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LARVICIDAL ACTIVITY OF POLYPHENOLS OF SOME ANACARDIACEAE ON MOSQUITOES (DIPTERA: CULICIDAE) AGENT OF PATHOGENIC DISEASES

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Abstract

Description of the Subject: As part of the search for a biological method against Culicidae vectors of pathogenic diseases, sensitivity tests are carried out on mosquitoes by the use of polyphenolic extract of pistachio: *Pistacia terebinthus, Pistacia lentiscus* and *Pistacia atlantica.*

Objective: This study contributes to the enhancement of plant extracts of the Pistacia genera. To this end, a reduction in the use of chemicals, as well as their imports; and finally to use an effective, natural and economical means of control.

Methods: In this work, the insecticidal activity of these extracts was studied on stage 1 and 4 larvae of *Culex pipiens* (Linnaeus). Toxicity tests revealed after 24/48 and 72 hours and after one week to determine LC50 and TL50, the results are confirmed by the ANOVA and Tukey test.

Result: In general, the polyphenolic extract has a strong larvicidal effect, in particular the polyphenolic extract of *Pistacia lentiscus* and Pistacia atlantica. Our results showed a very interesting larvicidal activity, with LD50 values of *Pistacia lentiscus* of 30.44 mg / ml and 92.30 mg / ml of L1 and L4 larval stages after 24 hours. On the other hand, the toxicity is well marked when the duration of exposure of the larvae is longer with a mortality rate reaching 100% for L1 and L3 after 72 hours for the polyphenolic extract of *Pistacia lentiscus* and *Pistacia atlantica*. The results show that the polyphenolic extract of the leaves of *Pistacia lentiscus* and *Pistacia atlantica* has a very significant toxic effect, is p=0.000 for the different larval stages, *Pistacia lentiscus* and *Pistacia atlantica* have a remarkable larvicidal activity.

Conclusion: As part of the fight against mosquitoes, the polyphenolic extract of the leaves of three plants of the genus *Pistacia* is used as a natural biocide.

Key words: Larvicidal activity; Polyphenolic extract; *Pistacia spp; Culex pipiens.*

ACTIVITÉ LARVICIDE DES POLY PHÉNOLS DE CERTAINES ANACARDIACÉES SUR LES MOUSTIQUES (DIPTERA : CULICIDAE) AGENT DE MALADIES PATHOGÈNES

Résumé

Description du sujet : Dans le cadre de la recherche d'une méthode de lutte biologique contre les Culicidae vecteurs de maladies pathogènes, des tests de sensibilité sont réalisés sur des moustiques par l'utilisation d'extrait polyphénolique de quelques pistachiers : *Pistacia terebinthus, Pistacia lentiscus* et *Pistacia atlantica*.

Objectifs : Cette étude contribue à la valorisation des extraits végétaux du genre Pistacia. A cet effet, une réduction de l'utilisation des produits chimiques, ainsi que leurs importations ; et enfin utiliser un moyen de lutte efficace, naturel et économique.

Méthodes : Dans ce travail, l'activité insecticide de ces extraits a été étudiée sur les larves du stade 1 et 4 de *Culex pipiens* (L.). Les tests de toxicité ont révélé au bout de 24,48 et 72 heures et après une semaine pour déterminer LC_{50} et $TL_{50,}$ les résultats sont confirmés par le test ANOVA et Tukey.

Résultats : En général, l'extrait polyphénolique a un effet larvicide important, en particulier l'extrait polyphénolique de *Pistacia lentiscus* et de *Pistacia atlantica*. Nos résultats ont montré une activité larvicide très intéressante, avec des valeurs de DL50 de *Pistacia lentiscus* de 30,44 mg/ml et 92,30 mg/ml de stades larvaires L1 et L4 après 24 heures. En revanche, la toxicité est bien marquée lorsque la durée d'exposition des larves est plus longue avec un taux de mortalité atteignant 100 % pour L1 et L3 après 72 heures pour l'extrait polyphénolique *de Pistacia lentiscus* et *Pistacia atlantica*. Les résultats montrent que l'extrait polyphénolique des feuilles *de Pistacia lentiscus* et *Pistacia atlantica* a un effet toxique très important, soit p=0,000 pour les différents stades larvaires, *Pistacia lentiscus* et *Pistacia atlantica* possèdent une activité larvicide remarquable.

