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Larvicidal Activities of Essential Oils Extracted from Five Algerian Medicinal Plants against *Culiseta longiareolata* Macquart. Larvae (Diptera: Culicidae).

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ABSTRACT

Objective: The use of essential oils in mosquito control is considered as a potential alternative of synthetic insecticides. The current study aimed to assess the larvicidal activity of the essential oils extracted from five medicinal plants collected from northeastern Algeria against the *Culiseta longiareolata* larvae, a vector of the *Plasmodium* species in birds and one of the most abundant mosquito species in Algeria.

Materials and Methods: The essential oils extracted from: *Thymus vulgaris*, *Artemisia herba-alba, Juniperus phoenicea, Rosmarinus officinalis*, and *Eucalyptus globulus* were tested against the 3rd and 4th instar *Culiseta longiareolata* larvae. The larvae were exposed to a series of concentrations of the tested essential oils for 24h. The concentrations that caused between 10% and 90% mortality were replicated four times, and the entire test was repeated three times. The collected data were used to determine the LC_{50} and LC_{90} values,

Results: The tested oils revealed an efficient larvicidal activity. *T. vulgaris* showed 100% mortality at 80ppm final concentration, while the other tested oils showed 100% mortality at 200ppm. Furthermore, the lethal concentrations that caused 50% and 90% mortality (LC_{50} and LC_{90}) were varying. *T. vulgaris* was the most efficient essential oil (LC_{50}=25.64ppm, LC_{90}=50.53ppm), followed by *J. Phoenicea* (LC_{50}=59.83ppm, LC_{90}=137.68ppm), *R. officinalis* (LC_{50}= 64.18ppm, LC_{90}= 96.55ppm), *A. herba-alba* (LC_{50}=86.67ppm, LC_{90}=139.55ppm), then *E. globules* (LC_{50}=95.83ppm, LC_{90}= 168.25ppm).

Conclusion: The use of essential oils or their principal active components as α-pinene, 1,8-cineole and Camphor may serve as an eco-friendly method to control mosquito larvae. Nevertheless, the field application of essential oils and their principal components remains a fundamental step to evaluate the field efficacy of these botanic extracts and to note their possible secondary effects on non-targeted organisms.

Keywords: Aromatic medicinal plants, *Culiseta longiareolata*, Essential oil, Larvicidal activity, Mosquitoes

INTRODUCTION

Culicidae, or mosquitoes as commonly known, is a family of Diptera insects that reproduce quickly and abundantly. Simultaneously, this family includes major vectors for many deadly and dangerous diseases. Therefore, the importance of the mosquito family in terms of public health makes mosquito control an important initiative to minimize the negative effects of mosquito-born-diseases. Mosquito control may depend on various strategies; the most common in the past decades was the use of synthetic insecticides as inexpensive and available products. However, the use of synthetic insecticides has over time created environment pollution and resistance problems (1, 2).

Recently, eco-friendly methods were developed to control mosquitoes. For instance, the enhancement

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of behavior-based control tools and the development of repellent and toxic products based on botanic components can target different mosquito life stages (3, 4). Essential oils (EOs) extracted from different parts of plants were frequently tested for their mosquitocidal activity (5). These primary botanic materials present various biological activities. They can act as insecticides where they can affect the oviposition, survival, larval duration, pupation and insect emergence (6, 7). However, the larvae stage appears to be more appropriate to control mosquito populations because of the high reproduction rates and larvae food mechanisms that allow a high number of mosquito individuals to be targeted simultaneously. Therefore, the assessment of the larvicidal efficacy of various plant derivatives was the main objective of many research papers (8-11).

