Mosquito larvicidal potential of ethanol leaf extract of the plant, *Annona reticulata* L. against *Aedes aegypti* L. and *Culex quinquefasciatus* Say (Diptera: Culicidae)

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Abstract The larvicidal potential of ethanol leaf extract of *Annona reticulata* L. (A. reticulata), ( Annonaceae) was evaluated against 1st - 4th instars larvae of *Aedes aegypti* L. (A. aegypti) and *Culex quinquefasciatus* Say (Cx. quinquefasciatus) mosquitoes at 24, 48 and 72 h exposure. The extract was found more effective against Cx. quinquefasciatus than Ae. aegypti larvae and its LC50 and LC90 values gradually decreased with increased period of exposure. LC50 values of the extract recorded after 24 h of exposure were 0.5021, 2.9374, 4.2048, 6.2245 ppm respectively against 1st - 4th larvae of Cx. quinquefasciatus and 6.8839, 5.9929, 14.5745, 19.8836 ppm against Ae. aegypti larvae. No mortality and any abnormal behavior up to 72 h post exposure were observed in aquatic non target organisms viz., Chironomus circmdatus larvae, Diplonychus annulatum and tadpoles of frog when exposed to 24 h LC50 dose of the extract against 3rd instar larvae of Cx. quinquefasciatus. In conclusion, the ethanol leaf extract of *A. reticulata* exhibited excellent larvicidal activity against Cx. quinquefasciatus and Ae. aegypti mosquitoes.

Keywords Aedes aegypti; Annona reticulate; Culex quinquefasciatus; larvicidal activity

1 Introduction Various diseases of human beings like malaria, filariasis, Japanese encephalitis, dengue/dengue haemorrhagic fever, etc are transmitted by mosquito species causing heavy morbidity and millions of death every year (Hotez et al., 2004; Rahuman et al., 2008). Biting of mosquitoes causes skin irritation and allergic reactions (Cheng et al., 2003). *Ae. aegypti* mosquito is associated with the transmission of dengue/dengue haemorrhagic fever, chikungunya and yellow fever. Dengue virus belongs to genus Flavivirus (family- flaviviridae) with four serotypes, viz., DEN 1, DEN 2, DEN 3 and DEN 4, and causes flu like illness, dengue fever to dengue haemorrhagic fever, a full-fledged illness, and then transforms into dengue shock syndrome, and ultimately death (Henchal and Putnak, 1990). Globally two fifth of human population is presently under the threat of dengue (Kovendan and Murugan, 2011). *Cx. Quinquefasciatus* mosquito is responsible for transmission of lymphatic filariasis, and at least 120 million people are infected in several countries of the tropics and subtropics. In addition to the morbidity and mortality, great economic loss and social disruption occur in developing countries due to these mosquito borne diseases (Hotez et al., 2004). Currently use of synthetic insecticides is the major tool used in controlling mosquitoes. However, use of many synthetic insecticides has been restricted because it creates problems such as food chain biomagnifications, high price, emergence of resistance in mosquitoes to chemical insecticides, bad effects on human health, and other beneficial organisms of the environment, non-biodegradability etc., thus, hampering sustainable development of environment, (Brown, 1986; Russell et al., 2009). The best alternative sources of synthetic insecticides are the botanical insecticides as they are easily degradable and renewable (Roel, 2001). The potentiality of botanical insecticides as mosquito larvicide has been reviewed by Ghosh et al., (2012). The present study was undertaken to evaluate mosquito larvicidal potentiality of ethanol leaf extract of *A. reticulata*. *A reticulata* is an evergreen plant, cultivated widely in India for its sweet fruits. In
English it is called custard apple and in Telugu it is called Ramphalam. It was reported that ethanol extract of leaf and stem of this plant has anticancerous properties. It is traditionally used for curing several diseases such as cardiac problems, worm infestation, dysuria, epilepsy, antifertility, dysentery, etc. (Kaleem et al., 2006; Suresh et al., 2006; Raj Sobiya et al., 2009).

