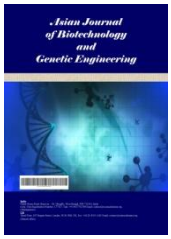

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Spores and Extracts of Entomopathogenic Fungal Isolate (*Paecilomyces formosus*) as Potential Biolarvicide of *Anopheles* Mosquitoes

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AIS and NSS designed the study, carried out the investigation and prepared the original draft. Authors MOI and RN carried out analysis of the data. Authors JLE and AN managed the literature searches and prepare the manuscript. Author AAI wrote the protocol, supervised and review the manuscript. All authors review the manuscript, read and approved the final manuscript.

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ABSTRACT

Introduction: *Paecilomyces formosus* is a geographically widespread entomopathogenic fungus that produces infectious conidia against *Anopheles* mosquito larvae, which curtail the uprising resistance of mosquitoes against synthetic insecticides. These mosquitoes are known vectors of human and animal pathogens, millions of people are killed by mosquito-borne diseases every year such as malaria, dengue, chikungunya, Zuka, yellow fever, encephalitis and filariasis.
Aim: This study investigated the spores and extract sourced from entomopathogenic (*Paecilomyces formosus*) fungal isolates as potential biolarvicide of *Anopheles* mosquitoes.
Methods: The conidia and extract bioassays were conducted according to WHO-2005 protocol with

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slight modification. The most active extract ethylacetate was characterized using Gas Chromatography-Mass Spectroscopy.

Results: From the conidia bioassay, The LC_{50} mortality of the larvae was found to be 1.4×10^4 conidia ml^{-1} at 24 hrs 6.1×10^5 conidia ml^{-1} at 48hrs 8.8×10^4 conidia/ml at 72 hrs. Solvents used for the extract bioassay includes; Diethyl-ether, Chloroform and Ethyl-acetate of which, Ethyl-acetate extract is found to be most active (LC50s; 101.5 µg/ml, 735.6 µg/ml, 769.0 µg/ml after 48-hours post exposure time.

Gas Chromatography-Mass Spectroscopic analysis of ethyl-acetate extract showed 6 major compounds (R.T) 3, 4-Altrosan (9.14), I, 6-anhydro-β-glucopyranose (9.30), Pentanoic acid (10.97), methylpropandioic acid (9.69), Cyclobutanol (10.97), and Diethylpropylmalonate (15.63).

Conclusion: These results indicated that *Paecilomyces formosus* spores and extracted secondary bioactive metabolites could serve as promising lead organism for the development of potential novel and effective insecticidal compounds.

Keywords: Entomopathogenic fungi; paecilomyces formosus; Anopheles mosquito; biolarvicide; gas chromatography-mass spectroscopy.

1. INTRODUCTION

Mosquitoes are known vectors of human and animal pathogens. Millions of people are killed by mosquito-borne diseases annually such as malaria, dengue, chikungunya, Zuka, yellow fever, encephalitis and filariasis [1]. Vector control sanitation, habitat disruption and personal protection from mosquito bites are the most adopted measures employed to control and protect people from infection of these diseases [2]. Over the past few decades, many countries organized official programs of mosquito vector control. Currently, synthetic chemical insecticides - adults or larva have been the mainstay and are the most widely used for malaria vectors control. Mosquito larvae are the attractive targets for these insecticides because mosquitoes breed in water and thus, it is easy to deal with them in this habitat [3]. The indiscriminate use of chemical insecticides to target adult mosquitoes causes problems such as mosquito resistance, environmental polution and health risk to humans and non-target organisms [3]. To reduce these problems, there is an urgent need to develop alternatives to conventional chemical insecticides, which are safe, effective, biodegradable and highly selective [3]. There has been an increasing awareness in the use biological control agents as alternative to chemical control of mosquitoes. Among the eminent biological control agents are entomopathogenic microorganisms such as fungi and bacteria [4]. Fungal bio control agents are the most essential among all the entomopathogenic microorganisms due to easy delivery, chances to improve formulation, a 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 pest with chewing mouth parts [4]. They include several phylogenetical morphological and ecologically diverse fungal species which evolve to exploit insects with their main route of entry being through the insect's integument, by ingestion or via wounds or trachea [5]. Most entomopathogenic fungi can be grown on artificial media; being natural mortality agents which are environmentally safe, there is a worldwide interest in the use and manipulation of entomopathogenic fungi for biological control of insects and other arthropod pests [6]. They display a higher degree of effectiveness in infecting their host, acting as regulators for numerous harmful insects including both domestic and forest insects [6]. In general, mosquitoes have shown susceptibility towards entomopathogenic fungi and their extracts. They have low toxicity to non-target organisms and using entomopathogenic fungi as larvicides may be a promising lead for biological control of mosquitoes due to their selective toxicity and ready decomposability in the ecosystem [7,8]. Also, unlike the dangers which are associated with the process of production of synthetic insecticides, the process for the manufacture of microbial products is safe and less toxic. Spores and extracts of different entomopathogenic fungi, notably *Paecilomyces formosus*, *Meterhizium anisopliae*, *Beaveria bassiana*, *Aspegillus niger*, *Aspergillus flavus, Lagenidium giganteum*, among others have been reported to exhibit promising larvicidal activity against mosquito larvae [9-12] in view of this, current research focuses on evaluating larvicidal efficacy of spores and extracts of *Paecilomyces formosus* on *Anopheles* mosquito.

