colony, including conidial size and shape, as well as mycelial growth rate, were examined using a light microscope at 4× and 10× magnification

Production of Conidial Suspension and Counting of Spores

Conidia suspensions were prepared from the surface culture of the isolated *I. fumosorosea* grown on a PDA medium. The collected hyphae and conidia were suspended in a plastic tube containing about 20ml of sterile distilled water in 0.05% Tween 80, vortexed for about 3 minutes, and filtered through four layers of muslin cloth to remove debris, resulting in a clean stock suspension. A hemocytometer was used to determine the conidia concentration under a compound microscope. Once the concentration was established, the suspension was diluted with sterile water to obtain final concentrations.

Collection and Rearing of *Anopheles* Mosquito

The sampling collections were carried out early in the morning. Upon arrival at the sites, potential breeding habitats, including stagnant water bodies, rivers, and streams, were identified. The breeding sites were approached carefully without casting shadows as any disturbance would cause the larvae and pupae to swim downwards and become inaccessible. All specimens collected were deposited in a transparent plastic container (10 liters') covered with muslin cloth and taken to the insectary, Department of Biological Sciences, UMYU, Katsina for adult rearing. The larvae were fed daily with grounded biscuits and 500mg yeast tablets.

The sample were checked daily for emerged adults which were aspirated and placed into adult rearing cages. A 10% glucose solution serves as food for adult mosquito which was soaked into cotton wool and placed at the top of the cages.

Laboratory bioassay

Bioassay of EPF against *Anopheles* larvae

Ten 4^{th} instars of *Anopheles* larvae were placed in a transparent plastic container (150ml) containing 20 ml of sterile distilled water. At each plastic container, 2 ml of conidia suspension (10^6, 10^7, and 10^8 conidia/ml) was inoculated. For the control treatments, ten fourth instar larvae of *Anopheles* were placed in a separate container having 20 ml of distilled water without conidia suspension. Larvae were fed with yeast powder and their mortality was observed in a 24h interval for 4 days.

Mortality assessment of the dead larvae

A mycosis test was conducted to determine how many of the dead larvae and adults had succumbed to fungal infection. Three Petri dishes were prepared: two containing distilled water and one containing 70% ethanol. Each dead larva or adult was sequentially dipped in distilled water, then in ethanol, and finally in distilled water again to remove any surface fungal contaminants.

Each plate contained larvae or adults from the three conidial concentrations, with different fungal treatments kept separately. This process was repeated for all replicates. If fungal growth subsequently appeared on the larvae or adults, it indicated that the fungal isolate had penetrated the larval cuticle, confirming that the larvae or adults had died due to the fungal infection

Bioassay of the EPF against adult *Anopheles* mosquito

Spore applications

A cellulose-based paper (Whatman #1 filter paper) was used for impregnation with a conidial suspension. The paper was cut to the desired size, and a handheld sprayer containing the conidial suspension was used to ensure uniform coverage, with the nozzle adjusted accordingly. A total of 10 mL/m² of the suspension was applied to each paper, which was then allowed to dry for 1 hour at room temperature. A suspensor lined on the inside with a white Whatman #1 filter paper, was sprayed with the conidial suspension contained in a 5 cm-high plastic tube. The conidia served as a mosquito attractant. Control suspensors were prepared similarly but without conidial treatment.

Inoculation

A 2-5 days old adult female *Anopheles* mosquitoes were used in the experiment. Each treatment was replicated 3 times with 20 individuals per replicate and the entire experiment

was repeated 3 times. Following the WHO procedure, mosquitoes were carefully placed into a WHO tube holder marked with green dots using an aspirator (sucking tube) before being moved to the exposure tubes. As the mosquitoes in the exposure tubes probed the spore-treated suspensors lined in the filter paper, they contracted fungal spore infections. Adult *Anopheles* in the control cages were also exposed to untreated suspensors. Both insects in the treated and untreated cage were transferred to sterile WHO tubes in order to monitor their daily survival after exposure.

Figure 1.. The impregnated paper containing conidia and WHO tubes used in laboratory bioassay.

Mortality assessment

Mortality post-exposure was assessed daily for 7 days. Dead mosquitoes from both the exposure and control tubes were removed and placed on Whatman #1 filter paper discs (7 cm in diameter) inside small glass Petri dishes (Ø = 6.5 cm). Each filter paper was moistened with distilled water to maintain high humidity (~90%) within the Petri dishes and to promote fungal growth.

