Evaluation of immature mosquitocidal properties of *Xanthium strumarium* Linn. plant extracts against *Culex* mosquitoes (Diptera: Culicidae)

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**ABSTRACT**

**Objective:** To evaluate immature mosquitocidal properties of *Xanthium strumarium* plant extracts against *Culex* mosquitoes at Entomology Laboratory, Maraki Campus, University of Gondar.

**Methods:** The immature mosquitocidal activity of plant extracts was tested by following World Health Organization recommended protocol. Acetone, methanol and water extracts were prepared at 50, 100, 150, 200 and 250 mg/L concentrations and tested against third and fourth instar larvae and pupae of *Culex* mosquitoes. The mortality rate of immature mosquitoes was recorded after 24, 48 and 72 h exposure period continuously.

**Results:** Third instar larvae after 24 h exposure period, maximum mortality of 77.80% was recorded at 250 mg/L concentration of acetone extract. After 48 h and 72 h exposure period, maximum mortality of 88.90% was recorded in acetone extract in all the tested concentration. The maximum mortality of fourth instar larvae was 88.90% in acetone extract at 200 and 250 mg/L concentrations. Pupal mortality was also greater in acetone extract. The percentage of mortality in all the stage of mosquitoes was higher in acetone extract followed by methanol and water extract.

**Conclusions:** The percentage of mortality is associated with concentration of the extracts tested and exposure period. This laboratory study confirmed immature mosquitocidal activity of *Xanthium strumarium* leaf extracts against *Culex* mosquitoes. The aqueous leaf extract can be used by applying on small man-made breeding places to prevent adult emergence.

1. Introduction

Mosquitoes belonging to the order Diptera, family Culicidae are responsible for transmission of disease causing organisms associated with many vector-borne diseases globally. Malaria, filariasis, Japanese encephalitis, dengue fever, chikunkunya and yellow fever are some of the diseases associated with mosquitoes which cause mortality and morbidity in affected people[11]. Among the various categories of mosquito species, *Culex* species in particular *Culex quinquefasciatus*, is a major vector for the transmission of *Wuchereria bancrofti* which is responsible for elephantiasis or disfiguring symptoms like hydrocoele and lymphedema[2-5]. In Ethiopia, Shiferaw et al., reported that Gambella region of Western Ethiopia is known to be endemic in lymphatic filariasis[6]. A total of 34 districts were found to be endemic and the prevalence rate is varied (> 20%, 10%–20% and 5%–9%). A total of 29 of 34 endemic districts were found in Gambella region (7 districts), Beneshangul-Gumuz region (13 districts) and Southern National, Nationalities and Peoples region (9 districts). The other five were from Amhara region (2 districts) and Oromia region (3 districts).

Chemical pesticides are proved to be effective in mosquito control program due to negative consequences. Many developed and developing countries are searching environmentally safe products for vector control program. Many control measures have been applied to reduce mosquito menace in which larvae are killed at different stages to prevent the establishment of mosquito population. Many plant products have been tried in earlier days before the discovery of chemical pesticides[7]. Several researchers reported that plant phytocides provide multiple modes of action on target organisms such as larvicides, insect growth regulators, repellents and oviposition attractants or deterrents[8-10]. Current scenario, several researches are searching locally available plant materials in order to find out eco-friendly products to manage different mosquito species[11-16].

Bio-potential of aqueous extract of *Xanthium strumarium* (*X. strumarium*) fruit extract against pulse beetle *Callosobruchus chinensis* was reported by observing mortality, repellency, reduced egg laying capacity and inhibited adult emergence[17]. According to Yanar et al., 20 eggs of *Tetranychus urticae* were sprayed with 0.5 mL of 10% of fruit and leaf extracts which caused
59.64% and 57.45% egg mortality respectively\[18\]. Toxic effect of the plant extracts against the larvae of grape berry moth and leaf roller larvae was reported\[19,20\]. The acetone extract at 10% and 20% and methanol extract at 20% was reported to cause death of the Helicoverpa armigera larva\[21\]. Sarmah \textit{et al.} observed 87.09% egg mortality at 10% concentration of aqueous extract against tea red spider mite\[22\]. Ethanol extract was most effective against four species of mosquito larvae and also had strong repellent activity against adult mosquitoes\[23\]. In Ethiopia, \textit{X. strumarium} is locally called as “bundaa” in Amharic (annual or a short lived perennial weed and growing extensively in many parts of the country). These plants are used for fungal treatment and also the leaf powder mixed with lime is used against tinea versicolor infection on skin\[24,25\]. There is no much work on this potential plant extract against \textit{Culex} mosquitoes. Therefore, present study was initiated to evaluate solvent extract of \textit{X. strumarium} leaves against immature stages of the \textit{Culex} mosquitoes.

