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MOSQUITOCIDAL ACTIVITY OF *Hedychium coronarium* RHIZOME EXTRACT AND COPEPOD *Megacyclops formosanus* FOR THE CONTROL OF DENGUE VECTOR *Aedes aegypti*

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Key words: *Hedychium coronarium*, *Megacyclops formosanus*, *Aedes aegypti*, larvicidal.

ABSTRACT

The bio-efficacy of *Hedychium coronarium* Rhizome extract and copepods *Megacyclops formosanus*, were tested against first to fourth-instar larvae and pupae of dengue Vector *Aedes aegypti* under laboratory conditions. Different solvent extracts of *H. coronarium*, combined with copepod *M. formosanus* showed considerable mortality against *A. aegypti*. The median lethal concentration value (LC_{50}) observed for the larvicidal and pupicidal activities against mosquito vector species *A. aegypti* value were 38.59, ppm; no mortality was observed in the control group. This study was also initiated to test the predatory efficiency of copepod against different larval instars and predatory efficiency was noticed at the laboratory and efficiency was higher after the combined treatment with *H. coronarium* extract. This is an ideal eco-friendly approach for the control of vector control programs.

I. INTRODUCTION

Diseases like dengue, malaria, lymphatic filariasis, leishmaniasis and Chagas' disease are caused by pathogens transmitted by insect vectors and represent a significant part of all morbidity and mortality records in tropical countries. In the last decades, urban areas in most of these countries have faced an accelerated and disorganized growth, with deficient sanitation and general infrastructure, a scenario that favors the expansion of insect vector populations WHO [53]. The main consequence is that two fifths of the world's population is potentially exposed to four infections by the dengue viruses, resulting in 50 million infections annually, as estimated by the World Health Organization (2011). *Aedes aegypti* (Linnaeus) transmits the bulk of dengue infections Phillips [41], and vector control is the only means of combating this disease for which no vaccine, prophylaxis, or therapeutant currently exists. The mosquito gets the virus by biting an infected person. The first symptom of the disease appears in about 5-7 days after the infected mosquito bites a healthy person. It is possible to become infected by dengue multiple times because the virus has four different serotypes. The dengue symptoms of dengue fever include high fever, rash, and a severe headache. Additional of *Chikungunya* fever symptoms include severe joint and muscular pain (break bone fever), nausea, vomiting, and eye pain. Although dengue fever itself is rarely fatal, it can be an extraordinary painful and disableing illness and may become epidemic in a population following the introduction of a new serotype Morena-Sanchez *et al*. [33]. *Aedes aegypti* populations appear to be currently well established in most households at almost every tropical urban setting and are also established in some sub-tropical areas. Indeed, the present recrudescence of these diseases is due to the higher number of breeding places in today's throw-away society and to the increasing resistance of mosquitoes to current commercial insecticides Ciccia *et al*. [4]. Mosquitoes develop genetic resistance to synthetic insecticides Wattal *et al*. [52] and even to biopesticides such as *Bacillus sphaericus* Tabashnik [49]. Years and millions of money have been spent on researches on the dengue vaccine but nothing much is produced.

Plants may be a source of alternative agents for control of

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mosquitoes, because they are rich in bioactive chemicals, are active against limited number of species including specific target-insects and are biodegradable Sukumar *et al.* [48]. *Hedychium coronarium* is an erect herb belonging to the family *Zingiberaceae*. The plant is widely cultivated in Taiwan and available in all tropical countries. The rhizome of the plant is used in the treatment of diabetes Bhandary *et al.* [3]. It is also used as antirheumatic, excitant, febrifuge and tonic Jain *et al.* [11]. Previous phytochemical investigations showed that the plant contains the diterpenes-coronarin A, coronarin B, coronarin C, coronarin D and isocoronarin D Nakatani *et al.* [38]. The plant is used in Chinese natural medicine, and has been prescribed for the treatment of headaches, lancinating pain and contusion inflammatory Hou [10]. In pharmacological studies of this natural medicine, it was reported that sesquiterpenes of *H. coronarium* showed inhibitory effects on the release of beta-hexosaminidase Morikawa *et al.* [34]. Terpenoids from *Hedychium* oil showed antioxidant and antimicrobial properties Joy *et al.* [14]; Joshi *et al.* [13]. Solvent organic extracts from aerial parts, bark, flowers, fruits, heartwood, leaves, twigs and root from medicinal plants have been investigated aiming to validate their ethnopharmacological use. Extracts from plants used to treat diarrhea (*Indigofera daleoides*, *Punica granatum*, *Syzygium cordatum*, *Gymnosporia senegalensis*, *Ozoroa insignis*, *Elephantorrhiza elephantina*, *Elephantorrhiza burkei*, *Ximenia caffra*, *Schotia brachypetala* and *Spirostachys africana*) contained agents against bacteria that cause gastrointestinal infections (*Vibrio cholerae*, *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysentery*, *Shigella sonnei*, *Shigella flexneri*, *Shigella boydii* and *Salmonella typhi*) and this strengths their usefulness in the treatment of diarrhea Mathabe [30].

