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Toxicity evaluation and effects on the development of a plant extract, the Saponin, on the domestic mosquito, *Culex pipiens*

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Abstract
Mosquitoes transmit serious human diseases, causing millions of deaths every year. Use of conventional insecticides to control vector mosquitoes has caused physiological resistance and adverse environmental effects in addition to high operational cost. Insecticides of botanical origin have been reported as promising alternatives for mosquito controls. The present study carried out in order to evaluate the larvicidal activity and the effects on development of saponin against mosquito larvae. The larval mortality was observed after 24 h of exposure. The product showed larvicidal effect with a concentration-dependent mortality. The highest larval mortality was found using the 20mg/l of saponin against the larvae of *Cx. pipiens* with the LC_{50} =11.07 mg/l and $LC_{90=}$ 32.85 mg/l for the fourth instar stage. The egg laid was observed after 48 h post-emergence. The percent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. The product showed moderate ovicidal activity. Saponin and its derived products have shown a variety of insecticidal properties.

Keywords: Saponin**,** plant extract, essential oils, mosquito, *Culex pipiens*

1. Introduction
Mosquitoes instead of causing a great annoyance are considered the most insect vector diseases. It is known that several mosquitoes belonging to the genera *Anopheles*, *Culex* and *Aedes* are vectors for pathogens of various diseases such as malaria, filariasis, yellow fever, dengue, Japanese Encephalitis (JE) and hemorrhagic fever [1] causing serious health problems to human beings. The domestic mosquito, *Culex pipiens* is considered among the most abundant species in Algeria. It is widely distributed in the urban areas due to the presence of many artificial breeding sites practically through all the year [2; 3].The insect populations control around the world is primarily dependent upon chemical insecticides and fumigants. These synthetic pesticides are expensive and have in many cases only produced moderate results along with major ecological damage [4]. Although effective, their repeated use has disrupted natural biological control systems and led to resurgence of these insects, resulted in the development of resistance and had undesirable effects on the environment, non-target organisms and human health concern [5; 6]. The increasing concern; over the level of pesticide residues in the different environmental sites and especially in food, has encouraged the scientists to propose a new alternatives of conventional pesticides [7].

An alternative approach for mosquito control is the use of natural products such as microorganisms and plants. The microbial pesticides have undergone extensive testing prior to registration. They are essentially nontoxic to humans, so there are no concerns for human health effects [8; 9]. Extensive testing shows that microbial larvicides do not pose risk to wildlife, non-target species or the environment and retain a good activity in polluted water [10]. Plants have generated extraordinary interest as potential alternatives natural insect control agents. Botanical extracts have several advantages over traditional pest control agents [11; 12; 13]; such as specificity, biodegradability and low mammalian toxicity [14]. The aim of the present study was to evaluate the larvicidal activity and biological effects of the saponin against mosquito larvae of *Culex pipiens*.

2. Materials and methods

2.1 Rearing of mosquito larvae of *Culex pipiens*

The toxicological essays were conducted in the laboratory following the standard methods for testing larval susceptibility [15]. Different instars larvae of *Cx. pipiens* were obtained from laboratory colonies of the department. Larvae were reared in storage jars containing 500 ml of stored tap water and maintained at a temperature between 25-27 °C, 85% of relative humidity (RH) and a photoperiod of 14:10 (L:D). Larvae were daily fed with fresh food consisting of a mixture of Biscuit-dried yeast (75:25 by weight), and water was changed every four days. The feeding was maintained till the larvae were developed into the pupa stage. The pupae were collected from the culture trays and were transferred to jars containing 500 ml of water with the help of a dipper. The jars were kept in (50×50×50 cm) size mosquito cage for adult emergence. The adults were fed with 10% sugar solution for a period of three days before they were provided by an animal for blood feeding.

2.2 The saponin product

Saponins are phytochemicals which can be found in most vegetables, beans and herbs. The best known sources of saponins are peas, soybeans, and some herbs with names indicating foaming properties such as soapwort, saoproot, soapbark and soapberry. Commercial saponins are extracted mainly from *Yucca schidigera* and *Quillaja saponaria*. The saponins are consisted of a polycyclic aglycones attached to one or more sugar side chains. The aglycone part, which is also called sapogenin, is either steroid (C27) or a triterpene (C30). The foaming ability of saponins is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part. In this work a commercial formulation of the saponin; extracted from the tomato and the potato, was used.

2.3 Toxicological assays

The toxicological essays were carried out using various concentrations 5; 10; and 20 mg / l, of a commercial formulation of the saponin against the different mosquito larval stage L1, L2, L3 and L4 of *Culex pipiens*. The treatment with the saponin of different larval stages was made on newly exuviated larvae. The bioassays and the control series were realized with three repetitions of 25 larvae for each used concentration, prepared in a separate jar containing 500 ml of breeding water. After an exposure time of 24 hours, according to the world health organization (WHO) recommendations [16], of the larvae to the product the water is changed and the food is added every three days until the emergence of the adults. The mortality of the treated stage is recorded daily and followed during the other developing stages until the emergence of adults.

