UTTAR PRADESH JOURNAL OF ZOOLOGY

43(6): 19-25, 2022
ISSN: 0256-971X (P)

TOXIC EFFECT OF A CERTAIN MEDICINAL PLANT EXTRACTS AGAINST LARVAE OF MOSQUITO SPECIES *Culiseta longiareolata* (MACQUART, 1838)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2022/v43i62974

Editor(s):
(1) Dr. Osama Anwer Saeed, University of Anbar, Iraq.
Reviewers:
(1) Maulana Yusuf Alkandahri, Buana Perjuangan Karawang University, Indonesia.
(2) V. Selvanarayanan, Annamalai University, India.

Received: 10 February 2022
Accepted: 14 April 2022
Published: 20 April 2022

Original Research Article

ABSTRACT

The domestic mosquito *Culex pipiens* and *Culiseta longiareolata* are considered to be the most important mosquito species in Algeria, due to their large geographical distribution and abundance. Instead of causing environmental nuisance, they transmit diseases to the animals. The mosquito control, using conventional insecticides becomes harmful to human and to the environment and in addition mosquitoes have developed a resistance against these products. These reasons have encouraged scientists to propose new eco-friendly and effective alternatives to control insects. Plants extracts have showed a promising agents for pest treatments. In order to test a botanical larvicide against mosquito, extracts were prepared from the leaves of *Marrubium vulgare, Laurus nobilis, Eucalyptus globulus,* and *Myrtus communis* and the extractions were done with methanol, using a Soxhlet extractor. Bioassay was carried out, using different concentrations against the fourth mosquito larvae of the *C. longiareolata.* The toxicity of the tested extracts was evaluated and the lethal concentrations, LC_{50} and $LC_{90)}$ values were estimated. The obtained results indicated a sensitivity of *Cs. longiareolata* larvae to the four plants species, with dose-response relationship mortality. The lethal concentrations were calculated with their confidence limits and their values were for LC_{50} of 6.13, 7.03, 9.60 and 17.84g/l, while the LC_{90}'s were 22, 37.19, 75.69 and 92.76g/l for *M. vulgare, L. nobilis, E. globulus,* and *Myrtus communis* respectively. These toxicological assays, using these autochthon plant extracts could be developed as an alternative compound for mosquito control programme.

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Keywords: Plant extracts; *Culiseta longiareolata;* insecticide; mosquito control.

1. INTRODUCTION

The mosquitoes are considered to be the most important insect vectors of diseases. The main transmitted diseases to human are malaria, yellow fever, dengue, West Nile, Zika, chikungunya fever, Japanese encephalitis, and lymphatic filariasis [1,2]. Therefore, World Health Organization has declared the mosquitoes are considered as the prime public health pest [3]. *Culiseta longiareolata* (Diptera: Culicidae) is one of the most common mosquitos prevalent in Algeria mainly in urban areas [4,5]. Previous study has reported that, *Cs. longiareolata* could increase its distribution range in temperate regions [6]. The hematophage female mosquito sucks the blood of their hosts that include humans, horses and other vertebrates [7]. The blood feeding is the pivotal factor in the continuation of both parasite and vector generations [8]. Some *Culiseta* species were mentioned to be implicated as virus vectors [9] and are suspected of being involved in the transmission of arboviruses and avian malaria [10]. *Cs. longiareolata* is considered as a primary vector of *Plasmodium circumflexum* (Kikuth1931), *Plasmodium relictum* and *Plasmodium polare* (Manwell 1934) in birds [11]. The pest control using chemical compounds is causing serious health problems to human and to environment. Moreover, insect control techniques present serious threats because of the emergence of resistance to widely used synthetic insecticides [12]. To improve the environmental quality and public health, more attention has been focused on botanical extracts, in order to reduce and restrain the evolution of further resistance with biodegradable and environmentally safe tools [13]. In general, the adverse effects of pesticide use and the increased restrictions on the application of pesticides have stimulated the exploration of eco-friendly pest control methods [14], such as plant essential oil application with the lowest risk and the highest compatibility with the environment [15]. As stated by the recommendation of WHO [16], the application of botanical extracts could be an alternative solution for the mosquito control. The aim of the present study is to evaluate the insecticidal activity of the methanolic extracts of *Marrubium vulgare, Laurus nobilis, Eucalyptus globulus,* and *Myrtus communis;* against the 4thinstar larvae of *Cs. longiareolata*; the most abundant mosquito species in Algeria, particularly in Setif region [17].

