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TOXIC EFFECTS OF SOME BOTANICAL SEED EXTRACTS AGAINST MOSQUITO LARVAE CULEX QUINQUEFASCIATUS SAY (DIPTERA: CULICIDAE)

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ABSTRACT

*Culex quinquefasciatus*is is responsible for biting nuisance and spreading some dreadful diseases among human beings. Hence, to protect the human in search of an alternative natural insecticide, an excellent study was designed to evaluate the larvicidal activities of *Phyllanthus emblica, Carica papaya* and *Brassica napus* seed extracts against *C. quinquefasciatus* Say larvae under laboratory condition. The larvicidal activity of *P. emblica* seed extracts was evaluated at different concentrations of distilled water (2000-8000 ppm), methanol (1000-3000 ppm), acetone (800-1400 ppm) and 50% ethanol (2500-4500 ppm), and LC_{50} of the four extracts were 4189.230 ppm, 1724.708 ppm,1030.483 ppm and 3354.829 ppm, respectively. In case of *C. papaya* seed extract larvicidal activity was checked at concentrations of distilled water (2000-8000 ppm), methanol (700-1500 ppm), acetone (200-400 ppm) and 50% ethanol (1000-2000 ppm) and LC_{50} were 4559.578 ppm, 1000.561 ppm, 265.929 ppm and 1406.930 ppm for the four extracts, respectively. For *B. napus* seed extracts larvicidal activity was recorded at concentrations of distilled water (7000-15000 ppm), methanol (1500-3500 ppm), acetone (500-1100 ppm) and 50% ethanol (2500-4500 ppm) and LC_{50} were 10118.017 ppm, 2146.948 ppm,700.689 ppm, and3180.144 ppm respectively. Relative position of the extracts on the basis of LC_{50} and relative potency values was acetone > methanol > 50% ethanol > distilled water, and that of the seed was *C. papaya* > *P emblica* >*B. nigrus*.

Key words: Toxicity, *Phyllanthus emblica, Carica papaya, Brassica napus,* mosquito larvae

Introduction

Across the world a great number of research works had been conducted on plant extracts against mosquito larvae. There are many such potential larvicidal phytochemicals extracted from various plant species which are rapidly biodegradable, eco-friendly, have broad spectrum target specific activities and less toxic to ecosystem (Ghosh *et al.*, 2012). The use of eco-friendly products for mosquito control is being encouraged and appreciated throughout the world. Siam *et al.* (2021) studied the larvicidal efficacy of three indigenous plant extracts against the mosquito larvae *Culex quinquefasciatus* Say (Diptera: Culicidae). Younoussa *et al.* (2020) reported on the potential mosquito larvicidal activity of CH_2CL_2-MeOH (30:70 v/v seed extracts of *Magnifera indica*, *Persea americana* and *Dacnyodes edulus* against 3rd and 4th instar larvae of *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles gambiae*. Nasiruddin *et al*. (2019) studied the larvicidal effects of some plant seed extracts on *Anopheles annularis* and *Culex quinquefasciatus* larvae. Nasiruddin *et al.* (2018) studied the potential larvicidal activity of leaf, bark and seed extracts of two indigenous plants, *Aegle marmelos* and *Pongamia pinnata* against *Culex quinquefasciatus* larvae. A study was designed by Ravaomanarivo *et al.* (2014) to examine the insecticidal and larvicidal property of aqueous and oil seed extracts of *Annona squamosa* and *Annona muricata* against the larvae and adults of *Aedes albopictus* and *Culex quinquefasciatus.* Ashfaq and Ashfaq (2012) assayed the larvicidal effect of the water extract of *Moringa oleifera* seeds against the 2nd and 4th instar of *Culex quinquefasciatus.* Nuchsuk *et al.* (2012) determined the larvicidal effect of *Jatropha curcas* seed against the 3rd instar larvae of *Culex quinquefasciatus.* Tennyson *et al.* (2012) screened twenty-five plant extracts for larvicidal activity against *Culex quinquefasciatus*. Batabyal *et al.* (2009) experimented petroleum ether, carbon tetrachloride and methanol extracts of *Azadirachta indica* fruit and seed extracts of bitter gourd (*Momordica charantia*) and castor (*Ricinus communis*) against the larvae of *Culex quinquefasciatus.* Martinez-Tomas *et al.* (2009) evaluated the larvicidal efficacy of aqueous neem seed extracts against the 1st, 2nd, 3rd and 4th instar larvae and pupae of *Culex quinquefasciatus*. In spite of having great potential of larvicidal activity, the application of plant extract as effective larvicide in mosquito control is quite limited. The present study regarding efficacy of botanical extracts against *Culex quinquefasciatus* will therefore strengthen this specific research sector. So, the present study explores the larvicidal potential of three locally available wild plant seeds of *Phyllanthus emblica*, *Carica papaya* and *Brassica napus* against *Culex quinquefasciatus* Say larvae under laboratory conditions. The outcome of this research work may introduce people to the insecticidal potential of indigenous plant parts and encourage them for using cheaper and safer way to control mosquito larvae.

