Ragavendran, C, Balasubramani, G, Tijo, C, Manigandan, V, Kweka, EJ, Karthika, P, Sivasankar, P, Thomas, A, Natarajan, D, Nakouti, I and Malafaia, G

Cladophialophora bantiana metabolites are efficient in the larvicidal and ovicidal control of Aedes Aegypti, and Culex Quinquefasciatus and have low toxicity in zebrafish embryo

Cladophialophora bantiana metabolites are efficient in the larvicidal and ovicidal control of Aedes Aegypti, and Culex Quinquefasciatus and have low toxicity in zebrafish embryo

Chinnasamy Ragavendran, Govindasamy Balasubramani, Cherian Tijo, Venkatesan Manigandan, Eliningaya J. Kweka, Pandi Karthika, Palaniappan Sivasankar, Adelina Thomas, Devarajan Natarajan, Ismini Nakouti, Guilherme Malafaia

PII: S0048-9697(22)05601-7

DOI: https://doi.org/10.1016/j.scitotenv.2022.158502

Reference: STOTEN 158502

To appear in: *Science of the Total Environment*

Received date: 14 July 2022

Revised date: 29 August 2022

Accepted date: 30 August 2022

Please cite this article as: C. Ragavendran, G. Balasubramani, C. Tijo, et al., Cladophialophora bantiana metabolites are efficient in the larvicidal and ovicidal control of Aedes Aegypti, and Culex Quinquefasciatus and have low toxicity in zebrafish embryo, *Science of the Total Environment* (2022), https://doi.org/10.1016/j.scitotenv.2022.158502

Cladophialophora bantiana METABOLITES ARE EFFICIENT IN THE LARVICIDAL AND OVICIDAL CONTROL OF *Aedes aegypti*, AND *Culex quinquefasciatus* AND HAVE LOW TOXICITY IN ZEBRAFISH EMBRYO

Chinnasamy Ragavendran[1,2]#, Govindasamy Balasubramani[3] Cherian Tijo[4], Venkatesan Manigandan[5], Eliningaya J. Kweka[6,7], Pandi Karthika[1], Palaniappan Sivasankar[8] Adelina Thomas[9], Devarajan Natarajan[1], Ismini Nakouti[10], Guilherme Malafaia[11]#

[1]Natural Drug Research Laboratory, Department of Biotechnology, School of Biosciences, Periyar University, Salem – 636 011, Tamil Nadu, India.

[2]Department of Cardiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai-600 077, India

[3]Department of Research and Innovation, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai 602105, Tamil Nadu, India.

[4]Department of Ocean Studies and Marine Biology, Pondicherry University, Port Blair campus, Brookshabad, Port Blair, Andamans- 744112.

[5]University of Texas Health Science Center, San Antonio, Texas, USA.

[6]Division of Livestock and Human Diseases Vector Control, Tropical Pesticides Research Institute, P.O. Box 3024, Arusha, Tanzania.

[7]Department of Medical Parasitology and Entomology, Catholic University of Health and Allied Sciences, P.O. Box 1464, Mwanza, Tanzania.

[8]Water Supply and Bioeconomy Division, Faculty of Environmental Engineering and Energy, Poznan University of Technology, Berdychowo 4, 60-965, Poznan, Poland.

[9]School of Pharmacy, Catholic University of Health and Allied Sciences, P.O. Box 1464, Mwanza, Tanzani.

[10]Centre for Natural Products Discovery (CNPD), School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool L3 3AF, UK.

[11]Laboratory of Toxicology Applied to the Environment, Goiano Federal Institute, Urutaí, GO,

Brazil. Post-Graduation Program in Conservation of Cerrado Natural Resources, Goiano Federal Institute, Urutaí, GO, Brazil. Post-Graduation Program in Ecology, Conservation, and Biodiversity, Federal University of Uberlândia, Uberlândia, MG, Brazil. Post-Graduation Program in Biotechnology and Biodiversity, Federal University of Goiás, Goiânia, GO, Brazil.

#**Corresponding Authors**: Dr. Chinnasamy Ragavendran, Natural Drug Research Laboratory, Department of Biotechnology, School of Biosciences, Periyar University, Salem – 636 011, Tamil Nadu, India. E-mail: ragavan889@gmail.com. Dr. Guilherme Malafaia. Laboratory of Toxicology Applied to the Environment, Goiano Federal Institution – Urutaí Campus. Rodovia Geraldo Silva Nascimento, 2,5 km, Zona Rural, Urutaí, GO, Brazil. CEP: 75790-000. Phone: +55 64 3465 1995. E-mail: guilhermeifgoiano@gmail.com.

ABSTRACT

Mosquitoes' current insecticide resistance status in available public health insecticides is a serious threat to mosquito control initiatives. Microbe-based control agents provide an alternative to conventional pesticides and insecticides, as they can be more targeted than synthetic insecticides. The present study was focused on identifying and investigating the mosquitocidal potential of *Clonophialophora bantiana,* an endophytic fungus isolated from *Opuntia ficus-indica*. The *Clonophialophora* species was identified through phylogenetic analysis of the rDNA sequence. The isolated fungus was first evaluated for its potential to produce metabolites against *Aedes aegpti* and *Culex quinquefasciatus* larvae in the 1-4th instar. The secondary metabolites of mycelium extract were assessed at various test doses (100, 200, 300, 400, and 500 µg/mL) in independent bioassays for each instar of selected mosquito larvae. After 48 h of exposure, *A. aegypti* expressed LC_{50} values of 13.069, 18.085, 9.554, and 11.717 µg/mL and LC_{90} = 25.702, 30.860, 17.275, and 19.601 µg/mL; followed by *C. quinquefasciatus* LC_{50} = 14.467, 11.766, 5.934, and 7.589 µg/mL, and LC_{90} = 29.529, 20.767, 11.192, and 13.296 µg/mL. The mean % of ovicidal bioassay was recorded 120 h after exposure. The hatchability (%) was proportional to mycelia metabolite concentration. The enzymatic level of

acetylcholinesterase in fungal mycelial metabolite treated 4th instar larvae indicated a dose-dependent pattern. The GC-MS profile of *C. bantiana* extracts identified five of the most abundant compounds, namely cyclobutane, trans-3-undecene-1,5-diyne, 1-bromo-2-chloro, propane, 1,2,3-trichloro-2-methyl-, 5,5,10,10-tetrachlorotricyclo, and phenol, which had the killing effect in mosquitoes. Furthermore, the *C. bantiana* fungus ethyl acetate extracts had a strong larvicidal action on *A. aegypti* and *C. quinquefasciatus*. Finally, the toxicity test on zebrafish embryos revealed the induction of malformations only at concentrations above 1 mg/mL. Therefore, our study pioneered evidence that *C. bantiana* fungal metabolites effectively control *A. aegypti* and *C. qunquefasciastus* and show less lethality in zebrafish embryos at concentrations up to 500 µg/mL.

Keywords: Biopesticide; endophytic fungal; vector control; mosquitoes; ecotoxicity.

1. INTRODUCTION

Mosquitoes are the most threatening insects to humans, as they are responsible for the spread of mosquito-borne diseases such as malaria, filariasis, Japan encephalitis (JE), chikungunya, and dengue hemorrhagic fever (DHF)(WHO, 2019). Mosquitoes are responsible for more than a million deaths annually and are accountable for the transmission of 17% of all severe diseases (WHO, 2014). Dengue fever (DF) is becoming a major public health problem, especially for the more severe form of the disease (WHO, 2017). *Aedes aegypti* is reported to be an effective species in spreading the dengue, yellow fever, and chikungunya virus. Most tropical and subtropical regions are affected by this disease, adding a strain on public health (Vega-Ra et al., 2014). The number of possible breeding environments for *A. aegypti* has been attributed to the high transmission frequency of these diseases (Borah et al., 2010). On the other hand, *Culex quinquefasciatus* Say, 1823 (Diptera, Culicidae) compounds these problems by acting as a vector of arboviruses, such as West Nile and St. Louis encephalitis (AMCA, 2005).

The demand for alternate and more effective mosquito control strategies is emphasized by the rise of new and re-emerging illnesses spread by the mosquito vector worldwide (de Oliveira et al., 2021).Thus, mosquito management has grown increasingly difficult due to the continued use of man-made chemical insecticides that have a negative environmental impact and have expedited the selection of insecticide-resistant mosquito vector populations (Chareonviriyaphap et al., 2013). An increase in mosquito populations resistant to insecticides used in public health has caused reduced efficacy of available insecticide classes such as pyrethroids, organophosphates, and carbamates (Hemingway, 2014, Moyes et al., 2017). Fungal metabolites are a significant source of bioactive compounds that may be effective for mosquito control (Daniel et al., 2017). More precisely, endophytic or soil habitat-specific fungus constitute a significant and underutilized source of novel natural compounds. Recently, biologically derived/based metabolites used to kill mosquitoes have been extracted from various microorganisms, including bacteria and fungi (Benelli et al., 2016; Singh et al., 2017; Derua et al., 2019a;Derua et al., 2019b;Vivekanandhan et al., 2020). Examples of biological agents considered mosquito larvicidal potential is derived from plants (Saxena et al., 1993), and microbes include *Streptomyces* sp. (Deepika et al., 2012), *S. avermectinius* (Molinari et al., 2010), and marine Streptomyces VITSVK5 sp. (Saurav et al., 2013), *Salinospora* sp. (Zafrir Ilan et al., 2013) *Bacillus sphaericus*, *B. thuringiensis* (Mulligan et al., 1980;Derua et al., 2019b), and *Cochliobolus lunatus* (Salunkhe et al., 2011). Furthermore, traditionally, plants and their derivatives have been used against mosquitoes. Phytochemicals such as alkaloids, steroids, terpenoids, essential oils, and phenolics have exhibited varying efficacies (Kweka et al., 2008a; Kweka et al., 2008b; Kweka et al., 2009; Sharma et al., 2009; Kweka et al., 2010; Kweka et al., 2012).

Approximately 1500 species in the cactus group are in the genus *Opuntia*, and various produce edible and ideal fruits (Anwar et al., 2016). In addition, *Opuntia* leaves and fruits are used for treating diabetes, gastritis, hyperglycemia, arteriosclerosis, and antioxidant and anti-

inflammatory agents (Park et al., 2000; Lee et al., 2002). Plant endophytes are known to coexist with their hosts in maximum situations. It is mostly nonpathogenic in wildlife, but it may extract secondary compounds that aid in their survival in the economically valuable biosphere of interstitial plant space (Stierle et al., 1993). Some endophytic fungal strains have been reported to produce secondary metabolites with potential larvicidal activity. Such fungal strains include *Graphiopsis chlorocephala* (Kondo et al., 2013), *Penicillium purpurogenum* (Li et al., 2013a;Li et al., 2013b), *A. fumigatus* (Li et al., 2013a), *Fusarium solani* (Shweta et al., 2013;Potshangbam et al., 2017), *Rhytidhysteron* sp. AS21B (Pudhom et al., 2014), *A. iizukae* (El-Elimat et al., 2014), *Pseudotaeniolina globosa* (Netala et al., 2016), *Aspergillus nidulans*, and *A. oryzae* (Sibero et al., 2017).

Endophytic fungi, in particular, produce abundantly bioactive ingredients such as alkaloids, chinones, flavonoids, isocoumarins, terpenoids, phenols, xanthones, peptides, steroids, quinines, enniatine, etc. (Wang et al., 2010) that are used in agriculture, medicine, and trades (Porras-Alfaro & Bayman, 2011). A species of endophytic fungi refers to *Cladophialophora bantiana,* which, although considered an emerging pathogen in animals, including humans (Prenafeta-Boldu et al., 2015; Mody et al., 2022), the secondary metabolite connected to central nervous system dysfunction may help control mosquitoes that act as important epidemiological disease vectors. As Velayutham et al. (2022) highlight, *D. rerio* is a species used worldwide in drug toxicity tests, secondary metabolites, and pollutants, among others. In addition, the genetic composition of zebrafish contains (84%) a gene related to human disease (Naomi et al., 2021). The present research aimed to isolate, molecularly identify and examine the mycelial metabolites of the endophytic fungus *Cladophialophora bantiana* from *Opuntia ficus-indica* to test them against *A. aegypti* and *C. quinquefasciatus* larvae and determine their ovicidal efficacy as well as to inhibit acetylcholinesterase (AChE). Furthermore, we evaluated their toxicity in zebrafish embryos (*Danio rerio*) to predict the ecotoxicological potential of *C. bantiana* fungal metabolites for aquatic organisms. We hypothesize that an

endophytic *C. bantiana* fungal extract may be employed to manage selected mosquitos' larvae with minimal damage on a vertebrate model.