2. MATERIALS AND METHODS

Fungal growth medias and selective proteins such as Potato dextrose agar, Czapek'sdox agar/ broth, Cetyl-trimthyl ammonium bromide (CTAB), Chloramphenicol, synthetic chemical larvicides/ insecticides such as Malathion (781.25 mg/L), Temephos (156.25 mg/L) as well as chemicals used for fungal identification procedures such as Tween-20, Lacto-phenol cotton blue were supplied by the department of biochemistry, Bayero University, Kano. DNA extraction kit and PCR reagents were purchased from Sigma-Aldrich Inc., USA while laboratory apparatus and machineries used in this research were obtained from Biochemistry department laboratories and Microbiology department laboratory complex, Bayero University, Kano, Nigeria.

2.1 Soil Sample Collection

Soil sample about (200 g) was collected from insect hibernation site including fields characterized by soil with a lot of leaf litters that typically cover the ground and grasses, shrubs and shades of trees at a depth of 0-20 cm using trowel after removing litter or weeds and placed in appropriately labelled plastic bags within Bayero University Kano premises (11.9836^{o}N 8.4753^{o}E). Before use, samples were thoroughly mixed and passed through 0.4 mm mesh sieve for breaking of soil lumps [13].

2.2 Isolation and Identification of *Paecilomyces formosus*

The fungus was isolated from soil using soil suspension procedures [14]. Soil suspension was prepared by weighing 0.1g of soil into 10mL 0.05% Tween-20. 100µL of the soil suspension was inoculated into a perti-dishes of solidified Czapek's media (3g $NaNO_3$, 0.5g $MgSO_4.7H_2O$, 1g K_2HPO_4, 0.5g KCl and $FeSO_4.7H_2O$), supplemented with 0.6 g/L of CTAB and 0.1 g/L of streptomycin. The plates were incubated at room temperature in the dark for 3-5days. Micro and macro morphological characteristics of the isolate was used for identification of fungal genus [15,16], while molecular characteristic of the ITS-region of the fungal genome amplified using TW81 (5'GTTCCGTAGGTGAACCTGC) and AB28 (5'ATATGCTTAAGTTCAGCGGGT) primers was used for specie identification [17, 18,19].

2.3 Formulation of Conidial Suspension

Fungal conidiosphore was harvested from 10 days old culture in 0.05% Tween-20 (used as negative control), its concentration was determined using hemocytometer, after which, four concentrations (6.6×10^4, 6.6×10^5, 6.6×10^6, 6.6×10^7conidia/ml) were formulated by serial dilution [20, 21].

2.4 Extract Production

Extract production was carried out following the method of Ragavendran and Natarajan [22]. Isolate of *Paecilomyces formosus* was cultured in conical flasks containing 150 mL of sterile Czapek's dox broth medium. The flasks were incubated in a rotary shaker at 28^{o}C and 130rpm for 7days. The mycelium was filtered through cheese cloth and washed several times with sterile water. 100 ml of each solvent (Chloroform, Diethyl-ether and Ethyl-acetate) was measured into a conical flasks and 5g of the harvested mycelium was transferred into each flask. The flasks were further incubated in a rotary shaker at 28^{o}C and 130 rpm for 7 days. The mycelium was then filtered to collect the crude extract. The extract was transferred into a round bottomed flask, concentrated using rotary evaporator at 45^{o}C and finally air dried at room temperature. 100 mg of the dried extract was dissolved into 100ml of DMSO to give a final concentration of 1mg/ml.

