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Research Article

Endophytic Fungi As Biocontrol Agent

Endophytic Fungi *Fusarium Equiseti* EF2 Isolated from *Leucas Aspera*: A Novel Biocontrol Agent Against *Culex* Sp

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Abstract: Among several life-threatening diseases, vector-borne diseases play a significant role. Mosquito-borne conditions are dangerous and are more prevalent. Culex is the most prevalent and causative agent of many zoonotic diseases among various mosquito species. Endophytic fungi that reside in the healthy plant produce an array of bioactive compounds against several conditions causing pathogens. Thus, this study aimed to identify a novel antilarval combination from the endophytic fungi present in *Leucas aspera* leaves. The objectives include the isolation of endophytic fungi from leaf samples, selection of potent antilarval endophyte, and characterization of its bioactive compound. The exploration of the least studied fungi, *Fusarium equiseti* as an endophyte in the leaves of *Leucas aspera* with potent antilarval properties is an inquisitive discovery. A total of 10 endophytic fungi (EF-1 to EF-10) were isolated and screened for the larvicidal activity of the fungal broth and its spore. The best isolate, EF-2 was identified as *Fusarium equiseti.* The crude sample and the active fraction of the ethyl acetate extract exhibited potent antilarval properties against *Culex* mosquito larvae with 90% mortality. Phytochemical analysis and characterization studies by UV-Vis spectroscopy and GC-MS revealed bioactive compounds in the active fraction of the extract. Overall, this study suggested a new option for biocide formulation that could aid in the effort to control mosquitoes. Among several discoveries of bioactive compounds from the plant extracts, this study has identified novel compounds from its endophytic fungi rather than the plant itself. The extracts of endophytic fungi Fusarium equiseti isolated from *Leucas aspera*, has antilarvicidal activity.

Keywords: *Leucas aspera, Fusarium equiseti, Culex* mosquito, Larvicidal activity and Endophytic fungi

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1. INTRODUCTION

In many South Asian countries, particularly India, mosquito-borne diseases have become one of the major threats to humans, causing high mortality and morbidity rates [1]. Of the various mosquito genera, *Aedes, Anopheles, Culex* and *Armigeres* were the predominant ones found in many parts of India and other South Asian countries causing dengue, malaria, Japanese encephalitis and filariasis respectively [2,3]. Though all the mosquito species are considered as vectors, *Culex* sp., is highly considered for scientific studies since this species can feed both on humans and animals. *Culex quinquefasciatus* and *Culex pipiens* are the commonly reported species [4]. In 2021, World Health Organization (WHO) released a statistical report on Lymphatic filariasis stating that around 859 million people residing in 50 different countries worldwide have been affected by this deadly disease, of which *Wuchereria bancrofti* alone contributed to 90% of the infection. The main vector of *Wuchereria bancrofti* is the *Culex* mosquito [5] and *Culex* mosquitoes were considered the primary target organism in this study. Mosquito control strategies all begin with treating their larval and pupal stages [6]. Usage of synthetic pesticides such as DDT, malathion, pyrethroids, and many other chemical agents exists. Mosquitoes are highly resistant to these pesticides, which are toxic and lethal to non-target organisms, including humans [7]. The promising alternative for mosquito control will be exploiting plants and microbes. From the biological control perspective, plants belonging to the families *Asteraceae, Fabaceae, Lamiaceae* and *Rutaceae* were highly reported. In contrast, in microbial communities the entomopathogenic species belonging to genus *Beauveria, Coelomomyces, Culicinomyces, Entomophthora, Lagenidium* and *Metarhizium* [8] and the use of bacterial toxins from *Bacillus thuringiensis* were reported against the mosquito[9]. Endophytic fungi in medicinal plants are used as potent larvicidal agents, especially against mosquito larvae [10]. As a beneficial bioresource, endophytic fungi act as the protector against biotic and abiotic stresses of plants and as a source of bioactive substances [11]. Flavonoids, alkaloids, terpenoids, steroids, xanthones, quinones, phenols, etc., are among the secondary metabolites produced by endophytic fungi [12]. *Fusarium* sp., belonging to the Ascomycota, are one of the endophytic fungi in plants. Among its 1000 different species, *Fusarium equiseti* has attained prominence due to its varying properties as beneficial endophytes. In addition to acting as probiotics, they produce several secondary metabolites that are beneficial for treating several illnesses, including hepatitis [13]. *Leucas aspera,* belonging to the family *Lamiaceae,* is an annual herb found in many parts of India. Ayurveda, Siddha, and other medicinal practices use this plant for its diverse phytochemicals, such as phenols, sterols, terpenoids, ursolic acid, diterpenes, oleanolic acid, nicotine, glucosides, apigenins, maslinic acid, *etc*., [14]. In addition, therapeutic properties of various extracts of different parts of the plant, such as hepatoprotective and cytotoxic activities of leaves, analgesic and antinociceptive activities of roots, anti-inflammatory activity of flowers, and anti-arthritic, anti-ulcer, anti-diabetic, anti-mutagenic and anthelminthic activities of the whole plant were reported [15]. This study is the first report on the antilarval properties of the endophytic fungi present in the leaves of the medicinal plant, *Leucas aspera* rather than the plant itself. The main objectives of the study are (i) isolation of endophytic fungi from different medicinal plants in and around Kanchipuram, Tamil Nadu, India; (ii) selection of the best endophytic fungal isolate based on their antilarval activities against the *Culex* mosquito larvae; (iii) partial purification of novel antilarval compounds from the extracts of endophytic fungi, *Fusarium equiseti*; (iv) characterization of the antilarval compounds in the partially purified fractions active against the *Culex* mosquito larvae.

