RESEARCH ARTICLE

Comparative evaluation of the effect of phytochemicals of garlic (*Allium sativum*) ethanolic extract against *Aedes albopictus* and *Culex quinquefasciatus* mosquitoes in Sri Lanka

W.R.G.W.N. Rajapaksha, W.A.P. P. De Silva and T.C. Weeraratne*

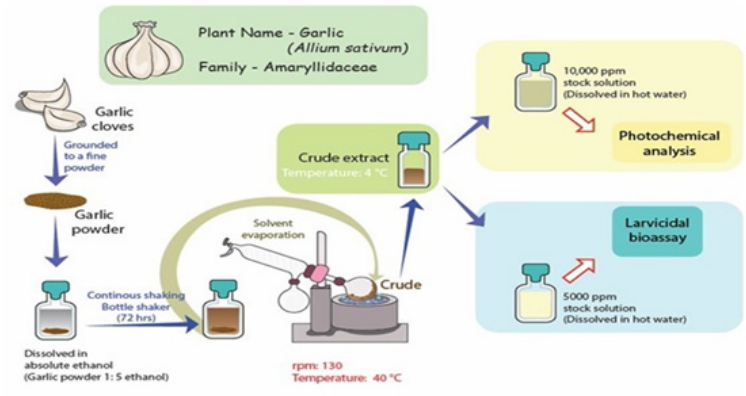
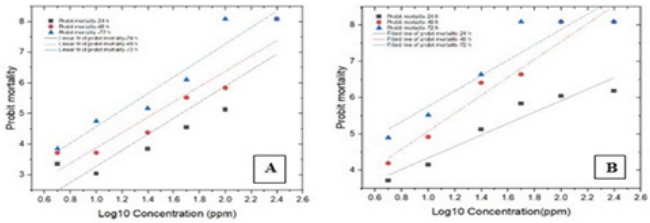


Illustration of the crude extraction from garlic and the experimental process



Results obtained for log-probit analysis from larvicidal bioassays for (A) *Aedes albopictus* and (B) *Culex quinquefasciatus*

Highlights

- Ethanolic garlic clove extract exhibits significant efficacy against *Aedes albopictus* and *Culex quinquefasciatus* larvae
- Larvae of both *Ae. albopictus* and *Cu. quinquefasciatus* show concentration-dependent mortality when exposed to garlic clove extract
- Flavonoids, saponins, and reducing sugars are the common bioactive compounds of garlic clove extract
- Effectiveness of bioactivity of garlic clove extracts remains over a year-long period

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Comparative evaluation of the effect of phytochemicals of garlic (*Allium sativum*) ethanolic extract against *Aedes albopictus* and *Culex quinquefasciatus* mosquitoes in Sri Lanka

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Abstract: Botanical extracts offer sustainable and eco-friendly alternatives to synthetic insecticides for managing insect pests, including mosquitoes. This research focuses on the potential of ethanolic garlic extract as a larvicide against *Aedes albopictus* and *Culex quinquefasciatus* mosquito species in Sri Lanka. Third to early fourth instar larvae were exposed to six concentrations of ethanolic garlic extract (ranging from 5 to 250 ppm) for 72 hours to assess efficacy. The experiment, repeated four times with controls, monitored daily mortalities. Lethal concentrations required to eliminate 50% (LC_{50}) and 90% (LC_{90}) of larvae at 24, 48, and 72 hours were determined through regression analysis. A phytochemical analysis was conducted to assess the compounds present in the garlic extract. Positive correlations were observed between garlic concentration and mortality percentages during each exposure period for both *Ae. albopictus* and *Cu. quinquefasciatus* larvae. LC_{50} values for *Ae. albopictus* larvae were 45.5 ppm, 28.0 ppm, and 14.4 ppm at 24, 48, and 72 hours respectively, with corresponding LC_{90} values of 140.0 ppm, 91.0 ppm, and 42.9 ppm. For *Cu. quinquefasciatus* larvae, LC_{50} values were 26.3 ppm, 9.4 ppm, and 4.4 ppm, while LC_{90} values were 169.8 ppm, 30.7 ppm, and 17.6 ppm for the same exposure periods. The garlic ethanolic extract retained its flavonoids, saponins, and reducing sugars even after a year of extraction, as revealed by phytochemical analysis. The study underscores the potent toxicity of garlic extract against mosquito larvae, with LC_{50} values below 50 ppm. These findings highlight the potential of garlic extracts as effective larvicides for combating mosquito vectors, contributing to environmentally friendly pest management strategies.

