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Larvicidal Effect of Spores and Metabolites Extracts of *Aspergillus Fumigatus* against *Culex* Mosquito Larvae

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Abstract

Culex mosquito species are known to transmit diseases such as dengue fever, West Nile virus infection, malaria, lymphatic filariasis, and Japanese encephalitis. An estimated 120 million people suffer from mosquito-borne diseases across the globe. Repeated use of chemical insecticides has led to the emergence of insecticide resistance by Culex mosquito species, pollution of the environment, and harmful impacts on non-target organisms. The purpose of this study is to evaluate the larvicidal potential of metabolites and spore extracts of Aspergillus fumigatus against Culex mosquito. The fungal spore concentrations were ascertained after 5 days of fungal culture by optical density measurements. An equal amount of methanol and ethyl acetate was used to extract metabolites at four different test concentrations (10, 20, 30, and 40 mg/mL). The chemical constituents of the extracted metabolites were characterized using GC-MS and FTIR analyses. The protocols enshrined by WHO (2005) were followed in conducting the larvicidal bioassay, whereas the lethal concentrations (LC_{50} and LC_{90}) were calculated by Probit analysis. The highest mortality rate (100%) was recorded at the highest concentration of metabolites extract (40 mg/mL) of Aspergillus fumigatus. Complete (100%) was recorded at spores concentration of 4.5×10^8 CFU/ml. The major bioactive compounds revealed by the GC-MS analysis include 9-eicosene, (E)-, 1-octadecene, 3-eicosene, (E)-, oleic acid, 1-nonadecene, cis-vaccenic acid, octadec-9-enoic acid, andsqualene. The outcomes of this study showed that Aspergillus fumigatus metabolites and spores extract have the potential to control mosquito vectors. Hence, there is a need for large-scale production of bioactive components, as revealed by GC-MS analysis.

Keywords: Aspergillus fumigatus spores, Culex mosquito, GC-MS analysis, Metabolites.

INTRODUCTION

Mosquitoes of the genera *Culex* is the main vector of viruses that kills millions of people annually (Cheek, 2020). Numerous diseases can be spread by mosquitoes, such as yellow fever, malaria, filariasis, chikungunya, and Japanese encephalitis, to humans and other tamed creatures (Budhiraja *et al.*, 2013). Humans contract malaria from female Anopheles mosquitoes. Just over half of the deaths related to malaria globally in 2021 occurred in four countries: Nigeria (31%), the Democratic Republic of the Congo (13%) United Republic of Tanzania (4%), and the Niger Republic (4%). Data provided by WHO on the monitoring of pesticide resistance between 2010 and 2020,78 nations out of 88 nations have verified that a single malaria vector is resistant to not less than one pesticide in at least one malaria carrier species from a single mosquito collecting site and 29 documented cases of resistance to four insecticide classes—organochlorines, carbamates, pyrethroids, and organophosphates, in at least one malaria vector species from multiple locations throughout the nation (WHO, 2022). *Culex quinquefasciatus* mosquitoes are carriers of diseases like Ross River viral infection, lymphatic filariasis, West Nile Virus infection, and Japanese encephalitis. There are over 120 million individuals infected by *Culex quinquefasciatus* worldwide. *Aedes Aegypti*are vectors of Zikaand Dengue virus, which are currently considered a primary human health risk (Naqqash*et al.*, 2016). Lack of reliable information explaining the intricate interactions between secondary metabolites and their target mosquito larvae and non-target organisms is a major barrier to the widespread use of these chemical substitutes. With the recent bio-guided computational techniques such as molecular docking, it is possible to identify physiological targets for secondary

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metabolites or look for potential binding approaches of the secondary metabolites along the receptors of various organisms. This has successfully helped to clarify the selective effects of alternative pesticides (Abideen *et al.*, 2021).

