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RESEARCH ARTICLE

BIOEFFICACY OF *Citrullus colocynthis* (L.) Schrad (Cucurbitaceae) WHOLE PLANT EXTRACTS AGAINST *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae)

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ABSTRACT

The whole plant crude extracts of *Citrullus colocynthis* were evaluated for larvicidal, pupal deformities and adult emergence inhibition activity against vector mosquitoes viz., *Anopheles stephensi, Aedes aegypti,* and *Culex quinquefasciatus*. Dichloromethane extract was found to be effective against *Culex quinquefascaitus* (LC_{50} value of 240.36 ppm) 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 with respect to all three vector mosquito species. Adult emergence inhibition activity was more pronounced in *Anopheles stepehensi*. Hatching was delayed and its rate was reduced when compared to control and significant ovicidal activity was observed in the eggs of *Anopheles stephensi*. Disrupted egg shells and dechitinized body walls were observed, indicating the anti-juvenile potential of the plant extract. The growth index was considerably reduced. These results suggest the extracts of *Citrullus colocynthis* 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.

INTRODUCTION

Insects are the most important living beings of the invertebrate group beneficial to human beings and other animals as well as detrimental causing several devastating diseases. The amenable tropical biotopes provide an ideal environment for many arthropods including the disease carrying arthropods, mainly insects. Among these, mosquitoes are the most important blood sucking group of insects (Rai *et al.,* 2007). WHO has declared the mosquito "Public enemy number one" as they are responsible for the transmission of various dreadful diseases (WHO, 1996a). Mosquitoes (Diptera: Culicidae) 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 *Anopheles, Aedes* and *Culex* are vectors for the pathogens of various diseases like Malaria, Japanese Encepahalitis, Dengue fever, Dengue haemorrhagic fever, Yellow fever, Schistosomiasis and Filariasis (Service, 1983; Gubler, 1998; Hubalek and Haluzka, 1999). One of the approaches for control of mosquito-borne diseases is the interruption of disease transmission by either killing, preventing mosquitoes to bite human beings (by using repellents) or by causing larval mortality in a large scale at the breeding centres of vectors.

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In recent years, mosquito control programmes have failed because of the ever increasing insecticide resistance (Brown, 1986; Georghion and Lagunestjida, 1991; WHO, 1992; Kelm *et al.,* 1997; Su and Mulla, 1998; Ranson *et al.,* 2001; Gericke *et al.,* 2002; Hargreaves *et al.,* 2003). Synthetic insecticides have created a number of ecological problems, ecological imbalance, harm to human and animals, environment ill effect, non-target organisms being affected in addition to the physiological resistance of vectors to synthetic insecticides (Wattal *et al.,* 1981; VCRC, 1989). Plant products have been used traditionally by human communities (Jacobson, 1958) and application of easily degradable plant compounds is considered to be one of the safest methods of control link of insect pests and vectors (Alkofahi *et al.,* 1989). Development of resistance by pests and vectors against the botanicals has not been reported (Sharma *et al.,* 1992). Botanical insecticides are generally pest specific, readily biodegradable, target specificity, lower bioaccumulation and lack toxicity to higher animals (Bowers, 1992; Mohan *et al.,* 2005; Sharma *et al.,* 2005). Many medicinally important plant extracts have been studied for their efficacy as mosquito cidal agent of different species of mosquito (Saxena and Sumithra, 1985; Kumar and Dutta, 1987; Chariandy *et al.,* 1999; Markouk *et al.,* 2000; Tare *et al.,* 2004). Not only can medicinal plant extracts be effective but also they may greatly reduce the risk of adverse ecological effects and do not induce pesticide resistance in mosquitoes. *Citrullus colocynthis* (L.) Schrad a medicinal plant belonging to the family Cucurbitaceae commonly called as 'bitter apple', 'wild water melon' (Mahmoud *et al.,* 2009) is reported to be used as an antidiabetic medication in tropical