2. MATERIALS AND METHODS

2.1 Collection of Plant Leaf Samples

Leaf samples of various plants were collected from different places in Kanchipuram District. The leaves were stored in sterile bags and kept in a cool place until they were processed. The leaf samples were arbitrarily named as PS-1 to PS-35. Dr.N.Karmegam, Assistant Professor, Department of Botany, Govt, authenticated the plants. Arts college, Salem, Tamil Nadu.

2.2 Media Preparation

Potato Dextrose Agar (PDA) was used to isolate endophytic fungi from the leaf samples. The plates were prepared using Potato Dextrose Agar (HiMedia Laboratories, Mumbai) containing 200 g of potatoes infusion form, 20 g of dextrose, and 15 g of agar per litre of distilled water. The media was boiled until it dissolved completely and sterilized at 121°C for 15 minutes. Once the media cooled down to 45-50°C, it was poured into sterile petriplates and was allowed to solidify. [16]

2.3 Isolation of Endophytic Fungi from Plant Leaves

The leaves were sequentially washed, rinsed with distilled water, surface sterilized for 1 minute with 75% ethanol, then treated with 1% sodium hypochlorite for 10 minutes, and finally rinsed with distilled water. The samples were air-dried before being cut into little 10 mm segments with a sterile blade. The sliced segments were placed on potato dextrose agar (PDA) plates and incubated for about 7 days in the dark at 25°C. Once mycelium formed around the sample segments, the hyphal tips were transferred onto fresh PDA plates, and the procedure was repeated until the pure culture was obtained[17].

2.4 Morphological Identification of Isolated Endophytic Fungi

The pure cultures of isolated endophytic fungi on potato dextrose agar (PDA) plates were used for morphological identification by the Lactophenol Cotton Blue (LPCB) staining method [18]. The hyphae and conidia from the purified colonies were stained with LPCB and observed under a microscope [19]. The micromorphology and colony morphology were used for identification. Periodical screening of the culture was done to identify the sporulation.

2.5 Mass Cultivation of Endophytic Fungi

Isolated endophytic fungi were mass-cultivated in 100 ml of Sabouraud dextrose broth and incubated at room temperature for three weeks at 150 rpm. After incubation, the cultures were recovered and strained through a sterile cheesecloth to remove the mycelial mats [17].

2.6 Assessment of Larvicidal Activity of Isolated Endophytic Fungi

2.6.1. Collection and Identification of Mosquito Larvae

Mosquito larvae were collected from the stagnant drainage

wastewater at Nellore, Kanchipuram, and identified based on morphological features. Various larvae instars were segregated and grown individually under control laboratory conditions at room temperature (26±2°C) with a natural photoperiod of 18:6 h (light: dark). Larvae were raised in fresh tap water and fed powdered meals comprising a 3:1 ratio of dog biscuit and baker's yeast [9].

2.6.2. Assessment of Larvicidal Activity of Isolated Endophytic Fungal Broth

After 15 days of mass cultivation, the fungal broth was filtered using Whatman filter paper No. 1. Every batch was introduced separately into 30 ml of test medium (fresh tap water), each containing 2 ml of the filtrate. Positive control of malathion (40 µg/ml) and negative control contained 30 ml of distilled water. Larvae were fed normally with the prepared meal. Larval mortality was recorded following 24 h of exposure to the filtrate [9].

2.6.3. Selection of Best Antilarval Compound Producing Endophytic Fungal Strain and Taxonomic Identification

Based on the 24 h mortality rate of the larvicidal assay, the best endophytic fungi, EF2 that caused maximum mortality was selected. The traditional method of staining the hyphae and conidia of the selected endophytic fungi from the purified colonies by lacto phenol cotton blue (LPCB) stain was employed for morphological identification [20]. The 18S rRNA ITS gene sequences of the endophytic fungi EF2 were amplified and sequenced. PCR amplifications were done as follows: initial denaturation at 94°C for 3 min, 35 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 60 s and a final extension of 72°C for 7 min. The nuclear ribosomal RNA Internal Transcribed Spacer (ITS) gene amplicon was sequenced using ABI 3730 automated sequencer. The forward and reverse sequences received were assembled using the CAP3 sequence assembly program [21]. The assembled contig sequence was analyzed through BLAST analysis in Blast-n at the NCBI server [22]. The top 10 similar sequences were used for phylogenetic tree construction using MEGA XI [23] by the UPGMA method.