Chemical pesticides have a negative effect on non-target organisms and result in environmental pollution when they are used frequently. Therefore, substitute environmentally favorable sources from fungi, bacteria, and plants are being investigated (Vivekanandhan *et al.*, 2020). Moreover, mosquitoes have emerged resistant due to continuous and regular use of these chemicals, making them more resilient to current control methods (Jayaraj, 2005). Biorational insecticides that are isolated from microorganisms appear to carry less risk to the environment and human health, which allows these alternative approaches to substitute or be incorporated into mosquito control programs (Cross, 2020). Among these alternative techniques, fungal cultures of secondary metabolites have demonstrated sufficient ability to suppress the larvae of mosquitoes without adversely affecting non-target organisms (Cuenca-Estrella, 2001).

Previously, *A. fumigatus* was virtually only known as a fungal infectious agent capable of causing lung disease (Dada & Aruwa, 2014). As such, studies with regard to the use of *A.fumigatus* spores extract as bio larvicide were limited. The purpose of this study is to investigate the potency of *Aspergillus fumigatus* spores in inducing mortality and compare the activity of spores and metabolites of *Aspergillus fumigatus* against *Culex* larvae. In Nigeria, the number of diseases transmitted by mosquitoes is on the upswing, yet little research has been done on the use of fungal metabolites as a substitute method of eliminating mosquito larvae. Therefore, this research aims to evaluate the larvicidal potential of spores and metabolite extract of *Aspergillus fumigatus* against *Culex* mosquito species.

MATERIALS AND METHODS

Sample collection

The samples were obtained from soil being a natural reservoir for many entomopathogenic fungi. The decomposition of insects in the soil provides a variety of nutrients for entomopathogenic fungi (Oghaz *et al.*, 2022). Soil samples were collected from Darawa village in Dutsin-Ma local government, Katsina state. About 50g of soil samples from a depth of 10 – 12cm were gathered aseptically from three different sites using clean and sterilized hand towels and aluminum foil paper. Samples were transported to the Microbiology laboratory, Faculty of Life Sciences, Federal University Dutsinma, Katsina state, in a clean polythene bag and processed immediately. Soil samples were then subjected to serial dilution technique for isolation of *Aspergillus fumigatus* (Dada & Aruwa, 2014; Amjad, 2016).

Serial dilution and inoculation

Ten grams (10 g) of soil sample was diluted in 90 ml of sterile distilled water, from 1st to 7th fold serial dilution (10^{-3} to 10^{-7}). One-tenth of a millilitre (1/10th ml) of the 1st and 4th to 7th fold dilutions was plated out in duplicates. The SabouraudDextrose Agarmedia was incubated at 25-27°C for 48–72 hrs (Dada & Aruwa, 2014). The fungi colonies were individually separated after the period of incubation and maintained in the fresh SDA medium for further studies (Balumahendhiran *et al.*, 2019).

Subculture and identification of isolated fungus

The fungal isolate was sub-cultured using the same isolation media and morphologically identified using the cotton-blue in lactophenol method (Abideen *et al.*, 2021).

Collection of fungal spores

Aspergillus fumigatus isolate was inoculated into the newly prepared media and cultured for five (5) days. Manual spores extraction was done usinga glass pipette to extract the spores from the culture plate. The media was incorporated with antibiotics to prevent bacterial growth (Abideen *et al.*, 2021).

Measurement of optical density

The larvicidal activity of spores was measured based on the number of spores formed per kilogram. Spores have to be suspended in sterilized distilled water. For each suspension, spores concentration was determined by measuring an optical density (OD) using a spectrophotometer (0.5 McFarland standard= 10^8 CFU/mL) (Sathish *et al.*, 2019).

