COMPARATIVE LARVICIDAL EFFICACY OF α-CYPERMETHRIN ALONE AND α-CYPERMETHRIN/*CITRUS SINENSIS* PEEL EXTRACT BINARY MIXTURES AGAINST *AEDES AEGYPTI* L.

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Aedes aegypti is a widely spread disease vector of great concern throughout the world. With continuous rise in cases of Zika, dengue and Chikungunya worldwide, control of *Ae. aegypti* has become a prime concern. The present study investigated the larvicidal effects of individual and various combinations of *Citrus sinensis* hexane peel extract and a synthetic pyrethroid, alpha-cypermethrin against *Ae. aegypti*. Larvicidal bioassays were performed using WHO protocol with minor modifications. The investigated compounds were found effective individually as well in binary mixtures indicating the efficient synergism. Hexane extract of *Citrus sinensis* peels assayed against *Aedes aegypti* larvae resulted in LC_{50} of 46.53 ppm after exposure for 24 h, while alpha-cypermethrin treatment resulted in LC_{50} value of 0.0063 ppm. The binary mixtures of both the compounds in 1:1, 1:5 and 1:10 ratios also showed significant larvicidal potential. The 1:1 mixture was found most effective with co-toxicity coefficient and synergistic factor as 23.456 and 3.865, respectively, for the LC_{50} at 24h. The binary mixtures showed synergism as well as additive effects in all the ratios tested except 1:5 ratio for LC_{90} at 48h which showed inconsequential antagonistic effect. Results showed decreased synergistic effects with increase in the citrus extract proportion in the binary mixtures. We suggest that phytoextract/cypermethrin mixtures can be more operative than insecticide/phytoextract alone, and can be used as a good ecofriendly approach in vector control programs. Such mixtures could reduce the costs, reduce insecticide dose, and regulate insecticide resistance as part of integrated vector management.

Keywords: *Citrus sinensis*, *Aedes aegypti*, synergism, additive, antagonism, binary mixtures.

INTRODUCTION

Mosquito-borne diseases are the major cause of concern worldwide, especially in tropical countries. Different mosquito vectors, *Aedes*, *Culex* and *Anopheles* transmit a range of disease pathogens causing dengue, Chikungunya, malaria, filariasis and Zika, etc. Though, different species of mosquitoes are playing havoc at global level, yet since last decade, outbreak of *Aedes*-borne diseases has taken a huge attention; especially notable increase in the worldwide preponderance of dengue fever. According to the World Health Organization (WHO); half of the world's population inhabit dengue-endemic regions and 50–100 million individuals may contract dengue infections annually (WHO, 2019). In India, a total of 40,868 dengue incidences and 30,121 suspected cases of Chikungunya were reported in 2018 (NVBDCP 2018a, b).

The most endorsed strategy to tackle and control mosquito-borne diseases principally lies on breaking the disease-transmission cycle by mosquito management below threshold level. Adoption of vector control measures has become the utmost important global strategy to battle and for better management of mosquito-associated diseases (Liu, 2015). Consistent human efforts and huge amount of capital resources have been channelized to reduce the incidence and prevalence of mosquito-transmitted diseases (Sunday *et al.*, 2016). Various integrated approach-based control measures programmed towards different mosquito life stages have been devised and practiced till date; such as, killing of mosquitoes at larval/adult stage, abolition of breeding places, use of biological control agents, release of genetically modified mosquitoes in the fields in competition with normal mosquitoes etc.; (Kumar *et al*., 2017). Nevertheless, use of chemical insecticides has always been the most favourite control method due to speedy action and easy application despite of mosquitoes developing resistance against these, negative impacts on the environment, adversities on human health and lethality to non-target organisms.

Researchers all over the world are making attempts to enlighten the insecticide resistance mechanism enabling the formulation of novel insecticide with enhanced efficacy (Liu, 2015). Previous studies have implicated the role of multiple resistance mechanism in individual mosquito species due to consistent and augmented insecticide pressure (Hemingway *et al*., 2002; Ranson *et al*., 2002; Li & Liu, 2014). However, widely accepted phenomena among research community are metabolic degradation of toxicants and reduced sensitivity of insecticide-target proteins (Li & Liu, 2014; Yang *et al*., 2014).

