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Theme :

Effect evaluation of *Juniperus phoenicea* essential oil on a certain biomarkers in mosquito larva

Presented by :

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Appearing in front of the committee

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Dedication

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لم تكن الرحلة قصيرة والطريق لم يكن محفوفا بالسهولة، لكنني فعلت ذلك. الحمد لله الذي يسر البدايات وبلغنا النهاية بفضله وكرمه.

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ونام

Dedication

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وصال

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ملخص

في إطار البحث عن طرق فعالة للمكافحة البيولوجية ضد البعوض، تم اختبار زيت أساسي مستخرج من نبات العرعر الفينيقي المزروع في ولاية تبسة (شمال شرق الجزائر) بتركيزات قاتلة مختلفة CL25 وCL50 على يرقات العمر الرابع لحشرة *C. longiareolata (L 4)* التي تم جمعها من المناطق غير المعالجة بولاية تبسة، تحت ظروف مخبرية حسب توصيات منظمة الصحة العالمية.

تم فحص التأثيرات على اثنين من المؤشرات الحيوية الأنزيمية (Glutathione-s-transferase، وcatalase) في أوقات مختلفة، 24 ساعة، 48 ساعة، و72 ساعة.

أظهر تحديد محصول استخلاص الزيت العطري عن طريق التقطير المائي للأوراق المجففة عائدًا قدره 0.56٪.

تكشف نتائج العلامات الحيوية عن تحفيز نظام إزالة السموم من زيت *Juniperus phoenicea* الأساسي.

من خلال زيادة نشاط كلا من GST و Catalase مقارنةً بالضوابط، بالإضافة إلى انخفاض كبير في مستويات البروتين الكلي بتركيزات (LC25) و(LC50) في فترات المعالجة المختلفة.

أثر الزيت العطري للعرعار الفينيقي (تركيزات LC25، LC50) على نمو وزن أنواع البعوض مع انخفاض كبير في وزن جسم يرقات *C. longiareolata* مقارنةً بالضوابط في فترات المعالجة المختلفة.

يعد البحث في المنتجات الطبيعية المستخرجة من النباتات القادرة على التحكم في أعداد *Culiseta longiareolata* أمرًا مهمًا للغاية لأنه يمكن أن يساعد في الحد من انتشار المرض من هذا الناقل. وفقًا للبيانات المستمدة من هذه الدراسات، يمكن استخدام زيت *Juniperus phoenicea* الأساسي كمبيد حشري حيوي وكبديل للمنتجات الاصطناعية في مكافحة البعوض.

الكلمات المفتاحية : البعوض، *C. longiareolata* ، زيت عطري، *Juniperus phoenicea* ، المؤشرات الحيوية

Résumé

Dans le cadre de la recherche de méthodes efficaces de lutte biologique contre les moustiques, une huile essentielle extraite de la plante de *Juniperus phoenicea* cultivée dans la wilaya de Tebessa (nord-est de l'Algérie) a été testée à différentes concentrations létales CL25 et CL50 sur des larves de quatrième stade de *Culesita longiareolata* (L 4) collectée dans les zones non traitées de Tébessa, dans des conditions de laboratoire selon les recommandations de l'Organisation Mondiale de la Santé.

Les effets ont été examinés sur deux biomarqueurs enzymatiques (Glutathion-s-transférase et catalase), le taux de protéines et sur la croissance pondérale des *Culiseta longiareolata* à différents périodes de traitement 24h, 48h et 72h.

La détermination du rendement d'extraction des huiles essentielles par hydrodistillation des feuilles séchées a montré un rendement de 0,56%.

Les résultats des biomarqueurs révèlent une induction du système de détoxification de l'huile essentielle de *Juniperus phoenicea* via une augmentation de l'activité GST et catalase, ainsi que le taux de protéine total est diminué significativement avec les deux concentration CL25 et CL50 a différents périodes de traitement.

L'huile essentielle de *Juniperus phoenicea* (CL25, CL50) a affecté la croissance pondérale des espèces de moustiques avec une réduction significative du poids corporel des larves de *C. longiareolata* par rapport aux témoins à différentes périodes de traitement.

La recherche sur les produits naturels extraits de plantes capables de contrôler les populations de *Culiseta longiareolata* est très importante car elle peut contribuer à réduire la propagation de la maladie causée par ce vecteur. Selon les données de ces études, l'huile essentielle de *Juniperus phoenicea* peut être utilisée comme bio-insecticide et comme alternative aux produits de synthèse dans la lutte contre les moustiques.

Mots clés : Moustique, *Culiseta longiareolata*, Huile essentielle, *Juniperus phoenicea*, Biomarqueurs

Abstract

As part of the search for effective biological control methods against mosquitoes, an essential oil extracted from the plant of *Juniperus phoenicea* cultivated in the wilaya of Tebessa (northeastern Algeria) was tested at different lethal concentrations CL25 and CL50 on fourth instar larvae of *Culesita longiareolata* (L 4) collected from untreated areas of Tebessa, under laboratory conditions according to the recommendations of the World Health Organisation.

The effects were examined on two enzymatic biomarkers (Glutathione-s-transferase, and catalase), total protein content and the weight growth of *Culesita longiareolata* at different treatment times, 24h, 48h, and 72h.

The determination of the essential oil extraction yield by hydrodistillation of the dried leaves showed a yield of 0.56%.

Biomarker results revealed an induction of the detoxification system of *Juniperus phoenicea* essential oil via an increase in GST and catalase activity compared to controls, as well as a significant decrease in total protein levels with both CL25 and CL50 concentrations at different treatment periods.

Juniperus phoenicea essential oil (LC25,LC50) affected the weight growth of the mosquito species with a significant reduction in the body weight of *C. longiareolata* larvae compared to controls at different treatment periods.

Research into natural products extracted from plants capable of controlling populations of *Culiseta longiareolata* is very important as it can help to reduce the spread of the spread of the disease from this vector. According to the data from these studies, *Juniperus phoenicea* essential oil can be used as a bio-insecticide and as an alternative to synthetic products in mosquito control.

Key words: Mosquito, Culiseta longiareolata, Essential oil, *Juniperus phoenicea*, Biomarkers

Introduction 

I. Introduction

Insects are an extremely successful and diverse group of animals that changing inhabit almost all habitats and ecosystems on earth. They play essential roles in the pollination of flowering plants, the production of silk, and in certain societies, can be a food source. Insects are also a significant cause of crop losses and food waste. Its success has been attributed in the past to their rapid rates of reproduction, their relatively short lifespan and their ability to rapidly adapt to environments **(Sheehan *et al*., 2020).**

Among the most significant branches of the animal kingdom are the arthropodes, which comprise over a million species, of which three quarters are insects. These latter account for almost half of the planet's diversity and around 60% of the diversity of the animal kingdom. Their remarkable diversity, exceptional multiplicity, and reduced size have allowed insects to colonize most continental environments **(Dris, 2018).**

Mosquitoes have been a big burden to human health for a long time, These insects can invade in different geographic locations and new habitats through global trade and travel **(Sengül demirak *et al*., 2022)**, There are about 3450 species of there divided into 38 genera and 3 subfamilies (Toxorhynchitines, Anophelines and Culicinae) making up the family *Culicidae* in the order *Diptera*.

Adult female haematophagous mammals and/or birds; the male is raised in a floricultural environment **(Soudani *et al.*, 2022)**. Due to the *Culicidae* fauna's significance in the spread of parasitic and viral diseases that can impact both humans and animals, The family is at the forefront of entomological news worldwide **(Chahed *et al*., 2021)**.

Mosquitoes (Diptera: *Culicidae*) are arthropods of high medical and veterinary importance, since they act as vectors for many pathogens and parasites, including malaria, dengue, yellow fever, Japanese encephalitis, West Nile encephalitis, lymphatic filariasis, and Zika virus **(Vaníčková *et al*., 2016)**. Malaria is responsable for more than 400000 deaths each year **(Jonesrt *et al*., 2020).**

The mosquito *Culiseta longiareolata* is the most interesting mosquito species in Algeria, particularly in Tebessa area **(Bouabida, 2012)**, They are easily distinguished from other *Culiseta* species because of their white stripes and the points on their legs, head, and thorax . They aggregate in agricultural and urban areas and have been described as an ornithophilic species that rarely bite humans . However, previous studies have reported that this mosquito species could be a possible vector of the bacteria that cause Malta fever (brucellosis) and western encephalitis virus **(Boumaza *et al*., 2022)**.

Historically, mosquito vector control has been an essential component of public health management. The basis of all vector control relies on environmental management of

mosquito. Many strategies have been used globally to aggressively manage mosquitoes, including chemical and biological control **(Bawin *et al*., 2014).**

Chemical control has been used on a large scale since the 1940s and benefited from the development of insecticide molecules with neurotoxic properties for the insect as part of crop protection or vector control . After more than half a century of agricultural inputs and intensive use of insecticides, concerns have arisen about the accumulation of these substances in natural and anthropogenic and man-made environments **(Lecollinet *et al*., 2021)**.

Their toxicity to the environment, animals and humans, as well as the increase in insecticide resistance, particularly in mosquitoes, call into question past practices and argue in favor of research and the deployment of innovations in this field, see innovations in the "Biological control" section **(Lecollinet *et al*., 2021)**. It includes fish, fungi, microorganisms, and even plant extracts, plant-based extracts **(Keffous, 2019)**.

Plants produce a remarkable set of secondary metabolites (SMs) with alleged health benefits, which form the basis of herbal remedies and even of pharmaceutical drugs. One of such examples is essential oils (EOs) **(Mansour *et al*., 2023).** Strong biological activity has been observed in the EOs of Cupressaceae family plant species, especially those from the genera *Cupressus* and *Juniperus* **(Kavetsou *et al*., 2021).**

The EOs of *J. phoenicea* origin are typically rich in monoterpenoids and their derivatives, although their specific chemical constituents vary significantly according to the plant organ, phenological stage, and geographical distribution, among other factors.

Distinct authors have previously reported the chemical composition of EO of *J. phoenicea* collected from several origins **(Mansour *et al*., 2023)**

Juniperus phoenicea L , also known as red juniper, is distributed in Mediterranean countries of North Africa, extending to the Arabian coast of the Red Sea in the east and to the Canary Islands and Madeira in the west. The species' wide geographic distribution allows for a tremendous amount of genetic variability. In folk medicine, this plant is considered a remedy that is commonly used in many countries for the treatment of diarrhea, bronchitis, rheumatism, acute gonococcal infection, eczema, hemorrhoids, dysmenorrheal, sunstroke, and depurative disinfectant **(Mansour *et al*., 2023)**.

Previous studies have confirmed that *Juniperus phoenicea L* essential oil has a toxic effect on larvea of the species *Culiseta longiareolata* .

In this context, our work aims to complete this study by evaluating the effect of *Juniperus phoenicea* essential oil (CL25,CL50) on certain biomarkers(GST,Catalase), total protein content, and on growth body weight in fourth larvae stage of *Culiseta longiareolata*.

Material and Methods

II. Material and Methods

II.1. Animal material

II.1.1. *Culicidae* family

Diptera (flies, mosquitoes, etc.) are insects that have only one pair of functional wings, the posterior wings are transformed into pendulums with a role their regression leads to changes in the thorax, which is formed almost exclusively of the mesothorax. The mouth parts form a proboscis of the biting-sucking type **(Ales & Amroun, 2021).**

Mosquitoes are insects that belong to the *Culicidae* family, classified in the order Diptera and the suborder Nematocera **(Ales & Amroun, 2021).**

They are found everywhere around the globe, except in permanently frozen areas, There are more than 3500 species. The earth plant is constantly undergoing climate change, depending on the extent of the increase in average temperature significant changes could occur in ecosystems, particularly in the distribution areas and ecological interactions **(Ales & Amroun, 2021).**

In 1878, mosquitoes were the first arthropods officially introduced as the intermediate hosts of vertebrate parasites; however, they are now recognized as the most important arthropods affecting human health **(Ghahvechi Khaligh *et al.*, 2020).**

In Algeria, only the two sub-families Culicinae and Anophelinae are represented, with six genera. That of Culicinae separated into 11 tribes Culicidian species currently known in Algeria is *Culex pipiens* and *Culiseta longiareolata* **(Ales & Amroun, 2021).**

II.1.2. *Culiseta longiareolata*

Culiseta longiareolata is the most interesting mosquito species in Algeria, particularly in Tebessa area **(Bouabida, 2012).** It was found in all types of natural habitats throughout the country. It is widely distributed in the south of the Palearctic and Mediterranean regions, as well as in Europe and Asia. More specifically, they have been found in diverse locations, from freshwater rocks and pools to plastic containers, casks, tire basins, and fountains.

They are easily distinguished from other *Culiseta* species because of their white stripes and the points on their legs, head, and thorax They aggregate in agricultural and urban areas and have been described as an ornithophilic species that rarely bite humans **(Boumaza *et al.*, 2022).**

C. Longiareolata is a species of the family *Culicidae*, the Culicinae sub-family and a vector of avian malaria tularemia, and arboviruses such as West Nile fever.

This mosquito species are readily distinguished from other *Culiseta* species and its morphological characters include white stripes and points on legs, head, and thorax **(Ghahvechi Khaligh *et al.*, 2020).**

It is multivoltine and ranges in size from 3 to 5 mm. It features long, thin legs, a slim torso, and long, narrow membrane-out swings.

The females are stenographic and autogenous, and they bite both people and household pets. Their siphon is conical in shape, with an index of 1.5 to 2, and they feature a basal siphonal tuft with unevenly implanted teeth. They also have a short antenna and smooth integument. Three longitudinal white stripes on the thorax, one patch of dark scales on the wing, and the absence of the long, powerful bristles from the basal lobe are all present in the adult **(Kharchi & Fares, 2023).**

Figure 01. *Culiseta longiareolata* **(personal photo).**

II.1.3. Systematic Position

The Systematic position of *Culiseta longiareolata* is as follows **(Aitken, 1954)**.

Table 01. Systematic position of *Culiseta longiareolata* **(Aitken, 1954)**.

Kingdom	Animalia
Subdomain	Metazoa
Phylum	Arthopoda
Sub-branch	Hexapoda
Superclass	Protostamia
Class	Insecta
Subclass	Pterygota
Infra-class	Neoptera
Super-order	Endopterygota
Order	Diptera
Sub-order	Nematocera
Infra-order	Culicomorpha
Family	Culicidae
Sub-family	Culicinae
Genus	*Culiseta*
Species	*Culiseta longiareolata*

II.1.4. Life cycle of *Culiseta longiareolata*

Mosquitoes are holometabolous insects, their life cycle comprising an aquatic phase and an aerial phase. The adults, or imago, are aerial, while the eggs, larvae and nymphs are the pre-imaginal stages and live in freshwater, usually brackish water. The total duration of development, strongly influenced by temperature, is 10 to 15 days in tropical areas **(Dris, 2018).**

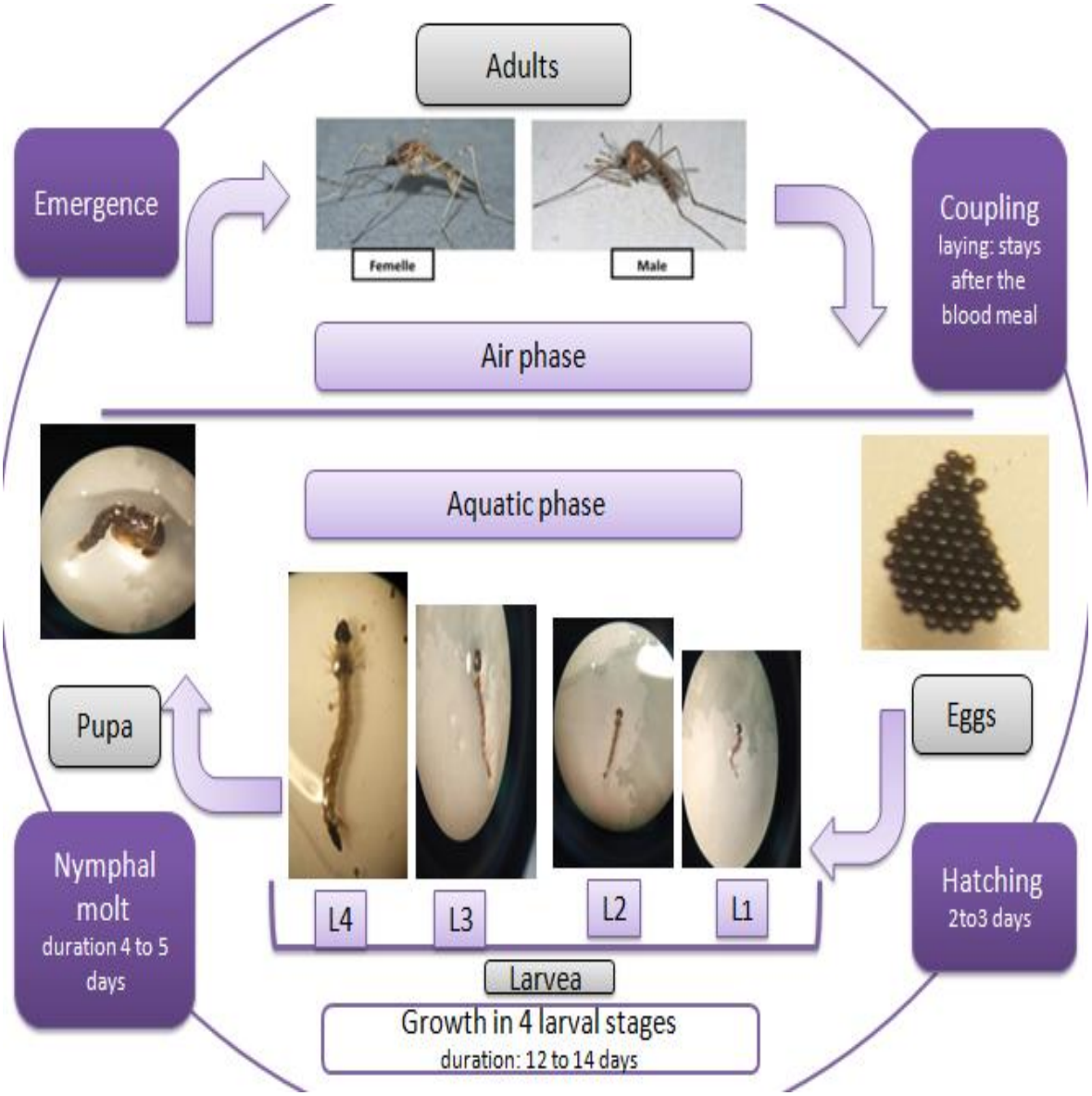

Figure 02. Life cycle of *Culiseta longiareolata* **(personal photo).**

II.1.4.1. Eggs

The females lay their eggs on the surface of various breeding sites (basins, abandoned wells, holes in rocks, seas, ponds, canals, cisterns, rainwater, etc.), where the water is always stagnant and rich in organic matter. These habitats may be permanent or temporary, shaded or sunny, filled with fresh or brackish, clean or polluted water. The eggs are fusiform and 0.5 to 1 mm in size **(Chalgou & Zerari, 2021).**

