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Thesiswith a view to obtaining a Master LMDDiploma
Domain : Science of Natural and Life
Sector : AppliedBiology
Speciality: AppliedBiochemistry

Theme

Effect of *Ruta montana* essential oil on *Culiseta longiareolata* larvae: Toxicity and Biomarkers.

Presented by
Messai Aasma **Guerti Youcef**

Before the jury

Pr. Bouabida Hayette	**Pr**	Larbi Tebesi University -Tebessa-	**President**
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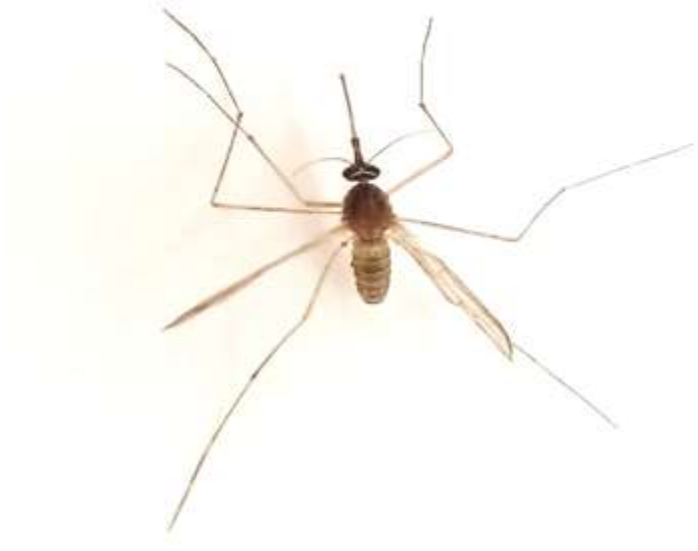
Dr. Dris Djemaa	**MCA**	Larbi Tebesi University -Tebessa-	**Promoter**

Date of discussion : 06/06/2024

Academic year : 2023/2024

بسم الله الرحمن الرحيم

"إِنَّ اللَّهَ لا يَسْتَحْيِي أَنْ يَضْرِبَ مَثَلًا مَا بَعُوضَةً فَمَا فَوْقَهَا" [البقرة:26]



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إهداء

كل الحروف تعجز عن إيجاد الكلمات المناسبة...
كل الكلمات تعجز عن التعبير عن الامتنان,
...الحب، الاحترام

إلى من حقت فيهم الطاعة بعد الله سبحانه وتعالى ورسوله الكريم

إلى روح والدي الطاهرة " **علي** " إلى من علمني أن أعطي بلا انتظار إلى من أحمل اسمه بفخر واعتزاز أسأل الله

أن يتغمدك برحمته وتبقى كلماتك نجوماً ترشدني اليوم وغداً وإلى الأبد .

إلى من لا نور الا نورها ولاعظمة الا عظمتها إلى من حملتني وهن على وهن وكان دعائها وحنانها بلسم جراحي إلى منبع

الحنان ونور العينان وأول من نطق بيها اللسان " **أمي نادية** " حفظها الله وأطال في عمرها .

إلى الروح الطيبة التي أنارت ولازالت تنير دربي دائما بوجودها في عقلي وقلبي

أخي الغالي " **شهاب** " رحمه الله وأسكنه فسيح جناته .

إلى من ساندوني حق السند ومنحوني الثقة والأمل خطوة بخطوة طيلة مسيرتي الدراسية وكان لهم بالغ الأثر في كثير من

العقبات سندي وعضدي ومشاطري أفراحي وأحزاني أختي **ريهام** وأخي **صهيب** حفظهم الله ورعاهم .

إلى شريكي ، أنا ممتنة جدا ولا يمكنني أن أشكرك بما فيه الكفاية على لطفك وكرمك ومساعدتك التي لا تقدر بثمن. أتمنى أن تجدوا في هذا العمل تعبيراً عن احترامي العميق ومودتي الصادقة.

إلى كل من ساهم من قريب أو بعيد في إنجاز هذا العمل ولو بكلمة أو بدعوة صالحة.

Dedication 02:

I dedicate this work to our brothers in Gaza,
praying to God to release their families and have mercy on their dead.
to the memory of my father, who instilled in me a lifelong love of learning. Though you are no longer here, your spirit continues to inspire me every day.
to my mom, who have always believed in me and encouraged me to follow my dreams. Their unwavering support has been theTo my brothers, who have always been there for me, offering guidance, wisdom, and a sense of security that only comes from knowing you are loved and cared for. Your selflessness, patience, and dedication to our family are the greatest gifts you could ever give.
To my partner, whose patience and understanding helped me sail through the toughest moments of this journey.
Finnaly I dedicate this thesis to coffee, for it kept me awake; and the late-night programming errors, for always keeping me on my toes.

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Abbreviation list

VBDS	**Victor-borne Diseases**
Eos	Essential oils
LD50	The median lethal dose of population
CAT	Catalase
GST	Glutathione-s-transferase
C	*Culiseta*
P	Plasmoduima
Cs	*Culiseta longiareolata*
Mm	Millimeter
L1	first-stage larva
L2	Second-stage larva
L3	Third-stage Larva.
L4	Fourth-stage larva
Ph	Hydrogen potential
Km/h	Killometer/hour
°C	Celsius
Cm	Centimetre
Rm	*Ruta montana*
R	*Ruta*
Mg/l	Milligrame/liter
G	Gram
Ml	Milliliter
%	Percentage
R	Essential oil yield expressed as a percentage: standard deviation
PB	Weight of oil in grams
Pa	Weight of plant dry metter in grams
∑	Total
Cdnb	1-chloro-2,4-dinitrobenzene
Nm	Nanometer
M	Mole
µl	Microliter

rpm	Révolution per minute
mM	Micromole
X	millimoles of subtrate hydrolysed per minute and per mg of protein
ΔD0	Slope of the régression line obtained after hydrolysis of the substrate as a fonction of time
Vt	Total volume in the tank
Vs	Volume of supernatant in the cuvette
Uv	Ultrat violet
Dmax	Maximum optical density obtained
Dmin	minimum optical density obtained
mg proteine	quantité of protein expressed in mg
BBC	Commassie brilliant blue
P	The risk
N	Namber of repetition
Ss	Somme square
DF	Degree of freedom
MS	Mean square
Lc 25	Lethal concentration of 25% of the population
Lc 50	Lethal concentration of 50% of the population
Lc 90	Lethal concentration of 90% of population

ملخص:

تهدف هذه الدراسة إلى اختبار تأثير الزيت الأساسي المستخرجمن *Ruta montana* على نوع من البعوض *Culiseta longiareolata* الاكثر انتشارا في منطقة تبسة، تم الحصول على الزيت الأساسي عن طريق التقطير باستخدام جهاز من نوع Clevenger اظهر تقطير *Ruta montana* عائدا قدره %0.57 من المادة الجافة للجزء الهوائي للنبات

تم اختبار الزيوت الاساسية لنبات *Ruta montana* بتركيزات مختلفة على يرقات البعوض *Culesita longiareolata* في ظروف معملية وفقا لتوصيات منظمة الصحة العالمية .تظهر الاختبارات السمية ل *Ruta montana* نشاط مبيد لليرقات ومبيد الجراثيم لهذه الزيوت الاساسية مع علاقة بين الجرعة و الاستجابة.التركيزات شبه المميتة والقاتلة لليرقات CL25=3.25 µL/mL و CL90=7.21 µL/ml و CL50 =4.24µL/m

تكشف نتائج المؤشرات الحيوية عن تحريض لنظام ازالة السموم من الزيت الاساسي لنبات الفجل الجبلي عن طريق زيادةفي نسبة GST , وتباين في افرازال CAT

كما أظهرت النتائج انخفاضاً ملحوظاً في كمية البروتين الكلي ووزن يرقات البعوض.

الكلمات المفتاحية , *Culiseta longiareolata* , *Ruta montan*: زيت عطري، سمية، مؤشرات حيوية.

Résumé :

Cette étude vise à tester l'effet de l'huile essentielle extraite de *Ruta montana* sur l'espèce de moustique *Culiseta longiareolata* la plus répandue dans la région de Tebessa., L'huile essentielle a été obtenue par distillation à l'aide d'un appareil de type Clevenger, la distillation de *Ruta montana* a montré un rendement de 0,57% de la matière sèche de la partie aérienne de la plante.

Les huiles essentielles de *Ruta montana* à différentes concentrations ont été testées sur des larves de moustiques *Culesita longiareolata* dans des conditions de laboratoire selon les recommandations de l'OMS. Les tests toxicologiques de *Ruta montana* montrent une activité larvicide et bactéricide de ces huiles essentielles avec une relation dose-réponse. Les concentrations sublétales et létales pour les larves CL25=3,25µL/mL, CL90=7,21µL/ml et CL50=4,24µL/ml.

Les résultats des biomarqueurs révèlent une induction du système de détoxification à partir de l'huile essentielle de la plante de raifort par une augmentation de la GST et une diminution de la sécrétion de CAT.

Les résultats ont également montré une diminution significative de la quantité de protéines totales et du poids des larves de moustiques.

Mots-clés : *Ruta montan*, *Culiseta longiareolata*, huile essentielle, toxicité, biomarqueurs.

Abstract :

This study aims to test the effect of essential oil extracted from *Ruta montana* on *Culiseta longiareolata* mosquito species most prevalent in the Tebessa region, The essential oil was obtained by distillation using a Clevenger-type apparatus, showing a yield of 0.57% of the dry matter of the aerial part of the plant.

Ruta montana essential oils at different concentrations were tested on *Culesita longiareolata* mosquito larvae in laboratory conditions according to WHO recommendations. Toxicological tests of *Ruta montana* show larvicidal and bactericidal activity of these essential oils with a dose-response relationship. Sub-lethal and lethal larval concentrations CL25=3.25 μL/mL, CL90=7.21 μL/ml and CL50=4.24 μL/m

The biomarker results reveal an induction of the detoxification system of horseradish essential oil by an increase in GST and a decrease in CAT secretion.

The results also showed a significant decrease in the amount of total protein and weight of mosquito larvae.

Keywords: *Ruta montan, Culiseta longiareolata*, essential oil, toxicity, biomarkers.

Introduction

I. Introduction

Nearly 700 million people suffer from mosquito-borne diseases each year resulting in over one million deaths globally **(Louis *et al*., 2020).**These are called vector-borne diseases (VBDs), which are infections caused by pathogens that are transmitted by arthropods such as mosquitoes, triatomine bugs, black flies, tsetse flies, sand flies, lice, and ticks **(Wilson *et al*., 2020).** Like most multicellular organisms, mosquitoes host a community of commensal, symbiotic, or pathogenic microbes known as the microbiota. These microbes, including bacteria, viruses, fungi, protozoa, nematodes, and mites, are present in varying stability within the exoskeleton, intestine, hemocoel, and/or within mosquito cells **(Heu & Gendrin, 2018).** Bites by these insects can induce a variety of diseases including malaria, filariasis, chikungunya, dengue fever, yellow fever, West Nile virus disease, and Zika virus disease **(Abagli *et al*., 2023).** According to the work of **(Bouabida, 2012),** Culiseta longiareolata is the most interesting mosquito species in Algeria, particularly in the Tebessa area, as this species is considered a bird Plasmodium vector.

A number of chemical products formulated to provide a high safety profile are commercially available, but their toxicity to human skin and the nervous system can lead to several serious problems, such as rashes, swelling, and eye irritation. The most important drawback of these products is the incidence of insecticide resistance, which has increased rapidly in recent years, and the extremely challenging or downright impossible task of finding and treating all mosquito breeding sites. New approaches and vector-control tools targeting aquatic stages and adults are urgently needed **(Dahmana & Mediannikov, 2020).** Due to these problems, researchers have been working on safer alternatives to control this vector **(Luz et *al*., 2020).** The advantages of natural insecticides are non-pollutant to the environment and safe for human health. Several natural products have been shown to have mosquito repellent, larvicidal, pupicidal, and ovicidal activities **(Louis *et al*., 2020).**

Biological control has many advantages as a pest control method, particularly when compared with chemical insecticides. One of the most important benefits is that biological control is an environmentally friendly method and does not introduce pollutants into the environment. Another great advantage of this method is its selectivity. Biological control of mosquito larvae using larvivorous fish has shown many advantages over chemicals, but exotic mosquito fish may have negative effects on other native fishes and destroy local habitats. Mermithid nematodes have been documented from at least 63 species of mosquitoes worldwide, but they have received little consideration until now **(Kennedy, 2020).**

Among these alternatives, essential oils are highly complex natural mixtures characterized by strong aromas and the presence of several secondary metabolites, mainly monoterpenes, sesquiterpenes, and phenylpropanoids. This mixture acts mainly on the defense mechanism, protecting the plant from attack by predators and pathogenic microorganisms, as well as attracting pollinators **(Luz *et al*., 2020).** Essential oils (EOs) are volatile oil components that impart distinctive flavors or scents and have a long history of commercial use, ranging from pharmaceuticals to flavor additives for foods. While recognized as non-toxic to humans, EOs often contain between 20–60 components. Of these, 2 or 3 components are present at distinctly high concentrations, and generally, it is these components that determine the biological activity of the EO. Terpenes or terpenoids are common primary constituents of EOs, as are aromatic or aliphatic molecules **(Workman *et al*., 2020).**

The genus *Ruta* L. belongs to the tribe Ruteae of the family Rutaceae Juss. and comprises 40 different accepted species, which are native to or naturalized in many countries worldwide, especially in African, Asian, and European countries, e.g., Algeria, China, Iraq, Italy, Libya, Morocco, Portugal, Spain, Syria, and Tunisia, and have been introduced in the countries of North and South America. However, the greatest distribution of *Ruta* species is found in the Mediterranean region **(Nahar *et al*., 2021).** It can also be noted that the number of *Ruta* species as claimed by various authors may vary from as few as eight to as many as 160 species. *Ruta chalepensis* L., *Ruta graveolens* L., and *Ruta montana* L are the three most widely distributed and most extensively studied species of the genus Ruta **(Nahar *et al*., 2021).** Belonging to different chemical classes such as alkaloids, coumarins, flavonoids, essential oils, saponins, triterpenes, phenols, and lignans. The richness of this plant in these compounds is probably a reason for its extensive use in traditional medicine. This plant is not used much anymore in Europe; however, it remains a plant appreciated by traditional practitioners, particularly in the Mediterranean basin and in South America, where it is used for various purposes such as an emmenagogue, aphrodisiac, abortive, antihelmintic, hypoglycemic, antirheumatic, antipyretic, in the treatment of hepatic diseases, hypertension, and vitiligo **(Ghedjati *et al*., 2022).** To our knowledge, there have been no scientific investigations concerning the toxicological studies of *Ruta montana* L. on mosquitoes, such as the determination of the LD50 value and the subchronic study.

