الجمهورية الجزائرية الديمقراطية الشعبية

People's Democratic Republic of Algeria

وزارة التعليم العالي والبحث العلمي

Ministry of Higher Education and Scientific Research

جامعة أمحمد بوقرة بومرداس

University of M'Hamed Bougara of Boumerdès

Faculty of Sciences

Biology department

Thesis presented for obtaining the diploma of

MASTER

Domain: Science of Nature and Life

Sector: Biological Sciences

Specialty: Biology of Populations and Organisms

Theme

Evaluation of the bioinsecticide effect of the essential oil of a Lamiaceae and a Cupressaceae on mosquito larvae

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Presented by:

M^{elle} MOUSSEK Rania & M^{elle} BOURABIA Wafa

Devant de jury composé de :

Mrs BEHIDJ Nassima	Prof (UMBB)	Presidente
Mrs BOUMAZA Sarah	MCA (UMBB)	Examinatrice
Mrs TOUBAL Souheyla	MCA (UMBB)	Promotrice

2023/2024

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Thanks

First and foremost, we thank Almighty God for giving us the courage, patience, and strength to complete this final project.

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Assistant Professor, Class A, at M'Hamed Bougara University in Boumerdes

For accepting to supervise and guide us throughout this work, as well as for her patience, availability, and above all, her advice.

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to the realization and success of this work

with their support and encouragement.

Rania & Wafa

Dedication

First and foremost, I would like to thank God for giving me the strength

and courage to successfully complete this work.

I dedicate this work:

To the man of my life, my eternal love, my moral support, and my hero, to the source of my joy,

The one who has always sacrificed to see me succeed, to you my dad ***"Rabah"***

To my paradise, the source of my efforts, the thread of hope that lights up my path,

my happiness, to my dear mom ***"Houria"***

To my dear brothers: ***"Ahmed"*** *and* ***"Sofiane"***

To my right-hand man, my little hero, my eternal support and source of joy and happiness,

to my little brother ***"Kamel"***

To all my family, near and far.

To the buds of the family: ***"Mohamed", "Daniel",*** *and* ***"Silane"***

To my best partner in the world: ***"Wafa",*** *thank you for your help.*

*To all my dear friends****: "Sarah", "Ikram", "Zina", "Manel", "Imane", "Siham",***

"Lydia", "Farida"

And to all the people I love and have not named in writing but keep in my thoughts,

I say thank you, I love you.

Rania

Dedication

Praise to Almighty God, who has allowed me to see this long-awaited day.

No expression, no matter how elaborate, could convey my deep gratitude

and appreciation for all these years.

I dedicate this work:

To the man of my life, my eternal role model, my moral support

and source of joy and happiness, the one who has always sacrificed to see me succeed,

*to you my father "**Sidali**".*

To the light of my life, the source of my efforts, the flame of my heart,

*my happiness, beloved mother "**Karima**".*

*To my dear brothers: "**Khaled**", "**Youcef**", "**Walid**", and "**Hamza**".*

*And my dear sisters: "**Hanane**" and "**Lamia**"*

Source of my joy and happiness, I always carry you in my heart,

I wish you happiness, health, success, and joy.

*To my dear grandmother: "**Zhira**", whom I wish a long life and good health.*

*To all my family "**Bourabia**" and "**Sid Ahmed**" small or large,*

whether close or distant, for their support, love, and generosity.

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*To all my friends, near and far: "**Zina**", "**Lydia**", "**Manel**", "**Imane**", "**Ikram**"*

And to all the people I love and

haven't named in writing

but keep in mind, I say thank you.

Wafa

Chapitre II : Material and methods

Chapitre III : Results and Discussions

Introduction

Mosquitoes are insects of the order Diptera (two wings) belonging to the family Culicidae, identifiable by a piercing-sucking mouthpart. Currently, there are more than 3500 species grouped into 41 genera **(Center for Disease Control and Prevention CDC, 2021)**. They have a worldwide distribution and occur in tropical and temperate regions **(Carnevale and Robert, 2009).** The Culicidae are divided into two subfamilies:

- The subfamily Anophelinae includes all genera responsible for transmitting the *Plasmodium* parasite that causes malaria. It is within this subfamily that the genus Anopheles (An.) is found, first described in 1818 by J.W. Meigen **(Reinert, 2001).**
- The subfamily Culicinae groups all genera other than those of the second subfamily. It includes genera such as *Culex* (Cx.) and *Aedes* (Ae.), which are the main vectors of dengue and chikungunya.

The mosquito is the most dangerous vector, transmitting parasitic diseases **(Cuervo-parra, 2016)** and having different habitat preferences **(Sithiprasana *and al*., 2003)**. Indeed, these hematophagous insects transmit numerous arboviruses **(Heu and Gendrin, 2018**) that can be dangerous for the human species, such as those causing yellow fever, dengue, chikungunya, Rift Valley fever, and many others **(Fontenille, 2017).** Mosquitoes also transmit nematodes responsible for lymphatic filariasis in humans, such as Bancroft's filarial worm, *Wuchereria* bancrofti, the Malaysia filarial worm, *Brugia malayi*, and the Timor filarial worm, *Brugia timor*.

Of all these diseases, malaria is certainly the most deadly, causing over 400,000 deaths each year **(O.M.S, 2020).** There are over 400 different species of mosquitoes belonging to the genus *Anopheles*, with around thirty of them being significant vectors of malaria **(Carnevale and Robert, 2009).**

The overuse of synthetic chemical products in mosquito control campaigns has several major disadvantages, including environmental pollution and toxicity to non-target organisms **(Ramkumar and Shivakumar, 2015; Benelli and Pavela, 2018).**

The application of botanical extracts could be an alternative solution for mosquito control **(Aissaoui *and al*., 2022).** Compounds derived from plants have a good prospect as safe, selective, economically viable, and biodegradable natural alternatives that are important in combating mosquito-borne diseases **(Damtie and Mekonnen, 2021).** Aromatic plants are known to produce essential oils, especially higher plants, angiosperms, and gymnosperms, belonging to around 50 families, most commonly Apiaceae, Lamiaceae, Myrtaceae, Pinaceae,

and Cupressaceae. Their essential oils are known for their culinary uses and especially in traditional medicine for treating various ailments **(AbdelMassih and El Beyrouthy, 2022).**

The main objective of this research is to study the larvicidal activity of essential oils from *Cupressus sempervirens* L. and *Lavandula stoechas* L. on an experimental population of *Culex pipiens*. In this work, we aimed to evaluate the effect of these two essential oils on mosquito larvae based on the dose used and the duration of exposure.

In this context, we organized our work into three chapters :

- Chapter I: Literature review on mosquitoes, plants, and the tested essential oils.
- Chapter II: Materials and methods used in our experimentation.
- Chapter III: Results and Discussion.

Followed by a general conclusion

Chapter I

Bibliographic synthesis

1. General overview of mosquitoes

Culicids or mosquitoes are insects belonging to the suborders of Nematocera, in the family Culicidae, which is divided into three subfamilies: Taxorhynchitinae, Anophelinae, and Culicinae. According to the most recent classification, the Culicidae family comprises 2 subfamilies, 11 tribes, 111 genera, and 3528 species worldwide. In Algeria, 50 species of Culicids from 6 different genera are grouped in the subfamilies Anophelinae and Culicinae **(Belkhiri, 2022)**. **(Table 1)**

Table 1 : general systematics of culicidea present in Algeria

Reign	**Animalia**	
Branch	Arthropoda	
Sub-branch	Hexapoda	
Class	Insecta	
Sub-class	Pterygota	
Order	Diptera Linné	
Sub-order	Nematocera Latreille	
Family	Culicidae Latreille	
Sub-family	Anophelinae	Culicinae
	Anopheles (Meigen, 1818)	*Culex* (Linné, 1758) *Aedes (*Meigen, 1818) *Culiseta* (Neveu-Lemaire,1902) *Orthopodomya* (Théobald, 1904) *Uranotaenia* (Lunch Arribalzaga, 1904

The Culicidae, widespread and common near water, fly in the summer evenings, mornings, and in open skies. They are insects with a holometabolous development, including the stages: eggs, larvae, nymphs, and adults. The life cycle is often short; in some species, the larval development lasts less than two weeks **(Belkhiri, 2022).**

1.1. Presentation of *Culex pipiens*

Culex pipiens is an extremely common mosquito in all temperate zones of Europe, Africa, Asia, North and South America, and Australia. This ubiquitous species can adapt to different habitats and thrives in both urban and rural environments, in polluted as well as clean waters. It is of particular interest due to its wide geographic distribution, abundance, and real nuisance, especially in urban areas (**Zeghib, 2022**).

1.1.1. Systematic position

According to **Zeghib (2022),** the systematic position of *Culex pipiens* is as follows **(Table 2) :**

Table 2 : systematic position of *Culex pipiens*

Reign	**Animalia**
Branch	Arthropoda
Sub-branch	Antennata
Class	Insecta
Sub-class	Pterygota
Order	Diptera
Sub-ordre	Nematocera
Family	Culicidae
Sub-family	Culicinae
Genus	*Culex*
Species	*Culex pipiens*

1.1.2. Characteristics of *Culex pipiens*

The abdomen, which contains the organs for digestion, reproduction, and egg development, significantly increases in size when the female takes a blood meal. The proteins found in the blood enable the maturation of the eggs **(Figure 1).**

On peut reconnaître un moustique *Culex*, qui appartient à la sous-famille des Culicinae grâce à certaines caractéristiques :

- The pulvilli (mosquito attachment system located on the legs) are at the end of the legs.
- The mouthparts are of the piercing-sucking type.
- The palps (sensory organs) are elongated in males (longer than the proboscis), slightly curved upwards, and have feathery antennae; the palps are shorter than the proboscis in females (about a quarter of its length). **(Tabti, 2017)**

- When at rest, the abdomen of adults is almost parallel to the surface.
- Elongated antennae and a fairly long breathing siphon in the larvae

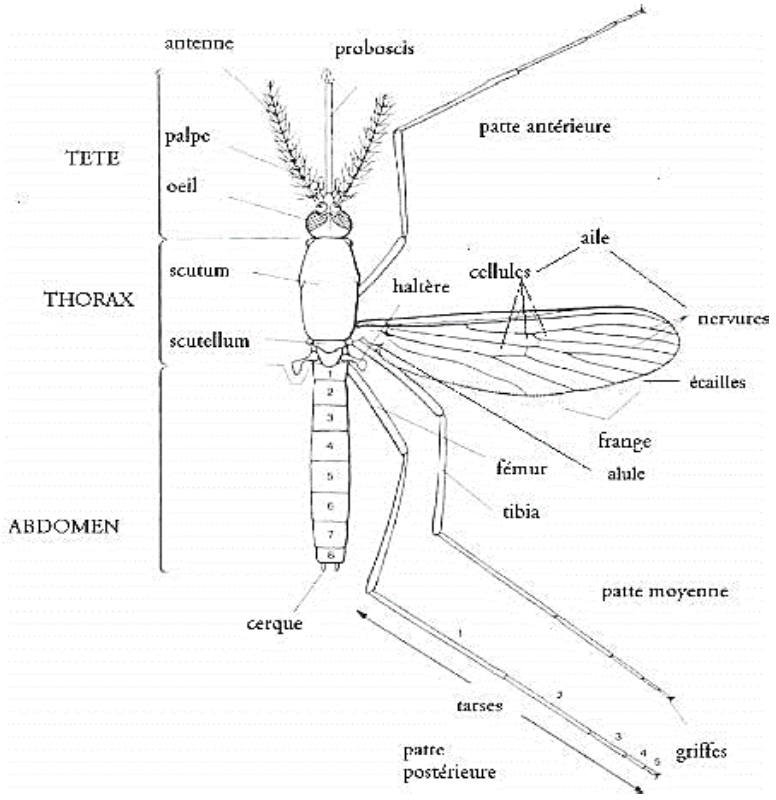


Figure 1 : general morphology of an adult culicid

1.1.3. Development cycle

Culicids are characterized by four stages of development in their life cycle: eggs, larvae, nymphs, and adults **(Figure 2).** The larval stage goes through three successive larval stages, characterized by a significant increase in size, which can be about 10 times from the first to the fourth stage. This life cycle includes a pre-imaginal phase that occurs in the water, involving the egg, larvae, and nymph, and an aerial phase involving the winged adult. During this latter phase, the period of reproduction and dispersion takes place **(Chahed, 2022).**

