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LARVICIDAL ACTIVITY OF EXTRACTS FROM SIX PLANT SPECIES ON LARVAE OF *Culiseta longiareolata* (DIPTERA; CULICIDAE)

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ABSTRACT

Because of the environmental problems and dangers to human health caused by chemical insecticides, the use of natural biocides seems to be imperative. In this context toxicity tests were carried out according to the protocol of the World Health Organization (WHO) for six aqueous extracts of leaves of: *Ambrosia Maritima, Hertia centifolia, Xanthium strumarium, Datura stramonium*, *Solanum elaeagnifolium,* and *Salvea verbena*, with a series of three doses for each extract. The evaluated extracts showed good larvicidal activity against the fourth instar larvae of *Culiseta longiareolata* mosquito, the decoction method is used for the preparation of extracts. The mortality rate increases depending on the concentration of the used extract and the exposure time, with interesting lethal concentrations LC50% and LC90%, *A. Maritima* (11.65 µg / ml and 52.40 µg / ml) after 120 hours and *D. stramonium* (16.94 µg / ml and 28.36 µg / ml) after 96 hours. While the lethal times LT50% and LT90% do not exceed (0,01 day to 2,14 day) at a dose of 219.9 µg / ml of *S. elaeagnifolium* (0,95 day to 1,34 day) with a dose of 160 µg / ml of *H. centifolia.*

S. elaeagnifolium, H. centifolia **and** ***D. stramonium*** **showed an excellent larvicidal activity of the aqueous extract of the leaves of the studied plants.**

Keywords: Biological activity; *Solanum elaeagnifolium; Hertia centifolia; Datura stramonium; Culiseta longiareolata;* lethal concentrations LC50%; lethal times LT50%.

INTRODUCTION

Many arthropods are vectors of diseases as malaria, lymphatic filariasis, and arboviruses such as yellow fever, dengue fever, viral encephalitis [1], and African horse sickness [2]. These characteristics give this fauna a high level of importance and sanitary interest [3]. Among these, mosquitoes are the most formidable because of their abundance rather than the diseases they transmit. *Culiseta longiareolata* is considered as a vector of bird plasmodium; it can experimentally transmit West Nile Virus. Given its trophic preferences, its role as a vector of human parasitosis can only be very limited [13].

For several years, the control methods practiced sporadically have been done by spraying chemicals. However, the massive use of these products was not long in experiencing several difficulties, in addition to the phenomena of resistance, the imbalance of ecosystems, the lack of specificity, and the residual effect in non-biodegradable insecticides are the most frequent [4, 5]. To ensure better intervention while protecting the natural environment as much as possible, new preventive methods and new products were constantly sought. Thus, to contribute to sustainable environmental management, implementing new mosquito control alternatives is further encouraged [6].

The use of plant extracts as insecticides has been known for a long time. Indeed, pyrethrum, nicotine, and rotenone are already known as insect control agents [7]. According to [8], more than 2000 plant species with insecticidal activity have already been identified. Recently, the litter of alder, a plant rich in polyphenols, has been shown to have critical toxic properties towards the larvae of *Culex pipiens* mosquitoes [9].

Algeria has one of the most diversified and original flora in the Mediterranean basin comprising 3139 species which 653 are endemic [10]. The Sahara includes about 500 taxa of higher plants [11], some of which are still used today by the natives as medicinal plants [12]. Within the framework of the valorization of the Algerian flora. We are focused on a study to determine, mainly in the laboratory, the toxicity of the aqueous extracts of the leaves of six plant species belonging to the Asteraceae, Solanaceae and Lamiaceae families (*Ambrosia Maritima*, *Hertia centifolia, Xanthium strumarium*, *Datura stramonium*, *Solanum elaeagnifolium* and *Salvea verbena*) on the fourth instar larvae of the mosquito *Culiseta longiareolata*.

MATERIALS AND METHODS

Biological Model: *Culiseta longiareolata*

The larvae of *Cs. longiareolata* submitted to toxicity tests come from untreated larval deposits located at a pond and a well in rural areas in the Wilaya of Batna (Algeria). They were kept in the laboratory in mass-rearing containers containing 250 ml of dechlorinated water and insect food. The latter is a mixture of cookies (75%) and yeast (25%). The containers of our breeding are placed in cages, and the breeding is conducted at a temperature of 25°C and a hygrometry of 70%.

The Plants Used

In total, six plant species were used for this work. The tested plants were harvested from 6 regions of Algeria (Table 1). The aerial part of each plant species was dried in the shade in a dry and airy place at an ambient temperature of 25°C for two weeks for each of them.

