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Toxicity Investigation of *Cestrum parqui* Saponins to *Culex pipiens* Larvae

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ABSTRACT

Cestrum parqui is a plant largely described for its insecticidal effect. This activity comes mainly from saponins. Our study shows an interesting toxicity of the crude saponic extract on *Culex pipiens* larvae. The LC_{50} values were 100 and 111 p.p.m. after 24 and 48 hours, respectively of treatment. The treated larvae showed a destroyed cuticle structure, a change in the colour, form and size of the fat body cells and a deterioration of the digestive walls with a separation of its peritrophic membrane.

Keywords: crude saponic extract, cytoxicity, insect vector, mosquito control

INTRODUCTION

Mosquitoes constitute a major health menace as vector of human and animal disease (malaria, arboviral viruses). *Culex pipiens* is the principal species distributed in Africa, especially in Tunisia (Krida *et al.* 1998b). This species is the vector of west Nile virus that causes fatal meningitis and encephalitis. To control this pest, we use synthetic insecticides that become progressively inactive against mosquitoes due to their resistance. Therefore, it will be necessary to find other insecticidal molecules in order to manage mosquitoes. Many recent studies investigated insecticidal proprieties of plant material and concluded that they are environment-friendly products, degradable and target-specific (Shalaibi *et al.* 1998; Traboulsi *et al.* 2002, 2005).

Cestrum parqui is a shrub originating from Chile used in Tunisia as an ornamental plant. The toxicity of this plant was shown against several insects. This activity was demonstrated for the first time on desert locust *Schistocerca gregaria* (Ammar *et al.* 1995). This plant is also toxic to some other lepidoptera (*Spodoptera littoralis*, *Helicoverpa armigera*, *Pieris brassicea*) (Chaieb *et al.* 2001) and diptera (*Ceratitis capitata*) (Zapata *et al.* 2006). Its toxicity is concentrated in the crude saponic extract (CSE) (Barbouche *et al.* 2001), which contains an insecticidal saponin efficient against *S. gregaria* larvae and adults (Barbouche *et al.* 2001; Chaieb *et al.* 2007a).

The aim of this work was i) to explore the larvicidal effect of CSE containing a potent insecticidal saponin and ii) to study its histological effect on *C. pipiens* larvae.

MATERIALS AND METHODS

CSE extraction

The saponin extraction method was described by Barbouche *et al.* (2001). *C. parqui* leaves were obtained from the garden of the National Tunisian Agronomic Institute and dried in a steamroom at 40°C during 4 days. Dried leaves were finely ground and 100 g of the powder was washed with petroleum ether (Carlo Erba Reactifs-SDS) then extracted three times with 300 ml methanol (Normapur). After filtration, the methanol was evaporated with a rotary evaporator at 40°C to obtain a dry residual weighing 6 g. Dissolution of 1 g of this residual in 100 ml of methanol followed by the addition of 100 ml of ethylic ether (Panreac Chimica) resulted in 0.06 g of a brown precipitate, CSE.

Test animals

C. pipiens larvae were collected from streams in the region of Chott Mariem in Tunisia and identified following an identification key (Krida *et al.* 1998a) to eliminate other mosquito species. Larvae were maintained at 25 ± 2°C and 70-80% relative humidity. The larvae were fed daily a mixture of 50% dog biscuit and yeast extract (Sigma) until bioassays were conducted.

Bioassays

All bioassays were conducted according to a standard method (WHO 1996) with slight modifications. 20 newly hatched L_4 larvae were exposed to different concentrations of tested extract (1000, 500, 250, 125, 62.5 ppm). Controls were maintained in water. For each dose, five replicates were run. Larval mortality was recorded after 24 and 48 h. Larvae were considered dead when they were unable to move. No food was offered to the larvae during treatment.

Data analysis

The median lethal concentration LC_{50} was calculated using a probit analysis program according to Finney (1971). Mortality of larvae treated with different concentrations was compared with Duncan's multiple range test using SPSS 11 for Windows ($P \leq 0.05$).

Microscopic observation

For light microscopic observation, L_4 *Culex* larvae sections were obtained according to Chaieb *et al.* (2007b) with slight modifications. Newly hatched L_4 larvae were used for histological studies. They were treated with 500 ppm CSE for 12 h. Only living larvae were used for the hematoxylin, phloxin, orange G (HPO) procedure (Chaieb *et al.* 2007b). Larvae were fixed in trichloroacetic Bouin liquid for 32 h followed by paraffin embedding and sectioning. The sections (7.5 µm) were placed on slides and stained with HPO.

