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Evaluation of the biological activity of crude latex and ethanolic leaves extract of *Calotropis procera* (Asclepiadaceae) against the mosquito vector *Culex quinquefasciatus* (Diptera: Culicidae)

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Abstract
In recent years, use of environmentally friendly and biodegradable natural insecticides of plant origin have received a great attention as agents for disease vector control. Crude latex and ethanolic extract of leaves from the Saudi plant *Calotropis procera* (Asclepiadaceae) were tested against 3rd instar larvae of *Culex quinquefasciatus* mosquito. The obtained results indicated that crude latex was more efficient than ethanolic leaves extract. The LC_{50} values of the crude latex and ethanolic leaves extract were found to be 57.3 and 388.7 ppm, respectively. Also, a remarkable reduction in both the pupation percent and adult emergence was obtained. Moreover, the latex showed a highly delayed toxic effect on the pupae and adults resulted from treated larvae, where the pupal mortality was 100% at the highest concentrations used (75 and 150ppm). The current results were considered promising to proceed in studying the bioactive plants which represent an environmentally sound alternative for the synthetic larvicides.

Keywords: *Calotropis procera*, Ethanolic extract, Toxicity, mortality, pupation, emergence, *Culex quinquefasciatus*

1. Introduction
Insect-transmitted disease remains a major cause of illness and death worldwide. Mosquitoes are important vectors of several tropical diseases, including malaria, filariasis, and numerous viral diseases, such as dengue, Japanese encephalitis and yellow fever (Curtis, 1992; Fradin *et al*.,) [6]. Mosquitoes alone transmit disease to more than 700 million people annually (Taubes, 2000) [29]. Therefore, the control of mosquitoes is an important public health concern around the world. For example, *Culex quinquefasciatus* Say (Diptera: Culicidae) is a pantropical pest and urban vector of *Wuchereria bancrofti*, Plasmodium (avian malaria), myxomatosis, and other diseases in some parts of the world (Goddard *et al*., 2002) [10]. The only efficacious approach to minimizing the incidence of these diseases is to eradicate and control mosquito vectors, mainly by applying insecticides to larval habitants, and educating the public (Corbel *et al*., 2004) [4].
Chemical control is an effective strategy used extensively in daily life. Synthetic insecticides are today at the forefront of mosquito controlling agents. Nevertheless, controlling the mosquitoes has become complicated because of their resistance to synthetic insecticides, as well as the toxicity of insecticides to fish and other non-target organisms (Wattanachai and Tintanon, 1999; Rohani *et al*., 2001, Mohan and Ramaswamy, 2007) [30, 21, 15].

2. Materials and Methods
2.1 Mosquito culture
Mosquito used in this study were *Culex quinquefasciatus*, the larvae were collected from a well within a farm in a village Sunbah, Saudi Arabia (10 km from Jazan city), then they were reared for several generations, in the Department of Biology faculty of science Jazan University, under room conditions at temperature of 27±2 °C, relative humidity 70±10% and 12-12 light-dark regime. Adult mosquitoes were kept in (30 x 30 x 30 cm) wooden cages and daily provided with sponge pieces soaked in 10% sucrose solution for a period of 3-4 days after emergence. After this period the females were allowed to take a blood meal from a pigeon host, which is necessary for laying eggs (anautogeny).

Plastic cup oviposition (15x15cm) containing dechlorinated tap water was placed in the cage. The resulting egg rafts picked up from the plastic dish and transferred into plastic pans (25 x 30 x 15 cm) containing 3 liters of tap water left for 24 h. The hatching larvae were provided daily with fish food as a diet. This diet was found to be the most preferable food for the larval development and a well female fecundity, (Kasap and Demirhan, 1992) [12].

2.2 Collection and extraction of plant materials

Freshly leaves of *C. procera* (Family: Asclepiadaceae) were collected in the month of Oct. 2011 from the Sabya, Saudi Arabia (desert road) and the taxonomic identification was made by Dr. Wael Kasem Ass. Prof. of Biology Department, Faculty of science, Jazan University. The leaves were washed and dried in the shade at room temperature (27–31 °C) for 7 days till they become brittle, then pulverized to powder in a hammer mill. The extraction was performed using 70% ethanol. One hundred grams of powder was extracted five times with 300 ml of aqueous 70% ethanol. After 24 h., the supernatants were decanted, filtrated through Whatman filter paper No. 5. and dried to obtain 75.0 g/kg (ethanol) of a semi solid crude extract. The dry extracts were kept in deep freezer (-4 ºC) till used for experiments.