2. MATERIALS AND METHODS

2.1 Isolation of endophytic fungus

The isolation of endophytic fungus from the *Opuntia ficus- indica* plant was carried out according to the protocol developed with minor modifications by Zeng et al. (2008). Healthy, disease-free samples were washed with tap water, cut into tiny pieces (3 x 3 cm), immersed in 70% ethanol for 3 min, washed 5 times with sterile water, soaked in magnesium chloride solution (at 0.1%) for 8 min, and rinsed with sterile water four times. The samples were chopped into small pieces (0.2 x 0.2 cm), placed on sterile potato dextrose agar medium (PDA) medium (50 µg/mL streptomycin and 40 µg/mL chloramphenicol), and stored upside down at 26 °C for 21 days (Kulkarni and Ganavalli, 2011, Kuan et al., 2016). The growth of the fungus was observed daily. Freshly developed mycelia were collected, moved to new PDA Petri dishes, and screened until pure strains were produced.

2.2 Identification of the endophytic fungus

2.2.1 Microscopic identification

Filamentous fungal isolates were separated into various species based on their colony color and texture, border type, and circular growth frequency on PDA agar (Frohlich, 2000). A stereoscopic microscope was used to study morphological aspects of the fungus, such as mycelia, conidia, and conidiophores.

2.2.2 Molecular identification

The cetyltrimethylammonium bromide (CTAB) technique was used to extract genomic DNA (Qadri et al., 2013). ITS-1 and ITS-4 were used to amplify the Internal Transcribed Spacer (ITS) region. The complete PCR reaction mixture (40 µL) and the reaction components containing 2 µL of each primer were employed (10 µM), 20 µL of Taq PCR mix (Amplicon),

12 µL of Milli-Q water, and 4 µL of template DNA. The amplified regions were then sequenced and purified using the QIA fast PCR purification kit from QIAGEN (Thermo Fisher Scientific).

2.3 Phylogenetic tree analysis

The 5.8s rDNA obtained during sequencing was edited using BioEdit software and aligned by the ClustalX multiple sequence editor (Hall, 1999). A total of 11 of the 5.8-s rDNA sequences that were closely associated were used to construct phylogenetic trees. The phylogenetic tree study was performed by the neighbor-joining (NJ) method (MEGA 5.0) (Tamura et al., 2011). The Kimura 2-parameter assessment was used to construct the NJ study. Gaps in the arrangement were regarded as omitted data. MEGA 5.0 bootstrap analysis with 1,000 replicates confirmed the phylograms' accuracy.

2.4 Recovery of secondary metabolites from endophytic fungus

The pure, isolated *C. bantiana* strain was placed into a 500 mL Erlenmeyer conical flask with broth and held at 26 ± 2 °C for 14 days purity of the cultures was determined by colony morphology. After well matured mycelium was filtered with Whatman No. 1 sieve paper (pore size: 11µM). The resulting mycelia were macerated in ethyl acetate. The resulting mixture was twice filtered through Whatman No. 1 sieve paper after 8 days. Immediately these mycelia metabolites were determined at low pressure in a removal of solvents from the metabolites by rotary evaporator. Finally, the remaining extracts were weighed to find the yield (Ragavendran and Natarajan, 2015).

2.5. Gas chromatography-mass spectroscopy analysis

Twenty microliter sample was introduced through a split glass injector at 32 cm/s fused silica capillary column (film thickness-25 m; length-30 m; ID-0.2 mm) (Devi and Singh, 2013).

At 70 eV, the electrical impact mode of the mass spectrometer was activated. The carrier gas was helium, with a mass scan of 50–600 Da with a split ratio of 10:1 and a movement frequency of 1 ml/min. The oven temperature was automatically set to 62 °C for 3 min, then increased to 300 °C at a rate of 10 °C/min, and finally maintained at 300 °C for 6 min. The entire path took 32 min. The mass spectrum was measured from 40 to 1000amu. Active metabolites were well known based on the assessment of their retention time (RT) and mass spectrum, VIT Library data from the GC–MS system (Perkin Elmer Clarus 500), and literature data (Rahuman et al., 2000).

2.6. Analysis of Fourier transform infrared (FTIR) spectroscopy

The secondary metabolites of the fungal *C. lantiana* were dried and pelleted for subsequent analysis on a Jasco FTIR 5300 spectrophotometer. 1 mg of the metabolites was mixed with 99 mg of KBr by a hygienic/fresh mortar and pestle to make a powder into a tablet (1:10 ratio) and recorded in mid-IR region (FT-IR) spectroscopy (Bruker, Germany) ranging from 400 to 4,000 cm^{-1} at a room resolution of 4 cm^{-1}. Measurements were supported to categorize possible bioactive molecules responsible for the death of mosquito larvae done fungal extract (Kumar et al., 2014).

2.7. Larvae collection and rearing

Healthy and active movement larvae were collected from rice fields and stagnant water locations in and around Karuppur Panchayat, Salem District, Tamil Nadu, India. The obtained larvae were placed in cages for growing mosquitoes in an insect house that was kept at 24 °C, 72% relative humidity, and light/dark cycle (14:10). The larvae of *Aedes aegypti* and *Culex quinquefasciatus* were maintained in separate enamel trays (24-cm length x 16-cm width x 6-cm height), each tray containing 200 larvae. Good powdered brewer's yeast and dog biscuits were used to feed the larvae (proportion 3:1). The larvae were raised to adults in distilled water with a

pH of approximately 7.0. To compensate for evaporative water loss, water from tap water was added daily.

2.8. Larval toxicity bioassay

The larvicidal activities of the isolated endophytic metabolites of *C. bantiana* mycelia were carried out according to a slightly modified procedure (Deepika et al., 2012; Du Sert et al., 2020). The larvicidal bioassay and concentration-response experiments used five concentrations (100, 200, 300, 400, and 500 µg/mL). The 1-4th instar larvae were placed in a sterile borosil glass beaker containing 100 mL of water and preferred concentrations of mycelium-derived metabolite. Three replicates were performed for each concentration, a total of 150 larvae/treatment. The percentage of death was determined (after 48 h of exposure), and no food was administered to the larvae during the experiment. For each concentration, a set of control groups (10% DMSO and dH_2O) were set up in triplicate for each treatment test. The probit analysis investigated the lethal concentrations (LC_{50} and LC_{90}) (Finney, 1971).

2.9. Concentration-response assay

The larvae were exposed to the metabolites of *C. bantiana* mycelia following the standard of the World Health Organization with slight modifications (WHO, 2005). Based on the initial screening described above, extracted metabolites were used for concentration-response bioassays to determine their larvicidal properties in selected larvae. Five groups (750 larvae) of larvae from the first instar to the last were transferred to a 250 mL glass container and exposed to various extract concentrations (100-500 µg/mL). Two negative controls (10% DMSO and dH_2O) were established for each concentration. Larval feeding was given during the tests (Dog biscuit and yeast powder 3:1 ratio), and the mortality rate was observed 48 h after exposure. Larval mortality was calculated in triplicates; mortality (%) was corrected via; Abbott's formula (Abbott, 1925) and used to calculate the LC_{50} and LC_{90} values.

2.10. Ovicidal bioassay

Adult females lay 100-150 eggs 3 days after blood feeding. The following procedure collects eggs for culture maintenance (Clemons et al., 2010). The filter paper is wet when collecting the eggs. A 3″ wide strip of filter paper lines the interior of a tiny bowel. There is a 2.5 cm layer of water added. The egg collection container is then put into an adult cage and left there for 48 h. The bowel is taken out, and any extra water is removed from it. The egg paper is left in the cage for an additional 24 h, removed, and let air dry for 4 days before being placed in a huge, sealed plastic container for storage. The ovicidal bioassay was carried out in accordance with the protocol of Su and Mulla, (1998). The two mosquito species of eggs were collected from the Centre for Research in Medical Entomology (CRME), Madurai, Tamil Nadu, India. At least 100 eggs for targeted mosquito species were collected in separate ovicidal cups at the desired concentrations (100-500 µg/mL) of *C. bantiana* mycelia extract. Currently, a cup with normal water with 10% DMSO acts as a negative group. The experiments were examined 3 times. Later in the experimental period, the eggs of each concentration were counted, and separate cups with water were removed for hatching. The percentage of egg hatchability was counted as the number of unhatched eggs (after 96 h post-treatment) (Prathibha et al., 2014).

2.11. Acetylcholinesterase inhibition assessment

AChE catalyzes the hydrolysis of acetylcholine, a neurotransmitter essential in cholinergic neurotransmission in insects. *A. aegypti* and *C. quinquefasciatus* 4th instar larvae were analyzed spectrophotometrically using whole body homogenates as controls and treated samples (Ellman et al., 1961) with slight modifications. Five different C. bantiana mycelial extract concentrations were diluted in methanol (100-500 µg/mL). In a 96-well plate, the experiment was conducted with 20 µL of metabolite solutions (100-500 µg/mL), 80 µL of phosphate buffer (to 100 mM), 40 µL of 5,5-dithiobis (2-nitrobenzoic acid) (to 2.5 mM), and 20 µL of the enzyme AChE (to 1.0U/mL) in each well. The mixture was then incubated at 37°C

for dark light. Then, 40 µL of acetylcholine iodide (at 10mM) was added, and the samples were kept in identical conditions. All the solutions were used along with negative control. The absorbance of the solutions was measured in triplicates at 405nm using a UV spectrophotometer.

2.12. Zebrafish maintenance and embryo toxicity test

Assay of zebrafish embryo toxicity using the OECD's standard procedures for chemical testing. In both the dark (12 h) and light (12 h) periods, the wild-type (AB strain) male/female 2:1 ratio was maintained. The embryos were carefully collected for further research. The viable embryos were placed in 24 well plates with 1 mL of Embryo medium (E3) distributed in each well (Faria et al., 2015). Dissolved stocks (5 mg/mL) of produced *C. banitana* were used to make test solutions. While preparing the dilutions, the magnetic stirrer was constantly checked to maintain the suspension. The embryo toxicity test design followed standard OECD procedures. Distinct concentrations of metabolites were employed in this experiment, including Control, 250, 500, 750 µg/mL, and 1 mg/mL with water served as a control in this study. Each treatment was conducted in triplicates. Finally, the samples were examined with a stereomicroscope (Karthik et al., 2019). The growth of embryos and larvae was studied using an inverted microscope (Nikan, Japan) at 24, 48, and 72 h after fertilization. Embryo/larval survival and embryo hatching rates were used to evaluate toxicity (Malafaia et al., 2020). The malformations in the embryos and larvae of the control and treatment groups were observed (Srinivasan et al., 2019).

2.13. Statistical analysis

Abbott's formula was used to determine the larval mortality rate. The findings of the concentration-response assay were exposed to probit analysis by IBM SPSS Statistics ver. 20 software to determine the LC_{50} and LC_{90} values (Finney, 1971). All data obtained were evaluated

regarding the assumptions for using parametric models. For this, we used the Shapiro-Wilk test to assess the distribution of residual data, and the Bartlett test was used to assess the homogeneity of variances. Thus, applying one-way ANOVA, the variances between the experimental embryo/larvae groups were identified (ANOVA). Additionally, correlations were performed using Pearson's correlation coefficients as well as linear regression analysis. Significance levels were set at Type I error (p) values lower than 0.05. IC_{50} values (50% inhibition) for enzymatic activity were measured using the software GraphPad Prism 5.0.

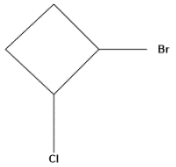
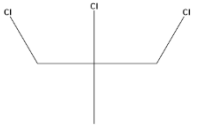
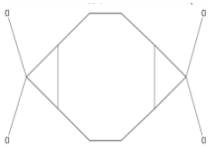
3. RESULTS

GC-MS was used in this study to evaluate and identify the bioactive metabolite profile of *C. bantiana*. The NIST database was used to analyze the GC-MS mass spectrum, and the active compounds' retention times (Rt), molecular formulae, weights, and percentage areas (percent) were listed in Table 1. Totally five main molecules were detected from the metabolites, i.e., trans-3-undecene-1,5-diyne (21.69%), cyclobutane, 1-bromo-2-chloro-(17.97%), propane, 1,2,3-trichloro-2-methyl- (14.85%),5,5,10,10-tetrachlorotricyclo [7.1.0.0 (4,6)] decane (20.74%) and phenol, 3,5-bis (1,1-dimethylethyl) - (23.99%), respectively (Table 1, Figure 1S-A).

