



Research Article

Utilization of *Penicillium* and Mycorrhizal Fungal Species for Controlling *Anopheles* Mosquitoes

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ABSTRACT

Mosquito-borne diseases remain a significant public health challenge in Nigeria, with insecticide resistance increasingly compromising conventional chemical control strategies. This study evaluated the potential of *Penicillium* and mycorrhizal fungal species as biological control agents against *Anopheles* mosquitoes, specifically assessing larvicidal and adulticidal efficacy, determining lethal concentrations and times, and characterizing bioactive secondary metabolites. Mosquito larvae and adults were exposed to three concentrations (0.25×10^8 , 0.5×10^8 , 1.0×10^8 CFU/ml) of fungal spore suspensions, with mortality recorded at 24, 48, and 72 hours. Bioactive compounds were identified using Gas Chromatography-Mass Spectrometry (GC-MS). Data were analyzed using one-way ANOVA and probit analysis. *Penicillium* species achieved 72% larval mortality at 1.0×10^8 CFU/ml after 72 hours, while mycorrhizal fungi achieved 63% under identical conditions. Adulticidal activity was lower, with *Penicillium* causing 57% and mycorrhizal fungi 42% mortality. LC_{50} and LC_{90} highlighted the effectiveness of both fungi at low doses, for larvae; *Penicillium* LC_{50} (1.24) is more potent than mycorrhiza (1.58). For adults both require a higher concentrations than for larvae but *Penicillium* (2.86) again outperforms *Mycorrhiza* (3.95). GC-MS analysis identified dibutyl phthalate as the predominant compound in both fungi (71.77% in *Penicillium*, 83.18% in mycorrhiza), along with hexadecanoic acid ethyl ester, 2, 4-di-tert-butylphenol, and various alkanes and fatty acid esters with documented mosquitocidal properties. All effects were dose-dependent and time-dependent ($p < 0.05$). *Penicillium* and mycorrhizal fungi demonstrate significant potential as biological control agents for mosquitoes.

Keywords: Adulticidal; *Anopheles*; Fungi; Larvicidal; Mycorrhizal; *Penicillium*

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INTRODUCTION

The global burden of mosquito-borne diseases specifically Malaria remain a significant public health issue particularly in sub-Saharan Africa, where these diseases are rampant (Galadanchi *et al.*, 2023). Over 95% of malaria cases globally occurred among 29 countries including Nigeria which is leading with highest percentage (27%) of the total cases, followed by Democratic Republic of the Congo (12%), Uganda (5%), and Mozambique (4%) (WHO, 2023). Insecticide resistance has emerged as a major challenge for vector control programs, driven by the

excessive use of chemical pesticides, which has led to increasing resistance in pests, nematodes, and other pathogens in the environment (Rosa *et al.*, 2021). As a result, researchers have turned to biocontrol as an attractive alternative to chemical pesticides in pest and vector management (Nguyen *et al.*, 2023). Biological control of mosquitoes involves utilizing their natural enemies, including predators and parasitoids, which offer effective strategies with minimal environmental impact (Sajjad & Arif 2019). Among the eminent biological control agents, plants extract and Entomopathogenic microorganisms such

as bacteria and fungi are the most widely used for mosquito control, and they are used throughout the world with great advantage and success (Ghaffari *et al.*, 2025).

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 pests with chewing mouth parts (Mushyakhwo *et al.*, 2025).

MATERIALS AND METHODS

Soil sample collection

The soil samples were collected from a microbial rich soil with nutrients from decomposed organisms. Approximately 100g of soil were collected gently using clean aluminium foil paper. Mycorrhizal fungi were collected by taking soil samples from around plant roots at a depth of 10-12 cm using a soil corer. The samples were placed in polythene bags and immediately transported to the laboratory for processing and isolation.

Fungal Isolation

Mycorrhizal Spores were extracted using wet sieving and decanting techniques. 10g of soil was suspended in water at a 1:1 ratio. After allowing heavier particles to settle, the liquid was decanted through a 2 mm sieve, followed by a 5 µm sieve to obtain pure spores (Lange, 2014). The cultures were then grown on PDA plates for 7–10 days at 25°C to observe colony morphology.