Conclusion : Dans le cadre de la lutte contre les moustiques, l'extrait polyphénolique des feuilles de trois plantes du genre Pistacia est utilisé comme biocide naturel.

Mots clés: Activité larvicide; extrait polyphénolique; *Pistacia spp.; Culex pipiens*

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INTRODUCTION

This work aims to evaluate the insecticidal effect of extracts of Anacardiaceae (Pistacia) against mosquitoes. Several mosquito species, mainly belonging to the genera Anopheles, *Aedes* and *Culex*, are vectors of pathogens as arboviruses, protozoa, and are the indirect cause of the greater morbidity and mortality for humans compared to other organisms [1 and 2]. According to Farajollahi et *al.* [3], the genus *Culex* is responsible for the transmission of filariasis, yellow fever, West Nile virus and viral encephalitis, particularly in the countries of the Mediterranean basin, *Aedes* is a vector of fever dengue, lacrosse, Chikungunya virus and encephalitis [4 and 5]. Anopheleses are vectors of malaria (yellow fever) [6 and 7].

At present, mosquitoes are generally controlled by chemical insecticides as carbamates, organophosphorus, pyrethroids that have a very serious and harmful impact on the environment and also on human health [8]. In parallel, Barbouche et *al.* [9], report that chemical control presents a pollution problem because of the significant accumulation of non-biodegradable active ingredients in the treated aquatic and terrestrial ecosystems. Moreover, these chemical insecticides are characterized by the lack of specificity that could kill non target organisms because the active substances of these products used have a broad spectrum of action [10 and 11]. Chemical control also presents the problem of appearance in treated insects of resistance to chemical insecticides [12, 13, 14 and 15]. More than 2000 plant species have insecticidal activity [16]. In Algeria, mosquitoes have been the subject of several studies, whether toxicological or taxonomic [17 and 18].

MATERIEL AND METHODS

1. Harvesting, drying and grinding the leaves of the plant

Samples harvested are spread on paper for drying then reduced to powder and then stored in cardboard bags away from light and heat, the period and regions of harvest are shown in the Table 1.

Table 1. The plant material

The harvest plant	harvest	Harvest location	Position
Pistacia lentiscus	Novembre 2016	M'kira,(Tizi Ghenif, Tizi Ouzou)	36°37′31″N. 03°47′33″E.
Pistacia atlantica	Septembre 2016	Baraki (Alger)	36°39′59″N. 03°05′45″E.
Pistacia vera	Septembre 2016	Baraki (Alger)	36°39′59″N. 03°05′45″E.

2. Presentation of the larva collection area of Culex sp.

The larvae subjected to the toxicity tests (Table 2) are obtained after the hatching of the eggs which are recovered from the lodgings domiciles in May 2017. These lodgings are in Màala, a mountainous region of Lakhdaria (Bouira), the Geographic coordinates of Màala is illustrated in Table 2 and fig. 1.

Table 2. Geographic coordinates of the region that is Màala.

Geographic Coordinates	Latitude : 36.4426. longitude 3.87112 36° 26′ 33″ N., 03° 52′ 16″ E.
Height	601m
Climate	Mediterranean climate with hot summer (koppen classification: csa)

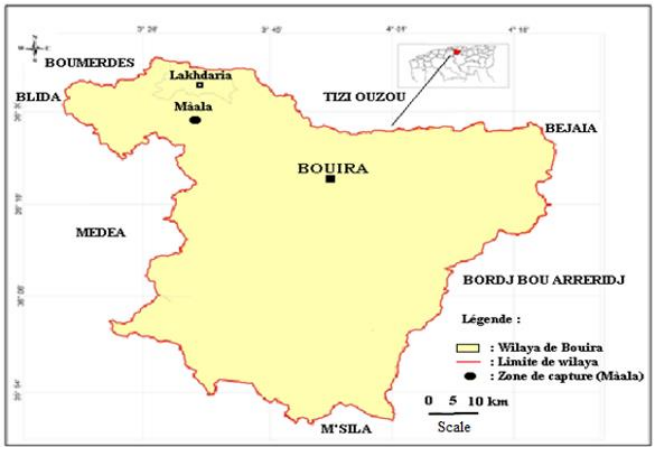

Figure 1: A map of Bouira geographical map by locating the area of Maala.