The integrated control of *A. aegypti* emphasizing the biological control of larvae by predators and parasites is a desirable alternative strategy to the traditional use of insecticides, due to the development of resistance and the negative impact on the environment Molyneux [31]. There are several types of biological control including the direct introduction of parasites, pathogens and predators to target mosquitoes. Copepods are widespread in ponds, lakes, streams, and small reservoirs in tropical and subtropical regions. Effective biocontrol agents include predatory copepods are one of the natural enemies that feed on mosquito larvae. These agents are microcrustacea, present in fresh water worldwide. *Mesocyclops thermocyclopoides* is a very common species and was evaluated as a biological control against *Aedes*. Several species of copepods, including *Mesocyclops aspericornis*, *M. thermocyclopoides*, *M. guangxiensis*, and *M. longisetus*, have been reported as potential biological control agents of *A. aegypti* Kay *et al.* [16]. Cyclopoid copepods are important predators of early-instar *A. aegypti* larvae Marten [28]; Marten *et al.* [29]. This copepod feeds on the 1st and 2nd instars of the mosquito larvae, fatally wounding about seven individuals pery day Shaper and Hernandez [46]. Copepods *Mesocyclops* has most been studied as an antagonist of mosquito larvae and whose effectiveness has been demonstrated in different countries, including the United States Marten [28], Honduras (Marten *et al*. [29], Vietnam Nam *et al*. [39], India (Murugan *et al.* [36, 37] and the French Polynesia (Lardeux *et al*. [23]. Inoculative copepod releases in natural and artificial small water containers at urbanized areas significantly reduced the population abundance of *A. aegypti* (Gorrochotegui *et al*. [7]; Schaper [45]. Prolonged efficacy of a combination of bacteria (*Bacillus thuringiensis* var. *israelensis* [Bti] and copepods (*Mesocyclops aspericornis*) in controlling immature forms of *Aedes aegypti* in peridomestic water containers was achieved by adding various products from local villages as supplementary food for copepods Kosiyachinda *et al.* [18]. The present study was conducted as a brief individual and combined laboratory experiment designed to *Hedychium coronarium* rhizome various solvents extracted and predatory copepod *Megacyclops formosanus* against *A. aegypti* in the search for an alternative natural product, which can be used in the control of recurrent dengue epidemics.

II. MATERIALS AND METHODS

1. Collection of Eggs and Maintenance of Larvae

The eggs of *A. aegypti* stock culture 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 of water for hatching. The mosquito larvae were fed with a 3:1 ratio of pedigree dog biscuits and yeast. The feeding was continued until the larvae entered the pupal stage.

2. Maintenance of Pupae and Adults

The pupae were collected from the culture trays and transferred to plastic containers (12 × 12 cm) containing 250 mL of water by using a dipper. The plastic jars were kept in a 30 × 30 × 30-cm mosquito cage for adult emergence. The mosquito larvae were maintained at 27 ± 2°C, at 75%-85% relative humidity under a light:dark photoperiod of 14:10 h. A 10% sugar solution was provided for a period of 3 d before blood feeding.

3. 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 to last 5 d. After blood feeding, enamel trays with water from the culture trays were placed in the cage as ovipositional substrates.

4. Collection of Plant and Preparation of Extract

The plant *Hedychium coronarium* rhizome was collected around from National Taiwan Ocean University, Taiwan**.** The *H. coronarium* rhizome was washed with tap water and shade-dried at room temperature (27 ± 2°C). An electrical blender was used to powder the dried rhizome. The 300 g of

rhizome powder was extracted with 1 L of the organic solvents petroleum ether, acetone, and methanol by using a Soxhlet apparatus, with a boiling point range of 60-80°C for 8 h. After extract dry to room temperature then different concentrations were prepared 10 to 900 ppm solutions.

