

EFFICACY OF FRUIT PULP SOLVENT EXTRACTS OF CASSIA FISTULA LINN. AGAINST THE FOURTH INSTAR LARVAE OF THE MOSQUITO CULEX QUINQUEFASCIATUS SAY

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Abstract

The efficacy of the fruit pulp extracts of *Cassia fistula* Linn (Caesalpiniodae: Leguminosae) extracted with three solvents (viz. water, acetone and n- hexanes) was studied against the 4th instar larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae) in the laboratory. Larval mortality was observed after 36 hours. The sun-dried extracts showed 18.67, 30.67, 41.33, 54.67 and 70.67 percent larval mortalities, respectively while the shade-dried extracts showed 6.67, 21.33, 42.67, 68.00 and 90.67 percent larval mortalities, respectively. The acetone based sun five extracts showed 18.67, 41.33, 60.00, 82.67 and 98.67 percent larval mortalities, respectively and the shade-dried extracts showed 17.33, 41.33, 73.33, 89.33 and 97.33 percent larval mortalities, respectively. The n-hexane based sun-dried extracts showed 21.33, 41.33, 59.67, 74.67 and 93.33 larval mortalities, respectively and the shade-dried extracts showed 33.33, 60.00, 68.00, 77.33 and 97.33 percent larval mortalities, respectively. The LC₅₀ values for n-hexane, acetone and water based sun-dried extracts were 1.087, 1.097 and 3.211 mg/ml, respectively and the shade-dried extracts were 0.808, 1.054 and 3.048 mg/ml, respectively. No mortality was observed in control treatment.

Key words: Toxicity, *Culex quinquefasciatus*, *Cassia fistula*, Mosquito, Larvae, LC₅₀

Introduction

Mosquitoes are dominant insects of tropical areas causing a great concern for the public health and are common nuisance pests throughout the urban areas of all over the world. There are over 3500 species of mosquitoes on the globe under 41 genera (Leisnham 2007). There are about 117 species of mosquitoes recorded in Bangladesh (Ahmed *et al.* 2009). So far 79 Culicines and 36 Anophelines have been recorded (Bashar *et al.* 2013) of which about 25 species are under the genus *Culex*, most common and predominant one being the species *Culex quinquefasciatus* (Ahmed 1987). In Dhaka city more than 90 per cent of the mosquitoes belong to *Cx. quinquefasciatus* (Ameen *et al.* 1994). Recently, (Khan *et al.* 2014 and 2015) reported 13 species of mosquitoes in five wards of Dhaka metropolitan city of which *Cx. quinquefasciatus* was the predominant one.

The mosquitoes serve as vectors in the transmission of important pathogens to mankind that cause fatal diseases. About 300 species of mosquitoes are responsible for the transmission of the human and animal diseases. They transmit the pathogens of malaria, filaria, yellow fever, dengue fever, dengue hemorrhagic fever, encephalitis, dermatobiasis etc. Among them *Culex* mosquitoes carry encephalitis, filariasis, and the West Nile virus etc. Mosquito borne diseases attack about 100 million people per year and more than one million of them die (Hill 1997).

That's why the mosquitoes create global health problem, particularly in the tropics (WHO 1999). So, the mosquito *Cx. quinquefasciatus* is a serious threat to the human health. As its population is increasing in an alarming rate it requires to be controlled.