3. Livestock

Larvae are cultured in containers containing 500 ml of dechlorinated water. The food is a mixture of one volume of biscuit powder and another volume of crystallized sugar or a few milliliters of sweet juice without preservatives. When the larvaes turn into nymphs placed in containers and placed in cubic cages with a wooden frame, 30 cm × 30cm ×30 cm covered with tulle with side, with a 15cm opening allowing the introduction of the hand. After mating, the females feed on blood from the exposed hand for 30 minutes in the light-protected cage twice a week [19]. In the culture vessel, the females lay eggs in clusters, which float on the surface of the water, once the eggs hatch the larvae are fed every 2 to 3 days with a renewal of the water.

4. Preparation of polyphenolic extracts

The leaves of *Pistacia lentiscus, Pistacia atlantica* and *Pistacia vera* plants are washed and then reduced to powder. 10 g of powder of each of the plants was macerated in 200 ml of absolute methanol for one week. The extract is filtered and evaporated to dryness under reduced pressure at 45°C. in the Rotavapor. The weighed dry residue is mixed with 10 ml of methanol and stored at -18°C [20].

4.1. Performance calculation

The percentage of dry crude methanolic and aqueous extract was calculated by the following formula:

R (%) = M / M × 100. whose: ***R (%):*** Yield expressed in%; ***M:*** mass in grams of the resulting solids; ***M0:*** Mass in grams of plant material to be treated.

4.2. Determination of total polyphenols

The total phenol content of the extracts (aqueous and methanolic) of the plants being analyzed was determined by the method of Singleton [21]. A volume of 0.5 ml of the extract solutions at different concentrations is introduced into test tubes; the mixture (0.5 ml of Folin-Ciocalteus diluted 10 times and 0.5 ml of 20% sodium carbonate) is added. The tubes are shaken and incubated for 1 hour at room temperature ; the absorbance is measured at 760 nm against a blank using a spectrophotometer. The amount of total polyphenols was determined according to a standard calibration curve of Gallic acid at different concentrations under the same conditions as the sample.

5. Tests of toxicity

The sensitivities tests that are adopted to test larval susceptibility by Who [22]. From the initial extract of each plant in concentration is C1=0.212g/ml (*Pistacia vera),* C1=0.272g/ml (*Pistacia atlantica)* and C1=0.138g/ml (*Pistacia lentiscus),* doses of D0 corresponds to the control (pure methanol), D1=1/75, D2=1/50, D3=1/25, D4=1/10 of the extract, were prepared. The tests are carried out in cups each containing 100 ml of solution and 10 mosquito larvae of the same species and the same lodging. We used first, second, third and fourth larval stage. For each of the concentrations of the extract as well as for the control, three repetitions are performed. The mortality rate is calculated in terms of the average of the three determinations, each of which covers 10 individuals. The statistical analysis of the means is carried out using the ANOVA 2 analysis of variance test (Statistica) [23 and 8].

The percentages of mortality are calculated by the following formula: ***% of Mortality = (ML / TL - N) × 100***. whose: ***ML:*** Number of dead or moribund larvae; ***TL:*** Total number of larvae; ***N:*** Number of nymphs.

If the percentage of mortality in the control does not exceed 5%, the test is considered invalid. If it is between 5% and 20%, the test is valid but a correction of the percentages of mortality must be carried out, this using the formula of Abbott [24 and 22]. ***CM%= (OM% - WM%) / (100 - WM%) × 100.*** whose: ***WM%:*** Mortality Witness; ***CM%:*** Corrected mortality; ***OM %:*** Observed mortality.

By treating and comparing mortality averages by the Tukey test at the *p*=0.05 threshold [25]. Determination of lethal concentrations LC_{50} will be made after 24, 48 and 72 hours of exposure; they are calculated using Spearman-Kaber software Probit analysis [23].

6. Determination of lethal concentration 50 LC_{50}

It is necessary to estimate the lethal dose LD_{50}. The dose needed to kill 50% of a population. The percentages of mortality observed are corrected by the Abbott [24]. Formula, which makes it possible to eliminate natural mortality and to know the true toxicity of the extract by probit analysis [26].