The fertilized females lay between 200 and 400 eggs, which have a lid that opens downwards when they hatch, and the larva emerges from it by means of a chitinousspine in the head. **(Dris, 2018).**

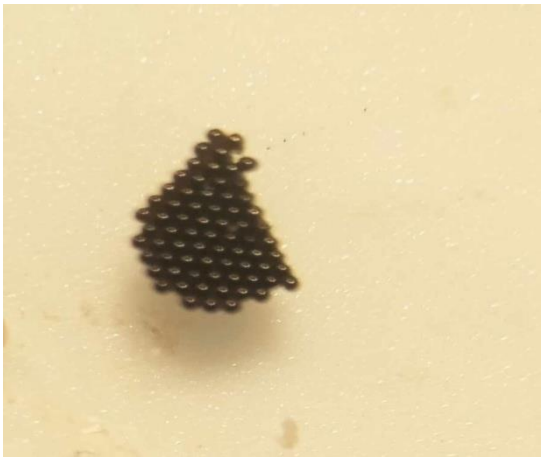

Figure 03. Eggs pods of *C. longiareolata* before hatching **(personal photo).**

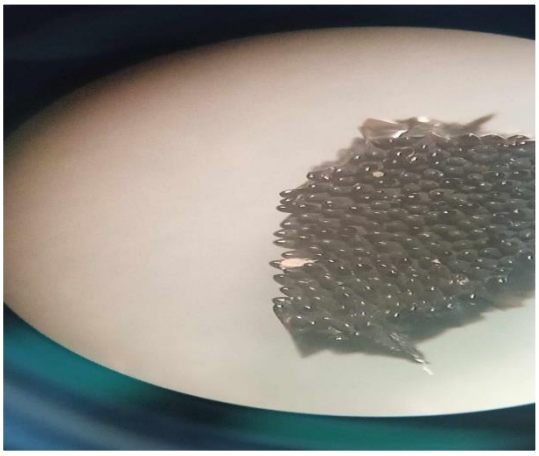

Figure 04. Eggs pods of *C. longiareolata* after hatching **(personal photo).**

II.1.4.2. larva

The larva goes through 4 stages of development: L1, L2, L3 and L4, separated by a moat. Enabling it to grow from around 2 to 12 mm. The larvae are mobile and breathe at the surface of the water via a respiratory siphon located at the end of the abdomen **(Dris, 2018).**

They move by saccades and feed on various micro-organisms (plant particles, bacteria and yeasts) the larvae are apodous, move rapidly and their mouthparts are of the crushing type **(Dris, 2018).**

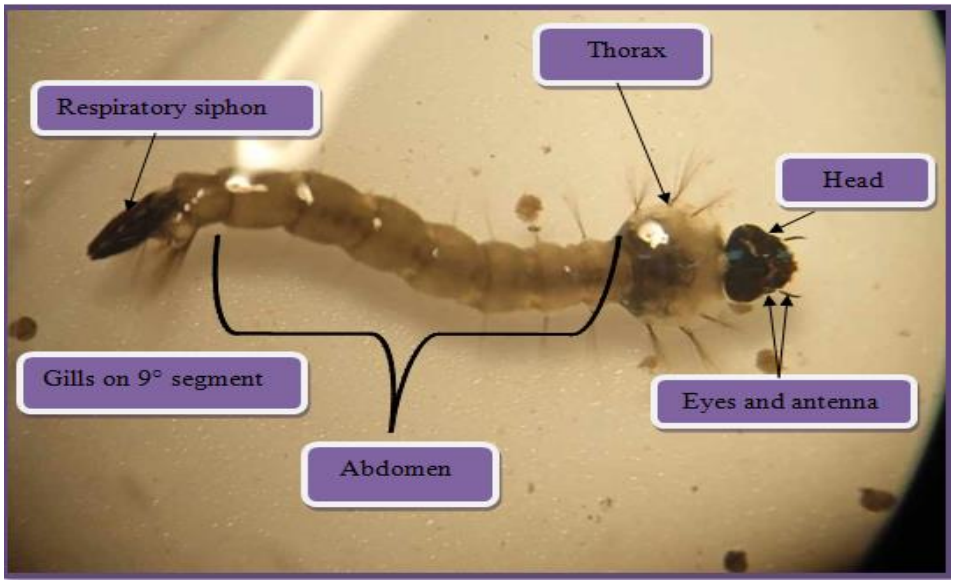

Figure 05. larva of *C. longiareolata* **(personal photo).**

II.1.4.3. Pupa

The nymph or pupa, also aquatic, is comma-shaped and mobile, but does not feed throughout this stage, which lasts from 2 to 5 days. It takes in atmospheric air via two respiratory trumpets located on the cephalothorax. Its body is made up of 2 parts: a large cephalothorax (antennae, proboscis, legs and wings) and an abdomen in the form of a tail, which distinguishes the sexes. Females have a shorter tail. The pupal stage is a transitional stage with an extremely active metabolism, during which the insect undergoes very profound morphological and physiological transformations that take it from the larval stage, aquatic and saprophytic, to the adult form **(Dris, 2018).**

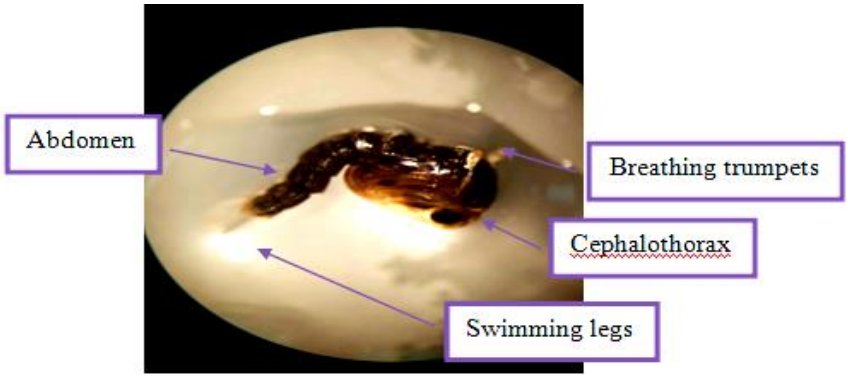

Figure 6. General appearance of the nymph **(personal photo).**

II.1.4.4. Adults

Adults, like all Diptera, have a pair of long, narrow, membranous wings with scales along the veins. which are folded horizontally at rest. The second pair is reduced to a pair of pendulums. They have a slender body divided into head, thorax and abdomen. On the head, adults have long slender, jointed antennae, unlike other members of the Diptera family. Females are easily distinguished from males by the presence of pinnate antennae. They have long, sucking mouthparts **(Dris 2018).**

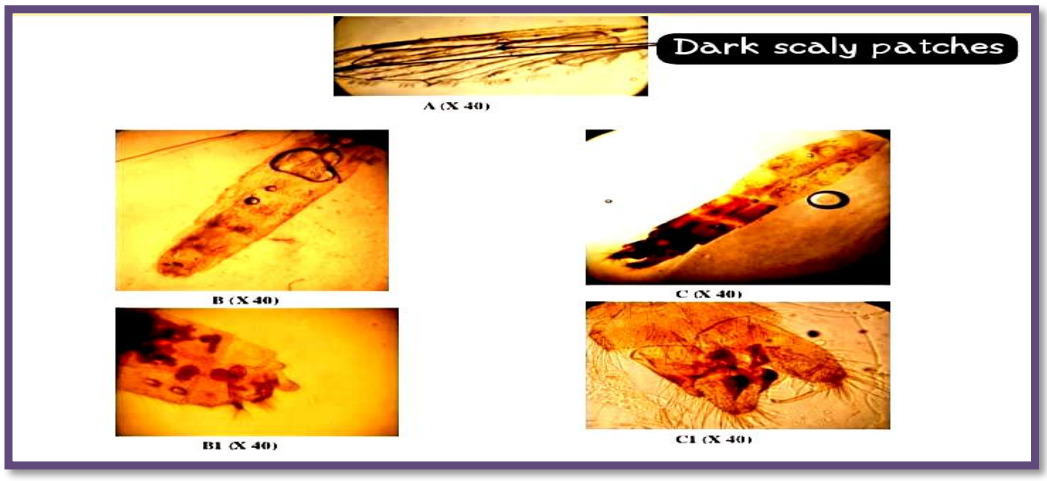

Figure 07. A: The wing of *C. Longiarelata*. B: Abdomen female of *C. Longiareolata*. B1: Female genetalia. C: Mal abdomen. C1: Mal genetalia **(Aissaoui, 2014).**

II.1.5. Structure of *Culicidae* larvae

II.1.5.1. Head

The structure of the head capsule comprises a median chitinous plate, the oclypeus front and two lateral epicranial plates. An attachment is attached to the oclypeus front. A narrow plate at the front (pre-clypeus) houses the mouth brushes. The teeth in the mouth are fragile, and consist mainly of thick mandibles with sharp points, and a triangular, serrated chin plate. The symmetrical setae of the preclypeus and frontoclypeus are coded from 0-C to 17-C (the letter C refers to the setae present on the head plates) **(Chalgoe & Zarari, 2021).**

These setae are of great taxonomic interest because of their shape and number of branches, particularly the peclypeal, clypeal, frontal and occipital setae. There are two pairs of eyes on the medio-lateral wall of the epicranial plates. The blackish spots in the two front eyes are the essential compound eyes of the future adult. The two small eyes of the larvae or stemmata are located behind there **(Chalgoe & Zarari, 2021).**

The antennae located in the anterolateral angles of the head are of variable thinness and slightly tapered. They may be shorter than the head and straight, slightly curved or even as long as the head, with a regular curve, Hairs and spicules are frequently present on the integument of the antennae **(Chalgoe & Zarari, 2021).**

II.1.5.2. Thorax

The thorax is large and marked by three successive series of more or less branched setae, which were otherwise indistinct. The symmetry of the pairs of setae is known, The numbers 0-P to 14 are used for the prothorax, 1-M to 14 for the mesothorax and 1-T to 13 for the metathorax . It should be emphasised that only the prothoracic setae are of taxonomic importance **(Chalgoe & Zarari, 2021).**

II.1.5.3. Abdomen

The abdomen of *Culicidae* larvae is elongated and sub-cylindrical. *Culicidae* larvae is made up of ten individualised segments. The first seven are similar to each, other each segment is adorned with 15 pairs of setae (except segment I, where only 13 pairs of setae). The majority of these setae are rarely used in taxonomy. The respiratory siphon, the main feature of *the* Culicinae, This is one of the most commonly used characters for identifying the species that make up the Culicinae. More This siphon, which varies in length, has a row of spines on either side (siphon comb) and, depending on the genus and species, one or more tufts of bristles **(Chalgoe & Zarari, 2021).**

The last segment or ventrally projecting anal segment, is not in line with the body, but forms an angle of 130° with it. It is surrounded on the dorso-lateral side by a chitinous reinforcement that forms the saddle, It is adorned with spines and a pair of setae (1-X), pairs of long setae arranged in a dorsal brush, a line of setae and a ventrally arranged brush. At the posterior edge of the saddle, four protruding anal papillae surround the anus, which is terminal **(Chalgoe & Zarari, 2021).**

II.1.6. Mosquito harvesting

II.1.6.1. Presentation of the study region

The wilaya of Tebessa is located in the east of Algeria (35°20' N, 8°6' E, altitude: 960 m). Its surface area is around 13878 km². It is bordered to the north by the wilaya of Souk Ahras, to the south by the wilaya of El Oued, to the west by the wilaya of Oum El Bouaghi and Khenchla, and to the east by the Algerian border. It is divided in to 28 communes **(Bouabida *et al.*, 2012).**

The stations of the site of Tebessa city are artificial environments, the specific richness of the selodgings is relatively insignificant, These sites is relatively low. They include stations where conditions are unfavourable for diverse Culicidian fauna. These sites are small and devoid of vegetation. The water is generally well oxygenated and rarely polluted. It would appear that this type of artificial shelter not the ideal habitat sought **(Tine Djebbar *et al.*, 2016).**

Figure 08. Presentation of Tebessa region **(Bougerra, 2019).**

II.1.6.2. Laboratory rearing

During the spring (March, April, May 2024), mosquito larvae and eggs are collected from sites in various regions of the town of Tebessa and are preserved in our laboratory. The larvae are reared in containers containing 150 ml of dechlorinated water . The food supplied consists of a biscuit-yeast mixture (75% -25%) **(Rehimi & soltani,1999)**, which provides protein for vellogenesis. The water is changed every two days. When the larvae reach the pupal stage, they are placed in cages where they transform into adults.

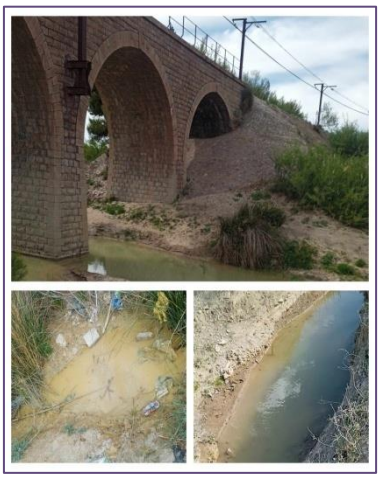

Figure 09. A water pool in the commune of-Al-Aouinet-Tebessa **(personal photo).**

Figure 10. A pond in the-El Wiam-district **(Personal photo).**

Figure 11. Fountain in university residence-Mekahlia Ibrahim-Tebessa**(Personal**

Figure 12. Sorting, counting and separating larva **(Personal photo).**

Figure 13. Laboratory rearing **(Personal photo).**

II.1.7. Mosquitoes control

II.1.7.1. Physical control

Physical action generally consists of undertaking works to regulate the water regime. In urban areas, it is often recommended to remove anything that could serve as a potential reservoir of stagnant water. This includes draining marshy areas for agricultural purposes planting trees or plants to provide shade, which will hinder the larvae development **(Chikh & Djema, 2020).**

The health authorities recommend that household waste be collected and treated, that flower pots and vases be kept to a minimum, that gutters be cleaned regularly, that dustbins be properly closed and that people be encouraged to keep their garbage clean, bins, fill pits with gravel and seal tree hollows with cement **(Chikh & Djema, 2020).**

II.1.7.2. Chemical control

Chemical control involves the use of synthetic chemical products to combat mosquito larvae and imagos, The first generation of insecticides are synthetic insecticides dating from before 1940 (e.g. potassium dinitro-o-cresolate, dinitro ortho cresol) and inorganic insecticides (copperaceto-arseniate), fluorescent (sodium fluoride), sulphur (carbon disulphide) **(Chikh & Djema, 2020).**

The second generation corresponds to synthetic organic insecticides divided into organochlorines (DDT, lindane, endosulfan), organophosphates (dichlorvos, chlorpyrifos,

temephos) and carbamates (carbaryl, aldicarb, propoxur). The third generation, which appeared later, includes synthetic pyrethroids phenylpyrazoles (fipronil), neonicotinoids (imidacloprid) and also insect growth regulators **(Chikh & Djema, 2020).**

II.1.7.3. Biological control

Biological control can be defined as "the reduction of a population through the use of competitors, predators, parasites, pathogens or toxin derived from them", the aims to maintain a population below an acceptable threshold in terms nuisance and epidemic risk (in the case of vector control) by means of an organism (known as an auxiliary) or substances of natural origin, while avoiding deleterious effects to the ecosystem **(Bawin *et al.*, 2014).**

This concept is an ancient one, dating back to in ancient Egypt, when cats were domesticated to protect foodstuffs from rodents. However, the term "biological control" as such was only used for the first time in the early the beginning of the 20th century, However, the development and massive use of synthetic organic insecticides from the Second world on wards were a considerable this practice. These products, which are inexpensive and easy to use, did not suffer from any competition in either and vector control. It was only when the major drawbacks of these insecticides in the early 1960s that the need for selective control agents is highlighted and renewed interest in biological control **(Bawin *et al.*, 2014).**

The biological method has been the subject of a new control method, which is safer, more selective and less toxic. It involves the use of micro-organisms, fungi fish and even plant extracts **(Chikh & Djema, 2020).**

II.1.7.4. Ecological control

This refers to all the environmental measures that prevent mosquitoes from reproducing or that lead to the elimination of larval breeding sites. It aims to destroy breeding sites and modify the environment in such a way as to make it unfavourable to the survival of the arthropod. Ecological control involves, for example, eliminating larval habitats, draining and drying up water sources, managing farm waste and fertilizers , finally ensuring that silage is properly managed **(Harrath & Benmedakhen, 2023).**

II.1.7.5. Genetic control

Genetic control (by alteration or replacement of hereditary-material of mosquitoes) using two strategies is also possible. Autocidal control consists of introducing sterile individuals whose mating will not produce offspring in the target population. This mosquito species-specific method is based on intensive breeding, sterilisation of the males (which do not take a blood meal), and the releasing them in to the environment. However, it has only been possible to obtain results insular (isolated) environment, due to the lack of-sterile males and the immigration of new strains of mosquitoes from untreated areas. In addition, a large

number individuals must be released for this technique to be effective, making it costly and unaffordable **(Bawin *et al.*, 2014).**

II.1.7.6. Microbiological control

Most micro-organisms have a narrow host range and a specific mode of action, which limits the effects on non-target organisms: this is their common advantage. The choice of a microbial control agent depends on the target insect species, and on the packaging and application possibilities of the agent itself. Several application strategies of these micro-organisms exist. These include promoting micro-organisms that already exist in the environment of the target insect (augmentation), or introducing them and acclimatizing them over the long term (inoculation). But micro-organisms are particularly suitable for application as biopesticides **(Bawin *et al.*, 2014).**

II.2. Vegetal material

II.2.1. Cupressaceae family

Cupressaceae belong to the conifers and are a family of the order Pinales . They are the most widely represented family of Gymnosperms in the world, according to phylogenetic classification, they comprise 160 species divided into 7 subfamilies and 29 genera distributed in the northern and southern hemispheres. Pollen grains are small, averaging between 20 and 40µm, They are abundantly pollinated, with pollen accounting for up to represent up to 60% of its spectrum**(Poncet *et al.*, 2021).**

Severe allergies have been reported throughout the Mediterranean basin, where the species Cupressus sempervirens and Hesperocyparis arizonica , in the United States due to *Juniperus ashei* and in Japan due to Cryptomeria japonica . Five groups of allergens have been described **(Poncet *et al.*, 2021).**

Their foliage is made up of compact, overlapping scales and is evergreen. The tree is anemophilous and most Cupressaceae are monoecious, with male and female reproductive organs on the same plant. and, in rare cases, dioecious (e.g.*Juniperus*). They bear small, incomplete, unisexual flowers borne on strobiliform catkins, called male and female cones. The male cones consist of 3 to 10 pollen sacs or microsporangia which when mature, split longitudinally and release large quantities of pollen into the air, an average of 400,000 grains per cone. smaller in the case of the Cupressus genus, intermediate for New World species and larger for Asian species. Because of this small size, both the pollen grains and the orbicles are small, fine particles 300 to 600 nm in diameter on their surface, can be transported over long distances **(Poncet *et al.*, 2021).**

The pollination period for Cupressaceae differs depending on the species and climatic conditions. In some regions, it covers a large part of the year from late summer to mid-spring,

or even all year round. The pollen grains of this family are morphologically very homogeneous, making it impossible to distinguish genus or species.

