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Kandasamy Kalimuthu
Institute of Marine Biology, National Taiwan Ocean University, Keelung, Taiwan, R.O.C. Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India.

Chia-Hsiang Wang
Institute of Marine Biology, National Taiwan Ocean University, Keelung, Taiwan, R.O.C.

Shiu-Mei Liu
Institute of Marine Biology, National Taiwan Ocean University, Keelung, Taiwan, R.O.C.

Li-Chun Tseng
Institute of Marine Biology, National Taiwan Ocean University, Keelung, Taiwan, R.O.C.

Kadarkarai Murugan
Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India.

See next page for additional authors

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Authors

Kandasamy Kalimuthu, Chia-Hsiang Wang, Shiu-Mei Liu, Li-Chun Tseng, Kadarkarai Murugan, and Jiang-Shiou Hwang

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MOSQUITO LARVICIDAL ACTIVITY OF *Broussonetia papyrifera* COMPOUND MARMESIN BY BLOCKING PROTEIN AESCP-2, DOCKING STRATEGIES, AND COMBINED EFFECT OF COPEPOD *Megacyclops formosanus* AGAINST DENGUE VECTOR *Aedes aegypti* (DIPTERA: CULICIDAE)

Kandasamy Kalimuthu[1, 2], Chia-Hsiang Wang[1], Shiu-Mei Liu[1], Li-Chun Tseng[1], Kadarkarai Murugan[2], and Jiang-Shiou Hwang[1]

Key words: *Aedes aegypti*, *Broussonetia papyrifera*, sterol carrier protein-2, marmesin, larvicidal.

ABSTRACT

Mosquito-borne diseases that have an economic impact create losses in commercial and labor outputs, particularly in countries with tropical and subtropical climates. The emergence of resistance to synthetic insecticides is a challenge to mosquito control. Cyclopoid copepods are important predators in many aquatic ecosystems and have been successfully used as biological agents to control mosquito larvae. For this study, we examined the larvicidal activity of the copepod *Megacyclops formosanus* in combination with the compound marmesin (which was purified from the methanol crude extract of the plant stem bark of *Broussonetia papyrifera*) against *Aedes aegypti* larvae. Their larvicidal activity and *in silico* docking analysis regarding the inhibition of the binding cholesterol sterol carrier protein-2 (AeSCP-2) against *A. aegypti* were evaluated. The significant larvicidal potential was recorded after the marmesin plant compound treatment against the dengue vector *A. aegypti*. Larval mortality was observed after 24 h of exposure. The LC_{50} and LC_{90} of marmesin against the first to fourth instar larvae and pupae were 0.104, 0.115, 0.137, 0.176, and 0.353 ppm, and 0.255, 0.270, 0.297, 0.365, and 0.643 ppm, respectively. This study showed that marmesin and copepods can be used effectively for mosquito larvae control programs. This is an ideal eco-friendly approach for controlling *A. aegypti* larvae.

I. INTRODUCTION

Millions of people suffered from insect-transmitted diseases yearly. One primary vector of yellow fever, chikungunya fever, dengue fever, dengue hemorrhagic fever, and dengue shock syndrome is the mosquito *Aedes aegypti* Grantz [7]. The WHO (2009) indicated that approximately two-fifths of the world's population is at risk of dengue, and the only way to prevent dengue virus transmission is to combat the disease-carrying mosquitoes. In India, 28292 cases and 110 deaths were reported to have been caused by dengue in 2010 National Vector Borne Disease Control Programme [32]. In the absence of effective vaccines and drugs, the dengue prevention and control programs have depended on vector control. Plants may be a source of agents for the control of mosquitoes, because they are rich in bioactive chemicals. They are active against a limited number of species, including specific target-insects Sukumar *et al.* [42]. New botanical natural products are effective, environment-friendly, biodegradable, inexpensive, and readily available in many areas of the world, produce no ill effects on non-target organisms, and have novel modes of action Su and Mulla [40]. Many studies have reported the effectiveness of plant extracts against mosquitoes Kalimuthu *et al.* [11]; Murugan *et al.* [27]; Kovandan *et al.* [14]; Mahesh Kumar *et al.* [20]; Ponarlselvam *et al.* [35]; Subramaniam *et al.* [41].

Paper submitted 11/18/13; revised 12/18/13; accepted 12/23/13. Author for correspondence: Jiang-Shiou Hwang (e-mail: jshwang@mail.ntou.edu.tw).
[1]Institute of Marine Biology, National Taiwan Ocean University, Keelung, Taiwan, R.O.C.
[2]Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India.