Penicillium specie 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 was placed aseptically above solidified media in petri dishes. The plates were prepared in triplicates and incubated at room temperature until growth of fungi is observed (Suleiman *et al.*, 2024).

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 and incubated at about 24°C, to obtain pure colonies of different fungal species. (Suleiman *et al.*, 2024).

Fungal Identification

Microscopic identification was done based on the reference text of morphological characters observed under a microscope (Yunusa *et al.*, 2025). The genus *Penicillium* is characterized by septate hyphae, conidia, sterigmata, and conidiophores (Rosa *et al.*, 2021) while mycorrhiza possesses hyphae, vesicle and

arbuscule. Macroscopic identification was focused on colony color and shape.

Determination of optical density

Actively growing cultures were transferred to potato dextrose broth (PDB) from potato dextrose agar (PDA) and incubated at 28 °C with shaking at 150 rpm to obtain uniform mycelial growth (Singh & Pandey 2026). Dilutions were prepared in sterile saline, spread on PDA plates, incubated at 28 °C for 48–72 h, and colonies were counted to calculate CFU/mL. Spore suspension was diluted with sterile water to the concentrations of (0.25×10^8 , 0.5×10^8 , 1.0×10^8 CFU/ml).

The optical density (OD) was determined using a spectrophotometer (Camspec M107) at 600 nm (OD_{600}) with 1 cm path length cuvette. Readings were corrected against sterile medium blanks to eliminate background absorbance. The control treatment contained sterile distilled water (Suleiman *et al.*, 2024).

Mosquito larval collection

Mosquito samples were collected from breeding sites within Katsina metropolis. Collection was done in the morning, specimens collected were deposited in a white plastic container and labeled appropriately with specific location of collection, time of collection, date of collection and initials of collector. The containers were covered with muslin cloth to allow proper gaseous exchange for the larvae. Larvae were transported to the insectary unit of Biology Department of Umaru Musa Yar'adua University (UMYUK) for rearing to the adult stage. This step is critical for ensuring the availability of adult mosquitoes for subsequent research analysis.

Mosquito Rearing

The insectary is equipped with climate-controlled rooms maintained at a temperature of $25 \pm 1^\circ\text{C}$ and a relative humidity of $80 \pm 10\%$, with a photoperiod of 10–12 hours of light and darkness per day to simulate natural conditions. The larvae were fed twice daily, rearing water was changed every 1-2 days and all dead larvae in the bucket were removed and larvae were not overcrowded to avoid competition for food and cannibalism. The larval containers were checked daily for emerged adults who were removed and placed into adult cages. The adults were fed with 10% glucose solution soaked into cotton wool and placed at the top of the cages.

Identification of Mosquitoes

Morphological identification serves as the foundational method for distinguishing mosquito species. The identification of *Anopheles* mosquitoes was based on keys by Gilles and Coetzee (2019). Key morphological features, such as palp which is as long

as proboscis and pointed, wing patterns with small blocks on the veins, and by number, length, and arrangement of dark and pale scales. Males have a feathery (plumose) antennae while females have short and inconspicuous antennal hairs (Supriyono *et al.*, 2022).

Larvicidal bioassay

Larvicidal bioassay was done following methods of Yunusa *et al.*, (2025) with some modifications; fungal spore suspension was added to a small transparent container containing 50 ml of distilled water and 20 larvae. Each container inoculated with fungal suspensions (0.25×10^8 , 0.5×10^8 , 1.0×10^8 CFU/ml). Control treatments were added with 50ml of distilled water only. Each assay was replicated three times. Larvae were fed with yeast and their mortality was observed in a 24 hours interval for 3 days. All test containers were tightly covered with mosquito nets and kept at room temperature. Subsequently, the dead larvae were counted. Mortality and survival rates were recorded after 24 hours of exposure (WHO, 2005) and the percentage mortality was determined.