Materials and Methods

To study the toxic effect of seed extracts of three plants like *Phyllanthus emblica, Carica papaya* and *Brassica napus* on *Culex quinquefasciatus* larvae, systematic short-term bioassays were performed in the Entomological Research Laboratory of Department of Zoology, University of Chittagong during the period of March 2022 to November 2022.

The larvae of the experimental mosquito species *Culex quinquefasciatus* were collected from stagnant drains, ditches, derelict ponds and holes containing dirty water. Collections were done in the morning between 7:00 am to 9:00 am using muslin net and were transferred into plastic jars filled with water of the breeding place, covered with netting and taken into the laboratory. Then, the 3rd and 4th instar larvae of *Culex quinquefasciatus* Say were sorted, separated by dropper and kept in petri dish. Identification of the larvae of *C. quinquefasciatus* was confirmed following the keys of Bram (1967).

Extract preparation of dry seed: Firstly, the dried seeds of *P. emblica, C. papaya* and *B. napus* were coarsely powdered using an electrical stainless blender. Then the seed powder was sieved through a sieve of mesh size 0.0025 cm^2 and stored in separate jars for subsequent solution preparation. The powder was weighted in a digital balance and added to the solvent following the ratio 10 gm of seed powder in 100 ml solvent in a conical flask. The flask was shaken vigorously on a magnetic stirrer for one hour to ensure maximum extraction of toxic components of the seed powder. The resultant solution was designated as "Stock Solution".

Preparation of experimental dose: The doses were applied directly from the freshly prepared stock solution. For each set of experiment a certain (calculated) volume of the stock solution of seed extract was added to the certain volume of tap water, so that the final volume in the beaker was always 250 ml in each of the replicates (APHA, 2005). The doses were prepared in terms of parts per million (ppm). From freshly prepared stock solution of *P. emblica, C. papaya* and *B. napus* seeds, five test concentrations of different solvents i.e., distilled water, methanol, acetone and 50% ethanol for each seed were prepared after performing preliminary bioassays.

Bioassay test for larvicidal activity: Bioassays were carried out on the 3rd and 4th instar larvae of *C. quinquefasciatus*. Prior to the final experiments several preliminary bioassays were conducted in the laboratory at room temperature to find out 01-99% mortality of different solvent extracts obtained from the seeds of *P. emblica, C. papaya* and *B. napus* against the mosquito larvae. Ten larvae were released by means of a dropper into each 500 ml beaker of 250 ml volume of each seed extract concentration. For each concentration, three replicates were conducted to check the mortality in a completely randomized design. Larvae were considered dead if they showed no sign of movement. The percentage of larval mortality was recorded after 24 hours.