Petri dishes were sealed with masking tape and all petri dishes were kept in the inoculation room and cadavers were monitored for 2- 4 days for evidence of fungal growth on the surface of infected mosquitoes. The species of fungal growth where detected was confirmed morphologically using a dissecting microscope (400X magnification).

Statistical analysis

Analysis of variance ANOVA and Excel have been used to find the mean values and their significant differences. The lethal concentrations required to kill 50 and 90% (LC_{50} and LC_{90}) were estimated by fitting mortality data to probit analysis by using IBM SPSS Statistics 20.

RESULTS AND DISCUSSION

Morphological identification of EPF isolated from mosquito cadavers

The morphological characteristics of EPF observed confirmed that one of the isolates is *I. fumosorosea.* In addition, other important characteristics of entomopathogens species such as *Aspergillus*, *Trichoderma*, and *Fusarium* species were morphologically identified, but special attention has been given to this one species due to its importance in the control of insect pests. On the PDA plate, the fungal colony of the *I fumosorosea* isolate appeared pinkish and cottony, with dense mycelia at the center and a light-yellow pigment forming a concentric ring pattern. The conidia of the *I. fumosorosea.* isolate was oval to spindle-shaped, measuring approximately 6 × 2 µm (length × width) (Figure 1)

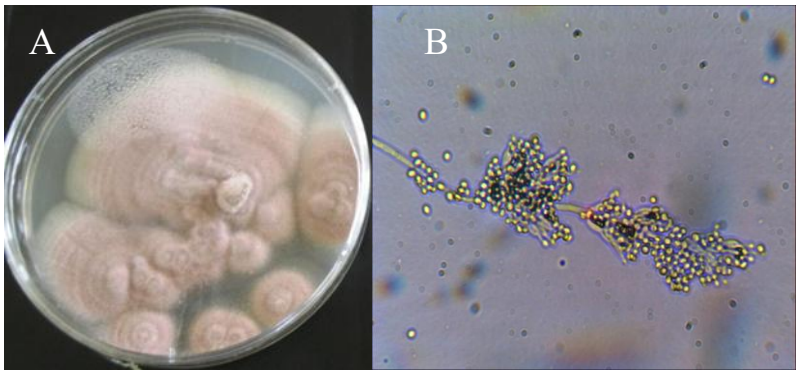

Figure 2: Colony morphology of *I. fumosorosea* isolated from infested mosquito (*Anopheles* spp) on PDA media after two weeks of growth **A** the Colony of *I. fumosorosea* colony **B.** the Conidia of *I. fumosorosea* (100x)

Virulence of *Isaria fumosorosea* isolate against larvae of *Anopheles* spp after 24, 48 and 72 hours

The present study's results showed that *I. fumosorosea* causes a significant efficacy to the 4th instar larvae of *Anopheles*. At 72 hours after exposure, the mortality was high in the highest concentration (1×10^8 conidia/ml) at 80%, and the lowest mortality was recorded at the other two concentrations, (1×10^7 and 1×10^6 conidia/ml (Table 1).

Table 1: Larvicidal effect of *Isaria fumosorosea* against *Anopheles* after 24, 48 and 72 hours

Fungal Concentrations	24 hours (Mortality)	48 hours	72 hours
$\times 10^6$	36.6	60.0*	76.7
$\times 10^7$	40.0	63.3	76.7
$\times 10^8$	43.3*	63.3	80.0*
Control	0	0	0

Key Mean Value± Standard deviation, (*) = statistically significant,

Virulence of *Isaria fumosorosea* isolate against Adult of *Anopheles* after 72 hours' interval

The results of the virulence assay of the isolated EPF against mosquitoes showed that *I. fumosorosea* significantly suppressed adult *Anopheles* mosquitoes at three different concentrations (1×10^6, 1×10^7, and 1×10^8 conidia/mL). Mortality in the control treatments was 0%. However, virulence varied depending on the conidia concentration and the duration of exposure. Mortality increased with exposure duration and conidia concentration for all three concentrations. As shown in Table 2, the highest mortality was recorded at the highest dose of 10^8 conidia/mL (68%). Mortality at the lowest doses of 10^6 and 10^7 conidia/mL was 45% and 56%, respectively.

