D-6265
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RESEARCH ARTICLE Agricultural Science Digest

Evaluating the Insecticidal Potential of Selected Plant Extracts Found in Upper Brahmaputra Valley against the Dengue Vector *Aedes aegypti* Linn. (Dipter: Culicidae)

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10.18805/ag.D-6265

ABSTRACT

Background: Mosquitoes act as vector for many pathogens that spread many diseases to human which causes millions of death every year. Dengue is one of the viral diseases transmitted by *Aedes aegypti*, with the fastest rate of growth across the globe. Control of mosquito relies primarily on synthetic insecticide. But synthetic insecticides have led us to emergence of insecticide resistance in mosquito and increase of their population over the years. Hence, in the past few years, major advances have been made in the area of biological mosquito control using bioactive compounds of plant secondary metabolites.

Methods: The present study assessed the larvicidal activity of different extracts of three plants *Polygonum hydropiper* L., *Litsea salicifolia* Roxb. (L.) and *Clerodendrum indicum* (L.) Kuntze found in upper Brahmaputra valley against larvae of *Aedes aegypti*. Petroleum ether, chloroform and methanolic extracts were prepared to check the mortality after 12 and 24 hours of exposure in all the extracts. Further, the adult inhibition assay was performed to check the growth period of the species with the most effective plant extract.

Result: The highest mortality was recorded in the petroleum ether extract of *P. hydropiper* with LC_{50} value 39.55 ppm and lowest mortality in chloroform extract of *L. salicifolia* with LC_{50} value 148.21 ppm after 24 hours of exposure. Further, emergence inhibition assay was conducted with petroleum ether extract of *P. hydropiper* (most effective extract). Present study found that petroleum ether extract of *P. hydropiper* prolongs the developmental stages while significantly reduces the adult life span of *A. aegypti*. Hence, *P. hydropiper* can be used effectively as potential larvicide against *A. aegypti* larvae.

Key words: *Aedes aegypti*, Dengue vector, Larvicide, Petroleum ether, *Polygonum hydropiper*.

INTRODUCTION

Mosquitoes act as vector for many pathogens. In the global health community, vector borne diseases (VBDs) are a major issue and the concerned about VBDs are also increasing drastically, especially in tropical and subtropical areas. Annually, more than 1 million people die from VBDs each year, making up more than 17% of all infectious illnesses (WHO, 2020).

Dengue is one of the viral diseases with the fastest rate of growth in the globe. With a 30-fold rise in occurrence over the previous 50 years, it is widely spread in tropical and sub-tropical regions. Globally, there are 3.9 billion people who are at risk of dengue virus infection in 90 countries (WHO, 2024). It is spread by mosquito species of genus *Aedes* from human to human. The primary vector of dengue is *Aedes aegypti* in India. Once mosquito is infected, the virus takes 7-8 days to develop in mosquito's body. Mosquito remains infected for life, transmitting the virus to susceptible individuals by probing (WHO, 2024). The vectors of dengue, *A. aegypti* and *A. albopictus* are very efficient vector which can build up high transmission potential because of its day time biting habitat where a female is oblighted to make repeated biting attempts for taking a single blood meal. There are 8 fold increases of dengue cases over the last two decades from 505,430 cases in 2000 to over 4.2 million in 2019 with increasing trend of death (WHO, 2020). The dengue hemorrhagic fever (DHF) and dengue shock syndromes (DSS) could be fatal when left untreated at proper time. At present, there is no specific antiviral treatment against both JEV and Dengue and implementation of vector control interventions is the most suitable measure to control and further spread of those diseases (WHO, 2020; NVBDCP, 2022).

The use of plant products as insecticides dates back to more than 3000 years. Europe used *Chrysanthemum* spp. against louses and house flies. A total 429 species of

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How to cite this article: Konwar, D., Singh, M.K. and Bordoloi, R. (2025). Evaluating the Insecticidal Potential of Selected Plant Extracts Found in Upper Brahmaputra Valley against the Dengue Vector *Aedes aegypti* Linn. (Dipter: Culicidae). Agricultural Science Digest. 1-8. doi: 10.18805/ag.D-6265.

