Research Article

ISSN: 2454-5023
J. Ayu. Herb. Med.
2023; 9(1): 7-12
Received: 13-01-2023
Accepted: 05-03-2023

www.ayurvedjournal.com
DOI: 10.31254/jahm.2023.9102

***Corresponding author:**
Dr. Piratheepkumar Rajadurai
Unit of Siddha Medicine, Faculty of Applied Science, Eastern University Sri Lanka, Batticaloa, Sri Lanka
Email: piratheepkumarr@esn.ac.lk

Evaluation of Larvicidal Activity of Aqueous Extracts of Leaves, Root, Stem of *Plumbago zeylanica* Plant on *Aedes aegypti*

Paba Senarath[1], Vijitha Paheerathan[1], Sivakanesan Ramiah[2], Piratheepkumar Rajadurai[1]

[1] Unit of Siddha Medicine, Faculty of Applied Science, Eastern University Sri Lanka, Batticaloa, Sri Lanka

[2] Department of Biochemistry, Faculty of Medicine, University of Peradeniya Peradeniya, Sri Lanka

ABSTRACT

The purpose of this study was to determine the larvicidal effect of aqueous extracts of different parts of the Plumbago zeylanica plant on the dengue vector, Aedes aegypti. This research assessed *P. zeylanica* for its larvicide activity against *A. aegypti* larvae. Five different concentrations of hot and cold aqueous extracts of root, leaf, and stem powders of *P. zeylanica* were separately prepared. The larvicidal effect of these extracts was assessed against early 1st, 3rd, and 4th instar larvae of *Aedes aegypti*. Mosquito larvae were sampled, larval populations were monitored before and after application of aqueous extracts, and larval mortality was monitored after 12, 24, and 48 hours. Three trials were carried out under the same conditions. The percentage mortality of larvae in the sample was calculated, and the mean mortality was obtained for the 3 trials. Then the LC_{50} value was calculated according to the mean mortality with the SPSS package. The lowest LC_{50} values were obtained for the hot aqueous extracts on 1st instar larvae: root 122.74 mg/l, leaf 274.95 mg/l and stem 275.92 mg/l, 3rd instar larvae: root 129.37 mg/l, leaf 205.74 mg/l and stem 286.21 mg/l, 4th instar larvae: root 165.52 mg/l, leaf 216.89 mg/l and stem 329.94 mg/l and the root was found to have the highest larvicide activity. Finally, it was identified that by using 165.52 mg/l all three larval instar stages could be killed up to 50% and 90% with 280.921 mg/l. The present study reports that *Plumbago zeylanica* could serve as a potential larvicidal agent.

Keywords: *Plumbago zeylanica*, *Aedes aegypti*, Mosquito lava, Traditional medicine, Medicinal plant.

INTRODUCTION

According to the National Dengue Control Unit, 2016, dengue fever is the most critical mosquito borne viral infection in Sri Lanka in recent years, with major global health importance. The global prevalence of dengue has increased dramatically in recent decades. Dengue fever and Dengue Shock Syndrome (DSS) occur in over 100 countries and threaten the health of more than 2.5 billion people in urban and peri-urban areas, especially in South and South-East Asia, including Sri Lanka [1].

Even though dengue fever is a critical disease, there are no specific antiviral agents for dengue, and there is no commercially available vaccine against it [2]. Therefore, prevention is the most important step to reduce the risk of dengue infection. There are several ways of preventing dengue fever currently practiced in Sri Lanka. In general, effective vector control that does not harm or produce residual negative effects on the ecology is critical. Widespread use of synthetic insecticides leads to many negative consequences not only for humans but also for the environment. As a result, a plant product will be more beneficial as a larvicide in every way.

Plambago zeylanica, which is commonly known as Ceylon leadwort, is one of the divine plants, having many therapeutic indications, including larvicidal activity. Many studies have been done in order to evaluate the larvicidal effect of *P. zeylanica* on different types of larvae. In 2009, hexane and chloroform extracts of *P. zeylanica* were found to have effective larvicidal activity against *Anopheles gambiae* [3] and in 2010, chloroform, dichloromethane, and methanol extracts with high larvicidal activity against *Anopheles stephensi* were reported [4]. Plumbagin, which is the most available constituent, has also been proven to have a high larvicidal effect on *Plasmodium falciparum*. Pradeepa (2014) tested *P. zeylanica* extracts on *A. stephensi* and conducted additional research on the chemical constituents involved in the larvicidal activity, clarifying its chemical compounds that are responsible for its larvicidal activity. But still, there have been no studies conducted to evaluate the larvicidal effect of aqueous extracts of different parts of *P. zeylanica* [5].