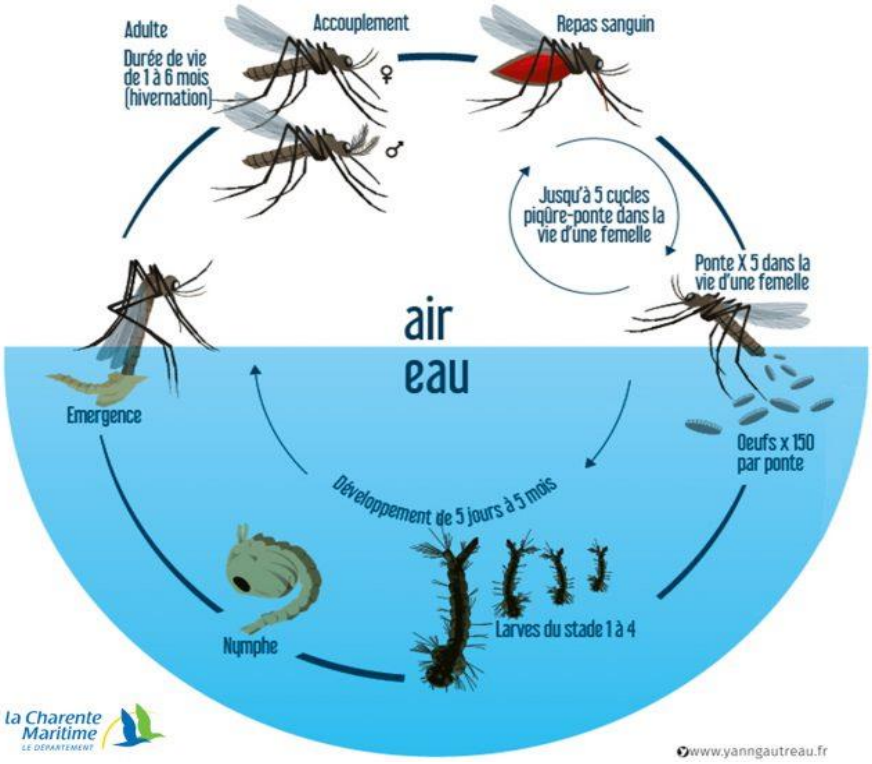


Figure 2 : mosquito development cycle **(Chahed, 2022)**

1.1.3.1. Egg

They are laid in water, usually clear, but can also be found in polluted waters with organic matter that will provide food for the larvae. They are deposited in clusters forming a raft that floats on the water. This raft measures 3-4 mm long and 2-3 mm wide. Hatching occurs approximately 24 to 48 hours after oviposition **(Larabi, 2017).**

1.1.3.2. Larva

The larva is aquatic, characterized by discontinuous growth, and undergoes three successive molts, leading to the development of four post-embryonic stages (L1, L2, L3, and L4). The larvae of Culicinae breathe at the water surface through a siphon, unlike Anophelinae, whose respiratory openings are at the cuticle level. *Aedes* and *Culex* larvae position themselves almost perpendicular to the water surface, holding vertically or obliquely, while *Anopheles* larvae rest horizontally on the water surface. The larval lifespan is one to two weeks depending on the species and ecological conditions (temperature), but it increases during hibernation. In *Ae.*

Albopictus, the larval cycle lasts about eight days to reach adulthood, with a lifespan of 4 to 5 weeks.

At the end of growth, the larva of the final stage transforms into a nymph, which, 48 hours later, emerges as a winged adult ready to take flight **(Chahed, 2022).**

1.1.3.3. Nymph

The nymph is aquatic, apodal, comma-shaped, and characterizes the resting stage that interposes between the larva and the adult, marking the transition from aquatic life to aerial life. It has a short lifespan of 2 to 3 days. If disturbed, it dives to the bottom of the water and breathes through two respiratory trumpets **(Chahed, 2022).**

1.1.3.4. Adult

Male mosquitoes emerge a day or two before females. After mating, the female seeks a blood meal, attracted by carbon dioxide (CO2) from a long distance. Some species bite indoors (endophagic), while others prefer outdoors (exophagic). The female *Ae. Albopictus* is anthropophilic and primatophilic, mainly biting at dawn, dusk, and sometimes at night. Females live on average thirty to forty days, while males live about a week, and adult *Ae. Albopictus* can live up to ten weeks. The biology of adult mosquitoes includes feeding and reproduction, with females dispersing to find a vertebrate host, an egg-laying site, and a resting place, integrating these behaviors into their gonotrophic cycle **(Chahed, 2022).**

1.1.4. Pathogenicity role

Most mosquitoes are annoying but not pathogenic. However, some species transmit serious diseases to humans and animals. These mosquitoes are vectors of parasitic, viral, or bacterial pathogens, causing diseases with high rates of mortality and morbidity. They are considered the most dangerous animals to human health. The most concerning species belong to the genera *Anopheles*, *Culex*, and *Aedes.* Females bite to take a blood meal, which can lead to bacterial secondary infections, local irritations, hypersensitivity, or the transmission of viruses, filariae, or protozoa **(Chahed, 2022).**

2. Means of combating mosquitoes

Based on mosquito control, they consist of chemical, biological, physical, and genetic methods.

2.1. Chemical control

The main measures taken against mosquitoes rely on chemical control through the use of insecticides. Depending on the situation, larval control measures (dispersing insecticides in breeding sites) or autocidal techniques (indoor spraying) can be adopted. Chemical control is carried out using synthetic or plant-based products that kill insects through ingestion or contact. The application method of these products depends on the ecology of the vector and the targeted stage. Insecticides used against mosquitoes include various molecules belonging to different chemical families (Organophosphates, carbamates, pyrethroids, bio-insecticides, etc.) with diverse modes of action **(Kharoubi, 2021)**.

2.2. Biological control

Introducing different species of organisms into the mosquito habitat to act as their enemies is known as biological control. This involves using larvivorous fish like Gambusia affinis, which are effective in permanent water bodies, and the bacterium Bacillus, which causes mortality in mosquito larvae of the *Culex* and *Anopheles* genera, to a lesser extent on *Aedes*. Herbivorous fish like carps are used in China to consume the vegetation that serves as breeding sites for mosquito larvae **(Merabti, 2016).**

2.3. Physical control

It is an intentional modification of the biotope aimed at eliminating or reducing surface water bodies where mosquitoes develop through physical means. Different methods include drainage, containerization, capturing resurgences, filling, and afforestation. Physical actions typically involve regulating water flow, managing drainage, or physically modifying the environment through various means **(Kharoubi, 2021).**

2.4. Genetic control

It involves the manipulation of the genetic heritage of mosquitoes to obtain transgenic individuals that can be either sterile or refractory to the parasites they usually transmit. The manipulations also target plants such as algae that reproduce in larval habitats. These genetically modified algae, through the integration of genes from bacterial toxins, act on mosquito larvae **(Kharoubi, 2021).**

3. General information about essential oils

The term Essential Oils "EOs" is a generic term that refers to the liquid and highly volatile components of plants, characterized by a strong and distinctive odor. Indeed, essential oils are

natural complexes of volatile and fragrant molecules, synthesized by the secretory cells of aromatic plants. Essential oils are contained in aromatic plants and are responsible for the different scents they emit. Hydrodistillation remains the most common method used to produce essential oils, especially for commercial and medicinal purposes **(Bastien, 2008).**

Secondary metabolites are extracted from plants by steam distillation. The volume of essential oil recovered depends on the distillation yield, which varies, in the same plant, depending on the season. Essential oils can also be obtained by cold pressing, as with citrus fruits. New techniques have been developed to increase production yield, such as extraction using low-temperature and high-pressure liquid carbon dioxide or extraction assisted by ultrasound or microwaves **(Toure, 2015).**

3.1. Classification of essential oils

The study of the chemical composition of essential oils shows that they are complex and variable mixtures of components due exclusively to two biogenetic groups: terpenoids and aromatic compounds derived from phenylpropane **(Bencheikh, 2017).**

3.1.1. Terpenoids

The term "terpene" originates from the first extraction of this type of compound in turpentine oil. Terpenoids are those with relatively low molecular weight, meaning they have more volatile molecules. They generally have the general formula (C5H8) n. Depending on the value of n, we have hemiterpenes (n=1), monoterpenes (n=2), sesquiterpenes (n=3), triterpenes (n=6), tetraterpenes (n=8), and polyterpenes. The components of essential oils are very diverse. In addition to terpenes, they contain hydrocarbons, esters, lactones, aldehydes, alcohols, acids, ketones, phenones, oxides, and others **(Bencheikh, 2017) (Figure 3).**

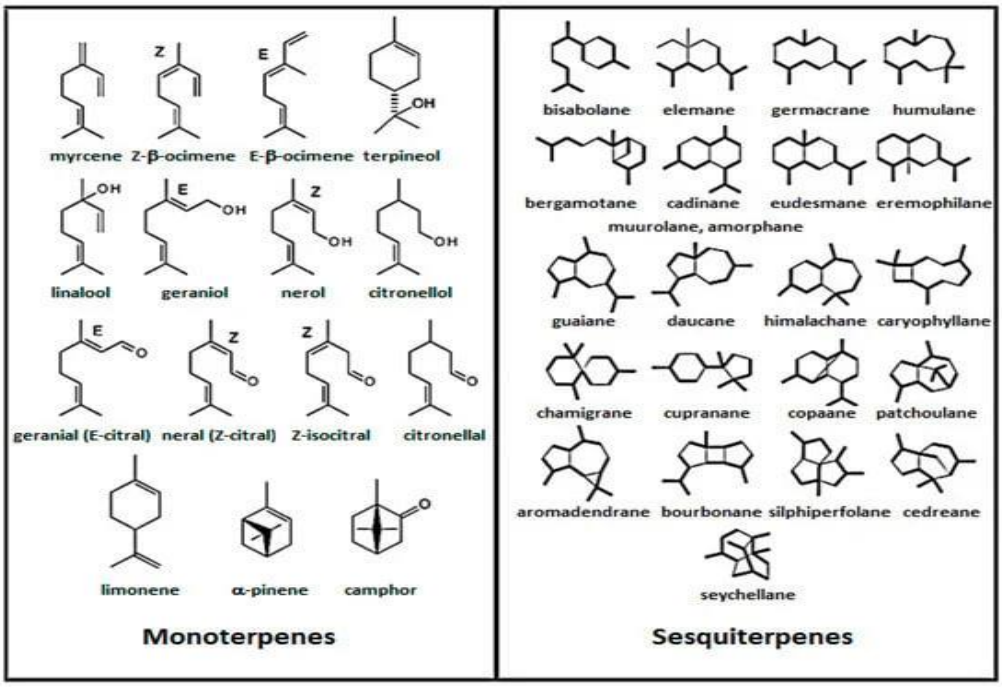


Figure 3 : structures of certain terpenes **(Dhifi *and al*., 2016)**

3.1.2. Aromatic compounds

Unlike terpenic compounds, aromatic compounds are less present in essential oils. However, they are considered an important group because they are generally responsible for the organoleptic characteristics of essential oils. They often consist of allyl and propenylphenol. An example is eugenol, which is responsible for the smell of cloves **(Figure 4) (Bencheikh, 2017).**

Figure 4 : chemical structure of some aromatic compounds extracted from essential oils **(Bencheikh, 2017).**

3.2. Location of essential oil

Essential oils are located in all living parts of the plant and are formed in the cytoplasm of certain specialized plant cells. They can be stored and accumulated in various plant structures

such as secretory hairs or trichomes, epidermal cells, internal secretory cells, secretory pouches, and secretory channels **(Farhat, 2010).**

Several categories of secretory tissues can coexist simultaneously in the same species, or even in the same organ. Glandular trichomes are the primary sites of essential oil biosynthesis, and plants that lack such specialized structures synthesize and accumulate only traces of monoterpenes.

As a result, the development dynamics of these structures as well as the secretion process and mechanism have an indirect impact on oil production and the functioning of the producing system **(Sharma *and al*., 2003).**

3.3. Uses of essential oils

There are several ways to use essential oils: they can be ingested, inhaled, or applied directly to the skin:

3.3.1. Oral route: it should only be used on the advice of a doctor. Never take a pure oil in your mouth as it can cause burns. Also, we advise never to take more than three drops.

3.3.2. Respiratory route: essential oils are quickly absorbed by all the small ciliary cells lining our respiratory tree from the nasal cavities to the ends of our lung alveoli.

3.3.3. Cutaneous route: This is the ideal route as it is safe and very effective. Essential oils are generally used at very dilute concentrations. They can be used in various ways such as through massage or simply by applying them according to the area and condition to be treated. Other forms are also possible: ointments, baths, etc.