Statistical analysis: Statistical analysis was done for the obtained data of the experiment. The dose concentrations were transferred to logarithms. The LC_{50} values of each seed extract for each plant and the experimental chemicals were determined by probit analysis. Values of LC_{50} were analyzed in a computer-based probit analysis program. The regression equation was calculated from empirical probit, working probit, weighting probit, the values of which were taken from the Tables given by Finney (1971). Values of Chi square at 0.05 level were calculated following Fisher and Yates (1963). Relative potency values were calculated by taking the highest LC_{50} value as unit.

Results and Discussion

The efficacy of *Phyllanthus emblica, Carica papaya* and *Brassica napus* seed extracts were observed on *Culex quinquefasciatus* larvae at different concentrations of distilled water, methanol, acetone and 50% ethanol extracts. Records in terms of mortality were taken at an interval of 24 hours exposure to the test plant seed extracts. The dose range, mortality rate, regression equation, P value of Chi-square of the extracts of the experimental plant seeds applied on *C. quinquefasciatus* larvae are given in Table 1. Probit mortality lines for the experimental seed extracts are shown in Figs. 1-3.

Effects of *P. emblica, C. papaya* and *B. napus* seed extracts on *Culex quinquefasciatus* larvae: *Culex quinquefasciatus* larvae showed different mortality rates in case of different extracts of the three experimental plants seed. The mortality rates varied from 13.33-96.67%, 6.67-96.67% and 16.67-96.67% for *P. emblica, C. papaya* and *B. napus* seeds respectively. The Chi-square values of almost all the seed extracts showed insignificant values at both 0.01 and 0.05 levels except for distilled water extract of *P. emblica* and methanol extract of *C. papaya* seed.

Table 1: Toxicity of distilled water, methanol, acetone and 50% ethanol extracts of *P. emblica, C. papaya* and *B. napus* seed on *Culex quinquefasciatus* Say larvae exposed for 24 hours

Experimental Plant	Solvent	Dose range (ppm)	Mortality range (%)	Regression equation	χ^2 Value
Phyllanthus emblica	Distilled Water	2000-8000	13.33-96.67	2.784 x – 4.896	33.581 $p < 0.01 < 0.05$
	Methanol	1000-3000	13.33-96.67	5.101x – 11.555	3.365 $p > 0.01 > 0.05$
	Acetone	800-1400	16.67-93.33	9.208x – 22.747	1.703 $p > 0.01 > 0.05$
	50% Ethanol	2500-4500	16.67-9.00	8.290x – 24.268	3.478 $p > 0.01 > 0.05$
Carica papaya	Distilled Water	2000-8000	6.67-90.00	4.395x – 11.052	3.891 $p > 0.01 > 0.05$
	Methanol	700-1500	16.67-96.67	9.731x – 24.367	34.02 $p < 0.01 < 0.05$
	Acetone	200-400	16.67 – 96.67	8.538x – 15.720	3.319 $p > 0.01 > 0.05$
	50% Ethanol	1000-2000	13.33 – 93.33	7.458x – 18.476	4.198 $p > 0.01 > 0.05$
Brassica napus	Distilled Water	7000-15000	16.67 – 90.00	7.400x – 24.630	3.149 $p > 0.01 > 0.05$
	Methanol	1500-3500	20.20-96.67	6.242x – 15.792	6.236 $p > 0.01 > 0.05$
	Acetone	500-1100	23.33- 93.33	6.035x – 12.207	4.799 $p > 0.01 > 0.05$
	50% Ethanol	2500-4500	16.67-96.67	10.710x – 32.512	5.14 $p > 0.01 > 0.05$