2. Materials and methods

2.1. Immature Culex larvae collection and maintenance

The immature \textit{Culex} mosquito larvae were collected from the stagnant water polluted with organic wastes in and around University of Gondar. The collected larvae were brought to the laboratory and maintained in a plastic container by providing powdered yeast and dog biscuits (3:1 ratio) as a source of feed. After acclimatization of the larvae in the laboratory condition, they were used for subsequent experiment.

2.2. Processing of plant materials for solvent extraction

The leaves of \textit{X. strumarium} were collected from Tewodros Campus, University of Gondar. The leaves were collected randomly from 20 plants, washed with water to remove unwanted debris attached to the leaves and dried under shade in order to prevent chemical denaturation. After complete drying, plant leaves were powdered using an electric blender. The powdered leaves were sieved through kitchen strainer to obtain fine powder for solvent extraction.

2.3. Solvent extraction of plant powder

About 30 g of leaf powder was taken into 500 mL conical flask and added 300 mL of solvents such as acetone, methanol and water individually. The mouth of the conical flask was tightly plugged with cotton followed by aluminum foil and kept in a shaker for 12 h for complete mixing of ingredients into solvents and to get homogenous solution. The liquid part was removed by using Whatman No. 1 filter paper and the residue was discarded. The filtrate of acetone and methanol was kept inside the oven at 55 °C for 2 days to evaporate the solvent. The water extract was kept in a water bath at 100 °C to evaporate water. After complete evaporation, solvent free residues were collected and stored in a refrigerator for subsequent experiment.

2.4. Preparation of stock and working concentration

From the solvent residue stock, solution of 1000 mg/L was prepared by adding 150 mg of plant extract mixed with 1 mL of methanol and 1 mL of soap solution (added for the purpose of emulsification). The final volume of 150 mL was prepared by adding distilled water. From the stock solution, working concentrations of 50, 100, 150, 200 and 250 mg/L were prepared by serial dilution method. The five concentrations prepared from the stock solution were tested against third and fourth instar larvae and pupae of the \textit{Culex} mosquitoes.

2.5. Evaluation of larvicidal activity of plant extract

Larvicidal activity of acetone, methanol and water extract of \textit{X. strumarium} was tested by following World Health Organization protocol with modifications\[26\]. The experiment was conducted at the Entomology Laboratory, Maraki Campus, University of Gondar. The larvicidal activity of plant extract was conducted by using 250 mL plastic container. In each container, 10 larvae of third and fourth instar were released. In each beaker, concentration of acetone, methanol and water extract was maintained at 50, 100, 150, 200 and 250 mg/L in 100 mL of final water volume. In control, except plant extracts, remaining materials were added as mentioned in preparation of concentration. The number of dead larvae was recorded continuously after 24, 48 and 72 h exposure period from three replication. The larval mortality was corrected and calculated by using Abbott’s formula\[27\].

\[
\text{Corrected percent of mortality} = \left(\frac{\text{mortality in test (\%)} – \text{mortality in control (\%)}}{100\% – \text{mortality in control (\%)}}\right) \times 100
\]

2.6. Evaluation of pupicidal activity of plant extract

The similar concentration of plant extracts tested for larvicidal activity was used to confirm pupicidal activity. In this experiment, freshly emerged pupae were used. In each concentration, 10 pupae were released individually and the mortality was recorded after 24, 48 and 72 h exposure period. The number of dead pupa was counted and following Abbott’s formula corrected percentage of mortality was calculated. The experiments were replicated three times.

2.7. Statistical analysis

The percentage of mortality of \textit{Culex} larvae and pupae obtained from the three replications at different concentrations and exposure periods was subjected to statistical analysis. The calculations were carried out by using Microsoft Excel program in order to obtain mean percentage values and standard deviation. The SPSS version 16 software was used to confirm statistical significant at 5% level ($P < 0.05$) by using Chi-square ($\chi^2$) analysis. The $L_{50}$ and $L_{90}$ values and 95% upper confidence limit (UCL) and lower confidence limit (LCL) were also calculated.