In this context, our work focuses on evaluating the responses of populations of a mosquito species, *Culiseta longiareolata*, to the impact of a new insecticide based on essential oil from a medicinal plant, *Ruta montana*, on the detoxification biomarkers Catalase (CAT) and glutathione S-transferase (GST) in fourth instar larvae of the mosquito species *Culiseta longiareolata*.

Material and Methods

II. Materials and Methods

II.1. Animal Materials

II.1.1. Generality of mosquitoes

Mosquitoes were the first arthropods officially introduced as the intermediate hosts of vertebrate parasites **(Khaligh *et al*., 2020).** These insects belong to the Diptera order within the Culicidae family; Diptera is the largest order of insects, consisting of two-winged flies. Mosquitoes are abundant in tropical and temperate regions worldwide. There have been reports of 3700 mosquito species from two families and 112 genera. The diversity and species composition of mosquitoes are crucial for the management and control of mosquito-borne diseases **(Kachhawa *et al*., 2021).**

Mosquitoes are blood-sucking insects due to their strong sense of smell and a heat-sensitive organ that detects the warm air emitted by mammals. This ability allows mosquitoes to locate their victims **(Hamaidia & Berchi, 2018; Belkhiri, 2022).**

Mosquitoes are a species of winged insects whose females feed on human blood. Female mosquitoes feed on blood because it is necessary for their eggs to ripen, while males feed on plant juices and flower nectar. The female mosquito's mouth is characterized by fine parts that help puncture the skin and absorb blood (perforated sucking tongue) **(Hussain, 2020).**

II.1.2. Generality of *Culicidae*

The *Culicidae*, or mosquitoes, are insects that belong to the order Nematocera in the family Culicidae, which is divided into three subfamilies: *Toxorhynchitinae*, *Anophelinae*, and *Culicinae*. According to the most recent classification, the Culicidae family includes 2 subfamilies, 11 tribes, 111 genera, and 3528 species worldwide **(Asloum, 2023).** The Culicidae fauna is distributed in Mediterranean regions, America, India, northern Europe, Mediterranean Europe, Asia, and Madagascar, due to their strong adaptability and flight capabilities; they are now present worldwide **(Haouari-Abderrahim, 2016).**

In Algeria, the study of Culicidian biodiversity has gained momentum in the last decade, with several studies being published. Notably, **Messai *et al*., (2011)** in Mila, **Berchi *et al*., (2012)** in Constantine, **Bouabida *et al*., (2012)** in Tébessa, **Lafri *et al*., (2014)** in 16 regions including Tizi-Ouzou, Boumerdès, Algiers, Blida, Tipaza, Médéa, Tlemcen, Mostaganem, Saida, El Tarf, Annaba, Naâma, Béchar, Tindouf, Ghardaïa, and Tamanrasset. Additionally, **Boudemagh *et al*., (2013),Amara Korba *et al*., (2015),** and **Houmani *et al*., (2017)** in El Tarf, **Lounaci *et al*., (2016)** in Tizi-Ouzou, **Messai *et al*., (2016)** in Oum El Bouaghi, **Benhissen *et al*., (2014, 2017)** in Biskra, **Benhissen *et al*., (2018)**, and **Asloum *et al*., (2021)** in M'Sila, **Hamaidia & Berchi (2018)** and **Hafsi *et al*., (2021)** in Souk Ahras, **Nabti & Bounechada**

(2020) in Sétif, **Arroussi *et al*., (2021)** in Annaba, and **Belkhiri *et al*., (2021)** in Batna, **(Chahed, 2022).**Fifty species of Culicidae from six different genera are grouped into the subfamilies Anophelinae and Culicinae. Culex pipiens and Culiseta longiareolata are the most important mosquito species in Algeria **(Belkhiri, 2022).**

The Anophelinae can be identified by characteristic floats at the egg stage, the absence of a siphon at the abdominal end during the larvae stage, and the presence of long maxillary palps in both male and female adults. In contrast, Culicinae larvae have a well-developed siphon at the abdominal end, and both male and female adults have short maxillary palps. Entomologists recognize forty-three different genera of mosquitoes, with forty belonging to the subfamily Culicinae **(Duvallet & Chabasse, 2022).**

Mosquitoes are significant due to their role as vectors of pathogenic organisms for certain species and the nuisance they cause to others **(Hamaidia & Berchi, 2018).** Mosquitoes of this species primarily obtain carbohydrate sources from plant sap. However, females require dietary protein for oviposition, which they obtain through blood-feeding. Infected female mosquitoes can transmit arboviruses through their saliva during a bite **(Luz *et al*., 2020).**

II.1.3. Introduction to the insect *Culiseta*

Culiseta longiareolata is a species of the family Culicidae, specifically in the Culicinae subfamily **(Khaligh *et al*., 2020).** It is a common mosquito species that primarily feeds on birds (ornithophilic), and is known to be a pest and a vector for various blood parasites and arboviruses **(Tsurim & Silberbush, 2016).** This species is multivoltine, meaning it can have multiple generations per year, and is capable of undergoing hibernal diapause in adult females in colder regions and in larvae in temperate regions. The females of *C. longiareolata* are stenogamous (mate only once in their lifetime) and autogenous (able to produce eggs without needing a blood meal first). They prefer to feed on vertebrates, especially birds, and rarely feed on humans. *C. longiareolata* is considered a vector for avian Plasmodium parasites **(Bouzidi, 2021).**

This species is widely distributed in Algeria, particularly in the southern part of the country **(Merabti *et al*., 2020).Nabti and Bounechada (2019)** confirmed that females of this species exhibit an adaptive response against the risk of predation and negative density effects by avoiding laying their eggs in predator pools. Additionally, *C. longiareolata* is considered a primary vector for avian Plasmodium species such as Plasmodium circumflexum, Plasmodium relictum, and Plasmodium polare. Its capacity to transmit P. relictum in Algeria has been experimentally proven.

II.1.4. Characteristics

- The species *Culiseta longiareolata* is multivoltine and has a wide distribution in hot zones. It exhibits a variety of adaptive and survival characteristics. The larvae of the first and second stages are typically found in shallow areas of ponds, while the third and fourth stage larvae, along with the chrysalis, are located in the deeper areas of the ponds **(Salem & Diaf, 2023).**

- *C. longiareolata* has the ability to develop in various types of sites, displaying a remarkable capacity to colonize both natural biotopes and artificial deposits that differ in their physical characteristics **(Benhissen *et al*., 2018).**

- In terms of its morphological features, *Culiseta longiareolata* is characterized by white stripes and spots on its legs, head, and thorax **(Khaligh, 2020).**
- This mosquito species typically ranges in size from 3 to 5 mm, with a slender body, long thin legs, membranous wings that are long and narrow. The eggs of *C. longiareolata* are attached at the time of laying and form a basket structure. These eggs have a cylindro-conical shape and are usually clustered in a structure known as a Nacelle, which can contain around 50 to 200 eggs **(Merkhi & Maifi, 2020).**

II.1.5. The systematic position of *Culiseta longiareolata*

The systematic position of *Cs longiareolata* according to **(Aitken, 1954)** is as follows :

Table 01 : Thesystematic position of *Culiseta longiareolata*(Bouzidi, 2021)

Reign	Animal
Branch	Invertebrate
Class	Insect
Subclass	Pterygote
Order	Diptera
Suborder	Nematocera
Family	Culicidae
Subfamily	Culicinae
Gender	*Culiseta*
Specie	*Culiseta longiareolata*

II.1.6.Development life cycle

The mosquito development cycle typically lasts from twelve (12) to twenty (20) days and consists of two main phases.

The first phase is the aquatic phase, which includes the egg, larval stage, and nymphal stage. The second phase is the aerial phase, which involves the adult stage after emergence **(Salem & Diaf, 2023; Matoug, 2018).**

Adults, also known as imagos, are aerial creatures, while eggs, larvae, and nymphs represent the pre-imaginal stages and are typically found in fresh water, although sometimes in brackish water **(Dris, 2019).**

Following hatching, the larval stage progresses through four aquatic stages separated by three molts. After these four larval stages, a nymph is formed. Once the nymph reaches maturity, its integument splits at one end, allowing the fully developed adult mosquito to emerge. The young adult mosquito initially remains motionless for the first few hours to allow for the hardening of its cuticle and the spreading of its wings **(Oussad, 2020).**

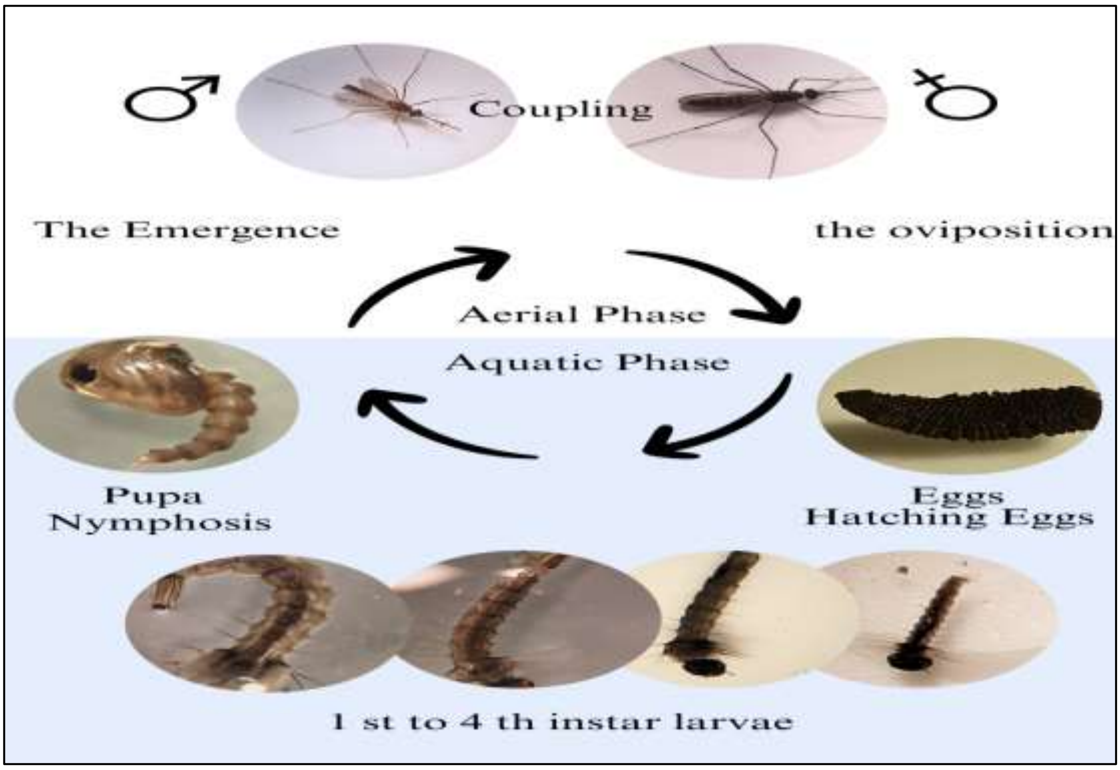

Figure 01.*Culesita longiareolata* development life cycle (personal photo, 2024)

II.1.7. Morphological characters

- Egg : The females of *Culiseta longiareolata* are stenogamous and autogenous. The eggs are attached at the time of laying and form a structure called a nacelle **(Tine-Djebbar *et al*., 2016).** Mating typically occurs shortly after emergence during a swarm flight. The female mates with the male only once and retains the sperm in her sperm banks throughout her life **(Touati, 2019).**

After emergence, fertilized females deposit between 200 and 400 eggs perpendicular to the water surface **(Dris, 2019).** Mosquito eggs are generally fusiform and measure around 0.5 mm **(Oussad, 2022).** Initially, the eggs are cylindrical and whitish in color, but they may turn greyish or blackish after a few hours due to the oxidation of certain chemical components upon exposure to air or water. The egg is equipped with an operculum that opens downward when it is time for hatching, and the larva emerges thanks to a chitinous spine located at the head **(Brahmi & Snoussi, 2021).**While egg development in blood-sucking insects typically relies on a blood meal, there are instances of female mosquitoes that can develop their eggs without requiring a blood meal, a phenomenon known as autogeny **(Khaligh *et al*., 2020).**

Figure 02. Eggs of *Culiseta longiareolata* (personal photo, 2024)

-Larvae : The larva of *Culiseta* mosquitoes progresses through four stages of development: L1, L2, L3, and L4, with each stage separated by molting, allowing the larva to grow from about 2 mm to 12 mm in size. These larvae are mobile and obtain their oxygen by breathing at the water's surface through a respiratory siphon located at the end of the abdomen. They exhibit jerky movements and primarily feed on various microorganisms such as plant particles, bacteria, and yeasts **(Dris, 2019; Eid, n.d.; "La Vie du Moustique").**

Culiseta larvae are distinguished by the absence of legs; The four larval stages, noted as L1, L2, L3, and L4, with only the L4 larvae possessing definitive taxonomic characteristics, facilitating easy differentiation **(Aissaoui & Moukher, 2020).**

The larval body is legless and consists of three segments: head, thorax, and abdomen. The head is well defined and capable of rotating 180° around its axis, with a distinct capsule bearing a pair of "eyes" composed of lateral ocelli clusters, antennae of varying shapes and lengths, and chewing mouthparts equipped with various structures like brushes, combs, and sweepers used for feeding **(Oussad, 2020; Foster & Walker, 2019).**

The thorax of *Culiseta* mosquitoes consists of three fused segments, while the abdomen is divided into nine segments, with the last abdominal segment bending graciously at its posterior end where the anus is located. After each molt, the larvae settle near the discarded exoskeletons and, at the end of this period, undergo metamorphosis into a nymph **(Dris, 2019).**

Cs longiareolata larvae can develop in water with pH levels ranging between 6 and 12. Fourth-stage larvae exhibit a slightly higher capacity to tolerate and thrive in water with varying pH levels compared to first-stage larvae. They become more resilient to their environments as they age and progress through their developmental stages **(Brahmi *et al*., 2021).**

These larvae are seldom found in natural water bodies but are predominantly found in temporary pools, rock pools, artificial containers, wooden and metallic barrels, and concrete tanks rich in decomposing organic matter. Early-stage larvae of *Cs longiareolata* are more commonly found in shallow pool regions, whereas later-stage larvae are typically located in deeper pool regions **(Hazratian *et al*., 2019).**

Figure 03.*Cs Longiareolata* Larva (personal photo, 2024)

-Pupae: The nymph or pupa of *Culiseta* mosquitoes is comma-shaped, mobile, and features a robust cephalothorax that is swollen and equipped with two breathing trumpets

(Messai &Touahria, 2021). This stage represents a transient phase of metamorphosis that leads to the emergence of the adult mosquito onto the water surface. The transitions enabling the mosquito to transition from the aquatic to terrestrial environment commence at the end of the larval stage with the breakdown of muscles and continue during the nymphal stage with the development of an entirely new system **(Foster &Walker, 2019).**

