Insecticidal and biological effects of three plant extracts tested against the dengue vector, *Stegomyia agyptii* (Diptera: Culicidae)

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Abstract

Development of resistant in vectors especially vector mosquitoes are becoming a challenge for the scientific community for management and control mosquito population. Vector mosquitoes are likely to withstand toxicity and develop resistant mechanism to single active compound hence, combining medicinal plants with rich active compounds stops resistant development and proliferation of mosquitoes. In this study we put effort to evaluate the effect of methanol extract of *Tagetes patula, Clerodentron phillomedis,* and *Catharanthus roseus* in individual and in combination against the dengue vector, *Stegomyia agyptii*. Lethal concentrations (LC_{50} and LC_{90}) were calculated to find out the effect of the test plants in individual and in combination. *T. patula* extract showed vaguely higher mortality rate when compared to *C. phillomedis,* and *C. roseus* but there was no significant variation among the three test plants. The median LC of combined treatment showed a significant difference between the combined (2.25 μg/mL/3rd instar) and individual treatment (6.41 μg/mL/3rd instar for *T. patula,* 6.85 μg/mL/3rd instar for *C. phillomedis* and 6.59 μg/mL/3rd instar for *C. roseus*). The combined efficacy of three test plants was also effective in controlling vector mosquitoes at fields with different agro-climatic conditions. The study proves that the combination of *T. patula, C. phillomedis,* and *C. roseus* is effective in different field conditions at lower concentrations.

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Introduction

Mosquitoes are medically and economically significant groups of insects among dipterans. They transmit disease-causing pathogens that can have devastating impacts on humans (Sivapriyajothi *et al*., 2014). Most important vector borne diseases such as malaria, lymphatic filariasis, Japanese encephalitis, dengue and chikungunya as well as yellow fever and other forms of encephalitis are transmitted by different mosquito species of Anopheles, Aedes and Culex genera (Jayapriya & Gricilda, 2015). Mosquito-borne infections have an economic impact, including death in countries with tropical and subtropical climates; however, no part of the world is free from vector-borne diseases (Amerasan *et al*., 2015). Dengue is an important mosquito transmitted viral disease, which is prevalent in more than 100 endemic countries (Marques & Kaplan, 2015). An estimated 2.5 billion people live in areas under threat of the disease. Nearly half a million cases of infections progress to more severe forms, such as hemorrhagic dengue fever leading to death (Guzman *et al.,* 2010). The disease is transmitted by *Stegomyia* (=Aedes) mosquitoes and constitutes a serious public health threat worldwide (WHO, 2009; De la Mora-Covarrubias *et al*., 2010; Fonseca-González *et al*., 2011; Khan *et al*., 2011; Brathwaite Dick *et al*., 2012).

In tropical countries like India, outbreak of dengue result in thousands of hospital admissions, human suffering and massive economic losses however, early recognition and prompt treatment can lower the risk of developing this severe disease (Epelboin *et al*., 2013). However, there are no available vaccines or effective antiviral agents against the virus. Hence in such case, mosquito control, assumes global importance (Nareshkumar *et al*., 2015). Synthetic and chemical insecticides resulted in environmental hazards and also in development of physiological resistance among vector mosquito species. Secondary metabolites of plants are considered to be a potential alternative approach for control insect pests as they are environmentally safe, target specific and biodegradable (Jayapriya & Gricilda, 2015).

Plant could be an alternative source for mosquito larvicides because they constitute a potential source of bioactive chemicals and generally free from harmful effects. Use of these botanical derivatives in mosquito control instead of syhthetic insecticides could reduce the cost and environmental pollution. Further analysis is required to isolate the active and its mode of action in inhibiting the developmental of *Stegomyia aegypti* larvae (Rajasekaran & Duraikannan, 2012).

The active metabolites of many plants were found to exhibit insecti-

cidal activity against mosquitoes and other vectors and also nontoxic to human and other non-target organisms. They are promising candidates to replace conventional insecticides to which insects are becoming more resistant (Cantrell *et al*., 2012; Vontas *et al*., 2012; Nareshkumar *et al*., 2013; Reegan *et al*., 2014; Miresmailli & Isman, 2014).