Submitted: 12-12-2024 **Accepted:** 24-02-2025 **Online:** 13-05-2025

plants have been studied against mosquito as insecticides across the globe. The importance of plant based insecticides is attributed to efficacy, varied mode of action, biodegradability under natural environments and low residual activity in environment (Pavela *et al.,* 2019). A large number of plant essential oils may be potential sources of mosquito larvicides, since they constitute a rich source of bioactive components. The development of technologies related to isolation and stabilization of plant products have increased the interest on botanicals as insecticides (Pavela, 2016). Many different types of plants, including huge trees, shrubs and herbs have been currently drawing interests for their insecticidal properties such as alkaloids, steroids, terpenes, essential oils and phenolics (Ghosh *et al.,* 2012). The pesticidal qualities function as anti-feedants, attractants, repellents, larvicides or adulticides that affect the olfactory receptor neurons by modifying or blocking its response in the host-seeking behavior of mosquitoes. Few of them cause destruction of epithelial cells in the midgut of mosquitoes and affect the process of metamorphosis (Shaalan *et al.,* 2005; Sharma *et al.,* 2006; Tyagi, 2016; Boulkenafet *et al.,* 2023). IGRs disrupt normal growth and development of insects (Abreu *et al.,* 2008). As molting is the biological process of growth and development of insects and it is controlled by hormonal processes, interference in homeostasis would result in disruption in developments leading to abnormal growth in target insect (Kamboj and Saluja, 2008). Another key factor of any control method lies in the reproductive potential and survivability of mosquitos' subsequent generations when treated with insecticides.

In the present study, an attempt has been made to investigate the larvicidal activity of petroleum ether, chloroform and methanol extracts of three plant species belonging to varied taxonomic group, *Polygonum hydropiper* (L.), *Litsea salicifolia* Roxb. (L.) and *Clerodendrum indicum* (L.) Kuntze against *Aedes aegypti* larvae.

MATERIALS AND METHODS

All laboratory experiments were performed in Department of Life-Sciences, Dibrugarh University, Assam during the year 2021-22.

Preparation of plant extract

Based on their local availability and traditional use with known medicinal properties, three plants *Polygonum hydropiper* (L.), *Litsea salicifolia* Roxb. (L.) and *Clerodendrum indicum* (L.) Kuntze were selected for the study (Fig 1, 2 and 3). The plants were collected from different sites of Sivasagar district of Upper Assam. The plant parts were washed, cut into small pieces, shade dried for 5-7 days and then coarsely powdered. The dried powder was extracted with petroleum ether, chloroform and methanol successively with Soxhlet apparatus (Jaglan *et al.,* 1997; Hari and Mathew, 2018). The solvent from each extract was removed under reduced pressure and extracts were dried under vacuum. There were 3 different extracts prepared for each plant. All dried crude extracts were separately re-dissolved in acetone to prepare a stock solution and stored in 4°C for further experiments.

Rearing of mosquito

Eggs of *Aedes aegypti* were collected from the Regional Medical Research Centre (RMRC), Dibrugarh, Assam. The eggs were allowed to hatch in tap water in a rearing plastic tray of 45 cm × 30 cm × 10 cm dimensions. It was covered with mosquito net. For each treatment set, there were four replicates used for each treatment (n=25). The newly hatched larvae were fed with 40% brewer's yeast and dog biscuits (1:1).

Bioassay

Larvicidal bioassay was performed using 4th instars larvae following WHO guidelines (2005). The stock solution of plant extract was serially diluted to obtain different concentrations for bioassay. To the treatment set, varying

Fig 1: *Polygonum hydropiper.*

Fig 2: *Litsea salicifolia.*

Fig 3: *Clerodendrum indicum.*

concentrations of plant extracts *viz.*, 1000 ppm, 500 ppm, 250 ppm, 125 ppm and 62.5 ppm, 31.25 and 15.6 were prepared from the stock solution (Devi and Bora, 2017). Four replica containing 25 larvae were prepared for each concentration and mortality was recorded after 12 hours and 24 hours of treatment respectively. After 12 and 24 hours of treatment both dead and moribund larvae were recorded. Control tests were conducted in acetone (negative) and Deltamethrin (positive). Mortality was corrected using Abbott's formula (Abbott, 1925) if mortality in control set were between 5-20%.