3. Results

3.1. Mortality of third instar larvae of Culex mosquitoes exposed 24 h in \textit{X. strumarium} plant extracts

Mean percentage of mortality of third instar larvae of \textit{Culex} mosquitoes exposed to \textit{X. strumarium} plant extracts after 24 h exposure period was present in Table 1. Results revealed that maximum percentage of mortality of 88.90% was observed in acetone extract at 250 mg/L. The calculated $L_{50}$ and $L_{90}$ values...
were 107.9 and 241.6 mg/L respectively. The \( \chi^2 \) analysis results showed statistical significance at 5% level \( (\chi^2 = 7.768; P < 0.05) \). The calculated range of 95% LCL and UCL of LC50 and LC90 value was 54.1–141.2 mg/L and 197.2–355.7 mg/L respectively. In methanol extract, maximum percentage of mortality of 74.10% was observed at 250 mg/L. The calculated LC50 and LC90 values were 209.7 and 333.3 mg/L respectively. The \( \chi^2 \) analysis results showed statistical significance at 5% level \( (\chi^2 = 18.659; P < 0.05) \). The calculated range of 95% LCL and UCL of LC50 and LC90 value was 159.6–371.6 mg/L and 252.6–863.5 mg/L respectively.

Table 1
Mean percentage of mortality of third instar larvae of Culex mosquitoes exposed to 24 h in X. strumarium plant extracts. %.

| Concentrations (mg/L) | Extracts tested | Values were presented as mean ± SD of three replications. |
|-----------------------|-----------------|----------------------------------------------------------|
| 50                    | Acetone         | 22.10 ± 11.16                                            |
|                       | Methanol        | 11.00 ± 0.00                                             |
|                       | Water           | 11.00 ± 0.00                                             |
| 100                   | Acetone         | 51.80 ± 16.97                                            |
|                       | Methanol        | 11.00 ± 0.00                                             |
|                       | Water           | 11.00 ± 0.00                                             |
| 150                   | Acetone         | 74.10 ± 6.41                                             |
|                       | Methanol        | 14.70 ± 6.47                                             |
|                       | Water           | 11.00 ± 0.00                                             |
| 200                   | Acetone         | 77.80 ± 0.00                                             |
|                       | Methanol        | 44.40 ± 11.11                                            |
|                       | Water           | 33.30 ± 11.11                                            |
| 250                   | Acetone         | 88.90 ± 0.00                                             |
|                       | Methanol        | 74.10 ± 6.41                                             |
|                       | Water           | 44.40 ± 11.11                                            |

LC50 (LCL-UCL) 107.9 (54.1–141.2) 209.7 (159.6–371.6) 284.9 (217.7–792.1)
LC90 (LCL-UCL) 241.6 (197.2–355.7) 333.3 (252.6–863.5) 488.9 (339.8–1846.9)

\( \chi^2 \) 7.768

Values were presented as mean ± SD of three replications.

3.2. Mortality of third instar larvae of Culex mosquito exposed 48 h in X. strumarium plant extracts

Mean percentage of mortality of third instar larvae of Culex mosquitoes exposed to X. strumarium plant extracts after 48 h exposure period was present in Table 2. Results revealed that maximum percentage of mortality of 88.90% was observed in acetone extract at all concentrations tested. The range of 95% LCL and UCL of LC50 and LC90 values was not calculated by SPSS software due to no variation in percentage of mortality. In methanol extract, maximum percentage of mortality of 88.90% was observed at 250 mg/L. The calculated LC50 and LC90 values were 161.9 and 249.3 mg/L respectively. The \( \chi^2 \) analysis results showed statistical significance at 5% level \( (\chi^2 = 20.965; P < 0.05) \). The calculated range of 95% LCL and UCL of LC50 and LC90 value was 116.2–215.4 mg/L and 201.5–415.2 mg/L respectively.

Table 2
Mean percentage of mortality of third instar larvae of Culex mosquitoes after 48 h exposure in X. strumarium plant extracts. %.

| Concentrations (mg/L) | Extracts tested | Values were presented as mean ± SD of three replications. |
|-----------------------|-----------------|----------------------------------------------------------|
| 50                    | Acetone         | 88.90 ± 0.00                                             |
|                       | Methanol        | 88.90 ± 0.00                                             |
|                       | Water           | 88.90 ± 0.00                                             |
| 100                   | Acetone         | 88.90 ± 0.00                                             |
|                       | Methanol        | 88.90 ± 0.00                                             |
|                       | Water           | 88.90 ± 0.00                                             |
| 150                   | Acetone         | 88.90 ± 0.00                                             |
|                       | Methanol        | 88.90 ± 0.00                                             |
|                       | Water           | 88.90 ± 0.00                                             |
| 200                   | Acetone         | 88.90 ± 0.00                                             |
|                       | Methanol        | 88.90 ± 0.00                                             |
|                       | Water           | 88.90 ± 0.00                                             |
| 250                   | Acetone         | 88.90 ± 0.00                                             |
|                       | Methanol        | 88.90 ± 0.00                                             |
|                       | Water           | 88.90 ± 0.00                                             |

LC50 (LCL-UCL) Not calculated Not calculated Not calculated
LC90 (LCL-UCL) Not calculated Not calculated 249.3 (201.5–415.2)

\( \chi^2 \) Not calculated

Values were presented as mean ± SD of three replications.

