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Antioxydant activity and combined toxic effect of *Ruta graveolens* and *Ruta montana* essential oils on *Culiseta longiareolata* larvae

By

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بسم الله الرحمن الرحيم

﴿وَمَا مِن دَابَّةٍ فِي الْأَرْضِ وَلَا طَائِرٍ يَطِيرُ بِجَنَاحَيْهِ إِلَّا أُمَمٌ أَمْثَالُكُم ۚ مَّا فَرَّطْنَا فِي الْكِتَابِ مِن شَيْءٍ ۚ ثُمَّ إِلَىٰ رَبِّهِمْ يُحْشَرُونَ﴾

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«A work even made of a single hand is never the work of unseul».

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Dedication

(وَآخِرُ دَعْوَاهُمْ أَنِ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ)

First of all , I am extremely grateful to Allah who helped me

to finish this work

To the most precious people who gave me strength, encouragement and hope;

To my dear father ***Mouhammed yazid***

And beloved mother ***Nadjah***

I dedicate this work

To my dear sister ***Sara****,* ***Amal*** *and* ***LaraChada***

To my beloved ones ***Adem Djihad*** *and* ***Mouhamed Djasser***

CHAIMAA

Dedication

﴿ وَأَنْ لَيْسَ لِلإِنسَانِ إِلَّا مَا سَعَى ﴾

Praise be to God at the beginning and at the end, for no path ends, no effort ends, andno effort is completed except by His grace.

With all love, I dedicate my graduation research to my strong self, who endured all the pitfalls and completed it despite the difficulties.

*To the great man, **my father**, my support and lifelong companion, to the one whosupported me without limits and gave me for free.*

To the one whose prayers were the secret of my success,

***My mother,** Idedicate this achievement to you, which without your sacrifices would not have beenachieved.*

*To my brother **Mohamed Al-Amin***

*To my sisters: Samia, **Farah, Nour houda, Rayan.***

My support in life, may God keep you as a steady side for me.

*To those who shared my joy, **my big family***

*Who was part of this achievement, **Chaima***

*To those who showered me with love and gave me strength, **my friends**.*

IkRAM

RESUME

Plusieurs études s'intéressent depuis plusieurs années aux différentes activités thérapeutiques et anti-insectes des plantes médicinales car elles contiennent la source de composés naturels bioactifs.

Dans ce contexte, nous avons mené cette étude, qui visait la recherche en laboratoire sur les activités antioxydantes des huiles essentielles *R graveolens* et *R montana* , et Evaluer l'antiL'activité des insectes des deux huiles essentielles de la plante contre les larves les plus abondantes de *Culiseta longiareolata* à Tébessa, pour déterminer l'effet toxique commun des deux huiles essentielles.

L'huile de base a été obtenue par distillation à l'aide d'un type clevenger donne une huile essentielle *R montana* et R *graveolens* avec un rendement de 0,55% et 0,48. % de matière sèche provenant respectivement de la partie aérienne des deux plantes.

Évaluer l'activité antioxydante. En utilisant une technique DPPH Test , *R graveolens* EO inhibe l'activité oxydative avec un pourcentage allant de 20,26% (à 0,1 mg/mL) à 37,01% (à 1,6 mg/mL). *R montana* EO inhibe l'activité oxydative avec un pourcentage allant de 18,49% (à 0,1 mg / mL) jusqu'à 43,65%.

Évaluer l'effet combiné de l'huile à l'aide de trois mélanges différents (75 % *R graveolens* et 25 % *R montana*) (50 *% R graveolens* et 50 % *R montana*) (25 % *R graveolens* et 75 *% R montana)* En utilisant plusieurs concentrations sur les larves de la quatrième phase, *Culiseta longiareolata,* dans des conditions de laboratoire, selon les directives de l'Organisation mondiale de la Santé ,Tests de toxicité selon lesquels ces huiles possèdent des propriétés pesticides avec la relation entre la dose et la réponse.

Nos résultats confirment que les huiles essentielles bénéficient de l'activité biologique des insecticides et des antioxydants et justifient leur efficacité comme alternative aux produits chimiques nocifs.

Mots clés : *R montana, R graveolens,* essentiel, huiles insecticides, pesticides, toxicité, activité antioxydante, *Culiseta longiareolata.*

ABSTRACT

Several studies have been concerned for several years with the various therapeutic and anti-insect activities of medical plants because they contain the source of bioactive natural compounds.

In this context, we conducted this study, which was aimed at laboratory research on the antioxidant activities of essential oils *R graveolens* and *R montana* , and Evaluate the anti-insect activity of both of the plant's essential oils against the most abundant *Culiseta longiareolata* larvae in Tebessa, to determine the common toxic effect of both essential oils.

The base oil was obtained by distilling using a clevenger-type gives an essential oil *R montana* and *R .graveolens* with a yield 0,55% and 0,48.% of dry matter from the aerial part of the two plants respectively.

Evaluate antioxidant activity. Using a technique DPPH Test , *R graveolens* EO inhibits oxidative activity with a percentage ranging from 20.26% (at 0.1mg/mL) up to 37.01% (at 1.6mg/mL). *R. montana* EO inhibits oxidative activity with a percentage ranging from 18.49% (at 0.1mg/mL) up to 43.65%.

Determine the lethal concentrations (CL25 and CL50and CL90) , Evaluate toxicity effect the combined oils essential using three different mixtures (75 %*R graveolens* and 25% *R montana) ,(*50 % *R graveolens* and 50% *R montana) , (*25% *R graveolens* and 75 *% R montana)* Using several concentrations on the fourth phase larvae *Culiseta longiareolata* in laborator conditions as directed by the World Health Organization ,Toxicity tests that these oils possess pesticide properties with the relationship between dose and response.

Our results confirm that essential oils enjoy biological activity of insecticides and antioxidants and justify their effectiveness as an alternative to harmful chemicals.

Keywords: *R.montana*, *R.graveolens*, essential oils, insecticides , pesticide, Toxicity, antioxidant activity.

ملخص

اهتمت العديد من الدراسات لعدة سنوات بالأنشطة العلاجية والمضادة للحشرات المختلفة للنباتات الطبية لأنها تحتوي على مصدر المركبات الطبيعية النشطة بيولوجيًا.في هذا السياق، أجرينا هذه الدراسة، التي استهدفت الأبحاث العلمية حول الأنشطة المضادة للأكسدة للزيوت الأساسية *R graveolens* و*R montana* ، وتقييم نشاط الحشرات للزيوت الأساسية للنبات ضد أكثر يرقات وفيرة في تبسة *Culiseta longiareolata* ، لتحديد التأثير السام المشترك للزيوت الأساسية.

تم الحصول على الزيت الأساسي عن طريق التقطير باستخدام جهاز من النوع clevenge ظهر تقطير *R montana* و *R graveolens*بعائد 0.55٪ و 0.48.٪ من المادة الجافة على التوالي.

تقييم النشاط المضاد للأكسدة باستخدام تقنية اختبارDPPH ، يثبط EO *R graveolens* النشاط التأكسدي بنسبة تتراوح من 20.26٪ (عندmg/mL 0.1) إلى 37.01٪ (عند 1.6 mg/mL).يثبط EO *R. montana* النشاط التأكسدي بنسبة تتراوح من 18.49٪ في (0.1mg/mL) إلى 43.65٪.

تقييم تأثير السمية المشترك للزيت باستخدام ثلاثة نسب مختلفة (75٪ *R graveolens* و 25٪ *R montana*) (50٪*R graveolens* و 50٪ 25٪*R montana*) (*R montan* 75٪ *R graveolens* باستخدام عدة تركيزات على يرقات المرحلة الرابعة، *Culiseta longiareolata*المبادئ التوجيهية للصحة العالمية، وفقًا للظروف المختبر.

تؤكد نتائجنا أن الزيوت الأساسية تستفيد من النشاط البيولوجي للمبيدات الحشرية ومضادات الأكسدة وتبرر فعاليتها كبديل للمواد الكيميائية الضارة.

الكلمات المفتاحية : *Ruta graveolens* , *Ruta montana* , *Culiseta longiareolata* , الزيوت الاساسية النشاط المضاد للاكسدة, السمية.

LIST OF ABRIVIATION

Cs : *Culiseta longiareolata*

R.Montana: *Ruta.montana*

R.Graveolens*: Ruta.graveolens*

EO : Essential oil

Eos : Essentials oils

L1: first larval instar

L2: Second larval instar

L3: Third larval instar

L4: Fourth larval instar

LC: Lethal concentraition

LC50: Lethal concentraition of 50% of Population

LC25: Lethal concentraition of 25% of population

LC90 : Lethal concentraition of 90% of population

EC50 : Efficient concentration 50

DPPH :2,2-diphenyl-1-picrylhydrazyl %: Percentage

D.D.T : Dichloro-diphenyl-trichloroethane

Cm: Centimeter

Mm: millimeter

Ml: milliliter

G: Geam

Mg: milligram

M: Mass in grams

Nm : nanometer

Min : minute

H: hour

Ppm: Parts

R(%): Essentiel oil yield in (%)

±: more or less

INTRODUCTION

I.INTRODUCTION:

In terms of human and animal epidemiology as well as ecosystems, mosquitoes are essential. These insects are not only a nuisance because of their bites, but they are also the primary carriers of viruses that can infect domestic animals and people. They therefore have an effect that goes well beyond simple annoyance **(Foster & Walker, 2019)**. Arthropods are one of the most important branches of the animal kingdom, with more than one million known species, three quarters of which are insects. The latter constitute more than 50% of the diversity of the planet and nearly 60% of that of the animal kingdom. Their immense variety, their extraordinary prolificity, as well as their reduced size allowed insects to colonize most continental environments. Among these insects can be found the Culicidae. They occupy the first place by their role as vector of pathogenic organisms or nuisance agent **(Dris, 2018)**.

