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Larvicidal Efficacy of Locally Available Plant Species in Sri Lanka for the Development of Potential Larvicidal Agents Against *Aedes aegypti* L

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Abstract

The *Aedes aegypti* mosquito transmits diseases such as dengue, Zika, and chikungunya, prompting the need for safe and effective larvicidal agents. Traditional insecticides pose risks, prompting the exploration of sustainable alternatives like local plants with bioactive compounds. This study evaluates the efficacy of these plants against *Aedes aegypti* larvae, aiming to develop new larvicidal agents. A larvicidal bioassay methodology recommended by the WHO was adopted. Following the initial screening of 32 plant species, those exhibiting promising larvicidal effects were selected for the follow-up studies using storable dried powder. A series of larvicidal bioassays was conducted to determine the effectiveness. Eight different concentrations derived from the dried powder of the most promising plants were tested to determine the effective concentration for mosquito controlling at their natural breeding sites. The egg hatching and survival rate of *Aedes aegypti* second instar larvae was monitored at each concentration. Out of 15 samples identified as potential larvicides against *Aedes*, 13 were leaf crude extracts. The time taken for the 100% mortality varied from 5 and 138 minutes. Positive retesting with the larvicidal bioassay was conducted for nine selected plant leaf crude extracts that demonstrated mortality within 60 minutes. The overall results showed that clover leaf powder was the most promising treatment for controlling dengue mosquito breeding. It inhibits larvae development by over 80% at a concentration of 0.01g/ml and can be recommended for application in water-holding containers where *Aedes* mosquitoes are likely to breed.

Keywords: Plant extracts, *Aedes aegypti*, Larvicidal, Dengue

1 Introduction

Aedes aegypti (L) (Diptera: Culicidae), is a mosquito species that originated in the African continent and later dispersed into other tropical geographical regions across the globe (Higa, 2011). It is remarkably adapted to thrive in urban and suburban areas (Kraemer et al., 2015) ovipositing an average of 100 eggs per blood meal and potentially being able to produce more than 500 eggs during its lifetime (Wilke et al., 2021). Most larvae reach maturity due to a lack of natural predators and larval competition, resulting in a significant increase in population size over a short time frame (Wilke et al., 2021). They have been identified as vectors for several viral diseases such as Yellow Fever, Dengue, Chikungunya, and Zika virus (Paixão et al., 2018). Although Yellow Fever is preventable through vaccination (Robertson, 1996), no effective vaccines or other specific treatments currently exist for Dengue and other diseases transmitted by *Ae. aegypti* (Thisyakorn & Thisyakorn, 2014). In addition to ongoing drugs discovery efforts for these diseases, the primary preventative measure to reduce the Dengue pandemic is controlling mosquito populations (Rather et al., 2017), as, the transmission of Dengue relies on mosquitoes as vectors; therefore, reducing mosquito populations can effectively restrict the spread of the disease

(Scott & Morrison, 2010).

There are several conventional methods currently used to control mosquito populations such as chemical control, source reduction, habitat modifications, personal protection, the use of traps, biological control, and emerging approaches such as genetic control (Fouet & Kamdem, 2019). Larvicides are products used to eliminate the early stages of the mosquitoes before they mature into adults, are introduced right into the water that holds mosquito eggs, larvae, or pupae. When this method is applied, the overall population of mosquitoes will be significantly reduced (Ghosh et al., 2012; Wang et al., 2013). As hundreds of mosquito larvae may exist in a single water body, routine mosquito control is typically more effective when targeted at the immature stages rather than targeting adult stages, due to the larger area that needs to be covered and the adverse effects of insecticide toxicity on humans and non-target organisms from ground and aerial application or fumigation (Killeen et al., 2002; Singh et al., 2018; Walker & Lynch, 2007). Temephos (George et al., 2015), larvicidal oils (Cavalcanti et al., 2004), insect growth regulators (Lau et al., 2015), monomolecular films (Webb & Russell, 2009), and *Bacillus thuringiensis* (Bti), a biological control agent (Federici et al., 2010) are some of the larvicidal techniques currently available. Despite the fact that they are effective against mosquito larval stages, there are several drawbacks such as toxicity to susceptible aquatic organisms, ineffectiveness against mosquito pupae, and some of their formulations cannot persist in the environment, and others are ineffective against certain mosquito species (Antonio-Nkondjio et al., 2018).

Among different novel philosophies, such as Sterile Insect Technique (SIT) and Incompatible Insect Technique (IIT), controlling maritime larval periods of vector mosquitoes by using their indigenous typical enemies (pathogens, parasites, and predators), known as natural control, remains one of the negligible exertion and eco friendly approaches of integrated vector control. Since the 1960s, the specialists working on dengue control in Sri Lanka have concentrated more on the chemical substance-based controlling methods, while efforts in natural control of *Ae. aegypti* has been largely limited to evaluating the larvicidal potential of only a few plants, primarily the *Cymbopogon citratus* (lemongrass). Frequent use of chemical insecticides creates the problem of evolving insecticide resistance among the mosquitoes and destabilizes the ecosystem. The Sri Lankan government is restricting and banning the use and the import of chemical fertilizers and other agrochemicals, including insecticides and herbicides, from May 6, 2021, with a view to making Sri Lanka the first country in the world that exclusively practices organic agriculture. Thinking beyond that, there is a possibility of implementing a government action plan to reduce reliance on chemical insecticides for vector control in Sri Lanka in the near future. Therefore, the intended research question is: If the government bans all chemical insecticides, "how can mosquito populations be controlled without the use of chemical pesticides?"

