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Research Article

Qualitative Phytochemicals Analysis and Larvicidal Activity of Hydro-Ethanolic Extract of Moroccan *Mentha pulegium* Against Larvae Mosquito *Culex pipiens* (Diptera: Culicidae) Vector of Infectious Diseases

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ABSTRACT

The mosquitos are vectors of many tropical diseases and subtropical. The main targeted mosquito vector in this study, *Culex pipiens*, causes serious clinical human diseases. In this work, which has never been realized before in the North center of Morocco, the phytochemical screening of the extract of *Mentha pulegium* and its insecticidal activity on larval stages 3 and 4 of *Culex pipiens* were studied. The phytochemical constituents were obtained using a simple qualitative analysis method and biological tests were realized according to a methodology inspired from standard World Health Organization protocol. The percent yield of the hydro-ethanolic extract from aerial part of *Mentha pulegium* was 8.7±0.2. The phytochemical screening of extract of *Mentha pulegium* grown in North center of Morocco revealed a presence of tanins, gallic tannins, flavonoids, sterols and terpenes, and mucilage components. However, catechic tannins, glycosides and coumarins were not detected. The highest larvicidal activity was observed in the hydro-ethanolic extract of *Mentha pulegium* against *Culex pipiens* with LC_{50} and LC_{90} values in the order of 0.38 (0.35 – 0.49) and 0.64 (0.50- 0.69) mg/ml respectively. From the results, it can be concluded that hydro-ethanolic extract had excellent potential for controlling mosquito larvae in Morocco.

Keywords: Hydro-ethanolic extract, Larvicidal activity, *Culex pipiens, Mentha pulegium,* North Central Morocco.

INTRODUCTION

Many insects transmit to man a wide variety of microbes and parasites, many of which can prove too pathogenic. The culicids were the leading role in the transmission of diseases which today represent the most serious health problems in the world[1]. These insects form very homogeneous groups have an important place in the terrestrial fauna as the aquatic fauna on the one hand and in the transmission of diseases due to their second shots. We list now more than 3500 species[2], three quarters of which are in humid subtropical and tropical areas. In Morocco, the statistical study also showed that the species of *Culex* and *Aedes* are found during the year and are among the species inventoried[3,4]. The fight against vector-borne diseases means using chemical insecticides. However, the use of insecticides faces several serious problems today. In addition to the negative effects of synthetic insecticides on the environment and non-target organisms, including man[5-7], the development of resistant mosquito populations in particular is one of the most serious problems[1]. The global mosquito control strategy aims at protecting individuals and communities using long-lasting impregnated nets, indoor-residual spraying in addition to prompt and effective clinical treatment[8]. Recent years Morocco has experienced the problem of resistance to chemical insecticides used in vector control, especially genus Culex[9-11]. This has led to research alternatives that respect the environment and avoids any sort of potential harm to public health. Botanical insecticides have many advantages, such as low mammalian toxicity; they are also biodegradable and there is practically no risk of developing resistance[12,13]. Recalling that the plant extracts on the mosquito species, especially Culex, were proved their larvicidal effects[14-17]. *Mentha pulegium* (Lamiaceae) are herbaceous plants, perennials, strongly scented that one finds particularly in the wetlands. They are very aromatic plants used in traditional medicine, culinary preparations, confectionery, cosmetics and perfumery[18]. Other authors have obtained very large effects of menthe spp on insect[19-21]. However,

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the insecticidal activity of *Mentha pulegium* against *Culex pipiens* has never been studied before in Morocco. Our contribution opts for the use of hydro-ethanolic extract as bio-insecticide for vector control. This approach has two advantages that converge enhancement of national plant heritage and the development of ecological bio-insecticide.

MATERIALS AND METHODS

Crop Plants and Ultrasound-Assisted Extraction.

The aerial parts (leaves, stems and roots) of *Mentha pulegium* were harvested during the April (2014) at the mountain of Timezgana falling within the rural community of Timezgana (area of Taounate, North center of Morocco) to an approximate altitude of 800m. In a 500mL beaker, 20 g of a dried plant powder was mixed with 150mL of hexane. The beaker was set in a Sonicator brand "ELMA" 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 plant incubator at a temperature of 40◦C for 30mins, the powder was reextracted again with ethanol at 80% for 45min under the same conditions[22].

Phytochemical screening.

The phytochemical constituents existing in the hydro-ethanolics are tannins, catechic tannins, gallic tannins, flavonoids, glycosides, mucilages, sterols and terpenes and coumarins, obtained using a simple qualitative analysis method, as described in the study[23,24].

Characteristics of larval Site.

The collection of larvae of *Culex pipiens* was performed in a breeding site located in the urban area of the city of Fez, named Ain Boukhnafer (1132 m altitude, 34°01'35" N and 5°11'44" E), with an area of 22 500 m^2. This site corresponded to reed beds with a very high density of Culicidae larvae.