Among various conventional chemicals used, pyrethroids, formulated in 80's as synthetic analogues of pyrethrins extracts from the flowers of *Chrysanthemum cinerariaefolium*, are still considered the safest, most successful and effective mosquito control agents. Synthetic pyrethroids have been utilized as Indoor Residual Sprays (IRS), and manufacture of insecticide-treated mosquito nets (ITNs) and long-lasting insecticidal nets (LLINs) (Liu *et al*., 2006; Kumar *et al*., 2012; Sharma *et al*., 2016). To date, several pyrethroids have been investigated against mosquitoes; among which α-cypermethrin has been reported as an effective larvicide as well as adulticide against *Aedes aegypti, Culex quinquefasciatus* and *Anopheles gambiae* (Floore *et al*., 1992; Hougard *et al*., 2003; Mosha *et al*., 2008; Pettit *et al*., 2010). However, a few reports have evidenced development of resistance to α-cypermethrin not only in laboratory-bred populations of *Ae. aegypti* (Geetha & Shetty, 2018) but also in the field-collected population (Luna *et al*., 2004; Lima *et al*., 2011).

One of the most effective alternate approaches in mosquito control programme is to explore the plant biodiversity and use plant-derived insecticides as the simple and sustainable method. According to Ghosh *et al*. (2012), plant-derived insecticides comprise botanical blends of compounds which act concertedly on life processes on mosquitoes, unlike conventional insecticides based on a single active ingredient. Botanical pesticides are considered environmentally safe, cost-effective, with potential against target species and low mammalian toxicity. Citrus plants have been known for their mosquitocidal potential apart from their use as food or food-flavoring agents. Different parts of the citrus plant; fruits, seeds, roots and leaves; have been tested for the presence of mosquitocidal components (Traboulsi *et al*., 2005; Akram *et al*., 2010). The ethanolic extract of the orange peel (*Citrus sinensis*) has been found effective against *Aedes aegypti* larvae (Amusan *et al*., 2005). The hexane leaf extracts of *C. sinensis* possessed moderate larvicidal efficiency against dengue vector with LC_{50} and LC_{90} value of 446.84 and 1370.96 ppm, respectively after 24 h of exposure (Warikoo *et al*., 2012). Earlier, Amer & Mehlhorn (2006) had reported efficacy of essential oil from *C. limon* against *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi*. Citrus peels, though waste, are rich in nutrients and contain many secondary metabolites. They can be efficiently used as safe alternate to synthetic chemical against mosquitoes. Kumar *et al*. (2012) investigated hexane and petroleum ether extract of *C. limetta* peels against *Ae. Aegypti*, and observed respective LC_{50} values of 96.15 ppm and 145.50 ppm.

The role of phytoproducts for synergistic activity along with synthetic pyrethroids is well known against different pests (Vastrad *et al*., 2002) and vectors (Mohan *et al*., 2007). Keeping in view the current scenario of α-cypermethrin resistance in *Ae. aegypti* and potentially effective use of *C. sinensis* to control them; the present study investigates the efficacy of α-cypermethrin and hexane extract of *C. sinensis* peels alone and in binary mixtures against dengue vector, *Ae. aegypti* L. The efficient synergistic action of the hexane extract of *C. sinensis* peels on the larvicidal efficacy of α-cypermethrin may provide useful information to formulate combinations which are safer than the insecticide alone and more effective than the peel extract alone.

MATERIAL AND METHODS

CULTURE OF *AEDES AEGYPTI*

Pure strain of dengue fever mosquitoes, *Aedes aegypti*, was procured from ICGEB (International Centre for Genetic Engineering and Biotechnology), New Delhi and was maintained under controlled conditions of 28± 1° C, 80 ± 5% RH, 14 L/10 D photoregime (Samal & Kumar, 2018). Adults were fed on sugary juice by providing them water-soaked raisins, while females were provided with occasional blood meals for egg maturation. The eggs collected in an ovitrap were transferred into dechlorinated water. The hatched larvae were given powdered dog biscuits and yeast (3:1) as food. The pupae, collected regularly, were kept in clothed cages for adult emergence.

INSECTICIDE USED IN THE STUDY

Present investigations employed a synthetic pyrethroid, α-cypermethrin (97.0% purity) which was procured from M/s Aimco Pesticides, Mumbai India (Fig. 1). A stock of 10 ppm α-cypermethrin was prepared using absolute ethanol as the solvent and stored at 4°C in the refrigerator for future use. During assays, the graded series of α-cypermethrin was prepared using ethanol.

