Bio-efficacy of insecticidal molecule emodin against dengue, filariasis, and malaria vectors

Ragavendran Chinnasamy

Periyar University

Balasubramani Govindasamy

Saveetha University Saveetha Medical College and Hospital

Manigandan Venkatesh

University of Texas Health Science Center at Tyler: The University of Texas Health Science Center at Tyler

Sivanandam Magudeeswaran

Periyar University

Arulbalachandran Dhanarajan

Periyar University

Natarajan Devarajan

Periyar University

Peijnenburg Willie

Leiden University Medical Center: Leids Universitair Medisch Centrum

Vivekanandhan Perumal (✉ mosqvk@gmail.com)

Chiang Mai University Faculty of Agriculture   https://orcid.org/0000-0002-3283-1306

Supamit Mekchay

Chiang Mai University

Patcharin Krutmuang

Chiang Mai University

Research Article

Abstract

Emodin, a chemical isolated from *Aspergillus terreus*, was studied using chromatographic and spectroscopic methods and compound purity (96%) was assessed by TLC. Furthermore, high larvicidal activity against *Aedes aegypti*-AeA (LC_{50} 5.08 and LC_{90} 8.23 mg.L^{-1}), *Culex quinquefasciatus*-CuQ (7.13 and 12.01 mg.L^{-1}), and *Anopheles stephensi*-AnS larvae (6.40 and 15.24 mg.L^{-1}) was recorded. The first isolated fraction showed higher pupicidal activity against AeA (0.349 and 0.872 mg.L^{-1}). Most emodin-treated larvae (ETL) involutate variations in acetylcholine esterase, α and β-carboxylesterases, and phosphatase activities in the 4th instar, indicating intrinsic differences in their biochemical changes. ETL had numerous altered tissues, including muscle, gastric caeca, hindgut, midgut, nerve ganglia, and midgut epithelium. Acute toxicity of emodin against brine shrimp *Artemia nauplii* (154.0 and 184.5 mg.L^{-1}) and the zebrafish *Danio rerio* (less toxicity observed) was evaluated. In docking studies, Emodin interacted well with odorant-binding-proteins of AeA, AnS, and CuQ with docking scores of -8.89, -6.53, and − 8.09 kcal/mole, respectively. Therefore, *A. terreus* is likely to be effective against mosquito larvicides.

Introduction

Malaria, dengue, chikungunya, and Zika are the most common diseases spread by mosquitoes, which kill millions of people worldwide (Ward and Benelli 2017). The most frequent mosquito-borne illnesses in India include malaria, chikungunya, encephalitis, filariasis, and dengue fever (Bhatia et al. 2014). Microbiological agents (with effective insecticidal activity) play a significant role in avoiding disease spread by eliminating disease vectors while being environmentally friendly and specialised for target species (Tabanca et al. 2013; Vivekanandhan et al. 2018a,b; Kalaimurugan et al. 2019; Balumahendhiran et al. 2019; Logeswaran et al. 2019). Many fungi produced substances, such as bioactive metabolites, industrial enzymes, and pigments, have multiple functions and are low toxic and biodegradable (Krutmuang and Mekchay 2005; Kulkarni and Gupta 2013; Rajula et al. 2020; Vivekanandhan et al. 2020; Vivekanandhan et al. 2018c).

Environmental toxicological assays (using brine shrimp) are useful for assessing pesticides and environmental toxicity of pesticides and other chemicals before they are employed in larger-scale studies (Minguez et al. 2016; Vivekanandhan et al. 2021a; Vivekanandhan et al. 2022a). Long-term usage of synthetic chemicals has the potential to affect non-target organisms and the environment (Songa and Okonkwo 2016; Vivekanandhan et al. 2021b). It is very difficult to find novel and more selective compounds to combat the toxicity of target species. There have been numerous bio-metabolite toxicity studies in the past against various model organisms such as *Oncorhynchus mykiss, Brachydanio rerio, Dicentrarchus labrax, Gambusia holbrooki* (Georgalas et al. 2007) and some invertebrates (*Artemia salina, Daphnia magna, Balanus amphitrite, Hippolyte inermis*) (Venkateswara Rao et al. 2007). Zebrafish can be used to test veterinary drugs, biocides, insecticides, feed additives, and any other new material for animal toxicity (*Danio rerio*) (Scholz et al. 2014). Acute fish embryo toxicity tests (EFT) are commonly

performed, according to the OECD testing guideline (TG) 203 (OECD, 1992). Because zebrafish embryos are transparent, scientists may study their development from the single-cell stage to the larval stage using stereomicroscopes (Braunbeck et al. 2015).

Insects communicate with their environment using biological signals such as pheromones, plant volatiles and animal odours (Ronderos and Smith 2009; Pratheeba et al. 2019) (Benton et al. 2009). Among insect species, odour is utilized to detect food (Foster and Hancock 1994), find a host (Takken 1991), complete the mating process (Cabrera and Jaffe 2007), complete the oviposition process (Bentley and Day 1989), and recognize predators (De Bruyne and Baker 2008). The odorant-binding proteins (OBPs) have an crucial role in pest and insect management, as well as olfactory signal transmission (Vivekanandhan et al. 2022b,c). They are released by accessory cells surrounding olfactory neurons and are found mostly in the sensillar lymph (Tegoni et al. 2004). Furthermore, the maxillary palp and proboscis are better adapted for sensing taste, carbon dioxide, octanol and crucial chemical markers that distinguish the human host (Lu et al. 2007). Arthropod OBPs are water-soluble proteins with molecular weights ranging from 10 to 30 kDa that are distinguished by a highly conserved six–helical domain that is specific to this protein family (Calvo et al. 2006). Previously, OBPs have been discovered in *Anopheles gambiae* (Vieira and Rozas 2011).

Molecular docking is an useful technique for determining how a ligand will interact with a protein with a known three-dimensional structure. Understanding structural type communication and calculating inhibitor efficacy require knowledge of binding modalities (Vijayakumari et al. 2016). In order to develop unique and strong mosquito repellents derived from fungal-based bioactive compounds, logistics predictions and hypotheses are generated using in silico and computer simulation methodologies. Emodin, also known as 1, 3, 8-trihydroxy-6-methyl anthraquinone, is an anti-cancer, anti-inflammatory, anti-oxidant, anti-ulcer, antifungal, antiviral, and antiparasitic bioactive pigment found in a variety of fungi, plants, and lichens (Lin et al. 2009). Isolating the bioactive chemical emodin and examining how it kills larvae, pupae, and adults were the goals of this work. After that, in silico docking studies were conducted. In the present study is to isolate and evaluate the insecticidal molecule from *Aspergillus terreus*. The more potent fraction was evaluated against target insect pest as well as non-target species. The insecticidal molecule Emodin were identified through UV and FTIR HPLC analysis Nuclear magnetic resonance (NMR) of protons (^{1}H NMR), Carbon nuclear magnetic resonance (^{13}C NMR), Liquid chromatography-electrospray ionization mass spectrometer (LC–ESI-MS) analysis.

Materials And Methods

The isolation, identification, and mosquitocidal capabilities of *Aspergillus terreus* mycelial ethyl acetate crude extract (ATMEAE) have been described (Ragavendran and Natarajan 2015) (Ragavendran et al. 2018). Chemicals and solvents were of analytical quality (Merck, Germany), and dechlorinated water was utilized throughout the bioassay. To clean glassware and Petri plates, diluted nitric acid (HNO_3) was rinsed with distilled water and dried in a hot air oven.

Thin-layer chromatography

ATMEAE was separated from the chemicals using thin-layer chromatography (TLC). Several solvents (methanol-hexane, ethyl acetate-acetone, methanol-ethyl acetate and chloroform-acetone) were utilized in the separation of ME using a TLC plate. ATMEAE solutions were diluted and coated onto Silica Gel60 F254 TLC plates (Merck) with a size of 20 x 20 cm and a layer thickness of 0.20 mm and eluted with more volume of methanol to chloroform (95:5, 96:4, 97:3, 98:2, 99:1, and 100 %), with each elution drying naturally. The solvent front of the plate was marked immediately after it was removed from the TLC chamber. The plate was then allowed to dry naturally. UV light was used to see the various zones/spots of ME on the TLC plate (254 nm). Rf was calculated as follows:

Rf = Distance moved by the solute/ Distance moved by the solvent

Rf values were calculated for each component of each fraction. Compounds with the same Rf values were mixed. Totally 3 fractions (F1, F2 and F3) were obtained from the ATMEAE. In addition to its weight (milligrams), each fraction also had a larvicidal potential (Pandey et al. 2011).

Preparative TLC

After separating the fraction with the highest larvicidal activity, its contents were separated on a 20 cm^2 silica gel plate using a chloroform-methanol mobile phase (97:3%). The zone eluted from the silica plate was scraped and collected separately. Each test tube received 100ml of ethyl acetate. After dissolving the compounds, they were filtered twice using Whatman filter paper No. 1 to eliminate any suspended silica powder. Following evaporation of the extract, the purified samples were concentrated and kept at 4°C for subsequent analysis. The pure component was obtained using an ethyl acetate extract (84 mg).

Structural elucidation of the compound

UV and FTIR characterization of F1 fraction

In the UV-Visible spectrophotometer (Shimadzu UV1800), the UV spectrum of the compound was measured at the λ between 300 and 700 nm with DMSO acting as a blank. An FTIR spectrometer (FT-IR; Brucker 4100) was used to determine the absorption spectrum of active fractions. The F1 fraction (1: 3 drop) was applied to KBr pellets that were dried at 50°C to make potassium bromide pellets. The FT-IR system was calibrated for background signal scanning with pure KBr (Deepika et al. 2012). Intensity versus wave number was used to create a spectrum and the compound analysis was conducted at St. Joseph's College in Trichy, Tamil Nadu, India.

HPLC analysis of the emodin

A modified earlier method (Anjum et al. 2012) was utilised to evaluate *A. terreus* MEAE fraction-1 using a modified HPLC method. The sample was eluted with methanol (HPLC grade, Sigma Aldrich, USA) and pre-filtered using a 0.22μm membrane filter after injection (20 μl). A UV detector was attached to an

instrument equipped with a Shimadzu LC solution No. 20 AD to measure peak purity in Shimadzu LC solution 20 AD, Japan. For isocratic resolution, an LCGC C18 column with methanol: water (50:50) mobile phase at 1.0 ml/min and a head pressure of 25 kgf/cm^2 was used.Room temperature (30°C) was maintained throughout the setup. Each HPLC analysis took 45 min to complete. After developing a plate, a plate drier was used to dry the plate and it was analyzed with a UV detector (254 nm wavelength) (Zhang et al. 2013).

Nuclear magnetic resonance (NMR) of protons (^{1}H NMR)

For the sample solution, the dried chemical was dissolved in deuterated DMSO. The solution was injected at a depth of 4.5-5 cm into the NMR tubes. The spectra were taken using the scale, and tetramethylsilane (TMS) was used as the internal standard on a 500 MHz Bruker Advance instrument (Khan et al. 2018).

Carbon nuclear magnetic resonance (^{13}C NMR)

The number of carbon atoms in the sample was determined using NMR ^{13}C. Spectra were produced using DMSO at 500 MHz (on scale) using a sample prepared in DMSO (Khan et al. 2018). The studies were carried out using an NMR spectrometer set at 100.52 MHz for ^{13}C and 400 MHz for ^{1}H, with DMSO as a solvent. This research was carried out at the Gandhigram Rural Institute (Deemed University) in Dindigul, Tamil Nadu, India, in the Department of Chemistry.

Liquid chromatography-electrospray ionization mass spectrometer (LC–ESI-MS) analysis

In this experiment, an electrospray ionization (ESI) source and an ion trap mass analyzer were used on a Bruker Dionex Ultimate (Thermo 3000) mass spectrometer. In brief, 20 μl of fraction 1 was introduced into the ESI. As a given gradient program was applied, the solvent was eluted at 1 ml/min as the mass spectra were scanned between 10–40 m/z. Fraction 1 was subjected to the following conditions: capillary temperature of 300 °C, source voltage of 5.0 kV, source current of 100 mA, and capillary voltage of 22 V. All analyses were conducted in positive mode for 20 min with MS scans. The HPLC system with auto-sampler (HD Brucker) was used in conjunction with the mass spectrometer. A Zorbax Eclipse reversed-phase analytical column (LC18, particle size 5.0 μm, 150 mm x 4.6 mm) was used (Silva et al. 2016). Analyses of the sample were performed by the Indian Institute of Science's (IISc) Division of Biological Sciences, Bangalore, India.

Larvicidal bioassay

To make the proper doses, all fractions (F1, F2, and F3) were dissolved in DMSO and diluted with water. The effectiveness of mosquito larvicidal fractions was assessed using the conventional WHO technique (WHO 1996) and, with minor modifications (Seetharaman et al. 2017). Twenty larvae of target mosquitoes were placed in a 150-ml glass beaker containing 100 ml of dechlorinated water and one ml of the relevant test sample concentrations (control, 50, 100, 150, and 200 mg.L^{-1}). After 12 hours of therapy, the proportion of patients that died was calculated in triplicate for each concentration examined.

A 10% DMSO solution in water was employed as a negative control. Using Abbott's approach, the mortality rate was corrected and calculated (Abbott 1925).

