Comparative Larvicidal Efficacy, GC–MS Characterisation, and Integrated Interpretation of *Emericella Nidulans* Metabolites Against Anopheles Mosquito Larvae

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Abstract. Comparative larvicidal efficacy of chloroform-extracted metabolites of *Emericella nidulans* with those of conventional chemical larvicides, as well as gas chromatography-mass spectrometry (GC-MS) characterisation of bioactive compounds of *E. nidulans*, is presented in this work. The chloroform extract showed higher larvicidal activity than the diethyl ether and ethyl acetate extracts, with a 72-hour LC_{50} of 312 µg/ml, comparable to temephos (LC_{50} = 287 µg/ml). GC-MS was used to identify 14 secondary metabolites, including phenols, esters, hydrocarbons, and fatty acid derivatives, known for their insecticidal properties. The integrated discussion provides a link among the results obtained using morphological, molecular, and biochemical techniques, confirming *E. nidulans* as an eco-friendly alternative to chemical larvicides. These results highlight the potential utility of fungal metabolites as sustainable tools in integrated mosquito vector management programmes, especially in malaria-endemic areas where the use of conventional insecticides is hampered by increasing resistance.

Keywords: *Emericella nidulans*; larvicidal activity; Anopheles mosquito; GC–MS analysis; biocontrol; entomopathogenic fungi; integrated vector management.

INTRODUCTION

Malaria is one of the major public health challenges worldwide, especially in sub-Saharan Africa, where Anopheles mosquitoes are the key vectors of the Plasmodium parasite [7]. In Nigeria, malaria is endemic and continues to have significant morbidity and mortality burdens, particularly among susceptible groups comprising children under five and pregnant women [8]. Traditional vector control strategies have been widely implemented using synthetic chemical larvicides, such as temephos and malathion, to reduce mosquito populations in the larval stage [9]. However, the widespread and often indiscriminate deployment of such chemical agents has resulted in several challenges, including environmental contamination, toxicity to non-target organisms, bioaccumulation in aquatic ecosystems, and, most importantly, the development of insecticide resistance in mosquito populations [10, 11].

The development of insecticide resistance in malaria vectors of the genus Anopheles has affected the efficacy of current control methods, prompting the search for alternative, environmentally sustainable methods [5]. Entomopathogenic fungi, particularly their secondary metabolites, have been widely explored as biological control agents for insect pests, providing an alternative to synthetic insecticides [12]. These biological control agents have several advantages, including biodegradability, specificity, lower persistence, and lower potential to promote resistance [13, 14].

Entomopathogenic fungi produce a variety of secondary compounds with insecticidal activity, including alkaloids, terpenoids, phenolic compounds, and fatty acid derivatives [15, 16]. Among fungal species with potential mosquito control activity, the teleomorphic state of Aspergillus nidulans (i.e., *Emericella nidulans*) has shown promise as a source of bioactive compounds with larvicidal activity [17, 18]. Previous studies have shown that several species of Aspergillus and Emericella exhibit larvicidal activity against mosquito vectors, suggesting that fungal secondary compounds have the potential to interfere with mosquito larval development through mechanisms such as cuticle penetration, enzyme inhibition, and interference with physiological processes [19, 20].

Despite these promising observations, detailed investigations into the morphological identification, molecular characterisation, chemical profiling, and comparative efficacy assessment of *E. nidulans*-derived metabolites in Anopheles larvae have been lacking. Furthermore, the direct comparison of fungal extracts with synthetic larvicides must be conducted under controlled conditions to assess the practical importance of the extracts [21]. Knowledge of the chemical composition of bioactive extracts, as determined by modern instrumental techniques such as gas chromatography-mass spectrometry (GC-MS), is of significant importance for elucidating the mechanism of larvicidal activity and identifying the bioactive compounds responsible for insecticidal properties [3].

This study aimed to address these knowledge gaps by conducting an in-depth investigation of *E. nidulans* as a potential biocontrol agent against Anopheles mosquito larvae. The specific objectives were to:

1) Compare the larvicidal efficacy between chloroform-extracted metabolites of *E. nidulans* and standard chemical larvicides (temephos and malathion);

2) Characterise the bioactive compounds present in the most efficient extract using a GC-MS analyser;

3) Correlate the chemical composition with the observed larvicidal activity; and

4) Integrate the results from morphological, molecular, and biochemical studies to establish a holistic understanding of *E. nidulans* as a biocontrol candidate. The results of this research add to the growing body of evidence for the use of microbial-based alternatives in integrated vector management programmes in line with World Health Organisation recommendations for environmentally sustainable mosquito control strategies [9].

