# CHAPTER 4

# INSECTICIDAL EFFECT OF ESSENTIAL OILS OF ORIGANUM MAJORANA AGAINST *CULEX PIPIENS*

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**ABSTRACT:** The use of conventional pesticides is one of the most widespread methods of combatting harmful insects and disease vectors. However, the side effects of these chemicals on the environment as well as on human health have encouraged the development of several alternative methods to chemical control, such as the

use of biopesticides. The objective of this work is to test the effectiveness of essential oils from the leaves of *Origanum majorana*, cultivated on the region of Ain Defla, against the disease vector *Culex pipiens.* Toxicological tests were conducted with a series of essential oil solutions (5, 10 and 20 µl) prepared in tubes containing 1 ml of ethanol. Three repetitions were performed in containers holding 15 newly ecdysed L3 stage larvae and 150ml of rearing water. The results revealed a toxic effect with a concentration-response. A very significant mortality rate was observed after treatment with *Origanum majorana*, especially the mortality rate reached with the highest concentration and with the lowest concentration. The $LC_{50}$ and $LC_{90}$ are respectively: 5.37µl/ml and 15.48 µl/ml.
**KEYWORDS :** biopesticide, essential oils, *Origanum majorana, Culex pipiens*.

## EFFET INSECTICIDE DE L'HUILE ESSENTIELLE DE L'*ORIGANUM MAJORANA* CONTRE *CULEX PIPIENS*

**RÉSUMÉ:** L'utilisation des pesticides conventionnels est l'un des types de lutte les plus répandus contre les insectes nuisibles et vecteurs de maladies. Cependant, les effets secondaires de ces produits chimiques sur l'environnement ainsi que sur la santé humaine, ont encouragés le développement de plusieurs méthodes alternatives à la lutte chimique comme l'utilisation des biopesticides. L'objectif de ce travail est de tester l'efficacité des huiles essentielles des feuilles d'*Origanum majorana*, cultivée dans la région d'Aïn Defla, à l'égard du vecteur de maladie *Culex pipiens.* Les essais toxicologiques ont été réalisés avec une série de solutions des huiles essentielles (5, 10 et 20 µl) préparé dans des tubes contenant 1 ml d'éthanol. Trois répétitions ont été effectués dans des récipients contenant 15 larves du stade L3 nouvellement exuviées et 150ml d'eau d'élevage. Les résultats ont révélé un effet toxique avec concentration-réponse. Un taux de mortalité très important a été constaté après le traitement avec l'*Origanum majorana,* notamment le taux de mortalité atteint avec la concentration la plus forte et avec la concentration la plus faible. Les $CL_{50}$ et $CL_{90}$ sont respectivement : 5.37µl/ml and 15.48 µl/ml.
**MOTSCLÉS:** biopesticide, huiles essentielles, *Origanum majoran, Culex pipiens*.

## INTRODUCTION

*Origanum majorana,* belonging to the Lamiaceae family, is an herbaceous plant found in Southern Europe and the Mediterranean region, known as marjoram, which can reach up to 60 cm. *O. majorana* is widely used as a garnish in food preparation, as well as being a medicinal plant used for various purposes in the traditional medicine of different regions.

Studies on this plant have identified a large number of its active compounds (Nasser, 2018); the most important of these bioactive constituents, which are mainly secondary metabolites, include alkaloids, flavonoids, tannins, phenolic compounds, and essential oils, which explain its biological properties. According to Fournier (2003), aqueous extracts, powders, and essential oils from plants contain some types of secondary metabolites have an insecticidal property.

For several years the fight against harmful insects and disease vectors has been mainly done using chemical pesticides. However, the repeated and continuous use of these products has encountered several difficulties, such as resistance phenomenon, ecosystem imbalances, lack of specificity, and persistent effects in non-biodegradable insecticides, which are the most common issues (WHO, 2018; Richards et al, 2020). Therefore, resorting to natural insecticidal molecules turns out to be an alternative approach. Currently, insecticides based on essential oils are under study to take the place of chemical insecticides in the field of phytoprotection.

