South African Journal of Botany 140 (2021) 103–109

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Synergistic effect of *Croton bonplandianum Baill.* with Cypermethrin and Lambda-cyhalothrin against *Aedes aegypti Linn*, a Dengue fever vector

Kalarikkal Venugopalan Lakshmi[a,b], Ambalaparambil Vasu Sudhikumar[a], Embalil Mathachan Aneesh[b,*]

[a] Department of Zoology, Christ College, Irinjalakuda, Kerala 680125, India
[b] Communicable Disease Research Laboratory, Department of Zoology, St.Joseph's College, Irinjalakuda, Kerala, India

ARTICLE INFO

Article History:
Received 21 July 2020
Revised 26 February 2021
Accepted 19 March 2021
Available online xxx

Edited by R Maharaj

Keywords:
Mosquito
Larvae
Aedes
Plant extract
Insecticide
Cypermethrin
Lambda-cyhalothrin
Synergy
LC50
LC90
GC-MS

ABSTRACT

Vector-borne diseases are increasing exponentially, and the mosquito vectors contribute to the majority of it. Dengue fever is considered one of the foremost causes of causalities around the globe, which is primarily transmitted by Aedes aegypti. Control sequences of this vector species using chemical insecticides are now come to be insufficient due to the rapid resistance development in the aimed species. To avoid this, a newer concept of synergy has been introduced by combining two or more compounds with pesticidal capacity. In this study, a hexane extract of the plant Croton bonplandianum are separately combined with two pyrethroids, Lambda-cyhalothrin and Cypermethrin against the dengue fever vector, *Aedes aegypti*. Individual larvicidal capabilities and the synergistic effects of these compounds were inquired, and synergism was found to provide better larvicidal activity.

1. Introduction

In the 1780s, a clinically recognized dengue outbreak was detailed in different continents, counting Asia, Africa, and North America. As per the records, the epidemics coincided in these regions (Gupta et al., 2012). Nowadays, dengue has swept into most of Asian countries and turn out to be a dominant cause of causalities counting both adults and children. Studies suggest that around 3.9 billion people in 129 countries are at the risk of Dengue infection, of which Asia constitutes as much as 70 percentage (Bhatt et al., 2013, Brady et al., 2012).

Aedes (Stegomyia) *aegypti* (Linneaus) is assigned as the worldwide primary vector of the Dengue fever virus as there was an increased distribution of this vector species relating to a similar pattern of regional virus transmission (Mackenzie et al., 2004). Aedes aegypti is also known to be the vectors of Chikungunya and yellow fever.

Plant extracts are used in the practice of effective insect pest repellents or pesticides from ancient times. Nevertheless, after synthetic or Chemical insecticides' invention, they have been elapsed or evaded for decades because they were far behind these chemical formulations when it comes to the concentration of application (Ghosh et al., 2012, McIndoo,1945). Nevertheless, after the substantial discharge of these chemicals to the environment, it has slowly started revealing its negative impacts. They have already upset our ecosystem, toxified the non-targeted species, and the major drawback was considered as the development of resistance in the targeted species. Studies revealed that mosquitoes are developing resistance to such formulations swiftly (Karunaratne and Hemingway, 2000 and Selvi et al., 2010)

In this field of mosquito vector control, intermediary strategies are being discussed and designed, which could moderate the negative attributes of both individual methods. For this, a synergistic effect of both plant and chemical insecticides are used in such a way that the minimum quantity of the chemical insecticide is benefited in proportion to the other (Bernard and Philogene, 1993).

Edited by R Maharaj.
* Corresponding author.
E-mail address: aneeshembalil@stjosephs.edu.in (E.M. Aneesh).

https://doi.org/10.1016/j.sajb.2021.03.034
0254-6299/

2. Methodology

2.1. Mosquito colony

Laboratory colonized *Aedes aegypti* larvae that were maintained in an insectary at the Communicable Disease and Research Laboratory were used for the tests. The adults were reared in sterilized cages fitted with mosquito netting and fed first on freshly water-soaked raisin at 27±2°C, 75–85% RH with 14:10 L/D photoperiod cycle. Female mosquitoes were allowed to feed on blood placed in resting cage on the third-day post-emergence. The conditions were sustained constant throughout the experiments.