Toxicity Tests

Preparation of aqueous plant extracts

To prepare the aqueous extracts of the six plant species, we used the method of decoction consisting in 3 steps: leaves soaked in distilled water, and boiled for 30 minutes on a basin spout.

Table 1. Characteristics of the harvest regions of the insecticide plants studied

Plants	Harvest region	Latitude	Longitude	Weather
A. Maritima	Bejaia (Algeria)	36° 45′ 00″ N	5° 04′ 00″ E	Humid
H. centifolia	Bordj-Bou-Arreridj (Algeria)	36° 04′ 00″ N	4° 46′ 00″ E	Semi arid
X. strumarium	M'Sila (Algeria)	35° 42′ 07″ N	4° 32′ 48″E	Arid
D. stramonium	Skikda (Algeria)	36° 52′ 0 ″ N	6° 54′ 0″ E	Humid
S. elaeagnifolium	M'Sila (Algeria)	35° 42′ 07″ N	4° 32′ 48″ E	Arid
S. verbena	M'Sila (Algeria)	35° 42′ 07″ N	4° 32′ 48″ E	Arid

The resulting mixture was filtered and stored in labeled bottles to the refrigerator at 4 °C. From each aqueous extracts, three concentrations (C1, C2 and C3) were prepared (Table 2).

Table 2. The concentrations of the aqueous extracts were tested on the larvae fourth Stage

Plant species	Applied concentration (µg/ml)		
	C1	C2	C3
A. Maritima	7,51	14,67	61,63
H. centifolia	38,09	72,72	160
X. strumarium	22,52	33	70,45
D. stramonium	16,58	38,7	69,66
S. elaeagnifolium	128,3	188,8	219,9
S. verbena	11,92	27,83	50,1

(C1: low concentration, C2: medium concentration, C3: high concentration).

Preparation of larvae for a controlled trial

In a beaker of 300 ml capacity, 20 larvae of the fourth instar (L4) of *Cs. longiareolata* were put with 200 ml of spring water and a dose (C) of the previously prepared aqueous extract added by a mixture of washer and cookie to ensure their nutrition. After preliminary trials, we administered the three concentrations (C1, C2, and C3) for each plant. Each concentration was applied on three replicates with a preparation of 20 control larvae. The number of dead individuals (L4 larvae, pupae, or adults) was recorded daily.

Statistical Analysis

Toxicity tests were calculated according to the mathematical procedures of [14]. The lethal concentrations (LC50% and LC90%) and the lethal times of each concentration used (LT50% and LT90%) for each of the bio-insecticides used.

The observed mortality rate is corrected by the Abott formula which allows to know the real toxicity of bio-pesticides. The different rates undergo an angular transformation according to the Bliss tables. The data are thus normalized and are the subject of an analysis of variance on XLStat 2009. The data obtained are then transformed into probits, which makes it possible to establish a regression line according to the decimal logarithms of the concentrations used. The Chi2 test allows a good fit of the regression line. From this line, we calculate the lethal concentrations. The same statistical analysis was used to calculate the lethal times for each concentration used (LT50% and LT90%).

RESULTS

Variation of the Mortality Rate

After exposing the fourth stage larvae of *Cs. longiareolata* species to different concentrations of 6 different aqueous extracts, the mortality rate varies according to the concentrations (Fig. 1). For the majority of extracts, larval mortality exceeds 50% of the mean concentration (C2). However, in the extract of *H. centifolia* and *S. elaeagnifolium*, mortality is reached at a percentage of more than 50% from the low concentration C1 (56.67% and 59.43%), respectively. On the other hand, in the extract of *S. verbena*, mortality does not exceed the threshold of 30% (30.56%) even at a high concentration (C3). From all these results, a first classification of the toxic effects of the tested extracts is highlighted.

Toxicological Parameters

Lethal concentrations

The results showed a strong positive correlation between the mortality rates recorded and the concentration of the extract used against mosquitoes (Table 3).