Most of the medicinal plants around our environment are found to possess various properties. The utilization of these resources will definitely contribute to control a wide range of pests (Vadeyar *et al.,* 2010). *Tagetes patula* Linn., (family Asteraceae), is used in folk medicine as an insect repellent, antiseptic, diuretic, blood purifier and also for cancer treatment (Vanessa *et al.,* 2015; Kashif *et al.,* 2015). The chemical constituents of *Tagetes patula* are β-karyophyllene, terpenes, hydrocarbons, alcohols, ethers, aldehydes, ketones, esters, carotenoids, flavonoids and thiophenes (Camarillo *et al*., 2007; Prakash *et al.,* 2012).

Clerodendrum phlomidis (family Lamiaceae) is an important medicinal plant contains secondary metabolites like tannins, alkaloids, polyphenols, terpenoids, and essential oils which have a wide range medicinal properties used in treatment of inflammation diabetes, nervous disorders, asthma, rheumatism, digestive disorders, urinary disorders and also has insecticidal activity (Ahmad, 2007; Lakshmi & Viji Stella Bai, 2015). *Catharanthus roseus* (L.) (family Apocynaceae) is another medicinal plant constitutes two important secondary metabolites *viz*. vincristine and vinblastine, the invaluable antitumor terpenoid indole alkaloids (Nejat *et al.,* 2015). It also has other medicinal properties, such as antibacterial, antifungal and antiviral activities (Jaleel *et al.,* 2007).

Enormous plant species *viz*. *Hippocratea excelsa*, *Hippocratea celastroides*, *Argemone mexicana*, *Tagetes lucida*, *Pseudosmodingium perniciosum* (Ruiz-Guerrero *et al*., 2015), *T. patula* (Munhoz *et al*., 2014), *C. phlomidis* (Kashte *et al*., 2015), *C. roseus* (Panneerselvam *et al*., 2013), *Blepharis maderaspatensis*, *Elaeagnus indica*, *Maesa indica*, *Phyllanthus wightianus*, *Memecylon edule* (Shivakumar *et al.,* 2013), *etc*. have been used to control different mosquito species in recent years.

The present investigation was carried out to examine the larval toxicity, pupal toxicity and adult toxicity of methanol extracts of *T. patula* (French marigold or Honey comb), *C. phillomedis* (Urni, Arna or Aarni), and *C. roseus* (Madagascar periwinkle or rosy periwinkle) against dengue vector, *St. aegypti* and their impact upon adult longevity and fecundity. The results of the present study may lead to the development of target specific and environmentally safer insecticidal products.

Materials and methods

Tested mosquitoes

All tests were carried out against the dengue vector, *Stegomyia* (*St*.) *aegypti* (L.).

Colony establishment and maintenance

The eggs of *St. aegypti*, were collected using oviposition traps placed in shaded areas at a height of less than 1.2 m. Traps were filled with water plus a few dried leaves placed at the bottom of the container, with a muslin strip placed vertically inside the container and half-submerged in the water. The eggs were then brought to the laboratory and transferred to 18×13×4 cm size enamel trays containing 500 mL water and kept for larval hatching. The *St. aegypti* larval culture was maintained in our laboratory at 27+2°C. The larvae were fed with dog biscuits and yeast at 3:1 ratio. The feeding was continued till the larvae transformed into the pupae. Water was changed alternate days. The breeding medium was regularly checked and dead larvae were removed at sight.

The pupae were collected from culture trays and were transferred to glass beakers containing 500 mL of water with help of a sucker. The pupae containing glass beaker were kept in (90 cm L×90 cm H×90 cm W) cm size mosquito cage for adult emergence. The emerged adults were maintained at 27.2°C, 75-85% relative humidity, under 14:10 light:dark photoperiod cycles. The adults were fed with 10% sugar solution for a period of three days before they were provided an animal for blood feeding. The adult female mosquitoes were allowed to feed on the blood of rabbit (exposed on the dorsal side) for two days. The males were provided with 10% glucose solution on cotton wicks. The cotton was kept moist with the solution and changed every day. An egg trap (cup) lined with filter paper containing water was placed in a corner of the egg collection cage.