Adulticidal bioassay

A Whatman filter paper was used for impregnation with spore suspension. A hand –held sprayer containing fungal suspension was used and sprayed on the papers. Nozzle was adjusted to ensure uniform coverage. The paper was allowed to dry for 1 hour at room temperature ($27 \pm 1^\circ\text{C}$, $80 \pm 10\%$ RH). The impregnated filter paper was placed into the tubes of 7 cm height. The dried spores on the paper acted as a mosquito attractant. Control suspensions were prepared similarly but without treatment.

Inoculation

Adult *Anopheles* 2-5 days old was used. Each treatment was replicated at least 3 times with at least 20 individuals per replicate and the entire experiment was repeated at least 3 times. All infections were carried out in the Insectary at the 5.5-6.5 humidity and a temperature range of 25-28 °C during exposure. Mosquitoes were gently introduced into tube holder using aspirator, then transferred to the exposure tubes based on the WHO protocol. Mosquitoes in the exposure tubes were infected with fungal spores as they touch the spore treated suspensions in the filter paper. Mosquitoes in the control cages were similarly exposed to untreated suspensions. After exposure, mosquitoes from the experimental and control cages were transferred to clean tubes in order to monitor daily survival post exposure. They were maintained on 10% glucose solution. Separate aspirators for

fungal treatments and controls were used to avoid contamination.

Data Analysis

The survival and mortality of mosquito species was monitored at 24-hours interval. One way analysis of variance (ANOVA) was used in finding the mean values and their significant differences. The Lethal concentration causing 50% and 90% mortality (LC_{50} and LC_{90}) were estimated by fitting mortality data in to probit analysis by using IBM SPSS Statistics 20. P-value of less than 0.05 (<0.05) was considered significant.

Bioactive secondary metabolites responsible for bio-insecticidal activity were analyzed using gas chromatography-mass spectrometry (GC-MS).

RESULTS

Larvicidal activity of *Penicillium* species against *Anopheles* mosquito larvae

At three different concentrations (0.25×10^8 , 0.5×10^8 , 1.0×10^8 CFU/ml) observed over 24, 48, and 72 hours. The results demonstrate a dose-dependent and time-dependent increase in larval mortality. The lowest concentration (0.25×10^8 CFU/ml), mortality increased progressively from 1.33 ± 0.58 at 24 hours to 7.33 ± 2.52 at 72 hours. The highest concentration (1.0×10^8 CFU/ml) at 72 hours exhibited the highest larvicidal activity with mortality exceeding 70% of the exposed larvae as shown in Table 1. **Larvicidal activity of mycorrhizal fungi against *Anopheles* mosquito larvae**

Similar to *Penicillium*, mycorrhizal fungi demonstrated concentration-dependent and time-dependent mortality patterns. At (0.25×10^8 CFU/ml) concentration, mortality increased from 1.33 ± 0.58 at 24 hours to 4.33 ± 0.58 at 72 hours. The highest concentration (1.0×10^8 CFU/ml) achieved 12.67 ± 4.16 mortality at 72 hours. Compared to *Penicillium* (Table 1), mycorrhizal fungi showed slightly lower overall larvicidal activity, with maximum mortality of 12.67 compared to 14.33 for *Penicillium* at the same concentration and time point. Control groups recorded zero mortality. Significant differences ($p < 0.05$) were observed between concentrations and across time intervals, confirming the biological activity of mycorrhizal fungi against mosquito larvae as shown in Table 2.

Adulticidal Efficacy of *Penicillium* fungal species against Adult *Anopheles*

The efficacy of *penicillium* species against adult mosquitoes indicates that adult mosquitoes require higher concentrations and longer exposure times to achieve comparable mortality to larval stages. At 0.25

$\times 10^8$ CFU/ml concentration, no adult mortality was recorded at 24 hours (0.00 ± 0.00), increasing to only 3.67 ± 0.58 at 72 hours. The highest concentration (1.0×10^8 CFU/ml) produced mortality of 1.33 ± 0.58 at 24 hours, 4.00 ± 1.00 at 48 hours, and 11.33 ± 2.52 at 72 hours. This represents approximately 57% mortality at the maximum concentration and exposure time (Table 3) which is lower than the larval mortality (72%) observed for *Penicillium* under similar conditions.