During the nymphal stage, which typically lasts between 24 and 48 hours, the nymph does not feed but relies on reserves accumulated during the larval stage. It breathes through two trumpets situated on the cephalothorax, unlike larvae that respire at the posterior end of the abdomen. Nymphs typically float on the water's surface but dive when disturbed **(Foster & Walker, 2019).**

The pupal stage consists of eight segments, with the eighth segment possessing two swim paddles while the ninth is atrophied. The cephalothorax displays the outlines of the eyes and various appendages such as antennae, trunk, legs, and wings. It also features two prothoracic respiratory trumpets, which come in various shapes and serve as physiological equivalents to the larval respiratory siphon **(Djabri & Lahmidi, 2021).**

Figure 04.*Cs Longiareolata* pupa (personale photo, 2024)

-Adult: The adult mosquito, also known as the imago, features an elongated body that typically ranges from 5 to 20 millimeters in length. It has an overall light brown coloration with distinct frontal stripes on the abdominal tergites. The exoskeleton is made up of rigid plates called sclerites, which are interconnected by thin chitinous membranes. Each segment of the body, known as a metamer, consists of a dorsal sclerite (tergite), a ventral sclerite (sternite), and lateral sclerites (pleurites) **(Brahmi &Snoussi, 2021).**

Following the transformation into an adult, the nymphal head ruptures, and the adult emerges onto the water's surface. Male mosquitoes reach sexual maturity within one day due to their shorter larval growth periods (Foster & Walker, 2019), while females take one to two days to achieve sexual maturity and are larger than males from the same emergence **(Djabri & Lahmidi, 2021).**

Upon emergence, the newly developed adult mosquito can fly short distances until its cuticle becomes fully sclerotized. The energy required for flight and survival for the first few days is sourced from lipids and glycogen carried over from larval reserves **(Foster & Walker, 2019).**

Similar to all dipterans, adult mosquitoes possess a single pair of elongated, narrow, membranous wings that fold horizontally at rest and are adorned with scales along their veins. Their slender bodies are divided into three main sections: the head, thorax, and abdomen **(Dris, 2019).**

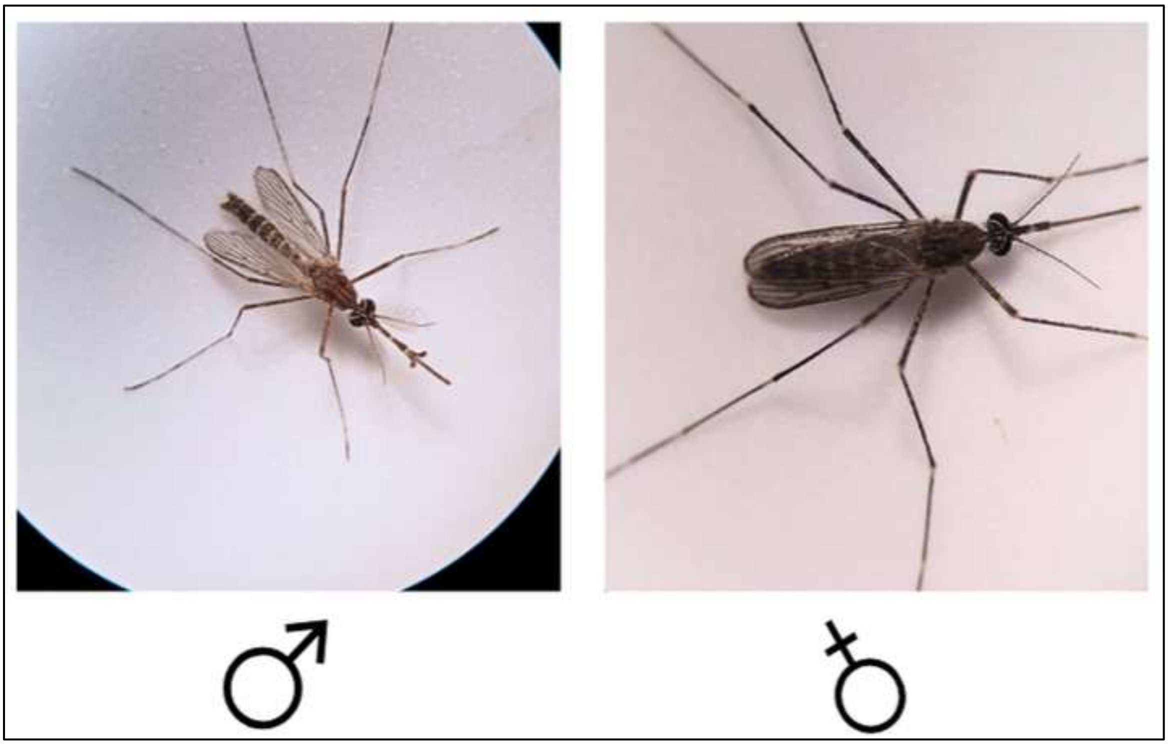

Figure 05 .*Cs Longiareolata* adult (personal photo,2024)

II.1.8. Mosquito anatomy:

- **Head:**

The sensory organs of adult mosquitoes are essential for collecting information and locating food sources, with the proboscis playing a key role in feeding. Antennae are crucial for detecting odors from potential hosts, breeding sites, and other environmental cues where females lay their eggs **(Beugin-Bizjak, 2022).**

In the Culicinae species, females have shorter palps compared to males. The antennae of male mosquitoes are typically more feathery in appearance than those of females. The eyes of mosquitoes are large and complex, composed of many ommatidia, allowing them to have a wide field of vision **(Lecollinet *et al*., 2022).**

- **The thorax:**

It allows for displacement **(Beugin-Bizjak, 2022).** The thorax is composed of three segments (prothorax, mesothorax, and metathorax) of unequal sizes, with each segment giving rise to a pair of legs. The mesothorax, being the most developed segment, carries two pairs of lateral respiratory stigmata on the mesothorax and metathorax, one single pair of wings on the mesothorax, one pair of halteres or balancers, and three pairs of legs. The legs, which are long and slender, sometimes feature characteristic scales and are comprised of nine segments. The thorax terminates with a scutellum, which is monolobed in Anophelinae and trilobed in Culicinae **(Lecollinet *et al*., 2022).**

The three pairs of legs are attached to the thorax, with two pairs oriented downwards and one pair upwards. Additionally, there is a pair of wings behind the wings are the halteres, small oscillatory sensory organs used for flight control **(Asloum, 2023).**This allows most species to fly at an average speed of 3 km/h **(Beugin-Bizjak, 2022).**

- **the abdomen :**

The abdomen of the Culicidae is elongated and much narrower than the thorax. It is formed of ten segments, but only the first eight are differentiated and visible externally **(Asloum, 2023),** with respiratory stigmas opening laterally on each segment. The abdomen contains scales - ventral, lateral, and dorsal - of varying sizes, shapes, positions, colors, and numbers, which are used for specific identification **(Lecollinet *et al*., 2022).**

This part of the mosquito's body is responsible for digesting its food. In females, the abdomen is where the eggs develop. Just after feeding, the abdomen becomes noticeably more visible as it expands to accommodate a maximum amount of blood **(Beugin-Bizjak, 2022).**

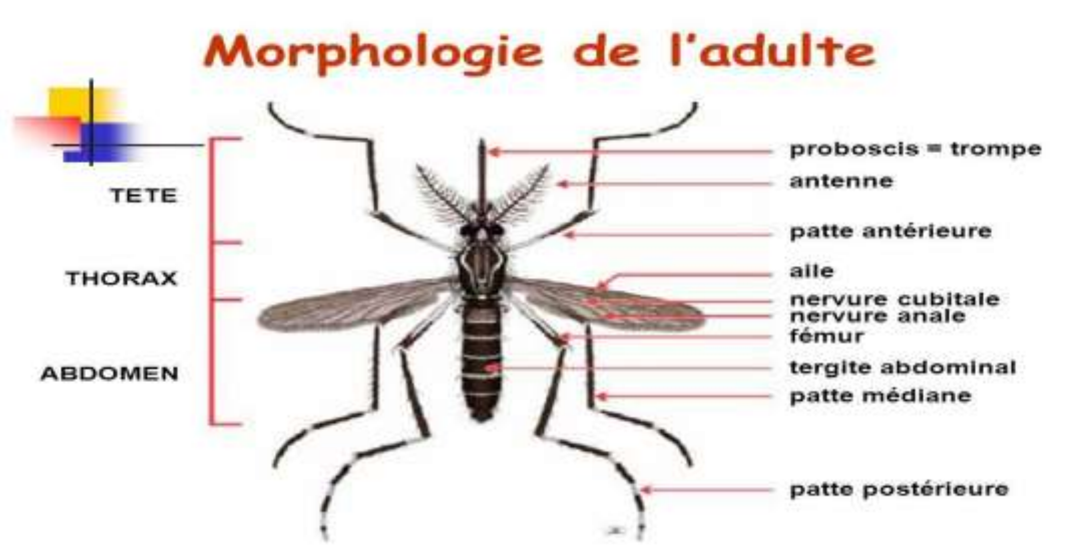

Figure 06. Adult mosquito morphology **(Beugin-Bizjak,2022)**

II.1.9. Biology of Culicidae:

a. The coupling:

Mosquitoes mate in flight or while in vegetation, with nematodes mating at night in solitary forms. Male Culicids press against the female's abdomen while the two insects continue flying together. Fertilization occurs quickly, typically requiring temperatures of at least 20°C. A solitary male has the ability to mate with multiple females at very close intervals **(Belkhiri, 2022).**

Mating usually takes place within forty-eight hours of female emergence **(Abbassi & Zemali, 2019).** In order to reproduce, a female mosquito typically only needs to be fertilized by one male. The spermatheca, a pocket where spermatozoa are stored, enables the preservation of sperm. Following mating, the female must take a first blood meal to obtain essential nutrients necessary for egg maturation **(Asloum, 2023).**

The majority of species that require a blood meal for egg maturation are known as anautogenous species. However, there are a few species, such as those in arctic regions where hosts are scarce or in urban areas, that can lay eggs without a blood meal; these are called autogenous species. It is important to note that the need for blood during fertilization in anautogenous species contributes to the aggressive behavior of infected females, making them highly dangerous **(Abbassi & Zemali, 2019).**

b. The oviposition:

After taking a blood meal, the female mosquito settles in a sheltered location to digest her meal **(Asloum, 2023).** Subsequently, she deposits eggs in various environments. These eggs can vary in shape, being fusiform, elongated, or swollen in their medium, and sometimes have tiny side floats, depending on the mosquito species. Typically, females lay between 100 to 400 eggs, and the egg stage lasts for 2 to 3 days under conditions of moderate temperature, water pH, and the nature and quantity of aquatic vegetation and associated wildlife **(Chenouf & Nacef, 2021).**

c. Hatching of eggs:

When the mosquito eggs reach maturity, they hatch into stage 1 larvae, which are typically 1 to 2 mm in size. These larvae then develop into stage 4 larvae, which can grow up to 1.5 cm in length. At this stage, the larvae feed on microbes, organic materials, and in some cases, live prey for carnivorous species. Despite evolving in an aquatic environment, mosquito larvae breathe air with the help of respiratory stigmas or a siphon **(Chenouf & Nacef, 2021).**

Among the aquatic stages, only the larvae feed, with some larvae moving actively by jerking on the water's surface or at the bottom of the larval habitat. They are voracious eaters as they require ample food for growth, going through four moults at irregular intervals **(Belkhiri, 2022).**

Stage 4 larvae are easily visible to the naked eye due to their size. After six to ten days or longer, depending on water temperature and food availability, the larvae undergo a transformation and emerge as nymphs in a process known as nymphosis **(Chenouf & Nacef, 2021).**

d. The nymphosis :

After completing its growth, the larva transitions into a nymph, also referred to as a pupa. In contrast to the larva, the nymph is more stocky and has a comma-like shape. While the nymph remains active, it tends to stay stationary closer to the water's surface, absorbing oxygen through its breathing tubes **(Belkhiri, 2022).** If disturbed, the nymph dives to the bottom to avoid predators. Interestingly, the Culicid nymph, despite its activity, does not engage in feeding **(Abbassi & Zemali, 2019).**

Following 2-3 days of aquatic life and significant anatomical changes, the nymph begins its transformation by immobilizing itself on the water surface. In conditions where the temperature is sufficiently high, metamorphosis occurs rapidly, typically within 1-2 days **(Asloum, 2023).**

e. The emergence:

After completing its development within the nymphal envelope, the adult mosquito emerges and begins to breathe at the water's surface. As the integument dries upon exposure to air, a T-tear forms on its dorsal side due to an increase in internal pressure. The adult insect gradually emerges as it inflates with air, eventually taking flight after allowing time for wing and leg unfolding by increasing the pressure of the hemolymph **(Belkhiri, 2022).**

Males often emerge before females as they require a longer period to establish their sexual glands. They typically assemble in large groups, often in dark areas or over tall grasses, bodies of water, visible objects, or open spaces **(Larbi-Cherif, 2015).** The females eventually join them, and couples form and depart the swarm to mate. In general, the lifespan of adult mosquitoes ranges from around one week to over 30 days. Some individuals have been known to live up to 2 months in breeding conditions. Females tend to live longer than males, who typically die shortly after mating **(Larbi-Cherif, 2015).**

Figure 07. The emergence of Adult mosquito (personal photo,2024)

II.1.10. Breeding in the laboratory

Culiseta mosquito larvae and eggs were collected from various regions of the city of Tébessa , El-aouinet, Boukhadra, Hammamet and El Dhokara.

Figure 08. Mosquito collection sites (personal photo, 2024).

