



Larvicidal efficacy of indigenous fungus *Aspergillus flavus* Against *Aedes aegypti*

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Abstract

Mosquito-borne diseases pose a substantial global health threat, necessitating effective vector control strategies. This study explores the potential of fungal metabolites as alternative larvicidal agents against *Aedes aegypti* larvae, a major vector for diseases such as dengue and filariasis. Soil samples from Nandi Hills, Bengaluru District, Karnataka, India, yielded five distinct fungal species, including *Penicillium* sp., *A. niger*, *A. flavus*, *Rhizopus* sp., and *Mucor* sp. The isolated fungi were subjected to various analyses, including morphological identification, confirming their abundance and diversity. Mycelial extracts from *A. flavus* exhibited notable larvicidal bioefficacy against 4th instar *A. aegypti* larvae, with mortality observed after 24 hours. These findings align with previous studies highlighting the mosquito larvicidal properties of fungal extracts, emphasizing their promise as environmentally friendly alternatives to chemical insecticides. The study underscores the potential application of fungal metabolites in filariasis and dengue control suggests a novel and effective approach to address vector-borne diseases.

Keywords: Vector-borne diseases, larvicidal activity, bioassay, *Aedes aegypti*

Introduction

The World Health Organization (WHO) reports that vector-borne illnesses account for about seven million fatalities annually, making them a significant worldwide health issue. In particular, illnesses transferred by mosquitoes provide a serious threat because of their high transmission frequency and general prevalence. Of the 3,300 service species in 41 genera that make up the Culicidae family, Anopheles, Culex, and Aedes are the most harmful ones (Chala & Hamde, 2021) [4]. About 390 million people worldwide are at risk of contracting dengue fever, and an estimated 3.9 billion people in 128 countries are thought to be infected with the dengue virus. The primary method of controlling mosquitoes and houseflies has been the use of artificial and commercial pesticides such as carbamates, dichloro diphenyl trichloroethane (DDT), organochlorines, and organophosphates (Bhatt *et al.*, 2013) [3]. The goal of this strategy is to reduce severe side effects and manage growing resistance. An important period in mosquito control history was ushered in by the discovery of DDT's insecticidal qualities in the late 1930s, which paved the way for the creation of organochlorine and organophosphate insecticides. Nonetheless, the limits of synthetic pesticides have been highlighted by the spread of mosquito species and the increase in disease frequency. The lack of specific insecticides, rising costs, ecological issues, detrimental effects on human health and non-target populations, persistence, and the emergence of pesticide resistance are some of the difficulties that need to be considered while reevaluating vector control methods (Senthil-Nathan, 2020) [13].

The development of resistance to DDT in *Aedes* species and the challenges posed by synthetic compounds like permethrin and malathion have posed setbacks to mosquito control programs. Mosquitocides of many types, including BHC, organophosphorus, carbamate, and pyrethroid, have been used in malaria control efforts. However, increased production of detoxifying enzymes such monooxygenases

(MFOs), glutathione-S-transferases (GST), and carboxyl-cholinesterase (CCE) has enabled mosquitos to resist the insecticidal effects of these substances, contributing to the phenomena of resistance. Additional insecticides like benzylphenyl urea and the larvicide *Bacillus thuringiensis israelensis* (Bti) have demonstrated only partial effectiveness, as documented by Govindarajan *et al.* (2016) [16]. Furthermore, unexpected ecological variations, whether arising from natural or anthropogenic factors, have the potential to significantly impact vector biology, posing challenges to current mosquito control strategies and influencing disease incidence. Recognizing these challenges, scientists worldwide are actively seeking alternative methods for vector control. Natural compounds or metabolites derived from microbial resources provide a promising ecologically safe, biodegradable, and cost-effective option for larvicidal control (Vora, 2008) [14]. Fungal-based products have shown considerable toxicity to mosquitos while having little effect on non-target creatures. Because of their selective toxicity, decomposability, and eco-friendliness, microbial insecticides are increasingly being evaluated as alternatives to chemical insecticides. Several fungal species have been shown to have larvicidal, cellulolytic, and cytotoxic properties, including *Penicillium* sp., *Aspergillus* sp., *Fusarium* sp., *Podospora* sp., *Mucor* sp., *Cladosporium* sp., and *Stoloniferum* sp. (Ragavendran *et al.*, 2019) [11]. In light of the foregoing, the current work was aimed to isolate and identify the powerful indigenous soil fungus and their metabolite for larvicidal impact on mosquitos.

Materials and methods

Fungal isolation

Soil samples were aseptically collected from Nandi Hills in the Bengaluru District of Karnataka, India, at a depth of 6–10 cm, using sterile polyethylene bags. The approximately 50-gram samples were then transported to the laboratory and refrigerated at 4°C for storage until further analysis.

Fungal isolation was carried out by employing Martin Rose Bengal Agar (MRBA) medium, supplemented with streptomycin (1 mg/ml) to hinder bacterial growth. 0.1 ml of the diluted soil sample was transferred onto the agar medium using a sterile pipette, and the mixture was uniformly spread with a sterile L-rod. Incubation of all MRBA plates occurred at room temperature ($27 \pm 1.5^\circ\text{C}$) for a period of 5 days to foster fungal growth. The fungi was identified based on its colony growth (length and width), presence of aerial mycelium, colony color, occurrence of wrinkles and furrows, and pigment production, among others.

