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# Larvicidal and adult emergence inhibition activity of *Abutilon indicum* (Linn.) (Malvaceae) leaf extracts against vector mosquitoes (Diptera: Culicidae)

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**ABSTRACT**

The crude leaf extracts of *Abutilon indicum* were evaluated for larvicidal, pupal deformities and adult emergence inhibition activity against vector mosquitoes *viz.*, *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. Larvicidal activity was carried out using WHO protocol. Hexane extract with $LC_{50}$ value of 261.31 ppm after a period of 24 hr was found to be effective against *Aedes aegypti* larvae. Larval and pupal development was arrested resulting in decreased pupal transformation and adult emergence. Larval and pupal periods were prolonged with appearance of larval–pupal and pupal–adult intermediates, with an overall increase in the developmental period. Hatching was delayed and its rate was reduced compared to control. Disrupted egg shells and dechitinized body walls were observed, indicating clearly the anti-juvenile potential of the extract. The growth index was considerably reduced. These results suggest the leaf extracts of *Abutilon indicum* as a promising adult emergence inhibitor against vector mosquitoes and might be used in small volume aquatic habitats or breeding sites of limited size in and around human dwellings.

**Key words:** *Abutilon indicum*, leaf extracts, *Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus*

## INTRODUCTION

World Health Organization has declared mosquito as "public enemy number one", because mosquitoes are responsible for the transmission of various dreadful diseases (WHO, 1996a). They represent a significant threat to human health because of their ability to vector pathogens that cause diseases that afflict millions of people worldwide (WHO, 1992, 1998; Pinheiro, 1997; Taubes, 1997). Mosquitoes constitute a major public health problem as vectors of serious human diseases (Hag *et al*., 1999). Several species belonging to genera *Aedes*, *Anopheles* and *Culex* are vectors for the pathogens of various diseases like Dengue fever, Dengue haemorrhagic fever, Malaria, Japanese Encepahalitis and Filariasis (Service, 1983; Gubler, 1998; Hubalek and Haluzka, 1999). Earlier efforts to control mosquito vectors concentrated mainly on the application of broad spectrum insecticides but it resulted in environmental pollution (Youdeowei and Service, 1983). The drawbacks of conventional insecticides have now aroused interest in the development of alternative insecticides such as plant derived products/compounds (Ascher, 1993; Talukder and House, 1995; Thacker *et al*., 2003). Plant derived materials are comparatively safer to humans and ecosystem and development of resistance by pests and vectors against the botanicals has not been reported (Sharma *et al*., 1992). Many plant extracts have been studied for their efficacy as mosquito agent of different species of mosquito. Plant extracts act as general toxicants like larvicides, oviposition attractants/ deterrents, insect growth regulators, repellents and adulticides (Venkatachalam and Jebanesan, 2001; Murthy and Rani, 2009; Kasturi Vadeyar *et al*., 2010).

Several plants have demonstrated toxic effects on mosquito larvae. *Tagetes* sp. (Green *et al*., 1991; Perich *et al*., 1994; Macedo *et al*., 1997; Pathak *et al*., 2000), ethanol extract of *Eclipta paniculata* (Macedo *et al*., 1997), leaf extract of *Polyalthia longifolia* (Murty *et al*., 1997), petroleum ether extract of *Thymus capitatus* (Mansour *et al*., 2000), *Murraya koengii* (Ramsewak *et al*., 1999; Pathak *et al*., 2000), crude and ethanol extract of leaves of *Solanum nigrum* (Ahmed *et al*., 2001; Singh *et al*., 2002), Alcoholic extracts of leaves and stems of *Vanilla fragrans* fractionated with ethyl acetate and aqueous butanol (Sun *et al*., 2001) exhibited larvicidal and growth inhibition effects. Saxena *et al*. (1992) discovered growth inhibitory and juvenile hormone mimicing activity in the larvae of *Culex quinquefasciatus* treated with acetone extracts of *Ageratum conyzoides*, *Cleome icosandra* and *Tridax procumbens* resulting in larval pupal intermediates, demelanised pupae, defective egg rafts and adult with deformed flight muscles. *Annona squamosa* exhibited larvicidal and growth inhibition in *Anopheles stephensi*. *Annona squamosa* and *Lansium domesticum* showed larvicidal

potential against *Aedes aegypti* and *Culex quinquefasciatus* (Monzon *et al*., 1994). However, very few reports on the impact of *Abutilon indicum* against mosquitoes are reported.

