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Effects of Aqueous Extracts of *Daphne gnidium* (Thymelaeaceae) Leaves on Larval Mortality and Reproductive Performance of Adult *Culex pipiens* (Diptera; Culicidae)

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Abstract *Daphne gnidium, better known as Garou or the Saint Wood, is a shrub of the Thymelaeaceae family. All parts of the plant are toxic, mainly the leaves and fruits. Our study aims to highlight the toxic effect of aqueous extracts from fresh leaves of Daphne gnidium (Thymelaeaceae) on fourth-stage larvae of Culex pipiens. A high mortality has been noticed due to this plant that varies according to the exposure time and used concentrations. The LC50 and LC90 values for Daphne gnidium were specified. We also demonstrated that aqueous extracts of fresh leaves of Daphne gnidium have greatly disturbed the fecundity and fertility of adults from treated larvae.*

***Key Words*:** *Culex pipiens, aqueous extract, Daphne gnidium, Mortality, Fecundity.*

Introduction:

The Thymelaeaceae are a small family of dicotyledons composed of about 1200 species divided into 67 genera (Borris et *al*., 1988). They are deemed toxic due to their content of diterpene esters of tigliane or daphnane types. The Thymelaeaceae have several bactericidal, molluscicidal, fungicidal and especially larvicidal properties, (Bruneton, 1999), and the best species of this family is *Daphne gnidium*.

Daphne gnidium (Thymelaeaceae) known as Garou is a shrub of Mediterranean scrubland and the Atlantic sands. It can be found throughout the Algerian Tell. This plant is widely used in traditional medicine and pharmacology since it has many properties: antiseptic, purifying, healing, sudorific and abortive. It is known for its cytotoxic, antioxidant and antimicrobial action. Several authors have demonstrated its toxicity for insects. (Mohammedi, 2013).

As part of our search for natural products used in the biological control, aqueous extracts of *Daphne gnidium* leaves were tested on larvae of *Culex pipiens*. It is a cosmopolitan species that acts as a major vector in the transmission of many diseases and considered as an important nuisance to human populations exposed to itsbites in most African countries (Subra 1981).

We are interested in the toxic effect of aqueous extracts of *Daphne gnidium* leaves on larval mortality and the effect on fecundity and fertility of adult female *Culex pipiens*.

2. Materials and Methods :

2.1. Mosquito Rearing:

The larva used in this study provokes a mass livestock of adults collected in urban areas of Annaba, East Algerian city. Livestock is kept in laboratory cages (20 x 20 x 20 cm) at a temperature of 25 ± 2 °C, humidity of 75 ± 10% and a 12-hour scotophase. A mixture of biscuit and dried yeast insures the nutrition of larvae while the adults feed on dried raisins.

2.2. Plant material:

Daphne gnidium was harvested at the flowering phase from the region of Annaba (Algeria) during the period that runs from March to October. Although all parts of the plant are toxic, seeds and barks of different Daphne species contain toxic diterpenes: the daphnetoxin (bark) and mezerein (seeds) (Mohammedi, 2013). The fruits ingestion triggers ulceration of the digestive tract, and the contact of barks with skin or mucous membranes causes significant irritation. (Mohammedi, 2013).

2.3. Preparation of plant extracts:

To prepare the aqueous extract of the plant, 300 g of *Daphne gnidium* fresh leaves, preferably cut into small pieces, are mixed with the distilled water and let to boil for 75 minutes on a hot plate at 180 ° C. The obtained mixture was filtered through filter paper and one liter of filtrate is recovered.

2.4. Treatment:

Treatment of *Culex pipiens* was inspired by the technique of standardized sensitivity tests of the World Health Organization (OMS, 1963). The tests are carried out in beakers with a capacity of 200 ml each containing 20 fourth-stage larvae of *Culex pipiens* (L4) in 100 ml of spring water. After a preliminary test, three concentrations of the L4 stage larvae (27 g/l, 60 g/l and 100 g/l) were administered. These values are chosen in order to get the lethal doses 100 (LD100). Each concentration is applied to 3 repetitions, with a preparation of 20 larvae of *Culex pipiens* as a control.