Mosquitoes are a major health problem **(Vivekanandhan *et al.*, 2022)**. Diseases transmitted by hematophagous arthropods represent a threat for animal and human health with the increase of their emergence outside the endemic areas. Mosquitoes (Diptera: *Culicidae*) are considered as the main vectors of pathogens of various important diseases such as malaria, filariasis, dengue fever, yellow fever, chikungunya, West Nile virus, Zika virus and other arboviruses responsible for encephalitis, bacteriosis, and helminthiasis, which are today among the greatest health problems in the world. Many arboviruses have been isolated from mosquito species belonging to the genera *Culex, Aedes* and *Culiseta* **(Arroussi *et al.*, 2021)**.

Which impact 700 million people each year and kill over one million people **(Vivekanandhan *et al.*, 2022)**. Unfortunately, there are no effective, commercially available vaccines for most mosquito-transmitted diseases, and crucially, malaria parasites are rapidly developing resistance to drugs , while drugs for arboviruses do not currently exist. For this reason, mosquito control has long been the most common strategy employed to limit disease transmission, and historically the most common approach has been to utilize different chemical insecticides to rapidly and effectively kill mosquitoes. These insecticides can be used to target both larval and adult mosquito stages and can be used synergistically with mosquito bite prevention strategies. Many commonly used insecticides, including pyrethroids and organophosphates, kill by targeting the mosquito central nervous system **(Caragata *et al.*, 2020)**.

For that, vector control remains the best method to lower the rate of disease transmission in the population. Unfortunately, insect vector control is also facing numerous difficulties because of the misuse of synthetic insecticides in agriculture and insect pest control programmes leading to environmental pollution, causing an ecological imbalance. Specifically, the synthetic insecticides in use are toxic not only to humans but also to nontarget organisms **(Younoussa *et al*., 2020)**, poisoning and death; cancer, by non-genotoxic mechanisms (immunesuppressants, cytotoxic) or by triggering the carcinogenic process in different ways; harmful effects on the nervous, renal, respiratory and reproductive systems and induction of oxidative stress **(Silvério *et al*., 2020)**without omitting the problem of the development of insecticide resistance and the resurgence of new pest species **(Younoussa *et al*., 2020)**. There has been a great interest in the use of biologically derived pesticides as an alternative to synthetic chemicals **(Vivekanandhan *et al*., 2022).**

Thus, in recent years around the world, natural products raised the attention of researchers to look for new alternative solutions to reduce the excessive use of these synthetic pesticides. Among these alternatives of which nature presents, botanical-derived products are of particular interest since they are less toxic, biodegradable, and target-specific Plants are abundant in secondary metabolites with bioactive properties that are known for their insecticidal capabilities(ovicidal, larvicidal, pupicidal, adulticidal, repellent and ovipositional effects) against the mosquito **(Younoussa *et al*., 2020)**. Numerous studies have reported the potential of essential oils as biopesticides for integrated weed or pest management **(Werrie *et al.*, 2020)**.

Essential oils are extracted from various aromatic plants generally localized in temperate to warm countries like Mediterranean and tropical countries where they represent an important part of the traditional present, approximately 3000 essential oils are known, 300 of which are commercially important especially for the pharmaceutical, armacopoeia big advantage of essential oils is the fact that they are usually devoid of long-term genotoxic risks. Moreover, some of them show a very clear antimutagenic **(Isman, 2020)**. However, the commercialization of essential oils as biopesticides faces several challenges, including reduced stability and efficiency of essential oils under environmental conditions. Further research is needed to address these challenges and to explore the potential of essential oils as eco-friendly and sustainable alternatives to synthetic pesticides **(Assadpour *et al*., 2022)**.

Rutaceae is a family of flowering plants in the order Sapindales, which includes the economically important genus Citrus, known for citrus fruits such as oranges, lemons, and grapefruits. Rutaceae plants are known for their bioactive compounds, such as polyphenols, terpenoids, sterols, carotenoids, vitamins, and amino acids, which contribute to their medicinal properties. Research has explored the insecticidal properties of essential oils from plants in the Rutaceae family **(Tan *et al.*, 2020 ; Appelhans *et al.*, 2021)**. The Rutaceae family consists of about 700 spontaneous species, present in temperate and warm regions. The *Ruta* genus has about sixty of which some are on the Mediterranean: *Ruta angustifolia* Pers. *Ruta chalepensis*; *Ruta montana*. However, *Ruta graveolens* is cultivated throughout the Maghreb **(Bouabida & Dris., 2020).**

Ruta graveolens and *Ruta montana* characterized and possess many bioactivities such us the properties of antimicrobial and antioxidant agents **(Drioiche *et al.*, 2020)**, anthelminitic and nematocidal activity. The three types of larval activity are insecticidal, repellent . Phytotoxic and herbicidal activity, antiparasitic inaction **(Nahar *et al.*, 2021)**, anti-Inflammatory Properties **(Boudjema *et al.*, 2018)**, antifungal and antibacterial action **(Coimbra *et al.*, 2020).**

Essential oils of *R. graveolens, R. montana* have insecticidal properties **(Grabsi *et al.*, 2023;bouabida &Dris, 2022 ; Wang *et al.*, 2022)** and contain natural and active substances of complex chemical composition, which gives them very interesting insecticidal properties and very slow resistance development compared to synthetic insecticides **(Bouabida & Dris, 2022)**.

In this context, The aim of our study is to evaluate the antioxidant activity of tow medicinal plants rutacea family (*Ruta montana* and *Ruta graveolens*). Also, to test the insecticidal properties of both plant's essential oils against *Culiseta longiareolata* larvae, however to set the combined toxic effect of both plant's essential oils .

MATERIALS AND METHODES

II. MATERIALS AND METHODES

II.1.Presentation of the insect (*Culiseta longiareolata*)

II.1.1. Generality of mosquitoes

The expression "mosquito" alludes to little flying bugs characterized under the request Diptera and the family Culicidae (**Zhao *et al.*, 2022**).

Only female mosquitoes feed on blood as they require the proteins for egg development, while male mosquitoes primarily feed on plant nectar. Mosquito larvae undergo development in water, progressing through various stages before emerging as adults. Understanding mosquito species is crucial for studying their behavior, ecology, and potential risks of disease transmission **(Zhao *et al*, 2022)**.

Mosquito-borne illnesses are a worldwide wellbeing problemgrowing, compromising over 40% of the total populace and it is normal that close to the portion of the total populace is in danger of arbovirus transmission by 2050 **(Baz *et al*., 2022)**. An existence without mosquitoes would be joy! Not any more irritating hums. No more biteswhich irritated. Or more all, no more dengue or intestinal sickness **(Merabti *et al*., 2021)**.

The *Culicidae* larvae play a very important role in trophic chains, they feed on microorganisms, algae, protozoa, invertebrates and detritus this very varied diet gives them the opportunity to participate in the self-purification process, by eliminating the organic matter accumulated in the bottom of aquatic environments. They are considered essential prey of several species of fish and amphibians. Whatever their role in trophic chains like predators or prey, they occupy a very important place in the functioning of aquatic ecosystems **(Chahed, 2022) ,** These bugs are famous for sending sicknesses like intestinal sickness, dengue fever, Zika infection, and West Nile infection. Mosquitoes have a thin body, long legs, and prolonged mouthparts known as proboscis, which they use to benefit from the blood of creatures and people **(Zhao *et al*, 2022)**.

II.1.2. Presantation of *Culicidae*

Arthropods in the class insecta, order Diptera, and family Culicidae comprise mosquitoes **(Foster & Walker., 2019)** Culicidae family is comprised of 113 genera and 3570 species with a broad distribution throughout the world, In 1878, mosquitoes were the first arthropods officially introduced as the intermediate hosts of verte- brate parasites; however, they are now recognized as the most important arthropods affecting human health **(Khaligh *et al*.,2020).** They are characterized by long and thin antennae with multiple articles, wings provided with scales, and females with long mouthpieces in the form of rigid proboscis of the type piqueur-suceur. They make up the majority of pathogen-transmitting vectors for both humans and animals **(Foster & Walker., 2019)**. Indeed, Typically, the development of eggs in blood-suck- ing insects is normally dependent on a blood meal nonetheless, there are some female mosquitos that can develop their eggs without a blood meal, a trait called autogeny.

In a number of females of this group of insects, the first group of eggs and possibly the next can grow without blood feeding . For the growth, they often use the last larval stage food. This characteristic has been observed in manyspecies of mosquitoes, **(Khaligh *et al*.,2020)** This group of hematophagous insects has benefited from the largest number of studies in Algeria and more particularly at the level of research laboratory **(Yssouf *et al*., 2016).**

Figer01: *Culiseta longiareolata* (Personal photo)

II.1.3. *Culicidae* in Algeria

In Algeria, only the two subfamilies *Culicinae* and *Anophelinae* are represented. fifty three species belonging to seven genera of mosquitoes are listed, including fifteen species of the genus Aedes, fifteen species of the genus Anopheles, fourteen species belonging to the genus *Culex*, five species of the genus *Culiseta,* two species for Coquillettidia and one species each for *Uranotaenia* and *Orthopodomyia* **(Oussad, 2022)**.

II.1.4. Life Cycle

Mosquitoes are insects with holometabolism . They go through a short nymphal stage and an aquatic larval stage before reaching adulthood . The adults or imago, are aerial while the eggs, larvae and nymphs constitute the pre-imaginal stages and live in fresh water most often or sometimes brackish **(Kaleka *et al.*, 2019)** . For tropical regions, the entire development period, which is greatly impacted by temperature, lasts 10 to 15 days **(Becker, 2008)**.

The life cycle consists of two phases :

The aquatic phase: includes the pre-imaginal or immature stages, egg, larvae (which have four stages), and nymph. The larval stages are characterised by a growing period during which there is a notable increase in size, which can occur up to ten times, from stage I to stage IV; this phenomenon of increase will not remain in the later phase **(Kaleka *et al.*, 2019)**.