Collection of plant materials and preparation of extracts

The leaves of *T. patula* (Asteraceae), flowers of *C. phlomidis* (Asteraceae), and leaves of *C. roseus* (Apocynaceae) were collected from Salem district, Tamil Nadu, India. The leaves were washed with tap water and dried at room temperature. The dried plant materials were powdered by an electrical blender. From the powder 100 g of the leaves were extracted with 300 mL of methanol for 8 h in a Soxhlet apparatus (Vogel, 1978). The crude plant extracts were evaporated to dryness in rotary vacuum evaporator. The test concentrations (2 mg/L, 4 mg/L, 6 mg/L, 8 mg/L and 10 mg/L) were prepared using distilled water.

Larval and pupal toxicity test

F2 larvae/pupae from the wild adult collection were used for the larvicidal/pupicidal activity. Twenty-five of first, second, third, fourth instar larvae and pupae were introduced into the 500 mL glass beaker containing 300 mL of de-chlorinated water with the desired concentrations (2 mg/L, 4 mg/L, 6 mg/L, 8 mg/L and 10 mg/L) of plant extracts. Food was provided for the test insects. Five replicates were made for each concentration. The control was setup using de-chlorinated water alone. The test mortalities were corrected by using Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100 \quad (1)$$

Biological observations

Mosquito fecundity, longevity and egg hatchability test

The effect of larval exposure to plant extracts on adult longevity, fecundity and egg hatchability of *St. aegypti*, was determined by placing 20 male and female mosquitoes of similar size and age emerged from each treatment group [twenty-five fourth instar larvae treated with desired concentrations of plant extracts as individual (4 mg/L and 6 mg/L) and in combination (0.5 mg/L, 1.0 mg/L and 1.5 mg/L)] and the respective control into separate screened cages (30 cm L×30 cm W×30 cm H). Sucrose solution was available to adults *ad libitum.* Commencing 3 d after blood feeding and until oviposition ceased, eggs or egg rafts were collected daily from plastic bowl (12 cm H×12 cm dia) containing 500 ml water that was placed in each cage. The average number of eggs laid by each female in each cage was calculated as the total number of eggs collected in each cage divided by the number of females originally placed in that cage.

To characterize adult longevity, the number of live male and female mosquitoes in each cage was recorded at the same time each day until all mosquitoes had died. This value was divided by the number of male or females originally placed in to the cage to obtain percent survival according to the number of days since emergence.

Hatchability was evaluated using filter papers with the test eggs were cut into portions and placed into a glass cavity block (50×50 mm) for hatching and counted. The percentage of hatchability was calculated as number of eggs hatched by number of experimented eggs.

Further the freshly emerged larvae were monitored for its survival rate and adult emergence.

$$\text{Hatchability (\%)} = \frac{\text{Number of hatched eggs}}{\text{Number of tested eggs}} \times 100 \quad (2)$$

Statistical analysis

All data were subjected to analysis of variance and the means were separated using Duncan's multiple range test (Alder & Rossler, 1977). The mean values of larvae, and pupae mortality were subjected to probit analysis (Finney, 1971) for calculating lethal concentrations at 50% and 90% (LC_{50}, LC_{90}) and other statistical parameters (95% confidence limits of upper confidence limit and lower confidence limit values), and chi-square test were calculated using the SPSS (Statistical Package of Social Sciences) software version 13 (IBM Corp., Armonk, NY, USA).