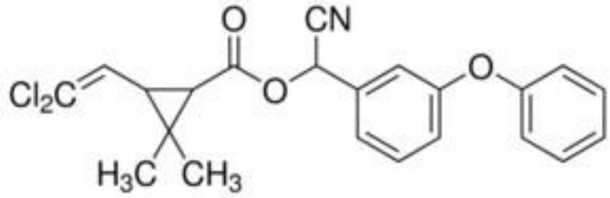

Fig. 1. Alpha-cypermethrin ([(*S*)-cyano-(3-phenoxyphenyl)methyl] (1*R*,3*R*)-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate).

COLLECTION OF PLANT MATERIAL

The fruits of *C. sinensis* collected from the surrounding areas in Delhi, India, were brought to the laboratory in sterile bags. The peels were separated and thoroughly washed under tap water followed by rinsing in distilled water to remove adhered dirt particles. The peels were examined for any kind of disease or infection. The healthy peels selected were dried under shade at room temperature (27 ± 2°C) for about 20 days ensuring non-occurrence of any fungal or bacterial growth.

PREPARATION OF *CITRUS SINENSIS* PEEL EXTRACT

The dried peels were mechanically grinded using a small blender and sieved to get fine powder. The 15 g of powdered citrus peels was extracted with 200 mL of hexane for 8 h per day using Soxhlet extractor at a temperature not exceeding the boiling point of the solvent. The extraction was continued till 3 days and the extracts obtained were concentrated at 45°C under low pressure using a vacuum evaporator. The stock solution of 1000 ppm was prepared and stored in a refrigerator at 4°C (Kumar *et al*., 2012).

LARVICIDAL BIOASSAY WITH *CITRUS SINENSIS* PEEL EXTRACT AND ALPHA-CYPERMETHRIN

The larvicidal bioassay was performed at 28 ± 1°C on the *Ae. aegypti* early fourth instars described by WHO with minor modifications (WHO, 2016). The graded series of α-cypermethrin and hexane peel extract of *C. sinensis* were prepared using ethanol as the solvent. The batches of 20 early fourth instars of

Aedes aegypti were exposed to 1 mL of α-cypermethrin or citrus peel extract (CPE), at a particular concentration, added to 199 mL of distilled water. For each dilution, three simultaneous replicates were carried out making a total of 60 larvae for each concentration. Controls were run simultaneously by replacing toxicant with 1 mL of ethanol. The dead and moribund larvae were recorded after 24 h and data was subjected to regression analysis.

DATA ANALYSIS

The control assays resulting in higher than 20% larval mortality or 20% pupal formation were performed again; while the control assays with mortality ranging between 5% and 20%, were rectified using Abbott's formula (Abbott, 1925). The data was subjected to the regression analysis using SPSS 19.0 Programme. The LC_{50} and LC_{90} values with 95% fiducial limits along with other statistical parameters, such as standard error and regression coefficient were calculated to measure difference between the test samples.

BIOASSAYS WITH BINARY MIXTURES OF ALPHA-CYPERMETHRIN AND *CITRUS SINENSIS* PEEL EXTRACT

The larvicidal bioassays were conducted with the binary mixtures of α-cypermethrin and citrus peel extract to formulate the combinations which are safer than the insecticide alone and more effective than the citrus peel extract alone. The α-cypermethrin and citrus peel extract were mixed in three different ratios (v/v) – 1:1, 1:5 and 1:10; and larvicidal assays were conducted as described above. The mortality counts were made and data was subjected to probit analysis as described earlier to calculate LC_{50} and LC_{90} values with 95% fiducial limits along with other statistical parameters.

The expected mortalities were calculated as described by Trisyono & Whalon (1999); whereas the co-toxicity coefficient was calculated as per the formula given by Sun & Johnson (1960).

$$\text{CTC (co - toxicity Coefficient)} = \frac{\text{Observed \% mortality} - \text{Expected \% mortality}}{\text{Expected \% mortality}} \times 100$$

CTC value ≥ 20 indicate synergism; ≤ (-) 20 indicate antagonism; intermediate value of ≤ (-) 20 to ≥ 20 indicate additive effect.

The synergistic factor was calculated after subjecting the data to regression analysis (Kalyanasundaram & Das, 1985).

$$\text{Synergistic factor (SF)} = \frac{LC_{50} \text{ value of insecticide alone}}{LC_{50} \text{ value of synergised insecticide}}$$

Values of SR > 1 indicate synergism; Value of SR < 1 indicate antagonism.