Adulticidal efficacy of mycorrhizal fungal specie against Adult anopheles

The adulticidal efficacy of fungal species mycorrhizal against adult mosquitoes the results demonstrate that mycorrhizal fungi were less effective as adulticides compared to *Penicillium* species. At 1.0×10^8 CFU/ml concentration, mycorrhizal fungi caused mortality rate of 1.00 ± 1.00 at 24 hours, 3.00 ± 0.00 at 48 hours, and 8.33 ± 1.53 at 72 hours. This represents approximately 42% mortality at the maximum concentration and exposure time, compared to 57% for *Penicillium* under identical conditions. At lower concentrations, mortality was minimal, with 0.25×10^8 CFU/ml producing only 3.67 ± 0.58 mortality at 72 hours. Control groups recorded zero mortality throughout the experiment. Significant differences ($p < 0.05$) were observed between concentrations and time intervals, confirming the adulticidal potential of mycorrhizal fungi, albeit at lower efficacy than *Penicillium* as shown in Table 4.

Lethal Concentrations (LC₅₀ and LC₉₀) of *Penicillium* specie and mycorrhizal Fungi against Mosquito Larvae and Adults at 72 Hours Post-Exposure

The LC₅₀ and LC₉₀ values provide critical quantitative measures of fungal potency against mosquito populations. For larval stages, *Penicillium* specie demonstrated an LC₅₀ of 1.24 indicating that this concentration killed 50% of exposed larvae within 72 hours. In comparison, mycorrhizal fungi required a higher concentration (LC₅₀ = 1.58) to achieve the same effect, representing approximately 27% lower potency. The LC₉₀ values showed an even greater disparity: *Penicillium* required 3.87 to kill 90% of larvae, while mycorrhizal fungi required 5.23, representing a 35% difference in potency at the higher mortality threshold. The adult stage exhibited markedly reduced susceptibility to both fungal species. *Penicillium* achieved an LC₅₀ of 2.86 against adult mosquitoes more than double the concentration needed for larvae (1.24). Mycorrhizal fungi required an LC₅₀ of 3.95×10^8 CFU/ml against adults, which is 2.5 times higher than its larval LC₅₀. The LC₉₀ values for adults were substantially elevated, with *Penicillium* requiring 7.94 and mycorrhizal fungi requiring 12.16 to achieve 90% adult mortality. The slope values of the probit regression lines ranged from 1.96 to 2.31, indicating relatively homogeneous susceptibility among test populations. Steeper slopes (higher values) suggest greater uniformity in response to increasing concentrations, while shallower slopes indicate greater individual variation in susceptibility as shown in Table 5.

Table 1: Mortality rate of mosquito larvae exposed to *Penicillium* species

Concentrations (CFU/ml)	24hours	48hours	72hours
0.25×10^8	1.33 ± 0.58^b	3.00 ± 0.00^b	7.33 ± 2.52^a
0.5×10^8	2.67 ± 1.15^b	5.33 ± 2.31^b	10.00 ± 1.73^a
1.0×10^8	4.33 ± 1.53^c	10.00 ± 1.00^b	14.33 ± 0.58^a
Control	0.00 ± 0.0	0.00 ± 0.00	0.00 ± 0.00

Mean Value $\pm$ Standard deviation, Means that do not share a letter are significantly different

Table 2: Mortality Rate of Mosquito larvae exposed to mycorrhizal fungi

Concentrations (CFU/ml)	24hours	48hours	72hours
0.25×10^8	1.33 ± 0.58^b	2.67 ± 1.53^{ab}	4.33 ± 0.58^a
0.5×10^8	2.67 ± 1.16^b	4.67 ± 1.16^b	9.00 ± 1.73^a
1.0×10^8	4.00 ± 0.00^b	9.67 ± 1.53^a	12.67 ± 4.16^a
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Mean Value $\pm$ Standard deviation, Means that do not share a letter are significantly different.