Now-a-days synthetic pyrethroid, carbamate, hydrochlorine and other organophosphorus compounds are used to control mosquitoes. These are harmful to human health and to other non-target populations because of their non biodegradable nature and higher rate of biological magnification through ecosystem. So, an alternative way should be found out which will be effective in the mosquito management strategies, along with it will focus on public health, monitoring and surveillance, source reduction and environmentally least-toxic larval control. These factors have resulted in an approach to look for environment friendly, cost-effective, biodegradable and target specific insecticides against mosquito species. Plant materials used as potential pesticides were first time studied in the country by Ameen *et al.* (1983a & b and 1985). They studied the solvent based root extracts of *Derris elliptica* plant on the larvae of two mosquito species of the genera *Aedes* and *Culex*; they also isolated rotenone, the principal toxicant ingredient of *D. elliptica* and the chemical was bioassayed as insecticide on the larvae of the mosquito species. The plant *Cassia fistula* is a semi wild slender tree. It is known as "Golden Shower Tree" or "Sonalu" which is well known in Bangladesh as an ornamental tree for its beautiful bunches of yellow flowers. This plant is also well known for its traditional use as medicine. There is little information regarding the use of *C. fistula* as the source of insecticides and application to mosquito control in the country. However some information are available in literature in which different parts of *C. fistula* have been reported to be used as crude extracts with solvents as well as individual toxic ingredients for the control of different species of mosquitoes and other insect species (Govindarajan *et al.* 2008, Govindarajan 2009, 2013 a & 2013 b, Barakat *et al.* 2004, Danish *et al.* 2011, Duraipandiyani *et al.* 2011, Kumar *et al.* 2014, and Misra *et al.* 1997). The chemical components of *C. fistula* vary with in different parts of the plant. The pulp of pod of *C. fistula* contains anthraquinone glycosides, sennosides A and B, rhein and its glucoside, barbaloin, aloin, formic acid, butyric acid and their ethyl esters, oxalic acid, pectin, tannin, emodin, sennidin, aloe-emodin (Agarwal and Paridhavi 2005, Dave and ledwani 2012, Liptak and Szentagali 1937 and Misra *et al.* 1997). Some of these chemicals have larvicidal properties among which rhein was found to be a very effective larvicide (Duraipandiyani *et al.* 2011). Ethanol and hexane crude extracts of *C. fistula* reduce pupation, egg production, hatchability and increased per cent sterility in the cotton leaf worm, *Spodoptera littoralis* (Barakat *et al.* 2004). Stearic acid has larvicidal properties and methanolic extracts of *C. fistula* are promising as larvicidal and ovicidal agents against mosquito (Danish *et al.* 2011).

The present study was conducted in the laboratory to determine the efficacy of three solvent based extracts of the fruit pulp of the plant *Cassia fistula* on the mosquito larvae of *Cx. quinquefasciatus*.

Materials and Methods

Test insect: The larvae of the mosquito *Cx. quinquefasciatus* Say (Diptera: Culicidae) were collected from the drains of Curzon Hall premises, Dhaka University in October 2015 and were carried to the Entomological Laboratory of the Zoology department, Dhaka University where these were reared and bioassay tests were conducted in the ambient environment of the laboratory at $27 \pm 2^\circ$ C and 75-85% RH. The larvae belonging to *Cx. quinquefasciatus* were identified by following the identifying key suggested by Bram (1967).

As the larvae of *Cx. quinquefasciatus* were required for the bioassay test with the plant extracts, a continuous supply of the larvae was maintained in the rearing room placed in the Animal Garden of the Department of Zoology, Dhaka University. The larvae were served with yeast powder, while the adults were provided with 10% glucose solutions as their food. After 3-4 days of emergence, the adult female mosquitoes were given a blood meal from a pigeon, *Columba livia* for egg maturation.

Test botanical: The fruits of the plant *C. fistula* were collected from the Curzon Hall campus area, Dhaka University and “Osmani Milonayoton” premises, Dhaka in November 2015 and carried to the Entomology laboratory. The extraction procedure was conducted at the Entomological laboratory of the Department of Zoology and CARS (Centre for Advanced Research Science) of the University of Dhaka. The collected plant parts were taken to the Botany Department of the University for the Species Identification and the species was confirmed as *Cassia fistula*.

Plant extraction: The fruits of *C. fistula* were collected and washed with tap water. Then one half of the collected materials was placed under the sun and the other half in the shade for drying. After drying, the shade-dried sample was found slightly sticky in comparison to the sun-dried sample. Then, the fruit pulps, excepting seeds and outer covering, were taken into an electric blender machine and ground into powder and stored.

Six conical flasks (500 ml) were taken and rinsed with respective solvents for the preparation of different solvent based extract solutions. Then, 50 g of sun and 50 g of shade-dried fruit pulp powders of *C. fistula* were taken separately in each of the six flasks (three for sun-dried and three for shade dried extracts). With these 300 ml fresh distilled water, Acetone, and n-Hexane were added separately and kept for 24 hours with periodic shaking in an Orbital Shaker Machine at 100 rpm and 30°C. The solutions were then collected and stored at temperature 4°C in an air tight glass bottle for dose preparation in bioassay tests.