These larvae were then reared in the laboratory using plastic cups filled with 150 ml of dechlorinated water. The larvae were fed a mixture consisting of 75% biscuit and 25% yeast**(Rehimi & Soltani, 1999).**

with the water being changed every two days, all at room temperature.The diet provided to the larvae plays a significant role in fecundity, as observed in studies by **(Wigglesworth, 1972).**

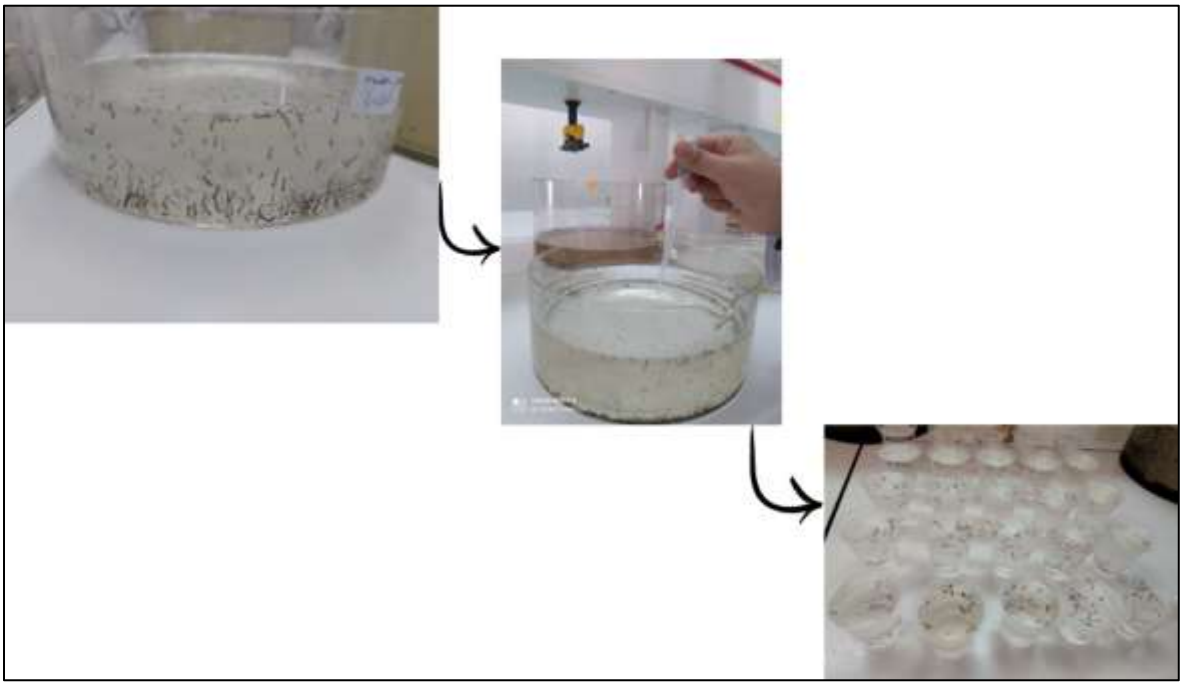

Figure 09. The different stages of preparing mosquitoes larvea in the lab(personal photos, 2024).

II.1.11. toxicity test

The principle is to treat *Culiseta longiareolata* larvae with essential oils preparations of *Ruta montana*. These preparations contain increasing concentrations, dissolved in 1ml ethanol. For each concentration, three replicates with 20 larvae each were carried out. A negative control series (the individuals were not treated) and a positive control series (the larvae received 1ml ethanol) were run in parallel. The treatment was applied in jars each containing 150ml of dechlorinated water for 24 hours, as recommended by the World Health Organization **(WHO, 1963).**

After this period, the larvae were rinsed and placed in new containers. Individual mortality was recorded after 24 hours of treatment.

II.2. Plant Materials

II.2.1.*Rutaceae* family overview

With approximately 2100 species in about 154 genera, *Rutaceae* is the largest family within the order Sapindales and is most well-known for the economically significant genus Citrus L **(Appelhans, 2021).** The family exhibits a wide diversity in morphological characteristics and has a nearly worldwide distribution **(Liu *et al*., 2023).** While primarily found in tropical and subtropical regions, there are also a few species that occur in temperate climates **(Moshood *et al*., 2023).**

One of the most notable morphological features of the Rutaceae family, easily observable in the field, is the presence of schizogenous secretory cavities that contain essential oils. These cavities manifest as transparent dots in the leaves and can also be found in other parts of the plant such as the pericarp, flowers, and young axes **(Appelhans, 2021).**

The Rutaceae family consists of aromatic herbs **(Yahya *et al*., 2020)** that have been historically utilized in perfumery, gastronomy, and traditional medicine. Various publications have documented the presence of secondary chemical constituents in these plants **(Fatema-T-Z *et al*., 2019).**

Numerous research groups have shown interest in exploring the therapeutic and pharmacological properties of *Ruta* species. *Ruta* species have been extensively used in traditional medicine for treating a variety of ailments, including menstrual disorders, skin inflammations, cramps, and earaches **(Bejaoui & Abderrabba, 2019).**

The aerial parts of the *Ruta* plant are utilized in the treatment of rheumatism and are known for their analgesic, antipyretic, and mental disorder-relieving properties. The plant's oily compounds have various uses, such as stimulating the nervous system and uterine functions. Fresh leaves are also used to extract juice, which is given to children to address issues like helminthic infections while also aiding in relieving otalgia (earaches) and odontalgia (toothaches) **(Yahya *et al*., 2020).**

II.2.2. *Ruta* genus overview

Ruta is indeed the most representative genus of the *Rutaceae* family, belonging to the tribe Ruteae and serving as the type genus of the subfamily Rutoideae **(Coimbra *et al*., 2020).** This medicinal plant genus comprises over 1800 species, with origins in the Mediterranean region but now also found in hot areas and tropics **(Slougui *et al*., 2023).** In Algeria, five species of the genus *Ruta* are present: *Ruta montana*, *Ruta chalepensis*, *Ruta graveolens*, *Ruta angustifolia*, and *Rutatuberculata***(Mohammedi *et al*., 2019).**

Ruta plants have a long history of use in traditional medicine for treating various ailments. They are employed as stimulants, abortifacients, anti-inflammatories, resolving agents, for eye problems, dermatitis, rheumatic conditions, hypertension, phototoxicity, bacteriostatic effects on skin diseases, and rhinitis **(Poonkodi *et al*., 2017).**These plants are valuable sources of diverse classes of natural products with biological activities, including antifungal, antioxidant, phytotoxic, abortifacient, depressant, anti-inflammatory, and antidote properties. Researchers worldwide have shown interest in studying *Ruta* species extensively **(Slougui *et al*., 2023).**

Despite their medicinal potential, *Ruta* plants are not commonly used in modern phytotherapy due to concerns about their perceived toxic properties. They naturally thrive in arid, sunny environments and can be propagated through methods such as seed sowing, cutting, or root division **(Bennaomi, 2018).**

II.2.3. Taxonomy of Rutaceae

Taxonomic hierarchy *of Rutaceae* family according to **(Quezel & Santa, 1963; Bonnier, 1999; Wiart, 2006 ; Takhtajan, 2009)** is as follows :

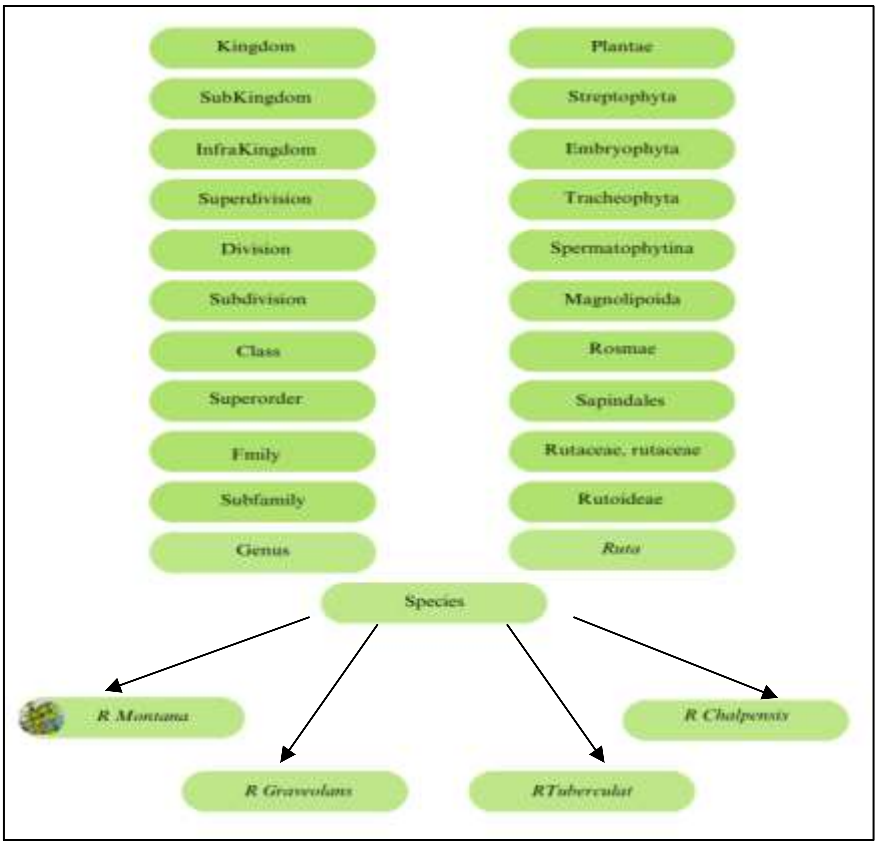

Figure 10. The taxonomy of *Rutaceae Family*

II.2.4. *Ruta montana* presentation

Ruta montana(R.m) is a spontaneous plant and one of the four species of the genus *Ruta* from the *Rutaceae* family **(Zeraib *et al*., 2021; Drioueche *et al*., 2019).** This species naturally thrives in arid regions, slopes, and rocky areas, with a wide geographical distribution including Portugal, Greece, Turkey, Algeria, Tunisia, and Morocco **(Mohammedi *et al*., 2019; Mahbed *et al*., 2023).** In Algeria, *RM* is known as '*mountain rue*' or 'Fidjel' **(Mohammedi *et al*., 2019).**

The population of Algeria uses the *R.m* plant for various purposes such as assisting with difficult births, alleviating toothaches, joint discomfort, and digestive issues, due to its biological activities including antifungal, antibacterial, antioxidant, insecticidal, and larvicidal properties **(Mohammedi *et al*., 2019).** The whole plant or the flowering tops of *R.m* may be infused internally, typically with a maximum of 1 to 2 grams per cup of boiling water. Externally, it is used as an antirheumatic and as an antiseptic on cuts and ulcers, as well as in mouthwashes to treat gum disease **(Hazzit *et al*., 2015).**

R. montana was chosen for study due to its abundance in Algeria and its well-recognized medicinal properties that have been utilized since ancient times.

Figure 11.*Ruta montana*(Personal photo, 2024)

II.2.5. Common names

Algeria: fidjel or montain rue **(Mohammedi *et al*., 2019 ; Merghem&Dahamna, 2020 ; Kara Ali *et al*., 2016).**

Berber: Awermi **(Drioiche *et al*., 2020).**

Morocco: Âwermï **(Ghadjati, 2023).** Fijel,Fidjel **(Lakhder, 2015 ; Drioiche *et al*., 2020).**

Tunisia: Figel **(Masri *et al*., 2015).**

France: rue des montagnes , rue sauvage **(Ghadjati, 2023).**

Spain: chocho de vieja (old woman's cunt) espiguilla (little spike),perejil borriquero (donkey's parsely),rtia,ruda, ruda montesina (wild rue),ruda silvestre(wild rue) **(Ghadjati, 2023).**

Turkey: sedefotu **(Ghadjati, 2023).**

II.2.6. Botanical description of *Ruta montana*

Ruta montana is an evergreen shrub that is 20–60 cm tall with triangular and slender leaves. Its flowers are small and yellow, with two whorls of stamens, and they are bisexual. The fruits are capsular with four rounded lobes. The plant is known for its strong, foul-smelling, nauseating odor, attributed to an essential oil contained in large sacs that house secretory glands **(Benkhaira *et al*., 2022; Kara Ali & Abidli, 2017).**The botanical characteristics of *Ruta montana* are summarized in Table 02.

Table 02 : The botanical traits of *R.m*

Type of plant	**Height**	**General characteristics**	**Flowering period**	**Reference**
Perennial shrub	20-60 cm	Spindly stems. Leaves are light green–obovate Segments. Flowers are small, yellow, borne in dichasial cymes. Fruits are rounded, small and lobulated.	May-August	**Drioiche *et al*., 2020** **Benkhaira *et al*.,2022**

Figure 12. Stems of Rue (Web 01)

Figure 13. Seeds of *Ruta montana* (Web 02)

Figure 14. Flowers of *Ruta montana* (Web 03)

Figure 15. Plant of *Ruta montana* (Web 04)

Figure 16. *Ruta montana* leaves (Web 05)

II.2.7. The pharmacological use of *R.m*

Table 03: **The** use of *R.montana* in folk medicine **(Khadhri *et al.*, 2017 ; Coimbra *et al*., 2020)**

Species	Uses	Part of use	Mode preparation	Country
Ruta montana	Hysteria, worms, or colic, as an antiseptic, stimulant, emmenagogue, and abortifacient and for its antifertility Activity.	ND	ND	Algeria
	Tonic, febrifuge, treatment of malaria, inflammatory, antioxidant, microbial processes, digestive, gastrointestinal motility, child fevers and as an abortive drug—but with great care because of the toxic effect due to the presence of xanthotoxin.	ND (not described)	Infusion/decoction	Many contries

II.2.8.Plant toxicity

Using *Ruta montana* as a remedy does not guarantee that it is always beneficial to human health **(Masri *et al*., 2015).** This plant is toxic to handle due to its essential oil content **(Allouni, 2018);** it contains alkaloids, flavonoids, coumarins, organic acids, tannins, vitamin C, and furocoumarins. While these compounds can have therapeutic effects, they also possess toxic properties, such as causing photodermatitis, kidney, and liver damage **(Szewczyk *et al*., 2023).** The leaves of Ruta montana are irritating and vesicant due to the essential oils present, particularly methylnonylketone, which acts as a rubefacient (**Allouni, 2018).** Therefore, caution should be exercised when using this plant, and its application should be regulated **(Coimbra *et al*., 2020).**

II.3. Essential oils (EOs)

II.3.1. Definition of EOs

Essential oils (EOs) are natural, volatile, and aromatic liquids extracted from specific plants **(Falleh *et al*., 2020).** They can originate from various parts of the plant, including the stem, flower, root, seed, bark, peel, fruit, leaf, wood, or the entire plant itself, and they are named based on the plant part they are obtained from **(Brahmi *et al*., 2016; Ríos, 2016; Khorshidian *et al*., 2018; El Sawi *et al*., 2019).** EOs are known for their complex composition, typically consisting of a few to several hundred components, notably hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (aldehydes, acids, alcohols, ketones, oxides, phenols, acetals, lactones, ethers, and esters). These chemical components determine the odor and flavor characteristics of EOs **(Yousefi *et al*., 2019).**

The variability of essential oils from the same botanical species is influenced by the chemical composition, which can be affected by geographical and climatic factors, as well as the extraction and purification methods used. Therefore, confirming the identity of essential oils can be a complex process **(Agatonovic-Kustrin *et al*., 2020).**

II.3.2. Essential Oil Distribution and Localization

Essential oils are produced and stored in specialized plant cells. They can be found in various plant organs throughout the plant. The synthesis and accumulation of essential oils are typically associated with the presence of histologically specialized structures **(Laurent, 2017).**

According to **Laurent (2017),** the synthesis and accumulation of essential oils are typically associated with the presence of histologically specialized structures that are often located on or near the surface of the plant. These structures vary depending on the plant family:

- **Essential oil cells: in Lauraceae and Zingiberaceae;**
- **Secretory hairs: in Lamiaceae;**
- **Secretory sacs: in Myrtaceae and Rutaceae;**
- **Secretory ducts: in Apiaceae and Asteraceae**