3.3. Mortality of third instar larvae of Culex mosquito exposed 72 h in X. strumarium plant extracts

Mean percentage of mortality of third instar larvae of Culex mosquitoes exposed to X. strumarium plant extracts after 72 h exposure period was present in Table 3. Results revealed that maximum percentage of mortality of 88.90% was observed in acetone and methanol extract at all the concentrations tested. The range of 95% LCL and UCL of LC50 and LC90 values was not calculated by SPSS software due to no variation in percentage of mortality. In water extract, maximum percentage of mortality of 88.90% at 250 mg/L followed by 81.60% at 200 mg/L concentration. The calculated LC50 and LC90 values were 161.9 and 249.3 mg/L respectively. The \( \chi^2 \) analysis results showed statistical significance at 5% level \( (\chi^2 = 20.965; P < 0.05) \). The calculated range of 95% LCL and UCL of LC50 and LC90 value was 116.2–215.4 mg/L and 201.5–415.2 mg/L respectively.

Table 3
Mean percentage of mortality of third instar larvae of Culex mosquitoes after 72 h exposure in X. strumarium plant extracts. %.

| Concentrations (mg/L) | Extracts tested | Values were presented as mean ± SD of three replications. |
|-----------------------|-----------------|----------------------------------------------------------|
| 50                    | Acetone         | 88.90 ± 0.00                                             |
|                       | Methanol        | 88.90 ± 0.00                                             |
|                       | Water           | 11.00 ± 0.00                                             |
| 100                   | Acetone         | 88.90 ± 0.00                                             |
|                       | Methanol        | 88.90 ± 0.00                                             |
|                       | Water           | 11.00 ± 0.00                                             |
| 150                   | Acetone         | 88.90 ± 0.00                                             |
|                       | Methanol        | 88.90 ± 0.00                                             |
|                       | Water           | 33.30 ± 0.00                                             |
| 200                   | Acetone         | 88.90 ± 0.00                                             |
|                       | Methanol        | 88.90 ± 0.00                                             |
|                       | Water           | 81.60 ± 6.35                                             |
| 250                   | Acetone         | 88.90 ± 0.00                                             |
|                       | Methanol        | 88.90 ± 0.00                                             |
|                       | Water           | 88.90 ± 0.00                                             |

LC50 (LCL-UCL) Not calculated Not calculated 161.9 (116.2–215.4)
LC90 (LCL-UCL) Not calculated Not calculated 249.3 (201.5–415.2)

\( \chi^2 \) Not calculated

Values were presented as mean ± SD of three replications.

3.4. Mortality of fourth instar larvae of Culex mosquito exposed 24 h in X. strumarium plant extracts

Mean percentage of mortality of fourth instar larvae of Culex mosquitoes exposed to X. strumarium plant extracts after 24 h exposure period was present in Table 4. Results revealed that maximum percentage of mortality of 77.80% was observed in acetone extract at 250 mg/L concentration. The calculated LC50 and LC90 values were 179.6 and 327.7 mg/L respectively. The \( \chi^2 \) analysis results did not show statistical significance at 5% level \( (\chi^2 = 6.005; P > 0.05) \). The calculated range of 95% LCL and UCL of LC50 and LC90 value was 147.9–224.2 mg/L and 267.1–485.1 mg/L respectively. In methanol extract, maximum percentage of mortality of 62.90% was observed at 250 mg/L. The calculated LC50 and LC90 values were 239.1 and 388.8 mg/L respectively. The \( \chi^2 \) analysis results showed statistical significance at 5% level \( (\chi^2 = 18.058; P < 0.05) \). The calculated range of 95% LCL and UCL of LC50 and LC90 value was 179.1–931.2 mg/L and 276.8–2192.6 mg/L respectively. The mean percentage of mortality in water extract was minimum (11.00%) in all the tested concentrations. The range of 95% LCL and UCL of LC50 and LC90 values was not calculated by SPSS software due to no variation in percentage of mortality.