Pupal toxicity test

Aspergillus terreus isolated fractions were tested for pupicidal activity against target mosquitoes. In a 150-ml beaker containing 99 ml of dechlorinated water, 20 freshly emerged pupae were kept with 1 ml of each of the desired concentrations (control, 50, 100, 150 and 200 mg.L^{-1}) from the sample. To set up the control, 1ml of 10% DMSO was added to 99ml of dechlorinated water. To calculate the pupae mortality after 24 hours, the Abbott formula was also used to correct the control mortality (Abbott 1925).

Ovicidal activity

The ovicidal bioassay of the material was done using a modified technique (Su and Mulla, 1998). Samples of various concentrations were produced from the stock solution (50, 100, 150, and 200 mg.L^{-1}). Before the treatment, each egg of the target insect was checked under a microscope. A total of 75 distinct mosquito species had their freshly developed eggs exposed to a concentration of fractions until they hatched or perished. Negative control was DMSO, while positive control was a commercial pesticide (Azadirachtin). The eggs were treated and counted under a microscope before being placed in distilled water for hatchability testing. Each test was carried out three times. Using the following calculation, the hatch rate after 48 hours was calculated (Chenniappan and Kadarkarai 2008).

Whole larval body homogenate preparation

In the 4th instar larvae treated and control groups, sterile double H$_2$O was used to wash the larvae and adhered water was removed from their surfaces using tissue paper. Individual larvae were homogenized, using a homogenizer, in Eppendorf tubes with ice-cold sodium phosphate buffer (pH 7.0, 20 mM) for determining enzyme activity. Upon centrifugation (8000 x g at 4°C) for 15 min, the homogenates were used to analyze enzymes in the subsequent steps.

Acetylcholinesterase (AChE) assay

Three types of mosquitoes were tested to determine whether emodin compounds inhibited the enzyme acetylcholinesterase. According to Ellman et al. (1961), a modified AChE assay was performed. The mosquitoes, *An. stephensi, Cx. quinquefasciatus,* and *Ae. aegypti* were used to test how well the compounds blocked acetylcholinesterase.

Carboxylesterase assays

A technique described previously was used to evaluate the activity of α- and β-carboxylesterases on the three larvae investigated (Van Asperen 1962). Incubate 100 µl of undiluted and diluted (1:3) homogenates for 30 minutes at 30°C in 1 ml sodium phosphate buffer (pH 7.0) containing 250 µM of α- and β-naphthyl acetate. The colour was formed in an aliquot reaction with 400 µl of freshly synthesised

0.3 % Fast Blue B in 3.3 % SDS for 20 min at 28 °C. Optical density was determined at 430 nm (α-carboxylesterase) and 588 nm (β-carboxylesterase) using the blanks used.

Acid and alkaline phosphatase assays

Acid and alkaline phosphatases were measured in the larvae of tested mosquitoes using the modified procedure (Asakura 1978). To evaluate acid phosphatase activity, 50 μl of larval homogenate was added to 450 μl of 50mM sodium acetate buffer at 4.6 pH. To test alkaline phosphatase activity, 20 μl of larval homogenate was mixed evenly with 50mM Tris-HCl buffer (pH 8.0) containing 12.5 mM p-nitrophenyl phosphate. The enzymatic reaction was arrested by adding 100μl of 0.5N NaOH solution in an incubation vessel for 15 min at 37°C in a hot water bath, followed by centrifugation (5000xg for 6 min). Using a Shimadzu UV-160A spectrophotometer, optical density was measured at 440 nm for a blank sample.

Histopathological study

The response of fraction1 was observed histopathologically by studying mosquito samples to observe the changes in morphological features. In addition, control larvae were initially fixed with 10% formalin and the 4th instar larvae were treated with the pure compound. After dehydration in ethyl alcohol, the tissues were cleared in xylene, fixed in para-plast and sectioned (5 μm). By using the standard staining procedure, the sections were stained with hematoxylin and eosin (HE staining) (Kaewnang et al. 2011). In the end, control and treated larvae of the midgut area were viewed under a light microscope (at x40 magnification) and photographs were taken (Seetharaman et al. 2017).

Bio-toxicity assay of fraction 1 against *Artemia nauplii*

The brine shrimp biotoxicity test of fraction 1 was performed following OECD guidelines 236 (Mannan et al., 2012)(Busquet et al., 2014). A beaker containing 32g of sea salt (32 g per liter) was used to hatch *Artemia* nauplii/shrimp cysts. The beaker was covered with black polythene and placed under constant oxygen and light for 48 hours. For this experiment, well-developed *Artemia* nauplii were collected and transferred into a glass container following an appropriate incubation period. A number of doses of fraction 1 (1 g.L^{-1} dissolved in 10% DMSO) were tested (2, 4, 6, 8, and 10 mg.L^{-1}). As a negative control, the equivalent volume of DMSO was added to the respective glass container. The LC_{50} value and percentage of dead larvae were calculated with SPSS 20.0 software after 24 hours of treatment. The formulae below mentioned was used to correct the data (Meyer et al. 1982).

% death = [(test-control)/100] ×100

Danio rerio embryo test

According to the OECD (2013), the embryonic acute toxicity test was conducted with some minor modifications based on the fish embryo toxicity test (FET). Various concentrations of emodin (0, 1.95, 15.6 and 62.0 mg.L^{-1}) were prepared using *D. rerio* water. Two fertilized eggs and a zebrafish embryo were mixed with 2.0 mL of these solutions and placed in a 24-well microplate. As a control, each plate

contained four wells filled with 10% DMSO and the remaining wells with four concentrations were equally distributed. For each replication, ten embryos per concentration were used. Every 24 hours, the emodin concentration and water quality were restored to maintain an appropriate level. At a temperature of 20±1.0°C and a 14:10 light/dark photoperiod, the embryos were monitored at intervals of 0, 24, 48, 72, 96, and 120 hours. The body length, hatching rate, and mortality rate of larvae were studied using an inverted microscope (Nikon TF2000-U) (Li et al. 2016).

Homology modelling

Modeler 9.20 was used to generate the mosquito protein (odorant-binding protein) homology model from *An. stephensi* (Eswar et al. 2006). *An. stephensi* FASTA sequence was obtained from UniProtKB (Accession: B5A5T7). Furthermore, the matching PDB IDs must be 2ERB, 3KIE, 3OGN, and 5DIC, as determined using the BLAST search engine. The best model protein was chosen based on the DOPE score. Furthermore, the AMBERTOOLS 14 package was used to minimize energy use (Case et al., 2014). We used a Structural Analysis and Verification System (SAVES) and a Ramachandran plot to figure out how to make the final structure as energy-efficient as possible (Ramachandran et al. 1963) (Ramachandran and Sasisekharan 1968).

Molecular docking study

Preparation of odorant-binding proteins

Ae. aegypti (PDB ID: 3K1E) and *Cx. quinquefasciatus* crystal structures were retrieved from the Protein Data Bank (PDB) (PDB ID: 3OGN). To produce unique species proteins, a mosquito Odorant Binding Protein structure is required. Hydrogen atoms and kollman charges were added to the 3D structures using Autodock 4.2, which were then saved as pdbqt files (Morris et al. 2009). Rotating bonds, torsional degrees of freedom, atomic partial charges, and non-polar hydrogen atoms have all been given to the ligands. Based on existing research, docking simulations with various grid sizes encompassing all residues involved in compound identification were carried out. The hybrid Lamarckian Genetic Algorithm was used for each docking simulation, with a grid spacing of 0.375 Å and 30 docking runs. To perform a molecular docking analysis, and implementation of the Lamarckian genetic algorithm (LGA) was adopted (Morris et al. 1998). As part of the LGA method, the following parameters will be used: a maximum number of 250,000 energy evaluations, 27,000 generational cycles, and crossover and mutation rates of 0.02 and 0.8, respectively.

Ligand preparation

From PubChem (http://pubchem.ncbi.nlm.nih.gov/) we retrieved the ligand structure of emodin ($C_{15}H_{10}O_5$). In the next step, the geometry was optimized using B3LYP/6-311G**, using the Gaussian 03 package (Frisch et al. 2004). The docking analysis was performed using the prepared molecule.

Molecular docking of the target protein with ligand

A molecular docking analysis has been conducted to understand how emodin binds to target proteins. *Ae. aegypti* (PDB ID: 3K1E), as well as *Cx. quinoquefasciatus* (PDB ID: 3OGN) and a homology-modeled protein (*An. stephensi*), were docked with the emodin ligand. By binding the ligand to the protein, the conformation of the protein structure is altered, which automatically alters the function of the protein.

Visualization and analysis

The docked complex was viewed with PyMol (DeLano 2002) and the Discovery Studio visualizer (Dassault Systems 2016) to find out about the key hydrogen and hydrophobic interactions between the related emodin-odorant binding protein complexes.

Statistical analysis

Probit analysis was used to calculate the median lethal concentrations (LC_{50} and LC_{90}) of the sample and Chi-square χ^2 values (Finney 1971). The significance level for the (ANOVA) analysis was established using the Tukey test at a *P<0.05*. The data were analyzed using IBM SPSS 20.0 software (IBM, Armonk, NY, USA) as the mean ± standard deviation.

Results

Thin-layer chromatography of sample

Using different solvent combinations, TLC plate separation of bioactive molecules from ATMEAE (hexane-ethyl acetate, hexane-methanol, chloroform-acetone, ethyl acetate-acetone and chloroform-methanol). The chloroform-methanol method was demonstrated to be ideal in extracting the most active 3 fractions based on the unique resolution of the extract's active components, with Rf values of 0.94, 0.81, 0.38, 0.62, 0.43, 0.28, and 0.26 cm (Fig. 1). Table S1 presents the Rf values for each fraction of *A. terreus*. In any of the tried concentrations of 1% to 5% hexane in methanol solvent, no spots were visible. There was no separation of the components of the mycelia extract sample during its movement through the mobile phase. The TLC purity of compound was determined by the Rf value of 0.38cm (chloroform: methanol, 97:3%); the short and long UV at 254 nm were used to evaluate the single band obtained; then the compound was weighed (84 mg).

Larvicidal activity of separated fractions

The 4th instar larvae of target mosquitoes were tested with purified fractions (F1, F2, and F3) of *A. terreus* (200, 150, 100, and 50 mg.L^{-1}) (Table 1). *Ae. aegypti* (5.08 and 8.23 mg.L^{-1}) had the lowest LC_{50} and LC_{90} values, followed by *Cx. quinquefasciatus* (7.13 and 12.01 mg.L^{-1}) and *An. stephensi* (7.13 and 12.01 mg.L^{-1}) (6.40 and 15.24 mg.L^{-1}). An isolated pure chemical proved 100 percent effective against the 4th instar larvae of *Ae. aegypti* (100 percent), *Cx. quinquefasciatus* (98 percent), and *An. stephensi* at a concentration of 200 mg.L^{-1} (99 percent). With higher concentrations of the compound (200 mg.L^{-1}), death occurs within 4 hours after exposure. Approximately 50% of deaths occurred within 6 hours of

exposure. It was found that selected mosquitoes failed to develop as normal adults and died as a result of morphological changes (Fig. S1a). A mosquito exposed to fraction 1 (200 mg.L^{-1} at maximum concentration) demonstrated aggressive behaviors such as behavioral alterations (Fig. S1b), interference with coordination, up and down writhing activity, and forceful self-biting. F2 was tested against three mosquito species' larvae: *Ae. aegypti* (LC$_{50}$= 6.30 and LC$_{90}$= 9.51 mg.L^{-1}), *Cx. quinquefasciatus* (LC$_{50}$= 9.11 and LC$_{90}$= 19.54c mg.L^{-1}), and *An. stephensi* (LC$_{50}$= 9.11 and LC$_{90}$= 19.54 mg.L^{-1}) (LC$_{50}$= 11.85 and LC$_{90}$= 21.64 mg.L^{-1}). The (F3) fraction performed the best against mosquitos, notably *Ae. aegypti* (6.75 and 9.38 mg.L^{-1}), *Cx. quinquefasciatus* (12.19 and 15.72 mg.L^{-1}), and *An. stephensi* (6.75 and 9.38 mg.L^{-1}) (13.45 and 16.24 mg.L^{-1}). A negative control showed a 100 percent larval survival rate. Azadirachtin (200 mg.L^{-1}) was the least toxic commercial insecticide (LC$_{50}$ = 3.65 and LC$_{90}$ = 5.12 mg.L^{-1}). Significant chi-square values were found at the *p≤0.05* level. At a low dose of compounds, fraction 1 showed the highest larvicidal activity among the three fractions. According to the obtained results, fraction 1 was selected for further spectral analysis.

Pupicidal toxicity test

In three isolated fractions exposed to target mosquitoes, the pupicidal toxicity assay results in increased mortality rates at different levels of concentration (50, 100, 150, and 200 mg.L^{-1}). The concentration of fraction1 of 200 mg.L^{-1} was found to be 80% effective against *Ae. aegypti* and *An. stephensi*. Based on the LC$_{50}$ and LC$_{90}$ values of fraction 1, better results were found for *Ae. aegypti* (LC$_{50}$=0.35 and LC$_{90}$=0.87 mg.L^{-1}), *An. stephensi* (LC$_{50}$=0.93 and LC$_{90}$=2.07 mg.L^{-1}), as well as *Cx. quinquefasciatus* (LC$_{50}$=1.93 and LC$_{90}$=4.09 mg.L^{-1}) (Table 2). Azadirachtin's LC$_{50}$ and LC$_{90}$ values against mosquito pupae were 4.22 and 6.05 mg.L^{-1}, respectively. During the experiment (doses of fraction 1), the pupal bodies moved restlessly, violently, and irregularly, and dead pupae were found in the bottom of the beakers (Figs. S2a and S2b). All mosquitoes tested showed activity in fractions 2 and 3 as well.