The emphasis on the use of natural plant products as larvicides has been introduced as a healthy alternative to synthetic insecticides to mitigate these issues. Biologically active plant extracts are well- documented for their role in developing an ecologically sound and environmentally accepted mosquito control program. Plant extracts provide a promising alternative to synthetic pesticides in an eco- friendly and ecologically sustainable manner. Plant extracts have low toxicity to humans and other higher animals, and contribute minimally to the environmental pollution compared to the recorded negative environmental and health impacts of synthetic insecticides.

Screening plant species as sources of metabolites for entomological control purposes provides new insights in the search for safer and more effective tools as mosquito larvicides (Govindarajan & Benelli, 2016). Studies indicate that various plant species have potential larvicidal effects against mosquito larvae and have recently been implemented to enhance mosquito control (Ghosh et al., 2012). Furthermore, development and use of botanical insect management products for controlling mosquitoes and other insect pests have strong market potential due to the increasing interest in biological pest control methods and the popular adoption of green concepts by communities worldwide. A number of such plant products have been used for insect control since ancient times. Numerous researchers have reported the effectiveness of plant extracts or essential oils as efficient mosquito larvicides and repellents without posing hazards of toxicity to humans (Amer

& Mehlhorn, 2006a, 2006b; Rahuman, Bagavan, Kamaraj, Saravanan, et al., 2009; Rahuman, Bagavan, Kamaraj, Vadivelu, et al., 2009). The efficacy of larvicidal botanical extracts against *Culex* species has been well-documented. Previous literature cited over 2000 plant species are capable of producing chemical agents effective against insect pests (Gunawardene et al., 2007). However, only 344 of these species have been reported to produce active agents effective against mosquitoes (Sukumar et al., 1991).

Sri Lanka is considered as a major biodiversity hotspot (Gunawardana & Jayasuriya, 2019). From mangroves to coastal wetlands, rivers to rain forests, dry grasslands to mixed evergreens, the tiny island hosts a vast variety of ecosystems, including human -modified environments such as plantations which comprise of many promising endemic, medicinal, and aromatic plants (Brady et al., 2012). However, sufficient studies about using plant extracts as larvicides remain limited to date, apart from traditional practices. Hence, there is a huge potential for research and development of natural, plant-based larvicidal products in Sri Lanka. Therefore, the present study aimed to evaluate the larvicidal effects on the *Ae. aegypti* (L) using the extracts of readily available local plant species to develop potential larvicidal agents against particular mosquito species. Additionally, attention was drawn to the use of the underutilized parts of these plants for such preparations.

Objective of the study:

Identification of local plant species for the development of potential larvicidal agents against the *Aedes aegypti* L. (Diptera: Culicidae) mosquito larvae.

2 Methodology

2.1 Research area and design

The research was conducted following the World Health Organization (WHO) recommendations for mosquito larvicidal bioassay (Innocent et al., 2014). Preselected freely available 32 plant species were evaluated for the potential larvicidal effect against *Ae. aegypti* mosquito from the bioassay.

Collection of plants:

The plant materials collected from different locations within Gampaha district, Western province, Sri Lanka are listed in Table 1.

2.1.1 Mosquito rearing

Adult susceptible colonies of *Ae. aegypti* were maintained in an insectary at the Animal Biotechnology section, Department of Bioprocess Technology, Faculty of Technology, Rajarata University of Sri Lanka. The mosquito colonies were fed on 10% sugar solution, and females were blood- fed on discarded blood taken from the Blood Bank at the Teaching Hospital, Anuradhapura, North Central Province, Sri Lanka. Larvae were reared in white plastic trays (24 x 36 x 6 cm) and were fed on sterilized shrimp powder (1 g/1 L).

2.2 First experiment: pre-screening of the identified plant crude extracts

2.2.1 Preparation of crude sample extracts

Fresh plant leaf samples were carefully gathered into separate containers, 10 g of each sample was weighed, and cleaned with detergent before being rinsed with chlorine-free water. After grinding with a mortar and pestle to extract fresh plant juice from each leaf sample, the fresh plant crude extract was filtered to eliminate large particles. The crude extract was then used for a pre-screening test of the larvicidal bioassay.

2.2.2 Pre-screening test

Pre-screenings of the plant crude extracts were conducted using 24 well cell culture plates. A drop of fresh plant crude extract was added to a labelled well with 1 mL of distilled water to make a constant dilution of the crude extract, while distilled water alone was used as a negative control (1 mL+a drop of water). Thereafter, two late 3rd (L3) and early 4th (L4) instar larvae (6 days old after hatched) were added to each well separately (Figure 1, a). After 24 hours, the mortalities of *Ae. aegypti* larvae were determined. Larvae with total absence of movement, even after touch, were considered dead. Two replicates were done for one sample at this stage, and the tests with more than 20% mortality in controls and pupae formation were discarded and repeated . Crude extract samples that showed promising results in the above larvicidal bioassay were chosen for further testing.

