Evaluation of Mosquito Larvicidal Activities of Seed Coat Extract of *Cassia sophera* L.

Mousumi Kundu, Anjali Rawani, Goutam Chandra

Mousumi Kundu
Corresponding author email: trmousumi_kundu@rediffmail.com

Abstract

The objective of the present finding, to evaluate the mosquito larvicidal activity of crude and ethyl acetate solvent extract of matured seed coat of *Cassia sophera* L. against *Culex quinquefasciatus* Say. The lethal concentration was determined and the LC$_{50}$ (i.e. half of absolute lethal concentrations) value at 24 h for both crude and ethyl acetate extract was also tested against some non target organisms such as Daphnia sp., *Diplonychus annulatum* (predatory water-bug) and *Chironomus circumdatus* larvae (insect). The presence of secondary metabolite in the crude extract of matured seed coat of *Cassia sophera* was also analysed. All the graded concentration i.e. 0.6%, 0.7%, 0.8%, 0.9% and 1% showed that the larval mortality was significant (P<0.05) and regression analysis revealed the positive correlation between larval mortality and concentration of extracts. LC$_{50}$ and LC$_{90}$ values were calculated at 24 h, 48 h and 72 h of exposure and the minimum value obtained at 72 h for first instar larvae. In ethyl acetate solvent extract the mortality rate was higher at 520 ppm against *Culex quinquefasciatus* than the other doses. There was no mortality of non-target organism within 72 h of post exposure to LC$_{50}$ concentration at 24 h of both crude and solvent extracts under the laboratory condition. The result of phytochemical analysis of presence of the secondary metabolite in crude extract of seed coat discovered the presence of some secondary metabolite such as saponin, alkaloid and cardiac glycosides. The results support that the tested plant extract can be used for control of larval form of *Culex quinquefasciatus*.

Keywords *Cassia sophera*; *Culex quinquefasciatus*; Mosquito larvae; Biocontrol

Introduction

Mosquitoes are the most nuisance creature of the nature that causes the transmission of mosquito borne diseases such as malaria, filaria, dengue fever, yellow fever, Japanese encephalitis by some major vectors viz. *Anopheles stephensi*, *Culex quinquefasciatus*, *Aedes albopictus* and *Culex vishnui* group (James, 1992; Gubler, 1998). The biting of mosquitoes also causes the skin allergy, the biting area becomes inflated and irritation persists (Peng et al., 1999). *Culex quinquefasciatus* is a vector of lymphatic filariasis. In tropical countries the lymphatic filariasis is widely distributed infecting 120 million people world wide and common chronic manifestation occur in about 44 million people (Ottesen et al., 1997). Previously mosquito borne diseases were controlled by application of chemical insecticides. For this purpose many chemical insecticides were developed and applied in field with significant success. But the development of resistance, non selective mode of action and harmful to another organisms of the environment are the major negative aspect of chemical insecticides. It may also cause the toxicity to non-target organisms and environment. These developments require efforts to prepare alternative insecticidal agents with high mosquito control activity that cause little or no harmful effect to human health and environment. The plant based herbal insecticides are found to more efficient, safe and best substitute for chemical insecticides (Ghosh, 2012; Chowdhury et al., 2008; Rawani et al., 2009; Rawani et al., 2010; Banerjee et al., 2011). Natural products of plant origin are safe to use than the synthetic insecticides (Kishore et al., 2011). Therefore biological and ecofriendly natural resources are broad search area for the control of vector of medical importance (Singha et al., 2012; Chowdhury, 2009).
**Cassia sophera** L. (Caesalpiniaceae), a medicinal plant commonly known as Kasaundi, is a shrub of 3 m in height. It is glabrous and having compound leaves with 8–12 paired acute and tapering leaflets; rachis with single gland at the base. It has carymbose racemes inflorescence with yellow flower. The plant is found in most tropical countries. Respiratory disorders medicines are prepared from root bark which is used by ancient Indian physicians. It is widely used as folk medicine for the treatment of many diseases like resolvent, ulcer, asthma, purgative, digestive, diaphoretic (Mostafa et al., 2007; Nagore et al., 2001; Nagore, 2009). In ethno botanical literature, it is mentioned to be effective in the treatment of inflammation, liver damage, asthma, acute bronchitis, cough, diabetes and convulsions of children (Nagore et al., 2010; Suhael et al., 2008; Attiqur et al., 2008).

The purpose of present study was made to evaluate the efficacy of crude and solvent extract of matured seed coat of *C. sophera* against the larvae of filarial vector *Cx. quinquefasciatus* as well as some non-target organisms. A preliminary phytochemical analysis was also done to get some idea about the active principle.

