Bangladesh J. Zool. ***52****(1): 119-132, 2024* *Article*
ISSN: 0304-9027
eISSN: 2408-8455

EFFICACY OF *AEGLE MARMELOS* (L.) CORRÊA LEAF EXTRACTS AGAINST 4TH INSTAR LARVAE OF THE MOSQUITO *CULEX QUINQUEFASCIATUS* SAY (DIPTERA: CULICIDAE)

Sumi Akter*, Humayun Reza Khan, Hafisha Khatun Anee and Shefali Begum

Faculty of Biological Sciences, Department of Zoology, University of Dhaka, Dhaka -1000, Bangladesh

ABSTRACT: Present study evaluated the toxicity of the leaf extracts of the plant *Aegle marmelos*(L.) Corrêa against the 4th instar larvae of the mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae). The experiment was conducted in the ambient environment of the laboratory at the temperature (29 ± 2)ºC and (75-85)% relative humidity. Three solvents were used (viz. ethanol, chloroform and distilled water). A total of 25 actively swimming 4th instar larvae of the mosquito were exposed to various dose concentrations of the plant leaf extracts for 24 hours. Larval mortality was observed after 24 hours of exposure. For ethanol based leaf extracts, the larval mortality of mosquito at five different concentrations, viz. 2.0, 3.0, 4.0, 5.0 and 6.0 mg/ml were 5.33%, 44%, 56%, 73.33% and 97.33%, respectively. Chloroform based leaf extracts were tested with five different concentrations viz. 0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml at which the extracts showed 9.33%, 25.33%, 56%, 94.66% and 100% larval mortality, respectively. For the water based extracts, the dose concentrations were viz. 2.0, 4.0, 6.0, 8.0 and 10.0 mg/ml at which the extracts exerted 30.66%, 54.66%, 60%, 68% and 90.66% larval mortality, respectively. With each dose 2 ml of dimethyl sulfoxide (DMSO) was added to make the extracts soluble in water. The LC_{50} and LC_{90} values for ethanol based leaf extracts were 3.530 mg/ml, 5.836 mg/ml and 8.793 mg/ml, respectively. For chloroform based leaf extracts, values were 1.184 mg/ml, 2.127 mg/ml and 3.430 mg/ml, respectively. For distilled water based leaf extracts, values were 3.730 mg/ml, 15.502 mg/ml and 49.521 mg/ml, respectively. The relative potency of three types of leaf extracts of *Aegle marmelos*against the larvae of *Culex quinquefasciatus* on the basis of , and values are as follows in decreasing order: chloroform based extract (= 1.184 mg/ml, = 2.127 mg/ml and = 3.430 mg/ml) > ethanol based extract (= 3.530 mg/ml, = 5.836 mg/ml and = 8.793 mg/ml) > water based extract (= 3.730 mg/ml, = 15.502 mg/ml and = 49.521 mg/ml). It was found that chloroform extracts showed greater mortality of larvae of *Cx. quinquefasciatus* than the ethanol extracts and that of the water extracts. From the results it can be concluded that the different solvent based crude extracts of the leaf of *A. marmelos*was an excellent potential for controlling *Cx. quinquefasciatus* mosquito larvae.

Key words: Aegle marmelos, *Culex quinquefasciatus*, larvicide, natural insecticides, mosquito control, botanical extracts.

*Author for correspondence: <ferdousisumi.du2196@gmail.com>
 DOI: https://doi.org/10.3329/bjz.v52i1.74733

INTRODUCTION

Insects are the most diverse species of animals living on earth and can be found in all habitats. Less than 0.5% of the total numbers of the known insect species are considered pests and only a few of these can be a serious menace to people (Hikal *et al.* 2017). Mosquitoes are one of those few pests which are tiny and midge like flies under order Diptera and family Culicidae. They comprise two sub-families (Anophelinae and Culicinae). Mosquitoes are widely distributed throughout the world; however, they are predominantly tropical insects (Alam *et al.* 2015). They are very important insect pests as well as major public vector throughout the world and about more than 3500 species are recorded throughout the world under 41 genera (Leisnham *et al.* 2007). Among them *Anopheles, Culex, Aedes, Lutzia, Culisea, Mansonia, Haemagogus* etc. are the most common types of mosquito genera. Of these around hundred species are capable of transmitting various diseases to human and other animals. Anopheles mosquitoes, as vectors of many zoonotic diseases, they are involved in human health problems in many countries including Bangladesh (Rahman and Howlader, 2018). About 117 species of mosquitoes have been recorded in Bangladesh (Ahmed *et al.* 2009). According to Bashar *et al.* 2013, 79 Culicines and 36 Anophelines have been recorded in Bangladesh. In our country total recorded mosquito species under genus *Culex* are about 25 in number (Ahmed, 1987). Among these *Culex* species, the most common is *Culex quinquefasciatus* (Say, 1823). The *Culex* is one of the important genus serve as vector of many important diseases of humans, birds, and other animals such as West Nile virus, Japanese encephalitis, or St. Louis encephalitis, filariasis, and avian malaria (Rahman and Howlader, 2018). A survey conducted by Khan *et al.* 2014, reported presence of 13 species of mosquitos but the *Cx. quinquefasciatus* became the predominant ones in Dhaka, Bangladesh. The peak population of the *Cx. quinquefasciatus* mosquito in Dhaka city was found to occur during the dry weather of November to December (Hamid, 1979). The environment of Dhaka city is very favorable for the breeding of the mosquito *Cx. quinquefasciatus* because there are vast areas of low land with stagnant and polluted water where mosquito can breed easily and get nourished as well (Ahmed, 1996). Mosquitos have four distinct stages in their life cycle: egg, larva, pupa and adult. The females usually mate only once but produce eggs at intervals throughout their life. In order to be able to do so, female mosquitos require a blood-meal. Males do not suck blood but feed on plant juices. The digestion of a blood-meal and the simultaneous development of eggs take 2–3 days in the tropics but longer in temperate zones. The gravid females search for suitable places to deposit their eggs, after which another bloodmeal is taken and another batch of eggs is laid. This process is repeated until the mosquito dies. The entire period from egg to