The corrected mortalities obtained make it possible to establish a probit curve as a function of the logarithms of the decimals of the doses. The corrected percentages are converted into their probits [27].The logarithms of the lethal doses (LD_{50} and LD_{90}) are determined from a regression line according to the mathematical method [y = ax + b] [14]. The table of probits of Bliss and cavelier [28]. Is used for this purpose.

The LD_{50} is calculated from the regression rights ***Probits = f (log dose)***, whose: ***Y:*** Probit value corresponding to the larvicidal effect (corrected mortality probabilities); ***X:*** dose of the extract tested; ***a:*** the slope.

7. Determining the TL_{50}

Lethal time $_{50}$ (TL_{50}) corresponds to the time required for 50% of individuals exposed to a specific concentration to perish [29]. It is calculated from the Probit regression line corresponding to the percentages of corrected mortalities as a function of the logarithms of the processing times (Probits = f (log time).

RESULTS

After evaporation of macerates, the polyphenol extract obtained leaves has a liquid, gelatinous and dark green. The table below shows the yield and the quantity in gram of the PT extract of each plant.

Table 3. Polyphenol yield of the powder of the leaves of *Pistacia lentiscus, Pistacia atlantica Pistacia vera*.

The plant	Yield in PT
Pistacia lentiscus	34.5%
Pistacia vera	53.1%
Pistacia atlantica	68.1%

The phenol content of the extract was calculated from the Gallic acid calibration curve A = 0.4121 [A. gal] +0.0378 / R^2 = 0.9882, the concentration of each sample is expressed in µg of Gallic acid equivalent per milligram of extract (µg EAG/mg extract). The Phenolic Compound is illustrated in Table 4.

Table 4. The Phenolic Compound Content of the *Powder Pistacia lentiscus, Pistacia atlantica Pistacia vera.*

The plant	The polyphenol content
Pistacia lentiscus	0.63mg/ml
Pistacia vera	0.98mg/ml
Pistacia atlantica	1.85mg/ml

After exposure of the larvae of the four *Culex pipiens* stages to the different concentrations of Polyphenol extract of *Pistacia lentiscus, Pistacia atlantica* and *Pistacia vera* for 24 h, 48 h and 72 h. Mortality varies by concentration and speciesis summarized in Table 5, 6 and 7. The results show that the minimum concentrations necessary to obtain 100% mortality of stage 1 and 2 *Culex pipiens* larvae were evaluated at D4 after 72 hours of exposure to the methanolate extract of *Pistacia lentiscus* and the same percentage is marked at the third stage. L3 for same dose also D4 of *Pistacia atlantica* after 72 hours of exposure. On the other hand, the polyphenol extract of *Pistacia vera* only affected 27.2% of mortality whereas this percentage is rather low compared with the toxic effect of *Pistacia lentiscus* and *Pistacia atlantica*.

Table 5. Corrected Mortalities for the different larval stages of *Culex pipiens* as a function of the dose of the Polyphenol extract (%) *Pistacia lentiscus, Pistacia. (*Average of 3 repetitions)

Larval stage	time (h)	D0 (methanol)	D1	D2	D3	D4
L1	24h	0	33	42	50	67
	48h	0	42	58	67	83
	72h	0	67	67	75	100
L2	24h	0	25	58	67	67
	48h	0	33	67	75	83
	72h	0	42	67	83	100
L3	24h	0	8	17	19	25
	48h	0	17	33	42	50
	72h	0	17	42	50	92
L4	24h	0	8	8	17	25
	48h	0	25	25	25	42
	72h	0	33	33	50	67

Table 6. Corrected Mortalities for the different larval stages of *Culex pipiens* as a function of the dose of the Polyphenol extract (%) *Pistacia atlantica. (*Average of 3 repetitions)

Larval stage	time (h)	D0 (methanol)	D1	D2	D3	D4
L1	24h	0	0	0	16.6	41.66
	48h	0	8.33	8.33	1.66	58.33
	72h	0	16.66	25	25	66.66
L2	24h	0	0	0	8.33	16.66
	48h	0	8.33	8.33	16.66	41.66