### 1 Result

Present study revealed highest mortality at 1% concentration of crude extract, tested against all larval instars and significantly (p<0.05) higher than 0.6%, 0.7%, 0.8% and 0.9% at 24 h, 48 h and 72 h of exposures (Table 1). Results of three-way factorial ANOVA were presented in Table 2. The result of regression analysis of crude extract of seed coat extract of *C. sophera* showed the positive correlation between mortality and exposed concentration with a regression coefficient value (R) values between 0.34 and 0.71. The result of probit analysis (Finney, 1982) showed the lowest value of LC50 and LC90 at 72 hours of exposure for the 1st instar larvae followed by 2nd, 3rd and 4th instar larvae (Table 3). The mortality of 1st, 2nd, 3rd and 4th instar larval forms with ethyl acetate is presented in Table 4. The result of preliminary qualitative phytochemical analysis of tested plant crude extract revealed the presence of some secondary metabolite which may be an active ingredient for larvicidal activity (Table 5). There is no any adverse effect on non target organism after 72 hours of exposure of respective LC50 values at 24 h.

**Table 1** Mean larval mortality of larvae of *Cx. quinquefasciatus* mosquitoes at different concentration of crude extracts of seed coats of *C. sophera* (mean of three experiments)

| Larval instar | Concentration (%) | 24 h | 48 h | 72 h |
|---------------|------------------|------|------|------|
| 1st           | 0.6              | 10.33 ± 0.88 | 13.00 ± 0.58 | 15.66 ± 0.33 |
|               | 0.7              | 12.33 ± 0.33 | 16.66 ± 0.33 | 17.00 ± 0.58 |
|               | 0.8              | 17.66 ± 0.88 | 19.33 ± 0.33 | 20.00 ± 0.00 |
|               | 0.9              | 18.33 ± 0.88 | 20.00 ± 0.00 | 19.66 ± 0.33 |
|               | 1                | 15.33 ± 0.33 | 19.33 ± 0.66 | 19.66 ± 0.33 |
| 2nd           | 0.6              | 8.00 ± 0.56 | 12.00 ± 0.58 | 13.66 ± 0.88 |
|               | 0.7              | 7.66 ± 0.33 | 13.66 ± 0.66 | 14.00 ± 0.58 |
|               | 0.8              | 9.33 ± 0.33 | 14.66 ± 0.33 | 15.66 ± 0.67 |
|               | 0.9              | 8.33 ± 0.88 | 14.67 ± 0.88 | 16.00 ± 0.58 |
|               | 1                | 14.00 ± 0.58 | 15.33 ± 0.89 | 17.33 ± 0.89 |
| 3rd           | 0.6              | 6.00 ± 0.58 | 14.00 ± 1.00 | 16.00 ± 0.58 |
|               | 0.7              | 7.00 ± 1.00 | 11.66 ± 0.33 | 17.66 ± 0.33 |
|               | 0.8              | 8.00 ± 1.00 | 16.66 ± 0.66 | 18.33 ± 0.33 |
|               | 0.9              | 7.66 ± 0.66 | 15.66 ± 0.33 | 17.67 ± 0.33 |
|               | 1                | 9.66 ± 0.66 | 17.66 ± 0.33 | 19.33 ± 0.67 |
| 4th           | 0.6              | 4.67 ± 0.33 | 11.33 ± 0.67 | 15.67 ± 0.33 |
|               | 0.7              | 8.00 ± 0.58 | 12.67 ± 0.67 | 16.33 ± 0.33 |
|               | 0.8              | 7.33 ± 0.33 | 8.67 ± 0.33 | 15.67 ± 0.33 |
|               | 0.9              | 7.67 ± 0.30 | 13.67 ± 0.89 | 16.00 ± 1.00 |
|               | 1                | 8.33 ± 0.67 | 16.67 ± 0.89 | 18.67 ± 0.33 |
Table 2: Completely randomized three-way factorial ANOVA using different concentrations, period of exposure and different instar as variables

| Source of variation | Sum of squares | df | Mean square | F value | P value |
|---------------------|----------------|----|-------------|---------|---------|
| Hours (H)           | 38.23          | 2  | 19.11       | 66.58   | 0.003   |
| Instar (I)          | 85.37          | 3  | 28.46       | 99.14   | 0.004   |
| Concentration (C)   | 68.72          | 2  | 34.36       | 119.71  | 0.005   |
| H × I               | 1.18           | 6  | 0.11        | 0.69    | 0.660   |
| H × C               | 8.22           | 4  | 2.05        | 7.16    | 0.001   |
| I × C               | 17.35          | 6  | 2.89        | 10.07   | 0.002   |
| H × I × C           | 3.25           | 12 | 0.27        | 0.95    | 0.507   |
| Residual            | 20.67          | 72 | 0.29        |         |         |
| Total               | 243.00         | 107|             |         |         |

