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LARVICIDAL EFFICACY OF SIX INSECTICIDES AGAINST CULEX QUINQUEFASCIATUS SAY (DIPTERA: CULICIDAE) LARVAE

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Abstract

Searching for potent larvicidal toxic effects of six synthetic organophosphate insecticides (Diazinon 60 EC, Solar 55 EC, Malathion 57 EC, Delathroit 40 EC, Parathrin 10 EC and Clorasid 20 EC) were evaluated in the laboratory against 3rd or 4th instar larvae of the mosquito species, *Culex quinquefasciatus* Say. The tested insecticides: Diazinon (Dizinol) 60 EC, Solar (Chloropyrics 50% + Cypermethrin 5%) 55 EC, Malathion (Sithion) 57 EC, Delathroit (Dimehoate) 40 EC, Parathrin (Cypermethrin) 10 EC and Clorasid (Chloropyrics) 20 EC showed LC_{50} values at application dosages 0.00250-0.05 ppm, 0.0001-0.0025 ppm, 0.00025-0.005 ppm, 0.0005-0.001 ppm, 0.0001-0.002 ppm and 0.00025-0.0025 ppm to be 8.609×10^{-3} ppm, 4.440×10^{-4} ppm, 9.094×10^{-4} ppm, 24.215×10^{-5} ppm, 4.797×10^{-4} ppm and 7.022×10^{-4} ppm respectively. Among the six insecticides, on the basis of LC_{50} and relative potency values, Delathroit 40 EC was the most toxic followed by Solar 40 EC > Parathrin 10 EC > Clorasid 20 EC > Malathion 57 EC > Diazinon 60 EC.

Key words: Larvicidal efficacy, Organophosphate insecticides, Toxicity, LC_{50}, *Culex quinquefasciatus* larva

Introduction

Mosquito control is necessary to prevent the transmission of mosquito-borne diseases and to protect people and livestock from their biting nuisance. But mosquito control is one of the major problems of the world in view of its ecology and vector behaviour. Effective mosquito control is often a complex and complicated task. The current mosquito control approach is based on synthetic insecticides. Insecticides especially organophosphorus and chlorinated hydrocarbons are extensively used as a control measure against the mosquitoes either as larvicides or as adulticides. The control of mosquito at the larval stage is necessary and efficient in integrated mosquito management. During the immature stage, mosquitoes are relatively less mobile, remaining more concentrated.

Several insecticides were tested in past against larvae of *Culex quinquefascatus, C. fatigans, C. pipiens, Aedes aegypti, Anopheles culifacies, An. stephensi, An. fluviatilis* and *An. gambiae* (Diptera : Culicdae) by Das *et al.* (1982), Verma and Rajvanshi (1983), Thavaselavam *et al.* (1993), Miyagi *et al*. (1994), Kalyanasundaran *et al*. (2003), Bansal and Singh (2006), Michaelakis and Koliopoules (2007), Kumar *et al*. (2010, 2011), Sarar *et al*. (2011) and Nkya *et al*. (2014) and at home by Begum and Mirdha (1975), Begum and Bhuiya (1983), Hossain *et al*. (1995), Shahjahan (1996), Ali *et al.* (1999), Zannat (2003) and Saha (2003).

The present study was undertaken to test the potency of some commercial insecticides i.e. Dizinon (Dizinol) 60 EC, Solar (Chlorpyrics 50% + Cypermethrin 5%) 55 EC, Malathion (Sithion) 57 EC, Delathroit (Dimethoate) 40 EC, Parathrin (Cypermethrin) 10 EC and Clorasid (Chloropyrics) 20 EC. In this investigation, toxic effects of the six synthetic organophosphorus insecticides were examined for their toxicity against the mosquito larvae *Culex quinquefasciatus* Say.

Materials and Methods

To study the toxic effect of six insecticides: Diazinon Dizinol) 60 EC, Solar (Chloropyrics 50% + Cypermethrin 5%) 55 EC, Malathion (Sithion) 57 EC, Delathroit (Dimethoate) 40 EC, Parathrin (Cypermethrin) 10 EC and Clorasid (Chloropyrics) 20 EC systematic short term bioassays were done in the Entomological research laboratory of Department of Zoology, Chittagong University. All the six insecticides were bought from the government-approved shop of Chittagong city. Larvae of the mosquito were collected by small hand sieve from drains and small stagnant water bodies of Chittagong University campus and reared in the laboratory in an aquarium containing stagnant water. The third or fourth instar larvae of *Culex quinquefasciatus* were sorted as experimental specimens. The experiments were conducted at 30 ± 2^{o}C room temperature.

