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Research Article

Synergistic Effects of Entomopathogenic Fungi (*Beauveria bassiana* and *Metarhizium anisoplae*) Extracts against the Developmental Stages of *Anopheles* Mosquitoes

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ABSTRACT

The increasing resistance of *Anopheles* mosquitoes to conventional insecticides has intensified the search for environmentally friendly biological control agents. Entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* have shown considerable potential for mosquito management. This study evaluated the efficacy of *B. bassiana*, *M. anisopliae*, and their combination against the egg, larval, and pupal stages of *Anopheles* mosquitoes at concentrations of 50 and 100g/mL. Bioassays were conducted using 50 and 100g/mL suspensions of *B. bassiana*, *M. anisopliae*, and their combined formulation. The percentage of egg hatchability and mortality of larval and pupal stages were determined and compared across treatments. At 50g/mL, *B. bassiana* recorded higher ovicidal activity (60% unhatched eggs) than *M. anisopliae* (45%), while the combined treatment resulted in 50% unhatched eggs. At 100g/mL, the combined treatment achieved the greatest ovicidal effect, with 80% unhatched eggs, compared with *B. bassiana* (60%) and *M. anisopliae* (35%). *M. anisopliae* induced greater larval mortality than *B. bassiana* when applied individually, whereas the combined treatment was less effective. Both fungi produced high pupal mortality, particularly at 100g/mL, although their combination was less effective. The combined application exhibited the strongest synergistic effect, particularly against the egg stage. In conclusion, *B. bassiana* and *M. anisopliae* are effective biocontrol agents against immature stages of *Anopheles* mosquitoes, with their combination showing enhanced ovicidal activity at higher concentrations. The combined formulation at 100g/mL is recommended for integrated mosquito management, while further field studies are needed to validate its efficacy under natural environmental conditions.

Keywords: *Anopheles; Beauveria bassiana;* Entomopathogenic; Fungi; Larvae; *Metarhizium anisopliae*; Mosquito

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INTRODUCTION

Malaria is one of the major tropical and subtropical diseases, worldwide with the highest burden acquiring in sub-sahara African, particularly among children under five year of age and pregnant woman. *Anopheles* mosquitoes are primary vectors responsible for the transmission of disease (WHO, 2023).

The malaria parasites consists of five *Plasmodium* species which include *Plasmodium falciparum* (P.f), *Plasmodium vivax* (P.v), *Plasmodium ovale* (P.o), *Plasmodium malariae* (P.m), *Plasmodium knowlesi* (P.k).In Nigeria, the most dominant species is *Plasmodium falciparum* and, then follow by *Plasmodium malariae* and *Plasmodium ovale* (NMSPN, 2025).

Vector surveillance and insecticide resistance monitoring activities conducted with support from PMI across the five ecological zones in Nigeria to determine the species composition, behaviour, seasonality, biting rates, infectivity rates, blood meal sources, and entomological inoculation rates (EIRs) of malaria vectors across sentinel sites, showed that *Anopheles gambiae s*.l. was the most abundant species across all sites of the country (NMSPN, 2025). There is currently renewed interest in the use of *B. bassiana* and *M. anisopliae* for malaria control (Enserink, 2005: Thomas and Read, 2007). And the first stable *An*. *funestus* laboratory colony (Hunt *et al*., 2005) enables an evaluation of the effectiveness of entomopathogenic fungi against *An*. *funestus a*nd also combined effects of fungi with other biological microorganisms on *Aedes* mosquitoes in Uganda (Katongole *et al*., 2022).

While both *B. bassiana* and *M*. *an*h*isopliae* are individually effective against mosquitoes, it remains unclear whether their extracts will induce effectiveness on the mosquito life cycle stages. The main aim of this research is to determine the synergistic effect using ethanol extracts of *Beauveria bassiana* and *Metarhizium anisoplae* against developmental stages of *Anopheles* mosquito.

MATERIALS AND METHODS

Entomopathogenic Fungal Isolation

Conidia of *M. anisopliae* and *B. bassiana* were isolated and preserved in the Biology laboratory of Umaru Musa Yar'adua University, Katsina (UMYUK).The physical apparent of *Metarhizium anisoplae* was greenish/black conidia and that of *Beaveria bassiana* is whitish conidia. After media preparation, sub culture of the two fungi species was carried out. This doneby inoculation of the fungi in prepared media of about 30 plates PDA media and incubated for 10 days to have massive growth of the two entomopathogenic fungi separately and used for the research (Shalaby, 2017).

Entomopathogenic Fungi Sample Preparation

The sample preparation was conducted in the Biology laboratory of UMYUK where two samples of *Beaveria bassiana* and *Metarhizium anisoplae* fungal extracts of 30.0 g each were placed into two clean beakers and 300 mL of 70% ethanol solvent were added separately, and shacked vigorously. The samples were gently sonicated in a bath 20–30 minutes at room temperature to avoid overheating and then centrifuge to remove the supernatant and decant/filter into a clean flask. The pellets were-extracted for the second time with another 300 mL 70%ethanol, and sonicated for 20–30 minutes, centrifuged and combined the supernatants. Filter paper was used to remove particulates from the supernatants. A 10mL medical syringe was used to measure three separate concentrations both *Beaveria bassiana* and *Metarhizium anisoplae* fungal extracts (Fernández-Larrea and Rangel, 2010).