In any case, essential oils penetrate our body to reach the bloodstream in order to be transported to the site of the illness **(Bencheikh, 2017).**

3.4. Methods of extracting essential oils

Essential oils are plant extracts that are subject to various processes and can vary depending on where they are grown, the climate, altitude, soil, agricultural methods, and the timing of the harvest. It is therefore very important that the starting material used to produce the essential oil represents the plant's natural product, in order to produce the highest quality essential oil. Due to their different distribution, there are several methods of producing essential oils **(Boukhatem *and al*., 2019).**

3.4.1. Steam distillation extraction

This is one of the official methods for obtaining essential oils **(Figure 5).** In this extraction system, the plant material is subjected to the action of a steam current without prior maceration. The vapors saturated in volatile compounds are condensed and then decanted in the essencier. The steam injection is done at the base of the still **(Boukhatem *and al.*, 2019).**

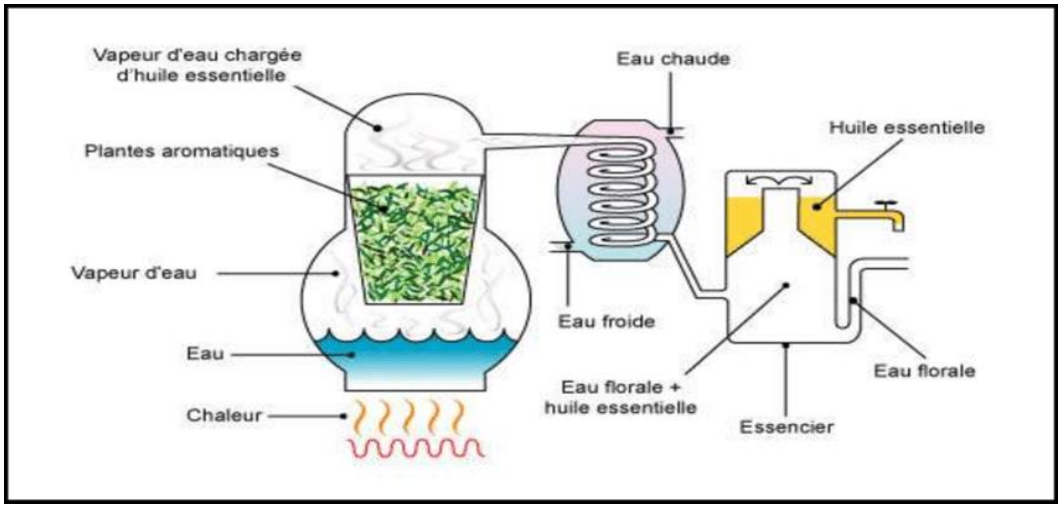


Figure 5 : steam distillation extraction setup

3.4.2. Extraction by hydrodistillation

This technique involves immersing the raw material in a water bath and bringing it to a boil, usually at atmospheric pressure. Distillation can be done with or without cohobation of the aromatic waters obtained during decantation. However, this method has disadvantages, including the impact of steam or boiling water on certain plants. In particular, flowers, which are often too delicate, do not withstand treatments well using steam distillation and hydrodistillation **(Figure 6) (Farhat, 2010).**

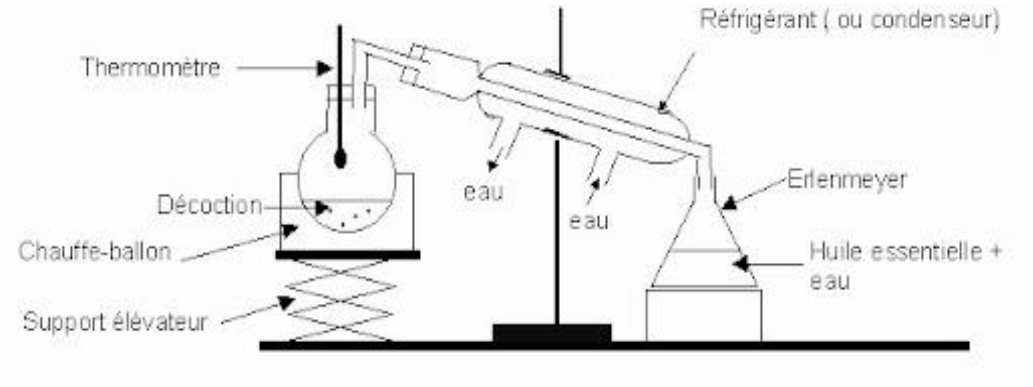


Figure 6 : hydrodistillation extraction setup

3.4.3. Microwave-assisted extraction

In the early 1990s, a completely new technique called vacuum microwave hydrodistillation appeared **(Figure 7).** In this process, the plant material is heated by microwaves in a closed chamber where the pressure is sequentially reduced. The volatile compounds are carried by the steam formed from the water present in the plant material. They are then recovered using traditional processes of condensation, cooling, and decantation **(Touhami, 2017).**

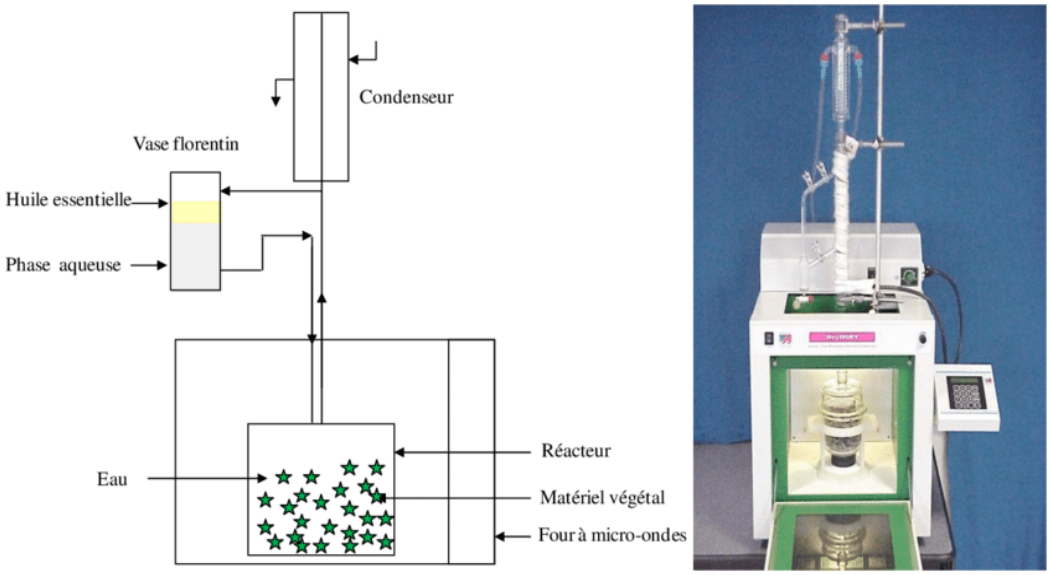


Figure 7 : microwave distillation setup **(Benaiche, 2022).**

3.4.4. Supercritical CO2 extraction

This technique, similar to solvent extraction, uses supercritical CO2 as a non-toxic solvent, leaving no trace in the essential oil obtained. It is based on the solubility of constituents in supercritical CO2, allowing extraction in liquid phase and separation in gas phase. The CO2 is liquefied by cooling, compressed, and then injected into the extractor containing the plant material. Then, it expands to return to gaseous form and is directed towards a separator to separate the extract from the solvent. This method allows for solvent recycling through simple compression and expansion and uses low extraction temperatures, preserving fragile constituents. It is ideal for essences that are difficult to distill **(Figure 8) (Benaich, 2022).**

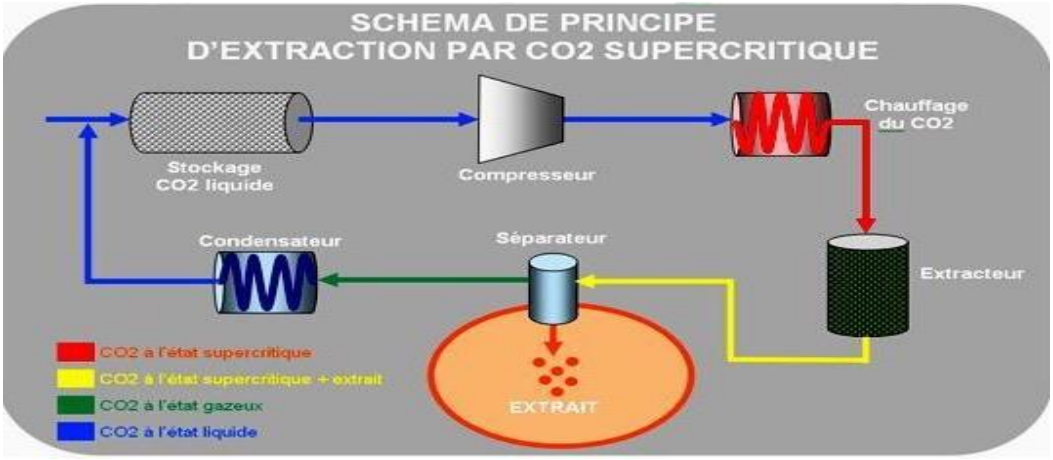


Figure 8 : Supercritical CO2 extraction method **(Benaiche, 2022).**

3.5. Essential oil analysis method

3.5.1. Gas chromatography (GC)

Gas chromatography (GC) is a method of separating volatile substances carried by an inert gas called carrier gas. The nature of the column determines the type of chromatography (partition or adsorption). The choice of column will depend on the physico-chemical properties of the compounds to be separated. A gas chromatograph is composed of an injector, a furnace (with programmable temperature) containing the column, and a detector **(Humbert and Lhermitte, 2005).**

3.5.2. Gas chromatography coupled with mass spectrometry (GC/MS)

The development of mass spectrometry for identifying complex constituents has been greatly facilitated by coupling gas chromatography with mass spectrometry (GC/MS). This coupling allows for obtaining an interpretable mass spectrum for amounts of substance ranging from micrograms to milligrams. Electron impact ionization (EI) uses a beam of electrons of about 70 eV to ionize and fragment substances, producing characteristic mass spectra. These spectra are compared to those in commercial reference libraries, such as the NIST Mass Spectral Library and the Wiley Registry of Mass Spectral Data **(Touhami, 2017).**

4. General information about plants: lavender and cypress

These are perennial aromatic plants whose medicinal properties are widely used in herbal medicine and aromatherapy. These therapeutic properties are linked to their primary and secondary metabolites, particularly their essential oils. Indeed, the latter have antispasmodic, antibacterial, antifungal, antioxidant, acaricidal **(Mohammedi and Fawzia, 2011; Bachiri *and***

***al.*, 2017)** and insecticidal effects. The insecticidal effect of these medicinal plants has been widely reported against stored product insects and as larvicidal and repellent agents against certain mosquitoes **(Ramzi *and al*., 2022).**

4.1. *Lavandula stoechas* L.

4.1.1. Botanical description

The genus *Lavandula*, belonging to the subfamily Nepetoideae, comprises approximately 39 species, numerous hybrids, and nearly 400 cultivars. Primarily Mediterranean, these plants are highly aromatic and used for their expectorant, antispasmodic, disinfectant, antimicrobial, anticarcinogenic, sedative, antidepressant, antioxidant, anti-inflammatory, and insecticidal properties **(Bachiri *and al*., 2016).**

Lavandula are perennial subshrubs or shrubs reaching up to one meter in height, in full bloom from mid-June to mid-July, preferring acidic soils. Their flowers, often violet, blue **(Figure 9)** or pink, are arranged in clusters of cylindrical or quadrangular panicles **(Ez Zoubi *and al*., 2020).**

Figure 9: photos of the aerial parts of the plant *Lavandula stoechas* L. (stems, leaves, and flowers)

4.1.2. Systematic position of *Lavandula stoechas* L.

According to **Chakoual (2019),** the systematic position of lavender is as follows **(Table 3):**

Table 3 : systematic position of lavender

Reign	Planta
Sub reign	Vascular plants
Branch	Spermaphytes
Sub-branch	Angiosperms
Class	Dicotyledonous
Order	Lamials
Family	Lamiaceae
Genus	*Lavandula*
Species	*Lavandula stoechas L.*

4.1.3. Origin and geographical distribution

In the world: *Lavandula stoechas* L. is found in the Mediterranean region and cultivated in France, Spain, and Italy. In Turkey, *Lavandula stoechas* L. and *Lavandula angustifolia* are the main species, and *L. stoechas* is also found in North Africa, Southwest Asia, tropical Africa, and India **(Ez Zoubi *and al.*, 2020).**

In Algeria: *Lavandula stoechas* L. is widely distributed throughout the northern periphery of the country **(Figure 10) (Ez Zoubi *and al.*, 2020).**

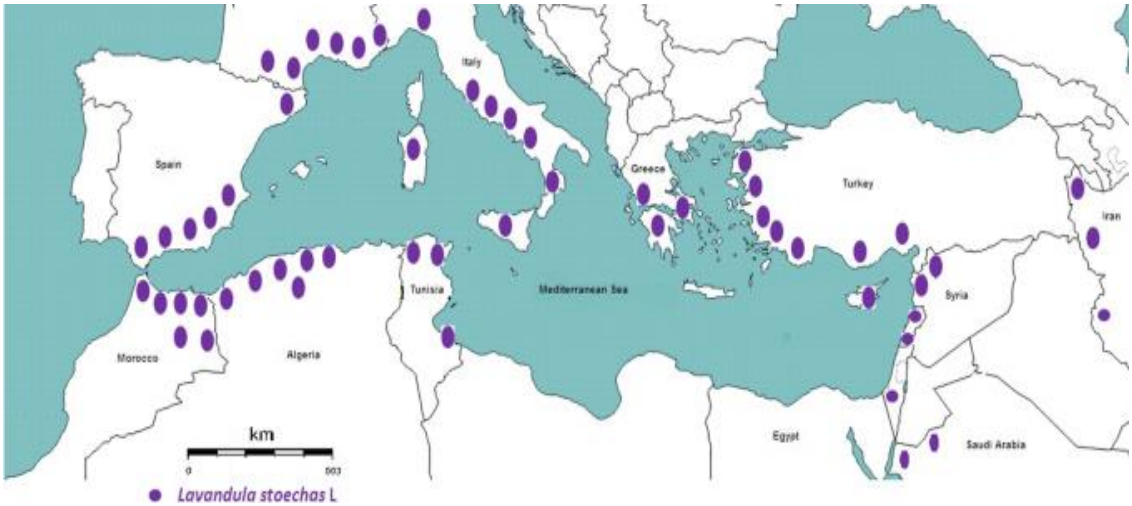


Figure 10: geographical distribution of L. stoechas **(Ez Zoubi *and al.*, 2020)**

4.2. *Cupressus sempervirens* L.

