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Larvicidal activity of essential oils of *Citrus sinensis* and *Citrus aurantium* (Rutaceae) cultivated in Morocco against the malaria vector *Anopheles labranchiae* (Diptera: Culicidae)

Fouad El-Akhal[1,2], Abdelhakim El Ouali Lalami[1,3*], Raja Guemmouh[2]

[1]*Regional Diagnostic Laboratory Epidemiological and Environmental Hygiene, Regional Health Directorate, EL Ghassani Hospital, Fez 30000, Morocco*

[2]*Sidi Mohamed Ben Abdellah University, Faculty of Sciences Dhar El Mahraz, Laboratory Analysis and Modelling of Ecosystems Continentals, Dhar El Mahraz, 30000, Morocco*

[3]*Institute of Nursinng Professions and Health Techniques, EL Ghassani Hospital, 30000 Fez, Morocco*

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ABSTRACT

Objective: To study the larvicidal activity of essential oils of two aromatic and medicinal plants, *Citrus aurantium* (*C. aurantium*) and *Citrus sinensis* (*C. sinensis*) (Rutaceae) cultivated in North Eastern Morocco, against the larvae of the malaria vector *Anopheles labranchiae* (*An. labranchiae*) (Diptera: Culicidae).

Methods: Biological tests were realized according to a methodology inspired from standard World Health Organization protocol. The mortality counts were made after 24 h and LC_{50} and LC_{90} values were calculated.

Results: Bioassays revealed that these oils had remarkable larvicidal properties. The minimum levels necessary to achieve 100% mortality of *An. labranchiae* larvae were evaluated at 160 mg/L for *C. aurantium* and 640 mg/L for *C. sinensis*. Essential oil of *C. aurantium* remained the most efficient (LC_{50} = 22.64 mg/L, LC_{90} = 83.77 mg/L), while those of *C. sinensis* was the least (LC_{50} = 77.55 mg/L, LC_{90} = 351.36 mg/L).

Conclusions: These results suggest that the essential oils isolated from *Citrus* plants have the potential to be used as an ideal ecofriendly approach for the control *An. labranchiae*.

1. Introduction

Mosquitoes are responsible for more diseases than any other group of arthropods[1]. The mosquito-borne diseases, such as malaria, filaria, dengue fever, yellow fever, and Japanese encephalitis, contribute significantly to disease burden, death, poverty, and social mobility in tropical countries[1,2]. Mosquitoes of genus *Anopheles* transmit malaria parasites to humans[3]. The various malaria vectors exhibit a wide variety of life history strategies, thus there is no simple and universal applicable form of vector control[3].

*Corresponding author: El Ouali Lalami Abdelhakim, Regional Diagnostic Laboratory Epidemiological and Environmental Hygiene, Regional Health Directorate, EL Ghassani Hospital, Fez 30000, Morocco.
Tel: +212 661937474
E-mail: eloualilalami@yahoo.fr

By its abundance, density, vectorial capacity and wide geographical distribution, *Anopheles labranchiae* (*An. labranchiae*) is the main malaria vector in Morocco[4-6]. The study of its ecology and relationship with malaria transmission has been extensively studied in the field[7-9].

The diseases transmitted by mosquitoes have an economic impact, including the loss of trade and labor outputs, particularly in countries with a tropical and subtropical climate[10]. Continued use of synthetic chemical insecticide based measures for vector control has resulted in a loss of efficacy with the appearance of resistance in mosquito populations[11], undesirable effects on non-target organisms, and damages to environment and human health[12]. Current control mainly involves the use of synthetic repellents, which also have a potential toxic effect on public health and environment. Their repeated use has disrupted natural biological systems and often resulted in the development of resistance[13]. This has propelled the search and use of eco-

friendly plant based products for the control of mosquitoes[14].