Culiseta longiareolata (Macquart 1838) constitutes with the *Culex pipiens* (Linnaeus 1758) complex the most abundant species in Algeria. It usually breeds near human habitations, however, the females prefer to feed on bird blood (12). *Cs longiareolata* has uniquely adaptive and survivor features. Kiflawi et al. (13) have confirmed that the females of this species showed an adaptive response against the risk of predation and negative density effects where they avoid laying their eggs in predator pools. Further, *Cs longiareolata* is considered as a primary vector of *Plasmodium* (*Giovannolaia*) *circumflexum* (Kikuth 1931), *Plasmodium relictum* (modified from Garnham 1966) and *Plasmodium polare* (Manwell 1934) in birds, and its capacity to transmit *P. relictum* in Algeria was proven experimentally (14, 15). In this context, we have assessed the larvicidal activity of EOs extracted from five aromatic medicinal plants, harvested from Northeastern Algeria, against *Cs longiareolata* larvae. The efficacy of the tested EOs will be evaluated by calculating the LC_{50} and LC_{90} values and by comparing them with the LC_{50} and LC_{90} values of the same EOs tested previously against other targeted mosquito species.

MATERIALS AND METHODS

Mosquito Collection

Culiseta longiareolata larvae were collected regularly from three clean fixed and controlled pools in Algeria, where the mosquitoes were not exposed to any insecticides. Larvae of the third and fourth instar were used directly in the test; eggs, first and second instar larvae were reared in room temperature (27°C±2°C), in a 12 h light: 12 h dark photoperiod, until the fourth instar was reached.

Essential Oils Extraction

The aerial parts of the tested plants were collected from different regions in the Mediterranean and semi-arid climate northeastern Algeria: *Thymus vulgaris* L. from Guelma, *Artemisia herba-alba* Asso from M'Sila, *Juniperus phoenicea* L. from Jijel, *Rosmarinus officinalis* Linn from Bouira and *Eucalyptus globules* L. from Batna. The plants' collection started at the beginning of the summer (June) in 2018. The samples were air-dried at room temperature. The dried plants were submitted to classical steam distillation for 3-6 h. The samples were exposed to the water vapor produced in the flask crosses, the vapor was charged with the EO, and then was condensed in the condenser. The EO floated on the water surface was then recuperated. The yield of the EOs was between 0.8 and 1.5%.

Larvicidal Bioassay

According to WHO guidelines for laboratory and field testing of mosquito larvicides (16), we tested the larvicidal activity of EOs extracted from the leaves of five aromatic medicinal plants *T. vulgaris*, *A. herba-alba*, *J. phoenicea, R. officinalis ,E. globulus* against *Culiseta longiareolata* larvae under laboratory conditions. The EOs were extracted by steam distillation, they were next serially diluted in ethanol to obtain 10%, 1%, 0.1% and 0.01% of stock solution, and 0.1-1ml of the previous dilutions were added to 100ml of water to obtain the final concentrations. A series of concentrations and controls were applied on 25 mosquito larvae distributed in five cups containing 100ml of water. A total of 8925 larvae were tested. We started the test with the lowest concentrations. The concentrations that showed less than 10% mortality were excluded. Concentrations that showed 10% mortality or more were replicated 4 times, and each test was run three times. After 24h of exposure, moribund and dead larvae were counted. We have chosen four concentrations which caused between 10% and 90% mortality to determine the LC_{50} and LC_{90} values. The data obtained from the four replicates in the three tests were pooled for analysis.

Statistical Analyses

Data were subjected to probit analysis using SPSS software V25 (Using probit model because of the normal distribution of data); and final concentrations were transformed to log10. Lethal concentration LC_{50} and LC_{90} with a 95% confidence limit (CL) suspected of killing 50% and 90% of the population respectively, were calculated and presented with the regression equations (Y= a +b*x) and regression coefficients (R^2).