4.2.1. Botanical description

The common cypress, an evergreen tree of the Cupressaceae family and Gymnosperms, is characterized by its short scale-like leaves in adulthood and larger needle-like leaves when young. The leaves are arranged in opposite pairs. The cones, globular or ovoid, mature 18 to 24 months after pollination. The seeds are small, with a narrow wing on each side. **(Figure 11) (Frezza *and al*., 2022).**

Figure 11 : morphological characteristics of *Cupressus sempervirens* L. **(Original 2024).**

4.2.2. Systematic position

According to **Al-Snafi (2016),** the systematic position of the Cypress is as follows **(Table 4):**

Table 4 : systematic position of the Cypress

Reign	**Planta**
Sub-reign	Viridiplantae
Branch	Tracheophyta
Sub-branch	Spermatophytin
Class	Pinosida
Sub-class	Pinidae
Order	Pinales
Family	Cupressaceae
Genus	*Cupressus*
Species	*Cupressus sempervirens*

4.2.3. Origin and geographical distribution

- **In the world**

Among the Cupressaceae found in the northern hemisphere, the genus *Cupressus*, with 29 species, is the most widespread. The Mediterranean cypress *(Cupressus sempervirens*), also known as Italian cypress, Mediterranean cypress, or common cypress, is endemic to the Mediterranean region and naturally extends to Iran. It has been introduced as an ornamental plant in many regions of the world, including Australia, New Zealand, Chile, India, England, and France. A symbol of Provence, it is commonly used in gardens and as a decorative plant in planters. **(Figure 12) (Poncet *and al*., 2022).**

- **In Algeria**

The species of endemic or naturalized cypresses include the Tassili cypress *(Cupressus dupreziana)*, the Atlas cypress *(Cupressus atlantica)*, and the Mediterranean cypress *(Cupressus sempervirens* L.). The latter, originating from the eastern part of the Mediterranean basin, has the widest natural distribution range and is also the most widely used species for windbreak, ornamental, or religious purposes **(Figure 12) (Bechir *and al*., 2004).**

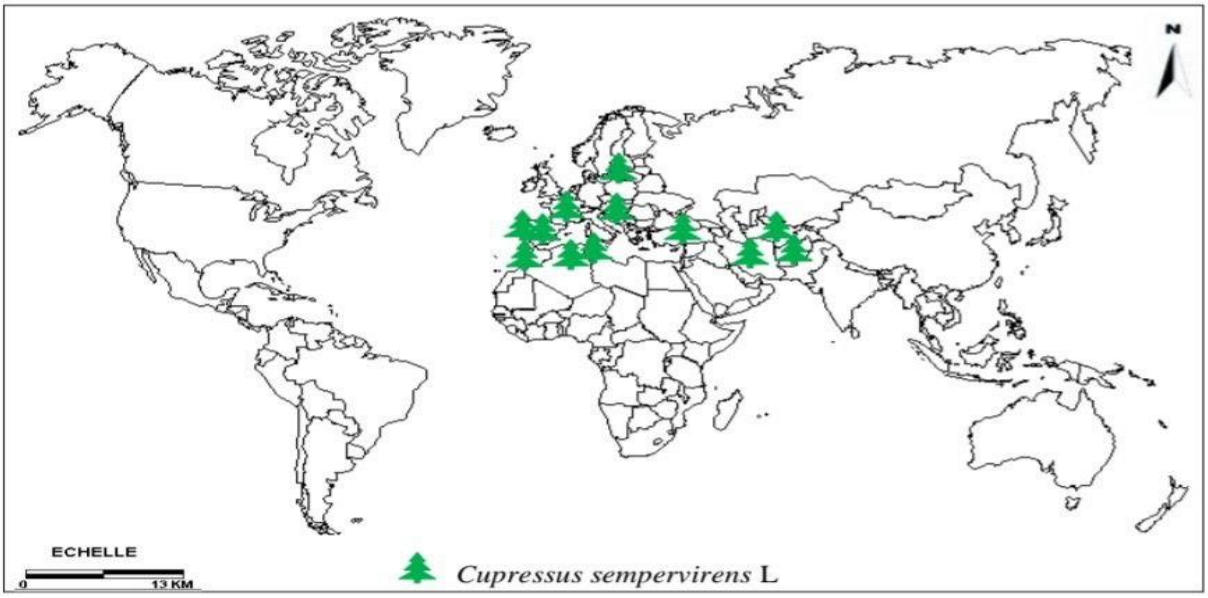


Figure 12 : distribution range of *Cupressus sempervirens* L. **(Nichane, 2015)**

Chapter II

Material and methods

The present study focuses primarily on the evaluation of the bioinsecticidal effect of the essential oils (EOs) of *Lavandula stoechas* L. and *Cupressus sempervirens* L. on the larvae of *Culex pipiens* mosquitoes at the L4 development stage.

The experimentation took place at the educational laboratory specializing in Population Biology and Organisms "BPO" at the M'Hamed Bougara University of Boumerdes, as well as at the molecular spectroscopy laboratory of Sonatrach (IAP).

1. Material

In this study, we used biological material from plants and animals (mosquito larvae) and non-biological material (devices, tubes...).

1.1. Biological material

1.1.1. Plant material

The plant material used in this study consists of two types of plants, the first belonging to the Lamiaceae family, called *Lavandula stoechas* L. and the second belonging to the Cupressaceae family, which is *Cupressus sempervirens* L.

1.1.2. Animal material

The study was conducted on a type of mosquito larvae, which is *Culex pipiens* at the 4th stage of development.

1.2. Non-biological material

The non-biological material used in this study is a set of standard equipment consisting of reagents, chemicals **(Annex 1).**

2. Study method

Our work was carried out in four steps:

- ✓ Harvesting of plants and obtaining essential oils from (*Lavandula stoechas* L. and *Cupressus sempervirens* L.) from a specialized company;
- ✓ Collection and identification of mosquito larvae (*Culex pipiens*);
- ✓ Spectral analysis by ATR infrared (IR-ATR);
- ✓ Gas chromatographic analysis by gas chromatography (GC);

- ✓ Evaluation of the bioinsecticide effect of *Lavandula stoechas* L. and *Cupressus sempervirens* L. essential oils on *Culex pipiens* L4 larvae;

The entire process of this study is summarized in the following figure **(Figure 13):**

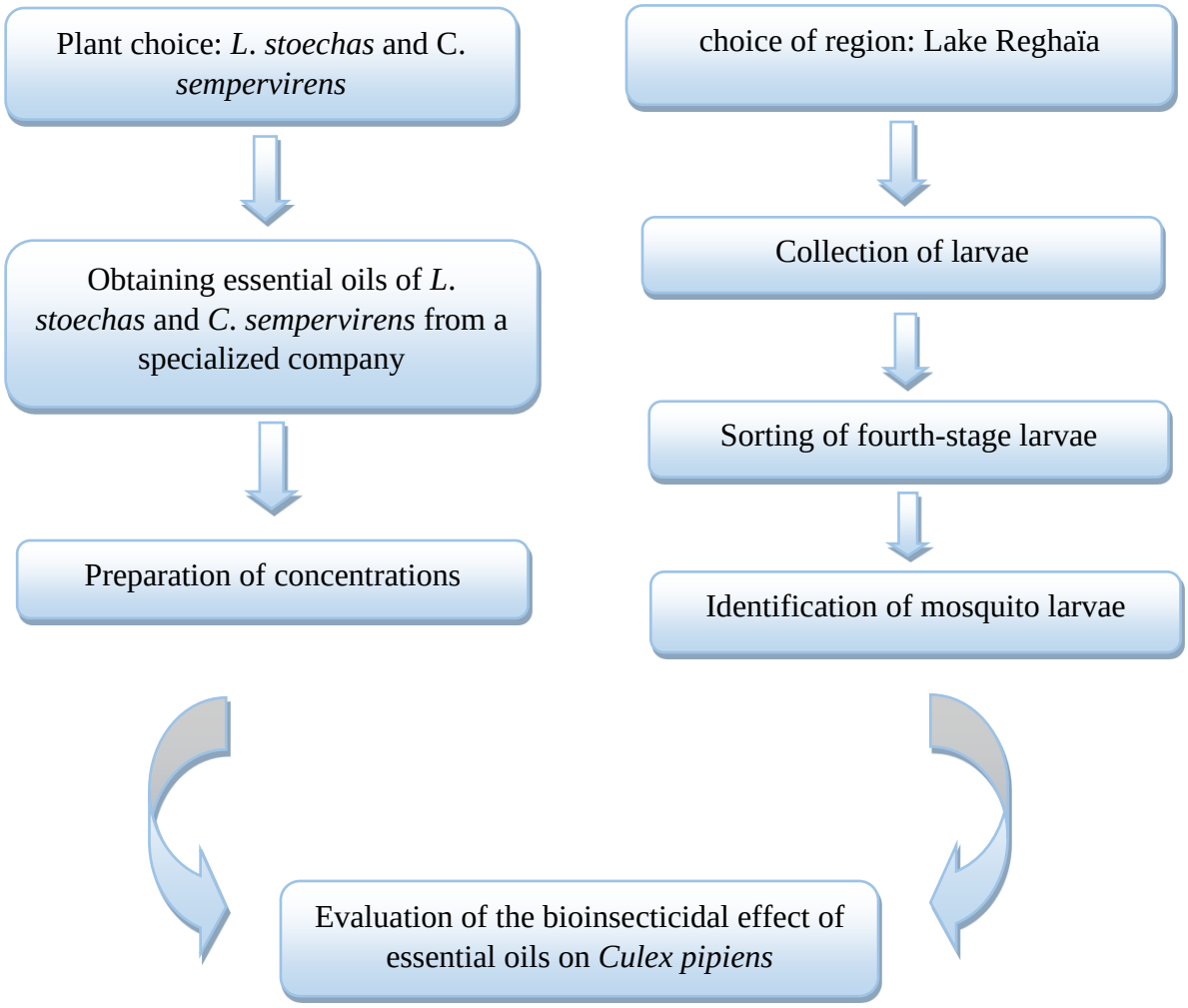


Figure 13 : General diagram of the various stages of work

2.1. Method of obtaining essential oils

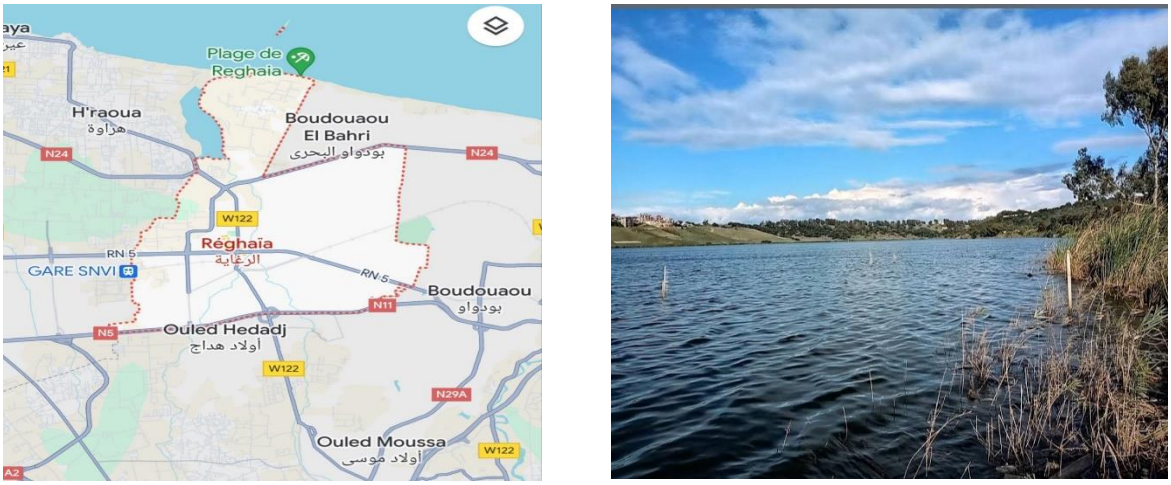
The essential oil of the two plants (*Lavandula stoechas* L. and *Cupressus sempervirens* L.) was extracted by the company "BIO ADYMA" specialized in the production of essential oils, located in Afir (Boumerdès). The essential oil was extracted from the aerial parts of the plant (stems, leaves, and flowers) in the month of March. The extraction process used was steam distillation.

2.2. Method of larval collection

The larvae were collected at Reghaia Lake on April 24, 2024, which is located within the territory of the municipality of Reghaïa, 30 km from Algiers, at the northeastern border of the

Mitidja plain, and covers an area of 842 hectares. It is bordered to the north by the Mediterranean Sea, to the south by the municipalities of Awlad Hadaj and Awlad Musa, to the east by the municipalities of Boudouaou EL-Bahri, and to the west by the municipalities of Rouiba and Harawa **(Figure 14).**

The larvae were collected using a plastic ladle, and we adopted the direct capture technique to facilitate larval collection.



(a) **(b)**

Figure 14 : Reghaïa lake

(a): geographic map ; (b): Reghaïa lake

Once in the laboratory, only fourth-stage (L4) larvae were separated from other larval stages and labeled. The younger larvae were raised in boxes to gradually collect L4 larvae. The latter are widely used in this field due to the ease of their collection and their chaetotaxy which allows for the identification of species and subspecies.