***keywords*:** *Aedes albopictus*; *Culex quinquefasciatus*; Garlic extract; Larvicidal; Lethal Concentrations; Phytochemical analysis

INTRODUCTION

Vector-borne diseases (VBDs) account for approximately 17% of the global burden of infectious diseases, resulting in over 700,000 deaths annually (WHO, 2014, 2020). Mosquito-borne diseases (MBDs) pose a significant threat to public health worldwide, and in Sri Lanka, with its tropical warm climate providing ideal conditions for mosquito breeding and disease transmission (Sirisena et al., 2017). The country is home to approximately 142 species of mosquitoes, representing high diversity and abundance (Chathuranga et al., 2018). These mosquitoes act as vectors for major MBDs such as dengue, chikungunya, malaria, and filariasis, which have caused significant morbidity and mortality in Sri Lanka. *Aedes aegypti* and *Ae. albopictus* are the principal vectors of dengue and chikungunya viruses in Sri Lanka (Sirisena & Noordeen, 2014). Dengue has been endemic in the country since the mid-1960s and continues to contribute to high fatality rates (Messer et al., 2002). In 2022, there were 42,729 reported dengue cases, with 39 fatalities, surpassing the number of cases in 2021 (33,000 cases and 26 deaths) (WHO, 2020). Chikungunya re-emerged in Sri Lanka in 2006 after a four-decade absence, affecting around 40,000 individuals in 2006 and 2007 (Hapuarachchi et al., 2010). *Culex quinquefasciatus* mosquitoes are major vector for filariasis. Sri Lanka successfully eradicated lymphatic filariasis as a public health issue in 2016, but the presence and abundance of the vector pose a risk of re-emergence (Chandrasena et al., 2018). Additionally, wild animals, such as birds, can act as reservoir hosts for these pathogens, which can be transmitted to humans via zoophilic mosquitoes (Chathuranga et al., 2018).

Considering the limited availability of specific drugs or vaccines for MBDs, vector control measures remain the most effective method for prevention (Erlanger et al., 2008; Achee et al., 2015). However, chemical interventions have drawbacks such as resistance development, environmental persistence, and adverse effects on non-target organisms. As a result, plant extracts have gained attention as promising alternatives owing to their bioactive properties and cost-effectiveness. Numerous plant species, including peppermint, lemongrass, rosemary, clove, citronella, thyme, spearmint, sweet orange, catnip, and basil, have shown insecticidal and repellent effects against various pests, including mosquitoes (Das et al., 2007; Hikal et al., 2017; Koul & Walia, 2009).

Garlic extracts (*Allium sativum*) has been identified as an effective larvicide against mosquitoes in several studies, particularly in South Asian and African regions. The earliest study reported was by Amonkar & Reeves (1970), where the efficacy of garlic extract was tested on larvae of *Aedes* sp. and *Culex* sp. Several examples include studies by Bilal et al. (2012), Rahmah et al. (2019), Yarsi & Munawaroh (2021) and Aminu et al. (2022) in which the efficacy of organic solvent extracts of garlic was demonstrated against larvae of *Aedes* sp. Other than that, Iqbal et al. (2018) revealed in their study that an aqueous

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extract of garlic was the most potent larvicide against *Cu. quinquefasciatus* among several other plant extracts. Kalu et al. (2010) also indicated that ethanolic extract of garlic is highly efficacious against the second, third and fourth instar stages of *Cu. quinquefasciatus*.