2.3 Morphological view and visualization

Late third and early fourth instar larvae of *Ae. aegypti* were observed under the optical microscope at magnifications ranging from 40x to 400x, and

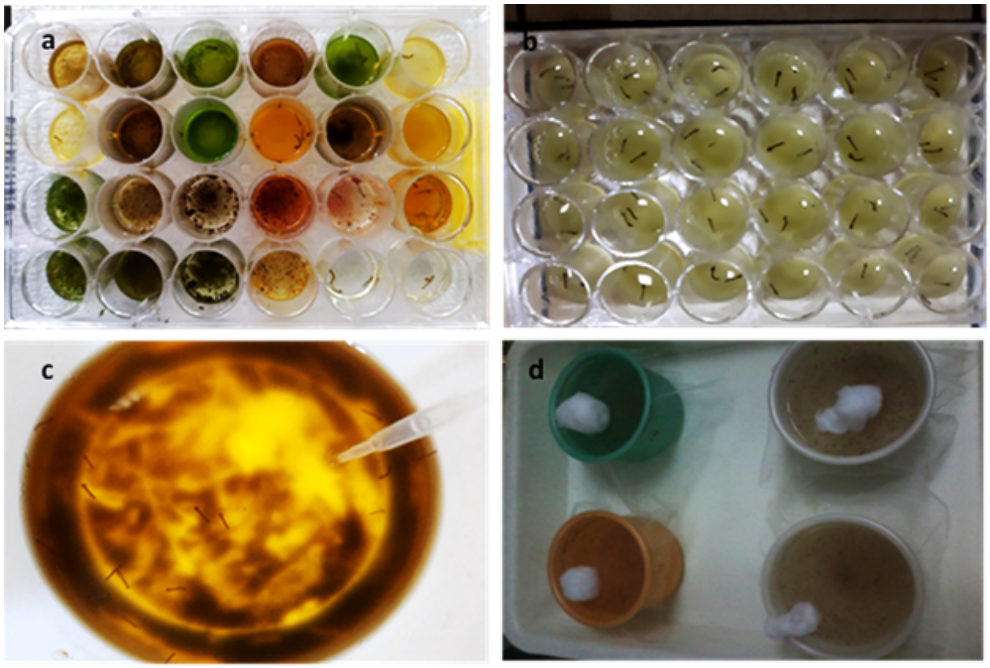

Figure 1: a. Pre-screening larvicidal bioassay (2 replicate/treatment); b. 1st larvicidal bioassay in 24 well cell culture plate (24 replicates/treatment); c. 2nd larvicidal bioassay in plastic containers; d. 2nd larvicidal bioassay in plastic containers (4 replicates/treatment)

their images were visualized on a computer screen attached to the microscope.

2.3.1 Preparation of dried samples for larvicidal bioassay

After sun drying 500 g of plant leaves from each selected sample, which showed promising results in the pre-screening step, for 2 to 3 days, finely powdered samples were prepared by grinding the dried leaves with an electrical blender for 10 – 30 seconds, followed by sieving. Then, fine powders were put into sealable bags (e.g.; Zipper bags) and stored at room temperature until used for bioassay. The stock crude extract solutions were prepared by adding 50 mL of distilled water to each sample, and the extracts were filtered separately through clean muslin cloths to eliminate large particles. Next, a concentration series was prepared (2%, 1%, 0.1%, 0.05% and 0.02%) from the crude extract mixing with distilled water. The prepared plant crude extract solutions in each concentration were aliquoted into six sterilized glass containers separately and 5 were stored inside the refrigerator (4 ^{0}C). The effect of each concentration was measured in bioassay for a six months research period as at the point of sample preparation and later once a month during the six months using the stored, aliquoted samples.

2.4 Second experiment: positive test re-testing with larvicidal bioassay

The crude extracts selected from the pre-screening test were used in five different concentrations (2%, 1%, 0.1%,0.05%, and 0.02%), and their efficacy was evaluated using the standard WHO method with slight modifications (Sujarwo et al., 2016). The standard 24 well cell culture plates (CLS3527-100EA, Merck KGaA, Darmstadt, Germany) were used for the bioassay, together with 1 mL of distilled water to make uniform dilution, and 1 mL+one drop of distilled water alone used as a negative control. Thereafter, two late L3 and early L4 stage larvae were added per well (Figure 1, b). Each concentration of the crude extraction was used per plate and counted as 24 replicates per treatment considering individual wells in the plate as one replicate. The larvae were exposed to extract for 6 hours for each treatment, with the pre-selected crude extracts in five different concentrations.