2.2. Plant extract bioassay

The plant selected for this study was collected from the Palakkal Kol situated in Thrissur, Kerala, India (10°33'22.2" N 76°10'41.6" E). The preliminary criteria for the selection were the aromatic nature of the plant species. It was identified as *Croton bonplandianum* Baill, which is coming under the family Euphorbiaceae. For the larval bioassay, the leaves of the plant were collected, cleaned with tap water, and was subjected to shade drying. Fine powder of the dried leaves was made by crushing it in a hand mixer grinder. The extractions were done in a Dionex ASE 150 accelerated solvent extractor at 100° C and 1700 PSI for 20 minutes using hexane as the solvent. The collected extracts were then subjected to HS-2005V-N Rotary Flash evaporator under reduced pressure at 40°C to remove the solvent. The rest of the extract was lyophilized for further drying procedures. Stock solutions were prepared by dissolving a specific quantity of extract in acetone to prepare the stock solution. WHO procedure (WHO, 2005) with slight modifications was followed to conduct larval bioassay.

Test concentrations were primed by adding 1ml of identified concentration of the plant extract. This was further made up to 250ml by means of dechlorinated tap water. A control was upheld with 1 ml of acetone in 249ml water per experiment.

Twenty-five *Aedes aegypti* larvae were released into each test concentrations as well as control, and three replicates were kept for both control and tests. The test was carried out for 24 hours without disturbing the whole set up. The larvae were not provided with food in both control and test during the experiment time. After completing the experiment period, larvae were analyzed, and it was anticipated as dead or moribund for not having any response to the gentle nudging with a fine needle. The experiment set up was done at the room temperature of 27±2°C and 75±5% humidity.

2.3. Insecticide bioassay

Two pyrethroid insecticides, Cypermethrin, Lambda-cyhalothrin, were selected for this study. To prepare the stock solution, 1milligram of insecticide was dissolved in 10ml acetone. A range of varying concentrations was prepared for each insecticide used for synergic study from this stock. An analogous method of plant extract bioassay was executed with the insecticides against fourth instar larvae of *Aedes aegypti.*

2.4. Synergistic assay of phyto-extracts and insecticides

Two different tests were organized with the insecticide and plant extract for finding out the Co-toxicity coefficient (CTC) and Synergistic factor SF.

2.4.1. Co-toxicity coefficient (CTC)

For finding the Co- toxicity coefficient (CTC), a test solution was prepared by combining the insecticide and plant extract in the same ratio (1:1). Both the LC25 concentrations of insecticide and plant extracts that were previously determined were selected for this test. 500μl of each solution were combined, and it was made up to 250 ml using tap water. Control for this test was made by adding the combination of 500 μl of alcohol and acetone in the same ratio and volume to the tap water.

$$Co-toxicity\ coefficient\ (CTC) = 100 \times \frac{(Observed\ \%\ mortality - Expected\ \%\ mortality)}{Expected\ \%\ mortality}$$

A positive factor or CTC >20 specifies synergism, a negative factor or CTC <0 specifies antagonism and the transitional values of 0 < CTC < 20 designates an additive effect

Expected mortality is estimated as the total of percentage mortalities at LC25 concentration of both the test materials independently.

The observed mortality is the documented mortality obtained at 24 h after exposure to the mixtures (Sun and Johnson, 1960).

2.4.2. Synergistic Factor (SF)

The LC25 value of the plant extract was made inflexible to document the synergistic factor of the insecticide-Plant extract combination, and varying concentrations of the insecticides were prepared independently.

$$Synergistic\ factor\ (SF) = \frac{(LC50\ or\ LC90\ of\ the\ insecticide\ alone)}{(LC50\ or\ LC90\ of\ the\ insecticide\ with\ plant\ extract)}$$

A value higher than one for synergistic factor denotes synergism and a value lower than one denotes antagonism (Kalyanasundaram and Das, 1985)

2.5. GC-MS analysis

GC-MS analysis was performed using a GC Clarus 500Perkin Elmer, including AOC-20i Autosampler and Gas Chromatograph intermingling with a Mass Spectrometer instrument. The environments upheld for the trial consist of a Column Elite-5MS glued silica capillary column (30mm x0.25mm x 0.25 μm df composed of 5% Diphenyl/95% Dimethylpolysiloxane), functioning in operating in electron impact manner at 70eV. A constant flow rate of 1 ml per minute and injection volume of 3 μl (split ratio 10:1) was sustained for carrier gas Helium (99.999%) at an injector temperature 250°C and ion-source temperature 280°C. The average peak area of each

Table 1
Mean and Percentage Mortality with Standard deviation of *Aedes aegypti* larvae against hexane extract of *Croton bonplandianum* Baill.

