



## Combined Larvicidal and Pupicidal Action of *Coriandrum sativum*, *Piper nigrum* and Synthetic Insecticide Cypermethrin Against the Dengue Fever Mosquito, *Aedes Aegypti* L.

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### ABSTRACT

After the adoption of green revolution, India has The combined action of cypermethrin and methanolic extracts of *Coriandrum sativum*, *Piper nigrum* were observed for their larvicidal and pupicidal activities against *Aedes aegypti*. When analyzed individually, Cypermethrin were found to be most effectual against the first instar larvae of *Aedes aegypti*, followed by methanolic extracts of *Piper nigrum*, *Coriandrum sativum* being least effective. The LC<sub>50</sub> values obtained with Cypermethrin and methanolic extracts of *Piper nigrum*, *Coriandrum sativum* against the first instar larvae were 0.61, 0.71 and 0.86%, respectively and the LC<sub>90</sub> values were 1.32, 2.73 and 3.71% respectively. The combination of Cypermethrin and *Coriandrum sativum* was studied at mixed with Cypermethrin 0.5% and *Coriandrum sativum* 0.5, 1.0, 1.5, 2.0 and 2.5. Similar mixtures were also used for the combination of Cypermethrin and *Piper nigrum*. The Cypermethrin and *Coriandrum sativum* seed extract combination acted antagonistically. The combination of Cypermethrin and *Piper nigrum* seed extract acted synergistically against the target organisms at a first instar larvae, which showed the best results of: LC<sub>50</sub> 0.58 and LC<sub>90</sub> 2.40% at 24 hours, respectively. The present study will be helpful in developing in a commercial formulation for effective vector management.

**Keywords:** *Cypermethrin*, *Coriandrum sativum*, *Piper nigrum*, *Aedes aegypti*, larvicide, pupicide and synergism

### INTRODUCTION

Mosquitoes are major public health pests throughout the World. Among the 3492 species of mosquitoes recorded Worldwide, more than a hundred species are capable of transmitting various diseases to humans and other vertebrates (Rueda, 2008). Many devastating diseases such as malaria, West Nile virus (WN), dengue, filariasis, yellow fever, Japanese encephalitis and chikungunya are transmitted to humans by vector mosquitoes. Also mosquito bite causes considerable pain and leads to loss of sleep. Mosquito attack on farm animals can cause loss of body weight and decreased milk production (Nour *et al.*, 2009). The *Aedes aegypti* mosquito is the primary vector of dengue. The *Aedes aegypti* mosquito lives in urban habitats and breeds mostly in man-made containers. Unlike other mosquitoes *Aedes aegypti* is a daytime feeder; its peak biting periods are early in the morning and in the evening before dusk. Female *Aedes aegypti* bites multiple people during each feeding period. As estimated 500 000 people with severe dengue require hospitalization each year, a large proportion of whom are children. About 2.5% of those affected die. Dengue has become the most important mosquito-borne viral disease affecting humans (WHO, 2009). Dengue fever is a severe, flu-like illness that affects infants, young children and adults, but seldom causes death. Dengue should be suspected when a high fever (40c-/104F) is accompanied by two of the following symptoms; severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands or rash.

Insect pests have been controlled with synthetic insecticide over 50 years but problems of pesticide resistance and negative effects on non-target organisms, including human and environment have been reported (FAO, 1992). Botanical insecticides are considered as alternatives to the synthetic chemicals for being biodegradable, pest specific, non-hazardous to human health and environment and leaving no toxic residue in nature (Periera and Wohlgemuth, 1982).

*Coriandrum sativum* coriander (Family: Apiaceae). It is a herb that is often used as a side dish in Iranian cooking is a soft, hairless plant growing upto 50cm in height and is native to Southwestern Asia and North Africa. All parts of the plant are edible, but the fresh leaves and the dried seeds are the most eaten parts of the plant (Burdock and Carabin, 2009). These seeds used to treat indigestion, aninexia and stomachache. Coriander seeds are ground into a paste for application to skin and mouth ulcers. Before the invasion of toothpaste, coriander seeds were chewed as a breath sweetener. Coriander is used in several ayurvedic remedies.