Metabolite Preparation

The mycelial mats of the isolated fungi were introduced into Potato Dextrose Broth (PDB) and incubated for duration of 3 weeks under dark conditions. Upon achieving maximal growth, the fungal mycelial mat was harvested through filtration. Subsequently, 10 g of fungal mycelium were extracted with 100 ml of methanol in a static condition over 5 days. The mixture underwent filtration, with the extraction steps repeated three times. The concentrated mixture was subjected to centrifugation at 12,000 rpm for 5 minutes to eliminate debris. The resulting mixture was then transferred to a round-bottom flask, dried under a rotary evaporator at 40°C , and kept at -20°C until further use.

Collection and Maintenance of Mosquito Larvae

In accordance with the morphological identification keys given in the photographic handbook of mosquito identification, mosquito larvae were collected from agricultural areas in Chikkaballapur, Bengaluru, and used for identification. The mosquito larvae were raised at 27°C in an enamel tray that held 1 L / 300 larvae. After 24 hours

Of exposure, mortality and survivor rates were noted. The larvae were not given any food during the trial. Each experiment was run three times in order to confirm the findings. The dead larvae were counted after all test containers were securely covered with mosquito net, kept at room temperature, and protected from sunlight.

Dose-Response Bioassay

Aedes aegypti larvae were collected in beakers filled with sterile deionized water. The metabolites were then prepared at different concentrations using 100 mL of water. The mycelial metabolite was dissolved in 10% DMSO at a concentration of 1 mg/ml (stock solution 5 ml) and metabolites from mycelium extract were used in the bioassay experiment at various doses (100, 200, 300, 400, and 500 $\mu\text{g/ml}$). Three tests were conducted on the DMSO-distilled water-treated negative control in each trial. After 24 hours of exposure, the mortality (using Abbott's formula) and survival rate were calculated.

Statistical Analysis

All the experiments were performed in triplicates, Statistical Package for Social Science (SPSS) Version 16.0 was used to interpret the following data in form of mean (\bar{x}) \pm standard error (SE).

Results and Discussion

Isolation of Fungi

Mosquitoes can be killed by the various compounds found in fungi, a very complex category including several species. The most abundant sources of many secondary metabolites are found in fungi and actinomycetes. They serve as a more reliable supply of potent chemicals that may be applied to the biocontrol of parasites and pests.

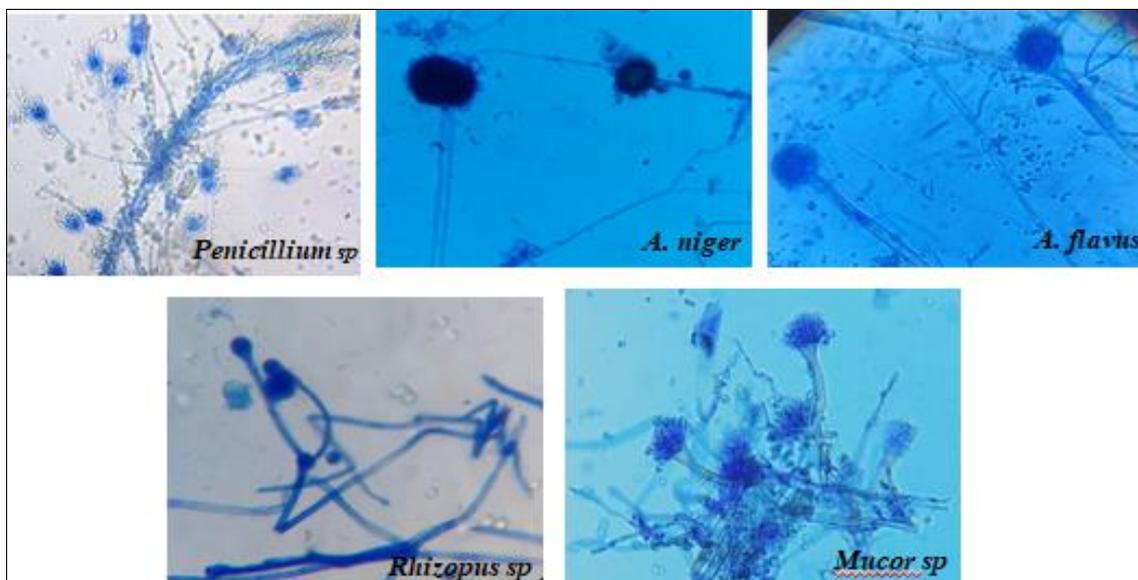


Fig 1: Types of fungus isolated from soil sample

In natural sources, the quantity of fungus is often much higher than that of other bacteria. They are being cut off from several sources through a variety of techniques. The majority of fungal species are recognized by their morphological characteristics. In the present study, *Penicillium sp.*, *A. niger*, *A. flavus*, *Rhizopus sp.*, and *Mucor sp.* are the five distinct fungal species that have been isolated from soil. Previously, Kostadinova *et al.* (2009)^[8]

Reported the isolation and identification of fungus from soils, with the most often found forms belonging to the genera *Penicillium*, *Aspergillus*, *Cladosporium*, *Alternaria*, *Geomyces*, and *Lecanicillium*. Interestingly, the current findings are consistent with the findings of Kumari *et al.* (2005)^[9], who identified 46 fungus from 40 distinct soil samples.