2.7 Partial Purification of Bioactive Substance

2.7.1. Solvent Extraction of Bioactive Substance

The endophytic fungi EF2 was cultured in 500 mL of Sabouraud dextrose broth at 27°C for 15 days in the shaker at 150 rpm. After 15 days the culture broth was filtered through Whatman filter paper no 1. The filtrate was stirred in the magnetic stirrer overnight with an equal volume of ethyl acetate and transferred into the separating funnel. It was shaken vigorously for 10 min, and it was allowed to stand for the cell mass to get separated. The solvent was collected by evaporation using the Soxhlet apparatus, and the resultant compound was dried and transferred to the vial [24].

2.7.2. Column Chromatography for Partial Purification

For partial purification, the column was packed with 10 g of silica gel dissolved in 100 ml of distilled water, after which the extract was poured into the column. The eluent was collected as different fractions at a rate of 1 ml/min in 30 test tubes [20].

2.8 Assessment of Antilarval Activity of the Crude Sample and the Fractions of Column Chromatography

The antilarval activity was assessed by exposing the larvae to the sample in triplicates. Batches (10 larvae in each batch) of the second instar larva were introduced into the 30 ml of the test medium containing 30 ml of distilled water along with 300 µl of 30 fractions, each in separate containers. 100 µl of malathion (40 µg/ml) was added as a positive control. 100 µl of ethyl acetate was added as a negative control. All the containers were maintained at room temperature with proper light and feed provided. The mortality rate of the larvae was assessed after 24 h [20].

2.9 Qualitative Profile of Phytochemicals

All the phytochemical tests for the identification of bioactive compound in the endophytic fungal extract was carried out using standard procedures.

2.9.1. Test for Amino Acids

To 2 ml of the fungal extract, 2 drops of ninhydrin reagent was added. The appearance of purple color indicates the presence of amino acids. [25]

2.9.2. Test for Tannin

The endophytic fungal extract was treated with $FeCl_3$ solution. The bluish-black color appears but disappears upon the addition of dilute H_2SO_4. The subsequent formation of yellowish-brown precipitate indicates the presence of tannin.[25]

2.9.3. Test for Protein

To 2 ml of the filtrate, 1 drop of 2% $CuSO_4$ solution, 1 ml of 95% ethanol, and potassium hydroxide pellets were added sequentially. The formation of pink layer indicates the presence of proteins. [26]

2.9.4. Test for Alkaloids

The dried fungal extract was dissolved in 2N HCl, mixed well, and filtered. To one part of the filtrate few drops of Mayers reagent was added. To another part of the filtrate, Dragondroffs reagent was added. The formation of cream white precipitate and orange precipitate indicated the presence of alkaloids, respectively[26].

2.9.5. Test for Flavonoids

To 0.5 ml of the fungal extract, a few drops of dilute HCl and a small piece of magnesium were added, and the solution was boiled for a few minutes. The appearance of a dirty brown precipitate indicates the presence of flavonoids. [27]

2.9.6. Test for Phenols

The fungal extract was dissolved in 5 ml of distilled water, to which a neutral 5% ferric chloride solution was added. The appearance of dark green color indicates the presence of phenols. [27]

2.9.7. Test for Steroids

To 0.2 g of dried fungal extract, 2 ml of acetic acid was added.

After cooling, concentrated H_2SO_4 was added slowly. The formation of blue-green ring indicates the presence of steroids. [27]

2.10 Characterization of Active Fraction from Column Chromatography

The active fraction from the column chromatography with potent antilarval activity was characterized by UV-Visible spectrophotometry and Gas Chromatography-Mass Spectrometry analysis [28].

2.10.1. UV-Visible Spectral Analysis

The active fraction was subjected to multi-wavelength scanning (190-900 nm) by UV-visible spectrophotometry (Cecil CE 7200) [29].

2.10.2. Gas Chromatography-Mass Spectrometry Analysis

The active fraction from column chromatography that exhibited good antilarval activity was characterized by Gas Chromatography-Mass Spectrometry (GC-MS). Agilent Gas chromatography system 7820A and mass detector 5977E were used to perform GC-MS. DB-5 column was used with oven temperature from 100°C to 270°C at 10°C increments per min with a flow rate of 1.2 ml/min. Helium was used as carrier gas [30].

3. STATISTICAL ANALYSIS

All the experiments were carried out in triplicates. Values corresponding to mortality of mosquito (in %) were represented as mean ± SEM, as analyzed by ANOVA with Sidak's multiple comparison test (α <0.05).

4. RESULTS

4.1. Isolation and Mass Cultivation of Endophytic Fungi from Leaf

Endophytic fungi from the collected leaf samples were isolated on Potato Dextrose Agar (PDA) medium. Based on morphology, a total of 10 different endophytic fungal isolates (EF1 – EF10) were obtained. In addition, Micromorphology was observed using LPCB staining (Fig. 1). Mass-cultivated mycelial-free fungal culture filtrates were collected and used for further processing.