*Piper nigrum* (Family: Piperaceae) contains approximately 2000 species, which are widely grown and commonly used in tropical regions as medicines, spile and condiments in regional cuisine (Numba, 1993). Pepper plants have also been prescribed for pest control as they contain potentially insecticidal compounds (Su and Horvat, 1981).

Cypermethrin belongs to a class of insecticides known as synthetic pyrethroids. Cypermethrin is commonly used as a crack and crevice or spot treatment for residual and contact control of spiders, ants, carpenter ants, scorpions, German cockroaches, ladybugs, carpenter bees, and yellow jackets. In laboratory tests, cypermethrin was highly toxic to mosquito larvae and pupae. It was more toxic at low temperatures after a 24 hours exposure. No significant mortality of caged stickleback fish occurred in these pools (Helson and Surgeoner, 1986).

Synergistic activity between synthetic pesticides and botanicals is powerful and effective tool for the development of an efficient, more eco-friendly and less hazardous insect pest control strategy (Bernard and Philogne, 1993). The application of synergists has been preferred as a strategy to enhance the eco-friendliness and cost effectiveness of an insecticide by reducing the quantity needed to kill the target

population and lengthen the residual activity. The role of synergists in resistance management is an accepted alternative for resistance management.

The importance of propel selection of plant extracts as synergists in mixed formulation with different synthetic insecticides is being increasingly recognized in mosquito management. The mixture may provide less toxicity, prevent the development of resistance, have economic benefits and could be more effective than individual components of the mixture.

In the view of the above fact, an attempt has been made to evaluate the individual and combined effect of *Coriandrum sativum*, *Piper nigrum* and synthetic insecticide Cypermethrin against the laticidal and pupicidal activity of the dengue vector, *Aedes aegypti*.

## METHODOLOGY

The plants material of *Coriandrum sativum* (Family: Apiaceae), *Piper nigrum* (Family: Piperaceae) seeds were collected from Namakkal market, Tamil Nadu, India. The seeds of *Coriandrum sativum* and *Piper nigrum* were dried in shade under normal environmental condition and powdered by on electrical blender to coarse powder and stored in opaque screw tight jars until use. The dried *Coriandrum sativum* and *Piper nigrum* seeds powder (30g) was extracted in Soxhlet apparatus with 300 ml of 95% methonal at controlled temperature (Vogel, 1978). The collected extract was concentrated under reduced pressure (<45°C) using a vacuum pump for complete removal of the solvent. Pure organic part of the sample thus prepared was stored at 4-5°C until used. 30 g of the plant residue was dissolved in 300 ml of methanol considered as 1% stock solution. Each different concentration was prepared from stock solution.

Cypermethrin (10%EC) or megathrin were obtained from the Agroform Namakkal District and diluted in dechlorinated tap water to obtain a concentration of 1% stock for cypermethrin. Different test concentrations ranging from 0.25 to 1.25% were prepared by diluting these stock solutions.

Keeping the cypermethrin as standard solution (0.5%), the stock was mixed with the different percentage of the phytoextract *Coriandrum sativum* such as 0.5, 1.0, 1.5, 2.0 and 2.5 and *Piper nigrum* such as 0.5, 1.0, 1.5, 2.0 and 2.5.

The egg rafts of *Aedes aegypti* were collected from local in and around Namakkal District rain water bodies, water stored container with help of 'O' type brush for the laboratory bioassay. These eggs were brought to the laboratory and were transformed to 18x13x4 cm size enamel trays containing 500ml of water and keep for larval hatching. The mosquito larval culture was maintained our laboratory at 27± 2°C, 75-80% relative humidity under a period of 11±0.5 hrs in the cycles. The mosquito larvae were feed with dog biscuits and yeast at 3:1 ratio. The feeding was continued till the larvae are transformed into the pupal stage. The pupae were collected from the culture trays and were transformed to conical flasks containing 500 ml of water with help of sucker.