The identification of the larvae was facilitated by using a software for the identification of Culicidae of the Mediterranean Africa established by IRD Montpellier **(Brunhes *and al.*, 1999)** followed by confirmation using the identification key of **Himmi *and al.*, (1995).**

2.3. ATR infrared spectral analysis method

The ATR spectra of the essential oils *Lavandula stoechas* L. and *Cupressus sempervirens* L. were analyzed for qualitative purposes to identify characteristic bands and various functional groups. The chemical structures of these essential oils were characterized using ATR spectroscopy with a JASCO-1400 spectrophotometer, Germany. The measurements were

conducted in a frequency range from 4,000 to 600 cm-1. For the analysis, a drop of oil was added and spread on an ATR crystal (diamond) before being analyzed **(Figure 15).**

Figure 15 : Fourier Transform Infrared Spectroscopy (FTIR)

2.4. Gas Chromatography (GC)

The gas chromatography (GC) analysis of *Lavandula stoechas* L. essential oil was performed using an Agilent 7890B-5799A USA system with the nonpolar HP 5MS column (30 m × 0.25 mm × 0.25 µm film thickness).

The conditions for the GC-MS spectra were as follows: the column temperature program was set at 60°C for 8 minutes, increasing at a rate of 2°C/min up to 280°C, and held at 280°C for 15 minutes. An undiluted sample (0.2 µl) was injected by split injection with a split ratio of 1:20. The injection was carried out at 250°C. A helium carrier gas flow rate of 0.5 ml/min and an electron ionization mode at 70 eV over a scan range of 30 to 550 atomic mass units were used **(Figure 16) (Kerbouche *and al*., 2015).**

Figure 16 : gas chromatography device coupled to a mass spectrometer (GC/MS)

2.5. Bio-test evaluation method

Biological tests were conducted following the protocol of the World Health Organization **(WHO, 1996),** with slight modifications **(Pavela and Sedlák, 2018).**

For the toxicity test, different concentration ranges were tested using *Lavandula stoechas* L. and *Cupressus sempervirens* L. essential oils, specifically 7%, 5%, 3%, and 1%, corresponding to 70 mg/L, 50 mg/L, 30 mg/L, and 10 mg/L respectively. These concentrations were prepared from a stock solution of 1g/L.

Therefore, 20 larvae of *Culex pipiens* were exposed to 50 ml of each concentration. The same number of larvae (20 larvae) were placed in a control tube containing 50 ml of breeding site water. Three repetitions were performed for each dilution (R1, R2, and R3). Results were read after each 24-hour time interval.

2.6. Rate of larval mortality

A larva is considered dead if it does not move or shows a slow reaction to different stimuli. The mortality rate is calculated as the following ratio:

Mortality rate % = (Number of dead larvae / Total number of larvae) × 100

The test is considered valid if the mortality percentage in the controls is between 5% and 20%.

If the mortality percentage in the controls is 20%, the post-exposure mortality should be corrected using the Abbott formula **(Abbott, 1925):**

$$Mc = [(M2 – M1) / 100\text{-}M1] \times 100$$

Mc : Corrected mortality percentage

M1 : Percentage of mortality in the control group

M2 : Percentage of mortality in the treated group

If the mortality in the controls is greater than 20%, the test is invalid and must be repeated **(O.M.S, 2004).**

2.7. Data analysis

The lethal dose 50 (LD50) is the dose that induces mortality in 50% of the target population, it is calculated from the probit regression line (y = ax + b) corresponding to the number of mortalities based on the treatment doses.

The lethal time 50 (LT50) is the time required for 50% of individuals exposed to a certain dose to perish **(Ramade, 2007).** It is calculated from the probit regression line corresponding to the number of mortalities based on treatment times.

2.8. Method for making natural repellent

The steps for making a natural repellent using essential oils are:

- Prepare a clean and empty sprayer;

- Fill the sprayer with distilled water;

- Add a few ml of white vinegar;

- Add a few drops of essential oils;

- Shake the sprayer well to mix the water and essential oils;

- Spray the mixture in areas where you want to repel mosquitoes;

Chapter III

Results and Discussions

This final chapter is dedicated to presenting and discussing the experimental results obtained during our study. This comparative experimental study focuses primarily on spectral analysis by ATR infrared of the essential oils *Lavandula stoechas* L. and *Cupressus sempervirens* L. chromatographic characterization by gas chromatography (GC) of *Lavandula stoechas* L. essential oil, and finally an evaluation of the bioinsecticidal effect of the essential oils from *Lavandula stoechas* L. and Cupressus sempervirens L. on fourth-stage Culicidae larvae. Toxicological tests were conducted on these larvae. The results of these tests are illustrated in various figures and tables.

1. Results of ATR infrared spectral analysis of essential oils

1.1. *Lavandula stoechas* L.

The results of the infrared analysis of the essential oil of *L. stoechas* are shown in the following figure **(Figure 17):**

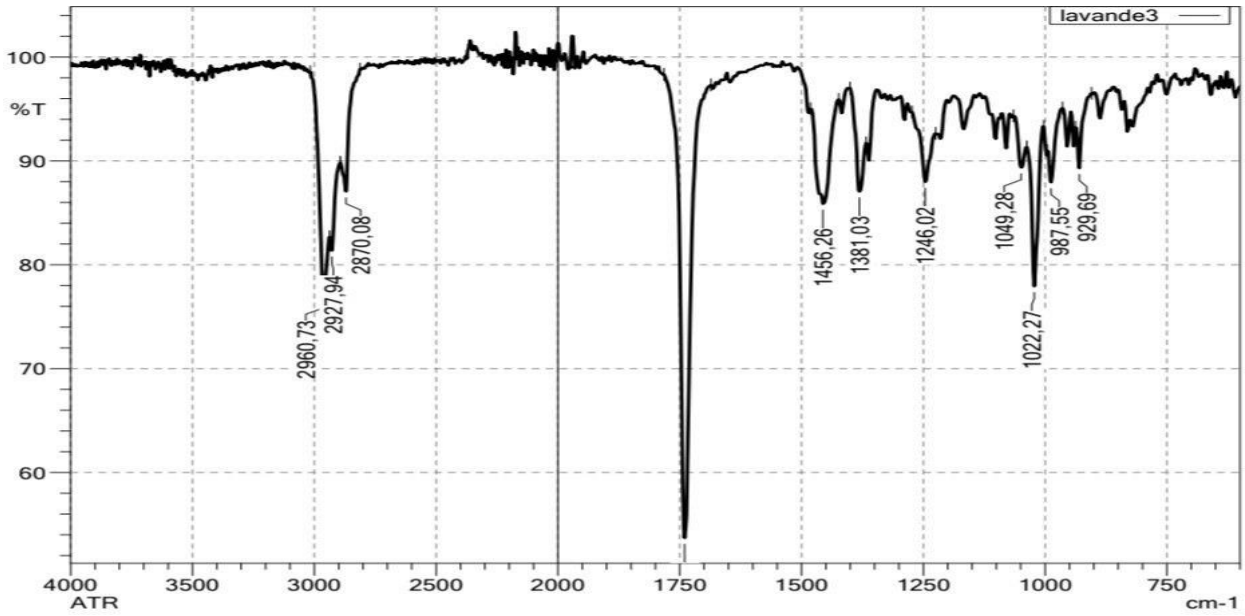


Figure 17: infrared analysis spectrum of essential oils of *L. stoechas*

The ATR infrared analysis of *Lavandula stoechas* L. essential oil provided a spectrum with several spectral bands. These were found at wave lengths ranging from 929.69 to 2960.73cm^{-1}.

The ATR infrared spectrum of *Lavandula stoechas* L. essential oil presents characteristic peaks indicating the presence of different functional groups in the biological compounds it contains **(Table 5).**

Table 5: corresponding chemical groups of the biomolecules in *Lavandula stoechas* L.

Wave number (cm-1)	Connection	Function
3400	O-H	Linalool (an alcohol)
2850-3000	C-H	Methylene et Methyl
1735	C=O	Aldehydes
1600-1500	C=C	Terpene compounds
1400-1300	C-H	Methylene et Methyl
1000-1250	C=O	Esters, Alcohols et ethers
650-900	C-H	Limonene

1.2. *Cupressus sempervirens* L.

The infrared spectrum representing the values of transmittance (T%) as a function of wave number (cm-1) of the essential oil of *Cupressus sempervirens* L. is shown in the following figure **(Figure 18).**

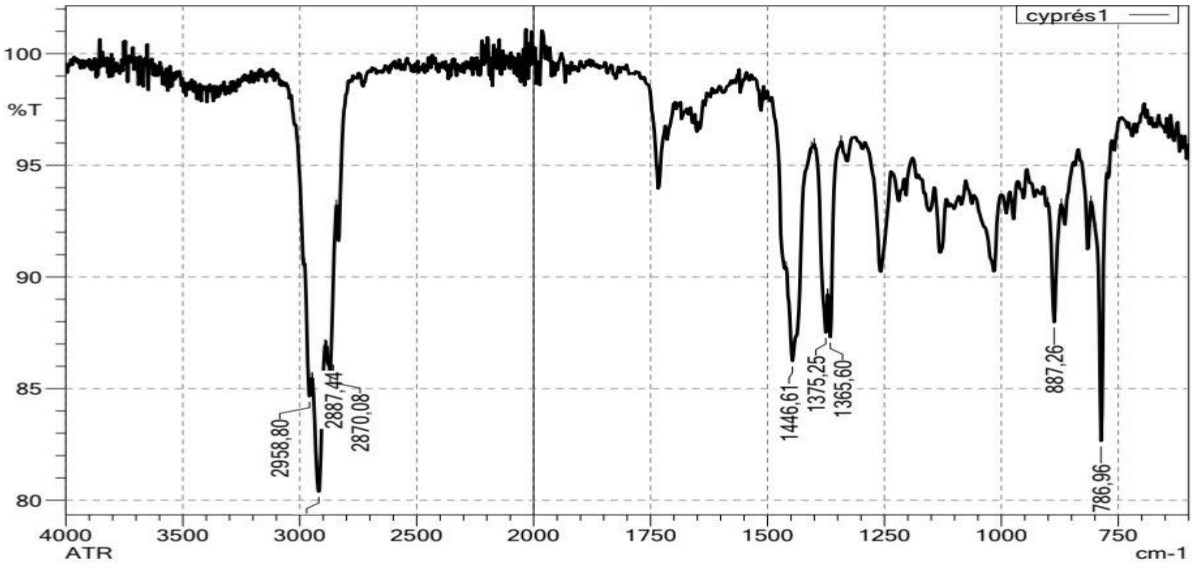


Figure 18 : Infrared analysis spectrum of essential oil of *Cupressus sempervirens* L.

The ATR infrared analysis of the essential oil of *Cupressus sempervirens* L. provided a spectrum with several spectral bands. These were found at wavelengths ranging from 786.96 to 2958.80 cm-1.

The analysis of the infrared spectrum of essential oil of *Cupressus sempervirens* L. reveals the presence of various functional groups in the biomolecular compounds of this essential oil **(Table 6).**

Table 6 : corresponding chemical groups of the biomolecules in *Cupressus sempervirens* L.

Nombre d'onde (cm-1)	Liaison	Fonction
3000-3100	C-H	Alkenes
2800-3000	C-H	Alkanes
1750-1700	C=O	Carbonyls
1640-1680	C=C	Terpene
1000-1200	C-O	Esters, ethers et alcohol

a. Comparison with reference spectra

By using databases of infrared spectra of essential oils, we can compare the observed bands on the provided spectrum with those typical of components of cypress essential oil (alpha-pinene, delta-3-carene, alpha-cedrene, etc.).

b. Interpretation of the present functional groups

- **Hydrocarbons** : The presence of bands between 3100-3000 cm^{-1} and 3000-2800 cm^{-1} indicates hydrocarbon structures (saturated and unsaturated).
- **Carbonyl compounds** : If bands are observed around 1750-1700 cm^{-1}, this would indicate the presence of carbonyls, although this is less common for cypress essential oil.
- **Aromatic rings and multiple bonds** : Peaks in the region of 1600-1500 cm^{-1} and 1680-1640 cm^{-1} may suggest the presence of aromatic rings or double bonds.

c. Specific analysis of the provided spectrum

• **Band between 3100-3000 cm^{-1}:** Indicates the presence of unsaturated hydrocarbons such as alkenes.

• **Band between 3000-2800 cm^{-1}:** Indicates saturated hydrocarbons (alkanes).

• **Notable absence of band around 1750-1700 cm^{-1}:** Absence of significant carbonyls.

• **Band between 1680-1640 cm^{-1}:** Confirmation of C=C double bonds, possible presence of terpenes.

• **Band around 1600-1500 cm⁻¹:** May indicate aromatic rings.

According to **Boukhris *and al*. (2012),** the main components of the different aerial parts of Cypress were α-pinene (37.14%), δ-3-carene (19.67%), limonene (5.43%), and α-terpinolene (4.69%). Monoterpene hydrocarbons were found to be rich in this oil. The dominant compounds in cypress oil were monoterpene hydrocarbons (84.38%), followed by oxygenated monoterpenes (3.81%), sesquiterpene hydrocarbons (3.07%), and oxygenated sesquiterpenes (1.69%).