Ovicidal bioassay

In Table 3, the results of ovicidal bioassays of *A. terreus* bioactive fractions (1, 2, and 3) are presented. In the present study, bioactive fractions caused embryonic death and prevented eggs from hatching. The eggs' mortality rate was largely determined by the relationship between the doses of bioactive fractions and the egg size. Fraction 1 exhibited a higher ovicidal effect than the other 2 fractions. At 150 mg.L^{-1} concentrations, *Ae. aegypti* hatched at a low rate (6.9%), followed by *Cx. quinquefasciatus* (13.4%), and *An. stephensi* (14 %). The maximum concentration (200 mg.L^{-1}) of fraction 1 attained 0% hatchability of eggs and *Ae. aegypti* had a hatchability of 39 %, *Cx. quinquefasciatus* had a hatchability of 36 % and *An. stephensi* had a hatchability of 32 % in the same concentration of fractions 2 and 3. Control eggs were reported to hatch at 97 % with 10 % DMSO in testing mosquitoes. The concentrations of 200 mg.L^{-1} of all components were very harmful to eggs as compared to other concentrations. In positive control trials, azadirachtin proved extremely harmful to mosquito eggs.

Biochemical assays

A general finding was that the tested larvae changed the activity of normal components either by increasing or decreasing compared to the control. Our study involved biochemical enzymatic assays of mosquito larvae as a result. In this procedure, acetylcholinesterase, α- and β-carboxylesterase, and acid and alkaline phosphatases are measured.

Ae. aegypti (F_4=1434.070; P<0.01) significantly suppressed the Acetylcholinesterase activity, which was measured using the control value of 2.61 mg protein/ml of homogenate for the larvae of *An. stephensi* (F_4=901.954; P<0.01) and *Cx. quinquefasciatus* (F_4=1266.187; P<0.01) (Fig. 2a). AChE was inhibited by emodin dose-dependently. A significant decrease in α-carboxylesterase activity was also observed following emodin treatment among larvae *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* (2.61 to 0.61, 2.06 to 0.39 and 3.21 to 0.50 mg protein/ml of homogenate), respectively (Fig. 2b). A similar type of activity was observed for the α-carboxylesterase (0.561 to 0.051, 0.521 to 0.151 and 0.621 to 0.097 μM β-naphthol released/mg/min) (Fig. 2c).

As a result of exposure to emodin, the activity of acid and alkaline phosphatases was reduced slightly in larvae of *An. stephensi*, *Cx. quinquefasciatus*, and *Ae. aegypti*, which decreased from 0.448 to 0.230, 0.421 to 0.213, and 0.484 to 0.211 μM p-nitrophenol released/min/mg protein, respectively (Fig. 2d). Furthermore, alkaline phosphatase significantly reduced the levels of target mosquito larvae (0.361 to 0.142, 0.301 to 0.101, and 0.324 to 0.161 mg protein/ml of homogenate) (Fig. 2e).

Histopathology profile of 4th instar larvae

The midgut epithelial columnar cells (EC) of *An. stephensi* larvae in their fourth instar were severely damaged after being exposed to fraction 1 of ATMEAE. The lumen was encompassed by thin peritrophic membranes containing food particles in control larvae, whereas in treated larvae, the midgut contents, epithelial cells (EC), and peritrophic membranes (PM) were ruptured (Fig. 3a-c). Similarly, *A. terreus* compound-treated larvae had broken mid-gut epithelium and vacuolated cells (Fig. 3d-f), whereas control larvae had a normal appearance in the mid-gut, hindgut, muscles, brush border, and epithelial cells. The muscles appear slightly damaged and the brush border is disorganized. In larvae treated with the bioactive compounds, the mid-gut was the most affected tissue. After treatment with the compound, the histopathological alterations of larvae of *Ae. aegypti* 4th instar were observed. Specifically, muscles, gastric caeca, hindgut, mid-gut, nerve ganglia, and mid-gut epithelium were damaged and collapsed. There were spoiled epithelial cells that contained the nuclei of the compound-treated larvae in vacuolation.

In a lethality study using brine shrimp, emodin was found to be moderately toxic to *Artemia nauplii*, with an LC_{50} of 153.97 mg.L^{-1} and an LC_{90} of 184.51 mg.L^{-1} (χ^2=9.891, p 0.05). Using emodin doses, the survival of *A. nauplii* was significantly decreased. In figure 4a, the maximum mortality rate (64%) is

reported at 10 mg.L^{-1}, while the controls do not exhibit mortality. On the other hand, *A. nauplii's* inside gut showed clusters of emodin after 24 hours (Figs 4b-d) (Table 4).

A development study was conducted between 0 and 126 hpf. Based on the results, the treated and untreated samples showed significantly different hatching rates at 96 hpf. As a control (96h), 99.5% of the eggs hatched, and at lower concentrations (1.95 and 15.6 mg.L^{-1}) of emodin, more than 50% of the eggs hatched. Emodin-treated embryos showed a dose-dependent reduction in body length. Compared to the control group, the maximum exposure (62.5 mg.L^{-1} of emodin) significantly reduced the length of embryos. Following 96 hours of treatment with 62.5 mg.L^{-1} of emodin, embryos showed underdeveloped head regions and closed tails, with no heartbeat remaining in 120-hpf embryos (unhealthy tail). The concentrations of emodin had a significant effect on the mortality rate. The entire toxicity test showed that the activity of compounds was primarily dependent on their concentration. The total body length of the embryos as well as hatchability, mortality, and body length of emodin-treated embryos (Fig. 5 and Fig. S3) were also significantly affected by the presence of concentrations of 15.6 mg.L^{-1} or higher of emodin (Fig. 5).

The UV spectra of isolated fraction (F1) was measured between 200-700 nm using DMSO as a blank. The solution of the compound (F1) has a wide band with maxima at 443nm and 291nm (Fig. S4). The greatest UV absorption wavelength for emodin (0.856 optical density value) according to the UV spectrum is 443 nm, indicating the presence of both aromatic and methyl groups. TLC revealed that the pure compound was yellowish-orange in color. A single fraction was obtained and tested for purity by HPLC. There is a single prominent peak (Fig. S5, Table S2) indicating maximum purity and purity as a result of the compound being separated into a single prominent peak with a retention time of 6.288 min and a peak area of 95.90%.

The FTIR spectrum of Emodin/fraction (F1) shows numerous unique peaks, including one at 3389.68 cm^{-1} that indicates stretching vibrations of OH alcohol (or) phenol groups. In the fractionated sample, a noticeable band forms at 3079.30 cm^{-1}, suggesting that the OH bond stretching frequency is most likely occurring in the protein or carbohydrates. The C-H alkanes are responsible for the peak of 2924.04 cm^{-1}. Anhydrides groups are detected in the sample as symmetrical stretching peaks at 1815.89cm^{-1}. At 2854.14 cm^{-1}, a peak was seen caused by methyl or methoxyl stretching vibrations. Based on the absorption peak at 1665. 98cm^{-1}, bending vibrations of N-H primary amines may be responsible for the medium-intense band 1623.58 cm^{-1}. It is possible that aromatics -C-C stretching caused the medium peak at 1416.97 cm-1. The strong band at 1272.84 cm^{-1} C-O stretching and 1216.96 cm^{-1} C-N stretching, respectively, represents alcohols, carbohydrates or esters, and aliphatic amines. A prominent peak associated with O-H bending vibrations of carboxylic acids may be seen at 907.00 cm^{-1}, whereas N-H primary amines can be found at 874.80 cm^{-1} (Fig. S6, Table S3).

To confirm, the compound's 1H NMR spectra in DMSO solution were obtained regarding Tetramethylsilane (TMS) as an internal standard. 1H-NMR data (400 MHz, D6-DMSO): δ 12.11 (s, Ar(O-H),

1H); 12.04 (s, Ar(O-H), 1H); 11.41 (br, s, Ar(O-H), 1H); 7.52 (s, ArH, 1H); 7.19 (s, ArH, 1H); 7.14 (s, ArH, 1H); 6.61 (s, ArH, 1H); 2.42 (s, Ar(CH3), 3H) ppm. The structure of the molecule was validated by ^{13}C NMR analysis, which was convincingly proven. The ^{13}C NMR spectra indicate characteristics of carbon position in the predicted area. ^{13}C-NMR data (100 MHz, D6-DMSO): δ 189.91 (C-9), 181.41 (C-10), 165.96 (C-8), 164.84 (C-1), 161.79 (C-6), 148.53 (C-3), 135.26 (C-12), 132.97 (C-14), 124.40 (C-4), 120.17 (C-2), 113.53 (C-13), 109.16 (C-11), 108.24 (C-5), 106.74 (C-7), 21.93 (C-15) ppm. The 1H and ^{13}C NMR spectra of the molecule showed a noticeable resonance shift in comparison to the analogous protons and carbons in the unbound compound. Fig. S7a shows the findings of measuring emodin using 1H NMR in D6-DMSO solvent. The phenolic proton has a wide singlet signal at 11.41 ppm (O-H). The phenolic protons caused peaks in the NMR spectra of emodin at 12.11 and 12.04 ppm (O-H). Aromatic protons in four-meta locations were discovered at 7.52, 7.19, 7.14, and 6.61 ppm. Only one methyl (Ar-CH3) functional group (at a quantity of 2.42 ppm) was expressed, according to data from emodin (Fig. S7b). Ketocarbons (C=O) had downfield shifts of 189.91 (C-9) and 181.41 (C-10) ppm, whereas phenolic carbons (Ar-C-(O-H)) had downfield shifts of 165.96 (C-8) and 164.84 (C-10) ppm, respectively (C-1). A forward shift of 21.93 ppm was observed for the methyl carbon (Ar (CH3)) (Fig. S8). All chemical structures and closely similar compounds were compared to previously published literature using spectral data analysis (Li et al. 2000 and Guo et al. 2011). A molecular ion peak at m/z 269.044 was discovered in emodin by LC-ESI-MS analysis (Fig. S9). Based on spectrum data, the molecule was identified as *A. terreus* trihydroxy-6-methyl anthraquinone (emodin), with the molecular formula $C_{15}H_{10}O_5$. The pure compound has a boiling temperature of 263-267 °C and a melting point of 529.0 K.

Docking studies look at the different ways ligands and receptors, enzymes, and other binding sites can bind to one other. The binding energy of the ligand inside the target protein was calculated using Autodock 4.2 study of binding energy. The findings of the Ramachandran plot for the *An. stephensi*, *Ae. aegypti* (3K1E), and *Cx. quinquefasciatus* (3OGN) model structures revealed that the most preferred residues are 93.6, 89.6, and 91.7 %, respectively (Fig. S10). Table S4 shows the values of the dope score of the *An. stephensi* Odorant binding protein. The emodin molecule interacts with odorant-binding proteins with the same active site perfectly, according to molecular docking research. *Ae. aegypti*-emodin (-8.89 kcal/mol) complex forms hydrogen and hydrophobic interactions with active site residues (Table S5), which is higher than the other two complexes (-6.53 and -8.09 kcal/mol). PyMOL was used to analyze intermolecular interactions, and the results are shown in Fig. S11. A summary of binding energy and related parameters can be found in Table S6**.** As best as can be determined from Fig.S12, the binding energy value of the emodin *An. stephensi* complex is -6.53kcal/mol. Four hydrogen bonds are formed between emodin and the amino acid residues Leu143 and His141. In the complex of emodin and *Cx. quinquefasciatus*, the binding energy is -8.09 kcal/mol. With the amino acid residues His111, Ala88, Met84 and Phe123, emodin forms four hydrogen-bonding interactions. In Fig. 6a-c, we show the interaction between proteins and ligands obtained from PyMol software.

Discussion

Bioactive compounds are being isolated from plants, and microbes and this technique have led to the discovery of many essential natural compounds (McRae et al. 2007). The importance of understanding the effects of fungal compounds on the diffusion of host cuticles and larvicidal toxins has greatly increased in recent years (Demain and Fang 2000). *A. terreus* mycelial ethyl acetate extract (ATMEAE) and its fractions were tested against the 4th instar larvae of *Ae. aegypti*, *Cx. quinquefasciatus*, and *An. stephensi*. *A. terreus* fractions with larvicidal activity against target vectors were screened. LC_{50} values of 5.08 to 7.13 mg.L^{-1} were observed for fraction1 (emodin) against all tested mosquitoes. In addition to having malformed pupae and deformed larvae, the treated mosquito larvae displayed restless and irregular movements. Ragavendran et al. (2018) also reported similar behavioral observations. Sharma et al. (2015) examined the effects of *Achyranthes asperaon* extracts against *Ae. aegypti* larvae, which caused behavioral changes and excitation of the biting anal gills. During larval death, the respiratory muscles paralyze causing the larvae to be unable to breathe properly, and they eventually die. Insect skin and cuticle pores allow emodin to enter the larval body, where the compound interferes with molting, malformation, and other metabolic processes. The toxic compounds cause the respiratory system, digestive system, and nervous system to collapse in mosquito larvae/pupae were reported (Lee et al. 2017). Various researchers identified malformed larvae in mosquitos treated with triterpenoids, limonoids, niloticin, and isonimocinolide (Sengottayan 2013) (Reegan et al. 2016). Pradeep et al. (2015) discovered 2, 3, 4, 5-tetrahydroisoquinolimidine-4-ol) generated by *Fusarium moniliforme* applied to third and fourth instar larvae of *Ae. aegypti* (LC_{50}= 237.0 and 276.4) and *An. stephensi* (LC_{50}= 335.6 and 258.1 mg.L^{-1}). When tested against *Cx. quinquefasciatus* larvae in the fourth instar, catechin compounds yielded LC_{50} values of 3.76 and LC_{90} values of 9.79 mg.L^{-1} (Elumalai et al. 2016). The *Streptomyces* sp. metabolite (5-(2, 4-dimethylbenzyl) pyrrolidin-2-one) was reported to be 100 percent effective against *An. stephensi* and *Cx. tritaeniorhynchus* (Saurav et al. 2013). Deepika et al. (2012) isolated (2S, 5R, 6R)-2-hydroxy3, 5, 6-trimethyloctan-4-one from *Streptomyces sp.*, which was found to have larvicidal activity against *An. subpictus* and *Cx. quinquefasciatus* at low doses. Using higher LC_{50} and LC_{90} values (110 and 200 mg.L^{-1}). Murugesan et al. (2009) reported that *Trichophyton mentagrophytes* extracellular metabolites were larvicidal against *Ae. aegypti* larvae in the third instar.