2. MATERIALS AND METHODS

The larval susceptibility tests were carried out according to the World Health Organization (WHO) procedure [16]. The toxicological assays were done using four medicinal plants extracts, *Marrubium vulgare* (Lamiaceae), *Laurus nobilis* (Lauraceae), *Eucalyptus globulus* and *Myrtus communis* (Myrtaceae) against *Cs. longiareolata* larvae under laboratory conditions.

2.1 Mosquito Rearing

The larvae of *Cs. longiareolata* were obtained from laboratory mass colonies. Larvae were reared in controlled temperature (25 ± 2°C) and photoperiod cycle of 12/12(light/dark) in Pyrex storage jars containing 500ml stored tap water.

2.2 Plant Collection and Aqueous Extract

Leaves of all the four selected plants *Marrubium vulgare, Laurus nobilis, Eucalyptus globulus* and *Myrtus communis*, were collected at the end of spring season from Setif region (Northeastern Algeria). In the laboratory, the material was washed with distilled water, to remove all the unwanted impurities, and dried at room temperature. Completely dried leaves were subjected to grinding with a commercial blender. Soxhlet extraction was carried out separately, using 100 g fine powder of each plant material which was put in a thimble made up of What man cellulose paper, using 350ml of aqueous solution of methanol (70%; 70 : 30, v/v), until exhaustion (till the solvent is seen completely colorless, then 2-3 additional drain-off to ensure complete extraction) for 72h (time of one drain off and accordingly total duration for 70-80 cycles) [18]. The crude extracts were concentrated in rotary evaporator under reduced pressure at 45°C and the residue obtained was weighed, labeled and stored in refrigerator at 4°C for the bioassays.

2.3 Toxicological Bioassays

The larvicidal activity of the four plants extracts were tested against newly exuviated fourth instar larvae of *Cs. longiareolata*, under laboratory conditions. All the plant extract residues after weighing (50g) individually were solubilized in 100ml of acetone. From the stock solution, four concentrations were considered, after preliminary tests (5g/l= 10 ml; 23g/l= 64ml; 40g/l= 80ml and 50 g/l= 100ml) and then prepared for toxicity treatments. Larvicidal activity was done according to World Health Organization method [16]. Twenty-five new exuviated fourth instar larvae of test mosquito species, were kept in three replicates, prepared in a separate jar containing 500 (ml) of breeding water for every test concentration. Controls received the same quantity of the solvent (acetone) only. The treated larvae series were exposed to the methanolic extract for 24 hours, than the water was changed and the food was added every three days. The mortalities, of the control and treated series, were daily recorded and were followed during the other developing stages until the adult emergence.

2.4 Statistical Analysis

Statistical analyzes were performed using Minitab and Prism 7.0 for Windows (Graph Pad Software Inc, USA). The results were subjected to correction using the Abott formula [19] when the mortality was observed in the control series. The data have been expressed by the mean± and the standard deviation (M ± SD). Additionally, all data were analyzed by one-way ANOVA analysis of variance with a significant level of $p<0.05$. The equation and the regression line were determined, after the transformation of the mortality to probit and the tested concentrations in decimal logarithms.

3. RESULTS

The data on larvicidal activity, of *M. vulgare, L. nobilis, E. globulus* and *M. communis,* against *Cs. longiareolata* larvae are shown in Table 1. After treatment, the observed mortality increased accordingly with the concentrations. The recorded mortality of the treated 4th instar larvae varies between 12% and 100%, which demonstrated that *M. vulgare, L. nobilis, E. globulus* and *M. communis* have a larvicidal effect on the mosquito species *Cs. longiareolata.* As it shown in Fig. 1 the mortality increased as much as the concentration was increased for all plants.