The objective of this study would be to test the insecticidal activity of *Origanum majorana* essential oils, on the L3 larvae of *Culex pipiens*; known as a significant vector of diseases.

## MATERIALS AND METHODS

### Plant material

The sampling method used involves randomly collecting leaves, cultivated in the region of Aïn Defla) 36.2634° N, 1.9679° E(. The collected sample is placed in a bag and then transported to the laboratory.

### Mosquito rearing

The larvae of *Culex pipiens* (Diptera: Culicidae) were obtained from a stock colony of the laboratory. Each 25 larvae were kept in Pyrex storage jar containing 150 ml of stored tap water and maintained at temperature between 25-27°C and a photoperiod of 14L:10D. Larvae were daily fed with fresh food consisting of a mixture of Biscuit Petit Regal-dried yeast (75:25 by weight), and water was replaced every four days (Bendali et al, 2001).

### Extraction and toxicity of essential oils of *Origanum majorana*

The extraction of essential oil from oregano is carried out by hydrodistillation using a Clevenger-type extraction. The process involves introducing 140g of dried plant material into a 1-liter flask, to which a quantity of distilled water equivalent to 2/3 of the flask's volume is added. The extraction operation is carried out for three hours from the start of boiling. Finally, the obtained oil is stored and conserved at a temperature of 4°C.The toxicity tests were conducted in accordance with the protocol recommended by the World Health Organization, adopted for testing the sensitibity of larvae to insecticides used in control campaigns (WHO, 2005). A preliminary test (1 ml of 96° ethanol and 99 ml of rearing water) is carried out to demonstrate that the solvent (ethanol) has no larvicidal effect. The solvent acts as a dispersing agent for the essential oil in the water. The treated series of newly exuviated L3 of *Cx. pipiens* were exposed to the product for 24 h and the control series were exposed to water only. After the exposure time, the larvae were removed and placed in untreated water. The tests were carried out with three replicates containing 15 larvae for each.

Mortality was registered daily until the end of the treated stage. The percentage of recorded mortality was corrected (Abott, 1925) and toxicity data were studied by probit analysis (Fisher & Yates, 1957) and $LC_{50}$ & $LC_{90}$ were calculated by the method of (Finney, 1971).

## Yield

The yield of essential oil is the ratio between the weight of the extracted oil and the weight of the dry matter of the plant (AFNOR, 1987). It is expressed as a percentage and is calculated using the following formula:

**Y=(WA/WB)x100, where:**

- Y: Yield of oil in %
- WA: is the Weight of the oil in g.
- WB: is the Weight of the dry matter of the plant in g.

# RESULTS

## Yield

The weight of the essential oil after extraction was 0.336g, therefore the yield is calculated as follows:

Y:(0.336g/140g) x100=0.24%.

The essential oil from *Origanum majorana* is yellow, clear with a pleasant smell, and with a yield of 0.24% of the dry matter of the leave part of the plant.

## Toxicity of *Origanum majorana* on *Culex pipiens* larve

Fig.01 illustrates the variation in the mortality rate of *Culex pipiens* larvae according to the 4 concentrations used ($p < 0.005$), after 24h of exposure. Indeed, the treatment of L3 with the essential oils show average mortalities correlated to the doses used. The mortality reaches (96%) for the high concentration 20 µl/ml compared to (58%) for the low concentration 5 µl/ml.

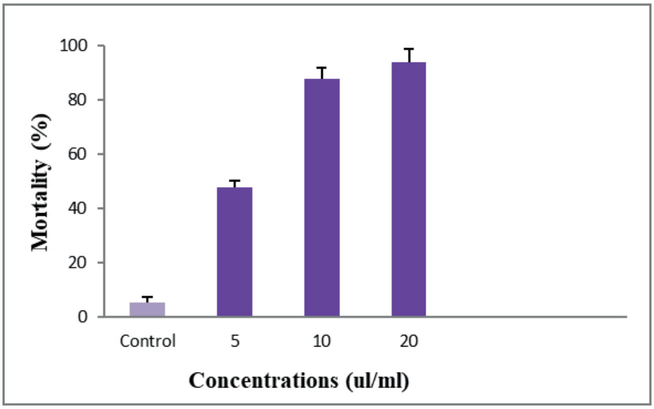


Figure 01: Concentration-response relationship for treatment of *Origanum majorana* on 3th instar larvae of *Culex pipiens* for 24h.