Latex was collected by cutting the fresh leaves using a razor blade and gathered into glass vials and dried.

2.3 Larvicidal activity

Different range of concentrations of each leaves extract and latex were prepared in order to detect mortalities. All tested materials were performed in 100ml. of dechlorinated tap water contained in 200ml plastic cups. Then, 3rd instar larvae were put immediately into plastic cups contained different concentrations of extract and latex. At least three replicates were usually used for each tested concentration. All plastic cups were incubated under controlled conditions at temperature of 27±2 °C, relative humidity 70±10% and 12-12 light-dark regime. Control larvae received 0.1 ml of 70% ethanol in 100ml water. Mortality was recorded daily and dead larvae and pupae removed until adult emergence.

3. Criteria studied

3.1. Biological activity of plant extracts against the larval stages: The larvae were observed daily until pupation and adult emergence to estimate the following parameters:

3.2. Larvicidal activity: Larval mortality percent was estimated by using the following equation (Briggs, 1960) [3]: larval mortality % = A – B / A × 100 where: A = number of tested larvae, B = number of tested pupa.

3.3. Pupation rate: The pupation percent was estimated by using the following equation: pupation % = A / B × 100 where: A = number of pupae, B = number of tested larvae.

3.4 Pupal mortality: The pupal mortality percent was estimated by using the following equation: pupal mortality % = A – B / A × 100 where: A = number of produced pupae, B = number of observed adults.

3.5 Adult emergence: The emerged males and females adults were counted and the adult emergence percent was calculated by using the following equation: Adult emergence % = A / B × 100 where: A = number of emerged adults, B = number of tested pupae.

3.6 Malformative effects: Pupal malformation was indicated by any change in color, size, shape or failure to develop into the adult stage (pupal-adult intermediate). All malformed pupae were counted and removed immediately. The pupal malformation percent was calculated using the following equation: Pupal malformation % = C / A × 100 (where: C = number of malformed pupae, A = number of tested pupae).

3.6 Statistical analysis

Statistical analysis of the data was carried out according to the method of lentner *et al*., (1982) [13]. LC_{50} was calculated using multiple linear regression (Finney, 1971) [8].

4. Results

4.1 Biological activity of plant extracts against the larval stage of *Culex quinquefasciatus*

The biological activity (larvicidal activity, pupal rate, pupal mortality, total larval and pupal mortality, adult emergence) of ethanolic extract and latex against the 3rd instar larvae of *C. quinquefasciatus* has been studied. The results may be arranged as follows:

4.2 Ethanolic extract

Data given in table (1) indicated the biological activity of ethanolic extract of *C. procera* (leaves) against the 3rd instar larvae of *C. quinquefasciatus.*

Results presented in table (1) indicated that the highest larval mortality percent (100%) occurred at the highest concentration (1000ppm), while the lowest mortality percent (30%) occurred at the lowest concentrations (1250ppm), compared to 6.7% for the control. The pupation percent decreased as the concentration level of ethanolic extract of *C. procera* increased. The pupation percent recorded 0.0% at (1000ppm) and 70% at the lowest concentrations (125ppm), compared to 93.3% of the control.

It is cleared from table (1), the ethanolic extract had moderate toxic effect against the pupae resulted from the treated larvae especially at the concentrations (750 and 500 ppm), where the pupal mortality percent was 50.0 and 33.3%; respectively compared to 0.0% at the control group. The total mortality of larvae and pupae were: 100, 100, 83.3 and 53.3% at the concentrations 750, 500, 250 and 125 ppm compared to 6.7 for control group.

The adult emergence percent was affected especially at the highest conc. (750ppm) where it reduced to 50.0%, compared to 100.0% for the untreated larvae. The lethal effect of the ethanolic extract extend to the adult stage at all concentration used. Moreover this extract not induce malformation effects on pupae resulted from treated larvae.

Table 1: Effect of ethanolic extract of *Calotropis procera* (leaves) on mortality percent of different stages of *Culex quinquefasciatus*.