With the highest concentration of 50g/l, the mortality increased for the four plants, when it was 88% for *M. communis*, 92% for *E. globulus*, 97.33% for *L. nobilis* and 100% for *M. vulgare*, after 24h (Tab. 1). At the highest concentration (50g/l), the larvae showed restless movement, without flickering, for sometimes with abnormal wagging and then died. Whereas, the lowest concentration was 5g/l caused a different mortality rate of the treated larvae 45.33, 41.33, 32 and 12%, after 24 hours for *M. vulgare*, *L. nobilis, E. globulus* and *M. communis* respectively (Tab.1). In Fig. 1, after using the same concentrations, all the plant extracts, exhibit a toxic effect, but *M. vulgare* and *L. nobilis* were found more effective than *E. globulus*. On the other hand, the lowest activity was exhibited by the leaf extracts of *M. communis*. The presented results reveal a dose-response effect ($p<0.005$) to four plant extracts.

The coefficient of determination (R^2 = 0.809, 0.764, 0.800 and 0.892 for plant extracts; *Marrubium vulgare*, *Laurus nobilis*, *Eucalyptus globulus* and *Myrtus communis* respectively) reveals an equally positive relation between the probit and the decimal logarithms of the tested concentrations (Fig. 2).

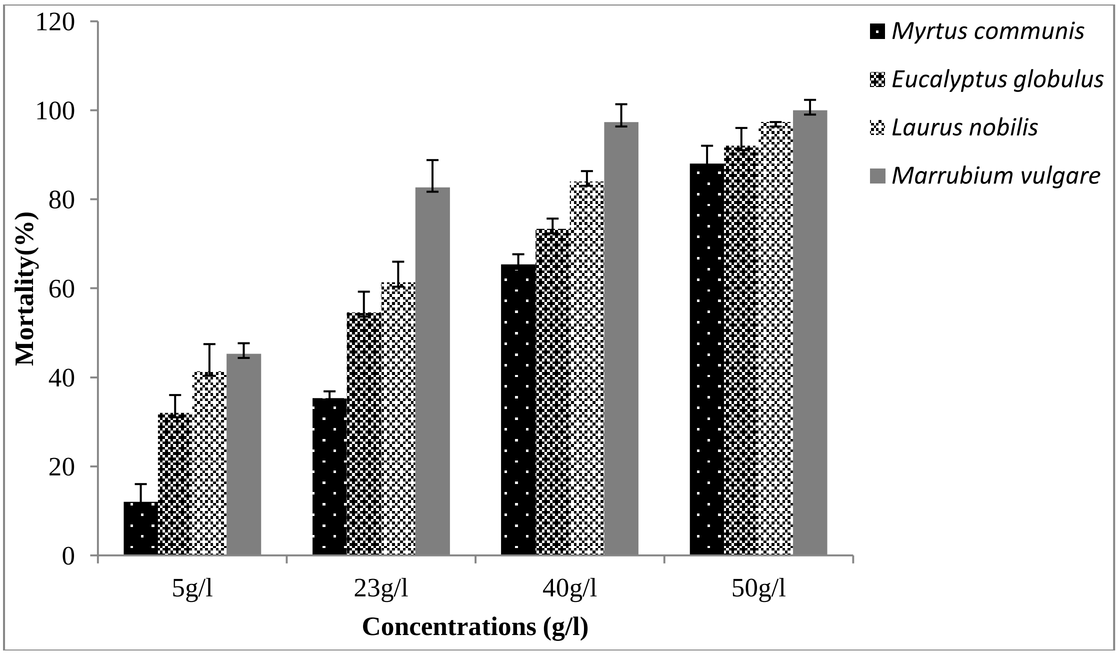

Fig. 1. Concentration-response relationship for treatment of methanolic extracts of *M. vulgare, L. nobilis, E. globulus* and *M. communis* applied to the newly exuviated fourth instar larvae of *C. longiareolata*

Table 1. The observed mortality (%) of the fourth larvae stage of *Cs. longiearolata* exposed to different concentrations of the plant extracts of *Marrubium vulgare, Laurus nobilis, Eucalyptus globulus* and *Myrtus communis*