Concentrations Preparation

The concentrations of fungal extracts was prepared using standard WHO (2022) concentrations preparation by using analytical balance to weight the three concentrations and small amount of ethanol solvent and shake until become homogenous solution. 10ml Medical syringe was to measure 50g/ml and 100g/ml. the following formulae were used to measure to preparation concentrations M=n/v where n is the number of solute and v is the volume (WHO, 2022).

Ovicidal and Larvicidal Bioassays

The ovicidal activity was assessed following WHO (2022) bioassay method. The eggs were collected from Dutsi-ma Dam in a transparent bucket. A minimum of 100 eggs of *Anopheles* mosquito was taken in into individual beaker concentration of (50, 100, and 100 mg/ml mixture each) of entomopathogenic fungi extracts label. Simultaneously, beaker was kept with normal water containing acetone that served as a control. After the treatment, the counted eggs from each concentration were individually transferred to stored water beaker for the assessment of hatchability. Hatchability was recorded after 24 hours of exposure for 4 days in both treatments and control. Eggs that did not hatch to larvae within 96 hours were recorded. Also, Larvae that were immobile and did not respond to the needle were counted as dead. Similarly, those pupae that did not reach the adults stage within 98 hours were recorded as dead pupa (WHO, 2022).

Data Analysis

All analyses were performed using one way-ANOVA in SPSS software version 22 to determine the variation between concentrations at significance level of 5%. The effectiveness of hatch, un-hatch eggs and synergistic effects of larvae and pupa was determined. The following formulae used to calculate the eggs hatchability percentage. Piepho formulae (2024) used for mortality:

$$\text{Eggs Hatchability (\%)} = \frac{\text{Number of Hatched Eggs}}{\text{Total Eggs}} \times 100$$

Eggs un-Hatchability (Mortality %)

$$= \frac{\text{Unhatched Eggs}}{\text{Total Eggs}} \times 100$$

$$\text{Synergistic Effect} = \frac{\text{Observed Mortality}}{\text{Expected Mortality}} \times 100\%$$

RESULTS

Synergistic Effect of *Beauveria bassiana* and *Metarhizium anisoplae* on Egg Hatchability of *Anopheles* Mosquito

The 50g/ml, *Beauveria bassiana* exhibited 60% un-hatched eggs with 40% hatched eggs, while *Metarhizium anisopliae* showed 45% un-hatched and hatched 55% . Their mixture showed 50% un-hatched eggs, for 100g/ml, un-hatched and hatched eggs in *Beauveria bassiana* (60%, 30%) and *Metarhizium anisopliae* (35%, 65%) the hatchment level is moderate, but their mixture (80%, 20%) (Table 1).

Larvicidal Activity of *Beauveria bassiana* and *Metarhizium anisoplae* against *Anopheles* Mosquito

For larvicidal activity, at 50g/ml concentration, *Metarhizium anisoplae* showed a higher corrected mortality of 56%, and Synergistic effect of 82% than *Beaveria bassiana* recorded corrected mortality as 29% and synergistic effect of 51% and the mixture resulted in 32% mortality with 44% synergistic effect. For 100g/ml concentration, *Metarhizium anisoplae* also indicate moderate performance of 50% corrected mortality and synergistic effect of.61%, than follow by *Beaveria bassiana* of 33% corrected and synergistic effect of 44% with is the lowest, the mixture of 71% and synergistic effect of 83% is the highest mortality performance (Table 2).

Pupa Activity of *Beauveria bassiana* and *Metarhizium anisoplae* against *Anopheles* Mosquito

At 50g/ml concentration in pupal activity of *Beaveria bassiana* recorded as higher effectiveness with is corrected mortality of 81% and synergistic effect of 93%,than by *Metarhizium anisoplae* corrected mortality 37% and synergistic effect is 43%, and lower mortality is in Mixture with a corrected mortality of 28%, Synergistic effect of 38%. While 100g/ml concentration, *Metarhizium anisoplae* with the corrected mortality of 84%, and synergistic effect of 85% very effective, *Beaveria bassiana* corrected mortality 39% and synergistic effect of 45% as lowest mortality, while their mixture recorded as most effective mortality with corrected mortality 88% and synergistic effect of 91% (Table 3).