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Short Communication

RESULTS AND DISCUSSION

Toxic effect of CSE

The susceptibility level of *C. pipiens* L_4 larvae to different concentrations of CSE is described in **Table 1**. CSE exhibited larvicidal activity against mosquito larvae after 24 and 48 h. This insecticidal propriety was higher when we used 1000 and 500 ppm. The mortality with these concentrations was total after 24 h.

LC_{50} values are listed in **Table 1**. The LC_{50} of CSE was 100 and 111 ppm, respectively after 24 and 48 h. These results show a potent insecticidal activity of CSE compared to other studies made on other saponins of other plant species (Weisman and Chapagain 2003; Chapagain and Weisman 2005; Weisman and Chapagain 2006).

The mosquitocidal activity of different plant species has been studied. Some were applied on larvae in aqueous solution and others were active on adults by fumigation. A few mosquitocidal plants were studied related to their saponin concentration. *Blanites aegyptiaca*, a common plant in dry land in Africa and south Asia, was tested for its mosquitocidal activity against *C. pipiens*: 100% of the larvae died in three days with 0.1% of root extract or 0.5% of bark extract (Chapagain and Weisman 2005). Further investigations demonstrated a relationship between saponin concentration and mosquitocidal activity of *B. aegyptiaca* fruit mesocarp extracts (Weisman and Chapagain 2006) whose saponins caused 100% *Aedes aegypti* mortality after 6 days of treatment (Weisman and Chapagain 2003).

Quillaja saponaria is a tree native of Peru and arid zones of Chile. Commercial saponin mixture extracted from this plant was tested for its toxicity to *A. aegypti* and *C. pipiens*, causing 100% mortality on *A. aegypti* and *C. pipiens* larvae after 5 days with 500 and 1000 ppm, respectively of *Quillaja* saponins (Pelah *et al.* 2002).

Ethyl acetate extract of *Achyranthes aspera* showed high mortality on 4th-instar larvae of *Aedes aegypti* and *Culex quinquefasciatus*. Bioassay-guided fractionation of *A. aspera* led to the separation and identification of a saponin as a potential mosquito larvicidal compound, with LC_{50} values of 18.20 and 27.24 ppm against *A. aegypti* and *C. quinquefasciatus*, respectively (Bagavan *et al.* 2008).

Table 1 Toxicity parameters of different doses of CSE to *C. pipiens* larvae.

Percent mortality			
Time		**24 h**	**48 h**
CSE concentration (ppm)	1000	0 ± 0 a	0 ± 0 a
	500	11.25 ± 2.5 b	0 ± 0 a
	250	25 ± 4.08 c	0 ± 0 a
	125	41.25 ± 4.79 d	15 ± 7.07 b
	62.5	63.75 ± 4.79 e	62.5 ± 6.45 c
	31.2	86.25 ± 2.5 f	81.25 ± 4.79 d
	0	98.75 ± 2.5 g	98.75 ± 2.5 e
LC50, Feducidal limits and X^2 calculation			
LC50 (ppm)		102.48	64.72
Feducidal limits (ppm)	Upper	119.81	72.44
	Lower	86.67	57.48
X^2		2.75	70.11

Means ± standard error followed by the same letter are not significantly different ($p \leq 0.05$) according to DMRT.

Histopathologic effects of CSE

The microscopic observation of transversal cuts of L_4 larvae showed a discontinuous and jagged cuticle with a significant separation of the cuticle from the external part of the fat body (**Fig. 1**). Therefore, saponins were able to destroy the *Culex* cuticle. However, Chaieb *et al.* (2007b) did not observe a CSE effect on *Spodoptera littoralis* and *Schistocerca gregaria*.

Fat body tissue was shredded. Moreover, cells changed colour and form and they were denser and smaller than the control. These results were confirmed by Chaieb *et al.* (2007b) on *Spodoptera littoralis* larva treated by *C. parqui* saponins. In fact, microsopic observation proved that fat body cells had a reduced dimension and a coloured cytoplasm after 6 h of injection. After 24 h, these same cells become destroyed and their membrane was damaged with the appearance of cellular exudates.