The reported active components in garlic cloves are allicin, diallyl disulfide, S-allyl cysteine, and diallyl trisulfide (Mikaili et al., 2013; Rahmah et al., 2019). Allicin, the most important compound, can interfere with RNA synthesis and lipid production in organisms, without affecting mammalian cells (Yarsi & Munawaroh, 2021). Garlic extracts are also claimed to contain flavonoids, which act as respiratory inhibitors (Rahmah et al., 2019). These properties make garlic a best candidate as a biopesticide for mosquito control.

Given Sri Lanka's tropical climate and high susceptibility to mosquito-borne diseases, it's crucial to assess the efficacy of plant extracts as replacements for synthetic insecticides. Despite the abundance of plant species in Sri Lanka, research on botanical biopesticides for mosquito control is limited compared to other pest management applications. Although there are several reported studies on application of garlic extracts to control pests of stored products and agricultural pests such as *Callosobruchus maculatus* (Niranjana & Karunakaran, 2019) and *Tribolium castaneum* (Karunakaran & Niranjana, 2019) in Sri Lanka, there are no reported studies conducted against mosquitoes up to date. Similar to synthetic insecticides, botanicals can inhibit acetylcholinesterase (the target-site of organophosphates and carbamates), GABA gated chloride channels and, disrupt sodium and chloride channels of the nervous system (target-sites of organochlorines and pyrethorids). Therefore, the response of mosquitoes to any biopesticide can vary depending on the insecticide exposure history, the target population and also on the environmental factors (Glunt et al., 2011; Gosh et al., 2012; Nkya et al., 2013; Owusu et al., 2017). Therefore, it is essential to conduct laboratory studies before implementing these botanical extracts in the field. Therefore, this study was conducted to evaluate the larvicidal activity of ethanolic garlic extracts against *Ae. albopictus* and *Cu. quinquefasciatus* mosquitoes in Sri Lanka under laboratory conditions and identify the secondary metabolites present in the extracts. These findings can serve as a foundation for further research and the development of novel mosquito control strategies using bioactive ingredients from plant materials.

MATERIALS AND METHODS

Mosquito larvae for larval bioassays

Mosquito larvae for bioassay experiments were obtained from *Ae. albopictus* and *Cu. quinquefasciatus* mosquito colonies established at the insectary of the Department of Zoology, University of Peradeniya. The colony was maintained at room temperature (28 ± 2) °C under 12L:12D photoperiod and ± 70% Relative Humidity (RH). Late third and early fourth instar larvae were used for the bioassay experiments.

Preparation of the ethanolic crude extract from garlic

Manually separated 1 kg of garlic cloves (*Allium sativum*), collected from the local market of Digana on 5th of January, 2022, were washed and hot air dried at 75- 85°C. Then, the dried cloves were ground in an electrical grinder to obtain a fine powder. The fine garlic powder was mixed with 100% ethanol in 1:5 ratios and loaded to a bottle shaker and was shaken for 72 h at room temperature (28 °C ± 2), at 100 rpm. The garlic–ethanol mixture was filtered using a double layer of cotton cloth and a Whatman No. 1 filter paper. Ethanol was removed using a vacuum rotary evaporator (HS-2005V-N, Hanshin Scientific, South Korea) under the inner temperature of 40- 41°C and 130 rpm speed to obtain a semisolid crude extract. The crude extract was stored at 4 °C temperature until later use.

Preparation of the concentration series

Stock solution of 5000 ppm was prepared by dissolving 50 mg of the crude garlic extract in 10 mL of hot (40 °C) distilled water, stored in screw-cap glass vials, and kept in the refrigerator at 4 °C. A series of concentrations determined by preliminary experiments; 250 ppm, 100 ppm, 50 ppm, 25 ppm, 10 ppm, and 5 ppm was prepared. The bioassays for *Ae. albopictus* and *Cu. quinquefasciatus* were conducted in a final volume of 5 mL and 20 mL by mixing the appropriate volume of the stock solution with distilled water.

