Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article doi: 10.12980/jclm.3.2015j5-109

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

Kasim Roba, Getinet Masresha, Wondmeneh Jemberie, Raja Nagappan*

Department of Biology, College of Natural and Computational Sciences, University of Gondar, Post Box-196, Gondar, Ethiopia

ARTICLE INFO

Article history:
Received 7 Jul 2015
Accepted 21 Jul 2015
Available online 28 Oct 2015

Keywords:
Culex mosquitoes
Larvicidal
Pupicidal
Solvent extract
Xanthium strumarium

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[1]. Among the various categories of mosquito species, *Culex* species in particular *Culex quinuefasciatus*, 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 phytochemicals 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

*Corresponding author: Raja Nagappan, Department of Biology, College of Natural and Computational Sciences, University of Gondar, Post Box 196, Gondar, Ethiopia.
Tel: 00251-918-711780
E-mail: nagappanraja@yahoo.com
Foundation Project: Supported by University of Gondar (UoG/Budget/no 6215/2015).

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 *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, *X. strumarium* is locally called as "bandaa" 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 *Culex* mosquitoes. Therefore, present study was initiated to evaluate solvent extract of *X. strumarium* leaves against immature stages of the *Culex* mosquitoes.

2. Materials and methods

2.1. Immature Culex larvae collection and maintenance

The immature *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 *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 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 1 000 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 *Culex* mosquitoes.

2.5. Evaluation of larvicidal activity of plant extract

Larvicidal activity of acetone, methanol and water extract of *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].

Corrected percent of mortality = [mortality in test (%) – mortality in control (%)]/[100% – mortality in control (%)] × 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 *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 (χ^2) analysis. The LC_{50} and LC_{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 X. strumarium plant extracts

Mean percentage of mortality of third instar larvae of *Culex* mosquitoes exposed to *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 LC_{50} and LC_{90} values

were 107.9 and 241.6 mg/L respectively. The χ^2 analysis results showed statistical significance at 5% level (χ^2 = 7.768; P = 0.05). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} 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 LC_{50} and LC_{90} values were 209.7 and 333.3 mg/L respectively. The χ^2 analysis results showed statistical significance at 5% level (χ^2 = 18.659; P < 0.05). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} 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 after 24 h exposure in *X. strumarium* plant extracts. %.

Concentrations (mg/L)	Extracts tested		
	Acetone	Methanol	Water
50	22.10 ± 11.16	11.00 ± 0.00	11.00 ± 0.00
100	51.80 ± 16.97	11.00 ± 0.00	11.00 ± 0.00
150	74.10 ± 6.41	14.70 ± 6.47	11.00 ± 0.00
200	77.80 ± 0.00	44.40 ± 11.11	33.30 ± 11.11
250	88.90 ± 0.00	74.10 ± 6.41	44.40 ± 11.11
LC_{50} (LCL-UCL)	107.9 (54.1–141.2)	209.7 (159.6–371.6)	284.9 (217.1–792.1)
LC_{90} (LCL-UCL)	241.6 (197.2–355.7)	333.3 (252.6–863.5)	488.9 (339.8–1846.9)
χ^2	7.768	18.659	8.328
P value	0.050	0.000	0.400