3.5. Mortality of fourth instar larvae of Culex mosquito exposed 48 h in X. strumarium plant extracts

Mean percentage of mortality of fourth instar larvae of Culex
mosquitoes exposed to X. strumarium plant extracts after 48 h exposure period was present in Table 5. Results revealed that maximum percentage of mortality of 88.90% was observed in acetone extract at 200 and 250 mg/L concentrations. The calculated LC50 and LC90 values were 42.8 and 105.1 mg/L respectively. The LC50 value was 51.4–170.5 mg/L and 203.9–480.1 mg/L respectively. In methanol extract, maximum percentage of mortality of 88.90% was observed at 250 mg/L concentration. The calculated LC50 and LC90 values were 105.1 and 249.4 mg/L respectively. The LC50 and LC90 values were 253.9 and 461.4 mg/L respectively. The calculated LC50 and LC90 values were 127.7 and 261.1 mg/L respectively. The LC50 and LC90 values were 170.5 and 265.4 mg/L respectively. The LC50 and LC90 values were 137.7–210.4 mg/L and 221.7–377.7 mg/L respectively.

### Table 4

Mean percentage of mortality of fourth instar larvae of Culex mosquitoes after 24 h exposure in X. strumarium plant extracts. %.

| Concentrations (mg/L) | Extracts tested | Acetone | Methanol | Water |
|-----------------------|----------------|---------|---------|-------|
| 50                    | 14.70 ± 6.47   | 11.00 ± 0.00 | 11.00 ± 0.00 |
| 100                   | 22.20 ± 11.11  | 11.00 ± 0.00 | 11.00 ± 0.00 |
| 150                   | 44.40 ± 11.1   | 11.00 ± 0.00 | 11.00 ± 0.00 |
| 200                   | 48.40 ± 6.42   | 33.30 ± 11.1 | 11.00 ± 0.00 |
| 250                   | 77.80 ± 0.00   | 62.90 ± 0.00 | 29.60 ± 6.41 |

**LC50 (LCL-UCL)**: 179.6 (147.9–224.2), 239.1 (179.1–931.2), Not calculated

**LC90 (LCL-UCL)**: 327.7 (267.1–485.1), 388.8 (276.8–2192.6), Not calculated

**P value**: 0.111, 0.000, Not calculated

Values were presented as mean ± SD of three replications.

### Table 5

Mean percentage of mortality of fourth instar larvae of Culex mosquitoes after 48 h exposure in X. strumarium plant extracts. %.

| Concentrations (mg/L) | Extracts tested | Acetone | Methanol | Water |
|-----------------------|----------------|---------|---------|-------|
| 50                    | 48.40 ± 6.42   | 33.30 ± 11.1 | 11.00 ± 0.00 |
| 100                   | 74.10 ± 6.41   | 44.40 ± 11.1 | 11.00 ± 0.00 |
| 150                   | 85.20 ± 6.41   | 66.70 ± 11.1 | 18.50 ± 6.48 |
| 200                   | 88.90 ± 0.00   | 81.50 ± 6.41 | 48.10 ± 6.42 |
| 250                   | 88.90 ± 0.00   | 88.90 ± 0.00 | 70.40 ± 6.41 |
| LC50 (LCL-UCL)        | 42.8 (8.4–63.9) | 105.1 (88.2–119.2) | 209.1 (170.3–292.3) |
| LC90 (LCL-UCL)        | 188.5 (165.9–225.8) | 249.4 (226.8–281.6) | 338.7 (267.9–592.8) |

**P value**: 0.220, 0.073, 0.014

Values were presented as mean ± SD of three replications.

### 3.6. Mortality of fourth instar larvae of Culex mosquito exposed 72 h in X. strumarium plant extracts

Mean percentage of mortality of fourth instar larvae of Culex mosquitoes exposed to X. strumarium plant extracts after 72 h exposure period was present in Table 6. Results revealed that maximum percentage of mortality of 88.90% was observed in acetone extract in all the tested concentrations. The range of 95% LCL and UCL of LC50 and LC90 values was not calculated by SPSS software due to no variation in percentage of mortality. In methanol extract, maximum percentage of mortality of 88.90% was observed at 200 and 250 mg/L. The calculated LC50 and LC90 values were 42.5 and 224.8 mg/L respectively. The LC50 and LC90 values were 209.1 and 338.7 mg/L respectively. The calculated LC50 and LC90 values were 170.5 and 265.4 mg/L respectively. The LC50 and LC90 values were 137.7–210.4 mg/L and 221.7–377.7 mg/L respectively.