and subtropical countries (Diwan *et al.,* 2000; Mahmoud *et al.,* 2009). In traditional medicine, the plant has been utilized as an abortifacient (Madari and Jacobs, 2004; Duke, 2006) and as purgative (Ageel *et al.,* 1987; Aburjai *et al.,* 2007). Further it is used as a cardiac depressant, smooth muscle relaxant (Lavie *et al.,* 1959), as a stimulant for hair growth (Hussein, 1985), and in curing tumors, leucoderma, elephantiasis, ulcers and in removing kidney stones (Bolous, 1983; Shah *et al.,* 1989). The plant is used as an anticancer agent in many drugs, as an antipyometra in animals (Chaudhary and Al-Jowaid, 1999) and as a local anaesthesia on frogs (Ramanathan *et al.,* 2011).

The dried pulp of the plant is used for treating constipation, oedema, bacterial infections, cancer (Arena and Drew, 1980; Ziyyat and Legsyer, 1987; Alkofahi *et al.,* 1996; Ziyyat *et al.,* 1997; Al-Ghaithi and El-Ridi, 2004; Madari and Jacobs, 2004, Duke, 2006). The powerful medicinal values of the pulp are due to the presence of an amorphous glucosid 'colocynth'. Many modern purgative pills contains the solid extract of colocynth in small doses which are used as expectorants in treating coughs, asthmatic attacks in children, jaundice, urinary disease and rheumatism (Anonymous, 1970; Ageel *et al.,* 1987). The plant also possesses antimicrobial (Gurudeeban *et al.,* 2010), antibacterial (Memon *et al.,* 2003; Dehghani *et al.,* 2008; Kumar *et al.,* 2008; Najafi *et al.,* 2010), anti-inflammatory (Yesilada *et al.,* 1988; Peters *et al.,* 1999; Rajamanickam *et al.,* 2010), antioxidant (Gebhardt, 2003), hypoglycemic, hypolipidaemic (AbdEl-Baky *et al.,* 2009), antihelmentic, antipyretic, carminative (Bolous, 1983; Shah *et al.,* 1989), antihepatotoxic (Agil *et al.,* 1999; Lavie and Glotter, 1971), antihypertensive (Ziyyat *et al.,* 1997), immunostimulant (Bendjeddou *et al.,* 2003), and hypocholesterolamic properties (Valette *et al.,* 1994). Some of the phytochemical compounds present in the plant include flavonoids, saponins, alkaloids, flavones, glycosides, terpenoids and cucurbitacin glycosides (Hatam *et al.,* 1989; Galal *et al.,* 1997; Abdel-Hassan et al. 2000; Seger *et al.,* 2005; Abbas *et al.,* 2006; Gulcan *et al.,* 2006; Tehila *et al.,* 2007; Asyaz *et al.,* 2010). Further, *Citrullus colocynthis* gained increasing attention as a natural insecticide and its activity have been evaluated against many important insect species. It possesses deterrent, antifeedant, growth regulating and fertility reducing properties in insects (Prabuseenivasan *et al.,* 2004), insecticidal effect against the aphid *Aphis craccivora* (Torkey *et al.,* 2009), and also the whole plant extracts of *Citrullus colocynthis* were assayed for their toxicity against the larvae of *Culex quinquefasciatus* (Rahuman *et al.,* 2008). Therefore, the present study was carried out to evaluate the mosquitocidal properties of *Citrullus colocynthis* whole plant extracts.

MATERIALS AND METHODS

Plant extracts

Citrullus colocynthis whole plants collected in and around Tamilnadu were brought to the laboratory, shade dried under room temperature and powdered using an electric blender. Dried and powdered whole plants (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 was refrigerated at 4 ^{0}C until testing for bioassays.