The aerial phase: for both males and females in the adult or imaginal stage. This is the time of dispersal and reproduction. The female consumes both sweet fluids, which give her the energy she needs to fly, and human or animal blood, which promotes the growth of her ovaries. The male solely consumes sweet juices. Only the female anopheles is hematophagous, meaning that it can absorb and/or spread the parasite from blood during a meal **(Kaleka *et al.*, 2019).**

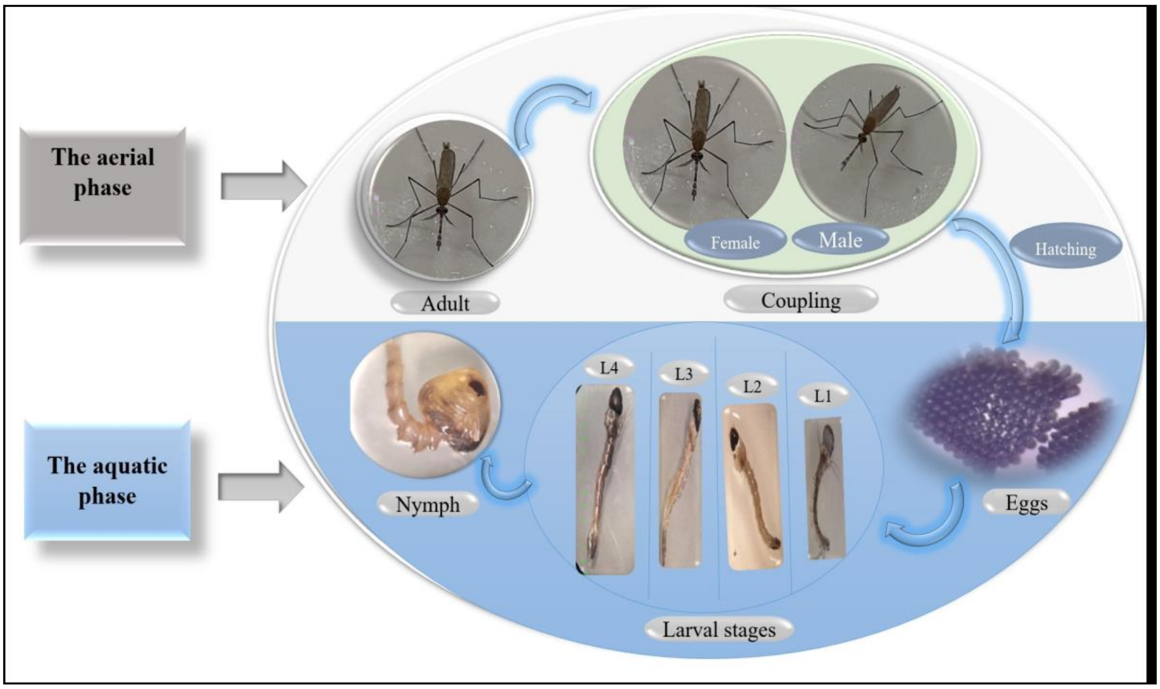

Figure02: Life cycle of *Culiseta longiareolata* (Personal photos)

II.1.5. Morphological characters

Mosquitoes are holometabolous insects, they have a complete metamorphosis, from so that the life stages (adult, larva and nymph) have very different morphologies, which allow identification of subfamilies, genera and species **(Chahed, 2022)**.

II.1.5.1. Egg

The egg after mating that took place shortly after its emergence at maturity, fertilized females lay between 200 and 400 eggs, perpendicular to the egg. Surface of the water. The eggs are cylindrical in shape and white at birth they spawn, and become gray or black after a few hours. This coloring is due to Oxidation of some chemical components of the bladder upon contact with water or air **(Dris, 2018)**.

The mosquito egg is generally spindle-shaped and measures approximately 0.5 mm . There outer layer of the egg bears lateral or apical expansions characteristic of the genera and species of more or less ovoid shape, and provided laterally with floats allowing them to maintain a horizontal position . Aedes eggs are elongated, shrunken and show a network of fine depressions. They float horizontally on the surface of the water, hovering above the water or resting there, they can be laid in rafts on the surface water as in *Culex, Culiseta* . The anterior pole of each egg has a cup-shaped corolla with a hydrophilic inner surface, which rests on the surface of water, while the outer surface is hydrophobic **(Oussad, 2022).**

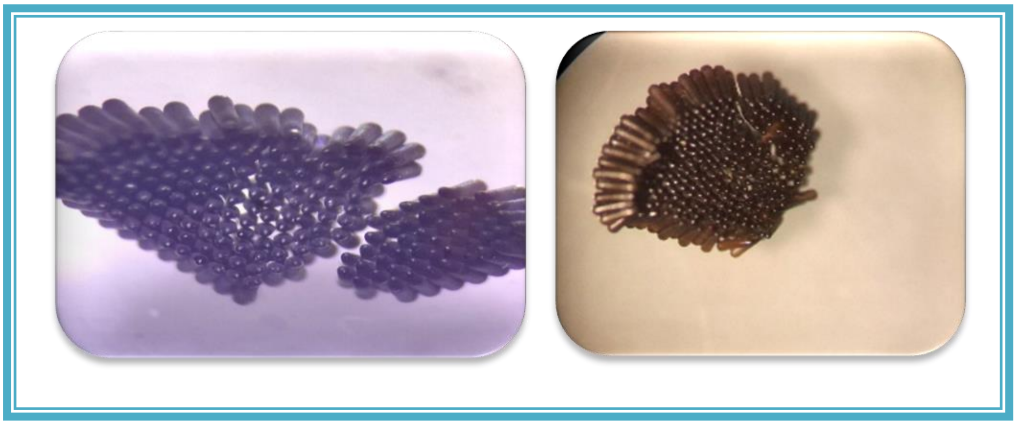

Figure03: ***Culiseta longiareolata*** egg pod (personal photos)

II.1.5.2. Larvae

This stage is aquatic. *Culiseta* larvae differ from other aquatic insects by the absence of legs. There are four larval stages generally noted L1, L2, L3, L4 ; whose first three stages do not have specific taxonomic traits, only the 4th stage larvae makes dichotomy easy; These larvae are clearly made up of three parts: head, Thorax, abdomen **(Arbaoui , 2017).**

The head: Of general globular shape, it carries faceted eyes, voluminous and almost joint.They are long and numerous. In the male (who therefore carries feathery antennae), while they are short and rare in females (hairless antennae).Females have long pieces oral characteristics of the pricking-sucking type.

Thorax: It is spherical and consists of three fused segments: prothorax, mediastinum andmediastinum, each having a dorsal and ventral part with the lateral parts of the pleura.A pair of legs is positioned on each of these segments .

The abdomen: Of its ten segments, eight are discernible. The tenth segment has the female's deer genitalia and the male's phallosoma genitalia. These segments are ornamented with hairs and scales of various colours and patterns **(Arbaoui , 2017).**

Figure04: *Culiseta longaireolata* larvae (Personal photo)

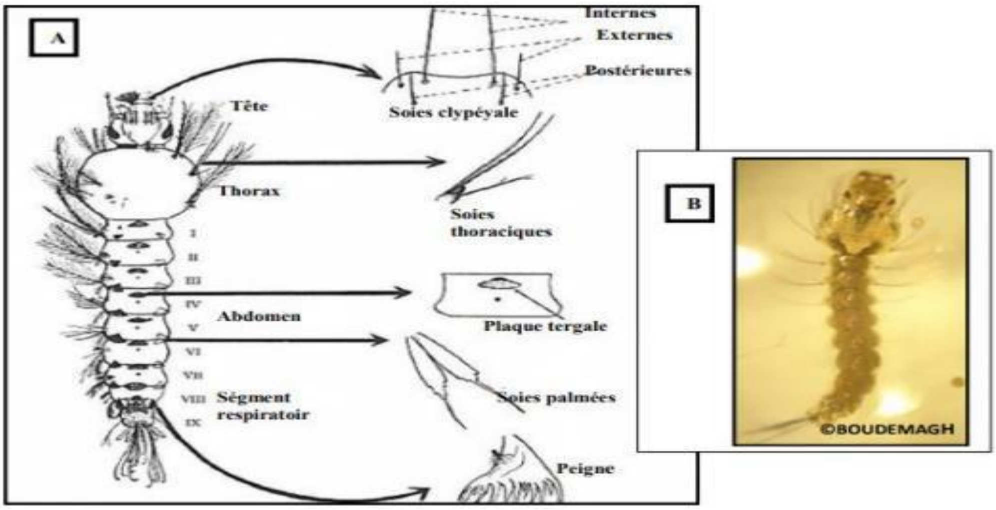

Figure05: Larval morphology (HOLSTEIN, 1949)

II.1.5.3. Nymph

The nymphal stage is a transitional stage with an extremely active metabolism, during which the insect undergoes very profound morphological and physiological transformations which take it from the larval, aquatic and saprophytic stage, to the adult, aerial and usually hematophagous form in the females. At the end of this stage, the integument then dries out on contact with air and a T-shaped tear forms on its dorsal side as a result of the increase in internal pressure. Through this opening, the adult mosquito will successively release its thorax, head, legs and abdomen, leaving the nymphal exuvia in the water. This emergence phenomenon lasts approximately 15 minutes during which the insect finds itself exposed without defenses to numerous surface predators **(Dris, 2018)**. A nymph is an animated young girl in the shape of a virgin, a body which accommodates two parts:

The cephalothorax and abdomen are reassembled into the cephalothorax Globular, joined by two respiratory tubes, and its hydrophobic plates are distinguished from The water surface, connected to the middle thoracic spiracles of developing adults to provide oxygen **(Oussad, 2022)** .

