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Research Article

Development of bioformulations using plant extracts for the control of dengue vector, *Aedes aegypti*

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Abstract
Dengue fever is a life-threatening illness in humans caused by the dengue virus belonging to Flaviviridae, affecting mainly in tropical and subtropical countries. The spread of this disease is through the dengue vector *Aedes aegypti*. Development of biodegradable, environmentally safe, low-cost larvicides is essential to overcome the resurgence effects of chemical insecticides. The present study included the development of bioformulations from larvicidal polar solvent extracts of the plant leaves. The solvents used for the extractions were ethyl acetate for *Aloe vera* (A), ethanol for *Carica papaya* (B), and methanol for *Parthenium hysterophorous* (C). Bioformulations in different combinations like A, B, C, A+B, B+C, C+A, and A+B+C with the concentrations of 50ppm, 100ppm, 150ppm, 200ppm, 250ppm and 300ppm were tested against the IVth instar larvae of *Aedes aegypti* and the results were recorded for every 24h 48h and 72h . The bioformulation extracts B+C (300ppm) showed 90 percent mortality of larvae after 72 h of treatment. It was concluded that the larvae's mortality was caused by the presence of bioactive compounds of B+C extracts. This formulation can be considered an effective biopesticide for the dengue vector *Ae. aegypti* IVth instar larvae.

Keywords: *Aedes aegypti, Aloe vera, Carica papaya,* Dengue*, Parthenium hysterophorous*

INTRODUCTION

World Health Organization has reported the global prevalence of dengue fever as an endemic disease in more than 100 countries in Africa, America, South-East Asia and Western Pacific. Worldwide distribution of dengue infection per year estimated currently by WHO ranges from 390 million. An estimated 96 million people with severe dengue require hospitalization each year, a large proportion of whom are children. About 3.5% of the mortality rate was reported (WHO 2023).

The dengue virus, a member of the Flaviviridae family and is responsible for this reemerging life-threatening disease in humans, mostly affects tropical and subtropical nations. Four separate, closely related serotypes of the dengue virus-DENV1, DENV2, DENV3, DENV4 and DENV 5 - cause infection. Due to a genetic similarity of 60–70% among the dengue serotypes, infection with one of them confers lifetime protection against that particular serotype and only transient immunity to the other serotypes. Severe dengue is more likely to occur if another serotype infects later (Lalithambika *et al*., 2023).

Aedes spp. is a daytime feeder whose peak biting periods are early in the morning and evening before dusk. The first symptom in dengue-infected people develops within 4-5 days or, at most 12 days (WHO, 2023). Closets, shadowy areas inside, and chilly, gloomy areas are where *Aedes* mosquitoes are found. Instead of a frigid climate, they want a warmer one. They often reside in and around the homes

where their eggs hatch for the entirety of their lives.

The female *Ae. aegypti* lays eggs on objects that retain water, such as containers, home water storage tanks, tyres, jars, and other waste deposit containers filled with rainwater. *Ae. aegypti's* propensity to feed on many human blood sources raises the likelihood of a dengue outbreak. To complete a gonotrophic cycle, the highly anthropophilic *Ae. aegypti* get more blood from feeding with multiple individuals (Rezza 2012). While *Ae. albopictus* and *Ae. polynesiensis* are endemic species in Asia found in natural habitats like tree holes, rock pools, and leaf litter, *Ae. aegypti* is the most significant vector distributed in several endemic countries. It is adapted to breed around human dwellings and prefers to lay its eggs in unpolluted water in artificial man-made containers (Gratz *et al*., 2008). Both rural and urban environments support the survival of the *Ae. albopictus* species in Europe's milder temperate regions. Controlling dengue and other mosquito-borne diseases is getting harder because mosquitoes are becoming resistant to the chemical insecticides that are now being utilised (Lalithambika and Vani, 2016). This has necessitated the search and development of biodegradable, environmentally safe, low-cost larvicides from natural sources for killing larvae of mosquitoes (Chakkaravarthy, 2011). Biopesticides include microbial organisms, nematodes, secondary metabolites from plants and microorganisms, insect pheromones, genes, insect predators and parasites received much attention for being environmentally friendly (Copping and Menn, 2000). It was understood that *Carica papaya* juice has been attracted worldwide for the control of dengue fever. The leaves of *C. papaya* contains alkaloids, flavinoids and phenolic acids and has high medicinal value (Teh *et al*., 2022). Total flavinoids can be isolated using ethlyacetate extract from the plants *C. papaya* (Pujiastuti and Andreana, 2022) and based on the various medicinal properties of the compounds present in the above plants, it was chosen for the study. It has been reported that *Aloe vera* has potential anti dengue compounds against Dengue virus, which was proved by docking studies against dengue virus (Mohsin and Bhandari, 2022).

