



©Biotechnology Society



www.bti.org.in
ISSN 0974-1453
Research article

EVALUATION OF LARVICIDAL PROPERTIES OF *ARGEMONE MEXICANA* LINN. SEED AND LEAF EXTRACTS AGAINST *CULEX* MOSQUITO (DIPTERA: CULICIDAE) SPECIES

Wondmeneh Jemberie, Getinet Masresha and Nagappan Raja*

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

*Corresponding author: nagappanraja@yahoo.com

ABSTRACT

Culex mosquito species transmit various human pathogens including encephalitis, Rift valley fever and lymphatic filariasis. To find out eco-friendly alternatives to replace synthetic chemical pesticides used in mosquito control program present was carried out to evaluate acetone, chloroform, methanolic and distilled water extracts of *Argemone mexicana* seeds and leaves against IVth instar larvae of *Culex* mosquito species. The larvicidal property of the plant extracts was tested following WHO method. Results revealed that maximum percentage mortality of 73.3% was observed in 500 ppm concentration after 96 hr exposure period in chloroform extract of leaves. Similar concentration and exposure period was recorded in methanolic extract of seeds with 86.6% mortality. The computed probit analysis results showed minimum LC₅₀ concentration of 567.3 ppm for methanolic extract of leaves after 24 hr exposure period followed by chloroform (407.4 ppm) extract after 48 hr exposure period and 190.1 ppm for acetone extract after 72 hr exposure period. In seed extracts, 670.9 ppm concentration for methanolic extract after 24 hr exposure period, 499.4 ppm concentration for acetone extract after 48 hr exposure period and 64.3 ppm and -289.6 ppm concentration for methanolic extract after 72 and 96 hr exposure period respectively. In general, dose and time dependent larval mortality was observed in the study among the plant extracts tested. These plant extracts may have toxic chemicals to kill the larvae of *Culex* mosquito species. In Ethiopia, these plants are growing extensively and it can be useful to control larvae of *Culex* mosquitoes in their breeding site. However, field validation of the plant extracts, characterization of bioactive molecules and formulations are important to utilize large scale field application.

Keywords: Botanicals, *Culex*, larvicidal, lethal concentration, solvent extracts.

INTRODUCTION

Mosquitoes belong to the order Diptera family Culicidae are the principal vector for several vector borne diseases. Mosquitoes belong to the genus *Anopheles*, *Culex* and *Aedes* transmit disease causal organism of malaria, filariasis, Japanese encephalitis, dengue fever, dengue haemorrhagic fever and yellow fever (Hubalek and Haluzka, 1999). *Culex* mosquito species transmit various human pathogens including encephalitis, Rift valley fever and lymphatic filariasis. More than 80 countries over 120 million people are affected with Lymphatic filariasis and over 40 millions are seriously affected with this disease. Synthetic chemical pesticides are extensively used in mosquito control program but those chemicals are toxic to human and other organisms living on the earth in addition to environmental pollution (Rahuman *et al.*, 2009).

Plant secondary metabolites are useful alternatives for vector control program because of rich source of potential bioactive chemicals. Phytochemicals played significant role to develop eco-friendly insecticides due to biodegradable nature and safer than synthetic insecticides (Moretti *et al.*, 2002; Cetin *et al.*, 2004). The exotic weed *Argemone mexicana* was indigenous in South America but widespread distribution in many tropical countries including West Africa (Ibrahim and Ibrahim, 2009). Earlier reports showed that *Argemone mexicana* seeds extracted with chloroform proved to have strong significant larvicidal activity compared with methanolic extract against 3 instar larvae of *Cx. pipiens* after 24

and 48 hr exposure period (Zeinab and Abou-Elnaga, 2015). In Ethiopia, particularly in Gondar these plants are growing extensively on the road side and waste lands as a common weed. The bioactivities of these plant extracts against *Culex* mosquito species are scientifically not well documented.