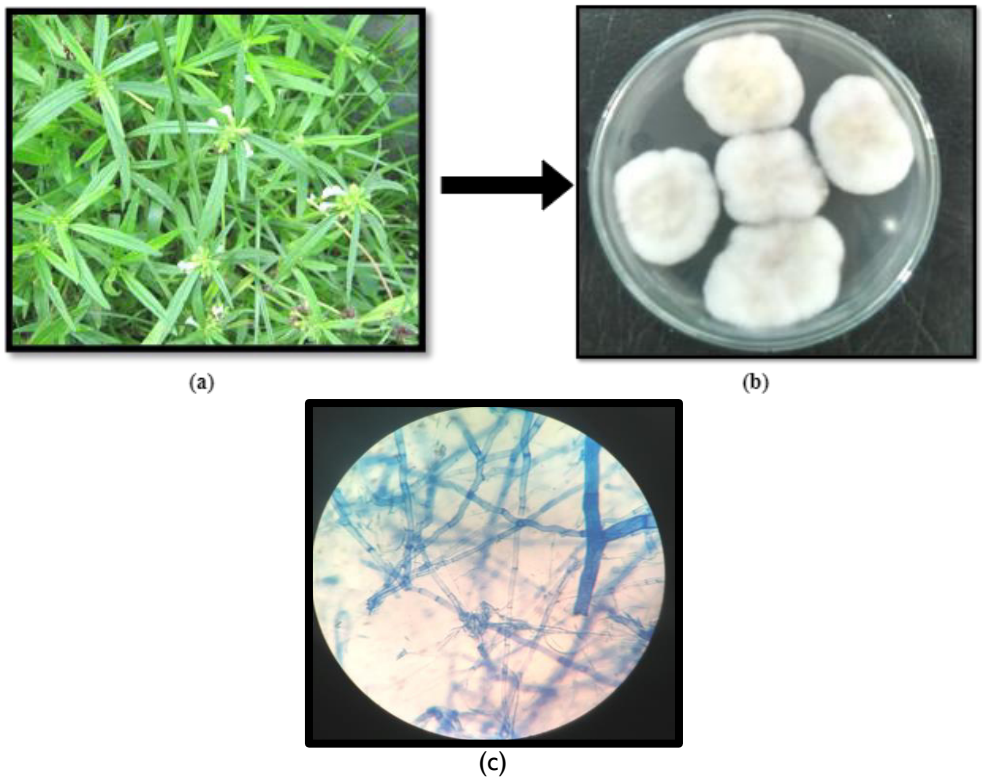
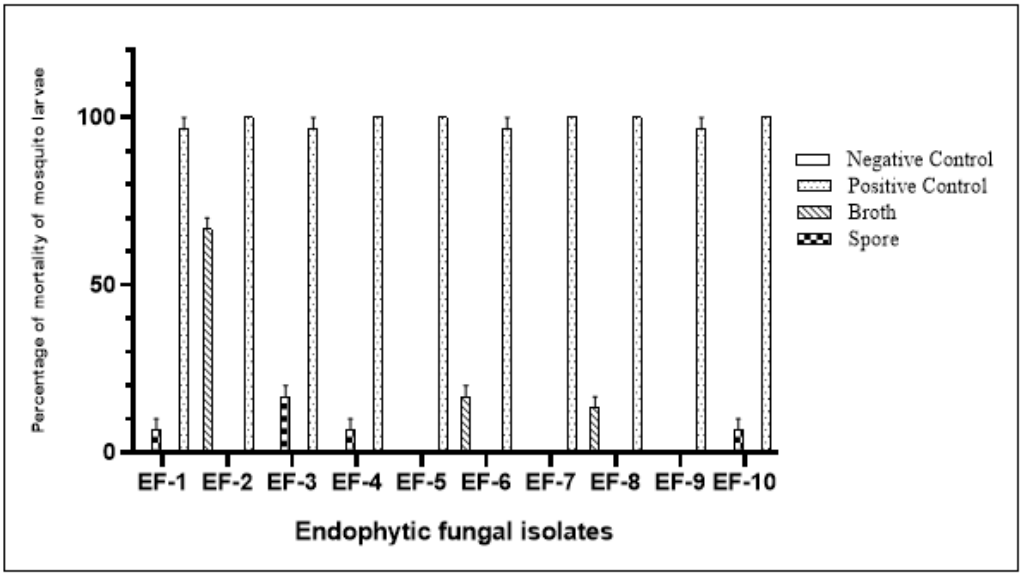


Fig 1: Isolation of endophytic fungi; (a) - Leaf sample of the plant *Leucas aspera*, (b) – isolation of endophytic fungi from *Leucas aspera* leaf, (c) - LPCB staining of EF2, showing filamentous structure with segmented hyphae.

4.2. Screening for the Antilarval Activity of Fungal Broth

Based on the morphological characteristics, *Culex* sp., larvae were identified and used for further study. Endophytic fungal broth and spores of all the 10 isolates (EF-1 to EF-10) were tested for antilarval activity against the tested mosquito larvae at 24 h. The percentage of mortality was calculated for each isolate. Broth culture of the isolates EF-2 (70%), EF-6 (20%), and EF-8 (10%) exhibited antilarval activity (Fig. 2). The endophytic fungi EF-2 isolated from the plant *Leucas aspera* was selected as the best isolate with potent antilarval properties.