2 Result

Table 1 and Table 2 depict the mortality percentages of all instars larvae (1st - 4th) of Ae. aegypti and Cx. quinquefasciatus at different concentrations respectively after 24, 48 and 72 h of exposure periods. From Table 1 and Table 2, it was observed that mortality percent increased with increase in concentrations and time of exposure. 1st instar larvae of Cx. quinquefasciatus were more susceptible than 2nd, 3rd and 4th instars larvae. LC50, LC90 values (95% confidence level), regression equations of ethanol leaf extract of the plant against 1st - 4th instars larvae of Ae. aegypti and Cx. quinquefasciatus after 24, 48 and 72 h of exposure are presented in Table 3 and 4. From Table 3 and 4, it was observed that LC50 and LC90 values gradually decreased with period of exposures in different larval forms of Ae. aegypti and Cx. quinquefasciatus. There was a strong correlation between concentrations of the extract and mortality percentages as R² (co efficient of determination) values were close to 1 in all cases. Three ways factorial ANOVA established statistical significance of larval mortality of Ae. aegypti and Cx. quinquefasciatus (p<0.05) in terms of instars, times and concentrations (Table 5 and 6). In case of tested non-target aquatic organisms, no mortality and abnormal behaviour were noticed upto 72 h of exposure period. No mortality was observed on control treatments of non-target organisms.

3 Discussion

Phytochemicals are suitable alternatives to chemical insecticides as their use in environment are relatively safer and such plants are available in many parts of the world (Bowers et al., 1995). Various parts of the plants have been reported by researchers for their larvicidal potency against different species of mosquitoes (Chowdhury et al., 2007, 2008; Hossain et al., 2011; Singha and Chandra, 2011; Mallick Halder et al., 2011; Adhikari et al., 2012; Mallick et al., 2014; Singha Ray et al., 2014; Singh et al., 2015; Mallick and Chandra, 2015). The present study indicates that larvae of Cx. quinquefasciatus were more susceptible to ethanolic leaf extract of A. reticulata as compared to Ae. aegypti. Nayak, (2014) reported the larvicidal activity of methanol leaf extract of A. reticulata against early 4th instar larvae of Cx. quinquefasciatus while Mallick et al., (2015) reported the larvicidal activity of acetone leaf extract of A. reticulata against 1st to 4th instars larvae of Ae. aegypti, Anopheles stephensi and Cx. quinquefasciatus. Mallick and Chandra, (2015) reported the larvicidal activity of extracts of stem bark of A. reticulata against Cx. quinquefasciatus mosquito. Mallick and Chandra, (2015) also reported the larvicidal potential of root extracts of A. reticulata against Cx. quinquefasciatus Singh et al., (2011) worked with petroleum ether, chloroform:methanol (1:1 v/v) and ethyl acetate extracts of mature leaves of Mesua ferra L. against 3rd instar larvae of Cx.quinquefasciatus having LC50 values 195.33, 27.28, 74.19 ppm respectively after 48 h of exposure. However, in this study, ethanol leaf extract of A. reticulata shows a remarkably low LC50 value (1.728 ppm) against 3rd instar larvae of Cx. quinquefasciatus after 48 h of exposure. Manzoor et al., (2013) reported the larvicidal activity of essential oil from Ocimum calamus, against 3rd instar larvae of Ae. aegypti and Cx. quinquefasciatus after 24 h of exposure having LC50 values 75.35 and 92.30 ppm respectively. In comparison, ethanol leaf extract of A. reticulata recorded very low LC50 values (14.57 and 4.2048 ppm) against 3rd instar larvae of Ae. aegypti and Cx. quinquefasciatus respectively after 24 h of exposure. Singh et al., (2006) reported that LC50 values of hexane extract of Momordica charantia against 4th instar larvae of An. stephensi, Cx. quinquefasciatus and Ae. aegypti were 66.05, 96.11 and 122.45 ppm respectively after 24 h of exposure. However, present study shows LC50 values of ethanol leaf extract of A. reticulata as 6.22 and 19.88 ppm against 4th instar larvae of Cx. quinquefasciatus and Ae. aegypti respectively after 24 h of exposure which are much lower values than other plants. Maheswaran et al., (2008) reported highest larvicidal activity of the hexane extract followed by chloroform and ethanol extracts of Leucus aspara leaves against Cx. quinquefasciatus and Ae. aegypti. The LC50 values of hexane extract of leaves of Leucus aspara against 1st - 4th instar larvae of Cx. quinquefasciatus were 122.50,
The results indicate that ethanol leaf extract of leaves of A. reticulata can be used effectively against Ae. aegypti and Cx. quinquefasciatus mosquito species. Because of non-toxic effect on tested non-target organisms, its use will also be safer. Further study is needed to identify the chemical structure of actual compound(s) involved in larvicidal activity.