adult takes about 7–13 days under good conditions. *Culex* sp. mosquitoes are the main vectors of bancroftian filariasis in Bangladesh (Ameen and Moizuddin, 1973). This disease manifested by enlargement of the limbs, scrotum, and other extremities, is not the initial effect but the result of longstanding infection (Khanum *et al.* 2013). Filariasis is found to occur frequently in the northern parts of Bangladesh. The highest incident of filariasis was in Dinajpur district (14%) and in the Mirpur area of Dhaka city was 1.5%. It also occurs in Thakurgaon, Rangpur and Nilphamari districts (Wolfe and Khan, 1972). As, *Cx. quinquefasciatus* is a threat to human health and its population is increasing in an alarming rate thus it requires to be controlled. At present, organophosphate, organochlorine, and synthetic pyrethroid insecticides are being used for mosquito control but they are harmful to human health and to other nontarget organisms because of their non-biodegradable nature and higher rate of biomagnification through the ecosystem. Successive changes in insecticides results in multiple insecticide resistance (Patil *et al.* 2010). Phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents, and oviposition attractants and can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level. Thus, one of the approaches for control of these mosquito borne diseases is the interruption of disease transmission by killing or preventing mosquitoes from biting human beings. The search for herbal preparations that do not produce any adverse effects on the organisms and are easily biodegradable remains a top research issue for scientists associated with alternative vector control (Redwane *et al.* 2002).

Aegle marmelos (L.) Correa is one of only three species in genus Aegle (Sharma *et al.* 2011), is a subtropical fruit-bearing, deciduous tree that grows throughout hills and plains of the sub-Himalayan countries of Nepal, India, Sri Lanka, and Bangladesh (Sekar *et al.* 2011). It is a spinous aromatic tree of family Rutaceae with typical aroma. *A. marmelos* is commonly known as bael. The tree is considered as herbal medicine for the treatment of various ailments infact, each and every part of its plant has its own medicinal property (Hema and Lalithakumari, 1999; Maity *et al.* 2009). In our country, the tree has been used for fertility control and anti-proliferative and in Srilanka it has been used for its hypoglycemic activity (Sharma *et al.* 2007). Besides the medicinal uses, this plant was also studied for their antimicrobial, antifungal and insecticidal properties (Satyal *et al.* 2012; Kumar *et al.* 2008). The effect of leaf extracts of *A. marmelos* was also studied against *An. subpictus* in their oviposition deterrent, ovicidal and repellent activities (Elango *et al.* 2009). Larvicidal activity of the essential oil of *A. marmelos* was also reported against *Cx. pipiens* and *Ae. aegypti* (Vineetha *et al.* 2009; Kumar *et al.* 2008). Snehlata *et al.* (2018), emphasized the efficacy of *A. marmelos* leaf extract as fumigant against insect infestations of

stored grains and strengthen the possibility of using it as an alternative to synthetic chemicals for preserving stored grains. Manimegalai and Annapoorani (2013), evaluated the effect of *A. marmelos* extract on the fourth instars of *Cx. quinquefasciatus* at different concentrations

The aims and objectives of present study were to examine the susceptibility of the 4th instar larvae against botanical insecticides prepared from bael leaf extracts using different solvents under ambient environment of the laboratory. So that it can be used as an eco-friendly insecticide instead of eco-enemy synthetic insecticides in low cost from the natural environment available to us.

MATERIAL AND METHODS

Sample collection: The larval samples were collected from drains, pits etc. of different areas of the University of Dhaka, such as Curzon Hall, Kabi Sufia Kamal Hall, Amer Ekushey Hall and also from the drains of Ananda Bazar area.