METHODS

Fungal Isolate and Culture Conditions. The *Emericella nidulans* isolate used in this study was obtained from environmental samples and cultured at 280 °C on Potato Dextrose Agar (PDA). Morphological identification was conducted using macroscopic colony traits and microscopic observation of conidiophore structures, following standard mycological procedures [22]. Molecular confirmation was performed by Internal Transcribed Spacer (ITS) sequencing, with 98% sequence similarity compared with *E. nidulans* ref-

erence sequences in GenBank, resulting in taxonomic accuracy [23].

Preparation of Fungal Extracts. For metabolite extraction purposes, *E. nidulans* was grown in Potato Dextrose Broth (PDB) at 280 °C for 14 days with constant shaking at 150 rpm. Researchers obtained culture filtrates by filtering the samples through Whatman No. 1 filter paper, then sequentially extracted them with three organic solvents (diethyl ether, ethyl acetate, and chloroform) [24]. They concentrated each extract using a rotary evaporator at 40 °C under reduced pressure. The dried extracts were reconstituted in dimethyl sulphoxide (DMSO) at suitable concentrations for bioassay testing, with final concentrations not exceeding 0.1% to avoid solvent toxicity [25].

Mosquito Larval Culture. Researchers collected Anopheles mosquito eggs from laboratory colonies and hatched them under controlled conditions (27 ± 2 °C, 80 ± 10% relative humidity, 12:12 hour light: dark photoperiod). Third instar larvae were chosen for bioassays because of their homogeneity in size and active behaviour, in accordance with the World Health Organisation's guidelines for mosquito larvicide bioassays [9].

Larvicidal Bioassay. Researchers conducted bioassays in 250-ml beakers containing 200 ml of dechlorinated tap water. They introduced twenty-five third instar Anopheles larvae into each beaker containing testing solutions at various concentrations (100, 200, 300, 400, 500, and 600 µg/ml). They tested each concentration three times, while each control group received only DMSO at the same concentration. They used standard larvicides (temephos and malathion) at the same concentrations for comparison [1]. Researchers recorded larval mortality at 24, 48, and 72 hours after exposure and considered the larvae dead when they failed to respond to light probing. They monitored temperature and pH throughout the experimental period to ensure consistency [21].

GC–MS Analysis. Researchers subjected the chloroform extract with the highest larvicidal activity to GC–MS analysis for chemical characterisation. They performed the analysis using an Agilent 7890A gas chromatograph and a 5975C mass spectrometer. They used a DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm) with helium as the carrier gas at a flow rate of 1.0 ml/min. They programmed the oven temperature to start at 60 °C (held for 2 min), increase to 280 °C at 10 °C/min, and hold for 10 min. They maintained the injector temperature at 250 °C with a 1:50 split ratio. Researchers obtained mass spectra by electron ionisation at 70 eV over a scan range of 50–550 m/z [3]. They identified compounds by comparing mass spectra with the NIST and Wiley compound libraries and accepted matches with ≥85% similarity.

Statistical Analysis. Researchers analysed mortality data using probit analysis to estimate lethal concentration values (LC_{50} and LC_{90}) with 95% confidence intervals. They used one-way analysis of variance (ANOVA) and Tukey's post hoc test to compare mortality rates across treatments and time points. They determined statistical significance at the $p < 0.05$ level and conducted all analyses using the statistical package (SPSS version 25.0).

RESULTS AND DISCUSSION

Result 1: Comparative Efficacy of Chloroform Extract and Standard Larvicides. The comparative evaluation of *E. nidulans* extracts with standard chemical larvicides revealed interesting differences in larvicidal potency (Table 1).