FTIR analysis of mycelial metabolites revealed a CG stretching of 2926.5 cm^{-1}. The prominent peaks at 2926 cm^{-1} correspond to the C-H stretching vibration of the alkanes. The bands at 1626 cm^{-1} are due to the N-H bending and can be assigned to the functional group in primary amine. The 1550cm^{-1} band appears due to the stretching of the nitro compounds. The observed peak of 1386 cm^{-1} was related to the stretching of the alkanes in the CH_3 bending. The strong peak vibrated at 1269 cm^{-1}, assigned to the C-N stretching of aromatic amines. The medium band appeared at 1154 cm^{-1}, linked to the C-N bending of the aliphatic amines. The bands appeared at 862 cm^{-1}, corresponding to NH wagging of secondary amines. The vibration of a broad peak observed at 671 cm^{-1} is due to the -CC-H alkynes groups (Table 2, Figure 1S-B).

Microscopic morphological characteristics initially identified the isolated fungal strain, i.e., odor, color, shape, conidial structure, and hyphae characteristics (Figure 1A-B). Based on colony characteristics, size, and lactophenol cotton blue stain endophytic fungus belonging to *Cladophialophora* sp. This was further confirmed in the 5.8s rDNA species level by molecular analysis. The colonies of *C. bantiana* colonies are gray olivaceous, suede-like to floccose, and grow at a temperature between 40-42 °C. Conidia are shaped in one-celled and long, thinly branched, acropetal chains of undifferentiated conidiophores, flexuose (occasionally two-celled), pale brown, and the walls are smooth, ellipsoid to the oblong ellipsoid (2-3 x 4-7 μm).

Table 1. Identification of secondary metabolites from *C. bantiana* mycelial ethyl acetate extract by GC-MS analysis.

ID	Name of the major compound	Molecular formula	Molecular weight (g/mol)	Retention time	Area (%)	Structure of compounds
1.	Trans-3-undecene-1,5-diyne	$C_{11}H_{14}$	146	19.37	21.69	
2.	Cyclobutane, 1-bromo-2-chloro-	C_4H_6ClBr	168	19.60	17.97	
3.	Propane, 1,2,3-trichloro-2-methyl-	$C_4H_7Cl_3$	160	20.41	14.86	
4.	5,5,10,10-tetrachlorotricyclo[7.1.0.0(4,6)]decane	$C_{10}H_{12}Cl_4$	272	20.55	20.74	

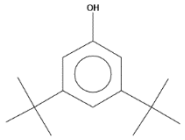
5.	Phenol, 3,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	21.40	24.73	

Table 2. Identification of functional groups from mycelial ethyl acetate extract of *C. bantiana* by FT-IR analysis.

ID	Observed wavelength numbers (cm^{-1})	Peak assignment	Visible intensity	Functional groups
1	3429.78	O-H stretching	Strong, sharp	Phenols
2	2926.45	C-H stretching	Medium	Alkanes
3	1626.66	N-H bending	Medium	Primary amines
4	1550.49	N-O Asymmetric stretching	Strong	Nitro compounds
5	1386.57	CH_3 bending	Medium	Alkanes
6	1269.9	C-N stretching	Strong	Aromatic amines
7	1154.19	C-N stretching	Medium	Aliphatic amines
8	862.025	N-H wagging	Strong	Secondary amines
9	671.106	-C≡C-H	Broad	Alkynes

The isolated DNA region (609bp) was amplified and compared with the standard molecular weight marker (1Kb). The sequences from the *C. bantiana* fungal strain's PCR product were deposited in the NCBI gene database (Access no: MH094277). The UPGMA algorithm was used to construct the phylogenetic tree. The ideal tree with the sum of the length of the division = 1.99. The proportion of the phylogenetic tree in which the connected taxa clustered according to the bootstrap test (1000 replicates). The Tajima-Nei method was used to calculate the evolutionary distances. About 11 nucleotide sequences were involved in the analysis, and phylogenetic tree analyzes were performed in MEGA5. An individual sequence with a closed related species was recovered from the gene bank using the phylogenetic analyses

of ten phylotypes. The ITS -1 and ITS -2 sequences of the phylotype (MH094277) show a similarity of 98% with *C. carrionii* (AF397182.1) and 63% with *Aspergillus* sp. *Fusarium oxysporum* (JN400702.1). However, the phylogenetic analysis (AF397182.1) showed a high difference in the nucleotide sequence range (2 to 11%) (Figure 1C).

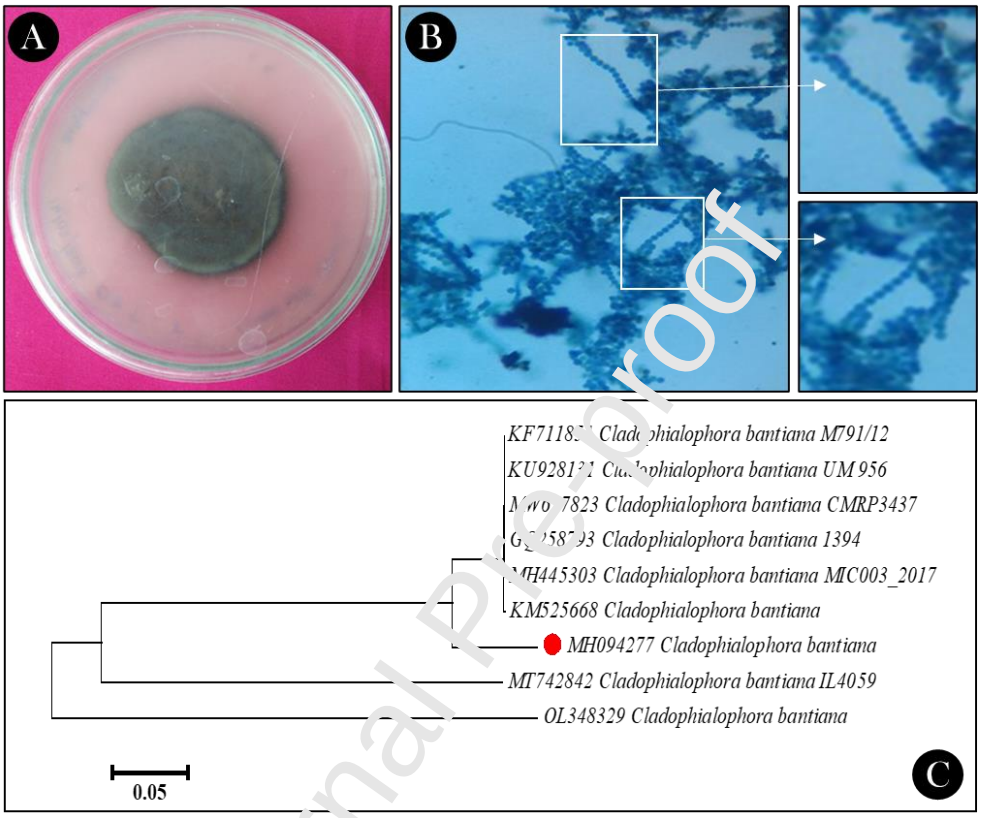


Figure 1. Morphology and microscopic view of isolated endophytic *fungus* (A) *C. bantiana,* (B) round-shaped coin structure of spore, and (C) phylogenetic tree analysis for evolutionary steps of *C. bantiana* was inferred using the Tajima-Nei method. The percentage of replicates trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is observed next to the branches.

The larvicidal potency of the mycelial metabolites of *C. bantiana* showed that increasing their concentration could effectively control the larval populations of the tested mosquito (Table 3-4). The LC_{50} values of the mycelial metabolites of the third and fourth instar larvae of *A. aegypti* were 9.554 and 11.717 µg/µL, while LC_{90} were17.275 and 19.601 µg/µL, compared to

LC_{50} of 5.934 and 7.589 µg/µL, and LC_{90} of 11.192 and 13.296 µg/µL for *C. quinquefasciatus*. Compared to unexposed groups, exposures to the mycelial metabolite of *C. bantiana* against *A. aegypti* and *C. quinquefasciastus* resulted in high mortality for all five different concentrations (Tables 3-4). The 400 and 500 µg/mL concentrations exhibited the minimum mortality, while the 100 and 200 µg/mL showed the highest mortality (*A. aegypti* with LC_{90}= 30.860 µg/mL and *C. quinquefasciatus* has LC_{90}=29.529 µg/mL). The most increased mortality in *A. aegypti* and *C. quinquefasciatus* was 97% and 86%, respectively. No mortalities were observed in the negative control cohorts (10% DMSO). During ovicidal bioassays, egg hatching was directly proportional to the concentrations of mycelial metabolites that caused ovicidal activity, failing to hatch egg rafts/eggs (Table 5). *C. bantiana* metabolites completely inhibited egg hatching at 400 and 500 µg/mL concentrations for both vector species. Both *A. aegypti* and *C. quinquefasciatus* control cohorts' eggs hatched fully (100%).

Table 3. Larval mortality of *A. aegypti* after 48 h-exposure to *C. bantiana* mycelial ethyl acetate extracts.

Larva stage	Concentrations (µg/mL)	48-h mortality (%) - Mean ± SD	LC_{50} (µg mL) (LCL-UCL)	LC_{90} (µg/mL) (LCL-UCL)	χ^2 (*df*=10)
1st instar	(control)	0.0±0.0	13.07 (8.24-18.11)	25.70 (6.13-53.73)	10.47 n.s
	100	30.00±1.0			
	200	34.44±0.5			
	300	41.11±0.5			
	400	56.66±1.0			
	500	72.22±0.5			
2nd instar	(control)	0.0±0.0	18.08 (5.70-35.73)	30.86 (11.75-54.80)	9.75 n.s
	100	31.11±0.5			
	200	37.77±0.5			
	300	56.66±1.0			
	400	68.88±1.5			
	500	81.11±0.5			
3rd instar	(control)	0.0±0.0	9.55 (2.03-22.63)	17.27 (4.66-35.99)	7.33 n.s
	100	36.66±1.0			

	200	47.77±0.5			
	300	66.66±1.0			
	400	75.55±0.5			
	500	83.33±1.0			
	(control)	0.0±0.0			
	100	40.00±1.0			
	200	54.44±0.5	11.71	19.60	
4th instar	300	67.77±0.5	(3.41-24.46)	(6.81-36.97)	8.630 n.s
	400	77.77±1.5			
	500	91.11±0.5			

LC_{50}: lethal concentration that kills 50% of the exposed larvae, LC_{90}: lethal concentration that kills 90% of the exposed larvae, SD: standard deviation, LCL: lower confidence limit, UCL: upper confidence limit, df: degree of freedom, n.s-not significance.

Table 4. Larval mortality of *C. quinquefasciatus* after 48 h-exposure to *C. bantiana* mycelial ethyl acetate extracts.

Larva stage	Concentrations (µg/mL)	48-h mortality (%) - Mean ± SD	LC_{50} (µg mL) (LCL-UCL)	LC_{90} (µg/mL) (LCL-UCL)	χ^2 (*df*=10)
1st instar	(control)	0.0±0.0	15.46	29.52	5.81 n.s

	100	26.66±0.1	(3.15-35.67)	(8.08-58.63)	
	200	32.22±0.5			
	300	46.66±1.0			
	400	60.00±1.0			
	500	66.66±1.0			
	(control)	0.0±0.0			
	100	36.66±1.0			
	200	44.44±0.5	11.76	20.76	
2nd instar	300	60.00±1.0	(2.93-26.01)	(6.42-40.75)	8.62 n.s
	400	73.33±1.0			
	500	83.33±1.0			
	(control)	0.0±0.0			
	100	42.22±0.5			
	200	55.55±0.5	5.93	11.19	
3rd instar	300	63.33±1.0	(0.88-16.35)	(2.21-26.65)	5.94 n.s
	400	74.44±1.5			
	500	85.55±0.5			

	(control)	0.0±0.0			
	100	44.44±0.5			
	200	57.77±0.5	7.58	13.29	
4th instar					6.74 n.s
	300	71.11±0.5	(1.62-18.21)	(3.53-28.34)	
	400	77.77±0.5			
	500	91.11±0.5			

LC_{50}: lethal concentration that kills 50% of the exposed larvae, LC_{90}: lethal concentration that kills 90% of the exposed larvae, SD: standard deviation, LCL: lower confidence limit, UCL: upper confidence limit, df: degree of freedom, n.s-not significance.