Isolation of fungal metabolites

The broth was prepared in triplicate for culturing of *Aspergillus fumigatus*. Sabouraud

dextrose broth was prepared in five 250 mL sterilized Erlenmeyer flasks, each containing 200 ml of sabouraudextrose broth were autoclaved for 30 min at 15 psi. The broth was supplemented with 50µg/ml chloramphenicol to suppress bacterial growth (Balumahendhiran *et al.*, 2019). The flask was incubated for 2 weeks at 28 °C together with periodical shaking at 150 rpm (Sharma *et al.*, 2016). After the incubation period, the broth culture was filtered using a clean Waterman No.1 filter paper to remove the fungal biomass. The filtrate was centrifuged at 5000 rpm for 30 mins, after which the pellet was discarded, and the supernatant was used for secondary metabolites extraction (Balumahendhiran *et al.*, 2019). An equal volume of ethyl acetate and methanol (1:1) was added to the supernatant filtrates, mixed well for 10 min, and then kept for 5 min till the two clear immiscible layers formed. The upper layer of solvent that contains the extracted compounds was separated using a separating funnel followed by solvent evaporation, and the resultant compound was dried in a rotator vacuum evaporator to yield the metabolite extract, which was subsequently measured (Sharma *et al.*, 2016). The weight of the dried metabolite extract was measured. The dried metabolite extracts were diluted using distilled water as a diluent to obtain various metabolite concentrations (40, 30, 20, and 10 mg/mL), which were afterward used to test the larvicidal activity. Twenty (20) larvae were separately exposed to each test concentration (Vyas, 2015).

Characterization and identification of metabolites

GC-MS sample analysis

The sample was analyzed using Agilent GC (7890B), equipped with a 30 m x 250 µm x 0.25 µm Column, coupled with Agilent MSD (5977A MSD). The carrier gas helium was at a flow rate of 1 ml/min. The temperature was then ramped at the rate of 5 °C/min to 230 °C and then held for another 5 min after the GC oven was initially set at 70 °C, and then ramped at the rate of 20 °C/min to 110 °C and held for 1 min. Finally, the oven temperature was ramped to 2800°C at the rate of 200°C/min and held for a further 5 minutes. Equilibration time, MSD Transfer Line, MS Source, and MS Quad were set at 0.5 minutes, 250 °C, 230 °C, and 150 °C respectively. The characterization and identification of chemical compounds in different samples was based on GC retention time. The mass spectra were computer-compared with those of standards available in NIST mass spectrum libraries. The percentage composition of the sample constituents was expressed as a percentage by peak area (Balumahendhiran *et al.*, 2019).

Fourier Transform Infrared Spectrophotometer (FT-IR) of metabolite extract

The dried extract was prepared by placing it in a sample holder or directly on the instrument's sample stage. The instrument emitted a beam of infrared radiation that passed through the sample and was detected by a detector. The instrument scanned a range of frequencies, typically in the mid-infrared region (4000 - 400 cm^{-1}), and recorded the amount of radiation absorbed or transmitted by the sample at each frequency in which a spectrum was produced in the process that represents the unique absorption pattern of the sample (Baskar *et al.*, 2020).

Collection of *Culex* mosquito larvae

The *Culex* mosquito larvae were collected from selected farms near Zobe Dam, Dutsin-Ma local government, Katsina state, Nigeria. The larvae were collected using the dipping method (ECDC, 2018). The collected larvae were taken to the laboratory of the Department of Biochemistry & Molecular Biology Federal University Dutsin-Ma.

Morphological Identification of *Culex* Mosquitoes

The larval identification was done using morphological characters observed under the ×20 objective of a Zeiss light microscope, as described by Coetzee (2020). The larvae were reared at room temperature(28±1°C)and then used for larvicidal bioassay (Coetzee, 2020).

Larvicidal Bioassay

For every concentration, four replicate cups were used. Additionally, 100 mL of deionized water was used as a control. The twenty larvae were introduced into the test containers at different concentrations to observe the mortality rate. 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). The percentage mortality was determined using the formula below:

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

Statistical Analysis

All experiments were performed in triplicates. LC_{50} and LC_{90} (and their upper and lower confidence limits) were calculated using Probit analysis. The chi-square test was also used to analyse the mortality data. All analyses were conducted using IBM SPSS (Version 20.0). Results were considered statistically significant at $P \leq 0.05$ confidence interval.