2.5 Third experiment: 2nd larvicidal bioassay to determine the efficacy of most promising Larvicides for dengue mosquito breeding control

A concentration series of clove plant powder (0.1g, 0.25g, 0.5g, 0.75g, 1.0g, 1.25g, 1.5g per

100 mL of distilled water) was used in the 2nd larvicidal bioassay, while utilizing seasoned water as a negative control. Four replicates (each with 25 eggs) were carried out according to the usual WHO larval susceptibility test methodology with slight modifications. After counting under a light microscope, 25 *Ae. aegypti* eggs were placed into each container. The survival rate of *Ae. aegypti* larvae were assessed after 48 hours of egg hatching. The results of the absolute mortality effect against the *Ae. aegypti* L3/L4 larvae were measured by the average time taken for all the mosquito larvae to die. The number of dead larvae was counted after 24 hours. The experiment was conducted under lab conditions at a temperature of 27± 2 ^{0}C and 80± 5% relative humidity.

2.5.1 Data analysis

The experimental data were analyzed through the SAS University Edition. Overall, positive treatments have been used to check the effect on larvae. The ANOVA procedure was used to determine the significant differences among the treatments, and Duncan's multiple range test was used to compare the means of the treatments in the 1st larvicidal bioassay. The data obtained from the 2nd larvicidal bioassay were subjected to probit analysis, and LC50 & LC99 values were calculated using SAS software. Additionally, chi-square analysis was conducted to check the homogeneity of the tested population.

2.6 Waste management

All study waste was discarded following appropriate health and environmental guidelines. Briefly excess *Ae. aegypti* larvae and eggs were placed in a microwaveable container with sufficient water and were microwaved for at least 10 minutes to destroy the larvae and egg viability. After that, it was kept another 2 or 3 hours to cool at room temperature and filtered through cotton cloth with mesh. Then the dried particle collected on top of the cotton cloth was placed in a garbage bag and discarded. Waste generated from the larvae tray was also treated with boiling water until the content and container were completely sterilized. It was followed by filtration, and the filtrate was removed and disposed in a garbage bag. Once the study's plant fresh and crude extracts, in liquid or powdered form, were delivered to the Bioprocess laboratory at the Department of Bioprocess Technology, Faculty of Technology, Rajarata University of Sri Lanka in Mihintale, for temporary storage and future use.

2.7 Ethical considerations

Ethical clearance for the current study was obtained from the Ethical Review Committee, Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka. Further, informed written consent was obtained from the heads of all households participating in the entomological and ovitrap surveys.

3 Results and Discussion

3.1 Adult and larval survey

3.1.1 First experiment: pre-screening of the identified plant crude extracts

Out of 32 pre-selected plant species, 15 plant species were identified which have a possible larvicidal action for *Aedes aegypti* larvae from at least one of their plant parts (leaves/rhizomes/seeds/bulbs). The summary of the results obtained for the pre-screening test was given in Table 2. The time taken for achieving 100% mortality was recorded as 5 minutes minimum and 2 hours and 18 minutes maximum.

Thirteen plant species out of 15 species having potential larvicidal effect represented the crude extract taken from plant leaves and it was followed by the 1 each representing a plant rhizome and a bulb (Figure 2).

According to the findings of the pre-screening test of larvicidal bioassay in this study, crude extracts obtained from 15 plant species out of 32 species screened showed potential of using *Ae. aegypti* larvae control irrespectively to the plant part used for the preparation of extract. However, results of the pre-screened larvicidal bioassay indicaated that the higher potential of the larvicidal effect was presented by the plant leaves than the other parts of the plant such as rhizomes, flowers, and bulbs. A similar study conducted by AhbiRami and colleagues (Soonwera & Phasomkusolsil, 2016) showed significant differences in larvicidal efficacy of different parts of the railway creeper plant (*Ipomoea cairica*) extract against dengue vector mosquitoes. In their study, *I. cairica* leaf demonstrated the most effective larvicidal action compared with other fractions of *I. cairica* extract obtained from the flower, and stem (Soonwera & Phasomkusolsil, 2016).