Test mosquitoes

All tests were carried out against laboratory reared vector mosquitoes viz., *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* free of exposure to insecticides and pathogens. Cyclic generation of vector mosquitoes was maintained at 25 – 29 ^{0}C and 80 – 90% R.H. in the insectariums. 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 instars 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 h. When control mortality ranged between 5.20 per cent, it was corrected using Abbott's (1925) formula,

$$\text{Percent mortality} = \frac{\%\ \text{Mortality in treated} - \%\ \text{Mortality in control}}{100 - \%\ \text{Mortality in control}}$$

DEVELOPMENTAL INDICES

Larval inhibition test

This test was performed according to the standard protocol described by WHO (1975b). The powdered plant material were put in cotton gauze sachets and immersed (for 6 h) in the 250 ml beaker containing water. Hundred early third instars larvae were exposed for 12 h to the aqueous 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 h. The pupae, which developed following this 12 h 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.

Pupal deformities

Dried and powdered leaves were dissolved in distilled water and stirred for 6 h at room temperature. The required concentration (500 and 1000ppm) was mixed thoroughly with 250 ml of beaker containing 200 ml of sample solution. Hundred late fourth instars larvae were released into beakers containing treated solution. A beaker containing only water (200 ml) served as control. Dead larvae and pupae were removed and counted after 24 h.

Observation on larval mortality, per cent pupation and adult emergence was recorded.

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.

Hatchability ratio

Freshly laid mosquito eggs were used for the treatments and were exposed for 12 h in desired concentrations of 500 and 1000 ppm. Hundred eggs collected on a filter paper for *Anopheles stephensi* and *Aedes aegypti* 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 are 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 I and II. Among the vector mosquito species, *Culex quinquefasciatus* larvae were found to be more susceptible followed by *Aedes aegypti* and *Anopheles stephensi*. The dichloromethane extract was found to be the most effective providing 100 per cent mortality at 1000 ppm against the larvae of *Culex quinquefasciatus* at 24 h followed by *Aedes aegypti* at 48 h. Dichloromethane extract showed the least LC_{50} value of 240.36 and 515.69 ppm against *Culex quinquefasciatus* and *Aedes aegypti* but it was diethyl ether in the case of *Anopheles stephensi* with a LC_{50} value of 503.39 ppm.

Developmental indices

During developmental metamorphosis, time taken for total larval and pupal developmental periods (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 for 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 *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* recorded at 500 and 1000 ppm was 53.0 ±2.12 and 21.4 ±2.51; 55.8 ±2.05 and 25.2 ±2.59; 70.4 ±1.52 and 36.2 ±2.49 respectively. Among the three species of vector mosquito, *Anopheles stephensi* was 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 III).

Morphogenetic variations

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, 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 its exuvia, which remained attached to its 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 *Citrullus colocynthis* extract against *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* at 500 and 1000 ppm was 41.2 ±1.92 and 19.6 ±2.51; 46.0 ±2.74 and 23.6 ±2.30; 55.2 ±3.03 and 28.8 ±2.39 respectively. The per cent hatchability of eggs in control medium was 90.8 ±3.83, 90.2 ±2.17 and 91.2 ±3.70 per cent respectively. The decrease in hatchability was found to be

Table I: Per cent larvicidal activity of *Citrullus colocynthis* whole plant extracts against vector mosquitoes at different concentrations