RESULTS

Five plant EOs were tested to evaluate their larvicidal activity, and the tested oils revealed various mortality percentages at different concentrations (Table 1). The majority of the tested oils showed 100% mortality at 200ppm final concentration, except for *T. vulgaris* that showed 100% mortality at 80ppm. Further, the oils started to affect the larvae life at different concentrations; the lowest concentration that caused equal or more than 10% mortality was 20ppm for *T. vulgaris* , 40ppm for *J. phoenicea*, 50ppm for *A. herba-alba* and *R. officinalis* and 70ppm for *E. globules* (Table1). The 24h LC_{50} and LC_{90} estimate, upper and lower values obtained from the larvicidal activity test of EOs extracted from the five plants in addition to the regression equations and regression coefficients are presented in Table 2. *T. vulgaris* was the most efficient with 25.64 (16.58-32.03) LC_{50} and 50.53 (40.15-82.43) LC_{90}, while *A. herba-alba* was the least efficient. Likewise, the influence degree of increasing one unit of EOs concentration on their larvicidal activity was different. Among the tested EOs, the augmentation of one unit of *R. officinalis* concentration showed the highest influence in increasing the LC_{50} and LC_{90} (b=7.16). The R^2 was close to 1 in all probit analysis, the minimal residuals obtained between the observed and expected values was shown by *E. globulus* EO (R^2=0.99) (Table 2; Figures 1-5).

Table 1: The mortality observed to the *Culiseta longiareolata* larvae, caused by the application of the tested essential oils at different concentrations, with the arithmetic mean (AM) and standard error (SE).

			Dead in a total of 300 larvae (AM±SE)				
IC (%)	**Aliquot (ml)**	**FC (ppm)**	***Thymus vulgaris***	***Juniperus phoenicea***	***Artemisia herba-alba***	***Rosmarinus officinalis***	***Eucalyptus globules***
1	0,2	20	93 (7.75±1.53)	-	-	-	-
	0,4	40	253 (21.08±1.97)	94 (7.83±1.23)	-	-	-
	0,5	50	257 (21.42±1.59)	103 (8.58±1.23)	24 (2±0.75)	75 (6.25±1.54)	-
	0,6	60	286 (23.83±0.42)	156 (13±1.58)	57 (4.75±1.52)	106 (8.83±1.56)	-
	0,7	70	-	-	-	-	68 (5.67±1.1)
	0,8	80	300 (7.75±1.53)	176 (14.67±1.91)	89 (8.17±1.36)	236 (19.67±0.85)	107 (8.92±1.02)
	0,9	90	-	-	-	-	134 (11.17±1.6)
	1	100	300 (25±0.0)	255 (21.25±1.55)	216 (18±2.03)	274 (22.83±1.21)	159 (13.25±1.69)
10	0,2	200	300 (25±0.0)	300 (25±0.0)	300 (25±0.0)	300 (25±0.0)	300 (25±0.0)

IC(initial concentration), FC (final concentration)

Table 2: The LC_{50} and LC_{90} values of essential oils extracted from *T. vulgaris, A. herba-alba, J. phoenicea, R. officinalis* and *E. globules* against the 3rd and 4th instar larvae of the *Culiseta longiareolata*, after 24 hours exposure period; with regression equations and regression coefficients (R2).

	LC50 (ppm) 95% CI			LC90 (ppm) 95% CI					
Essentialoils	**Estimate**	**Lower**	**Upper**	**Estimate**	**Lower**	**Upper**	**Sig (df)**	**Regression equation**	**R2**
Thymus vulgaris	25.64	16.58	32.03	50.53	40,15	82.43	p>0.05 (2)	y=-6.15+4.36*x	0.97
Juniperus phoenicea	59.83	45.36	75.81	137.68	97.21	<250	p>0.05 (3)	y=-6.49+3.66*x	0.9
Artemisia herba-alba	86.67	66.59	<250	139.55	98.03	<250	p>0.05 (2)	y=-11.77+6.08*x	0.93
Rosmarinus officinalis	64.18	55.41	72.56	96.55	82.73	139.84	p>0.05 (2)	y=-12.93+7.16*x	0.98
Eucalyptus globules	95.83	92.27	101.09	168.25	146.59	201.87	p>0.05 (2)	y=-10.45+5.28*x	0.99

Sig (significance level), df (degrees of freedom)