Results

Larval and pupal toxicity

Larva and pupal mortality of *St. aegypti* after treatment with acetone extract of *T. patula* leaf is shown in Table 1. 18% mortality was noted in I instar larvae after the treatment with 2 mg/L concentration whereas it has been increased to 93% at 10 mg/L concentration. The observed LC_{50} values (5.73 mg/L, 6.11 mg/L, 6.41 mg/L, 6.77 mg/L and 5.38 mg/L for 1st, 2nd, 3rd, 4th instar and pupae of *St. aegypti* respectively) indicates that *T. patula* is effective over the larvae and pupae of *St. aegypti* at lower concentration ranging between 5 mg/L to 7 mg/L. Early instars were susceptible to *T. patula* when compared with the later ones.

Table 2 provides the larval and pupal mortality of *St. aegypti* after treatment with methanol extract of *C. phlomidis* flowers at different concentrations (2 mg/L, 4 mg/L, 6 mg/L, 8 mg/L and 10 mg/L). The lowest mortality rate recorded was 12% in IV instar after the treatment with 2 mg/L concentration and the maximum mortality rate recorded was 85% in I instar after the treatment with 10 mg/L concentration. The LC_{50} values of *C. phlomidis* flower extract (6.05 mg/L, 6.41 mg/L, 6.85 mg/L, 7.50 mg/L and 7.71 mg/L for I-IV larval instars and pupal stage, respectively) were lower. There was no significant variation in median LC among the various developmental stages.

Larval and pupal mortality of *St. aegypti* after the treatment of methanol extract of *C. roseus* leaf extract at different concentrations (2 mg/L, 4 mg/L, 6 mg/L, 8 mg/L and 10 mg/L) is given in Table 3. A percentage of 89% mortality was observed in I instar at a treatment of 10 mg/L concentration. Significant variation in mortality was observed among the different concentrations tested. The lowest recorded LC_{50}

Table 1. Larvicidal and pupicidal activity of *Tagetes patula* leaf extract on dengue vector *Stegomyia aegypti*.

Larval instars and pupal stage	% Larval/pupal mortality (mean±SD) Concentration (mg/L)						LC_{50} (LCL/UCL)	LC_{90} (LCL/UCL)	χ^2 value
	Control	2	4	6	8	10			
I	2±0.4[a]	18±0.94[b]	36±1.63[c]	50±1.25[d]	63±0.82[e]	93±0.47[f]	5.73 (4.27/7.17)	10.71 (8.77/15.77)	8.63*
II	3±0.4[a]	15±0.82[b]	35±1.63[c]	45±0.82[d]	60±1.63[e]	90±0.47[f]	6.11 (5.29/8.28)	11.18 (9.18/16.33)	8.08*
III	2±0.2[a]	14±0.82[b]	33±0.47[c]	41±1.63[cd]	58±0.94[d]	87±0.82[e]	6.41 (5.13/7.86)	11.63 (9.62/16.57)	6.95*
IV	1±0.1[a]	11±0.82[b]	30±1.63[c]	39±0.82[cd]	55±0.82[d]	84±1.63[e]	6.77 (5.65/8.11)	11.92 (10.01/16.22)	5.78*
Pupae	1±0.1[a]	31±2.45[b]	49±1.25[c]	57±1.63[d]	61±2.05[e]	64±1.25[e]	5.38 (4.02/6.51)	18.19 (14.58/26.38)	3.75*

[a-f]Means±standard deviations (SD) followed by same letter within rows indicate no significant difference (Duncan's multiple range test, P<0.05). LC_{50}, LC_{90}, lethal concentration at 50% and 90%; LCL, lower confidence limits; UCL, upper confidence limits. *Significant at P<0.001 (heterogeneity factor used in calculation of confidence limits).

Table 2. Larvicidal and pupicidal activity of *Clerodendron phlomidis* flower extract on dengue vector *Stegomyia aegypti*.