A fine-tuned chemical approach is more practical against mosquitoes: only 1 compound is used, which functions for a short period and targets a specific insect. These chemicals must be starget-specific pesticides that kill only mosquitoes, with low residue time, and they must not go down the same road as DDT. Regarding the blocking of target proteins in insect (mosquito) physiology, and the development and identification of potential inhibitory effects should be a promising approach or a step in the right direction. Sterols are ubiquitous among eukaryotic organisms, and serve both as bulk membrane lipid components and as precursors for additional metabolites such as mammalian steroid hormones, plant-based steroid hormones, and insect ecdysteroids Nes and McKean [33]. The major sterols of plants and fungi contain alkyl substitutions at carbon 24, which is absent in cholesterol, the dominant sterol of virtually all animals. Cholesterol, a hydrophobic, sticky substance that accumulates on the lining of human arteries, is an important component of the cell membrane in vertebrates and invertebrates Nes and McKean [33]. Unlike humans, mosquitoes cannot synthesize cholesterol, but it is vital for their growth, development, and egg production. They must obtain it from the decomposed plants they eat during the larval stage in shallow water. Plants produce phytosterol, which mosquitoes convert to cholesterol in the gut. To transport it in a liquid medium, such as blood or cell fluids, the organisms must have a way to shield it from the watery environment through which it moves, which is studied typically in a carrier protein (sterol carrier protein two, SCP-2) Barani Kumar *et al*. [3]. This dictates the need to develop safe, less expensive, and preferably locally available materials for mosquito vector control, and plant-based products are such potential tools. These products are the compounds that have evolved in plants for defense against phytophagous insects. Modern researchers are equipped with the technology to exploit the toxic properties of these compounds and use them against organisms, despite the technology have never been intended for use on normal vector diseases in humans.

Biological control entails using the natural enemies of an organism (*Aedes*) for their regulation and management, seems to be an alternative approach to the systematic failure of insecticide use Lardeux *et al.* [16]. Copepods are small aquatic crustaceans. There is a vast array of agents used in the biological control of mosquitoes, including copepods. Various species of cyclopoid copepods prey on early mosquito larvae and have been successfully used in programs for controlling mosquito-transmitted diseases, such as dengue Nam *et al.* [29, 30]. Most are omnivorous and prey on mosquito larvae, especially first-instar larvae, but rarely for those in the later stages Marten *et al.* [21]; Williamson [44]. Several species of copepods, including *M. aspericornis*, *M. thermocyclopoides*, *M. guangxiensis*, and *M. longisetus*, have been reported as potential biological control agents of *A. aegypti* Rawlins *et al.* [38]; Jekow *et al.* [10]; Locantoni *et al.* [18]; Murugan *et al.* [27]; Mahesh Kumar *et al.* [20]. Only large-sized species of copepods can prey on mosquito larvae. These copepods play a similar role to larvivorous fish, which are particularly effective predators for biological control because of their broad diet that allows them to maintain large populations almost anywhere they are present, and they also independently prey on mosquito larvae for food. Cyclopoid copepods have been shown to be effective predators of *A. aegypti* larvae in both laboratory experiments and field trials Kay *et al.* [12].

However, to evaluate new natural insecticides, several factors need to be evaluated. Among these factors, it is important to know the time the toxicity of the products begin and how long they maintain lethal dosages for mosquito larvae. Moreover, it is important to know the lowest concentrations of sublethality in affecting mosquito development. Thus, the objective of this study was to increase the predictive capability, larvicidal activity, predation by the copepod *Megacyclops formosanus*, and the combined effect of the copepod with different concentrations of the compound marmesin obtained from *Broussonetia papyrifera* (L) against *A. aegypti* larvae.

II. MATERIALS AND METHODS

1. Collection of Eggs and Maintenance of Larvae

The stock culture of *A. aegypti* were collected from the Institute of Epidemiology, National Taiwan University, Taipei, Taiwan, by using an "O" type brush. These eggs were brought to the laboratory and transferred to 34 × 26 × 7-cm enamel trays containing 500 mL water for hatching. The mosquito larvae were fed with Pedigree dog biscuits and yeast at a 3:1 ratio. The feeding was continued until the larvae entered the pupal stage Murugan *et al*. [26]

2. Blood Feeding of Adult *A. aegypti*

The adult female mosquitoes were allowed to feed blood from mice for 2 d (1 mice per day, exposed on the dorsal side) to ensure adequate blood feeding. After blood feeding, enamel trays with water were placed in the cage as ovipositional substrates.