LC<sub>50</sub> (lethal concentration 50%) is the concentration of any toxic substance reducing by mortality the number of tested individuals to 50% in a prefixed time (Ravera, 1986). According to (Rand and Petrocelli, 1985) the LC<sub>50</sub> (median lethal concentration) is estimated to produce mortality in 50% of a test solution over a specific period of time.

Preliminary tests were carried out to find the toxicity of the median lethal to tolerance limit of *Aedes aegypti* larvae to (*Coriandrum sativum*, *Piper nigrum*) seeds and synthetic insecticide for 24 hours. For determining LC<sub>50</sub> concentration separate glass beaker of 100 ml of water capacity were taken. Then

different concentration of two plants seeds extract and synthetic insecticide were added to different glass beakers. Then 10 *Aedes aegypti* was introduced in to each glass beaker, a control beaker with 10 larvae were also maintained. The mortality/ survival of larvae in the treatment glass beaker were recorded after 24 hours. The concentration at which 50% mortality of larval occurred after 24 hours was taken as the median lethal concentration (LC<sub>50</sub>) for 24 hours. The LC<sub>50</sub> concentration for 24 hours was calculated by the profit analysis method of (Finney, 1971).

A laboratory colony of *Aedes aegypti* larvae were used for the larvicidal toxicity. 25 numbers of first, second, third and fourth instar larvae were kept in 300 ml of plastic cup containing 249 ml of dechlorinated water and 1 ml of desired concentration of plants extracts of (*Coriandrum sativum*, *Piper nigrum*) seeds and synthetic insecticide were added. Larval food was given for the test larvae (WHO, 1996). At each tested concentration 1 to 5 trials were made and each trial consisted of five replicates. The control was setup by 250 ml of dechlorinated water without plant extract and synthetic insecticide. In the larval toxicity, the larvae exposed to dechlorinated water without plant extract and synthetic insecticide served as control. Mortality was corrected by using Abbott's formula (Abbott, 1925).

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

$$\text{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae tested}} \times 100$$

The values of LC<sub>50</sub>, LC<sub>90</sub> and their 95% confidence limit of upper confidence limit (UCL) and lower confidence limit (LCL) and Chi-square values were calculated by using profit analysis (Finney, 1971). The level of significance by Duncan's Multiple Range Test (Duncan, 1963).

A laboratory colony of *Aedes aegypti* were used for pupicidal toxicity test and 25 number of freshly emerged pupae were kept in 300 ml plastic cup containing 249 ml of dechlorinated water and 1 ml of desired concentration of plants extracts of (*Coriandrum sativum*, *Piper nigrum*) seeds and synthetic insecticide were added. At each tested concentration 1 to 5 trials were made and each trial consisted of five replicates (WHO, 1996). The control was setup by 250 ml of dechlorinated water without plant extract and synthetic insecticide. In the pupal toxicity, the pupae exposed to dechlorinated water without plant extract and synthetic insecticide served as control. Mortality was corrected by using Abbott's formula (Abbott, 1925).

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

$$\text{Percentage of mortality} = \frac{\text{Number of dead pupae}}{\text{Number of pupae tested}} \times 100$$

The values of LC<sub>50</sub>, LC<sub>90</sub> and their 95% of confidence limit of upper confident limit (UCL) and lower confidence limit (LCL) and Chi-square values were calculated by using profit analysis (Finney, 1971). The level of significance by Duncan's Multiple Range Test (Duncan, 1963).