Table 5. Ovicidal effects of the *C. bantiana* mycelial ethyl acetate extract against *A. aegypti* and *C. quinquefasciatus*.

Mosquito species	Egg hatchability (%)					
	Concentration of metabolites (µg/mL)					
	0 (control)	100	200	300	400	500
A. aegypti	100±0.0	45.0±0.5	27.2±1.2	17.3±1.0	NH	NH
C. quinquefasciatus	97.5±1.0	37.5±1.0	24.5±0.0	16.5±0.5	NH	NH

NH: No hatchability

The level of AChE activity in targeted 4th instar larvae was reduced after a 24h-exposure. The AChE activity was dramatically reduced after exposure to fungal metabolites. *A. aegypti* larvae exhibited the most AChE at a concentration of 100 µg/mL of metabolites, then larvae of *C. quinquefasciatus*. Likewise, the maximum concentration of metabolites (500 µg/mL) had shown a lower amount of AChE activity in test larvae. Furthermore, correlation and regression analysis indicated that enzyme expression was mainly concentration-dependent (Figure 2).

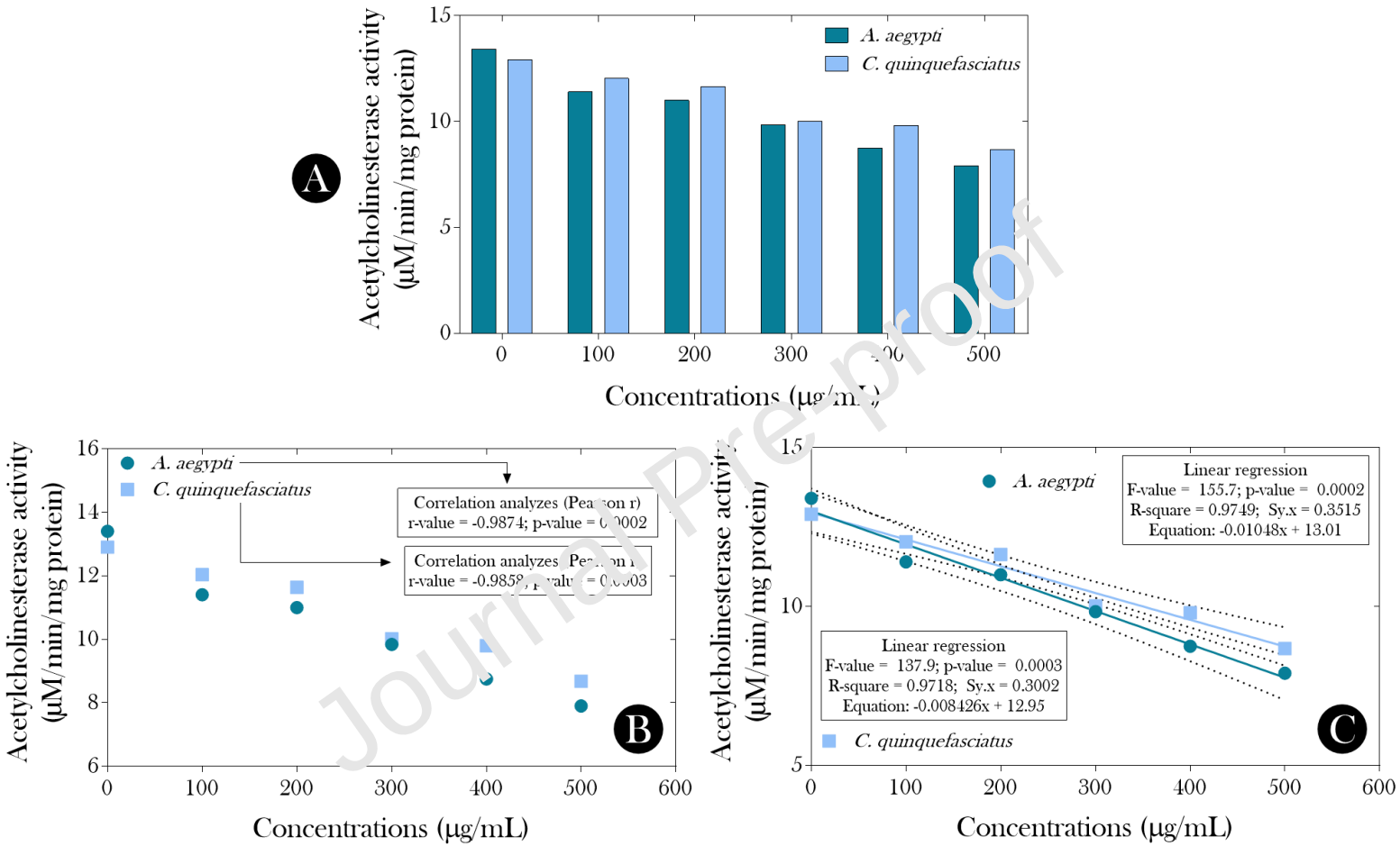


Figure 2. (A) Acetylcholinesterase activity of *A. aegypti* and *C. quinquefasciatus* exposed or unexposed to different *C. bantiana* mycelial ethyl acetate extract concentrations, followed by the results of (B) correlation and (C) linear regression analysis (concentrations vs. AChE activity).

One-way analysis of variance (ANOVA) was used to find the variations between the treated groups of embryo/larvae. *p≤0.05, ***p≤0.001, ****p≤0.0001 was regarded as statistically significant. On the other hand, the zebrafish embryo did not induce the morphology changes in behavior, internal organ shapes, and functions on the first day of exposure (24 h). The maximal test concentration indicated that death occurred on the first day. At (2 mg/mL), treated embryos showed abnormal tissue formation in the head region and yolk sac elongation on the 3rd day of treatment (Figure 3A). At the same concentration, cardiac bulging and pericardial fluid accumulation were observed. The embryo had a hemorrhage around the heart chamber and oral deformities (Figure 3B). The embryo survival rate was reduced after 72 h when metabolite concentrations were increased (Figure 4A). Metabolite concentrations mainly influence the survival rate. Based on the concentrations of mycelia extract, the body length was significantly shorter than in the unexposed groups (Figure 4B). The hatching rate of zebrafish embryos (after 72 h) was lower in the "750 µg/mL" and "1 mg/mL" groups when compared to the other experimental groups (Figure 4C). Furthermore, all the assessed groups had considerably lower heartbeat counts than the "1 mg/mL" group (Figure 4D).

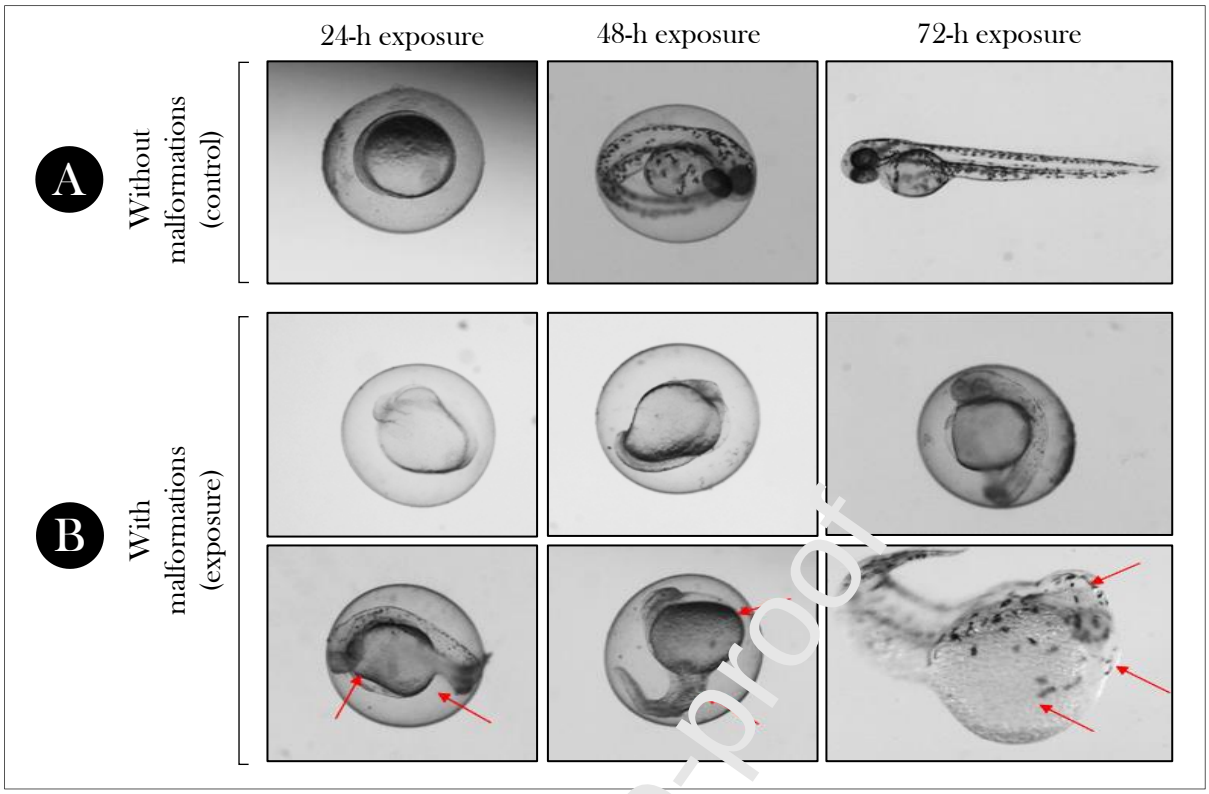


Figure 3. Morphological anomalies caused by *C. lunata* mycelial extract on zebrafish embryo (A) Control zebrafish embryo showing normal architecture, and (B) mycelial metabolite (at 2 mg/mL) caused the death of embryo and yolk sac edema and tail malformation. The red arrow indicates deformities.