### Table 6

Mean percentage of mortality of fourth instar larvae of Culex mosquitoes after 72 h exposure in X. strumarium plant extracts. %.

| Concentrations (mg/L) | Extracts tested | Acetone | Methanol | Water |
|-----------------------|----------------|---------|---------|-------|
| 50                    | 88.90 ± 0.00   | 48.10 ± 6.43 | 11.00 ± 0.00 |
| 100                   | 88.90 ± 0.00   | 66.70 ± 11.1 | 11.00 ± 0.00 |
| 150                   | 88.90 ± 0.00   | 81.50 ± 6.41 | 33.30 ± 11.1 |
| 200                   | 88.90 ± 0.00   | 88.90 ± 0.00 | 66.70 ± 11.1 |
| 250                   | 88.90 ± 0.00   | 88.90 ± 0.00 | 88.90 ± 0.00 |

**LC50 (LCL-UCL)**: Not calculated 42.5 (4.2–67.3), 170.5 (137.7–210.4) Not calculated 224.8 (199.5–263.9) 265.4 (221.7–377.7)

**P value**: Not calculated 0.210, 0.008

Values were presented as mean ± SD of three replications.

### 3.7. Mortality of pupal stage of Culex mosquito exposed 24 h in X. strumarium plant extracts

Mean percentage of mortality of pupal stage of Culex mosquitoes was maximum (81.50%) in acetone extract at 250 mg/L concentration (Table 7). The calculated LC50 and LC90 values were 127.7 and 261.1 mg/L respectively. The analysis results showed statistical significance at 5% level (χ² = 12.776; χ² = 179.6; χ² = 253.9). The calculated range of 95% LCL and UCL of LC50 and LC90 values was 49.8–130.8 mg/L and 348.6–653.3 mg/L respectively. The mean percentage of mortality in water extract was maximum (88.90%) at 250 mg/L. The calculated LC50 and LC90 values were 170.5 and 265.4 mg/L respectively. The LC50 and LC90 values were 137.7–210.4 mg/L and 221.7–377.7 mg/L respectively.

### Table 7

Mean percentage of mortality of pupa of Culex mosquitoes after 24 h exposure in X. strumarium plant extracts. %.

| Concentrations (mg/L) | Extracts tested | Acetone | Methanol | Water |
|-----------------------|----------------|---------|---------|-------|
| 50                    | 88.90 ± 0.00   | 48.10 ± 6.43 | 11.00 ± 0.00 |
| 100                   | 88.90 ± 0.00   | 66.70 ± 11.1 | 11.00 ± 0.00 |
| 150                   | 88.90 ± 0.00   | 81.50 ± 6.41 | 33.30 ± 11.1 |
| 200                   | 88.90 ± 0.00   | 88.90 ± 0.00 | 66.70 ± 11.1 |
| 250                   | 88.90 ± 0.00   | 88.90 ± 0.00 | 88.90 ± 0.00 |

**LC50 (LCL-UCL)**: Not calculated 42.5 (4.2–67.3), 170.5 (137.7–210.4) Not calculated 224.8 (199.5–263.9) 265.4 (221.7–377.7)

**P value**: Not calculated 0.210, 0.008

Values were presented as mean ± SD of three replications.
3.8. Mortality of pupal stage of Culex mosquito exposed 48 h in X. strumarium plant extracts

Mean percentage of mortality of pupal stage of Culex mosquitoes was maximum (85.20%) in acetone extract at 250 mg/L concentration (Table 8). The calculated LC50 and LC90 values were 77.6 and 385.1 mg/L respectively. The χ² analysis results did not show statistical significance at 5% level (χ² = 1.646; P > 0.05). The calculated range of 95% LCL and UCL of LC50 and LC90 value was 22.5–108.4 mg/L and 317.2–541.1 mg/L respectively. The mean percentage of mortality in water extract was maximum (85.10%) at 250 mg/mL. The calculated LC50 and LC90 values were 217.9 and 305.1 mg/L respectively. The χ² analysis results did not show statistical significance at 5% level (χ² = 9.205; P < 0.05). The calculated range of 95% LCL and UCL of LC50 and LC90 value was 7.9–138.4 mg/L and 173.1–345.0 mg/L and 289.2–805.1 mg/L respectively.

Table 8

| Concentrations (mg/L) | Extracts tested |
|-----------------------|-----------------|
|                       | Acetone | Methanol | Water |
|-----------------------|---------|----------|--------|
| 50                    | 25.90 ± 6.41 | 44.40 ± 11.12 | 14.70 ± 6.48 |
| 100                   | 51.90 ± 6.42 | 51.90 ± 6.42 | 18.50 ± 6.48 |
| 150                   | 66.70 ± 11.11 | 66.70 ± 11.11 | 22.20 ± 11.16 |
| 200                   | 81.50 ± 6.41 | 70.40 ± 6.41 | 40.70 ± 12.83 |
| 250                   | 85.20 ± 6.41 | 74.10 ± 6.41 | 66.70 ± 11.11 |
| LC50 (LCL-UCL)        | 106.9 (89.5–121.4) | 77.6 (22.5–108.4) | 217.9 (173.1–345.0) |
| LC90 (LCL-UCL)        | 255.8 (232.2–289.7) | 385.1 (317.2–541.1) | 383.1 (289.2–805.1) |

Values were presented as mean ± SD of three replications.