Plant Extract	Observed Mortality (M±SD) throughout the duration of the treated stage				
	Control	5g/l	23g/l	40g/l	50g/l
Marrubium vulgare	2.65±1.15	45.33±6.11	82.67±4.62	97.33±2.31	100±0.00
Laurus nobilis	2.65±1.15	41.33±2.31	61.33±6.11	84.00± 4	97.33±2.31
Eucalyptus globulus	2.65±1.15	32± 4	54.67±4.62	73.33±2.31	92 ± 4
Myrtus communis,	2.65±1.15	12± 4	35.33±1.15	65.33±2.31	88 ± 4

Values are expressed in M±SD

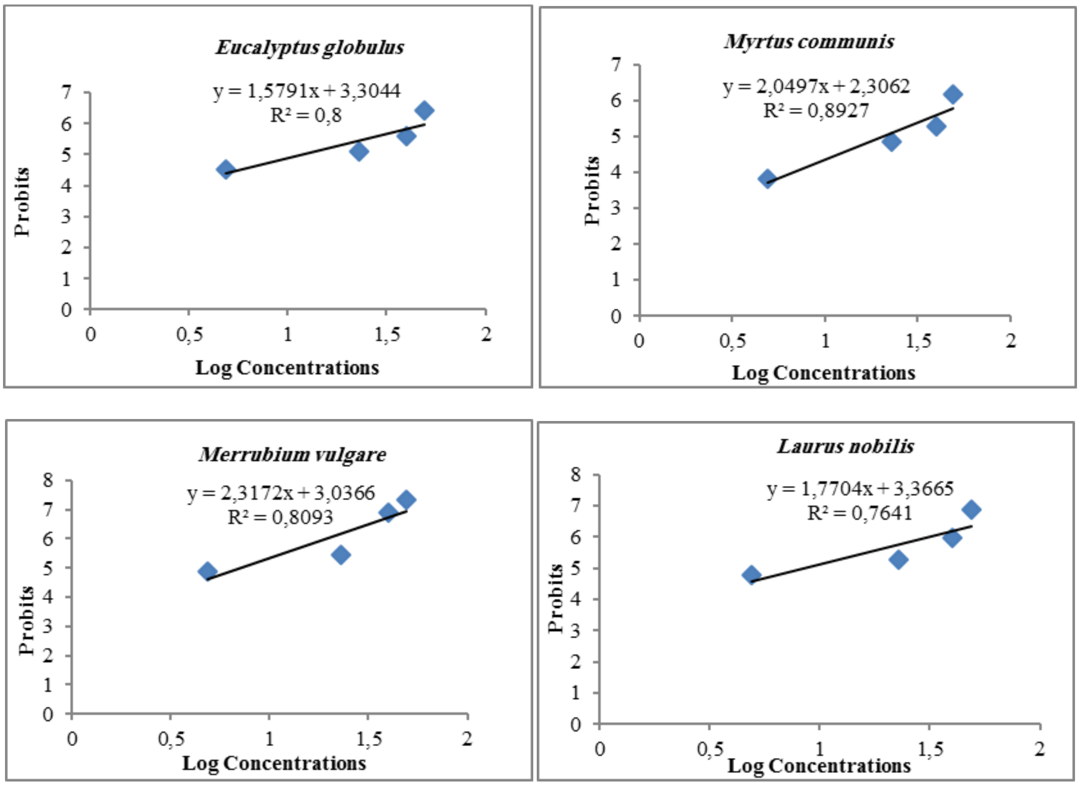

Fig. 2. Probit transformed responses with equation regression and coefficient of determination R^2 for four methanolic extracts tested on 4th instars larvae of *Culiseta longiareolata* for 24 h

Table 2. The LC_{50} and LC_{90} values of methanolic extracts of *Marrubium vulgare, Laurus nobilis, Eucalyptus globulus* and *Myrtus communis* against the 4th instar larvae of *Culiseta longiareolata*, after 24, hours exposure time; with regression equations and regression coefficients (R^2)

Plant extract	Linear regression	Slope	LCL>LC_{50}>UCL(g/l) Confidence limit 95%)	LCL>LC_{90}> UCL(g/l) Confidence limit (95%)	R^2
Marrubium vulgare	Y= 2.317x+3.036	2.78	7.1 2>6.13>8.25	25.52>22>29.60	0.809
Laurus nobilis	Y=1.770x+3.366	3.62	8.44 >7.03 >10.12	44.63>37.19>53.55	0.764
Eucalyptus globulus	Y=1.579x+3.304	4.26	12.1 >9.60 >15.24	79.08>92.76>99.62	0.80
Myrtus communis	Y=2.049x+2.306	2.52	21.06 >17.84 >24.85	89.32>75.69>105.39	0.892