The regression line of larval mortality after treatment with *Origanum majorana* is presented in Fig.02. The corresponding $LC_{50}$ and $LC_{90}$ values are 5.37µl/ml and 15.48 µl/ml after 24h of contact.

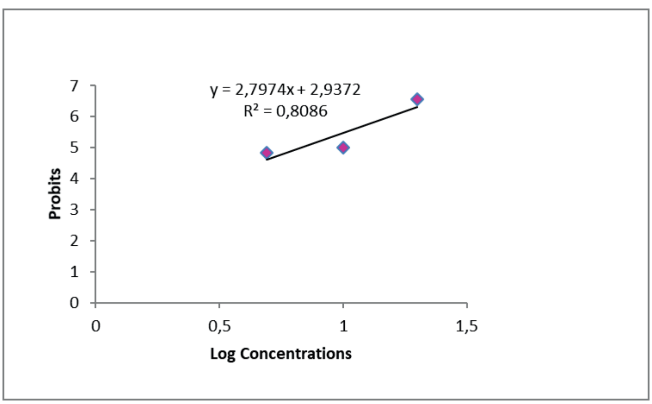


Figure 02: Probit transformed responses with equation regression and coefficient of determination R2 for essential oils extracted from Origanum majorana and tested on 3th instar larvae of Culex pipiens for 24h.

## DISCUSSION

Due to their chemical nature, pesticides are pollutants and toxic, and several harmful effects resulting from their use have been documented. The use of natural substances of plant origin, namely essential oils, constitutes a promising alternative (Ayed et al, 2017). In this study, we attempted to evaluate the bio-insecticidal effect of essential oils from *Origanum majorana* on the larvae of stage L3 of *Cx. pipiens,* depending on studied concentrations.

The yields of essential oil extracted from *Origanum majorana* was 0.24%. In general, this yield varies from one plant to another; it is 0.5% for *Artemisia mestlantica* and 0.2% for Artemisia campestris (Khebri, 2011), 0.5% and 0.9% for *Kaempferia galanga L.* (Manat & Weerachai, 2022). The insecticidal properties of certain essential oils have been specifically tested on mosquito larvae. In this context, the work of Pitarokili et al, (2011), demonstrates that oils extracted from three species of *Mentha: M. pulegium, M. piperita, and M. spicata,* possess larvicidal activity against *C. pipiens* with $LC_{50}$ values of 46.4, 40.28 and 27.23 ppm, respectively. Furthermore, Singh et al, 2003, revealed a larvicidal activity of essential oils (EOs) extracted from *Ocimum canum* with lethal concentrations (LC50=301, 340 and 234 ppm, respectively) that are higher than those found in our experimentation and this against three species of mosquitoes: *A. aegypti, C. quinquefasciatus* and *A. stephensi*. These extracts contain on average 20 to 60 compounds, most of which are relatively simple molecules. Their mechanism of action is unknown, and relatively few studies have been conducted on this subject (Isman, 2000). It is considered that these mechanisms are unique, and essential oil-based biopesticides can be tools of choice in pest resistance management programs. With these specific mechanisms of action, these biopesticides can be used alone and repeatedly without potentially inducing the development of resistance in pests (Windley et al, 2012).

The results obtained in this work show that the essential oils of *Origanum majorana* could constitute very interesting raw materials for the formulation of bio-insecticides to combat the mosquito larvae. However, field trials will be necessary to confirm the practical interest of these results.

## CONCLUSION

The very encouraging results obtained in this experiment deserve to be exploited through further research on these botanical species. In fact, this test was conducted only at the laboratory, and additional work would be necessary to be able to establish a means of biological control based on essential oils from plants that would be effective, economical, and environmentally friendly.

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