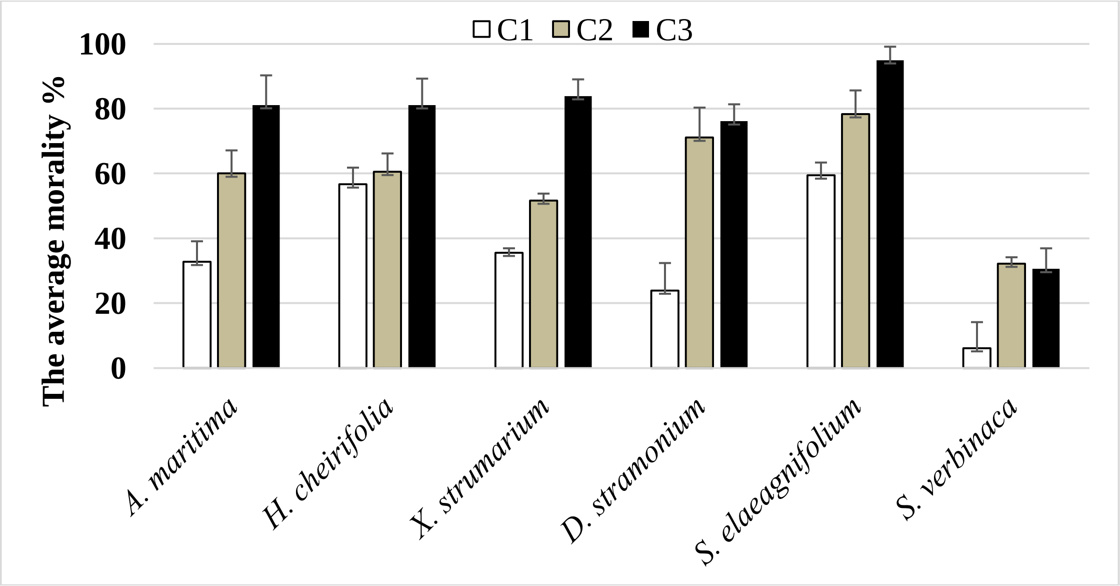

Fig. 1. The average corrected mortality of *Cs. longiareolata* larvae fourth stage treated with different plants and concentrations (µg/ml)

To ensure 50% mortality of insects after 72 h, the concentration of *A. Maritima* must be equal to 17.43 µg / ml, while 515.99 µg / ml of the leaves ensure 90% mortality. At 120 h, the LC50% was 11.65 µg / ml, while the LC90% was 52.40 µg / ml (Table 3).

After 24 hours of treatment, the lethal concentration for 50% of the population is 207.75 µg / ml, which decreased to 23.24 µg / ml after 72 hours of treatment. Mortality of 90% of the larvae caused with 47.28 µg / ml concentration (Table 3).

To ensure 50% mortality of insects after 120 h, the concentration of *X. strumarium* must be equal to 21.23 µg / ml. On the other hand, 90% of larvae die with a concentration of 53.19 µg / ml (Table 3).

The results showed a strong positive correlation between the mortality rates recorded and the concentration of *D. stramonium* used against mosquitoes. 50% of the larvae die after 48 h when the concentration of *D. stramonium* is 91.23 µg / ml, while 336.16 µg / ml ensures 90% of the sample. After 72 h of treatment, the LC50% and LC90% do not exceed 16.94 µg / ml and 28.36 µg / ml, respectively (Table 3).

In order to eliminate 50% of the mosquito population studied, the concentration of the extract prepared with *S. elaeagnifolium* should be 135.3 µg / ml in 48 h and 104.04 µg / ml after exposure of *Cs. longiareolata* larvae at 196 h (Table 3). However, a dose of 196.09 µg / ml is sufficient to achieve a 90% mortality rate of the insect after 196 h.

A concentration of 84.08 µg / ml can kill 50% of the larvae at 24 h and 39.16 µg / ml at 120 h, eliminating 90% of the Culicidian population after 120 h, the concentration must be equal to 166.18 µg / ml (Table 3).

Lethal times

The toxic effect of the analyzed extracts is clearly apparent through the TL50 values. These values decrease when the concentration of the tested extract increased, reflecting the excellent efficacy of the tested extracts (Table 4).

Results showed a positive correlation between the mortality rate and the time of exposure of the larvae to the *A. Maritima* extract. With 7,51 µg / ml of extract 50% of the population of *Cs. longiareolata* can be eliminated in about 34,85 days, and 90% of these mosquitoes can be eliminated in 30651,1 days of treatment. The LT50% and LT90% are respectively 2,23 days and 4,52 days when a concentration of 61,63 µg / ml of the extract is applied (Table 4).

The three doses of *H. centifolia* 38,09 µg / ml, 72,72 µg / ml, and 160 µg / ml confirms a positive correlation between the mortality rate of larvae of *Cs. longiareolata* and the exposure time to the extract. The death of 50% of the treated mosquito population is assured after 1,75 days with the low concentration of 38,09 µg / ml and after 0,95 days with the highest concentration 160 µg / ml. The LT90% reaches 1,34 days for the highest concentration.