Argemone mexicana belongs to the family Papaveraceae is called as Mexican poppy, prickly poppy, yellow thistle, Mexican thistle in vernacular name (Ownbey, 1997). The methanolic extract at 100 mg/ml proved to kill 100% mosquito larvae after 24 hr exposure period (Rothe *et al.*, 2016). Petroleum ether extracts was reported to have maximum oviposition deterrent (99.4%) and moderate ovicidal activity against *Aedes aegypti* mosquitoes (Warikoo and Kumar, 2014). The prolonged larval pupal period, promising larvicidal activity and decreased adult emergence due to the inhibition of molting process was observed from crude alkaloid of the leaves (Bapna *et al.*, 2016). The hexane extract of seeds reported to have larvicidal properties against the larvae of *Cx. Quinquefasciatus* and *Ae. aegypti* (Sivaraman *et al.*, 2016). Sharma *et al.* (2016) reported that ethanol and acetone extract of leaves showed LD₅₀ value of 1.878 ml/kg and 1.219 ml/kg respectively against *Heliothis armigera* after 96 hr exposure period. Malarvannan *et al.* (2008) observed reduced life span, fecundity rate and egg hatchability of *Helicoverpa armigera* treated with petroleum ether and water extracts. The combination *Nerium* and *Argemone* extract was observed with higher repellent activity (89.29%) compared to

commercial neem product Nimbicidine (78.58%) against *Helicoverpa armigera* (Kulkarni *et al.*, 2009). Mukhopadhyay *et al.* (2002) observed damaged malphigian tubules and midgut tissues of *Drosophila melanogaster* treated with seed oil. Feeding deterrent, insecticidal and insect growth regulatory activities of various parts of *Argemone mexicana* was confirmed against *Spodoptera litura*. Acetone extracts of the seeds was reported with higher feeding deterrence activity; methanolic extracts of seeds with maximum insecticidal activity and insect growth regulatory activity alone was noticed in ethyl acetate extract (Ramanan and Selvamuthu kumaran, 2016). Hence, the present study was conducted to check the larvicidal properties of acetone, chloroform, methanolic and distilled water extracts of leaves and seeds of *Argemone mexicana* against IVth instar larvae of *Culex* mosquito species.

MATERIALS AND METHODS

Collection and maintenance of mosquito larvae

Culex mosquito larvae were collected from the stagnant water polluted with organic wastes in Kehha River, Gondar. The larval collection was made by using kitchen strainer from the breeding site. The collected larvae were kept in the plastic container and brought to the laboratory. In the laboratory, yeast powder and powdered biscuit (1:3 ratio) was added in the container as a source of feed. After 24 hr, acclimatization of the larvae in the laboratory was used for experiment.

Plant source collection and processing

One kg of fresh leaves and 250 gm of seeds were collected from opened mature dry fruit of *Argemone mexicana* in and around Tewodros campus, University of Gondar. The leaves and seeds were collected randomly from more than 20 plants, pooled together and washed with water to remove unwanted debris. The washed leaves and seeds were dried under shade in order to prevent chemical denaturation due to sunlight. After complete drying, plant leaves and seeds were finely powdered using electric blender (RRH-A200 high speed multifunctional with the motor speed of 28000 rpm purchased from Shanghia Yuanya Industries and Trade Company Limited, China. The powdered leaves and seeds were sieved through kitchen strainer to obtain fine powder for solvent extraction.

Extraction of plant powder

Twenty gram of leaf powder was taken in to 250 mL conical flask and added 100 mL of solvents such as acetone, chloroform, methanolic (Laboratory reagents supplied by Loba chemicals private limited, India) and distilled water individually. After adding the solvent, mouth of the conical flask was tightly plugged with cotton followed by covering with aluminium foil. Then the conical flasks were kept in a shaker for 24 hr shaking in order to get homogenous mixing of solution. After shaking, liquid part was removed by filtration using Whatman No.1 filter paper and the residue was discarded. The liquid filtrate was kept inside the oven at 37° C for 2 days or up to the solvent evaporated completely. After complete evaporation of

the solvent, residue was collected and stored in a refrigerator at 4°C for subsequent experiment.

Preparation concentration

The residue collected from each solvent was used to prepare 10,000 ppm concentration (1 gm/100 mL basis). Based on the amount recovered from each residue the amount of water and solvent was adjusted. For each solvent extract 1 mL of soap solution was added for the purpose of emulsification. From the stock solution, working concentration of 50, 100, 250 and 500 ppm was prepared by serial dilution method and tested against IVth instar larvae of *Culex* mosquito species.