Values are expressed in terms of mosquito mortality (in %) as mean ± SEM, as analysed by ANOVA with Sidak's multiple comparison test. The sample was found to differ significantly as compared to the control (α <0.05); positive control- malathion (40 µg/ml), negative control-distilled water.

Fig. 2: Antilarval activity of broth and spores of various endophytic fungi isolated from leaves of different plants.

4.3. *Molecular Taxonomy of the Endophytic Fungal Isolate EF-2*

The taxonomic identification was done by 18S rRNA ITS region sequencing using the primers, ITS1-F and ITS4 followed by phylogenetic analysis. The sequences obtained were contig assembled and submitted to GenBank with accession number MK733980.1. The FASTA format files were used for BLAST analysis and phylogenetic tree construction using MEGA XI software by the UPGMA method (Fig. 3). The endophytic fungal isolate, EF2 was identified as *Fusarium equiseti.*

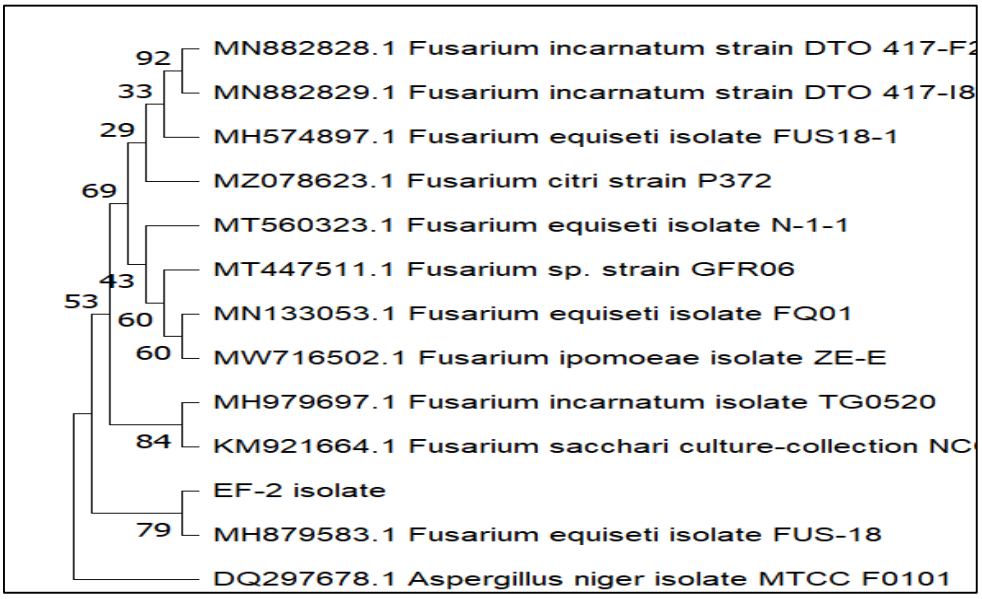


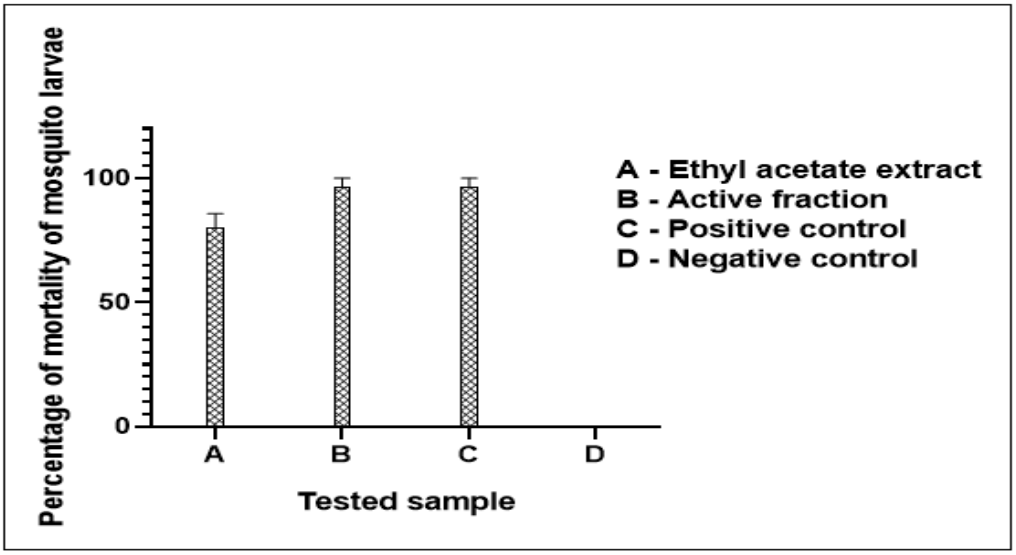
Fig. 3: The evolutionary history was inferred using the UPGMA method [31].

The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed [32]. The evolutionary distances were computed using the Maximum Composite Likelihood method [33] and are in the units of the number of base substitutions per site. This analysis involved 13 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1628 positions in the final dataset. There were a total of 1628 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [23].

