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Larvicidal Effects of Stem and Fruits of *Duranta repens* Against the Mosquito *Culex quinquefasciatus*

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Abstract: In the present study, the larvicidal activity of crude extracts from the stem and fruits, their fractions and fresh fruit juice of *Duranta repens* were assayed against the larvae of *Culex quinquefasciatus*. The highest larval mortality was found in chloroform soluble fraction of stem (LC_{50} = 10.75 ppm in 12 h) and ethanol extract of fruits (LC_{50} = 8.51 ppm 12 h) of *Duranta repens* against I instars larvae. Different concentrations of juice and fresh fruit juice also showed potent effects on *C. quinquefasciatus* and the larvae showed comparative tolerance with the increase of their age and time. These results suggest that the stem and fruits of *Duranta repens* are very effective natural larvicide and can be useful against *Culex quinquefasciatus*.
Key words *Duranta repens* Linn., *Culex quinquefasciatus* Say., larvicidal activity.

Introduction

Culex quinquefasciatus Say., a potential vector of *Wuchereria bancrofti*, is causative agent of human lymphatic filariasis (HLF) all over the world, including Bangladesh [1-3]. Mosquitoes transmit many deadly diseases like malaria, filariasis, yellow fever, dengue fever and Japanese B-encephalitis[4]. There are 26.42 million people have already been infected in filaria and 20-40 million chronic cases in India and Bangladesh[5]. Mosquitoes are also an important pest of humans, causing allergic responses that include local and systemic skin reaction such as angioedema and urticaria[6]. So, in order to prevent the transmission of mosquito borne diseases and also to protect people from biting nuisance, it is necessary to control mosquitoes for getting a healthy environment. But in recent years, mosquito control programs have been suffering from failures because of the ever-increasing insecticide resistance. Many mosquito species have already developed resistance against a number of chemical insecticides all over the world. In Bangladesh, *C. quinquefasciatus* is totally resistant to diazinon, fenitrothion, malathion, and primiphos-methyl and DDT[7,8]. Development of resistance to insecticides and widespread environmental pollution necessitate a continued search for alternative pest control as well as vector control strategies[9-14]. The phytochemicals have already been reported to be alternatives to synthetic pesticides and many of them are effective in mosquito control[15]. Plants or parts of plants possess a complex of chemicals with unique biological activity[16]. Over 2000 plant species contain chemicals with pest control properties[17] and among them several species of plants have been shown to have some degree of activity against mosquitoes[15, 18]. Thymol, an alkylated phenol derivative and essential oil extracted from *Lippia sidoides* were identified as the active larvicide against the mosquito *Aedes aegypti*[19]. It has been reported that the fruit juice of *D. plumieri* and the fruits of *D. repens* showed the larvicidal and antimalarial effects, respectively[20, 21].

The present study demonstrated the larvicidal action of stem and fruits (extracts and fractions) and fresh fruit juice of indigenous plant, *Duranta repens* (Verbenaceae) (locally named- Kata mehedi) on the vector mosquito, *C. quinquefasciatus*, under laboratory conditions. To best of our knowledge, this is the first report on larvicidal activity of extracts and fractions of

stem, fruits and fruits juice of *Duranta repens* against the mosquito *Culex quinquefasciatus*.

Materials and methods

Plant materials- The plant part (stem and fruits) of *Duranta repens* Linn. were collected from the adjoining areas of Rajshahi University Campus, Bangladesh during September to November and were identified in the Department of Botany, University of Rajshahi, Bangladesh where a voucher specimen number (Alam 78, collection date 19.09.1997) has been deposited.

Extraction and fractionation- *Duranta repens* stem were sun dried and pulverized into a coarse powder. The ground plant materials (1kg) were then extracted in cold with ethanol and then fractionated with chloroform and diethyl ether. Rotary evaporator at 40oC under reduced pressure to concentrate the solvents afforded a semisolid mass of ethanol extract (90.0 gm), diethyl ether soluble fraction (15.6 gm) and chloroform soluble fraction (20.8 gm). Similar extraction process was followed for the 1 kg of fruit to obtain a semisolid mass of ethanol extract, chloroform soluble fraction and petroleum ether soluble fraction (30.0, 8.0 and 6.0 gm), respectively.