Larvicidal bioassay

Larvicidal activity of acetone, chloroform, methanolic and distilled water extract of *Argemone mexicana* leaves and seeds was tested following the protocol recommended by WHO (WHO, 1996) with modifications. The experiment was conducted at Entomology laboratory, Maraki campus, University of Gondar. The larvicidal activity of the plant extract was conducted by using 250 mL plastic container. In each container, 10 IVth instar stage larvae of *Culex* mosquitoes were released. The concentration of acetone, chloroform, methanolic and distilled water extract of leaves and seeds was maintained at 50, 100, 250 and 500 ppm in 100 mL of water. In control, except plant extracts remaining materials used to prepare stock solution was added as mentioned in preparation of concentration. The number of dead larvae was recorded continuously after 24, 48, 72 and 96 hr exposure period. The

number experiment was replicated three times. The experiment was conducted by using completely randomized block design. The immovable larva when the water was disturbed and settled at the bottom of the container was considered as dead. The larval mortality was corrected and calculated by using Abbott's formula (Abbott, 1925).

Corrected per cent mortality = (% mortality in test - % mortality in control) / (100 - % mortality in control) X 100.

Statistical analysis

The number of dead larva recorded from three replications was subjected to calculate percentage mortality. The descriptive statistical analysis was carried out to calculate mean and standard error. The LC₅₀ and LC₉₀ values and 95% upper confidence limit (UCL) and lower confidence limit (LCL) was calculated. The Chi-square analysis [χ^2] was carried out to check the level of statistical significant at 5% level (p<0.05). All the statistical analysis was carried out by using SPSS version 16 software for windows.

RESULTS

Percentage mortality of IVth instar larvae of *Culex* mosquito species exposed to acetone extract of *Argemone mexicana* leaves was presented in Table 1. Results indicate that maximum percentage mortality of 73.3% was recorded at 500 ppm concentration after 96 hr exposure period. The exposure period and plant extract concentration increased percentage mortality rate was also increased. The percentage mortality after 96 hr was ranged from 60 to 73.3% from lower concentration to higher concentration. The calculated LC₅₀ and LC₉₀

value for 24 hr exposure period was 654.2 ppm and 1292.9 ppm respectively. The χ^2 analysis result was showed statistically

significant difference (P<0.05) after 48 and 72 hr exposure period.

Table 1. Mean percentage mortality of IVth instar larvae of *Culex* mosquito species exposed to acetone extract of *Argemone mexicana* leaves.

Concentration in ppm	Exposure period			
	24 hr	48 hr	72 hr	96 hr
50	10.0 ± 0.00	16.6 ± 3.33	40.0 ± 5.77	60.0 ± 0.00
100	13.3 ± 3.33	33.3 ± 3.33	46.6 ± 3.33	63.3 ± 3.33
250	23.3 ± 3.33	36.6 ± 3.33	56.6 ± 3.33	63.3 ± 3.33
500	36.6 ± 3.33	50.0 ± 0.00	63.3 ± 3.33	73.3 ± 3.33
LC ₅₀ value	654.2	477.2	190.1	-291.6
LCL-UCL	572.3-778.9	374.2-709.9	67.1-288.0	-967.1 - -91.7
LC ₉₀ value	1292.9	1240.0	1216.2	1410.7
LCL-UCL	1098.1-1600.2	916.3-2106.9	864.1-2335.9	973.4 – 2962.2
χ^2	14.42	26.37*	19.78*	10.45

Values are mean ± standard error, *indicates statistical significant (P<0.05); LC – Lethal concentration; LCL- Lower confidence limit; UCL-Upper confidence limit; χ^2 - Chi-square.

Table 2. Mean percentage mortality of IVth instar larvae of *Culex* mosquito species exposed to chloroform extract of *Argemone mexicana* leaves.

Concentration in ppm	Exposure period			
	24 hr	48 hr	72 hr	96 hr
50	10.0 ± 0.00	13.3 ± 3.33	26.6 ± 3.33	46.6 ± 3.33
100	26.6 ± 3.33	40.0 ± 5.77	43.3 ± 3.33	60.0 ± 5.57
250	33.3 ± 3.33	43.3 ± 3.33	56.6 ± 3.33	66.6 ± 3.33
500	40.0 ± 5.77	53.3 ± 3.33	66.6 ± 3.33	80.0 ± 5.57
LC ₅₀ Value	604.8	407.4	245.1	30.3
LCL-UCL	442.3-1200.1	285.5-805.5	173.5-325.1	-171.6- 21.7
LC ₉₀ Value	1387.8	1108.8	871.8	720.8
LCL-UCL	945.2-3208.4	747.5-2877.1	677.5-1311.2	539.3-1223.3
χ^2	41.6*	65.5*	29.1*	32.5*

Values are mean ± standard error, *indicates significant (P<0.05); LC – Lethal concentration; LCL- Lower confidence limit; UCL-Upper confidence limit; χ^2 - Chi-square.