*Abutilon indicum* belongs to the family Malvaceae and is distributed in the tropical and subtropical countries of America, Asia and Australia (Sikorska and Matlawska, 2008). It is an erect woody, shrubby plant found in outer Himalayan tracts of Jammu to Bhutan and extending through the whole of Northern to Central India (Rajurkar *et al*., 2009). It is commonly known as 'Country Mallow, Indian Mallow in English, 'Atibala', 'Kanghi', 'Kakahi' in Hindi, 'Petari' in Bengali, 'Dabi', 'Uram' in Malayalam, 'Khapat', 'Kansi', 'Dabli' in Gujarati, 'Mudra' 'Petari' in Marathi, 'Thutti', 'Pantara', 'Hutti' in Tamil and Tutturubenda' in Telugu. *Abutilon indicum* has been known and used for centuries for its medicinal properties. Traditionally various parts of the plant have been useful in treating several human ailments (Lakshmayya *et al*., 2003). The plant as a whole is used in inflammation, piles, gonorrhea treatment and immune treatment (Kirtikar and Basu, 1980).

The leaf is used for treating toothache, piles, lumbago, inflammation, ulcers, headache and bladder infections (Prajapati *et al*., 2003). The leaf juice is formulated into an ointment for quick ulcer healing. The leaf extract is used for relieving thirst, treating bronchitis, diarrhoea, gonorrhoea, vaginal infections, diabetes, haemorrhoids, enema, inflammation of bladder, reducing fever, cleaning wounds, ulcers, antinflammatory (Rajurkar *et al*., 2009). Further the plant possesses antimycotic, antifungal (Padma *et al*., 2009; Rajalakshmi and Senthil, 2009), antidiarrhoeal (Chandrashekhar *et al*., 2004), anticonvulsant (Golwala *et al*., 2010), antimalarial (Rahuman*et al*., 2008), immunomodulatory (Dashputre and Naikwade, 2010), *in vitro* anthelmentic (Venkatachalam *et al*., 2010), cytotoxic and antimicrobial (Abdul *et al*., 2010), antioxidant (Chakraborthy, 2009) and hypoglycemic activity in rats (Seetharam *et al*., 2002; Adisakwattana *et al*., 2009), The phytochemical constituents present in the plant include fatty acids, abutilin A, flavonoids, quercetin, glycosides, alkaloids, steroids, terpenoids, saponins, sesquiterpenes, lactones, gallic acid, â-sitosterol, geranoil, caryophylline and phenolic compounds (Irena and Maria, 2002; Pengelly, 2004; Singh and Gupta, 2008; Kashmiri *et al*., 2009). Therefore, the present study had been carried out to evaluate the larvicidal, pupal deformities, morphogenetic variations, behavioural changes, adult emergence inhibition and ovicidal activity of *Abutilon indicum* leaf extracts against vector mosquitoes *viz*., *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus.*

## MATERIALS AND METHODS

### Plant extracts

*Abutilon indicum* leaves collected in and around Tamilnadu, India were brought to the laboratory, shade dried under room temperature and powdered using an electric blender. Dried and powdered leaves (1 kg) was subjected to sequential extraction using 3 L of hexane, diethyl ether, dichloromethane and ethyl acetate for a period of 72 hours to obtain the crude extracts using rotary vacuum evaporator. The hexane, diethyl ether, dichloromethane and ethyl acetate crude extracts thus obtained were lyophilized and a stock solution of 1,00,000 ppm prepared from each crude extract by adding adequate volume of acetone, refrigerated at 4 $^0$C until testing for bioassays.

### Test mosquitoes

All tests were carried out against laboratory reared vector mosquitoes *viz*., *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* free of exposure to insecticides and pathogens. Cyclic generation of vector mosquitoes were maintained at 25 – 29 $^0$C and 80 – 90% R.H. in the insectarium. Larvae were fed on larval food (powdered dog biscuit and yeast in the ratio 3:1) and adult mosquitoes on 10 per cent glucose solution. Adult female mosquitoes were periodically blood-fed on restrained albino mice for egg production.

### Bioassays

A total of three trials were carried out with five replicates per trial against vector mosquitoes for the following bioassays.