The most studied genera of Cupressaceae are :

- Cryptomeria 1 single species: Cryptomeria japonica, Japanese cedar
- Taxodium 3 species including distichum, bald cypress
- Thuja cedars, 5 species including occidentalis, plicata
- Chamaecyparis 5 or 6 species including obtusa, Japanese cypress
- Cupressus cypresses, 29 species including sempervirens, macrocarpa, arizonica (renamed Hesperocyparis arizonica), ...
- *Juniperus* , around twenty species including ashei, oxycedrus, communis, Virginiana **(Poncet *et al.*, l 2021).**

II.2.2. *Juniper* genus

II.2.2.1. History

Juniperus is a Latin term with no clear etymology, one hypothesis is that it refers to the Celtic word Gen, meaning "bush" and Prus, meaning "acrid", another discovery shows that the name is made up of the Latin words Junior, meaning "younger", and Parere, meaning "to appear". Christian tradition regards juniper as a protective plant, the Greeks and Arabs have used juniper since ancient times **(Latrech-Douar, 2019).**

As president, François-Joseph Cazin described its anti-rheumatic, digestive and diuretic properties in the 19th century. Cade oil, which was produced by heating the wood of *Juniperus* oxycedrus, was used by the Romans. This oil was also used to wrap the dead. Juniper was used as a medicine in Antiquity and the Middle Ages, and its fumigations were considered medicinal **(Latrech-Douar, 2019).**

II.2.2.2. Origin

Native to America, Asia and Africa, junipers play an important role in the North African landscape. They are undemanding pioneer species, they can be found from the seaside to the summits of the Atlas mountains. *Junipers* are the only resinous species that can form genuine forest stands in the mountain **(Latrech-Douar, 2019)**

II.2.3. *Juniperus phoenicea*

Linne described *Juniperus phoenicea L* in 1753. They are known by vernacular names such as the *Phoenician juniper*, the *red juniper*, the *Lycian juniper* , or the *Fausse Sabine*, which are Provençal names for this species. The Libyan names for this species are Ara'ar in Morocco and in Algeria, Aifs and zimba (in the Chaouia language) **(Latreche-Douar, 2019).** Is a wild tree belonging to the *Cupressaceae* family **(Bouassida *et al.*, 2018).**

Is a small tree of about 8–12m tall or sometimes only a shrub. It is a pioneer species with highlight demands and relatively high resistance to a dry climate **(Dzialuk *et al*,. 2011).**

The species *Juniperus phoenicea* is considered as an important medicinal plant largely used in traditional medicine, its leaves are used in the form of decoction to treat diarrhea, rheumatism diabetes . The mixture of leaves and berries of this plant is used as an oral hypoglycaemic agent , whereas the leaves are used against bronco-pulmonary disease and us a diuretic **(Mazari *et al.*, 2010).**

Figure 14. Appearance of *Juniperus phoenicea* **(Abdelli, 2017).**

Figure 15. *Juniperus phoenicea* **(Personal photo).**

II.2.4. Botanical characteristics

II.2.4.1. Leaves

The leaves are all or almost all squamiform (0.7 to 1 mm), oval or rhomboidal, obtuse, convex, furrowed on the back, glandular and dark green in colour . They are non-articulated, grouped in threes and closely interlocked with each other in 4 or 6 rows, forming a single unit with the branch **(Abdelli, 2017).**

II.2.4.2. Flowers

Male and female flowers are often found together on the same plants (rarely on different individuals). The male flowers form numerous small oval or rounded catkins, with pedicellate scales, borne on short leafy peduncles and arranged laterally along the branches. The female flowers are much less numerous, their scales are thick, acute and arranged in 4 rows. Flowering lasts from February to April and eventually produces false spherical berries dark red when ripe **(Abdelli, 2017).**

II.2.4.3. Fruits

The fruits, known as berries, are initially green in colour, turning glossy reddish brown when ripe (after 2 years), globular and fleshy in shape, 7 to 10 mm in diameter, with an irregular surface. Their flesh is firm, dry, fibrous, yellow tinged with green then brown, with a strong odour and containing 4 to 9 oval seeds, with sharp ends and a hard shell. The fruiting period is from September to December **(Abdelli, 2017).**

Figure 16. Leaves and fruit of *Juniperus phoenicea* **(Abdelli, 2017).**

Figure 17. Flowers and Leaves of *Juniperus phoenicea* **(Abdelli, 2017).**

II.2.5. Taxonomy

Juniperus phoenicea belongs to the *Cupressaceae* family, two varieties are known for this species: *J. phoenicea* var. phoenicea (the seeds of the cones are globose) and *J. phoenicea* var turbinata (the seeds of the cones are turbinates) **(Abdelli, 2017).**

Table 02. The classification of *Phoenician juniper* is as follows **(Quezel & Santa, 1962)**.

Phylum	**Spermaphytes**
Subphylum	Gymnosperms
Class	Conifers
Order	Coniferales
Family	Cupressaceae
Genus	*Juniperus*
Species	*Juniperus phoenicea L*

II.2.6. Geographic distribution

Global climate change is having an unprecedented impact on forests around the world, causing changes in ecosystem functions and services, species abundance and biodiversity. Its impacts include phenological changes and altered local and global distribution of species, increasing the risk of local and global extinction of plant species **(Dakhil *et al.*, 2022).**

Figure 18. Chorological map for the distribution of *Juniperus phoneucia* **(Telaidji, 2018).**

II.2.6.1. In the world

Phoenician juniper is a plant found throughout the Mediterranean region, both on the coast and in low mountains at altitudes of up to 2,000 metres. Globally, it occurs in southern Europe (southern France, eastern Portugal, Spain), temperate and subtropical Asia (Turkey, Cyprus, western Saudi Arabia, Jordan), the Atlantic Ocean (Canary Islands) and North Africa (Algeria, Morocco, Tunisia, Libya and Egypt) **(Abdelli, 2017).**

II.2.6.2. In Algeria

Red juniper occupies an estimated area of 227,000 hectares, 10% of Algeria's forest area. It is common all along the coast, in the high plateaux and the Saharan Atlas of Oran, Algeria and Constantinois regions. It is fairly rare elsewhere, being found mainly on coastal dunes, in the hills, on the Barbary coasts and on the coast of the Mediterranean. And, alongside cedar, it is the main plant cover in the mountains of the Aurès, particularly in the south of this massif (Maafa and Beni Fodhala regions) where it covers an area of 1950 h **(Abdelli, 2017).**

II.2.7. Chemical composition

There are many papers report on the chemical composition of leaves and berries essential oils of *J. phoenicea* **(Ramdani *et al.*, 2013).**

In total, 63, 46 and 78 compounds were identified in fresh leaves, dried leaves and berries, respectively, accounting for 98.1%, 98.3% and 96.4% of the total oil. The EOs are mainly composed of monoterpene hydrocarbons, the major component found in fresh and dried leaves was β-phellandrene (43.9 - 44.9%), followed by α-pinene (25.1 - 20.3%), myrcene (8.5 - 8.2%), α-phellandrene (4.7 - 4.5%), p-cymene (2.7 - 3.0%) and limonene (2.3 - 2.5%) . The variations in EO content can be related to several factors such as the geographical

area of collection, climate, season, storage conditions, part of the plant studied and stage of development **(Abdelli *et al.*, 2018).**

Table 03. Oligo-element and mineral content of the aerial parts of *J. Phoneucia* **(Nedjimi *et al.*, 2015).**

Element							
	Ca (%)	Co (mg/g)	Cr (mg/g)	Fe (mg/g)	K(%)	Na (mg/g)	Zn (mg/g)
Values	**1.60**	**0.17**	**1.13**	**340**	**0.67**	**52.13**	**15.6**

Table 04. Chemical compositions of *J. Phoneucia* EO **(Abdelli *et al.*, 2018).**

Componets found in fresh and dried *juniperus* leaves	Componets found in juniper berry oil
β-phellandrene (43,9 – 44,9%)	α-pinea (43,7%)
α-pinea (25,1 - 8,2%)	P-cymene (5,8%)
Myrcene (8,5 - 8,2%)	β-phellandrene (4,6%)
α-phellandrene (4,7 - 4,5%)	α-terpineol (4,3%)
P-cymene (2,7 - 3,0%)	α-campholenal (4,0%)
Limonene (2,3 - 2,5%)	

II.2.8. Uses

Phoenician juniper is highly prized for its service wood, this is used for heating and to make charcoal, the leaves are sometimes used to feed livestock **(Abdelli, 2017).**

It has been utilized in household remedies for various ailments for centuries and is widely recognized as a medicinal plant. Various species of *Juniperus* have been utilized in traditional medicine to combat infectious and inflammatory ailments,these species are believed to contribute to overall well-being and good health. Besides, *Juniperus* exhibits various biological effects including antioxidant, antimicrobial, anti-cholinesterase, anti-tyrosinase, antiseptic, anthelmintic, hepatoprotective and cytotoxic properties **(Amokrane *et al.*, 2024).**

Studies on the plant's phytochemistry have revealed its high concentration of flavonoids, phenolic acids, and essential oil. Additionally, the berries in orange-brown colour are abundant in unsaturated lipids and minerals. Plant secondary metabolites have a wide variety of effects on insects defensive, repellent, or deterrent substances, and phagostimulants or digestion inhibitors, pollinator attractants, oviposition or capture inducers **(Amokrane *et al.*, 2024).**

II.2.9. Biological activities

The biological activity of an essential oil depends on its chemical composition, the functional groups of the main compounds and their synergistic effects **(Bougerra, 2019).**

The main applications for biological activities are in the fields of health, agriculture and the cosmetics and food industries **(Bouyahyaoui, 2016).**

II.2.9.1. Antibacterial activity

In recent years due to an upsurge in antibiotic-resistant infections, the search for new prototype drugs to combat infections is an absolute necessity and in this regard plant essential oils may offer great potential and hope. Volatile compounds from plants, especially essential oils have antimicrobial activitie **(Derwich *et al.*, 2010).**

The mechanisms by which EOs exert their antibacterial activity are poorly understood known, Because of the complexity of their chemical composition, it is difficult to give a precise idea of the mode of action of EOs. It is likely that their antibacterial activity is not attributable to a single mechanism, but to several sites of action at the cellular **(Bougerra, 2019).**

II.2.9.2. Antifungal activity

It is thought that the antifungal activity is due to the complexity of the composition of EOs. Phenols have a higher antifungal activity than aldehydes, and this varies according to the type of chemical function, as essential oils contain terpene compounds, particularly phenols and aldehydes, which interact with membrane enzymes and alter the plasma membrane of cells. Sequiterpene alcohols and lactones have also been shown to have antifungal properties **(Bougerra, 2019).**

II.2.9.3. Anti-Oxydant Activity

Oxidative stress, which occurs when the production of free radicals is out of balance with that of antioxidant enzymes, is linked to the emergence of diseases such as Alzheimer's. Certain alcohols, ethers, ketones, monoterpene aldehydes (tinalool, 1,8-cineole, geranial/neral, citronellal, isomenthone, menthone) and some monoterpenes such as α-terpinene, γ-terpinene and α-terpinolene are also responsible for the antioxidant activity of essential oils. Several

studies have confirmed that EOs have a tendency to hinder the oxidation of linoleic acid due to the presence or absence of phenolic compounds **(Bougerra, 2019).**

II.2.9.4. Bio-insecticidal activity

Insecticides are all substances that kill insects, prevent eggs from hatching and compromise the normal development of larvae or sexual maturation. The group of pesticides encompasses several families, including organochlorines, carbamates, organophosphates and plant insecticides **(Latrech-Douar, 2019).**

II.2.9.5. Biopesticides of plant origin

- ✓ A bio-pesticide is defined as any living organism or substance of natural origin. However, for some authors, the term bio-pesticide should be reserved for biological agents insect or control agents.
- ✓ EOs are used to protect plants and control harmful insects, and can therefore be considered as "biopesticides of plant origin"**(Latrech-Douar, 2019).**

II.3. Essential oil

II.3.1. History

Agriculture has been a field developed by all ancient civilisations, and most of the great physicians of the past were phytotherapists **(Kerbouche, 2009).**

EOs are natural compounds that have been around since ancient times. Perfumes and aromas were among the first elements of recognition that marked the lives of human beings **(Kerbouche, 2009).**

In 1879, Louis Poure invented the first industrial machine to extract perfume using a solvent. In 1877, Otto Wallach discovered the "isoprene rule" while Léopold Ruzicka highlighted the "polyterpenes" that are essential components of essences. Since the emergence of organic chemistry at the end of the 19th century, plant essences have gradually revealed their mysteries. At the beginning of the 20th century, their therapeutic properties emerged from scientific research **(Kerbouche, 2009).**

The term "essential oils" was coined by the Swiss physician paeascelsus von Hohenheim to designate the active compound in a natural remedy **(Kerbouche, 2009).**

II.3.2. Definition

The term essential oils, also known as volatile oils or ethereal oils, is used to designate aromatic plant extracts **(Bouyahyaoui, 2016)**, marked by a strong and characteristic odour. The terpenes (mainly monoterpenes) make up the majority (around 90%) of these components. These extracts contain an average of 20 to 60 compounds, most of which are not very complex molecules (monoterpenes, sesquiterpenes). Essential oils contain a considerable

number of biochemical families (chemotypes) including alcohols, phenols, esters, oxides, coumarins, sesquiterpenes, terpenols, ketones, aldehydes, etc **(Dris, 2018).**

Insecticidal properties are essentially due to the fraction of essential oils contained in the plant. EOs represent a promising avenue for the future, and there has been a great deal of research into oil extracts. However, the vast majority of these studies have focused on mosquitoes, either in terms of the repellent effect of these oils or their larvicidal effect. Aromatic plants are among the most effective insecticides, and essential oils often constitute the bioactive fraction of plant extracts **(Dris, 2018).**

Essential oils can be considered as bioinsecticides, acting either :

- on the nervous system, causing paralysis in particular.
- On cellular respiration: the cell becomes incapable of absorbing the oxygen supplied by the respiratory system.
- Affects the insect's development by blocking its moult, an essential stage in its growth.
- On the formation of its protective skin, the cuticle, which makes it sensitive to various environmental aggressions **(Dris, 2018).**

II.3.3. Location and synthesis site

There is a correlation between the production and accumulation of oils and the presence of specific histological structures **(Latrech-Douar, 2019).**

The formation of secretory cells in the cytoplasm varies according to the plant organ in question. These are then generally grouped together in specialised membrane-covered glandular cells. They are then preserved and stored in specialised histological structures, generally located on or near the surface of the plant, such as essential oil cells, epidermal glandular hairs that produce superficial essences, secretory pockets or secretory ducts **(Abdelli, 2017).**

Essential oils can be extracted from various plant organs,these can include barks, leaves, roots, flowers **(Abdelli, 2017).**

II.3.4. Physico-chemical properties

The physico-chemical properties of EO are usually linked to the following observations :

- ✓ In general, they are liquid and change at room temperature, they are rarely coloured, their density is generally lower than that of water **(Latrech-Douar, 2019)**.
- ✓ The refractive index depends essentially on the content of monoterpenes and oxygenated derivatives **(Latrech-Douar, 2019),** It is high and most of it deflects polarised (optically active) light **(Abdelli, 2017).**

- ✓ They are soluble in most organic solvents, they are also liposoluble but not very soluble in water **(Latrech-Douar, 2019).**
- ✓ They are also highly sensitive and oxidise in contact with air and light **(Abdelli, 2017).**
- ✓ Their boiling point varies from 160°C to 240°C
- ✓ EOs are stable at room temperature if they are stored properly: away from oxidation and polymerisation caused by air, light and heat **(Kerbouche, 2009).**

II.3.5. Physiological function

As a source of energy, essential oils facilitate certain chemical reactions, in desert climates, they help conserve plant moisture, they can also be used as a means of competing with environmental resources by inhibiting the germination of seeds from other plant species or by limiting the growth of certain species **(Abdelli, 2017).**

repel or attract insects to encourage pollination, inhibit the multiplication of infectious microbial flora **(Abdelli, 2017).**

II.3.6. Chemical composition

The chemical composition of many essential oils has been described. They are vary depending on various factors, including the stage of development of the plants, the organs harvested, and the period and geographical area of harvest. Chemical composition is generally studied using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) **(Dris, 2018).**