The use of natural alternatives such as plant extracts can provide the best option for environmentally safe natural larvicides with high efficiency and cost effectiveness. Even though it is able to fulfill the above requirements, it should be feasible even for the uneducated community. Hence, a study to evaluate the

larvicidal activity of the *P. zeylanica* plant on *Aedes aegypti* was conducted. *P. zeylanica* was selected to evaluate its larvicidal activity because it is a commonly available plant in home gardens in the Eastern Province of Sri Lanka. It was decided to use water as the medium to prepare the extract so that it can be added to natural water sources without causing harm to human health or non-target organisms and also to be more reliable, feasible, and easier to introduce to the public.

Traditional medicine is popular in Sri Lanka and is practiced by well-trained graduates. As a traditional practitioner, it is compulsory to know about the contemporaneous diseases that are common in Sri Lanka. As a medical officer, it is also your responsibility to find solutions to the country's pressing health issues in order to improve the country's health status.

MATERIALS AND METHODS

This is the laboratory based experimental study conducted at the Unit of Siddha Medicine, Trincomalee Campus, as a laboratory experiment.

Collection of plant material

Fresh plants of *P. zeylanica* were collected from Trincomalee district, Eastern Province, and authenticated by the Gunpadam division, Unit of Siddha Medicine. The plant parts were separated into leaves, roots, and stems. The collected plant parts were washed with distilled water and dried in the shade for about 2–3 days. Then it was powdered using the electric grinder and sieved to obtain the fine powder. Finally, the powder was stored in dry, cool, airtight containers and labeled.

Collection of larvae

A homogenous population of larvae suspected to be *A. aegypti* was collected from a drainage canal in the town premises, which was identified by the Public Health Inspector of the area during the field visits.

Identification of the *A. aegypti*

The entomologist of the Trincomalee district observed the sample under the microscope and confirmed the larvae as *A. aegypti* according to the WHO guidelines [6].

Mosquito colony and maintenance

An *A. aegypti* mosquito colony was set up at the MOH Uppuveli in a well-covered tank, and the mosquitoes were allowed to breed in the natural environment at a temperature of 30–370°C. The larvae were allowed to grow for one week, and safety precautions were taken to prevent the mosquitoes from escaping. They were collected into bottles and taken to the laboratory after one week.

Preparation of the extracts

Cold water extract

Each part of the plant powder weighing 10 mg was placed into a mortar, and 10 ml of distilled water was added and macerated to prepare a 1% solution. The solution was allowed to stand at room temperature for 30 minutes. Finally, the solution was centrifuged at 10000 rpm for 10 minutes, and the supernatant was carefully separated.

Hot water extract

A 10 mg plant powder was macerated in 10 ml of distilled water, and the solution was kept in a 1000 °C water bath for 10 minutes. Then the solution was centrifuged at 10000 rpm for 10 minutes, and the supernatant was carefully separated.

The plant extract was diluted to obtain 2%, 3%, 4%, and 5% with distilled water.

Larvicidal bioassay

The larvicidal bioassay was conducted according to the WHO guidelines (2005) [6]. To evaluate the biological activity of a mosquito larvicide, laboratory-reared mosquito larvae of known age or instars were exposed for 24 to 48 hours in water treated with the larvicide at various concentrations within its activity range, and mortality was recorded. The 1st, 3rd, and 4th instar larvae were identified according to the procedure described by Nishiura et al., 2005, and Ray et al., 2009.

Preparation of larvae samples

From the collected larvae, batches of 20 first-instar larvae were taken and transferred by means of a strainer and a dropper to small disposable test cups, each containing 90 mL of water collected from where the larvae were bred. The depth of the water in the cups or vessels was between 5 cm and 10 cm, as deeper levels may cause undue mortality. Small, unhealthy, or damaged larvae were removed and replaced.