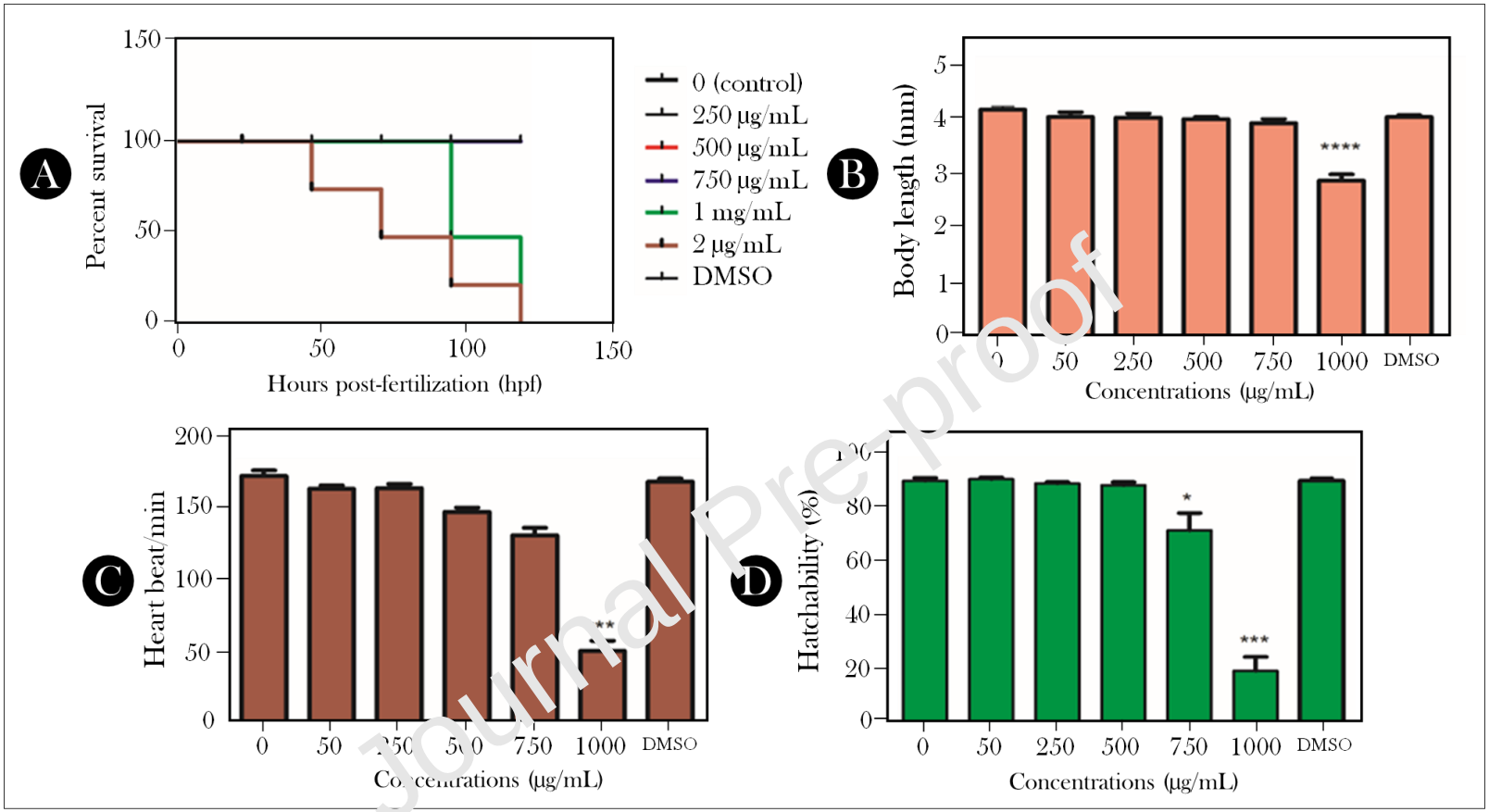


Figure 4. (A) Survival rate, (B) body length, (C) Heart-beat counts, and (D) percentage of hatchability of zebrafish embryos (*Danio rerio*) exposed or unexposed to different *C. bantiana* mycelial ethyl acetate extract concentrations. Statistical significance *p≤0.05, ***p≤0.001, **** p≤0.0001.

4. DISCUSSION

Microbially produced molecules have various benefits in vector control strategies, particularly high toxicity to larvae, pupae, and adults, as well as minimal toxicity to non-target species (Dhanasekaran and Thangaraj, 2014). This study demonstrated that in an unpolluted environment, various beneficial and economically important species of the microbial community might be present, including endophytic fungi, which have been proven to have a mortality effect on mosquito vector species while having minimal impact on non-targeted organisms. These results are consistent with earlier research that indicated toxicity to a range of arthropods (Prakash, 2003; Vyas et al., 2006). Presently, increasing attention is paid to the use of pesticides produced from microorganisms to decrease synthetic chemical pesticides (Bucker et al., 2013). These results suggest that the selection of endophytic fungi and their potent toxins are potential alternatives to synthetic pesticides for managing mosquitoes laboratory and field applications (Araujo et al., 2022). The findings of this study have shown that the active ingredients of *C. bantiana* natural extracts have insecticidal activity against mosquito larvae. Previous studies showed that lead and prime bioactive compounds from endophytes have insecticidal, antimicrobial, and anticancer activities (Aly et al., 2010; Kharwar et al., 2011). Endophytic fungi are a rich source of bioactive molecules, making them valuable in pharmacology, medicine, and agriculture (Chaturvedi, 2015). Molecular techniques have recently made it possible to quickly and easily distinguish between different fungal species with similar morphologies by identifying fungal isolates (Sibero et al., 2017). In this work, molecular identification of the fungus was carried out with primers ITS-1 and ITS-2. The *C. bantiana* fungus isolated during this study showed cylindrical to narrowly fusiform morphology. This morphological structure is similar to *C. minourae, C. minutissina, C. carrionii, C. derriesii, C. hachijoensis,* and *C. modesta* species identified in previous *studies* (Braun and Feiler, 1995; Zeng et al., 2008). In this work, the 5.8s rDNA sequence of the *C. bantiana* (609bp) sequence showed that it was closely related to the genus found by Badali et al. (2011).

The public health impact of *A. aegypti* and *C. quinquefasciatus* in tropical locations has drawn the attention of local authorities and the World Health Organization due to the extensive geographical range and severity of diseases transmitted by these mosquitos in recent decades, including dengue, chikungunya, Zika, Japanese encephalitis, and filariasis (Rodrigues-Alves et al., 2020). Novel insecticides and larvicides that can control this vector are thus of great interest. To reduce environmental pollution and avoid the breeding of mosquitoes that are resistant to chemical insecticides, naturally produced insecticides have been investigated as possible less hazardous alternatives (Arajo et al., 2020). Mosquito larvicidal activities of the mycelial extracts and the broth culture medium have been reported for other fungi such as *Stereum* sp. (Chirchir et al., 2013), *Trametes* sp. (Waweru et al., 2017), *Beauveria bassiana* (Ragavendran et al., 2017). The present study shows the larvicidal potential of *C. bantiana* crude mycelial ethyl acetate extract against the targeted larvae of *A. aegypti* and *C. quinquefasciatus*. The results showed that the targeted larvae of *A. aegypti* were more tolerant to the mycelial extract, recording high LC_{50} values (13.069 to 18.085 µg/mL) and LC_{90} (19.601 to 30.860 µg/mL) compared to *C. quinquefasciatus* with LC_{50} of 7.589 to 15.467 µg/mL, and LC_{90} of 13.296 to 29.529 µg/mL. The highest larvicidal activity was observed at 500 µg/mL dose against fourth instar larvae at 91%, and no mortality was recorded in the control group. Concentration-dependent mortality was highly corroborated with earlier reported studies (Muthu et al., 2012; Seetharaman et al., 2017). Significant development has been made in using fungal metabolites/compounds capable of penetrating the cuticle as mosquito larvicidal toxins (Demain and Fang, 2000). Beauvericin is a cyclic hexadep-sipeptide, a well-known mycotoxin produced by *Beauveria bassiana* and *Fusarium* sp. Grove and Pople reported the insecticidal mechanism of beauvericin and showed that it is an effective insecticide against *A. aegypti* (Grove and Pople, 1980). Likewise, after 48 h of exposure, Beauvericin demonstrated larvicidal action against *A. aegypti* larvae with an LC50 value between 15.83 and 68.06 µg/mL and *C. quinquefasciatus* with an LC_{50} value of 13.069 µg/mL (Kovendan et al., 2012). Recently, Santra

et al., (2022) reported the endophytic fungus *Cochliobolus* sp. APS1 ethyl acetate extract had larvicidal ability against 1-4th instar larvae of *A. aegypti* with the least LC_{50} value of 9.19 µg/mL and LC_{90} value of 34.16 µg/mL, respectively. Similarly, da Silveira et al., (2021) demonstrated that a chitinolytic enzyme produced from *Trichoderma asperellum* disrupts 100% mortality in *A. aegypti* at a concentration of 40 mg/mL.

Ovicidal experiments indicated that egg hatching in *A. aegypti* and *C. quinquefasciatus* is wholly inhibited (100%) with 500 µg/mL of *C. bantiana.* The fungal extracts markedly reduced lifespan, egg hatchability, and fecundity. However, these fungal-derived molecules may be a promising alternative to synthetic pesticides for reducing mosquito populations in the field (Asemoloye et al., 2022). According to a prior study by Broadbent and Pree (1984), chemicals and metabolites that enter the eggshell when the eggs are exposed to maximal concentrations hurt development (Broadbent and Pree, 1984). Similarly, prolonged exposure times permit chemicals and metabolites to enter the shells more efficiently, increasing their effects. Again, Luz et al., (2007) reported the ovicidal activity of 21 entomopathogenic fungi against *A. aegypti*. Diflubenzuron and penfluron, two chitin synthesis inhibitors, were shown to have ovicidal effects on four mosquito species (Prakash, 1993). Furthermore, at a concentration, the bioactive compound (azadirachtin) exposed to *C. tarsalis* and *C. quinquefasciatus* completely inhibited egg hatching (Su and Mulla, 1998). Likewise, Karthik and others reported no ovicidal activity of marine actinobacterial extract (at 1,000 ppm) against the mosquitoes *C. tritaeniorhynchus* and *C. gelidus* (Karthik et al., 2011).

Enzymatic inhibition routes have been investigated as one of the primary modes of action of insecticidal and larvicidal drugs or metabolites (Rodrigues et al., 2021). The modulation of central and peripheral nervous system transmission largely depends on the activity of the enzyme AChE, which hydrolyzes the neurotransmitter acetylcholine (Ragavendran et al., 2018). AChE inhibition raises acetylcholine levels in the brain, and several

pesticides use AChE inhibition as a therapeutic effect (Ragavendran et al., 2019). In this study, we examined the expression of AChE in larvae of *A. aegypti* and *C. quinquefasciatus* that had been treated with the mycelial extract of *C. bantiana*. The levels of AChE in the homogenates of the targeted larvae's entire body showed significant changes. Interestingly, Suryawanshi et al. (2015) investigated alterations in alkaline phosphatase activity in *A. aegypti* larvae treated with *S. marcescens* prodigiosin. Similarly, Kannan et al., (2020) found that an extract of *Aspergillus tamarii* significantly reduced carboxylesterase and AChE levels in 4th instar larvae of *A. aegypti* and *C. quinquefasciatus*.

The zebrafish embryo is the finest scientific model organism for toxicological evaluations of metabolites and nanoparticles. It is small in size, transparency, simplicity, rapid embryogenesis, and rapid reproductions (Baskar et al., 2020). The present study addressed the acute toxicity of *C. bantiana* mycelial extract on zebrafish embryos after 72 h hatching. The concentrations of mycelial extract exhibited reduced heartbeat count, body length, percentage of survival rate, and heartbeat count in exposed groups. No deformities were observed in the control groups. Likewise, Abutaha et al. (2015) and their team found that the *Cochliobolus spicifer* endophytic fungal extract slightly causes significant abnormalities or deformities in zebrafish (*D. rerio*) embryos. Recently, Taher et al., (2022) studied the endophytic *Phyllosticta fallopiae* molecules that had teratogenicity and low lethality in a dose-dependent manner in zebrafish models. Additionally, Cao et al., (2016) found morphological and physiological alterations in cyhalofop-butyl-treated zebrafish, which included lower embryonic hatching rate, delayed spontaneous movement, decreased heartbeat, and larvae with shorter body lengths (at maximum doses).

FTIR analysis of *C. bantiana* mycelia extract revealed nine functional groups based on C-H stretching vibration of alkanes, N-H bending, CH_3 bonding, C-N stretching, and N-H wagging patterns. According to Seetharaman et al. (2017), the functional groups found in the endophytic *Penicillium oxalicum* fungus that peak at 2925 cm-1 were a part of the stretching

vibration of methyl and methylene C-H. (Abutaha et al., 2015). Presently, FTIR spectral investigations revealed the presence of amide, phenolic group, and aromatic and aliphatic amine groups, all of which could be involved in mosquito toxicity (Vivekanandhan et al., 2022). The peaks at 2926 cm -1 belong to the vibration of alkanes. The 3429.7 cm^{-1} O-H stretching and strong peak value, respectively, groups between 3000-3500 cm-1 (NH bond) primary amine N-H bending (1626 cm-1) and 1400-1650 cm^{-1} (N-H bending) were previously reported by George and others (George et al., 2011).

The mycelial extract of *C. bantiana* was tested using GC-MS and detected constituents were identified: 1-bromo-2-chloro- (17.97%) (Propane), Trans-3-undecene-1,5-diyne a contributing cyclobutane (21.69%), 1,2,3-trichloro-2-methyl- (14.85%),5,5,10,10-tetrachlorotricyclo [7.1.0.0(4,6)] a decane (20.74%) and a Phenol, (3,5-bis (1,1-dimethylethyl) - (23.99%). According to Ravi et al., (2018), the Phenol, (3,5-bis (1,1-dimethylethyl) has been extensively studied for its larvicidal and repellant activities against mosquitos. Interestingly, da Silva et al., (2015) reported the phenoxy dimethyl exhibited larvicidal activity against *A. aegypti* larvae Similarly, Ragavendran and Natarajan reported six bioactive compounds extracted from *Aspergillus terreus* showing larvicidal and pupicidal effects against selected mosquito vectors (Ragavendran and Natarajan, 2015). Additionally, Teles et al. identified insecticidal chemicals in secondary metabolites from *Periconia atropurpurea* and an endophyte from *Xylopia aromatica* utilizing ethyl alcohol (Teles et al., 2006). Recently, Sharma studied the isolation of bioactive metabolites from the endophytic fungus *Pestalotiopsis neglecta* of *Cupressus torulosa* and tested its separated 'taxol' molecules showing much biological activity (Sharma et al., 2016). Further research on the identification of active chemicals, as well as semi-field experiments, are required before recommending the active component of microbial metabolites for the development of eco-friendly mosquito vector control approaches.

