Larvicidal activity and Histopathological changes of Cinnamomum burmannii, Syzygium aromaticum extracts and their combination on Culex pipiens

Nael Abutaha *, Fahd A. AL-mekhlafi, Mohammed S. Al-Khalifa, Mohamed A. Wadaan

Department of Zoology, College of Science, King Saud University, P.O. Box 2455 Riyadh, 11451, Saudi Arabia

A R T I C L E   I N F O

Article history:
Received 19 October 2021
Revised 21 November 2021
Accepted 14 December 2021
Available online 17 December 2021

Keywords:
Cinnamomum burmannii
Syzygium aromaticum
Larvicidal
Histopathological
Culex pipiens

A B S T R A C T

In order to develop an eco-friendly botanical larvicide alternative to the synthetic larvicides, extracts were prepared from the Cinnamomum burmannii (C.B.) and Syzygium aromaticum (S.A.) with hexane using a sonicator. The extracts were evaluated for larvicidal activity individually and in combination against the Culex pipiens larvae. The LC50 value of C.B. and the S.A. hexane extracts tested individually were 184.2 and 363.7 µg/mL against Cx. pipiens respectively. All the combinations of the extract of C.B. and S.A. showed synergistic factors higher than one. Among the different ratios of extracts, the SA25%:CB75% extract was found to be more toxic than the other combinations (LC50: 125.7 µg/mL). Midgut cells treated with S.A. 25%: C.B. 75% extract showed severe morphological alterations such as degradation of microvilli; degeneration of epithelial cells, and peritrophic membrane; loss of nuclei, irregular and damaged of microvilli. The extract has a promising larvicidal potential against Cx. pipiens, However, the extract was toxic against HUVEC cells, as evident from MTT and cell morphology. Further investigation is required to assess the toxicity of the extract on aquatic animals.

© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Mosquitoes are a significant vector for many illnesses that affect animals and humans (Kovenden et al., 2012), such as Malaria, Dengue, Chikungunya, Yellow Fever, Filariasis, Schistosomiasis, and Japanese encephalitis (James, 1992). Furthermore, mosquitoes cause allergic reactions, including local (skin allergy) and systemic reactions (angioedema) (Gubler, 1998).

Cx. pipiens (L.) (Diptera: Culicidae) is widely spread in tropical and subtropical nations and bites a wide range of hosts (ECDC, 2021). Cx. pipiens are well-known carriers of West Nile Virus (WNV), Usutu virus (USUV), Rift Valley fever virus (RVFV), Japanese encephalitis virus (JEV), Sindbis virus (SINV), Tahyna virus (TAHV), Batal virus, Dirofilarial worms, and Avian malaria (ECDC, 2021). Managing mosquito vectors relies largely on synthetic insecticide spraying, and due to higher insecticide resistance, controlling mosquitoes is a considerable challenge (Ali et al., 2012; Organization, 2021). Cx. pipiens mosquitoes collected and screened for resistance from three different localities in Riyadh city were found resistant to deltamethrin, lambda-cyhalothrin, and beta-cyfluthrin. However, no resistance was detected for fenitrothion (Al-Sarar, 2010).

Larvicidal agents derived from natural sources are alternative tools, especially from bioactive secondary metabolites extracted from the plants because they are cheap, biodegradable, and non-toxic to other non-target organisms (Ghosh et al., 2012). Secondary metabolites from plants such as steroids, alkaloids, phenolics, terpenoids, and essential oils have been documented for their insecticidal, repellent, and adulticidal activities (Senthil-Nathan, 2020). The larvicidal potential of Cassia fistula hexane-methanol soluble fraction showed a promising LC50 value. The LC50 value was 21.04 µg/ml after 24 h of exposure. The extract of C. fistula showed no toxicity to Danio rerio embryos, and BEAS-2B at the highest concentration tested. (Abutaha et al., 2020). In addition, previous research of (Al-Solami, 2021) illustrates that the acetone extracts of Lantana camara, Rhazya stricta, Ruta chalepensis, and Acalypha fruticosa showed different percentages of mortality rate against the early fourth instar of Cx. pipiens. The results showed that L. camara extract (LD50: 264 mg/l) was significantly higher as com-
pared to *R. stricta* (293.4 mg/l), *A. fruticose* (435.6 mg/l) and *R. chalepensis* (611.9 mg/l) extract. A previous report by (Maheswaran and Ignacimuthu, 2015) synthesized a new novel plant-based (based on *Pongamia glabra* and *Azadirachtaindica*) extract named “PONNEEM” commercially synthesized to control *Cx. quinquefasciatus*, and *An. stephensi*.