2.4 Statistical Analysis

The mortality percentage observed for each stage and concentration was corrected [17] and subjected to the probit analysis [18]. LC_{50}, LC_{90}, confidential limits and the slope were calculated [19]. Data from insecticidal tests were subjected to analysis of variance after angular transformation of observed mortality percentages.

2.5 Fecundity tests

The fecundity experiments were conducted on the eggs of *Cx. pipiens* collected from the breeding jars of the females emerged from the treated fourth larval stage with the lethal concentrations (LC_{50} = 11.05mg/l and the LC_{90} = 32.85mg/l) of the saponin of the mosquito species. For each concentration 20 females and 20 males were kept in separate breeding cage. The laying eggs for each series were collected, counted and transferred to a new jar containing 500 ml of water and kept for larval hatching. Different parameters of reproduction; the number of egg laying, hatching rate, the fecundity, were studied. The fecundity was calculated by the number of eggs laid in ovitrap divided by the number of females let to mate. The death of adults in the experiments was also considered. The obtained results were subjected to a statistical analysis using the *t* test of student. Hatching Rate (HR) was calculated according to the following formula [20].

$$HR = \frac{\text{number of hatching eggs}}{\text{total number of eggs}} \text{X100}$$

3. Results

3.1 Toxicity assays

The toxicity effect of the saponin against the different larval stages of *Cx. pipiens* was expressed by the mortality of the larvae during the treated developmental stage. The lower concentration of 5mg/l of the product caused mortality around 80% for the L1. This mortality, decreased with the older larval stages, where it was 30% and 27% for the L3 and L4 respectively. The highest concentration of 20mg/l caused 100% for L1 and L2 and a recorded mortality between 70% and 80% for the L3 and L4 of the mosquito species. The analysis of variance of the data showed a significant (p<0.001) insecticidal activity with a dose response relationship (Fig. 2). With probit analysis the lethal concentrations were estimated and were for the stage L3 as following; the LC_{50} = 7.46 mg/l and the LC_{90} = 19.98mg/l. For the stage L_4 the LC_{50} was estimated at 11.05mg/l and the LC_{90} was at 32.85mg/l (Table 2).

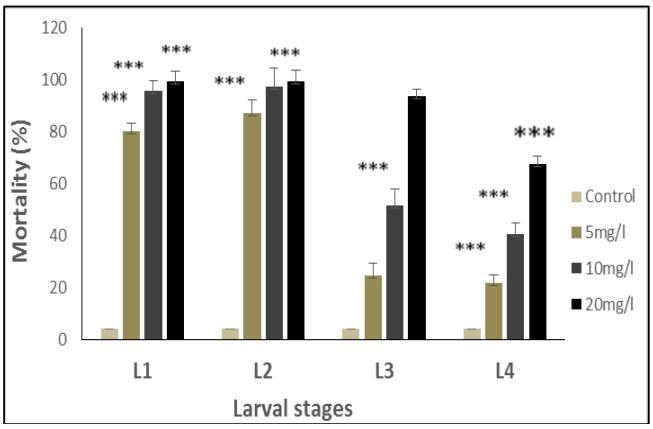

Fig 1: Concentration-response relationship of treatment of the saponin applied to newly ecdysed larvae of *Cx. pipiens.* (Means ± SD, n = 75).

Table 1: Toxicity of the saponin against *Cx. pipiens* larvae. (LC_{50} and LC_{90}, FL, mg/l).

Larval stage	Regression lenear	*Slope*	LC50 (mg/l) [LLC-ULC] Confidence limit (95%)	LC90 (mg/l) [LCL-UCL] Confidence limit (95%)
L1	y = 2.69 x +2.13	0.39	3.46 [2.11-5.23]	8.19 [5.55-12.18]
L2	y = 2.39 x +2.63	0.46	5.65 [3.18-8.56]	13.23 [11.45-18.30]
L3	y = 2.99 x +2.39	0.33	7.46 [5.16-9.35]	19.98 [15.16-23.76]
L4	y = 2.71x+2.17	0.36	11.07 [9.14-13.39]	32.85 [28.14-33.74]

3.2 Longevity of mosquito developmental stages

Table 2 shows the effect of the saponin on the adult longevity of the different developmental stages of *Cx. pipiens* after the treatment with their lethal concentrations (LC_{50} and LC_{90}) of both larval stages. Exposure of the instar larvae of both stages caused no difference in the duration of the all larval stages except with the LC_{90}. The following pupal stage no difference was recorded in the duration in all concentrations. However the adult longevity of mosquito species *Culex pipiens* was considerably reduced by the treatment of saponin. The longevity of adults of *Cx. pipiens* emerged from the treated 3rd instar larvae was reduced to 31 days with LC_{50} and 29 days with LC_{90}. However the adults emerged from L4, their longevity was reduced up 27 days with the LC_{90}.

