Struggle Against Vector-borne Diseases: Phytochemical Screening and Larvicidal Activity of Hydro-ethanolic Extract of Ocimum basilicum in North East of Morocco against the Larvae of Malaria Vector Mosquito Anopheles labranchiae (Diptera: Culicidae)

El-Akhal F1,3, Guemmouh R3, Zerrouq F4, Ez Zoubi Y5, El Ouali Lalami A1,2*

1Regional Diagnostic Laboratory Epidemiological and Environmental Hygiene, Regional Health Directorate, EL Ghassani Hospital, Fez 30000, Morocco
2Institute of Nursing Professions and Health Techniques Fez (Annex Meknès), Regional Health Directorate, EL Ghassani Hospital, Fez 30000, Morocco
3Sidi Mohamed Ben Abdellah University, Faculty of Sciences Dhar El Mahraz, Laboratory of Biotechnology, 30000 Fez, Morocco
4Laboratory of Catalysis, Materials and Environment, School of Technology, University Sidi Mohammed Ben Abdellah, 30000 Fez, Morocco
5Laboratory of Phytochemistry, National Institute of Medicinal and Aromatic Plants, Taounate 34000, Morocco

Available Online: 21st January, 2016

ABSTRACT
Mosquitoes are vectors for many tropical and subtropical diseases. They are also the most important single group of insects well-known for their public health importance. Usually the fight against vectors using synthetic pesticides generates the resistance amongst the target populations. The aim of this study was to assess the effectiveness of the extract larvicide hydro-ethanolic of Ocimum basilicum (Lamiaceae) against malaria mosquitoes, Anopheles labranchiae. The Biological tests methodology inspired from the standard WHO protocol has been used. The result of the phytochemical screening of the aqueous extract of Ocimum basilicum indicates the presence of flavonoids, tannins, mucilage and leucaonthocyanes. However, sterols, terpenes, triterpenes and coumarines were not detected. The LC50 and LC90 values found, after 24 hours of exposure of aqueous extract against Anopheles labranchiae larvae, were 23.72 and 30.78 mg/ml respectively. The study concluded that there was a lethal effect of Ocimum basilicum extract against mosquito Anopheles labranchiae larvae, which could be manipulated to develop a safe and effective larvicide.

Key words: Aqueous extract, Ocimum basilicum, Biological tests, Anopheles labranchiae, North East of Morocco.

INTRODUCTION
Mosquitoes being vector for many tropical and subtropical diseases are the most important single group of insects well-known for their public health importance. Indeed, odours of Ocimum basilicum (O. basilicum) and Corrigiola telephiifolia are very effective repellents. Ocimum basilicum is referred to as “king of the herbs” and was sourced originally from tropical and subtropical Asia for its medicinal, culinary, and ornamental properties. Biologically, it showed antibacterial, anti-thrombotic, anti-oxidant, anti-inflammatory, and antihypertensive activities. The aim of the present study was to investigate the phytochemical screening and assessing the larvicidal activity of hydro-ethanolic extract of O. basilicum on Anopheles labranchiae (An. Labrachiae). The insecticidal activity of O. basilicum plants against An. Labrachiae has never been studied before in the North East of Morocco.

MATERIALS AND METHODS
Collection and identification of plant material

*Author for Correspondence
The areal parts (leaves, stems and roots) of *O. basilicum* were freshly collected from the Medicinal Plants Farm of National Institute of Medicinal and Aromatic Plants, Taounate in June 2014. The Botanical identification and the Authenticated voucher specimens deposited in the Herbarium of The National Institute of Medicinal and Aromatic Plants, Sidi Mohamed Ben Abdellah University, Fez, Morocco.