5. CONCLUSION

In conclusion, *C. bantiana* fungal extract produced more than 90% mortality in larvae of *A. aegypti* and *C. quinquefasciatus.* The bioactive molecules of *C. bantiana* were effective larvicidal activity against tested larvae in a dose and time-dependent manner. The metabolites of *C. bantiana* mycelia completely inhibit the hatchability at a concentration of 400 and 500 µg/mL. The metabolic changes triggered by metabolites extracted from *C. bantiana* against selected mosquitoes were revealed in the effects of AChE inhibition. Furthermore, the toxicity assay of zebrafish embryo was done using *C. bantiana* metabolite after 72 hpf hatched embryo. The treated embryos revealed slightly reduced body length at maximum doses of mycelial extract compared to the control groups. GCMS analysis showed that the fungus *C. bantiana* produced a higher number of bioactive constituents against targeted larvae. These findings will also be helpful in the search for more biodegradable, environmentally friendly, selective, and lead-free mosquito larvicidal chemicals.

6. ACKNOWLEDGEMENTS

The authors thanked the Periyar University administration for providing the laboratory space needed to conduct this research. The DST-FIST [SR/FIST/LSIS-673/2016], a substantial award to the Biotechnology Department, is gratefully acknowledged by the authors of Periyar University for their assistance in boosting the instrumentation infrastructure. Furthermore, the authors thank the Goiano Federal Institute (Proc. No. 23219.001030.2022-05) for the financial support needed to conduct this research. Malafaia G. holds a productivity scholarship from National Council for Scientific and Technological Development (CNPq/Brazil) (Proc. No. 308854/2021-7).

7. AUTHORS' CONTRIBUTIONS

CR, GM, and DN designed the study; PK and CR participated in the experimental operations; DN, PS, and CR supervised the study; CR, EJK and AT contributed to data analysis; VM performed the embryotoxicity; EJK, CT, and AT provided scientific inputs; CR

wrote the first draft; CR, EJK, DN, IN, GM and AT revised the manuscript. All authors have read and agreed to the published version of the manuscript.

8. FUNDING

The Tamil Nadu State Council for Science and Technology, Chennai, India, provided financial assistance for this work through the Student Project Scheme (SPS) (TNSCST/SPS/AR/2017-2018 dated 30.01.2018, Project code: BS-027). Liverpool John Moores University supported the work.

9. DECLARATION OF COMPETING INTEREST

We confirmed no known conflicts of interest associated with this work, and there has been no significant financial support for this work that could have influenced its outcome. Furthermore, we assure you that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. Due care has been taken to ensure the integrity of the work.

10. ETHICAL ASPECTS

All experimental procedures were performed according to the ethical standards for animal experimentation, and meticulous efforts were made to ensure that the animals suffered as little as possible and to reduce external sources of stress, pain, and discomfort. The current study has not exceeded the number of animals needed to produce reliable scientific data. This article does not refer to any study with human participants performed by any authors.

11. AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

12. REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol* 18, 265-267.

Abutaha, N., Mashaly, A., Al-Mekhlafi, F. A., Farooq, M., Al-shami, M., Wadaan, M. A. 2015. Larvicidal activity of endophytic fungal extract of *Cochliobolus spicifer* (Pleosporales: Pleosporaceae) on *Aedes caspius* and *Culex pipiens* (Diptera: Culicidae). Appl Entomol Zoo. 50(3), 405-414.

Aly, A.H., Debbab, A., Kjer, J., Proksch, P., 2010. Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. Fungal diver 41, 1-16.

Amca 2005. "Mosquito Information: Control", in: *American Mosquito Control Association.* (USA).

Anwar, M., Sallam, E., Applications 2016. Utilization of prickly pear peels to improve quality of pan bread. Arab J. Nuclear Sci. 49, 151-163.

Araújo, I. F., Loureiro, H. A., Marinho, V. H., Neves, F. B., Sarquis, R. S., Faustino, S. M., Ferreira, I. M., 2020. Larvicidal activity of the methanolic, hydroethanolic and hexanic extracts from *Acmella oleracea*, solubilized with silk fibroin, against *Aedes aegypti*. Biocatalysis and Agricultural Biotechnology, 24, 101550.

Araujo, I. F., Marinho, V. H. D. S., Sena, I. D. S., Curti, J. M., Ramos, R. D. S., Ferreira, R., Ferreira, I. M., 2022. Larvicidal activity against *Aedes aegypti* and molecular docking studies of compounds extracted from the endophytic fungus *Aspergillus* sp. isolated from *Bertholletia excelsa* Humn. & Bonpl. Biotechnol Lett. 44(3), 439-459.

Asemoloye, M. D., Sunmola, N., Jonathan, G., Chikwem, J., 2022. Mycochemical screening reveals exopolysaccharide secretion, antioxidant and larvicidal activities of three oyster mushrooms. J. Sci. Food Agricult. 102(5), 2120-2126.

Badali, H., Prenafeta-Boldu, F.X., Guarro, J., Klaassen, C.H., Meis, J.F., De Hoog, G.S., 2011. *Cladophialophora psammophila*, a novel species of Chaetothyriales with a potential use in the bioremediation of volatile aromatic hydrocarbons. Fungal Biol. 115, 1019-1029.

Baskar, K., Chinnasamy, R., Pandy, K., Venkatesan, M., Sebastian, P. J., Subban, M., Devarajan, N., 2020. Larvicidal and histopathology effect of endophytic fungal extracts of *Aspergillus tamarii* against *Aedes aegypti* and *Culex quinquefasciatus*. Heliyon, 6(10), e05331.

Benelli, G., Jeffries, C.L., Walker, T., 2016. Biological control of mosquito vectors: past, present, and future. Insects 7**,** 52.

Borah, R., Kalita, M., Kar, A., Talukdar, A., 2010. Larvicidal efficacy of *Toddalia asiatica* (Linn.) Lam against two mosquito vectors *Aedes aegypti* and *Culex quinquefasciatus*. African J. Biotechnol. 9**,** 2527-2530.

Braun, U., Feiler, U., 1995. *Cladophialophora* and its teleomorph. Microbiol Res. 150**,** 81-91.

Broadbent, A.B., Pree, D.J., 1984. Effect of diflubenzuron and bay SIT8514 on the oriental fruit moth and oblique banded leaf roller. J. Econ Entomol. 77**,** 194–197.

Bucker, A., Bucker, N. C. F., Souza, A. G. L. D., Gama, A. M. D., Rodrigues-Filho, E., Costa, F. M. D., Tadei, W. P., 2012. Larvicidal effects of endophytic and basidiomycete fungus extracts on *Aedes* and *Anopheles* larvae (Diptera, Culicidae). Revista da Sociedade Brasileira de Medicina Tropical, 46, 411-419.

Cao, F., Liu, X., Wang, C., Zheng, M., Li, X., Qiu, L., 2016. Acute and short-term developmental toxicity of cyhalofop-butyl to zebrafish (*Danio rerio*). Environ Sci Pollut Res. 23(10), 10080-10089.

Chareonviriyaphap, T., Bangs, M.J., Suwonkerd, W., Kongmee, M., Corbel, V., Ngoen-Klan, R. 2013. Review of insecticide resistance and behavioral avoidance of vectors of human diseases in Thailand. Parasites Vectors 6**,** 280.

Chaturvedi, P., 2015. *Characterization of foliar endophytic fungi from Centella asiatica (L.) Urban as a promising source of bioactive metabolites.* PhD Thesis, GB Pant University of Agriculture and Technology.

Clemons, A., Mori, A., Haugen, M., Severson, D., Duman-Scheel, M., 2010. *Aedes aegypti* culturing and egg collection. Cold spring harbor protocols, 2010, pdb-prot5507.

da Silva, J. B. P., do AF Navarro, D. M., da Silva, A. G., Santos, G. K., Dutra, K. A., Moreira, D. R., Merz Jr, K. M., 2015. Thiosemicarbazones as *Aedes aegypti* larvicidal. European J. Med Chem. 100, 162-175.

da Silveira, A. A., Andrade, J. S. P., Guissoni, A. C. P., da Costa, A. C., de Carvalho e Silva, A., da Silva, H. G., Fernandes, K. F., 2021. Larvicidal potential of cell wall degrading enzymes from *Trichoderma asperellum* against *Aedes aegypti* (Diptera: Culicidae). Biotechnol Progress. 37(5), e3182.

Daniel, J. F., Silva, A. A., Nakagawa, D. H., Medeiros. L. S. D., Carvalho, M. G., Tavares, L. J., Rodrigues-Filho, E., 2017. Larvicidal activity of *Beauveria bassiana* extracts against *Aedes aegypti* and identification of Beauvericins. J. Brazilian Chemical Soci. 28, 1003-1013.

Dahl, J.A., Jung, I., Aanes, H., Greggains, G.D., Manaf, A., Lerdrup, M., Li, G., Kuan, S., Li, B., Lee, A.Y., 2016. Broad histone H3K4me3 domains in mouse oocytes modulate maternal-to-zygotic transition. Nature 537**,** 548-552.

Deepika, T.L., Kannabiran, K., Khanna, V.G., Rajakumar, G., Jayaseelan, C., Santhoshkumar, T., Rahuman, A.A., 2012. Isolation and characterisation of acaricidal and larvicidal novel compound (2S, 5R, 6R)-2-hydroxy-3, 5, 6-trimethyloctan-4-one from Streptomyces sp. against blood-sucking parasites. Parasitology Res. 111**,** 1151-1163.

Demain, A.L., Fang, A., 2000. "The natural functions of secondary metabolites," in *History of modern biotechnology I.* Springer), 1-39.

Derua, Y.A., Kweka, E.J., Kisinza, W.N., Githeko, A.K., Mosha, F.W. 2019a. Bacterial larvicides used for malaria vector control in sub-Saharan Africa: review of their effectiveness and operational feasibility. Parasit Vectors 12**,** 426.

Derua, Y.A., Kweka, E.J., Kisinza, W.N., Githeko, A.K., Mosha, F.W., 2019b. Bacterial larvicides used for malaria vector control in sub-Saharan Africa: review of their effectiveness and operational feasibility. Parasites vectors 12, 426-426.

Devi, N.N., Singh, M.S., 2013. GC-MS Analysis of metabolites from endophytic fungus Colletotrichum gloeosporioides isolated from Phlogacanthus thyrsiflorus Nees. Int J Pharm Sci. 23, 392-395.

de Oliveira, M. R., de Melo Katak, R., da Silva, G. F., Marinotti, O., Terenius, O., Tadei, W. P., de Souza, A. Q. L., 2021. Extracts of Amazonian fungi with larvicidal activities against *Aedes aegypti*. Front Micro. 12.

Dhanasekaran, D., Thangaraj, R., 2014. Microbial secondary metabolites are an alternative approaches against insect vector to prevent zoonotic diseases. Asian Pacific J. Trop Dis. 4(4), 253-261.

Du Sert, N. P., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., Browne, W. J., Würbel, H,. 2020. Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. PLoS biology, 18(7), e3000411.

El-Elimat, T., Raja, H.A., Graf, T.N., Faeth, S.H., Cech, N.B., and Oberlies, N.H. (2014). Flavonolignans from *Aspergillus iizukae*, a fungal endophyte of milk thistle (Silybum marianum). J. Natural Prod. 77, 193-199.

Ellman, G. L., Courtney, K. D., Andres Jr, V., Featherstone, R. M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 7(2), 88-95.

Faria, M., Garcia-Reyero, N., Padrós, F., Babin, P. J., Sebastián, D., Cachot, J., Raldúa, D. 2015. Zebrafish models for human acute organophosphorus poisoning. Scientific rep. 5(1), 1-16.

Finney, D. 1971. *Statistical methods.* Griffin, London.

Fröhlich, C. 2000. Observations of irradiance variations. Space Sci. Rev. 94, 15-24.