Morphological identification: The collected larvae were placed on slides with a few drops of water, covered with cover slip and were examined under a Stereo microscope (Leica EZ4) for identification (Fig. 1b). The identification of the larvae of *Cx. quinquefasciatus* was confirmed following the identification keys of (Mathews *et al.* 2017).

Mosquito rearing: The collected larvae were then brought to the laboratory and kept in a clean plastic bowl and washed gently with tap water for several times to clean the adherent dirt substances. The rearing bowl of the larvae was covered with mosquito net to avoid oviposition by other female mosquitoes. After pupation, the pupae were transferred to an adult rearing cage (30cm × 30 cm × 30 cm) for emergence. After emergence, the adults were fed for the first few days with glucose. After a pre oviposition period of 2-3 days, the female mosquitoes were given blood meal for the maturation of their eggs and the source of the blood meal was the Quail, *Coturnix coturnix*. A container containing tap water was placed inside the cage for females to oviposit (Fig. 1c). The oviposition of the females took place 1-2 days after a blood meal. After hatching, the larvae were fed with yeast powder. The water of the larval bowls was changed regularly and fresh tap water was added to avoid microbial contamination and formation of scum on the water surface. The eggs hatched later into 1st, 2nd, 3rd, 4th instar larvae and pupae respectively. The rearing temperature and relative humidity were 27 ± 2°C and 75-85%, respectively.

Collection and proper identification of the plant samples: The leaf sample of bael (*A. marmelos*) was collected from the trees located in Kaligonj, Gazipur village area (Fig. 2a). The plant species was identified following the guideline provided by Orwa *et al.* 2009. The voucher specimen was deposited for further application.

(a) (b) (c)

Fig. 1. (a) Laboratory reared different stages larvae (b) microscopic view (20x) of 4th instar larva of *Cx. quinquefasciatus* and (c) boat shaped egg clusters of *Cx. quinquefasciatus* under Stereo microscope (Leica EZ4) at 20x magnification.

(a) (b)

Fig. 2. (a) Leaves of *A. marmelos* and (b) collection of evaporated extracts using spatula.

Preparation of the plant samples: The plant leaves were collected in fresh condition. The leaves were sun dried for six-seven days. After drying the leaves were grounded into powder and stored in an airtight container.

Extraction procedure: The ethanol, cholorform and aqueous based extractions of the leaves of bael (*A. marmelos*) were prepared by taking 50g of sun dried leaf powder of bael in each of the three separate containers which were previously rinsed with ethanol, chloroform and water. With the powder, 250ml fresh volume of ethanol, chloroform, distilled water were added in separate three conical flasks and kept for 24 hours with periodic shaking in the Shaking Orbital Machine (Luckham Rotatest Shaker Model R100) at 80-90 rpm and 30°C, then filtered with Whatman‟s filter paper and the sample solutions were collected. That procedure was repeated three times with fresh volume of ethanol, chloroform and distilled water. Finally, the total volumes of the samples were evaporated at 40°C keeping the solutions in many petri discs to facilitate evaporation process in an incubator (BOD INCUBATOR Model-205). Then the evaporated samples were collected using spatula (Fig. 2b) and stored at 4°C in an air tight glass bottle.

Dose preparation: In the larvicidal assay, fourth instar larvae of *Cx. quinquefasciatus* were exposed to test doses of 200, 300, 400, 500 and 600 mg of ethanol, 200, 400, 600, 800 and 1000 mg distilled water and 50, 100, 150, 200 and 250 mg of chloroform based extracts of the leaves of *A. marmelos*. For each dose concentration, 25 larvae of *Cx. quinquefasciatus* were exposed in all experiments and each experiment was replicated three times. The measured plant extracts and Dimethyl sulfoxide (DMSO) were taken into vials for dose preparation. 1ml of tap water was taken in a series of 250 ml glass beakers. The measured amounts of the extracts were dissolved in 2 ml of DMSO which was used to solubilize the plant extracts in water. The dissolved plant extracts were added to contained water in a beaker. But, in case of organic based extracts, the plant extracts were made soluble by using distilled water only. After adding each of the solubilized extracts in water, the concentrations of water for the doses of 200, 300, 400, 500 and 600 mg of ethanol were 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml and 6 mg/ml, respectively. For doses of 200, 400, 600, 800 and 1000 mg of distilled water based extracts, the concentrations of water were 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml and 10 mg/ml, respectively. For doses of 50, 100, 150, 200, 250 mg of chloroform based extracts, the concentrations of water were 0.5 mg/ml, 1 mg/ml, 1.5 mg/ml, 2 mg/ml and 2.5 mg/ml, respectively. For mortality studies, 25 fourth instar larvae were introduced into 100 ml of water contained in a 250 ml glass beaker (Plate 25) and various concentrations of the bael (*A. marmelos*) extracts mentioned in the above paragraph were added. A set of control, using 2.0% DMSO as control 1 and an untreated set of larva in water (tap) as control 2, were run for the comparison. The beakers were stored at room temperature (29 ± 2°C) and humidity (75-85%).