Table 2: Effect of the saponin on the longevity of developmental stages; after treatment with the lethal concentrations (LC_{50} & LC_{90}), of 3rd and 4th instar larvae of *Cx. pipiens.* Means ± SD followed by the same letter indicate a significant difference (P<0.05). (n = 10-75).

Stage	Longevity of the developmental stages (day)after treatment of L3			Longevity of the developmental stages (day)after treatment of L4		
	Control	LC_{50} = 7.46 mg/l	LC_{90} = 19.98 mg/l	Control	LC_{50} = 11.07 mg/l	LC_{90}= 32.85 mg/l
L3	4.35±1.55	4.60 ±2.36	5.16 ±2,5			
L4	6.60±1.50	6.80±1.19	7.33±1.13[a]	6.60±1.88[a]	7.50±1.50[a]	8.50±2.33[a]
Pupae	3.53±1.66	3.43±1.43	3.43±1.33	3.53±1.76	3.33±1.66	3.43±1.21
Adult	32 ±1.23	31.53±2.33[b]	29.5±2.23[b]	32 ±1.23[b]	29±2.33[b]	27.53±3.66[b]

3.3 Effect of the saponin on reproduction

The effect of saponin on reproduction was evaluated on different parameters, of the females emerged from the treated fourth instar larvae of *Cx. pipiens* and is presented in table 3. Fecundity was highly reduced after the treatment with the saponin. The number of eggs laid was inversely proportional to the concentration in the treatment. For *Cx. pipiens* the number of eggs laid was reduced from 875 to 788 for LC_{50} and to 594 to LC_{90} (Table 3). Therefore the hatching rates showed a significant decrease according to the increase of the treatment with the saponin (Table 3). The essays showed also that the fecundity was highly reduced under the saponin effect, for the treated series compared to control.

Table 3: Effect of saponin on the reproduction of the females emerged from the treated fourth instar larvae of *Cx. pipiens* (n = 20 females). Means followed by the same letter indicate a significant difference (P<0.005).

Treatment	N° egg laid	Hatching rate (%)	Fecundity
Control	875[a]	98.66[a]	96.6[a]
CL_{50} = 16.21 µg/l	788[b]	73.33[b]	59.4[b]
CL_{90} = 75.85 µg/l	594[b]	55.02[b]	44.8[b]

4. Discussion

Mosquito control using conventional pesticides in the water sources introduces many risks to human health and the environment. In this context of integrated vector control, due to rapid development of mosquito resistance and the increase of environmental pollution, the use of conventional insecticides for mosquito control is no longer encouraged. Natural products of plant origin with insecticidal properties have been used in the recent past control of a different of insect pests and vectors. Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water, and thus, it is easy to deal with them in this habitat. During the last decades the effects of plant extracts were evaluated against different mosquito larvae species [21; 22]. The larvicidal activity of extract of *Leucas aspera* leaf, using hexane as a solvent, was tested for the on the larvae of *Culex quinquefasciatus* and *Aedes aegypti* [23; 24]. The leaf extract of *Acalypha indica* with different solvents, viz., benzene, chloroform, ethyl acetate and methanol, were found to have a larvicidal activity and ovicidal an effect on oviposition of *Anopheles stephensi* [25; 26]. The same it was reported that the leaf extract of *Citrullus vulgaris* with different solvents, have a larvicidal, ovicidal, and repellent activities against *Anopheles stephensi* [27]. The leaf extracts using acetone, chloroform, ethyl acetate, hexane and methanol of medicinal plants; *Aegle marmelos*, *Andrographis lineata*, *Andrographis paniculata*, *Cocculus hirsutus*, *Eclipta prostrate* and *Tagetes erecta* were tested against fourth-instar larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus. Andrographis paniculata* and according to their effectiveness they haves been used for pest control [28].

The bioassays using the saponin, against the larval stages of the mosquito species *Culex pipiens* showed a larvicidal activity expressed by a high larval mortality of the treated series compared to the control ones. The confirmed toxicity of the saponin was mentioned previously against other mosquito species [29; 30], such as *Aedes aegypti* [31], and *Anopheles stephensi* [32]. It was reported that some plant extracts have exhibit a toxic effect on mosquito larvae [33; 34; 35;] and other insect [36; 37].

In the present study**,** the saponin treatment reduced the larval duration, none the pupal and introverted the adult emergence. Those treated larvae escaped from mortality showed reduced longevity. The adult which emerged from treated larvae were morphologically normal but showed a great reduction in fecundity. The same results were mentioned against *Aedes aegypti* [31] and some insects [38]. Many reports showed changes in fecundity after treatment with biopesticide *B. thuringiensis* [8], insect growth regulators [39] and essential oil of plants [40].

5. Conclusion

From the present study it was concluded that the saponin proved good larvicidal agent against *Cx. pipiens* larvae in the laboratory and also reduced the longevity of different developmental stages, egg productions and fecundity.

6. References

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