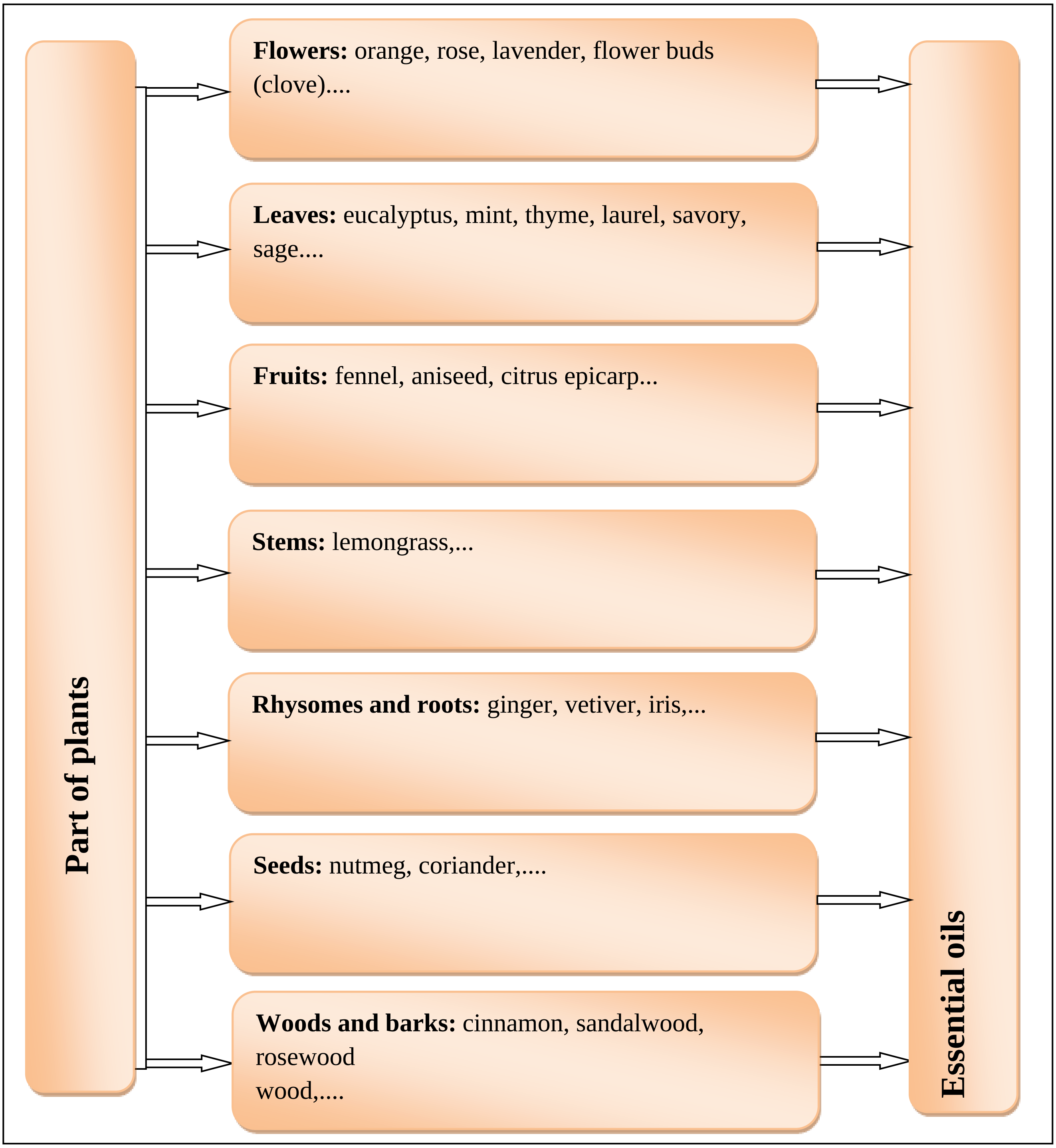

Figure 17. Essential oils sourced from different plant parts.

II.3.3.Essential oils' general properties

Essential oils' biological properties depend on their chemical composition and any synergism between their constituents. The value of essential oils lies in the completeness of their components, not just in the majority they contain **(Maamar, 2021).**

The use of essential oils from aromatic plants is becoming more precise and is based on a rational scientific basis, thanks to extensive research into their various activities such as antimicrobial, antioxidant, etc. **(Maamar, 2021).**

From a pharmacological perspective, all essential oils possess two very interesting properties: they are highly volatile and liposoluble. Due to these properties, they can be readily absorbed through the skin, respiratory tracts, and other common routes of administration **(Maamar, 2021).**

II.3.4. Physical characteristics of essential oils

- **Liquids at room temperature.**
- **Volatile (odorous character).**
- **Very rarely colored (except azulenes of dark blue color: in essential oil of blue Chamomile).**
- **Generally less dense than water (sassafras, cloves, and cinnamon are exceptions).**
- **High refractive index and most derive polarized light.**
- **Solubility:**
- **Soluble in fats; also soluble in most organic solvents.**
- **Entrainable to water vapor, very little soluble in water.**
- **Soluble alcohol with high levels (difference with lipids).**
- **Alterable and sensitive to oxidation (but not rancid).**
- **Tendency to polymerize and give birth to resinous products (conservation limited to one year) (Dalia & Bentchouala, 2022).**

II.3.5. EOs Toxicity

Even though essential oils have been shown to be effective, they are by no means harmless. An essential oil must first and foremost be labeled with the scientific name of the botanical species that it originates from to prevent any misunderstandings **(Mohamadi, 2023).**

Another essential condition for safe usage and maximizing the beneficial effects of essential oils is having complete knowledge of their chemical composition. It is generally accepted that the chemical profile of an essential oil directly determines its therapeutic activity **(Deschepper, 2017).**

Therefore, it is crucial that essential oils should not be readily available to everyone, and they should be used with care. Only those that are appropriately diluted should be sold over the counter to prevent accidents **(Dalia & Bentchouala, 2022).**

II.3.6. Essential oils' physiological functions in plants

We still have questions about the precise purpose of essential oils. They are produced by the secondary metabolism of plants and serve a variety of purposes, such as:

- **Protecting against pathogenic microorganisms;**
- **Repelling predators with a disagreeable taste or smell;**
- **Facilitating chemical reactions;**
- **Conserving moisture in the case of xerophilic plants;**
- **Reducing competition from rival plants by chemically blocking germination;**
- **Attracting or repelling specific insects (Mohamadi, 2023).**

II.3.7. Chemical composition of EOs

Essential oils are complex natural mixtures that might include a variety of components, both in type and quantity, making it extremely hard to identify and characterize their specific contents. However, in general, the data available indicated between 20 and 60 components at various concentrations ranging from 20% to 70% **(Baptista-Silva *et al.*, 2020).** These constituents are classified chemically based on four main characteristics:

- **Primary biosynthetic origin.**
- **Carbon atom size or number.**
- **Parent backbone or "skeleton".**
- **Kind of oxidation by electronegative atoms (Sadgrove et al., 2022).**

From a chemical standpoint, terpenes and phenylpropanoids are the two primary types of compounds that essential oils belong to. The predominant family is terpenes, and when phenylpropanoids are present, they give the plant its distinctive flavor and odor **(Sousa *et al.*, 2022).**

II.3.7.1. Terpenoids

The largest class of natural products is terpenes, also known as terpenoids, which contain a variety of structurally diverse molecules **(Sousa *et al.*, 2022).** They are generated through various biochemical modifications, including rearrangement and oxidation of terpenes, resulting in terpenoids that are oxygenated derivatives of terpenes, such as acids, alcohols, ketones, aldehydes, esters, and ethers **(Wani *et al*., 2021).**

Terpenes are organic molecules composed of multiples of five carbon atoms with the general formula (C5H8)n **(Bouyahya *et al*., 2016).** They are classified based on the number of isoprene units in their structures, which form carbon skeletons. The classifications include:

- **Hemiterpenes (1 isoprene unit; 5 carbons);**
- **Monoterpenes (2 isoprene units; 10 carbons);**
- **Sesquiterpenes (3 isoprene units; 15 carbons);**
- **Diterpenes (4 isoprene units; 20 carbons);**
- **Triterpenes (6 isoprene units; 30 carbons);**
- **Tetraterpenes (8 isoprene units; 40 carbons).**

Volatile essential oils primarily contain monoterpenes and sesquiterpenes. Terpenoids are terpenes that contain oxygen atoms and can exhibit aromatic, aliphatic, or cyclic structures **(Noriega, 2020).(Figure 17**).

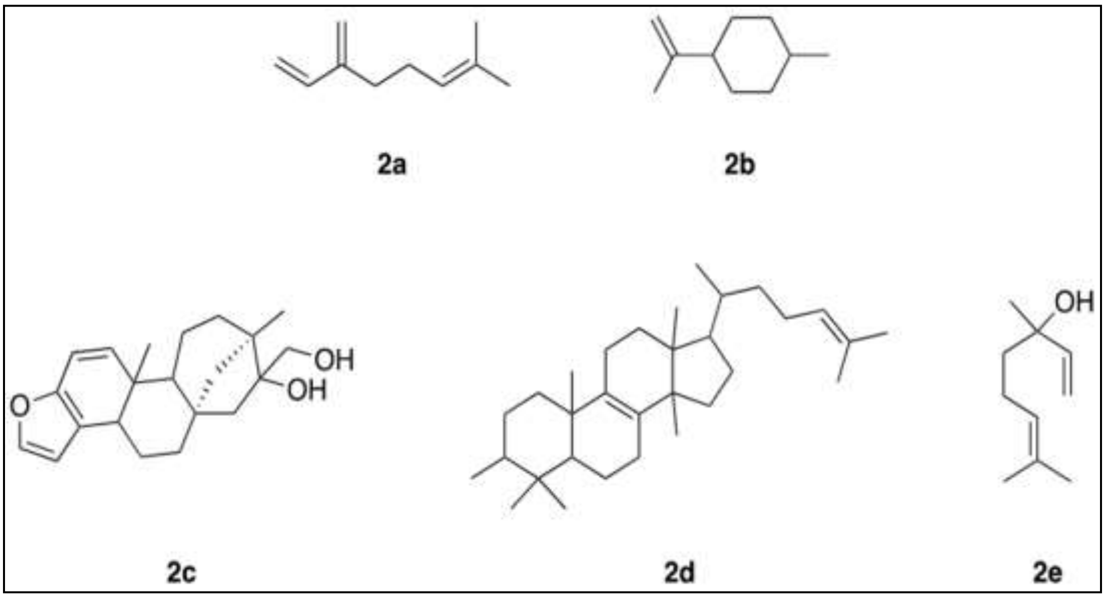

Figure 18. Structures of terpenes and terpenoids: acyclic monoterpenes (**2a**), cyclic monoterpenes (**2b**), diterpenes (**2c**), triterpenes (**2d**), and terpenoids (**2e**)**(Sousa *et al*., 2022).**

II.3.7.2.Phenylpropanoids

A six-carbon aromatic ring is connected to a three-carbon side chain to form the naturally occurring compounds known as phenylpropanoids, which are commonly found in plants. This side chain may contain a double bond, and the aromatic ring can be substituted. Shikimic acid serves as the precursor for cinnamic and p-coumaric acids, which are used in the biosynthesis of these compounds. Through oxidation of these units and degradation of the side chain, aromatic aldehydes are produced, and enzymatic reductions can yield propenylbenzenes and/or allylbenzenes **(Neelam & Sharma, 2020; Sousa *et al*., 2022).**

Table 04. Composition of compounds found in essential oils **(Sousa *et al*., 2022).**

Essential oils constituents	
Classes	Constituents
Terpenes	(−)-Camphene, p-cymene, (+)-limonene, β-ocimene α-phellandrene, α-pinene, α-terpinene, terpinoleneorange,
	(−)-β-isabolene, α-cadinene, β-caryophyllene, α-copaene, β-elemene, α-farnesene, α-humulene, α-zingiberene
Phenylpropanoids	(E)-Anethole, cinnamaldehyde, cinnamic acid cinnamic alcohol, eugenol, methyleugenol, myristicin

II.3.8. Extraction

The extraction of essential oils (EO) is a sensitive and complex process that must be carried out with great care. It aims to collect and preserve the most delicate, intricate, and volatile components produced by the plant without compromising their quality **(Mohamed**

Nadjib *et al*., 2019). There are various extraction methods available for EO extraction, including steam distillation, hydrodistillation, organic solvent extraction, expression, enfleurage, microwave-assisted distillation, microwave hydrodiffusion and gravity, high-pressure solvent extraction, supercritical carbon dioxide extraction, ultrasonic extraction, solvent-free microwave extraction, and the phytonic process **(Moghaddam & Mehdizadeh, 2017)**

II.3.8.1. Hydrodistillation

The process of hydrodistillation involves adding the plant material directly to the hot water container. Through a method called maceration, the oil components of the plant vaporize with the water, then condense and separate from the liquid phase in a separator, similar to the process in steam distillation. Following phase separation and condensation, the liquid phase is referred to as a hydrolate or hydrosol and may contain small amounts of oil (typically less than 50 mg/L) **(Uhl, 2024).**

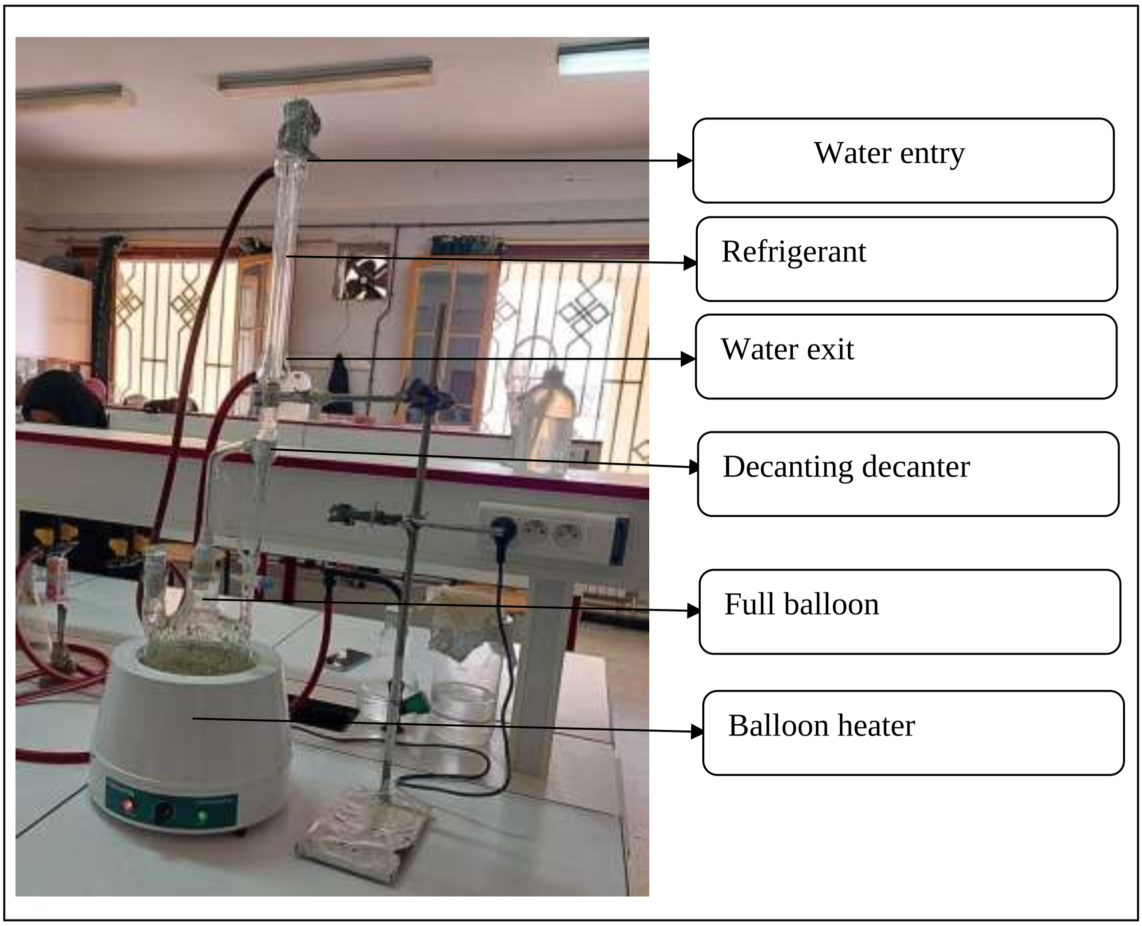

Figure 19. Assembly of the Clevenger hydrodistiller(personql photo, 2024)

II.3.8.2. Procedure

The essential oil extraction process involved hydrodistillation of *Ruta montana* using a Clevenger-type apparatus. The plant material was harvested from March to April 2024. After collection, the fresh plant material was dried in the shade in a well-ventilated, dry area for 15 days. The extraction process took place in the laboratory at Larbi-Tebessi University, Tebessa. Following the drying period, 100 g of the plant's aerial parts were placed in a round-bottomed flask with 1000 mL of distilled water for the extraction process.