	72h	0	25	25	25	41.66
L3	24h	0	0	0	8.33	16.66
	48h	0	8.33	8.33	16.66	16,66
	72h	0	16.66	16.66	25	41.66
L4	24h	0	0	0	8.33	16.66
	48h	0	9.08	9.08	18.18	18.18
	72h	0	10	18.18	18.18	27.27

Table 7. Corrected Mortalities for the different larval stages of *Culex pipiens* as a function of the dose of the Polyphenol extract (%) *Pistacia vera.*

Larval stage	time (h)	D0 (methanol)	D1	D2	D3	D4
L1	24h	0	16.5	16.5	25	33.25
	48h	0	19.5	25	28	44.33
	72h	0	50	28	50	60.34
L2	24h	0	16.5	16.75	16.75	16.75
	48h	0	41,5	25	25	33.25
	72h	0	36.23	25	41.75	75
L3	24h	0	8.25	25	33.25	75
	48h	0	25	48.5	60.5	83.5
	72h	0	25.4	58.25	83.25	100
L4	24h	0	8.99	8.99	9	18.25
	48h	0	18.25	18.25	36.23	63.48
	72h	0	25	40.5	63.5	90.73

Average of 3 repetitions

The toxic effect of the extracts analyzed is clearly demonstrated with the LC_{50} values. LC_{50} lethal concentrations of the polyphenolic are shown Table 8.

Table 8. LC_{50} lethal concentrations of the polyphenolic extract *Pistacia lentiscus, Pistacia atlantica* and *Pistacia vera* after 24h, 48h and 72h of exposure.

LC_{50}	24h				48h				72h			
	L1	L2	L 3	L4	L1	L2	L 3	L4	L1	L2	L 3	L4
P. lentiscus (mg/ml)	30.44	35.26	35.26	692.30	14.62	14.62	14.62	508.94	10.67	10.67	10.67	30.89
P. vera (mg/ml)	104.135	95.36	1067.29	135.53	67.51	182	104.14	5584.55	29.51	67.51	508.94	1019.08

Average of 3 repetitions.

The Table 9 shows Lethal times 50 (TL_{50}) of the polyphenolic extract for each dose treated.

Table 9. Lethal times of the polyphenolic extract of *Pistacia lentiscus, Pistacia atlantica and Pistacia vera* for each dose treated.

TL_{50}	stages	TL_{50} of *P. lentiscus* (h)	TL_{50} of *P. vera* (h)	TL_{50} of *P. atlantica* (h)
10%	L1	120.50	81.04	66.34
	L2	139.39	121.99	118.24
	L3	497.44	81.04	695.46
	L4	116.84	86.68	1199.74
20%	L1	33.84	76.55	354.61
	L2	22.32	76.55	108.86
	L3	94.53	81.04	59.46
	L4	116.84	79.84	82.65
40%	L1	23.90	695.46	91.01
	L2	9.61	191.30	121.99
	L3	64.54	135.88	35.43
	L4	83.55	410.69	57.78
100%	L1	22.32	33.84	52.94
	L2	9.50	79.85	52.16
	L3	37.77	191.30	19.87
	L4	50.17	811.65	38.38

From the analysis of ANOVA variance of the different doses of the polyphenolic extract of *Pistacia lentiscus*, *Pistacia atlantica* and *Pistacia vera* L. The results obtained are summarized in the tables below, Table 10.

Table 10. Variance analysis of the different polyphenol doses of *Pistacia lentiscus*, *Pistacia atlantica and Pistacia vera* on the four larval stages of *Culex pipiens.*

Pistacia lentiscus					
Sum	ddf	SSD	MS	Fobs	Pr
ord, origin	1	10.90613	10.90613	228,.409	0.000000
Model	3	0.89212	0.29737	6.2261	0.001283
Error	44	2.10155	0.04776		
Total corrected	47	2.99367			
Pistacia atlantica					
Sum	ddf	SSD	MS	Fobs	Pr
ord, origin	1	6.697378	6.697378	164.5764	0.000000
Model	3	0.818812	0.272937	6.7070	0.000795
Error	44	1.790564	0.040695		
Total corrected	47	2.609377			
Pistacia vera					
Sum	ddf	SSD	MS	Fobs	Pr
ord, origin	1	14.3858	14.38578	2.168298	0.148000
Model	3	26.6258	8.87527	1.337727	0.274322
Error	44	291.9222	6.63460		
Total corrected	47	318.5480			

ddf: degree of freedom**; SSD**: sum of squares of deviations; **MS**: middle square; **Fobs:** Fisher's F-value; **Pr**: probability.