Citrus fruits are the most important horticultural crops because of their nutritional value and unique flavour. Indeed, many members of the *Citrus* genus are well known for their medicinal, physiological and pharmacological properties including antimicrobial, antioxidant, anticancer, anti-inflammatory and hypoglycemic activities[15,16].

Citrus fruit cultivation is very important in Morocco, being the first exporting agricultural sector and playing a major role in the national economic development[17]. *Citrus aurantium* (*C. aurantium*) and *Citrus sinensis* (*Citrus sinensis*) are familiar through the use of their fruit for culinary purposes and traditional medicine.

In Morocco, only two observations have been published on larvicidal activity of medicinal plants against the malaria vector *An. labranchiae*. Indeed, in 2000 the larvicidal properties of 16 extracts of four Moroccan medicinal plants, *Calotropis procera* (Aiton) Dryand., *Brocchia cinerea* (Delile) Vis., *Solanum sodomaeum* L. and *Solanum elaeagnifolium* Cav. were studied against *An. labranchiae*[18]; in 2006 a preliminary evaluation of the larvicidal activity of aqueous extracts from leaves of castor bean (*Ricinus communis* L.) and cedar [*Tetraclinis articulata* (Vahl) Mast.] was realized on four mosquitos, *Culex pipiens* Linnaeus, *Aedes caspius* Pallas, *Culiseta longiareolata* Aitken, and *Anopheles maculipennis* Meigen[19].

The insecticidal activity of essential oils from *Citrus* plants against *An. labranchiae* has not been studied previously. Thus, the objective of this work was to research the larvicidal activity on *An. labranchiae* of essential oils of *C. aurantium* and *C. sinensis* grown in north east of Morocco as a potential production of biocides.

2. Materials and methods

Plant material (aerial part of the plant) Osbeck and *C. aurantium* L. (Rutaceae) was harvested in February and March 2014 near Taounate (north east of Morocco). A total of 200 g biomass of each species was subjected to hydrodistillation for 3 h, using a modified Clevenger type apparatus. Essential oils gathered by decantation at the end of the distillation were dried over anhydrous sodium sulfate to remove traces of residual water and were stored in small opaque vials at 4 ˚C before use.

By gas chromatography coupled with mass spectrometry, limonene was obtained as the major compound (90.00% and 95.36% for *C. aurantium* and *C. sinensis*)[20].

2.1. Characteristic of breeding site

The collection of larvae of *An. labranchiae* was performed in a breeding site located in the urban area of the city of Fez, named Ain Boukhnafer (1 132 m altitude, 34°01'35" N and 5°11'44" E), with an area of 22 500 m^2. This gite corresponded to reed beds with a very high density of Culicidae larvae.

2.2. Collecting An. labranchiae larvae

Larvae were collected using a rectangular plastic tray inclined 45 ˚C with respect to the water surface. Larvae harvested were maintained in breeding in rectangular trays at 23.42 ˚C in the Entomology Unit at the Regional Diagnostic Laboratory Epidemiological and Environmental Hygiene falling within Regional Health Directorate of Fez.

2.3. Morphological identification of larvae

Larvae were determined using a Moroccan Culicidae identification key[21] and the Mediterranean Africa mosquitoes identification software[22].

2.4. Protocol for larval susceptibility testing

Biological tests were inspired from standard World Health Organization protocol[23]. A stock solution (10%) of each essential oil in ethanol and dilution series: 10, 20, 40, 80, 160, 320 and 640 mg/L for *C. sinensis*; 5, 10, 20, 40, 80 and 160 mg/L for *C. aurantium* was prepared. Preliminary experiments were used to select a range of concentrations to be tested. About 1 mL of each solution prepared was placed in beakers containing 99 mL of distilled water in contact with 20 larvae of stadium 3 (L3) and 4 (L4)[23], and the same number of larvae was placed in a beaker containing 99 mL indicator of distilled water plus 1 mL ethanol. Three replicates were carried for each dilution and for the control. After 24 h contact, living and dead larvae were counted.