Plant extracts could enable the discovery of new larvicial agents for effective mosquito management. This research aimed to assess the larvicial activity of *C. burmannii* (Family: Lauraceae) and *S. aromaticum* (Family: Myrtaceae) extracts and their blend against insectary-reared *Cx. pipiens* larvae.

### 2. Materials and methods

#### 2.1. Preparation of extract

*C. burmannii* (C.B.) and *S. aromaticum* (S.A.) were purchased from the herbal shop in Riyadh and pulverized to powder using an electrical blender (SFstardust, Japan). The pulverized powder of the two plants was extracted using hexane as an extraction solvent in a sonicator (WiseClean, China) for 30 mins at 40°C. Each extract was filtered using Whatman filter paper No. 1 and evaporated under reduced pressure (Heidolph, Germany) at 45°C. The process was repeated twice for each solvent, and the extracts were combined. The yield was calculated, and stock solution (25 mg/mL) was prepared and kept at -4°C until used. Hexane extract from each extract was mixed using the different ratios (S.A. 75%; C.B. 25%, S.A. 50%; C.B. 50%, and S.A. 25%; C.B. 75%) and further tested to evaluate the synergistic potential of the combined extract using the following formula:

\[
\text{Synergistic factor (S.F.)} = \frac{\text{LC}_{50} \text{ of the plant extract alone}}{\text{LC}_{50} \text{ of the mixture}}
\]

Value of S.F. < 1 represents the antagonistic action, and S.F. > 1 represents synergistic action (Kalyanasundaram and Das, 1985).

#### 2.2. Mosquito culture

The larvae of *Culex pipiens* were maintained in the Zoology Department insectary, Riyadh, Saudi Arabia, and kept in plastic trays filled with de-chlorinated tap water. Tests were carried out at 30 ± 1°C and under a light/dark (14:10) phase. Larvae were fed with fish flakes (DAJANA, Czech Public).

#### 2.2.1. Larvicidal bioassay

The larvicidal bioassay was performed based on a previously carried out procedure (Al-Mekhlafi, 2018). The 20 larvae in each replica were placed into disposable plastic six-well plates (NIST, China) containing 8 mL of the test concentrations (500, 375, 250, 200, 125, 62.5 mg/mL). Larvae were recorded as dead if no response when the plates were disturbed or touched with a glass rod. The results were reported after 24hrs of exposure. Methanol was used as a negative control. LC50 and LC90 values were calculated at 24hrs, using SPSS 20.

#### 2.2.2. Histological assay

The treated and control of *Cx. pipiens* third instars were fixed in 10% formalin solutions overnight and processed as reported by (Al-Mekhlafi et al., 2021), followed by dehydration, mounting using paraffin, and. The prepared slides were sectioned using a microtome (Leica, Germany) and stained with eosin and hematoxylin.

### Table 1

Mortality percent of *Cx. pipiens* larvae treated with the combination of the hexane of *C. burmannii* and *S. aromaticum* LC50 as well as LC90 (mg) values 24 h postexposure.

