



leaves is used as a medicated bath for relieving rheumatic pains, and for cleaning foul ulcers and sores. The root juice is also used in the treatment of gonorrhoea. The roots and seeds are known to be used as fish-poison by the aborigines of Australia (Kirtikar and Basu 1935).

The seeds are mainly valued for the oil, which has many industrial and medicinal uses. Powdered seed is valued as a febrifuge and tonic, and also used in bronchitis and whooping cough, and the seed oil is used as a soap liniment to treat scabies, herpes and rheumatism (Burkill 1966). The stem bark is fibrous and is used for cordage. The fresh bark has a feebly sweetish and mucilaginous taste at first but it soon becomes bitter combined with a sort of pungency. It is also given internally in bleeding piles (Haemorrhoids). A decoction of bark is used for beri-beri. A very old well-known plant derived toxin, rotenone has been extracted from the roots of *Derris* sp. which is a contact and stomach poison, and acts as a repellent too. However, reports on the biological activity of this plant, especially on insect larvae are scanty, which led us to carry out this investigation.

### OBJECTIVE

The objective of this work was to determine whether or not the well known fish-toxic plant *D. indica* is potential against mosquito larvae.

### MATERIAL AND METHODS

*Preparation of plant materials for extraction:* The fresh leaves, fruit-shell, root-bark, root-wood, seeds, stem-bark, and stem-wood of *D. indica* were collected from the Rajshahi University Campus. *Leaves:* after collection the leaves were spread out to dry without heaping the material together. It was done under the shade avoiding direct sunshine. *Fruit-shell:* fruits were picked and the shells were opened to remove the seeds for the collection of fruit-shells and spread out to dry under the shade. *Root-bark:* roots were collected and were shaken and brushed to remove soil without washing them with water. Root-bark was collected by striping from the stem, and cut and dried thoroughly in a well-ventilated place. *Root-wood:* after removal of the root-bark the root-wood was cut into small pieces as thin as possible. Wood chips were dried thoroughly in a well-ventilated place. *Seeds:* after peeling out the fruit shells, seeds were cut into small pieces and spread out under shade to dry. *Stem-bark:* stem bark was collected by striping from the stem and cut into small pieces as thin as possible. After collection the bark was dry thoroughly in a well-ventilated place. *Stem-wood:* after removal of the stem-bark the stem-wood was collected and cut into small pieces as thin as possible and dried. After drying under the shade, the

plant materials were powdered in a grinder separately avoiding excessive heat during grinding.

*Chemical extraction of the collected materials:* The organic solvent chloroform was used for the extraction of seven different parts of *D. indica* separately. The ground dried materials, viz. leaves, fruit-shell, root-bark, root-wood, seeds, stem-bark, and stem-wood were extracted with sufficient amount of chloroform (500 g × 1500 ml × 3 times) for each of the items. Separate extracts were collected by the cool method after 72 hours of plunging for each of the materials. The extracts, thus, obtained through filtration and evaporation of the solvent as residue were kept in a refrigerator after proper labeling.

*Preparation of doses with the crude extracts for the larvicidal activity test:* The chloroform extracts of the plant materials were applied against *Cx. quinquefasciatus* larvae. For the fruit-shell, leaf and stem-bark two mg of each of the extracts was initially dissolved in 200 µl of pure dimethyl sulfoxide (DMSO) to make them hydrophilic before adding 19.98 ml of water to get a concentration of 200 ppm separately which were used as stock solution A. From the stock solution A, 10 ml was taken (that was with a concentration of 200 ppm) and diluted up to 20 ml with pond water to obtain a concentration of 100 ppm and this was used as stock solution B from which different other doses were made by the serial dilution method. Then a series of following concentrations was made from the stock solution A: 200, 100, 50, 25, 12.5 and 6.25 ppm. For root-bark, root-wood and stem-wood one mg of each of the extracts were dissolved in 100 µl of pure DMSO to make them hydrophilic before adding 19.98 ml of water to get a concentration of 100 ppm separately, which were used as stock solution A. From the stock solutions A, 10 ml was taken (that was with a concentration of 100 ppm) and diluted up to 20 ml with pond water to obtain a concentration of 50 ppm and this formed stock solution B from which different doses were made by the serial dilution method. Then a series of following concentrations were made from the stock solution A: 100, 50, 25, 12.5, 6.25 and 3.125 ppm. For the seed extract 0.5 mg was initially dissolved in 50 µl of pure DMSO for each before adding 19.98 ml of water to get a concentration of 50ppm which was used as the stock solution A. A series of following concentrations were made from the stock solution A for each of the samples: 50, 25, 12.5, 6.25, 3.125 and 1.5625 ppm.