Before the final experiments, several preliminary screenings on different concentrations (ppm) of the insecticides were done. These preliminary experiments helped to ascertain the dose ranges for obtaining 1-99% mortality. The bioassays were run in a series of glass beakers each containing 500 ml of required concentration of the insecticide and run for a period of 24 hours. The different insecticide extracts in different doses were added to the beakers to determine the LC_{50} and LC_{90} values for *C. quinquefasciatus* larvae. Five concentrations of each extract were used in the final experiments. Ten *C. quinquefasciatus* larvae of third or fourth instars were released in each beaker and kept for 24 hours. Three replicates were done for each concentration. In each experiment a control was maintained in which the same number of mosquito larvae were released in same volume of tap water and was replicated in the same way. No food was supplied to the insects during the test period. All the beakers were netted on top.

Statistical analysis was done for the obtained data of the experiment. The dose concentrations were transferred to logarithms. Probit analysis was used to determine the LC_{50} and LC_{90} values of each insecticide. Values of LC_{50} and LC_{90} with 95% confidence intervals were analyzed in a computer based probit analysis programme. 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). Expected probit was calculated from respective empirical probit. Values of chi square at 0.05 level and ANOVA- test at 0.01 and 0.05 level were calculated following Fisher and Yates (1963). The relative potency values were calculated by taking the highest LC_{50} value as unit.

Results and Discussion

Effects of the insecticides on Culex quinquefasciatus larvae: The effectiveness of Diazinon (Dizinol) 60 EC, Solar (Chloropyrics 50% + Cypermethrin 5%) 55 EC, Malathion

Table 1. Toxicities of the six experimental insecticides on *Culex quinquefasciatus* larvae exposed for 24 hours.

Solvents / Toxicity Parameters	Diazinon 60 EC	Solar 55 EC	Malathion 57 EC	Delathroit 40 EC	Parathrin 10 EC	Clorasid 20 EC
Dose range (ppm)	0.0025-0.05	0.0001-0.0025	0.00025-0.005	0.00005-0.001	0.0001-0.002	0.00025-0.0025
Mortality range (%)	13.33-96.67	10.00-96.67	13.33-93.33	10.00-93.33	13.33-93.33	13.33-96.67
Slope line values	0.76x+4.31	2.21x+3.60	1.79x+3.27	1.29x+2.98	1.94x+3.68	2.94x+2.51
Chi-square value	55.07	4.40	3.16	28.84	9.48	2.87
Degrees of freedom (χ^2)	4	4	4	4	4	4
P-value (χ^2)	P<0.05	P>0.05	P>0.05	P<0.05	P>0.05	P>0.05
ANOVA- value (Treatment)	213.99	62.89	448.28	120.98	134.30	46.00
Degrees of freedom (ANOVA- test) (Treatment)	F_1=4 F_2=8	F_1=4 F_2=8	F_1=4 F_2=8	F_1=4 F_2=8	F_1=4 F_2=8	F_1=4 F_2=8
P-value (Treatment)	P<0.01<0.05	P<0.01<0.05	P<0.01<0.05	P<0.01<0.05	P<0.01<0.05	P<0.01<0.05
ANOVA- value (Replication)	7.11	0.44	20.99	0.99	6.00	0.390
Degrees of freedon (ANOVA- test) (Replication)	F_1=4 F_2=8	F_1=4 F_2=8	F_1=4 F_2=8	F_1=4 F_2=8	F_1=4 F_2=8	F_1=4 F_2=8
P-value (Replication)	P>0.01 & P<0.05	P>0.01>0.05	P<0.01<0.05	P>0.01>0.05	P>0.01 & P<0.05	P>0.01>0.05
LC_{50} (ppm)	8.609×10^{-3}	4.440×10^{-4}	9.094×10^{-4}	24.215×10^{-5}	4797×10^{-4}	7.022×10^{-4}
Confidence limit (lower)	6.425×10^{-3}	3.388×10^{-4}	6.651×10^{-4}	18.156×10^{-5}	3.598×10^{-4}	5.788×10^{-4}
Confidence limit (upper)	11.342×10^{-3}	5.771×10^{-4}	12.240×10^{-4}	32.355×10^{-5}	6.360×10^{-4}	8.514×10^{-4}
LC_{90} (ppm)	38.450×10^{-3}	17.594×10^{-4}	46.855×10^{-4}	113.536×10^{-5}	22.146×10^{-4}	19.486×10^{-4}
Confidence limit (lower)	25.936×10^{-3}	12.168×10^{-4}	30.187×10^{-4}	74.239×10^{-5}	14.573×10^{-4}	14.620×10^{-4}
Confidence limit (upper)	72.840×10^{-3}	31.755×10^{-4}	98.103×10^{-4}	228.587×10^{-5}	44.694×10^{-4}	31.858×10^{-4}