RESULTS

Larvicidal bioassay of spores extracted from fungal species

The results of larvicidal activity of spores extracted from *Aspergillus fumigatus* at the tested concentrations (4.5×10^8 CFU/ml, 2.25×10^8, CFU/ml, 1.125×10^8 CFU/ml and 0.56×10^8 CFU/ml) was presented in Table 1. The results showed that spores extract of *Aspergillus fumigatus* was found to exhibit a larvicidal effect (100%) against *Culex* mosquitoes at the highest concentration. The LC_{50} and LC_{90} values obtained were 1.22×10^8 and 2.82×10^8 against the extracts of *Aspergillus fumigatus*, respectively. The results obtained were not statistically significant ($p = 0.61$).

Larvicidal bioassay of metabolites extracted from fungal species

The results of larvicidal activity of metabolites extracted from fungal species of *Aspergillus fumigatus* at the tested concentrations (10, 20, 30, and 40 mg/mL) were presented in Table 2. The results showed that the metabolites extract of*Aspergillus fumigatus* were found to exhibit larvicidal potential (100%) against *Culex* mosquitoes at the highest concentrations (40 mg/mL). The LC_{50} and LC_{90} values obtained were 13.275 mg/mL and 30.776 mg/mL. The results obtained were not statistically significant ($p = 0.20$).

GC-MS analysis of metabolites extract of *Aspergillus fumigatus*

The result of GC-MS analysis revealed 40 compounds having different retention times (Table 3).1-Docosene had the highest peak area (27.07%), and trans-13-octadecenoic acid had the lowest peak area (0.0029%). The major bioactive compounds found to exhibit larvicidal activity include 1-octadecene (0.71%), 3-eicosene, (E) - (0.72%), 1-nonadecene (1.01%), octadec-9-enoic acid (0.18%), oleic Acid (0.05%), squalene (3.47%) and cis-Vaccenic acid (0.02%). The chromatogram of metabolite extract of *Aspergillus fumigatus* showing different peaks and retention times is shown in Figure 1.

FTIR analysis of metabolites of *Aspergillus fumigatus*

The FTIR spectra of the metabolite extract of *Aspergillusfimigatus* are shown in Figure 2. The spectra reveal the presence of various functional groups of different compounds at their corresponding wave numbers. The functional groups include aromatic, carboxylic acids, amides, alkyl amines, alkynes, ether, and alcohol, as confirmed in Table 3.

Table 1: Larvicidal activity of spore extract of *Aspergillus fumigatus*

CONCENTRATION (CFU/ml)	Total larvae	Mean Mortality after 24 hours	%Mortality	LC_{50}(CFU/ml) (LCL-UCL)	LC_{90}(CFU/ml) (LCL-UCL)	x^2df = 2	P value
Control	20	00	00				
0.56×10^8	20	03	15	1.22×10^8	2.82×10^8	0.98	0.61
1.125×10^8	20	08	40	(0.95-1.55)	(2.11- 4.82)		
2.25×10^8	20	16	80				
4.5×10^8	20	20	100				

Key: control (deionized water) - nil mortality. LC_{50}, LC_{90}- lethal concentration that kills 50%, 90% of the exposed larvae, LCL, UCL = lower, upper confidence limit, df - degree of freedom, x2 - chi-square values. Data is expressed as the mean of triplicate measurements. P-values ≤ 0.05 are considered statistically significant.

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Table 2: Larvicidal activity of metabolites extract of *Aspergillus fumigatus*

CONCENTRATION (mg/mL)	Total larvae	Mean Mortality after 24 hours	%Mortality	LC_{50}(mg/mL) (LCL-UCL)	LC_{90}(mg/mL) (LCL-UCL)	x^2 df = 2	P value
Control	20	00	00				
10	20	08	40	13.28	30.78	3.22	0.20
20	20	12	60	(9.09-16.59)	(23.99-50.80)		
30	20	18	90				
40	20	20	100				

Key: control (deionized water) – nil mortality. LC_{50}, LC_{90}- lethal concentration that kills 50%, 90% of the exposed larvae, LCL, UCL = lower, upper confidence limit, df – degree of freedom, x2 - chi-square values. Data is expressed as the mean of triplicate measurements. P-values ≤ 0.05 are considered statistically significant.