**Ultrasound-assisted extraction**

In a 500 ml beaker, 20 g of dried plant powder was mixed with 150 ml of hexane. The beaker has been set in a Sonicator brand 'ELMA' at a frequency of 35 kHz, for 45 min, with a temperature of 25 °C. The extract was filtered through Whatman paper and the recovered solvent was rejected. Drying the powder in a plants incubator at a temperature of 40°C for 30 min, the powder was re-extracted with an ethanol / water mixture (4:1 (v/v)) for 45 min within the same conditions of the first extraction. The final extract was recovered after filtration using Whatman paper. Then it was dried using a rotary evaporator apparatus at a temperature of 40°C.

**Phytochemical screening**

The extract is screened for phytochemical constituents (tannins, flavonoids, sterols, terpenes, triterpenes, coumarins, leucoanthocyanins and Mucilages), using a simple qualitative method as described in the study of Paris et al. and Diallo.

The extract was concentrated and was dried under low pressure.

**Characteristic of larval site**

The collection of larvae of *An. labranchiae* was performed in a breeding site located in the urban area of the city of Fez, called Ain Bouknafer (1132 m altitude, 34°01´35´´N and 5°11´44´´E), with an area of 22500 m². This site is characterized by a high density of larvae belonging to Culicidae. The dominant vegetation in this site is composed of Roseau and Weed that promote the proliferation of larvae of *An. labranchiae*.

**Collection of larvae of *An. labranchiae***

Larvae were collected using a rectangular plastic tray that inclined 45° with respect to the water surface; the resultant tension force attracts the plate to the larvae. The larvae gathered were maintained in breeding in rectangular trays at an average temperature of 22.3 °C ± 2 °C in the Entomology Unit at the Regional Diagnostic Laboratory Epidemiological and Environmental Health (RDLEH) falling within the Regional Health Directorate of Fez.

**Identification of larvae**

The identification of morphological characteristics of larvae has been determined using the Moroccan key of identification of Culicidae and the identification software of mosquitoes of the Mediterranean Africa.

**Protocol of larval susceptibility testing**

The sensitivity tests were carried out in accordance with the WHO Protocol. From the initial extract (100 mg/ml stock solution) of each plant, concentrations of 10, 20, 30, 40 and 50 mg/ml were prepared. Preliminary experiments were used to select a range of concentrations for the tests previously mentioned. 1ml of each prepared solution was placed in beakers containing 99 ml of distilled water containing 20 larvae at stages 3 and 4. The same number of larvae was placed in a beaker containing 99 ml of distilled water plus 1 ml ethanol. Three replicates were carried out for each dilution and for the control. After 24 hours, we counted living and dead larvae. The results of susceptibility testing were expressed in the percentage of mortality versus the concentration of plant extract used. If the mortality percentage in control is greater than 5%, the mortality percentage in larvae exposed to the plant extract shall be corrected by using Abbott's formula. Mortality Percentage Corrected = [(Mortality Percentage Observed - Mortality Percentage Control) / (100 - Mortality Percentage Control)] × 100. If the control mortality exceeds 20%, the test is invalid and must be repeated.

**Processing of data**

For the entry and processing of data we used the log-probit analysis (Windl version 2.0) software developed by CIRAD-CA/MABIS. The analysis of the averages and standard deviation was also performed by using the test of analysis of variance ANOVA. Mean and standard deviation (± SD) were determined from at least three independent experiments.

**RESULTS**

Table 1 shows the outcomes of the phytochemical screening of the hydroethanolic extract of *O. basilicum*. The results revealed the presence of flavonoids, tannins, mucilage and leucaanthocyanes. However, sterols, terpenes, triterpenes and coumarins were not detected. The hydroethanolic extract of *O. basilicum* was used. The mortality rate varies between 10% and 100% (Figure 1). The lowest concentration necessary to achieve 100% mortality of larvae of *An. labranchiae* was evaluated at 40 mg/ml (Figure 1).