Figure06: *Culiseta longaireolata* nymph (Personal photo)

II.1.5.4. Adult

The grown-up, once transformed, causes a break in the headnymphal and arises on the outer layer of the water. Guys arrive at sexual development toward the finish of a day while females arrive at it following 1 to 2 days, and they are bigger than guys from a similar rise **(Djabri, 2021)**.They are slim bugs and moderately little, as a rule estimating around 3-6 mm long **(Mssais, 2017)**. Grown-ups, similar to all dipterans, have a solitary sets of long membranous wings.and limited with scales along its veins, collapsed evenly when very still.

There second pair is diminished to a couple of pendulums. They have a thin body that separated into three sections: the head, chest, and midsection **(Dris, 2018).**

Figure07: Adult of *Cs longiareolata* (Personal photo)

II.1.6. Presantation of *Culiseta longiareolata*

Is a pest with complete metamorphosis, more abundant in the regions hot. It is part of the Diptera, family Culicidae **(Foster & Walker., 2019)**. This mosquito ranges in size from 3 to 5 mm. Its legs are long and thin, and its wings are membrane-covered and long and narrow **(Sarwar, 2020)** . Multivoltine mosquitoes only lay at night in the laboratory, seldom during the day , Most *Culiseta longiareolata* (Diptera: Culicidae) females avoid laying eggs in pools that contain predatory retroswimmer Notonecta maculata. Females mainly bite birds, very rarely man; they penetrate occasionally in homes. The species is considered a vector of bird plasmodium **(Khaligh *et al*., 2020).**

II.1.7. systematic position of *Culiseta Longiareolata*

The systematic position of *Culiseta Longiareolata* was proposed by **(Aitken, 1954)**, as follows :

Table01: Systematic position of *Culiseta Longiareolata*

Reign	**Animalia**
Sub _ reine	Metazoa
Branche	Arthropoda
Sub _ branch	Hexapoda
Super _ class	Protostomia
Class	Insecta
Sub _ class	Pterygota
Sub _ class	Neoptera
Super _ ordre	Endopterygota
Ordre	Diptera
Sous _ ordre	Nematocera
Infra _ ordre	Culicomorpha
Family	Culicidae
Sub _ family	Culicinae
Genus	*Culiseta*
Species	*Culiseta longiareolata* (Aitken, 1954)

II.1.8. Rearing technique

Mosquitoes are found in various locations during their life stages ,such as the larvae and egg stages

1-Stagnant water : mosquitoes prefer to breed in stagnant water , such as ponds and open appliances that contain collected water .

2-Rain puddles : once rainwater accumulates in puddles and ponds , these places can become attractive to mosquito larvae .

3-pipes and sewers : stopped ,flowing or clogged sewer pipes may be ideal breeding sites for mosquitoes .

4- Hydroponices : in some cases , you may find mosquito larvae in hydroponic farms and their water bodies .

5-Water barrels and containers ; empty water barreles collected outdoors can become suitable sites for mosquito larvae to develop.

Mosquito eggs and larvae are collected from sites located in different districtes of tebessa (**Figure 08**)

Figure 08: Artificial larval deposits (Personal photo)

A mass breeding is carried out in the laboratory from the eggs of *Culiseta longiareolata* collected from sewage storage pits in Tébessa from March to April 2024.

Larvae were reared in containers containing 150 ml of dechlorinated water and fed with 0,04g of mixture of 75 %biscuit and 25 % yeast **(Rehimi & soltani, 1999).** At room temperature 25°C, the water is changed every two days . diet plays important role in fertility becouse protien allows the female to lay more eggs than females fed anly sugar **(dris,2018).**

Figure 09: Breeding in the laboratory (personal photo)

II.1.8. Control used against mosquitoes

Since antiquity, man has always sought to protect himself against harmful arthropods and vectors. In different tropical regions, some traditional practices allow the reduction of mosquito bites in homes. The means implemented at the time were essentially environmental planning measures **(Kaindoa *et al* .,2018)**.

As part of the fight against these vectors of parasitic diseases, very large quantities of insecticides in the form of synthetic chemicals are released every year in nature. However, some chemicals such as D.D.T (Dichloro-diphenyl-trichloroethane), organophosphates, pyrethroids and carbamates have become less effective due to the resistance developed by some species **(Khan *et al* .,2020).**

In order to take the lead in this battle, current research is looking for other readily available natural compounds (plants, fungi, and bacteria) that are less harmful.

II.1.8.1. Chemical control

Chemical control is the use of synthetic chemicals to control mosquito and larvae. The first generation of insecticides: are synthetic insecticides that date from before 1940 (ex: potassium dinitro-o-cresylate, dinitroorthocresol) and rubbed with inorganic insecticides (copper aceto-arseniate), fluorides (sodium fluorine), soufres (carbon sulphide). 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), neonicotinoides (imidacloprid) and also insect growth regulators (RCI:fenoxycarbe, lufenuron) **(Shroff *et al* .,2020).**

II.1.8.2. Biological control

The biological method, was the subject of a new method control, safer, more selective and less toxic. It is represented by the use of microorganisms, mushrooms, fish and even plant extracts.

II.1.8.2.1. Plant extracts

Plant extracts have toxic effects against different species of Diptera **(Muhammed *et al* .,2022).**The use of plant extracts as insecticides has long been known. Pyrethrum, nicotine and rotenone are already known as insect control agents **(Ahmed *et al*., 2021).**

In some parts of Africa, tobacco leaves mixed with water were used to control mosquitoes and odors of Basilic *Ocimum basilicum, Basil* (Labiee) and *Sarghina, Corrigiola telephiifolia* (Caryophyllacea) are very effective repellents **(Benelli *et al* ., 2019).**

The use of plant extracts as a type of insect control in Algeria has begun to develop, through a multitude of recent works . showed the effectiveness of extracts from five plants at different doses (1%, 2%, 3%, 4% and 5%), which were tested on four different species of mosquitoes such as *Culex pipiens* and *Culiseta longiareolata*. have demonstrated the activity of oleic and linoleic acids extracted from Citrullus colocynthis on mosquito larvae *(Aedes aegypti, Ae.stephensi* and *Culex quinquefasciatus*) **(Al-Snafi., 2016).**

II.1.8.2.2. Entomopathogenic bacteria

The use of entomopathogenic microorganisms is a very promising to ensure effective phytosanitary protection through the natural ubiquity of microbiological agents in ecosystems, their wide variety, easy dissemination, specificity, action and also persistence in the environment. *Bacillus thuringeinsis* (variety named Israelensis) and *Bacillus sphaericus* are the best known and most used bacteria to control mosquito larvae **(Valtierra-de-Luis *et al* 2020).**

II.1.8.2.3. Entomopathogenic fungi

Several species of entomopathogenic fungi have been isolated and tested on most disease-carrying mosquitoes that can kill both the larval and adult form **(Perumal *et al* .,2023).**

The species of entomopathogenic fungi most used in biological control belong to the genera Beauveria, Metharizium, Verticillium, Erynia, Hirsutella and Entomophtora **(Rajula *et al*., 2020).**

II.1.8.2.4. The larvivorous fish

In different parts of the world, native fish have been used to control mosquito larvae. This is the case for *Gambusia affinis, Poecilia reticulata*, *Oreochromis mossambicus*… , but

G. affinis remains the most widely used species for biological control of mosquito larvae

(Walshe *et al*., 2017).

II.1.8.2.5. Other natural predators

In the context of vector control, the use of natural predators is rather recurrent, in particular to limit populations of mosquito larvae. Some arthropods are also predators of mosquito larvae, such as copepods, which are small parasitic crustaceans. Thus, it has been observed in Vietnam that the use of copepods in large water tanks allowed to eliminate the larvae of Aedes and Anopheles **(Dambach , 2020).** The presence of *Notonecta* (which are small predatory aquatic bugs), may also deter female mosquitoes from laying eggs in the same pond. Finally, some non hematophagous mosquito larvae, such as *Toxorhynchites sp*. , are larvivores and eat larvae of other species, including *Aedes aegypti* **(Why *et al* ., 2016).**

Concerning the predators of adult mosquitoes, the best known remain insectivorous chiroptera. Thus in some French cities, nest boxes for bats have been installed to attract new populations that promote natural demoustication **(Collins *et al* .,2019).**

Among the aerial predators, there are also many birds such as swallows or swifts.

II.2.Botanical and pharmacogical data of the studied species *Ruta montana* and *Ruta graveolens*

II.2.1. Presentation of Rutaceae

The Rutaceae family, commonly known as the citrus family, is a large and diverse family of flowering plants. It includes a wide range of species, from small herbs to large trees, and is found in various regions around the world, **(Nathulal*et al*., 2022)** the genus *Ruta* belongs to the tribe *Ruteae* of the family Rutaceae Juss .40 different accepted species, which are native to or naturalized in many countries worldwide, especially in African, Asian, and European countries, Algeria, China, Iraq, Italy, Morocco, and Tunisia, the greatest distribution of *Ruta* species is found in the Mediterranean region. 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. *R chalepensis* ., *R graveolens* ., and *R montana* . are the three most widely distributed and most extensively studied species of the genus *Ruta***(Nahar *et al*., 2021).**

Pharmacognostic and phytochemical properties: Some species in the *Rutaceae* family, such as Atlantia monophylla, possess antispasmodic, stimulant, and antirheumatism properties and have been reported to have significant activity against immature stages of mosquito and non-target organisms **(Lillo *et al*., 2020).**

The species of this genus are also rich in essential oils, which contribute to their aromatic, agrochemical, and medicinal properties **(Nahar *et al*., 2021).**

In Algeria, this plant is widespread and used in traditional medicine as febrifuge, local anti-venom, against nausea and vomiting, constipation, malaria, anemia, rheumatism, gastric pains, intestinal worms, difficult childbirth, eye and ear pain, asthma, and neurosis **(Mokhtar*et al.*, 2022) .**

II.2.2. Systematic Position of *Rutaceae*

The systematic position of *Rutaceae* was proposed by (Bonnier, 1999), as follows