Observed parameters	Plant extract Test concentrations in ppm					
	10	20	30	40	50	60
Mean Mortality with SD	3.333333 ± 0.816497	8.5 ± 1.048809	13.16667 ± 0.752773	15.66667 ± 0.816497	20.83333 ± 0.752773	23.16667 ± 0.752773
Percentage Mortality with SD	13.33333 ± 0.816497	34 ± 1.048809	52.66667 ± 0.752773	62.66667 ± 0.816497	83.33333 ± 0.752773	92.66667 ± 0.752773

Table 2
Mean and percentage mortality with standard deviation of *aedes aegypti* larvae against cypermethrin.

Observed parameters	Cypermethrin Test concentrations in ppm					
	0.00004	0.00010	0.00016	0.00022	0.00028	0.00034
Mean Mortality with SD	4 ± 0.632456	8.333333 ± 0.516398	12.5 ± 0.547723	16.16667 ± 0.408248	20.16667 ± 1.169045	23.83333 ± 0.408248
Percentage Mortality with SD	16 ± 0.632456	33.33333 ± 0.516398	50 ± 0.547723	64.66667 ± 0.408248	80.66667 ± 1.169045	95.33333 ± 0.408248

Table 3
Mean and percentage mortality with standard deviation of *Aedes aegypti* larvae against Lambda-cyhalothrin.

Observed parameters	Lambda-cyhalothrin Test concentration in ppm					
	0.00002	0.00004	0.00006	0.00008	0.0001	0.00012
Mean Mortality with SD	3.5 ± 0.547723	8.833333 ± 0.752773	12.5 ± 0.547723	15.66667 ± 0.516398	20.66667 ± 1.36626	23.83333 ± 0.408248
Percentage Mortality with SD	14 ± 0.547723	35.33333 ± 0.752773	50 ± 0.547723	62.66667 ± 0.516398	82.66667 ± 1.36626	95.33333 ± 0.408248

chemical compound was premeditated by comparing the average peak area to the total area.

2.6. Statistical analysis

Abbott's formula (Abbot, 1925) was used to adjust the larval mortality counts if needed. The data were further subjected to regression analysis of probit mortality (Finney, 1971), and the SPSS software 24 version was used for the significant effect of plant extract and synergy. Figures arev designed using Microsoft excel 10.

3. Results

3.1. Larval bioassay results

Larvicidal activity of plant extract and two insecticides were individually assessed versus late third or early fourth instar larvae of Aedes aegypti. The mean and percentage mortality obtained are showed in Tables 1–3. The effective range of plant extracts observed was between 10 and 60ppm; that of cypermethrin and Lambda-cyhalothrin was noted as0.00004 to 0.00034ppm and 0.00002 to 0.00012ppm correspondingly. Figs. 1–3 illustrate the 'probit percentage mortality' of individual test material plotted against their corresponding' log of concentration.'

The hexane extract of Croton bonplandianum Baill was found to have a larvicidal effect on Aedes aegypti, with the lethal concentration values 15.90ppm (LC25), 26.337ppm (LC50), and 68.708 (LC90). The equivalent lethal concentration values of Cypermethrin were reflected as 0.00007274(LC25), 0.00013313(LC50), and 0.00041979 (LC90) and that for Lambda-cyhalothrin was 0.000031412(LC25), 0.000052068(LC50), 0.000136010(LC90) (Table 6). Co-toxicity coefficient tests and Synergistic studies were conducted using two different pyrethroids, Cypermethrin and Lambda-cyhalothrin, along with the Plant extract. The lethal concentration assay results with P and Chi square values are shown inTable 8.

3.2. Co-toxicity coefficient (CTC) test results

The values observed for Co-toxicity coefficient (CTC) tests performed by amalgamating the LC25 concentrations in 1:1 ratio of the

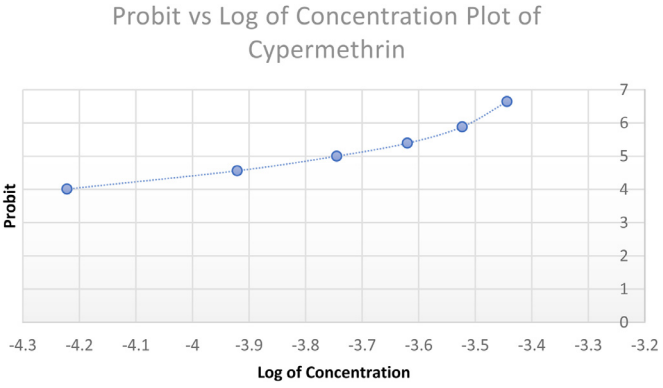

Fig. 2. Graph showing Probit vs Log of concentration of Cypermethrin against *Aedes aegypti* larvae