RESULTS

The present investigations were carried out with an aim to develop a safe and environmental-friendly strategy by using binary mixtures of a toxicant and waste fruit peels. The effective and safe combination will not only protect our environment from hazardous substances but also assist in waste minimization in fruit juice processing industry by utilizing peel waste of citrus fruits as an agent of mosquito control. Consequently, hexane extract of *C. sinensis* peels and α-cypermethrin were assessed for their larvicidal potential against *Ae. aegypti* separately and in binary mixtures of different combinations. The results of the larvicidal potential of *C. sinensis* and α-cypermethrin against *Ae. aegypti* are depicted in Tables 1 and 2.

Table 1

Larvicidal potential of hexane extract of *Citrus sinensis* peels against early fourth instars of *Aedes aegypti* L.

Duration of exposure	**LC_{50} (ppm)**	**95% Fiducial limit**	**LC_{90} (ppm)**	**95% Fiducial limit**	**χ^2 (df)**	**SE**	**RC**
24 h	46.53	40.32-52.97	85.37	72.89-109.34	4.79 (5)	0.54	4.47
48 h	39.57	35.22-43.66	61.92	54.77-74.83	2.23 (5)	0.85	6.49

No mortality was observed in the control, LC_{50} – Lethal Concentration that kills 50% of the exposed larvae; LC_{90} – Lethal Concentration that kills 90% of the exposed larvae; S.E. = Standard Error; χ^2 = Chi-square; df = degree of freedom; R.C. = Regression Coefficient; Test samples were transformed into log covariant ($\log_{10}$), p>0.05, level of significance is greater than 0.05, no heterogeneity factor is used in the calculation of confidence limits, Values are mean of three replicates.

Table 2

Larvicidal potential of α-cypermethrin against early fourth instars of *Aedes aegypti* L.

Duration of exposure	**LC_{50} (ppm)**	**95% Fiducial limit**	**LC_{90} (ppm)**	**95% Fiducial limit**	**χ^2 (df)**	**SE**	**RC**
24 h	0.0063	0.0034-0.0089	0.0235	0.0163-0.0472	5.763 (3)	0.495	2.241
48 h	0.0038	0.0010-0.0061	0.0157	0.0105-0.0358	1.757 (3)	0.587	2.066

No mortality was observed in the control, LC_{50} – Lethal Concentration that kills 50% of the exposed larvae; LC_{90} – Lethal Concentration that kills 90% of the exposed larvae; S.E. = Standard Error; χ^2 = Chi-square; df = degree of freedom; R.C. = Regression Coefficient; Test samples were transformed into log covariant ($\log_{10}$), p>0.05, level of significance is greater than 0.05, no heterogeneity factor is used in the calculation of confidence limits, Values are mean of three replicates.

The results demonstrate the considerable larvicidal efficacy of *C. sinensis* peels against early fourth instars of dengue vector resulting in respective LC_{50} values of 46.53 and 39.57 ppm after 24 h and 48 h of exposure (Table 1). It was also observed that the treatments resulted in complete mortality without any pupa or adult emergence. In comparison, the bioassays with the pyrethroid, α-cypermethrin against *Ae. aegypti* resulted in much higher larval toxicity with LC_{50} values of 0.0063 and 0.0038 ppm after 24 and 48 h of exposure, respectively (Table 2).

The larvicidal bioassays conducted with binary mixtures of α-cypermethrin and hexane extract of *C. sinensis* leaves against *Ae. aegypti* larvae are presented in the Table 3. The addition of equal parts of α-cypermethrin and hexane extract of *C. sinensis* leaves increased the larvicidal efficacy of cypermethrin by 3.865-fold at LC_{50} after 24 h. On the other hand, when 5 parts of peel extract were combined with 1 part of insecticide, the efficacy of extract increased by 8931-fold while that of α-cypermethrin by 1.209 at LC_{50} after 24 h (Fig. 2). Similar pattern, with 1:5 combination, was noticed after 48 h of exposure (Fig. 3). Nevertheless, the larvicidal potential of the mixture increased with rise in duration of exposure.