The per cent mortality was calculated using the following (WHO, 2005).

$$\text{Mortality (\%)} = \frac{\text{Total number of dead larvae}}{\text{Total number of treated larvae}} \times 100$$

Emergence inhibition

Further the most effective plant extract have been collected and bio-assayed to check the growth and development of mosquitoes. The 4th instar larvae were exposed for 24 hours to the lethal concentrations of LC_{50}, LC_{30} and LC_{10} (Shaalan *et al.,* 2005) to the most effective extract of previous bioassay. After 24 hours larvae were transferred to normal water and allowed to develop until adults emerge out. The mortality of 4th instar larvae was recorded after 24 hours of interval up to pupal stage. The treated insects that were failed to emerge out as adult were counted as inhibited insects. The growth period was counted as day/days between larval molting to pupal stage and pupal to adult. The mortality of adults was checked at an interval of 24 hours. The average adult life span was recorded in days to study the adult longevity.

The percentage of emergence inhibition (EI%) was calculated as (WHO, 2005).

$$\text{EI\%} = 100 - \left(\frac{\text{Insect emerged after treatment}}{\text{Insect emerged in control}}\right) \times 100$$

The Growth index (GI) was calculated as (Saxena and Sumithra, 1985)

$$\text{GI} = \frac{\text{Adult emergence (\%)}}{\text{Average developmental period (days)}}$$

Statistical analysis

All data were analyzed using latest MS excel and SPSS version 20. The data of mortality was subjected to Probit analysis to calculate the lethal concentrations (LC_{50} and LC_{90}) causing 50% and 90% of mortality after 12 and 24 hours of exposure (Finny, 1971). Similarly EI_{50} (the dose that caused 50% emergence inhibition) was calculated.

RESULTS AND DISCUSSION

The efficacies of different plant extracts against 4th stage larvae of *A. aegypti* were recorded (Table 1). The results revealed 100% mortality of larva in 1000 ppm and 500 ppm concentrations in all extracts of all three plants after 12 and 24 hours of exposure. The petroleum ether extract of *P. hydropiper* caused 5% and 12% mortality at concentration 15.6 ppm, chloroform extract showed 4% and 15% mortality at 31.3 ppm and methanolic extract showed 11% and 24% mortality at concentration 31.3 ppm after 12 and 24 hours

Table 1: Mortality of *Aedes aegypti* in different concentrations of plant extracts.

Plant extracts	Conc.	Petroleum ether		Chloroform		Methanol	
	(ppm)	12 hours	24 hours	12 hours	24 hours	12 hours	24 hours
Polygonum hydropiper	1000	25±0.00	25±0.00	25±0.00	25±0.00	25±0.00	25±0.00
	500	25±0.00	25±0.00	25±0.00	25±0.00	25±0.00	25±0.00
	250	25±0.00	25±0.00	25±0.00	25±0.00	25±0.00	25±0.00
	125	17±1.83	25±0.00	12±0.82	20±1.30	10±1.50	22±1.30
	62.5	11±1.5	17±2.35	9±0.82	15±1.30	7±0.96	12±1.00
	31.25	4±1.26	8±0.84	1±0.82	3±0.55	2±0.50	5±0.84
	15.6	0	3±0.84	0	0	0	1±1.10
Litsea salicifolia	1000	25±0.00	25±0.00	25±0.00	25±0.00	25±0.00	25±0.00
	500	25±0.00	25±0.00	25±0.00	25±0.00	25±0.00	25±0.00
	250	20±0.82	25±0.00	15±0.50	18±0.58	16±0.50	22±0.50
	125	8±1.00	10±0.96	2±0.50	7±0.82	3±0.82	11±0.58
	62.5	0	1±0.71	0	1±0.50	0	2±0.58
Clerodendrum indicum	1000	25±0.00	25±0.00	25±0.00	25±0.00	25±0.00	25±0.00
	500	25±0.00	25±0.00	25±0.00	25±0.00	25±0.00	25±0.00
	250	22±1.41	25±0.00	25±0.00	25±0.00	20±2.45	25±0.00
	125	7±0.82	12±1.82	9±2.38	13±1.41	4±1.73	11±1.70
	62.5	4±0.96	6±1.30	6±0.96	7±1.30	5±0.50	5±0.96
	31.25	0	1±0.57	0	2±0.50	0	1±0.00

Note: Each mean (+SD) represents four replicates of 25 larvae.