(Sithion) 57 EC, Delathroit (Dimethoate) 40 EC, Parathrin (Cypermethrin) 10 EC and Clorasid (Chloropyrics) 20 EC insecticides were bioassayed upon *C. quinquefasciatus* larvae at various concentrations. Records in terms of mortality were taken at an interval of 24 hours of exposure to the test insecticides. No control mortality was seen to occur. The values of the dose ranges and mortality ranges, slope line values, Chi square and ANOVA values, LC_{50} and LC_{90} values with their confidence limits of the six insecticides are given in Table 1. Probit mortality lines of the six insecticides for the species are shown in Fig 1.

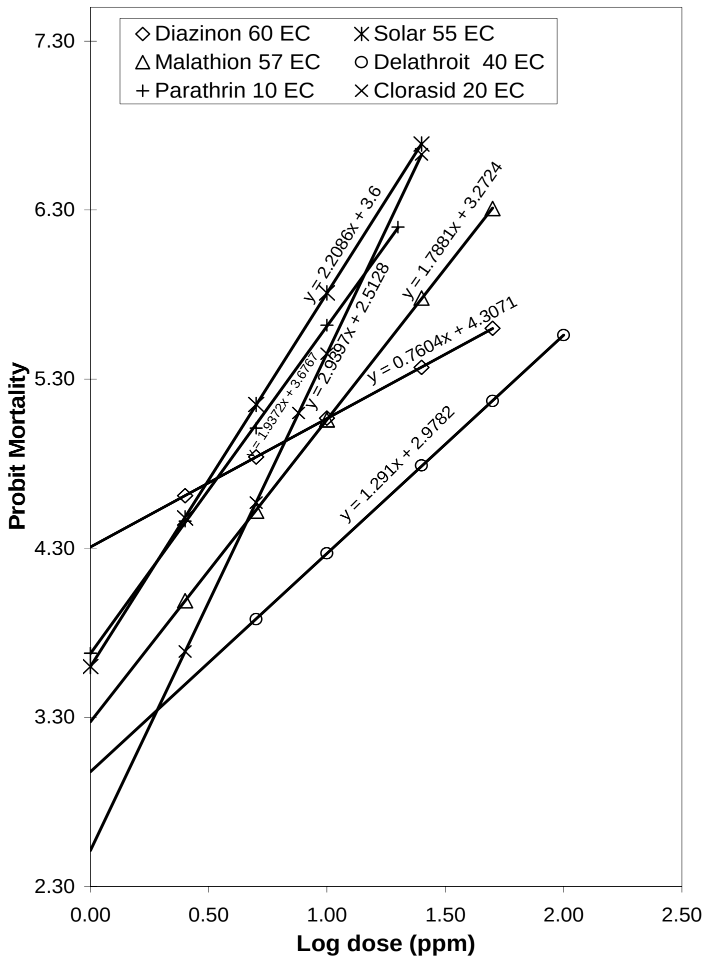

Fig 1. Regression lines for determining the LC_{50} of Diazinon 60 EC, Solar 55 EC, Malathion 57 EC, Delathroit 40 EC, Parathrin 10 EC and Clorasid 20 EC insecticides on *Culex quinquefasciatus* larvae after 24 hours of exposure.

Relative potency values of the six insecticides: The relative potency values of the six insecticides on *C. quinquefasciatus* larvae were calculated and are presented in Table 2.

Table 2. The LC_{50} and relative potency values of Diazinon 60 EC, Solar 55 EC, Malathion 57 EC, Delathroit 40 EC, Parathrin 10 EC and Clorasid 20 EC on *C. quinquefasciatus* larvae.