Evaluation of larvicidal activity

Ten healthy late-third instar larvae of *Ae. albopictus* and *Cu. quinquefasciatus* were separately exposed to each test concentration. The larvae were provided with ground fish food as the food source. Four replicates were conducted for each test concentration. Six controls were performed simultaneously with distilled water. All the tests were conducted at room temperature 28± 2 °C and a photoperiod of 12L:12D. Larval mortalities in treatments and control experiments were recorded at 24 h, 48 h, and 72 h exposure periods. Dead larvae were identified when they failed to move after probing with a glass rod in the siphon or cervical region. All the experiments were repeated if control mortality was more than 20%.

Data analysis

Percentage mortality values were calculated and corrected using Abbott's formula if the mortality value for controls were more than 5%. Mean percentage mortality values corresponding to each concentration were calculated and converted to probit values using Finney's table (Hamidi et al., 2014). The data were subjected to probit-log (dose) regression analysis using Minitab Software (version 18.0) to calculate lethal concentrations; LC_{50} and LC_{90} at 24 h, 48 h and 72 h.

Corrected mortality was calculated as follows using Abbott's formula;

$$\text{Corrected \% mortality} = \frac{(\text{\% mortality in treated} - \text{\% mortality in control})}{100 - \text{\% mortality in control}} \times 100$$

Correlation analyses were conducted using Minitab software (version 18.0) for each exposure period to determine the correlation between the mean percentage mortalities and concentrations of garlic extract.

Phytochemical analysis of ethanolic garlic extract

The qualitative phytochemical analysis was carried out as shown in Table 1, to identify different phytochemicals in ethanolic garlic extract following the protocols described by Soni & Sosa (2013), Banu & Catherin (2015), Esienanwan et al. (2020), Nazir & Chauhan (2019), Priska et al. (2019), and Singh & Kumar (2017). For all the tests, a 10 000 ppm stock solution of the ethanolic crude extract was prepared using distilled water.

RESULTS

Larvicidal effect of garlic extract on *Aedes albopictus* and *Culex quinquefasciatus*

The results indicated a gradual increase in *Ae. albopictus* and *Cu. quinquefasciatus* larval mortalities with increasing concentration and exposure period.

A positive correlation was observed between the concentration of garlic extract and the percentage mortalities of *Ae. albopictus* larvae at 24 h ($r= 0.983$, $p<0.05$) and 48 h ($r = 0.859$, $p<0.05$) exposure periods. Although *Ae. albopictus* mosquito larvae showed positive correlation after 72 h ($r= 0.732$, $p>0.05$) exposure period, this correlation was not significant. A non- significant positive correlation was observed between the concentration of garlic extract and the percentage mortalities of *Cu. quinquefasciatus* larvae at 24 h ($r=0.707$, $p=0.116$), 48 h ($r=0.592$, $p=0.216$) and 72 h ($r=0.676$, $p=0.140$) exposure periods.

The results derived from the regression analysis of *Ae. albopictus* and *Cu. quinquefasciatus* larvae are presented in Figure 1 with the obtained regression equations. The results revealed similarities and differences in *Ae. albopictus* and *Cu. quinquefasciatus* larvae responses to the treatments over different time intervals. For *Ae. albopictus* larvae, the LC_{50} values decrease progressively as the exposure time increases. At 24 hours, the LC_{50} value is 45.5 ppm, which decreases to 28.0 ppm at 48 hours and further decreases to 14.4 ppm at 72 hours. Similarly, the corresponding LC_{90} values for *Ae. albopictus* larvae show a decreasing trend, with values of 140.0 ppm, 91.0 ppm, and 42.9 ppm for 24 hours, 48 hours, and 72 hours, respectively. The concentration required to kill 90% (LC_{90}) of the population at all three-time points was approximately three times higher than that of the concentration needed to kill 50% of the larvae (LC_{50}).

In contrast to *Ae. albopictus* larvae, the LC_{50} values for *Cu. quinquefasciatus* larvae decrease significantly with each successive time interval. The LC_{50} values for *Cu. quinquefasciatus* larvae at 24 hrs, 48 hrs, and 72 hrs were 26.3 ppm, 9.4 ppm, and 4.4 ppm, respectively. The LC_{50} values for *Cu. quinquefasciatus* at all three time points were considerably lower than that for *Ae.*

Table 1: Procedures followed for phytochemical assays.