Adding the plant extract

Plant extract (10 ml) was added to 90 ml of water with larvae in the cups to obtain the desired target dosage, starting with the lowest concentration. The time of incubation was noted, and the cups were left undisturbed. Larvae behavior and mortality were verified after 12 hours, 24 hours, and 48 hours of treatment at room temperature. Larvae were considered dead if they were immobile and unable to reach the water surface (moribund larvae). For long exposures, larval food (fish food) was added to each test cup. The test containers were held at 25–280 °C with a photoperiod of 12 h light followed by 12 h dark.

Similar treatment was carried out for the third-instar larvae (5-day-old larvae) and fourth instar larvae (7-day-old larvae) of the mosquito.

Evaluation

An equal number of controls were set up simultaneously with distilled water, and 20 1st, 3rd, and 4th instar larvae were separately added to cups containing 90 ml of water where the larvae bred and 10 ml of distilled water was added in place of the plant extract. Hence, altogether, there were 33 test cups, each containing 20 larvae, added with 5 concentrations of hot and cold plant extract to the 3 stages of larvae and the 3 control samples for each instar. The test was carried out in a similar way for the root, stem, and leaf of the plant.

The mortality observations were made over 48 hours. Larvae were counted as dead when they were immobile and not coming to the surface for respiration. Dead larvae are those that cannot be induced to move when they are prodded with a needle in the siphon or the cervical region. Moribund larvae are those incapable of rising to the surface or not showing the characteristic diving reaction when the water is disturbed.

Safety measures taken

During the collection of larvae, breeding in the tanks, and larvicidal assay, steps were taken to prevent the mosquitoes from escaping into the environment. During the time of larval collection, only the instar stage larvae were collected, and the pupa stages were discarded. During the time of breeding, the tank was fully covered with nets, and the mosquitoes were not allowed to escape to the outside. When taking the samples to the laboratory, they were transferred to large bottles and closed with lids. During the 3 days of larvicidal bioassay, the samples were kept in a fully covered room, observed carefully, and the pupa stage that emerged during the final day of 4th-instar larvae was discarded. Finally, all the samples used for the experiment were filtered through filter papers, the water was discarded into the dry soil, and the larvae were put into a pit and burned with kerosene oil, and the pit was closed.

Analysis of data

The mosquito larvae were exposed to a wide range of test concentrations and distilled water (control) to find out the activity range of *Plumbago zeylanica* as a larvicide, as mentioned above. Data from all replicates was pooled for analysis. Bioassays were repeated three times, using new solutions and different batches of larvae each time. The results were recorded for the root, leaf, and stem at five concentrations in the hot and cold extracts for the three larval stages and each of the three replicates.

Larvae that pupated during the testing period will render the test invalid. If more than 10% of the control larvae pupated in the course of the experiment, the test was discarded and repeated. If the control mortality is between 5% and 20%, the treated group mortalities were corrected using the following formula:

$$\text{Mortality} = \frac{\text{X} - \text{Y}}{\text{X}} \text{ x } 100$$

Where X = percentage survival in the untreated control and Y = percentage survival in the treated sample.

The mortality percentage of each concentration in all three trials was taken separately, the mean value was taken as the dependent variable, and the different concentrations used were taken as the independent variable. Lethal concentration values, LC50 and LC90, were calculated for the 5 concentrations from a log dosage–probit mortality regression line using computer software programs (SPSS). The standard deviations or confidence intervals of the means of the LC50 values were calculated and recorded on the data recording form. If the relative standard deviation (or coefficient of variation) is less than 25% or the confidence limits of the LC50 overlap (significant level at P 0.05), the test series is valid. The potency of the chemical against the larvae of a particular vector and strain was then compared with the LC50 or LC90 values of other insecticides used against *Aedes aegypti*.

RESULTS AND OBSERVATION

The larvicidal activity of the aqueous extracts of the different parts of *Plumbago zeylanica* against the larvae of the dengue vector, *Aedes aegypti*, was determined through a mosquito larval bioassay. The mortality of the mosquito larvae was noted at 100 mg/l, 200 mg/l, 300 mg/l, 400 mg/l, and 500 mg/l concentrations of the plant extracts after 12 h, 24 h, and 48 h of exposure of 1st, 3rd, and 4th-instar larvae, respectively.

Larvicidal efficacy of plant extracts

The mean percentage mortality values were analyzed using probit analysis using SPSS, and the LC50 and LC90 values were calculated using the log dosage–probit mortality regression.