Table 2: Mean value of adulticidal effects of *Isaria fumosorosea* against *Anopheles*

Fungal Concentrations	24 hours (Mortality)	48 hours	72 hours
1×10^6	20	29.3	45*
1×10^7	25.3*	30.6	56
1×10^8	28.0	32.0	68.0
Control	0	0	0

Key: Mean± Standard deviation, (*) = statistically significant,

Figure 3. Fungal growth on the surface of mosquitoes dead after exposure to *I. fumosorosea*

For both virulent tests, the shortest LC_{50} and LC_{90} were obtained at the larvicidal test (Table 3). Therefore, the larvicidal test showed the highest efficacy in suppressing *Anopheles* spp

Table 3: Lethal Concentrations to kill 50 and 90% of larvae and adult *Anopheles* Mosquito

Bioassay	LC_{50}	LC_{90}
Larvicidal effect	14125.38	170794.57
Adulticidal effect	13152.24	18836.49

In the present study, *I. fumosorosea* was isolated and identified from adult mosquitoes. Previous studies have shown that *I. fumosorosea* could be detected from infected adult mosquitoes and some other insects and also has the potential to control various insect pests (Ramirez *et al*., 2018; Accoti *et al.,* 2021). To the best of our knowledge, *I. fumosorosea* was not previously identified from insects in Nigeria. However, different species of EPF had been previously isolated from various insect pests sampled from different fields in Nigeria, such as Variegated grasshopper, *Zonocerus variegatus* (Balogun andFagade, 2004), red flour beetle, *Tribolium castaneum* (Kalesanwo *et al.,* 2019). The first step in developing mycoinsecticides for insect pest control is to isolate fungi from a specific host. However, isolation, identification, and virulence testing of fungal strains are essential in developing EPF as biological control agents (Dayanti *et al*., 2018; Sani *et al*., 2023).

To date, little information has been found on the use of *I. fumosorosea* for the management of the *Anopheles* mosquito, but several studies have demonstrated the effectiveness of other species of EPF for the control of the *Anopheles* mosquito. For example, *Metarhizium anisopliae* and *Beauveria bassiana* have been demonstrated to infect and kill immature and adult stages of *Anopheles* mosquito (Middelman, 2008; Mnyone *et al*., 2009; Bukhari *et al.,* 2011; Sani *et al*.,, 2017; Accoti *et al*, 2021; Moosa-Kazemi *et al*., 2021; Ong'wen *et al*., 2024).

CONCLUSION

The EPF isolate, *I. fumosorosea* was isolated and identified from adult mosquitoes. It indicates that *I. fumosorosea* is a promising larvicidal and adulticidal agent against *Anopheles* mosquitoes, with significant mortality observed at various concentrations over 72 hours. The effectiveness of the fungus increases with both concentration and time, suggesting its potential in biological control strategies for managing mosquito populations. Continued research into optimizing fungal application techniques and integrating them with other control strategies will further enhance the effectiveness of this biocontrol agent.

REFERENCES

Accoti, A., Engdahl, C. S., and Dimopoulos, G. (2021). Discovery of Novel Entomopathogenic Fungi for Mosquito-Borne Disease Control. *Frontiers in Fungal Biology*, *2*(July), 1–13. https://doi.org/10.3389/ffunb.2021.637234

Balogun, S. A., and Fagade, O. E. (2004). Entomopathogenic fungi in population of *Zonocerus variegatus* in Ibadan, Southwest, Nigeria. *African Journal of Biotechnology*, *3*(8), 382–386. https://doi.org/10.5897/ajb2004.000-2074

Bichi, S., Alhassan, A., Ononamadu, C., Mahe, A., and Imam, A. (2024). Insecticides resistance profile of *Anopheles* mosquitoes from three different localities in Sudan savannah region of Kano state Nigeria. *International Journal of Mosquito Research*, *11*(1), 40–49. https://doi.org/10.22271/23487941.2024.v11.i1a.743

Bukhari, T., Takken, W., and Koenraadt, C. J. M. (2011). Development of *Metarhizium anisopliae* and *Beauveria bassiana* formulations for control of malaria mosquito larvae. *International Pest Control*, *53*(2), 84.