Table 02: Systematic Position of *Rutaceae*

Reign	**:Vegetal**
Sub _ branch	Angiosperms
Class	Rutales
Sub _ class	Dialypetals
Ordre	Dicotyledonous
Family	Rutaceace
Gender	*Ruta*
Species	*R.montana* *R.chalepensis* *R.graveolens* *R.tuberculata* (Bonnier, 1999)

II.2.3. Geographic distribution and habitat

The street is a plant native to southeastern Europe. It is widely cultivated worldwide for its ornamental and medicinal properties, and in gardens for its colourful and varied decorative qualities **(Bezanger *et al*., 1986)**. It was introduced in England, Spain, Italy and Yugoslavia. Spain is the main producer of essential oil in the street **(Rubin, 1988).**

The street grows naturally on rocks, dry places, ancient walls, dry hills and is rich in calcareous soils and exposed to the sun in some Mediterranean regions **(Doerper, 2008).**

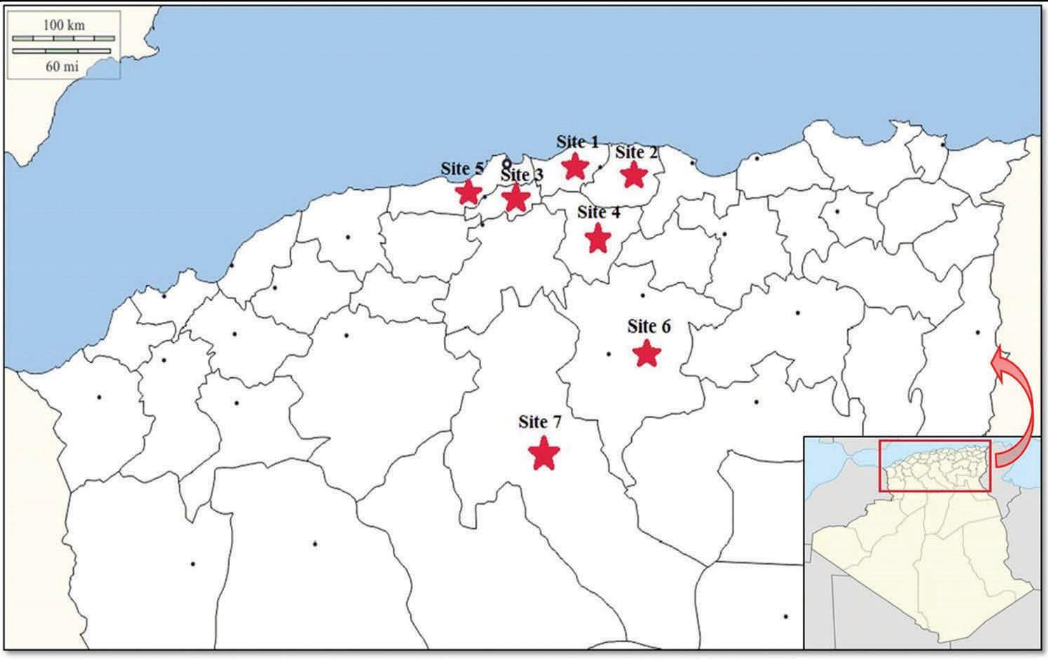

Figure 10: Collection sites of *R. montana* from Algeria **(Mohammedi *et al.*, 2020).**

II.2.4. Morphological of *Rutaceae*

Feature of the family that is easily observed in the field is the presence of schizogenous secretory cavitiescontaining essential oils. The cavities are visible as pelluciddots in the leaves, but also in other parts of the plants, suchas the pericarp, flowers, and young axes This character ispresent in nearly all *Rutaceae* except for some genera of theearly branching Cneoroideae Webb, and the cavities may beinconspicuous and less abundant in some genera **(Appelhans *et al.*, 2021).**

Rutaceae are quite variable in most morphological characters Fruits areoften baccate or dehiscent withseeds being either elastically discharged from the fruit or re-maining attached to the open fruit by the funicle. Other fruit types of **(Appelhans *et al*., 2021).**

II.2.5. Essential oils of *Rutaceae*

The essential oils derived from Ruta species are particularly notable for their bioactive compounds. They typically contain aliphatic ketones, such as 2-undecanone and 2-nonanone, which contribute to their bioactivities but are characterized by a lack of significant amounts of terpenes . These natural ointments show a variety of bioactivities **(Nahar *et al*., 2021)**, including anti-inflammatory **(Boudjema *et al*., 2018),** antimicrobial, antioxidant **(Drioiche *et al*., 2020)** , antiprotozoal ,cytotoxic ,herbicida , insecticidal , insectrepellent,larvicidal, nematocidal/anthelmintic ntibacterial Action and Antifungal Movement **(Coimbra *et al*., 2020),** and phytotoxic impactsThe varying potencies of these bioactivities among different

Ruta species can be attributed to differences in chemical composition, which in turn result from variables like geographical source, collection time, and the plant parts used **(Nahar *et al*., 2021).**

II.2.6. Presentation of *Ruta montana*

The medicinal plant *Ruta montana* . (*Rutaceae*) is known in Algeria as "mountain rue" or "Fidjel" .This plant grows in the arid regions in the Mediterranean zone, distributed in Spain,Portugal, Italy, Greece, Turkey, Algeria and Morocco .*R montana* . has several communally names (Fidjla el-djebeli in Arabic; rue des montagnes in French and mountain rue in English) **(Merghem, 2018).**

II.2.6.1. Botanical description

R.montana is a perennial aromatic herb with 20-40 cm of height **(Coimbra., 2020)**

Very branched and woody at the base, Grows spontaneously in the rockeries and pastures of Tell. It is a common species in mountainous areas up to the Saharan Atlas. It is known under the vernacular name «Fidjel el djebeli», belongs to the species of *Rutaceae* that attracted much attention because of their biological activities **(Allouni, 2018).**

The underground part

-Roots: White, fibrous and with many roots.

The aerial part

-Stems: Straight, cylindrical, very rowdy, glabrous and glaucous, 2 to 5 feet high.

-Leaves: Petioles, alternating, scattered, composed, glaucous green, with thick oval leaflets, slightly toothed at the edges or whole.

-the flowers are small yellow andin clusters

- The fruit: A globular capsule with a short peduncle does not exceed 4 mm and ends with 4 or 5 rounded, apparent lobes, releasing small blackish seeds at maturity **(Alamgir, 2017)**

Figure11: medicinal plant *Ruta Montana* (Personal photo)

II.2.6.2. Chimical composition of *Ruta montana*

Previous chemical studies on *R. montana*

Coumarines: Coumarins are a very broad class of substances that include flavonoids, chromones, and isocoumarin. They are members of the phenolic compound class.

Their primary structural characteristic is the existence of a noyaubenzopyrane **(Mekhelfi & Zaiter., 2016).**

The *Ruta montana* species' coumarins are found throughout the plant, but are most concentrated in the fruits and the essential oils of the seeds. They are often the source of heterosides **(Drioiche *et al* .,2020).**

Flavonoids: Glycosylated flavonoids from *Ruta*., specifically belonging to flavone groups like quercetin and rutin, which are both widely distributed in the Rutaceae family **(Ahmed *et al* .,2021).**

Lignans: Savinine and saventinin, two units phenylpropanoic, undergo intramolecular oxidative coupling to form lignans.

Volatile oils: The main compounds were oxygenated monoterpenes, characterized by the high prevalence of two ketone-undécane-2-one compounds (32.8%) and non-2-one (29.5%) and 2-acetate-nonanol-acetate (18,2%) **(Benali *et al* ., (2020).**

Alkaloids: are a general term for nitrogen-containing compounds derived from plants, frequently possessing a complicated structure and a large molecular weight. These are heterocyclic nuclei-containing quaternary ammonium hydrates, or primary, secondary, and tertiary bases **(Alamgir., 2018)**. Alkaloids are a second class of secondary metabolites that are common in the Rutaceae family, specifically in the genus *Ruta*, in addition to coumarins **(Szewczyk *et al*., 2023).**

II.2.7. Presentation of *Ruta graveolens*

Botanical Name : *Ruta graveolens Linn*.

Family : Rutaceae

English : The Garden Rue, Common Rue.

Arabic : Suddab

The name "Ruta" derives from Latin, taken from the Greek language, where "peganon" is still used today to refer to the plant. The Latin term "*graveolens*," meaning strong-smelling, describes the plant's potent and sharp scent emanating from its leaves **(Reza *et al*., 2020; Szewczyk *et al*., 2023; Colucci *et al*., 2020).**

R graveolens., commonly known as common rue or *sadab*, is a robust perennial shrub, The herb Suddab bears an ancient legacy of healing. The genus name reflects its healing prowess, . Its leaves are utilized for various medicinal purposes, having properties that range from being abortifacient to stomachic **(Reza *et al*., 2020)**

II.2.7.1. Botanical description

R graveolens, commonly known as *Rue* and part of the *Rutaceae* family, is a semi-shrub perennial that usually grows to a height of 65-70 cm. It is known to have a sharp and unpleasant odor,**(Reza *et al*., 2020; Badhusha *et al*., 2020).**

The leaves of *R graveolens* are blue-green, with dotted glandular leaves arranged alternately on the stem. The leaves are also triangular or bilateral, which contributes to their feathery appearance. Individual leaflets have a linear or oblong-oval shape **(Reza *et al*., 2020).** The plant produces bisexual flowers that have a regular shape and are yellow in color. These flowers have long stems and are usually born in cymbals. In these panicles, the terminal flowers are pentamerous, with five petals, while the other flowers are tetraploid, with four petals. The petals themselves are greenish-yellow in colour, spread widely and show a distinctive shape. It is covered above and held by a narrow claw below, and the edges of the

petals are wavy or serrated, **(Reza *et al*., 2020; Badhusha *et al.*, 2020)** The stem grows to a height One meter long, it is smooth, thin and pale in color Green **(Badhusha *et al.*, 2020).**

Figure12: medicinal plant *Ruta graveolens* (Personal photo)

II.2.7.2. Chimical composition of *Ruta graveolens*

Previous chemical studies on *R. graveolens* have reported the presence of coumarins, alkaloids, volatile oils, flavonoids, and phenolic acids in the plant **(Ainiwaer*et al*., 2022)** .The chemical composition of *Ruta graveolens* includes various components such as , ketones, aldehydes, esters, and sesquiterpene hydrocarbons **(Saeed *et al*., 2023)**

The essential oil derived from fruits, flowers, roots, leaves, or stems of *R graveolens* by steam distillation is mainly composed of oxygenated compounds like ketones, alcohols, and acetates. Reports show that the main components of essential oils are 2-nonyl acetate, 2-Undecanone, and 2-nonanone **(Badhusha *et al*., 2020).**

II.2.8. Traditional use of *Rutaceae*

Ruta plants are used as a topical salve for rheumatism, as a skin antiseptic and mosquito repellent, as a contraceptive, as an antihypertensive, to treat hysteria, and to reduce hangover symptoms such as headaches and ears.