Solvents	Concentration (ppm)											
	250			500			750			1000		
	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
						Anopheles stephensi						
Hexane	7.2 ±5.22 (15.6)^{ab}	15.2 ±5.22 (22.9)^{bc}	18.4 ±4.56 (25.4)^{b}	23.2 ±8.20 (28.8)^{b}	26.4 ±6.69 (30.9)^{b}	35.2 ±3.35 (36.4)^{c}	29.6 ±3.58 (32.9)^{c}	30.4 ±2.19 (33.5)^{c}	37.6 ±2.19 (37.8)^{c}	36.8 ±4.38 (37.4)^{c}	40.8 ±3.35 (39.7)^{c}	46.4 ±3.58 (42.9)^{c}
Diethyl ether	14.4 ±6.07 (22.3)^{b}	20.8 ±5.22 (27.1)^{cd}	28.8 ±5.22 (32.5)^{c}	24.8 ±7.16 (29.9)^{b}	28.8 ±7.69 (32.5)^{b}	37.6 ±4.56 (37.8)^{c}	30.4 ±2.19 (33.5)^{c}	34.4 ±3.58 (35.9)^{cd}	37.6 ±3.58 (37.8)^{c}	32.8 ±4.38 (34.9)^{c}	42.4 ±2.19 (40.6)^{c}	44.8 ±3.35 (42.0)^{c}
Dichloro-methane	25.6 ±7.27 (30.4)^{c}	29.6 ±3.58 (32.9)^{d}	30.4 ±3.58 (33.5)^{c}	29.6 ±2.19 (32.9)^{b}	35.2 ±3.35 (36.4)^{b}	37.6 ±3.58 (37.8)^{c}	33.6 ±3.58 (35.4)^{c}	40.8 ±3.35 (39.7)^{d}	45.6 ±2.19 (42.5)^{c}	53.6 ±11.87 (47.1)^{d}	63.2 ±11.10 (52.7)^{d}	70.4 ±10.43 (57.0)^{d}
Ethyl acetate	1.6 ±3.58 (7.3)^{a}	7.2 ±8.20 (15.6)^{ab}	8.8 ±9.12 (17.3)^{ab}	5.6 ±4.56 (13.7)^{a}	7.2 ±5.22 (15.6)^{a}	10.4 ±6.07 (18.9)^{b}	9.6 ±7.27 (18.1)^{b}	14.4 ±6.07 (22.3)^{b}	23.2 ±11.10 (28.8)^{b}	16.8 ±5.22 (24.2)^{b}	23.2 ±5.22 (28.8)^{b}	28.8 ±5.93 (32.5)^{b}
Control	0^{a}	0^{a}	0^{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}	0.8 ±1.79 (5.1)^{a}	1.6 ±2.19 (7.3)^{a}	4.0 ±2.83 (11.5)^{a}
						Aedes aegypti						
Hexane	5.6 ±4.56 (13.7)^{ab}	15.2 ±5.22 (22.9)^{bc}	18.4 ±4.56 (25.4)^{b}	23.2 ±8.20 (28.8)^{b}	27.2 ±7.69 (31.4)^{b}	38.4 ±10.81 (38.3)^{b}	29.6 ±3.58 (32.9)^{b}	36.8 ±8.67 (37.4)^{b}	52.8 ±3.35 (46.6)^{b}	48.8 ±11.10 (44.3)^{b}	62.4 ±14.03 (52.2)^{b}	82.4 ±11.52 (65.2)^{c}
Diethyl ether	14.4 ±6.07 (22.3)^{b}	21.6 ±6.07 (27.7)^{c}	31.2 ±7.69 (33.9)^{c}	25.6 ±4.56 (30.4)^{b}	34.4 ±8.29 (35.9)^{b}	45.6 ±7.27 (42.5)^{bc}	35.2 ±3.37 (36.4)^{b}	50.4 ±5.37 (45.2)^{b}	59.2 ±8.67 (50.3)^{b}	53.6 ±10.81 (47.1)^{b}	68.8 ±5.22 (56.1)^{b}	80.8 ±1.29 (64.0)^{c}