Fig 2: Larvae of *A. aegypti*

Larvicidal Activity

Due to its potential for more selectiveness than chemical insecticides, microbe-based control agents provide an

Alternative to chemical pest/insect management. Numerous researchers have developed various bio larvicidal compounds that have demonstrated effective LC₅₀ values against a wide range of diseases transmitted by mosquitoes (Şengül Demirak *et al.*, 2022) [12]. The fungal metabolite has caused the highest mortality rates in mosquito populations, especially in *Anopheles* and *Culex* species. Several researchers have recently focused on creating more feasible mosquitocidal therapies from biological resources in an effort to reduce the number of diseases transmitted by mosquito (Benelli *et al.*, 2016) [2]. Currently, the larvicidal activity of five distinct soil fungi's mycelial extracts against *A. aegypti* larvae is being studied. The larvicidal impact of fungal MEAE produced from *A. flavus* has demonstrated outstanding effectiveness against 4th instar *Aedes aegypti* larvae (Table 1), with larval mortality seen after 24 hours and no mortality observed in the control.

Table 1: Larvicidal efficacy of *A. flavus* on 4th instar larvae of *A. aegypti*

Fungal Species	LC ₅₀ Values				
	Concentration (µg/ml)				
	100	200	300	400	500
<i>Penicillium sp</i>	7.23	15.14	19.5	24.48	25.11
<i>A. niger</i>	2.12	2.90	10.74	13.97	28.94
<i>A. flavus</i>	11.45	18.24	27.58	39.11	44.96
<i>Rhizopus sp</i>	7.44	14.52	19.4	26.32	31.97
<i>Mucor sp</i>	8.11	11.23	17.88	22.19	27.32

At the highest dosage (500 g/ml), the metabolite had a strong effect on *Aedes aegypti* 4th instar larvae, and death began within 8 hours of exposure. Within 12 hours, more than half of the larvae died. As a result, larvae treated with isolated fungal mycelia metabolites died in the following order: *A. flavus*. > *Rhizopus sp.* > *A. niger* > *Mucor sp.* > *Penicillium sp.* When compared to the other fungi, *A. flavus* only showed better larvicidal activity.

Maurya *et al.* (2011) [10] assessed the larvicidal potential of *A. flavus*, *A. parasiticus*, *A. niger*, *F. sporotrichoides*, and *P. verrucosum* against *C. quinquefasciatus* and *A. stephensi* gives relevance to our results. *A. flavus* was shown to have

the best larvicidal bioefficacy against *An. stephensi* and *Cx. quinquefasciatus*, with LC₅₀ values of 9.54 and 10.98 ppm, respectively. According to Govindarajan *et al.* (2005) [6], mycelial extracts from numerous fungi, including *A. flavus*, *A. parasiticus*, *P. falcicum*, *F. vasinfectum*, and *T. viride*, produce higher toxicity against *Cx. quinquefasciatus*. The current study's findings indicate that fungal metabolites have significant potential for use as a supplement to current larval control strategies. Singh and Prakash (2012), who demonstrated that fungal metabolites might be used as a novel strategy for filariasis and dengue management, support this approach (Baskar *et al.*, 2020) [11].



Fig 3: Larvicidal activity of *A. flavus* extract

Conclusion

In conclusion, the diverse compounds found in fungi, particularly the isolated species *Penicillium sp.*, *A. niger*, *A. flavus*, *Rhizopus sp.*, and *Mucor sp.*, demonstrate significant potential for mosquito control. The abundance of secondary metabolites in fungi and actinomycetes establishes them as reliable sources of potent chemicals applicable to the biocontrol of parasites and pests. The morphological identification of these fungal species aligns with previous studies reporting the prevalence of genera such as *Penicillium* and *Aspergillus* in soil samples. The current study's focus on the larvicidal activity of mycelial extracts from *A. flavus* against *A. aegypti* larvae showcases

promising results. The demonstrated effectiveness, especially against 4th instar larvae, indicates the potential application of fungal metabolites as alternative larvicidal agents. The findings also align with existing research that highlights the mosquito larvicidal properties of fungal extracts, emphasizing their potential for controlling disease-transmitting vectors like *Anopheles* and *Culex* species. The study underscores the significance of exploring fungal metabolites as a viable supplement to existing larval control strategies, particularly in the context of filariasis and dengue control. The potential selectivity and reduced environmental impact of microbe-based control agents make them valuable alternatives to chemical insecticides. The findings contribute

to the growing body of research focused on developing effective and sustainable mosquitocidal treatments from biological resources, emphasizing the importance of continuing efforts in this direction for improved vector control strategies.

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