Rutin, an isolated street compound, is a flavonoid that is thought to have properties antioxidant and reduces triglyceride levels **(Ganeshpurkar & Saluja., 2017)**.

This genus of plants also contains flavonoids with cytotoxic and antibacterial activities, as well as antifungal and insecticidal qualities **(Nahar *et al* ., 2021).** In traditional medicine, many Ruta types from Africa and other continents are included into various therapeutic formulations.

Generally speaking, the various plant parts are widely utilised as analgesics, emmenagogues, antirhumatismals, antispasmodics, and antiparasitic drugs.

R. graveolens has traditionally been used in folk medicine to treat a wide range of ailments, including rheumatism, varicose veins, dislocations, eye issues, tendon strains, and skin disorders including psoriasis and eczema. Numerous inflammatory disorders, including some rashes, pain, and others **(Miguel., 2003 ; Monari *et al* ., 2022).**

The essential oil is spasmolytic, anti-inflammatory and antihistamine and dewormer **(Barhoumi & Abderraba., 2019).**It activates the uterine basal fibre and functions as an emmenagogue through the action of rutin.

This was not advised for use by women who were pregnant or nursing since high concentrations can result in uterine hyperemia and strong oxytocic activity, which can cause abortion and even result in a pregnant woman's death.

Ruta montana is a plant with amazing medicinal properties that is used in Algeria as an emmenagogue, antispasmodic rubéfiant, and echaroutic powder. Form and Roques, 1941 as an abandoned medication and against some paediatric fevers **(Merghem & Dahamna., 2020).**

The leaves and roots are the sections that are most frequently utilised. This plant is utilised in a number of preparations, including decoctions and infusions; also, its essential oil has therapeutic qualities. The best course of action is to take a pinch week to prevent any toxicity **(Douadi *et al.*, 2015).**It is applied externally to heal gum disease, relieve ulcers and pain, and act as an antirheumatic and antiseptic.

Its herbal tea is drunk to treat heart issues, earaches, fever, and stomach discomfort associated with colic. This plant's leaf juice acts as a preventative measure against insect and snake attacks **(Benziane., 2007).**

II.3. Essential oil

II.3.1. Presentation of essential oil

The term essential oil dates back to the sixteenth century and derives from the drug Quinta Essentia, named by Paracelsus von Hohenheim of Switzerland **(Brenner 1993).**

Essential oils (EOs) get their name because of their flammable characteristics. According to French Agency for Normalization: Agence Française de Normalisation (AFNOR), essential oils can be defined as: “The essential oil is the product obtained from a vegetable raw material, either by steam distillation or by mechanical processes from the epicarp of Citrus, or 'dry' distillation." EOs are insoluble in inorganic solvents (water) while soluble in organic solvents (ether, alcohol, fixed oils). They are volatile liquids, having a characteristic odor and density less than unity, except vetiver, sassafras, and cinnamon **(Hanif *et al*., 2019) .**

Essential oils (Burt) (ethereal or volatile oils) are known as essences, volatile oils, etheric oils or aetheroleum. They founded in aromatic plants as a mixture of volatile components produced as secondary metabolites **(Mossa, 2016)** are natural volatile compounds that large amount of them are produced from plant raw materials or plants organs like flowers, seeds, bud, leaves, fruits, wood, roots, barks and twigs.

They give a distinctive flavour, odour or scent to an aromatic plant. For example, citrus-derived essential oils included wide group of fruits from genus citrus, are the most well-known natural EOs and account for the largest spectrum of commercial available natural fragrances and flavours EOs are obtained from more than 17,500 aromatic flora or plants bearing many Angiospermic families such as Rutaceae, Lamiaceae, Myrtaceae, Asteraceae and Zingiberaceae . They are by-products observed from plant metabolism are referred to as secondary metabolites of volatile plant **(Eslahi *et al.*, 2017).**

II.3.2. Chemical compositions of essential oil

The main chemical constituents of essential oils include terpenes, phenylpropanoids, and aliphatic compounds.Terpenes are the most common compounds found in EOs, with monoterpenes, sesquiterpenes, and diterpenes being the most common types. Monoterpenes are the simplest terpenes, consisting of two isoprene units, and are the major constituents of many EOs. Sesquiterpenes are larger and more complex, consisting of three isoprene units, and are often found in smaller quantities in essential oils. Diterpenes are even larger, consisting of four isoprene units, and are less common in EOs **(Moumni *et al*., 2021; Dhifi *et al*., 2021).**

Phenylpropanoids : are another group of compounds found in EOs . These compounds are derived from the shikimate pathway and are synthesized from phenylalanine or tyrosine. Examples of phenylpropanoids found in essential oils include cinnamaldehyde, cinnamic acid, and coumaric acid.

Aliphatic compounds: such as aldehydes, ketones, and esters, are also found in essential oils. These compounds are often responsible for the characteristic odors and flavors of essential oils.

The specific chemical composition of an essential oil can vary depending on the plant species, extraction method, and environmental factors) **(Moumni*et al*., 2021;Dhifi *et al*., 2021).**

II.3.3. Uses of essential oil

The attraction of aromatic and medicinal plants grows continuously due to the increasing demand as well as interest of consumers in these plants for medicinal, culinary, and other anthropogenic applications. As consumers are increasingly informed about health, food, and nutrition issues, they are also realizing the potential and benefits of aromatic and medicinal plants and their metabolites. There are many secondary metabolites which are produced by these plants **(Hanif ., 2019).**

- They are extensively used in perfumery, aromatherapy, and cosmetics industry. Aromatherapy is a therapeutic technique which includes inhalations, massage, or baths by using essential oils (volatile oils) **(Hanif *et al* ., 2019).**
- Essential oils (EOs) also serve as chemical signals that allow the plant to control and regulate its environment (ecological role): repel predators, attract insects for pollination, inhibit seed germination, and communicate between different plants. **(Hanif *et al* ., 2019).**
- EOs can be applied in various situations including pharmaceutical and health industries (as antiseptic, antibacterial, anti-parasitic and anti-yeast agents), commercial industries, food sectors (as preservative and flavoring agents), as well as active agent. Plant insects, agricultural pests, weeds or biocides and viral fungicides. They grow their plants because of their requirement for natural marine ingredients, making it possible for these materials to be an integral part of our lives. Therefore, there is an increasing demand for it in the field of fraud **(Eslahi *et al*., 2017).**

II.3.4. Properties of essential oils for insecticides

The chemical structure of EOs is lipophilic, which can be the case It enters the insect and causes a biochemical imbalance . The toxicity of EOs does not depend solely on Chemical compounds that act as poisons but also on many Other factors play an important role in toxicity. The Poison entry point and molecular weights Mechanisms of action are EOs to induce toxicity. Common essential oils have been reported to contain pesticides Activities can be inhaled, ingested, or absorbed through the skin **(Mossa, 2016) .**

II.3.5. Mode action of bioinsecticides Of essential oils

- On the nervous system, which can lead to various conditions like paralysis regarding cellular respiration: at that point, the cell loses its ability to absorb the oxygen that the respiratory system supplies to it.
- Concerning the development of the insect's protective skin, the cuticula, which makes it vulnerable to many environmental aggressions; this is a crucial step in the insect's growth **(Dris, 2018).**

II.3.6. Techniques for extracting essential oils

II.3.6.1. Distillation

Distillation is a process that uses the volatile nature of the aromatic components to separate them from the rest of the plant **(Elguea-Culebras *et al* 2022).**There are three different processes using the distillation principle: hydrodistillation, hydrodiffusion and steam training.

II.3.6.1.1. The hydrodistillation

The essential oils were obtained by hydrodistillation in a device Clevenger type (Figure13). This is the simplest and oldest method used.

The principle of hydrodistillation corresponds to a heterogeneous distillation it consists in immersing the vegetable raw material in a flask during an extraction in the laboratory or in an industrial still filled with water placed on a heat source. Everything is then brought to a boil. Heat allows the bursting of plant cells and the release of odorous molecules contained therein.

These aromatic molecules form with water vapour, an azeotropic mixture. The vapours are condensed in a refrigerant and the essential oils separate from the water by difference in density.

The aromatic water is then recycled in the hydrodistiller to maintain the plant/water ratio at its initial level.

The duration of a hydrodistillation may vary considerably, up to several hours depending on the material used and the plant material to be treated.