Figure 20. Hydrodistillation steps (personal photos,2024)

II.3.8.3. Conservation of the extracted essential oils

Prior to being used for characterization testing, the essential oils were stored in hermetically sealed bottles, covered with aluminum foil, shielded from light, and maintained at a low temperature (stored in a refrigerator at 4°C) to prevent degradation. It has been noted that the biological activity of the altered oil is lost **(Dris *et al*., 2017).**

Figure 21.hermetically sealed bottles.

Figure 22. Bottle covered by Aluminum foil.

II.3.8.4. Yeild of EOs

The yield of essential oil is calculated as the ratio between the weight of the extracted oil and the weight of the dry plant matter **(Afnor, 1986).**

The yield, typically expressed as a percentage, is calculated using the formula:

$$\boxed{R = (PB / PA) \times 100}$$

Where:

R: Oil yield in %

PB: Weight of oil in grams

PA: Weight of plant dry matter in grams

Alternatively, for multiple samples: $R = [\Sigma PB / \Sigma PA] \times 100$.

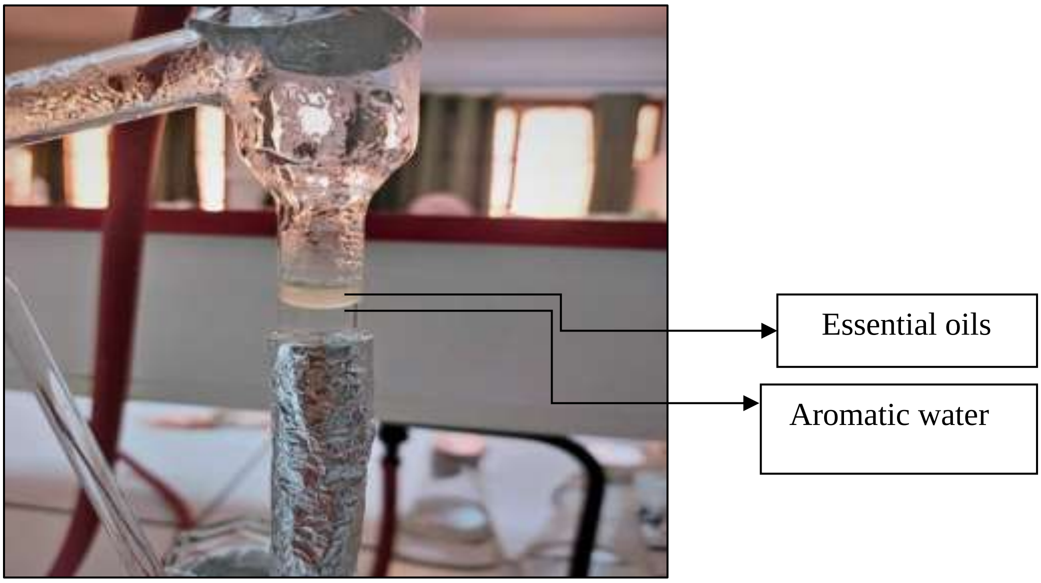

Figure 23. the final yield of *Ruta montana* essential oil (personal photo, 2024)

II.4. Biomarker assays

Glutathione S-transferases (GSTs) and the oxidative stress biomarker catalase (CAT) levels were measured in fourth instar larvae from both control and essential oil-treated series at different time points post-treatment: 24, 48, and 72 hours.

To determine the specific enzymatic activity and glutathione S-transferases levels, the total protein concentration of the samples had been previously measured using the **Bradford,(1976)** method. Parallel runs of the control series were conducted for comparison and consistency.

II.4.1. Glutathione S-transferase assay

Glutathione transferases (GSTs), also known as glutathione S-transferases, are part of the supergene family of phase II detoxification enzymes found ubiquitously in nearly all cellular life forms. They are involved in various intracellular transport and biosynthesis processes **(Mazari *et al*., 2023; Guenez, 2020).**

The activity of glutathione S-transferases (GSTs) is quantified using the technique described by **Habig *et al*., (1974).** This method involves the conjugation reaction of glutathione (GSH) with a substrate, CDNB (1-chloro-2,4-dinitrobenzene), and is measured at 340 nm using a spectrophotometer. The phosphate solution used has a pH of 0.1 M. The homogenate is then centrifuged for 30 minutes at 1400 rpm, and the resulting supernatant is collected and used as a source of enzymes **(Dris, 2019).**

The assay consisted 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 3 replicates, each comprising 20 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 distilled water replacing the quantity of supernatant **(Dris, 2019).**

The specific activity is determined according to the following formula:

$$\mathbf{X} = \frac{\Delta DO/mn}{9.6} \times \frac{Vt}{Vs} / \text{ mg de protéines}$$

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 hydrolysis of the substrate as a function of time.

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

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

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

mg protein: quantity of protein expressed in mg

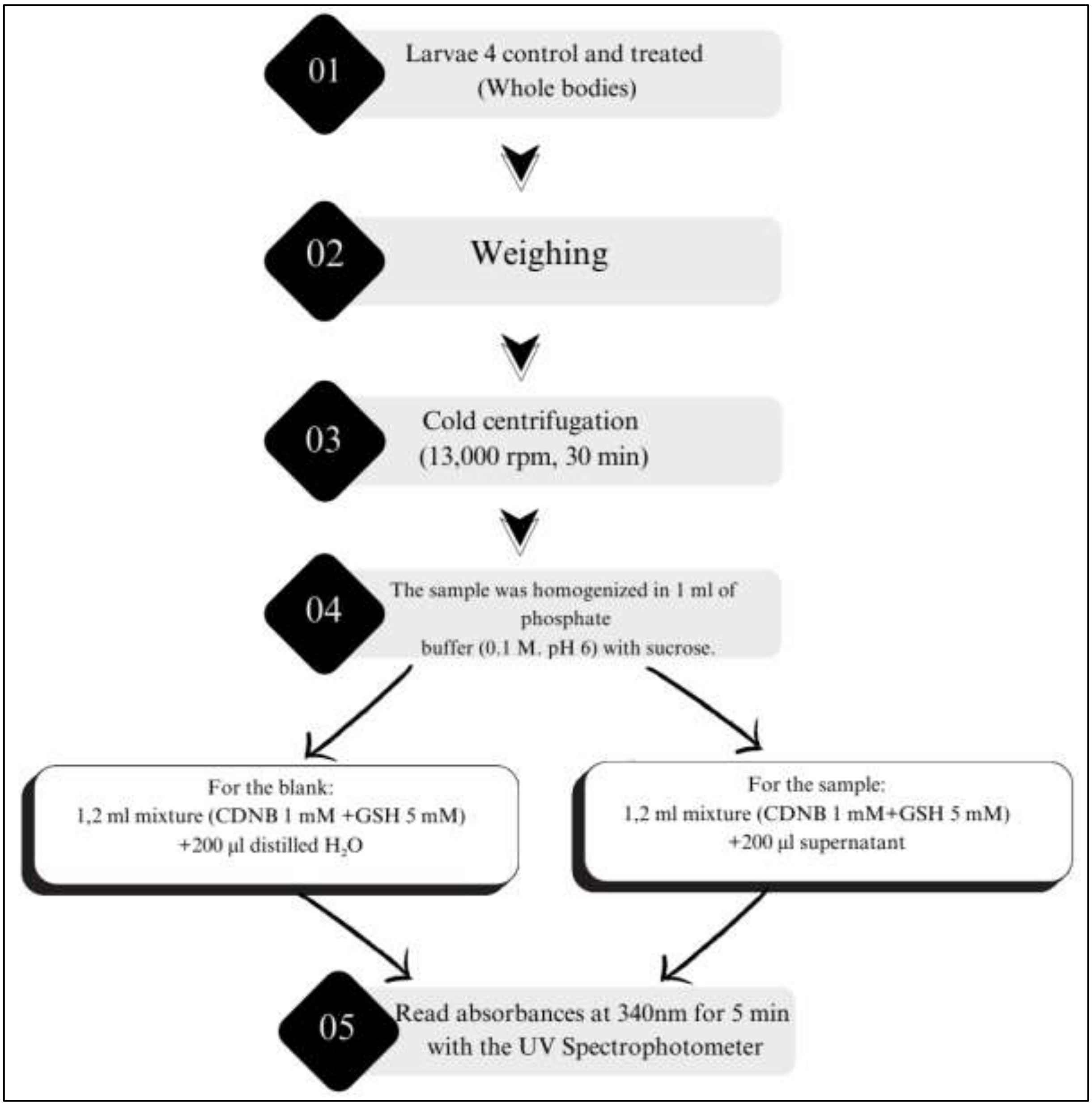

Figure 24. Extraction and assay of glutathione S-transferases **(Habig *et al*., 1974).**

Figure 25. GST assay (Personal photos,2024)

II.4.2.Specific catalase activity

Catalase assay

The **Claiborne, (1985)** method is used in the Catalase Assay (CAT). This method is based on the spectrophotometric detection of the reduction of hydrogen peroxide (H_2O_2) at a UV wavelength of 240 nm in the presence of the CAT into one oxygen molecule (O_2) and two water molecules (H2O). This reaction is described as follows:

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

Control and treated *C. longiareolata* fourth instar larvae are collected at different time points (24, 48, 72 h) and the experiment is performed with replicates of 20 animals each. The larvae are homogenised and centrifuged at 13,200 rpm for 10 minutes in 1 ml phosphate buffer (100 mM, pH 7.4). The supernatant obtained is used as an enzyme source.

The catalase activity is determined in a spectrophotometric cell at 25 °C on a 50 µl aliquot of the supernatant diluted to 1 to 1,5 mg protein per ml, i.e. 0,05 to 0,75 mg in the cell, to which 750 µl phosphate buffer (100 mM, pH 7,4) is added. After shaking, read the absorbance using a spectrophotometer. Read the absorbance against a blank of 800 µl phosphate buffer (100 mM, pH 7.4) and 200 µl H_2O_2 every 5 seconds for 30 seconds at a wavelength of 240 nm.
Specific activity is calculated according to the following formula :

$$\boxed{\boldsymbol{X} = \frac{D_{0max} - D_0 min}{0{,}04} mg\ of\ proteine}$$

X: micromole of substrate reduced per minute and per mg of protein (µM/mn/mg of protein).
D_0max :maximum optical density obtained.
D_0min :minimum optical density obtained.
0.04: molar extinction coefficient of H_2O_2(mM^{-1} cm^{-1}).
mg protein:quantity of protein expressed in mg

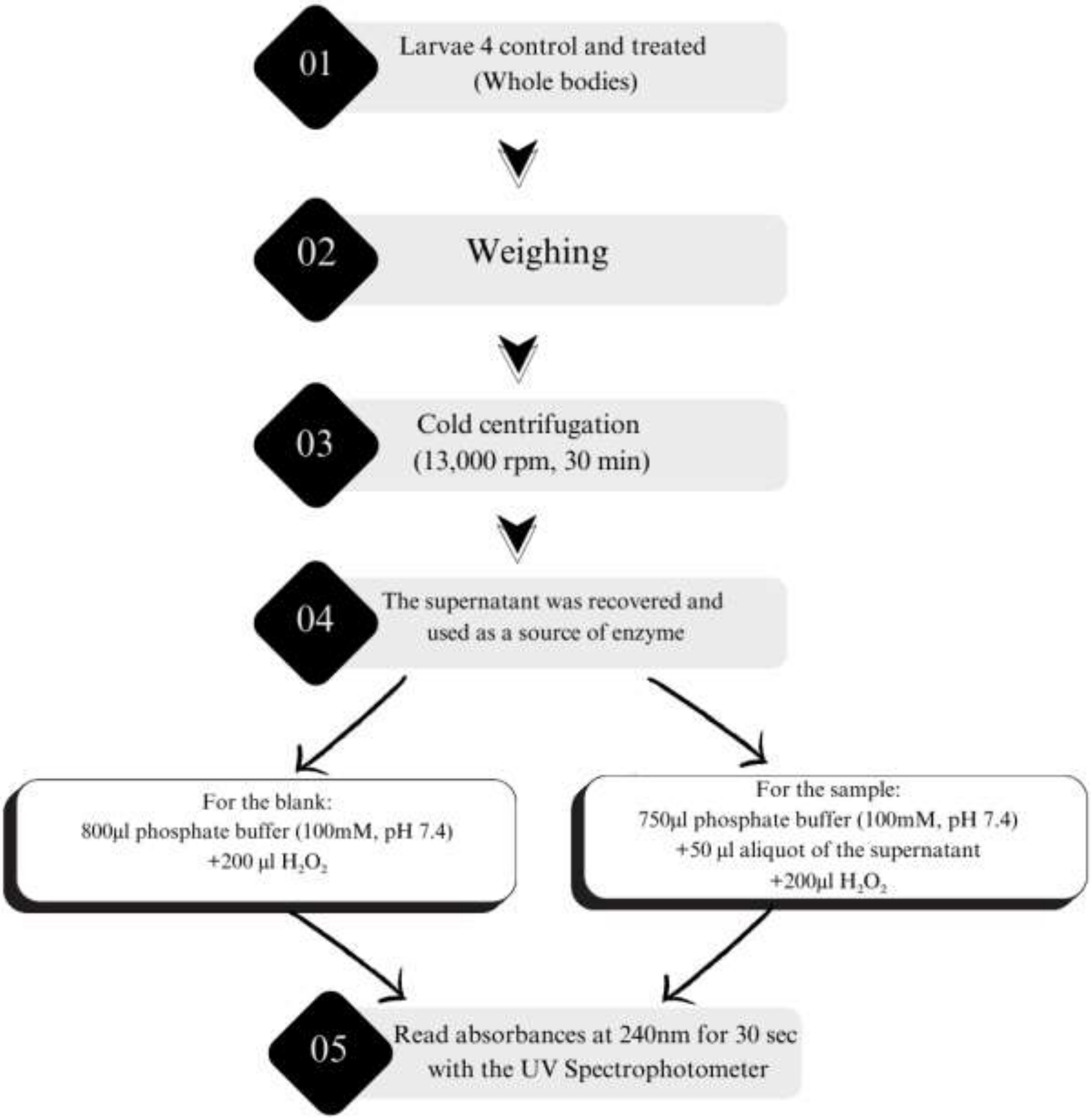

Figure 26. Extraction and assay of Catalase **(Claiborne, 1985)**

Figure 27. Catalaseassay (Personal photos)

II.4.3.Total protein content

The protein assay is performed in a 100 µl aliquot to which 4 ml of Commassie Brilliant Blue (BBC) G 250 reagent (Merck) is added, according to the method of **Bradford, (1976).** Preparation of the BBC solution shall be as follows Homogenise 100 mg of BBC in 50 ml of 95 % ethanol, add 100 ml of 85 % orthophosphoric acid and make up to 1000 ml with distilled water. The reagent is stable at 4°C for 2 to 3 weeks. A blue stain shows that proteins are present.