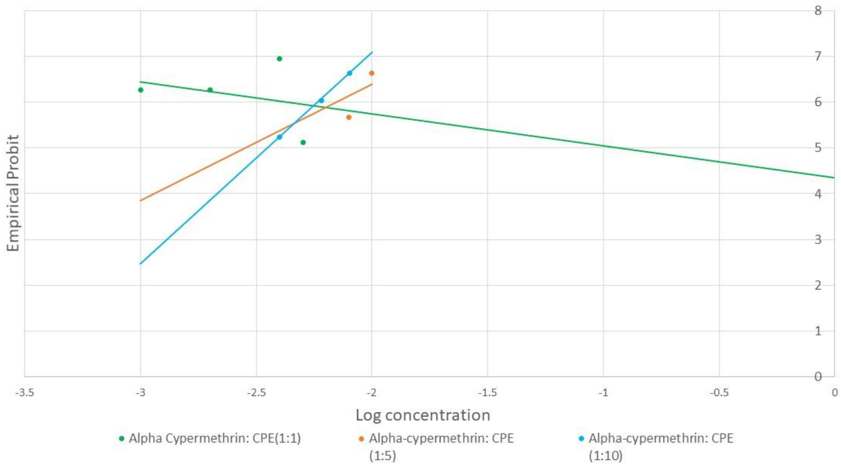

Fig. 2. Dosage-mortality regression lines obtained when larvae of *Aedes aegypti* were exposed with α-cypermethrin alone, and in binary combination with *Citrus sinensis* (1:1, 1:5 and 1:10) for 48 h. *CPE = *Citrus sinensis* peel extract in hexane.

The binary mixture of citrus peel extract and α-cypermethrin in 1:1 ratio proved to be the most effective against dengue larvae among all the ratios tested; it possessed 3.196-fold higher efficacy as compared to 1:5 ratio and 2.350-fold higher than 1:10 combination indicating the synergistic efficacy of citrus peel extract after 24h of exposure (Table 3).

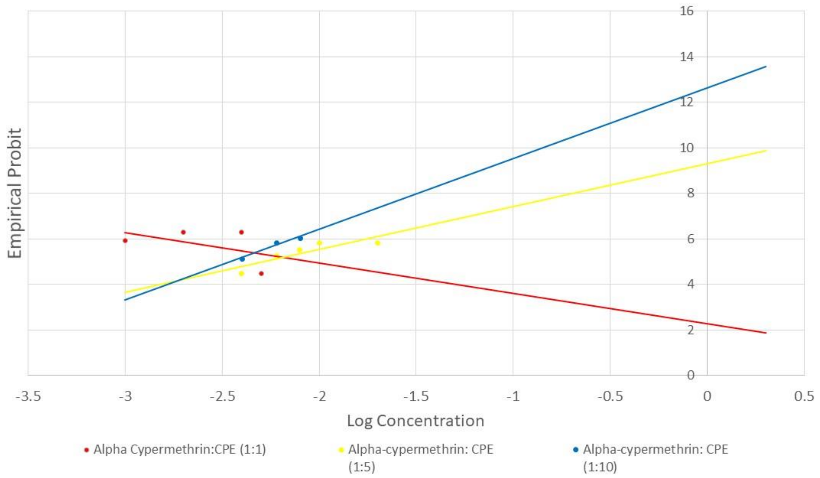

Fig. 3. Dosage-mortality regression lines obtained when larvae of *Aedes aegypti* were exposed with α-cypermethrin alone, and in binary combination with *Citrus sinensis* (1:1, 1:5 and 1:10) for 24 h. *CPE = *Citrus sinensis* peel extract in hexane.

Table 3

Larvicidal potential of binary mixtures of α-cypermethrin and hexane extract of *Citrus sinensis* peels (1:1, 1: 5 and 1: 10) against early fourth instars of *Aedes aegypti* L.

Insecticide: Extract Ratio	**Duration of exposure**	**LC_{50} (ppm)**	**95% Fiducial limit**	**LC_{90} (ppm)**	**95% Fiducial limit**	**χ^2 (df)**	**SE**	**RC**
1:1	24h	0.00163	0.00094-0.00353	0.00756	0.00134-0.01562	1.717 (4)	1.454	2.981
	48 h	0.00092	0.00078-0.00245	0.00679	0.00298-0.01432	2.713 (4)	2.782	5.082
1:5	24 h	0.00521	0.0004- 0.00802	0.02316	0.01269-1.81966	1.656 (3)	0.854	1.979
	48 h	0.00383	0.00067-0.00522	0.00858	0.00642-0.03309	1.805 (3)	1.346	3.200
1:10	24 h	0.00383	0.00088-0.00507	0.00766	0.00590-0.02254	0.926 (3)	1.673	4.261
	48 h	0.00346	0.00072-0.00472	0.00664	0.00505-0.01658	0.191 (3)	2.096	5.131