Table 3: Efficacy of *Penicillium* fungal specie against Adult *Anopheles* Mosquitoes

Concentrations (CFU/ml)	24hours	48hours	72hours
0.25×10^8	0.00 ± 0.00^c	1.67 ± 0.58^b	3.67 ± 0.58^a
0.5×10^8	0.67 ± 0.58^b	2.00 ± 1.00^b	5.67 ± 2.08^a
1.0×10^8	1.33 ± 0.58^b	4.00 ± 1.00^b	11.33 ± 2.52^a
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Mean Value $\pm$ Standard deviation Means that do not share a letter are significantly different.

Table 4: Efficacy of mycorrhizal fungi against Adult *Anopheles* Mosquitoes

Concentrations (CFU/ml)	24hours	48hours	72hours
0.25×10^8	0.33 ± 0.577^b	0.67 ± 0.577^b	3.67 ± 0.577^a
0.5×10^8	0.33 ± 0.58^b	1.67 ± 0.58^b	4.00 ± 1.00^a
1.0×10^8	1.00 ± 1.00^b	3.00 ± 0.00^b	8.33 ± 1.53^a
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Mean Value $\pm$ Standard deviation Means that do not share a letter are significantly different

Table 5: Lethal Concentrations (LC₅₀ and LC₉₀) of *Penicillium* and mycorrhizal Fungi against Mosquito Larvae and Adults at 72 Hours Post-Exposure

Fungal Species	Life Stage	LC ₅₀	95% Confidence Limits	LC ₉₀	95% Confidence Limits	Slope $\pm$ SE
<i>Penicillium</i> specie	Larvae	1.24	0.98-1.51	3.87	2.96-5.34	2.14 $\pm$ 0.18
Mycorrhizal fungi	Larvae	1.58	1.24-1.96	5.23	4.01-7.28	1.96 $\pm$ 0.21
<i>Penicillium</i> specie	Adults	2.86	2.31-3.52	7.94	6.12-11.03	2.31 $\pm$ 0.24
Mycorrhizal fungi	Adults	3.95	3.18-4.98	12.16	9.23-17.45	2.08 $\pm$ 0.27

Note: All probit models showed good fit with non-significant χ^2 values ($p > 0.05$), indicating adequate description of the concentration-mortality relationship. SE = Standard Error; df = degrees of freedom

Characterization of Bioactive Secondary Metabolites *Penicillium* specie and Mycorrhizal fungi using Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis of bioactive secondary metabolites presents in *Penicillium* species culture filtrates. Nine major compounds with peak area exceeding 1% were identified, with retention times (RT) ranging from 8.1662 to 22.7129 minutes. The predominant compound was Dibutyl phthalate (Peak 19, RT 13.9683 min), comprising 71.77% of the total identified compounds. This phthalate ester is known for its insecticidal properties. The second most abundant compound was Hexadecanoic acid, ethyl ester (Peak 21, RT 14.3902 min) at 4.80%, a fatty acid ester with documented larvicidal activity. 2,4-Di-tert-butylphenol (Peak 6, RT 8.5359 min) constituted 2.03%, followed by Heptadecane (Peak 13, RT 10.7136 min) at 1.85%, Octadecanoic acid, ethyl ester (Peak 26, RT 17.3791 min) at 1.69%, and Nonadecane (Peak 12, RT 10.6298 min) at 1.33%. Trichloroacetic acid, hexadecyl ester (Peak 22, RT 15.7066 min) and Diisooctyl phthalate (Peak 27, RT 22.7129 min) were

also identified at 1.39% and 1.13%, respectively. The quality scores ranging from 64 to 96 indicate high confidence in compound identification as shown in Figure 1.