Collecting larvae of Culex pipiens.

The larvae gathered were maintained in breeding in rectangular trays at an average temperature of 21.7°C ± 2°C in the Entomology Unit at the Regional Diagnostic Laboratory Epidemiological and Environmental Health (RDLEH) falling within Regional Health Directorate of Fez.

Identification of Larvae.

The identification of morphological characters of larvae has been determined using the Moroccan key of identification of Culicidae[25] and the identification software of mosquitoes of the Mediterranean Africa[26].

Larvicidal bioassays

The susceptibility tests were carried out in accordance with the standard protocol developed by WHO, in 2005[27]. From the initial extract (0.1 mg/mL stock solution) of

plant, concentrations of 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7mg/mL were prepared. Preliminary experiments were used to select a range of concentrations for the tests previously mentioned. 1mL of each solution prepared was placed in beakers containing 99mL of distilled water in contact with 20 larvae of stages 3 and 4; the same number of larvae was placed in a beaker containing 99mL of distilled water plus 1mL of ethanol. Three replicates were carried out for each dilution and for the control. After 24 hours of contact, we counted the 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 percentage of mortality in control is greater than 5%, the percentage of mortality in larvae exposed to the extract shall be corrected by using Abbott's formula[28].

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

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

Data Processing.

For the data processing we used the log-probit analysis (Windl version 2.0) software developed by CIRAD-CA/MABIS[29]. The analysis of the averages and standard deviation was also performed by using the test of analysis of variance ANOVA.

RESULTS

Percent yield and physical characters of Mentha pulegium.

The percent yield of the hydro-ethanolic extract from aerial parts of *Mentha pulegium* was 8.7±0.2.

As it is illustrated in Table 1, the phytochemical screening of extract of *Mentha pulegium* grown in North center of Morocco revealed a presence of tanins, gallic tannins, flavonoids, sterols and terpenes, and mucilage components. However, catechic tannins, glycosides and coumarins were not detected.

Variation of mortality

After exposing the larvae *Culex pipiens* to different concentrations of hydro-ethanolic extract of *Mentha pulegium* for 24h, the percentage of mortality varied according to concentrations. The mortality rate ranged between 8.33% and 100% (Figure 1). The minimum concentration of *Mentha pulegium* hydro-ethanolic extract required to achieve 100% of *Culex pipiens* larvae mortality was 0.7mg/ml.

Table 1: Phytochemical screening of hydro-ethanolic extract of *Mentha pulegium.*

Plants	tanins	catechic tannins	gallictannins	flavonoïdes	glycosides	mucilage	Sterols and terpenes	coumarines
Mentha pulegium	+	-	+	+	-	+	+	-

(+) = present; (−) = absent

Table 2: Lethal Concentrations LC_{50} and LC_{90} of larvae of *C. pipiens* after 24 hours of exposure.

Hydro-ethanolic extract plant	LC_{50} (mg/mL) (Ll-Ul)*	LC_{90} (mg/mL) (Ll-Ul)*	Equation of the regression line	Calculated Chi^2
Mentha Pulegium	0.38 (0.35 – 0.49)	0.64 (0.50- 0.69)	Y = 2.40893 + 5.88239 * X	18.679

∗Ll-Ul: Lower limit- Upper limit

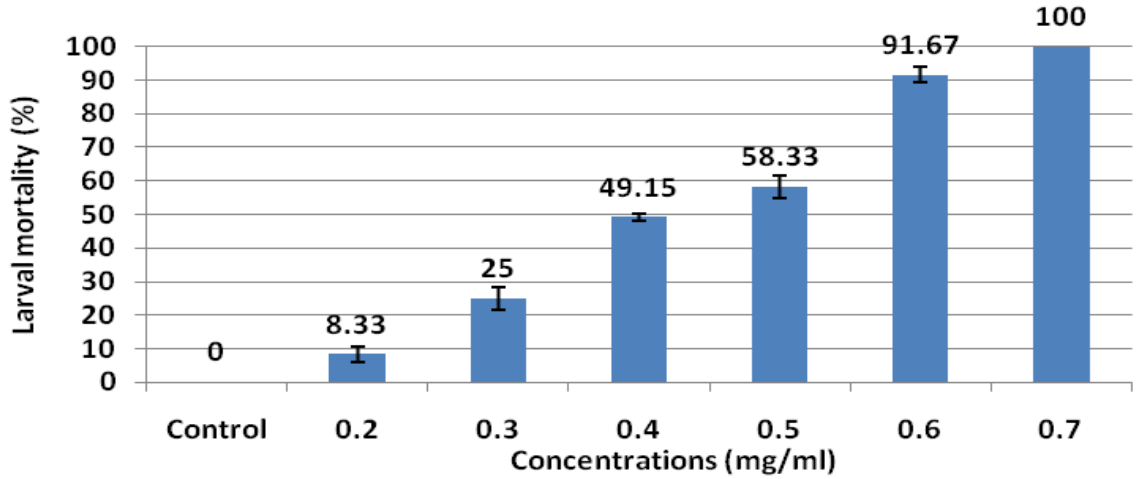

Figure 1: Percentage of mortality recorded in the test sensitivity by hydro-ethanolic extract of *Mentha pulegium* on *Culex pipiens*.