| Combinations       | Concentration (mg) | % mortality | LC50 (mg) | LC90 (mg) | df  | F    |
|--------------------|--------------------|-------------|-----------|-----------|-----|------|
| CB 100%            | Control            | 0.00 ± 0.00c| 184.28    | 263.92    | 4   | 152.00 |
|                    | 62.5               | 6.67 ± 3.33c|           |           |     |      |
|                    | 125                | 50.00 ± 5.77b|           |           |     |      |
|                    | 200                | 93.33 ± 3.33a|           |           |     |      |
|                    | 250                | 100.00 ± 0.00a|           |           |     |      |
|                    | 375                | 100.00 ± 0.00a|           |           |     |      |
|                    | 500                | 100.00 ± 0.00a|           |           |     |      |
| SA 100%            | Control            | 0.00 ± 0.00c| 363.70    | 483.50    | 4   | 166.80 |
|                    | 62.5               | 0.00 ± 0.00c|           |           |     |      |
|                    | 125                | 0.00 ± 0.00c|           |           |     |      |
|                    | 200                | 0.00 ± 0.00c|           |           |     |      |
|                    | 250                | 3.33 ± 1.33c|           |           |     |      |
|                    | 375                | 60.00 ± 5.77b|           |           |     |      |
|                    | 500                | 93.33 ± 3.33a|           |           |     |      |
| SA 75% : CB 25%    | Control            | 0.00 ± 0.00c| 145.13    | 202.60    | 4   | 1210.00 |
|                    | 62.5               | 6.67 ± 3.33c|           |           |     |      |
|                    | 125                | 10.00 ± 0.00b|           |           |     |      |
|                    | 200                | 100.00 ± 0.00a|           |           |     |      |
|                    | 250                | 100.00 ± 0.00a|           |           |     |      |
|                    | 375                | 100.00 ± 0.00a|           |           |     |      |
|                    | 500                | 100.00 ± 0.00a|           |           |     |      |
| SA 50% : CB 50%    | Control            | 0.00 ± 0.00c| 138.24    | 192.73    | 4   | 387.00 |
|                    | 62.5               | 0.00 ± 0.00c|           |           |     |      |
|                    | 125                | 30.00 ± 5.77b|           |           |     |      |
|                    | 200                | 100.00 ± 0.00a|           |           |     |      |
|                    | 250                | 100.00 ± 0.00a|           |           |     |      |
|                    | 375                | 100.00 ± 0.00a|           |           |     |      |
|                    | 500                | 100.00 ± 0.00a|           |           |     |      |
| SA 25% : CB 75%    | Control            | 0.00 ± 0.00d| 125.78    | 186.72    | 4   | 1024.00 |
|                    | 62.5               | 10.00 ± 0.00c|           |           |     |      |
|                    | 125                | 46.67 ± 3.33b|           |           |     |      |
|                    | 200                | 100.00 ± 0.00a|           |           |     |      |
|                    | 250                | 100.00 ± 0.00a|           |           |     |      |
|                    | 375                | 100.00 ± 0.00a|           |           |     |      |
|                    | 500                | 100.00 ± 0.00a|           |           |     |      |
The sections were observed for pathological alternations using a light microscope (Olympus, Japan). Midgut cells of *Cx. pipiens* were photographed, and the alternations in the midgut of treated larvae were observed and compared with control.

### 2.3. Cell culture

Normal Human Umbilical Endothelial Cells (HUV-EC) (ATCC, USA) were seeded in DMEM medium (UFC, KSA), supplemented with 10% and 1% of fetal bovine serum (Gibco, USA) and Antibiotic-Antimycotic solution (UFC, KSA), respectively. Plates were incubated at 37°C with 5% CO2 in a humidified incubator (BIN- DER, Germany).

### 2.4. Evaluation of cell viability

Cells (50,000 cells/well) were seeded in 24-well plates (NIST, China) and incubated at 37°C for 24 h. Plated cells were incubated with the five different concentrations of the extract (250–12.5 μg/mL), each dissolved in DMSO (Sigma, USA). DMSO (0.01%) was used as a negative control. After treatment (24 h), the cells were incubated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (Invitrogen, USA) at 37°C for 2 h. The crystals (purple) formed were dissolve in 0.01% HCL-methanol, and the optical density (O.D.) was read (595 nm) using a microplate reader (ChroMate, England). The graph was plotted using OriginPro 8.5.

### 3. Results

The total yield of hexane extract of S.A. and C.B. was 130 mg and 280 mg, respectively.

#### 3.1. Larvicidal activity

The larvicidal potential of hexane extract of *Cinnamomum burmannii* (C.B.) and *Syzygium aromaticum* (S.A.) against *Cx. pipiens* third instar was recorded after 24 h of treatment. The C.B. extract showed a higher larvicidal effect than the S.A. extract. The 50% (LC50) and 90% (LC90) mortality of larval treated with the plants with hexane extracts individually or in combinations against *Cx. pipiens* larvae are reported in Table 1. C.B. extract caused mosquito larval mortality with LC50 and LC90 values of 184.28 and 263.92 μg/mL against *Cx. pipiens*. Similarly, the hexane extract of S.A. was effective with respective LC 50 and LC 90 values of 363.70 and 483.50 μg/mL against *Cx. pipiens*. The combination of the C.B. and