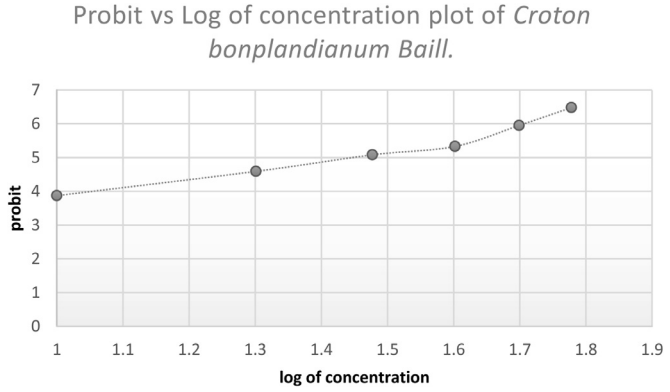

Fig. 1. Graph showing Probit vs Log of concentration of *Croton bonplandianum Baill* against *Aedes aegypti* larvae

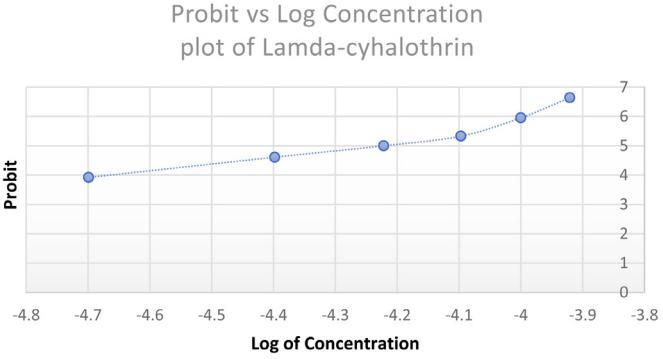

Fig. 3. Graph showing Probit vs Log of concentration of Lambda-cyhalothrin against *Aedes aegypti* larvae.

Table 4
Mean and percentage mortality with standard deviation of *Aedes aegypti* larvae against varying Synergistic concentration of Cypermethrin with constant concentration (LC25) of hexane extract of *Croton bonplandianum* Baill.

Observed parameters	Varying Synergistic concentration of Cypermethrin with constant concentration of plant extract (LC25)					
	0.00002	0.00005	0.00008	0.00011	0.00014	0.00017
Mean Mortality with SD	4.333333 ± 0.516398	8.333333 ± 0.516398	12.5 ± 0.547723	16.33333 ± 0.516398	20.5 ± 0.83666	23.5 ± 0.547723
Percentage Mortality with SD	17.33333 ± 0.516398	33.33333 ± 0.516398	50 ± 0.547723	65.33333 ± 0.516398	82 ± 0.83666	94 ± 0.547723

Table 5
Mean and Percentage Mortality with Standard deviation of *Aedes aegypti* larvae against varying Synergistic concentration of Lambda-cyhalothrin with constant concentration (LC25) of hexane extract of *Croton bonplandianum* Baill.

Observed parameters	Varying Synergistic concentration of Lambda-cyhalothrin with constant concentration of plant extract (LC25)					
	0.000002	0.000006	0.00001	0.000014	0.000018	0.000022
Mean Mortality with SD	4.5 ± 0.83666	9 ± 0.894427	12.83333 ± 0.752773	16.16667 ± 0.408248	20.16667 ± 1.169045	22.66667 ± 1.21106
Percentage Mortality with SD	18 ± 0.83666	36 ± 0.894427	51.33333 ± 0.752773	64.66667 ± 0.408248	80.66667 ± 1.169045	90.66667 ± 1.21106

plant extract with Cypermethrin, and Lambda-cyhalothrin were 34.66 and 44, respectively, signifying a positive factor for synergism for both insecticides (Table 7).

3.3. Synergy test result

Cypermethrin concentrations ranging from 0.00002 to 0.00017 and Lambda-cyhalothrin concentrations from 0.000002 to 0.000022 were combined separately with the LC25 concentration of the plant extract and tested against *Aedes aegypti* larvae. The mean and percentage mortality are given in Tables 4 and 5.