Lethal concentrations and relative potencies of *P. emblica, C. papaya* and *B. napus* seed on *C. quinquefasciatus* larvae: The LC_{50} and relative potency values of the extracts of *P. emblica, C. papaya* and *B. napus* seeds are given in Table-2. On the basis of LC_{50} and relative potency values the order of toxicity or larvicidal activity of dry seed extracts of *P. emblica, C. papaya* and *B. napus* on *C. quinquefasciatus* larvae was in the order: Acetone extract of *C. papaya* seed > Acetone extract of *B. napus* seed > Methanol extract of *C. papaya* seed > Acetone extract of *P. emblica* seed > 50% ethanol extract of *C. papaya* seed > Methanol extract of *P. emblica* seed > Methanol extract of *B. napus* seed > 50% ethanol extract of *B. napus* seed > 50% ethanol extract of *P. emblica* seed > Distilled water extract of *P. emblica* seed > Distilled water extract of *C. papaya* seed > Distilled water extract of *B. napus* seed, that of the extracts were: Acetone > Methanol > 50% ethanol > distilled water and that of the seeds were *C. papaya* > *P. emblica* > *B. napus*. Among all the twelve extracts, acetone extract of *C. papaya* seed showed highest toxicity at lowest dose concentration in terms of larvicidal activity.

Table 2: The LC_{50} and relative potency values for seed extracts of *Phyllanthus emblica*, *Carica papaya* and *Brassica napus* on *Culex quinquefasciatus* larvae

Plant	Extract	LC_{50} (ppm)	Relative potency
C. papaya	Distilled Water	4559.578	2.219
	Methanol	1000.561	10.112
	Acetone	265.929	38.048
	50% Ethanol	1406.930	7.192
B. napus	Distilled Water	10118.017	1.000
	Methanol	2146.948	4.713
	Acetone	700.689	14.440
	50% Ethanol	3180.148	3.182
P. emblica	Distilled Water	4189.230	2.415
	Methanol	1724.708	5.867
	Acetone	1030.483	9.819
	50% Ethanol	3354.829	3.016

The present research work was undertaken to study the toxicity of three plant seed extracts against *Culex quinquefasciatus* larvae using four chemicals as solvent under laboratory conditions and exposure period was 24 hours. Mortality rates of mosquito larvae were recorded at different concentrations of the seed extracts. The effects of various extracts were studied in a dose dependent manner. Among the seed extracts lowest toxicity was found in case of distilled water seed extract of *B. napus* having highest LC_{50} value (10118.017 ppm) and lowest relative potency value (1.000) and the acetone seed extract of *C. papaya* showed highest toxicity with the lowest LC_{50} value (265.929 ppm) and highest relative potency value (38.048). The order of toxicity or larvicidal activity of the four extracts of dry seeds of *P. emblica*, *C. papaya* and *B. napus* on *C. quinquefasciatus* larvae was in the order: Acetone extract > Methanol extract > 50% ethanol extract > Distilled water extract and of the three seeds, extracts of *C. papaya* were the most toxic followed by *P. emblica* and *B. nigrus* seed extracts.

The LC_{50} value of distilled water extract of *P. emblica* seed was 4189.230 ppm which showed close similarity with El Maghraby *et al.* (2012), where the LC_{50} of crude extract of corn was 4025.78 ppm. The methanol extract of *P. emblica* seed showed LC_{50} value of 1724.708 ppm which was almost similar to the LC_{50} value of acetone extract of *Capsicum annuum* seed (1604.852 ppm) of Nasiruddin *et al.* (2019). The acetone seed extract of *P. emblica* having LC_{50} value of 1030.483 ppm was almost related to the LC_{50} of 50% ethyl alcohol extract of *Aegele mermelos* seed (1104.960ppm) of Nasiruddin *et al.* (2018). Nika (2015) found the LC_{50} value of methanol extract of *M. nudiflorus* seed to be 2710.997 ppm which was quite close to the LC_{50} value of 50% ethanol extract of *P. emblica* seed (3354.829 ppm).

In case of distilled water extract of *C. papaya* seed, the LC_{50} value was 4559.578 ppm which disagreed with the LC_{50} value (705.599 ppm) of distilled water extract of *C. papaya* seed as observed by Nasiruddin *et al.* (2019). The LC_{50} value of methanol seed extract of *C. papaya* (1000.561 ppm) was somewhat similar with the ethanolic crude extract of *Solanum xanthocarpum* (1066.91 ppm) (Changbunjong *et al.*, 2010). Acetone seed extract of *C. papaya* having LC_{50} value of 265.929 ppm showed close similarity with the LC_{50} of CH_2Cl_2-MeOH seed extract of *Magnifera indica* (258.98ppm) of Younoussa *et al.* (2020).