Dose preparation: Several doses of the extracts were prepared for the experiment using a process of serial dilution. The plant extracts were taken into a screw capped vial and then weighed in a weighing machine. As the organic solvents based extracts were insoluble in water media, Di-methyl Sulfoxide (DMSO) was used to make them soluble in water as per the suggestion of Nour *et al.* (2012). The concentrations of the five doses for water based extracts were 2.0, 2.5, 3.0, 3.5 and 4.0 mg/ml, and for acetone based extracts were 0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml. But, in case of n-hexane solvent based extract, which was insoluble in DMSO solutions, were prepared by following the method proposed by Ravichandran *et al.* (2014). To prepare the stock solution, one gram of plant extract residue was dissolved in 5 ml of acetone, mixed well and then it was dissolved in 95 ml of distilled water. Each ml of this stock solution contains 10 mg of plant residue. By diluting this stock solution, a series of doses of different concentrations were prepared for bioassay tests and the concentrations of the prepared doses were 0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml.

Bioassay tests: A larvicidal bioassay method, suggested by Dua *et al.* (2009) was followed. The method was conducted with slight modification. Twenty five actively swimming mosquito larvae of 4th instar, were taken into a conical flask (250 ml) containing 100 ml water along with one of the five doses of the plant extracts. The flasks were stored at room 29 ± 2°C, 80 ± 5% RH and at 14L: 10D (photoperiod). The mortality of the larvae was recorded after 36 hours of exposures and the moribund larvae were counted as dead. A set of Control using 2.0% DMSO was taken as control 1, a set of Control using 5% Acetone was taken as

control 2 and an untreated set of larvae in water (tap) as Control 3. These three sets of control were run for calculating the corrected mortality.

The toxicity of the extracts was calculated as LC₅₀ and LC₉₀ representing the 50% and 90% of the test larvae died, respectively; both LC₅₀ and LC₉₀ values were calculated for 36 hours of exposures. The number of larvae died at each of the dose concentrations at the end of the stipulated exposure periods was recorded and the mortality percentage values were calculated by using the formula-

$$\text{Percentage of mortality} = \frac{\sum \text{LC}_{50} \text{ A } \text{larvae} \text{ LGRG}}{\sum \text{LC}_{50} \text{ SL } \text{larvae} \text{ L}} \times 100$$

When the mortality in control was more than 5%, the percentage mortality was corrected by using Abbott's (1925) formula-

$$\text{Corrected mortality} = \frac{\sum \text{larvae} \text{ ACS } \text{ARS} \text{ RaSCL } \text{SLaS} - \sum \text{larvae} \text{ ACS } \text{ARS} \text{ Ra} \lambda \text{aSCA}}{400 - \lambda \text{aSCACS } \text{ARS} \text{ Ra}} \times 100$$

Statistical analysis: Larval mortality data were observed and corrected mortality was obtained by applying Abbott's formula (Abbott 1925). LC₅₀ and LC₉₀ at 95% confidence intervals, lower and upper confidence limits were determined by the probit analysis method suggested by Finney (1971). Other statistics like chi-square values, regression at 95% confidence intervals of upper confidence limits and lower confidence limits and ttests were calculated using the IBM SPSS statistics 20 (Statistical Package of Social Science) software; here significance level were set at $p < 0.05$.

Results and Discussion

The larvicidal efficacy of different solvent based sun-dried and shade-dried extracts of the fruit pulp of *C. fistula* was tested against the 4th instar larvae of *Cx. quinquefasciatus*. Data were recorded and mean percentage mortality was calculated. Five different dose concentrations (viz. 2.0, 2.5, 3.0, 3.5 and 4.0 mg / ml) of water based sun-dried extracts showed 18.67, 30.67, 41.33, 54.67 and 70.67% larval mortality, respectively; while the shade-dried extracts showed 6.67, 21.33, 42.67, 68.00 and 90.67% larval mortality, respectively. Five different dose concentrations (viz. 0.5, 1.0, 1.5, 2.0 and 2.5 mg / ml) of acetone based sun-dried extracts showed 18.67, 41.33, 60.00, 82.67 and 98.67% larval mortality, respectively; the shade-dried extracts showed 17.33, 41.33, 73.33, 89.33 and 97.33% larval mortality, respectively. And five different dose concentrations (viz. 0.5, 1.0, 1.5, 2.0 and 2.5 mg / ml) of n-hexane based sun-dried extracts showed 21.33, 41.33, 59.67, 74.67 and 93.33% larval mortality, respectively and the shade-dried extracts showed 33.33, 60.00, 68.00, 77.33 and 97.33% larval mortality, respectively. Based on the above larval mortalities, values of LC₅₀, LC₉₀, Chi-square, parameter estimation and 95% confidence limits were calculated and the results are presented in Table 1.