2.5 Extract Bioassay

The bioassay was carried out according to the protocol of WHO [21] with slight modification. Different concentration of the extracts (of each solvent) ranging from 300 to 1200 µg/ml were prepared and tested against 15 healthy fourth instars larvae of *Anopheles* mosquito. Each experiment was conducted in triplicates and DMSO was used as a negative control, while Temephos and Malathion were used as positive controls. The number of dead larvae was counted after 24 h and 48 h, while the percentage mortality was reported as average of the three replicates.

2.6 Mosquito Larvae Collection, Identification and Maintenance

Mosquito larvae collected from stagnant water from Auyo Local Government Area of Jigawa State were brought and maintained in the

insectaria laboratory at a temperature of 27, relative humidity of about 70% and a photoperiod of 12L: 12D h. Anopheles larvae were identified using morphological and behavioural characteristics as described by Gilles and Coetzee (1987). Fourth instars of Anopheles larva were transferred into separate containers and are maintained according to WHO-2005 protocol [21].

2.7 Bioassay

Bioassay was conducted according to WHO-2005 protocol with slight modification.

2.7.1 Conidial bioassay

A set of 5 disposable cups each containing 15 fourth instars larvae was prepared. 4 cups were treated with one concentration of conidial suspension prepared as stated above, while the remaining cup was treated with 0.05% Tween-20 as negative control. The whole experimental set-up was prepared in triplicate and the result was reported as average of the three replicates [23].

2.7.2 Extract bioassay

Another set of 8 disposable cups was prepared containing 15 fourth instars larvae each, among which, 4 were treated with one of the four concentrations (300, 600, 900 and 1200 µg/ml) of the extracted metabolites, while the remaining three were used as controls; 2 positive controls treated with Temephos- 156.25µg/ml and Malathion- 781.25 µg/ml respectively, and the last cup was treated with DMSO as negative control. The whole experimental set-up was also prepared in triplicate and the result was reported as average of the three replicates [21, 23, 24, 25].

2.8 Gas Chromatography Mass Spectroscopy (GC-MS)

Dried ethyl-acetate extract was dissolved in n-hexane and then filtered through a 0.2 µm syringe filter before ingestion into the GC-MS column. The analysis was conducted with DB-5MS column (30 m×0.25 mm I.D., 0.25 µm film thickness). The oven temperature was programmed at 60°C for initial temperature for 2 min, which then rises at a rate of 10°Cmin^{-1} to 300°C and finally held isothermally at 300°C for 6min, to complete a total run time of 34min. Helium was used as carrier gas at 1 mlmin^{-1} flow rate, and the relative abundance of compounds that consists of the extract was expressed as percentage of peak area. Bioactive compounds were identified by comparing their mass spectra and retention indices with those of NIST mass spectral library and literature values [59].

2.9 Statistical Analysis

The data generated were analyzed using IBM SPSS Statistics version 20. The Average percentage mortality was determined using One-way ANOVA and Probit analysis was conducted to determine the lethal concentrations (LC50) of fungal conidiophores and bioactive extracts.