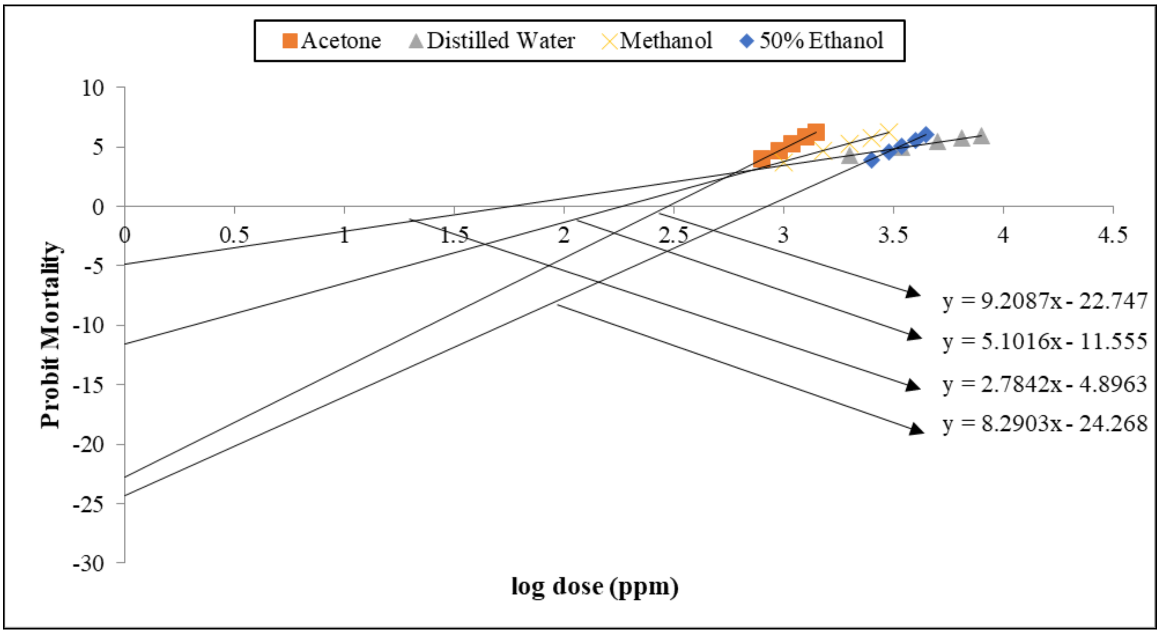

Fig. 1. Regression lines for determining the LC_{50} of distilled water, methanol, acetone and 50% ethanol extracts of *P. emblica* seed on *Culex quinquefasciatus* larvae after 24 hours of exposure