Bioactive components	Phytochemical assays
Phenols	2 mL of the stock was mixed with two drops of 5% ferric chloride and observed for the development of bluish-dark green colouration (Singh & Kumar, 2017; Nazir & Chauhan, 2019; Priska et al., 2019; Esienanwan et al., 2020).
Quinones	5 mL of the stock was mixed with a few drops of diluted sodium hydroxide and observed for blue, green or red colouration (Soni & Sosa, 2013).
Flavonoids	3 mL of the stock was treated with 2 mL of diluted sodium hydroxide. The formation of intense yellow colour, which becomes colourless upon the addition of a few drops of Hydrochloric acid was observed (Singh & Kumar, 2017; Esienanwan et al.,2020).
Cardiac glycosides	1 mL of the stock was treated with 1.5 mL of glacial acetic acid and 5% ferric chloride. Thereafter, about 2 mL of conc. Sulphuric acid was added carefully from the side of the test tube and the appearance of a brown ring at the interphase or formation of blue colouration in the acetic acid layer was observed (Singh & Kumar, 2017; Nazir & Chauhan,2019; Esienanwan et al., 2020)
Terpenoids	About 5 mL of 1% stock was mixed with 2 mL of chloroform and 3 mL of conc. Sulphuric acid was added carefully to form a layer. A reddish-brown colouration was observed for the presence of Terpenoids (Soni & Sosa, 2013).
Coumarin	About mL of 10% sodium hydroxide and chloroform was added to a small amount of the stock. A yellow colour was observed for the presence of Coumarin (Soni & Sosa, 2013).
Saponin	About 1 mL of the 1% stock was mixed with 5 mL of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication of the presence of Saponins (Banu & Cathrine, 2015; Singh & Kumar, 2017; Esienanwan et al., 2020)
Reducing sugar	About 1 mL of the 1% stock was mixed with a small amount of Benedict's reagent and heated in a water bath for 5-10 minutes. The appearance of green, yellow or red colouration was observed for the presence of reducing sugar (Singh & Kumar, 2017)

albopictus indicating relatively higher susceptibility of *Cu. quinquefasciatus* larvae to garlic crude extract. The corresponding LC_{90} values for *Cu. quinquefasciatus* larvae also demonstrate a similar decreasing trend, with values of 169.8 ppm, 30.7 ppm, and 17.6 ppm for 24 hours, 48 hours, and 72 hours, respectively. Similar to the LC_{50}, the LC_{90} at 48 h and 72 hours for *Cu. quinquefasciatus* sp. larvae were significantly lower than that for *Ae. albopictus* larvae. However, LC_{90} for *Cu. quinquefasciatus* after 24 h was slightly higher than that for *Ae. albopictus*. The concentration required to kill 90% (LC_{90}) of the population after a 24 h exposure period is approximately seven times higher than that of the concentration required to kill 50% (LC_{50}) of the population whereas, the LC_{90} value at 48 h and 72 h exposure is approximately three-four times higher than the corresponding LC_{50} value.

Phytochemical analysis of ethanolic garlic extract

The garlic extract was tested for the presence of phenolic compounds, quinones, flavonoids, terpenoids and steroids, cardiac glycosides, coumarin, saponins and reducing sugar. The results revealed the presence of three phytochemicals; flavonoids, saponins and reducing sugar in the ethanolic garlic extracts used for the study.

DISCUSSION

The importance of vector control in mitigating the spread of mosquito-borne diseases cannot be neglected. Although traditional chemical insecticides have been successful in keeping mosquitoes under control, their prolonged usage poses challenges that urge for innovation of eco-friendly and sustainable alternatives. One such alternative is the use of biological insecticides derived from plant sources. This study evaluated the potential larvicidal activity of garlic extract against *Ae. albopictus* and *Cu. quinquefasciatus* mosquito in Sri Lanka and the results confirmed the larvicidal properties of garlic extracts against *Ae. albopictus* and *Cu. quinquefasciatus* larvae in Sri Lanka.