### 4 Materials and methods

#### 4.1 Collection of plant materials

After proper identification of the plant, fresh mature leaves of A. reticulata (aged about one to four years) were collected from Burdwan town, West Bengal, India (23°16′N, 87°54′E) during the month of September and October, 2013, and the voucher specimen (voucher No. GCZSM-4) was deposited as herbarium to Mosquito, Microbiology and Nanotechnology Research Units, Parasitology Laboratory, Department of Zoology, The University of Burdwan, West Bengal, India. After collection, the leaves were washed gently with water and dried on paper towels.
Table 2 Percent mortality of different instars of *Cx. quinquefasciatus* exposed to different concentrations of ethanol leaf extract of *A. reticulata* (Mean mortality percent± standard error).

| Instar | Conc (ppm) | %Mortality (±SE) at different exposure periods |
|--------|------------|-----------------------------------------------|
|        |            | 24h               | 48h               | 72h               |
| 1st    | 1.5        | 86.66±3.33        | 86.66±3.33        | 100.0±00.0        |
|        | 3.0        | 90.00±5.88        | 93.33±3.33        | 100.0±00.0        |
|        | 6.0        | 100.0±00.0        | 100.0±00.0        | 100.0±00.0        |
|        | 12.0       | 100.0±00.0        | 100.0±00.0        | 100.0±00.0        |
|        | 18.0       | 100.0±00.0        | 100.0±00.0        | 100.0±00.0        |
| 2nd    | 1.5        | 26.66±3.33        | 60.00±5.78        | 83.33±3.33        |
|        | 3.0        | 36.66±3.33        | 83.33±8.88        | 90.00±5.8         |
|        | 6.0        | 83.33±3.33        | 93.33±3.33        | 100.0±00.0        |
|        | 12.0       | 100.0±00.0        | 100.0±00.0        | 100.0±00.0        |
|        | 18.0       | 100.0±00.0        | 100.0±00.0        | 100.0±00.0        |
| 3rd    | 1.5        | 10.00±5.78        | 43.33±3.33        | 70.00±5.78        |
|        | 3.0        | 23.33±3.33        | 76.66±3.33        | 86.66±3.33        |
|        | 6.0        | 63.33±3.33        | 96.66±3.33        | 100.0±00.0        |
|        | 12.0       | 100.0±00.0        | 100.0±00.0        | 100.0±00.0        |
|        | 18.0       | 100.0±00.0        | 100.0±00.0        | 100.0±00.0        |
| 4th    | 1.5        | 33.33±3.33        | 56.66±3.33        | 90.00±5.78        |
|        | 3.0        | 16.66±3.33        | 43.33±3.33        | 63.33±8.88        |
|        | 6.0        | 80.00±5.78        | 96.66±3.33        | 100.0±00.0        |
|        | 12.0       | 100.0±00.0        | 100.0±00.0        | 100.0±00.0        |

Note: Control No mortality (For all instars)

Table 3 Log probit and regression analyses of larvicidal activity of ethanol leaf extract of *A. reticulata* against different larval instars of *A. aegypti*

| Larval instar | Exposure period (h) | LC₅₀/ppm | LC₉₀/ppm | Regression equation | R² value |
|---------------|---------------------|----------|----------|--------------------|----------|
| 1st           | 24                  | 6.8839   | 23.3961  | Y= 6.6104 +4.1911 X| 0.9256   |
|               | 48                  | 3.0721   | 11.7153  | Y= 39.8459 + 2.9598 X| 0.8442   |
|               | 72                  | 2.3550   | 8.3395   | Y= 50.6641 + 2.5219 X| 0.7409   |
| 2nd           | 24                  | 5.9929   | 24.4299  | Y= 17.6836 +3.5229 X| 0.9271   |
|               | 48                  | 4.2606   | 19.7103  | Y= 29.6945 +3.1290 X| 0.8944   |
|               | 72                  | 2.9057   | 13.5762  | Y= 42.4162 + 2.7207X| 0.8348   |
| 3rd           | 24                  | 14.5745  | 106.0827 | Y= 7.9499 +2.5676 X| 0.9865   |
|               | 48                  | 6.5972   | 55.2839  | Y= 25.4283 +2.4923 X| 0.8511   |
|               | 72                  | 4.1226   | 33.5766  | Y= 34.9844 + 2.4818 X| 0.8565   |
| 4th           | 24                  | 19.8836  | 28.8044  | Y= -14.2149 + 3.1310 X| 0.8342   |
|               | 48                  | 19.4130  | 27.7418  | Y= -14.9512 + 3.3029X| 0.8332   |
|               | 72                  | 18.3592  | 25.0146  | Y= -17.0594 + 3.7575 X| 0.8343   |