Again fresh fruits (1 kg) of *Duranta repens* were washed gently with tap water to avoid foreign particles and then crushed in a mortar and pestle, filtered through Whatman no. 1 filter paper and thus obtained an orange colored clear juice (200 ml).

TLC screening- All extracts were run on pre-coated silica gel plate using hexane and ethyl acetate (2:1 and 1:1) as the mobile phase and vanillin-H_2SO_4 reagent as a spray reagent. Stem ethanol extract and diethyl ether soluble fraction gave positive tests for steroids and chloroform soluble fraction showed the presence of flavonoids and terpens. On the other hand, fruit ethanol extract gave positive test for steroids and glycosides but the chloroform and petroleum ether soluble fractions mainly showed the presence of flavonoids[22].

Mosquito culture- Larvae of the test mosquito were reared at 27 ± 1°C, 40-60% relative humidity and a 12:12 h light: dark photoperiod in laboratory. To rear larvae for toxicity assay, single egg rafts were placed in a number of 600-mL glass beakers containing 450 mL distilled water. The larvae were fed powdered Brewer's yeast at 10, 20, 40 and 80 mg per beaker everyday for I, II, III and IV instars larvae, respectively. Water was changed everyday to avoid scum formation, which might create toxicity.

Larvicidal bioassay

The larvicidal effect of crude ethanol extracts (stem and fruits) and their solvent fractions were determined by the WHO standard procedure[23]. The stock solutions were prepared by dissolving extracts and fractions (10 mg of each) in 1 ml of dimethyl sulphoxide (DMSO). After that twenty-five laboratory reared I, II, III and IV instars larvae were released into 100 ml glass beakers separately, containing 50 ml of distilled water to which 50, 100, 200 and 400 µl of each stock solutions were added using capillary micro-pipettes to get the desired test concentrations (w/v), viz, 10, 20, 40 and 80 ppm. Three types of control were maintained: i) distilled water; ii) distilled water plus food medium and iii) distilled water plus solvent (DMSO). Three replicates were made for each concentration and the experiment was performed under laboratory conditions at 27 ± 1°C and 40-60 % relative humidity. Brewer's yeast was supplied as a larval food during the test periods for larval feeding.

The larvicidal effects of fresh fruits juice were determined by the method of Carvalho[19]. 1%, 2%, 4%, 10% and 25% of test solutions (v/v) were prepared by distilled water. Then different concentrations of test solutions and fresh fruit juice (100%) were taken (25 ml of each) into separate glass beakers and twenty-laboratory reared I, II, III and IV instars larvae were placed on a filter paper separately and allowed to stand for few minutes. After the removal of water, the larvae were collected with a fine pointed camel hairbrush and transferred into glass beakers. Finally 50% and 100% of mortality were detected and recorded by timing for each concentration.

Statistical analysis- The cumulative mortality data were corrected by Abbott's formula[24]. Data were then subjected to Probit analysis for the determination of LC_{50} values[25].

Results

Statistical data obtained through toxicity bioassays are presented in Table 1 and Fig. 1, which clearly showed that the crude extracts (both stem and fruits), their fractions and fresh fruit juice of *Duranta repens* were highly effective against I, II, III and IV instars larvae of *C. quinquefasciatus*. Among the tested samples, the chloroform soluble fraction of stem and ethanol extract of fruit showed the highest toxicity and consequently, the lowest LC_{50} values (10.75 ppm, 14.06 ppm, 26.63 ppm and 36.53 ppm) and (8.51 ppm, 12.17 ppm, 14.37 ppm and 19.70 ppm), respectively in all instars larvae. The fruit extracts showed good potency over the stem extracts and the larvae showed comparative tolerance with the increase of their age and time, and LC_{50} values of all samples increased in all the instars tested (Table 1). The control showed no mortality against the larvae.