*Application of doses:* The second instar larvae of *Cx. quinquefasciatus* were collected from mass rearing beakers in the laboratory and different concentrations, viz. 200, 100, 50, 25, 12.50, 6.25, 3.125 and 1.5625 ppm were applied to the rearing medium in which the larvae were released. The crude extracts were measured by electronic balance and were taken in small vials. 100 µl of DMSO was used for 25 ml crude extracts for emulsification. Ten ml pond

water was taken in 25 ml test tubes, the crude extracts as different doses were added to test tubes and 10 larvae were released to the dose treated water. Three replicates for each dose and a control were maintained.

*Statistical analysis:* The mortality of *Cx. quinquefasciatus* larvae was corrected by the Abbott's (1925) formula:

$$P_r = \frac{P_0 - P_c}{100 - P_c} \times 100$$

Where,  $P_r$  = Corrected mortality (%),  $P_0$  = Observed mortality (%), and  
 $P_c$  = Control mortality (%).

Mortality data were subjected to statistical analysis according to Finney (1947) and Busvine (1971) and the dose-mortality relationship was expressed as a median lethal concentration ( $LC_{50}$ ).

## RESULTS AND DISCUSSION

The chloroform extracts of fruit-shell, leaves, root-bark, root-wood, seeds, stem-bark and stem-wood from *D. indica* samples were applied against *Cx. quinquefasciatus* larvae. According to toxic potency the extracts could be arranged in the order: seed > root-wood > root-bark > stem-wood > leaf > fruit-shell > stem-bark. The  $LC_{50}$  values of the extracts and the statistical analysis are given in Table 1.

This work demonstrates the potentiality of seven parts of the plant in the control of *C. quinquefasciatus*. *Croton spariflorus* proved effective against *Cx. quinquefasciatus* (Kalyanasundaram and Das 1985). Rahman *et al.* (2006) found aquatic plant extracts active against *Cx. quinquefasciatus*. Pandiyan *et al.* (1994) reported *Mentha piperita* to be highly effective in controlling the larvae of *Cx. quinquefasciatus*. Methanolic extracts of *Solanum suratense*, *Azadirachta indica* and *Hydrocotyl javanica* exhibited larvicidal activity against *Cx. quinquefasciatus* (Muthukrishnan *et al.* 1997, Muthukrishnan *et al.* 2001, Babu and Murugan 1998, Venkatachalam and Jebanesan 2001, Jaswanth *et al.* 2002).

Hossain *et al.* (1998, 2001) estimated the  $LC_{50}$  values of the different foliar extracts of neem against the larvae of *Cx. quinquefasciatus*. Hussain *et al.* (2002) reported that the chloroform extracts of *D. indica* were more effective against the 4<sup>th</sup> instar larvae of *Cx. quinquefasciatus* than petroleum ether extracts. The lowest dose recorded for a steam distilled crude extract of *Callitris glaucophylla* against *Aedes aegypti* was 0.69 mg/ml (Shaalan *et al.* 2003). Prabakar and Jebanesan (2004) evaluated the toxicity of methanolic leaf extracts of five species of Cucurbitaceous plants against the late 3<sup>rd</sup> instar larvae of *Cx. quinquefasciatus*. Chaithong *et al.* (2006) found that ethanolic extracts derived

from three species, viz. *Piper longum* L., *P. ribesoides* Wall. and *P. sarmentosum* Roxb. ex Hunt. of pepper family Piperaceae, were effective against the early 4<sup>th</sup> instar larvae of *Ae. aegypti*. The larvicidal effects of the aqueous extracts of *Hemidesmus indicus* roots, *Gymnema sylvestre* and *Eclipta prostrata* leaves against *Cx. quinquefasciatus* larvae have been noted by Khanna and Kannabiran (2007). The crude extracts of *M. charantia* against the larvae of *Anopheles stephensis*, *Cx. quinquefasciatus* and *Ae. aegypti* were studied by Singh *et al.* (2006). Mohan and Ramaswamy (2007) found that the acetone leaf extract of *Ageratina adenophora* was more toxic to both *Ae. aegypti* and *Cx. quinquefasciatus*. Govindarajan *et al.* (2008) demonstrated that the aqueous extract of the leaf of *C. fistula* was more lethal to the larvae of *An. stephensis* and *Cx. quinquefasciatus*. These results suggest that the bioactivity of phytochemicals against mosquito larvae can vary depending on plant species, plant part, age of plant part, solvent used in extraction and mosquito species.