The results of susceptibility testing sites were expressed in percentage of mortality versus concentrations of essential oils used. If the % mortality in controls is greater than 5%, the % mortality in larvae exposed to the essential oil is corrected by using Abbott's formula[24].

% Mortality Corrected = [(% Mortality Observed - % Mortality Control) / (100 - % Mortality Control)] × 100

If the control mortality exceeds 20%, the test is invalid and must be repeated.

2.5. Data processing

The log-probit analysis (Windl version 2.0) software developed by CIRAD-CA/MABIS was used[25].

Table 1
Lethal concentrations LC_{50} and LC_{90} of larvae of *An. labranchiae* after 24 h.

Plant species	LC_{50} (mg/L) (Ll-Ul)	LC_{90} (mg/L) (Ll-Ul)	Equation of the regression line	Calculated *Chi*-square (χ^2)
C. aurantium	22.64 (10.685-33.618)	83.77 (59.150-143.022)	Y = -3.056 89 + 2.256 05X	3.056
C. sinensis	77.55 (48.451-106.300)	351.36 (258.959-546.894)	Y = -3.690 86 + 1.953 29X	7.130

Ll-Ul: Lower limit-upper limit.

3. Results

3.1. Variation in mortality

A direct relation percentage mortality of larvae with the concentration of essential oils was observed (Figures 1 and 2). The minimum levels necessary to achieve 100% mortality of larvae of *An. labranchiae* were valued at 640 mg/L for *C. sinensis* and 160 mg/L for *C. aurantium*.

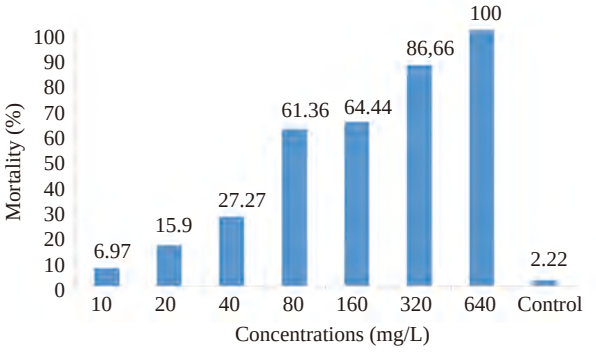

Figure 1. Mortality (%) of larvae of *An. labranchiae* depending on the concentration of essential oil of plant species *C. sinensis* after 24 h exposure.

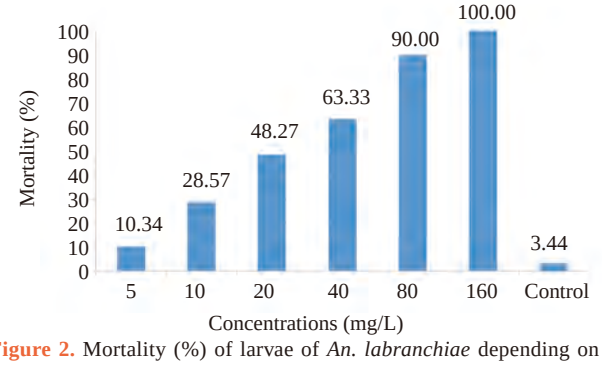

Figure 2. Mortality (%) of larvae of *An. labranchiae* depending on the concentration of essential oil of plant species *C. aurantium* after 24 h exposure.

3.2. LC_{50} and LC_{90} lethal concentrations

The essential oil of *C. aurantium* exhibited a lower LC_{50} and LC_{90} (22.64 and 83.77 mg/L) than that of *C. sinensis* (77.55 and 351.36 mg/L) (Table 1).

4. Discussion

Essential oils and extracts of many plants were observed to have mosquito larvicidal properties[26-28] and to have received attention as potentially controlling vectors of mosquito-borne disease. Some monoterpenes as geraniol and cuminaldehyde are very toxic to larvae[29].