The difference between the doses was tested using the Tukey test. This test allows a multiple comparison of the polyphenol extract doses of the leaves of these plants used during the treatment of *Culex pipiens* larvae, Table 11, 12 and 13 present the result of Tukey test of the different doses of the polyphenolic extract of *Pistacia*.

Table 11. Tukey test of the different doses of the polyphenolic extract *of Pistacia lentiscus, Pistacia atlantica and Pistacia vera* for the different larval stages.

Pistacia lentiscus	Larva 1	larva 2	Larva 3	Larva 4
Dose 1	-	0.411747	0.070413	0.000826
Dose 2	0.411747	-	0.771562	0.052197
Dose 3	0.070413	0.771562	-	0.340876
Dose 4	0.000826	0.052197	0.340876	-

Table 12. Tukey test of the different doses of the polyphenolic extract of *Pistacia atlantica* for the different larval stages.

Pistacia atlantica	Larva 1	Larva 2	Larva 3	Larva 4
Dose 1	-	0.969047	0.272188	0.001180
Dose 2	0.969047	-	0.517727	0.004142
Dose 3	0.272188	0.517727	-	0.127759
Dose 4	0.001180	0.004142	0.127759	-

Table 13. Tukey test of different doses of the polyphenolic extract of *Pistacia vera* for the different larval *stages.*

Pistacia vera	Larva 1	larva 2	Larva 3	larva 4
Dose 1	-	0.999999	0.999837	0.354121
Dose 2	0.999999	-	0.999905	0.361044
Dose 3	0.999837	0.999905	-	0.397321
Dose 4	0.354121	0.361044	0.397321	-

DISCUSSION

The yield of polyphenols obtained from 10 g of leaf powder of each species is variable; the highest *Pistacia atlantica* of a value is 68.1% *Pistacia vera* 53.1% and last *Pistacia lentiscus* with only 34.5%. These values confirm the richness of these species in total polyphenols. Compared to the study by Romani et *al.* [30], the species of *Pistacia lentiscus* harvested in Meknes revealed the presence of a percentage of phenolic compounds in the leaves that is greater than 7.5%.

On the other hand, the yield of the present study is low compared to the results of the total polyphenols of *Pistacia lentiscus* fruits harvested at El-Kala which is of the order of 79.35±0.01 [31]. The yield of the polyphenols of *Pistacia atlantica* obtained from 10 g of the leaf powder is high by comparing with those of Maameri [32]. This reports a yield of 44.12%.

Extracts from three plants is a promising source of phenolic compounds [32]. reports a concentration of total polyphenols of *Pistacia atlantica* leaves collected at Djelfa 48.92±0.04 mg/ml, using a hydroalcoholic mixture (methanol / water) (80/20: V/V) as a solvent extraction. For this purpose, the yield of total polyphenols varies not only according to the plant species, but also according to the solvent used and the extraction method adopted and according to the climatic and environmental factor, the geographical zone, the soil, aggressions and diseases [33], the genetic heritage, the harvest period and the plant's stage of development are also factors that affect the yield and the plyphenol content of the plant. According to Tahir et *al.* [34], the variation in the results of the amount of polyphenolic compounds in *P. atlantica* can contribute to the region and the harvest period, growing season, growth stage solvent, a method of extraction and organ of the plant.

The results show that methanol has no insecticidal effect on *culex pipiens*, and life cycle of tested mosquitoes is completed normal, it was noted that stage 1 to 4 larval developments is complete and also there is an emergence of nymph in adult.

The polyphenolic extract of *Pistacia lentiscus* can reach 100% mortality for stage 1 and 2 larvae for a D4 dose after 3 days, while the same time is marked by Bishnu & Zeev [35], with one dose 0.1% of the aqueous extract of *Balanitesae gyptiaca* del can reach 100% of mortality. These results present a first classification of the toxic efficacy of the polyphenolic extract of *Pistacia lentiscus* and *Pistacia atlantica* tested is highlighted, and also show that the sensitivity of the larvae is connected with the larval stage, the fourth stage is less sensitive than the other stages. The results show that the toxicity of the plant polyphenol extract increases with increasing exposure time and these results are in agreement with those of Ghebriel & Adugna [36].