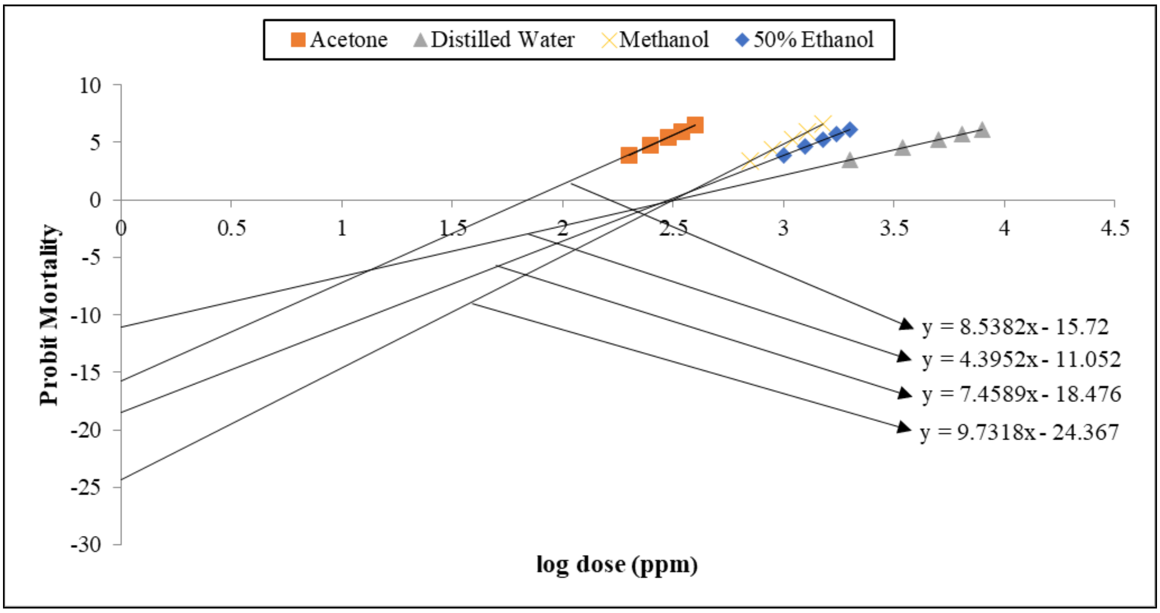

Fig. 2. Regression lines for determining the LC_{50} of distilled water, methanol, acetone and 50% ethanol extracts of *C. papaya* seed on *Culex quinquefasciatus* larvae after 24 hours of exposure

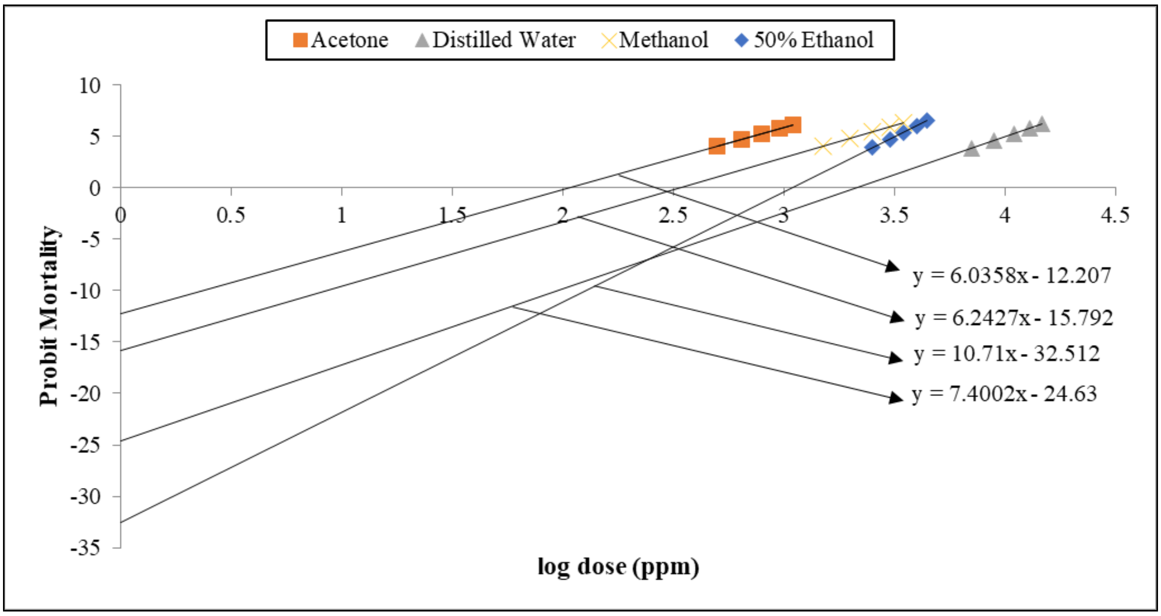

Fig. 3. Regression lines for determining the LC_{50} of distilled water, methanol, acetone and 50% ethanol extracts of *B. napus* seed on *Culex quinquefasciatus* larvae after 24 hours of exposure

LC_{50} of 50% ethanol seed extract of *C. papaya* (1406.930 ppm) was quite similar with the LC_{50} of methanol extract of *Capsicum annuum* seed (1311.538 ppm) (Nasiruddin *et al.*, 2019) and LC_{50} of methanol extract of *Brassica nigra* seed (1396.927 ppm) (Nika, 2015).