Results of larvicidal assay for 1st instar larvae

Table 1 indicates the LC50 and LC90 values (ppm) of aqueous extracts of *P. zeylanica* against 1st instars of *A. aegypti* after 12 h, 24 h, and 48 h of exposure. The LC50 values gradually decreased with time with increasing mortality, and the lowest LC50 values were observed at 48 h for all three plant parts. According to the probit analysis of mean mortality for 1st instar larvae, $p < 0.05$ was observed for most of the LC50 at 48 h. As a result, all three plant parts have a significant larvicidal effect after 48 hours.

Table 1: Larvicidal assay for 1st instar larvae

Plant part	Duration (hr)		LC50±SE (mg/l)	P value
Root	12	Cold	642.2±0.44	0.011
		Hot	566.8±1.25	0.103
	24	Cold	410.8±0.85	0.010
		Hot	265.2±0.80	0.000
	48	Cold	203.1±1.37	0.045
		Hot	122.7±0.25	0.000
Leaf	12	Cold	887.0±1.05	0.029
		Hot	607.6±1.34	0.141
	24	Cold	570.9±3.96	0.135
		Hot	558.1±3.05	0.190
	48	Cold	275.0±0.68	0.273
		Hot	185.1±0.56	0.009
Stem	12	Cold	715.1±0.60	0.028
		Hot	505.1±0.82	0.056
	24	Cold	501.5±0.82	0.316
		Hot	409.3±1.19	0.024
	48	Cold	349.0±1.06	0.020
		Hot	275.9±0.92	0.001

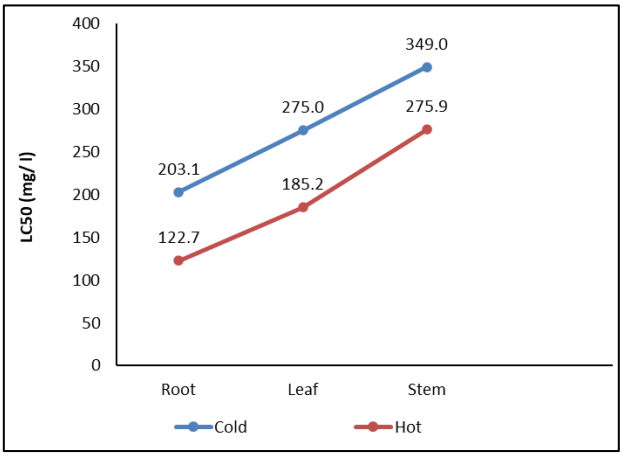

Figure 1: LC_{50} values of hot and cold extracts of root, leaf and stem at 48h.

According to Figure 1, hot extracts exhibit lower LC_{50} values compared to cold extracts, and the root has the least LC_{50} and the stem has the highest LC_{50} when 1st instar larvae are exposed to 48 h.

Results of larvicidal assay for 3rdinstar larvae

Table 2 shows the LC_{50} and LC_{90} values (ppm) of aqueous extracts of *P. zeylanica* against 3rd instars of *A. aegypti* after 12 h, 24 h, and 48 h of exposure. The LC_{50} values gradually decreased with time with increasing mortality, and the lowest LC_{50} values were observed at 48 h for all three plant parts. The p-value for most of the LC_{50} values at 48 h is less than 0.05, according to the probit analysis of mean mortality. As a result, all three plant parts have a significant larvicidal effect after 48 hours.

Table 2: Results of larvicidal assay for 3rdinstar larvae

Plant part	Duration (hr)		LC_{50}±SE (mg/l)	p value
Root	12	Cold	255.7±0.53	0.002
		Hot	216.8±0.77	0.161
	24	Cold	221.6±0.94	0.033
		Hot	203.1±1.37	0.045
	48	Cold	157.3±2.21	0.009
		Hot	129.4±3.53	0.001
Leaf	12	Cold	1049.9±1.02	0.022
		Hot	669.5±1.12	0.091
	24	Cold	894.2 ±1.19	0.075
		Hot	312.5±1.08	0.067
	48	Cold	339.7±0.71	0.002
		Hot	205.7±1.58	0.006
Stem	12	Cold	782.5±0.48	0.047
		Hot	707.2±1.30	0.050
	24	Cold	555.5±1.33	0.179
		Hot	484.0±0.95	0.057
	48	Cold	342.9±0.58	0.000
		Hot	286.2±0.96	0.003