Table 1. Efficacy of different solvent based sun-dried and shade-dried extracts of the fruit pulp of *Cassia fistula* against the 4th instar larvae of *Cx. quinquefasciatus*.

Types	Used Solvents	LC ₅₀ value (LCL-UCL)	LC ₉₀ value (LCL-UCL)	Parameter estimation		χ ² (df=13) value (significance)
				Concentration (LCL-UCL)	Intercept (LCL-UCL)	
Sun dried extracts	Water	3.211 (3.005-3.478)	6.074 (5.134-8.138)	4.630 (-2.669- -2.023)	-2.346 (0.968 ^a)	5.31
	Acetone	1.054 (0.945-1.163)	2.467 (2.144-2.982)	3.471 (-0.161- -0.001)	-0.08 (0.308 ^a)	14.995
	n-Hexane	1.087 (0.957-1.217)	3.041 (2.534-3.954)	2.869 (2.266-3.427)	-0.104 (-0.182- -0.026)	9.715 (0.717 ^a)
Shade dried extracts	Water	3.048 (2.937-3.166)	4.205 (3.956-4.570)	9.167 (7.520-10.814)	-4.436 (-4.847- -4.026)	9.124 (0.764 ^a)
	Acetone	1.097 (0.962-1.236)	3.187 (2.595-4.333)	2.768 (2.143-3.394)	-0.112 (-0.190- -0.033)	8.311 (0.760 ^a)
	n-Hexane	0.808 (0.663-0.938)	2.793 (2.271-3.823)	2.38 (1.800-2.960)	0.22 (0.145-0.295)	14.406 (0.346 ^a)

Here, LC= Lethal concentration, LCL= Lower confidence limit and UCL= Upper confidence limit of 95% confidence limits; df= Degree of freedom. a=Since the significance level is greater than 0.15, no heterogeneity factor is used in the calculation of confidence limits.

Comparison of the toxicity of different solvent based extracts of the sun-dried fruit pulp of C. fistula: The estimated LC₅₀ for water, acetone and n-hexane based sun-dried extracts were 3.211 mg / ml, 1.097 mg / ml and 1.087 mg / ml, respectively; whereas LC₉₀ values of these extracts were 6.074 mg / ml, 2.467 mg / ml and 3.041 mg / ml, respectively (Table 1). Here, the lowest LC₅₀ and LC₉₀ values belong to the n-hexane based extracts and the highest of these values belong to the water based extracts. This indicates that among all these three, the n-hexane based extracts showed highest toxicity and the water based extract showed lowest toxicity; On the contrary the acetone based extracts showed larval toxicity laying in-between the former two extracts (i.e. n-hexane and water based extracts). The relative potency of three types of sun dried fruit extracts of *C. fistula* against the larvae of *Cx. quinquefasciatus* on the basis of LC₅₀ values in decreasing order were as follows: n-hexane (1.087 mg / ml) > acetone (1.097 mg / ml) > water (3.211 mg / ml).

Comparison of the toxicity of different solvent based extracts of the shade-dried fruit pulp of C. fistula: The estimated LC₅₀ for water, acetone and n-hexane based extracts were 3.048, 1.054 and 0.808 mg / ml, respectively; whereas, the LC₉₀, values were 4.205, 3.187 and 2.793 mg / ml, respectively (Table 1). Thus, the n-hexane based extracts showed lowest LC₅₀ and LC₉₀ values and the water based extracts showed highest LC₅₀ and LC₉₀ values indicating that the shade-dried extracts showed the same results as that of the sun-dried extracts, i.e. among all these three, n-hexane based extract was highest in toxicity level and the water based extract was lowest and acetone based extract was moderate. The relative potency of three types of sun-dried fruit extracts of *C. fistula* against the 4th instar larvae of *Cx. quinquefasciatus*, on the basis of LC₅₀ values in decreasing order were as follows: n-hexane (0.808 mg / ml) > acetone (1.054 mg / ml) > water (3.048 mg / ml).