No mortality was observed in the control, LC_{50} – Lethal Concentration that kills 50% of the exposed larvae; LC_{90} – Lethal Concentration that kills 90% of the exposed larvae; S.E. = Standard Error; χ^2 = Chi-square; df = degree of freedom; R.C. = Regression Coefficient; Test samples were transformed into log covariant ($\log_{10}$), p>0.05, level of significance is greater than 0.05, no heterogeneity factor is used in the calculation of confidence limits, Values are mean of three replicates.

The results clearly showed that as the concentration of *C. sinensis* hexane peel extract in the binary combination increased, it imposed an antagonistic effect on the larvicidal potential of the α-cypermethrin against *Ae. aegypti*. The CTC (% suppression) and synergistic factor obtained in 1:1 combination citrus peel extract and cypermethrin showed more than 20 and 1 respectively (Table 4, Fig. 4).

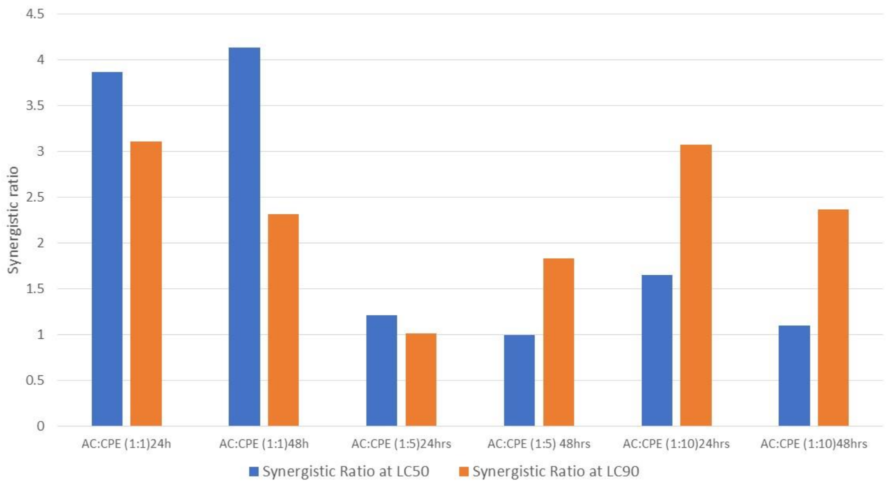

Fig. 4. Variations in synergistic factors when early fourth instars of *Aedes aegypti* were exposed to the 1:5 and 1:10 binary mixtures of α-cypermethrin and *Citrus sinensis* hexane peel extracts for 24 h and 48 h. *CPE = *Citrus sinensis* peel extract in hexane AC=Alpha-cypermethrin.

The data revealed that 1:1 ratio of *C. sinensis* hexane peel extract and α-cypermethrin exhibited 23.456 % CTC and 3.865 synergistic factor at LC_{50} after 24 h of exposure showing high level of synergism. Additive effect was also seen in 1:5 and 1:10 combination of extract and insecticide showing CTC % in a range of (-)3 to 17. However, antagonism was also seen in only of the case at LC_{90} level of exposure in 1:5 combination after 48 h, though that was inconsequential.

Table 4

Relative larvicidal efficacy of α-cypermethrin alone and in combination with *Citrus sinensis* hexane peel extract against early fourth instars of *Aedes aegypti*