### Larvicidal activity

Bioassay for the larvicidal activity was carried out using WHO (1996b) procedure with slight modifications. From the stock solution, concentration of 250, 500, 750, and 1000 ppm was prepared. Twenty five early third instar larvae were introduced in 250 ml beaker containing 200 ml of water with each concentration. A control was prepared by the addition of acetone to water. Mortality was recorded after 24, 48 and 72 hours and the control mortality was corrected using Abbott's (1925) formula when it ranged between 5-20 per cent,

$$\text{Per cent mortality} = \frac{\text{\% mortality in treated} - \text{\% mortality in control}}{100 - \text{\% mortality in control}} \times 100$$

### Developmental indices

This test was performed according to the standard protocol described by WHO (1975). The powdered plant material was put in cotton gauze sachets and immersed (for 6 hr) in the 250 ml beaker containing water. Hundred early third instar larvae

were exposed for 12 hr to the crude extracts at concentration of 500 and 1000 ppm. Thereafter, the larvae were transferred to clean water containing larval food, in which they were kept for 24 hr. The pupae, which developed following this twelve-hours exposure, were removed from the experiment. The number of adults that failed to emerge from the pupae was counted in order to calculate the per cent inhibition.

**For pupal deformities**

Dried and powdered leaves were dissolved in distilled water and stirred for 6 hr at room temperature. The required concentration (500 and 1000 ppm) was mixed thoroughly with 250 ml of beaker containing 200 ml of sample solution. Hundred late fourth instar larvae were released into beakers containing treated solution. Dried coconut midribs were placed over the water as substratum for pupation. A beaker containing only water (200 ml) served as control. Dead larvae and pupae were removed and counted after 24 hr. Observation on larval mortality, per cent pupation and adult emergence was recorded.

For, morphogenetic variation and behavioural changes, in continuation of bioassays done in respect of above experiments, larval, pupal deformities and inhibition of adult emergence, changes in morphological features such as deformed wings, mobility, flying nature, longevity and other behavioural aspects were also recorded.

To find out the hatchability ratio, freshly laid mosquito eggs were used for the treatments and were exposed for 12 hr in desired concentrations of 500 and 1000 ppm. Hundred eggs collected on a filter paper for *Aedes aegypti* and *Anopheles stephensi* and an egg raft containing approximately 100 eggs in the case of *Culex quinquefasciatus* were immersed in water treated with aqueous extract. After the exposure period, the eggs were carefully removed and thoroughly washed/rinsed in distilled water and were left separately on enamel trays containing distilled water for hatchability. Control experiments were performed using untreated water. The number of eggs hatched was counted and the per cent hatchability was calculated using the following formula:

$$\text{Per cent hatchability} = \frac{\text{Total number of hatched larvae}}{\text{Total number of eggs/egg raft}} \times 100$$

**Statistical analysis**

Probit analysis (Finney, 1971) was used for determination of $LC_{50}$ and $LC_{90}$. Data from mortality and effect of concentrations were subjected to analysis of variance. The percentage data obtained was angular transformed. Difference between the treatments was determined by Tukey's test ($P < 0.05$). The highest different values from average detected by statistical testing were marked with letter "a" the next text lower with "b" and continued accordingly (Snedecor and Cochran, 1989).

**RESULTS**

**Larvicidal activity**

The results of the larvicidal activity are presented in Table 1 and 2. Among the vector mosquito species, *Aedes aegypti* larvae were found to be more susceptible followed by *Culex quinquefasciatus* and *Anopheles stephensi*. The hexane extract was found to be the most effective providing 100 per cent mortality at 1000 ppm with $LC_{50}$ value of 261.31 ppm against the larvae of *Aedes aegypti* at 24 hr.

**Developmental indices**

During developmental metamorphosis, time taken for total larval and pupal developmental time (in days), per cent larval and pupal mortality, and adult emergence inhibition were recorded. Results revealed that treated individuals took prolonged larval and pupal period when compared to control in all species of vector mosquitoes. The larval period lasted 9 to 10 (control 8 days) and pupal period lasted 3 to 4 days (control 2 days) in treated individuals irrespective of all species of vector mosquitoes. Larval duration significantly increased in treated individuals and total developmental period (larval and pupal development) took 12 to 14 days (control 10 days) against all three vector mosquito species. The data also revealed gradual increase in pupal duration and decrease in adult longevity.

Adult emergence against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* recorded at 500 and 1000 ppm was 53.2 ±1.48 and 27.8 ±3.49; 45.4 ±1.95 and 18.4 ±1.67; 73.0 ±1.73 and 32.4 ±3.51 respectively. Among the three species of vector mosquito, *Anopheles stephensi* were most susceptible followed by *Aedes aegypti* and *Culex quinquefasciatus*. Student t-test analysis showed significant difference at $P < 0.001$ level on all three mosquito larval and pupal mortality treated with aqueous extracts and control (Table 3).