Conc. ppm	Larval Mortality %	Pupation %	Pupal Mortality %	Larval and pupal Mortality%	Adult Emergence %	Adult Mortality %
1000	100.0	—	—	—	—	—
750	80.0	20.0	50.0	90.0	50.0	66.7
500	66.7	33.3	30.0	76.7	70.0	42.9
250	40.0	60.0	27.8	56.7	72.2	30.8
125	30.0	70.0	19.0	43.3	81.0	11.7
Control	6.7	93.3	0.0	6.7	100.0	0.0

No. of tested larvae = 30; Conc. = Concentration; ppm = particle per million.

4.3 Latex

Data given in table (2) indicated the biological activity of latex of *C. procera* against the 3rd instar larvae of *C. quinquefasciatus*.

In table (2) Complete larval mortality (100%) was caused at the highest concentration (300ppm), meanwhile the lowest value (26.7%) occurred at the lowest concentration (25ppm) compared to 6.7% for the control group. At the highest and lowest concentrations: 300 and 25ppm the pupation percent was 0.0 and 73.3%; respectively vs. 93.3% for the untreated group (Table 2).

The lethal effect of the latex was extended to the pupal stage at the all concentrations used: 150, 75, 50 and 25ppm, where the pupal mortality percent was 100.0, 100.0, 68.8 and 36.4%; respectively, vs. 0.0% for the control. The total larval and pupal mortality were found to be highly affected by latex. The highest mortality (100.0%) was noticed at the concentrations 150 and 75ppm and the lowest mortality (53.3%) was noticed at the concentration 25ppm; respectively compared to 6.7% at the control group.

A remarkable reduction in the percentage of adult emergence from pupae produced by treated larvae with the latex was observed. The adult emergence percent (0.0%) was occurred at the concentrations 150 and 75ppm, meanwhile the percent increased to 31.2 and 63.6% at the concentrations 50 and 25ppm compared to 100.0% at the control group.

As shown from the results (table 2) the toxicity of latex extended to the adult stage, where the adult mortality percent was 100% at 50ppm, while at the lowest concentration (25ppm) the mortality percent was 64.3% compared to 0.0% at the control group.

The results recorded in table (2) showed that the latex induced highly % of malformation on the pupae developed from the treated larvae. The pupal malformation percent was 100.0% at the concentrations 150 and 75ppm compared to 0.0% for the control group.

From the aforementioned results it is obvious that the crude latex was more efficient than ethanolic leaves extract. In general, the toxicity values of tested materials of *C. procera* based on LC_{50} values (Tables 3) may be arranged in a descending order as follows: crude latex> ethanolic extract (leaves).

Table 2: Effect of latex of *Calotropis procera* on mortality percent of differen stages of *Culex quinquefasciatus*.

Conc. ppm	Larval Mortality %	Pupation%	Pupal Mortality%	Malformed pupa	Larval and pupal Mortality %	Adult Emergence %	Adult Mortality %	Pupal / adult intermediate %
300	100.0	—	—	—	—	—	—	—
150	86.7	13.3	100.0	100.0	100.0	0.0	—	—
75	63.3	36.7	100.0	72.7	100.0	0.0	—	—
50	46.7	53.3	68.8	54.5	83.3	31.2	100.0	80.0
25	26.7	73.3	36.4	37.5	53.3	63.6	64.3	35.7
Control	6.7	93.3	0.0	0.0	6.7	100.0	0.0	0.0

No. of tested larvae, Conc., ppm: see footnote of table (1)

Table 3: LC50 values (ppm) of ethanolic leaves extract, and latex of *Calotropis procera* against *C. quinquefasciatus* larvae.

Plant materials	LC_{50} (ppm)	Slope (b)	Correlation coefficient (r)
Ethanolic extract	388.7	0.109	0.798
Latex	57.3	0.418	0.093

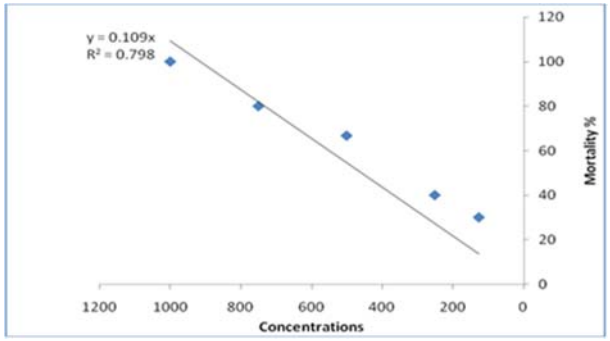

Fig 1: Regression line of larval mortality of *C. quinquefasciatus* treated with different concentrations from ethanolic extracts of *C. procera*.