The duration of distillation affects not only the yield but also the composition of the extract **(Pavida *et al*., 1976).**

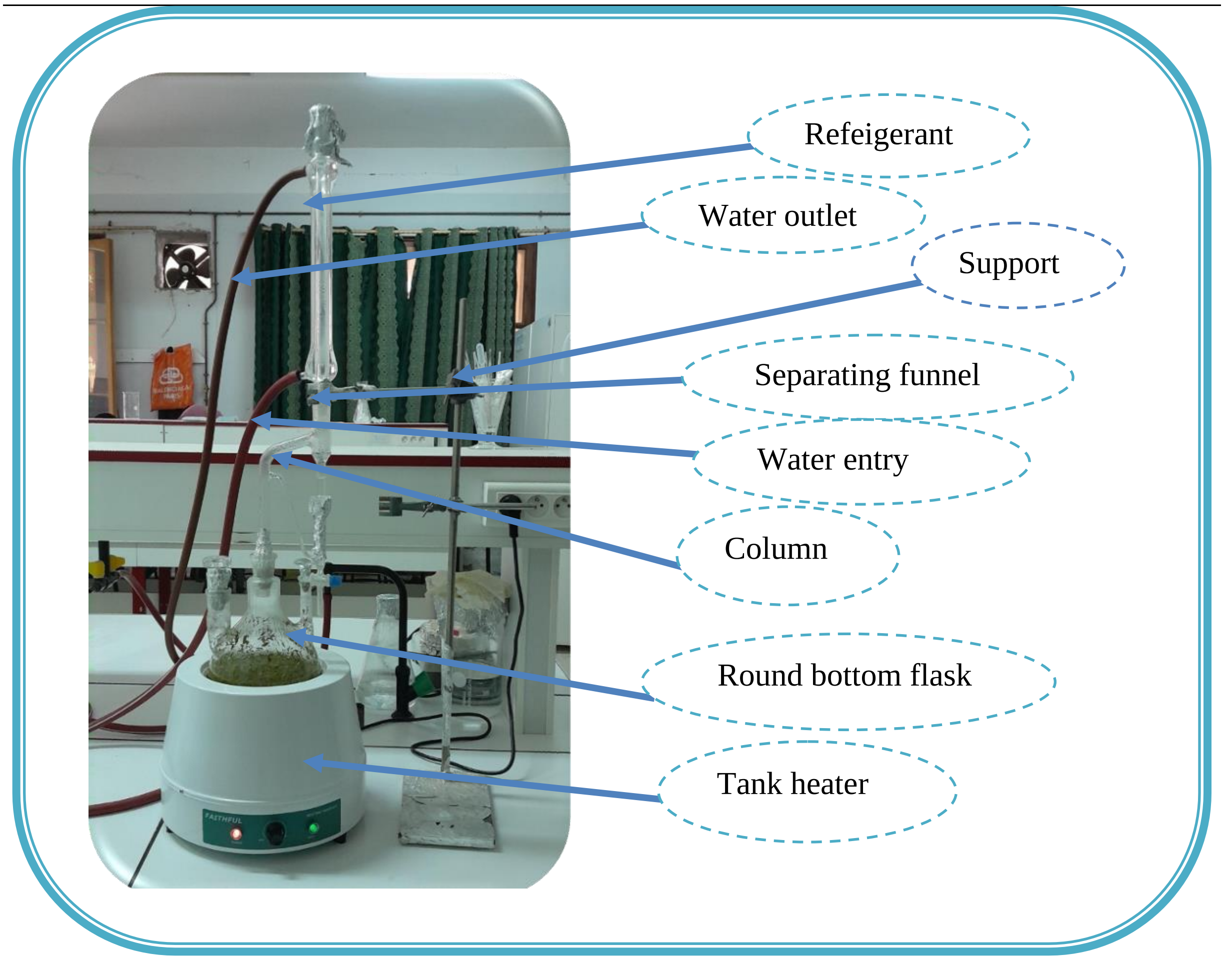

Figure13: Clevenger Hydrodistillation unit (Personal photo)

In our work we admit used the aerial part of 2 aroumatic plants, these last ones were harvested in the wilaya of Tebessa (region of Ouenza and Dhokara)

The extraction of essential oils from two plants was carried out by hydrodistillation using a Clevenger type device:

The operation consists of introducing 100g of vegetable material into a balloon, adding 1000 ml of distilled water. The mixture is brought to a boil, the vapours loaded with essential oil pass through the vertical tube and then into a refrigerant where the condensation of the oil droplets that accumulate in the tube filled with the paravant of distilled water will take place.

The extraction operation lasts two hours from the beginning of boiling.

Two liquid phases:

An aqueous phase (aromatic water)

An organic phase consisting of essential oil

The essential oil collected in glass bottles then refrigerated at 4°C.

Figure14 : vegetable material (*R.montana*)(Personal Photo)

Determination of yield :

The yield designates the mass of the extract determined after drying, and expressed as a percentage (%). It is calculated following the formula presented below (**Aberrane,2019)** .

$$R(\%) = [M/M\ ] \times 100$$

R% = (extract weight/ dry vegetable powder weight)   100

R(%): yield in (%)

M: mass in grams of crude extract

M: mass in grams of the vegetable powder used.

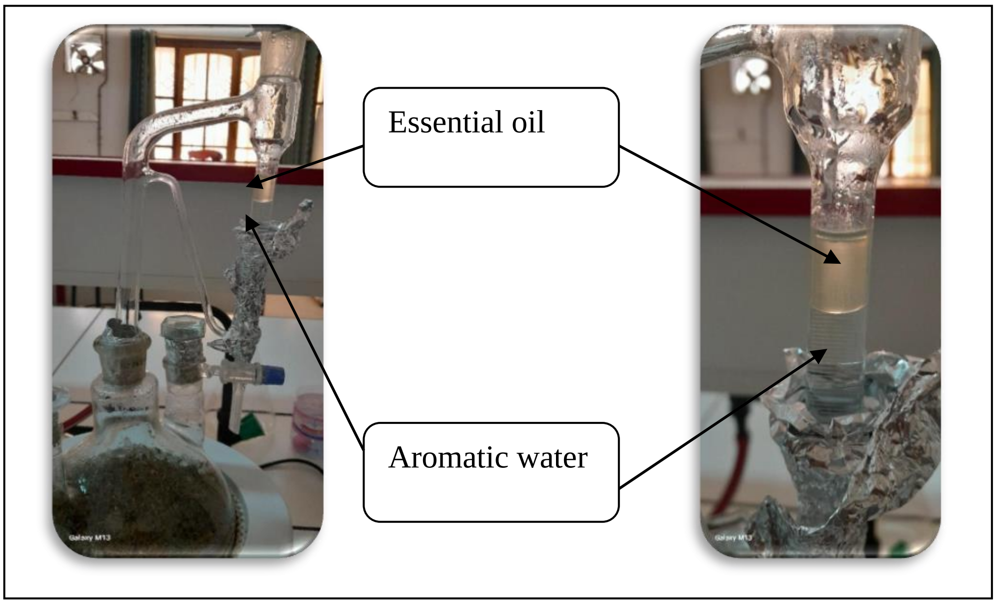

Figure15: Essential oil yield (personal photo)

Figure16: bottle with essential oil (Personal Photo)

II.4. Evaluation of the antioxidant activity: DPPH test

Principle

The DPPH° test measures the anti radical power of pure molecules or plant extracts in a model system (organic solvent, room temperature). It measures the ability of an antioxidant (AH, phenolic compounds generally) to reduce the chemical radical DPPH° (2,2-diphenyl-1-picrylhydrazyl) by hydrogen transfer. The DPPH°, initially purple, turns into DPPH-H, pale yellow (Fig.17).

DPPH reduction is easily measured by spectrophotometry at 517nm (λmax DPPH°). The reaction will be more or less rapid depending on the nature of the antioxidant, and the amount of DPPH-H formed will depend on the antioxidant concentration.

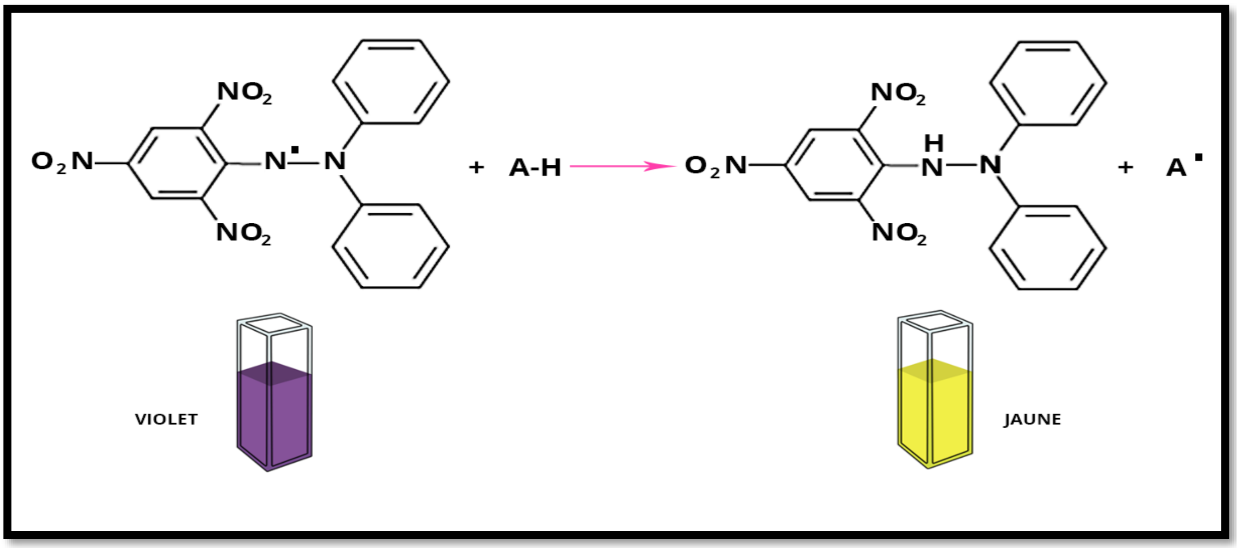

Figure17: Chemical transformation of DPPH

Oeraiting mode

a. Preparation of DPPH methanolic solution:

2.4mg of DPPH is added to 100ml methanol, then stirring for 20min.

b. Performing a test

400 µl of each solution extracts at different concentrations are added to 1.6 ml of the methanolic solution of the DPPH. At the same time, a negative control is prepared by mixing 400µl of methanol with 1.6 ml of DPPH methanolic solution. The absorbance reading is made against a blank prepared for each concentration at 517nm after 30 min incubation.