**Morphogenetic variations and behavioural changes**

Microscopic examination of dead larvae revealed that larval cuticle had started scleratization, which appeared to be a characteristic feature of the pupal cuticle. The dead pupa on the other hand, showed less scleratization of the cuticle compared to untreated ones and in majority of the partly emerged pupae with attached head capsule. The pupae that survived through larval treatment showed a variety of malformations like completely demelanized pupa with straight abdomen, partly melanized pupa with extended abdomen,

**Table 1.** Per cent larval mortality of *Abutilon indicum* leaf extracts against vector mosquitoes

| Solvents | Concentration (ppm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 250 | | | 500 | | | 750 | | | 1000 | | |
| | 24h | 48h | 72h | 24h | 48h | 72h | 24h | 48h | 72h | 24h | 48h | 72h |
| *Aedes aegypti* | | | | | | | | | | | | |
| Hexane | 56.8 ±1.79 (48.9)$^{b}$ | 65.6 ±2.19 (54.1)$^{c}$ | 71.2 ±3.35 (57.5)$^{c}$ | 59.2 ±1.79 (50.3)$^{c}$ | 71.2 ±5.22 (57.5)$^{c}$ | 80.8 ±4.38 (64.0)$^{c}$ | 70.4 ±9.21 (57.0)$^{c}$ | 87.2 ±8.67 (69.0)$^{d}$ | 97.6 ±5.37 (81.1)$^{d}$ | 100 0 (90.0)$^{d}$ | 100 0 (90.0)$^{d}$ | 100 0 (90.0)$^{d}$ |
| Diethyl ether | 3.2 ±4.38 (10.3)$^{a}$ | 13.6 ±6.07 (21.6)$^{b}$ | 20.8 ±7.69 (27.1)$^{b}$ | 17.6 ±6.07 (24.8)$^{b}$ | 24.8 ±9.55 (29.8)$^{b}$ | 28.8 ±11.80 (32.5)$^{b}$ | 28.8 ±9.96 (32.5)$^{b}$ | 32.8 ±9.55 (34.9)$^{b}$ | 36.8 ±9.96 (37.4)$^{b}$ | 33.6 ±6.69 (35.4)$^{b}$ | 42.4 ±9.21 (40.6)$^{b}$ | 48.8 ±7.16 (44.3)$^{b}$ |
| Dichloro-methane | 4.8 ±3.35 (12.7)$^{a}$ | 14.4 ±3.58 (22.3)$^{b}$ | 21.6 ±5.37 (27.6)$^{b}$ | 17.6 ±4.56 (24.8)$^{b}$ | 24.8 ±7.69 (29.8)$^{b}$ | 31.2 ±10.35 (34.0)$^{b}$ | 29.6 ±4.56 (33.0)$^{b}$ | 34.4 ±4.56 (35.9)$^{b}$ | 38.4 ±5.37 (38.3)$^{b}$ | 34.4 ±6.07 (35.9)$^{b}$ | 46.4 ±11.52 (42.9)$^{b}$ | 49.6 ±4.56 (44.8)$^{b}$ |
| Ethyl acetate | 6.4 ±6.07 (14.7)$^{a}$ | 9.6 ±8.29 (18.1)$^{a}$ | 16.8 ±3.35 (24.2)$^{b}$ | 22.4 ±6.69 (28.3)$^{b}$ | 28.8 ±4.38 (32.5)$^{b}$ | 33.6 ±6.07 (35.4)$^{b}$ | 36.8 ±5.22 (37.4)$^{b}$ | 54.4 ±4.56 (47.5)$^{b}$ | 62.4 ±5.37 (52.2)$^{c}$ | 59.2 ±6.02 (50.3)$^{c}$ | 71.2 ±11.52 (57.5)$^{c}$ | 83.2 ±4.56 (65.8)$^{c}$ |
| Control | 0$^{a}$ | 0.8 ±1.79 (5.1)$^{a}$ | 0.8 ±1.79 (5.1)$^{a}$ | 0$^{a}$ | 0.8 ±1.79 (5.1)$^{a}$ | 1.6 ±2.19 (7.3)$^{a}$ | 0$^{a}$ | 0.8 ±1.79 (5.1)$^{a}$ | 1.6 ±2.19 (7.3)$^{a}$ | 0$^{a}$ | 1.6 ±2.19 (7.3)$^{a}$ | 1.6 ±2.19 (7.3)$^{a}$ |
| *Anopheles stephensi* | | | | | | | | | | | | |
| Hexane | 33.6 ±4.56 (35.4)$^{c}$ | 39.2 ±3.35 (38.8)$^{d}$ | 46.4 ±3.58 (42.9)$^{e}$ | 39.2 ±3.35 (38.8)$^{c}$ | 42.4 ±2.19 (40.6)$^{d}$ | 49.6 ±4.56 (44.8)$^{d}$ | 40.8 ±3.35 (39.7)$^{d}$ | 46.4 ±5.37 (42.9)$^{cd}$ | 56.8 ±7.16 (48.9)$^{cd}$ | 48.8 ±9.55 (44.3)$^{d}$ | 70.4 ±4.56 (57.0)$^{d}$ | 89.6 ±4.56 (71.2)$^{d}$ |