Comparison of toxicity between sun-dried and shade-dried fruit pulps of C. fistula: Among all six types of solvent extracts, the n-hexane based shade-dried extract was found to be most effective ($LC_{50}=0.808$ mg/ml) larvicide against the tested larvae of *Cx. quinquefasciatus*; the acetone based sun-dried extract ranked 2nd in position of toxicity ($LC_{50}=1.054$ mg/ml); with the LC_{50} values of the n-hexane based sun-dried extract ($LC_{50}= 1.087$ mg/ml) and acetone based sun-dried extract ($LC_{50}=1.097$ mg/ml) were in the 3rd and 4th position, respectively. The water based extracts were found to be less effective than the acetone and n-hexane based extracts and the LC_{50} values were 3.048 mg/ml and 3.211 mg/ml for shade-dried and sun-dried extract, respectively. The relative potency of six types of extracts on the basis of the decreasing order of LC_{50} values were as follows: n-hexane shade (0.808 mg / ml) > acetone shade (1.054 mg / ml) > n-hexane sun (1.087 mg / ml) > acetone sun (1.097 mg / ml) > water shade (3.048 mg / ml) > water sun (3.211 mg / ml). So, for the same solvent based extract, effectiveness of the shadedried extracts was found better than the sun-dried extracts.

Comparison between sun and shade-dried extracts: Paired t-test was performed to show the comparative results between sun-dried and shade-dried extracts at all three solvents (Table 2). The water based shade-dried and sun-dried extracts, acetone based sun-dried and shade-dried extracts and n-hexane based sun-dried and the shade-dried extracts of the fruit pulp of *C. fistula* were found to be significant as shown in Table 2. The differences of the estimated means were 2.60, 1.2 and 2.8, respectively. The paired sample t-test values were 3.833, 1.39 and 4.221, respectively. And paired sample correlation values were 0.982, 0.974 and 0.983, respectively. As all the values were less than 0.005, the results were significant.

Table 2. Differences between the larvicidal efficacy of different solvent based sun dried and shade dried extracts of the fruit pulp of *C. fistula* against the 4th instar larvae of *Culex quinquefasciatus*.

Solvents	Type	Dead at each level (Mean)	Standard deviation	Standard error of mean	Paired t-test	Significance (2-tailed)	Degree of freedom	Paired Sample Correlation	Significance
	Sun	9.800	6.181	2.764					
Water	Shade	12.40	7.057	3.156	3.833	0.019	4	0.982	0.003
	Sun	14.60	7.603	3.400					
Acetone	Shade	15.80	8.258	3.693	1.395	0.235	4	0.974	0.005
	Sun	14.20	7.120	3.184					
n-Hexane	Shade	17.00	6.285	2.811	4.221	0.013	4	0.983	0.003

In the present study, the crude extracts of the fruit pulp of *C. fistula* were found to have effective larvicide against *Cx. quinquefasciatus*. The resulting larvicidal activities of the fruit pulp extracts of *C. fistula* may be comparable with some of the earlier reports. Govindarajan *et al.* (2008) studied the ovicidal and larvicidal efficacy of methanolic leaf extracts of *C. fistula* against two mosquitoes, viz. *Anopheles stephensi* and *Culex quinquefasciatus* and reported the LC_{50} values of 17.95 and 20.57 mg / L (=0.02057 mg / ml), respectively. Thus it seems that the methanolic extracts of the leaves of *C. fistula* are more toxic than all three solvent extracts of the fruit pulp of the same plant in the present study. Later Govindarajan

(2013) studied the efficacy of crude methanolic extracts of the flower of *C. fistula* against three mosquito species, viz. *Culex tritaeniorhynchus*, *Aedes albopictus* and *Anopheles subpictus*, and reported that the flower extracts of *C. fistula* were excellent larvicidal potential against the tested mosquito species showing the LC₅₀ values of 136.59, 118.64 and 96.51 ppm, respectively, and with the LC₉₀ values of 243.67, 231.79 and 174.39 ppm, respectively. In another earlier study, Govindarajan *et al.* (2009) reported the bioefficacy of *C. fistula* leaf extracts with different solvents like benzene, acetone and methanol against dengue vector *Aedes aegypti* and estimated LC₅₀ values of the above extracts which were 10.69, 18.27 and 23.95 mg / L, respectively; these values indicate that the benzene leaf extracts of *C. fistula* had highest efficacy on the larvae of *Ae. Aegypti*.

