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Laboratory study of the larvicidal efficacy of a local plant *Hertia cheirifolia* against the most abundant mosquito species, in Algeria

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Abstract
Mosquitoes are vectors of several diseases affecting humans and domestic animals worldwide. Plants seem to be alternative sources, instead of conventional pesticides, for mosquito control agents. Essential oils are used fumigants and have low toxicity to non target animals and toxicity to insect pests. The purpose of the present study was to assess the larvicidal activity of a medicinal plant, *Hertia cheirifolia,* against *Culex pipiens* mosquito, under laboratory conditions. The toxicological assays were carried out using a powder formulation of *Hertia cheirifolia,* with different concentrations (8, 16 and 48g/l), on the fourth instar larvae of *Culex pipiens,* for 24 hours as recommended by World Health Organization (WHO), to determine the two lethal concentration LC_{50} and LC_{90}. The obtained results showed a highly significant effect of this plant with all tested concentrations, with dose response relation-ship mortality, for all treated larval stages. Using the highest concentration of 48g/l of the plant extract the mortality increased throughout the stages; when it was 98.66% for the L1, 93.33% for L2, 86.66% for L3 and 82.66% for the oldest stage L4. The lethal concentrations LC_{50} and LC_{90} were estimated with confidence limits, and their values increased inversely to the larval stages.

Keywords: *Culex pipiens, Hertia cheirifolia,* insecticides, medicinal plant.

1. Introduction

Mosquitoes are the most important group of insects in terms of public health importance, which transmit a number of diseases not only the type that afflict humans, they also transmit several diseases and parasites that afflict animals too [1]. The insect populations control around the world is primarily dependent upon chemical insecticides and fumigants [2]. 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 environment, non-target organisms, and human health concern [3, 4]. The increasing concern; over the level of pesticide residues in the different environmental sites and especially in food, has encouraged researches to find new alternatives of conventional pesticides [5]. These synthetic pesticides are expensive and have in many cases only produced moderate results along with major ecological damage [6, 7, 8]. Plants have generated extraordinary interest in recent years as potential alternatives natural insect control agents. Botanical extracts have several advantages over traditional pest control agents; such as specificity, biodegradability and low mammalian toxicity [5].

The major mosquito control operations were carried out using synthetic insecticides, such as organochlorine and organophosphate compounds [9]. It though these products play a vital role in controlling insects, but from their abusive use some negative impacts were emerged on environment generally against the non-target organisms, and a harmful effect specially on human health [10-13].

The natural biodegradable insecticides are relatively harmless to non-target organisms including humans [14, 15]. Accordingly, the application of easily degradable plant compounds is considered one of the safest methods of control of insect pests and vectors [16, 17, 18]. Several plants used in traditional medicines were tested their mosquito larvicidal activities [19, 20]. These bioactive chemicals, due to their properties, may act as larvicides, adulticides, and ovipositional attractants against different mosquito species [21, 22]. In view of an increasing interest in developing plant origin insecticides, as an alternative to chemical insecticide, the present study was undertaken to evaluate the larvicidal activities of the medicinal plants, *Hertia cheirifolia,* against a medically important mosquito species, *Culex pipiens*.

2. Materials and methods

2.1 Mosquito rearing

The bioassays, using the plant extract of *Hertia cheirifolia,* were conducted in the laboratory according to the testing methods for larval susceptibility [23]. The larvae of *Culex pipiens* were obtained from laboratory mass colonies. Larvae were reared in storage jars containing 500 ml of stored tap water and maintained at temperature between 25-27 °C, 85% of relative humidity(R H) and a photoperiod of 14:10 (L:D). They were fed with fresh food consisting of a mixture of Biscuit-dried yeast (75:25 by weight), and water was changed every three days. During pupal stage, the pupae were transferred to other jars containing 500 ml of water with the help of a dipper and were kept in mosquito cage for adult emergence. The adults were fed with 10% sugar solution and supplied with blood, using small animals, for the egg maturation [24].