Table 3: GC-MS analysis of metabolites extract of *Aspergillus fumigatus*

S/N	RT	Area (%)	IUPAC	MF	MW
1	5.1712	5.3644	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322.53
2	8.5858	0.2166	Fluoroacetic acid, dodecyl ester	$C_{14}H_{27}FO_2$	246.36
3	13.2561	0.4489	9-Eicosene, (E)-	$C_{20}H_{40}$	280.53
4	13.4594	0.2008	Carbonic acid, eicosyl vinyl ester	$C_{23}H_{44}O_3$	368.59
5	17.6638	0.7057	1-Octadecene	$C_{18}H_{36}$	252.48
6	17.8398	0.2143	Carbonic acid, decylundecyl ester	$C_{22}H_{44}O_3$	356.58
7	19.1907	0.1385	9-Heptadecanone	$C_{17}H_{34}O$	254.45
8	20.2639	0.271	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45
9	21.6051	0.0866	Heptadecanoic acid, heptadecyl ester	$C_{34}H_{68}O_2$	508.90
10	21.72	0.7144	3-Eicosene, (E)-	$C_{20}H_{40}$	280.53
11	21.8433	0.1274	1-Dodecanol, 2-octyl-	$C_{20}H_{42}O$	298.55
12	23.3385	0.0571	13-Octadecenal, (Z)-	$C_{18}H_{34}O$	266.46
13	23.3735	0.037	Undec-10-ynoic acid, tetradecyl ester	$C_{25}H_{46}O_2$	378.63
14	23.5188	1.0147	1-Nonadecene	$C_{19}H_{38}$	266.51
15	25.4264	0.6044	Trifluoroacetoxy hexadecane	$C_{18}H_{33}F_3O_2$	338.45

To be continued next page

Table 3 continued

S/N	RT	Area (%)	IUPAC	MF	MW
16	33.6148	0.0928	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282.46
17	34.0639	0.0904	Oleic Acid	$C_{18}H_{34}O_2$	282.46
18	34.106	0.1825	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.46
19	34.1727	0.1223	Heptadecanoic acid, heptadecyl ester	$C_{34}H_{68}O_2$	508.90
20	35.0668	3.4677	Squalene	$C_{30}H_{50}$	410.72
21	35.3045	0.0509	Oleic Acid	$C_{18}H_{34}O_2$	282.46
22	35.4052	0.0029	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.46
23	35.434	0.0074	Oleic Acid	$C_{18}H_{34}O_2$	282.46
24	35.7803	0.0207	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.46
25	36.0057	0.0473	3-Eicosene, (E)-	$C_{20}H_{40}$	280.53
26	36.0583	0.0213	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.46
27	36.2309	0.0272	9-Octadecenal, (Z)-	$C_{18}H_{34}O$	266.46
28	36.4532	0.1308	5-Eicosene, (E)-	$C_{20}H_40$	280.53
29	36.4763	0.0641	3-Eicosene, (E)-	$C_{20}H_{40}$	280.53
30	36.5868	0.0074	1-Docosene	$C_{22}H_{44}$	308.58
31	36.6381	0.009	Erucic acid	$C_{22}H_{42}O_2$	338.57
32	36.6769	0.0187	Oleic Acid	$C_{18}H_{34}O_2$	282.46
33	36.8318	0.1154	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.46
34	37.0169	0.5065	Oxacycloheptadecan-2-one	$C_{16}H_{30}O_2$	254.41
35	37.7243	21.1865	1-Nonadecene	$C_{19}H_{38}$	266.51
36	37.7426	1.8951	Decanoic acid, 10-(2-hexylcyclopropyl)	$C_{19}H_{36}O_2$	296.49
37	37.7671	3.5105	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	296.53
38	37.8257	8.9926	9-Tricosene, (Z)-	$C_{23}H_{46}$	322.61
39	37.9949	18.0899	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282.46
40	38.1232	27.0743	1-Docosene	$C_{22}H_{44}$	308.58