Parthenium hysterophorous is the seventh most toxic weed in India. Still, various researches proved that there are bioactive compounds, anti-oxidant properties and more over the compounds from this plants have phytochemical properties. Jaiswal et al. (2022a) studied the phytochemical properties of *P. hysterophorous* and identified its ethanopharmacologicals implications against cancer, HIV-1 infection and immunological disorders. Hence, the present study aimed to include *A. vera, C. papaya* and *P. hysterophor* to develop bio formulations for the control of *Ae. aegypti* IVth instar larvae.

MATERIALS AND METHODS

Collection of plant species

The plant leaves of *A. vera, C. papaya* and *P. hysterophorous* were collected from in and around Karunya Nagar, Coimbatore. The identification of the plants was made in the Botanical Survey of India, Coimbatore (Identification number: BSI/SRC/5/23/2016/Tech.455).

Culturing of Dengue Vector and its maintenance

The *Ae. aegypti* mosquito eggs were collected from the Indian Council of Medical Research Madurai, Tamilnadu. The eggs were allowed to hatch in tap water. They were maintained at room temperature and the larvae were fed with yeast and pedigree biscuits. The relative humidity of 70% was maintained. The third and fourth instar larval stages of mosquitoes were taken for the bioassay process.

Extraction of solvents from plants

Ethanol, methanol and ethyl acetate were obtained from Sigma Aldrich. The collected leaves of the plants were washed with tap water and cut into pieces, then dried into room temperature. The plant materials were maintained at proper conditions to avoid contamination. The dried plant leaves were ground, made into powder, and stored at room temperature. Solvent extraction was performed as per the protocol (Jafari *et al*., 2021). The solvent extract obtained was slowly evaporated at 40°C and the concentrated extract was diluted with Dimethylsulfoxide (DMSO) at concentrations 50ppm, 100ppm, 150 ppm 200 ppm, 250 ppm and 300ppm were used in the study for the larvicidal activity against IVth instar larvae of *Ae. aegypti.*

Ethyl acetate-*Aloe vera*-A, Ethanol-*Carica papaya*-B, Methanol- *Parthenium hysterophorous*- C. The above solvents were used to extract secondary metabolites from plants due to the presence of bioactive compounds present in the extracts and by the previous research work done. Ethyl acetate was used for *A. vera* because Alonin A and B are secondary metabolites . The extract is rich in antioxidant properties. The *C. papaya's* ethanol extract was chosen because the phytochemicals such as alkaloids, saponins, and terpenoids were present in the *C. papaya* leaves (Igimoh, 2022). Jaiswal (2022a) reported the presence of medicinal compounds such as oils, polyphenols, flavonoids, flavones, alkaloids, terpenes pseudogauaianolides, and histamines in the methanolic extract of *P. hysterophorous* and was effective against cancer, HIV-1 infection, and immunological disorders. So methanolic extract of *P. hysterophorous* was used in present study.

Bioassay

Different concentrations 50ppm, 100ppm,150 ppm, 200ppm, 250 ppm and 300ppm of the solvent extracts

in the combinations of A, B, C, A+B, B+C, C+A, A+B+C were used for the bioassay against the IVth instar larvae of *Ae. aegypti*. The extracts of different combinations were added to the disposable cup containing 25ml of chlorine-free tap water and 10 *Ae. aegypti* larvae were added to each bioassay cup. Bioassay cup containing only chlorine-free tap water was used as control. Yeast and dog biscuit (1:4 by weight) were served as larval food sterilized by autoclaving at 121°C for 15 min and fine powder of the larval food was added to all bioassay cups. The mortality percentage was scored after 24h, 48h, and 72h of exposure and corrected for control mortality, if any, using Abbott's formula (Padmanabhan *et al*., 2005, Abbott, 1925). LC 50 was calculated using probit analysis (Postelnicu, 2014).

The combinations of plant species used in the study were :

Ethyl acetate extract of *Aloe vera*: A
Ethanolic extract of *Carica Papaya*: B
Methanol *Parthenium hysterophorous*: C
Aloe vera + *Carica Papaya*- A+B
Carica Papaya + *Parthenium hysterophorous*: B+C
Aloe vera + *Carica Papaya* + *Parthenium hysterophorous* : A+B+C

The larvicidal against IVth Instar larvae of *Ae. aegypti* was observed every 24,48, and 72 hrs of exposure.