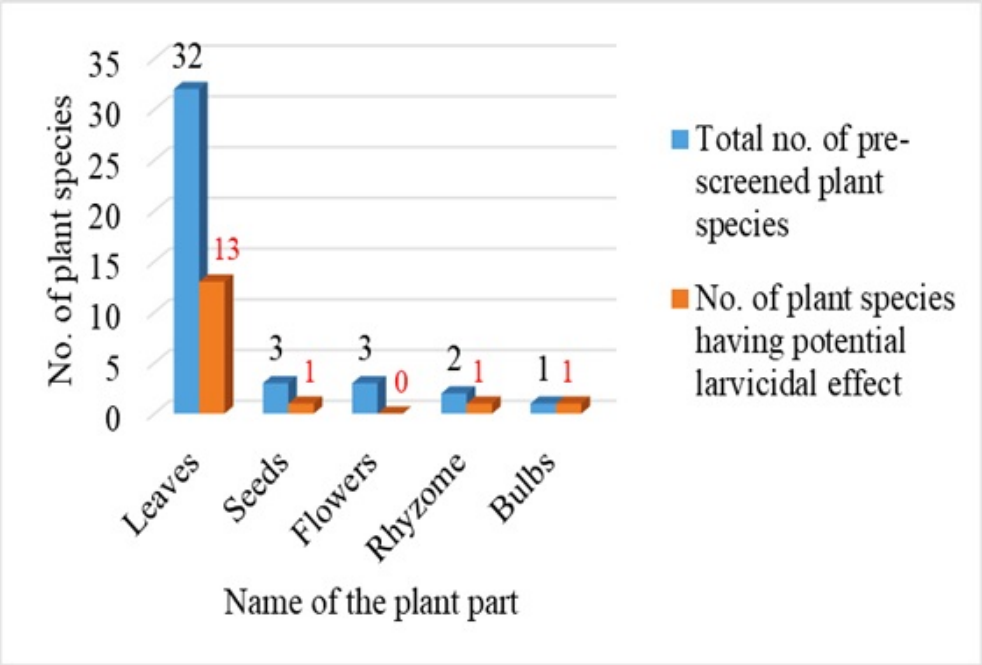

Figure 2: The total number of pre-screened plant species for potential larvicidal bioassay and the number of crude extracts (in water) exhibiting 100% mortality (within 48 hours) during preliminary laboratory trials, with respect to the plant parts used, are summarized below

3.1.2 Second experiment: positive test retesting with larvicidal bioassay

Response- Time Taken per 100% larvae mortality (min) Treatments:
1. T1- Garlic (*Allium sativum*)
2. T2- Dawul kurundu (*Neolitsea cassia*)
3. T3- Ginger rhizome (*Zingiber officinale*)
4. T4- Giant mexican sunflower (*Tithonia diversifolia*)
5. T5- Kapparawalliya leaves (*Plectranthus amboinicus*)
6. T6- Betel leaves (*Piper betle*)
7. T7- Kuppameniya leaves (*Acalypha indica*)
8. T8- Holybasil Maduruthala leaves (*Ocimum tenuiflorum*)
9. T9- Clove leaves (Syzygium aromaticum)
10. T10- Water (Negative control)

Total number of replicates per treatment- 24

According to the ANOVA table results (Table 3), the probability value of the treatment is approximately 0.0001 which is less than 0.05 (Here, the level of significance is 5%). Then, the null hypothesis was rejected, indicating that at least one treatment effect on larvae is different at a 5% significance level. According to Duncan's multiple range test results, the T10 (negative control) has the highest average time taken per larvae to die. And it was statistically significant to treatment 3, 4, 6, 7, 8, and 9 (Table 2). T9 (Clove) has the lowest average time taken per larvae to die and it was statistically significantly differ from all the other treatments except the treatment 1 (Garlic) (Table 2). The negative mean difference in larval mortality with a p-value of less than 0.05, indicating a significant difference between two plant species with lower larvae survival rate of treatment 9 (T9-Clove) (Table 2). Therefore, T9 (Clove) is significantly more effective than other plant crude extracts but not with Garlic.Treatment 3, 4 7 and 8 were statistically significantly differ from all the other treatments (Table 2). The effect of T3 and T6 (Ginger and Betel) have the same effect on larvae. Treatments 5, 2, 8, 1, and 9 (Kapparawalliya, Dawul kurundu, Maduruthala, Garlic, and Clove) have the same effect on larvae as well (Figure 3).

The average time taken for each plant material crude extract showed the higher concentration of active compounds against *Ae. aegypti* mosquito larvae were found in Garlic (*Allium sativum*) bulb and Ginger (*Zingiber officinale*) rhizome (Table 2). Hamada and colleagues also reported that the essential oil extracted from both these plant parts were very effective against dengue vector mosquito larvae (Soonwera & Phasomkusolsil,

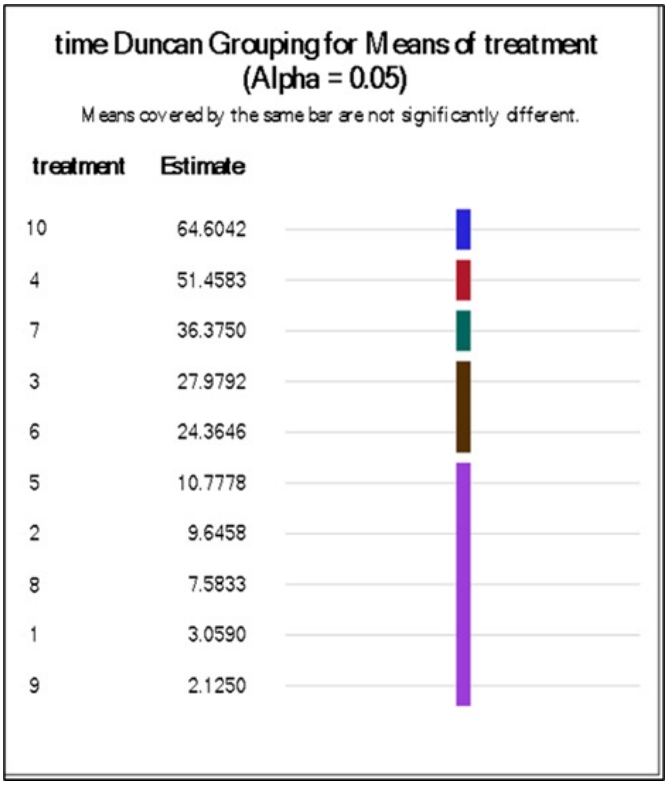

Figure 3: Time Duncan grouping for means of treatment

2016). However, both these plant parts have great economic value, both as herbal plants and for flavoring food or being consumed directly. Therefore, the use of these plant parts for developing *Ae. aegypti* mosquito larvicides has less economic potential. Thus, for the follow-up studies, these two treatments were not taken into consideration.