3. Collection of Plant and Preparation of Compound

The plant *Broussonetia papyrifera* was collected from a field around National Taiwan Ocean University, Keelung, Taiwan**.** Stem barks of *B. papyrifera* were washed using tap water and shade-dried at room temperature (27 ± 2°C). An electrical blender was used to powder the dried bark. From the raw powder, 300 g of bark powder was extracted with 1 L of the organic solvent methanol using a Soxhlet apparatus, with a boiling point range of 60-80°C for 8 h. Column chromatography was used to isolate the pure compound marmesin form the curd extract. The compound was dissolved in ethanol to prepare a stock solution. Reagents of different concentrations were prepared for the experiment by diluting the stock solution with water.

4. Larval and Pupal Toxicity Test

Laboratory colonies of mosquito larvae/pupae were used to

test the larvicidal/pupicidal activity. Twenty-five first to fourth instar larvae (I, II, III, and IV) and pupae were introduced into a 500 mL glass beaker containing 249 mL dechlorinated water, and 1 mL of the desired concentration of the compound marmesin was added. Larval food was given to the test larvae during the experimental period. At each tested concentration, 2 to 5 trials were performed and each trial consisted of 5 replicates. The control was set up by mixing 1 mL acetone with 249 mL dechlorinated water. The larvae and pupae exposed to dechlorinated water without ethanol served as the control. The control group's mortalities were corrected using Abbott's formula Abbott [1].

$$\text{Corrected mortality} = \frac{\text{observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Controlmortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{No. of dead larvae / Pupae}}{\text{Number of larvae / pupae introduced}} \times 100$$

The LC_{50} and LC_{90} were calculated from toxicity data using probit analysis Finney [6].

5. Copepod Culture

The stack culture of *M. formosans* were collected from zooplankton and coral reef laboratory, Institute of Marine Biology, National Taiwan Ocean University, Taiwan. The *M. formosanus* copepod colony was started by inoculating 10 gravid female copepods into a rectangular glass aquarium filled with 3 L of a culture medium consisting of ciliates, rotifers, and the alga *Chlorella vulgaris* Beyerinck [Beiierinck] 1890 as prey for the copepods. The copepods were reared at 27 ± 2°C, pH 7, and a photoperiod of 12:12 h in an incubator. They were fed mosquito larvae for 3 d, and were then starved for 24 h prior to the experiment.

6. Predatory Efficiency Test

Adult copepods were used to measure the predatory activity toward the 4 instars and pupae of the mosquito larvae. One hundred mosquito larvae of each instar and 10 adult copepods were introduced into 4 separate 500 mL glass beakers containing 250 mL dechlorinated water. The mosquito larvae were replaced daily with new ones. Each mosquito instar-copepod treatment was replicated 5 times. One hundred mosquito larvae were introduced to 250 mL dechlorinated water without copepods for the control group. The glass beakers were inspected after 24, 48, 72, 96, and 120 h, and the numbers of mosquito larvae consumed by the copepods were recorded.

7. Predatory Efficiency Test in Combination with Plant Compound Marmesin

Adult copepods were used to quantify the predatory activity toward the 4 instars and pupae of the mosquito larvae. One hundred mosquito larvae of each instar and 10 adult copepods were introduced into 4 separate 500 mL glass beakers containing 250 mL dechlorinated water and 1 mL of the desired concentration of the *B. papyrifera* compound marmesin. The mosquito larvae were replaced daily with new ones. Each mosquito instar-copepod treatment was replicated 5 times. The control group consisted of 249 mL dechlorinated water and 1 mL ethanol without any copepods. The glass beakers were inspected after 24, 48, 72, 96, and 120 h, and the numbers of larvae consumed by the copepods were recorded

8. Statistical Analysis

All data were subjected to variance analysis; the means were separated using Duncan's multiple range tests (DMRT). The average larval mortality data was subjected to probit analysis; to obtain LC_{50} and LC_{90}, the values were calculated using the Finney [6] method. Bioassay data and predation trials were analyzed using SPSS Statistics (Statistical Software Package) version 17.0, results with $P < 0.05$ were considered statistically significant.