![Fig. 1. The midguts of Cx. pipiens fourth instars treated with hexane extract of C. burmannii (75%) and S. aromaticum (25%). A and B are the midgut sections of the control group. C and D are sections of the treated larvae. Degenerating epithelial cells (DE), degraded microvilli (DMV), lumen (lu), degenerating nuclei (D.N.), degenerating peritrophic membrane (DPM).](image-url)
S.A. extracts using the different ratios (1:3, 1:1, 3:1) showed promising LC50 and LC90 values compared to the individual plant. Combination of C.B. and S.A. hexane extract enhanced the effectiveness of each extract by decreasing the LC50 values 145.13, 138.24 and 125.78 mg/ml for combinations of SA75%: CB25%, S.A. 50%: C.B. 50%, and S.A. 25%: CB75%, respectively compared to LC50 of C.B. (184.28) and S.A. (363.70) extracts tested singly. Table 2 illustrated the synergistic activity of the combination of the hexane extracts C.B. and S.A. against the Cx. pipiens larvae. All the combinations of the extract of C.B. and S.A. showed synergistic factors higher than one. Among the different ratios of extracts, the SA25%: CB75% extract was found to be more toxic than the other combinations. (Table 2). No larval mortality was detected in the control groups.

3.2. Gut-Histological activity

The midgut cells of Cx. pipiens third instar treated with sublethal dosages of S.A. 25%; C.B. 75% extract (125.7 ppm) (Fig. 1 C and D) and the control is shown in Fig. 1 A and B. The control midgut sections appeared normal, with intact microvilli (MV), nuclei (N), and normal peritrophic membrane (Pm). Midgut cells treated with S.A. 25%; C.B. 75% extract showed severe morphological alterations such as degradation of microvilli (DMV); degeneration of epithelial cells (D.E.); and peritrophic membrane (DPM); loss of nuclei, irregular and damage of microvilli.

3.3. Cell viability and cell morphology

The HUV-EC cells morphology was altered by C. cassia and Z. officinale extract, whereas the control (0.01% DMSO) cells retained normal morphology. Loss of cellular integrity, shrinkage of cytoplasmic materials, and cell detachment were noticed in HUV-EC cells by S.A. 25%; C.B. 75% extract (Figure 0000). The extract of S. A. 25%; C.B. 75% showed cytotoxic activity against HUV-EC cells screened, with the IC50 value of 32.4 µg/mL. The extract showed dose-dependent cytotoxic activity against the HUV-EC cell line with 47.8%, 50.3%, and 63.0 % cell viability at 62 µg/ml, 31 µg/ml, and 15 µg/ml concentration, respectively (Fig. 2).

4. Discussion

Botanical-based formulations are an economical approach to combat mosquito-borne diseases. Moreover, mosquito larvae are the ideal stage for insecticides screening using Botanical-based formulations (Benelli et al., 2017). C. burmannii and S. aromaticum extracts showed larvicidal activities individually and in combination, demonstrating the larvicidal capabilities of these two plants. The result showed that mortalities increased significantly with concentration and time (P ≤ 0.05). The present investigation is consistent with the reports that showed a positive correlation between concentration, time, and the percentage of larval mortality (Mehra and Hiradhar, 2000; Pelah et al., 2002). However, on an
individual basis, *C. burmannii* exhibited a higher larvicidal effect than *S. aromaticum*, as was observed with their LC50. The LC50 values of the two plants show that they can cause 50% larval mortality at 184.28 mg/ml and 363.70 mg/ml, which makes them promising botanical larvicides. Synergistic effects of larvicidal agents have been reported to be advantageous in the control of various pests (Seyoum et al., 2002).

Plant extracts combined formulations improve the larvicidal activity by decreasing the needed dose and lowering the time required to kill the larvae, making them more economical and practical (George and Vincent, 2005; Mohan et al., 2007). Mixtures of insecticide with a different mechanism of action are effective for managing resistant insects (Intirach et al., 2012; Ru et al., 1999). Therefore, they are very beneficial in mosquito control management (Mohan et al., 2010).

In a previous study, assessment of the larvicidal effectiveness of combinations Piper sermentosum, and *Zanthoxylum piperitum*, Foeniculum vulgare, *Myristica fragrans*, and *Curcuma longa* at different ratios (25%:75%, 50%:50%, and 75%:25%) revealed that at the highest ratio (75%:25%) the extracts displayed synergistic action. All combinations at the lower ratios (50%: 50% and 25%:75%) revealed antagonistic activity. Similarly, The mixture of *Pongamia glabra* and *Annona squamosa* extracts exhibited a synergistic effect against *Culex quinquefasciatus* larvae. Among the combined extracts (25%:75%, 50%:50%, and 75%:25%) used, the A 50%; P 50% extract was reported to be most effective extract than the other combinations and revealed the maximum synergism (Synergistic Effect:15.1) (George and Vincent, 2005).