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 at a concentration of 1 mg/ml (Table 05).

Table 05. Determination of total proteins in mosquitoes: Construction of the protein calibration range of proteins.

Tube	**1**	**2**	**3**	**4**	**5**	**6**
BSA stock solution(µl)	0	20	40	60	80	100
Distilated water (µl)	100	80	60	40	20	0
BBC reactif *(ml)*	4	4	4	4	4	4

Pellet

⇓

1 ml of NaOH (0,1N)

⇓

Agitation

⇓

AssayAliquot 100 µl

⇓

Proteins

Figure 28. Extraction and assay of Proteins **(Bradford, 1967)**

Figure 29. Protein assay (Persona photos, 2024)

II.5. 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 (GraphPad Software Inc., www.graphpad with a significant level p).

Results

III. Results

III.1. Yield of *Ruta montana* essential oil

The hydrodistillation of *R. montana* using an apparatus of the Clevenger gives an essential oil of yellow color, pleasant smell and with a yield 0,57% of dry matter from the aerial part of the plant.

III.2. Larvicidal tests of *Ruta montana* essential oil against *Culiseta longiareolata*

Our toxicological studies made it possible to determine the effectiveness of *R. montana* essential oil on the L4 larvae of *Cs longiareolata* evaluated from the mortality recorded in the target individuals with a direct effect.

Different concentrations of *R. montana* EO were applied to the fourth instar larvae of *Cs longiareolata* (2, 3, 4, 5, 6 and 8 µL/mL) for up to 24 hours. The observed mortality is corrected from natural mortality. It is mentioned in table 05.

After angular transformation of the mortality percentages, the analysis of variance with one factor reveals a very highly significant concentration effect ($p < 0.001$). It is mentioned in table 06

Table 06: Toxicity of *R. montana* EO applied to *Cs longiareolata* L4 larvae: Corrected mortality (%) (m ± SD, n = 3 repetitions each comprising 20 individuals).

Concentrations (µL/mL)	**2**	**3**	**4**	**5**	**6**	**8**
R1	10	20	40	60	85	100
R2	10	20	40	70	75	100
R3	5	15	55	65	75	100
m±SD	**8.33±2.88**	**18.33±2.88**	**45±8.66**	**65±5**	**78.33±5.77**	**100±0**

Table 07: Effect of *R. montana* essential oil (µL/mL) in *Culiseta longiareolata* larvae.

Analysis of variance with one factor after transformation analysis of recorded mortalities (%).

Source of variation	SS	DF	MS	F (DFn, DFd)	P value
Treatment	18763	5	3753	F (5, 12) = 150,1	P<0,0001
Residual error	300	12	25		
Total	19063	17			

The essential oil of *R. montana* was applied to L4 larvae at lethal concentrations, LC_{25}, LC_{50}, and LC_{90} (which causes mortality of 25%, 50%, and 90% of the targeted population). The LC_{25}, LC_{50} and LC_{90} concentrations determined are respectively 3.25µl/ml of the interval (2.86-3.61); 4.24 from the range 3.93-4.54 ; and 7.21 from the interval 6.26-8.58 with a Hill Slope of 4.13 and R^2 of 0.98.

Table 08: Effect of *R. montana* essential oil (µL/ mL) in L4 stage larvae of *Culiseta longiareolata* at lethal concentrations, LC_{25}, LC_{50}, and LC_{90}.

LC_{25} IC	LC_{50} IC	LC_{90} IC	Slope	R^2
3.25	**4.24**	**7.21**	**4.13**	**0.98**
2.86 to 3.61	3.93-4.54	6.26 to 8.58		

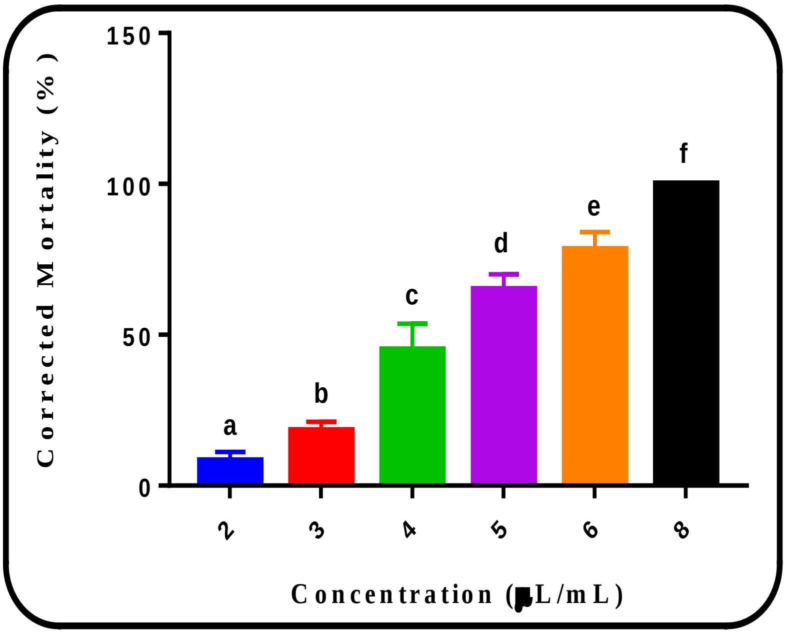

Figure 30: Bar chart presenting the mortality percentages of newly exuviated L4 stage *Culiseta longiareolata* larvae treated with different concentrations of *R. montana* essential oils. : Corrected mortality (%) (m±SD, n = 3 repetitions each comprising 20 individuals). Different letters indicate a significant difference between concentrations.

III.3. Effect of *Ruta montana* essential oil on enzymatic biomarkers

The essential oil of *R. montana* was applied to the newly exuviated L4 larval stage of *Cs longiareolata* at the lethal concentration 50 (LC_{50}=**4.24**) 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.3.1. 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_{50}) is recorded at 24, 48 and 72 hours after treatment.

The multiple comparison of the means by the Dunnett test shows a very highly 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, 48 hours and 72 hours ($p = 0.000$) compared to the control (Figure 28).

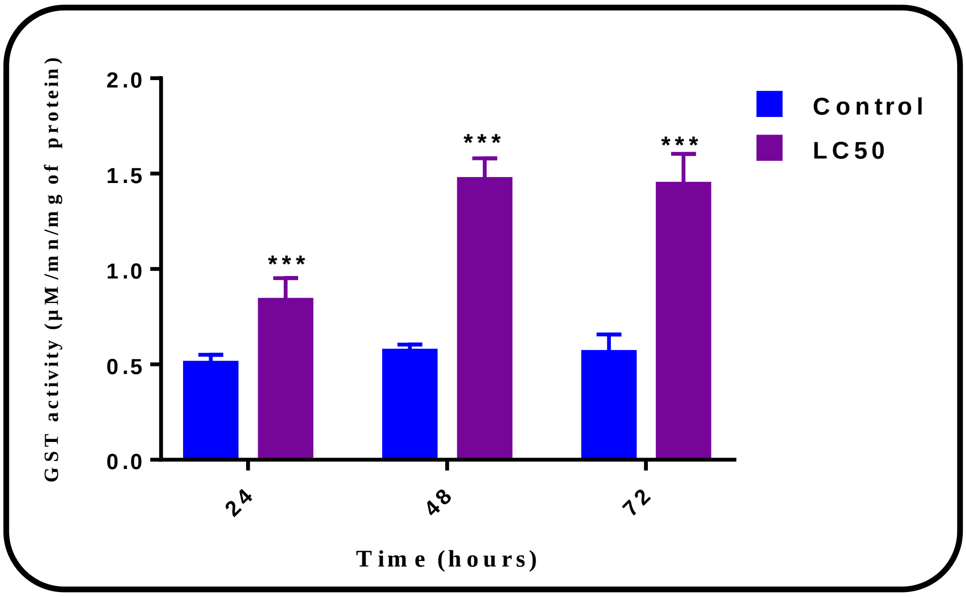

Figure 31. Effect of *R. montana* essential oil (LC_{50}) on the specific activity of GSTs (µM/min/mg of proteins) in *Cs longiareolata* larvae 4 (m ± SD, n=4). (** Highly significant difference (p<0.01) ; *** Very highly significant difference (p<0.001) between the control and treated series).

III.3.2. Effect on specific catalase activity

The specific activity of catalase was estimated in the control and treated series. The results obtained mark a highly significant increase (p=0.006) after 24 hours in *Culisetalongiareolata* larvae treated with LC_{50} of *R. montana* essential oil compared to the control series (Figure 29). This activity becomes insignificant after 48 and 72 hours (P>0.05).

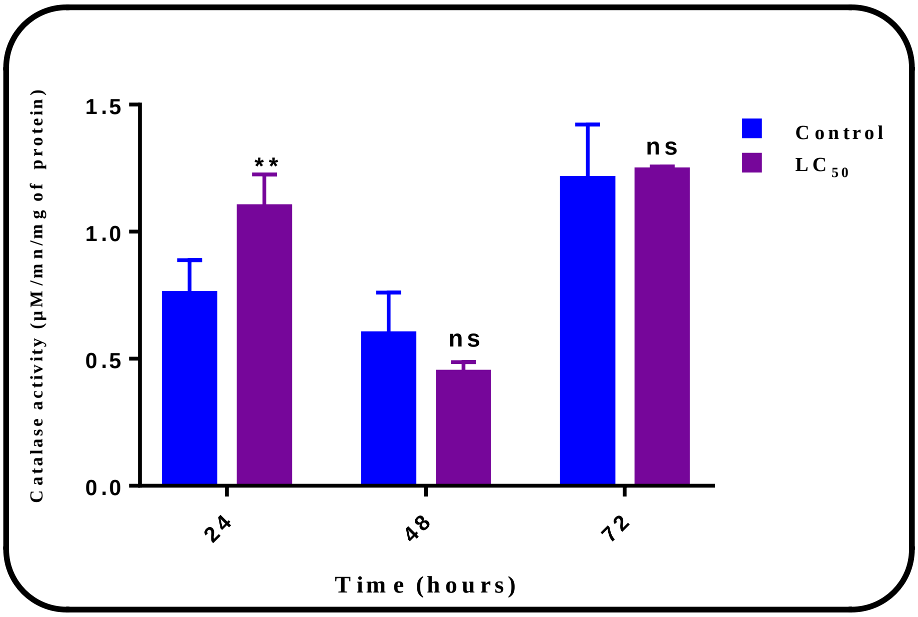

Figure 32. Effect of *R. montana* essential oil (LC_{50}) on specific catalase activity (µM/min/mg protein) in *Cs longiareolata* larvae 4 (m ± SD, n=4). (ns: Non-significant difference (p>0.05) ** highly significant difference (p<0.01) between the control and treated series).

III.4. 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 *R. montana* induces a very highly significant reduction in the total protein content of larvae at different period of treatment compared to the control.

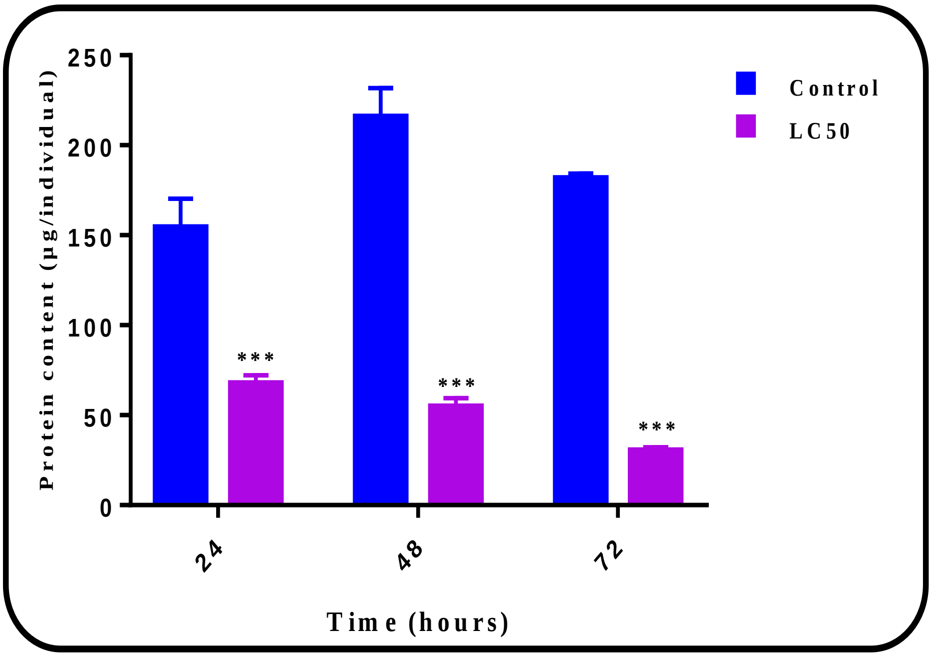

Figure 33. Effect of *R. montana* essential oil (LC_{50}) on protein content (µg/individual) in larvae 4 of *Cs longiareolata* (m ± SD, n=4). (*** Very highly significant difference ($p<0.001$) between the control and treated series).