5. Larval/Pupal Toxicity Test

The laboratory colonies of mosquito larvae/pupae were used to test the larvicidal/pupicidal activity. One hundred individual first to fourth instar larvae (I, II, III, and IV) and pupae were introduced into a 500 mL glass beaker containing 249 mL of dechlorinated water, and 1 mL of the desired concentration of rhizome extract was added. Larval food was given to the test larvae during the experimental period. At each tested concentration, 2 to 5 trials were performed, consisting of 5 replicates each. The two control groups was set up by mixing 1 mL of acetone with 249 mL of de-chlorinated water. The second control group larvae and pupae exposed to the dechlorinated water without acetone served as the control. The control group's mortalities were corrected using Abbott's formula Abbott [1]. The LC_{50} and LC_{90} were calculated according to the toxicity data by using probit analysis Finney [6].

6. Copepod Culture

The *M. formosans* stock culture 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 1890 in dechlorinated tap water. The copepods were reared at 27 ± 2°C temperature, pH 7, and a photoperiod of 12:12 h in an incubator

7. Predatory Efficiency Test

Adult copepods were used to measure the predatory activity toward the first to fourth instars (I, II, III, IV) and pupae of the mosquito larvae. One hundred individuals mosquito larvae of each instar and 10 adult copepods were introduced into separate 500 mL glass beakers containing 250 mL of dechlorinated water. The mosquito larvae were replaced daily with new ones. Each mosquito instar–copepod treatment was replicated 5 times. The control group consisted of 250 mL of dechlorinated water without copepods. The glass beakers were inspected after 24, 48, 72, 96, and 120 h, and the number of prey consumed by the predators was recorded.

8. Predatory Efficiency Test in Combination with *H. coronarium*

Adult copepods were used to quantify the predatory activeity toward the first to fourth instars larvae and pupae of the mosquito. One hundred individuals mosquito larvae of each instar and 10 adult copepods were introduced into separate 500 mL glass beakers containing 250 mL of dechlorinated water and 1 mL of the desired concentration of *H. coronarium* rhizome extract. The mosquito larvae were replaced daily with new ones. Each mosquito instar-copepod treatment was replicated five times. The controls consisted of 249 mL of dechlorinated water and 1 mL of acetone without any copepods. The glass beakers were inspected after 24, 48, 72, 96, and 120 h, and the numbers of prey consumed by the predators were recorded.

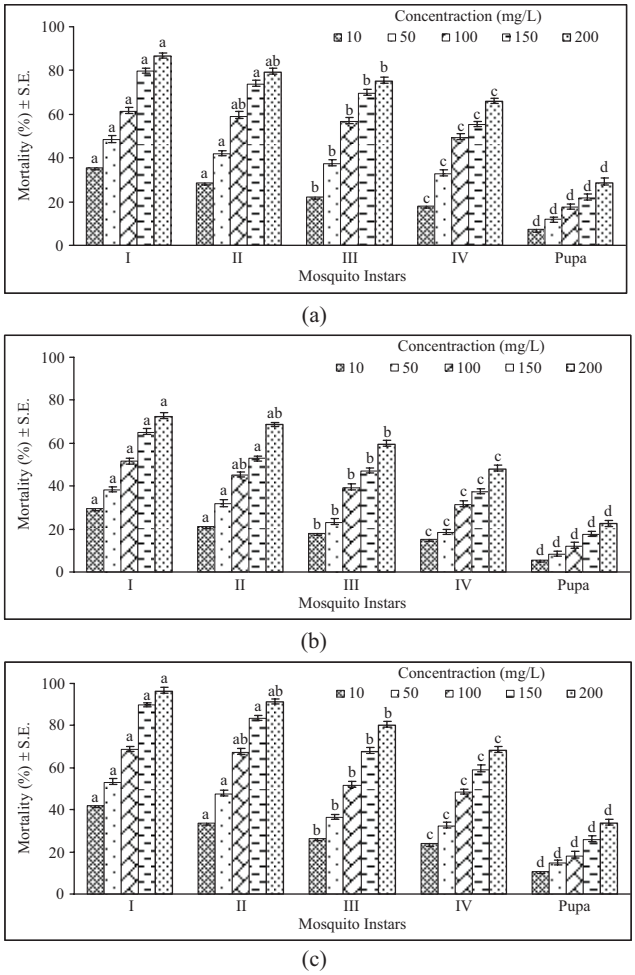

Fig. 1. larvicidal activity of different solvent extracts of *H. coronarium* against dengue vector *A. aegypti*. (a) petroleum ether extract (b) acetone extract (c) methanol extract value represents mean ± S.E. (standard error) of 5 replications. Mortality of the larvae observed after 24 h of exposure period. Different alphabets in the column are statistically significant at $p < 0.05$ level DMRT test. Control nil mortality.