Larval instars and pupal stage	% Larval/pupal mortality (mean±SD) Concentration (mg/L)						LC_{50} (LCL/UCL)	LC_{90} (LCL/UCL)	χ^2 value
	Control	2	4	6	8	10			
I	2±0.5[a]	16±0.82[b]	33±0.47[c]	49±1.25[d]	65±2.44[e]	85±1.63[f]	6.05 (5.54/6.55)	11.93 (10.39/12.68)	0.94*
II	2±0.5[a]	15±1.63[b]	30±0.82[c]	45±0.82[d]	61±2.45[e]	83±0.94[f]	6.41 (5.90/6.93)	11.81 (10.79/13.25)	1.25*
III	1±0.4[a]	13±1.63[b]	28±1.25[c]	41±1.25[d]	57±0.82[e]	79±0.94[f]	6.85 (5.79/8.59)	12.43 (9.71/16.57)	1.19*
IV	1±0.3[a]	12±2.05[b]	24±0.82[c]	37±0.47[d]	49±0.94[e]	74±0.47[f]	7.50 (6.94/8.17)	13.45 (12.13/15.40)	1.85*
Pupa	1±0.1[a]	24±0.82[b]	42±1.25[c]	45±2.45[d]	53±1.63[e]	55±0.82[e]	7.71 (6.52/9.59)	21.21 (16.62/32.33)	3.60*

[a-f]Means±standard deviations (SD) followed by same letter within rows indicate no significant difference (Duncan's multiple range test, P<0.05). LC_{50}, LC_{90}, lethal concentration at 50% and 90%; LCL, lower confidence limits; UCL, upper confidence limits. *Significant at P<0.001 (heterogeneity factor used in calculation of confidence limits).

was 5.89 mg/L for the I instar and highest was 6.87 mg/L for IV instar. The pupae were susceptible (6.53 mg/L) when compared to the III and IV instar of *St. aegypti*.

The synergistic effect of *T. patula* leaf extract, *C. phlomidis* flower extract and *C. roseus* leaf extract on the developmental stages of *St. aegypti* is shown in Table 4. The median LC (1.67 mg/L, 1.97 mg/L, 2.25 mg/L, 2.63 mg/L and 1.79 mg/L for the I, II, III, IV larval insatrs and pupa, respectively) were highly reduced when compared with the individual treatment. The maximum mortality of 97% was observed at 6 mg/L concentration.

Biological observations

Adult longevity and fecundity

The adult life span and number of eggs laid by *St. aegypti* after the treatment with *T. patula* leaf extract (TPLE), *C. phlomidis* flower extract (CPFE) and *C. roseus* leaf extract (CRLE) are shown in Table 5. There was significant reduction in adult life span of *St. aegypti* after the treatment with *TPLE, CPFE* and *CRLE* at 4.0 mg/L and 6 mg/L. The longevity and fecundity effect of individual treatment of *TPLE, CPFE* and *CRLE* were relatively close showing no significant difference. Whereas, the combined treatment was much effective than the individual treatment showing significant reduction in longevity and fecundity of *St. aegypti*.

Egg hatchability, survival rate and adult emergence

Table 6 shows egg hatchability, survival rate and adult emergence of *St. aegypti* after the treatment of TPLE, CPFE and CRLE in individual and in combination. Egg hatchability was reduced up to 40% after the treatment with TPLE at 6 mg/L, which was the highest reduction rate in individual treatment. Similarly the survival rate and adult emergence was reduced up to 36% and 29%, respectively in individual treatment of TPLE at 6 mg/L. Combined treatment of TPLE, CPFE and CRLE was conducted with a very low concentration (0.5 mg/L, 1.0 mg/L and 1.5 mg/L) where hatchability has been markedly reduced to 15%. The survival rate and adult emergence has been greatly reduced to 10% and 9% respectively after the treatment of TPLE, CPFE and CRLE in combination.

Discussion

Dengue fever is an arboviral disease caused by the DENV serotypes 1-4. It is most prevalent in tropical and subtropical regions around the world, predominantly in urban and semi-urban regions (Muhammad *et al.,* 2015). In tropical and subtropical countries, outbreak of dengue results in thousands of deaths, human suffering and massive economic losses (Epelboin *et al*., 2013). Since, there are no effective vaccines

Table 3. Larvicidal and pupicidal activity of *Catharanthouse* roseus leaf extract on dengue vector *Stegomyia aegypti*.