| Diethyl ether | 4.8 ±3.35 (12.7)$^{a}$ | 8.8 ±3.35 (17.3)$^{b}$ | 11.2 ±3.35 (19.6)$^{b}$ | 7.2 ±5.22 (15.6)$^{a}$ | 12.8 ±1.79 (21.0)$^{b}$ | 18.4 ±2.19 (25.4)$^{b}$ | 8.8 ±5.93 (17.3)$^{b}$ | 15.2 ±6.57 (22.9)$^{b}$ | 22.4 ±5.37 (28.3)$^{b}$ | 12.8 ±3.37 (21.0)$^{b}$ | 15.2 ±3.35 (22.9)$^{b}$ | 28.8 ±3.35 (32.5)$^{b}$ |
| Dichloro-methane | 6.4 ±4.56 (14.7)$^{a}$ | 13.6 ±4.56 (21.6)$^{b}$ | 18.4 ±5.03 (25.4)$^{c}$ | 20.8 ±4.38 (27.1)$^{b}$ | 28.8 ±1.79 (32.5)$^{c}$ | 33.6 ±3.58 (35.4)$^{c}$ | 29.6 ±4.56 (32.9)$^{c}$ | 41.6 ±8.29 (40.2)$^{c}$ | 54.4 ±6.69 (47.5)$^{c}$ | 38.4 ±4.56 (38.3)$^{c}$ | 50.4 ±6.69 (45.2)$^{c}$ | 65.6 ±11.52 (54.1)$^{c}$ |
| Ethyl acetate | 15.2 ±4.56 (22.9)$^{b}$ | 24.8 ±4.56 (29.9)$^{c}$ | 32.8 ±5.03 (34.9)$^{d}$ | 33.6 ±4.38 (35.4)$^{c}$ | 40.8 ±1.79 (39.7)$^{d}$ | 52.8 ±3.58 (46.6)$^{d}$ | 40.8 ±4.56 (39.7)$^{d}$ | 57.6 ±8.29 (49.4)$^{d}$ | 65.6 ±6.69 (54.1)$^{d}$ | 63.2 ±4.56 (52.7)$^{e}$ | 73.6 ±6.69 (59.1)$^{d}$ | 87.2 ±11.52 (69.0)$^{d}$ |
| Control | 0.8 ± 1.79 (5.1)$^{a}$ | 0.8 ±1.79 (5.1)$^{a}$ | 0.8 ±1.79 (5.1)$^{a}$ | 0$^{a}$ | 0.8 ±1.79 (5.1)$^{a}$ | 1.6 ±2.19 (7.3)$^{a}$ | 0$^{a}$ | 1.6 ±2.19 (7.3)$^{a}$ | 2.4 ±2.19 (8.9)$^{a}$ | 0$^{a}$ | 1.6 ±2.19 (7.3)$^{a}$ | 4.0 ±2.83 (11.5)$^{a}$ |
| *Culex quinquefasciatus* | | | | | | | | | | | | |
| Hexane | 30.4 ±4.56 (33.5)$^{d}$ | 36.8 ±5.22 (37.4)$^{e}$ | 47.2 ±6.57 (43.4)$^{e}$ | 35.2 ±3.35 (36.4)$^{c}$ | 42.4 ±4.56 (40.6)$^{c}$ | 50.4 ±6.69 (45.2)$^{c}$ | 54.4 ±7.27 (47.5)$^{d}$ | 59.2 ±10.35 (50.3)$^{b}$ | 70.4 ±8.29 (57.1)$^{d}$ | 71.2 ±10.35 (57.5)$^{c}$ | 82.4 ±6.07 (65.2)$^{d}$ | 88.8 ±5.93 (70.5)$^{d}$ |
| Diethyl ether | 2.4 ±3.58 (8.9)$^{ab}$ | 8.8 ±1.79 (17.3)$^{b}$ | 12.8 ±6.57 (21.0)$^{b}$ | 7.2 ±7.16 (15.6)$^{a}$ | 13.6 ±7.27 (21.6)$^{b}$ | 21.6 ±4.56 (27.7)$^{b}$ | 8.8 ±5.93 (17.3)$^{b}$ | 15.2 ±9.96 (22.9)$^{a}$ | 22.4 ±8.29 (28.6)$^{a}$ | 15.2 ±10.35 (22.9)$^{a}$ | 21.6 ±6.07 (27.7)$^{b}$ | 30.4 ±6.69 (33.5)$^{b}$ |
| Dichloro-methane | 13.6 ±3.58 (21.6)$^{c}$ | 24.8 ±3.35 (29.9)$^{d}$ | 32.8 ±3.35 (34.9)$^{d}$ | 36.8 ±5.22 (37.4)$^{c}$ | 42.4 ±6.07 (40.6)$^{c}$ | 50.4 ±6.07 (45.2)$^{d}$ | 41.6 ±3.58 (40.6)$^{c}$ | 57.6 ±3.58 (49.4)$^{b}$ | 62.4 ±7.80 (52.2)$^{cd}$ | 63.2 ±5.22 (52.7)$^{c}$ | 73.6 ±5.37 (59.1)$^{cd}$ | 80.8 ±5.22 (64.1)$^{cd}$ |
| Ethyl acetate | 8.8 ±3.35 (17.3)$^{bc}$ | 16.8 ±5.22 (24.2)$^{c}$ | 23.2 ±4.38 (28.9)$^{c}$ | 20.8 ±3.35 (26.9)$^{b}$ | 33.6 ±8.29 (35.4)$^{c}$ | 37.6 ±10.81 (37.8)$^{d}$ | 30.4 ±4.56 (33.5)$^{b}$ | 47.2 ±5.93 (34.4)$^{b}$ | 55.2 ±7.69 (47.9)$^{c}$ | 42.4 ±10.81 (40.6)$^{b}$ | 63.2 ±7.16 (52.6)$^{c}$ | 72.8 ±9.12 (58.6)$^{c}$ |
| Control | 0.8 ±1.79 (5.1)$^{a}$ | 0.8 ±1.79 (5.1)$^{a}$ | 0.8 ±1.79 (5.1)$^{a}$ | 0.8 ±1.79 (5.1)$^{a}$ | 1.6 ±2.19 (7.3)$^{a}$ | 2.4 ±2.19 (8.9)$^{a}$ | 0$^{a}$ | 1.6 ±2.19 (7.3)$^{a}$ | 1.6 ±2.19 (7.3)$^{a}$ | 0.8 ±1.79 (5.1)$^{a}$ | 2.4 ±2.19 (8.9)$^{a}$ | 4.0 ±2.83 (11.5)$^{a}$ |