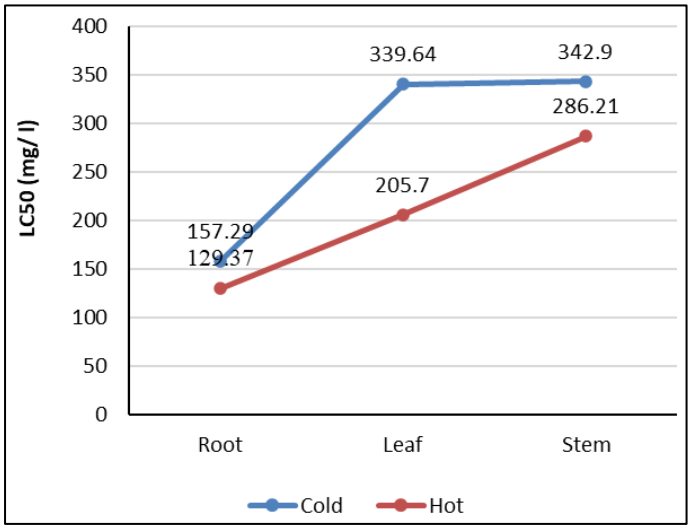

Figure 2: LC_{50} values of hot and cold extracts of 3 plant parts at 48h.

According to the LC_{50} values obtained for the hot and cold extracts, the hot extracts exhibit lower LC_{50} values than cold extracts, the root has the lowest LC_{50} during 48 hours of exposure, and the stem has the highest LC_{50} value of 3rd instar larvae.

Results of larvicidal assay for 4thinstar larvae

Table 3 shows the LC_{50} and LC_{90} values (ppm) for aqueous extracts of *Plumbago zeylanica* against the 4th instar of *Aedes aegypti* after 12 h, 24 h, and 48 h of exposure. The LC_{50} values gradually decreased with time with increasing mortality, and the lowest LC_{50} values were observed at 48 h for all three plant parts. The probit analysis of mean mortality yielded p 0.05 for the majority of the LC_{50} values at 48 h. As a result, all three plant parts have a significant larvicidal effect after 48 hours.

Table 3: Results of larvicidal assay for 4thinstar larvae

Plant part			LC_{50}±SE	p value
Root	12h	cold	621.003±1.417	0.021
		hot	589.127±0.693	0.118
	24h	cold	251.248±1.268	0.013
		hot	299.737±1.819	0.000
	48h	cold	190.691±0.636	0.003
		hot	165.525±3.128	0.009
Leaf	12h	cold	1371.045±1.91	0.008
		hot	648.495±0.751	0.032
	24h	cold	890.429 ±0.78	0.015
		hot	581.382±0.699	0.119
	48h	cold	515.611±1.811	0.159
		hot	216.891±0.309	0.030
Stem	12h	cold	783.248±1.478	0.270
		hot	635.42±0.905	0.119
	24h	cold	554.473±0.893	0.098
		hot	473.234±1.082	0.000
	48h	cold	523.155±1.072	0.027
		hot	329.944±1.165	0.030

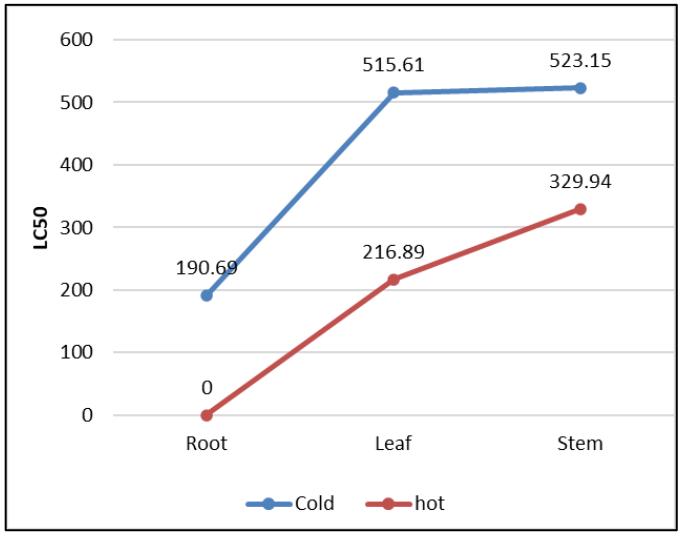

Figure 3: LC_{50} values of hot and cold extracts of 3 plant parts at 48h

According to the LC_{50} values obtained for the hot and cold extracts, the hot extracts exhibit the lowest LC_{50} values, the root has the lowest LC50 within 48 hours of exposure of 3rd instar larvae, and the stem has the highest LC_{50} of all the three parts.