Insecticide investigated	LC_{50} (ppm)	Relative Larvicidal Efficacy	CTC	Type of action (Based on CTC)	SF	Type of action (Based on SF)	LC_{90} (ppm)	Relative Larvicidal Efficacy	CTC	Type of action (Based on CTC)	SF	Type of action (Based on SF)
Duration of exposure: 24 h												
α-cypermethrin alone	0.0063	1.0	-	-	-	-	0.0235	1.0	-	-	-	-
α-cypermethrin: CPE (1:1)	0.0016	0.258	23.456	Synergism	3.865	Synergism	0.0076	0.322	31.754	Synergism	3.108	Synergism
α-cypermethrin: CPE (1:5)	0.0052	0.827	16.959	Additive	1.209	Synergism	0.0232	0.985	-18.802	Additive	1.015	Synergism
α-cypermethrin: CPE (1:10)	0.0038	0.607	-3.232	additive	1.645	Synergism	0.0077	0.326	31.368	Synergism	3.068	Synergism
Duration of exposure: 48 h												
α-cypermethrin alone	0.0038	1.0	-	-	-	-	0.0157	1.0	-	-	-	-
α-cypermethrin: CPE (1:1)	0.0009	0.242	19.325	Additive	4.130	Synergism	0.0068	0.432	24.567	Synergism	2.312	Synergism
α-cypermethrin: CPE (1:5)	0.0038	1.008	-0.715	Additive	0.992	Antagonism	0.0086	5.465	27.065	Synergism	1.829	Synergism
α-cypermethrin: CPE (1:10)	0.0034	0.910	-7.339	Additive	1.098	Synergism	0.0066	4.229	33.274	Synergism	2.364	Synergism

CTC= Co-toxicity coefficient; SF = Synergistic Factor; CPE = *Citrus sinensis* peel extract in hexane.

DISCUSSION

Extensive epidemic of *Aedes*-borne diseases and notable increase in the worldwide occurrence of dengue, Chikungunya, and yellow fever has attracted researchers to find eco-friendly and effective approaches. Reduction of mosquito larval source and larval population has always been the prime strategy of successful dengue vector control around the world. Though various chemical insecticides are employed to control mosquito larvae to keep them under threshold level and prevent their breeding, the frequent use of insecticides has been damaging our environment and non-target organisms including humans. Consequently, researchers are exploring plant diversity to formulate and employ plant-derived insecticides as one of the effective and eco-safe alternate approaches in mosquito control programme. With the aim of controlling dengue larvae with nominal use of environmentally-perilous insecticides, the larvicidal activity of peel extract of *C. sinensis* and alpha-cypermethrin was studied alone as well as in binary combinations against *Aedes* larvae.

Our investigations showed appreciable larvicidal efficacy of *C. sinensis* peels against dengue larvae resulting in respective LC_{50} values of 46.53 and 39.57 ppm after 24 h and 48 h of exposure. Our results are in agreement with that of Bagavan *et al*. (2008) who investigated the larvicidal efficacy of the chloroform and methanol extracts of *C. sinensis* peels against *Anopheles subpictus* and *Culex tritaeniorhynchus* and obtained LC_{50} values of 58.25 ppm and 38.15 ppm, respectively. The essential oils extracted from the peels of *C. limon* and *C. sinensis* possessed toxicity against *Culex pipiens* larvae exhibiting LC_{50} values in the range of 30.1 to 51.5 mg/L (Michaelakis *et al*., 2009). Assays performed by Murugan *et al*. (2012) with *C. sinensis* extracts against all the four instars of three mosquito vectors revealed these extracts most effective against *Ae. aegypti.* They reported LC_{50} values of 92.27, 106.60, 204.87, 264.26 ppm against *Ae. aegypti;* 182.24, 227.93, 291.69, 398.00 ppm against *An. stephensi;* and 244.70, 324.04, 385.32, 452.78 ppm against *Cx. quinquefasiatus* I-IV instars, respectively. The larvicidal efficacy of citrus peel extracts and essential oils against mosquito vectors have also been observed in the past (Mwaiko, 1992; Mwaiko & Savaeli 1994; Ezeonu *et al*., 2001; Amusan *et al*., 2005).

We also conducted larvicidal bioassays with α-cypermethrin against early fourth instars of *Ae. aegypti* and obtained LC_{50} values of 0.0063 and 0.0038 ppm after 24 and 48 h of exposure, respectively. Cypermethrin is a pyrethroid which is still considered the major class of highly active insecticides due to their anti-mosquito potential and comparatively low toxicity in relation to organochlorines and organophosphates. Though a number of reports exist about the efficacy of pyrethroids against mosquito vectors, limited work is reported about the efficacy of α-cypermethrin. In Australia, Pettit *et al.* (2010) investigated the efficacy of α-cypermethrin against *Ae. notoscriptus*, *Ae. aegypti* and *Ae. albopictus* in order to inhibit colonization of larvae in water-filled receptacles and reported high efficacy. Sunday *et al*. (2016) conducted larval and adult bioassays and revealed the susceptibility of *Culex* to cypermethrin. The larvicidal efficacy of cypermethrin has been advocated by Mohan *et al*. (2007) revealing LC_{50} value of 0.0369 ppm after 24 h of exposure which is much higher than observed by us. However, efficacy of alpha-cypermethrin reported by Samal & Kumar (2018) showed LC_{50} of 0.0005 ppm which was 13 times lower than the present investigation. This variation may be because of the status of resistance level in the *Aedes aegypti* larvae which need to be taken into consideration. Similarly, Aivazi & Vijayan (2010) obtained much higher LC_{50} value of 0.4 ppm when dengue larvae were assayed with cypermethrin.