Phytochemical studies reveal that *J. phoenicea* contains a large variety of compounds, However numerous studies in the literature have been reported the composition of the oil isolated from the leaves , berries and wood of *J. phoenicea* (subspecies and varieties) from different parts of world **(Dob *et al.*, 2008).**

The constituents of essential oils may be broadly classified as volatile and non-volatile fractions. The overall chemical composition of the aromatic oil volatile fraction includes mono- and sesquiterpene components, and several oxygenated derivatives along with alcohols, aliphatic aldehydes, and esters **(Zarith *et al.*, 2018).**

II.3.6.1. Hydrocarbons

Hydrocarbon is a chemical compound found in essential oils with their building blocks connected by hydrogen and carbon bonding . An example of basic hydrocarbons found in essential oils is isoprene as shown in Fig. **(Zarith *et al* 2018).**

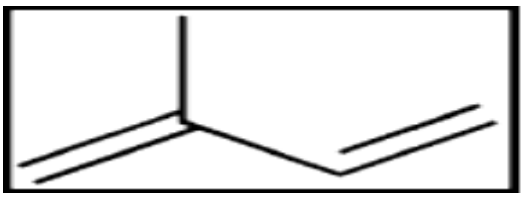

Figure 19. Chemical structure of isoprene **(Zarith *et al* 2018).**

- **Terpene compounds**

Terpenes are compounds formed by the assembly of two or more isoprenic units (2-methylbuta-1,3-diene). Only the most volatile terpenes whose molecular weight is not too high (monoterpenes and sesquiterpenes) are found in the composition of essential oils **(Latrech-Douar, 2019).**

✓ **Monoterpenes**

Monoterpenes are the simplest terpenes, most of which are found in essential oils (90%). Two isoprene units (C_5H_8) are present in these products, using the "head-tail" coupling mode. They can be acyclic, monocyclic or bicyclic. These terpenes are associated with several natural products that have specific chemical functions **(Dris, 2018).**

✓ **Sesquiroterpenes**

Sesquiterpenes are isoprene compounds derived from $C_{15}H_{22}$ hydrocarbons. The most varied class of terpenes is made up of different structural categories such as acyclic, monocyclic, bicyclic, tricyclic and polycyclic. Hydrocarbons, such as alcohols, ketones, aldehydes, acids and lactones, occur in nature in different forms **(Dris, 2018).**

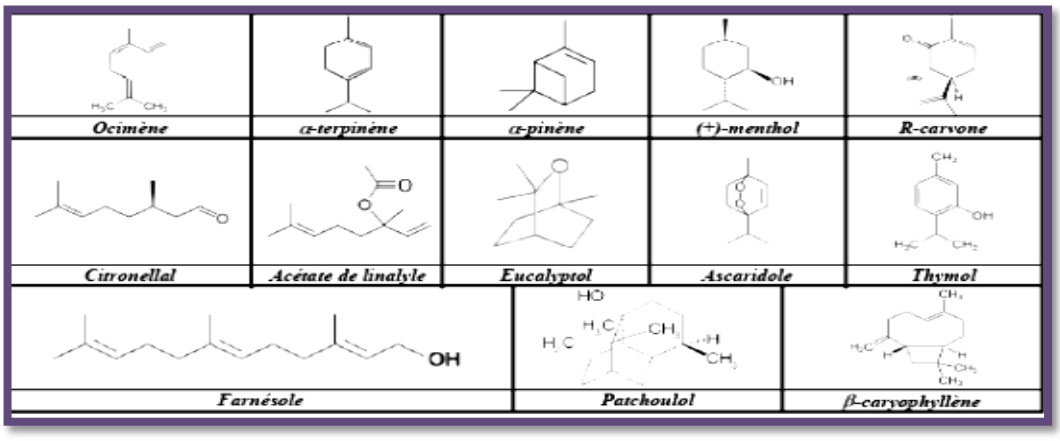

Figure 20. Representation of molecules characteristic of the different structures found in mono and sesquiterpenoids **(Abdelli, 2017).**

II.3.6.2. Alcohols

Alcohols in essential oils provide some excellent properties like anti-septic, anti-viral, anti-bacteria, and germicidal, it is naturally occur as a single component or combined with a

terpene or ester. The attachment of terpenes with oxygen and hydrogen atom can result in the formation of alcohols. The term monoterpenol is used to describe a monoterpene that contains hydroxyl groups inside its hydrocarbon structure. Alcohols are considered safe to be used since their amounts are extremely low or totally absent of toxic reaction in the body or onto the skin **(Zarith *et al.*, 2018).**

II.3.6.3. Esters

The formation of esters is due to the interaction between alcohols and acids. The ester component inside essential oils offers soothing and balancing effects due to the presence of alcoholic groups inside esters, they are able to provide anti-inflammatory activities. In medical sciences, esters are considered to have antifungal and sedative properties, with balancing action on the nervous system. They are generally free from precautions with the exception of methyl salicylate found in birch and wintergreen which is toxic within the system. The comon esters found in essential oils are linyl acetate and geranyl formate **(Zarith *et al.*, 2018).**

II.3.6.4. Ketones

Ketones have anti-catarrhal, cell proliferant, expectorant, and vulnerary properties and are often found in plants that are used for upper respiratory tract complaints. Essential oils with ketone group are advantageous for wound healing and improve scar tissue. **(Zarith *et al.*, 2018).**

II.3.6.5. Aromatic compositions

Another class of volatile compounds frequently encountered are aromatic compounds derived from phenylpropane **(Dris, 2018),** including aldehydes (cinnamaldehyde), alcohols (cinnamic alcohol), phenols (chavicol, eugenol), methoxy derivatives (anethol, estragol) or methylene dioxy derivatives (myristicine, safrol) **(Abdelli, 2017).** However, it is possible to find C_6-C_1 compounds, such as vanillin (fairly common) and methyl anthranilate, as well as lactones derived from cinnamic acids (coumarins, for example), at least the simplest of which, can be carried away by water vapour **(Abdelli, 2017).**

Table 05. Phenolic compound content of aerial parts of *J. Phoneucia* **(Dane *et al.*, 2015).**

phenolic compounds	Percentage (%)
Catechin	41.97
Myricetin-hexose	11.11
Myricetin-rhamnoside	1.23
Quercetin-3-o-rhamnoside	45.67

II.3.7. Variability factors in the composition of essential oils

The composition of an EO is highly fluctuating and variable, involving a large number of parameters **(Latrech-Douar, 2019).**

This variability can be explained by different factors of intrinsic origin, specific to the plant's genetic make-up, or extrinsic, linked to the plant's growth and development conditions **(Abdelli, 2017).**

II.3.7.1. Intrinsic

First and foremost, an essential oil must be related to the botanical material from which it to avoid any misleading names for the plant material. An essential oil can be extracted from several parts of the plant. However, the quantity and quality differ, hence the need to specify the name of the part used **(Abdelli, 2017).**

The yield and chemical composition of an essential oil also vary according to the vegetative cycle. In fact, for a given species, the content of the various components of the essential oil can vary remarkably throughout the vegetative cycle in relation to the age of the plant and the harvesting period or season. In other words, certain constituents are more abundant at certain times of the year than at others **(Abdelli, 2017).**

II.3.7.2. Extrinsic

The composition of an essential oil depends on environmental conditions, more specifically soil and climate. With regard to the soil, the growth of the aerial part of the plant depends on the growth and activity of the aerial part of the plant. The shape of the roots, their distribution in the soil, the speed at which they spread and the changes in the chemical composition of essential oils depend on the structure, water content and temperature of the soil **(Abdelli, 2017).**

This means, for example, that two plants of the same species harvested at the same time in different soils will have a different chemical composition and a different essential oil content **(Abdelli, 2017).**

In terms of climate, the combination of temperature, rainfall and light not only affects plant growth and development, but also the quality of the substances produced by the plant. Other factors such as forestry techniques (plant density, irrigation, fertiliser application, phytosanitary treatments, harvesting techniques, etc.), extraction methods and storage of raw materials before distillation can also affect the composition and production of essential oils **(Abdelli, 2017).**

II.3.8. Fields of Application

There is a wide variety of known essential oils in the world and several thousand have been characterized. However, of these, only a small proportion is of commercial interest. This is due to the chemical composition of essential oils, which gives them both fragrant and aromatic as well as antimicrobial properties, but also the different possible uses and the cost of production. These characteristics offer major opportunities in a wide range of industrial cosmetics industry, the health sector, the agri-food industry and the food industry, food processing and agriculture **(Abdelli, 2017).**

II.3.8.1. Perfumes and cosmetics

Thanks to their antimicrobial properties, essential oils are used as preservatives in perfumes and cosmetics to extend the shelf life of products. However, due to their high volatility and lack of fatty trace, they are mainly used in the formulation of perfumes and personal or household cleaning products **(Aburjai & Natsheh, 2003).**

II.3.8.2. Health: Pharmacy and cosmetics

Essential oils have been known and used for a long time, particularly in Asia, where they form part of traditional medicine. So the return of essential oils to the health field with pharmaceutical and aromatherapy applications is logical. Essential oils are mainly used in pharmacies to flavour oral medicines. They can also be used for their antiseptic effect, particularly in hospital environments. Given the ability of these oils to penetrate the skin easily, many products such as ointments, creams and gels based on essential oils can facilitate the administration of medication transdermally. These products are generally designed to relieve sprains, aches and pains, joint or muscle allergies **(Abdelli, 2017).**

While essential oils have important applications in so-called conventional or scientific medicine, their benefits form the basis of another area of health care which is closer to traditional medicine: aromatherapy **(Fillatre, 2011).**

The latter refers to the use of odours and volatile substances to treat, alleviate or prevent infections and internal ailments solely by means of inhalation or the use of essential oils to treat certain external illnesses by massaging them into the skin. The synergy of the different constituents of essential oils determines their balancing effect **(Abdelli, 2017).**

II.3.8.3. Food industry

Essential oils are used in the food industry as flavourings and spices for carbonated and alcoholic beverages, condiments, confectionery, dairy products, meat products and bakery products, as well as in nutrition. confectionery, and also for animal nutrition . The most commonly used are mint, vanilla, pepper, basil, ginger, eucalyptus, etc **(Abdelli, 2017).**

At present, essential oils or their active compounds represent a very interesting tool for increasing the shelf life of food products. while ensuring better organoleptic quality , by enhancing the taste of the food. These natural substances are rich in antimicrobial and antioxidant compounds. They could therefore be used as food preservatives, all the more so as they are that most of them are classified as generally recognised as GRAS' or approved as food additives by the US Food and Drug Administration (FDA). As a result, they do not require authorisation for use in foodstuffs, but prior studies are required to better define their activity without being toxic to humans **(Abdelli, 2017).**

II.3.8.4. Agricultural

The desire to reduce the use of synthetic pesticides in modern agriculture, in the interests of ecology and sustainable development and planning, has become stronger in recent years. As far as pesticides are concerned, one of the draft laws aims to reduce the consumption of plant protection products by 50% over ten years. In this environmental context, natural pesticides based in particular on essential oils represent an interesting alternative to protect crops against insects, weeds and fungi.

They can be applied in a wide variety of ways, including fumigation, attractants added to pheromone traps, repellents or by contact **(Abdelli, 2017).**

In addition to their biological activities, essential oils have other characteristics that make them suitable for pest control. Among these include**:**

- ✓ their low price and assured supply thanks to large-scale worldwide production for many essential oils.
- ✓ their multiple modes and sites of action on insects .
- ✓ their low toxicity to mammals (with a few rare exceptions).
- ✓ their low persistence in the environment due to their volatility (half-life outdoors 24 hours on surfaces, in soil or water) **(Abdelli, 2017).**

II.3.9. Toxicity of essential oils.

II.3.9.1. Toxicity by ingestion

Some authors base their conclusions on the composition of essential oils and the relative toxicity of the biochemical families to which they belong. As the aromatic molecules present are very powerful, ingestion can, depending on the category and the quantity absorbed, generate high toxicity or even death **(Latrech-Douar, 2019).**

Neurotoxic EOs are those containing ketones (wormwood, aniseed, fennel, rosemary, mint). These ketones induce epileptic and tetaniform seizures, and serious mental and sensory disorders **(Latrech-Douar, 2019).**

II.3.9.2. Dermal toxicity

All books dealing with EOs give maximum concentrations. Thyme, oregano and savory are known for their irritating power, angelica and bergamot are photosensitizing, cinnamon is dermocaustic and allergenic for sensitive skin **(Latrech-Douar, 2019).**

II.3.9.3. Toxicity on animal or human cells

Thyme and lavender EOs are cytotoxic for Chinese hamster cells. EO of different varieties of oregano showed strong cytotoxicity in human cells derived from cancer. derived from human cancers **(Latrech-Douar, 2019).**

II.3.10. Extraction methods

Extracting an essential oil (EO) is necessarily a complex and delicate operation, its aim is to capture and collect the most volatile, subtle and fragile products that plants produce, without altering their quality. To appreciate the difficulty of the task, we need only think of the speed with which the plant's volatile are released, then disappear or disappear again **(Boukhatem *et al.*, 2019).**

The fragrance of even the most fragrant flower, once the petals have been crumpled. Once the waxy cuticle of the epidermal pockets has been broken , the essence escapes and several odorous molecules are dispersed in the air **(Boukhatem *et al.*, 2019).**

Such extraction techniques can be categorized into two categories: classical methods and innovative methods. The application of innovative techniques, such as ultrasonic and microwave enhanced processes, has improved the efficiency of extraction process in terms time required for isolation of the essential oil and energy dissipation, as well as improvement in production yield, and high quality of essential oils **(Zarith, *et al.*, 2018).**

II.3.10.1. Innovation of extraction methods

II.3.10.1.1. Supercritical fluid extraction

Supercritical carbon dioxide (CO_2) extraction is an innovative method using fluidized CO_2, has been available since the 1980s and has made significant in the world of flavours,

fragrances and aromatherapy. It is a method that does not requires no heat or chemical solvents. When the temperature of the CO_2 is maintained at around 31°C, under pressure, it acts like a fluid and dissolves the soluble part of the plant in the CO_2 . As a result, the yield of supercritical CO_2 extraction is much higher than with other extraction techniques, but the disadvantage is that this method is more expensive **(Bouyahyaoui, 2016).**

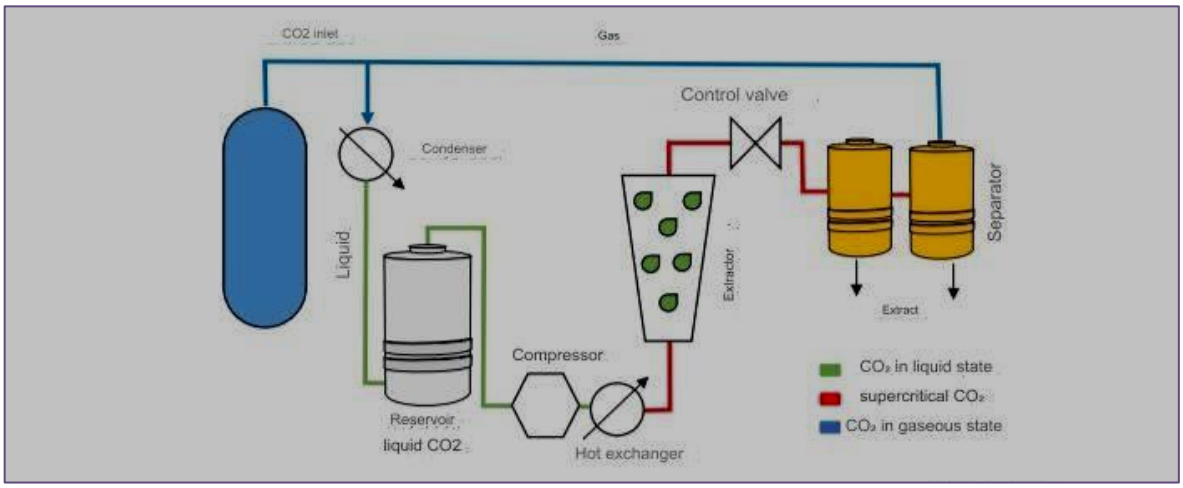

Figure 21. Schematic of supercritical CO_2 extraction process **(Boufares, 2020).**

II.3.10.1.2. Subcritical Extraction Liquid

The use of water at subcritical state has been reported by many researchers and found that this is a better and powerful alternative of essential oils extraction technique. The definition of subcritical stage of liquid is the time when liquid reaches pressure higher than the critical pressure. The fluids that are used to extract essential oils using this method are water and CO_2. The subcritical state of fluid offers several superior characteristics such as lower viscosity, lower density, and enhanced diffusivity between gas and liquids. This extraction technique is considered the best alternative approach as it enables a fast essential oil isolation process, conducted at a low working temperature. Moreover, it is a costefficient extraction, simple and environmental friendly process **(Zarith et al, 2018).**

II.3.10.1.3. solvent free Microwave extraction

This uses a source of microwave radiation. The plant material is immersed in a microwave-permeable solvent. The microwaves cause the water contained in the plant material to heat up and distension causes the essence pockets to burst. The products released are dissolved in the solvent. This process is faster than conventional extraction systems and the oils obtained are of good quality **(Bougerra, 2019).**

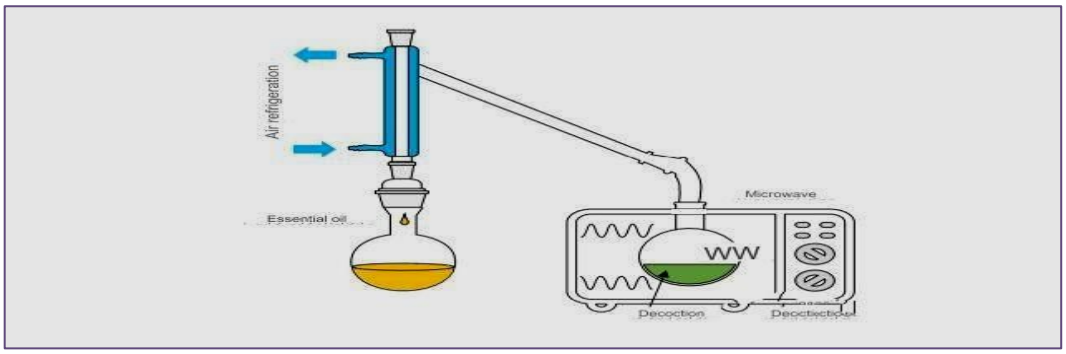

Figure 22. Schematic of the microwave extraction method **(Boufares, 2020).**

II.3.10.1.4. Cold expression

This technique is used to extract the volatile essences contained in citrus pericarps by tearing them open mechanical treatment. It consists of breaking or dilacerating the walls of the contained in the mesocarp located just under the peel of the fruit, the epicarp, to collect the contents which have not the unaltered contents **(Boukhatem *et al.*, 2019).**