3.1.3 Third experimental results

The probability value related to the treatment effect was approximately 0.0001, which was less than 0.05, leading to the rejection of the null hypothesis that there is no difference between treatments in terms of survival time. The survival of larvae with respect to each treatment was shown in Figure 4a. Accordingly, larvae treated with T1 had higher survival rates, while larvae treated with T8 had lower survival rates. The survival time of larvae with T5 and T6, as well as T7 and T8, were the same. The probability value related to the treatment effect was 0.2389, which was greater than 0.05, so the null hypothesis, which states that there is no difference between generations in survival time, cannot be rejected (Figure 4b). The effect of the survival of larvae in each generation is the same, while F0 has the highest and F2 has the lowest survival time. Since there are no validated specific criteria for classifying the larvicidal potential of new products, the values of the minimum lethal concentration that eliminates 50% and 90% of the population (LC50 and LC90) are used as a criterion for activity. Larvicidal effects observed with Treatments 9 (Clove) have LC50 = 133.4 ppm and LC90 = 659.1 ppm, respectively.

4 Conclusion

There are underutilized plant varieties, such as, Garlic, Dawul Kurundu, Ginger, Giant Mexican Sunflower, Kapparawalliya, Betel Leaves, Kuppameniya, Maduruthala, and Clove, which have the larvicidal potential of its leaf extracts against dengue vector, *Ae. Aegypti*. However, their plant parts are not widely known or utilized in industry. None of these plant leaves has economic value, except for betel leaves. Therefore, larvicidal preparations from these plant leaves create value addition for these underutilized plants. Clover leaf powder concentration above the 1 gram per 100 ml of water reduced the larvae development by more than 80% and could be recommended for application to water-holding containers with potential *Ae. aegypti* mosquito breeding. This study focused only on crude extracts prepared