The concentration-response curves clearly demonstrated the significant effectiveness of garlic extracts, even at lower concentrations. Previous studies conducted by Aminu et al. (2022) in Nigeria and Amonkar & Reeves (1962) in California, USA, also reported similarly high efficacy against *Aedes* larvae following 24-hour exposure. Despite the geographical differences in these studies, the consistent results underscore the potential of garlic as an effective biopesticide, provided further research is conducted.

Laojun et al. (2020) reported that an experimental material is highly effective if the LC_{50} value is less than 50 ppm. In our study, we received, low LC_{50} values (<50ppm) for *Ae. albopictus* and *Cu. quinquefasciatus* larvae confirming the high effectiveness of the ethanolic extract of garlic as a potential larvicide. Aminu et al. (2022) reported an LC_{50} value of 42.5 ppm for *Aedes* larvae exposed to aqueous garlic extract for 24 hours which was almost similar to what was observed in the present study.

Ghosh et al. (2012) conducted a comparative analysis of over 150 plant extracts against *Aedes, Culex,* and *Anopheles* mosquitoes and stated that the efficacy of phytochemicals against mosquito larvae may also vary depending on the solvent used for extraction, which is attributed to differences in solvent polarity. This statement is further confirmed with the differences in LC_{50} values between the present study and study by Amonkar & Reeves (1962), where methanol is used to prepare the garlic extract. The later study demonstrated a LC_{50} of 33.7 ppm against *Aedes* larvae which is lower than the present study.

Moreover, significant disparities in the LC_{50} and LC_{90} values of garlic extract against *Aedes* larvae have been reported from different parts of the world. For example, Susheela et al. (2016) observed much higher LC_{50} values ranging from 10 000 ppm to 15 000 ppm for fourth instar stages of *Ae. aegypti*. The extraction method followed by these authors is, however, different as they have used simple cold extraction for garlic extraction. These variations highlight the influence of extraction methods on the efficacy of garlic extract.

Numerous studies have discovered that garlic's aqueous and ethanolic extracts are both very efficient against *Cu.*

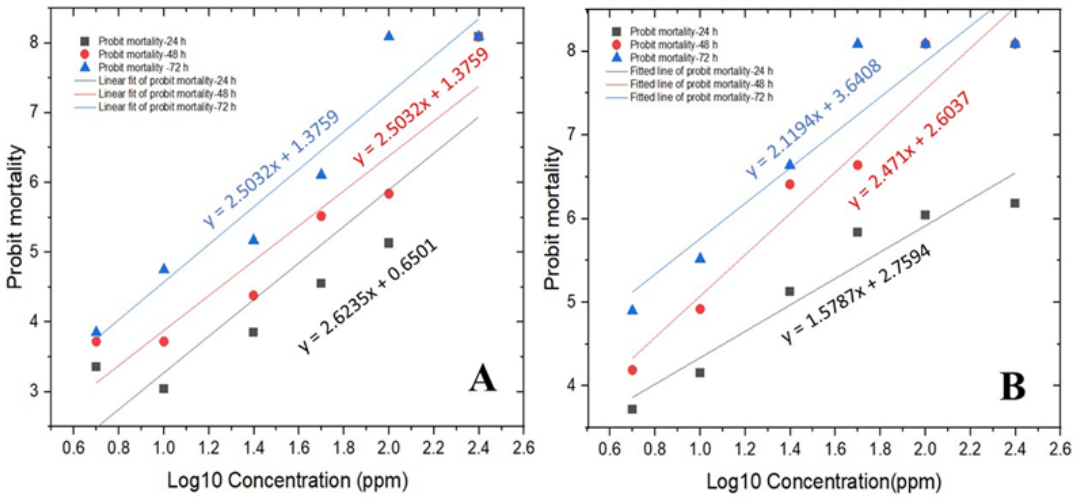

Figure 1: Log-probit dosage curves obtained for (A) *Aedes albopictus* and (B) *Culex quinquefasciatus* larvae exposed to six different concentrations (250 ppm- 5 ppm) of garlic extract for 24 h, 48 h and 72 h