9. Statistical Analysis

All data were subjected to analysis of variance; the means were separated using Duncan's multiple range tests (DMRT) by Alder and Rossler [2]. The average larval mortality data were subjected to probit analysis; to obtain the LC_{50} and LC_{90}, the values were calculated using the Finney (1971) method.

Table 1. Larvicidal activity of different solvent extracts of *H. coronarium* against dengue vector *A. aegypti*.

Solvent	Instars	LC_{50} (LC_{90})	LC_{50} LCL (UCL)	LC_{90} LCL (UCL)	χ^2 df = 3	Rogation
Petroleum ether	I	56.81 (216.75)	37.52 (72.23)	190.03 (257.11)	0.68	X = +0.008 Y = –0.455
	II	77.09 (247.53)	59.30 (92.52)	215.81 (296.70)	1.454	X = +0.008 Y = –0.580
	III	93.83 (259.39)	78.06 (108.87)	227.05 (308.99)	3.091	X = +0.008 Y = –0.726
	IV	126.35 (322.83)	108.81 (147.02)	274.77 (403.543)	3.004	X = +0.007 Y = –0.824
	Pupa	323.94 (612.90)	255.06 (497.55)	457.146 (1019.55)	0.328	X = +0.004 Y = –1.437
Acetone	I	116.63 (304.24)	98.21 (115.03)	257.28 (384.85)	0.386	X = +0.006 Y = –0.593
	II	127.97 (327.55)	110.14 (149.33)	277.96 (411.54)	0.662	X = +0.006 Y = –0.822
	III	158.30 (366.32)	137.82 (187.35)	307.25 (469.93)	0.708	X = +0.006 Y = –0.975
	IV	208.87 (455.29)	176.98 (301.79)	365.70 (635.28)	0.689	X = +0.005 Y = –1.086
	Pupa	370.65 (666.63)	283.25 (620.74)	483.58 (1204.01)	0.072	X = +0.004 Y = –1.605
Methanol	I	38.59 (160.96)	21.76 (51.92)	143.32 (185.51)	2.717	X = +0.010 Y = –0.404
	II	54.08 (187.55)	37.91 (67.41)	167.12 (216.46)	0.212	X = +0.010 Y = –0.519
	III	92.51 (254.93)	77.01 (107.27)	223.51 (302.77)	0.029	X = +0.008 Y = –0.730
	IV	117.49 (318.98)	99.57 (137.54)	270.68 (400.84)	0.609	X = +0.006 Y = –0.747
	Pupa	301.02 (600.97)	239.07 (455.24)	449.77 (988.48)	0.145	X = +0.004 Y = –1.292

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

Bioassay data and predation trials were analyzed using the SPSS Statistical Software Package version 17.0. Results with $P < 0.05$ were considered statistically significant.

III. RESULTS

The activity of crude *H. coronarium* rhizome extracts is often attributed to their complex mixture of active compounds. Preliminary screening is an effective and widely used means for evaluating the potential larvicidal activity of plant. The larvicidal activity of different solvent crude plant extracts are noted and presented as follows. The larvicidal and pupicidal activity of the *H. coronarium* petroleum ether (HPE) extract at various concentrations is shown in Fig. 1(a). Considerable mortality was evident after *H. coronarium* treatment for all larval instars and pupae. Mortality increased with the concentration. For example, the mortality at the first instar stage at a 10 ppm concentration was 35.6%; however, mortality increased to 86.6% when the concentration was increased to 200 ppm. The mortality in the pupal stage was 7.2% at a 10 ppm concentration, but it increased to 28.6% at a 200 ppm concentration (Fig. 1(a)). The LC_{50} and LC_{90} values were shown as follows: the LC_{50} values of the first instar, second instar, third instar, and fourth instar were 56.81, 77.09, 93.83 and 126.35 ppm, respectively; and the LC_{90} values of the first instar, second instar, third instar, and fourth instar were 216.75, 247.53, 259.39 and 322.83 ppm, respectively. The LC_{50} and LC_{90} values for pupae were 323.94 and 612.90 ppm, respectively (Table 1).