Larval instars and pupal stage	% Larval/pupal mortality (mean±SD) Concentration (mg/L)						LC_{50} (LCL/UCL)	LC_{90} (LCL/UCL)	χ^2 value
	Control	2	4	6	8	10			
I	3±0.7[a]	17±0.82[b]	35±0.82[c]	49±1.63[d]	64±0.82[e]	89±0.47[f]	5.89 (5.39/6.37)	11.05 (10.13/12.31)	3.79*
II	3±0.5[a]	15±0.82[b]	34±1.63[c]	48±0.82[d]	61±0.82[e]	85±1.25[f]	6.18 (5.67/6.69)	11.58 (10.58/12.99)	2.92*
III	2±0.4[a]	14±1.25[b]	31±0.82[c]	44±0.82[d]	59±0.82[e]	80±2.45[f]	6.59 (6.06/7.14)	12.26 (11.15/13.86)	1.33*
IV	2±0.5[a]	14±1.25[b]	29±2.45[c]	41±1.63[d]	55±0.82[e]	79±0.82[f]	6.87 (6.33/7.45)	12.67 (11.48/14.40)	2.08*
Pupa	1±0.2[a]	22±1.63[b]	45±2.45[c]	50±2.45[cd]	59±0.82[d]	62±0.82[e]	6.53 (3.89/10.35)	17.00 (12.06/49.70)	5.83*

[a-f]Means±standard deviations (SD) followed by same letter within rows indicate no significant difference (Duncan's multiple range test, P<0.05). LC_{50}, LC_{90}, lethal concentration at 50% and 90%; LCL, lower confidence limits; UCL, upper confidence limits. *Significant at P<0.001 (heterogeneity factor used in calculation of confidence limits).

Table 4. Larvicidal and pupicidal activity of a mixture of *Tagetes patula* leaf extract, *Clerodendron phlomidis* flower extract, *Catharanthouse roseus* leaf *extract on* dengue vector *Stegomyia aegypti*.

Larval instars and pupal stage	% Larval/pupal mortality (mean±SD) Concentration (mg/L)					LC_{50} (LCL/UCL)	LC_{90} (LCL/UCL)	χ^2 value
	Control	1.5	3	4.5	6			
I	1±0.1[a]	51±0.47[b]	66±1.63[c]	77±0.82[d]	97±0.82[e]	1.67 (–929.24/3.21)	5.43 (3.80/1272.94)	5.80*
II	3±0.8[a]	47±1.63[b]	62±0.47[c]	75±1.63[d]	96±0.47[e]	1.97 (–12.56/3.38)	5.65 (4.08/30.13)	5.27*
III	1±0.4[a]	43±0.55[b]	59±2.45[c]	71±1.63[d]	93±2.45[e]	2.25 (–3.13/3.50)	6.14 (4.57/17.28)	3.95*
IV	2±0.4[a]	39±1.70[b]	53±0.94[c]	67±1.24[d]	89±1.63[e]	2.63 (2.06/3.06)	6.72 (5.95/7.95)	2.76*
Pupa	1±0.2[a]	49±2.45[c]	65±1.25[c]	75±1.25[d]	96±1.25[e]	1.79 (–55.17/3.30)	5.64 (3.99/92.39)	5.51*

[a-e]Means±standard deviations (SD) followed by same letter within rows indicate no significant difference (Duncan's multiple range test, P<0.05). LC_{50}, LC_{90}, lethal concentration at 50% and 90%; LCL, lower confidence limits; UCL, upper confidence limits. *Significant at P<0.001 (heterogeneity factor used in calculation of confidence limits).

Table 5. Effect of *Tagete patula* leaf extract, *Cleridendron phlomidis* flower extract and *Catharanthus roseus* leaf extract on adult longevity and fecundity of *Stegomyia aegypti* females.