Table 2 indicates percentage mortality of IVth instar larvae of *Culex* mosquitoes exposed to chloroform extract of

Argemone mexicana leaves. Result showed that maximum mortality rate of 80% was recorded at 500 ppm concentration after 96

hr exposure period. The calculated LC₅₀ and LC₉₀ concentration for 24 hr exposure period was 604.8 ppm and 1387.8 ppm respectively. The χ^2 analysis results for the exposure period and concentration tested was showed statistically significant difference (P<0.05). The calculated LC₅₀ concentration after 96 hr exposure period was 30.3 ppm. The percentage mortality rate was below 50% after 24 hr exposure period for all the concentration tested.

Table 3 demonstrates percentage mortality of IVth instar larvae of *Culex*

Table 3. Mean percentage mortality of IVth instar larvae of *Culex* mosquito species exposed to methanolic extract of *Argemone mexicana* leaves.

Concentration in ppm	Exposure period			
	24 hr	48 hr	72 hr	96 hr
50	13.3 ± 3.33	13.3 ± 3.33	23.3 ± 3.33	36.6 ± 3.33
100	26.6 ± 3.33	33.3 ± 3.33	43.3 ± 3.33	56.6 ± 6.66
250	33.3 ± 3.33	36.6 ± 3.33	56.6 ± 3.33	56.6 ± 3.33
500	43.3 ± 3.33	50.0 ± 0.00	66.6 ± 3.33	76.6 ± 3.33
LC ₅₀ Value	567.3	471.3	254.2	132.9
LCL-UCL	439.1-895.6	363.2-735.1	178.1-343.6	61.6-213.1
LC ₉₀ Value	1336.1	1171.3	842.7	781.3
LCL-UCL	971.9-2375.9	851.7-2118.6	647.7-1312.7	586.7-1307.7
χ^2	27.6*	36.5*	37.3*	36.6*

Values are mean ± standard error, *indicates significant (P<0.05); LC – Lethal concentration; LCL- Lower confidence limit; UCL-Upper confidence limit; χ^2 - Chi-square.

Table 4 revealed percentage of mortality of IVth instar larvae of *Culex* mosquito species exposed to different concentration of distilled water extract of *Argemone mexicana* leaves. Result demonstrates that percentage of mortality after 24, 48 and 72 hr exposure period was less than 50% at all the concentration tested. The maximum percentage mortality of 76.6% was recorded in 500 ppm concentration after 96 hr

mosquito species exposed to methanolic extract of *Argemone mexicana* leaves. The results revealed that maximum percentage of mortality of 76.6% was recorded at 500 ppm concentration after 96 hr exposure period. The calculated LC₅₀ and LC₉₀ concentration for 24 hr exposure period was 567.3 ppm and 1336.1 ppm respectively. The χ^2 analysis result was showed statistically significant difference among the concentration and exposure period.

exposure period. The calculated LC₅₀ and LC₉₀ concentration for 24 hr exposure period was 1820.4 ppm and 3281.3 ppm respectively. The SPSS 16 version software was unable to calculate upper and lower confidence limits for LC₅₀ and LC₉₀ values due to wide range of difference. The χ^2 analysis result was showed statistically significant difference (P<0.05) among the concentration and exposure period.

Table 4. Mean percentage mortality of IVth instar larvae of *Culex* mosquito species exposed to distilled water extract of *Argemone mexicana* leaves.

Concentration in ppm	Exposure period			
	24 hr	48 hr	72 hr	96 hr
50	3.3 ± 3.33	6.6 ± 3.33	23.3 ± 3.33	30.0 ± 5.77
100	6.6 ± 6.67	6.7 ± 3.30	36.6 ± 3.33	43.0 ± 3.33
250	13.3 ± 3.33	20.0 ± 5.77	40.0 ± 0.00	56.6 ± 3.33
500	10.0 ± 0.00	30.0 ± 5.77	46.6 ± 3.33	76.6 ± 3.33
LC ₅₀ Value	1820.4	711.0	535.7	203.8
LCL-UCL	Not calculated	534.1-1290.2	394.9-996.4	150.9 – 254.7
LC ₉₀ Value	3281.3	1289.4	1684.3	698.3
LCL-UCL	Not calculated	915.8-2602.1	1140.7-3759.9	580.0-907.9
χ ²	78.7*	50.1*	19.4*	23.5*