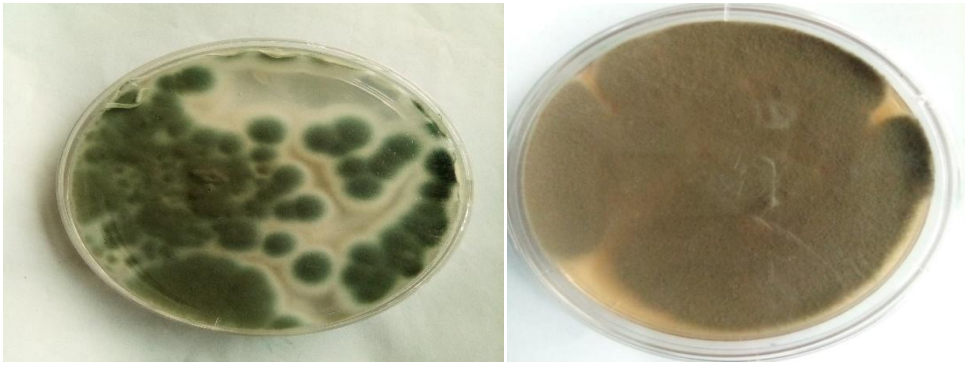
3. RESULTS AND DISCUSSION

Entomopathogens are microorganisms capable of infecting and invading live insects at various developmental stages (larvae, pupae and adult) and ultimately killing their host through feeding on their body nutrients and secreting biochemical toxins. This brings about reduction in the population of pest and vector to a level that does not cause economic or health impact [26]. Bacterial and fungal entomopathogens are widely employed as bio-control agents of mosquito worldwide, where fungi are most preferred because they are relatively easier to deliver. These entomopathogens also have higher chances of improving formulations, vast number of pathogenic strains, and wider range of host and are easily subjected to molecular transgenesis [27]. *Paecilomyces* species is a geographically widespread group of many entomopathogens that can infect different orders of insects in all stages of development and can be frequently isolated from soil [28]. *Paecilomyces formosus* is a thermophilic, filamentous and saprophyic fungus that is characterized by high level of sporulation [29]. A thermophilic fungus is one that has a minimum temperature of growth at or above 20℃ and a maximum temperature for growth at or above 50℃ [30]. Like most entomopathogenic fungi, *Paecilomyces formosus* infects its host by breaching the cuticle. Various metabolites allow the pathogen to physically penetrate the host as well as inhibit its regulatory system [31]. The drug resistance and increasing insecticidal resistance have stimulated the use of alternative larvicides. The European Union (EU) has withdrawn many pesticides due to the risk they pose to humans and the environment [32].

In recent years, interest on mosquito-killing fungi is reviving, mainly due to continuous and increasing levels of insecticide resistance and increasing global risk of mosquito-borne diseases. Historically, both environmental and biological controls of mosquitoes were exclusively aimed at larval stages and as such have been successful in a variety of geographical and ecological settings within the class of Dueteromycetes, especially Ascomycetes that have entomopathogenic fungi such as *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces formosus* species [33]. The basic mechanism of pathogenesis behind was entrance through the external integument. Besides, infection through digestive tract was also possible [34]. Conidia attach to the cuticle, germinate and penetrate the cuticle. Once in the hemocoel, the mycelium grows and spreads throughout the host, forming hyphae and producing blastospores. Humidity is a key factor for high and rapid killing of insects by entomopathogenic fungi, and further development on cadavers [35-37].

Identification of the fungal isolate was based predominantly upon the morphology of the conidia and conidiophores. Fig. 1 shows the macroscopic characteristics of the fungal isolate. Colonies were rounded; central bulged, dense, floccose and thick with orderly margins and radiating ring. The colony colour was white, later changing to dark green for young culture; while the mature culture gives an ash colour.

Fungal isolate is characterized by verticillate clusters of conidiophores bearing divergent whorls of phialides with a cylindrical or inflated base tapering to distinctly narrowed neck as shown in Fig. 2. The conidia are typically hyaline, one-celled, and smooth walled, and produced in basipetal chains [38-40]. DNA of fungal isolates amplified by ITS1&4 primers specifically for *paecilomyces formosus* isolates shows a similar result with that of ITS1-5.8S and ITS2 region that was amplified, hence the PCR product were found to be 534bp, 560bp and 569bp which is in line with result of *paecilomyces lilacinus, paecilomyces nostocoides,* and *paecilomyces variotii* as shown in Fig. 3. Nevertheless*n Paecilomyces lilacinus* can grow at 37°C and is usually considered as a biological control agent for root-knot nematodes [41,42,43].



(A) **(B)**

Fig. 1. (A) Five days old (B) Two weeks old mono-cultured plates of fungal isolate