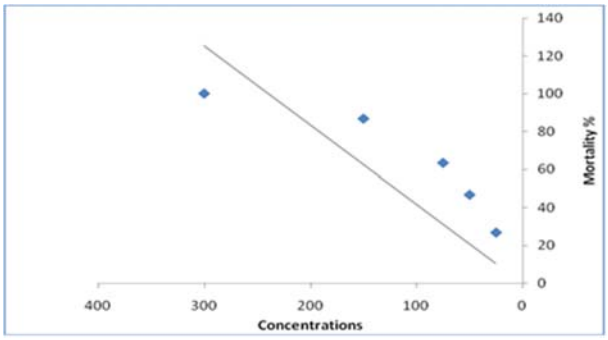

Fig 2: Regression line of larval mortality of *C. quinquefasciatus* treated with different concentrations from latex of *C. procera*.

4.4 Morphogenetic effects

The different forms of morphogenetic effects as induced by the latex tested against the 3rd instar larvae of *C. quinquefasciatus* are illustrated in Fig. (3) from A to D and can be summarized as follows:

A. Deformed decolorized pupa.
B. pupal- adult intermediate.
C. Incompletely emerged adult with thoracic appendages and abdomen attached with the pupal skin.
D. Incompletely emerged adult with legs attached to the pupal skin, wings unequal and abdomen not completely segmented.

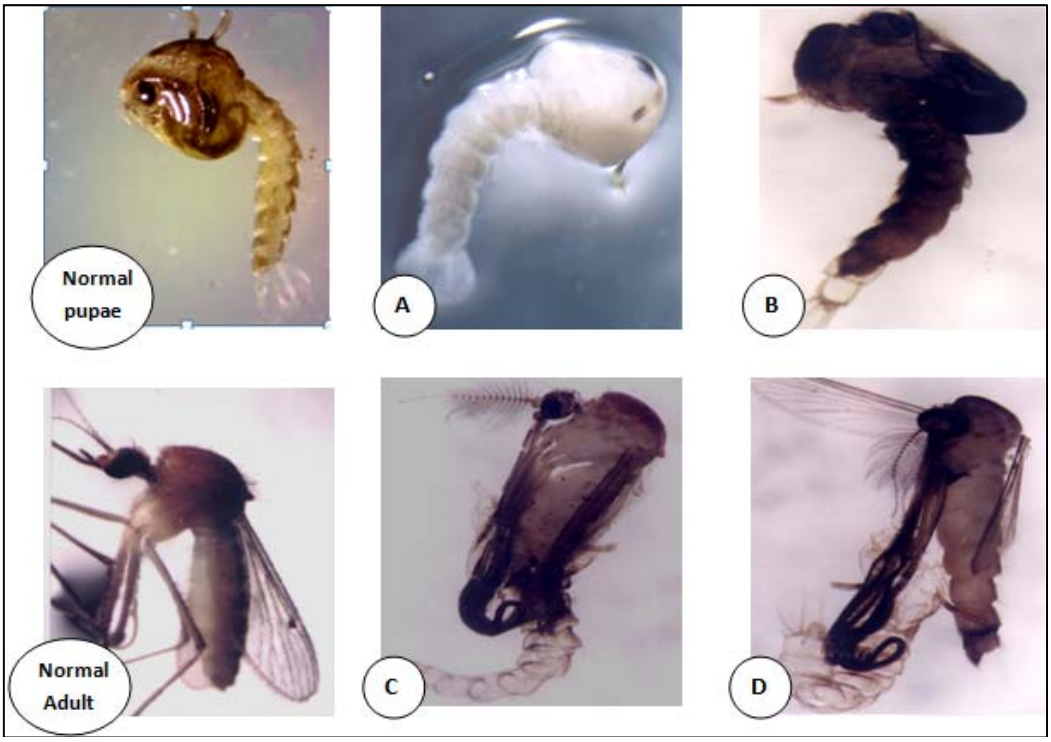

Fig 3: Malformative effects in *C. quinquefasciatus* pupae and adult as induced by the latex and plant extract tested.