Values are mean ± standard error, *indicates significant (P<0.05); LC – Lethal concentration; LCL- Lower confidence limit; UCL-Upper confidence limit; x²- Chi-square

Table 5 revealed the percentage mortality of IVth instar larvae of *Culex* mosquitoes exposed to different concentration of acetone extract of *Argemone mexicana* seeds. Result revealed that maximum percentage mortality of 76.6% was recorded in 500 ppm concentration after 96 hr exposure period. The percentage mortality after 24 and 48 hr exposure period was less than 50%. The calculated LC₅₀ and LC₉₀ concentration after 24 hr exposure period was 713.7 ppm and 1783.5 ppm respectively. The calculated concentration range for upper and lower confident limit of LC₅₀ and LC₉₀ values was 451.7-9941.0 ppm and 1021.6-31767.6 ppm respectively. The percentage mortality after 72 and 96 hr exposure period was ranged

from 36.6 -76.6. However, χ² analysis value indicates that after 72 and 96 hr exposure period within the concentration tested the result was statistically not significant (P>0.05).

Table 6 highlights the percentage mortality of IVth instar larvae of *Culex* mosquitoes exposed to chloroform extract of *Argemone mexicana* seeds. Result revealed that maximum percentage mortality of 63.3% was recorded in 500 ppm concentration after 96 hr exposure period. The calculated LC₅₀ and LC₉₀ concentration after 24 hr exposure period was 820.1 ppm and 1804.7 ppm respectively. The χ² analysis result was showed statistically significant difference (p<0.05) within the concentration and exposure period.

Table 5. Mean percentage mortality of IVth instar larvae of *Culex* mosquito species exposed to acetone extract of *Argemone mexicana* seeds.

Concentration in ppm	Exposure period			
	24 hr	48 hr	72 hr	96 hr
50	10.0 ± 0.00	13.3 ± 3.33	36.6 ± 3.33	43.3 ± 3.33
100	33.3 ± 3.33	36.6 ± 3.33	43.3 ± 3.33	53.3 ± 3.33
250	33.3 ± 3.33	40.0 ± 5.77	53.3 ± 3.33	66.6 ± 3.33
500	36.6 ± 3.33	46.6 ± 3.33	63.3 ± 3.33	76.6 ± 3.33
LC ₅₀ Value	713.7	499.4	239.1	82.9
LCL-UCL	451.7- 9941.0	345.4 - 1305.8	188.2-292.6	-13.9-145.3
LC ₉₀ Value	1783.5	1332.8	1214.6	756.6
LCL-UCL	1021.6-31767.6	854.8 -4543.6	922.3-1497.7	607.8-1053.5
χ ²	54.5*	57.5*	13.2	17.7

Values are mean ± standard error, *indicates significant (P<0.05); LC – Lethal concentration; LCL- Lower confidence limit; UCL-Upper confidence limit; χ²- Chi-square.

Table 6. Mean percentage mortality of IVth instar larvae of *Culex* mosquito species exposed to chloroform extract of *Argemone mexicana* seeds.

Concentration in ppm	Exposure period			
	24 hr	48 hr	72 hr	96 hr
50	13.3 ± 3.33	20.0 ± 0.00	23.3 ± 3.33	43.3 ± 3.33
100	20.0 ± 5.77	36.6 ± 3.33	40.0 ± 0.00	56.6 ± 3.33
250	23.3 ± 6.66	40.0 ± 5.77	53.3 ± 3.33	60.0 ± 5.77
500	33.3 ± 3.33	46.6 ± 3.33	60.0 ± 5.77	63.3 ± 3.33
LC ₅₀ Value	820.1	521.2	307.6	57.8
LCL-UCL	548.4-2805.3	370.6-1153.8	222.4-442.5	-967.2-206.5
LC ₉₀ Value	1804.7	1544.3	1002.6	1498.1
LCL-UCL	1119.9-7148.0	1008.1-4280.3	739.0-1752.6	900.3-8931.6
χ ²	39.8*	32.2*	36.2*	26.3*

Values are mean ± Standard error, *indicates significant (P<0.05); LC – Lethal concentration; LCL- Lower confidence limit; UCL-Upper confidence limit; χ²- Chi-square.