George, T.S., Guru, K.S.S., Vasanthi, N.S., Kannan, K.P., 2011. Extraction, purification and characterization of chitosan from endophytic fungi isolated from medicinal plants. World J. Sci Technol. 1, 43-48.

Grove, J.F., Pople, M., 1980. The insecticidal activity of beauvericin and the enniatin complex. Mycopathologia 70, 103-105.

Hemingway, J. 2014. The role of vector control in stopping the transmission of malaria: threats and opportunities. Philosophical Transactions of the Royal Society B: Biological Sciences 369, 20130431.

Karthik, L., Gaurav, K., Rao, K.B., Rajakumar, G., Rahuman, A.A., 2011. Larvicidal, repellent, and ovicidal activity of marine actinobacteria extracts against Culex tritaeniorhynchus and Culex gelidus. Parasitol Res. 108, 1447-1455.

Karthik, R., Manigandan, V., Ebenezar, K. K., Kavitha, M., Saravanan, R., 2019. Structural characterization, teratogenicity and *in vitro* avian antimicrobial activity of posterior salivary gland (PSG) toxin from cuttlefish, *Sepia prashadi*. Inter J. Biolog Macromol. 124, 1145-1155.

Kharwar, R.N., Mishra, A., Gond, S.K., Stierle, A., Stierle, D., 2011. Anticancer compounds derived from fungal endophytes: their importance and future challenges. Natural Product Rep. 28, 1208-1228.

Kondo, T., Asai, M., Tsukita, K., Kutoku, Y., Ohsawa, Y., Sunada, Y., Imamura, K., Egawa, N., Yahata, N., Okita, K., 2013. Modeling Alzheimer's disease with iPSCs reveals stress phenotypes associated with intracellular Aβ and differential drug responsiveness. Cell Stem Cell 12, 487-496.

Kovendan, K., Murugan, K., Vincent, S., Barnard, D.R., 2012. Studies on larvicidal and pupicidal activity of *Leucas aspera* Willd.(Lamiaceae) and bacterial insecticide, *Bacillus sphaericus*, against malarial vector, *Anopheles stephensi* Liston.(Diptera: Culicidae). Parasitol Res. 110, 195-203.

Kumar, D.A., Palanichamy, V., Roopan, S.M., 2014. Green synthesis of silver nanoparticles using Alternanthera dentata leaf extract at room temperature and their antimicrobial activity. Spectrochimica Acta Part A: Molecular Biomol Spectr. 127, 168-171.

Kuan, C. S., Cham, C. Y., Singh, G., Yew, S. M., Tan, Y. C., Chong, P. S., Ng, K. P., 2016. Genomic analyses of *Cladophialophora bantiana*, a major cause of cerebral phaeohyphomycosis provides insight into its lifestyle, virulence and adaption in host. PloS ONE, 11(8), e0161008.

Kulkarni, R. D., Ganavalli, A. S., 2011. *Cladophialophora bantiana* the neurotropic fungus–a mini review. J. Clin Diagnostic Res. 2011 November (Suppl-1), Vol-5(6): 1301-1306

Kweka, E.J., Lyatuu, E.E., Mboya, M.A., Mwang'onde, B.J., Mahande, A.M. 2010. Oviposition Deterrence Induced by *Ocimum kilimandscharicum* and *Ocimum* suave Extracts to Gravid *Anopheles gambiae* s.s (Diptera: Culicidae) in Laboratory. J. global infect dis. 2, 242-245.

Kweka, E.J., Mosha, F., Lowassa, A., Mahande, A.M., Kitau, J., Matowo, J., Mahande, M.J., Massenga, C.P., Tenu, F., Feston, E., Lyatuu, E.E., Mboya, M.A., Mndeme, R., Chuwa, G., Temu, E.A., 2008a. Ethnobotanical study of some of mosquito repellent plants in north-eastern Tanzania. Malaria Journal. 7, 152-152.

Kweka, E.J., Mosha, F.W., Lowassa, A., Mahande, A.M., Mahande, M.J., Massenga, C.P., Tenu, F., Lyatuu, E.E., Mboya, M.A., Temu, E.A., 2008b. Longitudinal evaluation of *Ocimum* and other plants effects on the feeding behavioral response of mosquitoes (Diptera: Culicidae) in the field in Tanzania. Parasites vectors. 1, 42-42.

Kweka, E.J., Munga, S., Mahande, A.M., Msangi, S., Mazigo, H.D., Adrias, A.Q., Matias, J.R. 2012. Protective efficacy of menthol propylene glycol carbonate compared to N, N-diethyl-methylbenzamide against mosquito bites in Northern Tanzania. Parasites vectors 5, 189-189.

Kweka, E.J., Nkya, H.M., Lyaruu, L., Kimaro, E.E., Mwang'onde, B.J., Mahande, A.M., 2009. Efficacy of *Ocimum kilimandscharicum* plant extracts after four years of storage against Anopheles gambiae ss. J. Cell Animal Biol. 3, 171-174.

Lee, J.-C., Kim, H.-R., Kim, J., Jang, Y.-S., 2002. Antioxidant property of an ethanol extract of the stem of Opuntia ficus-indica var. saboten. J. Agricul Food Chem. 50, 6490-6496.

Li, B., Shan, C.-L., Zhou, Q., Fang, Y., Wang, Y.-L., Xu, F., Han, L.-R., Ibrahim, M., Guo, L.-B., Xie, G.-L., 2013a. Synthesis, characterization, and antibacterial activity of cross-linked chitosan-glutaraldehyde. Marine drugs. 11, 1534-1552

Li, Y.-X., Himaya, S., Dewapriya, P., Zhang, C., Kim, S.-K., 2013b. Fumigaclavine C from a marine-derived fungus *Aspergillus fumigatus* induces apoptosis in MCF-7 breast cancer cells. Marine drugs. 11, 5063-5086.

Luz, C., Tai, M., Santos, A., Rocha, L., Alberaz, D., Silva, H., 2007. Ovicidal activity of entomopathogenic hyphomycetes on *Aedes aegypti* (Diptera: Culicidae) under laboratory conditions. J. medical entomol. 44, 799-804.

Malafaia, G., de Souza, A. M., Pereira, A. C., Gonçalves, S., da Costa Araújo, A. P., Ribeiro, R. X., Rocha, T. L., 2020. Developmental toxicity in zebrafish exposed to polyethylene microplastics under static and semi-static aquatic systems. Sci The Total Environ. 700, 134867.

Mody, P., Wada, P., Bloch, K. C., Lionakis, M. S., White, K. D., Maris, A. S., ... & Humphries, R. (2022). Gram stain to the rescue: a case report of cerebral phaeohyphomycosis by Cladophialophora bantiana in an immunocompetent 24-year-old. BMC Infectious Diseases, 22(1), 1-5.

Molinari, G., Soloneski, S., Larramendy, M., 2010. New ventures in the genotoxic and cytotoxic effects of macrocyclic lactones, abamectin and ivermectin. Cytogenetic genome Res. 128, 37-45.

Moyes, C.L., Vontas, J., Martins, A.J., Ng, L.C., Koou, S.Y., Dusfour, I., Raghavendra, K., Pinto, J., Corbel, V., David, J.-P., 2017. Contemporary status of insecticide resistance in the major Aedes vectors of arboviruses infecting humans. PLoS neglected tropical diseas. 11.

Mulligan Iii, F., Schaefer, C., Wilder, W., 1980. Efficacy and persistence of Bacillus sphaericus and B. thuringiensis H. 14 against mosquitoes under laboratory and field conditions. J. Economic Entomol. 73, 684-688.

Muthu, C., Daniel Reegan, A., Kingsley, S., Ignacimuthu, S., 2012. Larvicidal activity of pectolinaringenin from *Clerodendrum phlomidis* L. against *Culex quinquefasciatus* Say and *Aedes aegypti* L.(Diptera: Culicidae). Parasitology Res. 111(3), 1059-1065.

Netala, V.R., Kotakadi, V.S., Domdi, L., Gaddam, S.R., Bobbu, P., Venkata, S.K., Ghosh, S.B., Tartte, V., 2016. Biogenic silver nanoparticles: efficient and effective antifungal agents. Applied Nanosci. 6, 475-484.

Naomi, R., Bahari, H., Yazid, M.D., Embong, H., Othman, F., 2021. Zebrafish as a model system to study the mechanism of cutaneous wound healing and drug discovery: advantages and challenges. Pharmaceuticals. 14 (10), 1058.

Park, D.H., Kim, S.K., Shin, I.H., Jeong, Y.J., 2000. Electricity production in biofuel cell using modified graphite electrode with neutral red. Biotechnol Lett. 22, 1301-1304.

Porras-Alfaro, A., Bayman, P., 2011. Hidden fungi, emergent properties: endophytes and microbiomes. Annual Review Phytopathol. 49, 291-315.

Potshangbam, M., Devi, S.I., Sahoo, D., Strobel, G.A., 2017. Functional characterization of endophytic fungal community associated with Oryza sativa L. and Zea mays L. Front. Microbiol. 8, 325.

Prakash, A. 1993. Ovicidal action of certain chitin synthesis inhibitors in mosquitoes. *ENTOMON-TRIVANDRUM*- 17, 15-15.

Prakash, S., 2003. Laboratory efficacy tests for fungal metabolites of *Chrysosporium tropicum* against *Culex quinquefasciatus*. J. American Mosquito Control Assoc. 19(4), 404-407.

Prathibha, K., Raghavendra, B., Vijayan, V., 2014. Larvicidal, ovicidal, and oviposition-deterrent activities of four plant extracts against three mosquito species. Environ Sci. Pollut Res. 21, 6736-6743.

Prenafeta-Boldú, F. X., Medina-Armijo, C., & Isola, D. (2022). Black fungi in the built environment—The good, the bad, and the ugly. In Viruses, Bacteria and Fungi in the Built Environment (pp. 65-99). Woodhead Publishing.

Pudhom, K., Teerawatananond, T., Chookpaiboon, S., 2011. Spirobisnaphthalenes from the mangrove-derived fungus Rhytidhysteron sp. AS21B. Marine drugs. 12, 1271-1280.

Qadri, M., Johri, S., Shah, B.A., Khajuria, A., Sidiq, T., Lattoo, S.K., Abdin, M.Z., Riyaz-Ul-Hassan, S., 2013. Identification and bioactive potential of endophytic fungi isolated from selected plants of the Western Himalayas. SpringerPlus 2, 8.

Ragavendran, C., Natarajan, D., 2015. Insecticidal potency of Aspergillus terreus against larvae and pupae of three mosquito species *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti*. Environ Sci. Pollut. Res. 22, 17224-17237.

Ragavendran, C., Manigandan, V., Kamaraj, C., Balasubramani, G., Prakash, J.S., Perumal, P., Natarajan, D., 2019. Larvicidal, histopathological, antibacterial activity of indigenous fungus *Penicillium* sp. against *Aedes aegypti* L and *Culex quinquefasciatus* (Say)(Diptera: Culicidae) and its acetylcholinesterase inhibition and toxicity assessment of zebrafish (*Danio rerio*). Front. Microbiol. 10:427. doi: 10.3389/fmicb.2019.00427

Ragavendran, C., Srinivasan, R., Kim, M., Natarajan, D., 2018 *Aspergillus terreus* (Trichocomaceae): A natural, eco-friendly mycoinsecticide for control of malaria, filariasis, dengue vectors and its toxicity assessment against an aquatic model organism *Artemia nauplii*. Front. Pharmacol. 9:1355. doi: 10.3389/fphar.2018.01355

Ragavendran, C., Dubey, N. K., Natarajan, D., 2017. *Beauveria bassiana* (Clavicipitaceae): a potent fungal agent for controlling mosquito vectors of *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). RSC advan. 7(7), 3838-3851.

Rahuman, A.A., Gopalakrishnan, G., Ghouse, B.S., Arumugam, S., Himalayan, B., 2000. Effect of *Feronia limonia* on mosquito larvae. Fitoterapia 71**,** 553-555.

Ravi, R., Zulkrnin, N.S.H., Rozhan., N.N., Nik Yusoff, N.R., Mat Rasat, M.S., Ahmad, M.I., et al. 2018. Chemical composition and larvicidal activities of *Azolla pinnata* extracts against *Aedes* (Diptera: Culicidae). PLoS ONE 13(11): e0206982. https:// doi.org/10.1371/journal.pone.0206982

Rodrigues, A. M., da Silva, A. A., de Freitas, J. C. C., Martins, V. E. P., Ferreira, M. A. P., Ferreira, A. C. S. de Morais, S. M., 2021. Larvicidal activity of *Annona mucosa* Jacq. extract and main constituents rolliniastatin 1 and rollinicin against *Aedes aegypti* and *Aedes albopictus*. Industr Crops Product. 169, 113678.