III.5. Effect of essential oil of *R. montana* 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 (31). 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 mean values by the Dunnett test shows that the EO extracted from *R. montana* induces a non-significant effect in the body weight of L4 larvae at 24h and a highly reduction at 48h ($p=0.007$) and at 72h ($p=0.008$) compared to control.

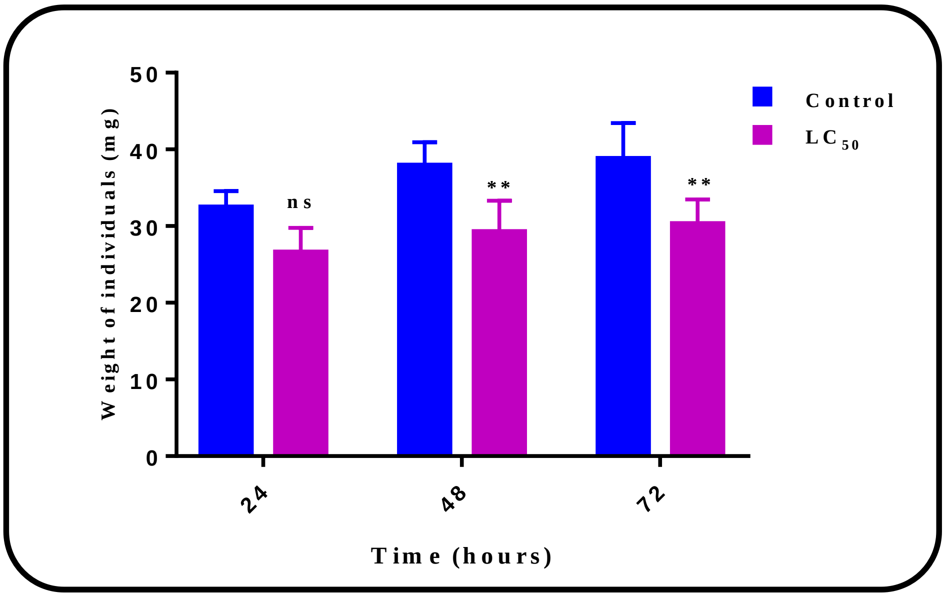

Figure 34. Effect of *R. montana* essential oil (LC50) on the weight (mg) of 4th instar larvae in *Culiseta longiareolata* (m ± SD, n = 4)

Discussion

IV. Discussion :

IV.1. Yield of *Ruta montana* essential oil

The essential oil yield from *Ruta montana* is 0.57% of the dried aerial parts of the plant.This result is lower than those reported in certain regions of Algeria, in Tizi ouzou(1.04 %)**(Daoudi *et al*., 2016),** Djelfa (1.45 %), msila (0.89%) and blida (0.71%) (**Mohammedi *et al*., 2020),** wilaya of Souk Ahras (0.67%) **(Slougui *et al*., 2023)**, medea 0.98 % **(Ammad *et al*., 2023)** and constantine (0.62 %) **(Djarri *et al*., 2013).** The steam-hydrodistillation of *R. montana* aerial parts yielded 2.5% of yellowish essential oil with a strong and penetrating odor by **(Zeraib *et al*., 2021)** in Batna . This yield also varies from country to country, (1.6 %) in morocco by **(Drioiche *et al*., 2020)**, in Tunisia (1.21%) **(Bejaoui et al 2019)** and (0.66 %) **(khadhri *et al*., 2014).** and elevated than that reported by **(Mohammadi *et al.*, 2020)** in tizi ouezou Algeria (0.38 %) and **(Barbosa *et al*., 2012)** (0.50%).

Previous studies show that variability in HE yield is due to factors of intrinsic origin factors of intrinsic origin specific to the genetic banding of the plant, or of extrinsic origin related to the period of collection, conservation of plant material and extraction method extraction method **(Khajeh *et al*., 2004; Khajeh *et al*., 2005; Viljoen *et al*., 2006; Sefidkon et al., 2007).** Geographic variation had a significant influence on essential oil yields of *R. montana* essential oil yields could be influenced also by climatic conditions including temperature and humidity as well as edaphic factors including altitude and soil characteristics **(Mohammedi *et al*., 2019).**

IV.2.Toxicity of EOs to mosquitoes

The study aimed to assess the toxicity of *Ruta montana* essential oil against newly exuviated fourth instar larvae of *C. longiareolata*. The results demonstrated a concentration-response larvicidal effect, with calculated LC25, LC50, and LC90 concentrations of 3.25, 4.24, and 7.21 µl/ml, respectively.

Various studies have highlighted the larvicidal properties of specific essential oils. For example, **(Dris *et al*., 2017)** found that *Lavandula dentata L*. essential oil exhibited larvicidal effects against mosquito larvae, with LC50 and LC90 values of 77.09 and 104.45 ppm for *Cs. longiareolata* and 113.38 and 150.38 ppm for *Cx. pipiens*. **(Bouabida & Dris, 2020)** reported that the essential oil of *Ruta graveolens* had an LC50 of 10.11 ppm and an LC25 of 6.96 ppm, showing toxic effects against *Culiseta longiareolata* larvae. Another study by **(Bouabida &**

Dris, 2022) illustrated the larvicidal efficacy of *Ruta graveolens* EO against mosquitoes and fruit flies at different concentrations. Additionally, volatile oil from *Mentha pulegium* exhibited larvicidal activity against fourth instar larvae of *Cx. pipiens* and *Aecaspius*, with Pulegone identified as the main compound **(Guenez *et al*., 2018).**

Previous studies have explored the toxicity of essential oils on *Cx. pipiens* larvae, such as the research by **(Bouguerra *et al*., 2018)** on *Thymus vulgaris* EOs, and **(Seghier *et al*, 2020)** on *Petroselinum crispum* oil. Furthermore, **(Abo El-kasem Bosly, 2022)** highlighted the larvicidal activities of *Lavandula angustifolia*, *Mentha* x *piperita*, and *Rosmarinus* officinalis essential oils against Cx. pipiens, with rosemary oil displaying the most potent larvicidal activity. **(Ezli *et al*., 2024)** showcased that *Myrtus* communis essential oil exhibited varying larvicidal activity percentages across different concentrations.

The efficacy of plant-based insecticides can vary depending on several factors, including the quality and quantity of active constituents, the plant species, age and parts used, the physicochemical characteristics of the essential oil, environmental conditions, extraction techniques, drying processes, cultivation practices, and growing environment. Additionally, criteria such as the plant's yield of essential oil and the price of the oil as an active substance for insecticide production should also be considered when selecting plants for the development of plant-based insecticides. **(Suttthanont *et al*., 2010; Pavela, 2016; Mustafa & Hussein, 2020; Sukumar *et al*., 1991; El Ouali Lalami *et al*., 2016).**

IV.3.Effect of EOs on specific GST activity

The definition of a biomarker is a measurable change in a biological or biochemical response **(Joshi*et al*., 2016)**, reflecting the interaction between a biological system and an environmental agent, which can be chemical, physical, or biological **(Winfield *et al*., 2012).** Glutathione S-transferases (GSTs, EC 2.5.1.18) are versatile enzymes involved in various cellular physiological activities, such as detoxification of endogenous and xenobiotic compounds, hormone biosynthesis, and protection against oxidative stress **(Adeyi *et al*., 2015).** They play a crucial role in xenobiotic detoxification **(Tang *et al*., 2020).**

Our findings indicate an increase in the specific activity of GSTs following treatment with *Ruta montana* essential oil compared to control groups. Similar results have been reported in *Cx. pipiens* larvae after treatment with *Basil*,*Mint*, and *Lavender***(Dris *et al*., 2018)** and with *Origanum vulgare* and *Thymus vulgaris***(Bouguerra, 2019).** Additionally, research on *Cx.*

pipiens after treatment with M. communis essential oil demonstrated a significant increase in enzyme levels in the treated samples **(Ezli *et al*., 2024).Bouabida & Dris (2020)** observed a significant increase in GST specific activity in *C. longiareolata* following treatment with *Ruta graveolens* essential oil. Furthermore, **Shojaei *et al*., (2017)** reported an elevation in GST activity in *Tribolium castaneum* larvae after exposure to Artemisia dracunculus essential oil.

These results may be attributed to the induction of a detoxification process as a response by the organism to the entry of essential oils **(Ezli *et al*., 2024).** Previous studies by **Zeghib *et al*,(2020)** also demonstrated an increase in GST activity after treating *Cx. pipiens* larvae with *Rosmarin*us officinalis essential oil. Similar observations were reported regarding the essential oil derived from Piper betle against Ae. aegypti **(Vasantha Srinivasan *et al*., 2017).**

IV.4. Effect of EOs on specific catalase activity

Catalase is an antioxidant enzyme found in almost all living organisms that catalyzes the decomposition of hydrogen peroxide (H2O2) into water and oxygen **(Ighodaro & Akinloye, 2018).** Hydrogen peroxide (H2O2) is produced during cellular respiration in all living cells **(Phaniendra *et al*., 2015).** H2O2 is dangerous and must be disposed of as soon as possible. Cells containing a small amount of catalase are very susceptible to oxidation by H2O2. Therefore, catalase plays an important role in the cell's defense mechanism against the oxidative attack of H2O2 **(Ighodaro and Akinloye, 2018).**

The results obtained in larvae 4 of *C. longiareolata* revealed a significant increase in catalase activity in treated larvae with the LC50 of *ruta montana* essential oil compared to controls. Similar observations were reported regarding the essential oils derived from *Lavandula dentata*, *Mentha piperita*, and *Ocimum basilicum* against *Culiseta longiareolata* and *Culex pipiens* **(Dris *et al*., 2018).** Another study by **Pinho *et al*., (2014)** demonstrated an increased catalase activity in *Drosophila melanogaster* treated with *Psidium guajava* essential oil. Additionally, catalase activity was significantly increased when exposed to the LC50 concentration of essential oils such as Bay *(Laurus nobilis)*, *Lemongrass (Cymbopogon citratus)*, and Tea tree *(Melaleuca alternifolia)* in *M. domestica* larvae **(Chintalchere *et al*., 2021)**. Similarly, the essential oil from Boswellia carteri caused a significant elevation in catalase activity of Callosobruchus chinensis and Callosobruchus maculatus **(Thapa *et al*., 2019).** This increase in activity reflects the onset of the detoxification process, which is a form of defense against the pesticide exposure **(Clark, 1989).**

IV.5. Effect on total protein content

Proteins play crucial roles in various biological processes such as hormonal regulation and serve as important structural components within cells alongside carbohydrates and lipids **(Sugumaran et al., 2010).**

In our study, we evaluated the total protein content in *C. longiareolata* larvae under different treatments at various time points (24, 48, and 72 hours post-treatment). Our results revealed a significant reduction in total protein content in the larvae treated with an essential oil extracted from *R. montana* compared to the control group.

Furthermore, when *C. longiareolata* larvae were treated with *Ruta graveolens* essential oil at LC25 and LC50 concentrations, a significant decrease in protein levels was observed at 24, 48, and 72 hours post-treatment compared to the control group **(Bouabida & Dris, 2020).** These findings align with previous studies by **Bouguerra & Boukoucha (2021),** which also reported reduced protein levels following the application of *Origanum glandulosum* essential oil on *Culex pipiens* larvae. Similar observations were noted in studies involving other essential oils such as *lavender*, *basil*, *mint*, *thyme*, *oregano*, *spearmint*, and *laurel* on various mosquito species **(Dris, 2019; Bouguerra, 2019; Guenez, 2020; Bouzidi, 2020).**

The decrease in protein levels could potentially be attributed to the insect's physiological adaptation to stress induced by the insecticides **(Zamani et al., 2010).** It is also possible that the increased energy demand under stressful conditions led to the stimulation of protein catabolism **(Ribeiro et al., 2001).**

IV.6. Effect of *R. montana* essential oil on the weight growth of *Culiseta longiareolata*

The body size is a pivotal trait for mosquitoes because it influences their blood-feeding ability, host attack rate, and fecundity. All of these traits are important determinants of their potential to transmit diseases **(Farjana *et al*., 2013).**

Our results show the evolution of body weight in individuals during the fourth larval stage. In both the control and LC50-treated series, there was a significant decrease in body weight from 24 hours to 72 hours. Previous studies have reported similar observations using other plant essential oils such as *Ocimum basilicum*, *Lavandula dentata*, *Mentha piperita* **(Dris *et al*., 2017***)*, *Laurus nobilis* **(Bouzidi *et al*., 2020)**, and *Petroselinum crispum* **(Seghier *et al*., 2020)** against *Culex pipiens* and *Culiseta longiareolata*.The treatment with lethal concentrations LC50 (70.95, 39.41, and 10.85 µL/mL) of *R. graveolens* EO for *Cx. pipiens*, *Cs. longiareolata*, and *D. melanogaster* species, respectively, induces a significant reduction in larval weight **(Bouabida *et al*., 2022).** A study by **(Yahia *et al*., 2023)** showed that the body weight was affected by treatment with *Eucalyptus globulus* essential oil at its LC25 and LC50, with the treatments significantly decreasing larval weight. Furthermore, *Laurus nobilis* and *Mentha pulegium* significantly reduce the body weight and body volume of fourth instar larvae of *Culiseta longiareolata*, *Culex pipiens*, and *Aedes caspius* **(Guenez *et al*., 2020).**

The decrease in larval weight and body size may be due to an impaired absorption process caused by the effects of essential oils on larval digestive cells **(Procópio *et al*., 2015).** Additionally, several studies have demonstrated that botanical insecticides inhibit the activity of several digestive enzymes, which convert complex food materials into micromolecules necessary to provide energy and metabolites for growth and development **(Sahayaraj *et al*., 2014).**

Conclusion :

Given the challenges associated with the use of chemical insecticides and their detrimental effects on health and the environment, the exploration of natural alternatives that can serve as effective substitutes to synthetic insecticides while offering ecological and economic benefits has become imperative.

Ruta montana essential oil, with a yield of 0.57% of aerial dry matter, was examined for its impact on the detoxification system, specifically the activity of GSTs and catalase, effect on total protein content and on in fourth instar larvae of a prevalent mosquito species *Culiseta longiareolata* from the Tebessa area. The essential oil derived from *Ruta montana* exhibited a reduction in the rate of increase in GST activity and a differential boost in catalase activity in treated *C. longiareolata* L4 larvae compared to untreated controls, showcasing promising properties of the essential oil.

The findings suggest potential applications of *Ruta montana* essential oil in biopesticide production due to its insecticidal properties and concentration-dependent response.

Future research aims to:

- **specify the nature of the compound(s) responsible for insecticidal activity.**
- **measure the biomarkers AChE and MDA for *Cs longiareolata* .**
- **Study the toxicity of *Ruta montana* oil on other species *Culex pipiens*.**

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