Bioassay test: A larvicidal bioassay method, suggested by WHO guidelines 2005 and Dua *et al.* (2009), was followed. The method was conducted with a slight modification. A number of 25 actively swimming larvae of 4th instar were taken into beaker (250 ml) containing 100 ml water along with the leaf extracts of different doses. The mortality of the larvae was recorded after 24 hours of exposure and moribund larvae were counted as dead. The toxicity of plant extracts was calculated in the form of LC_{50} and LC_{90} values which indicate 50% and 90% of death of test larvae, respectively in 24 hours of exposure. The recorded mortality percentage values were calculated by using the formula, Percentage mortality = (Number of larvae dead/Total number of test larvae) × 100. When mortality in control treatment was more than 5%, the percentage mortality was corrected by using Abott¨s (1925) formula,

Corrected mortality % = ((Larval mortality in the treatment% - Larval mortality in the control%)/(100 - Control mortality%)) × 100

Statistical analysis: Mortality data were observed and corrected mortality was obtained by applying Abott‟s formula (Abott, 1925). LC_{50} and LC_{90} values Chi-square values, regression at 95% confidence intervals of upper confidence limits and lower confidence limits were calculated by probit analysis using the IBM SPSS statistics 23 (Statistical Package of Social Science) software; here significance level were set at $p< 0.05$. Different charts and graphs were made using Microsoft Ex-cel software.

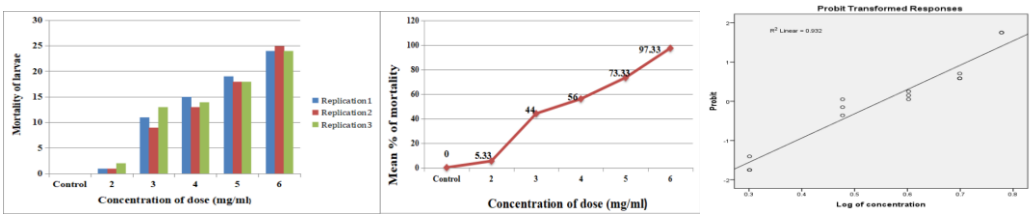
RESULTS AND DISCUSSION

Susceptibility of the larvae of ethanol based extracts of bael leaf: The mortality of 4th instar larvae of *Cx. quinquefasciatus* exposed to different dose concentrations of the ethanol extracts of the bael leaf (*A. marmelos*) for 24 hours shown in Table 1. After 24 hours of exposure, the larval mortalities of mosquito at the five different concentrations of the ethanol extracts of *A. marmelos,* viz. 2.0, 3.0, 4.0, 5.0, 6.0 mg/ml were 5.33%, 44%, 56%, 73.33% and 97.33%, respectively. Here the highest number of larvae killed at 6.0 mg/ml concentration and the lowest number of larvae was killed at 2.0 mg/ml (Fig 3a.). Each of the three replications for each dose concentration shows almost similar mortality. In case of Figure 3b, as the concentration of the plant extracts increased, the larval mean percentage of mortality was also found to be increased. The line diagram shows no mortality for control. Probit transformed responses and predicted Regression line of the mosquito *Cx. quinquefasciatus* against different concentration of ethanol based extracts of the bael, *A. marmelos* leaf, is shown in Figure 3c.

Table 1: Mortality of 4 th instar larvae of *Cx. quinquefasciatus* exposed to different doses of ethanol based extracts of the bael, *Aegle marmelos* leaf for 24 hours

Dose (mg/ml)	Total no. of larvae exposed in three replications	Total no. of larvae died in three replications	Mean % of larvae died
2.0	75	4	5.33
3.0	75	33	44.00
4.0	75	42	56.00
5.0	75	55	73.00
6.0	75	73	97.00
Control	75	0	0.00

Susceptibility of the larvae of chloroform based extracts of bael leaf: The mortality of 4th instar larvae of *Cx. quinquefasciatus* exposed to different dose concentrations of the chloroform extracts of the bael leaf (*A. marmelos*) for 24 hours shown in Table 2. After 24 hours of exposure, the larval mortalities of mosquito at the five different concentrations of the ethanol extracts of *A. marmelos*, viz. 0.5, 1.0, 1.5, 2.0, 2.5 mg/ml were 9.33%, 25.33%, 56%, 94.66% and 100% respectively. Here the highest number of larvae killed at 2.5 mg/ml concentration and the lowest number of larvae was killed at 0.5 mg/ml. Each of the three replications for each dose concentration shows almost similar mortality. In case of Figure 4b,as the concentration of the plant extracts increased, the larval mean percentage of mortality was also found to be increased. The line diagram shows no mortality for control. Probit transformed responses and predicted Regression line of the mosquito *Cx. quinquefasciatus* against different concentrations of chloroform extracts of the bael, *Aegle marmelos* leaf, is shown in Fig. 4c.