The mortality of *A. aegypti* larvae and pupae (I to pupae) following *H. coronarium* rhizome acetone extract (HAE) treatment at different concentrations (10 to 200 ppm) is shown in Fig. 1(b). A 29.4 % mortality was noted in I instar larvae following 10 ppm concentration HAE treatment, which increased to 72.2% with the 200 ppm concentration HAE treatment. A 5.6% mortality was noted in pupae following 10 ppm concentration HAE treatment, which increased to 22.4% with the 200 ppm concentration. A similar trend was observed for all of the instars of *A. aegypti* at different concentrations of HAE treatment (Fig. 1(b)). The LC_{50} and LC_{90} values were shown as follows: LC_{50} values of I instar, II instar, III instar, and IV instar were 116.63, 127.97, 158.30 and 208.87 ppm,

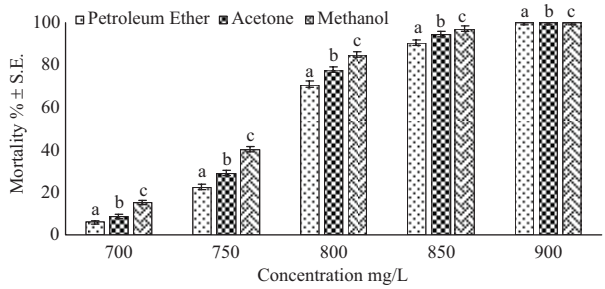

Fig. 2. The activity of different solvent extracts of *H. coronarium* against *Megacyclops formosanus*.

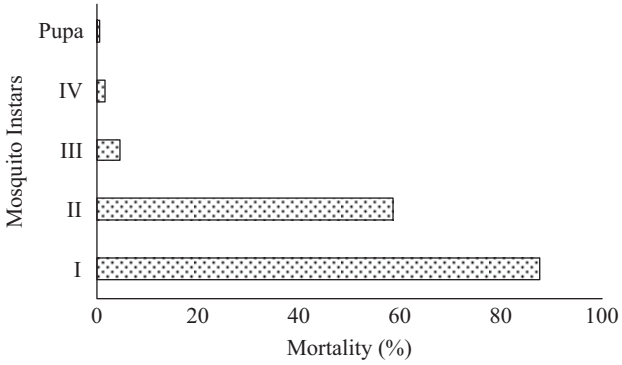

Fig. 3. Predatory efficiency of Copepods, *Megacyclops formosanus* on *A. aegypti*.