Histopathology evaluation of the third instar of *Cx. pipiens* exposed to S.A. 25%: CB. 75%. The result reveled that the midgut was affected by the extract. Midgut was severely damaged, especially the basal membrane, epithelium cells, and microvilli. These damages could be attributed to the larvicidal secondary metabolites (phenols, cinnamonaldehyde, flavonoids, alkaloids, eugenol, coumarin, tannins, steroids, and saponins) that were reported previously in the plants used (Davis and Stout, 1971). Phytochemicals have the potential as a larvicide and work as an insect growth regulator, a feeding deterrent, and by interrupting nerve impulses, blocking respiration and stomach poison (Al-Mekhlafi, 2018).

The mosquito midgut plays a significant role in the enzymes secretion, absorption of nutrients (Christophers, 1960), ion transport, osmoregulation (Sina and Shukri, 2016), and defense against pathogens (Terra, 2001). In addition, the regenerative cells in the midgut play a vital role in metamorphosis (Procopio et al., 2015). Botanical extracts have been reported to alter the midgut and the survivability of insects. Mosquito larvae treated with *Capparis cartilaginea, Melia azedarach, Derris iruca*, and *Averrhoa bilimbi* extracts have been reported to alter the midgut epithelium, microvilli, peritrophic matrix, enlargement of intercellular spaces, and cytoplasmic vacuolization (Abutaha and Al-Mekhlafi, 2014; Al-Mehmadi and Al-Khalaf, 2010; Gusmão et al., 2002). Previously, (Rey et al., 1999) and (Amala et al., 2021) stated that the primary target of any plant metabolite is the midgut epithelium cells and peritrophic membrane, in which the latter is mainly accountable for growth stimulus in the insects. The damage of the midgut region found in the larvae treated with S.A. 25%: CB. 75% could be related to the damage to digestive absorption processes and the regenerative cells in the larval midgut, disrupting larval mosquito development and compromising survival.

Although the *C. burmannii* and *S. aromaticum* extract have a promising larvicidal potential against *Cx. pipiens* (LC50: 0000 µg/ ml), it possesses higher toxicity (IC50: µg/ml) against HUVEC cells. The cell morphology also confirmed the cytotoxicity of the extract against HUVEC cells. The extract was toxic to human HUVEC cell lines, and hence precaution should be considered when using the extract. This finding does not agree with those reported by Silva et al. for *Eugenia calycina* leaf extract against *Ae. aegypti* (199.3 µg/mL), which was reported to show high toxicity against the third instar compared to Hela cells (240.3 µg/mL) (Silva et al., 2021).

5. Conclusion

The hexane extract of the two plants tested individually or in combinations resulted in a promising larvicidal potential against *Cx. pipiens* larvae. Tested singly, the hexane extract of *Cinnamomum burmannii* was revealed to be the most active compared to *Syzygium aromaticum* against mosquito larvae. However, the blending of the two hexane extracts of the two plants showed a synergistic effect when applied to mosquito larvae. This makes it a promising candidate for developing a new eco-friendly larvicidal agent in the larvae breeding sites.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Researchers Supporting Project number (RSP-2021/112), King Saud University, Riyadh, Saudi Arabia.

References

Abutaha, N., Al-Mekhlafi, A., 2014. Evaluation of the safe use of the larvicidal fraction of *Capparis cartilaginea* Decne, against *Aedes caspius* (Pallas) (Diptera: Culicidae) larvae. African Entomol. 22 (4), 838–846.

Abutaha, N., Al-Mekhlafi, F.A., Farooq, M., 2020. Target and nontarget toxicity of *Cassia fistula* fruit extract against *Culex pipiens* (Diptera: Culicidae); lung cells (BEAS-2B) and *Zebrafish* (Danio rerio) embryos. J. Med. Entomol. 57 (2), 493–502.

Al-Mehmadi, R.M., Al-Khalaf, A.A., 2010. Larvicidal and histological effects of *Melia azedarach* extract on *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). J. King Saud Univ.-Sci. 22 (2), 77–85.