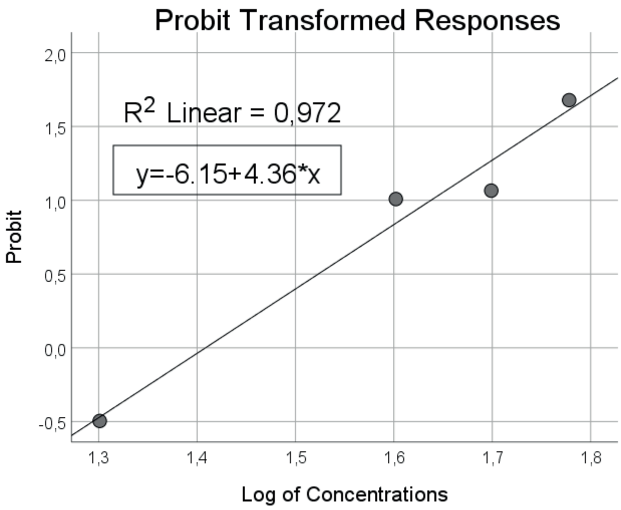

Figure 1. Probit transformed responses with equation regression and coefficient of determination R^2 for *Thymus vulgaris* essential oil tested on 3rd and 4th instars larvae of *Culiseta longiareolata* for 24 h.

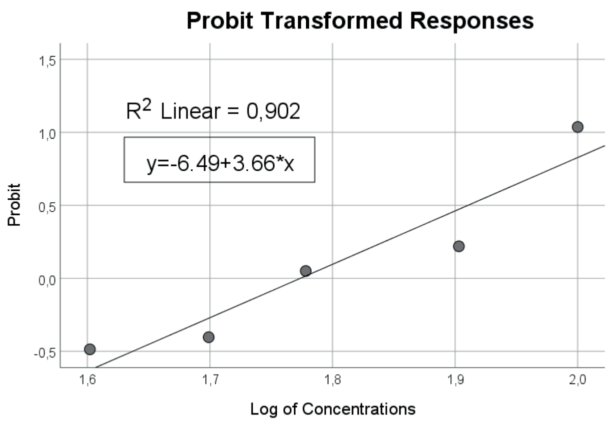

Figure 2. Probit transformed responses with equation regression and coefficient of determination R^2 for *Juniperus Phoenicia* tested on 3rd and 4th instars larvae of *Culiseta longiareolata* for 24 h.

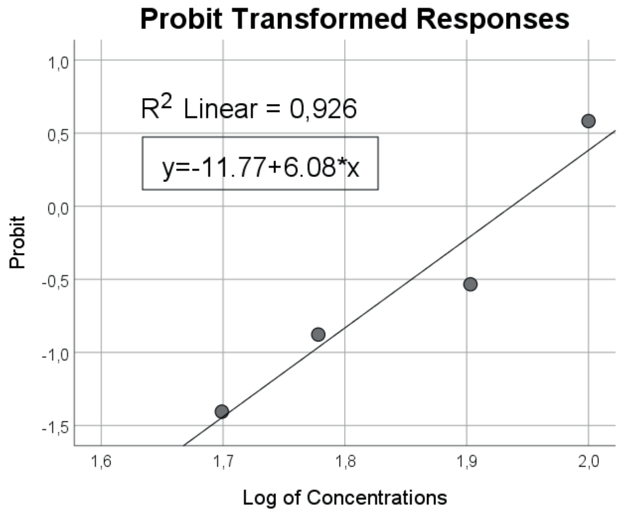

Figure 3. Probit transformed responses with equation regression and coefficient of determination R^2, for *Artemisia* herba-alba essential oil tested on 3rd and 4th instars larvae of *Culiseta longiareolata* for 24 h.