In Mycorrhizal fungal filtrate eight major compounds with peak area exceeding 1% were identified. Dibutyl phthalate (Peak 8, RT 13.9754 min) was again the dominant compound (83.18%) even higher than in *Penicillium* sp. 2,4-Di-tert-butylphenol (Peak 4, RT 8.5367 min) constituted 3.35%, followed by Undecanoic acid, ethyl ester (Peak 9, RT 14.3987 min) at 1.48%, Docosanoic acid, ethyl ester (Peak 15, RT 17.3837 min) at 1.25%, 8-Hexadecenal, 14-methyl-, (Z)- (Peak 13, RT 16.9440 min) at 1.15%, 1-Methoxymethoxy-oct-2-yne (Peak 12, RT 16.8670 min) at 1.03%, Bis(2-ethylhexyl) phthalate (Peak 16, RT 22.7136 min) at 1.08%, and Oleic Acid (Peak 17, RT 31.3153 min) at 1.05%. The presence of multiple phthalate derivatives and fatty acid esters suggests diverse mechanisms of mosquitocidal action as shown in Figure 2.

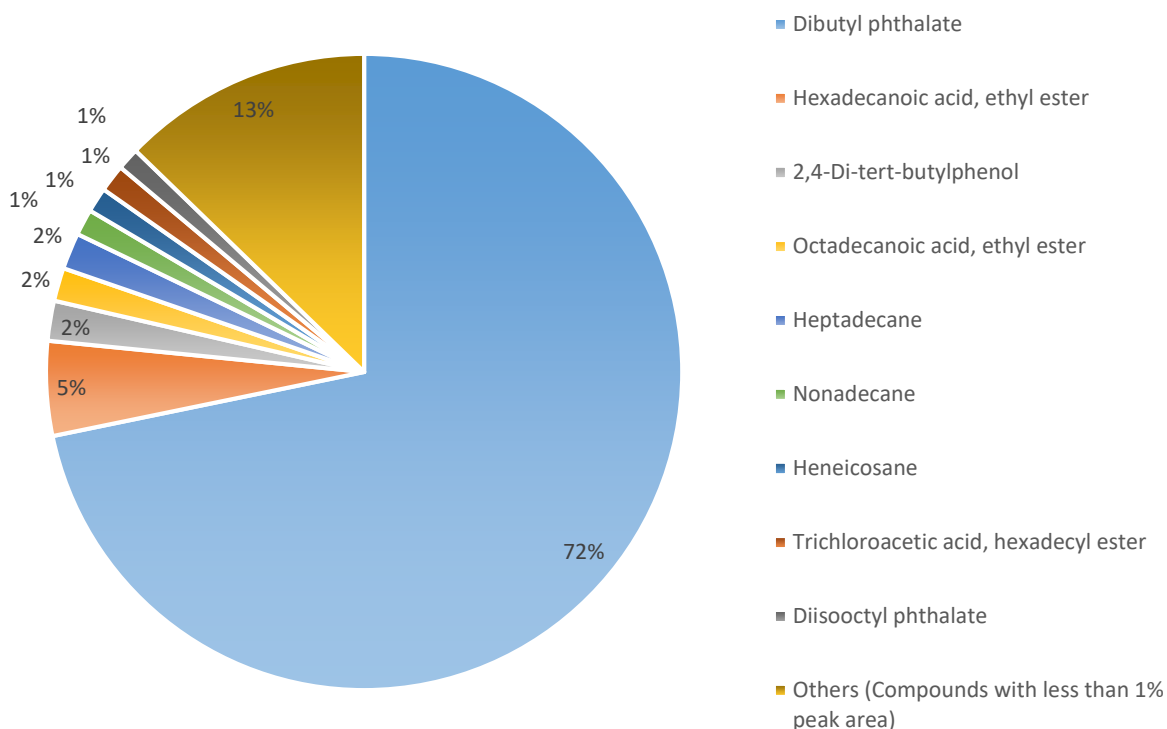


Figure 1: Pie chart showing bioactive secondary metabolites *Penicillium* specie using gas chromatography-mass spectrometry (GC-MS)