Insecticides	Extract	LC_{50} (ppm)	Relative Potency
Diazinon 60 EC	Distilled water	$8.6087\times10^{-3} = 86.087\times10^{-4}$	1.000
Solar 55 EC	Distilled water	4.440×10^{-4}	19.389
Malathion 57 EC	Distilled water	9.097×10^{-4}	9.463
Delathroit 40 EC	Distilled water	24.215×10^{-5} $= 2.422\times10^{-4}$	35.544
Parathrin 10 EC	Distilled water	4.797×10^{-4}	17.946
Clorasid 20 EC	Distilled water	7.022×10^{-4}	12.259

From the Table 2 it is evident that amongst the six insecticides Delathroit 40 EC was the most toxic insecticide having low LC_{50} value ($2.422\text{x}10^{-4}$ ppm) and high relative potency value (35.544). The lowest toxic insecticide was Diazinon 60 EC having a high LC_{50} value ($86.087\text{x}10^{-4}$ ppm) and low relative potency value (1.000). Hence, from the relative potency values it is suggested that Delathroit 40 EC was 35 times more toxic than Diazinon 60 EC extract. The relative position of the six insecticides on the basis of their LC_{50} and relative potency values was in the order: Delathroit 40 EC > Solar 55 EC > Parathrin 10 EC > Clorasid 20 EC > Malathion 57 EC > Diazinon 60 EC. From the results it may be concluded that of the six tested insecticides Delathroit was the most toxic, followed by Solar, Parathrin, Clorasid, Malathion and Diazinon. A comparison of the relative potency values amongst the six insecticides is shown in Table 3.

The effects of the six insecticides (Diazinon 60 EC, Solar 55 EC, Malathion 57 EC, Delathroit 40 EC, Parathrin 10 EC and Clorasid 20 EC) were studied in a dose dependent manner. In the present study, analysis of the data showed that Delathroit 40 EC was most toxic at 0.001 ppm, whereas 0.00005 ppm was found to be the least toxic dose. Diazinon 60 EC, Solar 55 EC, Malathion 57 EC, Parathrin 10 EC and Clorasid 20 EC insecticides were highly toxic at 0.5 ppm, 0.0025 ppm, 0.005 ppm, 0.002 ppm and 0.0025 ppm respectively whereas 0.0025 ppm, 0.0001 ppm, 0.00025 ppm, 0.0001 ppm and 0.00025 ppm were found to be the least toxic doses respectively.

From the mortality data it was also observed that the mortality of the experimental mosquito larvae increased with the gradual increase of the dose concentrations of different insecticides. Different mortality within the concentrations used, ranged between 10 and 97 percent. The order of larvicidal activity or toxicity of the six insecticides on *C. quinquefasciatus* observed in the present study was: Delathroit 40 EC > Solar 55 EC > Parathrin 10 EC > Clorasid 20 EC > Malathion 57 EC > Diazinon 60 EC.

Table 3. Comparison of the relative potency values among the six insecticides.

Comparison between the insecticides	Relative potency
Diazinon 60 EC relative to Solar 55 EC	19.389
Diazinon 60 EC relative to Malathion 57 EC	9.463
Diazinon 60 EC relative to Delathroit 40 EC	35.544
Diazinon 60 EC relative to Parathrin 10 EC	17.946
Diazinon 60 EC relative to Clorasid 20 EC	12.259
Solar 55 EC relative to Malathion 57 EC	0.488
Solar 55 EC relative to Delathroit 40 EC	1.833
Solar 55 EC relative to Parathrin 10 EC	0.926
Solar 55 EC relative to Clorasid 20 EC	0.632
Malathion 57 EC relative to Delathroit 40 EC	3.756
Malathion 57 EC relative to Parathrin 10 EC	1.896
Malathion 57 EC relative to Clorasid 20 EC	1.295
Delathroit 40 EC relative to Parathrin 10 EC	0.505
Delathroit 40 EC relative to Clorasid 20 EC	0.345
Parathrin 10 EC relative to Clorasid 20 EC	0.683