A study of fraction 1 showed that even when administered at low doses, it had remarkable pupicidal activity (with high mortality rates) against the tested mosquitoes. Furthermore, Gandhi et al. (2016) discovered that alizarin had good pupicidal activity against mosquito pupae (LC_{50} and LC_{90} values of 1.97, 4.79, 2.05, and 5.50 mg.L^{-1}). According to Geetha et al. (2010), *Bacillus subtilis* lipopeptide produces cyclic lipopeptides which show superior pupicidal properties against *An. stephensi*. According to the present study, emodin isolated from *A. terreus* had zero hatchability (higher concentration 200 mg.L^{-1}) against selected mosquitoes. It has also been found that diflubenzuron and penfluron are ovicidal against four species of mosquitoes (Prakash 1993). Karthik et al. (2011) reported that the *Streptomyces* sp. metabolite had no effect on the hatchability of eggs against *Cx.triaeniorhynchus* and *Cx.gelidus* when used at 1000 mg.L^{-1} concentrations. Researchers Su and Mulla (1998) reported that azadirachtin had zero ovicidal activity in the eggs of *Culex* species when administered at 10 mg.L^{-1}.

In mosquitoes, acetylcholinesterase plays a major role in resistance mechanisms against chemical insecticides (Solairaj and Rameshthangam 2017). AChE activity was significantly inhibited in three different mosquito larvae exposed to emodin in the current study. It should be noted that emodin is toxic because it inhibits the activity of AChE in the nerve junction, which catalyzes the hydrolysis of acetylcholine. Koodalingam et al. (2011) showed a significant decrease in AChE levels in *Ae. aegypti* larvae that were treated with *Sapindus emarginatus* soap nut extract. The *Aedes* and *Culex* larvae AChE enzyme level was inhibited by stigmasterol and hexacosanol compounds tested by Gade et al. (2017).

Esterase enzymes are involved in dissolving carboxyl ester and phosphodiester bonds to develop resistance to insecticides. Studies with a variety of mosquito vectors have used the detoxification activities of α- and β-carboxylesterase as biomarkers (Agra-Neto et al. 2014); (Selin-Rani et al. 2016). During the development of the larvae, the levels of detoxifying enzymes decreased significantly due to the emodin and the reduction of activity of α and β-carboxylesterase. *Serratia marcescens* (prodigiosin) metabolites likewise inhibit their enzymatic activity and acetylcholinesterase activity in *Ae. aegypti* and *An. stephensi* larvae (Suryawanshi et al. 2015). Edwin et al. (2016) examined the effects of andrographolide from *Andrographis paniculata* on the larvae of *Ae. aegypti,* particularly the inhibition of carboxylate esterase activity. It has been found that acid and alkaline phosphatases play a crucial role in metabolism and signaling processes, and their expression decreases during the developmental stages of insect larvae (Nathan et al. 2007). Compared to control larvae, larvae exposed to emodin showed a decrease in acid and alkaline phosphatase activity. Additionally, salicylic acid-derived nanoparticles inhibited acid and alkaline phosphatase activity in larvae of *Ae. aegypti* (Ga'al et al. 2018).

The molting of midgut cells has been observed to be disrupted by bioactive components generated from natural resources (Kihampa et al., 2009) (da Silva et al. 2013). The cuticle layer, fat body, brush boundary, and nuclei in the midgut and hindgut regions of treated 4th instar larvae were damaged, according to the histological profile of larvae treated with compound/fraction. In larvae treated with *Bacillus licheniformis* exopolysaccharides, Abinaya et al. (2018) looked at histological damage in the midgut, muscles, and abdominal regions shrinking. Similarly, Seetharaman et al. (2017) found that untested *Culex* sp mosquito larvae showed damage in their microvilli, midgut lumen, peritrophic membranes and epithelial cells after exposure to limonoid compound from *Penicillium oxalicum*. As reported by Déciga-Campos et al. (2007), the toxicity of brine shrimp for microbial metabolites or compound fractions above 1000 concentrations is non-toxic, between 500 and 1000 mg.L^{-1} is weakly toxic, and below 500 concentrations is toxic. LC$_{50}$ value for emodin in this study was 153.97 mg.L^{-1}, showing moderate toxic activity. Furthermore, Lee et al. (2002) found that phrymarolin and ursolic compounds exhibited greater toxicity on brine shrimp larvae, and their LD$_{50}$ values were 0.0013 and 27.0 mg.L^{-1}, respectively.

In genetics, cell biology, and embryology, zebrafish (*D. rerio*) embryos are ideal models (Bakkiyanathan et al. 2012). Several investigations on embryotoxic and teratogenic substances, as well as prospective dietary ingredients, have been conducted. The embryogenesis of zebrafish is comparable to that of higher vertebrates, such as humans (Busquet et al. 2008). At a concentration of 15.6 mg.L^{-1} in 96hpf, an isolated

emodin from *A. terreus* inhibited zebrafish embryo body length, hatching rate, and tail deformity somewhat compared to the control. Furthermore, Fan et al. (2015) reported that secondary metabolites from the marine-derived fungus *Penicillium expansum* Y32 inhibited the heartbeat rate during zebrafish embryo culture depending on the doses applied and the period of the treatment. Abutaha et al. (2015) studied the endophytic fraction of *Cochliobolus spicifer* for larvicidal effects and toxicity to zebrafish embryos. The metabolites produced by the fungal fraction did not cause any symptoms of toxicity.

For hypothesizing and assembling logistic predictions, in silico and computational simulation methods can be quite useful (Gaddaguti et al. 2012); (Koech and Mwangi 2013). As a result, it is also possible to identify the binding residues responsible for biological functions. In this study, potential fungal metabolites derived from fungi were examined for their repellent activity against an odorant-binding protein (OBP) of tested mosquitoes. Carvacrol, camphor ocimene, α- and β-pinene, citronellal, geraniol, and linalool were shown to have a higher binding potential for the OBPs associated with mosquitoes' repellent effect (Müller et al. 2009). When compared to two other complexes, the emodin - *Ae. aegypti* combination has high binding energy (-8.89 kcal/mol). It's followed by *An. stephensi* (-6.53 kcal/mol, with hydrogen bonds formed by Leu143 and His141 amino acid residues) and *Cx. quinquefasciatus* (-8.09kcal/mol, with hydrogen bonds formed by His111, Ala88, Met84, and Phe123 amino acid residues). Gaddaguti et al. (2016) discovered that several chemicals from *Ocimum* sp., such as licopersin, γ-sitosterol, and benzene, 1, 2-dimethoxy-4-(2-propenyl)-, exhibited strong binding with high affinity to OBP of (3Q8I), with a G-score of -7.14 and TH57 amino acid residues. Similarly, OBP of (3N7H) revealed G-score of -4.54, ASN56, and CYS53 *Anopheles gambiae* amino acid residues. According to Gopal and Kannabiran (2013), the camphor of *Nilaparvatha lugens* has a binding energy of -136 kcal/mol with OBP1 protein. The oleic acid showed the least binding energies with 1OOF, 2ERB, 3R1O and OBP1. Carvacrol showed the least binding energies with 1QWV and 1TUJ proteins with -117.45 kcal/mol and -21.78 kcal/mol, respectively. *Calotropis gigantea* components (β-amyrin) were studied by Dhivya (2014), who discovered that it had high glide scores (-6.73A°), made 1 H-bond with the target OBP, and comprised HIS111 amino acid residues. Di (2- ethylhexyl) phthalate (-8.66 A°) and alpha amyrin (-5.7A°) are two more components that show substantial binding characteristics with mosquito OBP of *Cx. quinquefasciatus*. Hydrophobic activity and hydrogen bonding were also seen in the isolated molecule when it was tested against comparable amino acids. High negative binding energy values showed the strongest binding affinity between the ligand and the target proteins, according to the current study. We also wanted to know more about the molecular processes driving natural mosquito repellents' interactions with OBP.

Conclusion

The study's findings clearly show that an emodin bioactive component (from *A. terreus*) was effectively extracted and identified using spectrum methods. Interestingly, the isolated emodin showed strong larvicidal activity against *Ae. aegypti* when compared to other mosquitos (LC_{50} = 5.084 and LC_{90} = 8.230 mg.L^{-1}). Hyper-excitation, severe paralysis, and aggressive self-biting movement were seen in emodin-

treated larvae with anal gills that form a circle or ring structure. Fraction 1 had the lowest LC_{50} and LC_{90} values (LC_{50} = 0.349 and LC_{90} = 0.872 mg.L^{-1}) and had a pupicidal efficiency of over 80% against *Ae. aegypti*. This (emodin) metabolite generated by *A. terreus* appears to activate biochemicals such as acetylcholine esterase, and-carboxyl esterases, and phosphatases. Histological changes in emodin-treated 4th instar larvae of examined mosquitos included mildly injured muscles, gastric caeca cell rupture, disordered brush border, and discharges of cytoplasmic debris in the gastric caeca lumen. At higher doses (200 mg.L^{-1}), the emodin chemical showed no percentage of egg hatchability on target mosquitos. *Artemia nauplii* was shown to be reasonably hazardous in a bio-toxicity experiment of non-target organisms evaluated with emodin. The chemical demonstrated a high binding contact with the odorant-binding proteins of all examined mosquitos, according to the computational study. The bioassay of emodin treated with *A. nauplii* revealed that the toxicity is mostly determined by compound dosages. The survival and hatching rates of zebrafish embryos treated with emodin were found to be significantly lower. The obtained results will give better avenues for selecting the most relevant compounds for the design and development of efficient, safe, and environmentally friendly mosquito repellents soon than the current harmful synthetic repellents.

Declarations

Acknowledgments

Author contribution

The general design and planning of the experiment, larval data processing, and result interpretation were all provided by CR, GB, MS, PW, V.P, SSN, P.K and DN. The molecular docking investigation and interpretation were carried out by MS and CR. The brine shrimp test toxicity experiments were carried out by CR. The embryo toxicity test was carried out by VM. The manuscript was co-written by CR, GB, PW, V.P, SSN, P.K and DN. The final document has been thoroughly examined and authorised for publication by all of the writers and special contribution by DA, P.K and DN.

Compliance with Ethical Standards

The present research entities does not contain any studies with human participants or animals performed by any of the authors.

Data availability statements

During the present research entities, the datasets gathered and generated from the analysis after extraction/ separation/ isolation of compound emodin and evaluated biological results are available from the corresponding author on reasonable request.

 Ethics approval and consent to participate: N/A

Consent for publication: N/A

Conflict of interests

The authors declare no conflict of interests for publication of this original article.

References

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

2. Abinaya, M., Vaseeharan, B., Divya, M., Vijayakumar, S., Govindarajan, M., Alharbi, N.S., Khaled, J.M., Al-Anbr, M.N., Benelli, G., 2018. Structural characterization of *Bacillus licheniformis* Dahb1 exopolysaccharide—antimicrobial potential and larvicidal activity on malaria and Zika virus mosquito vectors. Environ. Sci. Poll. Res. 25, 18604-18619.

3. 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, 405-414.

4. Agra-Neto, A.C., Napoleão, T.H., Pontual, E.V., de Lima Santos, N.D., de Andrade Luz, L., de Oliveira, C.M.F., de Melo-Santos, M.A.V., Coelho, L.C.B.B., do Amaral Ferraz Navarro, D.M., Paiva, P.M.G., 2014. Effect of *Moringa oleifera* lectins on survival and enzyme activities of Aedes aegypti larvae susceptible and resistant to organophosphate. Parasitol. Res. 113, 175-184.

5. Anjum, A., Shetty, S.K.A., Ahmed, M., Sridhar, B., Vijaya, K., 2012. Development and validation of RPHPLC method for the quantitative estimation of cefadroxil monohydrate in bulk and Pharmaceutical dosage forms. Int. J. Chem. Sci 10, 150-158.

6. Asakura, K., 1978. Phosphatase activity in the larva of the euryhaline mosquito, Aedes togoi Theobald, with special reference to sea-water adaptation. J. Exp. Mar. Biol. Ecol. 31, 325-337.

7. Bakkiyanathan, A., Nathan, J.R., Ravikumar, S., Gopalakrishnan, T.S., Aruldas, F.M.M., Malathi, R., 2012. Anti-angiogenic effects of theophylline on developing zebrafish (*Danio rerio*) embryos. Biomed. Prevent. Nutrit. 2, 174-178.