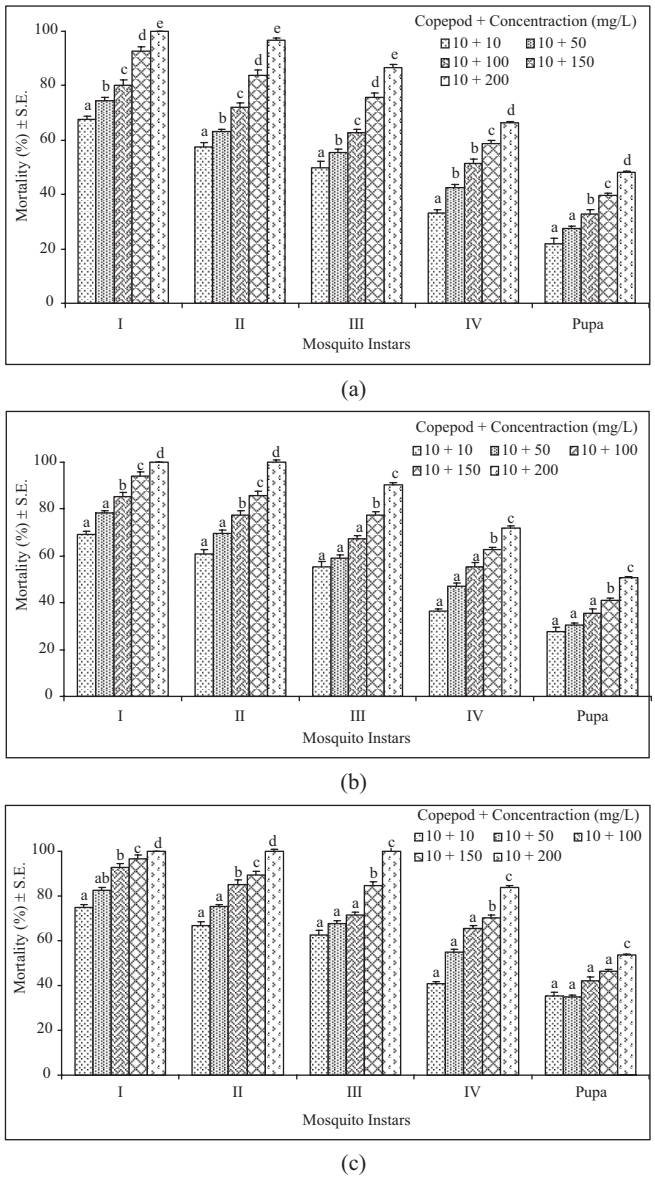

Fig. 4. Combined effect of predatory copepods, *M. formosanus* and seaweed *H. coronarium* different solvent crude extract against *A. aegypti*. (a) Petroleum ether Extract (b) Acetone Extract (c) Methanol Extract. Value represents mean ± S.E. (Standard error) of 5 replications. Mortality of the larvae observed after 24 h of exposure period. Different alphabets in the column are statistically significant at $P < 0.05$ level DMRT test. Control nil mortality.

respectively. The LC_{90} values of I instar, II instar, III instar, and IV instar were 304.24, 327.55, 366.32 and 455.29 ppm, respectively. The LC_{50} value of pupae was 370.65 ppm and the LC_{90} value of pupae was 666.63 ppm (Table 1).

The larval and pupal mortality results of *A. aegypti* following treatments at different concentrations (10 to 200 ppm) are shown in Fig. 1(c). A 41.8% mortality was noted in the first instar larvae following *H. coronarium* rhizome methanol extract (HME) treatment at 10 ppm concentration, which increased to 96.4% at 200 ppm concentration. A 10.6% pupal mortality was noted with the 10 ppm concentration HME treatment. A similar trend was observed for all the instars of *A. aegypti* for all the different concentrations of HME treatment. The LC_{50} and LC_{90} values were shown as follows: the LC_{50} values of the first instar, second instar, third instar, fourth instar, and pupae were 38.59, 54.08, 92.51, 117.49 and 301.02 ppm, respectively. The LC_{90} values of the first instar, second instar, third instar, fourth instar, and pupae were 160.96, 187.55, 254.93, 318.98 and 600.97 ppm, respectively (Table 1).

The activity of the plant extracts showed a moderate toxic effect on the copepod *M. formosanus* after 24 h of exposure at a 700 to 900 ppm concentration. However, the mortality was found when using the petroleum ether, acetone, and methanol extracts of *H. coronarium* rhizome (LC_{50} = 780.158, 769.695 and 755.461 ppm; LC_{90} = 841.596, 830.202 and 818.245 ppm respectively) against *M. formosanus* (Fig. 2). The copepod *M. formosanus* demonstrated effective predation against *A. aegypti* larval instars. The predation percentage decreased as the mosquito larvae grew older (Fig. 3). The predation percentage decreased as the mosquito larvae grew older. The early instars were more susceptible and considerably preferred by the copepods. Extremely low predation was observed in the IV instars of *A. aegypti*. The predatory efficiency of a single adult copepod was 8.77, 5.84, 0.45, 0.15, and 0.52 larvae/d in the I, II, III, IV, and pupal instars, respectively.

The predatory efficiency of *M. formosanus* increased when the mosquito larvae were treated with the petroleum ether, acetone, and methanol extracts of *H. coronarium* rhizome.

Fig. 4(a, b, c), provides the predatory efficiency of *M. formosanus* against the larval instars of *A. Aegypti* treated with the biopesticide, *H. coronarium* rhizome. The predatory efficiency percentage of copepods on treated larvae was higher compared with that on untreated larvae. The I and II instars were much preferred compared to the later instars. The predatory efficacy of a single copepod on HPE treated larvae were 8.30, 7.46, 6.6, 5.04 and 3.39; that on HAE treated larvae were 8.53, 7.87, 6.98, 5.46 and 3.70; and that on HME treated larvae were 8.94, 8.32, 7.73, 6.30 and 4.24 larvae/d for the I, II, III, IV, and pupal instars, respectively.