Dichloro-methane	29.6 ±8.29 (32.9)^{c}	38.4 ±8.29 (38.3)^{d}	46.4 ±9.21 (42.9)^{d}	31.2 ±6.57 (33.9)^{b}	37.6 ±8.29 (37.8)^{b}	55.2 ±5.93 (47.9)^{c}	67.2 ±8.20 (55.1)^{c}	76.8 ±5.22 (61.2)^{c}	85.6 ±10.43 (67.7)^{c}	82.4 ±11.87 (65.2)^{c}	100 0 (90.0)^{c}	100 0 (90.0)^{d}
Ethyl acetate	1.6 ±2.19 (7.3)^{a}	4.8 ±4.38 (12.7)^{ab}	4.8 ±4.38 (12.7)^{a}	4.8 ±1.79 (12.7)^{a}	5.6 ±2.19 (13.7)^{a}	6.4 ±2.19 (14.7)^{a}	5.6 ±5.37 (13.7)^{a}	6.4 ±6.69 (14.7)^{a}	6.4 ±6.69 (14.7)^{a}	5.6 ±5.37 (13.7)^{a}	8.8 ±6.57 (17.3)^{a}	15.2 ±3.35 (22.9)^{b}
Control	0^{a}	0^{a}	0^{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}	0^{a}	1.6 ±2.19 (7.3)^{a}	3.2 ±3.35 (10.3)^{a}
						Culex quinquefasciatus						
Hexane	36.8 ±5.23 (37.4)^{c}	40.8 ±5.93 (39.7)^{d}	48.8 ±5.93 (44.3)^{d}	37.6 ±4.56 (37.8)^{c}	42.4 ±4.56 (40.6)^{c}	50.4 ±6.69 (45.2)^{c}	54.4 ±7.27 (47.5)^{c}	56.8 ±5.93 (48.9)^{c}	70.4 ±8.29 (57.0)^{c}	70.4 ±9.21 (57.0)^{c}	81.6 ±6.07 (64.6)^{c}	87.2 ±5.93 (69.0)^{d}
Diethyl ether	23.2 ±3.35 (28.8)^{b}	28.8 ±3.35 (32.5)^{c}	32.8 ±3.35 (34.9)^{c}	34.4 ±7.79 (35.9)^{c}	42.4 ±6.07 (40.6)^{c}	51.2 ±7.16 (45.7)^{c}	35.2 ±5.93 (36.4)^{b}	59.2 ±7.15 (50.3)^{c}	60.8 ±7.15 (51.2)^{c}	61.6 ±4.56 (51.7)^{c}	70.4 ±5.37 (57.0)^{c}	78.4 ±3.58 (62.3)^{c}
Dichloro-methane	58.4 ±3.58 (49.8)^{d}	65.6 ±2.19 (54.1)^{e}	71.2 ±3.35 (57.5)^{e}	64.8 ±5.22 (53.6)^{d}	71.2 ±5.22 (57.5)^{d}	80.8 ±4.39 (64.0)^{d}	70.4 ±9.21 (57.0)^{c}	83.2 ±11.10 (65.8)^{d}	95.2 ±5.22 (77.3)^{d}	100 0 (90.0)^{d}	100 0 (90.0)^{d}	100 0 (90.0)^{e}
Ethyl acetate	5.6 ±5.37 (13.7)^{a}	14.4 ±3.58 (22.3)^{b}	20.8 ±6.57 (27.1)^{b}	15.2 ±9.12 (22.9)^{b}	24.8 ±7.69 (29.9)^{b}	30.4 ±11.87 (33.5)^{b}	24.8 ±14.53 (29.9)^{b}	28.8 ±16.36 (32.5)^{b}	34.4 ±11.52 (35.9)^{b}	32.8 ±7.69 (34.9)^{b}	46.4 ±11.52 (42.9)^{b}	51.2 ±3.35 (45.7)^{b}
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}	2.4 ±2.19 (8.9)^{a}	0^{a}	1.6 ±2.19 (7.3)^{a}	2.4 ±2.19 (8.9)^{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 II: Probit analysis of larvicidal efficacy of *Citrullus colocynthis* whole plant extracts against vector mosquitoes

