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Research Article

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)

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ABSTRACT
Mosquitoes are vectors for many tropical and suptropical diseases. They are also the most important single group of insects well-known for their puplic 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 extractof *Ocimum basilicum* indicates the presence of flavonoids, tannins, mucilage and leucaonthocyanes. However, sterols, terpenes, triterpenes and coumarines were not detected. The LC_{50} and LC_{90} 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[1]. In addition, the cost of mosquito born diseases is not restricted to the loss of human life, but also includes the loss of labor and productivity which affects the overall social and economic progress of a country[2]. Among the known species in the transmission of diseases to humans, we include those belonging to the *Culex*, *Aedes* and *Anophele*[3,4]. Repeated use of a single synthetic pesticide ingredient can result in resistance amongst the target population[2]. Insecticide applications although highly efficacious, against the target vector species, are facing a threat due to the development of resistance to chemical insecticides, resulting from rebounding vectorial capacity[5]. Therefore, since few decades researchers have diverted their attention towards the plant world, which are ecofriendly and cost effective[6]. In the African tradition, the exploitation of plant insecticide use has been known for long[7]. In Morocco, the use of plants against mosquito invasions is a very common practice, especially in rural areas. Indeed, odours of *Ocimum basilicum* (*O. basilicum)* and *Corrigiola telephiifolia* are very effective repellents[8]. *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[9]. Biologically, it showed antibacterial, anti-thrombotic, anti-oxidant, anti-inflammatory, and antihypertensive activities[10,11]. 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. Labranchiae)*. The insecticidal activity of *O. basilicum* plants against *An. Labranchiae* has never been studied before in the North East of Morocco.

MATERIALS AND METHODS

Collection and identification of plant material

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Table 1: Phytochemical screening of hydro-ethanolic extract of *O. basilicum*

Phytochemical constituents / Plants	Tanins	flavonoïdes	Sterols and terpenes	triterpenes	coumarines	leucaonthocyanes	mucilage
O. basilium	+	+	-	-	-	+	+

Presence of chemical components is: (+) = present; (-) = absent

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[12].

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[13] and Diallo[14]. 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 Boukhnafer (1132 m altitude, 34°01´35´´N and 5°11´44´´E), with an area of 22500 m^2. 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 an at 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[15] and the identification software of mosquitoes of the Mediterranean Africa[16].

Protocol of larval susceptibility testing

The sensitivity tests were carried out in accordance with the WHO Protocol[17]. 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[18]. 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[19]. 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 leucaonthocyanes. However, sterols, terpenes, triterpenes and coumarines were not detected.

Variation in mortality rate

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).

LC_{50} and LC_{90} lethal concentrations

Figure 1 confirms the analysis performed in the order of effectiveness of hydroethanolic extract tested. The *O. basilicum* exhibits the lowest LC_{50} of 23.72 mg/ml (Equation of the regression line: Y = -4.25561 +

Table 2: Concentrations LC_{50} and LC_{90} lethal larvae of *An. Labranchiae* after 24 hours of exposure.

Plant species	LC_{50} (mg/ml) (Ll-Ul)*	LC_{90} (mg/ml) (Ll-Ul) *
O. basilicum	23.72 (21.61-25.71)	30.78 (28.11- 35.77)

* Ll-Ul: Lower limit - Upper limit

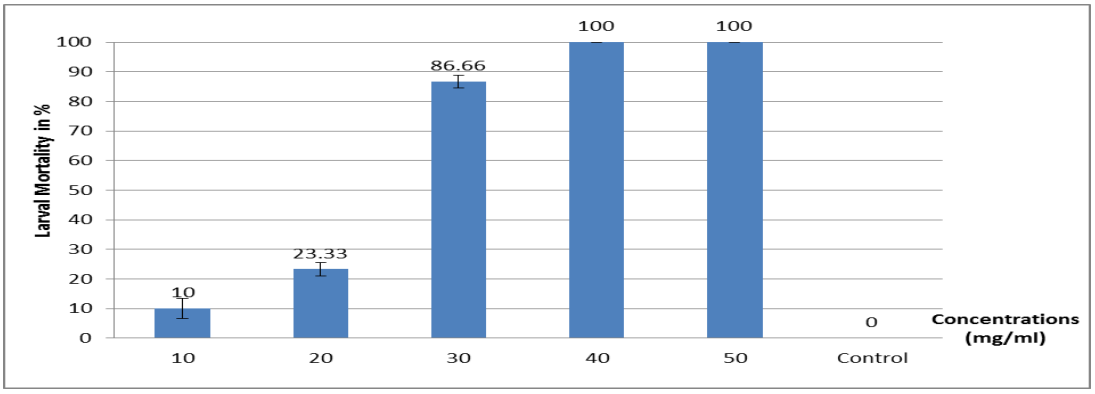

Figure 1: Percentage of mortality recorded in the sensitivity test by aqueous extract of the plant on *An. labranchiae*.

11.34007 * X; calculated Chi_2: 3.090) and LC_{90} = 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[20-22]. Our results are in accordance with other studies revealing to the presence of flavonoids and tannins[23, 24]. The larvicidal activity of the extracts of aromatic plants was also confirmed in the works of Jang et al[25]. 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[26-28]. 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 leucaonthocyanes) against the *An. labranchiae*. The values of LC_{50} and LC_{90} (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[29], 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[30]. Indeed, Stephens et al[31] and White[32] 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*[2], extracted from the leaves of *O. basilicum* has been effective on *Anopheles stephensi* and *Cx. Quinquefasciatus* with LC_{50} values of 8.29, 4.57; 87.68, 47.25 ppm and LC_{90} 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[33] noted the larvicidal nature of the essential oil of *O. basilicum*, which induced 100% mortality against *C. quinquefasciatus* at a concentration of 0.12%. These results strongly support the use of hydroethanolic extract of *O. basilicum* against the mosquitos, which may act as potential bio insecticide agent. These results also promise for further investigations.

CONCLUSION

This study demonstrated that hydroethanolic extract of *O. basilicum* containing: flavonoids, tannins, mucilage and leucaonthocyanes possesses larvicidal activity against harmful mosquitoes (*An. labranchiae*). They have more efficient effect on larvae of *An. labranchiae* with respective values of LC_{50} and LC_{90}, of 23.72 mg/ml and 30.78 mg/ml. Other studies of the tested plants, including 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 *O. 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.

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