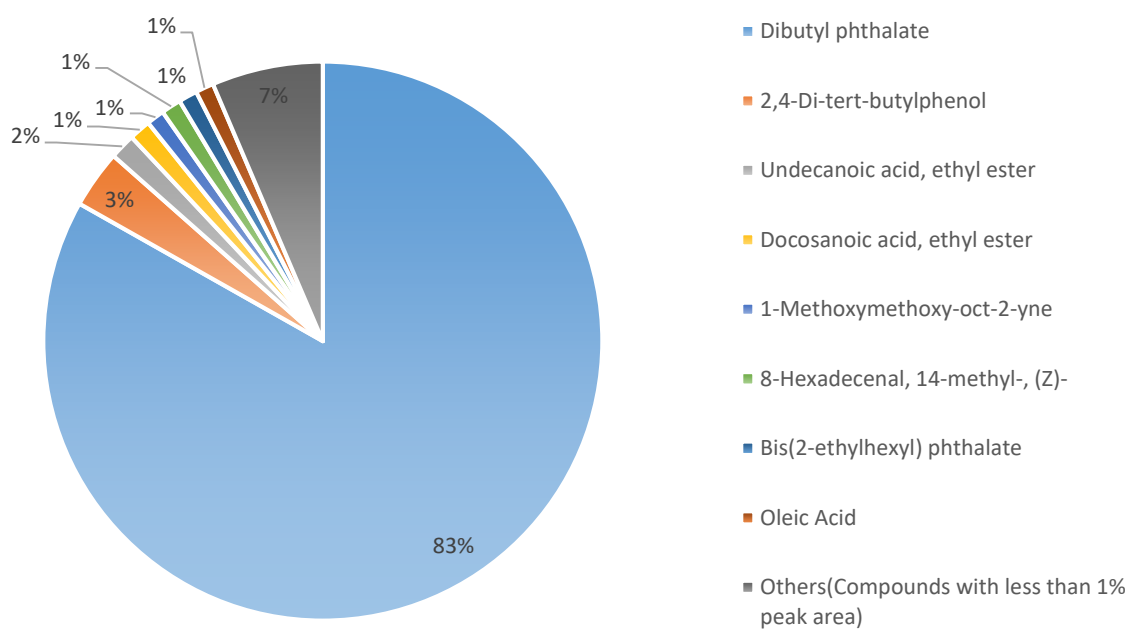


Figure 2: Pie chart showing bioactive secondary metabolites Mycorrhizal fungi using gas chromatography-mass spectrometry (GC-MS)

DISCUSSION

The findings from this study demonstrate that both *Penicillium* specie (72%) and mycorrhiza fungi (63%) possess significant larvicidal activity against *Anopheles* mosquito larvae, with *Penicillium* showing superior efficacy. The observed dose and time-dependent mortality patterns align with the established understanding of entomopathogenic fungal infection mechanisms, where higher spore concentrations increase the probability of cuticle contact and subsequent infection (Lovett & St Leger, 2025). Ragavendran *et al.* (2019) reported that *Penicillium* species exhibited better larvicidal effects compared to other fungal genera, with exposure resulting in severe damage to the larval midgut epithelium.

The slightly lower efficacy of mycorrhizal fungi (approximately 63%) in the present study may be attributed to differences in their primary ecological role as plant symbionts rather than obligate entomopathogens (Silva *et al.*, 2025). *Penicillium* specie are saprophytic fungi that have evolved secondary metabolic pathways producing diverse bioactive compounds, including mycotoxins and antibiotics, which contribute to their entomopathogenic potential (Rosa *et al.*, 2021).

In contrast, mycorrhizal fungi are primarily mutualistic symbionts that enhance plant nutrient uptake and secondary metabolite production in host plants rather than directly parasitizing insects (Hernández-Ortega *et al.*, 2025). However, the present study demonstrates that mycorrhizal fungi possess direct larvicidal activity independent of plant hosts. This finding is supported by Rai and Maurya (2024), who documented that mycorrhizal association aids plant survival and enables the application of phytochemicals as biocontrol agents showing larvicidal activities.

The mechanisms of direct larvicidal activity by mycorrhizal fungi remain incompletely understood but may involve the production of secondary metabolites with insecticidal properties, as backed by the GC-MS identification of dibutyl phthalate and other bioactive compounds in mycorrhizal culture filtrates (Table 7).