**Table 1. LC<sub>50</sub>, 95% confidence limits and regression equations of *D. indica* extracts against *C. quinquefasciatus* larvae.**

Test extract	Time exposed	LC <sub>50</sub> value (ppm)	95% Confidence limits		x <sup>2</sup> Value(df)
			Lower limits	Upper limits	
Fruit-shell	30 min	30762.54	16.09	5.88E+07	0.28(4)
	24 h	220.60	79.48	612.27	0.59(4)
	48 h	49.97	33.04	75.56	2.37(4)
Leaf	30 min	60922.83	5.79	6.41E+08	0.55(4)
	24 h	141.56	68.58	292.17	0.70(4)
	48 h	34.00	23.69	48.81	0.64(4)
Root bark	30 min	70070.31	0.89	5.49E+09	0.33(4)
	24 h	59.54	35.43	100.04	0.40(4)
	48 h	26.27	16.93	40.71	0.51(4)
Root-wood	30 min	3867.32	34.36	435254.20	0.39(4)
	24 h	36.95	25.75	53.03	0.16(4)
	48 h	18.33	13.39	25.11	3.19(4)
Stem-bark	30 min	453810.10	0.22	9.26E+11	0.43(4)
	24 h	229.92	80.99	652.74	0.73(4)
	48 h	68.88	38.82	122.23	0.53(4)
Stem-wood	30 min	225860.20	0.12	4.13E+11	0.42(4)
	24 h	82.84	40.01	171.53	0.53(4)
	48 h	30.15	18.35	49.54	1.15(4)
Seed	30 min	361.89	36.61	3577.46	0.60(4)
	24 h	21.52	14.35	32.26	2.02(4)
	48 h	8.64	6.42	11.62	4.24(4)