Rodrigues-Alves, M. L., Melo-Junior, O. A. D. O., Silveira, P., Mariano, R. M. D. S., Leite, J. C., Santos, T. A. P., Gimenchetti, R. C., 2020. Historical perspective and biotechnological trends to block arboviruses transmission by controlling *Aedes aegypti* mosquitos using different approaches. Front Med. 7, 275.

Santra, H. K., Maity, S., Banerjee, D., 2022. Production of bioactive compounds with broad spectrum bactericidal action, bio-film inhibition and antilarval potential by the secondary metabolites of the endophytic fungus *Cochliobolus* sp. APS1 isolated from the Indian medicinal herb *Andrographis paniculata*. Molecules. 27(5), 1459.

Salunkhe, R.B., Patil, S.V., Patil, C.D., Salunke, B.K., 2011. Larvicidal potential of silver nanoparticles synthesized using fungus *Cochliobolus lunatus* against *Aedes aegypti* (Linnaeus, 1762) and *Anopheles stephensi* Liston (Diptera; Culicidae). Parasitol Res. 109**,** 823-831.

Saurav, K., Rajakumar, G., Kannabiran, K., Rahuman, A.A., Velayutham, K., Elango, G., Kamaraj, C., Zahir, A.A., 2013. Larvicidal activity of isolated compound 5-(2, 4-dimethylbenzyl) pyrrolidin-2-one from marine *Streptomyces* VITSVK5 sp. against *Rhipicephalus* (Boophilus) microplus, *Anopheles stephensi*, and *Culex tritaeniorhynchus*. Parasitol Res. 112**,** 215-226.

Saxena, R., Harshan, V., Saxena, A., Sukumaran, P., Sharma, M., Kumar, M.L., 1993. Larvicidal and chemosterilant activity of *Annona squamosa* alkaloids against *Anopheles stephensi*. J Am Mosq Control Assoc. 9**,** 84-84.

Seetharaman, P., Gnanasekar, S., Chandrasekaran, R., Chandrakasan, G., Syed, A., Hodhod, M.S., Ameen, F., Sivaperumal, S., 2017. Isolation of limonoid compound (Hamisonine) from endophytic fungi *Penicillium oxalicum* LA-1 (KX622790) of *Limonia acidissima* L. for its larvicidal efficacy against LF vector, *Culex quinquefasciatus* (Diptera: Culicidae). Environ Sci. Pollut Res. 24**,** 21272-21282.

Sharma, D., Pramanik, A., Agrawal, P.K., 2016. Evaluation of bioactive secondary metabolites from endophytic fungus *Pestalotiopsis neglecta* BAB-5510 isolated from leaves of Cupressus torulosa D. Don. 3 Biotech. 6**,** 210.

Sharma, P., Mohan, L., Srivastava, C., 2009. *Amaranthus oleracea* and *Euphorbia hirta*: natural potential larvicidal agents against the urban Indian malaria vector, *Anopheles stephensi* Liston (Diptera: Culicidae). Parasitol Res. 106**,** 171-176.

Shweta, S., Gurumurthy, B.R., Ravikanth, G., Ramanan, U.S., Shivanna, M.B., 2013. Endophytic fungi from Miquelia dentata Bedd., produce the anti-cancer alkaloid, camptothecine. Phytomed. 20**,** 337-342.

Sibero, M.T., Sabdaningsih, A., Cristianawati, O., Nuryadi, H., Radjasa, O.K., Sabdono, A., Trianto, A. (Year). "Isolation, identification and screening antibacterial activity from marine sponge-associated fungi against multidrug-resistant (MDR) Escherichia coli", in: *IOP Conference Series: Earth and Environmental Science*: IOP Publishing), 012028.

Srinivasan, M., Venkatesan, M., Arumugam, V., Natesan, G., Saravanan, N., Murugesan, Pugazhendhi, A., 2019. Green synthesis and characterization of titanium dioxide nanoparticles (TiO2 NPs) using Sesbania grandiflora and evaluation of toxicity in zebrafish embryos. Process Biochem. 80, 197-202.

Singh, R., Kumar, M., Mittal, A., Mehta, P.K., 2017. Microbial metabolites in nutrition, healthcare and agriculture. 3 Biotech 7, 15.

Stierle, A., Strobel, G., Stierle, D., 1993. Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of Pacific yew. Sci. Total Environ. 260, 214-216.

Su, T., Mulla, M.,1998. Ovicidal activity of neem products (azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). J. American Mosq Cont Assoc. 14, 204-209.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. "Molecular Evolutionary Genetics Analysis (MEGA)". Version).

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biol Evolut. 28, 2731-2739.

Taher, M. A., Tan, W. N., Chear, N. J. Y., Leong, C. R., Rashid, S. A., Tong, W. Y., 2022. Metabolites characterisation of endophytic *Phyllosticta fallopiae* L67 isolated from Aloe vera with antimicrobial activity on diabetic wound microorganisms. Natural Product Res. 1-6.

Tayung, K., Barik, B., Jagadev, P., Mohapatra, U., 2011. Phylogenetic investigation of endophytic Fusarium strain producing antimicrobial metabolite isolated from Himalayan Yew Bark. Malaysian J. Microbiol. 7, 1-6.

Teles, R.P., Haffajee, A.D., Socransky, S.S., 2006. Microbiological goals of periodontal therapy. Periodontol. 42, 180-218.

Vega-Rúa, A., Zouache, K., Girod, R., Failloux, A.-B., Lourenço-De-Oliveira, R., 2014. High level of vector competence of *Aedes aegypti* and *Aedes albopictus* from ten American countries as a crucial factor in the spread of Chikungunya virus. J. Virology. 88, 6294-6306.

Velayutham, M., Sarkar, P., Rajakrishnan, R., Kuppusamy, P., Juliet, A., Arockiaraj, J., 2022. Antiproliferation of MP12 derived from a fungus, *Aphanomyces invadans* virulence factor, cysteine-rich trypsin inhibitor on human laryngeal epithelial cells, and *in vivo* zebrafish embryo model. Toxicon, 210, 100-108.

Vivekanandhan, P., Swathy, K., Kalaimurugan, D., Ramachandran, M., Yuvaraj, A., Kumar, A.N., Manikandan, A.T., Poovarasan, N., Shivakumar, M.S., Kweka, E.J., 2020. Larvicidal toxicity of *Metarhizium anisopliae* metabolites against three mosquito species and non-targeting organisms. PLoS One. 15, e0232172.

Vivekanandhan, P., Swathy, K., Murugan, A. C., Krutmuang, P., 2022. Insecticidal efficacy of *Metarhizium anisopliae* derived chemical constituents against disease-vector mosquitoes. J. Fungi. 8(3), 300

Vyas, N., Dua, K. K., Prakash, S. 2006. Laboratory efficacy of metabolites of *Lagenidium giganteum* (Couch) on *Anopheles stephensi* (Liston) after filtrations by Column Chromatography. J Commun Dis. 38(2), 176.

Wang, L., Ying, G.-G., Zhao, J.-L., Yang, X.-B., Chen, F., Tao, R., Liu, S., Zhou, L.-J., 2010. Occurrence and risk assessment of acidic pharmaceuticals in the Yellow River, Hai River and Liao River of north China. Sci. Total Environ. 408, 3139-3147.

WHO 2005. "Guidelines for laboratory and field testing of mosquito larvicides". World Health Organization).

WHO 2017. "Update on the dengue situation in the Western Pacific Region. Update,(522). WHO.(2005)". (WHO/HQ. Geneva: WHO).

WHO. 2014. A global brief on vector-borne diseases

WHO 2019. "World malaria report 2019". (WHO/HQ, Geneva: World Health Organisation).

Zafrir Ilan, E., Torres, M.R., Prudhomme, J., Le Roch, K., Jensen, P.R., Fenical, W., 2013. Farnesides A and B, sesquiterpenoid nucleoside ethers from a marine-derived *Streptomyces* sp., strain CNT-372 from Fiji. J. Nat Product. 76, 1815-1818.

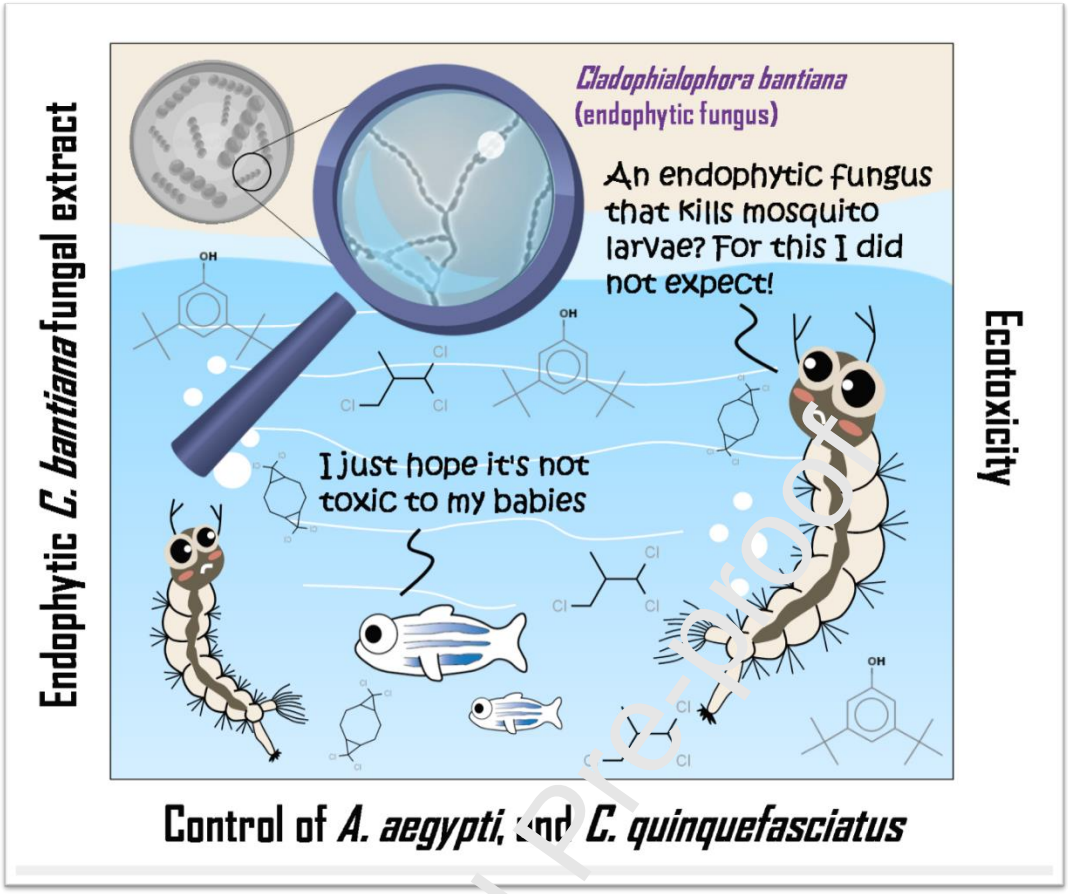
Zeng, Z.-Q., Yu, H.-B., Xu, H.-R., Xie, Y.-Q., Gao, J. (Year). "Fast training support vector machines using parallel sequential minimal optimization", in: *2008 3rd international conference on intelligent system and knowledge engineering*: IEEE), 997-1001.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical abstract



Cladophialophora bantiana METABOLITES ARE EFFICIENT IN THE LARVICIDAL AND OVICIDAL CONTROL OF *Aedes aegypti*, AND *Culex quinquefasciatus* AND HAVE LOW TOXICITY IN ZEBRAFISH EMBRYO

HIGHLIGHTS

- ✓ Highest mortality (91%) observed in *Ae. aegypti* and *Cx. quinquefasciatus* 4th instar larvae at 500 µg/mL concentration.
- ✓ The mean % of ovicidal bioassay was recorded 120 h after exposure.
- ✓ The AChE activity was reduced in the targeted larvae exposed to *C. bantiana* metabolites.
- ✓ Ecotoxicity of the *C. bantiana* metabolites was evaluated in zebrafish embryos.