Duraipandiyan *et al.* (2011) reported the antifeedant and larvicidal activities of the chemical rhein isolated from *C. fistula* and the LC₅₀ value were 606.50 ppm (0.607 mg / ml) for *Heliothis armigera* and 1192.55 ppm (1.193 mg / ml) for *Spodoptera littoralis* and the larvae survived showed malformed adults. Kumar *et al.* (2014) reported the LC₅₀ of *Cassia occidentalis* leaf petroleum ether and butanol extract against *Cx. quinquefasciatus* 3rd instar larvae were 98.4 and 161.6µg / ml, respectively. Barakat *et al.* (2004) reported that the ethanol and hexane crude extracts of *Cassia fistula* reduced pupation, egg production and hatchability, and increased per cent sterility; the dominant constituents were fatty acids, linoleic acid, hexadecanoic acid, and octadecanoic acid and their alkyl esters.

From the present findings and the relevant information available from literature it may be said that the plant *C. fistula* is a potential resource for the extraction of toxic ingredients which may be used as insecticides for the control of mosquito. Further research may be initiated to isolate the toxic ingredients from different plant parts of this species and bioassayed on mosquito species.

References

- Abbott, W.S. 1925. A method of computing the effectiveness of insecticides. *J. Econ. Entomol.* **18**(2): 267-269.
- Agarwal, S.S. and M. Paridhavi. 2005. *Clinically useful herbal drugs*. Ahuja Publishing House. 281-282 pp.
- Ahmed, T.U. 1987. Checklist of mosquitoes of Bangladesh. *Mosquito systematic*. **19**:191-204.
- Ahmed, T.U., J. Nur and A.F. Naved. 2009. Mosquito populations, including new record, in the tea gardens of Bangladesh. *Bangladesh J. Entomol.* **19**(2): 45-60.
- Ameen, M., M.A. Chowdhury and I. Hossain. 1994. *Survey of mosquito breeding site in the city of Dhaka*: a report submitted to the Dhaka City Corporation, Dhaka. 77pp.
- Ameen, M., R.M. Shahjahan, H.R. Khan and A.K.A. Chowdhury. 1985. Larvicidal effects of indigenous *Derris elliptica* root on *Aedes aegypti* (Diptera: Culicidae). *Intern. Quart. Entomol.* **1**:39-43.
- Ameen, M., A.K.A. Chowdhury. H.R. Khan and R.M. Shahjahan. 1983 a. Insecticidal properties of *Derris elliptica* (Wall.) (Leguminosae: Papilionaceae) roots against the larvae of *Culex fatigans* Wied. (Diptera: Culicidae). *Dhaka Univ. Stud.* **B 31**(1):1-11.
- Ameen, M., R.M. Shahjahan., H.R. Khan, and A.K.A. Chowdhury. 1983 b. Toxicity of rotenone extracted from indigenous *Derris* roots on mosquito larvae. *J. Bangladesh Acad. Sci.* **7**(1&2):39-47.
- Barakat, A.A., A.S. El-mahy, K. Omaima, K. Moustafa, F.A. Mansour and K.M. El-hadek. 2004. Biological effect of *Cassia fistula* (L.) seeds against the cotton leaf worm *Spodoptera littoralis* (Boisd.) with special reference to chemical constituents. *Bull. Entomol. Soc. Egypt. Econ.* **30**: 1-14.