9. Retrieval of the Protein

The 3-D crystal structure of the sterol carrier protein of *A. aegypti* (AeSCP-2) was obtained from the protein data ank (PDB) (www.rcsb.org/pdb/), (PDB ID: 1PZ4). The coordinate file of AeSCP-2 was obtained by the molecular visualization viewer SPDB viewer (www.expasy.org/spdbv/). The amino acids in an active site of AeSCP-2 were from SER-18 to HIS-28 (Dyer *et al*. 2003), and it was confirmed with the help of binding pocket detection server tools such as pocket finder and Q-site finder (www.modelling.leeds.ac.uk/qsitefinder). The predicted binding sites, based on the binding energy and 17 amino acids, comprise this binding cavity.

10. Selection of Chemical Compounds

The selected chemical structures are generated from the SMILES notation by using the Chemsketch Software (www.acdlabs.com). After building the structures successfully, geometry optimization and energy minimization were completed. The energy minimization process was performed for 100 cycles using the chimera software.

11. Protein Preparation

Autodock 4.0 is used for the docking process. The initial step for protein preparation involves adding polar hydrogen's to the target protein AeSCP-2. Thereafter, the appropriate partial atomic charges are assigned. The charged protein is converted to the 'PDBQ' format so that Autogrid can read it. It is noted that in most modeling systems, polar hydrogen's are added in a default orientation, assuming that each new torsion angle was 0° or 180°. Without some form of refinement, the hydrogen-bonds form in spurious locations. One refinement option involves relaxing the hydrogen's, and then a molecular mechanics minimization is performed on the structure. Another option involves using a program such as "pol_h", where the default-added polar hydrogen structure is

Table 1. Larvicidal activity of *B. papyrifera* compound marmesin against dengue vector *A. aegypti*.

Compound	Instars	$LC_{50}(LC_{90})$	LC_{50} LCL(UCL)	LC_{90} LCL(UCL)	χ^2 df = 3	Regression
Marmesin	I	0.104(0.255)	0.086(0.119)	0.231(0.290)	0.299	X = +8.496 Y = –0.886
	II	0.115(0.270)	0.098(0.130)	0.244(0.308)	0.238	X = +8.265 Y = –0.953
	III	0.137(0.297)	0.121(0.168)	0.268(0.341)	2.599	X = +7.998 Y = –1.094
	IV	0.176(0.365)	0.159(0.196)	0.320(0.436)	2.691	X = +6.810 Y = –1.201
	Pupa	0.353(0.643)	0.292(0.497)	0.498(0.996)	0.200	X = +4.430 Y = –1.566

Control: nil mortality, LCL: lower confident limit, UCL: upper confident limit, χ^2: chi-square value, df: degrees of freedom.

taken as the input. The favorable locations for each movable proton are sampled and the best position is selected for each one. This 'intelligent' placement of movable polar hydrogen would be particularly important for the tyrosine, serine, and threonine amino acids.

12. Ligand Preparation

The hydrogen's were initially added to all the atoms in the ligand and it was ensured that their valences were completed. This was achieved using ADT, a molecular docking package. It was ensured that the atom types were correct before adding the hydrogen's. Depending on whether charged or neutral carboxylates and amides were desired, the PH was specified automatically. Thereafter, the partial atomic charges were assigned to the ligand molecule. These charges were written in the 'PDBQ' format, which had columns similar to a Brookhaven PDB format, but with an added column for partial atomic charges.

13. Setting and Running of the Auto Grid

Pre-calculated grid maps (one for each atom type present in the ligand being docked) were required for the Autodock to achieve extremely fast docking calculations. These maps were calculated by the Autogrid. A grid map was created with a 3-D lattice of regularly spaced points, surrounding (entirely or partially) and centered on the active site of the macromolecule (i.e., the 17 amino acids of AeSCP-2). The typical grid point spacing varied from 0.2 Å to 1.0 Å, although the default was 0.375 Å (roughly a quarter of the length of a carbon-carbon single bond). An input grid parameter file, which usually had the extension ".gpf", was required for the Autogrid. The maximum and minimum energies found during the grid calculations for AeSCP-2 were stored in the log file. With these important features of the Autogrid, it was set exactly on the active site of the AeSCP-2 (1PZ4), and the grid parameter file was written as a result of this process.

14. Running of the Auto Dock

Molecular docking was performed using a genetic algorithm - the Least Square (GA-LS) algorithm used in Auto dock 4.0. Once the grid maps had been prepared by the Autogrid and the docking parameter file (dpf) was ready, the user could run an Auto Dock job. The docking results, called "lig.macro.dlg", were viewed using 'get-docked', and all the docked conformation outputs were viewed and analyzed. From the several poses of docking, the complex formed with the least energy and a stable conformation was taken.