The LC_{50} values and relative potency of the insecticides to the larvae as observed in the present investigation revealed that Delathroit 40 EC was the most toxic to the test larvae and was about thirty five times more toxic than Diazinon 60 EC. Solar 55 EC and Parathrin 10 EC also showed more or less same relative potency and were two times less potent than Delathroit 40 EC. Malathion 57 EC and Clorasid 20 EC showed more or less similar relative potency and were 3-4 times less potent then Delathroit 40 EC. The LC_{50} values were found to be 2.424×10^{-4}, 4.440×10^{-4}, 4.797×10^{-4}, 7.022×10^{-4}, 9.097×10^{-4} and 86.087×10^{-4} ppm for Delathroit 40 EC, Solar 55 EC, Parathrin 10 EC, Clorasid 20 EC, Malathion 57 EC and Diazinon 60 EC respectively. The relative potency of the insecticides to larvae of *C. quinquefasciatus* in order of decreasing toxicity was as follows: Delathroit 40 EC > Solar 55 EC > Parathrin 10 EC > Clorasid 20 EC > Malathion 57 EC > Diazinon 60 EC. Pal and Karla (1958) and Ramkrishnan *et al*. (1960) respectively observed LD_{50} values of Malathion as 0.032 ppm and 0.031 ppm on *Culex* sp. larvae. In the present test LC_{50} values of Malathion, 9.097×10^{-4} ppm indicated that the larvae of *C. quinquefasciatus* strain in Bangladesh is very susceptible than those tested by Pal and Karla (1958) and Ramkrishnan *et al.* (1960).

The dose ranges in the present findings were 0.0025-0.05 ppm for Diazinon 60 EC, 0.0001-0.0025 ppm for Solar 55 EC, 0.00025-0.005 ppm for Malathion 57 EC, 0.00005-0.001 ppm for Delathroit 40 EC, 0.0001-0.002 ppm for Parathrin 10 EC and 0.00025-0.0025 ppm for Clorasid 20 EC which were somewhat similar to the dose ranges found by Hossain *et al.* (1995) in case of Cypermethrin 10 EC (0.00006-0.00390 ppm), and Deltamethrin 2.5 EC (0.00006-0.00195 ppm) and Begum and Mirdha (1975) in case of Gusathion 20 EC (0.001-0.0001 ppm), Sumithion 50 EC (0.0002-0.0009 ppm), Lebaycid

50 EC (0.0004-0.0007 ppm), Folithion 50 EC (0.0007-0.0014 ppm) and Birlane 50 EC (0.002-0.010 ppm). The mortality range of the present observation was 10-97% which was more or less similar to the findings of Hossain *et al.* (1995) and Kumar *et al.* (2011) on *Culex quinquefasciatus* larvae, whereby mortality ranged from 0-100% with Cypermethrin 10 EC and Deltamethrin 2.5 EC and 8-100% with Propoxur and Bocide (Spinosad) respectively and Begum and Mirdha (1975) on *Culex fatigans* larvae whereby mortality ranged from 13-93% with Gusathion 20 EC.

The range of the LC_{50} values of the present finding with *C. quinquefasciatus* was 0.0086 (86.087×10^{-4}) ppm with Diazinon 60 EC to 0.00024 (2.422×10^{-4}) ppm with Delathriot 40 EC which was similar to the findings of Sarar *et al*. (2011) with Temephos (LC_{50} = 0.0079 mg/L or ppm), Kumar *et al*. (2011) with Propoxur (LC_{50} = 0.00013 ppm), Bansal and Singh (2006) with Alphamethrin (LC_{50} = 0.00021 mg/L or ppm), Kalyanasundaran *et al*. (2003) with Dursban (Chloropyrifos-ethyl) (LC_{50} = 0.00011 mg/L or ppm), Hossain *et al.* (1995) with Cypermethrin 10 EC (LC_{50} = 0.00034 ppm) and with Deltamethrin 2.5 EC (LC_{50} = 0.00029 ppm), Begum and Mirdha (1975) with Gusathion 20 EC (LC_{50} = 0.00033 ppm) and with Sumithion 50 EC (LC_{50} = 0.00038 ppm) on *Culex quinquefasciatus* and *Culex fatigans* larvae.

Results obtained from the present study clearly indicate that Diazinon 60 EC is least effective among the six insecticides tested. Under the consideration of above investigation, it is apparent that if we apply more insecticides at over dose, our environment will be polluted by these insecticides and the larvae will attain resistance.

References

Ali, A., M.A. Chowdhury, M.I. Hossain, M. Ameen, D.B. Habiba and A.F.M. Aslam**.** 1999. Laboratory evaluation of selected larvicides and insect growth regulators against field-collected *Culex quinquefasciatus* larvae from urban Dhaka, Bangladesh. *J. Am. Mosq. Control Assoc.* **15 (1)**: 43-47.

Bansal, S.K. and K.V. Singh. 2006. Laboratory evaluation for comparative insecticidal activity of some synthetic pyrethroids against vector mosquitoes in arid region. *Journal of Environmental Biology* **27**: 251-255.