**LC50 and LC90 lethal concentrations**

Figure 1 confirms the analysis performed in the order of effectiveness of hydroethanolic extract tested. The *O. basilicum* exhibits the lowest LC50 of 23.72 mg/ml (Equation of the regression line: Y = -4.25561 + ...
El-Akhal et al. / Struggle Against Vector-borne…

Table 2: Concentrations LC50 and LC90 lethal larvae of An. Labranchiae after 24 hours of exposure.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>LC50 (mg/ml) (LI-UI)*</th>
<th>LC90 (mg/ml) (LI-UI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. basilicum</td>
<td>23.72 (21.61-25.71)</td>
<td>30.78 (28.11-35.77)</td>
</tr>
</tbody>
</table>

* LI-UI: Lower limit - Upper limit

![Figure 1: Percentage of mortality recorded in the sensitivity test by aqueous extract of the plant on An. labranchiae.](image)

11.34007 * X: calculated Chi²: 3.090) and LC90 = 30.78 mg/ml (Table 2).

**DISCUSSION**

Many phytochemicals found in plants are either the product of plant metabolism or synthesized for defense purposes. Several studies have demonstrated that Ocimum species is an excellent source of the following components: alkaloids, glycosides, flavonoids, phenols and tannins which have exhibited different biological activities. Our results are in accordance with other studies revealing to the presence of flavonoids and tannins. The larvicidal activity of the extracts of aromatic plants was also confirmed in the works of Jang et al. Plant extracts have been suggested as alternative for insect control because some are selective, biodegradable and nontoxic products, and have few effects on nontarget organisms and the environment as well. After exposing larvae of the species An. labranchiae to different concentrations of aqueous extracts for 24 hours, the mortality rate varies according to the concentrations (Figure 1). Larval mortality rate reached 100% at a concentration of 40 mg/ml. This efficiency could be explained by the action or effect of phenolic component (flavonoids, tannins, mucilage and leucaonthenes) against the An. labranchiae. The values of LC50 and LC90 (23.72 and 30.78 mg/ml) that we found in this study are lower than those (57.57 and 166.35 mg/ml) recorded recently in another work, conducted also by the action of another plant “Nerium oleander”, known equally rich in flavonoids, against a Culicidae mosquito, Culex pipiens. Phenolic components are also known to have ovicidal, larvicidal, nymphocidal and adulticidal properties against various insect species. Indeed, Stephens et al. and White have investigated the use of Ocimum spp, freshly harvested, from kivumbasi, branches of Ocimum suave and Ocimum canum traditionally placed in the corners of rooms to prevent mosquitoes from entering houses freshly cut in Tanzania. According Maurya et al., extracted from the leaves of Ocimum basilicum has been effective on Anopheles stephensi and Cx. Quinquefasciatus with LC50 values of 8.29, 4.57; 87.68, 47.25 ppm and LC90 values of 10.06, 6.06, 129.32, 65.58 ppm being observed after 24 and 48h of treatment, respectively. In addition, Chavan and Nikam noted the larvicidal nature of the essential oil of Ocimum basilicum, which induced 100% mortality against C. quinquefasciatus at a concentration of 0.12%. These results strongly support the use of hydroethanolic extract of Ocimum basilicum against the mosquitoes, which may act as potential bioinsecticide agent. These results also promise for further investigations.

**CONCLUSION**

This study demonstrated that hydroethanolic extract of Ocimum basilicum containing: flavonoids, tannins, mucilage and leucaonthenes possesses larvicidal activity against harmful mosquitoes (An. labranchiae). They have more efficient effect on larvae of An. labranchiae with respective values of LC50 and LC90, of 23.72 mg/ml and 30.78 mg/ml. Other studies of the tested plants, containing the mode of action, synergy with biocides, field studies are needed. Therefore, we strongly recommend that African policymakers promote the use of plants such as Ocimum basilicum, as natural biocides in the implementation of the national policy-could they be an-effective control of malaria vectors.

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interests.

**ACKNOWLEDGMENTS**

We thank everyone who contributed to this work. Notably Mr. Ba Sidi EL idrissi, the proof- reader.

**REFERENCES**


17. WHO. Guidelines for Laboratory and Field testing of Mosquito larvicides, Website (e.g. Who/cds/whopes/gcdpp/2005.13.)., 2005.