From the results, it was evident that both Cypermethrin and Lambda-cyhalothrin showed a synergistic effect on *Aedes aegypti*. The larvicidal activity of both the insecticides were elevated when combined with the plant extract (Figs. 4 and 5). When compared, Lambda-cyhalothrin with the plant extract was more efficient than

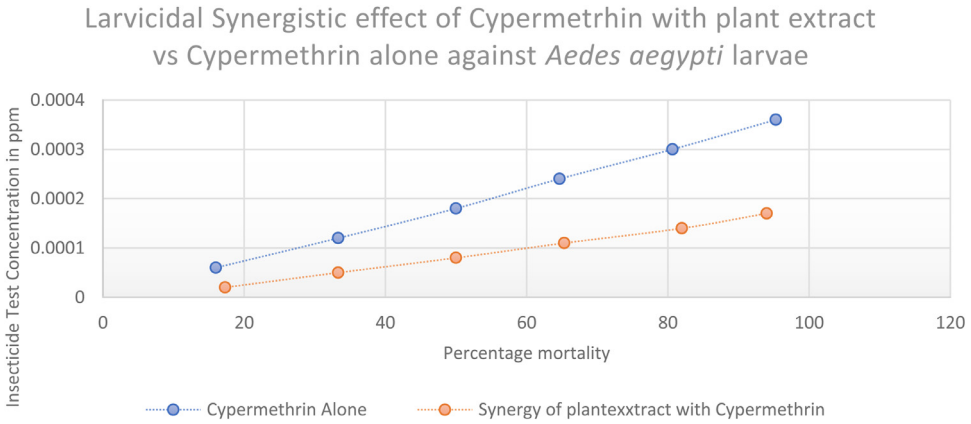

Fig. 4. Comparing the Larvicidal synergistic effect of cypermethrin with the hexane extract of *Croton bonplandianum* Baill., and the larvicidal activity of cypermethrin alone against *Aedes aegypti* larvae.

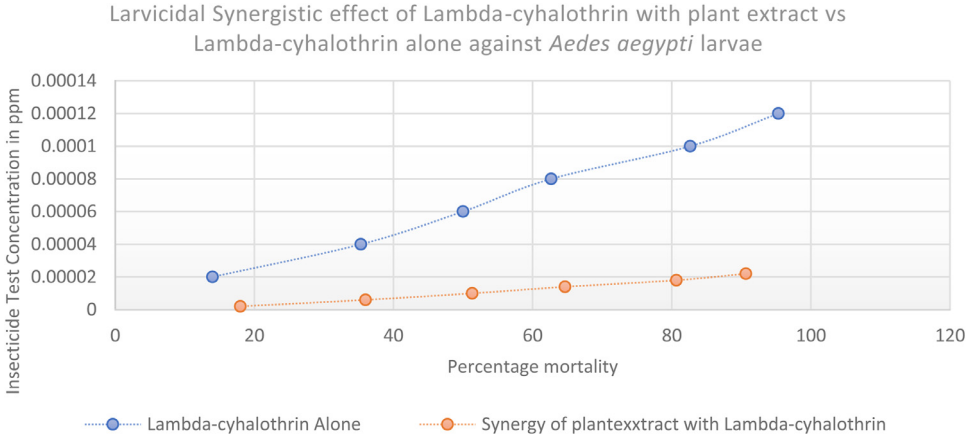

Fig. 5. Comparing the Larvicidal synergistic effect of Lambda-cyhalothrin with the hexane extract of *Croton bonplandianum* Baill., and the larvicidal activity of Lambda-cyhalothrin alone against *Aedes aegypti* larvae.