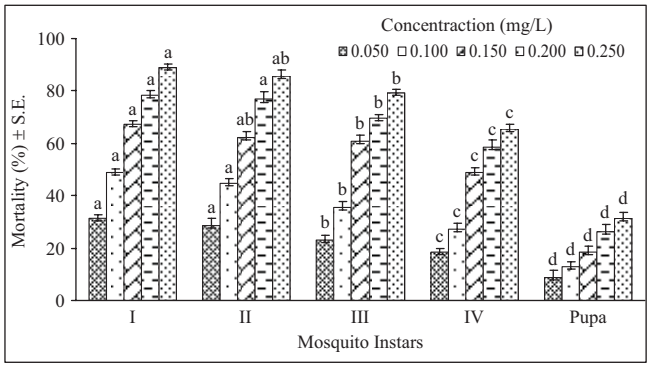

Fig. 1. Larvicidal activity of compound Marmesin from *B. papyrifera* against dengue vector *A. aegypti*. Value represents mean ± S.E. (Standard error) of 5 replications. Larvae mortality observed after 24 h of exposure. Different alphabets in the column are statistically significant at $P < 0.05$ (DMRT test). No mortality was observed in the control group.

III. RESULT

The compounds obtained from *B. papyrifera* has been studied for use as a natural insecticide rather than an organic phosphorus material or synthetic agent. The larvicidal effect of the compound on the I, II, III, and IV instar larvae and pupae of *A. aegypti* was presented on Table 1. The entire tested compound exhibited larvicidal activity. The most potent larvicidal compound was marmesin. After 24 h of exposure, the LC_{50} value of the first instar, second instar, third instar, fourth instar, and pupae were 0.104, 0.115, 0.137, 0.176, and 0.353 ppm, respectively. The LC_{90} value of the first instar, second instar, third instar, fourth instar, and pupae were 0.255, 0.270, 0.297, 0.365, and 0.643 ppm, respectively (Fig. 1).

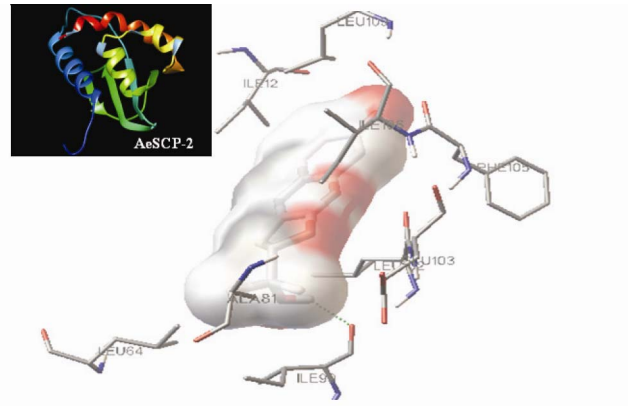

Fig. 2. Interactions of *B. papyrifera* compound Marmesin with AeSCP-2.

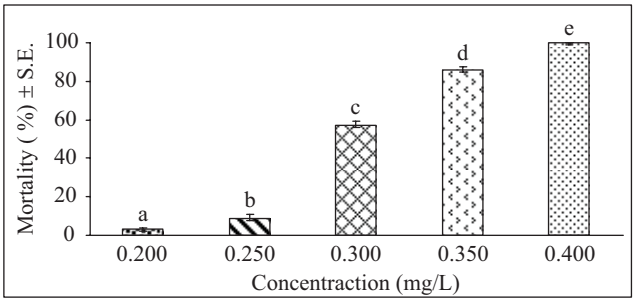

Fig. 3. The activity of compound Marmesin from *B. papyrifera* against *Megacyclops formosanus*.

The result was docking with AeSCP-2, the best docked ligand molecules are selected based on the docking energy and good interaction with the active site's residues. The predicted binding sites (based on the binding energy) and the 17 amino acids (VAL 8, PHE 9, ILE 12, ARG15, LEU 16, SER 18, ILE 19, ASP 20, ARG 24, GLN 25, VAL 26, TYR 30, PHE 32, MET 46, LEU 48, LEU 64, and MET 66) make up the binding cavity. The energy of the ligand with the target AeSCP-2 was -7.09 Kcal/mol and 9th conformation. The interaction of the natural compound marmesin with AeSCP-2 was shown in Fig. 2.