2. results of the chromatographic characterization by GC-MS

To determine the chemical composition of the essential oil of the plant *Lavandula stoechas* L. a gas chromatograph (GC) was used. The results are mentioned in the following figure **(Figure 19).**

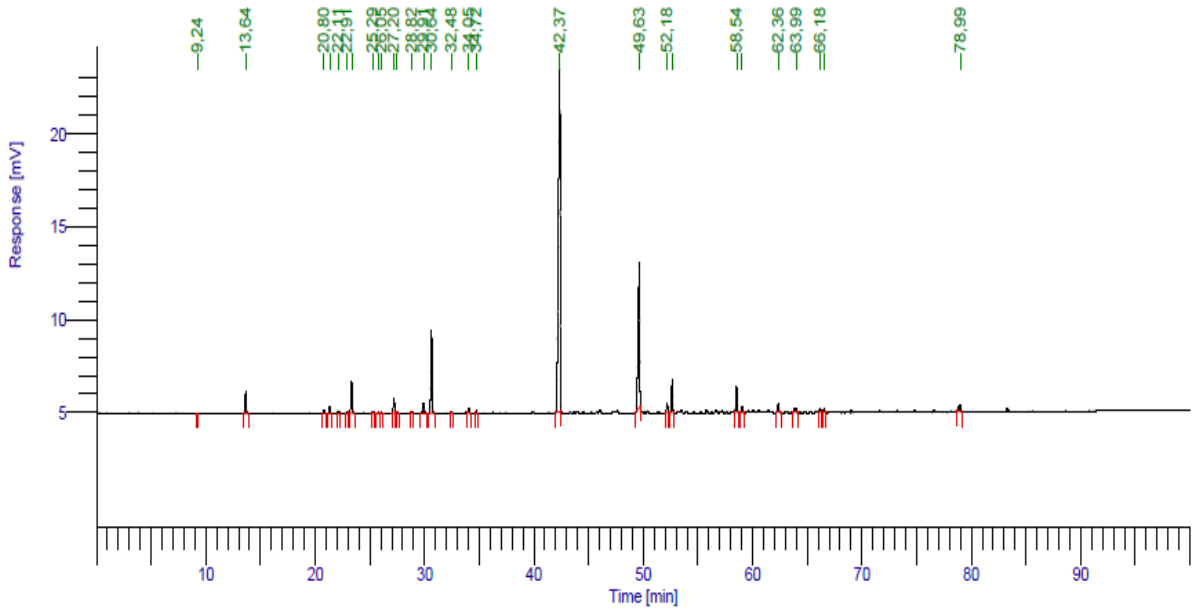


Figure 19 : GC chromatogram of *Lavandula stoechas* L. essential oil.

The chromatogram shows that the *Lavandula stoechas* L. essential oil is composed of several compounds. The analysis revealed a total of 29 distinct compounds, representing 100% of the total oil composition. Retention times range from 9.23 min to 78.995 min.

A characteristic peak appeared at 42.37 minutes, representing the major compound in this sample. This compound is likely attributable to camphor (terpene) or 1,8-cineole (eucalyptol), which are the most known and commonly identified.

According to **Kirmizibekmez *and al.* (2009)**, the different chemical compositions of the essential oils obtained from the leaves and flowers of *L. stoechas* are as follows:

• Leaves: alpha-fenchone (41.9%), 1,8-cineole (15.6%), camphor (12.1%), and viridiflorol (4.1%).

• Flowers: alpha-fenchone (39.2%), myrtenyl acetate (9.5%), alpha-pinene (6.1%), camphor (5.9%), and 1,8 cineole (3.8%).

3. insecticide test results

The initial results of the insecticide test conducted with different concentrations of *L. stoechas* oil (1%, 2%, 3%, 5%, 7%) and *C. sempervirens* oil (0.5%, 1%, 2%, 3%, 5%, 7%) are noted in **Annex 2**.

3.1. The effect of *L. stoechas* essential oil on *Culex pipiens* L4

The figure below shows a curve representing the evolution of mortality of 4th stage larvae of *Culex pipiens* over time **(Figure 20).**

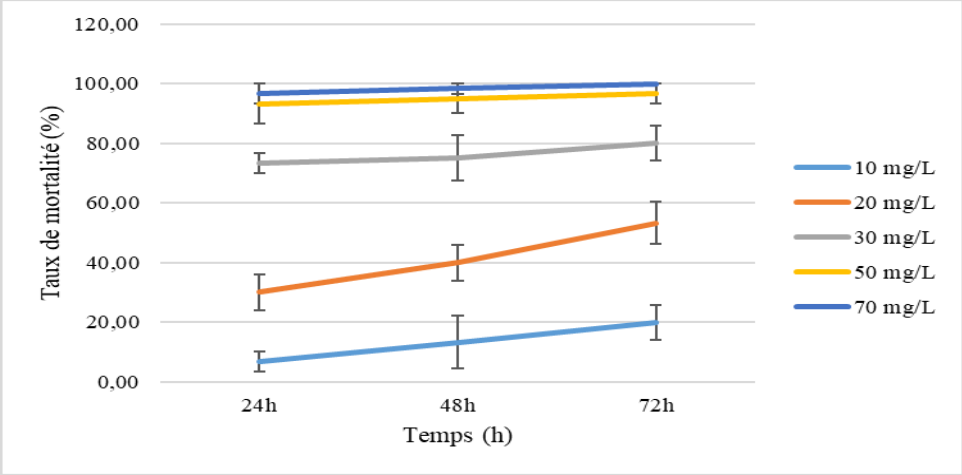


Figure 20 : Mortality kinetics of 4th stage larvae of *Culex pipiens* over time at different doses of *L. stoechas* essential oil.

The treatment results show significant larvicidal activity of *Lavandula stoechas* L. essential oil against 4th stage larvae of *Culex pipiens*, with sensitivity observed in the entire population over the treatment duration (24h, 48h, 72h) at doses of 1% (10 mg/L), 2% (20 mg/L), 3% (30 mg/L), 5% (50 mg/L), and 7% (70 mg/L). However, no mortality was noted in the control groups. These observations highlight the promising larvicidal potential of *Lavandula stoechas* L. essential oil against *Culex pipiens* larvae.

3.2. The effect of *Cupressus sempervirens* L. essential oil on *Culex pipiens* L4

The percentage of mortality of 4th stage larvae of *Culex pipiens* over time at different doses of *Cupressus sempervirens* L. essential oil is depicted in the following figure **(Figure 21).**

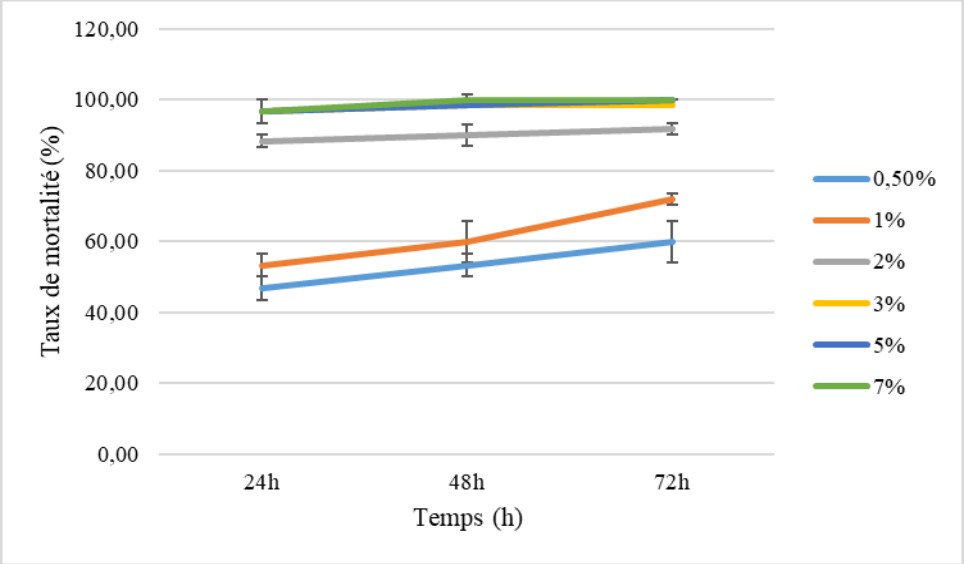


Figure 21 : Mortality kinetics of 4th stage larvae of *Culex pipiens* over time at different doses of *Cupressus sempervirens* L. essential oil

According to figure 21, the results of the insecticide tests conducted with *C. sempervirens* essential oil indicate a direct relationship between the mortality percentages of L4 larvae and the EO concentration. Indeed, the higher the concentration, the higher the mortality rate. The maximum mortality (100%) is obtained at doses of 5% and 7% within 48 hours.

3.3. Comparative study of mortality rates of *L. stoechas* and *Cupressus sempervirens* on L4 larvae of *Culex pipiens*

A comparative study was conducted to evaluate the efficacy of *Lavandula stoechas* and *Cupressus sempervirens* essential oils on L4 larvae of *Culex pipiens*.

The aim of this study was to assess which of the two essential oils was the most effective. Indeed, a plotting of the mortality rate curve of the two essential oils over time at the highest concentration of 7% **(Figure 22).**

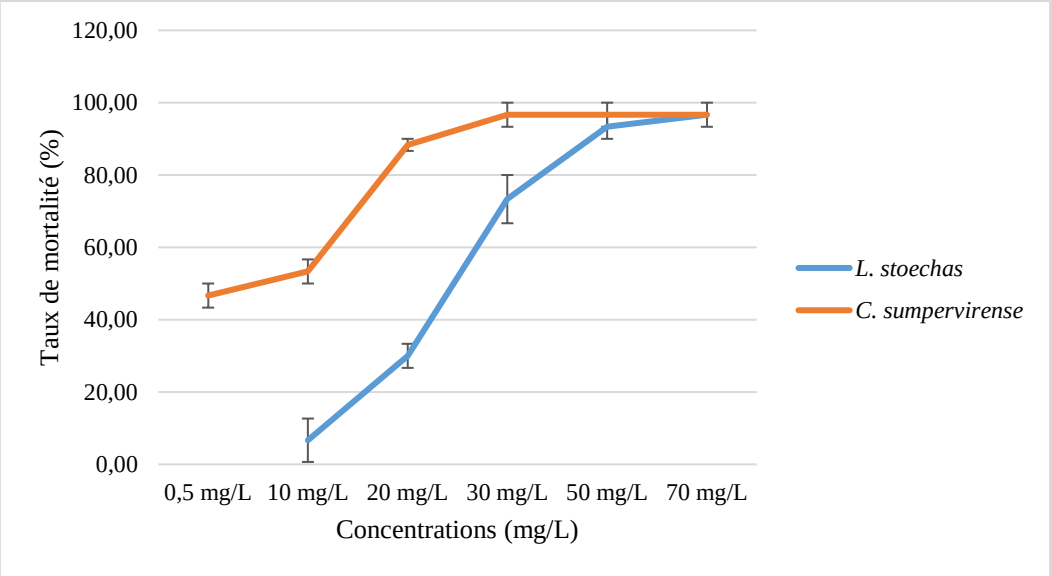


Figure 22 : Curve of the mortality rate (±SEM) of L4 larvae of *Culex pipiens* after 24 hours of treatment with *L. stoechas* and *C. sempervirens* essential oils at different concentrations.

From **figure 22**, the results of the observation suggest a significant evolution of the mortality rate according to the concentration used, highlighting a dose-response relationship. Additionally, it is also noted that *Cupressus sempervirens* L. essential oil exhibits superior efficacy compared to *Lavandula stoechas* L. on L4 larvae of *Culex pipiens*, with higher mortality rates at all tested concentrations.

4. Estimation of LC50 and LT50

Estimating the LC50 involves plotting a regression line of probits of corrected mortality rates against the logarithms of the applied essential oil concentrations. The LT50 was deduced from the regression lines of probits against the logarithm of time.

4.1. For *Lavandula stoechas* L.

4.1.1. LC50 (Lethal Concentration 50) : This is the concentration of a substance estimated to cause the death of 50% of a given population **(Figure 23).**

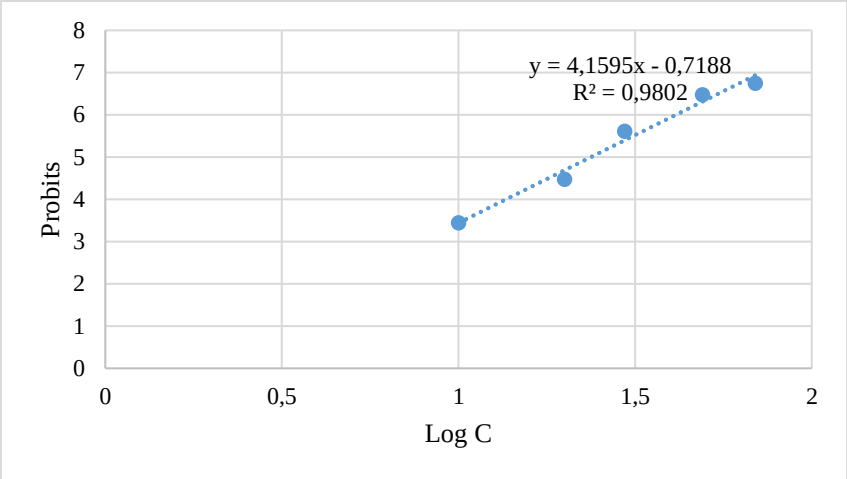


Figure 23 : Linear probit regression of *Culex pipiens* mortality as a function of concentrations of *Lavandula stoechas* L. essential oil after 24 hours of exposure.