Values are mean (%) of the five-replication of three trials ± standard deviation and figures in parentheses are angular transformed. ANOVA followed by TUKEY test performed; Different superscripts in the column indicate significance difference at $P < 0.05$ levels

**Table 2.** Probit analysis of larvicidal efficacy of leaf extracts of *Abutilon indicum* against vector mosquitoes

| Extracts | 24 h $LC_{50}$ (ppm) | 24 h $LC_{90}$ (ppm) | Chi-square value | Regre-ssion value |
|---|---|---|---|---|
| *Aedes aegypti* | | | | |
| Hexane | 261.31 | 1196.20 | 31.35 | 1.94 |
| Diethyl ether | 1442.34 | 5691.63 | 0.99* | 2.15 |
| Dichloromethane | 1434.59 | 5932.17 | 0.59* | 2.08 |
| Ethyl acetate | 898.87 | 2580.90 | 1.57* | 2.80 |
| *Anopheles stephensi* | | | | |
| Hexane | 1411.16 | 7276.48 | 0.59* | 0.58 |
| Diethyl ether | 725.09 | 2198.88 | 0.24* | 0.81 |
| Dichloromethane | 1376.03 | 6285.00 | 0.17* | 1.94 |
| Ethyl acetate | 790.27 | 3236.78 | 3.40* | 2.09 |
| *Culex quinquefasciatus* | | | | |
| Hexane | 585.65 | 3226.73 | 6.74* | 1.80 |
| Diethyl ether | 395.90 | 1406.15 | 0. 69* | 1.38 |
| Dichloromethane | 768.55 | 2922.22 | 3.80* | 2.21 |
| Ethyl acetate | 1992 .36 | 6016.58 | 0.23* | 1.99 |