Citrus essences have long been extracted by hand, but the mechanisation and industrialisation of the cold expression technique only took place in the early century, in order to reduce production costs and improve yields in the face of rising demand. Increased demand. Recent systems, such as the Food Machinery Corporation-in-line (FMC), allow fruit juice and essence to be extracted almost simultaneously, with no contact between the two **(Boukhatem *et al.*, 2019).**

Figure 23. Extraction by cold expression **(Guerrouf, 2017).**

II.3.10.2. Conventional extraction methods

II.3.10.2.1. Steam distillation

This is one of the official methods for obtaining EO. In this extraction system, the plant material is subjected to the action of a stream without prior maceration. The vapours, saturated with volatile compounds, are condensed and then decanted into the essencier, before

being separated into an aqueous phase (HA) and an organic phase (HE). The absence of direct contact between the water and the plant material, and then between the water and the aromatic molecules, avoids certain hydrolysis or degradation phenomena that could adversely affect the quality of the oil. What's more, the fragrance of the EO obtained is more delicate and distillation, which is regular and faster, means that the top notes of the oil are less intense and rich in esters **(Boukhatem *et al.*, 2019).**

The so-called "head" fractions, highly volatile fragrances due to light molecules, appear first. In most cases, half an hour is enough time to collect 95% of the volatile molecules, which is enough to meet the needs of industry and perfumery, as in the case of lavender. For use in aromatherapy to prolong the operation for as long as necessary in order to recover all the volatile aromatic components **(Boukhatem *et al.*, 2019).**

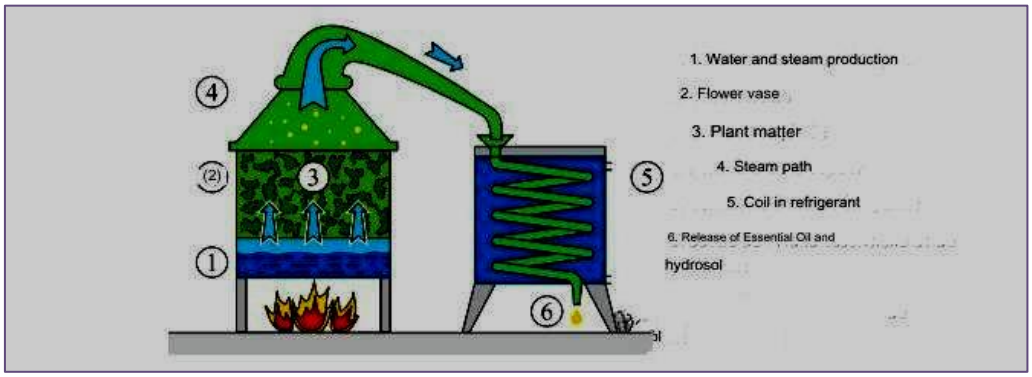

Figure 24. Summary of the principle of steam distillation **(Boufares, 2020).**

II.3.10.2.2. Hydrodiffusion

Hydrodiffusion extraction method is an extraction process in which steam is supplied to a container which holds plant materials. This technique is only applied on dried plant samples that can be damaged at boiling temperature. In the steam distillation process, steam is applied from the bottom of the steam generator, whereas in the hydrodiffusion method, steam is supplied from the top of the generator. This process was carried out at low pressure or vacuum and steam temperature can be reduced below 100°C. This steam diffusion method was further enhanced by adding microwave technology **(Zarith *et al.*, 2018).**

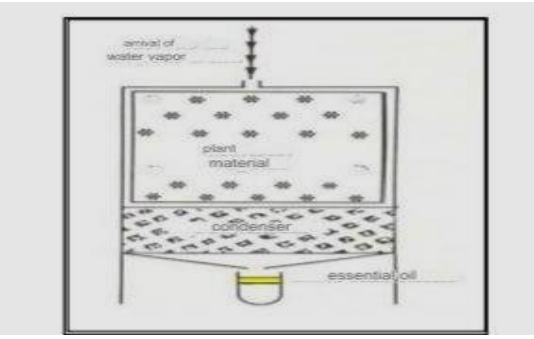

Figure 25. Diagram of the principle of the hydrodiffusion technique **(Abdelli, 2017).**

II.3.10.2.3. Solvent extraction

Solvent extraction was first used on flowers by French chemist and pharmacist Pierre Jean Robiquet in 1835 and quickly became a popular technique. Extraction could take place at room temperature. However, this is a complex process requiring expensive equipment **(Bouyahyaoui, 2016).**

The method is performed in three stages :

- **1st stage:** Solvents such as benzene, petroleum ether and, more recently, hexane have been used to extract the volatile parts of plants. This first stage leads to a mixture of waxes called concretes (50% essential oil and 50% wax). Hexane has considered a safe solvent and used in many food extraction processes. However, it is impossible to remove all the solvent after extraction **(Bouyahyaoui, 2016).**
- **2nd step:** a solvent, usually ethanol, is used to dissolve the wax. This step is repeated several times **(Bouyahyaoui, 2016).**
- **3rd step:** The alcohol/wax mixture is evaporated under vacum. Solvent extraction offers the possibility of obtaining a constant product, but its major drawback is the disadvantage that it is impossible to remove the residual solvents **(Bouyahyaoui, 2016).**

II.3.10.2.4. Hydrodistillation

Hydrodistillation remains the most widely used and fastest extraction technique for obtaining the best yields, without altering the fragile essential oils. Its principle corresponds to heterogeneous distillation involving the application of two physical laws (Dalton's law and Raoult's law).

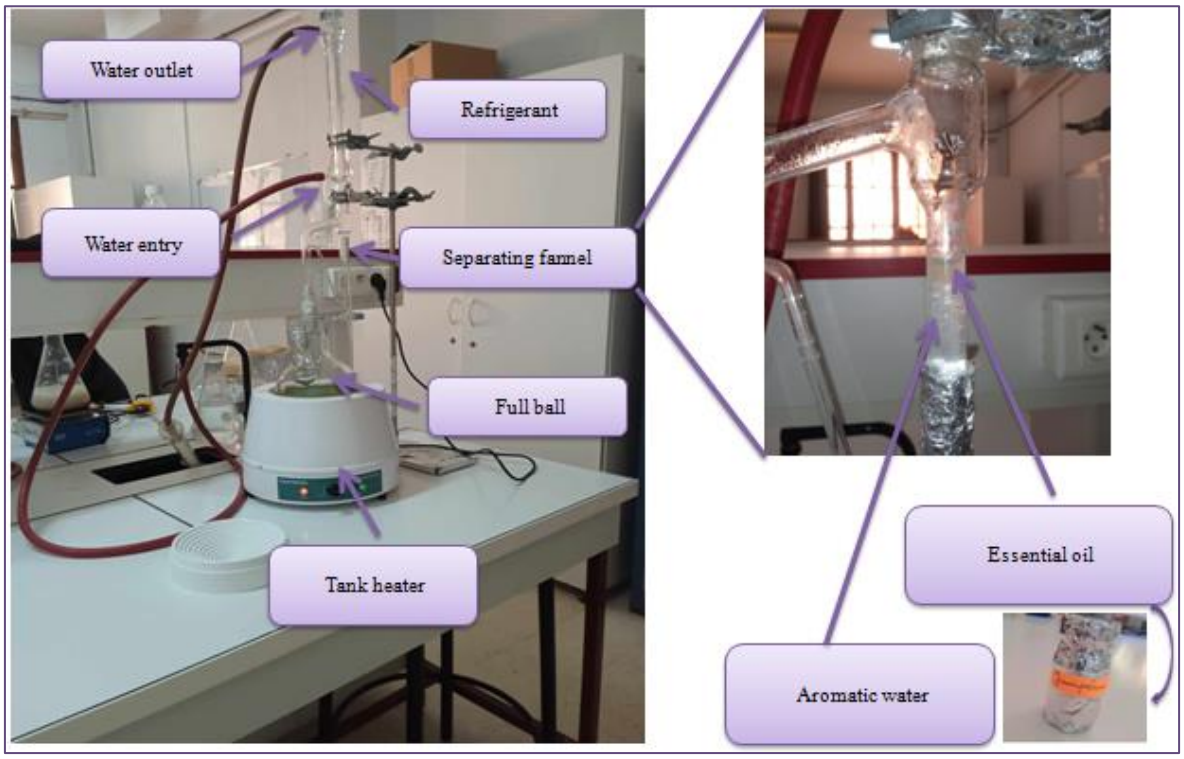

Figure 26. Clevenger type hydrodistillation setup **(personal photo).**

II.3.10.2.5. Extraction and essential oil yield

The extraction was carried out in our microbiology laboratory at the University of Tebessa using a clevenger hydrodistiller **(Dris, 2018).**

The plants were collected in January 2024 in the Tebessa region. After drying the plant material in the open air and in the shade, 100 g of the dry matter from the aerial part of the plant and 1000 ml of distilled water were introduced into a round-bottomed flask with a capacity of two litres, topped by a 60 cm long column. The flask is heated to around 100°C and connected to the rest of the extraction apparatus. The mixture is brought to boiled for 3 hours, during which time the vapour is directed towards the swan neck and then where it condenses rapidly and falls into the settling funnel in the form of oil.

They are then collected and stored at 4°C in the dark in a glass bottle, hermetically sealed and covered with aluminium foil to protect them from air and light. The quantity of oil obtained is weighed to calculate the yield **(Dris, 2018).**

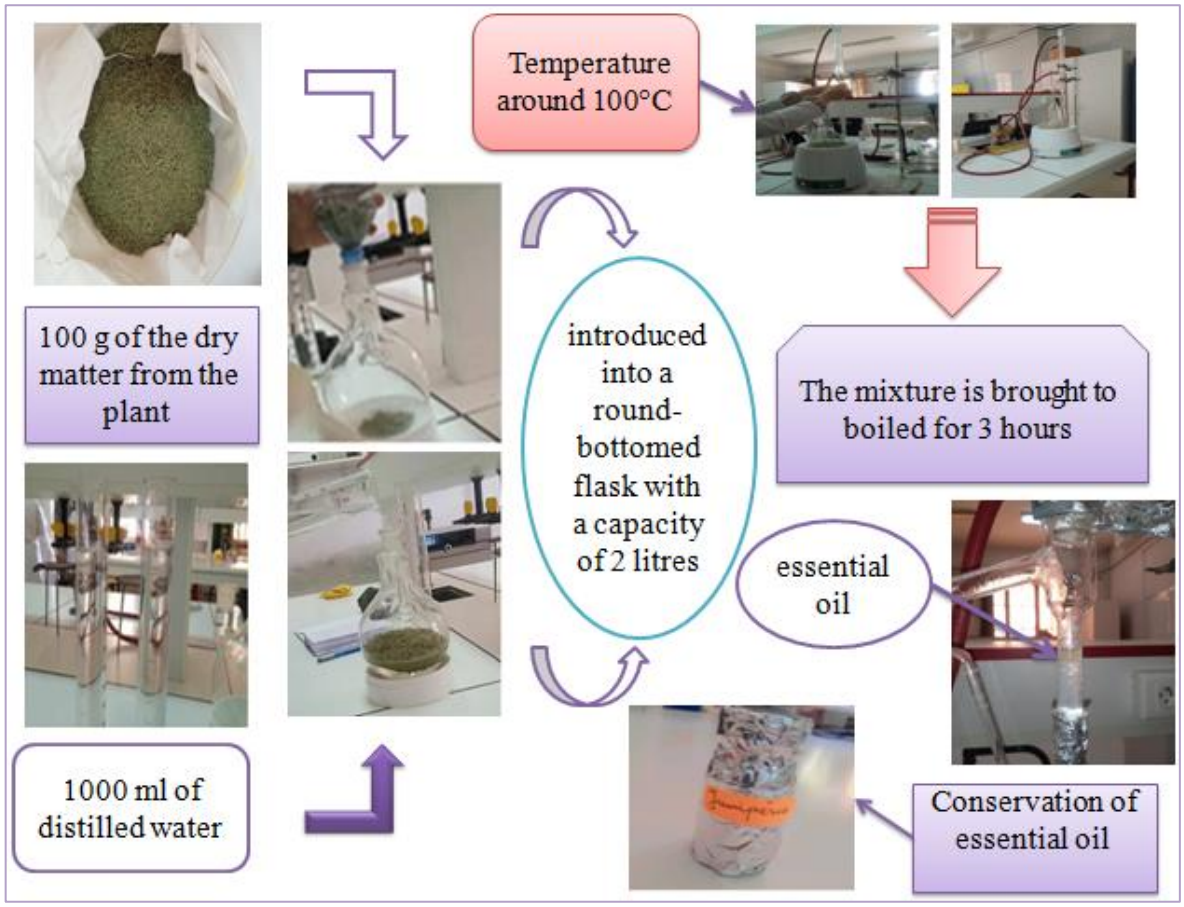

Figure 27. stages of hydrodistillation Extraction method **(personal photo).**

Only the secretory or most concentrated parts of the are harvested at the optimum yield period: before flowering , during and after flowering (seed plants) or after the morning dew (fragile flowers).

The quantities of EO produced by the plants are minimal, resulting in extremely low extraction yields, generally less than 2% Essential oil yield **(Boukhatem *et al.*, 2019).**

The yield of essential oil is calculated as the ratio between the weight of the extracted oil and the weight of the plant's dry matter using the following formula:

$$R = PB / PA \times 100$$

R: oil yield (%)

PA: plant dry matter weight in g

PB: oil weight in g **(Bougeraa, 2019).**

II.3.13. Biomarkeurs assay

The lethal concentrations CL_{25} and CL_{50} of *Juniperus phoneucia* essential oil for the fourth larval stage of *Culiseta longiareolata* are determined by a previous study.

Fourth larvae stage from the control series and those treated with *Juniperus phoneucia* essential oil corresponding to LC25 and LC50 were assayed for a detoxification biomarker, glutathione S-transferase (GST) and an oxidative stress biomarker, catalase (CAT) at different times after treatment: 24, 48 and 72 hours.

II.3.13.1. Glutathione S-transferases assay(GSTs)

The activity of glutathione S-transferases (GSTs) is determined using the method of Habig et al (1974). It is based on the conjugation reaction between the GST and a substrate, CDNB (1-chloro 2, 4 dinitrobenzene) in the presence of a cofactor, glutathione (GSH) and measured at a wavelength of 340 nm in a spectrophotometer.

Newly exuviated fourth stage (L4) larvae of *Culiseta longiareolata* and treated with *Juniperus phoneucia* essential oil at two concentrations, corresponding to LC_{25} and LC_{50}.

The larvae were homogenised in 1 ml of phosphate buffer (0.1 M; pH 6), The homogenate was centrifuged at 13200 rpm for 30 min and the supernatant recovered was used as an enzyme source.

The assay consists of reacting 200 µl of the supernatant with 1.2 ml of the CDNB (1mM)/GSH (5mM) mixture [20.26 mg CDNB, 153.65 mg GSH, 1 ml ethanol, 100 ml phosphate buffer (0.1 M, pH 6)]. The trial was conducted with 4 replicates, each comprising 10 individuals with control series. Absorbance readings were taken every minute for 5 minutes at a wavelength of 340 nm against a blank containing 200 µl of distilled water replacing the quantity of supernatant.

The specific activity is determined according to the following formula:

$$\mathbf{X} = \frac{\Delta Do/min}{9{,}6} \times \frac{Vt}{Vs} / mg\ of\ protein$$

X: millimoles of substrate hydrolysed per minute and per mg of protein (mM/min/mg of protein).

Δ Do: slope of the regression line obtained after substrate hydrolysis as a function of time.

9.6: molar extinction coefficient of CDNB (mM-1cm-1).

Vt: total volume in the tank: 1.4 ml [0.2 ml supernatant + 1.2 ml CDNB/GSH mixture].

Vs: volume of supernatant in the cuvette: 0.2 ml.

mg protein: quantity of protein expressed in mg.