Dayanti, A. K., Sholahuddin, Yunus, A., and Subositi, D. (2018). First record of entomopathogenic fungi on autumn leaf Caterpillar (*Doleschallia bisaltide*). *IOP Conference Series: Earth and Environmental Science*, *142*(1). https://doi.org/10.1088/1755-1315/142/1/012040

Eba, K., Duchateau, L., Olkeba, B. K., Boets, P., Bedada, D., Goethals, P. L. M., Mereta, S. T., and Yewhalaw, D. (2021). Bio-control of anopheles mosquito larvae using invertebrate predators to support human health programs in Ethiopia. *International Journal of Environmental Research and Public Health*, *18*(4), 1–10. https://doi.org/10.3390/ijerph18041810

Kalesanwo, A. O., Adebola, M. O., and Borisade, O. A. (2019). Characterization of Growth and Virulence of Five Nigerian Isolates of Entomopathogenic Fungi Using *Galleria mellonella* Larvae for Pathogenicity Testing. *Annual Research & Review in Biology*, *32*(5), 1–8. https://doi.org/10.9734/arrb/2019/v32i530098

Mahe, A., Alhassan, A. J., Ononamadu, C. J., Lawal, N., Bichi, S. A., Haruna, S. A., Sani, F. A., and Imam, A. A. (2022). Larval density and physicochemical properties of three different breeding habitats of *Anopheles* mosquitoes in Sudan Savannah Region of Jigawa State, Nigeria. *Dutse Journal of Pure and Applied Sciences*, *7*(4b). https://doi.org/10.4314/dujopas.v7i4b.6

Middelman, A. (2008). Biological control of *Anopheles stephensi* and *An. gambiae* larvae with the entomopathogenic fungus *Metarhizium anisopliae* Biological control of *Anopheles stephensi* and *An . gambiae* larvae with the entomopathogenic fungus *Metarhizium anisopliae* (Issue November).

Mnyone, L. L., Kirby, M. J., Lwetoijera, D. W., Mpingwa, M. W., Knols, B. G., Takken, W., and Russell, T. L. (2009). Infection of the malaria mosquito, *Anopheles gambiae*, with two species of entomopathogenic fungi: Effects of concentration, co-formulation, exposure time and persistence. *Malaria Journal*, *8*(1), 1–12. https://doi.org/10.1186/1475-2875-8-309

Moosa-Kazemi, S. H., Asgarian, T. S., Sedaghat, M. M., and Javar, S. (2021). Pathogenic fungi infection attributes of malarial vectors *Anopheles maculipennis* and *Anopheles superpictus* in central Iran. *Malaria Journal*, *20*(1), 1–9. https://doi.org/10.1186/s12936-021-03927-4

Ong'wen, F., Njoroge, M. M., Fillinger, U., Lutermann, H., and Bukhari, T. (2024). Efficacy of *Metarhizium anisopliae*, Isolate ICIPE 7, against *Anopheles arabiensis, Glossina fuscipes,* and *Rhipicephalus* spp. *Insects*, *15*(6). https://doi.org/10.3390/insects15060449

Raghavendra, K., Barik, T. K., Reddy, B. P. N., Sharma, P., and Dash, A. P. (2011). Malaria vector control: from past to future. *Parasitology Research*, *108*(4), 757–779.

https://doi.org/10.1007/s00436-010-2232-0

Ramirez, J. L., Muturi, E. J., Dunlap, C., and Rooney, A. P. (2018). Strain-specific pathogenicity and subversion of phenoloxidase activity in the mosquito *Aedes aegypti* by members of the fungal entomopathogenic genus *Isaria*. *Scientific Reports*, *8*(1), 1–13. https://doi.org/10.1038/s41598-018-28210-6

Sani, I., Jamian, S., Saad, N., Abdullah, S., Mohd Hata, E., Jalinas, J., and Ismail, S. I. (2023). Identification and virulence of entomopathogenic *fungi, Isaria javanica* and *Purpureocillium lilacinum* isolated from the whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in Malaysia. *Egyptian Journal of Biological Pest Control*, *33*(1), 1–12. https://doi.org/10.1186/s41938-023-00657-4

Sani I, I., Yusuf, U., and Suleiman, M. (2017). Larvicidal potentials of *Metarhizium anisopliae* and *Paecilomyces* sp on *Culex quinquefasciatu*s (Say) (Diptera: Culicidae). *Journal of Zoological And Bioscience Research*, *4*(1), 1. https://doi.org/10.24896/jzbr.2017411

Watanabe, T. (2010). Pictorial Atlas of Soil and Seed Fungi Morphologies of Cultured Fungi and Key to Species. In *Pictorial Atlas of Soil and Seed Fungi* (3rd ed.). CRC Press. https://doi.org/10.1201/ebk1439804193-c3