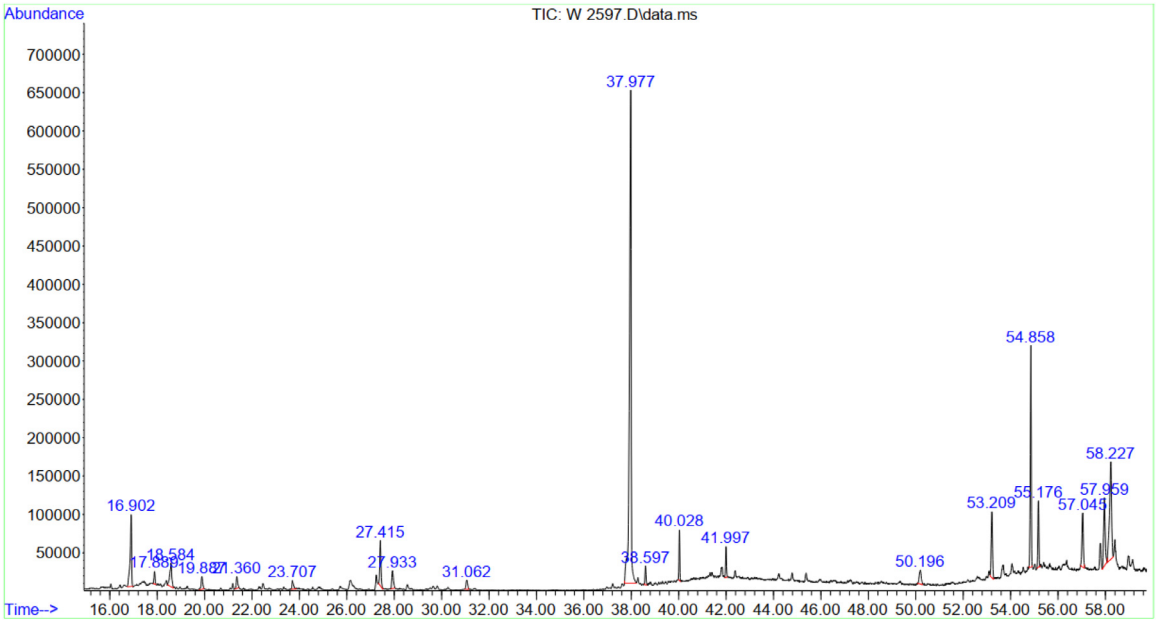

Fig. 6. GC-MS Chromatogram of Hexane extract of *Croton bonplandianum* Baill.

Cypermethrin combination, with a synergistic factor of 11.2746 and 2.065 correspondingly as shown in Table 8.

3.4. GC-MS Result

The hexane extract of *Croton bonplandianum* Baill was subjected to GC-MS analysis to ascertain its chemical composition. The analysis revealed 20 compounds that were interpreted using the National Institute Standard and Technology (NIST) database. All the compounds that are identified have been listed in Table 9 with their Peak Number Real-time, Area percentage, molecular formula, and mass in gram per mol, and Fig. 6 stand for the chromatogram of the analysis.

4. Discussion

Mosquito-borne diseases are budding as a global dispute as they are hastily increasing and grueling to control due to the resistance development, which stemmed in the failure of chemical insecticides. Phytoextracts alone are also proved to be scarce in this field as they have to be applied in large volumes to attain the desirable efficiency. When they applied together as a compound, they conveyed a better control measure and also resulted in the reduction of resistance development and also proved to be economically and environmentally advantageous (Khalequzzaman and Khanom, 2006).

In this study, *Croton bonplandianum* was selected for the plant extract counterpart for synergistic effect with two pyrethroids, Cypermethrin and Lambda-cyhalothrin. This plant is considered as a weed species in the wastelands of Kerala and used as a traditional medicine for various diseases like scabies, venereal sores, headache, cholera (Pal and Jain, 1998), etc. and found to have larvicidal (Jeeshna et al., 2010), antiseptic (Chaudhuri,2007), antimicrobial (Rajakaruna,2002) and antitumor capabilities (Islam et al., 2011). An investigation of the larvicidal activity of this plant against *Aedes aegypti* comprehended that the methanolic extract was adequate with an LC50 value of 124 ppm (Jeeshna et al., 2010).

Various studies are being carried out in this aspect of synergy and found to be a promising method (Kalyanasundaram and Das, 1985,

Table 6
Larvicidal activity of Test materials alone against *Aedes aegypti.*

Test substance	LC25 (LCL-UCL)	LC50 (LCL-UCL)	LC90 (LCL-UCL)	CHI SQUARE VALUE	P VALUE	R^2 VALUE
Plant Extract	15.900(10.590-20.080)	26.337(21.053-31.721)	68.708(52.702-111.476)	9.657	0.047[a]	0.947
Cypermethrin	0.00007274(0.00003915-0.00009988)	0.00013313(0.00009602-0.00017227)	0.00041979(0.00029416-0.00087118)	12.872	0.012[a]	0.923
Lambda-cyhalothrin	0.000031412(0.000017915-0.000041474)	0.000052068(0.000038765-0.000065634)	0.000136010(0.000099541-0.000265019)	14.636	.006[a]	0.910

P<0.05, significant p value, LCL-Lower Concentration Limit, UCL-Upper Concentration Limit.