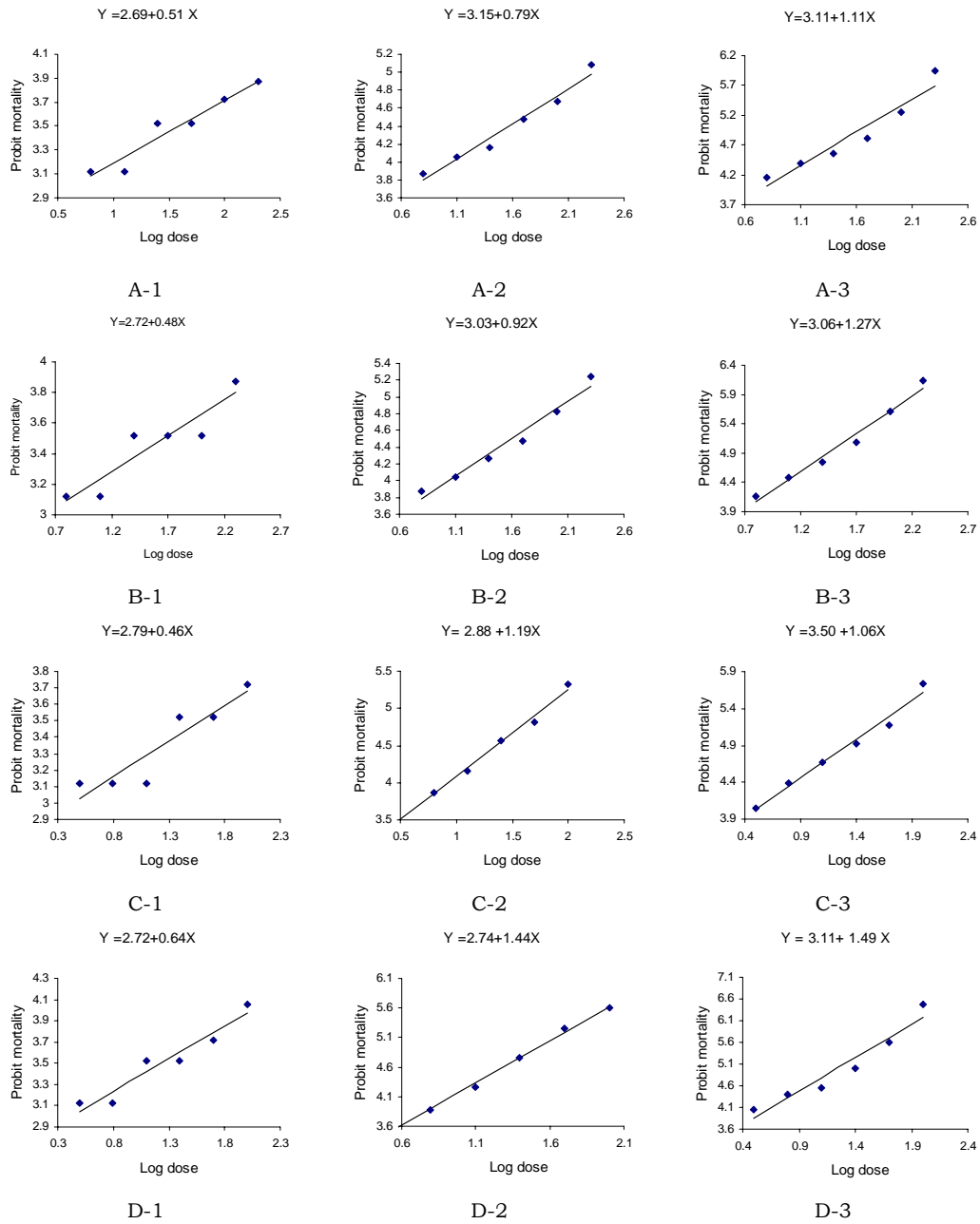


Fig. 1. Probit mortality lines of the chloroform extracts of fruit-shell (A-1, A-2, A-3); leaf (B-1, B-2, B-3); root-bark (C-1, C-2, C-3) and root-wood (D-1, D-2, D-3) of *D. indica* after 30 min, 24h and 48h of exposure to *Cx. quinquefasciatus* larvae.