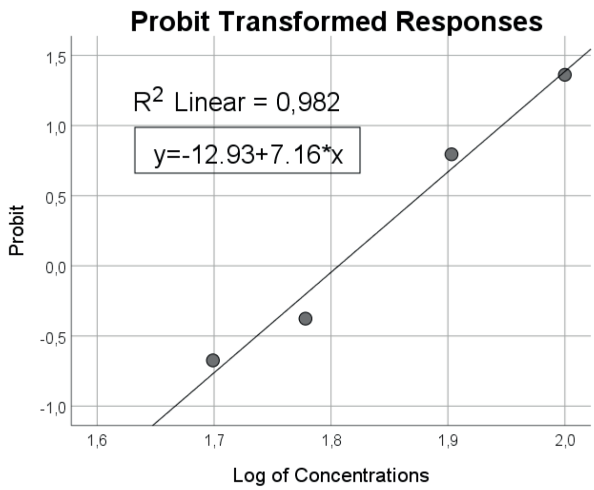

Figure 4. Probit transformed responses with equation regression and coefficient of determination R^2 for *Rosmarinus officinalis* essential oil tested on 3rd and 4th instars larvae of *Culiseta longiareolata* for 24 h.

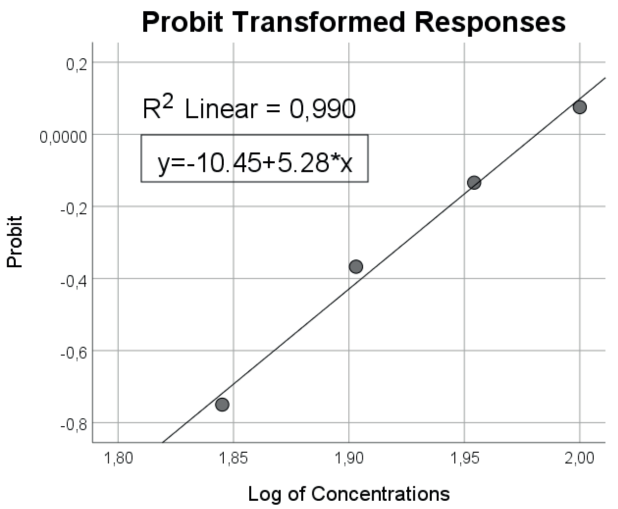

Figure 5. Probit transformed responses with equation regression and coefficient of determination R^2 for and *Eucalyptus globulus* essential oil tested on 3rd and 4th instars larvae of *Culiseta longiareolata* for 24 h.

DISCUSSION

The current study has confirmed that the EOs extracted from the aromatic medicinal plants *T. vulgaris*, *A. herba-alba*, *J. phoenicea, R. officinalis* and *E. globulus* present an efficient larvicidal activity against the *Culiseta longiareolata* larvae; however, the mortality responses obtained were varying.

T. vulgaris is a flowering herb that has a worldwide distribution (17). From the total of the tested oils, the *T. vulgaris* EO was the most efficient. This EO was previously assessed by Knio et al. (18) against the *Ochlerotatus caspius* (Pallas 1771) larvae; however, its toxicity against *Oc caspius* (LC_{50}=33.65ppm; LC_{90} =50.85ppm) was less than that shown by our *T. vulgaris* EO. Likewise, the larvicidal activity of the EOs extracted from the *Juniperus* species was tested in previous studies: *J. Phoenicea* against *Aede salbopictus* (Skuse 1894) (LC_{50}= 55.5ppm; LC90= 77ppm), and *J. virginiana* L. against *Ae aegypti* (Linnaeus 1762) and *Cx pipiens* (19, 20). Comparing our results, our *J. phoenicea* EO showed lower larvicidal activity against *Cs longiareolata*. Moreover, the larvicidal activity of *R. officinalis* EO was assessed against *Ae albopictus* (LC_{50}<250ppm), *Cx tritaeniorhynchus* (Giles 1901) (LC_{50}= 115.38ppm; LC90= 211.53ppm) and *Anopheles subpictus* Grassi (LC_{50}= 64.5ppm; LC90= 113.74ppm) (21, 22). The *R. officinalis* EOs tested against *Ae albopictus, Cx tritaeniorhynchus* and *An subpictus* in the previous researches showed lower values than the toxicity results that we obtained by testing the same EO against *Cs longiareolata*.