Key: RT: Retention time, MF: Molecular formula, MW: Molecular weight

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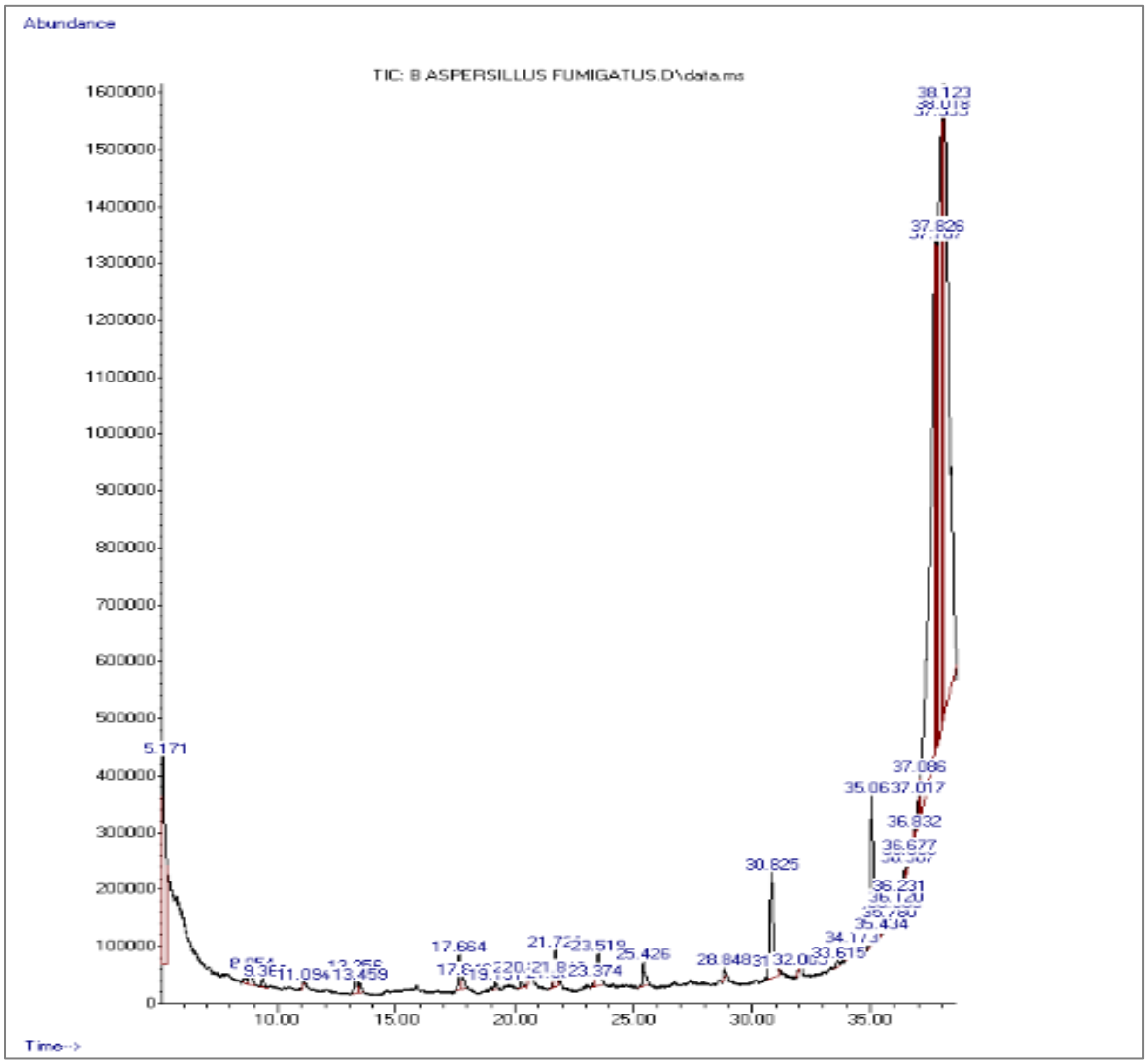