The lethal concentrations (LC50 and LC90) and the slope values, for each plant, were also estimated from the curves of Fig. 2 and presented in table2. The lethal concentrations LC50 and LC90 of *M. vulgare* (6.13,22 g/l), *L. nobilis* (7.03, 37.19), *E. globulus* (9.60, 92.76) and *M. communis* (17.84, 75.69 g/l) extracts were estimated from the equation and the linear regression with their fudicial limits (FL) (Table 2).

4. DISCUSSION

Plants still have a hopeful future, as a phytochemical composition and the potential health benefits of many species have not yet been studied or still need to be more deeply investigated [20]. A lot of medicinal plants such as *M. vulgare, L. nobilis, E. globulus,* and *M. communis* have gained popularity for the treatment or prevention of a lot of disorders. The antimicrobial and antioxidant power reported in different studies confirm the therapeutic value of *M.vulgare, L. nobilis* [21], *E. globulus*[22], and *M. communis* and support the use of this plant in folk medicine [23,24].

Several studies were conducted in order to evaluate the biological effects of different plant extracts against a wide range of mosquito vectors [25,26]. in Algeria The use of plant extracts against mosquitoes were tested and the toxic effect was reported [27,28]. Previous studies confirmed that the use of plant extracts against mosquito species such as *Culex pipiens* [29] and *Cs. longiareolata* [30] have shown the high efficacy for controlling the larval stages. This study indicates that *M. vulgare, L. nobilis, E. globulus,* and *M. communis* extracts present an interesting larvicidal activity against *Cs. longiareolata.*

The mortality percentage of the treated larvae was the first indicator to evaluate the larvicidal effect of the four methanol extracts *of M. vulgare, L. nobilis, E. globulus,* and *M. communis* in the control of *Cs. longiareolata* mosquito larvae. The present work demonstrates the potency of *M. vulgare, L. nobilis, E. globulus,* and *M. communis* extract in the control of *Cs. longiareolata* mosquito larvae with LC_{50} value 6.13, 7.03, 9.60 and 17.84g/l respectively ; However, for the LC_{50} of *M. communis* (17.84 g/l) was higher than that register for *E. globulus* (9.60 g/l), *L. nobilis* (7.03 g/l), and *M. vulgare* (6.13 g/l); This results indicate that the leaf extracts of *M. vulgare*, was the most efficient. These results concord with the results obtained previously [31], when the study show that the extracts of the plant *M. vulgare* is toxic to fourth stage larvae of *C. pipiens.* Other study confirmed the mortality responses obtained from the treatment of the essential oils extracted from the medicinal *plant E. globulus* against the same species of mosquito, present an efficient larvicidal activity [32]. In the other study the larvicidal activity of the essential oil extracted from *L nobilis, Eucalyptus spp* against *Culex pipiens molestus* have examined and the lethal concentrations 117.0 and 120.0 mg litre^{-1}, respectively [32]. In addition, previous studies on Myrtles *(Myrtus communis)* are in agreement with our study; [31] found that the methanolic extract and essential oil of different Myrtles exhibits an insecticidal activity against adults of *S. oryzae* (L.) and *T. confusum* (Duv), using direct contact application and fumigation methods. The larvicidal activity of plant extracts does not depend only on species, but also on used plant parts, environmental conditions and time of cultivation, but also on the type of the solvents polarity [33].

5. CONCLUSION

Because of the abusive and repeated use of the conventional pesticides, the World Health Organization recommended to reduce the using of chemical pesticides in mosquito control, in order to preserve the environment and human health, and encouraged a new alternatives without effects on non target organisms. The present results showed the efficacy of methanolic extracts of *Marrubium vulgare*, *Laurus nobilis*, *Eucalyptus globulus,* and *Myrtus communis* against larvae of *Culiseta longiareolata*, these plants can be used to develop environmentally safe vector and pest managing agents.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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