The methanolic extracts of *Coriandrum sativum* and *Piper nigrum* seed extract was tested for larvicidal and pupicidal efficacy of with and without insecticide (Cypermethrin). A control was setup by 250 ml dechlorinated water. The synergistic factor was calculated from LC<sub>50</sub> value of plant extract with insecticide. The synergistic (SF) is greater than one is considered to be synergism of SF value is less than one is considered to be antagonism.

$$\text{Synergistic factor (SF)} = \frac{\text{LC}_{50} \text{ value of insecticide alone}}{\text{LC}_{50} \text{ value of plant extract with insecticide}} \times 100$$

The tests with more than 20% mortality in control and pupae formed were discarded and repeated again. If the control mortality ranged between 5 -20% it was corrected using Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality} = \frac{\% \text{ Test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

The data were analysed using computerized SPSS programme. The LC<sub>50</sub> and LC<sub>90</sub> values with 95% of fiducial limits were calculated in each bioassay to measure difference between the test samples. The results obtained with different extracts were analysed using student's t-test with statistical significance considered for P ≤ 0.05.

## RESULTS

In our study, *Coriandrum sativum* gave an LC<sub>50</sub> of 0.86, 1.21, 1.54, 2.04 and 2.40% at 24 hours after the exposure. It gave on LC<sub>90</sub> value of 3.71, 4.30, 5.13, 5.85 and 6.22% at 24 hours after the exposure. *Piper nigrum* gave an LC<sub>50</sub> of 0.71, 0.94, 1.14, 1.41 and 1.82% at 24 hours after the exposure. It gave on LC<sub>90</sub> value of 2.73, 3.19, 3.41, 3.90 and 4.54% at 24 hours after the exposure. Cypermethrin gave an LC<sub>50</sub> of 0.61, 0.69, 0.82, 0.94 and 1.06% at 24 hours after the

exposure. It gave on LC<sub>90</sub> value of 1.32, 1.41, 1.60, 1.77 and 1.80% at 24 hours after the exposure. These data were used to determine the synergistic factor (Table 1).

The larvicidal activity of *Coriandrum sativum* and cypermethrin is shown in Table 2. At a larval stage of I, II, III, IV and pupae the LC<sub>50</sub> was 1.12, 1.41, 1.55, 1.73 and 2.04% and the LC<sub>90</sub> was 3.28, 3.61, 3.88, 4.13 and 4.61% at 24 hours after the treatment. They gave synergistic factors of 0.553, 0.489, 0.529, 0.543, 0.519 and 0.402, 0.390, 0.412, 0.428, 0.390 for LC<sub>50</sub> and LC<sub>90</sub> respectively, at 24 hours; which shows antagonistic activity of the combination.

The larvicidal activity of *Piper nigrum* and cypermethrin is shown in Table 2. At a larval stage of I, II, III, IV and pupae the LC<sub>50</sub> was 0.58, 0.66, 0.91, 1.04 and 1.66 and the LC<sub>90</sub> was 2.40, 2.66, 2.95, 3.27

and 4.34% at 24 hours after the treatment. They gave synergistic factors of 1.051, 1.045, 0.901, 0.903, 0.638 and 0.55, 0.530, 0.542, 0.541, 0.414 for LC<sub>50</sub> and LC<sub>90</sub> respectively, at 24 hours; which shows minimum synergistic activity and maximum antagonistic activity of the combination.

Table 1: Larvicidal activity of metanolic seed extracts of *Coriandrum sativum*, *Piper nigrum* and Cypermethrin against dengue vector, *Aedes aegypti*.