In this study, the 50% and 100% mortality of I, II, III and IV instars larvae have examined after application of different concentration of fruit juice and it has been found that the percentage of mortality of all instars larvae depends on time, age and concentration of juice (Fig. 1). We have also found that the concentration of juice is reciprocally related with the time of the larvicidal activity. When we applied 1% and 25% of fruit juice, 100% mortality of larvae have been observed within 15–28 hours and 3-9.30 hours, respectively for all instars larvae. But, in case of fresh juice (100%), it took only 35 min to 2 hours. On the other hand, 50% mortality of larvae in the above concentrations of fruit juice (1%, 25% and 100%)

has been occurred within 9-12 hours, 1-4 hours and 15-35 minutes, respectively.

Discussion

In this study, crude extracts (both stem and fruits), their fractions and fresh fruit juice of *Duranta repens* were found as highly effective larvicidal agents against I, II, III and IV instars larvae of *C. quinquefasciatus*. The increase in mortality with increase in exposure period could be due to several factors, which may be acted either separately or jointly. For example, the uptake of the active moiety of the compound could be time dependent, leading to a progressive increase in the titre of the plant-derived compounds tested and its effect on the larval body, the active moiety of the compound could be converted into more toxic metabolites in the larval integument and alimentary canal, resulting in time-dependent effects. Similarly, the larvicidal properties of the latex and stem of *Euphorbia tirucalli* plant and extracellular secondary metabolites of different fungi and various Euro-Asiatic plants against *Culex quinquefasciatus* larvae have been reported[26-28].

The extracts from *Duranta repens* had antifeedant and insecticidal properties against the larave of *Culex pipiens* and *Spodoptera littoralis*, and against the adults of *Musca domestica* and *C. pipiens,* respectively[29]. But as far we awarded there is no report found on the toxicity of *Duranta repens* against *Culex quinquefasciatus* Say.

We have also found the antibacterial, antifungal, MIC, brine shrimp lethality, acute toxicity test on rats and insecticidal activity on *Tribolium Castaneum* (Herbst) of stem and fruits of *Duranta repen* Linn.[30, 31]. The findings of the present study suggested that the stem and fruits of *Duranta repens* could be explored as potent natural larvicidal agent. Therefore we think that fruits compare to the stem is more convenient for larvacidal activity as both fresh juice and crude extract of fruit have shown their larvacidal activity.

Further study is needed to identify the compounds present in the fruits and stem of *Duranta repens* that have larvicidal activities. Finally the compounds from the stem and fruits of *Duranta repens* can be recommended as effective eco-friendly agents for the mosquito breeding control.

Table 1. Larvicidal activity of stem and fruit extracts and fractions of *D. repens* against *Culex quinquefasciatus* larvae.

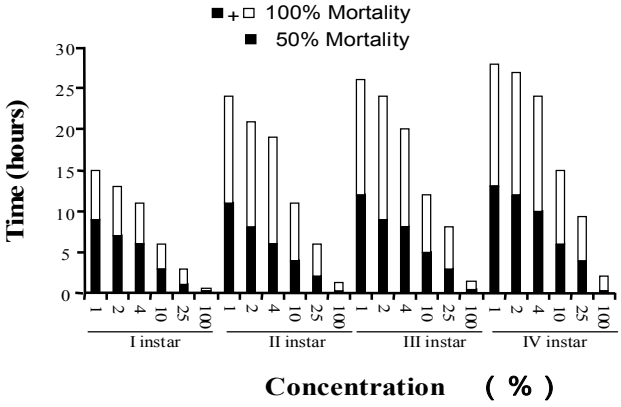
Table 1.