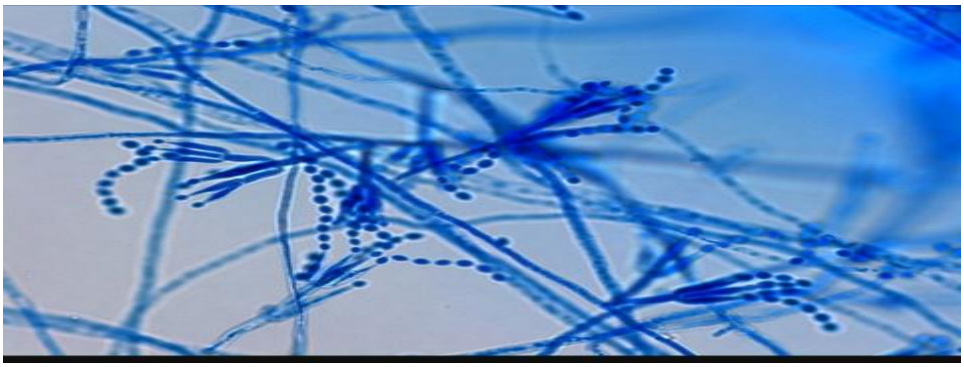


Fig. 2. Micro Slide Image of the Fungal Isolate viewed under X100 magnification

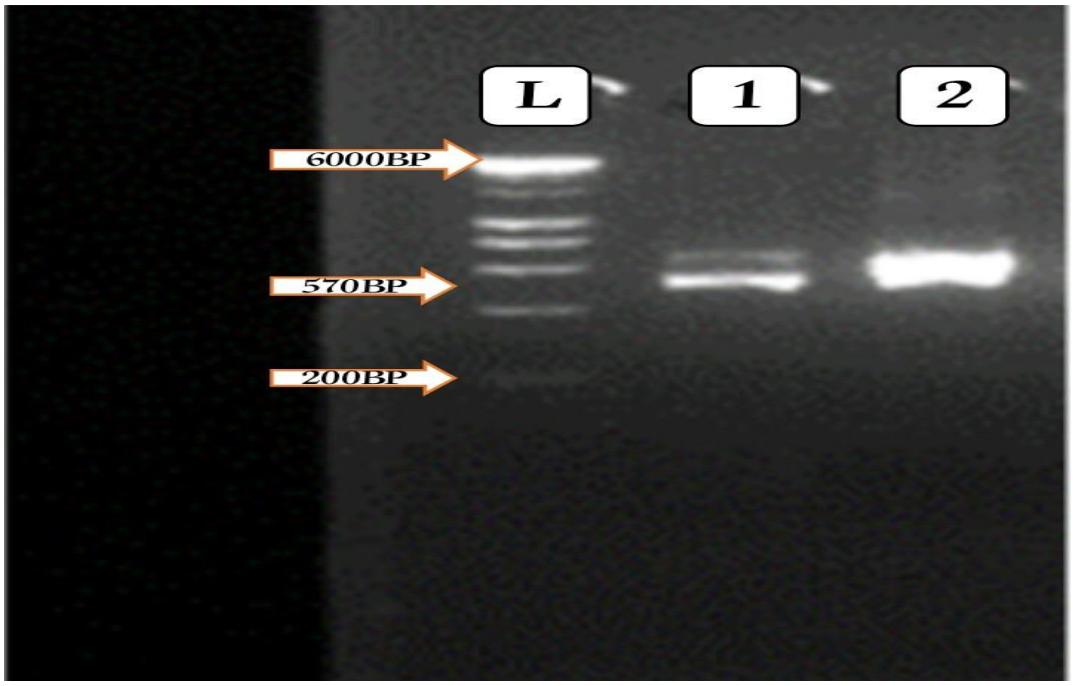


Fig. 3. PCR product of the isolate on 1.5% agarose gel electrophoresis. The fungal isolate produced a product of about 570bps-600bps. L is the ladder (hyperladder1kb) from 200bps-6000bps, lane 1 and 2 are the analyzed DNA of the isolate

Based on their morphological characteristics, mosquito larvae have the absence of breathing tube (siphon) which is unique characteristics of *Anopheles*. They breathe through a cluster of small abdominal plates and possess palmate hairs. Mosquito larvae are observed to lie flat just below the water surface when not diving or swimming [25]. However, a review of studies has elucidated the larvicidal activity of fungi and revealed that they could successfully infect and kill larvae of a wide range of mosquito genus with varying rates of mortality [6]. Furthermore, a research conducted in East Africa to determine the pathogenicity of entomopathogenic fungi against several strains of adult *Anopheles gambiae* revealed a high infection rates ranging from 46 to 88% with *Metarhizium anisopliae* being the most pathogenic strain [14]. In recent years, there is a considerable amount of attention focusing on identifying potential mosquitocidal fungus from natural sources for effective control of mosquitoes as a key measure to curtailing the vector borne diseases in human [15]. A study was also conducted in Asia researching the larvicidal potential of *Lagenidium giganteum*, a water weed, leading to its efficacies in killing the tested vectors with appreciable safety to non-target organisms and good biological stability [15]. According to a large-scale field trial conducted in the United States, mycelium extract of *Lagenidium giganteum* caused 40-90% infection rates in *Culextarsalis* and *Anopheles freeborn sentinel* larvae [16]. The potentials of many fungi have been established for mosquito control, nevertheless, only a few have received commercial attention and are marketed for use in vector control programmes globally [16].