Extracts	LC_{50}	LC_{90}	Chi-square value	Regression value
		Anopheles stephensi		
Hexane	1451.29	7895.79	0.77*	1.74
Diethyl ether	503.39	3678.13	0.23*	1.01
Dichloromethane	1209.59	8213.79	5.60*	1.09
Ethyl acetate	3467.26	7258.59	0.21*	1.84
		Aedes aegypti		
Hexane	1087.64	3752.82	1.90*	2.38
Diethyl ether	1022.36	5122.13	2.24*	1.83
Dichloromethane	515.69	1725.59	19.01	2.44
Ethyl acetate	1212.96	4682.91	0.28*	1.18
		Culex quinquefasciatus		
Hexane	542.09	4544.48	6.63*	1.39
Diethyl ether	875.04	6686.83	7.57	1.45
Dichloromethane	240.36	1140.37	27.71	1.90
Ethyl acetate	1731.96	8550.25	0.01*	1.85

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

dose dependent. Among the three species of vector mosquito, *Anopheles stephensi* was most susceptible followed by *Aedes aegypti* and *Culex quinquefasciatus* (Table III).

DISCUSSION

Vector-borne diseases constitute the major cause of morbidity in most of the tropical and subtropical countries and have

always been a challenge to the medical professionals struggling for the welfare of humanity. Mosquitoes are the most deadly vector for several of these disease causing organisms. In many parts of the world, plant-derived natural products have traditionally been used against mosquitoes (Curtis *et al.,* 1991). In the search for safer insecticide technologies, more selective modes of action and reduced risks for non-target organisms and the environment, progress has been made in the last twenty years with the development of natural and synthetic compounds capable of interfering with the processes of growth, development and metamorphosis of the target insects (Alstein *et al.,* 1993). Phytochemicals may serve as suitable alternatives to synthetic insecticides as they are relatively safe, inexpensive, and are readily available in many areas of the world. According to Bowers *et al.* (1995) screening of locally available medicinal plants for mosquito control would reduce dependence on expensive imported products and stimulate local efforts to enhance public health.

The results of present study are comparable with earlier reports. The toxicity to the late third instar larvae of *Culex quinquefasciatus* by methanolic leaf extract of *Memordica charantia*, *Trichosanthus anguina*, *Luffa acutangula*, *Benincasa cerifera* and *Citrullus vulgaris* showed the LC_{50} values of 465.85, 567.81, 839.81, 1189.30 and 1636.04 ppm respectively (Prabakar and Jebanesan, 2004). Mullai and Jebanesan (2006, 2007) also reported the larvicidal efficacy of the leaf extracts of *Cucumis pubescens* with four different solvents against late third instar larvae of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* and *Cucurbita maxima* against *Culex quinquefasciatus*. Likewise, Arivoli *et al.* (1999) reported that the hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extracts of shoot with leaves of *Leucas aspera* and leaves of *Vitex negundo* when tested against the larvae of *Culex quinquefasciatus*, ethyl aceate and hexane extract provided maximum mortality respectively.

Insect growth regulators (IGR) or third-generation insecticides (Williams, 1967; Staal, 1977) exert their insecticidal effects through their influence on development, metamorphosis and reproduction of mosquitoes by disrupting the normal activity of the endocrine system. The effects of *Citrullus colocynthis* extracts on the biology, reproduction and adult emergence of the mosquitoes are compared with other plants. For example 50 per cent inhibition of the emergence of the adult mosquitoes was observed by the use of *Calophyllum inophyllum, Solanum suratense, Samadera indica* and *Rhinocanthus nasutus* leaf extracts (Muthukrishnan and Puspalatha, 2001). Exposure of *Anopheles stephensi* larvae to sub-lethal doses of neem leaves extract prolonged larval development and reduced pupal weight (Murugan *et al.,* 1996; Su and Mulla, 1999). Several morphogenetic abnormalities in the present study, have been observed in *Anopheles stephensi* larvae when treated with methanol extract of *Ageratum conyzoides* (Saxena and Saxena, 1992). Larval period was not affected at lower concentrations but was lengthened to 10 days. This finding was in accordance with the phytoextract-induced lengthening of *Aedes aegypti* larval period due to interference in normal hormonal activity (Supavarn *et al.,* 1974). The failure in adult emergence could be due to insufficient availability of chitin during metamorphosis resulting in death of larvae and pupae entangled in the weak integument. Similar phytoextract induced deformities was noted in *Anopheles stephensi* (Saxena and Sumithra, 1985). Degenerative effects on the life cycle of *Anopheles stephensi* as observed in the present study have also been reported by Dhar *et al.* (1996).