The activity of the plant compound marmesin exhibited a moderate toxic effect on the *M. formosanus* copepods after 24 h of exposure in 0.2 to 0.4 ppm concentration. However, the LC_{50} and LC_{90} were 0.148 and 0.240 ppm against *M. formosanus*, respectively (Fig. 3). *M. formosanus* showed effective predation against *A. aegypti* larval instars. Fig. 4 shows the predatory efficiency percentage of copepods toward the different instars and pupae of *A. aegypti*. The predation percentage decreased as mosquito larvae grew older. The early instars were more susceptible and preferred by the copepods. The lowest predation was observed in the IV instars. The predatory efficiency of a single adult copepod was 8.68, 5.93, 0.50, 0.15, and 0.04 larvae/d for the I, II, III, and IV instar larvae and pupae, respectively (Fig. 5).

The predatory efficiency of *M. formosanus* increased when treated with marmesin. The predatory efficiency of

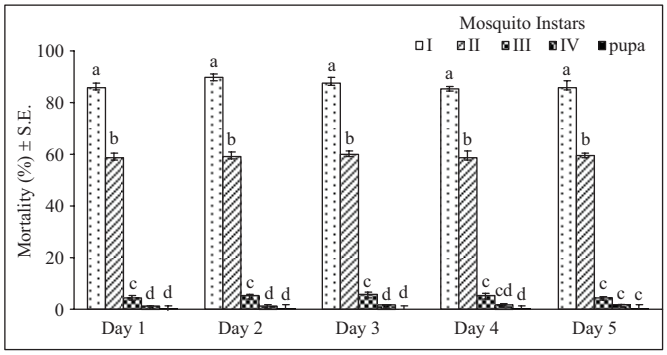

Fig. 4. Predatory efficiency of *M. formosanus* copepods on dengue vector *A. aegypti*.

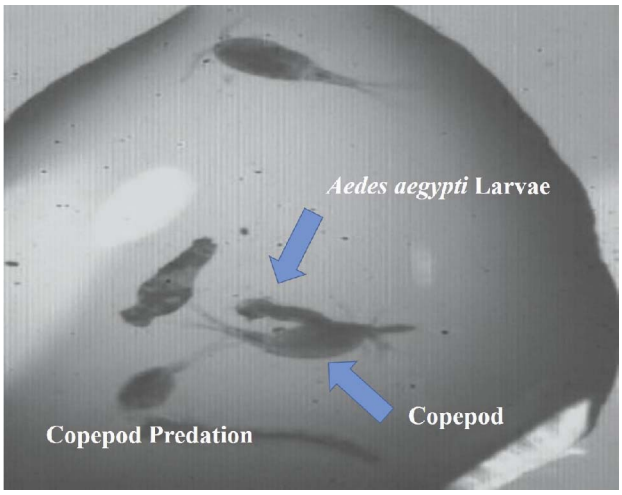

Fig. 5. Optical microscopic image of an *M. formosanus* copepod against dengue vector *A. aegypti* larvae.

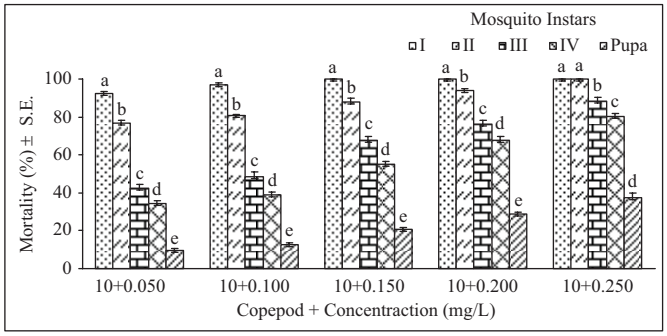

Fig. 6. Combined effect of the predatory *M. formosanus* copepods and compound marmesin from *B. papyrifera* against dengue vector *A. Aegypti*. Value represents mean ± S.E. (Standard error) of 5 replications. Larvae mortality observed after 24 h of exposure. Different alphabets in the column are statistically significant at $P < 0.05$ (DMRT test). No mortality was observed in the control group.

M. formosanus against larval instars with the biopesticide from *B. papyrifera* was shown in Fig. 6. The predatory efficiency of copepods on treated larvae increased compared to the untreated larvae. The I and II instars were much preferred

compared to the latter ones. The predatory efficacy of a single copepod on marmesin-treated larvae were 9.79, 8.78, 6.46, 5.52, and 2.17 larvae/d for the I, II, III, and IV instar larvae and pupae, respectively.