8. Balumahendhiran, K., Vivekanandhan, P. and Shivakumar, M.S., 2019. Mosquito control potential of secondary metabolites isolated from Aspergillus flavus and Aspergillus fumigatus. Biocatalysis and Agricultural Biotechnology, 21, p.101334.

9. Bentley, M.D., Day, J.F., 1989. Chemical ecology and behavioral aspects of mosquito oviposition. Annual Review Entomol 34, 401-421.

10. Benton, R., Vannice, K.S., Gomez-Diaz, C., Vosshall, L.B., 2009. Variant ionotropic glutamate receptors as chemosensory receptors in *Drosophila*. Cell 136, 149-162.

11. Bhatia, R., Ortega, L., Dash, A., Mohamed, A.J., 2014. Vector-borne diseases in South-East Asia: burdens and key challenges to be addressed. WHO South-East Asia J. Pub. Health 3, 2-4.

12. Braunbeck, T., Kais, B., Lammer, E., Otte, J., Schneider, K., Stengel, D., Strecker, R., 2015. The fish embryo test (FET): origin, applications, and future. Environ. Sci. Poll. Res. 22, 16247-16261.

13. Busquet, F., Nagel, R., von Landenberg, F., Mueller, S.O., Huebler, N., Broschard, T.H., 2008. Development of a New Screening Assay to Identify Proteratogenic Substances using Zebrafish *Danio rerio* Embryo Combined with an Exogenous Mammalian Metabolic Activation System (m Dar T). Toxicol. Sci. 104, 177-188.

14. Busquet, F., Strecker, R., Rawlings, J.M., Belanger, S.E., Braunbeck, T., Carr, G.J., Cenijn, P., Fochtman, P., Gourmelon, A., Hübler, N., 2014. OECD validation study to assess intra-and inter-laboratory reproducibility of the zebrafish embryo toxicity test for acute aquatic toxicity testing. Reg.Toxicol. Pharmacol. 69, 496-511.

15. Cabrera, M., Jaffe, K., 2007. An aggregation pheromone modulates lekking behavior in the vector mosquito *Aedes aegypti* (Diptera: Culicidae). J. Ameri. Mosq. Cont. Assoc. 23, 1-10.

16. Calvo, E., Mans, B.J., Andersen, J.F., Ribeiro, J.M., 2006. Function and evolution of a mosquito salivary protein family. J. Biol. Chem. 281, 1935-1942.

17. Case, D.A., Babin, V., Berryman, J., Betz, R., Cai, Q., Cerutti, D., Cheatham Iii, T., Darden, T., Duke, R., Gohlke, H., 2014. Amber 14.

18. Chenniappan, K., Kadarkarai, M., 2008. Oviposition deterrent, ovicidal and gravid mortality effects of ethanolic extract of *Andrographis paniculata* Nees against the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). Entomol. Res. 38, 119-125.

19. da Silva, O.S., da Silva, F.C., de Barros, F.M.C., da Silva, J.L.R., de Loreto Bordignon, S.A., Eifler-Lima, V.L., von Poser, G.L., Prophiro, J.S., 2013. Larvicidal and growth-inhibiting activities of extract and benzopyrans from *Hypericum polyanthemum* (Guttiferae) against *Aedes aegypti* (Diptera: Culicidae). Ind. Crop. Prod. 45, 236-239.

20. De Bruyne, M., Baker, T.C., 2008. Odor detection in insects: volatile codes. J. Chem. Ecol. 34, 882-897.

21. Déciga-Campos, M., Rivero-Cruz, I., Arriaga-Alba, M., Castañeda-Corral, G., Angeles-López, G.E., Navarrete, A., Mata, R., 2007. Acute toxicity and mutagenic activity of Mexican plants used in traditional medicine. J. Ethnopharm. 110, 334-342.

22. 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. Parasitol. Res. 111, 1151-1163.

23. DeLano, W.L., 2002. Pymol: An open-source molecular graphics tool. CCP4 Newsl. Protein Crystallogr. 40, 82-92.

24. Demain, A.L., Fang, A., 2000. The natural functions of secondary metabolites. Hist. Mod. Biotech. I, 1-39.

25. Dhivya, R., 2014. In silico molecular docking and molecular dynamics applications in the designing of a new mosquito repellent from the plant *Calotropis gigantea* targeting the odorant binding protein of *Culex quinquefasciatus*. Int. J. Pharm. Phytopharm. Res. 3.

26. Edwin, E.-S., Vasantha-Srinivasan, P., Senthil-Nathan, S., Thanigaivel, A., Ponsankar, A., Pradeepa, V., Selin-Rani, S., Kalaivani, K., Hunter, W.B., Abdel-Megeed, A., 2016. Anti-dengue efficacy of bioactive andrographolide from *Andrographis paniculata* (Lamiales: Acanthaceae) against the primary dengue vector *Aedes aegypti* (Diptera: Culicidae). Acta Trop. 163, 167-178.

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

28. Elumalai, D., Hemavathi, M., Hemalatha, P., Deepaa, C.V., Kaleena, P.K., 2016. Larvicidal activity of catechin isolated from *Leucas aspera* against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol. Res. 115, 1203-1212.

29. Eswar, N., Webb, B., Marti-Renom, M.A., Madhusudhan, M., Eramian, D., Shen, M.y., Pieper, U., Sali, A., 2006. Comparative protein structure modeling using Modeller. Curr. Protocol. Bioinfo. 15, 5.6. 1-5.6. 30.

30. Fan, Y.-Q., Li, P.-H., Chao, Y.-X., Chen, H., Du, N., He, Q.-X., Liu, K.-C., 2015. Alkaloids with cardiovascular effects from the marine-derived fungus *Penicillium expansum* Y32. Marine Drug. 13, 6489-6504.

31. Foster, W.A., Hancock, R., 1994. Nectar-related olfactory and visual attractants for mosquitoes. J. Amer. Mosq. Cont. Assoc. 10, 288-296.

32. Frisch, M., Trucks, G., Schlegel, H.e.a., Scuseria, G., Robb, M., Cheeseman, J., Montgomery Jr, J., Vreven, T., Kudin, K., Burant, J., 2004. Gaussian 03, revision C. 02. Gaussian 03 Rev. E. 01. *http://www. gaussian. com/*. Gaussian, Inc., Wallingford, CT.

33. Ga'al, H., Fouad, H., Tian, J., Hu, Y., Abbas, G., Mo, J., 2018. Synthesis, characterization and efficacy of silver nanoparticles against *Aedes albopictus* larvae and pupae. Pest. Biochem. Physiol. 144, 49-56.

34. Gaddaguti, V., Mounika, S.J., Sowjanya, K., Rao, T., Chakravarthy, M., Allu, R., 2012. GCMS analysis and in silico molecular docking studies of mosquito repellent compounds from *Hyptis suaveolens*. Int J Bioassay. 1, 36-41.

35. Gaddaguti, V., Venkateswara Rao, T., Prasada Rao, A., 2016. Potential mosquito repellent compounds of Ocimum species against 3N7H and 3Q8I of *Anopheles gambiae*. 3 Biotech 6, 1-8.

36. Gade, S., Rajamanikyam, M., Vadlapudi, V., Nukala, K.M., Aluvala, R., Giddigari, C., Karanam, N.J., Barua, N.C., Pandey, R., Upadhyayula, V.S.V., 2017. Acetylcholinesterase inhibitory activity of stigmasterol & hexacosanol is responsible for larvicidal and repellent properties of *Chromolaena odorata*. Biochim. Biophys. Acta. 1861, 541-550.

37. Gandhi, M., Reegan, A., Ganesan, P., Sivasankaran, K., Paulraj, M., Balakrishna, K., Ignacimuthu, S., Al−Dhabi, N., 2016. Larvicidal and pupicidal activities of alizarin isolated from roots of *Rubia cordifolia* against *Culex quinquefasciatus* Say and *Aedes aegypti* (L.)(Diptera: Culicidae). Neotrop. Entomol. 45, 441-448.

38. Geetha, I., Manonmani, A., Paily, K., 2010. Identification and characterization of a mosquito pupicidal metabolite of a *Bacillus subtilis* subsp. *subtilis* strain. Appl. Microbiol. Biotech. 86, 1737-1744.

39. Georgalas, V., Malavasi, S., Franzoi, P., Torricelli, P., 2007. Swimming activity and feeding behaviour of larval European sea bass (*Dicentrarchus labrax* L): Effects of ontogeny and increasing food density. Aquaculture 264, 418-427.

40. Gopal, J.V., Kannabiran, K., 2013. Studies on interaction of insect repellent compounds with odorant binding receptor proteins by in silico molecular docking approach. Interdiscipl. Sci.: Comput. Life Sci. 5, 280-285.

41. Kaewnang-O, E., Ngampongsai, A., Subhadhirasakul, S., Srichana, T., 2011. Toxicity of fixed oil and crude extract from sa-dao-thiam, *Azadirachta excelsa* (Jack) seed kernel to *Aedes aegypti* (L.). Songklanakarin J. Sci. Technol. 33.

42. Kalaimurugan, D., Vivekanandhan, P., Sivasankar, P., Durairaj, K., Senthilkumar, P., Shivakumar, M.S. and Venkatesan, S., 2019. Larvicidal activity of silver nanoparticles synthesized by Pseudomonas fluorescens YPS3 isolated from the Eastern Ghats of India. Journal of Cluster Science, 30(1), pp.225-233.

43. Karthik, L., Gaurav, K., Rao, K., 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.

44. Khan, M.S.A., Ahmed, N., Arifuddin, M., Zakaria, Z.A., Al-Sanea, M.M., Khundmiri, S.U.K., Ahmed, I., Ahmed, S., Mok, P.L., 2018. Anti-nociceptive mechanisms of flavonoids-rich methanolic extract from *Terminalia coriacea* (Roxb.) Wight & Arn. leaves. Food Chem. Toxicol. 115, 523-531.

45. Kihampa, C., Joseph, C.C., Nkunya, M.H., Magesa, S.M., Hassanali, A., Heydenreich, M., Kleinpeter, E., 2009. Larvicidal and IGR activity of extract of Tanzanian plants against malaria vector mosquitoes. J Vect. Born. Dis. 46, 145.

46. Koech, P.K.a., Mwangi, R.W., 2013. Repellent activities of *Ocimum basilicum*, *Azadirachta indica* and *Eucalyptus citriodora* extracts on rabbit skin against *Aedes aegypti*. J. Entomol. Zool. Stud. 1 (5), 84-91.

47. Koodalingam, A., Mullainadhan, P., Arumugam, M., 2011. Effects of extract of soapnut *Sapindus emarginatus* on esterases and phosphatases of the vector mosquito, *Aedes aegypti* (Diptera: Culicidae). Acta Trop. 118, 27-36.

48. Krutmuang, P. and Mekchay, S., 2005, October. Pathogenicity of entomopathogenic fungi *Metarhizium anisopliae* against termites. In Conference on International Agricultural Research for Development. Stuttgart-Hohenheim (pp. 1-4).

49. Kulkarni, P., Gupta, N., 2013. Screening and evaluation of soil fungal isolates for xylanase production. Recent Res. Sci. Technol. 5 (2) 33-36.

50. Lee, B.-M., Kacew, S., Kim, H.S., 2017. Lu's basic toxicology: fundamentals, target organs, and risk assessment. CRC press.

51. Lee, S., Min, B., Kho, Y., 2002. Brine shrimp lethality of the compounds from *Phryma leptostachya* L. Arch. Pharm. Res. 25, 652-654.

52. Li, Q., Wang, P., Chen, L., Gao, H., Wu, L., 2016. Acute toxicity and histopathological effects of naproxen in zebrafish (*Danio rerio*) early life stages. Environ. Sci. Poll. Res. 23, 18832-18841.

53. Lin, S.-Y., Lai, W.-W., Ho, C.-C., Yu, F.-S., Chen, G.-W., Yang, J.-S., Liu, K.-C., Lin, M.-L., Wu, P.-P., Fan, M.-J., 2009. Emodin induces apoptosis of human tongue squamous cancer SCC-4 cells through reactive oxygen species and mitochondria-dependent pathways. Anticanc. Res. 29, 327-335.

54. Logeswaran, C., Vivekanandhan, P. and Shivakumar, M.S., 2019. Chemical constituents of thermal stress induced Ganoderma applantum (Per.) secondary metabolites on larvae of Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus and histopathological effects in mosquito larvae. Biocatalysis and Agricultural Biotechnology, 20, p.101253.

55. Lu, T., Qiu, Y.T., Wang, G., Kwon, J.Y., Rutzler, M., Kwon, H.-W., Pitts, R.J., van Loon, J.J., Takken, W., Carlson, J.R., 2007. Odor coding in the maxillary palp of the malaria vector mosquito *Anopheles gambiae*. Curr. Biol. 17, 1533-1544.

56. Mannan, H.A., Ahmed, I., Hussain, I., Jamil, M., Miza, B., 2012. Antibacterial activity and brine shrimp toxicity of *Artemisia dubia* extract. Pakistan J. Bot. 44, 1487-1490.

57. McRae, J., Yang, Q., Crawford, R., Palombo, E., 2007. Review of the methods used for isolating pharmaceutical lead compounds from traditional medicinal plants. Environmental. 27, 165-174.

58. Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D., McLaughlin, J.L., 1982. Brine shrimp: a convenient general bioassay for active plant constituents. Plant. Med. 45, 31-34.