Larval Stage	Exposure Time (h)	Plant Extract/Fractions					
		Stem LC_{50} (ppm)			Fruits LC_{50} (ppm)		
		Chloroform	Diethyl Ether	Ethanol	Chloroform	Pet. Ether	Ethanol
Fisrt instar	3	35.58	-	-	-	-	30.03
	6	24.32	99.35	-	90.81	-	14.05
	12	10.75	76.78	285.68	59.04	245.58	8.51
	24	-	43.38	133.92	37.78	103.82	-
	48	-	-	92.65	-	61.63	-
Second instar	3	-	-	-	-	-	-
	6	55.29	-	-	-	-	51.84
	12	36.38	90.83	-	88.26	-	22.53
	24	14.06	78.49	399.59	70.76	325.85	12.17
	48	-	45.76	249.47	42.53	124.28	-
Third instar	12	65.82	-	-	-	-	38.90
	24	26.63	134.66	463.60	121.26	492.88	14.37
	48	-	60.09	230.73	48.83	161.11	-
Fourth instar	12	83.96	-	-	-	-	72.79
	24	36.53	175.99	788.56	154.53	858.05	19.70
	48	-	90.76	290.67	80.69	268.94	-

Values were based on three replicates with 25 larvae each.

Figure 1. Larvicidal effect (50% and 100% mortality) of different concentrations and fresh fruit juices of *D. repens* on four instars larvae of *Culex quinquefasciatus*.

Fig. 1.



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References

1. Birley M.H., A historical review of malaria, kala-azar and filariasis in Bangladesh in relation to the flood action plan, Ann. Trop. Med. Parasitol., 1993, 87, 319-334.
2. Ahmed S.S., Human filariasis in South and South-East Asia: A brief appraisal of our present knowledge, Proc. Pakistan Congr. Zool., 1994, 14,1-23.
3. Pailey K.P., Hoti S.L., Manonmani A.M., Balaraman K., Longevity and migration of *Wuchereria bancrofti* infective larvae and their distribution pattern in relation to the resting and feeding behaviour of the vector mosquito, *Culex quinquefasciatus;* Ann. Trop. Med. Parasitol., 1995, 89, 39-47.
4. Service M.W., Management of vectors, In Youdeowei A, Service MW (Eds). Pest and vectors management in Tropics, 1983, 265-280.
5. Sharma V.P, Health hazards of mosquito repellents and safe alternatives. Curr. Sci., 2001, 80 (3), 341-343.
6. Peng Z., Yang J., Wang H., Simons F.E.R., Production and characterization of monoclonal antibodies to two new mosquito *Aedes aegypti* salivary proteins, Insect Biochemistry and Molecular Biology, 1999, 29, 909-914.
7. Georghiou G.P., Lagunes-Tejeda A., The Occurrence of Resistance to Pesticides in Arthropods. Rome. FAO, 1991, 318.
8. WHO Vector resistance to pesticides, Fifteenth Report of the WHO Expert Committee on Vector Biology and Control. WHO Tech. Rep. Ser., 1992, 818, 1-62.
9. Pimental D., Acquay H., Biltonen M., Rice P., Silva M., Nelson J., Lipner V., Giordano S., Horowitz A., D'Amoer M., Environmental and economic costs of pesticide use. Bioscience, 1992, 42, 750-760.
10. Heckman C.W., The fate of pesticides in heavily polluted aquatic system. In: Hypertrophic and Polluted Freshwater Ecosystems: Ecological Bases for Water Resource Management. Ed. by Tilzer M.M., Khondker M., 1993, 39-90. Proc. Int. Symp. Limonol., Department of Botany,

University of Dhaka, Bangladesh, 1991, 25-28 November.