IV. DISCUSSION

Many studies had been focused on determining the distribution, nature, and practical use of plant-derived substances that have mosquito larvicidal activity Kalimuthu *et al.* [11]; Murugan *et al.* [26, 27]; Ponarulselvam *et al.* [35]; Kovendan *et al.* [14]; Subramaniam *et al.* [41]. Various compounds (e.g. phenolics, terpenoids, and alkaloids) existing in plants either jointly or independently contribute to behavioral efficacy (e.g., repellency and feeding deterrence) and physiological efficacy, and/or as acute toxicity and developmental disruption against various arthropod species Isman [9]. *A. aegypti* and *O. togoi* larvae were slightly more tolerant than *C. pipiens pallens* larvae to 3-carene, ethyl cinnamate, ethyl p-methoxycinnamate, p-meth oxycinnamic acid, fenthion, and temephos Nam *et al.* [31].

The toxicity effect of the ethanolic extract from the peel of the *Citrus sinensis* orange was tested on the larvae of the yellow fever mosquito *A. aegypti* Amusan *et al.* [2]; Murugan *et al.* [28]. The leaf extracts from *Sphaeranthus indicus*, *Cleistanthus collinus*, and *Murraya koenigii* were tested against the third instar larvae of *Culex quinquefasciatus* Kovendan *et al.* [14]. An earlier report indicated that compounds, such as diterpenoid furans, 6alpha-hydroxyvouacapan-7beta, 17beta-lactone (1), 6alpha, 7beta-dihydroxyvouacapan-17beta-oic acid (2) and methyl 6alpha, and 7beta-dihydroxyvouacapan-17beta-oate (3) from the seeds of *Pterodon spolygalaeflorus*, exhibited LC_{50} values of 50.08, 14.69, and 21.76 µg/ml against fourth instar *A. aegypti* larvae, respectively Omena *et al*. [34]. Siddiqui *et al.* [39] reported that the compounds spipnoohine (1) and pipyahyine (2), isolated from the petroleum ether extract of dried ground whole fruits of *Piper nigrum*, exhibited toxicity at 35.0 and 30.0 ppm against fourth instar *A. aegypti* larvae, respectively. Park *et al.* [36] reported that the compounds retrofractamide A (0.039 ppm), pipercide (0.1 ppm), guineensine (0.89 ppm), pellitorine (0.92 ppm), and piperine (5.1 ppm), derived from the fruits of *P. nigrum*, could efficiently eradicate third instar *A. aegypti* larvae.

Adult mortality occurred when the ethanol extract of *C. sinensis* was used, producing the following LC_{50} and LC_{90} values: 272.19 and 457.14 ppm, 289.62 and 494.88 ppm in *A. stephensi*, and 320.38 and 524.57 ppm in *A. aegypti*, respectively Murugan *et al.* [28). Eight of the 11 plant extracts studied exhibited toxicity against *A. aegypti* larvae (LC_{50} < 500 µg/ml). Dichloromethane extracts of *Abuta grandifolia* and *Minthostachys setosa* demonstrated high larvicidal activity, and the most active was the dichloromethane extract of *A. grandifolia*, with LC_{50} = 2.6 µg/ml (LC_{100} = 8.1 µg/ml). Conversely, the dichloromethane extract of *M. setosa* was potent against *A. aegypti* larvae, showing LC_{50} = 9.2 µg/ml Lyege *et al.* [19]. There are several studies regarding the larvicidal potential of natural products for controlling *Aedes* mosquitoes. However, varying results were obtained. Previous studies showed that ethanol extracts from the fruit endocarps of *Melia azedarach* and *Azadirachta indica*, 2 members of the family Meliaceae, had lethal effects on *A. aegypti* larvae, with LC_{50} values ranging from 0.017 to 0.034% (Wandscheer *et al.* [43]. In the present study, percentage mortality was elevated as the concentration of marmesin was increased. In addition, the mortality was higher in early instar larvae than those of later stages.