$LC_{50:}$ Lethal concentration required to kill 50 per cent of the population exposed

$LC_{90:}$ Lethal concentration required to kill 90 per cent of the population exposed

* Significant at $P < 0.05$ level

dwarf pupa with retarded abdomen, dechitinized pupa with distorted terminalia and pupa with defective genitalia. With reference to behavioral aspects, larvae treated with aqueous extract showed several morphological aberrations, like circular movements near the periphery of the beakers for longer period. The morphogenetic anomalies, during development and after adult emergence, suggested a general toxic effect of the extract, which was found to be dose dependent. The metamorphic abnormalities like larval inability to moult to next stage, larval pupal intermediates and small larvae noticed were higher when compared to control (untreated) groups.

Inability of adults to shed completely their exuvia, which remained attached to their appendages, was also noticed. The treated adult could not fly above normal level and rested for longer period on the water surface when compared to untreated adult mosquitoes. In this context of observation, exposure of third instar larvae (all three vector mosquito species) to aqueous plant extract, resulted in death at larval-pupal moult and pupal-adult eclosion suggesting inhibition of moulting process.

**Hatchability ratio**

Ovicidal activity in aqueous leaf extract of *Abutilon indicum* against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* at 500 and 1000 ppm was 53.6 ±3.36 and 24.4 ±2.07; 42.4 ±2.88 and 26.2 ±1.48; 63.2 ±2.86 and 30.6 ±2.51 respectively. The per cent hatchability of eggs in control

**Table 3.** Effect of *Abutilon indicum* leaf aqueous extract on the growth and metamorphosis of vector mosquitoes

| Mosquito species | Concen-tration (ppm) | Larval mortality (%)* | Total Larval period in days | Pupal mortality (%)* | Total pupal period in days | Adult emergence (%) (a) | Hatch ability (%) | Total develop-mental period in days (b) | Growth index (a/b) |
|---|---|---|---|---|---|---|---|---|---|
| *Aedes* | 500 | 40.4± 1.14 | 10 | 6.4±0.89 | 4 | 53.2±1.48 | 53.6 ±3.36 | 14 | 3.8 |
| *aegypti* | 1000 | 59.6± 2.07 | 10 | 12.6±2.07 | 4 | 27.8±3.49 | 24.4 ± 2.07 | 14 | 1.9 |
| Control | - | 8.2± 0.84 | 8 | 1.2±0.84 | 2 | 90.6±0.89 | 90.2 ± 2.17 | 10 | 9.1 |
| *Anopheles* | 500 | 47.2± 1.79 | 10 | 7.4± 0.55 | 4 | 45.4± 1.95 | 42.4 ± 2.88 | 14 | 3.2 |
| *stephensi* | 1000 | 70.0± 1.58 | 10 | 11.6± 1.14 | 4 | 18.4± 1.67 | 26.2 ± 1.48 | 14 | 1.3 |
| Control | - | 9.2±1.48 | 8 | 1.4±0.89 | 2 | 89.4±2.30 | 90.8 ± 3.83 | 10 | 8.9 |
| *Culex* | 500 | 21.2±1.48 | 9 | 5.8±0.84 | 3 | 73.0±1.73 | 63.2 ± 2.86 | 12 | 6.1 |
| *quinquefasciatus* | 1000 | 56.8±2.59 | 9 | 10.8±1.48 | 3 | 32.4±3.51 | 30.6 ± 2.51 | 12 | 2.7 |
| Control | - | 4.4±1.82 | 8 | 1.8±0.84 | 2 | 93.8±2.28 | 91.2 ± 3.70 | 10 | 9.4 |

* Significant at the level of $P < 0.001$ level

medium was 90.2, 90.8 and 91.2 per cent respectively. The decrease in hatchability was found to be dose dependent. Among the three species of vector mosquito, *Anopheles stephensi* were most susceptible followed by *Aedes aegypti* and *Culex quinquefasciatus* (Table 3).

**DISCUSSION**

Today, the environmental safety of an insecticide is considered to be of paramount importance. An insecticide does not have to cause high mortality on target organisms in order to be acceptable (Kabaru and Gichia, 2001). Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive, and are readily available in many areas of the world. In the search for an eco-friendly pesticide, researchers have considered pesticides of biological origin, and the replacement of chemical pesticides with biopesticides as a generally acceptable one. Plant derived products have received increased attention and more than two thousand plant species are already known to have insecticidal properties (Sukumar *et al*., 1991). Botanical derivatives have drawn attention as potential insect control agents and as a source of new pesticides for the insecticide industry in the last three decades.