4.1.2. LT50 (Lethal Time 50) : This is the time required to cause the death of 50% of a given population at a certain concentration. The LT50 is reported to be 24 hours for a concentration of 20 mg/L **(Figure 24).**

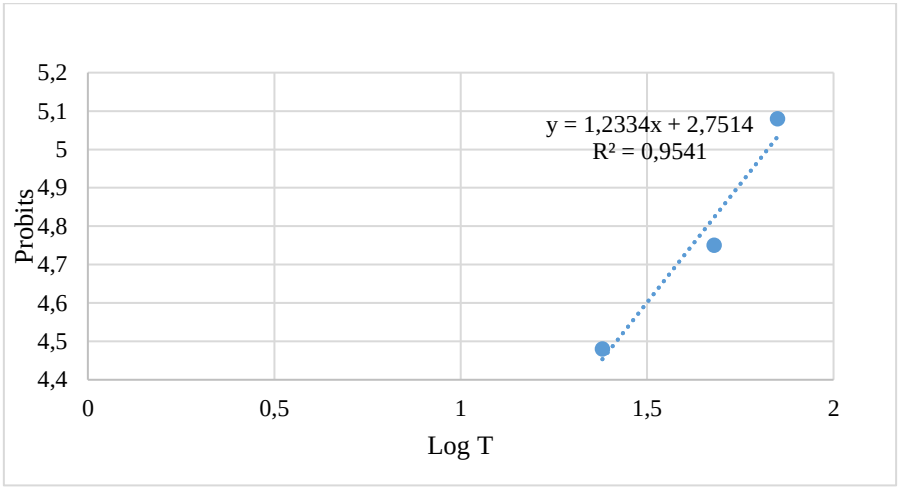


Figure 24 : Linear probit regression of *C. pipiens* mortality as a function of the logarithm of time

From **figure 23** and **figure 24**, the LC50 after 24 hours is 23.44 mg/L, meaning this concentration kills half of the exposed population. On the other hand, the LT50 is 66.06 hours, indicating the time required for half of the population to be killed by this substance. These values depend on the tested substance and the studied population.

4.2. for *Cupressus sempervirens* L.

4.2.1. LC50 (Lethal Concentration 50)

The figure below shows the relatively significant effect of doses of *Cupressus sempervirens* L. essential oil on *Culex pipiens* larvae mortality after 24 hours of treatment. The probit analysis conducted indicates that *Cupressus sempervirens* L. essential oil is effective on the *C. pipiens* population, with an LC50 of 5.57 mg/L **(Figure 25).**

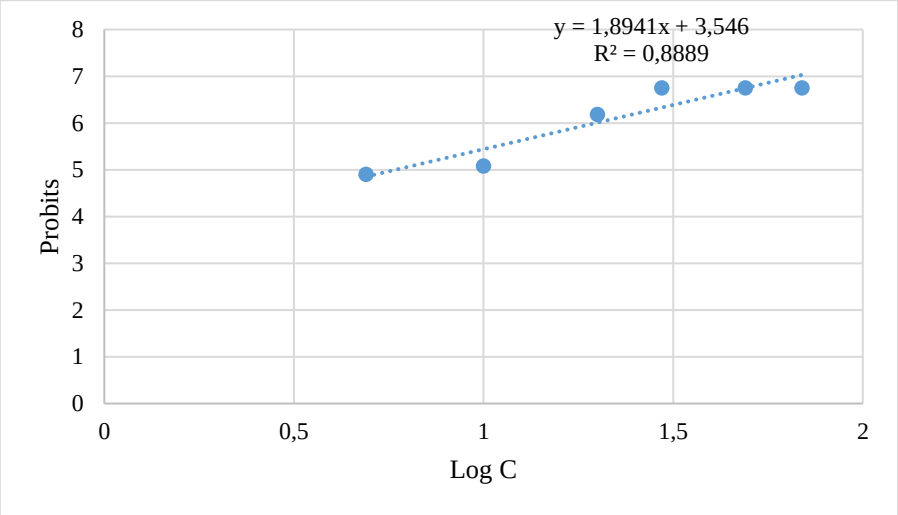


Figure 25 : Linear probit regression of *C. pipiens* mortality as a function of concentrations of *Cupressus sempervirens* L. essential oil after 24 hours of exposure.

4.2.2. LT50 (Lethal Time 50)

This is the time required to cause the death of 50% of a given population at a certain concentration. LT50 is reported to be 24 hours for a concentration of 10 mg/L **(Figure 26).**

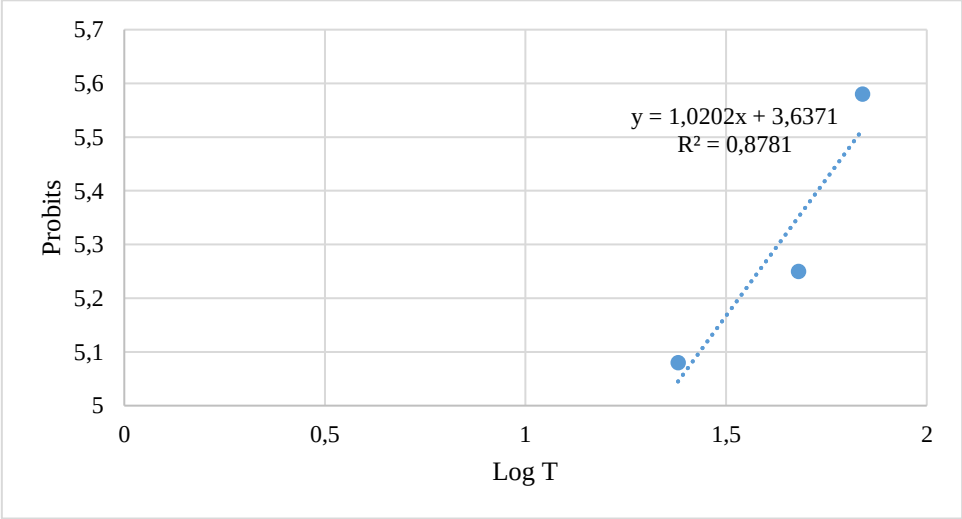


Figure 26 : Linear probit regression of *C. pipiens* mortality as a function of the logarithm of time

Based on Figure 25 and Figure 26, the LC50 after 24 hours is 5%, meaning this concentration kills half of the exposed population. On the other hand, the LT50 is 1.33 hours, indicating the time required to kill half of the population with this substance. Through these values, it is observed that *Cupressus sempervirens* L. essential oil is very effective in eliminating *Culex pipiens* larvae in a short period of time and at a low concentration value.

We can also deduce that the essential oil of the plant *Cupressus sempervirens* L. has more significant insecticidal properties compared to the essential oil of *Lavandula stoechas* L. with respective values of LC50 (5.57 mg/L, 23.44 mg/L) and LT50 (1.33 h, 66.06 h).

According to **Sayah *and al*. (2014)**, the larvicidal activity of essential oils could be of great interest in the field of vector control. This is due to the issues arising from the use of chemical insecticides (environmental pollution, resistance, risks to human health).

Similar results were obtained by **Traboulsi *and al*. (2002)** while working on the insecticidal activity of the essential oil from leaves and flowers of the aromatic plant *Lavandula stoechas* L. against 4th stage larvae of *Culex pipiens*, with an LC50 of 36%.

According to the results of **Ramzi *and al*. (2022)**, they revealed that the essential oils of *Lavandula stoechas* and *Lavandula officinalis* showed insecticidal activity against adult females of *Culex pipiens*. *Lavandula stoechas* L. was the most effective with 68.42 ± 1.54% mortality at 0.03125% after 48 hours of treatment, and the LD50 value was determined to be 83.99 µL/L of air (R2 = 0.93; P value = 0.008).

5. Results of manufacturing a repellent

We have also formulated a mosquito repellent with anti-mosquito effects; the repellent we obtained is shown in the figure above **(Figure 27).**

Figure 27 : the mosquito repellent with anti-mosquito effects

Conclusion

The fight against mosquitoes has become imperative due to its significant impact on human health and the environment, hence the need to find more effective and natural methods to combat mosquitoes.

This present study aims to perform an ATR infrared spectral analysis of *Lavandula stoechas* L. and *Cupressus sempervirens* essential oils, a chromatographic characterization using gas chromatography (GC) of *Lavandula stoechas* L. essential oil, and to evaluate larvicidal activities against the most abundant mosquito species, 4th stage *Culex pipiens*.

The ATR infrared analysis of *Lavandula stoechas* L. essential oil provided a spectrum with several spectral bands indicating the presence of different functional groups in the biological compounds it contains, namely aromatic anhydrides (C=O), aldehydes (C-H), carboxylic acids (O-H), and carboxylic acids (O-H). As for *Cupressus sempervirens* essential oil, the detected functional groups correspond to aldehyde function (C-H), alkenes (C-H), and carboxylic acids (O-H).

The gas chromatograph (GC) shows that *Lavandula stoechas* L. essential oil is composed of several compounds. The analysis revealed a total of 29 distinct compounds, representing 100% of the total oil composition, with a major compound appearing at 42.37 minutes.

The essential oils extracted from *Lavandula stoechas* L. and *Cupressus sempervirens* L. were tested at different concentrations on 4th stage larvae of *Culex pipiens* for 24 hours, 48 hours, and 72 hours.

The results obtained showed that both essential oils have a very significant insecticidal effect, leading to the complete mortality of the *Culex* population after 48 hours for doses of 50 mg/L and 70 mg/L of *Cupressus sempervirens* essential oil, and at 70mg/L for *Lavandula stoechas* L. essential oil.

We can also deduce that the essential oil from the *Cupressus sempervirens* L. plant has more significant insecticidal properties compared to *Lavandula* essential oil, with respective values of LC50 (5.57 mg/L, 23.44 mg/L) and LT50 (1.33h, 60.06h).

The present study shows that both essential oils exhibit insecticidal properties and could serve as preliminary data for the development of new natural products that could be used as mosquito repellents (bio-insecticide) in integrated mosquito control programs and to manage resistance to chemical insecticides. In perspective, the following are proposed:

- Develop a formulation or an insecticide based on lavender and cypress essential oils.

- Isolate, identify, and purify the active compounds from the extracts using chromatographic and spectral techniques such as GC-MS and NMR.

This work has been the subject of a scientific publication submitted for peer review in class A scientific journal.

References

A

- **Al-Snafi, AE (2016).** Importance médicale de la revue Cupressus sempervirens-A. Journal de pharmacie IOSR, 6 (6), 66-76.

B

- **Bachiri, L., Echchegadda, G., Ibijbijen, J. et Nassiri, L. (2016).** Etude phytochimique et activité antibactérienne de deux espèces de Lavande Autochtones Au Maroc :« Lavandula stoechas L. et Lavandula dentata L. ». Revue scientifique européenne, 12 (30), 313-333.
- **Benaiche H, (2022).** Etude ethnobotanique, phytochimique et activités biologique de certaines espèces végétales poussant dans le littoral ouest algérien et gestion des risques liés à leur utilisation. Thèse de doctorat en toxicologie, université des sciences et de la technologie d'Oran Mohamed-Boudiaf, pp 23.
- **Belkhiri N, (2022).** Etude des Culicidae des Aurès : inventaire et lutte. Thèse de doctorat en biodiversité, production et protection des végétaux, université Hadj Lakhdar Batna, pp 12-13.
- **Bechir, A., El Mousadik, A., & Pichot, C. (2004).** Diversité allozymique de peuplements de cyprès naturels et introduits au Maroc : conséquences pour la gestion des ressources. Annals of forest science, 61(7), 669-676.
- **Bencheikh S, (2017).** Etude de l'activité des huiles essentielles de la plante *Teucrium polium ssp Aurasianum Labiatae*. Thèse de doctorat en génie des procédés et environnement, université Kasdi Merbah- Ouargla, pp 8-9.
- **Boukhris, M., Regane, G., Yangui, T., Sayadi, S. et Bouaziz, M. (2012).** Composition chimique et potentiel biologique de l'huile essentielle de Cupressus sempervirens L. Tunisien. Journal of arid land Studies, 22 (1), 329-332.
- **Boukhatem, M. N., Ferhat, A., & Kameli, A. (2019).** Méthodes d'extraction et de distillation des huiles essentielles : revue de littérature. Une, 3(4), 1653-1659.

- **Brunhes, J. (1999).** Culicidae du Maghreb. Description d'Aedes (Ochlerotatus) biskraensis n. sp. D'Algérie (Diptera, Nematocera). *Bulletin de la Société entomologique de France*, *104*(1), 25-30.