The distilled water seed extract of *B. napus* showed LC_{50} value of 10118.017 ppm which was quite similar to the LC_{50} values of distilled water seed extract of *Swietenia mahagoni* (10595.604 ppm) of Siam *et al.* (2021) and distilled water seed extract of *Momordica charantia* (10593.241 ppm) of Nasiruddin *et al.* (2019). The LC_{50} of methanol extract of *B. napus* seed (2146.948) was almost similar with the LC_{50} of methanol extract of *Sinapis alba* seed (2269.975 ppm) (Nasiruddin *et al.*, 2019) and methanol extract of *Albizzia lebbeck* seed (2792.641 ppm) (Nika, 2015). The LC_{50} value of acetone extract of *B. napus* seed was 700.689 ppm which can be compared with the LC_{50} of acetone seed extract of *Sinapis alba* (569.701 ppm) of Nasiruddin *et al*. (2019). The 50% of ethanol seed extract of *B. napus* seed showed LC_{50} value of 3180.148 ppm having somewhat close relation with the methanol extract of *Polygonum hydropiper* seed having LC_{50} of 3355.353 ppm (Bhuiyan, 2013).

As *Carica papaya* seed extract showed highest toxicity, it is recommended that the effectiveness of the seed extract of *C. papaya* should also be evaluated against other mosquito species especially against the *Anopheles stephensi* and *Aedes aegypti* which are posing threat to public health.

Environmental aspects of the study: Plants are a renewable resource, making botanical extracts a sustainable option for mosquito control. Botanical extracts thus offer an environmentally friendly approach to mosquito control, using natural compounds from plants to repel or eliminate mosquitoes. Their use is gaining popularity as a sustainable alternative to synthetic pesticides, minimizing harm to other organisms and reducing the risk of resistance development. It can reduce the need for synthetic insecticides, which can have negative effects on the environment. Many botanical extracts are biodegradable, minimizing the risk of long-term environmental contamination.

Conclusion

The results observed in this study open the possibility for further studies to examine the larvicidal activity of the seed extracts against other mosquito species and determine the active ingredients in the plant extracts that stimulate the larvicidal activity. These active ingredients should be identified and utilized as commercial product so as to be used as larvicidal and adult mosquitocidal formulations. This strategy may save millions of dollars that are invested for importing or producing chemical insecticides as well as can protect the environment from these hazardous non-biodegradable pollutants. Again, if mosquitoes are controlled in the least hazardous way, economic and public health benefits can be procured.

References

APHA (American Public Health Association). 2005. *Standard Methods for the Examination of Water and Wastewater.* 21st edition. AWWA, WPCF, Washington DC. 1368 pp.

Ashfaq, M. and Ashfaq, U. 2012. Evaluation of mosquitocidal activity of water extract of *Moringa oleifera* seed against *Culex quinquefasciatus* (Diptera: Culicidae) in Pakistan. *Pak. Entomol.*, 34(1): 21-26.

Batabyal, L., Sharma, P., Mohan, L., Maurya, P. and Srivastava, C. N. 2009. Relative toxicity of neem fruit, bitter gourd, and castor seed extracts against the larvae of filaria vector *Culex quinquefasciatus* (Say). *Parasitology Res.*,105(5): 1205-1210.

Bhuiyan, S. Y. 2013. Evaluation of toxic effects of six insecticides and three plant seed extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae) larvae. MS Thesis. Department of Zoology, University of Chittagong, Bangladesh. 115 pp.