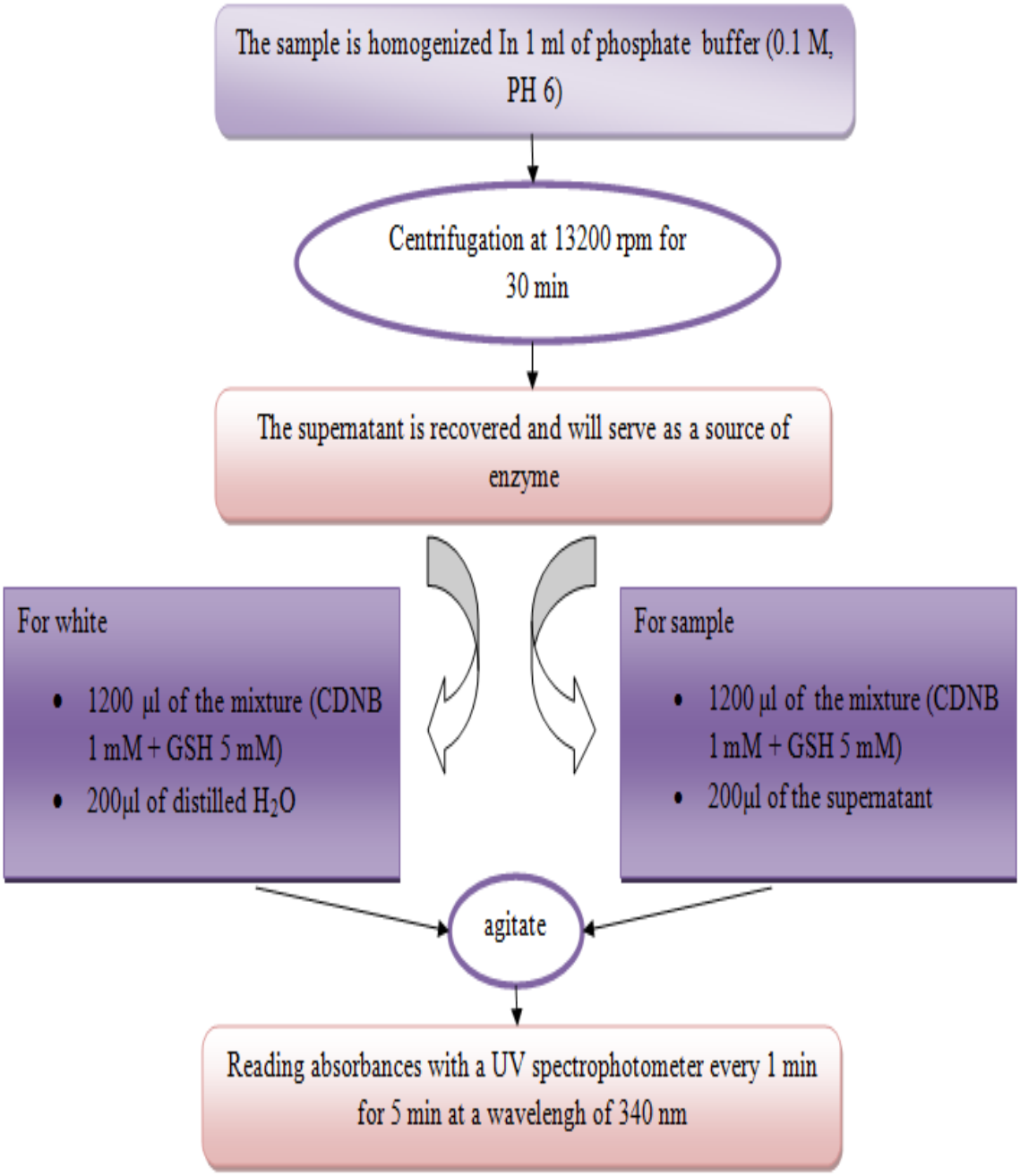

Figure 28. Procedure of GST assay **(personal photo).**

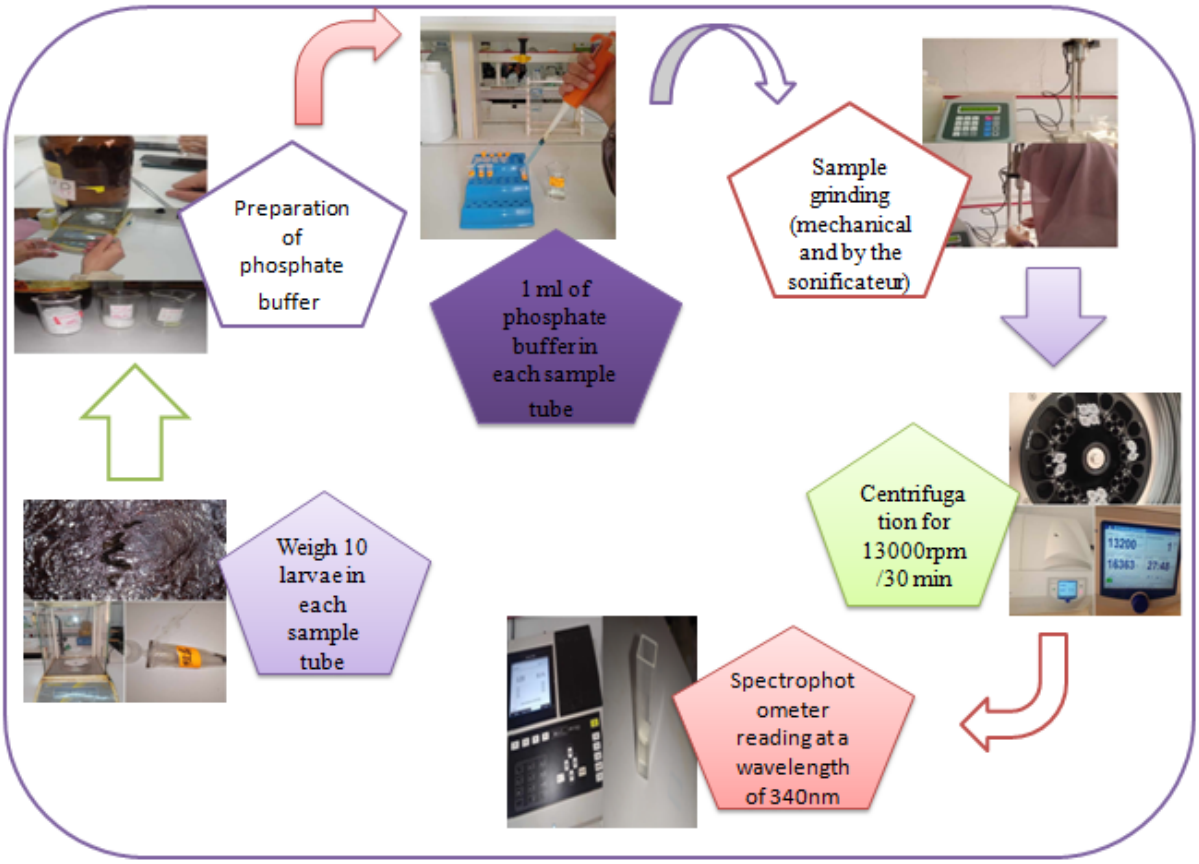

Figure 29. GST assay **(personal photo).**

II.3.13.2. Catalase assay

Catalase (CAT) is determined using Claiborne (1985) technique. This technique is based on spectrophotometric measurement of the reduction of hydrogen peroxide (H_2O_2) into one molecule of oxygen (O_2) and two molecules of water (H_2O) in the presence of the CAT at a UV wavelength of 240 nm, according to the following reaction:

$$H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$$

Control and treated fourth instar C. longiareolata larvae were collected at different times (24, 48, 72 hours), the trial was conducted with 4 replicates each containing 20 individuals. The larvae were homogenised in 1 ml of phosphate buffer (100 mM, pH 7.4), then centrifuged at 13200 rpm for 10 min. The recovered supernatant was used as a source of enzyme. The assay of catalase activity is carried out in a quartz spectrophotometer cuvette at 25°C, on a 50 µl aliquot of the supernatant diluted to between 1 to 1.5 mg protein/ml, either 0.05 to 0.75 mg in the cuvette, to which 750µl phosphate buffer (100mM, pH 7.4) and 200µl H_2O_2 are added. After shaking, a spectrophotometer reading was taken. Absorbance readings were taken every

5 seconds for 30 seconds at a wavelength of 240nm against a blank with 800µl phosphate buffer (100 mM, pH 7.4) and 200µl H_2O_2.

The specific activity was calculated using the following formula:

$$X = \frac{Do\ max \quad Do\ min}{0.04} \quad mg\ de\ proteines$$

X: micromole of substrate reduced per minute and per mg of protein (µM/mn/mg of protein).

D0 max: maximum optical density obtained.

D0 min: minimum optical density obtained.

0.04: molar extinction coefficient of H_2O_2 (cm-1. mM-1).

mg protein: quantity of protein expressed in mg

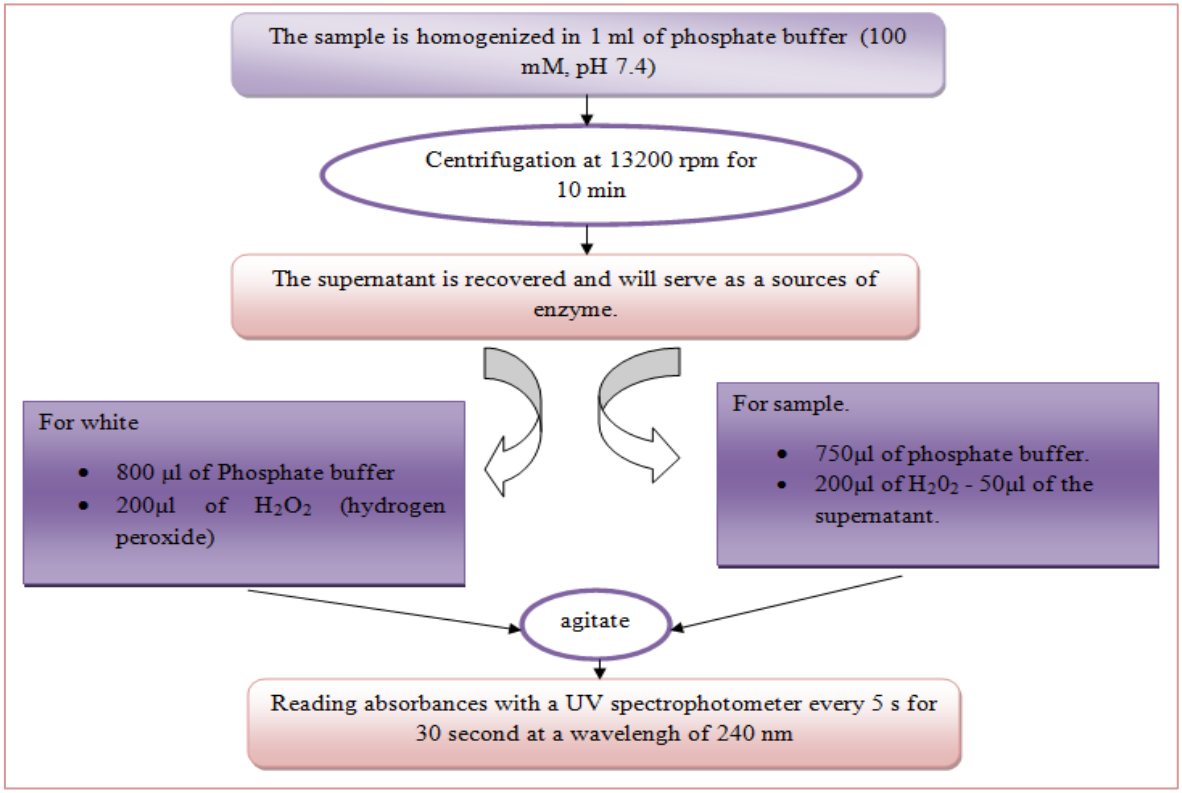

Figure 30. Procedure of catalase assay **(personal photo).**

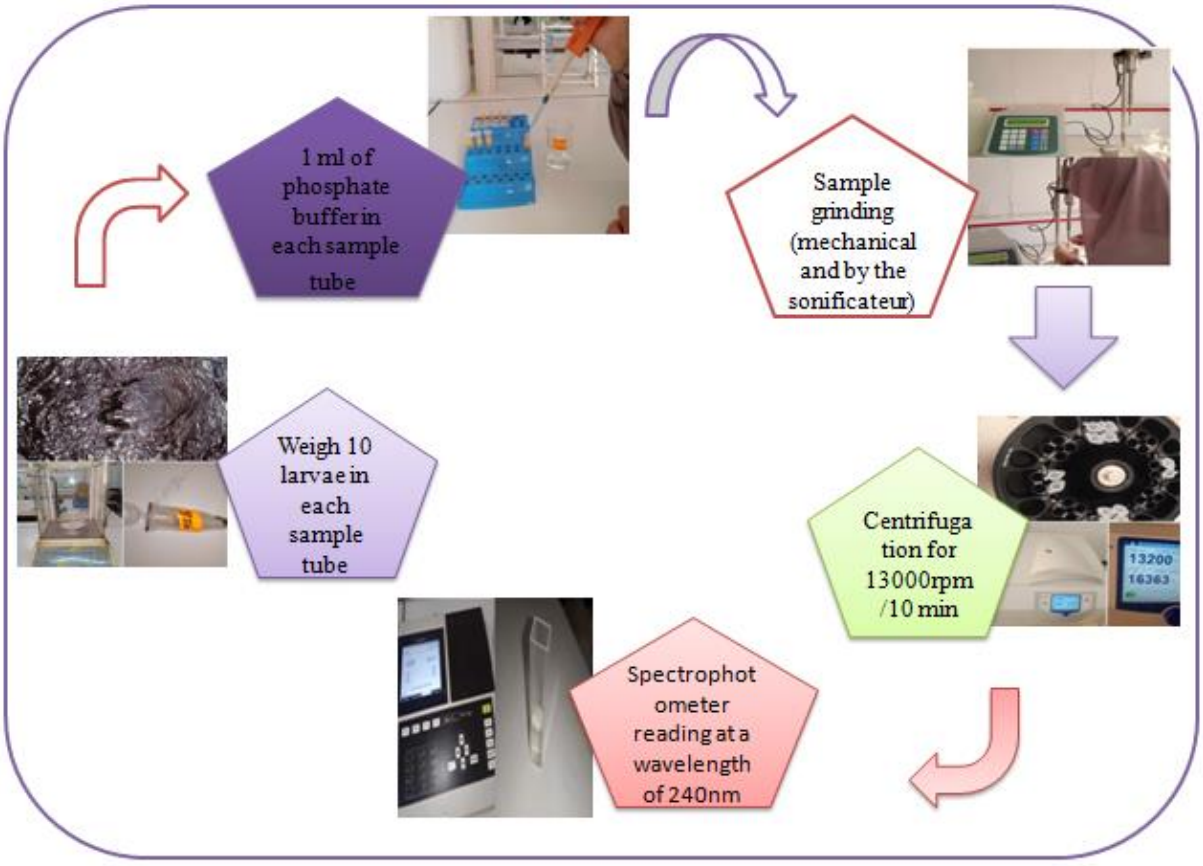

Figure 31. Catalase assay **(personal photo).**

II.3.14. Total protein assay

The protein assay is carried out according to the method of Bradford (1976), in a aliquot of 100 µl to which is added 4 ml of Brilliant Blue Commassie reagent, This reveals the presence of proteins by staining them blue.

The absorbance is read on a spectrophotometer at a wavelength of 595 nm. The calibration range is based on a solution of bovine serum albumin (BSA) at 1 mg/m

Table 06. determination of total proteins in mosquitoes: creation of a protein calibration range of protein

Tubes	1	2	3	4	5	6
BSA stock solution (µl)	0	20	40	60	80	100
Distilled water (µl)	100	80	60	40	20	0
BBC reagent (ml)	4	4	4	4	4	4

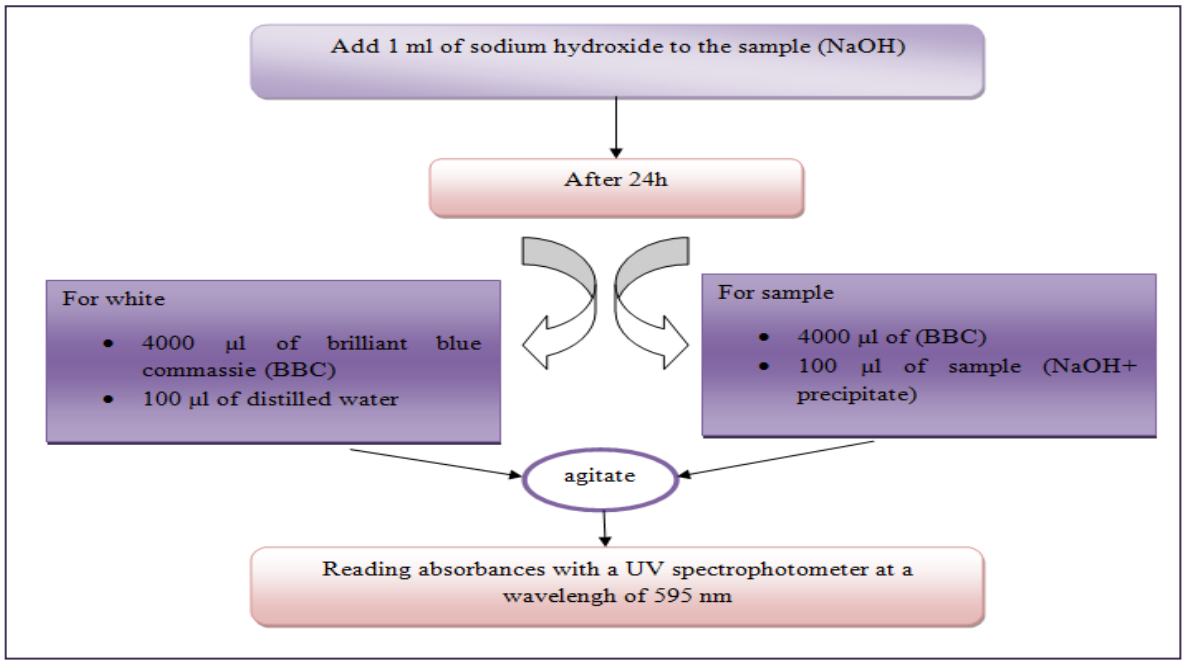

Figure 32. Procedure of total protein assay **(personal photo).**

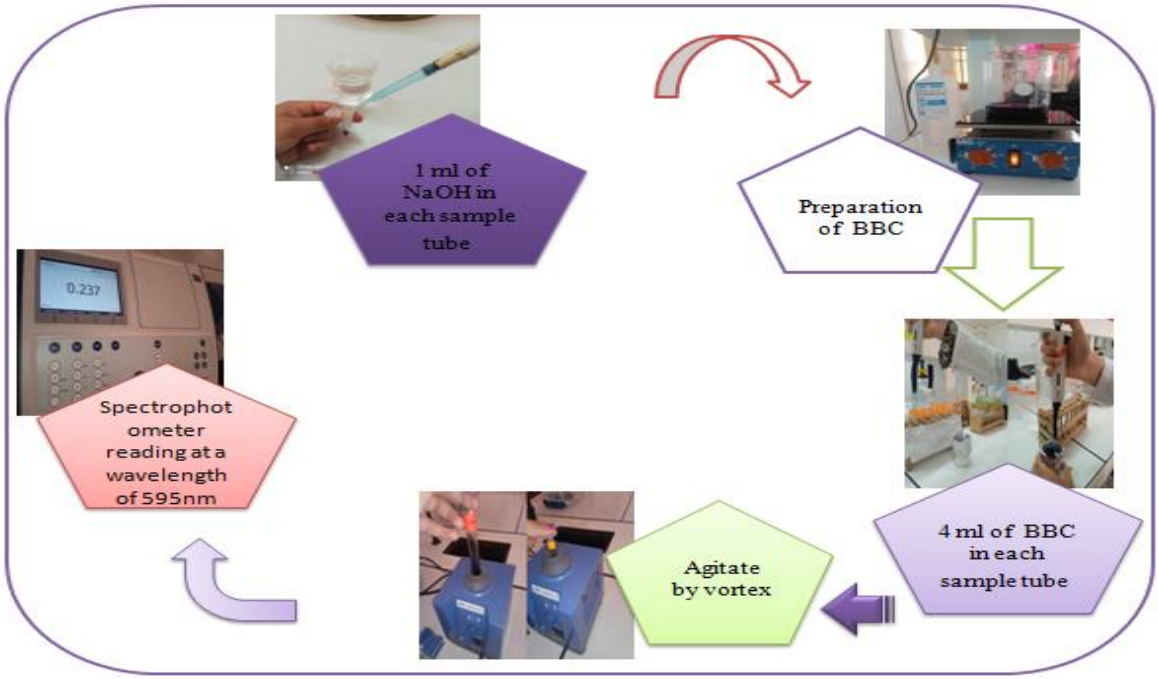

Figure 33. Total protein assay **(personal photo).**

II.3.15. Statistical analysis

The number of individuals tested in each series is given with the results. Data are presented as the mean ± standard deviation (SD). The significance between different series was tested using one-way analysis of variance (ANOVA) at 5% level followed by Tukey's multiple comparaison test. All statistical analyses were performed using Prism 8.0 for Windows (Graph Pad Software Inc., www.graphpad with a significant level p.