Al-Mekhlafi, F.A., 2018. Larvicidal, ovicidal activities and histopathological alterations induced by *Carum copticum* (Apiaceae) extract against *Culex pipiens* (Diptera: Culicidae). Saudi J. Biol. Sci. 25 (1), 52–56.

Al-Mekhlafi, F.A., Abutaha, N., Al-Daiss, A.A., Al-Keridis, L.A., Alsayadi, A.A., Mohamed, R.A.E.H., Wadaan, M.A., Ibrahim, K.E., Al-Khalif, M.S., 2021. Target and Non-target Effects of *Foeniculum vulgare* and *Matricaria chamomilla* combined extract on *Culex pipiens* mosquitoes. Saudi J. Biologic. Sci.

Al-Sarar, A.S., 2010. Insecticide resistance of *Culex pipiens* (L.) populations (Diptera: Culicidae) from Riyadh city, Saudi Arabia: Status and overcome. Saudi J. Biol. Sci. 17 (2), 95–100.

Al-Solami, H.M., 2021. Larvicidal activity of plant extracts by inhibition of detoxification enzymes in *Culex pipiens*. J. King Saud Univ.-Sci. 33, (3), 101371.

Ali, M.S., Ravikumar, S., Beula, J.M., 2012. Bioactivity of seagrass against the dengue fever mosquito *Aedes aegypti* larvae. Asian Pacific J. Tropical Biomedicine 2 (7), 570–573.

Amala, K., Karthi, S., Ganesan, R., Radhakrishnan, N., Srinivasan, K., Mostafa, A.-E.-Z., Ali, M.S., Ravikumar, S., Beula, J.M., 2012. Bioefficacy of *Epipactis divaricata* (L.)-Hexane Extracts and Their Major Metabolites against the Lepidopteran Pest *Spodoptera littoralis* (Fab.) and Dengue Mosquito *Aedes aegypti* (Linn.). Molecules 26 (12), 3695.

Benelli, G., Govindarajan, M., Rajeswary, M., Senthilmurugan, S., Vijayan, P., Alharbi, N.S., Kadaikunnan, S., Khaled, J.M., 2021. Larvicidal activity of *Blumea eriantha* essential oil and its components against six mosquito species, including Zika virus vectors: the promising potential of *4 E, 6 Z*-allo-ocimene, carvotanacetone and dodecyl acetate. Parasitol. Res. 116 (4), 1175–1188.

Christophers, S.R., 1960. *Aedes aegypti*: the yellow fever mosquito. CUP Arch. Vector Biot. 570–573.

ECDC, 2021. European Centre for Disease Prevention and Control, *Culex pipiens* - Factsheet for experts. https://www.ecdc.europa.eu/en/all-topics-z/disease-vectors/facts/mosquito-factsheets/culex-pipiens-factsheet-experts.

ECDCP, 2021. European Centre for Disease Prevention and Control *Culex pipiens* - Factsheet for experts. https://www.ecdc.europa.eu/en/all-topics-z/disease-vectors/facts/mosquito-factsheets/culex-pipiens-factsheet-experts.
George, S., Vincent, S., 2005. Comparative efficacy of Annona squamosa Linn. and Pongamia glabra Vent. to Azadirachta indica A Juss against mosquitoes. J. Vector Borne Dis. 42 (4), 159–163.

Ghosh, A., Chowdhury, N., Chandra, G., 2012. Plant extracts as potential mosquito larvicides. Indian J. Med. Res. 135 (5), 581.

Gubler, D.J., 1998. Resurgent vector-borne diseases as a global health problem. Emerg. Infect. Dis. 4 (3), 442–450.

Gusmão, D.S., Páscoa, V., Mathias, L., Vieira, I.J.C., Braz-Filho, R., Lemos, F.J.A., 2002. Derris (Lonchocarpus) urucu (Leguminosae) extract modifies the peritrophic matrix structure of Aedes aegypti (Diptera: Culicidae). Memórias do Instituto Oswaldo Cruz 97, 371–375.

Intirach, J., Junkum, A., Tuetun, B., Choochote, W., Chaithong, U., Jitpakdi, A., Riyong, D., Champakaew, D., Pitasawat, B., 2012. Chemical constituents and combined larvicidal effects of selected essential oils against Anopheles cracens (Diptera: Culicidae). Psyche 2012.

James, A.A., 1992. Mosquito molecular genetics: the hands that feed bite back. Science 257 (5066), 37–39.