Reports exist in the literature which reveal that cypermethrin possess considerable toxicity against non-target organisms. The insecticide has been found toxic against *Ceriodaphnia dubia* (Chen *et al*., 2015). Thus, we attempted to formulate a binary mixture of α-cypermethrin with *C. sinensis* peel extract which may prove a good eco-friendly approach by reducing the dose of cypermethrin but increasing its efficacy by adding eco-safe compound to be applied in vector control programs. In addition, such mixtures could extend lifetime of available insecticides, reduce the costs, and manage insecticide resistance (Aivazi & Vijayan, 2009).

We observed that 24 h exposure of *Ae. aegypti* early fourth instars with 1:1 binary mixture of cypermethrin and citrus peel extract reduced the LC_{50} of cypermethrin by 4-fold resulting in 3.865 synergistic factor. Studies conducted by Mohan *et al*. (2007) reported the synergistic action of the different combinations of *Solnaum xanthocarpum* chloroform and methanol extract and cypermethrin against *An. stephensi* larvae; stating the 1:1 combination with chloroform extract as the most effective one. They also reported a negative correlation between the amount of extract and the synergistic factor suggesting that synergistic activity of the binary mixture investigated decreases with the increasing concentrations of plant extract. In 2010, Mohan *et al*. investigated the larvicidal activities of the binary mixtures of temephos, fenthion and petroleum ether extract of *S. xanthocarpum* (1:1, 1:2, and 1:4 ratios) against *Cx. quinquefasciatus*. Interestingly, they found antagonistic effect with temephos/*S. xanthocarpum* extract combination; while in agreement to our results, the fenthion/*S. xanthocarpum* extract combination acted synergistically at 1:1 ratio. Likewise, the synergism of some plant extracts mixed with phenthoate and fenthion was reported against *An. stephensi* by Kalayanadundaram & Das (1985).

In Mysore, India, Aivazi & Vijayan (2009) obtained promising results with binary combination of Rue (*Ruta graveolens*) plant leaf methanol extract and cypermethrin (1:1) against *An. stephensi*. They reported the respective co-toxicity coefficient and synergistic factor as 119.4 and 9.94 after 24 h. Significant synergism has also been obtained by assaying the binary mixtures of fenthion and *Leucus aspara, Turnera ulmifolia, Vinca rosea, Clerodendron inerme, Pedalium murax, Parthenium hysterophorus* extract revealing respective synergistic factors of

1.31, 1.38, 1.40, 1.48, 1.61, and 2.23, respectively. Our observations are also supported by the findings of Thangam & Kathiresan (1991) who advocated the synergistic activity of three plant extracts; *Caulerpa scalpelliformis*, *Rhizophora apiculata* and *Dictyota dichotoma* when added to DDT.

Our investigations showed that the hexane peel extract of *C. sinensis* is a potential mosquito larvicide and it can increase the efficacy of cypermethrin by acting as an effective synergist. It has been cited that the plant extracts may possess some factors which may inhibit certain factors, such as detoxifying enzymes, thus resulting in synergistic activity and increasing the efficacy of pyrethroids (Thangam & Kathiresan, 1991). Our study is of high significance as this approach not only minimizes the amount of both the constituents but also make the binary compound more operative, cost-effective and comparatively less harmful to the environment. In addition, utilization of citrus waste for mosquito control would help to reduce waste and the pollution load boosting the environmental profile of fruit juice processing industry.

CONCLUSIONS

Current studies showed that binary combination of extracts prepared from the waste peels of *C. sinensis* and alpha-cypermethrin can be utilized as an effective agent in control of *Ae. aegypti* by exerting synergistic and additive effects. This will not only reduce the environment load of waste peels, but also protect the environment and non-target organisms including human beings, from the harmful effects of synthetic insecticide. However, further evaluation performed with the bioactive constituent of peel extract is required for the formulation of an efficient and eco-friendly combination which can be used effectually in dengue vector control programs.

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