59. Minguez, L., Pedelucq, J., Farcy, E., Ballandonne, C., Budzinski, H., Halm-Lemeille, M.-P., 2016. Toxicities of 48 pharmaceuticals and their freshwater and marine environmental assessment in northwestern France. Environ. Sci. Poll. Res. 23, 4992-5001.

60. Morris, G.M., Goodsell, D.S., Halliday, R.S., Huey, R., Hart, W.E., Belew, R.K., Olson, A.J., 1998. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J. Comput. Chem. 19, 1639-1662.

61. Morris, G.M., Huey, R., Lindstrom, W., Sanner, M.F., Belew, R.K., Goodsell, D.S., Olson, A.J., 2009. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J. Comput. Chem. 30, 2785-2791.

62. Müller, G.C., Junnila, A., Butler, J., Kravchenko, V.D., Revay, E.E., Weiss, R.W., Schlein, Y., 2009. Efficacy of the botanical repellents geraniol, linalool, and citronella against mosquitoes. J. Vect. Ecol. 34, 2-8.

63. Murugesan, A., Sathesh Prabu, C., Selvakumar, C., 2009. Biolarvicidal activity of extracellular metabolites of the keratinophilic fungus Trichophyton mentagrophytes against arvae of Aedes aegypti—a major vector for Chikungunya and dengue. Fol. Microbiol. 54, 213-216.

64. Nathan, S.S., Choi, M.-Y., Paik, C.-H., Seo, H.-Y., 2007. Food consumption, utilization, and detoxification enzyme activity of the rice leaffolder larvae after treatment with Dysoxylum triterpenes. Pest. Biochem. Physiol. 88, 260-267.

65. OECD, 1992. Guidelines for the Testing of Chemicals: 203—Fish, Acute Toxicity Test. OECD Library.

66. OECD, 2013. Guideline for testing of chemicals, 236. In: Fish embryo acute toxicity (FET) test. Organization for Economic Cooperation and Development.

67. Pandey, V., Chopra, M., Agrawal, V., 2011. In vitro isolation and characterization of biolarvicidal compounds from micropropagated plants of *Spilanthes acmella*. Parasitol. Res. 108, 297-304.

68. Pradeep, F.S., Palaniswamy, M., Ravi, S., Thangamani, A., Pradeep, B., 2015. Larvicidal activity of a novel isoquinoline type pigment from *Fusarium moniliforme* KUMBF1201 against Aedes aegypti and Anopheles stephensi. Proc. Biochem. 50, 1479-1486.

69. Pratheeba, T., Vivekanandhan, P., Faeza, A.N. and Natarajan, D., 2019. Chemical constituents and larvicidal efficacy of Naringi crenulata (Rutaceae) plant extracts and bioassay guided fractions against Culex quinquefasciatus mosquito (Diptera: Culicidae). Biocatalysis and Agricultural Biotechnology, 19, p.101137.

70. Prakash, A., 1993. Ovicidal action of certain chitin synthesis inhibitors in mosquitoes. Entomon-Trivandrum- 17, 15-15.

71. Rajula, J., Rahman, A. and Krutmuang, P., 2020. Entomopathogenic fungi in Southeast Asia and Africa and their possible adoption in biological control. Biological control, 151, p.104399.

72. 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. Poll. Res. 22, 17224-17237.

73. 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.

74. Ramachandran, G.N., Ramakrishnan, C., Sasisekharan, V., 1963. Stereochemistry of polypeptide chain configurations. J. Mol. Biol. 7, 95-99.

75. Ramachandran, G.N., Sasisekharan, V., 1968. Conformation of polypeptides and proteins. Adv. Prot. Chem. 23, 283-438.

76. Reegan, A.D., Stalin, A., Paulraj, M.G., Balakrishna, K., Ignacimuthu, S., Al-Dhabi, N.A., 2016. In silico molecular docking of niloticin with acetylcholinesterase 1 (AChE1) of Aedes aegypti L.(Diptera: Culicidae): a promising molecular target. Med. Chem. Res. 25, 1411-1419.

77. Ronderos, D., Smith, D., 2009. Diverse signaling mechanisms mediate volatile odorant detection in Drosophila. Fly 3, 290-297.

78. 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.

79. Scholz, S., Ortmann, J., Klüver, N., Léonard, M., 2014. Extensive review of fish embryo acute toxicities for the prediction of GHS acute systemic toxicity categories. Reg. Toxicol. Pharmacol. 69, 572-579.

80. 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. Poll. Res. 24, 21272-21282.

81. Selin-Rani, S., Senthil-Nathan, S., Revathi, K., Chandrasekaran, R., Thanigaivel, A., Vasantha-Srinivasan, P., Ponsankar, A., Edwin, E.S., Pradeepa, V., 2016. Toxicity of *Alangium salvifolium* Wang chemical constituents against the tobacco cutworm *Spodoptera litura* Fab. Pest. Biochem. Physiol.126, 92-101.

82. Sengottayan, S.-N., 2013. Physiological and biochemical effect of neem and other Meliaceae plants secondary metabolites against Lepidopteran insects. Front. Physiol. 4, 359.

83. Sharma, A., Kumar, S., Tripathi, P., 2015. Impact of Achyranthes aspera leaf and stem extracts on the survival, morphology and behaviour of an Indian strain of dengue vector, *Aedes aegypti* L.(Diptera: Culicidae). J. Mosq. Res. 5(7) 1-9.

84. Solairaj, D., Rameshthangam, P., 2017. Silver nanoparticle embedded α-chitin nanocomposite for enhanced antimicrobial and mosquito larvicidal activity. J. Poly. Environ. 25, 435-452.

85. Songa, E.A., Okonkwo, J.O., 2016. Recent approaches to improving selectivity and sensitivity of enzyme-based biosensors for organophosphorus pesticides: A review. Talanta 155, 289-304.

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

87. Suryawanshi, R.K., Patil, C.D., Borase, H.P., Narkhede, C.P., Salunke, B.K., Patil, S.V., 2015. Mosquito larvicidal and pupaecidal potential of prodigiosin from *Serratia marcescens* and understanding its mechanism of action. Pest. Biochem. Physiol. 123, 49-55.

88. Tabanca, N., Bernier, U.R., Ali, A., Wang, M., Demirci, B., Blythe, E.K., Khan, S.I., Baser, K.H.C., Khan, I.A., 2013. Bioassay-guided investigation of two Monarda essential oils as repellents of yellow fever mosquito *Aedes aegypti*. J.Agricul. Food Chem. 61, 8573-8580.

89. Takken, W., 1991. The role of olfaction in host-seeking of mosquitoes: a review. Int. J. Trop. Ins. Sci. 12, 287-295.

90. Tegoni, M., Campanacci, V., Cambillau, C., 2004. Structural aspects of sexual attraction and chemical communication in insects. Trend. Biochem. Sci. 29, 257-264.

91. Van Asperen, K., 1962. A study of housefly esterases by means of a sensitive colorimetric method. J. Insect. Physiol. 8, 401-416.

92. Venkateswara Rao, J., Kavitha, P., Jakka, N., Sridhar, V., Usman, P., 2007. Toxicity of organophosphates on morphology and locomotor behavior in brine shrimp, *Artemia salina*. Arch. Environ. Contam. Toxicol. 53, 227-232.

93. Vieira, F.G., Rozas, J., 2011. Comparative genomics of the odorant-binding and chemosensory protein gene families across the Arthropoda: origin and evolutionary history of the chemosensory system. Gen. Biol. Evol. 3, 476-490.

94. Vijayakumari, B., Sasikala, V., Radha, S.R., Rameshwar, H.Y., 2016. In silico analysis of aqueous root extract of *Rotula aquatica* Lour for docking analysis of the compound 3-O-acetyl-11-keto-β-boswellic acid contents. SpringerPlus 5, 1-8.

95. Vivekanandhan, P., Kavitha, T., Karthi, S., Senthil-Nathan, S. and Shivakumar, M.S., 2018a. Toxicity of *Beauveria bassiana*-28 mycelial extracts on larvae of Culex quinquefasciatus mosquito (Diptera: Culicidae). International journal of environmental research and public health, 15(3), p.440.

96. Vivekanandhan, P., Karthi, S., Shivakumar, M.S. and Benelli, G., 2018b. Synergistic effect of entomopathogenic fungus Fusarium oxysporum extract in combination with temephos against three major mosquito vectors. Pathogens and global health, 112(1), pp.37-46.

97. Vivekanandhan, P., Deepa, S., Kweka, E.J. and Shivakumar, M.S., 2018c. Toxicity of Fusarium oxysporum-VKFO-01 derived silver nanoparticles as potential inseciticide against three mosquito vector species (Diptera: Culicidae). Journal of Cluster Science, 29(6), pp.1139-1149.

98. Vivekanandhan, P., Bedini, S. and Shivakumar, M.S., 2020a. Isolation and identification of entomopathogenic fungus from Eastern Ghats of South Indian forest soil and their efficacy as biopesticide for mosquito control. Parasitology international, 76, p.102099.

99. Vivekanandhan, P., Swathy, K., Kalaimurugan, D., Ramachandran, M., Yuvaraj, A., Kumar, A.N., Manikandan, A.T., Poovarasan, N., Shivakumar, M.S. and Kweka, E.J., 2020b. Larvicidal toxicity of *Metarhizium anisopliae* metabolites against three mosquito species and non-targeting organisms. Plos one, 15(5), p.e0232172.

100. Vivekanandhan, P., Thendralmanikandan, A., Kweka, E.J. and Mahande, A.M., 2021a. Resistance to temephos in Anopheles stephensi larvae is associated with increased cytochrome P450 and α-esterase genes overexpression. International Journal of Tropical Insect Science, 41(4), pp.2543-2548.

101. Vivekanandhan, P., Swathy, K., Thomas, A., Kweka, E.J., Rahman, A., Pittarate, S. and Krutmuang, P., 2021b. Insecticidal efficacy of microbial-mediated synthesized copper nano-pesticide against insect pests and non-target organisms. International journal of environmental research and public health, 18(19), p.10536.

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

103. Vivekanandhan, P., Swathy, K. and Shivakumar, M.S., 2022b. Identification of insecticidal molecule aucubin from *Metarhizium anisopliae* ethyl acetate crude extract against disease mosquito vector. International Journal of Tropical Insect Science, pp.1-16.

104. Ward, M., Benelli, G., 2017. Avian and simian malaria: do they have a cancer connection? Parasitol. Res. 116, 839-845.

105. WHO, G., 1996. Report of the WHO Informal Consultation on the Evaluation and Testing of Insecticides. World Health Organization Geneva.

106. Zhang, L.-h., ZHANG, J.-l., LIU, Y.-c., CAO, Z.-y., HAN, J.-m., Juan, Y., DONG, J.-g., 2013. Isolation and structural speculation of herbicide-active compounds from the metabolites of *Pythium aphanidermatum*. J. Integ. Agricul. 12, 1026-1032.

Tables

Table 1 Larvicidal efficacy of bioactive fractions from *A. terreus* against *Ae. aegypti, Cx. quinequefasicatus* and *An. stephensi,* IVth instars larvae (after 12 hr of exposure).

Mosquito species	Name of the fractions	Concentration of fractions (mg.L^{-1})	Percent Mortality ±SD	LC$_{50}$ (LCL-UCL)	LC$_{90}$(LCL-UCL)	χ^2 (df=3)
Ae. aegypti	F1	50	54.0±1.0	5.084	8.230	3.736
		100	66.0±1.0	(0.233-14.380)	(0.609-19.903)	
		150	88.0±1.1			
		200	100±1.3			
		control	0.00±0.00			
Cx. quinquefasciatus		50	30.0±1.0	7.129	12.008	3.093
		100	46.1±1.0	(0.129-19.203)	(0.849-27.149)	
		150	62.1±0.5			
		200	98.1±1.3			
		control	0.00±0.00			
An. stephensi		50	22.1±0.5	6.395	15.240	2.034
		100	38.0±0.5	(0.000-27.994)	(0.000-43.997)	
		150	86.00±1.0			
		200	99.1±2.3			
		control	0.00±0.00			
Ae. aegypti	F2	50	28.0±0.5	6.297	9.612	3.094
		100	30.1±1.0	(0.231-18.171)	(0.651-40.141)	
		150	38.0±0.5			
		200	42.0±1.0			
		control	0.00±0.00			
Cx. quinquefasciatus		50	22.0±0.5	9.111	19.538	2.324
		100	26.4±0.5	(0.000-30.797)	(0.000-47.315)	
		150	34.0±1.5			
		200	44.0±0.5			
		control	0.00±0.00			
An. stephensi		50	20.1±0.5	11.85	21.643	2.356
		100	28.1±0.5			

		150	30.0±1.0	(0.000-15.371)	(0.002-30.417)	
		200	40.0±0.5			
		control	0.00±0.00			
Ae. aegypti	F3	50	22.0±0.5	6.748	9.381	2.531
		100	26.1±0.5	(0.002-18.177)	(0.231-27.305)	
		150	28.0±0.5			
		200	30.0±1.7			
		control	0.00±0.00			
Cx. quinquefasciatus		50	16.0±0.5	12.189	15.717	2.821
		100	20.0±0.5	(0.123-19.371	(0.221-40.171	
		150	26.0±0.5			
		200	30.0±1.0			
		control	0.00±0.00			
An. stephensi		50	20.0±1.0	13.451	16.237	3.817
		100	24.1±1.0	(0.000-21.171	(0.000-42.171)	
		150	28.1±1.1			
		200	32.0±0.5			
		control	0.00±0.00			
		Azadirachtin	100±0.00	3.652	5.118	4.554
				(0.126-10.808)	(0.253-13.535)	

Control (deionized water with DMSO) - nil mortality, Reference- Azadirachtin (200ppm) LC_{50} – Lethal concentration that kills 50% of the exposed larvae, LC_{90} – Lethal concentration that kills 90% of the exposed larvae, LCL = Lower confidence limit, UCL = Upper confidence limit, *df* degree of freedom, χ^2 – Chi-square values are significant at *P*<0.05 levels. [a]Mean value of triplicates.