- Bashar, K., K.H. Mahmud, M.S. Reza, P. Goda, F.F. Rain, M. Jesmin and A. Asaduzzaman. 2013. Revised checklist and distribution maps of *Anopheles* (Insecta: Diptera: Culicidae: Anophelinae) mosquitoes of Bangladesh. *J. Biodiv. 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.
- Danish, M., P. Singh, G. Mishra, S. Srivastava, K.K. Jha and R.L. Khosa. 2011. *Cassia fistula* Linn. (Amulthus)- An Important Medicinal Plant: A Review of Its Traditional Uses, Phytochemistry and Pharmacological Properties. *J. Nat. Prod. Plant Resour.* **1**(1): 101118.
- Dave, H. and L. Ledwani. 2012. A review on enthaquinones isolated from *Cassia* species and their application. *Indian J. Nat. Prod. Resour.* **3**(3): 291-319.
- Dua, V.K., A.C. Pandey, K. Raghavendra, A. Gupta, T. Sharma and A.P. Dash. 2009. Larvicidal activity of neem oil (*Azadirachta indica*) formulations against mosquitoes. *Malaria J.* **8**(1):124.
- Duraipandiyar, V., S. Ignacimuthu and M.G. Paulraj. 2011. Antifeedant and larvicidal activities of Rhein isolated from the flowers of *Cassia fistula* L. *Saudi J. Biol. Sci.* **18**(2): 129–133.
- Finney, D.J. 1971. Probit Analysis. Cambridge University press, London. 68-78 pp.
- Govindarajan, M. 2013 a. Larvicidal activity of *Cassia fistula* flower against *Culex tritaeniorhynchus* Giles, *Aedes albopictus* Skuse and *Anopheles subpitius* Grassi (Diptera: Culicidae). *J. Pure Appl. Zool.* **1**(2): 117-121.
- Govindarajan, M. 2013 b. Bioefficacy of *Cassia fistula* Linn. (Leguminosae) leaf extract against chikungunya vector, *Aedes aegypti* (Diptera: Culicidae). *Eur. Rev. Med. Pharmacol. Sci.* **13**: 99-103.
- Govindarajan, M. 2009. Bioefficacy of *Cassia fistula* Linn. (Leguminosae) leaf extract against chikungunya vector, *Aedes aegypti* (Diptera: Culicidae). *Eur. Rev. Med. Pharmacol. Sci.* **13**:99-103.
- Govindarajan, M., A. Jebanesan and T. Pushpanathan. 2008. Larvicidal and ovicidal activities of *Cassia fistula* Linn. leaf extract against filarial and malarial vector mosquitoes. *Parasitol. Res.* **102**: 289-292.
- Hill, D.S. 1997. *Economic importance of insects* (1st ed.) Chapman and Hall, London. 83 pp.
- Khan, H.R., M. Mujahid, T. Akter, M.R. Karim and M.S. Farid. 2014. Mosquito diversity in the Two wards of Dhaka city. *Dhaka Univ. J. Biol. Sci.* **23**(1): 17-26.
- Khan, H.R., T. Akter, M.S. Farid, M.M. Islam and M.R. Karim. 2015. Seasonal fluctuations of various mosquito species in two wards of Dhaka city. *Physiol. Ecol. & Environ. Sci.* **5**(1& 2):51-58.
- Kumar, D., R. Chawla, P. Dhamodaram. and N. Balakrishnan. 2014. Larvicidal Activity of *Cassia occidentalis* (Linn.) against the Larvae of Bancroftian Filariasis Vector Mosquito *Culex quinquefasciatus*. *J. Parasitol. Res.* **2014**.
- Leisnham, P.T., D.P. Slaney, P.J. Lester, P. Weinstein and A.C.G. Heath. 2007. Mosquito density, associated macro invertebrate biodiversity, and water chemistry in water filled containers: Relationships to land use. *New Zealand J. Zool.* **37**:203-218.
- Liptak, P. and I. Szentagali. 1937. *A Cassia fistula* hatóanyaga. *Ber. Ungar. Pharm. Ges.* **13**: 61-63.
- Misra, T.R., R.S. Singh, H.S. Pandey and B.K. Singh. 1997. A new diterpene from *Cassia fistula* pods. *Fitoterapia.* **LXVIII**(58):375.
- Nour, A.H., J. Sandanasamy and A.H. Nour. 2012. Larvicidal activity of extracts from different parts of the Neem (*Azadirachta indica*) against *Aedes aegypti* mosquitoes' larvae. *Sci. Res. Essays.* **7**(31): 2810-2815.
- Ravichandran, R., D. Thandaraj and M. Alwarsamy. 2014. Antimosquito Activity of Leaf Extract of Neem (*Melia azedarach*) and Papaya (*Corica papaya*) detected against the larvae *Culex quinquefasciatus*. *Int. J. Inno. Res. Sci. Eng. Tech.* **3**(4):11928-11935.

WHO. 1999. *Tropical Disease Research Progress 1997-1998*. Fourteenth Programme Report. UNDP/World Bank/WHO Special Programme for Research and Training in tropical Disease (TDR), Geneva, Switzerland, TDR/PR14/FIL/199.1.26 pp.

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