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 LC_{50} and LC_{90} 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 LC_{50} and LC_{90} values were 145.7 and 236.8 mg/L respectively. The χ^2 analysis results showed statistical significance at 5% level (χ^2 = 14.224; P < 0.05). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 106.4–182.9 mg/L and 195.9–344.1 mg/L respectively. The mean percentage of mortality in water extract was maximum (88.90%) at 250 mg/L concentration. The calculated LC_{50} and LC_{90} values were 167.9 and 259.0 mg/L respectively. The χ^2 analysis results showed statistical significance at 5% level (χ^2 = 17.063; P < 0.05). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 127.9–217.3 mg/L and 211.8– 406.3 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		
	Acetone	Methanol	Water
50	88.90 ± 0.00	11.00 ± 0.00	11.00 ± 0.00
100	88.90 ± 0.00	14.70 ± 6.47	11.00 ± 0.00
150	88.90 ± 0.00	62.90 ± 16.97	29.60 ± 6.41
200	88.90 ± 0.00	81.40 ± 6.41	74.10 ± 6.41
250	88.90 ± 0.00	88.90 ± 0.00	88.90 ± 0.00
LC_{50} (LCL-UCL)	Not calculated	145.7 (106.4–182.9)	167.9 (127.9–217.3)
LC_{90} (LCL-UCL)	Not calculated	236.8 (195.9–344.1)	259.0 (211.8–406.3)
χ^2	Not calculated	14.224	17.063
P value	Not calculated	0.003	0.001

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 LC_{50} and LC_{90} values of acetone and methanol extract was not calculated by SPSS software due to no variation in percentage of mortality. In water extract, maximum percentage of mortality was 88.90% at 250 mg/L followed by 81.60% at 200 mg/L concentration. The calculated LC_{50} and LC_{90} values were 161.9 and 249.3 mg/L respectively. The χ^2 analysis results showed statistical significance at 5% level (χ^2 = 20.965; P < 0.05). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} 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		
	Acetone	Methanol	Water
50	88.90 ± 0.00	88.90 ± 0.00	11.00 ± 0.00
100	88.90 ± 0.00	88.90 ± 0.00	11.00 ± 0.00
150	88.90 ± 0.00	88.90 ± 0.00	33.30 ± 0.00
200	88.90 ± 0.00	88.90 ± 0.00	81.60 ± 6.35
250	88.90 ± 0.00	88.90 ± 0.00	88.90 ± 0.00
LC_{50} (LCL-UCL)	Not calculated	Not calculated	161.9 (116.2–215.4)
LC_{90} (LCL-UCL)	Not calculated	Not calculated	249.3 (201.5–415.2)
χ^2	Not calculated	Not calculated	20.965
P value	Not calculated	Not calculated	0.000

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 LC_{50} and LC_{90} values were 179.6 and 327.7 mg/L respectively. The χ^2 analysis results did not show statistical significance at 5% level (χ^2 = 6.005; P > 0.05). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} 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 LC_{50} and LC_{90} values were 239.1 and 388.8 mg/L respectively. The χ^2 analysis results showed statistical significance at 5% level (χ^2 = 18.058; P < 0.05). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 179.1–931.2 mg/L and 276.8–2 192.6 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 LC_{50} and LC_{90} 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 LC_{50} and LC_{90} values were 42.8 and 188.5 mg/mL respectively. The χ^2 analysis results did not show statistical significance at 5% level (χ^2 = 3.032; $P > 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 8.4–63.9 mg/L and 165.9–225.8 mg/L respectively. In methanol extract, maximum percentage of mortality of 88.90% was observed at 250 mg/L concentration. The calculated LC_{50} and LC_{90} values were 105.1 and 249.4 mg/L respectively. The χ^2 analysis results did not show statistical significance at 5% level (χ^2 = 1.326; $P > 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 88.2–119.2 mg/L and 226.8–281.6 mg/L respectively. The mean percentage of mortality in water extract was maximum (70.40%) at 250 mg/L. The calculated LC_{50} and LC_{90} values were 209.1 and 338.7 mg/L respectively. The χ^2 analysis results showed statistical significance at 5% level (χ^2 = 10.647; $P < 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 170.3–292.3 mg/L and 267.9–592.8 mg/L respectively.