From the results showed in the (Table 4) also, it appears that there is also a strong correlation between the mortality rate and the time of exposure of the larvae to the different concentrations of *X. strumarium* (R^2 = 0,910 to 0,995). Calculated lethal times are 56.29 h 2,34 days to 5,07 days for 50% mortality and vary between 4,20 days and 12,14 days for 90% LT.

The results also confirm that treatment of the mosquito sample with different concentrations of *D. stramonium* shows a significant correlation between mortality rate and exposure times with a regression line of form Y=-7,32+6.20X (R^2=0,986) at dose 16,58 µg / ml. A period of 4,04 days is necessary to kill 50% of the larvae at 16,58 µg / ml and 2,16 days at a high concentration of 69,66 µg / ml, while to eliminate 90% of the Culicidian population with the concentration of 69.66 µg / ml it is necessary to expose the larvae to the product for 2,68 days (Table 4).

Regarding the dose of 128.3 µg / ml of *S. elaeagnifolium*, the calculations show a strong correlation between mosquito mortality and exposure time since the correlation coefficient is 0,998. Whose regression line is given by the formula Y=3,2 +1,04X. The calculated lethal times are 2,24 days and 38,1 days for 50% and 90% control. For the highest concentration, 219,9 µg / ml. The regression line is of the form Y=5,27+0,59X (R^2=0,994), showing a correlation between mortality and exposure time. LT50% and LT90% are much lower since they do not exceed 0,01 days and 2,14 days respectively (Table 4).

Regarding lethal times, the lowest concentration being 11,92 µg / ml of *S. verbena*, can eliminate 50% of the *Cs. longiareolata* population in about 161,53 h and 90% during 11,17 days of treatment. When a dose of 27,83 µg / ml of *S. verbena* solution is applied. The LT50% is 4,90 days. While the LT90% is 303,6 h. The higher concentrations of 50,1 µg / ml of the calculated lethal times (LT50% and LT90%) are 4,56 days and 15,21 days, respectively (Table 4).

Table 3. Lethal concentrations (µg / ml) LC50% and LC90% of aqueous extracts of 6 plant species concerning L4 larvae of *Cs. Longiareolata*

Concentration	Family	Species used	T1 (h)	T2 (h)	T3 (h)
LC 50%	**Asteraceae**	*A. Maritima*	17,43	12,05	11,65
		H. centifolia	207,75	35,28	23,24
		X. strumarium	46,41	31,88	21,23
	Solanaceae	*D. stramonium*	91,23	25,37	16,94
		S. elaeagnifolium	135,3	105,46	104,04
	Lamiaceae	*S. verbena*	84,08	54,95	39,16
LC 90%	**Asteraceae**	*A. Maritima*	515,99	76,07	52,4
		H. centifolia	813,11	63,32	47,28
		X. strumarium	141,74	78,74	53,19
	Solanaceae	*D. stramonium*	336,16	48,85	28,36
		S. elaeagnifolium	197,25	208,3	196,09
	Lamiaceae	*S. verbena*	142,6	239,88	166,18

(T1: minimum time, T2: medium time, T3: maximum time).

Table 4. Lethal times (day) LT50% and LT90% of the aqueous extracts of 6 plant species concerning L4 larvae of *Cs. Longiareolata*

Time	Family	Species used	C1(µg / ml)	C2(µg / ml)	C3(µg / ml)
LT 50%	**Asteraceae**	*A. Maritima*	34,85	3,40	2,23
		H. centifolia	1,75	1,71	0,95
		X. strumarium	5,07	3,79	2,34
	Solanaceae	*D. stramonium*	4,04	2,24	2,16
		S. elaeagnifolium	2,24	1,41	0,01
	Lamiaceae	*S. verbena*	6,73	4,90	4,56
LT 90%	**Asteraceae**	*A. Maritima*	30651,7	15,45	4,52
		H. centifolia	2,90	2,90	1,34
		X. strumarium	12,14	6,57	4,20
	Solanaceae	*D. stramonium*	6,50	3,42	2,68
		S. elaeagnifolium	38,1	6,16	2,14
	Lamiaceae	*S. verbena*	11,17	12,65	15,21

(C1: low concentration, C2: medium concentration, C3: high concentration)

DISCUSSION

As in public health (vector control programs) and veterinary medicine (livestock pest control treatments), the increasing use of insecticides over the last 40 years has resulted in a steady increase in the number of resistant species. In addition to compromising the effectiveness of control measures, this phenomenon of resistance can have worrying economic and health, and ecological repercussions through increased doses of insecticides [15]. To contribute to sustainable environmental management, the introduction of new mosquito control alternatives is further encouraged.