Treatment (mg/L)		Adult longevity		Fecundity
		Male	Female	(No. of eggs)
	Control	10±0.2[a]	13.5±0.2[a]	180±0.4[a]
TPLE (mg/L)	4.0	7.6±0.6[b]	8.3±0.4[b]	94±1.5[b]
	6.0	6.2±0.4[bc]	6.9±0.5[c]	73±1.1[bc]
CPFE (mg/L)	4.0	9.0±0.2[ab]	9±0.2[b]	97±1.6[b]
	6.0	8.0±0.5[b]	7±0.9[c]	80±1.1[bc]
CRLE (mg/L)	4.0	8.5±0.4[b]	9±1.1[b]	97±0.9[b]
	6.0	7.8±0.4[bc]	7±0.9[c]	80±0.6[bc]
	Control	10±0.2[a]	13.5±0.2[a]	180±0.4[a]
	0.5	8±0.9[b]	6±0.6[b]	55±1.9[b]
TPLE+CPFE+CRLE (mg/L)	1	6±0.6[c]	4±1.5[c]	43±2.1[c]
	1.5	4±0.9[d]	3±0.9[d]	25±1.6[d]

[a-d]Means±standard deviations (SD) followed by same letter within column indicate no significant difference (Duncan's multiple range test, P<0.05). TPLE, *Tagete patula* leaf extract; CPFE, *Cleridendron phlomidis* flower extract; CRLE, *Catharanthus roseus* leaf extract.

Table 6. Egg hatchability, survival rate and adult emergency of *Stegomyia aegypti* after the treatment of *Tagete patula* leaf extract, *Cleridendron phlomidis* flower extract and *Catharanthus roseus* leaf extract.

Treatment (mg/L)		Egg hatchability (%)	Survival (%)	Adult emergence
	Control	100±0.5[a]	100±0.4[a]	180±2.4[a]
TPLE (mg/L)	4.0	54±1.1[b]	49±0.6[b]	44±2.1[b]
	6.0	40±1.1[bc]	36±0.8[c]	29±2.9[c]
CPFE (mg/L)	4.0	64±0.2[b]	56±1.1[b]	49±1.1[b]
	6.0	57±0.4[bc]	48±1.6[bc]	37±1.4[bc]
CRLE (mg/L)	4.0	55±0.9[b]	50±0.9[b]	43±1.9[b]
	6.0	47±0.2[bc]	39±0.6[bc]	32±1.1[bc]
	Control	100±0.5[a]	100±0.4[a]	180±2.4[a]
TPLE+CPFE+CRLE (mg/L)	0.5	85±0.1[b]	31±0.4[b]	26±1.5[b]
	1.0	34±0.9[c]	20±1.6[bc]	15±0.9[bc]
	1.5	15±1.6[d]	10±1.1[c]	9±2.1[c]

[a-d]Means±standard deviations (SD) followed by same letter within column indicate no significant difference (Duncan's multiple range test, P<0.05). TPLE, *Tagete patula* leaf extract; CPFE, *Cleridendron phlomidis* flower extract; CRLE, *Catharanthus roseus* leaf extract.

available for dengue virus so that its control solely depends on effective vector control measures (Suresh *et al*., 2015).

Dengue virus is primarily transmitted by *Aedes* mosquitoes, particularly *A. aegypti,* which is frequently dependent on applications of conventional synthetic insecticides which are toxic to human and other non-target organisms (Remia & Logaswamy, 2010; Tabanca *et al.,* 2013). A good control measure lies on personal protection, public awareness for removal of mosquito breeding sites and controlling mosquito immatures through environment friendly and target specific larvicides (Ghosh *et al.,* 2012; Poopathi, 2012; Govindarajan & Sivakumar, 2014; Patil *et al.,* 2014).

In present study, as an alternative to synthetic pesticides, three plant extracts (*T. patula, C. phlomidis* and *C. roseus*) have been tested against *St. aegypti*. Recent research works show that the active metabolites present in different parts of plants exhibit a wide range of toxicity against various mosquito species. Dass & Mariappan (2014) reported the larvicidal effect of the extract of *Lawsonia inermis* and *Murraya exotica* leaves on III and IV larval instars and pupa of *Culex quinquefasciatus*. Shivakumar *et al.* (2013) reported the larvicidal potential of leaf extracts of five plants (*Blepharis maderaspatensis*, *Elaeagnus indica*, *Maesa indica*, *Phyllanthus wightianus* and *Memecylon edule*) extracted with different solvent crude (hexane, chloroform, ethyl acetate, acetone and methanol) against the fourth-instar larvae of *Aedes aegypti*.