| Product                   | Stage      | Exposure period (Hours) | Regression co efficient | Chi-square | LC <sub>50</sub> (Confidence limits %) | LC <sub>90</sub> (Confidence limits %) |
|---------------------------|------------|-------------------------|-------------------------|------------|--|--|
| <i>Coriandrum sativum</i> | I instar   | 24                      | 0.45                    | 0.27       | 0.86<br>(0.40-1.14)                    | 3.71<br>(3.07-5.03)                    |
|                           | II instar  | 24                      | 0.41                    | 1.26       | 1.21<br>(0.84-1.48)                    | 4.30<br>(3.48-6.12)                    |
|                           | III instar | 24                      | 0.36                    | 0.43       | 1.54<br>(1.21-1.90)                    | 5.13<br>(3.99-8.06)                    |
|                           | IV instar  | 24                      | 0.34                    | 1.04       | 2.04<br>(1.70-2.68)                    | 5.85<br>(4.43-9.77)                    |
|                           | Pupae      | 24                      | 0.34                    | 0.16       | 2.40<br>(2.00-3.32)                    | 6.22<br>(4.68-10.56)                   |
| <i>Piper nigrum</i>       | I instar   | 24                      | 0.63                    | 0.23       | 0.71<br>(0.37-0.93)                    | 2.73<br>(2.39-3.28)                    |
|                           | II instar  | 24                      | 0.57                    | 0.39       | 0.94<br>(0.63-1.16)                    | 3.19<br>(2.75-3.95)                    |
|                           | III instar | 24                      | 0.57                    | 0.48       | 1.14<br>(0.88-1.35)                    | 3.41<br>(2.94-4.24)                    |
|                           | IV instar  | 24                      | 0.52                    | 1.32       | 1.41<br>(1.20-1.66)                    | 3.90<br>(3.30-5.02)                    |
|                           | Pupae      | 24                      | 0.42                    | 0.39       | 1.82<br>(1.58-2.13)                    | 4.54<br>(3.74-6.14)                    |
| Cypermethrin              | I instar   | 24                      | 1.79                    | 8.74       | 0.61<br>(0.33-0.80)                    | 1.32<br>(1.05-2.14)                    |
|                           | II instar  | 24                      | 1.79                    | 5.81       | 0.69<br>(0.51-0.85)                    | 1.41<br>(1.16-1.99)                    |
|                           | III instar | 24                      | 1.64                    | 2.41       | 0.82<br>(0.75-0.90)                    | 1.60<br>(1.44-1.84)                    |
|                           | IV instar  | 24                      | 1.55                    | 6.07       | 0.94<br>(0.76-1.22)                    | 1.77<br>(1.40-2.84)                    |
|                           | Pupae      | 24                      | 1.43                    | 2.37       | 1.06<br>(0.97-1.19)                    | 1.80<br>(1.72-2.34)                    |

Chi-square value P<0.05, Significant level.

Table 2: Combined action of binary mixtures of Cypermethrin and metanolic seed extracts of *Coriandrum sativum*, *Piper nigrum* against dengue vector, *Aedes aegypti*.

| Product                                  | Stage                              | Exposure period (Hours) | Regression coefficient | Chi-square | LC <sub>50</sub> (Confidence limits %) | SF               | Type of action | LC <sub>90</sub> (Confidence limits %) | SF               | Type of action |
|--|------------------------------------|-------------------------|------------------------|------------|--|------------------|----------------|--|------------------|----------------|
| Cypermethrin + <i>Coriandrum sativum</i> | I instar                           | 24                      | 0.59                   | 0.67       | 1.12 (0.87-1.32)                       | 0.55<br>3        | Antagonistic   | 3.28 (2.84-4.02)                       | 0.40<br>2        | Antagonistic   |
|  |                                    | 24                      | 0.58                   | 1.90       | 1.41 (1.20-1.61)                       | 0.48<br>9        | Antagonistic   | 3.61 (3.11-4.46)                       | 0.39<br>0        | Antagonistic   |
|  | III instar                         | 24                      | 0.55                   | 0.89       | 1.55 (1.34-1.77)                       | 0.52<br>9        | Antagonistic   | 3.88 (3.31-4.92)                       | 0.41<br>2        | Antagonistic   |
|  |                                    | 24                      | 0.54                   | 0.74       | 1.73 (1.52-1.98)                       | 0.54<br>3        | Antagonistic   | 4.13 (3.49-5.29)                       | 0.42<br>8        | Antagonistic   |
|  | IV instar                          | 24                      | 0.50                   | 0.84       | 2.04 (1.80-2.40)                       | 0.51<br>9        | Antagonistic   | 4.61 (3.83-6.14)                       | 0.39<br>0        | Antagonistic   |
|  |                                    | Pupae                   |                        |            |  |                  |                |  |                  |                |
|  | Cypermethrin + <i>Piper nigrum</i> | I instar                | 24                     | 0.70       | 0.19                                   | 0.58 (0.24-0.80) | 1.05<br>1      | Synergistic                            | 2.40 (2.13-2.82) | 0.55<br>0.53   |
| 24                                       |                                    |                         | 0.64                   | 0.26       | 0.66 (0.32-1.89)                       | 1.04<br>5        | Synergistic    | 2.66 (2.34-3.20)                       | 0                | Antagonistic   |
| II instar                                |                                    | 24                      | 0.63                   | 0.22       | 0.91 (0.62-1.11)                       | 0.90<br>1        | Antagonistic   | 2.95 (2.59-3.57)                       | 0.54<br>2        | Antagonistic   |
|  |                                    | 24                      | 0.57                   | 0.01       | 1.04 (0.76-1.25)                       | 0.90<br>3        | Antagonistic   | 3.27 (2.83-4.05)                       | 0.54<br>1        | Antagonistic   |
| IV instar                                |                                    | 24                      | 0.48                   | 0.81       | 1.66 (1.42-1.93)                       | 0.63<br>8        | Antagonistic   | 4.34 (3.60-5.81)                       | 0.41<br>4        | Antagonistic   |
|  |                                    | Pupae                   |                        |            |  |                  |                |  |                  |                |