2.2 Plant material

Hertia cheirifolia is a small plant with yellow flowers, which grows in the border fields in the eastern part of Algeria and Tunisia. The genus *Hertia* with its twelve species is distributed over South and North Africa and South-west Asia [25]. It is used in traditional medicines for the treatment of spasm, inflammation, diarrhea and haemorrhoid [26]. Various extracts of *Hertia cheirifolia* were also tested for their spasmolytic and anti-inflammatory activities [26, 27]. The vegetable matter of *Hertia cheirifolia* was collected during the spring period from a semi-arid region, Tebessa district (North-East of Algeria). The areal parts of the plant were washed with distilled water, shade-dried and ground. The finely ground plant powder (500 g/l hot water) was extracted with infusion using hot water the extraction was continued till visibly no further extraction is possible. The crude extracts and stored at 4 °C. Standard stock solutions were prepared at 1% by dissolving the residues in ethanol. From this stock solution, different concentrations were prepared and these solutions were used for larvicidal bioassays.

2.3 Toxicity bioassay

The larvicidal efficacy of *Hertia cheirifolia* was determined against *Culex pipiens* with a powder extracts. The toxicological essays were carried out with three concentrations equivalent to 8, 16 and 48 g/l of the medical plant *Hertia cheirifolia* against the different mosquito larval stage L1, L2, L3 and L4 of *Culex pipiens*, under the laboratory conditions. The treatment, with this extract plant, 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 of the larvae, according to the world health organization recommendations [28], to the extract the water is changed and the food is added every three days until the emergence of the adults. The mortality of the control and treated series is recorded daily and followed during the other developing stages until the emergence of adults.

2.4 Statistical analysis

The mortality percentage was subjected to correction using the Abott formula [29] when the mortality was observed in the control series. After the toxicity data were subjected to probit analysis [30], to determine the lethal concentrations (LC_{50} and LC_{90}) of the treated larvae of the tested species, with confidence limits (95%) of upper confidence limit (UCL) and lower confidence limit (LCL) values, slope, and regression equation of the concentration-mortality lines were calculated [31].

3. Results

The results of the larvicidal activity of the plant extract *Hertia cheirifolia*, against the four larval stages of the most abundant mosquito *Culex pipiens* are expressed by the recorded mortality observed during the age of the treated stage. The mortality percentage for all stages is presented in Table 1. Because of the mortality was observed in the control series with highest result of 6.66% for L2, it was corrected in the treated ones and is presented in table 2.

Table 1: The observed mortality (%) of different mosquito larval stages of *Culex pipiens* exposed to different concentrations of the plant extract *Hertia cheirifolia. SD=* Standard deviation. Values are mean±SD of 3 replicates.

Instar larvae	observed Mortality (mean±SD) throughout the duration of the treated stage			
	Control	**8 g/l**	**16 g/l**	**48 g/l**
L1	5.33± 1.88	68.00±8.00	77.33±8.32	98.66±2.30
L2	6.66±1.88	53.33±8.32	81.33±10.06	93.33±2.30
L3	4.00±0.00	34.66±6.11	50.66±10.06	86.66±8.32
L4	4.00±0.00	28.00±8.00	37.33±6.11	82.66±2.30

The mortality throughout the stage of each instar larvae of *Culex pipiens* with different concentrations of *Hertia cheirifolia* is varying between stages (table 2). Under the lower concentration of 8g/l the mortality mean was 65% for L1 and was decreasing with the older stages up to 28% for the stage L4. Using the highest concentration of 48g/l of the plant extract the mortality increased for all stages, when it was 98.66% for the L1, 93.33% for L2, 86.66% for L3 and 82.66% for the oldest stage L4. It was noticed that the most stage is younger the most mortality is higher. Therefore we can conclude that the younger larvae are more sensitive than the oldest ones. The results of the statistical analyzes reveal a highly significant effect ($P<0.000$).

Table 2: The corrected mortality (%), using the Abott formulation, of different mosquito larval stages of *Culex pipiens* exposed to different concentrations of the plant extract *Hertia cheirifolia. SD=* Standard deviation. Values are mean±SD of 3 replicates.