Table 7
Co-toxicity coefficient of Cypermethrin and Lambda-cyhalothrin with hexane extract of plant against *Aedes aegypti.*

Test substance	Concentration in ppm	Observed % mortality	Expected % mortality	Co-toxicity coefficient
Plant Extract(alone)	LC25(15.900)	25.33333	25	-
Cypermethrin (alone)	LC25(0.00007274)	24	25	-
Lambda-cyhalothrin(alone)	LC25(0.000030964)	26	25	-
Plant extract + Cypermethrin	LC25+LC25(1:1)	67.33333	50	34.66
Plant extract+ Lambda-cyhalothrin	LC25+LC25(1:1)	72	50	44

Table 8
Synergistic effect of the hexane extract of plant with Cypermethrin and Lambda-cyhalothrin against *Aedes aegypti.*

Test substance	LC50 (LCL-UCL)	SF at LC50	CHI SQUARE VALUE	P VALUE	R^2 VALUE
Plant Extract+Cypermethrin	0.000064466 (0.000041320-0. 000089295)	2.065	18.095	0.001	0.888
Plant Extract+Lambda-cyhalothrin	0.000004614(0.000002608-0.000007028)	11.2746	14.765	.005[a]	0.902

Significance between plant extract+ insecticides, plant extract alone and insecticides alone:($P<0.05$), LCL lower concentration limit, UCL upper concentration limit, LC50 Lethal concentration that kills 50% of the tested larvaeLC90 Lethal concentration that kills 90% of the tested larvae.

Table 9
GC-MS spectral analysis of Hexane Extraction of *Croton bonplandianum* Baill.

Peak number	Real time	Area%	Compound name	Molecular formula	Mass(g/mol)
1	16.902	5.162%	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	$C_{15}H_{24}$	204.3511
2	17.889	0.675	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1α,2α,5α)-	$C_{10}H_{18}O$	154.2493
3	18.584	2.198%	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-	$C_{15}H_{24}$	204.35 g/mol
4	19.887	0.988	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	$C_{11}H_{16}O_2$	180.2435
5	21.360	0.793	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.24
6	23.707	0.656	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.5
7	27.415	2.812	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268.4778
8	27.933	1.367	Phthalic acid, isobutyl octyl ester	$C_{20}H_{30}O_4$	334.4
9	31.062	0.768	Isophytol	$C_{20}H_{40}O$	296.5
10	37.977	42.295	Phytol	$C_{20}H_{40}O$	296.5
11	38.597	0.946	(2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-methanol	$C_{11}H_{20}O$	168.28
12	40.028	1.994	Oxirane, hexadecyl-	$C_{18}H_{36}O$	268.4778
13	41.997	1.322	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324.5
14	50.196	1.344	Nonadecane	$C_{19}H_{40}$	268.5
15	53.209	4.288	Squalene	$C_{30}H_{50}$	410.7
16	54.858	10.606	Octacosane	$C_{28}H_{58}$	394.8
17	55.176	3.265	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)-	$C_{30}H_{50}O$	426.7174
18	57.045	3.619	Octadecanal	$C_{18}H_{36}O$	268.5
19	57.959	5.016	Cholesterol	$C_{27}H_{46}O$	386.6535
20	58.227	9.884	1-Octacosanol	$C_{28}H_{58}O$	410.8

GC-MS: Gas Chromatography- Mass Spectrometer.

Thangam and Kathiresan, 1990). *Eugenia jambolana* Linn. and *Solidago canadensis* Linn. leaf extracts with deltamethrin against *Aedes aegypti* Linn. was conducted at Mysore for their synergstic effect, and the results were found to be operative with an LC50 Synergistic factor 4.090 and 1.80, respectively (Raghavendra et al., 2013). An analysis steered by Mohan etal., in which they tested the synergistic activity of Solanum *xanthocarpum* extracts with cypermethrin evidenced to be effective in the control of the malarial vector, *Anopheles stephensi* (Mohan etal.,2007).

The present study also supports the effectiveness of synergism than the individual test substances used in larvicidal activity against *Aedes aegypti*, as it was evidenced that a lesser concentration of LC50 value of both the counterparts indicated more efficacy.

5. Conclusion

Any strategy designed to control any pests or vector should achieve some criteria like lesser environmental hazards with more efficient control measures. In Synergistic studies, an environment-friendly component plant-extract is used to reduce the negative impact on the environment in combination with an insecticide, which would increase the larvicidal efficiency of the compound against the vector. It would also condense the resistance development in the targeted species owing to the novel combinations used. From the results of the present study, synergism could be upheld as a better method of vector control than the individual efficacy of the test substances.

Declaration of Competing Interest

The Authors declare no conflict of interest.

Acknowledgment

The authors are gratified to the Principal, St.Joseph's College, Irinjalakuda for the laboratory amenities provided. We recognize E-grants government of Kerala for providing funds.