of exposure. Similarly, the petroleum ether extract of *L. salicifolia* caused 34% and 6% mortality at concentration 125 ppm and 62.5 ppm, chloroform extract showed 11% and 5% mortality at 125 ppm and 62.5 ppm and methanolic extract showed 16% and 10% mortality at 125 ppm and 62.6 ppm after 12 and 24 hours of treatment. Likewise, the results of *C. indicum* showed that petroleum ether extract caused 21% and 9% mortality at 62.50 ppm and 31.25 ppm, chloroform extract caused 27% and 9% mortality at 62.50 ppm and 31.25 ppm and methanolic extract of *C. indicum* showed 21% and 4% mortality at 62.50 ppm and 31.25 ppm after 12 and 24 hours of treatment respectively. The highest (12%) mortality of petroleum ether extract of *P. hydropiper* was recorded at the lowest concentration of 15.6 ppm and it has been increased upto 100% at concentration 125 ppm after 24 hours of treatment. However, chloroform and methanolic extracts of *P. hydropiper* were found to be the most effective compared to its respective extract of *C. indicum* and *L. salicifolia.* The commercial and synthetic plant based insecticide Deltamethrin was compared as positive control where 100% mortality was found in even in the lowest concentration same as experimental concentration. Acetone was used as negative control and zero mortality was recorded in negative control. Extracts from more than 429 plants have been evaluated earlier as insecticide against mosquito. Essential oils extracted from neem (Batra *et al.,* 1998) and *T. patula* (Dharmagadda *et al.,* 2005) was effective on the larvae of *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*. Amusan *et al.* (2005) studied the larvicidal efficacy of ethanolic extracts and the petroleum ether extract of *C. sinensis* on *A. aegypti*. The insecticidal properties of *Z. nitidum* stem bark was reported earlier (Bhattacharya and Zaman, 2009; Gogoi and Bora, 2012) and also found excellent result in a study by Devi and Bora (2017) against *A. aegypti.* Sonowal and Rahman (2010) reported the larvicidal activity of leaf extracts of *P. hydropiper* against *C. quinquefasciatus.* The larvicidal potential of many plants like *Pongamia pinnata, Azadirachta indica, Croton tiglium, Cascabela thevetia, Ricinus communis, Datura stramonium, Jatropha curcas, Pedilanthus tithymaloides, Phyllanthus amarus, Euphorbia hirta, Euphorbia tirucalli, Stichopus horrens* and *Lantana camara* were already studied against *A. aegypti* (Rahuman *et al.,* 2008; Borah *et al.,* 2012; Rajasekaran and Duraikannan, 2012; Hari and Mathew, 2018; Sharawi, 2024). Noosidum (2014) mixed two essential oils extracted from *L. cubeba* and *L. salicifolia* and the result showed potential repellent activity against *A. aegypti*. Patil (2014) studied insecticidal properties of organic solvent extracts of *C. inerme* leaves against larval stages of *A. aegypti* and *C. quinquefasciatus* where hexane extract was reported as effective one. Extract of *Zingiber officinale* showed excellent results in controlling different stages of *A. albopictus* (Nasir *et al.,* 2017).

Probit analyses of all extracts were carried out in order to determine the respective LC_{50} and LC_{90} values after 12 and 24 hours of treatment (Table 2, 3, 4). The LC_{50} and LC_{90} in *P. hydropiper* for petroleum ether extract were recorded as 39.55 ppm and 77.25 ppm whereas for chloroform extract 56.29 ppm and 118.23 ppm and for

Table 2: Larvicidal efficacy of *Polygonum hydropiper* on *Aedes aegypti* (after 24 hours).

Solvent	LC_{50} (ppm) at 95% CI (LL-UL)	LC_{90} (ppm) at 95% CI (LL-UL)	Chi-square	dfb	Sig.	Y= a+bX
Petroleum ether	39.55 (36.21-43.23)	77.25 (68.35-90.22)	2.53	7	0.925	Y=-6.7+4.2x
Chloroform	56.29 (51.25-61.76)	118.23 (104.20-138.39)	9.15	7	0.242	Y=-7.5+4.2x
Methanol	52.77 (47.82-58.27)	121.76 (106.01-144.51)	4.24	7	0.752	Y=-5.3+3.33x

Note: UL-Upper Limit; LL-Lower Limit; Sig.-Significance; df-degree of freedom.

Table 3: Larvicidal efficacy of *Litsea solicifolia* on *Aedes aegypti* (after 24 hours).