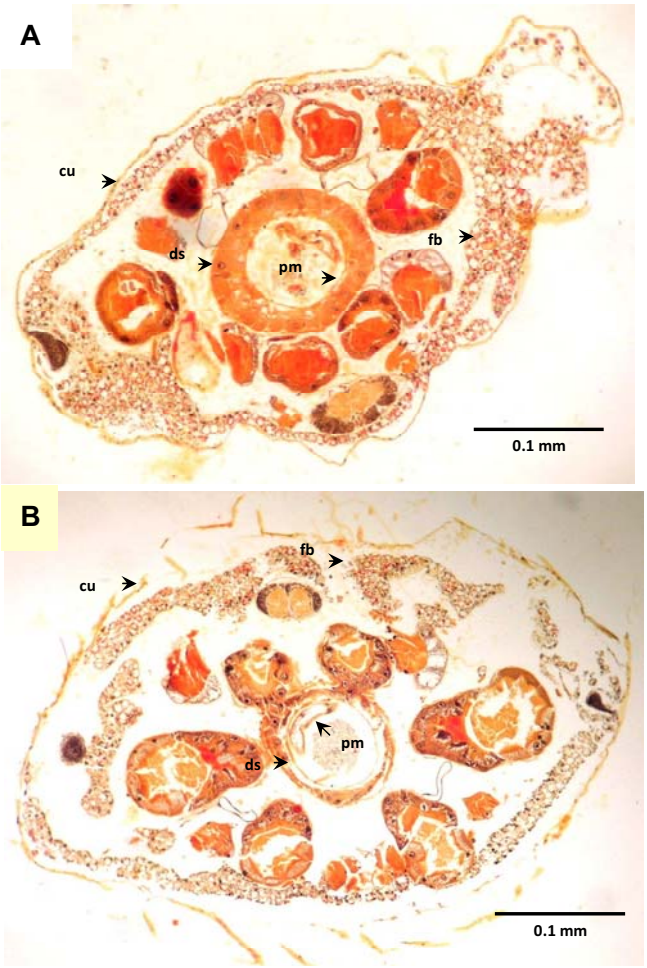

Fig. 1 Microscopic observation of transversal section of *Culex pipiens* L_4 larvae body. (**A**) Control larva; (**B**) saponin-treated larva; (cu): cuticle; (ds): digestive tract; (pm): peritrophic membrane; (fb): fat body (Gx100).

Furthermore, the digestive wall width of *Culex* larvae was reduced by a separation between the peritrophic membrane and the tract. Similar results were obtained by Chaieb *et al.* (2007b) on *Schistocerca gregaria* adults and larvae having a deterioration of the foregut structure and the destruction of the gastric caeca cells.

Saponins were largely studied for their cytotoxicity by their disturbing effect on biological and synthetic membranes. This effect was based on the interaction property between saponins and membrane cholesterol. This interaction modified the phospholipid bi-layer structure which allows the disturbance of cellular exchanges leading to cytotoxicity (Keukans *et al.* 1995).

Sung *et al.* (1995) showed that the application of soybean saponins on human cancerous cells induced plasmic and nuclear membrane deformation. They reported that *Gypsophila* saponins destroyed the whole plasma membrane.

Digitonin (spirostanic saponin with a 5-sugar chain) caused the rupture and disintegration of synthetic giant vesicle containing cholesterol in their membrane (Menger and Keiper 1998) whereas vesicles deprived of cholesterol were not sensitive to this saponin. Fibrous tubules formed on the surface of membranes of treated vesicles. Digitonin also caused deformations on the surface of membrane and formation of hemi-tubes on intestinal epithelium and pancreatic cells (Miller 1984).

Commercial saponins induced lesions of erythrocyte membranes (Baumann *et al.* 2000). The formation of hemitubes on the membranes was also largely described on synthetic vesicles treated by alcaloidic saponins (α-choacine and α-tomatine) (Keukens *et al.* 1995). Hu *et al.* (1996) reported that these saponins also had an effect on membrane structure by modifying their permeability.

Saponins from *C. parqui* have potent spermicidal activity. Sperm treated with saponins showed many modifications, especially in the head. The plasma membrane covering the head expanded and separated from the nucleus and the acrosome appeared interrupted (Kammoun *et al.* 2007).

Our results confirmed these phenomena. In fact, cells observed in the fat body of *C. pipiens* decreased considerably in size and became denser probably by losing their contents.

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