Table 7 indicates percentage mortality of IVth instar larvae of *Culex* mosquitoes exposed to different concentration of methanolic extract of *Argemone mexicana* seeds. Result revealed that maximum percentage mortality of 86.6% was recorded in 500 ppm

concentration after 96 hr exposure period. The percentage mortality after 96 hr exposure period was ranged from 60-86.6%. The calculated LC₅₀ and LC₉₀ concentration after 24 hr exposure period was 670.9 ppm and 2136.8 ppm respectively. The result of χ² analysis was showed statistically

significant difference (P<0.05) within the hrexposure period. concentration tested after 72 and 96

Table 7. Mean percentage mortality of IVth instar larvae of *Culex* mosquito species exposed to methanolic extract of *Argemone mexicana* seeds.

Concentration in ppm	Exposure period			
	24 hr	48 hr	72 hr	96 hr
50	26.6 ± 3.33	30.0 ± 0.00	46.6 ± 3.33	76.6 ± 3.33
100	33.3 ± 3.33	36.6 ± 3.33	50.0 ± 0.00	60.0 ± 5.77
250	36.6 ± 3.33	40.0 ± 5.77	70.0 ± 5.77	76.6 ± 3.33
500	43.3 ± 3.33	46.6 ± 3.33	73.3 ± 3.33	86.8 ± 3.33
LC ₅₀ value	670.9	577.3	64.3	-289.6
LCL-UCL	513.1-1083.1	404.9-1396.9	-107.2-150.2	-3574.4- -37.2
LC ₉₀ value	2136.8	2083.4	839.5	660.3
LCL-UCL	1520.0-3860.4	1317.9-6302.8	627.6-1412.2	421.6-3725.8
χ ²	14.0	15.9	25.9*	47.5*

Values are mean ± standard error, *indicates significant (P<0.05); LC – Lethal concentration; LCL- Lower confidence limit; UCL-Upper confidence limit; χ²- Chi-square.

Table 8 explained about percentage mortality of IVth instar larvae of *Culex* mosquitoes exposed to different concentration of distilled water extract of *Argemone mexicana* seeds. Result revealed that only after 96 hr exposure period at 500 ppm concentration showed above 50%

larval mortality. The calculated LC₅₀ and LC₉₀ concentration after 24 hr exposure period was 864.5 ppm and 1357.2 ppm respectively. The result of χ² analysis was showed statistically significant difference (P<0.05) within the concentration and exposure period.

Table 8. Mean percentage mortality of IVth instar larvae of *Culex* mosquito species exposed to distilled extract of *Argemone mexicana* seeds.

Concentration in ppm	Exposure period			
	24 hr	48 hr	72 hr	96 hr
50	0.0 ± 0.00	13.3 ± 3.33	16.6 ± 3.33	26.6 ± 3.33
100	0.0 ± 0.00	13.3 ± 3.33	30.0 ± 0.00	36.6 ± 3.33
250	13.3 ± 3.33	23.3 ± 3.33	43.3 ± 3.33	50.0 ± 5.77
500	13.3 ± 3.33	23.3 ± 3.33	56.6 ± 3.33	66.6 ± 3.33
LC ₅₀ value	864.5	1235.7	390.1	284.1
LCL-UCL	609.9-2717.7	747.0-11994	325.2-492.4	228.9-352.5
LC ₉₀ value	1357.2	2652.2	974.4	867.8
LCL-UCL	897.4-4885.2	1495.5-28751.8	784.5-1345.4	705.3-1175.7
χ ²	68.5*	24.5*	27.7*	21.3*

Values are mean ± standard error, *indicates significant (P<0.05); LC – Lethal concentration; LCL- Lower confidence limit; UCL-Upper confidence limit; χ²- Chi-square.

DISCUSSION

To develop eco-friendly products from plant secondary metabolites to control mosquitoes is one of the emerging fields of research in recent times among the scientific communities. To complement in this field of research present study was conducted to check the larvicidal activity of acetone, chloroform, methanolic and distilled water extract of *Argemone mexicana* leaves and seeds against IVth instar larvae of *Culex* mosquito species. In the present study percentage mortality of mosquito larvae was varied significantly based on the concentration and exposure period. The maximum percentage mortality was observed at higher concentration for all the tested solvent extracts. Among the solvent extracts tested, methanolic extract of the seeds at higher concentration proved to be highly toxic against IVth instar larvae of *Culex* mosquitoes. Among the leaf extract, maximum percentage mortality was observed in chloroform extract. The study clearly demonstrates that the dissolving nature of bioactive plant secondary metabolites varied from different polarity of the solvents used for extraction. The dose-dependent larval mortality of *Culex* mosquito species was observed in *Argemone mexicana* seeds and leaves extracts. Several earlier researchers also observed similar type of results against different species of mosquitoes (Choochote *et al.*, 2004; Singh *et al.*, 2006; Kaushik and Saini, 2008; Fred-Jaiyeseimi and Anthony, 2011).