3.9. Mortality of pupal stage of Culex mosquito exposed 72 h in X. strumarium plant extracts

Mean percentage of mortality of pupal stage of Culex mosquitoes was maximum (85.90%) in acetone extract at 250 mg/L concentration (Table 9). The calculated LC50 and LC90 values were 84.3 and 236.3 mg/L respectively. The χ² analysis results showed statistical significance at 5% level (χ² = 10.025; P < 0.05). The calculated range of 95% LCL and UCL of LC50 and LC90 value was 32.2–127.7 mg/L and 183.2–434.7 mg/L respectively. In methanol extract, maximum percentage of mortality was observed in acetone extract followed by methanol and water. The chemical substances of the plant powder may dissolve maximally in acetone extract compared to others. This will guide selection of solvent which is important for plant extraction. The percentage of mortality was observed in acetone extract by multiple modes of action [8-10].

Table 9

| Concentrations (mg/L) | Acetone | Methanol | Water |
|-----------------------|---------|----------|--------|
| 50                    | 29.63 ± 6.41 | 44.49 ± 11.12 | 22.20 ± 0.00 |
| 100                   | 66.70 ± 11.11 | 55.60 ± 0.00 | 25.90 ± 6.41 |
| 150                   | 70.37 ± 6.41 | 70.40 ± 6.41 | 29.60 ± 6.41 |
| 200                   | 85.20 ± 6.41 | 74.10 ± 6.41 | 62.90 ± 6.41 |
| 250                   | 88.90 ± 0.00 | 81.50 ± 6.41 | 85.10 ± 6.41 |
| LC50 (LCL-UCL)        | 84.3 (32.2–127.7) | 97.6 (7.9–138.4) | 164.9 (106.6–250.6) |
| LC90 (LCL-UCL)        | 236.3 (183.2–434.7) | 286.4 (223.5–506.3) | 305.1 (230.8–706.3) |

Values were presented as mean ± SD of three replications.

4. Discussion

Mosquitoes are nuisance insects that transmit various diseases from organism to human and animal. Prevention and control of mosquitoes are important to reduce the vector-borne disease incidence. Mosquito control in the larval stage is worthwhile to minimize the emergence of adult population and also easy to handle in small breeding habitats. These mosquitoes breeding in small ponds, marshes, ditches, pools, drains, water containers and any other utensils holding water is easily manageable. Synthetic chemicals are proved to be effective, but they cause adverse effects on the environment and human health [28]. In this situation, eco-friendly alternatives are important for safer control of mosquitoes. The phytochemicals from plant origin were proved to be effective due to multiple modes of action [8-10]. To complement in this research program, solvent extracts of X. strumarium were tried in the laboratory against immature stage of Culex mosquitoes.

In the present study, larval and pupal mortality was varied significantly. Among the three solvents used, maximum mortality was observed in acetone extract followed by methanol and water. The percentage of mortality was varied among the stage of the immature mosquitoes. The maximum percentage of mortality was observed in third instar larvae compared to fourth instar larvae and pupae. This will further suggest that before application it is important to monitor the stage of the larvae to determine the concentration of the biopesticides. The percentage of mortality of all the stages of immature mosquitoes was increased in increased concentration and period of exposure. This highlights that selection of appropriate concentration and exposure period is important for maximum benefits of plant extract in mosquito control program. Several studies also reported that dose dependent on mortality of mosquito species [13-16,29].