SF, Synergistic Factor, P<0.05, Significant level.

## DISCUSSION

The strategy of combining different vector control agent has proven to be advantageous in various pest management programs (Caraballo, 2000; Seyoum *et al.*, 2002). Synergistic formulation may be more bioactive than individual pesticides against different pests. A lot

of more work has been done on the synergistic activity of synthetic-synthetic pesticides than plant-plant and plant-synthetic pesticide combinations against various insect pests.

The individual bioefficacy of petroleum ether seed extract of *Coriandrum sativum* was studied and noted their LC<sub>50</sub> value of 20.57 ppm and LC<sub>90</sub> value of 47.35 ppm had significant toxic effects against the IV instar larvae of *Aedes aegypti* (Nagella *et al.*, 2012). In this present study showed LC<sub>50</sub> and LC<sub>90</sub> values of 2.04 and 5.85%.

Sarita Kumar *et al.* (2011) investigated the laboratory study of bio control potential of hexane extracts of three species of Peppercorns; Long Pepper, *Piper longum L.*, Black Pepper, *Piper nigrum* and White Pepper, *Piper nigrum* against larval forms of *Aedes aegypti* (Diptera: Culicidae), the vector of dengue haemorrhagic fever. The LC<sub>50</sub> and LC<sub>90</sub> values obtained with hexane extracts of *Piper nigrum* against the fourth instar larvae were 0.024 and 0.081 ppm. In this present study showed LC<sub>50</sub> and LC<sub>90</sub> values of 1.41 and 3.90.

Waseem akram *et al.* (2010) studied the citrus seed extracts from 10 varieties against 4th instar larvae of dengue fever mosquito, *Aedes albopictus* (Skuse). The results indicate that the extracts from rough lemon and lemon were more effective as larvicides with lowest LC<sub>50</sub> (119.993 and 137.258 ppm respectively, after 24h of exposure and 108.85 and 119.853 ppm respectively, after 48h of exposure) and LT<sub>50</sub> values (2.51 and 4.91h, respectively). In the present study, the results indicate that the methanolic extracts from *Coriandrum sativum* and *Piper nigrum* seed were more effective as larvicides with lowest LC<sub>50</sub> (0.86 and 0.71% respectively, after 24h of exposure).

The benzene, hexane, ethyl acetate, methanol and chloroform leaf extract of *A. paniculata* was found to be more effective against *Cx. quinquefasciatus* than *Ae. aegypti*. The LC<sub>50</sub> values were 112.19, 137.48, 118.67, 102.05, 91.20 ppm and 119.58, 146.34, 124.24, 110.12, 99.54 ppm was reported (Govindarajan, 2011). In the present study, methanolic seed extracts of *Coriandrum sativum* and *Piper nigrum* was found to be more effective against *Ae. aegypti*. The LC<sub>50</sub> values were 0.86, 1.21, 1.54, 2.04, 2.40% and 0.71, 0.94, 1.14, 1.44, 1.82% respectively.