Table 2 Pupicidal efficacy of *A. terreus* fractions (F1, F2 and F3) against *Ae. aegypti, An. stepheni* and *Cx. quinquefasciatus* (after 24 hr of exposure).

Mosquito species	Name of the fractions	Concentrations ($mg.L^{-1}$)	Percent[a] Mortality ±SD	LC_{50} (LCL-UCL) ppm	LC_{90} (LCL-UCL) ppm	χ^2 (df=3)
Ae. aegypti		50	60.0 ±1.0	0.349	0.872	1.161
		100	72.0 ±1.0	(0.00-5.692)	(0.00-9.184)	
		150	76.0± 0.5			
		200	82.1± 0.5			
		control	0.0± 0.0			
An. stephensi	F1	50	56.0± 0.5	0.934	2.069	1.717
		100	64.0± 0.5	(0.00-11.469)	(0.00-12.512)	
		150	72.0± 1.0			
		200	82.2 ±1.1			
		control	0.0 ±0.0			
Cx. quinquefasciatus		50	46.0 ±1.0	1.926	4.094	1.643
		100	54.0 ±1.0	(0.00-11.469)	(0.00-17.680)	
		150	66.0 ±1.0			
		200	72.0 ±1.0			
		control	0.0 ±0.0			
Ae. aegypti		50	34.0 ±0.5	1.288	3.718	0.693
		100	42.0 ±1.0	(0.00-10.469)	(1.341-18.411)	
		150	46.0 ±0.5			
		200	52.0 ±0.5			
		control	0.0 ±0.0			
An. stephensi	F2	50	34.0± 0.5	0.488	1.791	1.185
		100	42.0 ±1.0	(0.00-8.451)	(0.00-20.141)	
		150	46.1 ±0.5			
		200	52.0± 0.5			
		control	0.0 ±0.0			
		50	26.0± 1.1	1.897	5.894	2.836
		100	30.0 ±1.0			

Mosquito species	Fraction	Concentration	Egg hatchability (%)[a]	LC$_{50}$ (LCL-UCL)	LC$_{90}$ (LCL-UCL)	χ^2
Cx. quinquefasciatus		150	38.0 ±0.5	(0.00-12.415)	(0.00-21.541)	
		200	42.0 ±1.0			
		control	0.0 ±0.0			
Ae. aegypti		50	26.0 ±1.1	4.680	10.920	2.422
		100	30.0± 1.1	(0.00-22.438)	(0.00-35.387)	
		150	34.0 ±0.5			
		200	42.0 ±1.0			
		control	0.0 ±0.0			
An. stephensi	F3	50	22.0 ±0.5	2.285	8.338	1.163
		100	26.0± 0.5	(0.00-19.514)	(0.00-39.141)	
		150	30.0 ±1.0			
		200	34.0 ±1.5			
		control	0.0 ±0.0			
Cx. quinquefasciatus		50	26.0± 0.5	3.548	8.823	1.242
		100	38.0 ±0.5	(0.00-20.569)	(0.00-33.007)	
		150	40.0 ±1.1			
		200	41.0 ±0.5			
		Control	0.0 ±0.0			
		Azadirachtin	100±0.00	4.216 (0.262-11.495)	6.052 (0.521-14.731)	5.809

Mosquito species Egg hatchability (%)

Control-(deionized water with DMSO) - nil mortality, Reference- Azadirachtin (200ppm) LC$_{50}$ − Lethal concentration that kills 50% of the exposed pupae, LC$_{90}$ − Lethal concentration that kills 90% of the exposed larvae LCL = Lower confidence limit, UCL = Upper confidence limit, *df* degree of freedom, χ^2 − Chi-square values are significant at *P*<0.05 levels. [a]Mean value of triplicates.

Table 3 Ovicidal activity of bioactive fractions (F1-F3) from *A. terreus* against *An. stephensi, Cx. quinequefasicatus* and *Ae. aegypti.* Concentrations (mg.L^{-1})

	Name of the fraction	Control	50	100	150	200
An. stephensi	F1	100.0±0.0	56.0±1.5	40.0±1.5	14.0±0.2	NH
Cx. quinquefasciatus		97.5±0.0	61.0±0.7	42.7±0.7	13.4±0.7	NH
Ae. aegypti		98.3±0.0	51.3±1.1	32.2±0.5	6.9±0.0	NH
An. stephensi		99.0±1.2	68.6±0.5	55.2±1.3	44.7±0.0	32.8±1.2
Cx. quinquefasciatus	F2	100.0±0.2	74.2±1.1	63.2±1.3	43.5±1.2	36.6±0.4
Ae. aegypti		98.0±0.3	71.2±1.5	60.0±1.5	47.8±0.5	39.0±0.7
An. stephensi		99.8±0.0	86.1±0.4	74.5±1.2	68.9±0.6	56.1±1.2
Cx. quinquefasciatus	F3	96.2±1.2	88.0±0.9	77.2±0.0	55.0±0.9	40.0±1.6
Ae. aegypti		99.5±0.4	76.3±0.0	64.0±0.0	57.1±0.0	43.2±0.9
	Azadirachtin	100.0±0.0	89.2±0.5	97.01±0.2	NH	NH

Each value (X ±SD) represents mean of triplicate values

NH -No hatchability (100% mortality), Azadirachtin- Positive control

Table 4 Bio-toxicity assay of Brine shrimp lethality using emodin

Concentrations (ppm)	Percentage of mortality			
	24hrs			
		LC_{50} (LCL-UCL) (mg.L^{-1})	LC_{90} (LCL-UCL) (mg.L^{-1})	χ^2 (df=11)
2.0	8.6±0.5			
4.0	24.6±2.5			
6.0	42.0±1.0			
8.0	54.0±1.0	154.0 (119.5-184.9)	184.51 (148.4 -216.3)	9.891
10	64.0±2.0			
control	0.0±0.0			

Figures
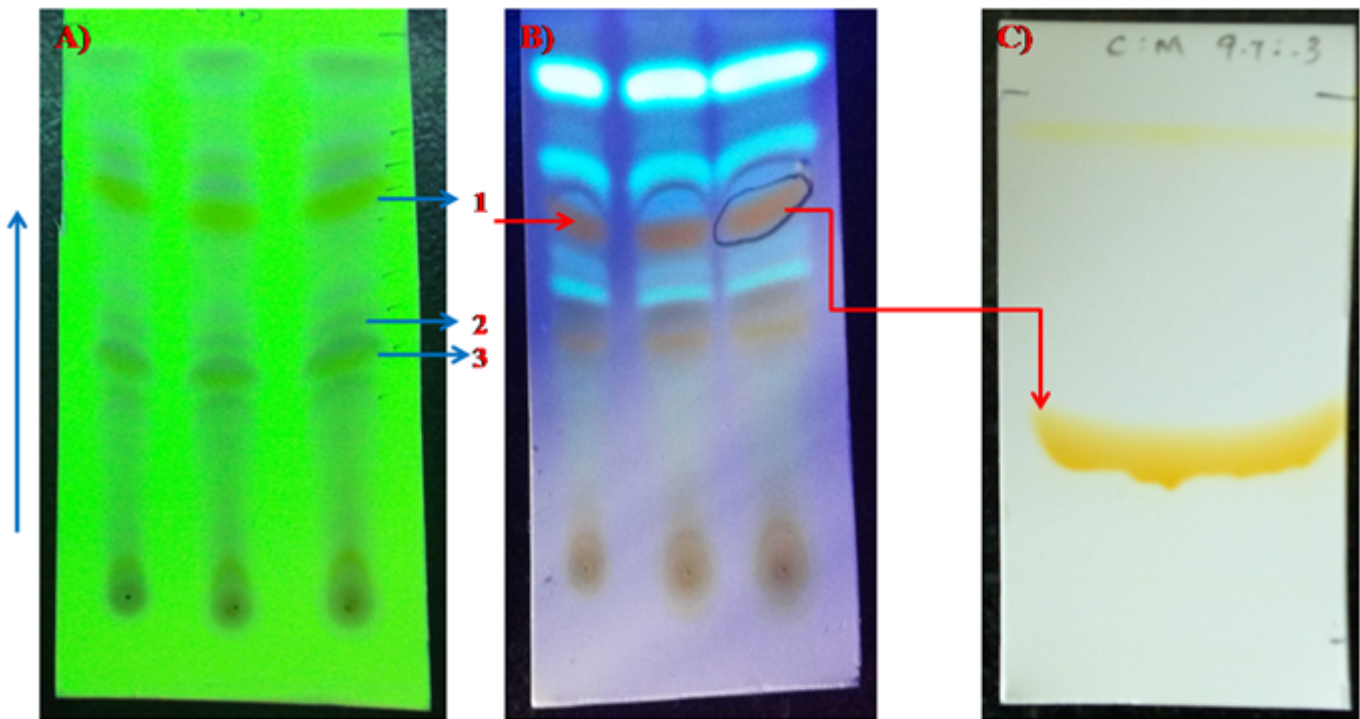
Figure 1

1a). Thin-layer chromatography of *A. terreus* MEAE. Mobile phase (chloroform:methanol, 97: 3%). **b).** The obtained band was visualized by short and long UV 254nm. **c).** UV-visible spectrum of single band (emodin) and its Rf value is 0.38cm.
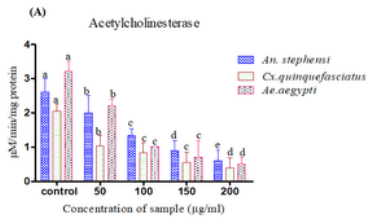
Fig.2A
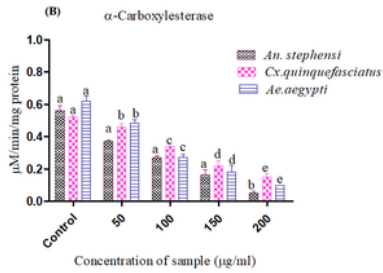
Fig. 2B
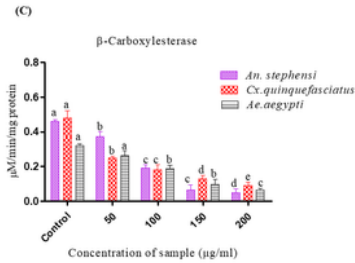
Fig. 2C
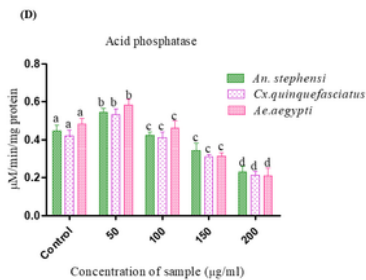
Fig. 2D
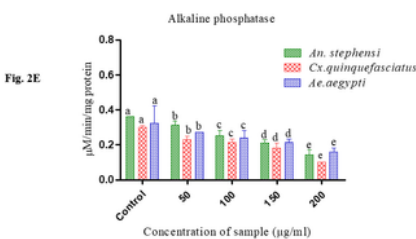
Fig. 2E

Figure 2

Biochemical profile of emodin treatment on the larval enzyme activities. (**A**) Acetylcholinesterase, (**B**) α-carboxylesterase, (**C**) β-carboxylesterase, (**D**) Acid phosphatise and (**E**) Alkaline phosphatise. Each bar represents mean ± SE of three replicates.
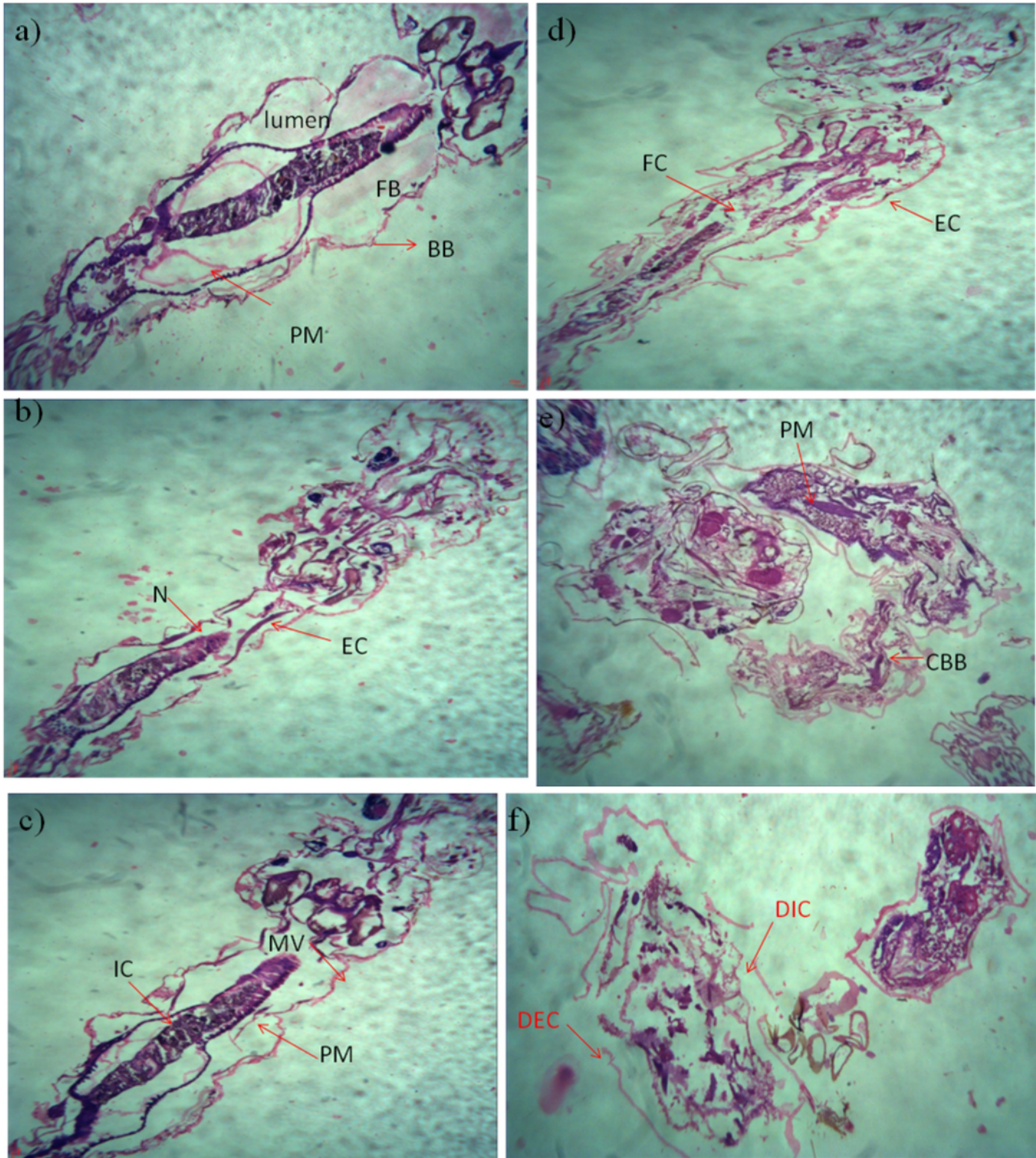
Figure 3