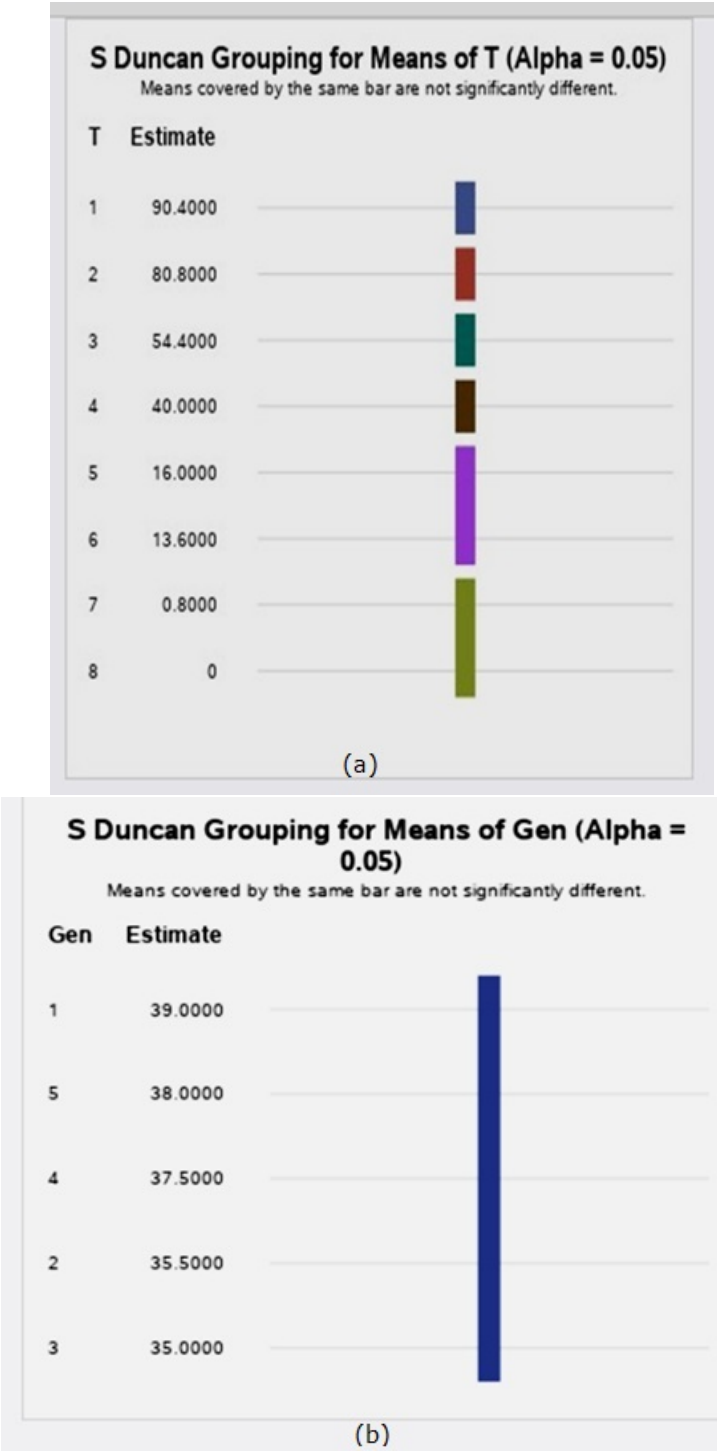

Figure 4: a. Survival Duncan grouping for means of treatment; b. Survival Duncan grouping for means of generations

from different plant parts. Therefore, further studies of larvicidal activities of organic solvent extracts of the same plant species, and analysis of their chemical composition using a GC-MS, are recommended for future research work.

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Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

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Table 1: Selected local plant species for the study

Ref. no.	Common Name	Botanical Name
1	Kapukinissa	*Abelmoschus moschatus*
2	Kuppameniya	*Acalypha indica*
3	Garlic	*Allium sativum*
4	Snap ginger (Heen araththa/Katu keeriya)	*Alpinia calcarata*
5	Indian birthwort	*Aristolochia Indica*
6	Yaki naran	*Atalantia ceylanica*
7	Neem (Kohomba)	*Azadirachta indica*
8	Papaya	*Carica papaya*
9	Chrysanthemum	*Chrysanthemum indicum*
10	Val kurundu	*Cinnamomum dubium*
11	Sour orange	*Citrus aurantium*
12	Lime	*Citrus hystrix*
13	Turmeric (Kaha)	*Curcuma longa*
14	Sea holly (Andhu)	*Eryngium foetidum*
15	Mexican lilac (vetamaara)	*Gliricidia sepium*
16	Fowl Manna Grass	*Glyceria striata*
17	Shrub verbena (Gadapana)	*Lantana camara var. aculeata*
18	Maduka	*Madhuca longifolia*
19	Mint	*Mentha spicata*
20	Lime berry (Etteriya)	*Murraya paniculata*
21	Dawul kurundu	*Neolitsea cassia*
22	Holybasil (Maduruthala)	*Ocimum tenuiflorum*
23	Pamburu	*Pamburus missions*
24	Pawatta	*Pavetta indica*
25	Betel	*Piper betle*
26	Pepper	*Piper nigrum*
27	Kapparawalliya	*Plectranthus amboinicus*
28	Akmalla	*Spilanthes paniculata*
29	June plum (Ambarella)	*Spondias dulcis*
30	Clove	*Syzygium aromaticum*
31	Giant mexican sunflower	*Tithonia diversifolia*
32	Ginger	*Zingiber officinale*

Table 2: Crude extracts (in water) exhibiting 100% mortality (within 48 hours) during preliminary laboratory trials

	Plant species	Voucher number (e.g., collected part-#/yr/mm/dd)	Plant part	Mortality time (hr. min)
1	*Lantana camara*	L-9/20/12/17	Leaves	1 hr 21 min
		F-1/20/12/17	Flowers	Negative
2	*Azadirachta indica*	L-8/20/12/17	Leaves	2 hr 18 min
3	*Zingiber officinale*	L-7/20/12/17	Leaves	Negative
		R-1/20/12/19	Rhizome	**0 hr 20 min**
4	*Eryngium foetidum*	L-6/20/12/17	Leaves	1 hr 11 min
5	*Spondias dulcis*	L-11/20/12/18	Leaves	Negative
6	*Cinnamomum verum*	L-5/20/12/17	Leaves	1 hr 17 min
7	*Pamburus missions*	L-20/20/12/24	Leaves	Negative
8	*Plectranthus zatarhendi*	L-4/20/12/17	Leaves	Negative
9	*Syzygium aromaticum*	S-2/20/12/24	Seeds	**0 hr 45 min**
		L-21/20/12/24	Leaves	**0 hr 05 min**
10	*Atalantia ceylanica*	L-10/20/12/17	Leaves	Negative
11	*Alpinta calcarata*	L-22/20/12/24	Leaves	Negative
12	*Ocimum tenuiflorum*	L-12/20/12/19	Leaves	**0 hr 13 min**
		F-2/20/12/18	Flowers (Yellow colour)	Negative
13	*Chrysanthemum indicum*	F-3/20/12/18	Flowers (Orange colour)	Negative
		L-13/21/12/19	Leaves	Negative
14	*Curcuma longa*	R-2/20/12/19	Rhizome	Negative
15	*Aristolochia indica*	L-3/20/12/17	Leaves	Negative
16	*Citrus aurantium*	L-2/20/12/17	Leaves	Negative
17	*Murraya paniculata*	L-19/20/12/23	Leaves	Negative
18	*Neolitsea cassia*	L-24/21/01/01	Leaves	**0 hr 26 min**
19	*Cinnamomum dubium*	L-25/21/01/01	Leaves	Negative
20	*Plectranthus amboinicus*	L-26/21/01/02	Leaves	**0 hr 07 min**
21	*Gliricidia sepium*	L-14/20/12/19	Leaves	1 hr 21 min
22	*Piper betle*	L-15/20/12/19	Leaves	**0 hr 40 min**
23	*Acalypha indica*	L-1/20/12/15	Leaves	**1 hr 00 min**
24	*Piper nigrum*	L-16/20/12/19	Leaves	Negative
		S-3/20/12/24	Seeds	Negative
25	*Madhuca longifolia*	S-1/20/12/19	Seeds	Negative
26	*Tithonia diversifolia*	L-17/20/12/19	Leaves	**0 hr 31 min**
27	*Carica papaya*	L-18/20/12/19	Leaves	1 hr 19 min
28	*Citrus hystrix*	L-27/21/04/06	Leaves	Negative
29	*Mentha spicata*	L-28/21/04/06	Leaves	Negative
30	*Allium sativum*	B-1/21/01/01	Bulbs	**0 hr 18 min**
31	*Abelmoschus moschatus*	L-29/21/05/03	Leaves	Negative
32	*Glyceria striata*	L-30/21/05/03	Leaves	Negative