In this study, the conidia from the entomopathogenic fungus (*Paecilomyces formosus*) exhibited larvicidal activity, these *Paecilomyces formosus* was isolated from soil using soil suspension and selective media, four different concentration of conidial suspension; 6.6×10^{7}, 6.6×10^{6}, 6.6×10^{5} and 6.6×10^{4} conidia ml^{-1} were tested, and the results show that; mortality increases with increase in conidial concentration and exposure time. The lowest mean percentage mortality (44%) was recorded at 6.6×10^{4} conidia/ml and the highest mean percentage mortality (98%) was recorded at 6.6×10^{7} conidia/ml at 72-hours post exposure. The lethal concentration of conidial suspension causing 50% and 90% mortality of the larvae was found to be 1.4×10^{4} and 2.2×10^{12} conidia ml^{-1} at 24-hours; 6.1×10^{5} and 4.6×10^{8} conidia/ml at 48-hours; 8.8×10^{4} and 1.2×10^{7} conidia/ml at 72-hours as shown below.

Table 1. Larvicidal efficiency of *Paecilomyces formosus* conidial suspension against *Anopheles* mosquitoes

Exposure Time	Concentration (conidia ml^{-1})	Percentage Mortality (%)	Probit equation	Lethal Concentration (LC_{50}) (conidia/ml)	P value	$\chi 2$ Chi Square
24 Hrs	6.6×10^{7}	47±3.8				
	6.6×10^{6}	33±3.8	y = 0.5x – 3.0	1.4×10^{8}	0.004	0.005
	6.6×10^{5}	25±2.2				
	6.6×10^{4}	16±5.9				
48 Hrs	6.6×10^{7}	82±2.2				
	6.6×10^{6}	67±7.7	y = 0.5x – 2.75	6.1×10^{5}	0.002	0.001
	6.6×10^{5}	51±4.4				
	6.6×10^{4}	33±6.7				
72 Hrs	6.6×10^{7}	98±2.2				
	6.6×10^{6}	80±7.7	y = 0.5x – 2.50	8.8×10^{4}	0.001	0.001
	6.6×10^{5}	78±2.2				
	6.6×10^{4}	44±8.0				

3.1 Positive Control Group (Treated with 1 ml 0.05% Tween-20 and Distilled Water) Records no Mortality

This study produced results which corroborate the findings of Sani et al. [23] reporting the mortality percentage of *Paecilomyces* spp against *culex* mosquito larvae to be up to 80% after 96 h post treatment. Thomas et al. [38] also in his findings reported the mortality percentage of *Aspergillus fumigatus* against *culex* mosquito reaching up to 96% after 72 h post treatment. Gayathri et al*.* [54] reported the pathogenicity of *Paecilomyces fumosoroseus* against *Culex quinquefasciatus* with 97.73% mortality on 8^{th} day after treatment with (10^{8} conidia ml^{-1}) which is similar to this research with mortality reaching 98% at concentration of 6.6×10^{7} conidia/ml after 72 h exposure time. In this study, mortality in the control was recorded zero percentage, pathogenicity varied according to concentration of conidial suspension and period of exposure. For the four concentrations of the conidial suspension isolate tested (Table 2). These findings further support the idea of Al-Hussaini and Hergian [44] and Benserradj and Mihoubi [45] who reveal that larval mortality percent and LC50 of *C. quinquefasciatus* increased as exposure periods increased. The results from extract bioassay of chloroform, diethyl ether and ethyl acetate revealed that the extracts of *P. formosus* were pathogenic to 4^{th} instars larvae of *Anopheles* mosquitoes with four different concentrations of the extracts tested; 300, 600, 900, and 1200 µg/ml. The results also show increased mortality with increased extract concentration. The LC50 were found to be 735.6 µg/ml for chloroform extract, 769.0 µg/ml for diethyl-ether extract and 101.5 µg/ml for ethyl-acetate extract as presented in Table 2.