Kalyanasundaram, M., Das, P., 1985. Larvicidal and synergistic activity of plant extracts for mosquito control. Indian J. Med. Res. 82 (1), 19–23.

Kovenden, K., Murugan, K., Shanthakumar, S., Vincent, S., 2012. Evaluation of larvicidal and pupicidal activity of Morinda citrifolia L. (Noni)(Family: Rubiaceae) against three mosquito vectors. Asian Pacific J. Tropical Dis. 2, 5362–5369.

Maheswaran, R., Ignacimuthu, S., 2015. A novel biopesticide PONNEEM to control human vector mosquitoes Anopheles stephensi L. and Culex quinquefasciatus Say. Environ. Sci. Pollut. Res. 22 (17), 13153–13166.

Mehra, B.K., Hiradhar, P.K., 2000. Effect of crude acetone extract of seeds of Annona squamosa Linn. (Family: Annonaceae) on possible control potential against larvae of Culex quinquefasciatus Say. J. Entomol. Res. 24 (2), 141–146.

Mohan, L., Sharma, P., Srivastava, C., 2007. Comparative efficacy of Solanum xanthocarpum extracts alone and in combination with a synthetic pyrethroid, cypermethrin, against malaria vector Anophes stephensi. Southeast Asian J. Trop. Med. Public Health 38 (2), 256–260.

Mohan, L., Sharma, P., Srivastava, C., 2010. Combination larvicidal action of Solanum xanthocarpum extract and certain synthetic insecticides against filarial vector, Culex quinquefasciatus (Say). Southeast. Asian J. Trop. Med. Public Health 41 (2), 311–319.

Organization, W.H., 2021. Lymphatic Filariasis. http://www.who.int/mediacentre/factsheets/fs102/en/.

Pelah, D., Abramovich, Z., Markus, A., Wiesman, Z., 2002. The use of commercial saponin from Quillaja saponaria bark as a natural larvicidal agent against Aedes aegypti and Culex pipiens. J. Ethnopharmacology 81 (3), 407–409.

Procopio, F.A., Fromentin, R., Kulp, D.A., Brehm, J.H., Bebin, A.-G., Strain, M.C., Richman, D.D., O’Doherty, U., Palmer, S., Hecht, F.M., 2015. A novel assay to measure the magnitude of the inducible viral reservoir in HIV-infected individuals. EBioMedicine 2 (8), 874–883.

Rey, D., Pautou, M.-P., Meyran, J.-C., 1999. Histopathological effects of tannic acid on the midgut epithelium of some aquatic Diptera larvae. J. Invertebr. Pathol. 73 (2), 173–181.

Ru, L., Rui, C., Fan, X., Zhao, J., Wei, C., 1998. Realized heritability analysis of resistance to single and multiple insecticides in Lipaphis erysimi (Kaltenbach) and Helicoverpa armigera (Hubner). Acta Entomologica Sinica 41, 243–249.

Senthil-Nathan, S., 2020. A review of resistance mechanisms of synthetic insecticides and botanicals, phytochemicals, and essential oils as alternative larvicidal agents against mosquitoes. Front. Physiol. 10, 1591.

Seyoum, A., Pålsson, K., Kung’a, S., Kabiru, E., Lwande, W., Killeen, G., Hassanali, A., Knots, B., 2002. Traditional use of mosquito-repellent plants in western Kenya and their evaluation in semi-field experimental huts against Anopheles gambiae: ethnobotanical studies and application by thermal expulsion and direct burning. Trans. R. Soc. Trop. Med. Hyg. 96 (3), 225–231.

Silva, M.V., Silva, S.A., Teixeira, T.L., De Oliveira, A., Morais, S.A., Da Silva, C.V., Espindola, L.S., Sousa, R.M., 2021. Essential oil from leaves of Eugenia calycina Cambes: Natural larvicidal against Aedes aegypti. J. Sci. Food Agric. 101 (3), 1202–1208.

Sina, I., Shukri, M., 2016. Larvicidal activities of extract flower Averrhoa bilimbi L towards important species mosquito, Anophes barbirostris (Diptera: Culicidae). Int. J. Zoolog. Res. 12 (1), 25–31.

Terra, W.R., 2001. The origin and functions of the insect peritrophic membrane and peritrophic gel. In: Archives of Insect Biochemistry and Physiology: Published in Collaboration with the Entomological Society of America, pp. 47–61.