In the present study, among the four solvent extracts of leaves, minimum LC₅₀ concentration was observed in methanolic

extracts after 24 hr exposure period. After 48 and 96 hr exposure period minimum LC₅₀ concentration was calculated for chloroform extract. After 72 hr, minimum LC₅₀ concentration was calculated for acetone extract. Among the solvent extracts tested, minimum LC₉₀ concentration was calculated for acetone extract after 24 hr; chloroform extract after 48hr; methanolic extract after 72 hr and distilled water extract after 96 hr exposure period. Among the seed extracts, the minimum LC₅₀ concentration was calculated for methanolic extract after 24 hr, 72 hr and 96 hr; acetone extract after 48 hr exposure period. The minimum LC₉₀ concentration was calculated for methanolic extract of seeds after 72 and 96 hr. This will indicate that accumulation of plant secondary metabolites varied in leaves and seeds. Banerji *et al.* (1969) reported that plant phytochemicals or secondary metabolites are accumulated in the form of the mixture and also their concentration varied among the plant part and the developmental stage. Earlier author reported that the plant contains terpenoids that have insecticidal and antifeedant activity against *H. armigera* and rice leaf folder larvae (Lagoet *al.*, 2002; Nathan *et al.*, 2005). The biological activity of some of the plant metabolites such as terpenoids, limonoids, phenolic and alkaloids already confirmed by earlier workers (Bilal and Hassan, 2012; Lame *et al.*, 2014). The bio-potential of *Argemone mexicana* plant extract confirmed against IVth instar larvae of *Culex* mosquito species in the present findings also in agreement with the earlier reports.

CONCLUSION

The present study confirmed larvicidal properties of *Argemone mexicana* solvent extracts of leaves and seeds against IVth instar larvae of *Culex* mosquito species. Several earlier literatures also confirmed bio-potential of this plant extracts against various agricultural pests and vectors. These plants may be useful to develop eco-friendly products to control larvae of *Culex* mosquito species in their breeding sites. In Ethiopia, these plants are growing extensively throughout the country. However, further isolation, characterization of bioactive molecules and formulations are important to develop eco-product to utilize large scale field application.

REFERENCES

- Abbott W (1925). A method for computing effectiveness of an insecticide. *J Econ Entomol.* 18: 265-267.
- Banerji A, Chadha MS, Malshet VG (1969). Isolation of 5-hydroxy-3, 6, 7, 3', 4'-pentamethoxyflavone from *Vitex negundo*. *J Phytochem.* 8: 511-512.
- Bapna S, Shinde RR, Satvaker T, Ramaiya M (2016). Larvicide and growth retarding activity of alkaloidal extract of two plants towards *Aedes aegypti* (Diptera: Culicidae). *World J Pharma Res.* 5(1): 998-1004.
- Bilal H and Hassan SA (2012). Plants secondary metabolites for mosquito control. *Asian Pacific J. Trop. Dis.* 169.
- Cetin H, Erler F, Yanikoglu A (2004). Larvicidal activity of a botanical natural product, AkseBio2, against *Culex pipiens*. *Fitoterapia,* 75:724-728.
- Choochote W, Tueton B, Kanjanapothi D, Rattanachanpichoi E, Chaithong U, Chainong P (2004). Potential of crude seed extract of celery, *Apium graveolus* L. against the mosquito, *Aedes aegypti* (L) (Diptera: Culicidae). *J Vect Ecol.* 29(2):340-346.
- Fred-Jaiyeseimi AA, Anthony O (2011). Larvicidal activities of the extract and fractions of *Paullinia pinnata* Linn leaf. *Pharmacog Comm.* 1(2): 37-40.
- Hubalek Z and Halouzka J (1999). West Nile Fever: A reemerging mosquito-borne viral disease in Europe. *Emerg Infect. Dis.* 2: 519-529.
- Ibrahim HA and Ibrahim H (2009). Phytochemical screening and toxicity evaluation on the leaves of *Argemone mexicana* Linn. (Papaveraceae). *Inter J Appl Sci.* 3:39-43.
- Kaushik R, Saini P (2008). Larvicidal activity of leaf extract of *Millingtonia hortensis* (Family: Bignoniaceae) against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. *J Vect Borne Dis.* 45:66-69.
- Kulkarni J, Kapse N, Kulkarni DK (2009). Plant-based pesticide for the control of *Helicoverpa armigera* on *Cucumis sativus*. *Asian Agri-History,* 13(4): 327-332.