3.2 Negative Control Group (Treated with DimethylSulfoxide) Records no Mortality. Positive Control Group 1 and 2 Treated with Standard Chemical Larvicides Temephos (176.25 µg/ml) and Malathion (781.25 µg/ml) Respectively) Recorded 100% Mortality

The findings of this study produced results similar to the reports on comparative study on larvicidal efficacy of mycelium; ethyl-acetate and methanolic extract of *A. Terreus* against *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti* larvae [46] and *Beauveria bassiana* against *Aedes aegypti* larvae [47], also shows higher larvicidal activity of ethyl-acetate extracts.

Ethyl-acetate extract; having recorded the least LC_{50} based on this result, ethyl-acetate extract is considered the most pathogenic and considered a better solvent for extraction of *P. formosus*-mycelium extract for control of *Anopheles* mosquito larvae. In the same vein, Vivekanandhan et al*.* [48] reported on larvicidal toxicity of ethyl-acetate mycelium extract of

Beauveria bassiana-28 grown in potato dextrose broth (PDB), reported lower LC_{50} which is in tandem with the result of this research. The concentration of the most pathogenic extract; ethyl acetate was further compared to standard chemical insecticide, Temephos and Malathion. The result, as presented in (Fig. 4) shows that the chemical insecticides are more toxic to the larvae than the extract, at 156.25 µg/ml Temephos and Malathion causes 100% mortality after 48hrs post exposure time, while the extract causes 80% mortality at 1200 µg/ml.

This shows that Temephos and Malathion are 20% more efficient, thus corroborate with a study on the evaluation of synergistic effect of *Aspergillus flavus* on Temephos, reported similar scenario. The fungal extract was reported to have LC50 of 13.61, 14.37, and 10.02 ppm after 24, 48 and 72 hrs, respectively while that of Temephos were 0.0060, 0.0055 and 0.0042 ppm after 24, 48 and 72 hrs respectively [49]. Bioactive compounds identified by GC-MS analysis of *Paecilomyces formosus* ethylacetate extract, 9 peaks were detected in the GC-MS chromatograms. However, 6 out of these compounds have been reported to exhibit certain biological activity. Most of the compounds identified are carbohydrates; 1, 6-anhydro-β-D-Glucopyranose, 3,4-Altrosan, organic acids; pentanoic acid, diethylpropylmalonate, propylpropandioic acid and cyclobutanol among others as shown in Table 3.

Table 2. Larvicidal efficiency of *Paecilomyces formosus* extracts against *Anopheles* mosquitoes

Solvent	Concentration (µg/ml)	Percentage Mortality (%)	Probit equation	LC_{50} (µg /ml)	P value	$\chi 2$
Chloroform						
24_Hours	300	27±0.01				
	600	33±7.70	y = 7.143x – 5.11	2135.5	0.04	0.001
	900	33±8.84				
	1200	47±3.84				
48_Hours	300	40±3.87				
	600	40±7.68	y = 2.1429x – 5.8	735.6	0. 018	0.001
	900	47±3.84				
	1200	67±3.84				
Ethylacetate						
24_Hours	300	33±0.01	y = 1.7857x – 4.7857	778.9	0.001	0.003
	600	40±3.86				
	900	47±3.84				
	1200	67±3.83				
48Hrs	300	67±3.84				
	600	60±2.83	y = 1.0x – 2.20	101.5	0.004	0.002
	900	73±3.83				
	1200	80±2.40				
Diethyl ether	300	20±1.24	y = 1.1429x – 3.2429	2605		0.002
24 _ Hours	600	27±2.31				
	900	31±2.21				
	1200	40±6.70				
48 _ Hours	300	36±2.23	y = 1.4286x – 4.4286	769. 0	0.002	
	600	47±3.84				
	900	51±2.20				
	1200	57±2.23				