References

Abbot, WS., 1925. A method of computing of the effectiveness of an insecticide. J. Eco Entomol. 18, 265–267.
Bernard, C.B., Philogene, B.J., 1993. Insecticide synergistic role, importance and perspectives. J. Toxicol. Environ. Health 38, 199–223.
Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., Drake, J.M., Brownstein, J.S., Hoen, A.G., Sankoh, O., Myers, M.F., 2013. The global distribution and burden of dengue. Nature 496 (7446), 504–507.
Brady, O.J., Gething, P.W., Bhatt, S., Messina, J.P., Brownstein, J.S., Hoen, A.G., Moyes, C.L., Farlow, A.W., Scott, T.W., Hay, S.I., 2012. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLOS Neglect. Tropical Dis. 6 (8), 1760.
Chaudhuri, A.B., 2007. Endangered Medicinal Plants. Daya Publishing House, Delhi, p. 226.
Finney, D.J., 1971. Probit analysis, 3rd Ed. Cambridge University Press, Cambridge.
Ghosh, A., Chowdhury, N., Chandra, G., 2012. Plant extracts as potential mosquito larvicides. Indian J. Med. Res. 135 (5), 581.
Gupta, N., Srivastava, S., Jain, A., Chaturvedi, U., 2012. Dengue in India. Indian J. Med. Res. 136 (3), 373–390.
Islam, M.S., Rahman, M.M., Rahman, M.A., Qayum, M.A., Alam, M.F., 2011. *In vitro* evaluation of *Croton bonplandianum* Baill. as potential antitumor properties using *Agrobacterium tumefaciens*. J. Agricult. Technol. 7, 711–719.
Jeeshna, M.V., Mallikadevi, T., Paulsamy, S., 2010. Screening of the weed plant species, *Croton bonplandianum* Baill. For larvicidal activity of *Aedes aegypti*. J. Biopesticides 3, 192–194.
Mackenzie, J.S., Gubler, D.J., Petersen, L.R., 2004. Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. Nat. Med. 10, 98–109.
Kalyanasundaram, M., Das, P.K., 1985. Larvicidal and synergistic activity of plant extracts for mosquito control. Ind. J. Med. Res. 82, 19–23.
Karunaratne, S.H.P.P., Hemingway, J., 2000. Insecticide resistance spectra and resistance mechanisms in populations of Japanese encephalitis vector mosquitoes, *Culex tritaeniorhychus* and *Culex gelidus* in Sri Lanka. Med. Vector Entomol. 14, 430–436.

Khalequzzaman, M., Khanom, M., 2006. Effects of Cypermethrin Alone and in Combination with Leaf and Seed Extracts of Neem Against Adult *Tribolium Castaneum* (Herbst), 25. Univ J Zoo Rajshahi Univ., pp. 45–49.
McIndoo, N.E., 1945. Plants of Possible Insecticidal Value: A Review of the Literature up to 1941. US Department of Agriculture, Agricultural Research Administration, Bureau of Entomology and Plant Quarantine.
Mohan, L., Sharma, P., Srivastava, C.N., 2007. Comparative efficacy of *Solanum xanthocarpum* extracts alone and in combination with a synthetic pyrethroid, cypermethrin, against malaria vector *Anopheles stephensi*. Southeast Asian J. Tropical Med. Public Health 38 (2), 256.
Pal, D.C., Jain, S.K., 1998. Tribal Medicine. Naya Prokash, Calcutta, India, p. 317.
Raghavendra, B.S., Prathibha, K.P., Vijayan, V.A., 2013. Synergistic effect of *Eugenia jambolana Linn*. and *Solidago canadensis Linn*. Leaf extracts with deltamethrin against the dengue vector *Aedes aegypti Linn*. at Mysore. Environ. Sci. Pollut. Res. 20 (6), 3830–3835.
Rajakaruna, N., Harris, C.S., Towers, G.H.N., 2002. Antimicrobial activity of plants collected from serpentine outcrops in Sri Lanka. Pharmaceut. Biol. 40, 235–244.
Selvi, S., Edah, M.A., Nazni, W.A., Lee, H.L., Tyagi, B.K., Sofian-Azirum, M., Azahari, A.H., 2010. Insecticide susceptibility and resistance development in malathion selected *Aedes albopictus (Skuse)*. Trop. Biomed. 27 (3), 534–550.
Sun, Y.P., Johnson, E.R., 1960. Analysis of joint action of insecticides against house flies. J. Eco. Entomol 53, 887–892.
Thangam, T.S., Kathiresan, K., 1990. Synergistic effects of insecticides with plant extracts on mosquito larvae. J. Trop. Biomed. 7, 135–137.
WHO, 2005. Guidelines for laboratory and field testing of mosquito larvicides. WHO Geneva 13, WHO_CDS_WHOPES_GCDPP_2005.