IV. DISCUSSION

Dengue is an arboviral disease mainly transmitted by the mosquito *A. aegypti*. More than 50 million people are at risk of dengue virus exposure worldwide. Annually, there are two million infections; 500,000 cases of dengue hemorrhagic fever; and 12,000 deaths Guha-Sapir and Schimme [8]. Plant extracts and phytochemicals have potential as products for mosquito control because many of them are selective, may often biodegrade into non-toxic products, and may be applied to mosquito breeding places in the same way as conventional insecticides (Sukumar *et al.* [48]; Murugan *et al.* [35]. The activity of crude plant extracts is often attributed to the complex mixture of active compounds. The preliminary screening is a good mean of evaluation of the potential larvicidal activity of plants popularly used for this purpose. Larvicidal activity of different solvent crude extracts plant are noted and presented in Table 1 and Fig. 1(a, b, c). The larvicidal activity of the essential oil aqueous solutions of the stalks and leaves of *Croton argyrophylloides*, *Croton nepetaefolius*, *Croton sonderianus*, and *Croton zehntneri* showed 100% mortality at 50 mL against *A. aegypti* Lima *et al.* [25]. Morais *et al.* [32] also reported that the main components methyleugenol and alpha-copaene for *C. nepetaefolius* (LC_{50} of 84 ppm); alpha-pinene and beta-pinene for *Croton argyrophyloides* (LC_{50} of 102 ppm); and alpha-pinene, betaphelandrene, and transcaryophyllene for *C. sonderianus* (LC_{50} of 104 ppm) and *Croton zenhtneri* exhibited higher larvicidal activity with an LC_{50} of 28 ppm against *A. aegypti.* Oleic and linoleic acids isolated from the whole plant petroleum ether extract of *C. colocynthis* were quite potent against fourth-instar larvae of *A. aegypti* (LC_{50} 8.80, 18.20, and LC_{90} 35.39, 96.33 ppm), respectively Rahuman *et al*. [43]. The methanol extract of *Clerodendron inerme* and *Acanthus ilicifolius* at different concentrations (20-100 ppm) against the I-IV instars larvae and pupae produced the LC_{50} values of 45.74%, 51.04%, 57.17%, 68.16%, and 56.44%, respectively; the LC_{50} values for the *A. ilicifolius* leaf extract against I-IV instars larvae and pupae were 69.579%, 76.635%, 82.692%, 88.230%, and 87.287%, respectively Kovendan and Murugan [19]. The compound beta-sitosterol isolated from petroleum ether extract of *Abutilon indicum* showed LC_{50} value of 11.49, 3.58, and 26.67 ppm against *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus*, respectively Rahuman *et al.* [42]. Many studies have reported the effectiveness of plant extracts against mosquito larvae (Murugan *et al*. [35, 36]; Kovendan *et al.* [20, 21]; Subramaniam *et al.* [47]; Kalimuthu *et al*. [15].

The biological control of mosquito larvae with predators and other biocontrol agents would be a more effective and ecofriendly approach compared to using synthetic chemicals, for reducing the concomitant damage of insecticide applications on the environment Kumar and Hwang [22]. The probability and frequency of encounters between prey and predator are influenced by their behavior and the presence of refuges Trochine *et al.* [51]. The laboratory predation rates observed in these experiments compare favorably with those observed by Marten [28] who reported single-copepod predation rates of 90% on first instar mosquito larvae after 24 h. A significant difference between the two experiments, however, is that Marten's copepods were starved for 24 h prior to prey exposure whereas ours were not. Williamson [54] showed that attack and consumption rates by the copepod *Mesocyclops edax* on various prey increased after starvation for periods as short as 24 h of exposure. *Mesocyclops* has been studied as an antagonist of mosquito larvae, and its effectiveness has been demonstrated in different countries, Marten [28], Honduras Marten *et al*. [29], Vietnam Nam *et al.* [39] and French Polynesia Lardeux *et al.* [23]. This work demonstrates that the predatory efficacy of *M. thermocyclopoides* is substantial against the different larval instars of *A. aegypti*. The predator *M. thermocyclopoides* consumed first and second instars in greater numbers than third and fourth instars. The active movements and large size of the older larval instars may have reduced the predation rate of the copepods. Though there was little consumption of the late instars, punctures and injuries to late instars of mosquitoes lead to constrained development and death. As a support report from earlier work states that *M. thermocyclopoides* is a very common species in Costa Rica Collado *et al.* [5]; Hernández-Chavarría and Schaper [9] and was evaluated as a biological control agent against *Aedes*. This copepod feeds on the first and second instars of the mosquito larvae, fatally wounding about seven individuals per day Schaper [45]. Results of cage simulated experiments on the efficacy of some species of copepods against *A. aegypti* larvae conducted by Jennings *et al.* [12]; Kay *et al.* [17] and Schaper [45] were different from our results. *M. guangxiensis* and *M. aspericornis* eliminated all mosquito larvae produced by 25 pairs of *A. aegypti* in 3-L tins placed in screen cages that were inoculated by 50 gravid female copepods 6 wk after the start of the experiment Jennings *et al*. [12]. In the present results, the predatory efficiency of a single adult copepod was 8.77, 5.84, 0.45, 0.15, and 0.52 larvae/day on I, II, III, and IV instars, respectively.