The dose required to kill 50% of *Culex pipiens* stage 1 larvae (Table 6) is 30.44 mg/ml, 104.155 mg/ml and 1067.29 mg/ml after 24 h of the polyphenol extract of *Pistacia lentiscus.* ,*Pistacia vera* and *Pistacia atlantica* successively. These results show that the polyphenolic extract of *Pistacia lentiscus* is more effective in terms of toxicity to other species since it gives the lowest LD_{50}% for all larval stages as a function of time. LC_{50} calculated for the larval stages of *Culex pipiens* (Table 6), showed that the polyphenol extract, proved to be interesting in terms of toxicity. These results show the interest shown by the extracts of the *P. atlantica* and *P. vera* in the fight against larvae. These results are comparable the most vulnerable larvae to the polyphenolic extract of the leaves of *P. lentiscus* are those of the (L1) stage of the LC_{50} (24 h) of 173 mg.l-1. Moreover, for stage (L4), the LC_{50}=407.38 $mg.l^{-1}$ is higher than those obtained by Aouinty et *al.* [8] who found LC_{50} (24 h) on mosquito larvae equal to 200 mg. l-1. The results show that in the tests carried out, the LC_{50}s are lower for the first larval stage (L1) than for the fourth stage (L4) are in agreement with the results of Aouinty et *al.* [8] and Ghebriel & Adugna [36] and whatever the species considered and the extract used.

The results show that the shortest and most toxic lethal time 50 for the four larval stages L1, L2, L3 and L4 corresponding respectively to 22.3 hours, 9.50 hours are marked for the extract of *Pistacia lentiscus*, 19, 87 hours, 38.38 hours are marked for *Pistacia atlantica* extract for the same treatment dose (D1=100%). The longest TL_{50} is recorded in all larval stages for the dose D1=10%, for L1 it is 120.50 hours, L2 is 497.44 hours, L3 is 695.46 hours and for L4 is 1199.74 hours whereas these results are invalidated since the duration of larval stage is 6 to 10 days before the pupation according to the temperature of the water and the availability of the food.

This variance has two factors, namely the time and dose of the present study, which indicates the existence of a significant effect of the four doses on the different larval stages. The results of the ANOVA test on the four larval stages of *Pistacia lentiscus* and *Pistacia atlantica* show: a highly significant difference for the different larval stages $p \leq 0.000$. The ANOVA test results on the larval stages of *Pistacia vera* show a non-significant difference for the four larval stages $p > 0.05$. From these results the polyphenolic extract of the leaves of *Pistacia lentiscus* and *Pistacia atlantica* could be used as a natural bio-insecticide, it is effective for the fight against mosquitoes by the use of a natural product because it has a good larvicidal activity. Tukey test shows that there is a difference between the first and the last dose of the polyphenol extract of *Pistacia lentiscus*,

Pistacia atlantica and *Pistacia vera* D1≠D4, which is a value of 0.0001 after the treatment of larvae *Culex pipiens*. The Tukey test confirms the ANOVA test results that the polyphenolic extract of leaves of *P. lentiscus, P. atlantica* and *P. vera*, has a larvicidal effect on larvae of *Culex pipiens*, so this extract is effective as a bio-insecticide against mosquitoes.

CONCLUSION

The use of plant extracts in the fight against mosquito disease agents can reduce vector transmission to these pathogenic diseases such as malaria thus reducing the transmission of parasites, This due to the problems found by the use of insecticides to chemical base (the appearance of resistant insects, environmental pollution and human health hazards). The results of the larviciding tests obtained show a variable sensitivity translated by low to very high mortality rates from one concentration to another, but especially from one larval stage to another. The results also reveal that larvicidal activity is progressive since an increase in mortality is reported for the different larval stages. The polyphenolic extract of these plants could have a very important insecticidal activity and it could be a good natural bio-insecticide. The results of this work show that all polyphenols tested have a significant larvicidal effect all samples showed larvicidal activity, particularly the polyphenols of *Pistacia lentiscus.*

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