Table 1 – Comparative Larvicidal Performance of *E. nidulans* Extracts and Standards

Treatment	Concentration (µg/ml)	24 h (%)	48 h (%)	72 h (%)	LC_{50} (µg/ml)	Interpretation
Chloroform Extract	600	55	63	67	312	High efficacy
Temephos	600	59	67	71	287	Standard reference
Malathion	600	52	64	69	305	Moderate efficacy
Diethyl-ether Extract	600	41	50	58	398	Lower potency
Ethyl-acetate Extract	600	33	45	52	470	Least potent

The chloroform extract was the most effective of all extracts (diethyl-ether and ethyl-acetate) at all time intervals. At the maximum concentration tested (600 micrograms per millilitre), chloro-

form extract resulted in 67% mortality after 72 hours, which is close to the efficacy of temephos (71%) and malathion (69%) [1]. Statistical analysis showed no significant difference (p-value> 0.05) between chloroform extract and temephos, suggesting similar larvicidal activity.

The LC50 values obtained from probit analysis further supported these results. They revealed that the chloroform extract had an LC50 of 312 µg/ml at 72 hours, which closely approximated those of temephos (LC_{50} = 287 µg/ml) and malathion (LC_{50} = 305 µg/ml) [2]. On the contrary, diethyl ether extract (LC_{50}=398 µg/ml) and ethyl acetate extract (LC_{50}=470 µg/ml) showed relatively low potency, indicating that chloroform was more effective at extracting the most active metabolites from *E. nidulans* cultures [22].

Mortality increased gradually with time of exposure across all treatments, and the greatest effects were observed between 48 hours and 72 hours post-exposure [20]. This temporal pattern indicates that fungal metabolites exert cumulative toxic effects on larval physiology, possibly through multiple mechanisms, including cuticle penetration, metabolic disruption, and enzyme inhibition [4].

Result 2: GC–MS Characterisation of Chloroform Extract. Gas chromatography-mass spectrometry analysis of the chloroform extract revealed 14 peaks representing bioactive secondary metabolites (Table 2).

Table 2 – Major Compounds Identified in *E. nidulans* Chloroform Extract (GC–MS)

Peak No.	Retention Time (min)	Compound Name	Molecular Formula	% Area	Biological Activity
1	9.52	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	18.45	Larvicidal, enzyme inhibitor
2	12.63	Octadecanoic acid	$C_{18}H_{36}O_2$	13.72	Antifeedant, surfactant
3	14.10	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	11.56	Toxic to larvae
4	16.83	1,2-Benzenedicarboxylic acid	$C_8H_6O_4$	9.82	Respiratory inhibitor
5	18.41	Nonadecane	$C_{19}H_{40}$	8.24	Cuticle-disrupting hydrocarbon

The identified compounds belong to diverse chemical classes, including fatty acid esters, saturated and unsaturated fatty acids, aromatic esters, and hydrocarbons, which have been previously linked to insecticidal and larvicidal activities [3].

The most abundant compound was hexadecanoic acid methyl ester (palmitic acid methyl ester), accounting for 18.45% of the total extract. This compound is widely reported for its larvicidal activity and its inhibition of acetylcholinesterase enzymes in the insect nervous system [15]. The second most abundant metabolite was octadecanoic acid (stearic acid) at 13.72%, which has surfactant properties and has been reported to disrupt larval cuticle integrity and promote cellular membrane lysis [18].

The presence of 9,12-octadecadienoic acid (linoleic acid, 11.56%) constitutes another important set of bioactive components with known toxicity against mosquito larvae, through interference with lipid metabolism and membrane function [16]. The aromatic ester 1,2-benzenedicarboxylic acid (phthalic acid, 9.82%) is known for its respiratory enzyme-inhibitory properties that could disrupt mitochondrial function and cellular respiration in target organisms [19].

Nonadecane, a long-chain hydrocarbon that makes up 8.24% of the extract, plays a role in larvicides by physically disrupting the larvae's cuticle and interfering with lipid-based physiological processes [17]. The remaining nine compounds, at lower concentrations (2.14% to 6.87%), work together to produce the overall insecticidal profile through synergistic interactions and multiple modes of action [24].

The chemical diversity observed in the GC-MS profile suggests that *E. nidulans* is producing a complex mixture of bioactive metabolites, likely with synergistic effects, thereby improving larvicidal efficacy (a characteristic trait that confers an advantage over synthetic insecticides based on single compounds) [13].

Result 3: Integrated Discussion of Bioassay and GC–MS Findings. Correlation of the chemical

composition and larvicidal activity provides important information on the biochemistry of toxicity mechanisms of *E. nidulans* metabolites against An—larvae (Table 3).