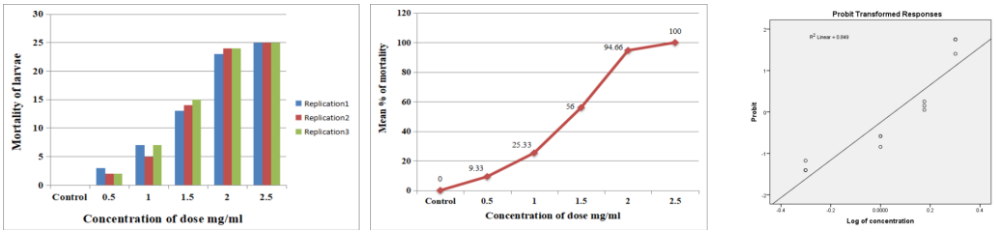
(a) (b) ©

Fig. 3. (a) Mortality of the 4th instar *Cx. quinquefasciatus* treated with different dose concentrations of the ethanol based bael, *A. marmelos* leaf Extracts, exposed to 24 hours (b) Mean percentage of mortality of the 4th instar larvae of *Cx. quinquefasciatus and* (c) Probit mortality lines of the ethanol based extracts of the bael, *A. marmelos* leaf.

Table 2. Mortality of 4th instar larvae of *Cx. quinquefasciatus* exposed to different doses of chloroform based extracts of the bael (*A. marmelos*) leaf for 24 hours

Dose (mg/ml)	Total no. of larvae exposed in three replications	Total no. of larvae died in three replications	Mean % of larvae died
0.5	75	7	9.33
1.0	75	19	25.33
1.5	75	42	56.00
2.0	75	71	94.66
2.5	75	75	100.00
Control	75	0	0.00

Susceptibility of the larvae of distilled water based extracts of bael leaf: The mortality of 4th instar larvae of *Cx. quinquefasciatus* (exposed to different dose concentrations of the water extracts of the bael leaf (*A. marmelos*) for 24 hours shown in Table 3. After 24 hours of exposure, the larval mortalities of mosquito at the five different concentrations of the ethanol extracts of *A. marmelos*, viz. 2.0, 4.0, 6.0, 8.0, 10.0 mg/ml were 30.66%, 54.66%, Here the highest number of larvae was killed at 10.0 mg/ml concentration and the lowest number of larvae was killed at 2.0 mg/ml. Each of the three replications for each dose concentration shows almost similar mortality 60%, 68% and 90.66% respectively.In case of Figure 5b, as the concentration of the plant extracts increased, the larval mean percentage of mortality was also found to be increased. The line diagr m shows no mortality for control. Probit transformed responses and predicted Regression line of the mosquito *Cx. quinquefasciatus* against different concentration of distilled water based extracts of the bael, *A. marmelos* leaf, is shown in Figure 5c.



(a) (b) (c)

Fig. 4. (a) Mortality of the 4th instar *Cx. quinquefasciatus* treated with different dose concentrations of the chloroform based bael leaf extract of *A. marmelos*. (b) Mean percentage of mortality of the 4th instar larvae of *Cx. quinquefasciatus* exposed to 24 hours at different doses of chloroform based leaf extracts of bael (*A. marmelos) and* (c) Probit mortality lines of the chloroform based extracts of the bael, *A. marmelos* leaf.

Table 3. Mortality of 4th instar larvae of *Cx. quinquefasciatus* exposed to different doses of distilled water based extracts of the bael (*A. marmelos*) leaf for 24 hours

Dose (mg/ml)	Total no. of larvae exposed in three replications	Total no. of larvae died in three replication	Mean % of larvae died
2.0	75	23	30.66
4.0	75	41	54.66
6.0	75	45	60.00
8.0	75	51	68.00
10.0	75	68	90.66
Control	75	0	0.00

Comparison of the toxicity of different solvent based extracts of leaf of A. marmelos: The powders of the leaf of *A. marmelos* were extracted with three different solvents, viz. ethanol, chloroform and water. These different solvents extracted different components at different percentages from the leaf powder. As different components have different toxicity level of their own, differences in the effectiveness among the different solvent based extracts were very natural.

