Larvicidal effect of *Marrubium vulgare* on *Culex pipiens* in eastern Algeria

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**Abstract**

Plant extract of *Marrubium vulgare* was tested against 4th instar larvae of the mosquito *Culex pipiens* L. The obtained results indicated a sensitivity of *Culex pipiens* larvae for the plant species aroused. This sensitivity is even higher when exposure of the larvae to insecticides is extended in time. Generates the greatest mortality rate 94% for 900 mg/l after 72 h of exposure against 59% for 900 mg/l after 72 h exposure for the extract of *Marrubium vulgare*. For LC50 values, *Marrubium vulgare* acted at low concentrations with an LC50 of 668mg/l after 72 h of exposure. Thus, these results may provide an opportunity to develop alternatives to environmentally hazardous chemicals with some available cheap plants which are usually environmentally safe to different living organisms.

1. Introduction

The insects Culicidae are probably the best known and the most feared for both parasitic diseases they can inoculate during their blood meal and the nuisance their presence constitutes. According to [1], *Culex pipiens* is one of the main vectors of St. Louis encephalitis virus in the United States, it was also considered to be the principal vector of West Nile virus in Romania[2], Israel [3], United States[4], Bulgaria and the Czech Republic [5]. Morocco was hit in 1996 [6] and 2003 [7]. This species occurs in the amplification of
the transmission cycle of the virus to birds [8]. It also has as pathogen vector responsible for malaria, yellow fever, dengue, filariasis [9] and some encephalitis [10]. In Algeria, the West Nile virus caused a major outbreak in the region Timimoune in 1994; isolated cases of encephalitis in humans with fatal cases are reported by [11] and [12]. In Algeria, *Culex pipiens* is the mosquito which presents the greatest interest because of its abundance and real nuisance it constitutes in urban areas [13]. Moreover, this species is suspected to be involved in West Nile and Rift Valley Fever Viruses transmission; according to this context, we define the importance of *Culexpipiens* as vector of both viruses in the Maghreb region [14].

To fight against this scourge, considerable quantities of chemical insecticides have been used worldwide [15]. Unfortunately, the mosquito, principal vector of the transmission of these pathogens has developed a resistance against the insecticides most commonly used in different programs, such as organophosphates or pyrethroids [16], [17] and [18]. These means of vectorial control have adverse effects on humans, animals and the environment due to their accumulation in the environment [19]. Diversification of approaches to integrated pest management is required for better environmental protection. Among the alternative strategies, the use of plants, insecticidal allelochemicals appears to be promising. Aromatic plants, and their essential oils, are among the most efficient botanicals [20]. This method is safe, selective and biodegradable.

It is in the context of control that our study was undertaken on the main nuisance in Constantine, represented by *Culex pipiens*. The introduction of new alternatives to control *Culex pipiens* may be particularly interesting; therefore the use of plant extracts with insecticidal effects offers promising potentialities.

2. Materials and methods

1.1. Plant collection and extraction

*Marrubium vulgare* (white horehound or Common Horehound) is herbaceous plant belonging Lamiaceae family, widespread in North Africa, they cover vast territories valued at more than ten million hectares. The plant was collected at Constantine (Algeria) during April and May, 2012 and brought to the laboratory. The separated leaves (from the twig) were washed and dried under shade at ambient room temperature during 12 hours; they were ground coarsely using a mechanical grinder. One hundred grams of leafpowder of plant was dissolved in 350ml of methanol (solvent) and extracted in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under a reduced pressure at 50°C and the residue obtained was stored at 4°C.

1.2. Insect rearing

Mosquito larvae of *Culexpipiens* collected from sampling sites near Constantine University (Algeria) are reared in the laboratory and fed with a mixture composed of 75% powder of biscuit and 25% dry yeast [21]. When the larvae reach the pupal stage, they are placed in other containers and placed in a cage until emergent adults were fed with dried grape.

1.3. Larvicidal test

In accordance with the recommendations of the World Health Organization (who) and after the preliminary examinations, three concentrations of plant extract (200, 500 and 900 mg/l) were prepared and tested against the fourth instar larvae of *Culex pipiens*. Ninety-nine milliliters of water are placed in a plastic cup to which are added 25 larvae and one milliliter of the insecticide. The experiments were conducted with four replicates and a concurrent control group. The number of larvae surviving at the end of 24, 48 and 72 hours of exposure was recorded and the mortality values percentage were calculated.

To prevent mortality caused by hunger, larvae are fed after 24 hours of exposure.

1.4. Statistical analysis
the highest mortality. Exploitation of the results was made using the XLSTAT “the data analysis software and statistics”. The mortality percentages were corrected using Abbott’s formula [22] and software to calculate probit analysis according to Finney [23] was used.

\[
\text{Percentage of Mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}}
\]

\[
\text{Observed Mortality (\%)} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}}
\]

The determination of lethal concentrations (LC\(_{50}\)) value of leaf extract of \textit{Marrubium vulgare} on \textit{Culex pipiens} is based on logistic regression to model the impact of concentrations of extract on the mortality using a log-probit analysis statistical software “XLSTAT”.