After 24 hours the water of treated groups has been changed including the control group. The variable measured daily, during 15 days, is the number of dead individuals (L4 larvae, nymphs and adults).

Lethal concentrations and lethal times (LC50% LC90%, LT50% and LT 90%) were calculated using the Finney's (1971) mathematical method. Data are normalized and processed according to the tables of Bliss and calculations were performed on XLStat 2009.

2.5. Effects of *Daphne gnidium* on fecundity and female fertility:

Adults from larvae treated with the lowest dose (27 g / l), having completed their development were isolated and separated in couples (male and female) in cages (20 x 20 x 20 cm) containing water containers. After coupling, the number of laid and hatched eggs for each female is counted. The results were subject to statistical description and comparison of variances.

3. Results and discussion:

3.1. *The effect of Daphne gnidium on the mortality of Culex pipiens:*

The fourth-stage larvae of Culex pipiens are sensitive to Daphne gnidium. This sensitivity is reflected by higher or lower mortality rates depending on the concentrations used, and especially according to the time of exposure to the extract (Tab. I). The mortality rate ranges between 5.11% and 75.31% for the lowest concentration (27 g/l) whileit reaches 84.26% when the larvae are exposed to the highest concentration (100 g/L) (Tab. I). The 4th stage larvae of Culex pipiens exposed during two, five and ten days to the treatment have mortality rates correlated with the doses used. Mortality rates were not significantly different (Tab. I). On the contrary, the mean mortality of individuals after fifteen days of treatment is significantly different (Tab. I).

Table I: Mortality rate (%) of *Culex pipiens* individuals (Diptera: Culicidae) treated with different concentrations of *Daphne gnidium* (Thymelaeaceae) at different exposure times.

	48h	**120h**	**240h**	**360h**	**F_{obs}**	***p***
27g/l	5,11	25,65	56,28	75,31	**1,33**	**0,33**
60g/l	10,23	33,53	77,86	84 ,26	**5,61**	**0,02**
100g/l	20,54	49,42	81,06	84 ,26	**7,92**	**0,009***
F_{obs}	**2,01**	**4 ,60**	**1,98**	**16**		
p	**0,21**	**0,06**	**0,21**	**0,004***		

The comparison of variances is done at the significance level α = 0.05 and mortality rates are corrected by the Abbott formula

The results also show that there is a strong positive correlation between recorded mortality rates and the exposure time and/or the concentration of the extract used against mosquitoes (Tab. II).

To ensure a 50% mortality of the insects after 2 days, the concentration of *Daphne gnidium* must be equal to 380.19 g/l, on the contrary, 1862.08 g/l of *Daphne gnidium* insures the mortality of 90% (Tab .II A). When it is the fifth day, the calculations show that the LC50 is 89,12g/l %, while the LC90% is of 457,09g/l (Table IIA.) and that the direct effect during 10 days resulted in an LC50 of 18.62% g/l, while the LC90% is of 51.29 g/l. After the 15th day of treatment, the LC50 and LC90% did not exceed 6.08 g/l and 4.48 g / l (Tab. II A). On the lethal times, the concentration 27 g/l *Daphne gnidium* can eliminate 50% of the population of *Culex pipiens* in the 13th day and 90% during 47 days of treatment (Tab. II B). When 60 g/l of *Daphne gnidium* extract is applied, LT50% is 5 days, while the LT90% is 8 days (Tab. II B). On the highest concentration (100 g/l), the calculated lethal times (LT50% and LT90%) are less important since they do not exceed 3 days and 6 days respectively (Tab. II B). Several studies investigated the toxicity of products generated from plants against mosquito larvae. We include in this regard, the works of Alouani et *al.* (2006) who showed larvicidal activity of azadirachtin (extract of Azadirachtaindica tree) on the fourth stage larvae *Culex pipiens*. On *Aedes aegypti*, Sujatha and Bollipo (2013) have successfully tested the larvicidal activity of essential oils of Artemisia vulgaris (Asteraceae). Our results confirm the findings of Benayad (2008) who showed a toxic effect of *Daphne gnidium* on the pests of stored products.