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.
	T2	−398.958*	148.923	0.01
	T3	−1497.292*	148.923	0
	T4	−2903.542*	148.923	0
	T5	−463.792*	148.923	0
T1	T6	−669.083*	148.923	0
	T7	−1998.542*	148.923	0
	T8	−3666.042*	148.923	0
	T9	59.792	148.923	0.69
	T10	−267.708	148.923	0.07
	T1	398.958*	148.923	0.01
	T3	−1098.333*	148.923	0
	T4	−2504.583*	148.923	0
	T5	−64.833	148.923	0.66
T2	T6	−270.125	148.923	0.07
	T7	−1599.583*	148.923	0
	T8	−3267.083*	148.923	0
	T9	458.750*	148.923	0
	T10	131.25	148.923	0.38
	T1	1497.292*	148.923	0
	T2	1098.333*	148.923	0
	T4	−1406.250*	148.923	0
	T5	1033.500*	148.923	0
T3	T6	828.208*	148.923	0
	T7	−501.250*	148.923	0
	T8	−2168.750*	148.923	0
	T9	1557.083*	148.923	0
	T10	1229.583*	148.923	0
	T1	2903.542*	148.923	0
	T2	2504.583*	148.923	0
	T3	1406.250*	148.923	0
	T5	2439.750*	148.923	0
T4	T6	2234.458*	148.923	0
	T7	905.000*	148.923	0
	T8	−762.500*	148.923	0
	T9	2963.333*	148.923	0
	T10	2635.833*	148.923	0
	T1	463.792*	148.923	0
	T2	64.833	148.923	0.66
	T3	−1033.500*	148.923	0
	T4	−2439.750*	148.923	0
T5	T6	−205.292	148.923	0.17
	T7	−1534.750*	148.923	0
	T8	−3202.250*	148.923	0
	T9	523.583*	148.923	0
	T10	196.083	148.923	0.19
	T1	669.083*	148.923	0
	T2	270.125	148.923	0.07
	T3	−828.208*	148.923	0
	T4	−2234.458*	148.923	0
T6	T5	205.292	148.923	0.17
	T7	−1329.458*	148.923	0
	T8	−2996.958*	148.923	0
	T9	728.875*	148.923	0
	T10	401.375*	148.923	0.01
	T1	1998.542*	148.923	0
T7				

	T2	1599.583*	148.923	0
	T3	501.250*	148.923	0
	T4	−905.000*	148.923	0
	T5	1534.750*	148.923	0
	T6	1329.458*	148.923	0
	T8	−1667.500*	148.923	0
	T9	2058.333*	148.923	0
	T10	1730.833*	148.923	0
	T1	3666.042*	148.923	0
	T2	3267.083*	148.923	0
	T3	2168.750*	148.923	0
	T4	762.500*	148.923	0
T8	T5	3202.250*	148.923	0
	T6	2996.958*	148.923	0
	T7	1667.500*	148.923	0
	T9	3725.833*	148.923	0
	T10	3398.333*	148.923	0
	T1	−59.792	148.923	0.69
	T2	−458.750*	148.923	0
	T3	−1557.083*	148.923	0
	T4	−2963.333*	148.923	0
T9	T5	−523.583*	148.923	0
	T6	−728.875*	148.923	0
	T7	−2058.333*	148.923	0
	T8	−3725.833*	148.923	0
	T10	−327.500*	148.923	0.03
	T1	267.708	148.923	0.07
	T2	−131.25	148.923	0.38
	T3	−1229.583*	148.923	0
	T4	−2635.833*	148.923	0
T10	T5	−196.083	148.923	0.19
	T6	−401.375*	148.923	0.01
	T7	−1730.833*	148.923	0
	T8	−3398.333*	148.923	0
	T9	327.500*	148.923	0.03

Table 3: The mean difference is significant at the 0.05 level.*
Results of the Post Hoc tests (Multiple comparison with dependent variable of larvae motility time.