Instar larvae	Corrected Mortality (mean±SD) throughout the duration of the treated stage		
	8 g/l	**16 g/l**	**48 g/l**
L1	65.09±7.99	75.90±9.48	98.61±2.40
L2	50.11±7.63	80.13±10.36	92.87±2.41
L3	31.94±6.36	48.61±10.48	86.10±8.67
L4	24.99±8.33	34.71±6.36	81.94±2.40

The equation and the regression line were determined, after the transformation of the corrected mortality to probit and the tested concentrations in decimal logarithms (Figure 1). The coefficient of determination (R^2 = 0.916, 0.840, 0.987 and 0.950 for larval stages; L1, L2, L3 and L4 respectively) reveals an equally positive relation between the probit and the decimal logarithms of the tested concentrations (Figure 1).

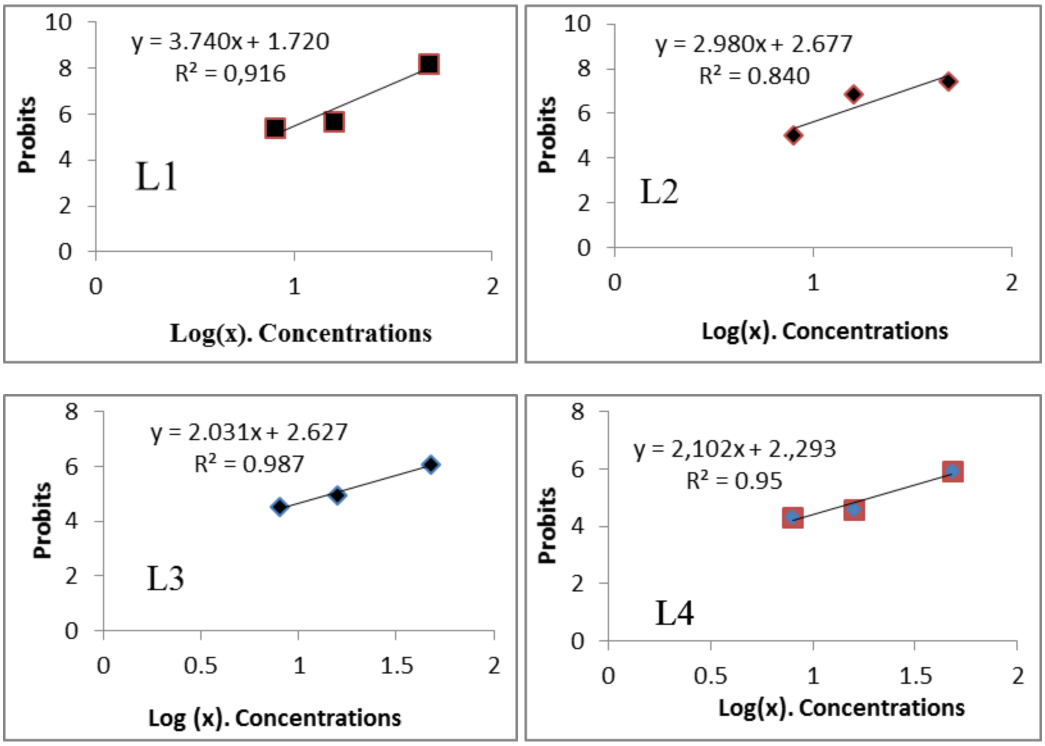

Fig 1: The equation of regression of probits (Mortality %) according to the decimal logarithms (log.x) of concentrations for the different larval stages (L1, L2, L3 and L4) of *Culex pipiens*.

For the concentrations tested, the highest larvicidal activity was observed with the highest one 48 g/l of *Hertia cheirifolia* extract against *Culex pipiens larvae* with the LC_{50} and LC_{90} values of 6.16, 6.76, 14.45 and 19.05 g/l and LC_{90} values of 21.37, 34.67, 61.63 and 77.65 g/l (Table 3). The lethal concentrations (LC_{50} and LC_{90}) were estimated at the 95% confidence limits LC_{50} (LCL=Lower CL- UCL=Upper CL) and LC_{90} (LCL-UCL) estimated from the linear regression (Figure 1). The LC_{50} and LC_{90} with their confidence limits, the regression equation and the slope values were also calculated and presented in table 3. The plant extract showed dose-response relationship mortality. At higher concentrations, the larva showed restless movement, without flickering, for some times with abnormal wagging and then died.