All insects lack the enzymatic pathway to synthesize their own cholesterol Zdobnov *et al*. [45]; thus, they obtain this vital nutrient from dietary sources. Cholesterol is a highly hydrophobic molecule, absorbing cholesterol from the gut and transporting it requires a specific carrier protein. Sterol carrier protein two (SCP-2) is at least partially responsible for this role Blitzer *et al*. [5]. Cholesterol uptake is the most important step for larval population, its conversion/uptake is performed in the presence of AeSCP-2. Kim *et al*. [13] reported that 5 SCPIs, namely N-(4{4-(3-4-dihidrophenyl)-1,3-thiazol-2-yl] amino} phenyl) acetamide hydro bromide, 8-chloro-2-(3-methoxy phenyl)-4,5-dihydroisothiazolo{5,4-c] quinonoline-1 (2H_tri une,3-(4-bromophenyl)-5-methoxy-7-nitro-4H-1, 2, 4-benzo xzdiazine, 4, 4, 8-trimethyl-5-(3-emthylbutanoyl)-4, 5dihydro-]H-[1, 2]dithiolo[3, 4-c]quinoline-1-thione3-bromo - N-{2-[4-chloro-2-nitrophenyl)amino] ethyl}-4-ethoxy benzamide, were compared with cholesterol for AesCP-2 and found that AeSCP-2-specific inhibitors exhibited physiological effects on cholesterol metabolism in cultured insect cells, which were similar to the effects of AeSCP-2 knockdown. The potential inhibitors, namely alpha-mangostin and panthenol, had effective interactions on AeSCP-2 binding sites Barani Kumar *et al*. [3]. Similarly, the botanical SCPI mangostin was found to possess larvicidal activity against various mosquito species Larson *et al*. [17]. The zoosterol 7-dehydrocholesterol is more similar to cholesterol but does not inhibit the binding of NBD-cholesterol to AeSCP-2 Radek *et al*. [37]. The chemical interaction between the selected ligands (marmesin) and the target protein (AeSCP-2) has been found to be good, and has the best binding energy and interaction scores. Similar to the identified ligands of phytochemical origin, it indicated that these extracts are safer to the environment.

This study indicates that natural agents can be used on biological control of mosquito larvae. Combination with animals such as competitors and predators is more effective and can alleviate the frequent use of synthetic chemicals. Many biological control agents disperse by themselves, which enhances the ability to spread and build up viable populations Bellows [4]. Similar to many predators in aquatic environments, cyclopoid copepods are known to strongly influence the structural and functional organization of the prey communities on which they feed (Matsumura-Tundisi *et al.* [24]; Irvine and Waya [8]. Cyclopoids copepods offer high promise as bio-

logical control agents for *A. aegypti* Marten *et al.* [23], and are abundant in eutrophic waters and play important roles in their trophodynamics. Some cyclopoids have long been known use mosquito larvae as food Marten [22]; Marten *et al.* [23]; Kay *et al.* [12]. Kumar and Rao [15] conducted a series of behavioral observations on the handling and predating of mosquito larvae by the cyclopoid *M. thermocyclopoides*. In the laboratory, many species of *Mesocyclops* have been shown to prey on *A. aegypti* or *Anopheles* larvae Marten *et al.* [23]; Kay *et al.* [12]; Mittal *et al.* [25]; Kumar and Rao [15]. Numerous *A. albopictus* larvae that inhabited untreated tires at the beginning of an experiment virtually disappeared within 2 months. The adults disappeared approximately 1 month later and remained scarce for at least another year Marten [23]; Marten *et al.* [23]; Nam *et al.* [29]. Copepods prey on mosquito larvae as well, and therefore can be applied efficiently as biocontrol agents of mosquitos (Murugan *et al.* [27]; Mahesh Kumar *et al.* [20]. The present study demonstrated that the predatory efficacy of *M. formosanus* is substantial against the different larval instars of *A. aegypti*. Furthermore, the number of first and second instars consumed by the *M. formosanus* copepods was greater than those of the third and fourth instars. Similar investigations have also been performed using *M. aspericornis* in conjunction with other controlling methods and resulted in the eradication of *A. aegypti* Locantoni *et al.* [18]; Murugan *et al.* [27], Mahesh Kumar *et al.* [20].

Our findings regarding the *B. papyrifera* plant include: (1) the toxic components responsible for in silico predicting AeSCP-2; (2) the laboratory level larvicidal effect found from *B. papyrifera* is the compound marmesin; and (3) marmesin is concentrated in the stem bark and is extractable using methanol. The results from our study revealed that the larvicidal potential of the *B. papyrifera* bark extract is more efficient compared to the natural products examined in previous studies. The most appropriate copepod for application as a biological control agent on *A. Aegypti* is *M. formosanus.* They can prey on all the instars of the *A. Aegypti* mosquito and maintain a steady predation rate over time. *M. formosanus* can be artificially cultured by mass production methods and adapt to various environments such as man-made water-containing habitats. Our results suggest that combining *M. formosanus* and the compound marmesin, obtained from the *B. papyrifera* extract, could be broadly applicable against mosquitoes as a larvicidal agent. This method may be successful for controlling *A. aegypti* dispersal.

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