The percentage of mortality was calculated by the formula:

(Number of larva dead /Number of larvae) x 100 Eq.1

Statistical analysis

Statistical analyses were performed with Graph Pad Prism 4 software, using two-way ANOVA followed by Tukey Kramer multiple comparison tests. All the values are expressed as Mean±Standard Deviation (SD). All the experiments were performed in triplicates. The significance was set at $p < 0.05$ (Guedes *et al*., 2014).

RESULTS AND DISCUSSION

Bioassay of the extracts against IVth instar larvae of *Ae. aegypti* at 24h duration of exposure

The mortality rate was observed in the larvae after 24h of treatment with the solvent extracts of the combinations. The mortality rate of the larvae was from 16 to 33.3 %. Maximum mortality per cent of 33.3 was recorded at 300 pm in A+B+C combination (Table 1). No significant mortality rate was recorded after 24h treatment of exposure. The present results were not on par with Sharawi (2023) results which reported that the aqueous plant extracts of *E. cardamomum* and M. chamomilla showed 60% mortality after 24h of treatment. This may be due to the effect of the bioactive compounds of the plants.

Riaz, (2023) analyzed the acetylcholinesterase activity of *Ae. aegypti* and *Musca domestica* adults using *Calotropis procera*, *Eucalyptus globulus* plant extracts and *Mentha spicata*. In this study the bioassay of 4th instar larvae recoded maximum of 33% mortality when treated with 300PM of A+B+C formulation. The mortality rate after 24h was 70% and the enzyme activity of the treated groups decilined after treatment of the extracts at a concentration of 0.6mg/ml. From this, it is understood that the duration of the treatment was to be increased for the effectiveness of the formulations developed.

Bioassay of the extracts against IVth instar larvae of *Ae. aegypti* at 48h duration of exposure

Table 2 represents the per cent mortality of IVth Instar larvae of *Ae.aegypti* when treated with different concentrations of the plant extracts. It was observed that maximum mortality was recorded as 43 % cent when treated with A+B+C extracts at 300 ppm concentration and 46 % in B+C. At 300 ppm, the range of mortality was from 36 to 46 percent. Samuel et al., 2023 reported that the biocontrol efficacy of apigenin from Anisomelis indica (L.) against Culex mosquito was 72% after 48h. The present study deviated from their research. This may be due to the secondary metabolites of *Anisomelis indica* plant. In the present study, the mortality was not maximum in the individual plant extracts (the A,B and C) when compared to the combinations such as A+B, B+C, A+B+C. Leandro et al., (2023) observed the effect of *Himatanthus drasticus* IVth instar larvae using various 100, 200, 300, 400 and 500 ppm concentrations. They reported that the ethanolic extract of *H. drasticus* (500 ppm) showed 66.7% mortality in *Ae. aegypti* larvae. In out study it is observed that, after 48h of the treatment of B+C formulation of 300ppm, recorded 46% mortality in *Ae. aegypi* larvae. From this, it can be concluded that the present results were not on par with their study. Further, the treatment of exposure was extended up to 72h.

Bioassay of the extracts against IVth instar larvae of *Ae. aegypti* at 72h duration of exposure

Maximum mortality of the *Ae. aegypti* larvae were recorded after 72h was 90 per cent when treated with B+C at 300 pm concentration. Minimum mortality was recorded in the A, B, C. The combination of B and C at 300 PPM concentration showed a maximum mortality rate (Table 3). LC 50 value (B+C for 72 hours) was 79 ppm and the LC 50 value (A+B+C for 72 h) was 63.09 ppm.

The present study revealed that the plants' extracts played an important role in the biological control of the IVth instar Larvae of *Ae.*aegypti larvae. Plant extracts can be used as biocontrol agents and as a substitute for synthetic insecticides which could diminish the production cost and environmental pollution (Soares *et al*., 2022). Repeated application of chemical insecticides

Table 1. Per cent mortality of IVth instar larvae when treated with different concentrations of the plant extracts after 24h of exposure