Begum, A . and M.M.B. Bhuiya. 1983. The susceptibility of *Culex fatigans* larvae to insecticides. *Bangladesh J. Zool.* **11 (1)**: 39-41.

Begum, A. and S.R. Mirdha. 1975. Toxicities of insecticides to larvae of *Culex fatigans* Wiedemann. *Bangladesh J. Zool.* **3 (2)**: 139-144.

Das P.K., T. Mariappan and C.B.S. Reddy. 1982. Susceptibility of *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles culicifacies* against insecticides. *J. Med. Res*. **75**: 529-533.

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

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

Hossain, M I., M. Ameen and A.K.M.R. Ahmed. 1995. Efficacy of two pyrethroid insecticides against *Culex quinquefasciatus* Say larvae in Dhaka City. *Bangladesh J. Zool.* **23 (2)**: 187-192.

Kalyanasundaran, M., P. Jambulingam, S.S. Sahu, P.S. Doss, D.D. Amalraj and P.K. Das. 2003. Efficacy of two organophosphorous insecticides, Reldan and Dursban against the larvae of *Culex quinquefasciatus. The Indian Journal of Medical Research* **117**: 25-29.

Kumar, R., A.K. Sharma, A.K. Gupta and M.P. Kaushik. 2010. Larvicidal activity of novel organophosphorus compounds against the *Culex quinquefasciatus. International Journal of Drug Design and Cover* **1 (3)**: 189-194.

Kumar, T.S., R.S. Kavitha, C.N. Nag, G.K. Navya, B.S. Raghavendra and V.A.. Vijayan. 2011. Present susceptibility status of *Culex quinquefasciatus* Say to four insecticides at Mysore, India. Research in Zoology, Univ. of Mysore. **1 (1)**: 8-11.

Michaelakis, A. and G. Koliopoulos. 2007. Attract and kill strategy. Laboratory studies on hatched larvae of *Culex pipiens. Journal of Medical Entomology* **63 (10)**: 954-959.

Miyagi, I., T. Toma, N. Zayasu and Y. Takashita. 1994. Insecticide susceptibility of *Culex quinquefasciatus* larvae (Diptera: Culicidae) in Okinawa prefecture, Japan in 1989. Japaneese. *J. San. Zool.* **45 (1)**: 7-11.

Nkya, T.E., F.W. Mosha, S.M. Magesaa and W.N. Kisinza. 2014. Increased tolerance of *Anopheles gambiae* S.S. to chemical insecticides after exposure to agrochemical mixture. *Tanzania Journal of Health Research.* **16 (4)**: 1-6.

Pal, R. and R.L. Karla. 1958. Comparative susceptibility of DDT resistant and normal *Culex quinquefasciatus* larvae to diphenyl methane derivatives and chlorinated insecticides. *Indian J. of Malariol.* **11 (4)**: 439-446.

Ramkrishnan, S.P., M.I.D. Sharma and R.L. Karla. 1960. Laboratory and field studies on the effectiveness of organophosphorus insecticides in the control of *Culex quinquefasciatus. Indian J. Malariology.* **11**: 545-565.

Saha, P. 2003. Effectiveness and residual action of five selected organophosphate larvicides against third instar larvae of *Culex quinquefasciatus.* M.Sc. Thesis, Dept. Zooogy., Jahangirnagar University. 84 pp.

Sarar, A.S., Y. Abobakr and H.I. Hussein. 2011. Laboratory and field evaluation of some chemical and biological larvicides against *Culex* spp. (Diptera: Culicidae) immature stages. *International J. of Agriculture and Biology.* **13 (1)**: 115-119.

Shahjahan, R.M. 1996. Effects of insecticides selection pressure on the larvae of *Aedes aegypti* (L.) (Diptera: Culicidae). *Bangladesh J. Zool.* **24 (2)**: 97-102.

Thavaselavam, D., A. Kumar and P. K. Sumodan 1993. Insecticide susceptibility status of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* in Punjab, Goa. *Indian J. Malariology.* **30 (2):** 75-79.

Verma, B.L. and A.C. Rajvanshi. 1983. Larvicidal efficacy of two synthetic pyrethroids against mosquitoes. *J. Com. Dis.* **15 (3)**: 219-221.

Zannat, T. 2003. Susceptibility levels of larvae of *Culex quinquefasciatus* against commonly used insecticides in Dhaka city. M..Sc. Thesis, Dept. Zoology Jahangirnagar University. 161 pp.

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