5. Discussion

During the last three decades, pest control methods were directed to the use of insecticides of plant origin. This trend appeared as a results of the accumulated side effects and environmental contamination from a long term extensive application of toxic synthetic insecticides. The good example of these botanical insecticides was those derived from the neem tree (*Azadirachta indica*) and tried against more than 400 species of pests (Schumutterer *et al*., 1995) [22]. Also, Stoll (2000) [27] mentioned about 65 plant species that showed insecticidal activity against a large number of insect pests. However, several studies were carried out in Sudan using plant extracts against a number of insect pests of agricultural or medical importance (e.g., Siddig, 1991; Ahmed, 1993; El-Kamali, 2001; Osman, 2003, and Ali, 2004) [26, 1, 7, 18, 2].

Latex and ethanolic leaves extract of the *C. procera* against the 3rd larval stage of *C. quinquefasciatus* were clearly affected the various biological aspects as follows:

5.1 Larvicidal activity

The present study showed that, the toxicity of the tested plant extract and latex against 3rd larval instar was varied according to the concentrations used. The larval mortality percent was increased by increasing concentration for both latex and ethanolic extract. Also we found latex a strong killer against *Cx. quinquefasciatus* larvae compared to extract (Table 1&2).The toxicity of latex and ethanolic extract based on LC_{50} was latex > ethanolic extract. These results are in consistent with the previously mentioned suggestions of (Sukumar *et al*. 1991; Maurya *et al*. 2009, Shahi *et al* 2010) [28, 14, 23].

Several plant extracts other than those used in the present study had been tested against different species of mosquitoes by many authors worldwide. The tested plant extracts on larval mortality of *C. pipiens* were in agreement with the results obtained by Pelah *et al*. (2002) [20], Jeyabalan *et al* (2003) [11], Nathan *et al*. (2005 & 2006) [16, 17], Sharma *et al*. (2006b) [25], Coria *et al*. (2008) [5], Maurya *et al*. (2009) [14].

5.2 Pupation percent, pupal mortality and adult emergence

In the present study, a remarkable decrease in the larval pupation percent was induced by all tested material. The pupation% was decreased as the concentration of the plant extract increased. Moreover, the pupation rate was found to be plant part - and solvent used in extraction – dependent.

The present study showed that the toxicity of plant extracts tested had been extended to the pupae, where 100% pupal mortality was induced by latex. In addition, the ethanolic leaves extract and latex tested induced a remarkable reduction in the % of adult emerged from the pupae produced from treated larvae. The reduction was concentration– dependent. These results are comparable to earlier results of Jeyabalan *et al* (2003) [11] using water extracts of *E. crassipes* and *Ar. Monosperma* against *C. pipiens* larvae, Nathan *et al*. (2006) [17] using methanolic extracts of leaves and seeds of *Melia azedarach* against *A. stephensi* larvae, Sharma *et al*. (2006 a & b) [24, 25] using petroleum ether extract of *Artemisia annua* against *An. stephensi* and *Culex quinquefasciatus* larvae, respectively and Pavela (2009) [19] using essential oils from 22 aromatic plant species against *Culex quinquefasciatus* Say (Diptera: Culicidae).

5.3 Survivorship of the resulted adults

Results obtained in the present study indicated that the toxicity of latex against the 3rd instar larvae of *C. quinquefasciatus* was extended to the produced adults causing mortality reached to 100%. Similar results were obtained by Jeyabalan *et al*. (2003) [11] using methanol extract of *Pelargonium citrosa* leaf against *A. stephensi*, Nathan *et al*. (2005) [16] using the neem *Azedirachta indica* extract against *A. stephensi* and Nathan *et al*. (2006) [17] using methanolic extracts of leaves and seeds from the chinaberry tree *Melia azedarach* against *A. stephensi*.

6. Conclusion

From the results, it can be concluded that *C. procera* ethanolic extract and latex possess good larvicidal activity against *C. quinquefasciatus* mosquito and more studies are indicated to extract the active compounds for future studies and use in mosquito control.

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