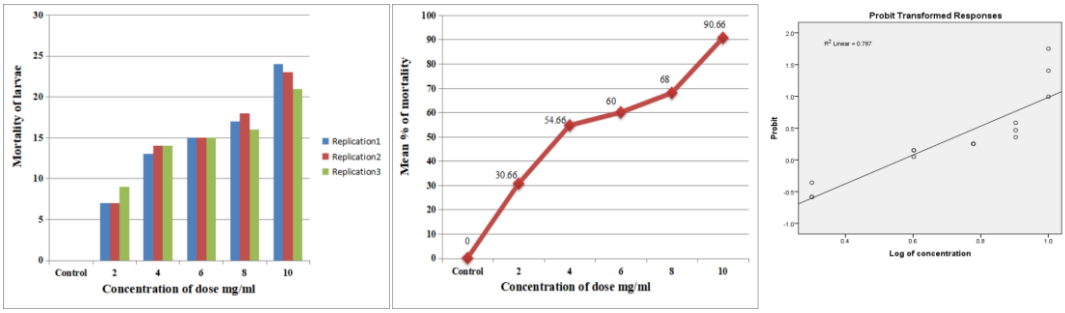


Fig. 5. (a) Mortality of the 4th instar *Cx. quinquefasciatus* treated with different dose concentrations of the distilled water based bael, *A. marmelos* leaf extract (b) Mean percentage of mortality of the 4th instar larvae of *Cx. quinquefasciatus* exposed to 24 hours at different doses of distilled water based leaf extracts of bael, *A. marmelos* (c)Probit mortality lines of the distilled water based extracts of the bael, *A. marmelos* leaf.

Table 4. Estimated LC values and confidence limits for Ethanol based extracts

Point	Estimate	95% Confidence Limits for concentration		Estimate	95% Confidence Limits for Log (concentration)[a]	
		Lower Limit	Upper limit		Lower limit	Upper limit
LC_{50}	3.530	3.315	3.743	.548	.520	.573
LC_{90}	5.836	5.355	6.548	.766	.729	.816
LC_{99}	8.793	7.646	10.696	.944	.883	1.029

Five dose concentrations were applied against the mosquito larvae of *Cx. quinquefasciatus.* For the ethanol based extract, the dose concentrations were 2.0, 3.0, 4.0, 5.0 and 6.0 mg/ml. The estimated LC_{50},LC_{90} and LC_{99} value for ethanol, chloroform and water based extracts were 3.530 mg/ml, 5.836 mg/ml and 8.793 mg/ml; 1.184 mg/ml, 2.127 mg/ml and 3.430 mg/ml; 3.730 mg/ml, 15.502 mg/ml and 49.521 mg/ml respectively (Table 4-6). Here, the lowest LC_{50},LC_{90} and LC_{99} value belongs to chloroform based extracts and the highest LC_{50},LC_{90} and LC_{99} value belongs to the water based extracts. This indicates that among all these three, chloroform based extract is highest in toxicity level, water

based extract is lowest in toxicity level and ethanol based extract is in between them. Because, lower LC_{50},LC_{90} and LC_{99} value indicates greater toxicity. So, it is suggested that chloroform extracts show greater mortality of the larvae of *Cx. quinquefasciatus* than the ethanol extracts and that of the water extracts, and the ethanol extracts show greater mortality of the larvae of *Cx. quinquefasciatus* than the water extracts.

Table 5. Estimated LC values and confidence limits for chloroform based extracts

Point	Estimate	95% Confidence Limits for concentration		Estimate	95% Confidence Limits for Log (concentration)[a]	
		Lower limit	Upper limit		Lower limit	Upper limit
LC_{50}	1.184	1.027	1.341	0.073	0.011	0.127
LC_{90}	2.127	1.821	2.697	0.328	0.260	0.431
LC_{99}	3.430	2.703	5.123	0.536	0.432	0.710

Table 6. Estimated LC values and confidence limits for water **based extracts**

Point	Estimate	95% Confidence Limits for concentration		Estimate	95% Confidence Limits for Log (concentration)[a]	
		Lower Limit	Upper limit		Lower limit	Upper limit
LC_{50}	3.730	3.048	4.354	0.572	0.484	0.639
LC_{90}	15.502	11.775	24.378	1.190	1.071	1.387
LC_{99}	49.521	29.886	117.714	1.695	1.475	2.071

According to, Dass and Mariappan (2014), LC_{50} values of *A. marmelos* leaf extracts against II, III, IV instar larvae and pupa of *Cx. quinquefasciatus*, were 91.52 ppm, 105.16 ppm, 151.43 ppm and pupa 203.78 ppm, respectively. Joseph *et al.* (2004), reported LC_{50} and LC_{90} values of *A. marmelos* against *An. gambiae,* were 0.082 mg/l and 0.152 mg/l, respectively. Sarma *et al.* (2017) reported the LC_{50}=121.88 ppm against larva of *Cx. quinquefasciatus* at 72 hour exposure while against *Ae. aegypti* the LC_{50} value was 278.82 ppm at 72 hour exposure. Vineetha and Murugan (2009), documented the LC_{50} values of *A. marmelos* plant extracts against first, second, third and fourth instar larvae of *Ae. aegypti* were 50.960, 52.979, 56.653 and 60.778 ppm, respectively.

The differences between these studies and the present investigation are due to variations in extraction solvents, mosquito species or exposure periods (Umar *et al.* 2002). The differences may also be due to different plant parts that were used, different geographical distributions, larval stage differences etc. It reveals that as the concentration of the plant extracts increased, the total larval mortality of the mosquito was also found to be increased.