Figure 1: Chromatogram of metabolites extract of *Aspergillus fumigatus*

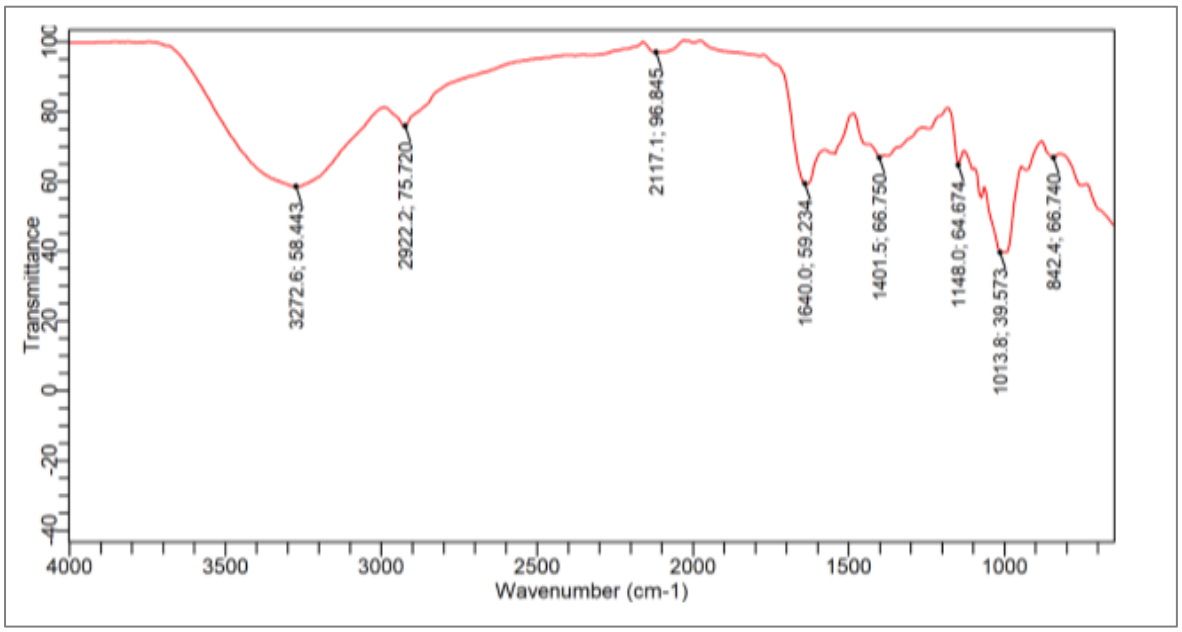

Figure 2: FTIR spectra of metabolites extract of *Aspergillus fumigatus*

DISCUSSION

The larvicidal efficacy of spores suspension of *Aspergillus fumigates* has been tested against *Culex* mosquito larvae. The results showed that spores extract of *Aspergillus fumigatus* was found to exhibit larvicidal effect (100%) against *Culex* mosquito at the concentrations of 4.5 × 10^8 CFU/ml and 2.25 ×10^8 CFU/ml. This is possibly due to fungal spores attaching to the vulnerable host's outer cuticle layer making it possible for germination and penetration (Singh *et al.*, 2017). The LC_{50} and LC_{90} values obtained were 1.224 × 10^8 and 2.822 × 10^8 against the extracts of *Aspergillus fumigatus*. The results obtained were not statistically significant (P value = 0.612). Similar results demonstrated the larvicidal efficacy of spores of *Aspergillus flavus* exhibiting 100% mortality at higher concentrations (Abideen *et al.*, 2021). In some organisms, such as *Beauveria bassiana*, the insect dies as a result of the release of toxins by the organisms (Charnley, 2003). However, a minor effect was observed at the lowest concentration of 0.56 × 10^8CFU/ml.