Bram, R. A. 1967. Contribution to the mosquito fauna of South East Asia|.The genus *Culex* in Thailand (Diptera: Culicidae). *Contribution American Entom. Ins.*, 2(1): 01-296.

Changbunjong, T., Wongwit, W. Leemingsawat, S. Tongtokit, Y. and Deesin, V. 2010. Effect of crude extract of *Solanum xanthocarpum* against snail and mosquito larvae. *Southeast Asian J. Trop. Med. Public Health,* 41(2): 320-325.

Finney, D. J. 1971. *Probit Analysis*. 3rd edition. Cambridge University Press, London. UK. 333 pp.

Fisher, R. A. and Yates, F. 1963. *Statistical Tables for Biological, Agricultural and Medicinal Research*. 6th edition, Oliver and Boyd Ltd. Edinburgh, UK. 47-50 pp.

Ghosh, A., Chowdhury, N. and Chandra, G. 2012. Plant extracts as potential mosquito larvicides. *Indian J. Medical Res.,*135(5): 581-598.

Martinez-Tomas, S. H., Perez-Pacheco, R., Rodriguez-Hernandez, C., Ramirez-Valverde, G. and Ruiz-Vega, J. 2009. Effects of an aqueous extract of *Azadirachta indica* on the growth of larvae and development of pupae of *Culex quinquefasciatus. African J. Biotechno.*, 8(17): 4245-4250.

Nasiruddin, M., Azadi, M. A. and Akter, M. 2018. Performance evaluation of two plant parts in controlling *Culex quinquefasciatus*Say (Diptera: Culicidae) larvae. *Bangladesh J. Environ. Sci*., 35: 97-110.

Nasiruddin, M., Azadi, M. A., Chowdhury, M. R., and Tonni, R. A. 2019. Larvicidal effects of some plant seed extracts on *Anopheles annularis* Vander Wulp and *Culex quinquefasciatus* Say (Diptera: Culicidae). *J. Biodivers. Conserv. Bioresou. Manag.*, 5(2): 41-52.

Nika, S. 2015. Larvicidal efficacy of six plant seed extracts against *Culex quinquefasciatus* Say larvae. MS Thesis. Department of Zoology, University of Chittagong, Bangladesh. 127 pp.

Nuchsuk, C., Wetprasit, N., Roytrakul, S. and Ratanapo, S. 2012. Larvicidal activity of a toxin from the seed of *Jatropha curcas* Linn. against *Aedes aegypti* Linn. and *Culex quinquefasciatus* Say. *Tropical Biomedicine*, 29(2): 286-296.

Ravaomanarivo, L. H. R., Razafindraleva, H. A., Raharimalala, F. N., Rasoahantaveloniana, B., Ravelonandro, P. H. and Mavingui, P. 2014. Efficacy of seed extract of *Annona squamosa* and *Annona muricata* (Annonaecae) for the control of *Aedes albopictus* and *Culex quinquefasciatus* (Culicidae). *Asian Pacific J. Tropical Biomedicine,* 4(10): 798-806.

Siam, M. A. H., Nasiruddin, M., Azadi, M. A. and Chowdhury, M. R. 2021. Larvicidal efficacy of three indigenous plant extracts against the mosquito larvae *Culex quinquefasciatus* Say (Diptera: Culicidae). *Bangladesh J. Environ. Sci.,* 40: 60-69.

Tennyson, S., Rabindran, K. J. and Arivoli, S. 2012. Screening of twenty-five plant extracts for larvicidal activity against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Asian Pacific J. Tropical Biomedicine,* 2(2): 1130-1134.

Younoussa, L., Oumarou, K. M., Kowa, T. K., Enama, S. T., Agabor, G. A., and Nukenine, E. N. 2020. Effectiveness of three fruit seed extracts as larvicide against three major mosquito vectors *Aedes aegypti* L, *Culex quinquefasciatus* Say and *Anopheles gambiae* Giles (Diptera: Culicidae). *Int. J. Tropical Disease and Health,* 41(23): 16-29.