CONCLUSION

The information of present work may be helpful in developing mosquito management project, as the results of the present experiment and evidences of previous works which is related to the present work showed that the larvae of the mosquito *Cx. quinquefasciatus* can be controlled by using the extractions of *A. marmelos*. It is a biological measure, biodegradable and less harmful to the environment. It requires very little or no cost. So, it will bring us a positive aspect in the mosquito controlling sectors which can be used economically in future.

LITERATURE CITED

ALAM, N., T FARJANA, T., KHANOM, T. F., LABONY, S. S., ISLAM, K. R., AHMMED, S., MONDAL, M. M. H. 2015. Prevalence of mosquitoes (diptera: culicidae) in and around Bangladesh Agricultural University campus of Mymensingh in Bangladesh. *Progressive Agriculture*. **26**: 60-66.

AHMED, T.U., NUR, J. and NAVED, A. F. 2009. Mosquito populations, including new record, in the tea gardens of Bangladesh. *Bangladesh J. Entomol.* **19**(2): 45-60.

AHMED. T. U. 1996. Mosquito problem in Bangladesh with special emphasis on urban mosquito situation and it's control. Third biennial conference of Bangladesh Entomological Society proceeding. Pp 10-17.

AHMED. T. U. 1987. Checklist of mosquitoes of Bangladesh. *Mosquito systematic*. **19**: 191-204.

AMEEN, M. and MOIZUDDIN, M. 1973. Bionomics of the common mosquitoes of Dacca. *Journal of Natural History*. **7**: 1-21.

BASHAR, K., MAHMUD, K. H., REZA, M. S., GODA, P., RAIN, F. F., JESMIN, F. and ASADUZZAMAN, A. 2013. Revised checklist and distribution maps of Anopheles (Insecta: Diptera: Culicidae: Anophelinae) mosquitoes of Bangladesh. *The Journel of Biodiversity Data*. **9**(2): 211-224.

BRAM, A. R. 1967. Contributions to the mosquito fauna of Southeast Asia-11. The genus *Culex* in Thailand (Diptera: Culicidae). *Am.Ent. Inst.* **2**(1): 192-201.

DASS, K. and MARIAPPAN, P. 2014. Larvicidal activity of *Colocasia esculendum*, *Eclipta prostrata* and *Wrightia tinctoria* leaf extract against *Culex quinquefasciatus*. *Proc. Natil. Acad Sci India Sect. B Biol Sci.* **14**: 423-427.

DUA, V.K., PANDEY and A.C., RAGHAVENDRA, K. 2009. Larvicidal activity of neem oil (*Azadirachta indica*) formulation against mosquitoes. *Malar Journal.* **8:** 124.

DAS, N., GOSWAMI, D. and RABHA, B .2007. Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. *Journal of Vector Borne Diseases*. **44**: 145-148.

ELANGO, G., BAGAVAN, A., KAMARAJ, C., ZAHIR, A. A., and RAHUMAN, A. A. 2009. Oviposition-deterrent, ovicidal, and repellent activities of indigenous plant extracts against *Anopheles subpictus* Grassi (Diptera: Culicidae). *Parasitol Res*. **105**: 1567–1576.

GHOSH, A., CHOWDURY, N. and CHANDRA, G. 2012. Plant extracts as potential mosquito larvicides. *Indian J Med Res*. **35**: 581-598.

HIKAL, W. M., BAESHEN, R. S. and SAID-AL AHL, H. A. H. 2017. Botanical I insecticide as simple extractives for pest control. *Cogent Biology*. **3**: 1404274.

HAMID, M. A. 1979. Studies on the seasonal fluctuation of *Culex quinquefasciatus* larvae in Dhaka city. M.S. thesis. Dept. Zool. Univ. Dhaka. Pp 126.

KHANUM, H., NATH, R., MUSA, S., ZAMAN, R.F. and HOSSAIN, M. 2013. Lymphatic filariasis in northern part of Bangladesh. *Univ. J. Zool. Rajshahi Univ*. **32**: 59-65.

KUMAR, R.A., PRASAL, C. S., DUBEY, N. K.and SAMANT, R. 2008. Insecticidal activity *Aegle marmelos* (L.) Correa essential oil against four stored grain insect pests. *International Journal of Food Safety*. 39-49.

LEISNHAM, P. T., SLANEY, D. P., LESTER,P. J., WEINSTEIN, P. and HEATH, A.C. G. 2007. Mosquito density associated macro invertebrate biodiversity and w waterchemistry in water-filled containers: Relationships to land use. *New Zealand Journal of Zoology*. **37**: 203-218.

NADYATARA, K. and NURDIAN, Y. 2018. Farmers are the main occupation that is easy to suffer from lymphatic filariasis. *Research Gate*.

PATIL, S.V., PATIL, C.D., SALUNKHE, R.B., SALUNKE, B.K., 2010. Larvicidal activities of six plants extracts against two mosquito species, *Aedes aegypti* and *Anopheles stephensi*. *Tropical Biomedicine*. **27**(3): 360-365.