The adulticidal efficacy results (Tables 3 and 4) reveal that adult mosquitoes were less susceptible to fungal infection than larval stages, requiring higher concentrations and longer exposure times to achieve comparable mortality. This differential susceptibility between life stages may be explained by several factors. First, the larval cuticle is thinner and more

permeable than the adult cuticle, facilitating easier fungal penetration (Khan *et al.*, 2012). Second, larvae inhabit aquatic environments with high humidity, which is optimal for fungal spore germination and hyphal growth, whereas adults are exposed to drier conditions that may reduce fungal viability (Ondiaka, 2024). Third, the larval immune system is less developed compared to adults, making larvae more vulnerable to infection (Scholte *et al.*, 2024).

The GC-MS analysis revealed that both fungal species produce diverse secondary metabolites with documented Mosquitocidal properties. The identification of dibutyl phthalate as the predominant compound in both *Penicillium* (71.77%) and mycorrhiza (83.18%) is particularly significant. Phthalate esters are known to possess insecticidal, larvicidal, and repellent activities against various insect pests (Balumahendhira *et al.*, 2025). The high abundance of this compound suggests it is a primary contributor to the observed Mosquitocidal activity. The presence of hexadecanoic acid, ethyl ester (4.80% in *Penicillium*) and undecanoic acid, ethyl ester (1.48% in mycorrhiza) is consistent with findings from Suleiman *et al.* (2024), who identified fatty acid esters as major Mosquitocidal compounds in fungal extracts. These compounds disrupt cellular membranes and interfere with metabolic processes in insect larvae. De Sousa Oliveira *et al.* (2025) similarly reported that fatty acid derivatives from *Penicillium* species exhibited potent larvicidal activity against *Aedes aegypti*.

2, 4-Di-tert-butylphenol (2.03% in *Penicillium*, 3.35% in mycorrhiza) is a phenolic compound with well-documented insecticidal and antimicrobial properties. Ragavendran *et al.* (2019) identified this compound as a key bioactive metabolite responsible for larval midgut epithelial damage in *Culex* and *Aedes* Mosquitoes. The identification of alkanes including heptadecane (1.85%), nonadecane (1.33%), and heneicosane (1.26%) in *Penicillium* is noteworthy, as long-chain alkanes are known to penetrate insect cuticles and disrupt their protective wax layer, enhancing susceptibility to desiccation and pathogen entry (Benelli & Beier, 2017). Oleic acid (1.05% in mycorrhiza) is a monounsaturated fatty acid that has been shown to cause cuticular disruption and mortality in mosquito larvae (Benelli *et al.*, 2016). The presence of bis (2-ethylhexyl) phthalate (1.08%) and diisooctyl phthalate (1.13%) in both fungal species further supports the conclusion that phthalate derivatives are key Mosquitocidal agents. Banduwardena *et al.* (2025) recently reported that phthalate esters from fungal sources exhibit selective

toxicity against mosquito larvae while showing minimal toxicity to non-target aquatic organisms.

CONCLUSION

This study successfully demonstrated the potential of *Penicillium* and mycorrhizal fungi as biological control agents for *Anopheles* mosquitoes. Both fungi exhibited significant dose-dependent and time-dependent larvicidal activity, with *Penicillium* achieving 72% larval mortality at 1.0×10^8 CFU/ml after 72 hours, while mycorrhizal fungi achieved 63% under identical conditions. Adulticidal activity was lower than larvicidal activity, with *Penicillium* causing 57% adult mortality and mycorrhizal fungi causing 42% mortality at the highest concentration and exposure time. LC_{50} and LC_{90} highlighted the effectiveness of both fungi at low doses. GC-MS analysis identified dibutyl phthalate as the predominant bioactive compound in both fungi (71.77% in *Penicillium*, 83.18% in mycorrhiza), along with other mosquitocidal compounds including fatty acid esters, phenolic compounds, and alkanes. The multiple modes of action of these fungal agents, combined with their environmental safety profile, make them promising candidates for integration into vector management programs, particularly in areas where insecticide resistance has compromised conventional chemical control.

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