Table II: Toxicological parameters of *Daphne gnidium* aqueous extract (Thymelaeaceae) in individuals treated with Culex pipiens (Diptera: Culicidae) (A: Exposed Time; B: used concentration)

A				
Time (Hours)	**48**	**120**	**240**	**360**
Regression line	Y= 0,22 + 1,85 X R^2= 0,86	Y= 1,49+ 1,80X R^2= 0,94	Y= 1,33 + 2,89 X R^2= 0,95	Y=1,45+4,54X R^2=0,85
LC50% (g/l)	380,19 g/l	89,12g/l	18,62 g/l	6,08 g/l
LC90% (g/l)	1862,08 g/l	457,09g/l	51,29 g/l	4,48 g/l

B			
Concentration (g/l)	**27**	**60**	**100**
Regression line	**Y=2,48+2,27X** R^2 =0,73	**Y=1,04+6,02X** R^2=0,92	**Y=2,16+5,16X** R^2=0,93
LT50% (j)	12,88J	4,57J	3,54J
LT90% (j)	46,77J	7,41J	6,16J

3.2. Daphne gnidium effect on fecundity and female fertility treatment:

After processing the fourth stage larvae by sublethal concentration of 27g/l, a longer development time and impaired fecundity and fertility are noticed on adults issued from this treatment.

Daphne gnidium effect on the number of eggs: The results in the table (Tab III) show that females treated with Daphne gnidium lay an average of 37.70 ± 27.52 eggs with a minimum of 16 and a maximum of 52 eggs. However, healthy females lay between 39 and 87 eggs. The comparison of egg averages shows the significant differences between the fertility of the two batches (tobs = 0.82; p = 0.43) (Table III.).*Culex pipiens* females lay 50 to 200 eggs, which usually hatch within 2 to 5 days (Schaffner et *al.,* 2001).

Table III: Comparison between eggs from control *Culex pipiens* females and eggs from females treated with *Daphne gnidium* (n = 10)
(A: Number of eggs from the control female; B: Number of eggs from females treated with *Daphne gnidium)*

	AVR ± S	**Min**	**Max**	t_{obs}	***p***
A	58,80 ±14,56	39	87	**3,86**	**0,001***
B	37,30 ±9,87	16	52		

AVR ± S : *Average ± standard deviation ;* ***Min :*** *Minimum ;* ***Max :*** *Maximum ;* * : Significantly different

***Daphne gnidium* Effect on the outbreak:** Eggs from control females take less time to hatch; their average time is 4.7 ± 1.16 days with a maximum duration of six days and a minimum one of 3 days (Tab. IV). Females treated with *Daphne gnidium* take longer time (4.8 ± 1,03jours) to lay eggs with a range of 3 days (Tab. IV). A hatching rate was recorded among control females 80% while the rate in treated females is 50% (Tab. IV).
This plant's effect on the fecundity and hatching rate of the insect could be due to the flavonoid compounds of the Daphne gnidium extract (Mohammedi, 2013).

Table IV: Effect of *Daphne gnidium* on the hatching of *Culex pipiens* female eggs (n = 10)
(A: The eggs of the control females; B: The eggs of females from larvae treated with *Daphne gnidium*)

	Hatching time			Hatching rate(%)		
	Avr ± s	**Min**	**Max**	**Avr**	**Min**	**Max**
A	4,7 ± 1,16	3	6	91,29	80%	100%
B	4,8 ± 1,03	3	6	86,68	50%	100%

AVR ± s : *Average ± standard deviation;* ***Min :*** *Minimum ;* ***Max :*** *Maximum*

Conclusion:

Daphne gnidium can be considered among the plants with important insecticidal effects in biological control against mosquitoes. Preliminary toxicity tests on *Culex pipiens* have confirmed its toxicity for these vectors. Moreover, using a sub-lethal dose from the aqueous extract of the *Daphne gnidium* shows a reduction in fertility and fecundity of female adults from treated larvae. It will also be interesting to test the extracts of this plant on other mosquito species.

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