Table 3: Toxicity of of *Hertia cheirifolia* extract against *Culex pipiens larvae* (LC_{50}, LC_{90}, LCL, UCL g/l)

Instar larvae	Linear regression	*Slope*	LCL >LC_{50}> UCL(g/l) Confidence limit (95%)	LCL >LC_{90}> UCL (g/l) Confidence limit (95%)
L1	y = 3.740x + 3.0965	2.63	5.54 >6.16 >6.83	19.25>21.37>23.72
L2	Y=2.980x + 3.4762	3.54	5.25>6.76>8.78	26.66>34.67>45.07
L3	y = 2.031x + 2.6276	3.09	11.03>14.45>18.92	47.66>61.63>80.76
L4	y = 2.1027x + 2.2939	2.95	14.43>19.05>25.14	58.85>77.65>102.45

4. Discussion

The plant bioactive compounds show promising alternatives for the conventional pesticides and subsequently can be used to develop environmentally safe vector and pest managing agents. Phyto-extracts are emerging as potential mosquito control agents, with low-cost, easy-to-administer and risk-free properties [31]. Simple crude extracts from plants have tested as insecticides exhibited a toxic effect against different mosquito species [32, 18, 33]. The screening of local medicinal plants for mosquito larvicidal activity may eventually lead to their use in natural product-based mosquito abatement practices [31].

In the present work, the larvicidal effect of a local medicinal plant *Hertia cheirifolia* was evaluated against the different larval stages of the most abundant mosquito species *Culex pipiens* in Algeria. The treatment with this plant extracts at different concentrations show a toxic effect expressed by a high mortality compared to the controls. The search of natural products, specifically the plant extracts that can be used as product in insect control, in Algeria, is emerging through a multitude of many works [34, 35, 18, 33]. The use of plant extracts against mosquito species such as *Culex pipiens* and *Culiseta longiareolata* have shown the high efficacy for controlling the larval stages [36]. The activity of oleic and linoleic acids extracts of *Citrullus colocynthis* on larvae of mosquitoes of *Aedes aegypti,* and *Culex quinquefasciatus* was demonstrated by a different mortality rate of the treated larvae and its was concluded that these products have a larvicidal effects [37]. Other studies have documented the efficacy of plant extracts as bioactive toxic agents against mosquito larvae; such as tested plants extracts; *Melia azedarach*, *Rhazya stricta*, *Jatropha curcas*, *Artemisia herba alba*, *Calotropis procera* and *Matricharia chamomella*, against *Aedes aegypti* larvae [38]. These results show an inhibition of adult emergence and the recorded mortality of the treated fourth instar larvae was increasing differently according to the mode of action of each plant extract. The larvicidal activity of ethanol extracts from leaves of three plants; castor bean (*Ricinus communis*), vinca (*Vinca rosea*) and lantana *(Lantana camara*), using 5 concentrations for each plant, against the third instar larvae of the main mosquito vector of malaria *Anopheles arabiensis*, showed that all higer concentration recorded 100% mortality of the tested larvae [39]. Previous work confirmed that plants like *Lantana camara* and *catharanthus roseus* have insecticides properties and seemed to be better as vector control agents than the synthetic pesticides [40]. Also, the use of phytochemicals, derived from 36 species of plants, in the control of mosquito vectors, 29 plants extracts showed a larvicidal activity, with 100% mortality, against the third instar larvae of the treated mosquitoes at varying concentrations [41]. These plants have the potential to be used as effective larvicides, signifying an eco-friendly approach for the control of mosquito vectors.

5. Conclusion

In Algeria, mosquito control program is based mainly on chemical pesticide applications and only against the adult stage. The adulticidal control is applied indoor throughout the year, by the citizens. The government interventions are carried out particularly when a reduction of the mosquito density is needed, especially during the hot periods, to avoid the transmission of a mosquito-borne infection or to control pest and nuisance mosquitoes from inaccessible breeding areas; such as building caves. The presence of mosquitoes affects public events (tourism, festivals, sport competitions, etc.). For the mosquito control, portable foggers or truck-mounted fogging equipment are used and areas are treated unfortunately with conventional pesticides or carburant. According to the toxicological effect of the plant extract, *Hertia cheirifolia,* it is recommended to be used as an alternative for mosquito control and better against larval stages.

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