4.4. *Antilarval Activity of the Extract and The Column Chromatographed Fraction*

The bioactive substances in the ethyl acetate extract and the partially purified fractions obtained from the column chromatography were used for the antilarval assessment

against mosquito larvae. In addition, the 8th fraction from the column chromatography that exhibited good antilarval activity of 90% mortality against *Culex* sp., (Fig. 4) was considered as the active fraction and subjected to further characterization studies.



Values are expressed in terms of mortality of mosquito (in %) as mean ± SEM, as analyzed by ANOVA with Sidak's multiple comparison test. The sample was found to differ significantly as compared to the control (α <0.05); positive control-malathion (40 µg/ml); negative control-ethyl acetate.

Fig. 4: Antilarval activity of extract and active fraction of the endophytic fungal isolate, EF-2;

4.5. Phytochemical Profile of Extract and Active Fraction

Qualitative analyses of the phytochemicals in the extract of endophytic fungi, EF-2 revealed the presence of various vital phytochemicals, *viz*., alkaloids, flavonoids, steroids along with amino acids and proteins. However, the active fraction contained alkaloids and flavonoids only.

4.6. Characterization of The Active Fraction

4.1. UV-Vis Spectroscopy Scanning of Extract and Active Fraction

The extract and the active fraction were subjected to multi-wavelength scanning (190-900 nm) by UV-Vis spectrophotometry. Both spectra showed a distinct peak at 301 nm (Fig. 5a and b).

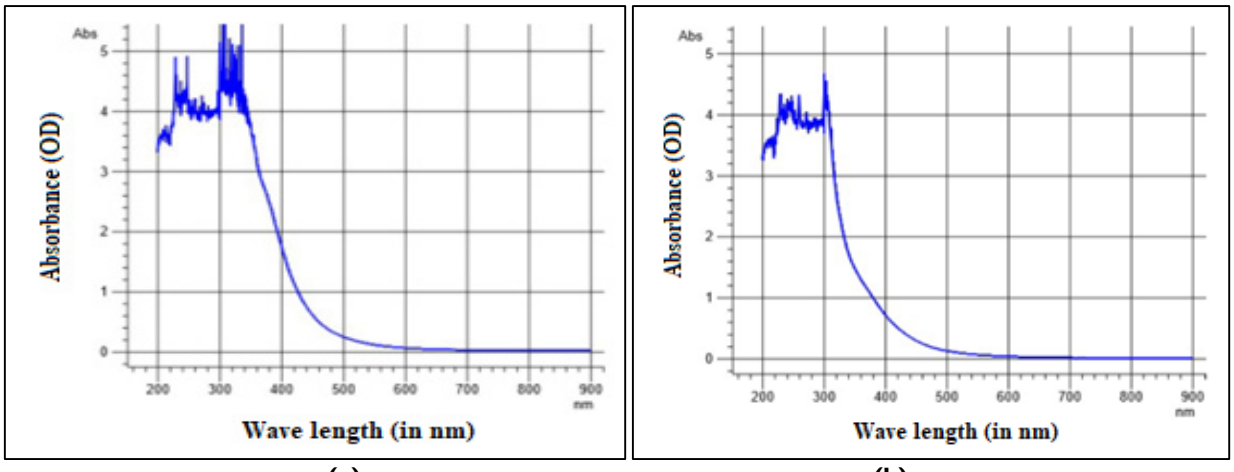
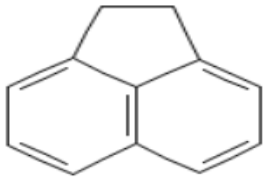


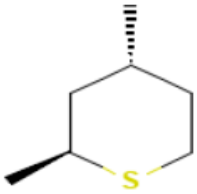
Fig. 5: UV-Vis spectroscopic profile of the endophytic fungal isolate, EF-2; (a) – Extract, (b) - active fraction. Both show the peak at 301 nm.

4.2. Gas Chromatography-Mass Spectrometry Analysis of Active Fraction

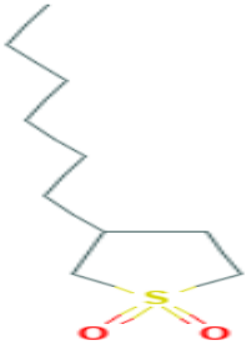
The GC-MS profile of the active fraction revealed the presence of major compounds Acenaphthene, Trans-2,4-Dimethylthiane, 3-n-Hexylthiolane,S,S-dioxide, 2,5-Cyclohexadiene-1,4-dione, 6-Fluoro-2-trifluoromethylbenzoic acid, Oleyl alcohol, trifluoroacetate, 4-Amino-7-diethylamino-chromen-2-one, Benzene,1,1'-(1,3-propanediyl)bis, Dodecyl acrylate, Phthalic acid, butyl nonyl ester, 7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione, Pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(phenylmethyl)-, and Phthalic acid, 2-pentyl undecyl ester (Fig. 6).