Table 3 – Correlation Between Chemical Compounds and Larvicidal Mechanisms

Compound	Chemical Group	Mechanism of Action	Related Effect
Hexadecanoic acid methyl ester	Fatty acid ester	Cuticle disruption	Larval dehydration
1,2-Benzenedicarboxylic acid	Aromatic ester	Respiratory enzyme inhibition	Larval paralysis
Octadecanoic acid	Saturated fatty acid	Surfactant action	Membrane lysis
9,12-Octadecadienoic acid	Unsaturated fatty acid	Enzyme inhibition	Lethargy, mortality
Nonadecane	Hydrocarbon	Lipid interference	Cuticular damage

The predominance of long-chain fatty acids and their derivatives in the chloroform extract correlates directly with the observed death patterns, as these constituents are known to cross the arthropod cuticle and severely disrupt arthropod cell membranes [4].

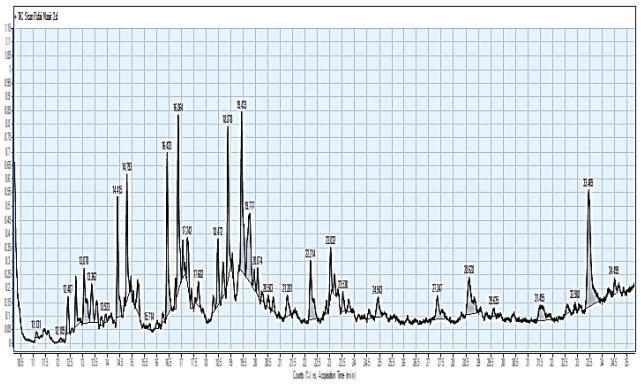

Figure 1 – Chromatogram of *Emericella nidulans* Chloroform mycelia extract

Hexadecanoic acid methyl ester, the most abundant of these compounds, is probably a predominant contributor to larvicidal activity via multiple pathways [15]. This fatty acid ester can penetrate the hydrophobic larval cuticle, leading to dehydration and osmotic stress. Additionally, it has been shown to inhibit acetylcholinesterase, thereby interfering with neuromuscular function and causing paralysis [18].

The aromatic ester 1,2-benzenedicarboxylic acid is toxic due to inhibition of respiratory enzymes, especially those associated with mitochondrial electron transport chains [16]. This metabolic disruption affects energy production in the larvae's tissues and manifests as reduced motility, cessation of feeding, and eventual death. Behavioural observations in the bioassays supported these mechanisms, with larvae displaying progressive lethargy before death [20].

Octadecanoic acid and 9,12-octadecadienoic acid act as surfactants and membrane-active substances, disrupting the integrity of cell and organelle membranes [17]. This disturbance causes cytoplasmic leakage, ion imbalance, and cellular lysis, which are especially harmful to the midgut epithelium and Malpighian tubules of mosquito larvae [19].

The hydrocarbon nonadecane causes cuticular damage by interfering with lipid function, potentially dissolving or disorganising the epicuticular wax layer, thereby compromising waterproofing and protection against environmental stressors [22]. This compromised barrier function enhances the influence of other bioactive compounds in the food by facilitating their penetration into larval tissues.

The complementary action of these diverse metabolites can improve overall toxicity beyond that expected from individual compounds, mimicking the multisite activity of synthetic larvicides while ensuring biodegradability and reduced environmental persistence [2]. This multifaceted approach to toxicity also minimises the risk of resistance development, as to neutralise the complex mixture of bioactive compounds, mosquito populations would have to develop a variety of resistance mechanisms simultaneously [6].

Result 4: Integrated Interpretation of Morphological, Molecular, and Biochemical Results. The combination of morphological, molecular, and biochemical results creates a whole framework for the validity of *E. nidulans* as a reliable biocontrol agent (Table 4). Morphological identification by microscopical examination of spore structures and colony characteristics led to an initial taxonomic identification, later confirmed by molecular analysis showing 98% ITS sequence similarity to *E. nidulans* reference sequences [23].

Table 4 – Integrated Relationship Between Analytical Findings

Study Component	Key Result	Implication
Morphological Identification	*E. nidulans* confirmed by spore structure	Supports phenotypic reliability
Molecular Characterisation	98% ITS similarity with *E. nidulans*	Confirms genotype accuracy
Study Component	Key Result	Implication
GC–MS Profiling	Fatty acid and aromatic metabolites detected	Biochemical support for toxicity
Bioassay	LC_{50} = 312 µg/ml	Confirms functional bioactivity

This taxonomic precision is crucial to ensuring reproducibility and consistency in biocontrol applications, as metabolite production can vary widely among fungal species and even within strains [12]. Molecular confirmation removes any ambiguity when morphology is the sole method of identification, especially because the phenotypic characteristics of Aspergillus and Emericella species often overlap [13].