Solvent	LC_{50} (ppm) at 95% CI (LL-UL)	LC_{90} (ppm) at 95% CI (LL-UL)	Chi-square	dfb	Sig.	Y= a+bX
Petroleum ether	121.05 (109.63-133.64)	188.68 (171.49-216.21)	3.33	3	0.344	Y=-13.13+6.25x
Chloroform	162.34 (148.21-177.79)	326.55 (287.74-383.77)	3.54	3	0.315	Y=-9.28+4.3x
Methanol	128.47 (117.19-140.60)	254.32 (224.47-298.78)	0.893	3	0.827	Y=-8.78+4.3x

Note: UL-Upper Limit; LL-Lower Limit; Sig.-Significance; df-degree of freedom.

Table 4: Larvicidal efficacy of *Clerodendrum indicum* on *Aedes aegypti* (after 24 hours).

Solvent	LC_{50} (ppm) at 95% CI (LL-UL)	LC_{90} (ppm) at 95% CI (LL-UL)	Chi-square	dfb	Sig.	Y=a+bX
Petroleum ether	91.63(68.44-122.92)	203.09(145.97-393.72)	15.36	4	0.004	Y=-5.2+2.57x
Chloroform	84.83(63.23-113.01)	197.76(141.93-377.30)	14.22	4	0.007	Y=-5.5+2.85x
Methanol	98.10(71.89-135.22)	209.92(148.86-440.98)	18.09	4	0.001	Y=-7+3.5x

Note: UL-Upper Limit; LL-Lower Limit; Sig.-Significance; df-degree of freedom.

methanolic extract, it was 52.77 ppm and 106.71 ppm respectively after 24 hours of treatment. The efficacy of plant extracts were categorized into high (LC_{50}<50), moderate (LC_{50}< 500) and low (LC_{50}>500) (Cheng *et al.,* 2003). The lowest LC_{50} was calculated as 39.55 ppm in *A. aegypti* (LC_{50}<50). Hence, the insecticidal efficacy of petroleum ether extract of *P. hydropiper* can be noted as the highest during the study. The efficacy was higher than that of *Azadirachta indica* which is an effective insecticide against mosquito. Previously, the larvicidal efficacy of essential oil of leaves of *P. hydropiper* against *C. quinquefasciatus* and *A. aegypti* were reported (Maheswaran and Igcinumuthu, 2013;). A study conducted by Duraipandiyan (2006) revealed the potentiality of compound confertifolin with bactericide and fungicidal properties isolated from *P. hydropiper*. The efficacy of petroleum ether extracts of *C. occidentalis* and *O. basilicum* were reported by Kumar *et al.* (2014) and Maurya *et al.* (2009).

In present study, emergence inhibition assay was conducted by the most effective extract of our larval bioassay (petroleum ether extract of *P. hydropiper*). A total of 47% of emergence inhibition (Table 5) was recorded and EI_{50} in *A. aegypti* was recorded as 41.07 ppm (Table 6). The mean duration of immature stages in *A. aegypti* were significantly increased after treatment of petroleum ether extract of *P. hydropiper* for 24 hours. The developments of pupal and adult stages were also influenced by the treatment of petroleum ether extract of *P. hydropiper* for 24 hours. The study recorded the mortality of 4th instar larvae as 5.0±0.95, pupae as 13±0.50 and adult as 30±0.50 in *A. aegypti* after treatment of EI_{50} dose for 24 hours. The mean time duration for developmental period was 6.0±0.50 days. The mean developmental period of the immature stages of untreated larvae (untreated EI_{50}) was recorded as 5.0± 0.50 days (Table 7). The results of petroleum ether extract of *P. hydropiper* were also found significant in reducing adult life span. The adult life span of *A. aegypti* was recorded as 24.0±0.95 days in post EI_{50} treatment for 24 hours (Table 8). The growth index has decreased in treated group of *A. aegypti* (GI-8.87) in compared to untreated group (GI-10.6). Increased malformations have been recorded in pupal and adults by the presence of larval-pupal and pupal-adult intermediates and formation of unmelanised larvae and pupae. Plant extract induced changes in growth period and longevity of insects have been reported by many workers (Kraiss and Cullen, 2008; Ibanez *et al.,* 2012; Granados *et al.,* 2014; Devi and Bora, 2017; Masih and Ahmed, 2019). The normal growth and development of insects are interrupted various ways by the chemical compounds present in different plant extracts (Bede *et al.,* 1999; Divekar *et al.,* 2022). The mode of action can be the inhibition of chitin synthesis or interference with hormonal system during molting. Numerous bioactive compounds in plant extracts can affect the endocrine regulation of molting and metamorphosis and thereby act as IGRs (Kabir *et al.,* 2013). The larval bioassay tests of *A. aegypti* was carried out using 16 IGRs which resulted that the juvenile hormone (EI_{50}= 0.010-0.229 ppb; EI_{95} = 0.066-1.118 ppb) and chitin synthesis hormone (EI_{50} = 0.240-2.412 ppb; EI_{95} = 0.444-4.040 ppb) showed higher inhibition in adult *A. aegypti* emergence, blood feeding rate and fertility (Fansiri