DISCUSSION

According to the results obtained by using extracts of 5 different concentrations of three plant parts of *Plumbago zeylanica (*root, leaf, and stem) and exposing 1st, 3rd, and 4th instar larvae of *Aedes aegypti* the mean mortality and lethal concentrations were calculated and tabulated.

The LC_{50} value is the lethal concentration at which 50% of the population gets killed in a given period of time. Cold extracts root LC_{50}=203.12 ppm, p=0.045; leaf LC_{50}=274.95 ppm, p=0.237; stem LC_{50}=349.04 ppm, p=0.002 and hot extracts root LC_{50}=122.74 ppm, p=0.000; leaf LC_{50}=185.17ppm, p=0.009; stem LC_{50}=275.92ppm, p=0.001 and for the This shows that the *Plumbago zeylanica* is having a larvicidal effect on the *Aedes aegypti* larvae, similar to the previous study by Patil (2010) on the larvicidal effect of different types of mosquito larvae. As a result of plumbagin (5-hydroxy-2-methyl-1, 4-naphthoquinone, C11-H8-O3), which has been reported as a toxic compound present in plant roots [7], the root has shown the most effective larvicidal effect in all three stages of larvae compared to the leaf and the stem.

During the study, among the 4 instar larvae, the 1st, 3rd, and 4th instars were taken for the study because the 1st and 2nd instars bear the same appearance and it was difficult to identify the two instars separately. According to the study, the lowest LC_{50} values among the 3 instars of larvae were observed for the 1st instar larva, and the highest LC_{50} values were observed for the 4th instar. Previous studies have also shown that the LC_{50} value is highest for the 4th instar larvae, and a similar trend has been noted for all the instars of *A. aegypti* at different concentrations with *T. purpurea* treatment. The lethal concentrations (LC_{50} and LC_{90}) presented as the LC_{50} value of the 1st instar were 139.24 ppm, 176.24 ppm, 219.28 ppm, and 256.27 ppm, respectively [8]. According to Humarya (2014), late larval instars (3rd and 4th instars) receive higher doses than 1st and 2nd instar larvae, and 3rd and 4th instar larvae are more radiotolerant than 1st and 2nd instar larvae. Adult emergence rates in irradiated first instar larvae were significantly lower than in other instars [9]. Accordingly, among the 3 stages of larvae, the 1st instar showed the highest mortality with the lowest LC_{50}.

Hence the first instar larvae were the weakest of the three larval instars. At an extract concentration of 165 mg/l, if added to a water source, it will kill all 3 stages of larvae, but the 1st instar larvae will be killed first and will not transform into the 2nd instar. Therefore, when larvae are exposed to *Plumbago zeylanica,* the emergence of the 2nd and 3rd instars will be minimized.

The LC_{50} of *Plumbago zeylanica* root at 48 hours of exposure was 122 mg/l for the 1st instar and 165 mg/l for the 3rd instar. After a 48-hour exposure to *Persicaria hydropiper, Aedes aegypti* had a LC_{50} of 773.69 mg/l, and *Plectranthus hadiensis* had a LC_{50} of 485.791 mg/l [10]; *Anona reticulata* had a LC_{50} of 161.44 mg/l [11]. Hence, this reveals that the LC_{50} of aqueous extracts of the root of *Plumbago zeylanica* is a more effective larvicide than that of the other plants.

CONCLUSION

With the lowest calculated lethal dose, the root had the greatest larvicidal effect. For the first instar larvae, hot extract showed the highest mortality with the lowest LC_{50}. The lowest $LC_{50}$to kill all three stages of the larvae was found to be 165.53 mg/l. Hence, 165.53 mg/l of the hot aqueous extract of *Plumbago zeylanica* added to water containers with *Aedes aegypti* will kill 50% of the larvae in their 3 instar stages, and 280.92 mg/l will kill 90% of the total population without producing any deleterious effect on the natural ecosystem.