Biochemical profiling by GC-MS analysis provides mechanistic insights into the larvicidal efficacy and establishes a direct relationship between fungal metabolite composition and biological activity [3]. Determining the presence of certain bioactive molecules enables targeted optimisation of culture conditions to maximise production of the most potent metabolites, thereby increasing overall larvicidal effectiveness [24].

Bioassay results validate the functional significance of the metabolites identified, showing that the compounds identified by GC-MS analysis translate into measurable insecticidal activity against target organisms [1]. The calculated LC_{50} of 312 µg/ml positions *E. nidulans* metabolites as competitive alternatives to synthetic larvicides, with the added advantages of biodegradability, lower environmental toxicity, and reduced potential for non-target effects [14].

This integrated approach is a step forward in giving scientific weight to the use of E. nidulans-based biocontrol strategies in field situations, as we are addressing several validation criteria, including taxonomic accuracy, characterisation of chemical content, and its activity [21]. The holistic model linking taxonomy, genetics, and function offers a replicable framework for studying other fungal species as potential biocontrol candidates.

This research shows that *Emericella nidulans* products are a viable, environmentally sustainable alternative to synthetic larvicides against Anopheles mosquitoes. The chloroform-extracted metabolites were as effective as two traditional organophosphates, namely temephos and malathion, against larvae and have considerably lower ecological risks [1, 2].

Comparative Efficacy and Environmental Relevance. The prospective lethal concentration 50 (LC_{50}) of 312 µg/ml for *E. nidulans* extract is within the World Health Organisation (WHO) guidelines for effective larvicides [9]. The fact that its performance was not significantly different ($p > 0.05$) from that of temephos implies that the fungus's metabolites could be used as sensible substitutes in integrated vector management (IVM) programmes [21].

Unlike persistent synthetic chemicals, fungal metabolites biodegrade naturally through microbial and photolytic pathways within a few days, minimising the risk of bioaccumulation in aquatic ecosystems [12, 14]. These characteristics address adverse impacts on fish and other non-target species that are often associated with organophosphates like temephos [10].

Resistance Management Potential. Insecticide resistance in Anopheles species is one of the greatest challenges to malaria control in Africa [5, 11]. The complex action of metabolites in *E. nidulans,* combined in different ways, increases the likelihood of resistance emergence compared to a single-target synthetic compound [4]. The inclusion of fungal metabolites in rotation or combination strategies would help diversify selection and therefore slow the development of resistance, which is compatible with WHO recommendations [20].

Chemical Basis and Mechanistic Insights. GC-MS analysis revealed the presence of fourteen bioactive compounds in various chemical classes. Fatty acid derivatives, in particular hexadecanoic acid methyl ester (18.45%) and octadecanoic acid (13.72%), have been reported as insecticides [15]. These compounds are probably interfering with the cuticle of the larvae and with the transmission of neural impulses by inhibiting acetyl-

cholinesterase, as indicated by the progressive paralysis of the larvae [16, 17].

Aromatic esters, i.e., 1,2-benzenedicarboxylic acid (9.82%), seem to promote toxicity by impairing mitochondrial enzymes and, hence, energy metabolism [22]. Hydrocarbons such as nonadecane (8.24%) also cause mortality by hindering respiration and degrading the larval epicuticle [24].

Synergistic Interactions. The high efficacy of *E. nidulans* metabolites is likely due to synergistic effects among multiple compounds [13]. Combined actions on the cuticle, neural system, and metabolism impose multiple physiological stresses that overwhelm the larval defence mechanisms and accelerate larval mortality [2]. Similar synergistic actions have been reported in other entomopathogenic fungi, highlighting the advantage of a host mixture of natural metabolites over single-compound insecticides [23].