In the case of ovicidal activity, exposure to freshly laid eggs was more effective than to the older eggs. It has been shown that the age of the embryos at the time of treatment played a crucial role with regard to the effectiveness of the chitin synthesis inhibitor, dimilin to *Culex quinquefasciatus* (Miura *et al.,* 1976). The petroleum ether extract of *Artemisia annua* had significant influence on hatching and post-hatching development of *Anopheles stephensi* (Sharma *et al.,* 2006). Jarial (2001) reported similar effects with garlic extract against *Aedes aegypti* displaying attached shell caps with trapped larvae within the shell and arresting the hatching eggs.

Table III: Effect of *Citrullus colocynthis* aqueous extract on the growth and metamorphosis of vector mosquitoes

Mosquito species	Concentration (ppm)	Larval mortality (%) ***	Total larval period in days	Pupal mortality (%) ***	Total pupal period in days	Adult emergence (%) (a)	Hatchability (%)	Total developmental period in days (b)	Growth index (a/b)
Anopheles stephensi	500	38.8 ± 1.92	9	8.2 ± 0.84	3	53.0 ± 2.12	41.2 ± 1.92	12	4.4
	1000	64.8 ± 3.11	9	13.8 ± 0.84	3	21.4 ± 2.51	19.6 ± 2.51	12	1.8
Control		9.2 ±1.48	8	1.4 ±0.89	2	89.4 ±2.30	90.8 ± 3.83	10	8.9
Aedes aegypti	500	35.8 ± 1.79	10	8.4 ±0.55	4	55.8 ±2.05	46.0 ± 2.74	14	3.9
	1000	61.2 ± 1.92	10	13.6 ±1.34	4	25.2 ±2.59	23.6 ± 2.30	14	1.8
Control		8.2 ± 0.84	8	1.2 ±0.84	2	90.6 ±0.89	90.2 ± 2.17	10	9.1
Culex quinquefasciatus	500	22.4 ± 1.82	10	7.2 ±0.84	4	70.4 ±1.52	55.2 ± 3.03	14	5.1
	1000	50.2 ±2.39	10	13.6 ±1.14	4	36.2 ±2.49	28.8 ± 2.39	14	2.6
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

The seed extract of *Atriplex canescens* showed complete ovicidal activity at 1,000 ppm concentration in eggs of *Culex quinquefasciatus* (Ouda *et al.,* 1998). The finding of the present investigation was comparable with the above ovicidal studies and reveals that the *Citrullus colocynthis* extracts possesses ovicidal activity against vector mosquitoes. It is

clearly proved that crude plant extracts are less expensive and highly efficacious for the control of mosquitoes (Jang *et al.,* 2002; Cavalcanti *et al.,* 2004). Undoubtedly, plant derived toxicants are a valuable source of potential insecticides. These and other naturally occurring insecticides may play a more prominent role in mosquito control programs in the future (Mordue and Blackwell, 1993). The results of this study will contribute to a great reduction in the application of synthetic insecticides, which in turn increase the opportunity for natural control of various medicinally important pests by botanical pesticides. Since these are often active against a particular species including specific target insects, less expensive, easily biodegradable to non-toxic products, they can be potentially suitable for use in mosquito control programme (Alkofahi *et al.,* 1989), and could lead to development of new classes of possible safer insect control agents. Plant allelochemicals may be quite useful in increasing the efficacy of biological control agents because plants produce a large variety of compounds that increase their resistance to insect attack (Berenbaum, 1988; Murugan *et al.,* 1996; Nathan *et al.,* 2005).

Recently, bio-pesticides with plant origins are given for use against several insect species especially disease-transmitted vectors, based on the fact that compounds of plant origin are safer in usage, as they leave no scum in the environment (Schmutterer, 1990; Nathan *et al.,* 2004). The present study clearly proved the bioefficacy of *Citrullus colocynthis* extracts on *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Citrullus colocynthis* extracts might lead to better application of botanical derivatives during the suitable developmental period and could also be helpful in usage of a natural mosquitocide which in future might be used directly as a larvicidal, insect growth regulator, adult emergence inhibition and ovicidal agent in small-volume aquatic habitats or breeding sites of limited size around human dwellings. Further studies such as mode of action, synergism with the biocides under field condition are needed.

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