11. Coats J.R., Risks from natural versus synthetic insecticides; Ann. Rev. Ent., 1994, 39, 489-515.
12. Mulrennan Jr. J.A., Vector control without chemical: a public health perspective; J. Am. Mosq. Contr. Assoc., 1995, 11, 256-257.
13. Sugiyama A., Takagi M., Maruyama K., A laboratory experiment of the predation by possible predators on *Culex tritaeniorhynchus* larvae, Trop. Med., 1996, 38, 7-12.
14. Peng Y., Song J., Tian G., Xue Q., Ge F., Yang J., Shi Q., Field evaluations of *Romanomermis yunanensis* (Nematoda:Mermithidae) for control of Culicinae mosquitoes in China; Fundam. Appl. Nematol., 1998, 21, 227-232.
15. Sukumar K., Perich M.J., Boobar L.R., Botanical derivatives in mosquito control: a review J. Am. Mosq. Cont. Assoc. 1991, 7, 210-237.
16. Farnsworth N.R., Bingel A.S., Natural products and plant drugs with pharmacological, biological or therapeutic activity. Springer-Verlag, Berlin, 1977.
17. Ahmed S., Grainge M., Hylin J.W., Mitchell W.C., Listsinger J.A., Some promising plant species for use as pest control agents under tradional farming systems.Proc 2nd Int Neem Conf Rauischolzhausen, Germany. GTZ, Eschborn, Germany, 1984, 565-580.
18. Kumar A., Dutta G.P., Indigenous plant oils as larvicidal agent against *Anopheles stephensi* mosquitoes, Curr. Sci., 1987, 56, 959-960.
19. Carvalho A.F., Melo V.M., Craveiro A.A., Machado M.I., Bantim M.B., Rabelo E.F., Larvicidal activity of the essential oil from *Lippia sidoides* Cham. against *Aedes aegypti* Linn.; Mem. Inst. Oswaldo. Cruz., 2003, 98, 569-71.
20. Rao C.B., Rao T.N., Vijayakumar E.K.S., Chemical examination of the fruits of *Duranta plumieri* Jacq., Ind. J. Chem., 1978, 16, 844-845.
21. Castro O., Barrios M., Chinchilla M., Guerrero O., Chemical and biological valuation of the effect of plant extracts against *Plasmodium berghei*., Rev. Biol. Trop., 1996, 44, 361-367.
22. Harborne, J.B., Phytochemical Methods. Champmann and Hall, London, 1984, 134.
23. WHO Instruction for determining the susceptibility or resistance of mosquito larvae to insecticides. Mimeographed document. WHO/VBC/1975, 583.
24. Abbott W.S., A method of computing the effectiveness of an insecticide; J. Econ. Entomol., 1925, 18, 265-267.
25. Busvine J.R., A Critical Review of the Techniques for Testing Insecticides. London, U.K: CAB international, 1971, 395.
26. Rajeshwari Y., srivastava V.K., Ramesh C., Ajay S., Larvicidal activity of latex and stem bark of *Euphorbia tirucalli* plant on the mosquito *Culex quinquefasciatus*; J. Comm. Dis., 2002, 34, 264-269.
27. Govindarajan M., Jebanesan A., Reetha D., Larvicidal effect of extracellular secondary metabolites of different fungi against the mosquito, *Culex quinquefasciatus* Say., Trop. Biomed., 2005, 22, 1-3.
28. Pavela R., Larvicidal effects of various Euro-Asiatic plants against *Culex quinquefasciatus* Say larvae (Diptera: Culicidae); Parasitol. Res. 2008, 102 (3), 555-559.
29. El-Naggar M.E.A., Mosallam S.S., Insecticidal properties of some isolates from *Duranta repens* L. J. Egypt. Soc. Parasitol. 1987, 17, 243-249.
30. Nikkon F., Habib M.R., Karim M.R., Hossain M.S., Mosaddik M.A., Haque M.E., Antishigellosis and cytotoxic potency of crude extracts and isolated constituents from *Duranta repens,* Mycobiol., 2008, 36 (3), 173-177.
31. Nikkon F., Hasan S., Rahman M.H., Hoque M.A., Mosaddik M.A., Haque M.E., Biochemical, hematological and histopathological effects of *Duranta repens* stems on rats, Asi. J. Biochem., 2008, 3 (6), 366-372.

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