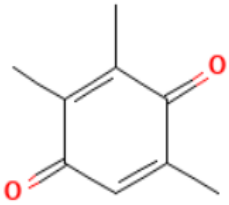
Acenaphthene



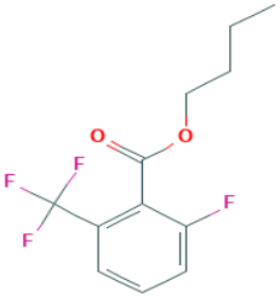
Trans-2,4-Dimethylthiane



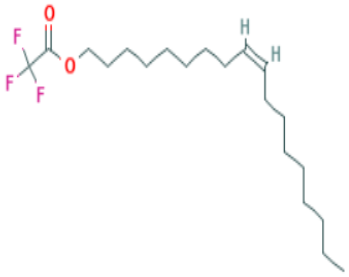
3-n-Hexylthiolane, S, S-dioxide



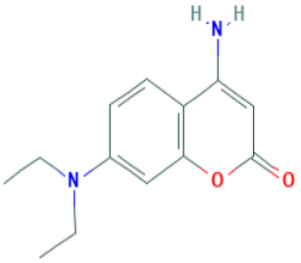
2,5-Cyclohexadiene-1,4-dione



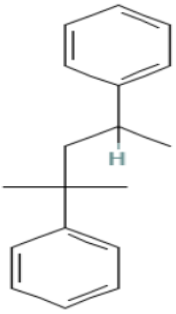
6-Fluoro-2-trifluoromethylbenzoic acid



Oleyl alcohol, trifluoroacetate

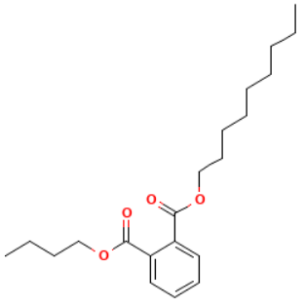


4-Amino-7-diethylamino-chromen-2-one

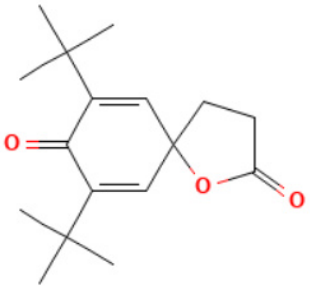


Benzene, 1,1'-(1,3-propanediyl)bis-

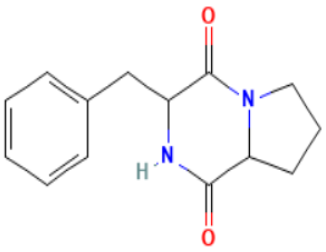
Dodecyl acrylate



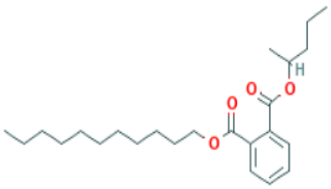
Phthalic acid, butyl nonyl ester



7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione



Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-



Phthalic acid, 2-pentyl undecyl ester

Fig. 6: Chemical structures of compounds identified through GC-MS of active fraction of Aqueous extract of the endophytic fungus *Fusarium equiseti* EF-2.