Table1: *Beauveria bassiana* and *Metarhizium anisoplae* on Eggs Hatchability of *Anopheles* Mosquito

Extracts Concentration Levels	Entomopathogenic Fungi	Un-Hatched (%)	Hatched (%)
50 g/ml	*Beaveria bassiana*	60	40
	Metarhizium anisoplae	45	55
	Mixture *B. bassiana* and *M. anisoplae*	50	50
100 mg/ml	*Beaveria bassiana*	70	30
	Metarhizium anisoplae	35	65
	Mixture *B. bassiana* and *M. anisoplae*	80	20

Table2: Larvicidal Activity of *Beauveria bassiana* and *Metarhizium anisoplae* against *Anopheles* Mosquito

Extracts Concentration Levels	Entomopathogenic Fungi	Observed Mortality	Corrected Mortality (%)	Synergistic Effects (%)
50 mg/l	*Beaveria bassiana*	0.60	53	72
	Metarhizium anisoplae	0.45	39	54
	Mixture *B. bassiana* and *M. anisoplae*	0.50	41	60
100 mg/l	*Beaveria bassiana*	0.70	68	73
	Metarhizium anisoplae	0.35	47	57
	Mixture *B. bassiana* and *M. anisoplae*	0.80	78	83

Table 3: Synergistic Pupa Activity of *Beauveria bassiana* and *Metarhizium anisoplae* against *Anopheles* Mosquito

Extracts Concentration Levels	Entomopathogenic Fungi	Observed Mortality	Corrected Mortality (%)	Synergistic Effects (%)
50 mg/l	*Beaveria bassiana*	0.85	81	93
	Metarhizium anisoplae	0.40	37	43
	Mixture *B. bassiana* and *M. anisoplae*	0.35	28	38
100 mg/l	*Beaveria bassiana*	0.45	39	45
	Metarhizium anisoplae	0.85	84	85
	Mixture *B. bassiana* and *M. anisoplae*	0.90	88	91

DISCUSSION

The research showed synergistic effects of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* had differ ovicidal effects on the eggs and larvicidal and pupal effect on developmental stages of *Anopheles* mosquitoes. The percentage of un-hatched eggs induced with some concentrations, showing that exposure of fungal is reduced significantly in the eggs hatchability. *Beaveria bassiana* at 50 g/m were recorded with 60% un-hatched and 40% hatched eggs, this is a concise ovicidal activity. The un-hatched eggs at 100 g/ml showed an increased to about 70% while decreased to 30% in the hatchability. This showed effectiveness depend on the concentrations, considering *B. bassiana* at higher concentration enhanced inhibit the ability of eggs development. The *B. bassiana* ovicidal strong effectiveness observed in this research correspond with the finding from previous studies globally Scholte *et al.* (2004) highlighting *B. bassiana* was highly pathogenic to malaria mosquitoes and reduced survival at all development stages significantly. Similar finding by Luz *et al.* (2011) reported that exposure of mosquitoes to fungal spores can reduced hatchability due to penetrations of the eggs shell and lead to embryonic damage. A finding in Brazil, indicated *B. bassiana* also has highly pathogenic against *Aedes aegypti* with decreased significantly in ovicidal viability and larval survival. Generally the ovicidal table in the research showed that *B. bassiana* is support due to effective biological control agent against mosquito vectors.

In larvicidal bioassays indicated *Metarhizium anisopliae* was more effective than *Beaveria bassiana* at all concentrations. *Metarhizium anisopliae* at 50 g/ml recorded the higher mortality and synergistic index, while at mixed concentration indicated reduction in the fungal performance with revealed to antagonistic interactions. These studies align with the finding of Blanford *et al*. (2011) and Thomas and Read (2007), reported that *Metarhizium anisopliae* is particularly effective in the mosquito larva due to its rapid germination and ability to penetrated through the cuticular and ingestion. In contrast, the mixture fungal concentration reported that application indicated competitive inhibition, where both fungal species compete for nutrient from the host, generally decreasing virulence at 100 g/ml, larval mortality increased for all treatments; however, still *M. anisopliae* remained more effective. This showed pattern that globally evidence that higher fungal concentrations induced synergistic mortality, especially in actively feeding stage such in larvae (Scholte *et al*., 2004).

The pupal activity recorded that both fungal were highly effective when applied individually**.** *B. bassiana* produced higher synergistic mortality at 50 g/ml while *M. anisiplea* showed superior performance. The mixture concentration consistently produced less action at both concentrations. This observation align with earlier studies indicating susceptible to individual fungal infection in the pupae due to in active and non-feeding stage and reliance on cuticular penetrations as the primary infection route (Lacey *et al*., 2015). The mixture concentration of fungal may lead to competitive exclusions, sporation limitation and enzymatic reactions, as induced and reflected the synergistic mortality and higher variability at lower treatment. Generally these studies reinforce global conclusions that pupal stage is best to achieved biocontrol with targeting single treatment application at moderated doses.

CONCLUSION

This research concluded that the two fungal *B. bassiana* and *M. metarhizium* show differences significantly in effectiveness across all mosquito life cycle stages. The study demonstrated entomopathogenic fungal reduced hatchability of *Anopheles* mosquito eggs *B. bassiana* indicated strong treatment dependent ovicidal activity, whereas *M. metarhizium* showed lower effect comparatibility. In larvicidal stage displayed more individual concentration application especially in *M. metarhizium* with showed consistency at highest larvicidal activity at both treatment doses. In pupal activity both two fungal were highly effective when applied individually, were *B. bassiana* performing best at 50 g/ml and 100 g/ml *M. metarhizium*

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