Plant Extracts	Concentrations of the plant extracts (ppm)					
	50	100	150	200	250	300
	Mortality (%)					
A	30±0.5	6±0.57	13±0**	20.3±0.57**	23±0.57**	23±0.57**
B	6±0.57	10±0*	23.3±0.57	26±0.57**	30±0**	30±0**
C	10±0*	16±0.57**	20±0	20±0**	23±0.57**	26.6±0.57**
A+B	13±0.57*	16±0.57**	20±0**	23±0.57**	26±0.47**	33±0.57**
B+C	16±0.57**	20±0**	23±0.47**	26.6±0.47**	26±0.57**	30±0**
C+A	13±0.57*	16±0.57**	20±0**	23±0.37**	26±0.47**	30±0**
A+B+C	16±0.57**	20±0**	23±0.27**	26±0.47**	30±0**	33±0**

Ethyl acetate extract of *Aloe vera*: A, Ethanolic extract of *Carica Papaya*: B, Methanol *parthenium hysterophorous*: C, *Aloe vera* + *Carica Papaya*- A+B, *Carica Papaya* + *Parthenium hysterophorous*: B+C, *Aloe vera* + *Carica Papaya* + *Parthenium hysterophorous* : A+B+C. The Values are expressed in mean ±SD. Significant * at $p \leq 0.05$; ** $p \leq 0.01$; highly significant*** $p \leq 0.001$; not significant^{ns} $p \geq 0.05$

Table 2. Per cent mortality of IVth instar larvae of *Aedes aegypti* when treated with different concentrations of the plant extracts after 48 h of exposure

Plant Extracts	Concentrations of the plant extracts (ppm)					
	50ppm	100ppm	150ppm	200ppm	250ppm	300ppm
A	23±0.5**	30±0.0**	33±0.7**	33±0.45**	33±0.51**	36±0.24**
B	20±0.0**	23±0.24**	30±0.23**	33±0.23**	36±0.48**	40±0.35**
C	23±0.5**	26±0.46**	33±0.46**	33±0.34**	40±0.23**	43±0.37**
A+B	23±0.57**	23±0.56**	30±0.24**	33±0.43**	36±0.48**	40±1.54**
B+C	26±0.57**	26±0.52**	30±0.13**	36±0.36**	40±0.53**	46±0.24**
C+A	10±0.57*	23±0.46**	23±0.57**	33±0.43**	33±0.43**	36±0.26**
A+B+C	26±0.57**	30±0.12**	33±0.57**	36±0.53**	40±0.25**	43±0.38**

Ethyl acetate extract of *Aloe vera*: A, Ethanolic extract of *Carica Papaya*: B, Methanol *parthenium hysterophorous*: C, *Aloe vera* + *Carica Papaya*- A+B, *Carica Papaya* + *Parthenium hysterophorous*: B+C, *Aloe vera* + *Carica Papaya* + *Parthenium hysterophorous* : A+B+C.The Values are expressed in mean ±SD. Significant * at $p \leq 0.05$; ** $p \leq 0.01$; highly significant*** $p \leq 0.001$; not significant^{ns} $p \geq 0.05$.

leads to the development of resistance in the *Ae. aegypti* larvae may be due to the knockdown of the gene which encodes for the target binding site of the insecticides and the upregulation of detoxifying enzymes in mosquitos (Priya et al., 2023).

The selection of these solvents were done based on the work of Anitha and Geethapriya (2012). They reported that the mortality per cent increases in the *Ae.aegypti* larvae when treated with polar solvents such as chloroform and ethyl acetate. The mortality was due to bioactive compounds in the extracts tested.

It is reported that the non-polar solvents gave moderate results in larvicidal activities. However, polar solvents provided better results only in higher concentrations (Anitha and Geethapriya, 2012). Polar solvents such as chloroform and ethyl acetate extracts are used to extract steroids and alkaloids. The strong polar solvent absolute alcohol is used to extract the biochemicals with higher molecular weights, such as proteins and glycans. However, significant results were obtained using polar solvents such as ethanol, ethyl acetate and methanol. Subramaniam *et al*. (2012) reported that saponin, tannic acid, lignin, minerals, enzymes, sugars, anthraxquinone, phenolic compounds in the petroleum ether leaf extract of *Aloe vera* plant caused the 70 % mortality of *Ae. aegyptI* after 72 h of treatment. Destrianto et al. (2023) worked with the ethanolic leaf extracts of *Aloe vera* and *Carica papaya recorded* 75% mortality in *Ae. aegypti* larvae. The B+C (*Carica Papaya* + *Parthenium hysterophorous*) formulation in present study resulted in 90% mortality after 72 h (300 PPM) of exposure in the IVth Instar larvae of *Ae. aegypti* when compared to their work. This may be due to bioactive molecules in the B+ C formulations. The increased mortality rate of 90 per cent was due to the B+C combinations with a concentration of 300 ppm after 72h of treatment. The *C. papaya* extract was obtained using ethanol and methanolic extracts of *Parthenium hysterophorus*