In the present study, X. strumarium plant extracts cause significant mortality on immature Culex mosquitoes. These plant parts contain steroids, alkaloids, flavonoids, triterpenoids (terpenoids), saponin, coumarin and quinine and several compounds like myrcene, limonene, xanthatin, xanthinin etc [30,31]. Any of these compounds or mixtures may be toxic to the immature Culex mosquitoes. These plants are growing well in many parts of Ethiopia and it can be used to reduce the mosquito population by applying on small man-made breeding places. Further, isolation and characterization of the active molecules will lead to development of novel botanical pesticide for vector control program.
Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Jang YS, Kim MK, Ahn YS, Lee HS. Larvicidal activity of Brazilian plants against Aedes aegypti and Culex pipiens (Diptera: Culicidae). Agric Chem Biotechnol 2002; 45: 131-4.
[2] Nanduri J, Kuzura JW. Clinical and laboratory aspects of filariasis. Clin Microbiol Rev 1989; 2(1): 39-50.
[3] Hettiarachchi UP, Munasinghe DH, Chandrasekharan NV, Karunanyake EH, Jayasekera N. A polymerase chain reaction based method for the detection of Culex quinquefasciatus (Diptera: Culicidae). Bull Entomol Res 2000; 90: 63-8.
[4] Freeman AR, Lammi PJ, Houston R, LaPointe MD, Streit TG, Jooste PL, et al. A community-based trial for the control of lymphatic filariasis and iodine deficiency using salt fortified with diethylcarbamazine and iodine. Am J Trop Med Hyg 2001; 65(6): 865-71.
[5] Goodman DS, Orrels JN, Roberts JM, Lammi PJ, Streit TG. PCR and mosquito dissection as tools to monitor filarial infection levels following mass treatment. Filaria J 2003; 2: 11.
[6] Shiferaw W, Kebede T, Graves PM, Golasa L, Gebre T, Mosher AW, et al. Lymphatic filariasis in Western Ethiopia with special emphasis on prevalence of Wuchereria bancrofti antigenaemia in and around onchocerciasis endemic areas. Trans R Soc Trop Med Hyg 2012; 106(2): 117-27.
[7] Mittal PK, Subbarao SK. Prospects of using herbal products in the control of mosquito vectors. Icmr Bull 2003; 33: 1-10.
[8] Ghosh A, Chowdhury N, Chandra G. Laboratory evaluation of a phytostroid compound of mature leaves of Day Jasmine (Solanaceae: Solanales) against larvae of Culex quinquefasciatus (Diptera: Culicidae) and nontarget organisms. Parasitol Res 2008; 103: 271-7.
[9] Bagavan A, Rahuman AA, Kamaraj C, Geetha K. Larvicidal activity of saponin from Achyranthes aspera against Aedes aegypti and Culex quinquefasciatus (Diptera: Culicidae). Parasitol Res 2008; 103: 223-9.
[10] Mathew N, Anitha MG, Bala TS, Sivakumar SM, Narmadha R, Kalyanasundaram M. Larvicidal activity of Saraca indica, Nycanthus arbor-tristis, and Clitoria ternatea extracts against three mosquito vector species. Parasitol Res 2009; 104: 1017-25.
[11] Rajkumar S, Jebanesan A. Larvicidal and oviposition activity of Cassia obtusifolia Linn. (Family: Leguminosae) leaf extract against malarial vector, Anopheles stephensi Liston (Diptera: Culicidae). Parasitol Res 2009; 104: 337-40.
[12] Karunamoorthi K, Ilango K. Larvicidal activity of Cymbopogon citratus (DC.) Stapf. and Croton macrostachyus Del. against Anopheles arabiensis Patton, a potent malaria vector. Eur Rev Med Pharmacol Sci 2010; 14: 57-62.
[13] Govindarajan M, Sivakumar R, Rajeswari M. Larvicidal efficacy of Cassia fistula Linn. leaf extract against Culex tritaeniorhynchus Giles and Anopheles subpictus Grassi (Diptera: Culicidae). Asian Pac J Trop Dis 2011; 1: 295-8.
[14] Arjunan N, Murugan K, Madhiyazhagan P, Kovandan K, Prasannakumar K, Thangamani S, et al. Mosquitocidal and water purification properties of Cydonon dactylon, Aloe vera, Hemidesmus indicus and Coleus amboinicus leaf extracts against the mosquito vectors. Parasitol Res 2012; 110: 1435-43.
[15] Yu J, Liu XY, Yang B, Wang J, Zhang FQ, Feng ZL, et al. Larvicidal activity of essential extract of Rosmarinus officinalis against Culex quinquefasciatus. J Am Mosq Control Assoc 2013; 29(1): 44-8.
[16] Girmay K, Fikre B, Asmelash A, Getachew B, Tekle E, Raja N. Evaluation of water and ethanol extracts of Schinus molle Linn. against immature Culex quinquefasciatus Say (Diptera: Culicidae). J Coast Life Med 2014; 2(6): 471-7.
[17] Roy B, Jalal S, Sarkar BC, Amin MR, Jeon YH, Chun IJ. Evaluation of insecticidal activities of common cocklebur fruit extract against pulse beetle Callosobruchus chinesis (Coleoptera: Bruchidae). Korean J Appl Entomol 2012; 51(4): 325-9.