Studies on the insecticidal activity of *Citrus* plants against larvae of genus *Anopheles* are limited. Crude extracts of *C. sinensis* peels against *Anopheles subpictus* larvae gave LC_{50} of 58.25 ppm and LC_{90} of 298.31 ppm[30]. Lemon oil (*Citrus limon*) and orange oil (*C. sinensis*) exhibited at 250 ppm maximum larvicidal activity of 99.20% against *Anopheles stephensi* with LC_{50} and LC_{90} values of 35.95 and 138.86 ppm[31]. These results are almost similar to those found in our study. We did not encounter in literature any work that treats larvivide activity of *C. aurantium* on *An. labranchiae*.

Similar studies on the insecticidal activity of *Citrus* plants, but this time against mosquito larvae of *Culex* and *Aedes* vectors of disease, have been conducted.

Some show LC_{50} higher compared to those reported in studies against *Anopheles*, including ours. Essential oil *C. aurantium* grown in Tunisia has effective larvicidal activity on *Culex quinquefasciatus* with LC_{50} of 179.8 mg/mL and LC_{90} of 351.1 mg/mL[32]. Extracts of *C. aurantium* has a significant effect on larvae of *Aedes albopictus* with LC_{50} of 322.36 ppm[33]. Hexane extracts of leaves of *C. sinensis* have insecticidal activity against *Aedes aegypti* (*Ae. aegypti*), with LC_{50} of 446.84 ppm and LC_{90} of 1 370.96 ppm[34].

Other authors have obtained very large larvicidal effects. The essential oils of *Citrus hystrix* and *Citrus reticulata* were found very effective against *Ae. aegypti* with LC_{50} respectively of 30.07 and 15.42 ppm[17]. That of *Citrus grandis* showed high larvicidal activity on *Ae. aegypti* mosquito larvae[35]. That of *C. sinensis* exhibited the highest larvicidal activity against *Ae. aegypti* (LC_{50} of 85.93 ppm) [36], and *Culex pipiens* (LC_{50} of 20 to 160 ppm)[16].

It appears across all of this work that the *Citrus* species show significant larvicidal activity against mosquito larvae, disease vectors such as *Anopheles*, *Culex* and *Aedes*.

Doubtless this could be explained by the presence of limonene. The analysis of volatile oil constituents of many varieties of *Citrus* revealed that limonene is often a major component of the essential oil of peels[16,37-39].

Nethertheless, this work shows that the essential oil of *C. aurantium* exhibits a higher larvicidal activity than that of *C. sinensis* while the content in limonene is similar (95.4% and 90.0%). The larvicidal activity could also be partly searched within other monoterpenes as linalool or γ-terpinene[16,39] and medicinally

active components as sesquiterpenes and some coumarins.

Although the last case of indigenous malaria was recorded in Morocco in 2004[40], this disease continues to pose a public health problem in some parts of the Kingdom including northern center through imported malaria[41,42]. Furthermore, the emergence of an early resistance of *An. labranchiae*[40] and the cases of resistance of *Culex*[11] against organophosphates as temephos has been reported in Morocco.

Therefore, the use of plant oils as mosquitocidal agents in insect/mosquito control is an alternative pest control method for minimizing the noxious effects of some pesticidal compounds on the environment[36]. The results of our study suggest that the essential oils isolated from *Citrus* plants have the potential to be used as an ideal ecofriendly approach for the control of malaria vector *An. labranchiae*.

The essential oils examined in this study offer great potential as new control agents against *Anopheles* larvae especially *An. labranchiae*, which is considered as a serious threat to human health in Morocco.

The essential oil of *C. aurantium* remains the largest efficient, with respective values of LC_{50} of 22.64 mg/L and LC_{90} of 83.77 mg/L, while the essential oil of *C. sinensis* is the least effective, with LC_{50} of 77.55 mg/L and LC_{90} of 351.36 mg/L.

Conflict of interest statement

We declare that we have no conflict of interests.

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