Note: LC = Lethal Concentration, X = Concentration, Y = mortality, R² = Coefficient of determination
Table 4 Log probit and regression analyses of larvicidal activity of etanol leaf extract of A. reticulata against different larval instars of Cx. quinquefasciatus

| Larval instar | Exposure period (h) | LC_{50} (ppm) | LC_{90} (ppm) | Regression equation | R^2 value |
|---------------|---------------------|---------------|---------------|---------------------|-----------|
| 1st           | 24                  | 0.5021        | 2.0977        | Y = 89.3157 + 7.428X | 0.6111    |
|               | 48                  | 0.5300        | 1.9153        | Y = 90.7165 + 6.520X | 0.6111    |
|               | 72                  | -             | -             |                     |           |
| 2nd           | 24                  | 2.9374        | 7.43          | Y = 32.8936 + 4.498X | 0.7628    |
|               | 48                  | 1.2057        | 4.0729        | Y = 71.6242 + 1.9392X| 0.6286    |
| 3rd           | 24                  | 0.6295        | 2.2741        | Y = 87.6987 + 8.602X | 0.5878    |
|               | 48                  | 4.2048        | 9.4812        | Y = 61.6924 + 2.671X | 0.5618    |
|               | 72                  | 1.0528        | 2.887         | Y = 79.8209 + 1.4211X| 0.5383    |
| 4th           | 24                  | 6.2245        | 15.8776       | Y = -0.3996 + 5.8925X| 0.9800    |
|               | 48                  | 3.6999        | 10.488        | Y = 22.9820 + 4.8988X| 0.8850    |
|               | 72                  | 2.3474        | 5.5919        | Y = 46.0531 + 3.6969X| 0.6488    |

Note LC = Lethal Concentration, X= Concentration, Y= mortality, R^2 = Coefficient of determination. First instar larvae showed 100% mortality at 72 h of exposure with all tested concentrations, hence, no LC_{50}, LC_{90} values, Regression equation and R^2 value were obtained.

Table 5 Completely randomized three ways ANOVA using instars (I) of Ae. aegypti, hours (H), and Concentrations (C) of ethanol leaf extract of A. reticulata as three independent parameter

| Source of variation | Sum of squares(SS) | Degree of freedom (df) | Mean of squares (MS) | F value | p-level |
|---------------------|--------------------|-----------------------|----------------------|---------|---------|
| Instars(I)          | 705.81             | 3                     | 235.27               | 259.28  | 0       |
| Time(H)             | 120.04             | 2                     | 60.02                | 66.14   | 0       |
| Conc.(C)            | 1399.98            | 5                     | 279.99               | 308.57  | 0       |
| IxH                 | 34.85              | 6                     | 5.81                 | 6.40    | 0       |
| IxC                 | 141.69             | 15                    | 9.45                 | 10.41   | 0       |
| HxC                 | 18.69              | 10                    | 1.87                 | 2.06    | 0.03    |
| IxHxC               | 39.98              | 30                    | 1.33                 | 1.47    | 0.07    |
| Within groups       | 130.67             | 144                   | 0.91                 | -       | -       |
| Total               | 2591.70            | 215                   | 12.05                | -       | -       |

4.2 Preparation of solvent extract
Leaves of A. reticulata were dried in shade for 9-10 days and then chopped finely. Two hundred grams of finely chopped leaves were packed in a Soxhlet apparatus and the plant extract was prepared using 2000 ml of ethanol. The period of extraction was 72 h. The pooled extract was evaporated by rotary evaporator to obtain semisolid extract. Semi solid extract was stored in refrigerator at 4°C for further use. The semi solid extract (0.05 g) were dissolved in 100 ml of 5 % ethanol to prepare stock test solution. After preliminary trial, required graded concentration i.e. 1.5, 3, 6, 12, 18 and 24 ppm were prepared adding required volume of stock test solution with required volume of distilled water, making 100 ml of final test solution of different concentrations which were used in bioassay experiments. Stock test solution was prepared freshly on the same day of bioassay experiments.

4.3 Test mosquitoes
The present study was conducted at Mosquito, Microbiology and Nanotechnology Research Units, Parasitology Laboratory, Department of Zoology, The University of Burdwan, Burdwan (23°16’N, 87°54’E) West Bengal, India. Larvae of Ae. aegypti and Cx. quinquefasciatus mosquitoes were taken for bioassay experiments from laboratory mosquito colonies which
were maintained in the laboratory. Mosquito colonies were kept free from insecticides, repellents and exposure to pathogens. The mosquito larvae were fed with artificial food i.e. mixture of dog biscuits and dried yeast powder in the ratio of 3:1.