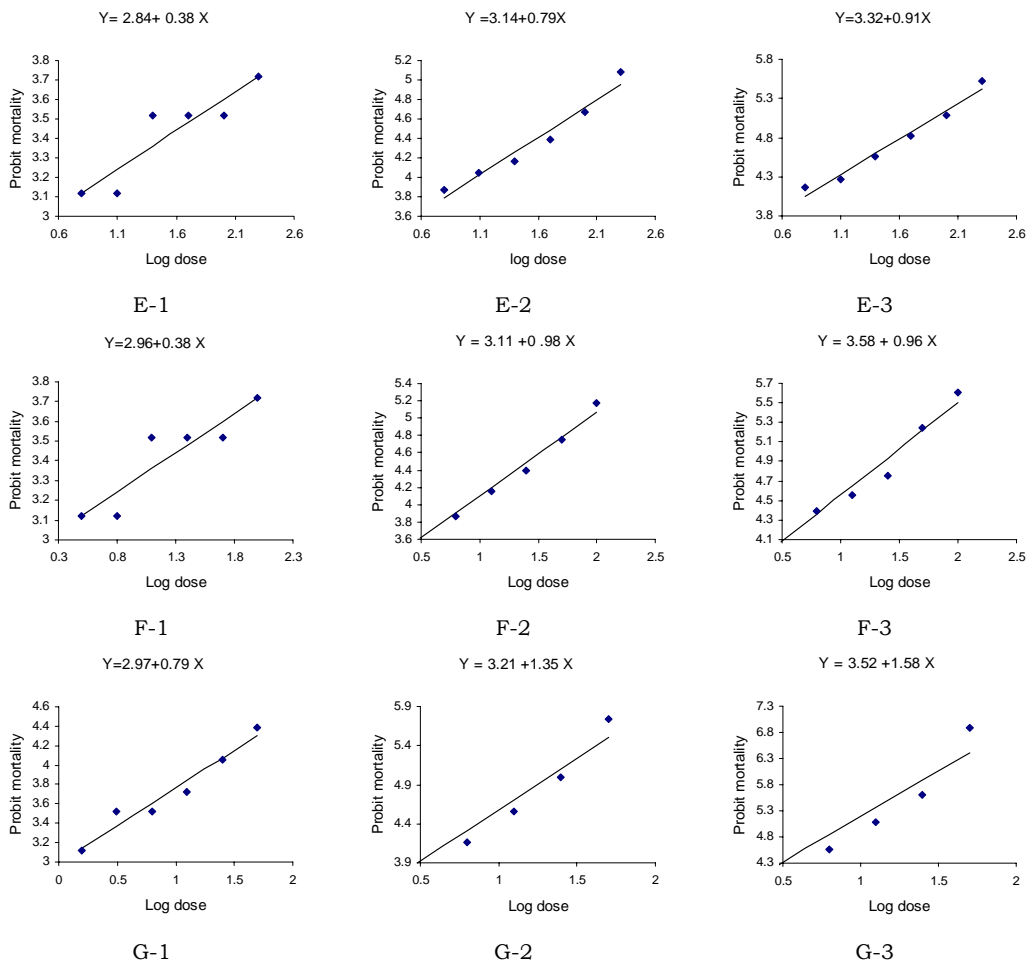


Fig. 2. Probit mortality lines of the chloroform extracts of stem-bark (E-1, E-2, E-3); stem-wood (F-1, F-2, F-3) and seeds (G-1, G-2, G-3) of *D. indica* after 30 min, 24h and 48h of exposure to *Cx. quinquefasciatus* larvae.

Mosquito control programs have been failure because of the ever increasing insecticide resistance. Besides resistance, increased costs of insecticide application and increased concern over environmental pollution have necessitated a continued search for alternative vector control methods which would be environmentally safer and specific to their action. Phytochemicals may be the potential choice in this regard.

It is clearly evident from the investigation that the extracts of different parts of *D. indica* are significantly potent against *Cx. quinquefasciatus* larvae. The seed extract produced the highest mortality at lower concentrations. Future comprehensive works are solicited in this line.

### CONCLUSION

From the present investigation it may be concluded that the test plant *D. indica* contains insecticidal properties for the control of mosquito larvae with the extracts especially of its seeds, root-wood, root-bark and stem-wood; their LC<sub>50</sub> values were 21.52, 36.95, 59.54 and 82.84 ppm for 24h of exposure, respectively and 8.64, 18.33, 26.26 and 30.15 ppm for 48h of exposure, respectively.

*Acknowledgement:* The authors are grateful to the University Grants Commission of Bangladesh for offering a grant for the present research and to the Chairman, Department of Zoology, University of Rajshahi, Rajshahi-6205, Bangladesh for providing laboratory facilities.

### LITERATURE CITED

- ABBOTT, W.S. 1925. A method of computing the effectiveness of an insecticide. *J. econ. Ent.* **18**: 265-267.
- AHMED, S.S. 1994. Human filariasis in South and South-East Asia: A brief appraisal of our present knowledge. *Proc. Pakistan Congr. Zool.* **14**: 1-23.
- ANON. 1969. *The wealth of India- a dictionary of Indian raw materials and industrial products*. CSIR, New Delhi **8**: 210.
- BABU, R. and MURUGAN, K. 1998. Interactive effect of neem seed kernel and neem gum extracts on the control of *Culex quinquefasciatus* Say. *Neem News Lett.* **15**(2): 9-11.
- BIRLEY, M.H. 1993. A historical review of malaria, kala-azar and filariasis in Bangladesh in relation to the flood action plan. *Ann. Trop. Med. Parasitol.* **87**: 319-334.
- BURKILL, J.H. 1966. *A dictionary of economic products of the Malay peninsula*. Art Printing Works, Kuala Lumpur, 2 vols.
- BUSVINE, J.R. 1971. *A critical review of the techniques for testing insecticides*. Commonwealth Agricultural Bureux, London. 345pp.
- CHAITHONG, U., CHOOCHOTE, W., KUMSUK, K., JITPAKDI, A., TIPPAWANGKOSOL, P., CHAIYASIT, D., CHAMPAKAEW, D., TUETUN. B. and PITASAWAT, B. 2006. Larvicidal effect of pepper plants on *Aedes aegypti* L. (Diptera: Culicidae). *J. Vec. Ecol.* **31**(1): 138-144.
- FINNEY, D.J. 1947. *Probit analysis: a statistical treatment of the sigmoid response curve*. Cambridge University Press, London. 333 pp.
- GOVINDARAJAN, M., JEBANESAN, A. and PUSHPANATHAN, T. 2008. Larvicidal and ovicidal activity of *Cassia fistula* Linn. leaf extract against filarial and malarial vector mosquitoes. *Parasit. Res.* **102**(2): 289-92.
- HOSSAIN, M.I., AMEEN, M., MRIDHA, M.A. and FERDOUS, A.J. 1998. Insecticidal action of neem seed extracts on the larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae). *J. Asiatic Soc. Bangladesh* **24**: 119-125.
- HOSSAIN, M.I., RAHMAN, G.M.S., AMEEN, M. and ISLAM, S.N. 2001. Effects of different foliar extracts of neem (*Azadirachta indica* A. Juss.) on the larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae). *Dhaka Univ. J. Bio. Sci.* **10**(2): 121-127.
- HUSSAIN, A.A., MOSTAFA, S.A., GHAZAL, S.A. and IBRAHIM, S.Y. 2002. Studies on antifungal, antibiotic and bioinsecticidal activities of some actinomycete isolates. *African J. Mycol. Biotechnol.* **10**: 63-80.