In more recent work, the aqueous extracts, powders, and essential oils of plants contain molecules with insecticidal properties [19]. The results on the larvicidal activity of aqueous extracts of castor-oil leaves (*Ricinus communis* L.) and cedar wood (*Tetraclinis articulata* (Vahl) Mast.) [16] and *Ruta chalepensis* L. (Rutaceae) [17, 18] on the larvae of four Culicidae mosquitoes, namely *Cx. pipiens, Aedes caspius, Cs. longiareolata,* and *Anopheles maculipennis* confirmed their insecticidal efficacy on Culicidae larvae.

In our research, the toxicity of aqueous extracts of six plants *A. Maritima, H. centifolia,* and *X. strumarium* (Asteraceae) *D. stramonium* and *S. elaeagnifolium* (Solanaceae) and *S. verbena* (Lamiaceae) was tested on *Cs. longiareolata*.

Our results showed that the six plants caused mortality of the larvae depending on the used concentration and the treatment time. We have shown that lethal concentrations (LC50%, LC90%) decrease with the duration of treatment.

At the 120h treatment time based on the aqueous extract of the leaves of *A. Maritima*, the average mortality rate of the larvae increases and can reach 81,13% when using the highest concentration (61,63 µg / ml), whose LC50% is equivalent to 11,65 µg / ml. In contrast, the LC90% is equal to 52,40 µg / ml. Whereas the lethal times LT50% and LT90% are respectively 2,23 days and 4,52 days. In fact *A.Maritima* is cultivated in parts of Africa for medical use [20]. Much recent work indicates molluscicidal effects against *Lymnaea cailliaudi* [21], *Limnaea natalensis,* and *Bulinus guernei* [22].

Treatment with *H. centifolia* showed a high toxicity on *Cs. longiareolata* with 81,10% of dead larvae after 72h when a high dose of the extract was applied (160 µg / ml). This result is in concordance with that of [23, 38] in which they approved the insecticidal action of *H. centifolia* on all instars larvae of *Cx. pipiens* in Algeria and on mites in Tunisia. The spasmolytic and anti-inflammatory effects of crude extracts from the vegetative part of this plant have been reported by [24]. In the other hand, the extract of *X. strumarium* causes a high mortality of *Cs. longiareolata* (83, 90%) after 120h. Studies of [25, 26, 27] have reported that *X. strumarium* induces intoxication and can be fatal to cattle, sheep, pigs, and humans [27]. Other works of [39, 40] showed the antibacterial and antifungal activities of this plant.

The Solanaceae family is one of the most prominent plant families, with more than 2500 species scattered over all continents, in both tropical and temperate climates. The chemical diversity of this family is essential and formidable poisons are derived from it [28]. Thus, the aqueous extract of *D. stramonium* leaves showed a toxic action against larvae of *Cs. longiareolata* with a 76.13% larval mortality at the 96-hour treatment time based by C3.

According to [29], *Datura* spp, are toxic and produce tropane alkaloids, bicyclic organic compounds, and nitrogenous compounds that significantly affect human and animal physiology. Ethanol extracts from *D. stramonium* leaves have been evaluated for larvicidal and repellent activities against the *mosquitoes Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* [30]. Further, the treatment by *S. elaeagnifolium* extracts showed a high mortality rate (94,97%) after 196h when a high dose of the extract (219,9 µg / ml) was applied. This result is in concordance with that of [31], in which they showed the molluscicidal, nematicidal and anticancer properties of *S. elaeagnifolium*. As far as [32], recorded the highest larval mortality of the flour beetle (*Tribolium castaneum*) treated with methanolic seed extract of *S. elaeagnifolium*. In Algeria, the Lamiaceae family is represented by 146 species [33] with 40% known for their aromatic properties [35]. In this work, the extract of *S. verbenaca* causes 30.56% mortality of *Cs. longiareolata*, after 120 hours. Indeed, *S. verbena* could be considered as a potential source of natural antihemolytic, enzyme modulating, antioxidant and antibacterial agents [36].

CONCLUSION

Although preliminary, these results showed an excellent larvicidal activity of the aqueous extract of the leaves of the studied plants, mainly *S. elaeagnifolium* and *H. centifolia*. They can be considered larvicide promoters for mosquitos control. Toxicological tests were used to determine the LC50%, LC90%, LT50%, and LT90% for the aqueous solution. The extract acts on mortality depending on the concentration used and the exposure time of the larvae. The toxicity process is essential, and it seems that the active substances of the plants have been put in solution against the digestion, which causes the death of the larvae. It is necessary to test other concentrations and other extraction methods that may give better results.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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