- Lago JHG, Brochini CB, Roque NF (2002). Terpenoids from *Guarea guidonia*. *Phytochem.* 60:333–338.
- Lame Y, Nukenine EN, Pierre DYS, Esimone CO (2014). Larvicidal activity of *Annona senegalensis* and *Boswellia dalzielii* leaf fractions against *Aedes aegypti* (Diptera: Culicidae). *Inter J Mosq Res.* 1(4): 25–29.
- Malarvannan S, Senthil Kumar S, Girigharan R, Sudha Nair (2008). Bioefficacy of *Argemone mexicana* against American bollworm *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera). *Hexapoda.* 15(1): 49-55.
- Moretti MD, Sanna-Passino G, Demontis S, Bazzoni E (2002). Essential oil formulations useful as a new tool for insect pest control. *AAPS Pharma Sci Technol.* 3: E13.
- Mukhopadhyay I, Nazir A, Mahmood K, Saxena DK, Das M, Khanna SK, Chowdhuri DK (2002). Toxicity of Argemone oil: Effect on hsp70 gene expression and tissue damage in transgenic *Drosophila melanogaster* (hsp70-lacZ) Bg⁹. *Cell Biol Toxicol.* 18: 1-11.
- Nathan SS, Kalaivani K, Murugan K, Chung PG (2005). Efficiency of Neemlimnoids on *Cnaphalocrocis medinalisi* (Guenee) (Lepidoptera: Pyralidae) the rice leaf folder. *Crop Protection.* 8:760–763.
- Ownbey GB (1997): *Argemone*. *Flora of North America.* Vol.3, Oxford University Press, New York-Oxford, pp 314-322.
- Rahuman AA, Bagavan A, Kamaraj C, Saravanan E, Zahir AA, Elango G (2009). Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitol Res.* 104:1365-1372.
- Ramanan M, Selvamuthukumar T (2016). Anti-insect properties of *Argemone mexicana* L. plant part solvent extracts against *Spodoptera litura* Fab. *Int J Recent Sci Res* 7(12): 14498-14501.
- Rothe SP, Ruchita Gandhi, Maheshwari AA (2016). Screening of local plant species for larvicidal activity. *Int J Adv Res Inn Idea Edu.* 2(5): 1077-1082.
- Sharma CT, Patil GP, Sharma NS, Zambare SP (2016). Effect of *Argemone mexicana* leaves extract at different solvents on gut of *Heliothis armigera* (Hub.). *Int J Life Sci Scientific Res.* 2(3):293-296.
- Singh RK, Dhiman RC, Mittal PK (2006). Mosquito larvicidal properties of *Momordica charantia* Linn. (Family: Cucurbitaceae). *J Vect Borne Dis.* 43:88–91.
- Sivaraman G, Daniel Reegan A, Rajiv Gandhi M, Ignacimuthu S (2016). Larvicidal activity of *Argemone mexicana* (Linn.) seed extracts against *Culex quinquefasciatus* and *Aedes aegypti* larvae (Diptera: Culicidae). *Int J Res Nat Appl Sci.* 6(1): 1-6.

Warikoo R and Kumar S (2014). Impact of the *Argemone mexicana* stem extracts on the reproductive fitness and behaviour of adult dengue vector, *Aedes aegypti* l. (Diptera: culicidae). *Int J Insect Sci.*6: 71–78.

WHO (World Health Organization) (1996). Report of the WHO informal

consultation on the evaluation and testing of insecticides. CTD (WHOPES). Ic 196.1 Geneva p.69.

Zeinab SH, Abou-Elnaga, (2015). Strong larvicidal properties of *Argemone Mexicana* L. against medically important vectors *Culex pipiens* and *Aedes aegypti*. *Int J Mosq Res.* 2(1):09-12.