Tagetes patula extract have exhibited an increased toxicity on different larval instars and pupae of *St. aegypti* when compared with the extracts of *C. phlomidis* and *C. roseus*. The mosquitocidal activity of the test plants are due to the presence of active secondary metabolite such as phenols, flavonoide, alkaloids, saponins, and terpenes in *T. patula* (Ramya *et al*., 2012); pectolinaringenin, scutellarein, clerodin, clerodendrin, *etc*. in *Clerodendron phlomidis* (Mohan & Mishra, 2010); Catharanthine, serpentine, tabersonine, vindoline, vinblastine, vincristine, ajmalicine, tryptophan, tryptamine and secologanine in *Catharanthus roseus* (Tikhomiroff & Jolicoeur, 2002).

In this study the synergistic activity of *T. patula, C. phlomidis* and *C. roseus* combination was evaluated on the larval instars and puape of *St. aegypti* and revealed that the combined treatment is much effective than the individual treatment. Recent research has showed that the synergistic activities of the secondary metabolites of plants are much toxic against mosquitoes and other insect vectors. For instance, Yankanchi *et al.* (2014) reported that maximum synergistic activities were found in combining extracts of *V. negundo* and *P. glabra*. The combined effects of cypermethrin and extracts of *Calotropis procera* root, *Piper longum* root and *Polygonum hydropiper* leaves were most effective against the housefly, *Musca domestica* (Islam & Aktar, 2013).

The active compounds of *T. patula, C. phlomidis* and *C. roseus* were also responsible for the reduction in duration of *St. aegypti* life cycle and inhibition of adult emergence. Kumar & Chapman (1984) observed reduction in adult longevity of *P. xylostella* when larval stages were treated with permethrin and fenvalerate. Similar response were also observed for *Culex pipiens* and *Culesita longiareolata* treated with *Bacillus thuringiensis* (Aissaoui & Boudjelida, 2014) and *A. aegypti* and *C. quinquefasciatus* treated with *Bacillus sphaericus* (Nareshkumar *et al.*, 2012). The adult which emerged from treated larvae and pupae showed a great reduction in fecundity. The numbers of eggs laid were reduced to around 50% in individual treatment of the test plants whereas; it has been reduced to 13% in the combined treatment. This may be due to the synergistic behaviours of active ingredients present in the plant materials. Senthil Nathan *et al.* (2004) reported the synergistic activity of *Bacillus thuringiensis* with neem and pongamia resulting in reduction of fecundity in rice leaffolder, *Cnaphalocrocis medinalis*.

The study shows that the active ingredients influenced the egg hatchability. Results showed that *T. patula* leaf extract, *C. phlomides* flower extract, and *C. roseus* leaf extract inhibited egg hatching from 40-50% whereas the combined treatment inhibited around 85%. The adult emergence and survival of larvae from the eggs of treated larvae were also greatly reduced when compared with control. Insects with abnormal behaviours such as slow movement and loss of equilibrium were also observed during the study. Rajkumar *et al.* (2011) reported reduced egg hatchability in malarial mosquito, *Anopheles stephensi* treated with leaf essential oil of *Coccinia indica*.

Conclusions

From the present study we conclude that the *T. patula* leaf extracts, *C. phlomides* flower extract, and *C. roseus* leaf extract can be used as an effective mosquito control agent. The three plant extracts can be used in combination to target mosquitoes at very low concentration. Additional research is needed to produce target specific bio-pesticides by screening the active compounds that actually cause impairment and death to mosquitoes. This may increase the biological activity of the product against mosquito larvae to levels proportionate with commercially available pesticides.

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