Table 4

Mean percentage of mortality of fourth insatar 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 ± 11.11	11.00 ± 0.00	11.00 ± 0.00
200	48.40 ± 6.42	33.30 ± 11.11	11.00 ± 0.00
250	77.80 ± 0.00	62.90 ± 6.41	11.00 ± 0.00
LC_{50} (LCL-UCL)	179.6 (147.9–224.2)	239.1 (179.1–931.2)	Not calculated
LC_{90} (LCL-UCL)	327.7 (267.1–485.1)	388.8 (276.8–2 192.6)	Not calculated
χ^2	6.005	18.058	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 ± 11.11	11.00 ± 0.00
100	74.10 ± 6.41	44.40 ± 11.11	11.00 ± 0.00
150	85.20 ± 6.41	66.70 ± 11.11	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
LC_{50} (LCL-UCL)	42.8 (8.4–63.9)	105.1 (88.2–119.2)	209.1 (170.3–292.3)
LC_{90} (LCL-UCL)	188.5 (165.9–225.8)	249.4 (226.8–281.6)	338.7 (267.9–592.8)
χ^2	3.032	1.326	10.647
P value	0.220	0.723	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 LC_{50} and LC_{90} 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 LC_{50} and LC_{90} values were 42.5 and 224.8 mg/L respectively. The χ^2 analysis results did not show statistical significance at 5% level (χ^2 = 4.525; $P > 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 4.2–67.3 mg/L and 199.5–263.9 mg/L respectively. The mean percentage of mortality in water extract was maximum (88.90%) at 250 mg/L. The calculated LC_{50} and LC_{90} values were 170.5 and 265.4 mg/L respectively. The χ^2 analysis results showed statistical significance at 5% level (χ^2 = 11.921; $P < 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 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 ± 16.9	11.00 ± 0.00
100	88.90 ± 0.00	66.70 ± 11.11	11.00 ± 0.00
150	88.90 ± 0.00	81.50 ± 6.41	33.30 ± 11.11
200	88.90 ± 0.00	88.90 ± 0.00	66.70 ± 11.11
250	88.90 ± 0.00	88.90 ± 0.00	88.90 ± 0.00
LC_{50} (LCL-UCL)	Not calculated	42.5 (4.2–67.3)	170.5 (137.7–210.4)
LC_{90} (LCL-UCL)	Not calculated	224.8 (199.5–263.9)	265.4 (221.7–377.7)
χ^2	Not calculated	4.525	11.921
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 LC_{50} and LC_{90} values were 127.7 and 261.1 mg/L respectively. The χ^2 analysis results showed statistical significance at 5% level (χ^2 = 12.776; $P < 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 51.4–170.5 mg/L and 203.9–480.1 mg/L respectively. In methanol extract, maximum percentage of mortality of 70.00% was observed at 250 mg/L concentration. The calculated LC_{50} and LC_{90} values were 100.5 and 438.2 mg/L respectively. The χ^2 analysis results did not show statistical significance at 5% level (χ^2 = 0.675; $P > 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value 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 (55.60%) at 250 mg/L. The calculated LC_{50} and LC_{90} values were 253.9 and 461.4 mg/L respectively. The χ^2 analysis results showed statistical significance at 5% level (χ^2 = 8.059; $P < 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 195.9–562.8 mg/L and 326.4–1 419.3 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	14.70 ± 6.48	40.70 ± 16.97	14.70 ± 6.48
100	48.10 ± 16.90	51.90 ± 6.42	14.70 ± 6.47
150	66.70 ± 11.11	55.60 ± 11.11	22.20 ± 11.16
200	77.80 ± 11.11	66.70 ± 0.00	29.60 ± 6.41
250	81.50 ± 6.41	70.00 ± 6.41	55.60 ± 11.12
LC_{50} (LCL-UCL)	127.7 (51.4–170.5)	100.5 (49.8–130.8)	253.9 (195.9–562.8)
LC_{90} (LCL-UCL)	261.1 (203.9–480.1)	438.2 (348.6–653.3)	461.4 (326.4–1419.3)
χ^2	12.776	0.675	8.059
P value	0.005	0.879	0.045