ResultS

III. Results

III.1. Yield of *Juniperus phoenicea* essential oil

The hydrodistillation of *J. phoenicea* using an apparatus of the Clevenger gives an essential oil of yellow color, pleasant smell and with a yield 0.56% of dry matter from the aerial part of the plant.

III.2. Effect of *Juniperus phoenicea* essential oil on enzymatic biomarkers

The essential oil of *J. phoenicea* was applied to the newly exuviated L4 larval stage of *Cs longiareolata* at the lethal concentration 25 and 50 (LC25= 5µl and LC_{50}= 9µl.) according to the studies of the previous year with control series. The effect of this oil was evaluated at different periods (24, 48 and 72 hours after treatment).

It was tested on a detoxification biomarker, glutathione S-transferases (GSTs) and an oxidative stress biomarker catalase. The results were expressed in relation to the quantity of proteins (mg) obtained from a reference curve.

III.2.1. Effect on the specific activity of glutathione S-transferases

The specific activity of GSTs (µM/min/mg of proteins) in control and treated *Cs longiareolata* L4 larvae (LC_{25} and LC_{50}) is recorded at 24, 48 and 72 hours after treatment.

The multiple comparison of the means by the Dunnett test shows a significant increase between the control series and the series treated with the essential oil (LC_{50}) in the specific activity of GSTs at 24 hours ($p = 0.000$) and non significant effect at 48 hours and 72 hours ($p>0.05$) and with LC25 at all periods of treatment compared to the control (Figure 34).

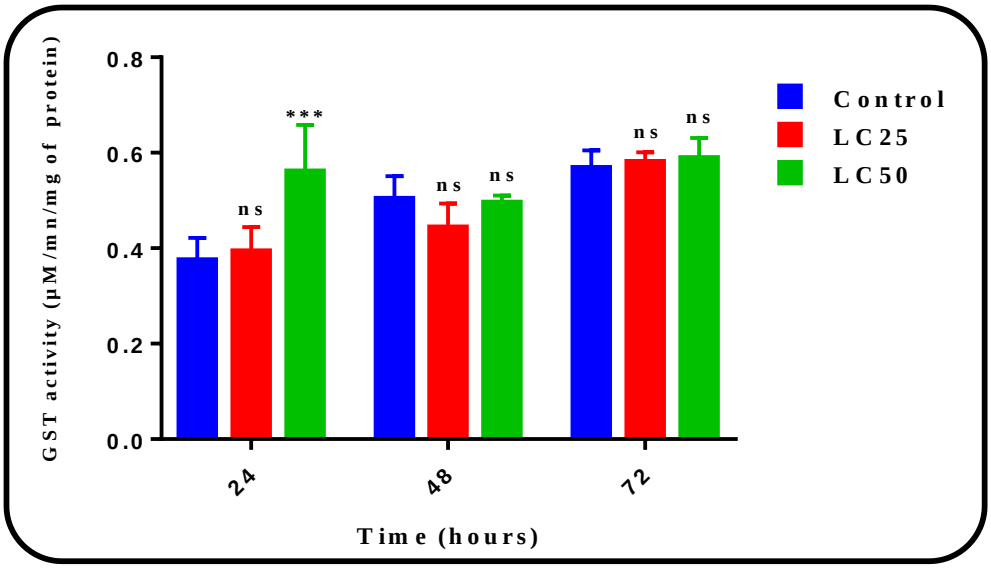

Figure 34. Effect of *J. phoenicea* essential oil (LC_{50}) on the specific activity of GSTs (µM/min/mg of proteins) in *Cs longiareolata* larvae 4 (m ± SD, n=4). (ns non significant difference (p>0.05) ; *** Very highly significant difference (p<0.001) between the control and treated series).

III.2.2. Effect on specific catalase activity

The specific activity of catalase was estimated in the control and treated series with LC25 and LC50.

The multiple comparison of the means by the Dunnett test shows a non significant effect between the control series and the series treated with the essential oil (LC_{25}). The results obtained mark a significant increase after 24 (p=0.003) and 48 hours (p=0.023) in *Culiseta longiareolata* larvae treated with LC_{50} of *J. phoenicea* essential oil compared to the control series (Figure 35). This activity becomes insignificant after 72 hours (P>0.05).

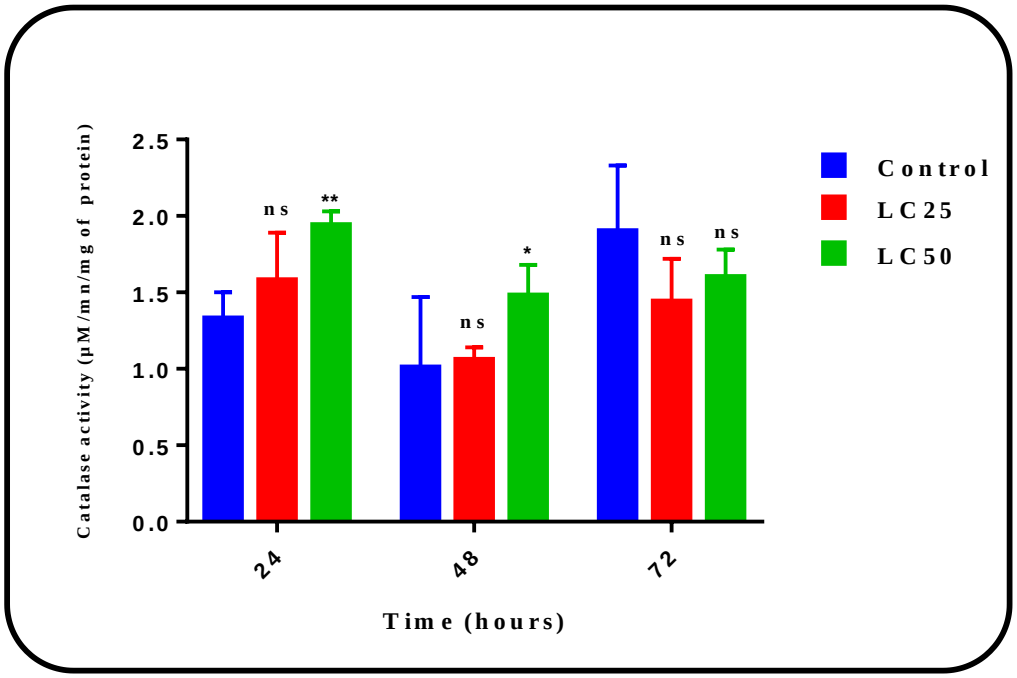

Figure 35. Effect of *J. phoenicea* essential oil (LC_{50}) on specific catalase activity (µM/min/mg protein) in *Cs longiareolata* larvae 4 (m ± SD, n=4). (ns: Non-significant difference ($p>0.05$) *** Very highly significant difference ($p<0.001$) between the control and treated series).

III.3. Effect on total protein content

Total protein content was determined in 4 control and treated *C. longiareolata* larvae at different periods (24, 48 and 72 hours after treatment).

Comparison of the mean values by the Dunnett test shows that the EO extracted from *J. phoenicea* induces a very highly significant reduction in the total protein content of larvae ($p=0.000$) at different treatment periods with both lethal concentrations.

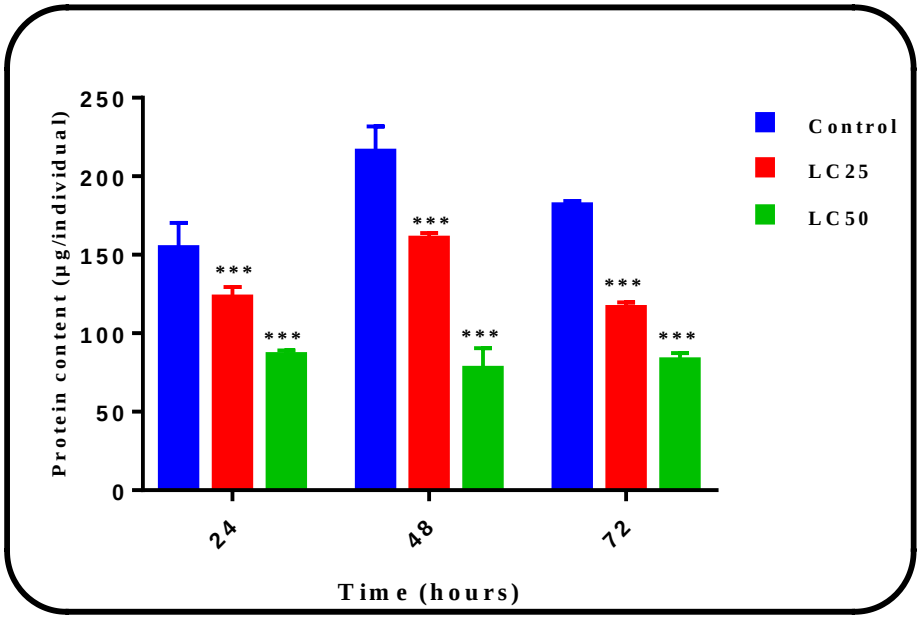

Figure 36. Effect of *J. phoenicea* essential oil (LC_{50}) on protein content (µg/individual) in larvae 4 of *Cs longiareolata* (m ± SD, n=4). (*** Very highly significant difference ($p<0.001$) between the control and treated series).

III.4. Effect of essential oil of *Juniperus phoenicea* on the weight growth of *Culiseta longiareolata*

The essential oil is used at a sublethal concentration (LC_{50}) on newly exuviated fourth instar larvae.

The results of the evolution of the body weight of the individuals during the fourth larval stage studied are mentioned in the (Figure 37) For the control and LC_{50}-treated series, there was a significant increase in body weight from 24 hours to 72 hours.

Comparison of the means between the control and treated series shows that the essential oil of *J. phoenisea* (LC_{25} and LC_{50}) affects the body weight of L4 larvae significantly at 24 h ($p>0.05$; $p=0,031$), 48 h ($p= 0,042$; $p=0,0017$) and 72 h ($p>0.05$; $p= 0.004$) with both concentrations respectively.

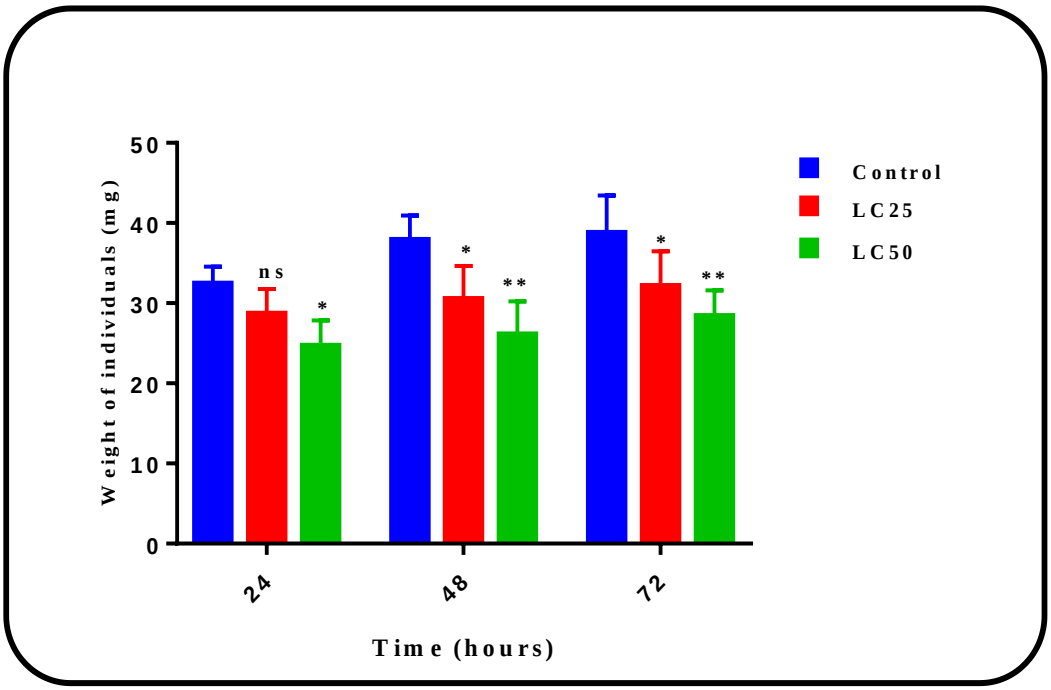

Figure 37. Effect of *J. phoenicea* essential oil (LC_{25} and LC_{50}) on the weight (mg) of 4th instar larvae in *Culiseta longiareolata* (m ± SD, n = 4)

Discussion

IV. Discussion

IV.1. *Juniperus phoenicea* essential oil yield

The yield of essential oil extracted from the leaves of the aerial part of *Juniperus phoenicea* is 0.56%.

In relation to the results of the study carried out, we note that, the yield of essential oil extracted from the dried leaves of *Juniperus phoenicea* according to **(Bouzouita *et al.*, 2008)** harvested in the Medenine region (southern Tunisia) is 0.5%. Almost the same yield from Algerian *Juniperus phoenicea* obtained by steam distillation from the leaves (0.52) according to **(Mazzari *et al.*, 2010)**,These results are similar to our yield study.

The yield of our research is clearly low and lower than those obtained from the same species harvested from different regions of Algeria. We cite the study of **(Ramdani *et al.*, 2013)**, which obtained the following yields from the east of the country: 0.7% (Menâa station in Batna), 0.75% (Boussâada station in M'sila), 0.8% (Boutaleb station in Sétif) and 0.92% (T'Kout station in Batna and Elhadjab station in Biskra). According to **(Akermi *et al.*, 2017)** and **(Boukhaloua *et al.*, 2022),** the yield of essential oils extracted from the aerial part of *juniperus phoenicea* from south-west and western Algeria is 2.004% and 0.8% respectively, which is still higher than that obtained from our study.

Another study by **(Bekhechi *et al.*, 2012),** based on several stations throughout the country, showed highly variable levels of essential oil, as follows: in the west: 0.32 - 0.89% (Aïn Sefra station in Naâma) and 0.79 - 1.32% (Honaïne station in Tlemcen), in the centre: 0.87 - 1.52% (Berine station in Djelfa) and in the east: 0.59 - 1.13% (Fesdis station in Batna). However, according to the same study, the plant taken from the Ben M'Hidi station in El Tarf, in the east of the country, gave lower yield than those of our which is 0.034 - 0.060%. Numerous other studies carried out on *Phoenician juniper* from Portugal, Spain, Greece, Egypt, Tunisia and Morocco, show highly variable yields of essential oil . They are respectively 0.41%, 0.66% and 0.58%. **(Robert *et al.*, 1996),** 0.36% **(El-Sawi *et al.*, 2007)**, 0,70 **(Akrout, 2004)** and 1.91% **(Achir et al, 2021).**

Several factors influence the variation in yield **(Akermi *et al.*, 2017),** in particular, the choice of harvesting, this is crucial in terms of the yield and quality of the essential oil.

Other factors can also have an influence, such as the geographical area of collection, climate, plant genetics, the organ used, the stage of development, the degree of freshness, the drying period and the extraction method and equipment used **(Abdelli, 2017).**

IV.2. Effect of EO on biomarkers

It is often necessary to adopt a multifactorial approach to assess the ecotoxicological impact of contaminants, using different biological markers. As far as biochemistry is concerned, there are various types of biomarkers that are classified according to their cellular function **(Badiou, 2007).**

The main biomarkers used are enzymatic markers such as catalase and glutathione S-transferase **(Dris, 2018).**

Despite the chemical diversity of insecticides and the biological diversity of insects, a number of resistance mechanisms are capable of detoxifying insecticides into less toxic metabolites, These mechanisms are :

- ✓ Behavioural resistance is observed at the level of the insect, which displays a different behaviour, preventing the toxicant from acting.
- ✓ Physiological resistance is expressed at the level of tissues and organs, characterised by reduced penetration or increased excretion of insecticides.
- ✓ Biochemical resistance occurs at the cellular level. On the one hand, it consists of an increase in the enzymatic activity of the detoxification systems (Cytochromes P450, GSTs and carboxylesterases) and, secondly, a reduction in the affinity of the sites of action for insecticides **(Dris, 2018).**

To help understand these mechanisms, we assessed the effect of *Juniperus phoenicea* EOs on the activity of biomarkers of detoxification and oxidative stress, glutathione s-transferases and catalase in the mosquito *C. longiareolata* at different periods after treatment: 24, 48 and 72 hours.