quinquefasciatus larvae. Kalu et al. (2010) reported LC_{50} values of 144.54 ppm, 165.70 ppm, and 184.18 ppm for second, third, and fourth instar *Culex* larvae, respectively, when exposed to ethanolic garlic extract. In contrast, Iqbal et al. (2018) reported a significantly higher LC_{50} value of 13,700 ppm for *Culex* larvae exposed to cold aqueous garlic extract. The lower LC_{50} values obtained in the present study indicate a higher susceptibility of *Cu. quinquefasciatus* larvae to garlic extract compared to these already published data. This could be due to the relatively lower resistance levels observed in the Sri Lankan population of *Cu. quinquefasciatus* mosquitoes, likely resulting from their reduced historical exposure to insecticides (Karunaratne & Hemingway, 2001).

In comparison to the LC_{50} values obtained for *Ae. albopictus* of the present study, *Cu. quinquefasciatus* shows significantly lower LC_{50} values for all the exposure periods. Furthermore, 86.25% of the tested *Cu. quinquefasciatus* larvae were dead by the third day of exposure to the treatments. The cumulative larval mortality for *Ae. albopictus* after third day of exposure was 66.67%. This confirms that *Cu. quinquefasciatus* larvae are relatively more susceptible to garlic extract compared to *Ae. albopictus* larvae.

The presence or absence of phytochemical compounds in a plant extract may differ according to the extraction solvent, the plant part used for extraction, and the method of extraction used for analysis (Esienanwan et al., 2020; Nazir & Chauhan, 2019; Singh & Kumar, 2017). Phytochemical analysis revealed the presence of flavonoids, saponins, and reducing sugars in the ethanolic garlic extracts used in this study. Similar studies by Priska et al. (2019), Singh & Kumar (2017), and Nazir &Chauhan (2019) have also identified these phytochemicals in garlic extracts. Flavonoids are water-soluble polyphenolic molecules, belonging to the polyphenol family, that can act as respiratory inhibitors by inhibiting the electron transport system and ATP production causing possible larvicidal effects (Singh & Kumar, 2017; Yarsi & Munawaroh, 2021). Xu & Lee (2001) stated in their study that flavonoids can disrupt bacterial growth by inhibiting protein synthesis. This antibacterial property of flavonoids could also contribute to the larvicidal activity of garlic.

Saponins are steroid or triterpenoid glycosides distinguished by their bitter or astringent flavour and foaming effects (Singh & Kumar, 2017). Studies conducted to test the efficacy of several plant extracts, which are positive for saponins as the major compound, have demonstrated high larvicidal activity against *Ae. aegypti* (Jawale, 2014; Bagavan et al., 2008) and *Cu. quinquefasciatus* (Bagavan et al., 2008) larvae. These studies further demonstrate that the saponin content of garlic accounts for its larvicidal effect against *Ae. albopictus* and *Cu. quinquefasciatus* mosquitoes. In future investigations, the phytochemical analysis may be used to assist in further analyzing the components of garlic extracts and developing new, efficient insecticides.

To further enhance the understanding of garlic extract components and develop more efficient insecticides, future investigations could involve in-depth phytochemical analyses. Additionally, comparative studies on extraction methods and solvents can be conducted to determine the most effective approach for obtaining garlic extracts with optimal larvicidal properties against *Ae. albopictus* and *Cu. quinquefasciatus* species. The knowledge gained from this study can also be extended to combat agricultural and stored product pests. However, further comprehensive research and continuous surveillance, such as quasi-field or pilot studies, are essential before applying garlic extracts to natural ecosystems.

CONCLUSION

The present study revealed the high efficacy of garlic ethanolic extract as a larvicide against mosquitoes of *Ae. albopictus* and *Cu. quinquefasciatus* in Sri Lanka with LC_{50} values lower than 50 ppm. Moreover, the garlic ethanolic extracts proved to contain several important secondary metabolites with larvicidal potential such as flavonoids and saponins. In conclusion, garlic could be considered a promising eco-friendly biopesticide to be used in mosquito control programmes in the future.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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