Sulaiman *et al.* (2002) evaluated Cynoff 25ULV® (cypermethrin 25 g/l) and Solfac UL015® (cyfluthrin 1.5% w/v) against the sentinel sugar-fed adults and 4th-instar larvae of *Aedes aegypti* in a housing estate endemic of dengue in Malaysia. The

impact of both pyrethroids on field populations of *Aedes albopictus* and *Aedes aegypti* larvae was monitored weekly using bottle containers. Both Cynoff 25ULV® and Solfac UL015® showed adulticidal effects and larvicidal effects. This field trial using Cynoff 25ULV® against dengue vectors showed its potential for use in dengue vector control programs. In the present study, the cypermethrin (10% EC) showed larvicidal and pupicidal effects.

The combined effect of cypermethrin and *Coriandrum sativum* possessed antagonistic activity in all observed larval stages (I, II, III, IV and pupae). The synergistic value of LC<sub>50</sub> and LC<sub>90</sub> was 0.553, 0.489, 0.529, 0.543, 0.519 and 0.402, 0.390, 0.412, 0.428, 0.390 for LC<sub>50</sub> and LC<sub>90</sub> respectively, at 24 hours. The combined effect of cypermethrin and *Piper nigrum* possessed synergistic activity in larval stages (I, II) at LC<sub>50</sub> value and acted antagonistic activity at LC<sub>90</sub> value after the treatment of 24 hours and antagonistic activity in observed larval stages (III, IV and pupae). The synergistic value of LC<sub>50</sub> and LC<sub>90</sub> was 1.051, 1.045, 0.901, 0.903, 0.638 and 0.55, 0.530, 0.542, 0.541, 0.414 respectively, at 24 hours. Similar observations were noted by (George and Vincent, 2005). It was also seen that synergistic activity was directly proportional to exposure period. This finding is supported by the observations of (George and Vincent, 2005) and (Mohan *et al.*, 2006; 2007).

The seaweeds *Caulerpa scalpelliformis* and *Dictyota dichotoma* and mangrove *Rhizophora apiculata* were extracted in acetone, combined with synthetic insecticides (DDT, BHC, HCH and malathion) and evaluated for activity against fourth instar larvae of *Aedes aegypti*. The extract showed synergism with the insecticide. The highest synergistic activity with all three insecticides especially HCH (Thangam and Kathinesan, 1991). Kalayanasundaram and Das (1985) was tested the larvicidal efficacy of some plant extracts in combination with phenthoate and fenthion against *Anopheles stephensi* and synergism was observed. Thangam and Kathinesan (1991) reported synergistic activity may be due to plant extract inhibiting some factors, such as detoxifying enzymes, in mosquito larvae, which can act against synthetic pesticides as observed in the larvae of *Aedes aegypti*. Cobel *et al.* (2003) observed synergistic activity between permethrin and propoxur giving a LC<sub>50</sub> of 0.26 mg/litre and a synergistic factor of 1.54 against *Culex quinquefasciatus* larvae. The synergism between DEET (N, N-diethyltoluamide) and propoxur against *Aedes aegypti*

was reported by Pennetier *et al.* (2005). Mohan *et al.* (2010) investigated the joint action of *Solanum xanthocarpum* and the synthetic pesticides, fenthion and temphos were evaluated against the larvae of the filarial vector, *Culex quinquefasciatus*.

## CONCLUSION

In conclusion, the findings of the present study indicate that the combined action of methanolic extracts of *Coriandrum sativum*, *Piper nigrum* seeds and synthetic insecticide cypermethrin could be used as a potential larvicidal agent. Combinations of synthetic pesticides are generally more effective and more economical which higher bioefficacy against target organisms but may be more environmentally.

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