IV.2.1. Effects of Eo on the specific activity of GST

Glutathione S-transferases (GSTs) are a diverse family of enzymes involved in a wide range of biological processes **(Ranson & Hemingway, 2005),** are multifunctional enzymes involved in phase II detoxification, catalysing the conjugation of reduced glutathione to a large number of xenobiotics **(Boyer, 2006).** They are mainly located in the cytoplasm of cells, fat bodies and wing muscles. They play an important role in the detoxification of xenobiotics

by catalysing the conjugation of these substances with the thiol group of endogenous glutathione **(Bougerra, 2019).** This results in the synthesis of mercapturic acid, which is then easily eliminated. The major role of GSTs is therefore to convert lipophilic compounds into hydrophilic molecules that are easily excreted **(Habig *et al.*, 1974).** GSTs enable the development of resistance to chemotherapeutic agents, insecticides, herbicides and microbial antibiotics. They play an important role in stress physiology, intracellular transport and various biosynthetic pathways **(George, 1994; Sun *et al.*, 2001).**

In this study, their multiple comparison by Dunnett's test showed a significant increase between the control series and the essential oil-treated series (LC50) in GST-specific activity at 24 hours ($p=0.000$) and a non-significant effect at 48 hours and 72 hours ($p>0.05$).) and with LC25 in all treatment periods compared to control.

The increase in GST activity is reflected in the the detoxification process. Excessive enzyme production may also be due to changes in the regulatory genes that control enzyme expression levels and to an increase in the number of copies of the genes coding for these enzymes **(Cedric, 2008).**

The increased activity in GST is in agreement with those reported by **(Shahat *et al.*, 2020)** in order to investigate the effect of median lethal concentration (LC50) of essential oil from leaves of Origanum syriacum, Pergularia tomentosa, Senna italica, and Otostegia fruticose against biological aspects of the 3rd instar larvae ofCulex pipiens, in culesita longiareolata treated with essential oil Artemisia Absinthium **(Redjel, 2023),** in culex pipiens treated with artemisia compestris essential oil **(Sehili et Boualleg, 2021),**in. Cx. pipiens treated with basilic **(Dris *et al.*, 2017 b),** in Culex and Culiseta treated with lavender and mint **(Dris, 2018),** in Xanthogaleruca luteola treated with neem oil **(Valizadeh *et al.*, 2013),** in culex pipiens treated with Rasmarinis officinolis (**Boukhroufa et Hafiane, 2021),** in Zonocerus variegatus exposed to Ocimum gratissimum extract **(Adeyi *et al.*, 2014),** in Glyphodes pyloalis treated with Artemisia annua extract **(Khostavi *et al.*, 2011).**

IV.2.2. Effect of EO on the specific activity of catalase

Antioxidant catalases are present throughout aerobic biological systems, and many anaerobic aerotolerant enzymes contribute to maintaining cellular equilibrium and adaptation to oxidative stress. Metalloenzymes play a crucial role in endogenous antioxidant defence by catalysing the degradation of H_2O_2. In this way, they protect cells from the harmful consequences of H_2O_2. Peroxisomes contain haemoproteins and synthetic compounds that neutralise ROS superoxide and H_20_2 hydrogen peroxide into less reactive H_2O gas and oxygen depending on availability **(Fares & kharchi, 2023).**

In the present study, The multiple comparison of the means by the Dunnett test shows a non significant effect between the control series and the series treated with the essential oil (LC25). The results obtained mark a significant increase after 24 (p=0.003) and 48 hours (p=0.023) in *Culiseta longiareolata* larvae treated with LC50 of *J. phoenisea* essential oil compared to the control series (Figure…). This activity becomes insignificant after 72 hours (P>0.05).

The increase in catalase activity translates into the implementation of the detoxification process, which is a form of defence by the insect against the pesticide **(Dris, 2018).**

Our results are consistent with those of **(Krzyzowski *et al.*, 2020)** of Callosobruchus maculatus larvae treated with Rosmarinus officinalis Essential Oil, also with (Bouabida,2013) of fourth instar larvae in Culiseta longiareolata and Culex pipiens treated with spiromesifene at two lethal doses: (LD50 and LD90) , with those of **(Pinho *et al.*,2014),** which demonstrated an increase in CAT activity in flies treated with Psidium guajava EO, and in *C. longiareolata* and *C. pipiens* treated with *O. Basilicum* **(Dris, 2018)**, An increase in the CAT rate of the order of 30.29% and 38.82%, was observed after 24 h of exposure to the LC50 of Boswellia carterii EO in *C. chinensis* and *C. maculatus* respectives **(Kiran *et al.*, 2017).**

On an other hand, a decrease in CAT activity was observed in R. dominica treated with Gaultheria procumbens EO **(Kiran & Prakash, 2015)** , this decrease induces an accumulation of H2o2 toxic to the cell which can lead to cytotoxicity **(Dris, 2019., Zini *et al.*, 2002).**

IV.3. Effect of EO on the specific total protein

In physiological studies, the determination of the total protein is important, are major biochemical components necessary for an organism to develop, grow and perform its vital activities **(Yazdani et al, 2014).** are an important source of nitrogen and amino acids, which are important regulators and resistors of environmental stress factors **(Bilbao *et al.*, 2020).** Proteins play an important role in metabolism, acting as catalysts for chemical reactions and are involved in tissue structure, storage and transport of small molecules and ions, cell communication, movement through muscle contraction and the body's defence against external factors **(Rodrigue *et al.*,2018).**

The protein assay was carried out in the whole body of fourth instar *C.longiareolata* larvae.The essential oil of *Juniperus phoenicea* (LC25 and LC50) induced a very highly significant reduction in the total protein content of larvae (p=0,000) at different treatment periods in treated compared with controls. Protein reduction is a frequent phenomen in insects treated with toxic products **(Nathan *et al.*, 2008).**

The reduction in protein content in the larvae was attributed to one or a combination of factors, like a reduction in the synthesis of proteins or an increase in the breakdown of proteins to detoxify the active principles present in the plant extracts or essential oils **(Vijayaraghavan *et al.*, 2010).**

The breakdown of proteins into amino acids aims to facilitate their incorporation into the Krebs cycle as ketone acids in order to compensate for the low energy levels caused by stress **(Dris, 2018)**, In addition, the reduction in protein reserves may also be due to the insect's physiological adaptation to a state of stress caused by insecticides **(Ribeiro *et al.*, 2001).** Also, it could be due to their metabolic degradation, altered incorporation of amino acids into polypeptide chains or inhibition of protein synthesis **(Bouabida *et al.*,2017).**

Protein synthesis and accumulation should be one of the main priorities of larval growth. It has been shown to be a useful indicator of the nutritional and growth status of larvae. **(Díaz *et al.*, 2008).**

The results obtained in our research are similar to those obtained in the same species of mosquito C.longiareolata treated with basilic **(Bouzidi & Ziani, 2015),** culex pipiens treated with Eucalyptus globulus **(Kheled & Dib, 2015),** Ocimum basilicum (Sayada & Messai **(2015),** and Lavandula dentata **(Sahbi & Aouni, 2015),** all showed a decrease in protein content over the periods tested. However, an increase in protein content was reported in Cx pipiens after treatment with Ocimum basilicum (Khamene, 2014), in white grubs, rhizotrogini after treatment with extracts of Nerium oleander (Apocynaceae) **(Madaci *et al.*, 2008)** and in Culiseta longiareolata treated with Lavandula dentata **(Gouasmia & Bouchagoura, 2013).**

Variations in protein values can be explained by changes in environmental conditions such as temperature, salinity and nutrients **(Banerjee *et al.*, 2009).**

IV.4. Effect of EO on the specific weight growth

The study of new pest control strategies using insect growth inhibitors is an environmentally friendly option, actions harmful to insect life, actions that do not cause immediate mortality during the treatment period, but cause mortality and morphogenetic anomalies in the post-treatment period **(Kharchi et fares, 2023).**

The amount of blood consumed in each meal, the amount used in metabolic pathways and the number of eggs that reach maturity can all be affected by the mosquito's body size. The presence of insect habitat, environmental factors and, above all, the hereditary characteristics of each species affect body weight. Studies have shown that the energy reserves of mosquitoes depend on their size **(Djedouani, 2023).**

Research into bio-pesticides of plant origin has shown that they have a deleterious effect on the growth and development of insects, by reducing the weight of their young, of larvae, pupae, adults and lengthening developmental stages**,(Dris, 2018).**

In the present study, the comparison of the means between the control and treated series shows that the essential oil of *J. phoenisea* (LC25 and LC50) affects the body weight of L4 larvae significantly (a reduction on weight growth) at 24 h ($p>0.05$; $p=0,031$), 48 h ($p= 0,042$; $p=0,0017$) and 72 h ($p>0.05$; $p= 0.004$) with both concentrations respectively. our results are similar to those obtained by **(Bouzida *et al.*, 2022),** in the same species of *C. longiareolata* treated with the essential oil of the plant Artemisia absinthium which causes a reduction in the weight and volume of the species, Also (Tine Jabbar, 2009) revealed that halophenoside applied to the third and fourth instar larvae of *Cs.longiareolata,* disturbs the morphological parameters of the individuals.

The same observation was made in the work of **(Bouabida, 2017)** after the use of spiromycefin. According to **(Degaichia& sehailia, 2017),** the application of essential oils extracted from Eucalyptus globulus (LC25 and LC50) caused a decrease in thorax width, body volume and weight of the L4 in the same species of mosquito *Cs. longiareolata*. In the same results with **(Dris, 2018),** The application of essential oils extracted from the plants Mentha piperita, Lavandula dentata and Ocimum basilicum on the larvae of *c. longiareolata* and culex pipiens showed a reduction in the biometric parameters, body volume, of the larvae, pupae and male and female adults.

The results of Qin et al **(2010)** showed that Piper sarmentosum EO and myristicin (a major compound) had an inhibitory effect on the growth and development of Brontispa longissima (Coleoptera: Hispidae) with a marked effect of myristicin.

Conclusion

V . Conclusion and perspectives

The presence of mosquito species is a serious health problem, and because of the dangers associated with the use of chemical insecticides and their harmful impact on health and the environment, the use of natural alternatives to synthetic insecticides such as essential oils is proving necessary.

The essential oil of *Juniperus phoeneucea* belonging to the Cupressaceae family has a yield of 0.56% of the aerial part of the dry matter obtained by a Clevenger type hydrodistiller. The essential oil of *Juniperus phoeneucea* is tested on newly exuviated fourth instar larvae of *Culiseta longiareolata*, the most abundant mosquito species in the Tébessa region.

Their effects were evaluated on several aspects: biomarkers, biochemical, morphometric, with two lethal concentrations (LC50 and LC25).

The essential oil of *Juniperus phoeneucea* treated on *Culiseta longiareolata* shows an increase in the specific activity of a detoxification biomarker GST and an oxidative stress biomarker catalase at different treatment periods of the treated series compared to the controls.

Exposure of an organism to an essential oil can modify the synthesis of certain metabolites and disrupt its functionality. *Juniperus phoeneucea* essential oil marked a highly significant decrease in total protein cough of treated series compared with the control at different treatment periods 24 h, 48 h and 72.

Juniperus phoeneucea essential oil affects the weight growth of individuals, significantly reducing the body weight of *Culiseta longiareolata* larvae in the treated series compared with the control.

Juniperus phoeneucea essential oil with superior larval insecticidal properties could be an alternative to synthetic insecticides in the control of *Culiseta longiareolata* mosquitoes. This study, based on the use of aromatic plants as insecticides, opens up wide prospects in the field of fundamental knowledge on the one hand, and in the applied field on the other.

In the future, it would be interesting to supplement the present work with: evaluation of the effect on other biomarkers such as MDA or acetylcholine esterase, evaluation of antioxidant activity, identification of the active compounds responsible for the repellent effect, evaluation of efficacy on other mosquito species.

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Intitulé : Effet evaluation of Juniperus phoenicea essential oil on certain Biomarkers in mosquito larvae.

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Département de: Sciences exactes et sciences de la nature et de la vie
Filière : Biologie appliquée
Spécialité : Bio chimie appliquée

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Formulaire de levée de réserves après soutenance d'un Mémoire de Master

Données d'identification du candidats (es) :

Nom et prénom du candidat : Mansouri Wiam

Intitulé du Sujet : Effect evaluation of Juniperus phoenicea essential oil on certain Biomarkers in mosquito larvae

Données d'identification du membre de jury :

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grade : Pr

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المكتبة الجامعية المركزية

معلومات حول الأطروحة أو المذكرة

الاسم: wiam

اللقب: Mansouri

الكلية: العلوم الدقيقة وعلوم الطبيعة والحياة

القسم: Biologie appliqué

التخصص: Biochimie appliqué

المستوى: Master

عنوان المذكرة أو الأطروحة:

Effect evaluation of juniperus phoenicea essential oil on certain biomarkeurs in mosquito larva.

المؤطر: Dris. D

تاريخ المناقشة (اليوم والشهر والسنة): 06/06/2024

السنة الجامعية: 2024/2023

الملخص كاملا بجميع اللغات المتوفرة

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الملخص:

في إطار البحث عن طرق فعالة للمكافحة البيولوجية ضد البعوض، تم اختبار زيت أساسي مستخرج من نبات العرعر الفينيقي المزروع في ولاية تبسة (شمال شرق الجزائر) بتركيزات قاتلة مختلفة CL25 وCL50 على يرقات العمر الرابع لحشرة (L 4) C. longiareolata التي تم جمعها من المناطق غير المعالجة بولاية تبسة، تحت ظروف مخبرية حسب توصيات منظمة الصحة العالمية.

تم فحص التأثيرات على اثنين من المؤشرات الحيوية الانزيمية(Glutathion-s-transferase، وcatalase) في أوقات مختلفة، 24 ساعة، 48 ساعة، و72 ساعة.

أظهر تحديد محصول استخلاص الزيت العطري عن طريق التقطير المائي لألوراق المجففة عائدًا قدره 0.56٪.

تكشف نتائج المؤشرات الحيوية عن تحفيز نظام إزالة السموم من زيت Juniperus phoenicea الأساسي من خلال زيادة نشاط كال من GST و Catalase مقارنةًبالضوابط، باإلضافة إلى انخفاض كبير في مستويات البروتين الكلي بتركيزات)LC25(و)LC50(في فترات المعالجة المختلفة.

أثر الزيت العطري للعرعار الفينيقي)تركيزات LC25، LC50(على نمو وزن أنواع البعوض مع انخفاض كبير في وزن جسم يرقات C. longiareolata مقارنةًبالضوابط في فترات المعالجة المختلفة.

يعد البحث في المنتجات الطبيعية المستخرجة من النباتات القادرة على التحكم في أعداد longiareolata Culiseta أمرًا مهمًا للغاية ألنه يمكن أن يساعد في الحد من انتشار المرض من هذا الناقل. وفقًا للبيانات المستمدة من هذه الدراسات، يمكن استخدام زيت Juniperus phoenicea ألساسي كمبيد حشري حيوي وكبديل للمنتجات الاصطناعية في مكافحة البعوض.

الكلمات المفتاحية : البعوض، C. longiareolata ، زيت عطري، Juniperus phoenicea ، المؤشرات الحيوية

Abstract

As part of the search for effective biological control methods against mosquitoes, an essential oil extracted from the plant of Juniperus phoenicea cultivated in the wilaya of Tebessa (northeastern Algeria) was tested at different lethal concentrations CL25 and CL50 on fourth instar larvae of Culesita longiareolata (L 4) collected from untreated areas of Tebessa, under laboratory conditions according to .the recommendations of the World Health Organisation

The effects were examined on two enzymatic biomarkers (Glutathione-s-transferase, and catalase), total protein content and the weight growth of Culesita longiareolata at different treatment times, 24h, 48h, .and 72h

The determination of the essential oil extraction yield by hydrodistillation of the dried leaves showed a .yield of 0.56%

Biomarker results revealed an induction of the detoxification system of Juniperus phoenicea essential oil via an increase in GST and catalase activity compared to controls, as well as a significant decrease in .total protein levels with both CL25 and CL50 concentrations at different treatment periods

Juniperus phoenicea essential oil (LC25,LC50) affected the weight growth of the mosquito species with a significant reduction in the body weight of C. longiareolata larvae compared to controls at different .treatment periods

Research into natural products extracted from plants capable of controlling populations of Culiseta longiareolata is very important as it can help to reduce the spread of the spread of the disease from this vector. According to the data from these studies, Juniperus phoenicea essential oil can be used as a bio- .insecticide and as an alternative to synthetic products in mosquito control

Key words: Mosquito, Culiseta longiareolata, Essential oil, Juniperus phoenicea, Biomarkers

Résumé:

Dans le cadre de la recherche de méthodes efficaces de lutte biologique contre les moustiques, une huile essentielle extraite de la plante de Juniperus phoenicea cultivée dans la wilaya de Tebessa (nord-est de l'Algérie) a été testée à différentes concentrations létales CL25 et CL50 sur des larves de quatrième stade de Culesita longiareolata (L 4) collectée dans les zones non traitées de Tébessa, dans des conditions de laboratoire selon les recommandations de l'Organisation Mondiale de la Santé.

Les effets ont été examinés sur deux biomarqueurs enzymatiques (Glutathion-stransférase et catalase), le taux de protéines et sur la croissance pondérale des Culiseta longiareolata à différents périodes de traitement 24h, 48h et 72h.

La détermination du rendement d'extraction des huiles essentielles par hydrodistillation des feuilles séchées a montré un rendement de 0,56%.

Les résultats des biomarqueurs révèlent une induction du système de détoxification de l'huile essentielle de Juniperus phoenicea via une augmentation de l'activité GST et catalase, ainsi que le taux de protéine total est diminué significativement avec les deux concentration CL25 et CL50 a différents périodes de traitement.
L'huile essentielle de Juniperus phoenicea (CL25, CL50) a affecté la croissance
pondérale des espèces de moustiques avec une réduction significative du poids corporel des larves de C. longiareolata par rapport aux témoins à différentes périodes de traitement.

La recherche sur les produits naturels extraits de plantes capables de contrôler les populations de Culiseta longiareolata est très importante car elle peut contribuer à réduire la propagation de la maladie causée par ce vecteur. Selon les données de ces études, l'huile essentielle de Juniperus phoenicea peut être utilisée comme bio-insecticide et comme alternative aux produits de synthèse dans la lutte contre les moustiques.

Mots clés : Moustique, Culiseta longiareolata, Huile essentielle, Juniperus phoenicea, Biomarqueurs