3. Results

Mortality of the larvae \textit{Culex pipiens} exposed to different doses of \textit{Marrubium vulgare} varies with exposure time (table 1). So after 24 hours of contact with the insecticide the maximum of mortality (39\%) is recorded for the dose of 900mg/l against 20\% of death for 200mg/l. After 48 hours of exposure, the concentration 900mg/l given 45\% of mortality. The highest mortality (59\%) was observed after 72 hours of exposure at a dose of 900mg/l.

<table>
<thead>
<tr>
<th>Exposure time</th>
<th>200 mg/l</th>
<th>500mg/l</th>
<th>900mg/l</th>
<th>Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours</td>
<td>20%</td>
<td>31%</td>
<td>39%</td>
<td></td>
</tr>
<tr>
<td>48 hours</td>
<td>24%</td>
<td>37%</td>
<td>45%</td>
<td></td>
</tr>
<tr>
<td>72 hours</td>
<td>31%</td>
<td>40%</td>
<td>59%</td>
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</tbody>
</table>

Larval mortality of \textit{Culex pipiens} after the treatment with \textit{Marrubium vulgare} is shown in Table 2; the exposure to \textit{Marrubium vulgare} \textsubscript{LC\(_{50}\)} and \textsubscript{LC\(_{90}\)} values were represented as follows; \textsubscript{LC\(_{50}\)} and \textsubscript{LC\(_{90}\)} values after 24 hours contact are respectively equal to 1893mg/l and 2924mg/l; after 48 hours, the values corresponding to 903mg/l and 1674mg/l and finally after 72 hours of exposure the values are equal to 668mg/l and 1665mg/l. The dermination coefficient corresponding to 24h, 48h and 72h are respectively 0,66, 0,43 and 0,52.
Table 2: Larvicidal activity of *Marrubium vulgare* at various concentration, applied for 24, 48 and 72h against *Culex pipiens*.

<table>
<thead>
<tr>
<th>Plants name</th>
<th>Concentration (mg/l)</th>
<th>Exposure time</th>
<th>LC50 (mg/l)</th>
<th>95% fiducial limits</th>
<th>RC90 (mg/l)</th>
<th>R²</th>
<th>Regression equation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Marrubium vulgare</em></td>
<td>200 mg/500 mg/l</td>
<td>24hours</td>
<td>1893</td>
<td>1737 1914</td>
<td>2924 0.66</td>
<td>Y=0.006x +3.93</td>
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<tr>
<td></td>
<td>900 mg/l</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>200 mg/l</td>
<td>48hours</td>
<td>903</td>
<td>869 954</td>
<td>1674 0.43</td>
<td>Y=0.008x +4.68</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500 mg/l</td>
<td></td>
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<td></td>
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<td></td>
<td>900 mg/l</td>
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</tr>
<tr>
<td></td>
<td>200 mg/l</td>
<td>72hours</td>
<td>668</td>
<td>598 709</td>
<td>1665 0.52</td>
<td>Y=0.010x +5.45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500 mg/l</td>
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<tr>
<td></td>
<td>900 mg/l</td>
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P< 0.05 significance level, R²: The determination coefficient.

4. Discussion

The results of this study show that the extracts of the plant *Marrubium vulgare* is toxic to fourth stage larvae of *Culex pipiens*. This toxicity is well marked when the exposure time of the larvae is longer. However, this sensitivity is even more enhanced with increasing concentration of the extract. The results show a high mortality rate (59%) after 72 h of exposure for the dose of 900 mg / l. For LC50 values, *Marrubium vulgare* acted at low concentrations with an LC50 of 668mg /l after 72 h of exposure. Exposure times of 72 hours are the most convincing for plant extract. This result is explained by the fact that the content in the extracts active ingredient is released slowly. Reference [24] reported this fact with neem powder tested on pre-imaginal stages of *Culex quinquefasciatus*.

The essential oil of *Marrubium vulgare* extract is mentioned by several authors as a good larvicide against different culicid species. The work of [25] indicates that the essential oil of this plant is mainly composed of thymol which has a larvicidal and nymphicidal action. The results obtained in this study corroborate those of [26] showing a positive larvicidal effect of the plant extracts against larvae culicidae.

Plants are a source of active substances with great potential of wide application range. This application diversity could be due to the diversification of bioactive molecules that make up the herbs. Indeed, several compounds are often cited as responsible for their larvicidal properties.
Many authors have tested extracts from several other plant species on different species of mosquitoes [27], [28]. When using aqueous extracts of leaves of *R. Communis*; Reference [29] reported a high larvicidal activity against the second and fourth instar larvae of four species of mosquitoes, *Culex ppienis* (L.), *Aedes caspius* (Pallas), *Culiseta longiareolata* (Aitken) and *Anopheles maculipennis* (Meigen). The pesticidal activity reported by [30], [31] and [32] could be explained by the molecular interaction of the functional groups of components extracts with the tissues of organisms targeted. This interaction could be the result of a singular action of a component, or of a synergistic effect of several compounds of the aqueous extract.

5. Conclusion

Our results suggest that the extract of this plant tested may be an alternative, a substitute to synthetic insecticides. It would be interesting to continue the investigations on this plant such that isolating and identifying molecules responsible for this insecticidal action.

6. References