Copepods are effective predators of first and second instars of mosquitoes but are not effective against the late instars; hence, a combined approach using botanicals to increase the predatory efficiency of copepods against the late instars was effective. In conjunction with rhizome, the copepods showed

higher predation against *A. aegypti* larvae when compared with predation without the addition of rhizome extract. The number of identified compounds was 30 in the leaves and 32 in the rhizomes, representing 98.3% and 97.8% of the total composition. *H. coronarium* rhizome active compound such as β - Pinene (33.9%), α - pinene (14.7%), 1,8-cineole (13.3%), r-elemene (11.0%) and carotol (9.1%) were the main components of the leaf oil, including 82.0% terpenoid compounds. The major constituents of the rhizome oil were 1,8-cineole (37.3%), β - pinene (23.0%), α - terpineol (10.4%) and α - pinene (9.9%), comprising 80.6% of the oil. The marker compounds of Zingiberaceae family, i.e., β - pinene, α - pinene, and 1,8-cineole were present in two organs(Joy *et al.* [14]; Joshi *et al.* [13]. It is reported that β - pinene, α - pinene and 1,8-cineole present larvicidal effects (LC_{50} values 15.4, 12.1 and 57.2 ppm, respectively) on *A. aegypti* larvae (Lucia *et al.* [26], might have interrupted the development and active movement of mosquito larvae, which increased the predatory efficacy of copepod on the early and also late instars. The active chemical compounds in *H. coronarium* rhizome also showed no effect on the survival and development of the copepod. Similar investigations have also been done using *M. aspericornis* in conjunction with other controlling methods and resulting in the eradication of *A. aegypti* (Kay *et al.* [17]; Nam *et al.* [39]; Lardeux *et al.* [23]. *Bacillus thuringiensis var. israelensis* has been used in conjunction with *M. aspericornis* because of its high toxicity and high specificity of Bti to mosquito larvae (Riviere *et al.* [44]; Tietze *et al.* [50]. In the present results, The predatory efficacy of a single copepod on HPE treated larvae were 8.30, 7.46, 6.6, 5.04 and 3.39; that on HAE treated larvae were 8.53, 7.87, 6.98, 5.46 and 3.70; and that on HME treated larvae were 8.94, 8.32, 7.73, 6.30 and 4.24 larvae/d for the I, II, III, IV, and pupal instars, respectively. Our study demonstrates that the predatory efficacy of *M. formosanus* is substantial against the different larval instars of *A. aegypti*. The predator *M. formosanus* consumed greater numbers of first and second instars than third and fourth instars.

Copepods are effective predators of the first and second instars of mosquitoes, but they are ineffective against later instars; hence, a combined approach that involves using plant to increase the predatory efficiency of copepods against the late instars proved effective. Combined with plant, the copepods showed greater predation against *A. aegypti* larvae compared with predation without the addition of *H. coronarium* rhizome. Similar investigations have been conducted that have successfully eradicated *A. aegypti* by using *M. aspericornis* combined with other controlling methods (Lardeux *et al.* [24]; Nam *et al.* [40]; Murugan *et al.* [36]; Mahesh Kumar *et al.* [27].

In conclusion, we evaluated the role of *H. coronarium* rhizome extracts in *A. aegypti* larvicidal activity. Based on the results, we recommend further investigation to enhance the control efficacy of natural product extracts on larvicidal properties. The most appropriate copepod to be used as a biological control agent for *A. aegypti*. *M. formosanus* can prey on all of the instars of mosquito species and maintain a steady predation rate over time. The *M. formosanus* copepod can be artificially cultured using mass production methods, and is able to persist various environments within human-made water-containing habitats. Despite *M. formosanus and H. coronarium* rhizome being broadly applicable against mosquitoes.

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