The larvicidal efficacy of *Abutilon indicum* leaf extracts is comparable to well established insecticidal plant species. Arivoli and Samuel (2011) reported the dichloromethane extract of *Citrullus colocynthis* whole plant extracts to be effective against the larvae of *Culex quinquefasciatus* with a $LC_{50}$ value of 240.36 ppm at 24 hr. Sharma *et al*. (2009) reported the petroleum ether extract of *Ageratum conyzoides* leaves exhibited larvicidal activity with $LC_{50}$ value of 425.60 and 267.90 ppm after 24 and 48 hr of exposure, respectively. Its carbon tetrachloride and methanol crude extracts, however, were not effective larvicides, with comparatively higher $LC_{50}$ values of 3,139.3 and 2,726.5 ppm for the former and 5,105.0 and 3,380.5 ppm for the latter after 24 and 48 hours of treatment respectively. In the case of leaves of *Argemone mexicana*, petroleum ether and methanol extracts displayed better larvicidal potential with $LC_{50}$ 140.15 and 137.70 ppm for the former and 977.24 and 382.29 ppm for the latter after 24 and 48 hours, respectively. The carbon tetrachloride crude extract of the plant showed the least larvicidal action with $LC_{50}$ 1,044.7 and 569.05 ppm after 24 and 48 hours of exposure. All four crude extracts of *Abutilon indicum* showed larvicidal activity however, the hexane extract exhibited maximum mortality against the larvae of *Aedes aegypti*.

Insect growth regulators offer considerable potential for the control of vectors of human disease. A number of synthetic compounds and plant derivatives are being examined for insect growth regulatory activity (Schaefer and Wilder, 1972; Wright and Schwarz, 1972; Wright and Spates, 1972; Wright and Sonnet, 1973; Wright, 1974). In this study, leaf extracts of *Abutilon indicum* showed insect growth regulatory activity against vector mosquitoes. It will be evident from these findings that plants could provide one of the biggest sources of IGRs.

The crude extracts of the leaves of *Abutilon indicum* has been found to possess adult emergence inhibition activity against vector mosquitoes. The biological activity of the plant extract might be due to the presence of various bioactive phytocompounds, including phenolics, terpenoids, and alkaloids, existing in plants, may jointly or independently contribute to produce adult emergence inhibition activity. The adult emergence inhibition activity of *Abutilon indicum* is also comparable to different species of plant extract in different families (Muthukrishnan *et al.*, 1999; Pushpalatha and Muthukrishnan, 1999). The lower dose treatments inhibited growth and caused mortality in a dose-dependent manner and also growth inhibiting effects on the various developmental stages of different mosquito species. A range of pre-emergent effects occur such as delays in larval development and extended pupal durations, moulting inhibition, morphological abnormalities, and mortality especially during moulting and melanization processes (Shaalan *et al*., 2005).

Furthermore, larval progress was affected showing several deformities, including dechitinized body wall, and body length of matured larvae was reduced as compared to controls. Deformities that developed in the body wall of larvae may be attributed to the dechitinizing effect of extract as reported by Saxena and Sumithra (1985). Tabassum *et al*. (1993) observed that phytoextracts affect larval morphology, resulting in pigmentation and alterations in head and abdomen shape. Adults emerging from physically deformed pupae remained trapped in the pupal eclusion. Likewise, Murty *et al*. (1997) have reported significant inhibition in adult emergence in *Culex quinquefasciatus* when treated with *Polyalthia longifolia* leaf extract.

Eggs and egg shells treated with plant extracts become damaged, probably due to endosmosis. After the initial phase of swelling, eggs become desiccated, followed by shrinkage and death of larvae trapped within. This lowered the percentage of hatching from 90.8 for controls to 42.4 per cent at 500 ppm, indicating a positive correlation between the concentrations of the extract and its ovicidal effect. Successive inhibition in hatching as a result of ovicidal action of phytoextract along with high mortality rates at larval and pupal stages reduced adult emergence. The growth index was considerably reduced at higher concentrations signifying the

anti-juvenile effect of the extract. It is hoped that more work would be undertaken to evaluate the utility of this plant extracts for field applications considering the promising leads given by the present study. Further investigations are needed to elucidate this activity against a wide range of mosquito species and also the active ingredient(s) of the extract responsible for adult emergence inhibition activity against vector mosquitoes should be identified and utilized, if possible, in preparing a commercial product/formulation to be used as a mosquitocidal agent.

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