4.4 Larvicidal bioassay

The bioassay experiments were done according to standard WHO procedure (1981) with slight modification. All instars larvae of Ae. aegypti and Cx. quinquefasciatus were used during bio assay experiments. Thirty larvae were put in different plastic bowls of 225 ml capacity and 9 cm in diameter containing each with 100 ml of test solution of different concentrations. After preliminary trial, 1.5, 3, 6, 12, 18 and 24 ppm and 1.5, 3, 6, 12 and 18 ppm dosages were used against Ae. aegypti and Cx. quinquefasciatus respectively for larvicidal bioassay experiments. Ethanol treated controls were run concurrently by mixing 100 ml of tap water with 0.5 ml of ethanol. Larval mortalities were recorded after 24, 48, and 72 h of exposure cumulatively. Dead larvae were detected when they fail to move after touching with fine brush on cervical or siphon region. All bioassays and control experiments were replicated three times on three separate days under laboratory conditions at 25-30°C and 80-90% relative humidity.

4.5 Effect on non target organisms

Ethanol leaf extract of A. reticulata were tested against non target organisms viz., Chironomus circundatus larvae, Diplonychus annulatum and tadpoles of frog with LC50 value of the extract against 3rd instar larva of Cx. quinquefasciatus at 24 h of post exposure. Each tested non target organism was kept in an environment similar to their natural habitat for acclimation in the laboratory as per procedure used by Suwannee et al., (2006). Ten early 4th instar Chironomus circundatus larvae, 10 3rd instar nympha of Diplonychus annulatum and 10 tadpoles of frog were kept separately in 500 ml beaker containing 200 ml of pond water and treated at the dosages mentioned. Number of dead non-target organisms was noted after 24, 48 and 72 h of exposures. A set of control (200 ml of pond water with 0.5 ml of ethanol) of each organism was run parallel. Each experiment (including control) was replicated thrice on separate three days.

4.6 Statistical analyses

The computer software STAT PLUS 2009 - trial version was used to calculate the LC50, LC90 values through Log Probit analyses (95% confidence level) regression equations, coefficient of determination (R2) and completely randomized three way ANOVA.

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Conflict of interest

We have no conflict of interest.

References

Adhikani U., Singha S., and Chandra G. 2012. In vitro repellent and larvicidal efficacy of Swietenia mahagoni against the larval forms of Culex quinquefasciatus Say, Asian Pac. J. Trop. Biomed., S260-S264

http://dx.doi.org/10.1016/S2221-1691(12)60171-3

Bhattacharya K., Burman S., Nandi S., Roy P., Chatterjee D., and Chandra G., 2014, Phytochemical extractions from the leaves of Raventala madagaskariensis from Sundarban area and its effect on southern house mosquito (Culex quinquefasciatus Say 1823) larvae, J. Mosq. Res., 4 (12): 1-6

Activity of Turkish medicinal plants against mosquitoes Aedes aegypti and Anopheles gambiae, Insect Sci. Appl. 16(3/4): 339-342

http://dx.doi.org/10.1017/s1742738400017379

Table 6 Completely randomized three ways ANOVA using instars (I) of Cx. quinquefasciatus, hours (H), and Concentrations (C) of ethanol leaf extract of A. reticulata as three independent parameters

| Source of variation | Sum of squares(SS) | Degree of freedom(df) | Mean of squares(MS) | F value | p-level |
|---------------------|--------------------|-----------------------|--------------------|---------|---------|
| Instars(I)          | 285.69             | 3                     | 95.23              | 300.73  | 0       |
| Time(H)             | 158.14             | 2                     | 79.07              | 249.70  | 0       |
| Conc.(C)            | 625.36             | 4                     | 156.34             | 493.70  | 0       |
| I×H                 | 39.68              | 6                     | 6.61               | 20.88   | 0       |
| I×C                 | 193.31             | 12                    | 16.11              | 50.87   | 0       |
| H×C                 | 96.08              | 8                     | 12.01              | 37.93   | 0       |
| I×H×C               | 57.66              | 24                    | 2.40               | 7.59    | 0       |
| Within groups       | 38                 | 120                   | 0.32               | -       | -       |
| Total               | 1493.91            | 179                   | 8.35               | -       | -       |