- JASWANTH, A., RAMANATHAN, P. and RUCKMANI, K. 2002. Evaluation of mosquitocidal activity of *Annona squamosa* leaves against filarial vector mosquito, *Culex quinquefasciatus* Say. *Indian J. Exp. Biol.* **40**: 363-365.
- KABIR, K.E., KHAN, A.R. and MOSADDIK, M.A. 2003. Goniiothalamine- a potent mosquito larvicide from *Bryonopsis laciniosa* L. *J. Appl. Ent.* **127**: 112-115.
- KALYANASUNDARAM, M. and DAS, P.K. 1985. Larvicidal and synergistic activity of plant extracts for mosquito control. *Indian J. Med. Res.* **82**:19-23.
- KHANNA, G.V. and KANNABIRAN, K. 2007. Larvicidal effect of *Hemidesmus indicus*, *Gymnema sylvestre* and *Eclipta prostrata* against *Culex quinquefasciatus* mosquito larvae. *African J. of Biotechnol.* **6**: 307-311.
- KIRTIKAR, K.R. and BASU, B.D. 1935. *Indian Medicinal Plants*, Vol. III (2nd ed). Basu, L.M., Allahabad, India. pp.1593-2393.
- MOHAN, D.R. and RAMASWAMY, M. 2007. Evaluation of larvicidal activity of the leaf extract of a weed plant, *Ageratina adenophora*, against two important species of mosquitoes, *Aedes aegypti* and *Culex quinquefasciatus*. *African J. Biotech.* **6**(5): 631-638.
- MUTHUKRISHNAN, J. and PUSHPALATHA, E. 2001. Effects of plant extracts on fecundity and fertility of mosquitoes. *J. Appl. Ent.* **125**: 31-35.
- MUTHUKRISHNAN, J., PUSPALATHA, E. and KASTHURIBHAI, A. 1997. Biological effect of four plant extracts on *Culex quinquefasciatus* Say larval stages. *Insect Sci. & Appl.* **7**(3/4): 389-394.
- PAILEY, K.P., HOTI, S.L., MANONMANI, A.M. and BALARAMAN, K. 1995. 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.* **89**: 39-47.
- PANDIYAN, R.S., ABRAHAM, M.G. and MANOHARAN, A.C. 1994. Susceptibility of the larvae of *Culex quinquefasciatus* Say to extracts of medicinal plants. *Environ. Pollut.* **1**(3&4):109-112.
- PRABAKAR, K. and JEBANESAN, A. 2004. Larvicidal efficacy of some Cucurbitaceous plant leaf extracts against *Culex quinquefasciatus* Say. *Bioresource Technol.* **95**(1): 113-114.
- RAHMAN, G.M., HOSSAIN, M., ISLAM, S.K.M. and AMEEN, M. 2006. Efficacy of aquatic plant extracts on the larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae). *Bangladesh J. Zool.* **34**: 1-5.
- SHAALAN, E., CANYON, D.V. FARIED, M.W., ABDEL-WAHAB, H., and MANSOUR, A. 2003. Efficacy of a highly active phytochemical (*Callitris glaucophylla*) against mosquito vectors of dengue and Japanese encephalitis. *The Annual Queensland Health and Medical Scientific Meeting, "Making It Better: Encouraging Health Research and Innovation"*, 25-26 Nov.
- SINGH, R.K., DHIMAN, R.C. and MITTAL, P.K. 2006. Mosquito larvicidal properties of *Momordica charantia* Linn. (Family: Cucurbitaceae). *J. Vec. Borne Dis.* **43**: 88-91
- VENKATACHALAM, M.R. and JEBANESAN, A. 2001. Repellent activity of *Ferronia elephantum* Corr. (Rutaceae) leaf extract against *Aedes aegypti* L. *Bioresource Technol.* **76**: 287-294.
- WORLD HEALTH ORGANIZATION 1997. Lymphatic filariasis: reasons for hope. Document No.: WHO/CTD/FIL/97.4 Rev.1.

(Manuscript received on February 2, 2010; revised on January 1, 2011)