5. DISCUSSION

About 112 mosquito genera are comprising of around 3500 species [34]. Mosquitoes are notorious for acting as the vector of several deadly diseases, thereby remaining a threat to human life [35]. Of the several genera, *Aedes* and *Anopheles* are highly considered as important disease vectors due to their greater adaptability and proficient vectorial capabilities [36]. The studies on *Culex* are comparatively less as they are not vectors of major outbreaks such as chikungunya or dengue. But the diseases caused by them such as filariasis and Japanese encephalitis have serious impacts on people's daily living unknowingly. The method used to kill adult mosquitoes includes chemicals, organophosphates like malathion and naled, organochlorines like dichlorodipenyltrichloroethane (DDT), natural pyrethrins, and synthetic pyrethroids are the commonly used mosquito repellents [37]. But these impose several threats to the non-target organisms, environment, and human health. Further, the resistance to these chemical pesticides was developed and dissemination among the mosquitoes was also reported [38]. A review of mosquito larvicidal properties of plants turned up around 429 plant extracts of various solvents of different parts of the plant belonging to different families [8]. But the present study focused on exploring the efficiency of the endophytes inhabiting medicinal plants to act as potent antilarval agents. Though other microorganisms are also present in the plants as endophytes, many studies focus on the endophytic fungi owing to their rich secondary metabolite production compared to other microbial species [39]. Though the larvicidal activities of *Leucas aspera* has been studied earlier [40], the new insight of analyzing the larvicidal potency of endophytic fungi was raised based on a study that discovered the ability of silver nanoparticles synthesized from *Leucas aspera* to act as the mosquito larvicide against the most threatening mosquito vector, *Aedes aegypti* [41]. The endophytic fungal isolate EF-2 screened in this study was identified as *Fusarium equiseti* belonging to the fungal division Ascomycota. This correlates with the earlier study that reported the phylum Ascomycota and Basidiomycota were the most common sources of endophytic fungus [11]. Being considered as a weak pathogen, *Fusarium equiseti* is one of the poorly studied fungi. Despite the few discoveries, the studies on the beneficial properties of *F. equiseti* are seemingly less. One of the greatest discoveries from *F. equiseti* was the identification of the presence of two unique primary metabolites, Formyl Fusarochromanone and Diacetyl Fusarochromanone [42] from rice culture, which is recently proved to possess anticancer properties [43]. A recent study revealed the presence of another two unique compounds, equisetin and epiequisetin in the endophytic isolate present in the leaves of *Carica papaya* [44]. The silver nanoparticles derived from *Fusarium equiseti* exhibited antilarval properties against *Anopheles stephensi, Aedes aegypti,* and *Culex quinquefasciatus* [45]. The cytotoxic activity of secondary metabolites of *F. equiseti* against hepatocarcinogenesis in rats was also reported [46]. But there are no elaborated studies on the potential use of this endophytic fungus, especially as a larvicide. The ethyl acetate extract of *Fusarium equiseti* ef-2, an endophytic fungus, had significant larvicidal activity against *Culex* mosquito larvae. This complements recent findings that used the same ethyl acetate extract of another endophytic fungus, *Aspergillus tamarii*, against two mosquito larvae, *Aedes aegypti* and *Culex quinquefasciatus* [47]. The ethyl acetate extract of *Cochliobolus spicifer*, an endophyte from the date palm *Phoenix dactylifera*, has larvicidal activity against *Aedes caspius* and *Culex pipiens* [48]. These reveal the presence of a bioactive compound that has the potential to kill mosquito larvae. The phytochemical analysis revealed the presence of alkaloids and flavonoids in both the crude and the active fraction.This is in accordance with the study that reported the presence of alkaloids, flavonoids along with phenols in the endophytic *Fusarium proliferatum* in *Cissus quadrangularis,* which belongs to the same genus as that of *F. equiseti* [49]. The maximum absorbance of alkaloids was reported to be around 300 nm, while that of flavonoids [50] is around 400 nm. This is evident that the alkaloid present both in the crude and the active fraction, which showed a distinct peak at 301 nm, is the potent larvicidal compound of *Fusarium equiseti* EF-2. This study is consistent with the other researchers, who identified similar phytochemicals from several solvent extracts but remained novel by exploring the antilarval properties of the endophyte, *F. equiseti,* against the *Culex* mosquito larvae. Alkaloids from the plant extract of *Evodia rutaecarpa* were previously identified to possess mosquito larvicidal activity against *Aedes aegypti* [51]. Similarly, several flavonoids were known to possess antilarval activity against *Aedes aegypti* and their mechanisms were also understood [52]. Acenaphthene has been described as a pesticide belonging to the polycyclic aromatic hydrocarbon group of compounds [53,54]. This compound has been listed as the major compound with antilarval activity in the active fraction in the present study. The compounds 2,5-Cyclohexadiene-1,4-dione, Cycloheptasiloxane and 1-hexadecanol were previously isolated from the fungi *Aspergillus terreus, Nigrospora sphaerica,* and *Alternaria* sp., respectively [55–57]. Cycloheptasiloxane is a cyclic dimethyl polysiloxane compound reported to be used in cosmetic agents [58]. Oleyl alcohol, a high molecular weight aliphatic amine, acts as the diluting agent for the tertiary amine Alamine 336 [59]. Owing to its role as a fuel and in other fatty acid-related applications, 1-hexadecanol has been synthesized by metabolic engineering of *Saccharomyces cerevisiae* [60]. This study reports the novel properties of these active substances to act as antilarval agents, which has not been documented elsewhere. *Fusarium equiseti* has been isolated from various natural sources. But its presence as endophyte in the leaves of *Leucas aspera* adds value to it.Since *Leucas aspera* is an widely available plant, it can be exploited to boost up mosquito eradication measures. The outcomes of this study to use these novel antilarval compounds from naturally occurring endophytic fungi opens the way to explore natural compounds for the eradication of mosquitoes in a safe and eco-friendly method. Further, the negative impacts of the pesticides and larvicides used for mosquito control on human health and the environment could ultimately be prevented. The present study ultimately focused on environmental protection by eradicating disease-causing mosquito vectors through a biological yet effective approach.

6. CONCLUSION

This study revealed that the plant, *Leucas aspera* is the natural habitat of the endophytic fungi, *Fusarium equiseti,* which is a sparingly studied species of the fungal kingdom. Along with the habitat discovery, the dynamic antilarval properties of *F. equiseti* was also uncovered against the most life-threatening disease vector, *Culex* mosquito larvae. The compound responsible for this property is possibly identified through partial purification and characterization by UV-Vis spectroscopic analysis of both the crude and the active fraction of ethyl acetate extract of the fungi. Further, the presence of active compounds was characterized by GC-MS also. Thus, this study has stumbled upon a new candidate, the endophytic

fungi *Fusarium equiseti,* from *Leucas aspera* for the formulation of larvicidal biocides.

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8. AUTHORS CONTRIBUTION STATEMENT

Kuppuswamy Kavitha – Methodology, Validation, Investigation, Writing – Original Draft Review & Editing; Paneerselvam Aarthi – Investigation, Data curation, Visualization, Mani Prakash – Validation, Supervision, Methodology, Writing-Review & Editing of the final manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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