MATHEWS, G., DERRAIK, J., WALKER, M., KNOX, R., and BARRACLOUGH, R.. 2017. Morphological variation in invasive mosquito Culex quinquefasciatus Say (Diptera: Culicidae) larvae from an urban site in Auckland, New Zealand. *New Zealand Journal of Zoology*. **44:** 1-12.

MANIMEGALAI, K. and ANNAPOORANI, C. A. 2013. *Aegle marmelos* as an effective agent against the fourth instar larvae of *Culex quinquefasciatus* (Diptera: Culicidae). *Int. Res J Pharm. App Sci*. **3**(4): 64-68.

MAITY, P., HANSDA, D., BANDYOPADHYAY, U., and MISHRA, D. K. 2009. Biological activities of crude extracts and chemical constituents of bael, *Aegle marmelos* (L.) Corr. *Indian Journal of Experimental Biology*. **47**: 849–861.

ORWA, C., MUTUA, A., KINDT, R., JAMNADASS, R. and SIMONS, A. 2009. Agroforestree Database: A Tree Reference and Selection Guide, version 4.0. *World Agroforestry Centre ICRAF, Nairobi, KE.*

RAHMAN, M. M. and HOWLADER, M. T. H. 2018. Laboratory evaluation of two organophosphate and one pyrethroid insecticide against the *Culex quinquefasciatus* (Say) (Diptera: Culicidae) mosquito larvae. *International Journal of Mosquito Research*. **5**(1): 121-124.

REDWANE, A., LAZREK, H.B., BOUALLAM, S., MARKOUK, M., AMAROUCH, H. and JANA, M. 2002. Larvicidal activity of extracts from *Querus Lusitania* var infectoria galls (Oliv). *Journal of Ethnopharmacology*.**79**: 261-263.

SNEHLATA, K., SHEEL, R., KUMAR, B. and SABITA. 2018. Larvicidal activity of methanol extract of leaves of *Aegle marmelos* (L.) against four insect pests causing severe damage to grains on storage. *Int.J.Curr.Microbiol.App.Sci.***7**(6): 12211235.

SARMA, R., MAHANTA, S., KHANIKOR, B. 2017. Insecticidal Activities of the Essential oil of *Aegle marmelos* (Linnaeus, 1800) against *Aedes aegypti* (Linnaeus,1762) and *Culex quinquefasciatus* (Say, 1823). *Universal Journal of Agricultural Research*. **5**(5): 304-311.

SHARMA, G.N., DUBEY, S. K., SHARMA, P. and SATI, N. 2011. Medicinal values of Bael (*Aegle marmelos*) (L.) Corr.: A Review. *Int. J. Curr. Pharma. Rev. Res*. **1**: 12-22.

SEKAR, D.K., KUMAR, G., KARTHIK, L. and RAO, K.V.B. 2011. A review on pharmacological and phytochemical properties of *Aegle marmelos* (L.) Corr. Serr. (Rutaceae). *Asian J. Plant Sci. Res*. **1**:8-17.

SHARMA, P. C., BHATIA, V., BANSAL, N. and SHARMA, A. 2007. A review on bael tree. *Natural Products Radiance*. **6**: 171-178.

SATYAL, P., KATHERINE, E. W., NOURA, S. D., SANJAYA, N. and WILLIAM, N. S. 2012. Biological Activities and Volatile Constituents of *Aegle marmelos* (L.) Corrêa from Nepal. *Journal of Medicinally Active Plants***. 1**(3): 114-122.

TANTELY, L. M., CÊTRE-SOSSAH, C., RAKOTONDRANAIVO, CARDINALE, E. and BOYER, S. 2017. Population dynamics of mosquito species in a West Nile virus endemic area in Madagascar. *Parasite*. **24**: 3.

TANJINA, T. 2017. The effectiveness of the extracts of the fruit pulp of the plant *Cassia fistula* Linn (Liguninaceae: Ceasalpinioideae) on the larvae of the mosquito*Culex quinquefasciatus* Say (Diptera:Culicidae). M. S. thesis. Dept. Zool. Univ. Dhaka. Pp 102.

UMAR, A., KELA, SANTA, L. and OGODI, JONATHAN, A. 2006. Susceptibility of pupae to Neem seed kernel extracts Animal research International. **3**(1): 403- 406.

VINEETHA, A., and MURUGAN, K. 2009. Larvicidal and smoke repellency effect of *Toddalia asiatica* and *Aegle marmelos* against the dengue vector, *Aedes aegypti*(Insecta: Diptera: Culicidae). *Entomological Research*. **39**: 61-65.

WOLF, M. S. and KHAN, M. A. 1972. Filariasis in East Pakistan. *Trans. R. Soc. Trop.Med. Hyg*. **65**: 63-69

(Manuscript received on 1 March 2024 revised on 25 April 2024)