The other EOs *E. globules* and *A. herba-alba* were less efficient; however, their lethal concentrations were notable. *E. grandis* L. EO and its major components were assessed for their larvicidal activity against *Aedes aegypti* by Lucia et al. (23). The EO showed 32.4ppm LC_{50} and the principal components α-pinene (52.71%) and 1,8-cineole (18.38%) showed 15.4ppm and 57.2ppm LC_{50} respectively. The principal leaf oil components of *E. globules* harvested from Algeria are α-pinene and 1,8-cineole, according to Samir et al. (24). However, our *E. globules* EO tested against *Cs longiareolata* was less efficient (LC_{50}= 95.83ppm). Furthermore, EOs extracted from *Artemisia* genus were assessed for their larvicidal activity against various mosquito species. Our *A. herba-alba* EO tested against *Cs longiareolata* larvae was more efficient (LC_{50}= 86.67ppm) than *A. vulgaris* L. that was tested by Ilahi and Ullah (25) against *Cx quinquefasciatus* (LC_{50}= 803.2ppm), but less efficient than *A. absinthium* L. tested by Govindarajan and Benelli (26) against *An stephensi* (Liston 1901), *An subpictus*, *Ae aegypti*, *Ae albopictus*, *Cx quinquefasciatus* (Say 1823), and *Cx*

Table 3: Principal component percentages of *T. vulgaris, A. herba-alba, J. Phoenicea, R. officinalis and E. globules* harvested from Algeria, according to previous works.

Principalcomponents	***T. vulgaris (29)***	***J. phoenicea (30)***	***A. herba-alba (31)***	***R. officinalis (32)***	***E. globules (24)***
Carvacrol	11.41	-	-	-	-
Thymol	25.57	-	-	-	-
α-Pinene	12.1	34.5	Tr	5.4	8.8
α-Terpinylacetate	-	14.7	-	-	-
p-Cymene	26.36	-	-	-	-
Thymoquinone	10.5	-	-	-	-
β-Phellandrene	-	22.4	-	-	-
Camphor	-	-	19.4	14.6	-
1,8-Cineole	-	-	Tr	12.2	71.3
β –Caryophyllene	-	-	-	10.9	-
Borneol	-	-	-	10.6	-
γ-terpinene	-	-	23.8	-	-
β-thujone	-	-	15.0	-	-
chrysanthenone	-	-	15.8	-	-
trans-pinocarveol	-	-	16.9	-	-

tritaeniorhynchus (LC_{50}=41.85, 52.02, 46.33, 57.57, 50.57, and 62.16 ppm respectively). Various mosquito species were targeted in the previous researches to assess the larvicidal activity of EOs. However, *Cs longiareolata* was not previously targeted by EOs, but by the lichen metabolites evaluated by Cetin et al. (27), that showed high larvicidal activity against *Cs longiareolata*.

The results obtained confirm the previous studies; the use of EOs can serve as an eco-friendly method to control mosquito larvae. However, the noted variability in the efficacy level of the tested oils may be due to their chemical composition and the percentages of their principal components as α-Pinene, Camphor and 1,8-Cineole (Table 3); whereas, the direct use of the principal components of EOs may produce a higher efficacy in mosquito control. This hypothesis was proven in the study conducted by Lucia, Gonzalez-Audino (23), where the principal components of Turpentineand *E. grandis* EO showed lower LC_{50} than that obtained by the use of the entire *E. grandis* EO. Moreover, the repellency effect of the thyme EO compounds against *Culex pipiens* mosquito evaluated by *Park et al. (28)* showed higher repellent efficacy of α-Terpinene and Carvacrol than the commercial formulation diethyltoluamide (DEET), and an equal efficacy between the Thymol component and the DEET.

CONCLUSION

The EOs extracted from the aromatic medicinal plants and their principal components may serve as safe products to control the *Culiseta longiareolata* larvae in Algeria; nevertheless, their practical application remains a fundamental step to evaluate their field efficacy and to note their possible secondary effects on non-targeted organisms.

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Conflict of Interest: The authors declare that they have no conflicts of interest.

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