Histological sections of 4th instar larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* after exposure to emodin compound : **a, b & c**) untreated 4th instar larvae; **d**) midgut of *An. stephensi* 4th instar larvae, **e**) midgut of *Cx. quinquefasciatus* 4th instar larvae, **f**) midgut of *Ae. aegypti* 4th instar treated larvae, after 12 h of exposure to pure compound. (Epithelial cells (EP), Microvilli (MV), Peritropic membrane (PM), Food column (FC), Collapsed brush border (CBB), Degenerated epithelial cells (DEC), Intestinal cells (IC), Degenerated Epithelial cells (DIC) and Nucleus (N).
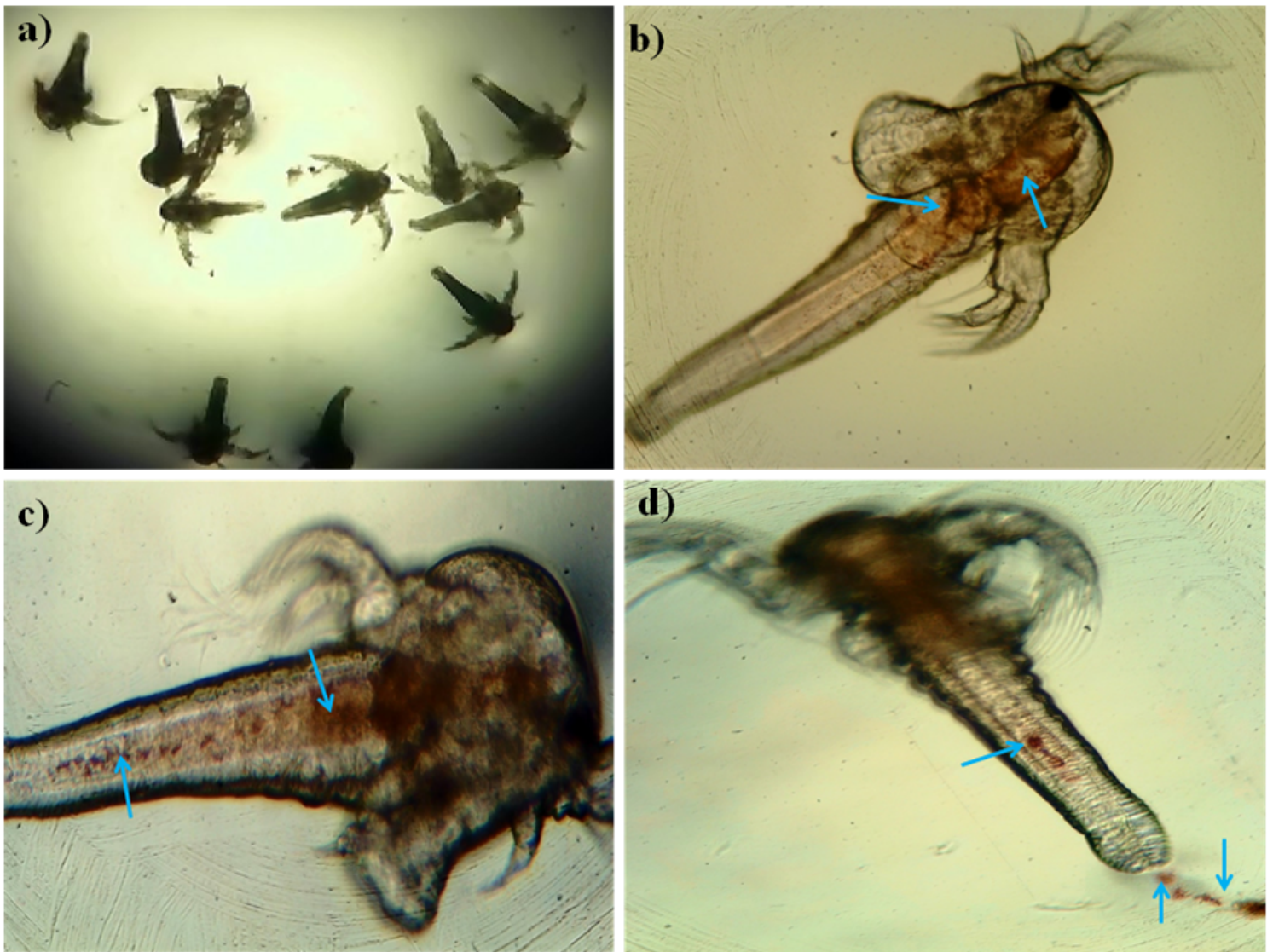

Figure 4

Toxicity assessment of emodin on *A. nauplii* **a**). Control *A. nauplii*, **b**) Treated with emodin. Blue arrow indicates the cluster of emodin compound.
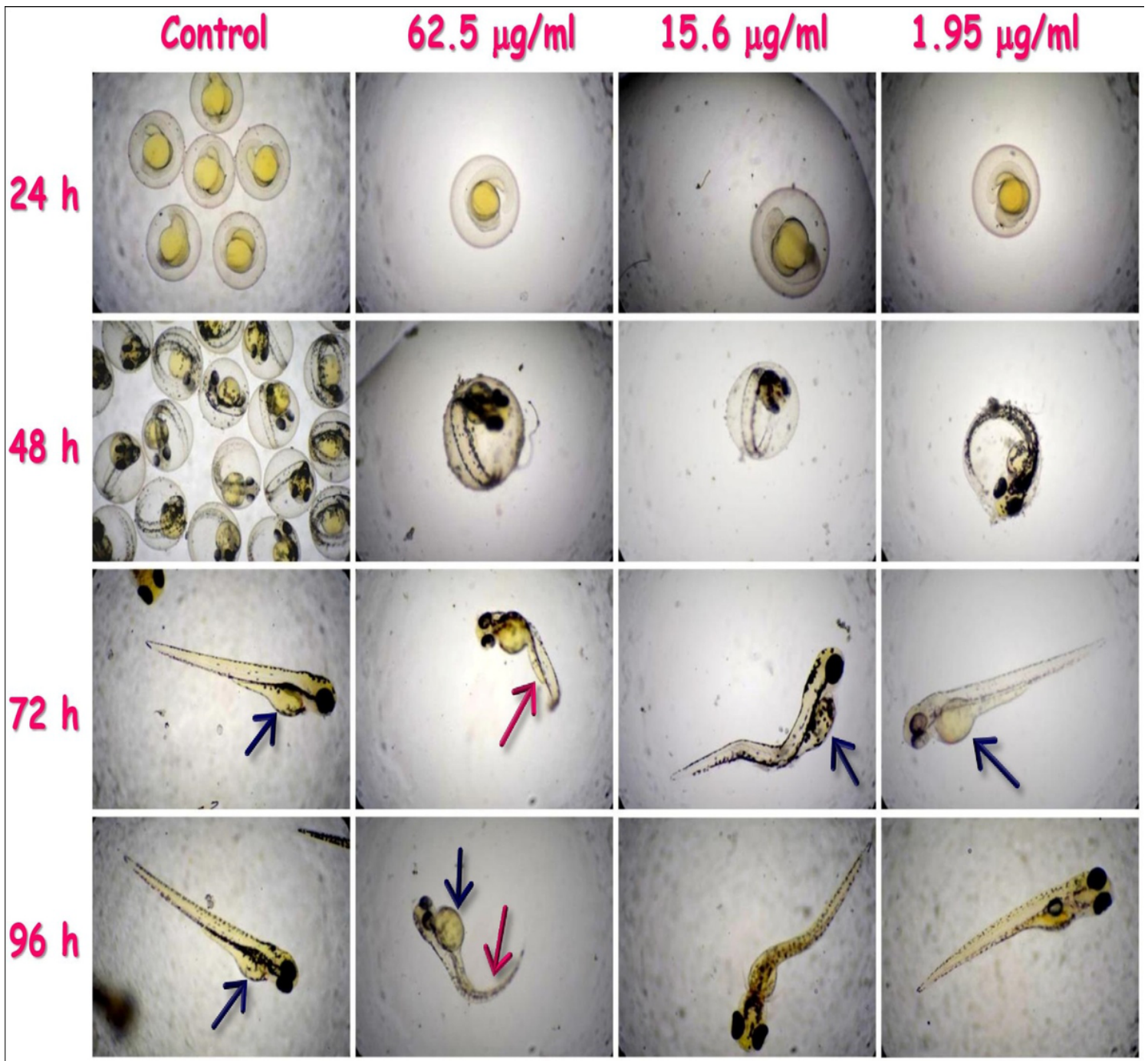
Figure 5

Bio-toxicity of Zebrafish 96hpf embryos induced by emodin isolated from *A. terreus* at various concentrations (control, 1.95, 15.6 and 62.5 mg.L^{-1}). Note: Pink arrow indicates malformation and blue arrow indicates yolk cysts.
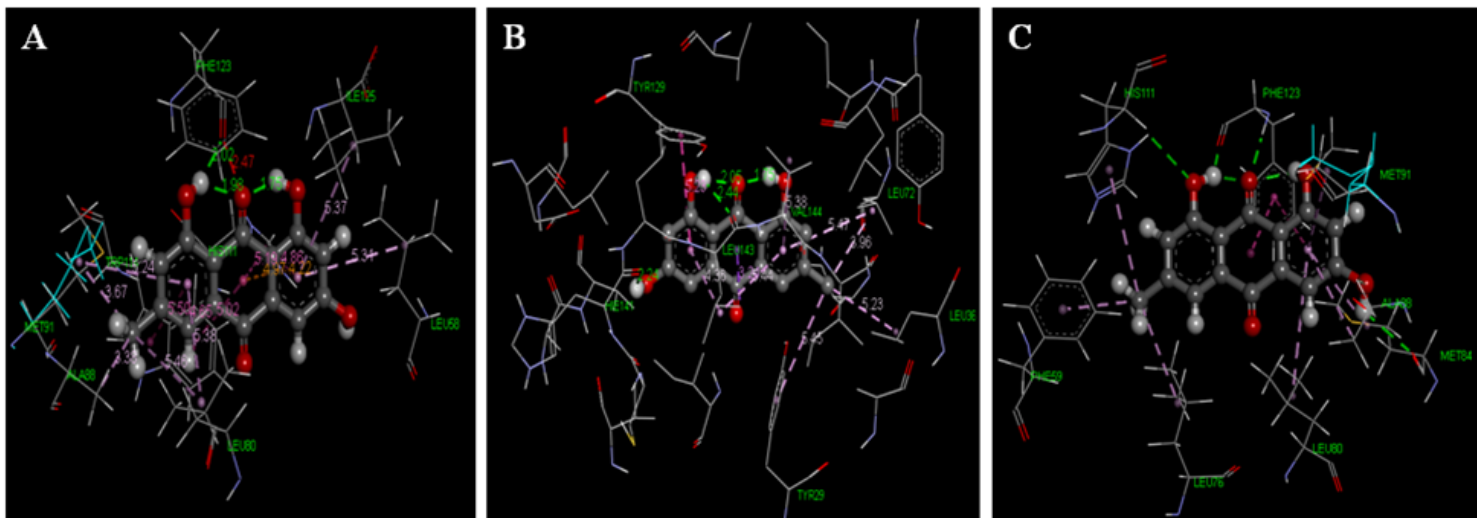
Figure 6

In silico molecular docking analysis of emodin against three mosquito vectors **A**). *Ae. aegypti* **B**). *An. stephensi* **C**). *Cx. quinquefasciatus* odorant binding proteins (OBPs).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterials.doc