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 LC_{50} and LC_{90} values were 106.9 and 255.8 mg/L respectively. The χ^2 analysis results did not show statistical significance at 5% level ($\chi^2 = 4.599$; $P > 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 89.5–121.4 mg/L and 232.2–289.7 mg/L respectively. In methanol extract, maximum percentage of mortality of 74.10% was observed at 250 mg/L concentration. The calculated LC_{50} and LC_{90} values were 77.6 and 385.1 mg/L respectively. The χ^2 analysis results did not show statistical significance at 5% level ($\chi^2 = 1.646$; $P > 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} 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 (66.70%) at 250 mg/L. The calculated LC_{50} and LC_{90} values were 217.9 and 383.1 mg/L respectively. The χ^2 analysis results showed statistical significance at 5% level ($\chi^2 = 9.205$; $P < 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 173.1–345.0 mg/L and 289.2–805.1 mg/L respectively.

Table 8

Mean percentage of mortality of pupa of *Culex* mosquitoes after 48 h exposure in *X. strumarium* plant extracts. %.

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
LC_{50} (LCL-UCL)	106.9 (89.5–121.4)	77.6 (22.5–108.4)	217.9 (173.1–345.0)
LC_{90} (LCL-UCL)	255.8 (232.2–289.7)	385.1 (317.2–541.1)	383.1 (289.2–805.1)
χ^2	4.599	1.646	9.205
P value	0.204	0.649	0.027

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 observed maximum (88.90%) in acetone extract at 250 mg/L concentration (Table 9). The calculated LC_{50} and LC_{90} values were 84.3 and 236.3 mg/L respectively. The χ^2 analysis results showed statistical significance at 5% level ($\chi^2 = 10.025$; $P < 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 32.2–127.7 mg/L and 183.2–434.7 mg/L respectively. In methanol extract, maximum percentage of mortality of 81.50% was observed at 250 mg/L concentration. The calculated LC_{50} and LC_{90} values were 97.6 and 286.4 mg/L respectively. The χ^2 analysis results did not show statistical significance at 5% level ($\chi^2 = 6.356$; $P > 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 7.9–138.4 mg/L and 223.5–506.3 mg/L respectively. The mean percentage of mortality in water extract was maximum (85.10%) at 250 mg/mL. The calculated LC_{50} and LC_{90} values were 164.9 and 305.1 mg/L respectively. The χ^2 analysis results showed statistical significance at 5% level ($\chi^2 = 15.774$; $P < 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 106.6–250.6 mg/L and 230.8–706.3 mg/L respectively.

Table 9

Mean percentage of mortality of pupa of *Culex* mosquitoes after 72 h exposure in *X. strumarium* plant extracts. %.

Concentrations (mg/L)	Extracts tested		
	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
LC_{50} (LCL-UCL)	84.3 (32.2–127.7)	97.6 (7.9–138.4)	164.9 (106.6–250.6)
LC_{90} (LCL-UCL)	236.3 (183.2–434.7)	286.4 (223.5–506.3)	305.1 (230.8–706.3)
χ^2	10.025	6.356	15.774
P value	0.018	0.096	0.001

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. Those 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 chemical substances of the plant powder may dissolve maximumly in acetone extract compared to others. This will guide selection of solvent which is important for plant extraction. 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 bio-pesticides. 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 depended 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.

Acknowledgments

Authors are thankful to University of Gondar for financial assistant (UoG/Budget/no 6215/2015) for the successful completion of this research work. The acknowledgment is also extended to Melkamsew Gezahegn, Alebachew Maru, Bayih Abebaw, Banchamlak Diress and Eftu Moti (undergraduate students final year), Department of Biology, College of Natural and Computational Sciences for assisting larval collection, preparation of plant materials and data collection during the experimental period.

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, Kazura JW. Clinical and laboratory aspects of filariasis. *Clin Microbiol Rev* 1989; **2**(1): 39-50.