Acknowledgments

I would like to thank for the Entomologist, Trincomalee District and the Medical Office of Health, Trincomalee Town for their valuable support to collect the mosquito lava and identify properly.

Disclosure

I am declared that there were no conflicts of interest in this work.

ORCID ID

Dr. Piratheepkumar Rajadurai: https://orcid.org/0000-0002-8210-668X

REFERENCES

1. National dengue control unit (NDCU), 2016, available at http://www.dengue.health.gov.lk/ on 11.11.2016.
2. Tyler M. Sharp, Janice Perez-Padilla, Stephen H. Waterman, 2017, Dengue, Infectious diseases related to travel, chapter 3, Centers for disease control and prevention available at wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/dengue.
3. Ndiege, Cornelius C Wanjala, Teresa Ayuko Akenga, Larvicidal activity of extracts from three Plumbago sppagainst Anopheles gambiae. Journal of Microbes and their vectors causing human infections. 2009;104(6):813-817, Available from: https://www.researchgate.net/publication/38054994_Larvicidal_activity_of_extracts_from_three_Plumbago_spp_against_Anopheles_gambiae.

4. Patil SV, Patil CD, Salunkhe RB, Salunke BK. Larvicidal activities of six plants extracts against two mosquito species, *Aedes aegypti* and *Anopheles stephensi*, Tropical Biomedicine 2010;27(3):360-65
5. Pradeepa V, Sathish-Narayanan S, Kirubakaran SA, Senthil-Nathan S. Antimalarial efficacy of dynamic compound of plumbagin chemical constituent from *Plumbago zeylanica* Linn (Plumbaginaceae) against the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). Parasitology research. 2014;113:3105-9.
6. WHO (2015). Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCDPP/2005.13
7. Tyagi R, Menghani E. A Review on *Plumbago zeylanica*: a compelling herb. Int J Pharma Sci Res. 2014;5:119-26.
8. Venkadachalam R, Subramaniyan V, Palani M, Subramaniyan M, Srinivasan P, Raji M. Mosquito larvicidal and pupicidal activity of *Tephrosia purpurea* Linn.(Family: Fabaceae) and *Bacillus sphaericus* against, dengue vector, Aedes aegypti. Pharmacognosy Journal. 2017;9(6):737-42.
9. Akter H, Khan SA. Sensitivity of immature stages of dengue causing mosquito, *Aedes aegypti* (L.) to gamma radiation. Journal of entomology. 2014;11(2):56-67.
10. Anoopkumar AN, Sreedev Puthur, Sharrel Rebello and Embalil Mathachan Aneesh, Screening of a Few traditionally used Medicinal Plants for their Larvicidal Efficacy against *Aedes aegypti* Linn (Diptera: Culicidae), a Dengue Fever Vector. SOJ Microbiology & Infectious Diseases. 2017;5(4):1-5 DoI: 10.15226/sojmid/5/4/00181
11. Govindarajulu B, Srimathi A, Bhuvana R, Karthikeyan J. Mosquito larvicidal efficacy of the leaf extracts of Annona reticulata against Aedes aegypti. International Journal of Current Microbiology and Applied Sciences. 2015;4(8):131-40.
12. Anoopkumar AN, Sreedev Puthur, Sharrel Rebello and Embalil Mathachan Aneesh, Screening of a Few traditionally used Medicinal Plants for their Larvicidal Efficacy against *Aedes aegypti* Linn (Diptera: Culicidae), a Dengue Fever Vector. SOJ Microbiology & Infectious Diseases. 2017;5(4):1-5 DoI: 10.15226/sojmid/5/4/00181
13. Nishiura JT, Ray K, Murray J. Expression of nuclear receptor transcription factor genes during *Aedes aegypti* midgut metamorphosis and the effect of methoprene on expression. *Insect Biochemistry and Molecular Biology* 2005;35:561-573.
14. Ray K, Mercedes M, Chan D, Choi C, Nishiura JT. Growth and differentiation of the larval mosquito midgut. Journal of Insect Science, 2009;9:13.

HOW TO CITE THIS ARTICLE

Senarath P, Paheerathan V, Ramiah S, Rajadurai P. Evaluation of Larvicidal Activity of Aqueous Extracts of Leaves, Root, Stem of *Plumbago zeylanica* Plant on *Aedes aegypti*. J Ayu Herb Med 2023;9(1):7-12. DOI: 10.31254/jahm.2023.9102