Table 3. Per cent mortality of IVth instar larvae of *Aedes aegypti* when treated with different concentrations of the plant extracts after 72 h of exposure

	Concentrations of the plant extracts (ppm)					
Plant Extracts	**50**	**100**	**150**	**200**	**250**	**300**
A	36±0.27**	33±0.43**	36±0.33**	36±0.22**	43±0.25**	46±0.21**
B	26±0.53**	33±0.24**	33±0.57**	36±0.35**	53±0.57**	66±0.44**
C	33±0.27**	36±0.34**	43±0.45**	43±1.36**	56±7**	70±0.13**
A+B	36±0.31**	40±0.37**	46±0.48**	46±0.35**	66±0.57**	73±0.34**
B+C	43±0.33**	46±0.43**	56±0.35**	63±1.23**	76±0.57**	90±27**
C+A	33±0.45*	36±0.23**	53±0.54**	56±0.25**	73±0.43**	83±0.35**
A+B+C	46±0.56**	50±0.34**	56±0.43**	66±0.23**	73±0.46**	86±0.54**

Ethyl acetate extract of *Aloe vera*: A, Ethanolic extract of *Carica Papaya*: B, Methanol *parthenium hysterophorous*: C, *Aloe vera* + *Carica Papaya*- A+B, *Carica Papaya* + *Parthenium hysterophorous*: B+C, *Aloe vera* + *Carica Papaya* + *Parthenium hysterophorous* : A+B+C. The Values are expressed in mean ±SD. Significant * at $p \leq 0.05$; ** $p \leq 0.01$; highly significant*** $p \leq 0.001$; not significant^{ns} $p \geq 0.05$

showed significant results. Whereas the combinations A+B+C recorded 86 per cent mortality. This may be due to saponin, flavonoid, and triterpenoids in the C. papaya. Active enzymes lignin and tri terpenoids of *P. hysterophorus* may be the reason for the larvicidal activity. The LC 50 of B+C was 79.32 ppm, whereas A+B+C was 63.09. This result deviated from the previous work of Ali et al. (2013) who reported that the sea weed's solvent extracts showed decreased mortality rate of *Ae. aegypti* and *Culex quinquefasciatus*. A study on the 15 species of plant extracts showed 50 per cent mortality at 250PPM (Kumar *et al*., 2012) but in the present research, 86% per cent mortality was obtained in the A+B+C in 300 PPM. The results were better when compared to the above study. The recent report on the larvicidal activity of mosquito vectors of avian plasmodium using *Ruta graveolens, R. montana and Artemisia absinthium* recorded LC 50 value of 199.5 PPM (Bouabida and Dris, 2022). Compared to this study, the present results showed better with LC 50 value of 79.32 ppm in B+C combination. A study on the herbal formulation for preparation of facial creams reported that *C. papaya* has antibacterial, antitumor and antioxidant properties because of saponins and alkaloids (Okafo *et al*., 2020), and it is rich in flavonoids (Endra and Andreana, 2022). *C. papaya* is Igimoh et al. (2022) recorded 76.7 %, mortality in Ae. aegypti after 72h when treated 1000 PPM of ethanolic extract of C. papaya. It was interesting to note that 300 ppm of the ethanolic extract of C. papaya (C) showed 70 % mortality in present study. The mortality rate increased to 90% in the formulation of (B+C) (LC 50 value of 79.32 ppm) ethanolic extract of *C. papaya* and methanolic extract of *P. hysterpphorus* at 300 PPM after 72 h of treatment, respectively. Jaiswal *et al*. (2022b) reported that *P. hysterpphorus* had potential medicinal properties because of the presence of oils, polyphenols, flavones, flavonoids, alkaloids, terpenes, pseudoguaoanolids and histamines. From the present study, it was understood that the mortality per cent increased in *Ae. aegypsti* larvae only because of the combinations of plant extracts than treated with individual plant extracts.

Conclusion

The present study concluded that the formulations of the bioactive compounds of B+C (ethanolic extract of *C. papaya* and methanolic extract of *P. hysterophorous*) were an effective biocontrol agents for the control of *Ae. aegypti* IVth instar larvae. This combination of bioformulations can be used for the biocontrol of dengue vector *Ae. aegypti* larvae and is cost-effective. Further identification of the compounds is necessary to understand the mode of action of B+C combination of the extract.

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Conflict of interest

The authors declare that they have no conflict of interest.

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