Table 5: Per cent emergence inhibition of *Aedes aegypti* in different concentrations of *Polygonum hydropiper.*

Sub lethal concentrations	Concentration used (ppm)	Emergence inhibition (%)
LC_{50}	39.55	47%
LC_{30}	30.07	24%
LC_{10}	20.20	6%

Table 6: EI_{50} and EI_{90} concentrations of petroleum ether extract of *Polygonum hydropiper.*

Mosquito species	EI_{90} (ppm) at 95%CI (LL-UL)	EI_{50} (ppm) at 95% CI (LL-UL)	Chi square	Sig.	Y=a+bX
Aedes aegypti	73.33 (59.56-107.55)	41.07 (37.51-47.47)	0.03	0.880	Y=-10.66+5.66x

Note: UL-Upper Limit; LL-Lower Limit; Sig.-Significance; df-degree of freedom.

Table 7: Effect of EI_{50} on different stages of *Aedes aegypti* after 24 hours of treatment.

Developmental stage	*Aedes aegypti* (n=212)		
	Mortality (%)	Treated group (Mean±SE)	Control group
4th instar larvae	8%	5.0±0.95	0
Pupae	12%	13±0.50	0
Adult	57%	30±0.50	0
Total	77%	48±0.65	0

Table 8: Effect of EI_{50} on growth period and adult longevity of *Aedes aegypti.*

Mosquito species	Growth period (days)		Adult life span (days)		Growth Index (GI)	
	Treated	Control	Treated	Control	Treated	Control
Aedes aegypti	6.0±0.50	5.0±0.50	24±0.95	26±0.00	8.83	10.6

et al., 2022). In another experiment, exposure to phenolic extracts of *Z. jujuba* leaves significantly prolonged the larval duration and reduced the adult lifespan of *A. aegypti* (Devi and Bora, 2017). The ovicidal, larvicidal, adulticidal, repellent and the decrease in fecundity and fertility of *A. aegypti, A. stephensi* and *C. quinquefasciatus* were also reported by the effect of leaf extracts of *Lippia alba* and *Piper longum* (Mahanta *et al.,* 2019; Dey *et al.,* 2020). Similarly, the extracts of *Ipomoea cairica* reduced the fecundity and hatching rates of *A. aegypti* and *A. albopictus* (Zuharah *et al.,* 2016).

CONCLUSION

The larval mortality caused by different doses of extracts of all three plants was screened in the present study. Different extracts prepared from same plant showed different efficacy while tested in the same environment. The bioactivity of photochemical vary according to the extraction method and plant part used. Diverse genetic condition of plants and differential rate of production of chemical compounds may also be the caused behind this. Variety of plant extracts have been reported against mosquito vectors, depending on laboratory assays, but there are many potential limitations for their efficacy and applicability in the field. Environmental factors, mosquito fitness, plant parts, solvent selection, extraction process, exposure dose and time have overall effect on the efficacy of any insecticide. Selective toxicity of any plant products towards a mosquito species and different developmental stages of any species is an important component for designing successful control measure. Present study concludes that insecticidal activity of *Polygonum hydropiper* against *Aedes aegypti* is promising and the plant can be further developed as a potential insecticide in future. The data generated from the present study will help different agency of medical science, public health department, other stakeholders and as well as the common people on mosquito control.

ACKNOWLEDGEMENT

The authors are thankful to ICMR-RMRC, Dibrugarh for providing *Aedes aegypti* larvae and department of Life Sciences, Dibrugarh University, Assam for providing necessary facilities to carry out this work.

Funding

The authors declare that no funds, grants or other support were received during the preparation of this manuscript.

Data availability

All data are reported in the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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