LC_{50} and LC_{90} lethal concentrations

Table (2) demonstrates that *Mentha pulegium* hydro-ethanolic extract remains effective while using concentrations of 0.38mg/ml for LC_{50} (which varies between a lower limit 0.35 mg/ml and an upper limit of 0.49 mg/ml) and 0.64mg/ml for LC_{90} (which also varies between a minimum of 0.50mg/ml and a maximum value of 0.69 mg/ml). Table (2) shows also the regression equation and the Chi^2 analyses results. The regression analysis indicates that the mortality rate is positively correlated with *Mentha pulegium* concentration. The adjustment model of tested data (Chi^2 Test), which is not significant at 5%, showed a good model fit.

DISCUSSION

In the present work, realized for the first time in Morocco, we are interested to examining the phytochemical screening of the hydro-etnanolic extract of one genus menthe from Morocco (*Mentha pulegium*), as well as studying its larvicidal activity against the Weste Nile vector *Culex pipiens.* We have not found similar work regarding the yield of hydro-ethanolic extract of *Mentha pulegium.* However, some authors[30] have observed for a species of Menth (*Mentha viridis*), a yield of the aqueous extract at 10%, followed by the methanolic extract at 9% then the hydro-methanolic extract at 6%. Generally the yields vary from sample to another. The results of our phytochemical screening of hydro-ethanolic extract studied (*Mentha pulegium*) showed the presence of flavonoids, tannins, gallic tannins, mucilages, sterols and terpenes. However, catechic tannins, glycosides and coumarins were not detected. The phytochemical screening of *Mentha pulegium* has been the subject of many studies[19,31]. Indeed, in recent work realized in 2014 by Geronutti et al[32] have found the lyophilized plant extract Mentha containing: 13.04% of polyphenols, 5.22% of flavonoids totals, 10.85% ± 0.0064 of total ashes, 0.96% ± 0.0081 of insoluble ashes, 0.4% by hydrodestilation of volatile oil content. The results obtained by Jain, in 2012[33] indicated that Phytochemical screening of the plant shows the presence of flavonoids in ethanolic extract. Various factors as the environmental conditions, the extraction technique, the drying, the period and gathering sites, the plant age, the concentration of the extract and the concentration of its active components, could be influenced, the physicochemical characteristics and the chemical composition of the extract. The hydro-ethanolic extract of *Mentha pulegium* exhibited an LC_{50} and LC_{90} concomitant with 0.38 and 0.64 mg/ml respectively (Table 2). This efficiency could be explained by the action or effect of phytochemical components (flavonoids, tannins, sterols and terpenes, and mucilage, against the *Culex pipiens.* Previous investigations have indicated that various Mentha spp. plant extracts displayed larvicidal effect on *Cx. pipiens*, *Cx. quinquefasciatus*, *Aedes aegypti*, *Anopheles stephensi* and *Anopheles tesselatus*[34-39].

Thus, Cetin et al., in 2006[40], concluded that there was a high to low lethal effect of extracts of *Mentha pulegium* against mosquito larvae (Diptera: Culicidae), with LC_{50} values of 81.0 ppm. The work of Koliopoulos et al., in 2010[41] confirmed the toxicity *Mentha ssp* (labiate) on Culicidae larvae (*Culex pipiens).* This aromatic species is very fluently seen and used traditionally in Africa; this finding opens interesting perspectives for its application in the production of biocides. All the results obtained in this work could be alternative or complementary

solutions to the use of bio-insecticides to fight against the mosquito disease vectors. The development of bio-insecticides and plant extracts the selection of resistant cultivars to the mosquitoes are part of the fight against mosquitoes and sustainable development.

CONCLUSION

This study concluded that the hydro-ethanolic extract of *Mentha pulegium* possesses larvicidal activity with values of LC_{50} and of LC_{90}; 0.38 (0.35–0.49) mg/mL and 0.64 (0.50-0.69) mg/mL, respectively. This would include the flavonoids, the tannins, the gallic tannins, the sterols and terpenes, the mucilage and the coumarins. Further studies are needed on formulations against mosquitoes and their efficacy and cost effectiveness, the products based on these hydro-ethanolic extract may contribute greatly to reduction in environmental chemicalisation and to an overall reduction of the population density of significant vectors of Weste Nile virus such as *Culex pipiens.*

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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