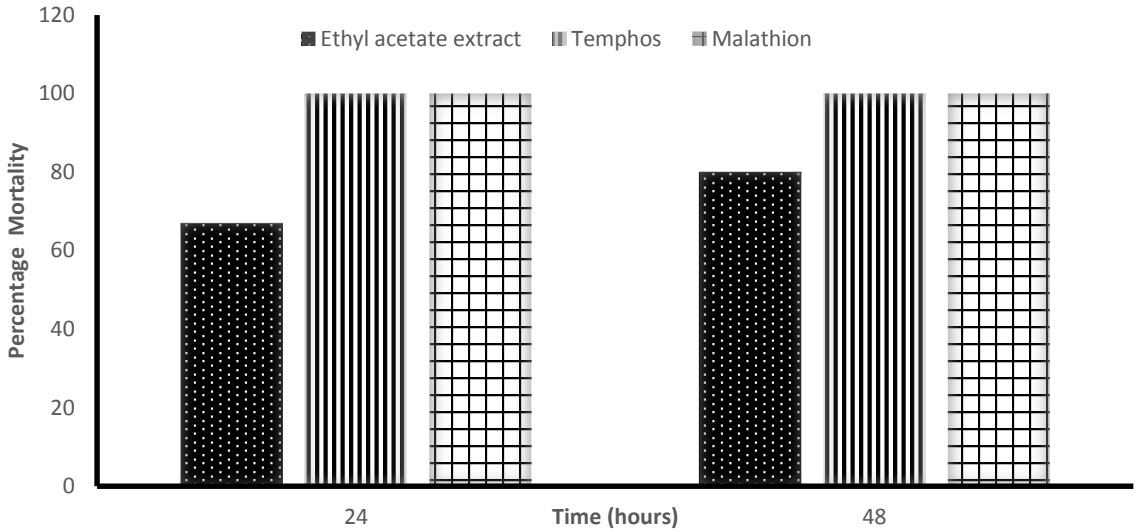
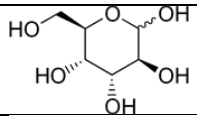
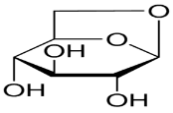
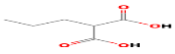
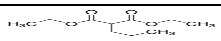
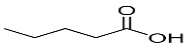


Fig. 4. Comparison between ethyl acetate extract and standard chemical larvicides

Table 3. Major bioactive compounds of *Paecilomyces formosus* ethylacetate extract identified by GC-MS

S/N	R. Time (Mins)	Molecular Weight (g/mol)	Compound	Structure	Biological activity	Reference
1	9.14	162	3,4- Altrosan		Bacteriostatic, Fungicide.	[50]
2	9.30	162	1,6-Anhydro-β-D-Glucopyranose		Antioxidant activity	[51]
3	9.50	146	Propyl-Propanedioic acid		Insecticidal, Antimicrobial	[55]
4	9.69	202	Diethylpropyl malonate		Antiplasmodial, pesticide.	[29]
5	10.97	102	Pentanoic acid		Insecticidal, Antimicrobial.	[49]
6	15.63	72	Cyclobutanol		Antimicrobial	[58]

3, 4-Altrosan a component of ethylacetate extract of *P. formosus* has been reported to exhibit biologically and pharmacologically important activities such as bacteriostatic, fungicide [50, 51]. Fatty acids generally have been reported to have larvicidal potential. Pentanoic acid, diethylpropylmalonate and methylpropandioic acid found on the ethylacetate extract of *P. formosus* have several biological activities such as hepatoprotective, antimicrobial, antiplasmodic and insecticidal effects. The mechanism of action of some of these metabolites may be as a result of them accumulating naturally in the spinal fluid during sleep deprivation and induce sleep in animals [52,53], the compounds are presumed to execute their biological activity through interaction with multiple neuro transmitter system [54,55] Senthil-Kumar et al. [56] have reported

the larvicidal and insecticidal activity of *Phomopsis* spp because of the presence of dodecanoic acid in *Phomopsis* spp, has better insecticidal property. 9,12-Octadecanoic acid has been widely reported as an important component of ethyl-acetate-mycelium extracted metabolites of *Beauveria bassiana*, exhibiting larvicidal activity against a wide range of insect larvae including mosquito (Vivekanandhan et al. [48], Ragavendran, [57,9]. Senthilkumar et al. [56] reported that ethyl acetate mycelium extract of *Beauveria bassiana* showed the presence of prominent functional groups which may be involved in the mosquitocidal activity.

4. CONCLUSION

Spores and extracts of *Paecilomyces formosus* have promising larvicidal activity against *Anopheles* mosquito larvae, the vector of *Plasmodium* parasite that causes malaria which is widely distributed in the Northern guinea savannah vegetation of Nigeria. GC-MS characterization of ethyl-acetate extract showed 6 major compounds and the effect of some of *these* compounds might to be responsible for the observed larvicidal activity of the extract.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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