Table 3: Log probit analysis and regression analysis of larvicidal activity of crude extracts of seed coats of *C. sophera* against all instar larvae of *Cx. quinquefasciatus*

| Larval instars | Period of bioassay(h) | LC50 | LC90 | Regression equation | R value |
|----------------|------------------------|------|------|---------------------|---------|
| 1st            | 24                     | 0.60 | 1.02 | Y=16x+2             | 0.49    |
|                | 48                     | 0.54 | 0.78 | Y=16x+4.86          | 0.71    |
|                | 72                     | 0.50 | 0.72 | Y=10.66x+9.86       | 0.68    |
| 2nd            | 24                     | 0.83 | 2.41 | Y=12.66x-0.66       | 0.52    |
|                | 48                     | 0.42 | 1.81 | Y=7.66x+7.93        | 0.45    |
|                | 72                     | 0.42 | 1.19 | Y=9.33x+8           | 0.57    |
| 3rd            | 24                     | 1.13 | 5.36 | Y=8x+1.26           | 0.46    |
|                | 48                     | 0.47 | 1.17 | Y=11.33x+6.06       | 0.49    |
|                | 72                     | 0.32 | 0.79 | Y=6.66x+12.46       | 0.54    |
| 4th            | 24                     | 1.25 | 6.54 | Y=7x+1.6            | 0.45    |
|                | 48                     | 0.58 | 1.77 | Y=11.66x+3.26       | 0.34    |

Table 4: Result of larval mortality of different concentration of ethyl acetate solvent extract of seed coat of *C. sophera* on all instar of *Cx. quinquefasciatus*

| Larval instar | Concentration (ppm) | 24 h | 48 h | 72 h |
|---------------|---------------------|------|------|------|
| 1st           | 480                 | 7.67 ± 0.33 | 9.33 ± 0.67 | 10.00 ± 0.00 |
|               | 500                 | 8.67 ± 0.33 | 9.67 ± 0.33 | 10.00 ± 0.00 |
|               | 520                 | 10.00 ± 0.00 | 10.00 ± 0.00 | 10.00 ± 0.00 |
| 2nd           | 480                 | 7.33 ± 0.33 | 8.67 ± 0.33 | 9.67 ± 0.33 |
|               | 500                 | 8.33 ± 0.33 | 8.67 ± 0.33 | 9.33 ± 0.33 |
|               | 520                 | 9.67 ± 0.33 | 10.00 ± 0.00 | 10.00 ± 0.00 |
| 3rd           | 480                 | 5.33 ± 0.33 | 7.33 ± 0.33 | 8.00 ± 0.58 |
|               | 500                 | 8.33 ± 0.33 | 8.67 ± 0.33 | 9.33 ± 0.33 |
|               | 520                 | 8.67 ± 0.33 | 9.00 ± 0.00 | 10.00 ± 0.00 |
| 4th           | 480                 | 4.33 ± 0.33 | 5.67 ± 0.33 | 6.33 ± 0.33 |
|               | 500                 | 6.67 ± 0.33 | 7.67 ± 0.33 | 8.00 ± 0.00 |
|               | 520                 | 7.67 ± 0.33 | 8.67 ± 0.33 | 9.33 ± 0.33 |
Table 5 Result of qualitative phytochemical analysis of the crude extract of the tested plant

| Phytochemicals                  | Absent/ Present |
|--------------------------------|-----------------|
| Tannin                         | Absent          |
| Saponin                        | Present         |
| Flavonoid                      | Absent          |
| Alkaloid                       | Present         |
| Steroid                        | Absent          |
| Cardiac glycosides             | Present         |
| Terpenoid                      | Absent          |
| Free glycosides bound anthaquinones | Absent     |