[3] Hettiaratchi UP, Munasingha DH, Chandrasekharan NV, Karunanayake 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, Lammie 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, Orelus JN, Roberts JM, Lammie 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 phytosteroid 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*, *Nyctanthes 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, Kovendan K, Prasannakumar K, Thangamani S, et al. Mosquitocidal and water purification properties of *Cynodon 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, Sarker BC, Amin MR, Jeon YH, Chun IJ. Evaluation of insecticidal activities of common cocklebur fruit extract against pulse beetle *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Korean J Appl Enttomol* 2012; **51**(4): 325-9.

[18] Yanar D, Kadıoğlu I, Gökçe A. Ovicidal activity of different plant extracts on two-spotted spider mite (*Tetranychus urticae* Koch) (Acari: Tetranychidae). *Sci Res Essays* 2011; **6**: 3041-4.

[19] Gökçe A, Stelinski LL, Whalon ME, Gut LJ. Toxicity and antifeedant activity of selected plant extracts against larval obliquebanded leafroller, *Choristoneura rosaceana* (Harris). *Open Entomol J* 2010; **4**: 18-24.

[20] Gökçe A, Isaacs R, Whalon ME. Ovicidal, larvicidal and anti-ovipositional activities of *Bifora radians* and other plant extracts on the grape berry moth *Paralobesia viteana* (Clemens). *J Pest Sci* 2011; **84**: 487-93.

[21] Alkari S, Chaturvedi A. *Xanthium strumarium* a possible biocontrol agent against *Helicoverpa armigera*. *J Entomol Res* 2010; **34**(2): 143-5.

[22] Sarmah M, Rahman A, Phukan AK, Gurusubramanian G. Effect of aqueous plant extracts on tea red spider mite, *Oligonychus coffeae*, Nietner (Tetranychidae: Acarina) and *Stethorus gilvifrons* Mulsant. *Afr J Biotechnol* 2009; **8**(3): 417-23.

[23] Singh SP, Raghavendra K, Dua VK. Evaluation of larvicidal and repellent activity of *Xanthium strumarium* (Asteraceae) ethanol extract against mosquito vectors. *J Commun Dis* 2009; **41**: 263-9.

[24] Wondimu T, Asfaw Z, Kelbessa E. Ethnobotanical study of medicinal plants around 'Dheeraa' town, Arsi Zone, Ethiopia. *J Ethnopharmacol* 2007; **112**: 152-61.

[25] Abebe D, Debella A, Urga K. *Illustrated checklist: medicinal plants and other useful plants of Ethiopia*. 1st ed. Singapore: Camerapix Publisher International; 2003.

[26] World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. Geneva: World Health Organization; 2005. [Online] Available from: http://apps.who.int/iris/bitstream/10665/69101/1/WHO_CDS_WHOPES_GCDPP_2005.13.pdf [Accessed on 15th June, 2014]

[27] Abbott WS. A method for computing the effectiveness of an insecticide. *J Econ Entomol* 1925; **18**: 265-7.

[28] Becker N, Petrić D, Zgomba M, Boase C, Dahl C, Lane J, et al. *Mosquitoes and their control*. New York: Kluwer Academic/Plenum Publishers; 2003; p. 498.

[29] Kumar MS, Maneemegalai S. Evaluation of larvicidal effect of *Lantana camara* Linn. against mosquito species *Aedes aegypti* and *Culex quinquefasciatus*. *Adv Biol Res* 2008; **2**(3-4): 39-43.

[30] Scherer R, Wagner R, Meireles MAA, Godoy HT, Duarte MCT, Filho JT. Biological activity and chemical composition of hydrodistilled and supercritical extracts of *Xanthium strumarium* L. leaves. *J Essent Oil Res* 2010; **22**(5): 424-9.

[31] Farooq U, Waseem B, Muzaffar R, Tripathi J, Tharani M, Sharma M. A comparative study of phytochemical investigation of *Xanthium strumarium* medicinal plant. *Int J Res Pharm Chem* 2014; **4**(1): 96-100.