C

- **Chahed S, (2022).** Biodiversité des moustiques (Diptera : Culicidae) de la région de Tizi-Ouzou (Nord d'Algérie). Thèse de doctorat en biologie de la conservation, université Mouloud Mammeri Tizi-Ouzou, pp 15.
- **Chekoual L, (2019).** Etude du pouvoir antimicrobien et antioxydant des huiles essentielles de *lavandula stoechas* L. du Nord de l'Algérie extraites par Hydrodistillation et Ultrasons. Thèse de doctorat en biotechnologie des plantes aromatiques, médicinales et des produits naturels, université Saad Dahlab – Blida, pp 20.

D

- **Dhifi, W., Bellili, S., Jazi, S., Bahloul, N. et Mnif, W. (2016).** Caractérisation chimique des huiles essentielles et étude de certaines activités biologiques : une revue critique. Médicaments, 3 (4), 25.

E

- **Ez Zoubi, Y., Bousta, D. et Farah, A. (2020).** Une revue phytopharmacologique d'une plante méditerranéenne : Lavandula stoechas L. Clinical Phytoscience, 6, 1-9.

F

- **Farhat A, (2010).** Vapo-Diffusion assistée par Micro-ondes : conception, optimisation et application. Thèse de doctorat en sciences des procédés, sciences des Aliments,

université d'Avignon et des Pays de Vaucluse & L'Ecole Nationale d'Ingénieurs de Gabès, pp 24.

- **Frezza, C., De Vita, D., Sciubba, F., Toniolo, C., Tomassini, L., Nicoletti, M., ... et Foddai, S. (2022)**. Il n'y a pas que Cupressus sempervirens L. : une revue de la phytochimie et des bioactivités des autres espèces de Cupressus L. Sciences appliquées, 12 (14), 7353.

H

- **Himmi O., Dakki M., Trari B. & Elagbani M.A. 1995.** Les Culicidae du Maroc. Clés d'identification avec données biologiques et écologiques. Travaux de l'Institut Scientifique série Zoologie, 44-50.
- **Humbert, L., & Lhermitte, M. (2005).** Molécules indétectables par chromatographie en phase gazeuse. In Annales de Toxicologie Analytique (Vol. 17, No. 1, pp. 57-62). EDP Sciences.

K

- **Keffous B, (2023).** Lutte biologique contre le moustique Aedes capius (Pallas, 1771) : Aspect toxicologique, biochimique et histologique. Thèse de doctorat en parasitologie, université Ferhat Abbas Sétif 1, pp2.
- **Kharoubi R, (2021).** Méthodes alternatives de lutte contre les moustiques : Efficacité des extraits d'une plante médicinale la menthe *(Mentha sp)* et effets secondaires sur *Gambusia affinis.* Thèse de doctorat en physiologie et environnement, université Badji Mokhtar Annaba, pp 15.
- **Kırmızıbekmez, H., Demirci, B., Yeşilada, E., Başer, KHC et Demirci, F. (2009).** Composition chimique et activité antimicrobienne des huiles essentielles de Lavandula stoechas L. ssp. Stoechas poussant à l'état sauvage en Turquie. Communications sur les produits naturels, 4 (7), 1934578X0900400727.

L

- **Lakhdari A, (2023).** Optimisation de rendement et étude des activités des principes actifs de quelques plantes médicinales de la wilaya de Ghardaïa (sud Algérie). Thèse de doctorat en Génie Chimique, université Kasdi Merbah Ouargla, pp 31.
- **Larabi F, (2017).** Composition phytochimique des organes aériens de Tetraclinis articulata (Vahl). Master. Et pouvoir insecticide. Thèse de doctorat en biodiversité végétale méditerranéenne : inventaire, valorisation et restauration, université Djillali Liabes de Sidi Bel Abbes, pp 30.

M

- **Menaceur F, (2015).** Contribution à l'étude phytochimique et biologique de l'érigeron, du fenouil commun, de la lavande et du genévrier. Thèse de doctorat en sciences agronomiques, école nationale supérieure agronomique EL-HARRACH-ALGER, pp 5.
- **Merabti B, (2016).** Identification, composition et structure des populations Culicidénne de la région de Biskra (Sud-est Algérien). Effets des facteurs écologiques sur l'abondance saisonnière. Essais de lutte. Thèse de doctorat en écologie animale, université Kasdi Merbah – Ouargla, pp 38.

N

- **Nichane M, (2015).** Contribution à l'étude du dépérissement de Cyprès vert *(Cupressus sempervirense L.)* dans les monts des Traras Occidentaux (Wilaya de Tlemcen). Thèse de doctorat en écologie animale, université de Tlemcen, pp 28.

O

- **Oussad N, (2022).** Biodiversité des moustiques (Diptera, Culicidae) dans la région de Tizi-Ouzou, et essais de lutte. Thèse de doctorat en entomologie appliquée à la Médecine, Agriculture et Foresterie, université de Mouloud Mammeri Tizi-Ouzou, pp 1.

P

- **Pavela, R. et Sedlák, P. (2018).** Post-application temperature as à factor influencing the insecticidal activity of essential oil from Thymus vulgaris. Industrial Crops and Products 113 : 46-49.
- **Poncet, P., Charpin, D., & Sénéchal, H. (2022).** Republication de : Les Cupressacées des cinq continents. Revue Française d'Allergologie, 62(3), 249-252.

R

- **Ramzi, A., Farah, A., Ez Zoubi, Y., Annemer, S. et El Ouali Lalami, A. (2022).** Profil aromatique et toxicité fumigante des huiles essentielles de deux espèces de Lavandula marocaines contre Culex pipiens (Diptera : Culicidae). Journal international de la science des insectes tropicaux, 42 (3), 2663-2672.

S

- **Sayah, M. Y., Lalami, A. E. O., Greech, H., Errachidi, F., El Kandri, Y. R., & Chahdi, F. O. (2014).** Activité Larvicide des Extraits de Plantes Aromatiques sur les Larves de Moustiques Vecteurs de Maladies Parasitaires [Larvicidal Activity of Aromatic Plant Extracts on Larvae of Mosquitoes Vectors of Parasitic Diseases]. International Journal of Innovation and Applied Studies, 7(3), 832.

T

- **Tabti N, (2017).** Etude comparée de l'effet de *Bacillus thuringiensis* sur les populations purifiées et des populations des gites artificiels de *Culex pipiens* (Diptera – Culicidae) dans la ville de Tlemcen. Thèse de doctorat en écologie animale, université de Tlemcen, pp 6.
- **Touhami A, (2017).** Etude chimique et microbiologique des composants des huiles essentielles de différentes genres *Thymus* récoltées dans les régions de l'Est Algérien

pendant les deux périodes de développement. Thèse de doctorat en chimie organique, université Badji Mokhtar Annaba, pp 23-25.

- **Toure D, (2015).** Etude chimique et biologique des huiles essentielles de quatre plantes aromatiques médicinales de COTE D'IVOIRE. Thèse de doctorat en chimie biologie, université Félix HOUPHOUET-BOIGNY, pp 7

W

- **WHO (World Health Organization) (1996).** Report of the WHO Informal Consultation on the Evaluation and Testing of Insecticides. CTD/ WHOPES/IC/96.1.

Z

- **Zeghib F, (2022).** Efficacité comparée des extraits de quelques plantes médicinales sur une espèce de moustique *Culex pipiens* : Aspect toxicologique, biochimique et biomarqueurs. Thèse de doctorat en biologie animale, université de Badji Mokhtar Annaba, pp 5.

ملخص

بسبب المشاكل المرتبطة باستخدام المبيدات الحشرية الكيميائية وتأثيرها الضار على الصحة والبيئة، من الضروري استخدام البدائل الطبيعية التي تؤدي الدور نفسه للمبيدات الحشرية الاصطناعية وتقدم مزايا بيئية واقتصادية.

تهدف هذه الدراسة إلى إجراء تحليل طيفي بالأشعة تحت الحمراء ATR للزيوت العطرية .L *stoechas* و*C. sempervirens*، والتوصيف الكروماتوغرافي بواسطة كروماتوغرافيا الغاز (CPG) للزيت العطري .L *Lavandula stoechas* وتقييم النشاطات اليرقية ضد أنواع البعوض *Culex pipiens*في الطور الرابع .يشير التحليل بالأشعة تحت الحمراء (IR-ATR) ATR للزيت العطري .L *stoechas* إلى وجود مجموعات وظيفية مختلفة وهي أنيدريدات عطرية(C=O) ، ألدهيدات(C-H) ، أحماض كربوكسيلية(O-H) ، أحماض كربوكسيلية .(O-H) أما بالنسبة للزيت العطري لـ*Cupressus sempervirens*، فقد تم الكشف عن مجموعات وظيفية تتوافق مع الألدهيدات(C-H) ، الألكينات (C-H) والأحماض الكربوكسيلية-O) .(Hوكشفت الكروماتوغرافيا الغازية (CPG) عن وجود 29 مركبًا مميزًا في الزيت العطري.L *stoechas* ، مع ظهور مركب رئيسي عند 42.37 دقيقة.

بعد القيام بالاختبار باستخدام تركيزات مختلفة بدلالة الوقت، اكدت النتائج ان الزيوت الأساسية للخزامة والسرو لها تأثير فعال للغاية ضد يرقات الطور الرابع. تبلغ قيمة LC50 لزيت الخزامة الأساسي بعد 24 ساعة هي 23.44. ملغم/لتر وقيمة LT50 هي 66.06 ساعة للزيت العطري، أما بالنسبة للزيت الأساسي للسرو فإن قيمة LC50 بعد 24 ساعة هي 5.57 ملغم/لتر وقيمة LT50 هي 1.33 ساعة

الكلمات المفتاحية: الزيت الأساسي للخزامة، الزيت الأساسي للسرو، يرقات البعوض، تأثير مبيد الحشرات.

Résumé

En raison des problèmes liés à l'utilisation des pesticides chimiques et de leurs effets nocifs sur la santé et l'environnement, il est nécessaire d'avoir recours à des alternatives naturelles qui remplissent le même rôle que les pesticides de synthèse et offrent des avantages environnementaux et économiques.

Cette étude a pour but d'effectuer une analyse spectrale par infrarouge ATR des HEs *L. stoechas* L. et *C. sempervirens*, une caractérisation chromatographique par chromatographie en phase gazeuse (CPG) de l'HE *Lavandula stoechas* L. et d'évaluer les activités larvicides à l'égard de espèces de moustique *Culex pipiens* de 4éme stade.

L'analyse par infrarouge ATR (IR- ATR) de l'HE *L. stoechas* L. indique la présence de différents groupes fonctionnels à savoir la fonction anhydrides aromatiques (C=O), aldéhydes (C-H), acides carboxyliques (O-H), acides carboxyliques (O-H). Concernant l'HE de *Cupressus sempervirens* les groupements fonctionnels détectés correspond à la fonction aldéhydes (C-H), alcènes (C-H) et acides carboxyliques (O-H). Le chromatographe en phase gazeuse (CPG) a révélé la présence de 29 composés distincts dans l'HE de *L. stoechas* L., avec l'apparition d'un composé majoritaire à 42,37 min.

Après avoir testé différentes concentrations en fonction du temps, les résultats ont confirmé que les huiles essentielles de lavande et de cyprès ont un effet très efficace contre les larves du quatrième stade de *Culex pipiens*. La valeur CL50 de l'huile essentielle de *lavandula stoechas* L. après 24 heures est de 23,44 mg/L et la valeur TL50 est de 66,06 heures, et pour l'HE de *Cupressus sempervirens* L, la valeur CL50 après 24 heures est de 5,57 mg/l et la valeur TL50 est de 1,33 heures.

Mots clés : *Lavandula stoechas* L. ; *Cupressus sempervirens* L. ; *Culex pipiens* ; huile essentielle ; effet insecticide.

Summary

Due to the problems associated with the use of chemical pesticides and their harmful effects on health and the environment, it is necessary to resort to natural alternatives that fulfill the same role as synthetic pesticides and offer advantages environmental and economic.

This study aims to perform an ATR infrared spectral analysis of the essential oils (EOs) of *L. stoechas* L. and C. sempervirens, a chromatographic characterization by gas chromatography (GC) of the EO *Lavandula stoechas* L., and to evaluate the larvicidal activities against the fourth-stage larvae of the mosquito species *Culex pipiens.*

The ATR infrared analysis (IR-ATR) of the EO *L. stoechas* L. indicates the presence of various functional groups, namely aromatic anhydrides (C=O), aldehydes (C-H), and carboxylic acids (O-H). Regarding the EO of *Cupressus sempervirens*, the detected functional groups correspond to aldehydes (C-H), alkenes (C-H), and carboxylic acids (O-H).

Gas chromatography (GC) revealed the presence of 29 distinct compounds in the EO of L. stoechas L., with the appearance of a major compound at 42.37 minutes.After testing different concentrations over time, the results confirmed that lavender and cypress essential oils have a very effective effect against fourth instar larvae of *Culex pipiens*, since the CL50 value of *lavandula stoechas* L. essential oil after 24 hours is 23.44. mg/l and the TL50 value is 66.06 hours for essential oil, for *Cupressus sempervirens* L, the CL50 value after 24 hours is 5.57 mg/l and the TL50 value is 1.33 hours.

Key words: *Lavandula stoechas* L.; *Cupressus sempervirens* L.; *Culex pipiens*; essential oil; insecticidal effect.