2 Discussion

The transmission of mosquito-borne diseases can be interrupted by the potential insecticides of herbal origin at the individual as well as at the community level (Campbell et al., 1993). Recently the natural insecticides of plant origin have been given importance due to their ecofriendly nature and biodegradability as a substitute of synthetic insecticides for the control of vectors of public health importance. Different types of phytochemical of plant either from the whole part or from the specific parts come out with solvent during chemical extraction depending on the polarity of the solvent (Rawani et al., 2012; Chowdhury et al., 2007). These phytochemical generally act as a toxicant for adult, pupa as well as larval form of mosquitoes, while some interfere with the growth (growth inhibitory) and reproduction (ovicide deterrent). The present study evaluate biocontrol efficacy of crude extract and ethyl acetate extract of seed coat of *C. sophera* against *Cx. quinquefasciatus*. Highest mortality was recorded in 1% concentration of crude extract against 1st instar larvae. The ethyl acetate extract showed 100% mortality at 520 ppm against 1st instar larvae after 24 h. The phytochemical analysis of crude extract of seed coat of *C. sophera* indicates the presence of some secondary metabolite which either in single form or in combination with other responsible for larval death. There is no any abnormal behaviour of non-target organisms when they exposed to LC50 value so it is safe to use in natural condition. Some other authors also reported the efficacy of ethyl acetate extract of plant parts against mosquito larvae. Senthil Nathan et al. (2008) reported the activity of ethyl acetate extract of the leaves of *Dysoxylum malabaricum* against the larvae of *Anopheles stephensi*. Highest mortality occurred in 4th instar larvae. Matasyoh et al (2008) reported the efficacy of the ethyl acetate extract of leaves of *Aloe turkanensis* against *Anopheles gambiae* where 100% mortality occurred at a concentration of 0.2 mg/L with a LC50 value of 0.11 mg/mL. The ethyl acetate extract of leaves of *Ocimum sanctum* produced significant mortality against *Aedes aegypti* and *Cx. quinquefasciatus*, with LC50 values of 425.94 ppm and 592.60 ppm, respectively (Anees, 2008). Rawani et al. (2010) studied on the ethyl acetate solvent extract of *Solanum nigrum* showed its highest mortality (100%) against *Culex quinquefasciatus* at 50 ppm dose having LC50 value 17.04 ppm after 24 h of exposure period.

In conclusion, crude and ethyl acetate extracts of *C. sophera* can be effectively used as a potent mosquito larvicide. Furthermore there is more investigation necessitates to identify the active ingredient and their mode of action and field application which become establishes the *C. sophera* as a new insecticide in a mosquitoes control program.

3 Material and Method

3.1 Plant material

Fresh and matured seeds of *C. sophera* were harvested randomly during April–June, 2011, from plants growing at the outskirts of Burdwan (23°16’N, 87°54’E). Initially collected seeds were washed with distilled water and soaked on paper towel.

3.2 Collection of larvae

Mosquito larvae of the species *Cx. quinquefasciatus* used during the present piece of work were taken from an established mosquito colony of Mosquito Research Unit, Department of Zoology, Burdwan University, maintained at (27±1)°C temp and 85% RH.

3.3 Preparation of crude extracts

Seed coats were crushed with a mechanical blender and the juice was filtered by Whatman no-1 filter paper. The filtrate was used as stock solution (100% concentration) for further bioassay experiment.

3.4. Preparation of solvent extracts

Dried seed coats (250 g) were put in a Soxhlet apparatus and the plant extract was prepared using
ethyl acetate as solvent (extraction period 72 hour and the temperature was < 40℃). The extract was collected in a separate beaker.

3.5 Larvicidal bioassay
According to world health organization standard protocols (WHO, 1981), the larvicidal bioassay with suitable modification was done. Each of the previously made concentration of crude extract was transferred into a sterilized glass petridishes (9 cm diameter, 150 mm capacity). Twenty larvae of each larval instar (1st, 2nd, 3rd, and 4th) of Cx. quinquefasciatus were separately transferred into different petridishes containing appropriate concentration. Larval food (20 mg dried yeast powder) was added in each Petridish. Mortality rates were recorded at 24 hr, 48 hr and 72 hr of post exposure. Dead larvae were recognized when they failed to move after probing with a needle in the siphon or cervical region. The experiments were repeated three times on different days and maintained at 25~30℃ and 80%~90% relative humidity.

3.6 Effect on non-target organisms
Effect of crude and ethyl acetate extracts were tested against non-target organisms like Daphnia sp., Diplonychus annulatum (predatory water-bug) and Chironomus circumdatus larvae (insect). The predators were exposed to half lethal concentrations of crude and solvent extracts at 24 h to observe the mortality and other abnormalities such as sluggishness and reduced swimming activity up to 72 h of exposure.

3.7 Phytochemical analysis of the plant extracts
Phytochemical analysis of the crude extract of seed coat of C. sophera was carried out according to the methodologies of Harbone (1984) and Stahl (1989). The phytochemicals included under study were saponins, terpenoids, alkaloid, steroids, tannin, flavonoids, cardiac glycosides and free glycoside bound anthraquinones.

3.8 Statistical analysis
The percentage mortality (%M) was corrected using Abbott’s formula (1925). Statistical analysis included the probit analysis (calculating LC₅₀ and LC₉₀ values), regression equations (Y=mortality; X = concentrations) and regression coefficient values. ANOVA was carried out to justify the significance between different variables such as different concentrations, different instars, hours and mortality rate.

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