In the present experiment, the GC-MS analysis revealed the 40 chemical compounds. The metabolite extract of *Aspergillus fumigatus* was tested against *Culex* mosquito larvae. The metabolite extracts showed larvicidal efficacy at LC_{50} value of 13.28 mg/mL and LC_{90} value of 30.78 mg/mL, respectively. The highest mortality rate (100%) was observed at a test concentration of 40 mg/mL following 24 hours post-treatment exposure to the fungal metabolites extract of *Aspergillus fumigatus*, which may be due to the presence of bioactive compounds revealed by GC-MS analysis. This result supported the findings of Cheek (2020), which revealed high susceptibility of *Aspergillus fumigatus* crude metabolites against *Culex* mosquito larvae at 24 hours post-treatment. The mortality rate tends to decrease with a decrease in concentration, as the lowest mortality rate (40%) was observed at a concentration of 10 mg/mL. The secondary metabolites of entomopathogenic fungi may either in concert or separately show their larvicidal potential on the mosquitoes by halting their growth (Vivekanandhan *et al.*, 2018).

GC-MS analysis results of *Aspergillus fumigatus*crude metabolite revealed 40 compounds. Among these constituents, about 10 compounds may be involved in mosquito larvicidal activity, as shown in Table 3. Prior research on GC-MS analysis of *Aspergillus fumigatus* isolate showed the presence of different fatty acids such as pentadecanoic acid, 4,7-octadecadienoic acid, and 8,11-octadecadienoic acid(Baskar *et al.*, 2020). The observed larvicidal potential of the metabolite extract may be due to the presence of 1-nonadecene (Ragavendran *et al.*, 2019). Moreover, 1-Octadecene has been shown to be effective against mosquito larvae (Jayaseelan *et al.*, 2018). Insecticidal and larvicidal activity of 3-eicosene, (E)- and squalene was revealed by Banakar & Jayaraj (2018). Another study by Rawani *et al.* (2017) demonstrated the larvicidal potency of Octadec-9-enoic acid. A similar report has been documented with regard to the larvicidal potency of cis-vaccenic acid against *Aedes* specie (Ravindran *et al.*, 2020).Another study demonstrated the larvicidal potency of Oleic Acid against *Culex quinquefasciatus* (Rahuman & Venkatesan, 2008).

The FTIR analysis of the metabolites extract of *Aspergillus fumigates* shows the existence of various functional groups such as alkenes, alkynes, amines, alcohol, aromatic rings, carboxylic acids, and esters. An investigation using FTIR revealed a band at 842.38 cm^{-1}which may be due to C-H bending of arenes, and a band at 1013.84cm^{-1} due to C-O Stretching of ether. Another band for C≡C Stretching of alkynes was observed at 2117.13 cm^{-1}, and the C-H Stretching band was noted at 2922.23 cm^{-1}. The bands ranged from 1400-1450 cm^{-1} and 1650-1755 cm^{-1} for C=O Stretching of carboxylic acids and esters, respectively, and finally, the presence of alcohol between 3200-3500 cm^{-1}due to H-bonded-O-H. Vivekanandhan *et al.*, (2018) demonstrated the existence of aromatic and aliphatic amines in the extracts of mycelia of *Beauveria bassiana*. A related study on *Metarhizium anisopliae* identified the major functional groups such as as carboxylic acids and alcohols (Vivekanandhan *et al.*, 2020).

CONCLUSION

Our findings clearly demonstrated that *Aspergillus fumigatus* metabolites and spores extracts are effective against the larvae of *Culex* mosquitoes and, therefore, have potential for use in controlling mosquito vectors. Bioassay-guided fractionation can be beneficial in elucidating bioactive insecticidal molecules, which could have commercial benefits. Ultimately, fungal metabolites can be developed

which can serve as substitutes for chemical insecticides for mosquito larva control.

CONFLICT OF INTEREST

We declare none.

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