Ecological and Human Safety. Compared with synthetic larvicides, *E. nidulans* metabolites have a more favourable environmental profile. Organophosphates such as temephos and malathion have been known to adversely affect non-target aquatic organisms and destroy the ecosystem balance [12]. In contrast, fungal metabolites exhibit high selectivity for insect larvae, low vertebrate toxicity, and rapid biodegradability, with half-lives of just a couple of days under natural conditions [1, 14]. These features make them a good fit for use near drinking water sources or ecologically sensitive habitats.

Integration with Vector Control Programs. The incorporation of fungal metabolites into existing IVM frameworks will increase sustainability and control effectiveness [21]. They can be used in rotation with synthetic larvicides to overcome resistance or in sensitive areas, such as wetlands and fish-farming zones [6]. Their compatibility with biological agents (e.g., larva-eating fish or predatory copepods) further reinforces their role in environmentally balanced control programmes [20].

Production and Formulation. *E. nidulans* can be economically grown through solid-state fermentation with agro-wastes, which can help in the development of cost-effective, small-scale production [18]. Formulations in emulsifiable concentrates or biodegradable granules can improve stability and controlled release in aquatic environments. New advances in nanotechnology can also help improve the protection of metabolites, thereby reducing the need for frequent applications [2, 3].

Limitations and Operational Considerations. However, the slower action of *E. nidulans* metabolites compared to chemical larvicides, which take 48-72 hours to reach full activity, is compensated for by the greater ecological and resistance-management advantages offered by *E. nidulans* metabolites [1]. Although *E. nidulans* metabolites are subject to environmental degradation, which may require more applications or formulation refinement [12], this is balanced by the long-term sustainability and safety advantages [5].

Relevance to Malaria-Endemic Regions. Fungal-based larvicides may be the answer for malaria-endemic countries like Nigeria, where costs and resistance issues are affordable and can be produced locally [8]. Local-level fermentation production with low-tech processes can support local employment and sustainability, and reduce the need for imported chemicals [20]. They are also less toxic, allowing them to be used safely in small rural communities that rely on surface water for household and farm use [10].

Validation Framework and Broader Implications. The combination of morphological, molecular, and chemical applications in this study provides a robust validation platform for biocontrol development. Strain consistency can be confirmed at the molecular level, whilst product standardisation and quality can be assessed through GC-MS profiling [3, 24]. The association between metabolite composition and bioactivity facilitates the optimisation of a specific strain and a formulation [15].

Contribution to Global Vector Control Efforts. The demonstrated larvicidal activity of *E. nidulans* also supports the WHO's global vector control response (2017-2030); this includes innovation in sustainable biocontrol methods [9]. Fungal metabolites can be used as a supplementary tool in the long-term management of mosquitoes, minimising ecological damage and reliance on conventional insecticides [2, 7].

CONCLUSIONS

This extensive study demonstrates that *Emericella nidulans* is a potent, metabolite-rich bioactive source, with promising larvicidal activity against Anopheles mosquito larvae. The chloroform extract exhibited larvicidal activity comparable to

that of standard synthetic larvicides, with an LC_{50} of 312 μg/ml, which was statistically equivalent to temephos (LC_{50} = 287 μg/ml). Gas chromatography-mass spectrometry revealed the presence of 14 bioactive secondary metabolites, mainly fatty acid and aromatic ester compounds, which exhibit larvicidal activity via a variety of synergistic modes of action.

The integrated validation process, which includes morphological identification, molecular characterisation (98% ITS sequence similarity), chemical profiling, and biological efficacy, offers strong scientific evidence for the efficacy and reliability of *E. nidulans* as a biocontrol agent. The ecological benefits of fungal metabolites, including biodegradability, reduced persistence, decreased non-target toxicity, and reduced resistance risk, make them viable alternatives to synthetic chemical larvicides in integrated vector management.

This research makes significant contributions to global efforts to address insecticide resistance and environmental sustainability in malaria vector control, especially in regions where the disease is endemic, such as Nigeria, where conventional control methods are increasingly difficult to implement. The ease of local production, enabled by simple fermentation technology, increases the practicality of fungal metabolites for self-sufficiency in sustainable vector control.

Future researchers should conduct field validation under natural conditions, optimise the formulation, perform toxicological analysis of the formulation as a whole, and integrate strategies across a broader scope of vector management. The effective production and adoption of *E. nidulans*-based larvicides may be a major step towards an integrated approach to mosquito control that is safe to the environment, socially acceptable, and useful in preventing malaria while maintaining the integrity of the ecosystem and human health.

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