The positive control is represented by a solution of a standard antioxidant; BHT and BHA whose absorbance was measured under the same conditions as the samples and for each concentration the test is repeated 2 times. The results were expressed as % inhibition (I%)(Fig.18)

c. Expression of results

• Calculation of 50 "IC50" concentrations

IC50 (also called EC50 for Efficient concentration 50), allows to calculate the concentration of the tested sample necessary to reduce 50% of DPPH radicals. It is calculated graphically by linear regression of plotted graphs, inhibition percentages according to different concentrations of fractions used **(Arous, 2012).**

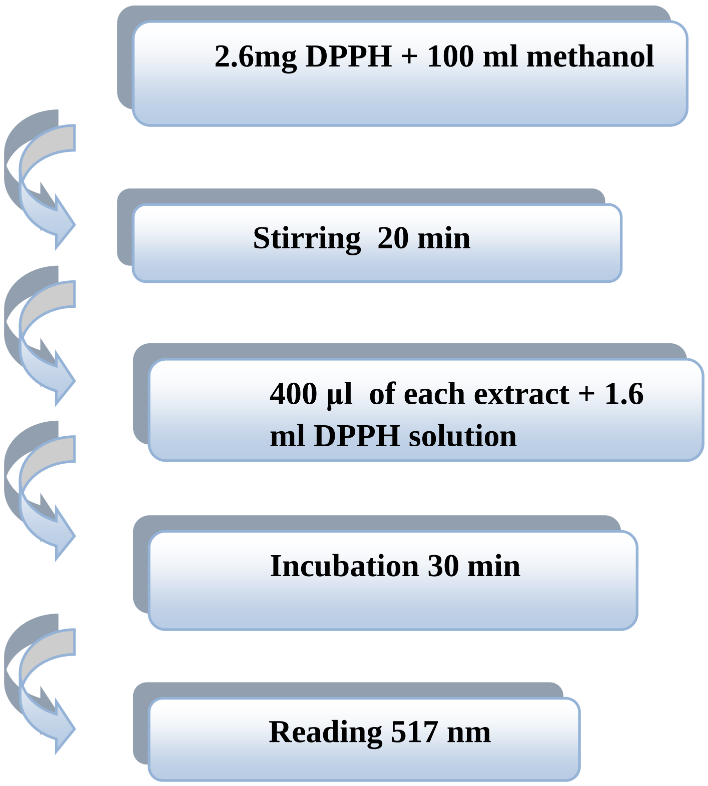

Figure 18: Diagram shows protocol applied for DPPH test

Figure 19: Operaiting mode of Antioxidant Test DPPH (Personal photo)

II.5. Toxicity test

Larvicidal activity was assessed using the previously recommended method (who,2005) of combined toxic effect of *Ruta graveolens* and *Ruta montana* essential oils on culiseta longiareolata larvae in three different mitures.

1. 75% *R graveolens* and 25% *R montana*
2. 50 % *R montan* and 50% *R graveolens*
3. 75% *R montan* and 25% *R graveolens*

Cups containing 150 ml of dechlorinated water in contact with 20 larvae (L4) *Culiseta longiareolata* separately, thus prepared solutions in eppindorf tubes containing 1 ml of diluted ethanol for each given concentration three repetitions at each ,the mortality was observed after a 24h exposure period . the larvae were considered dead when there was no movement.

Figure20: Toxicity test application (Personal photo)

II.6Statistical 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. Yieldof *Ruta graveolens*and *R. montana*essential oils

The hydrodistillation of *R. graveolens*and *R. Montana* using an apparatus of the Clevenger gives an essential oil of yellow color, pleasant smell and with a yield 0.48% and 0.55% of dry matter from the aerial part of the two plants respectively.

III.2. Antioxidant activity test of *Ruta graveolens*and *R. Montana* essential oils

R. graveolens EO inhibits oxidative activity with a percentage ranging from 20.26% (at 0.1mg/mL) up to 37.01% (at 1.6mg/mL). *R. Montana* EO inhibits oxidative activity with a percentage ranging from 18.49% (at 0.1mg/mL) up to 43.65% (at 1.6mg/mL) (Table 03).

Table03: Percentage of EOs inhibition of *R. graveolens* and *R. Montana* to oxidative activity

Concentration (mg/mL)	**1.6**	**0.8**	**0.4**	**0.2**	**0.1**
Percentage of inhibition of *R. graveolens*	37.01±0.43	26.72±0.65	23.98±0.52	20.69±0.13	20.26±0.65
Percentage of inhibition of *R. montana*	43.65±1.19	30.49±2.10	26.17±2.97	20.08±0.28	18.49±0.37

III.3. Combined effect of *Ruta graveolens* and *Ruta montana* essential oils with the percentage 75% and 25% respectively against *Culiseta longiareolata*

Our toxicological studies made it possible to determine the effectiveness between *R. graveolens* and *R. montana*essential oils with percentage 75% and 25% respectively on the L4 larvae of *Cs longiareolata* evaluated from the mortality recorded in the target individuals with a direct effect.

Different concentrations of this mixture were applied to the fourth instar larvae of *Cs longiareolata* (2, 3, 4, 6, 7 and 8 µL/mL) for up to 24 hours. The observed mortality is corrected from natural mortality. It is mentioned in table 04

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 05

Table 04: Toxicity of the mixture 75% and 25% respectivelyof *R. graveolensand R. Montana* EOs applied to *Cs longiareolata* L4 larvae: Corrected mortality (%) (m ± SD, n = 3 repetitions each comprising 20 individuals).

Concentrations (µL/mL)	2	3	4	6	7	8
R1	10	30	55	80	95	100
R2	10	25	55	80	95	100
R3	15	30	70	80	100	100
m±SD	**11.66±2.88**	**28.33±2.88**	**60±8.66**	**80±0**	**96.66±2.88**	**100±0**

Table 05: Effect of of the mixture 75% and 25% respectivelyof *R. graveolensand R. Montana* EOs (µL/mL) in *Culiseta longiareolata* larvae. Analysis of variance with one factor after transformation analysis of recorded mortalities (%).

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment	19911	5	3982	F (5, 12) = 238,9	P<0,0001
Residual	200	12	16,67		
Total	20111	17			

The mixture 75% and 25% respectivelyof *R. graveolens* and *R. Montana* Eos 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 2.78µl/ml of the interval (2.33-3.19); 3.69 from the range 3.31-4.09; and 9.51 from the interval 5.26-8.19 with a Hill Slope of 3.87 and R^2 of 0.98.

Table 06: Effect ofthe mixture 75% and 25% respectively of *R. graveolens* and *R. Montana* EOs (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
2.78	**3.69**	**9.51**	**3.87**	**0.98**
2.33-3.19	3.31-4.09	5.26-8.19		

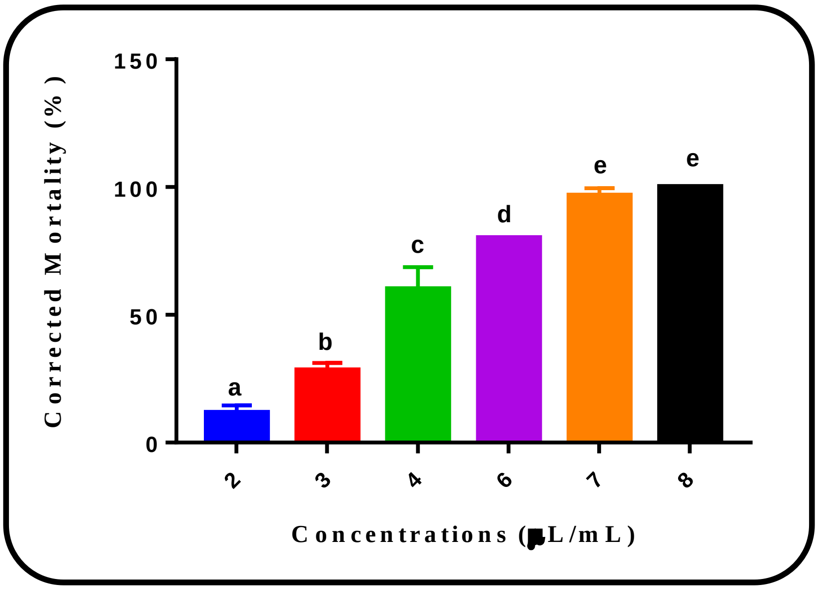

Figure21: Bar chart presenting the mortality percentages of newly exuviated L4 stage *Culiseta longiareolata* larvae treated with different concentrations of the mixture 75% and 25% respectively of *R. graveolens* and *R. Montana* EOs : Corrected mortality (%) (m±SD, n = 3 repetitions each comprising 20 individuals). Different letters indicate a significant difference between concentrations.

II.3. Combined effect of *Ruta graveolens* and *Ruta montana* essential oilswith the percentage 50% and 50% against *Culiseta longiareolata*

Our toxicological studies made it possible to determine the effectiveness ofthe mixture 50% and 50%of *R. graveolens* and *R. Montana* EOs on the L4 larvae of *Cs longiareolata* evaluated from the mortality recorded in the target individuals with a direct effect.

Different concentrations of the mixture 50% and 50% of *R. graveolens* and *R. Montana* Eos were applied to the fourth instar larvae of *Cs longiareolata* (1, 2, 3, 4, 6 and 8 µL/mL) for up to 24 hours. The observed mortality is corrected from natural mortality. It is mentioned in table 07.

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 08

Table 07.: Toxicity of the mixture 50% and 50%of *R. graveolens* and *R. Montana* EOs applied to *Cs longiareolata* L4 larvae: Corrected mortality (%) (m ± SD, n = 3 repetitions each comprising 20 individuals).

Concentrations (µL/mL)	1	2	3	4	6	8
R1	10	40	65	75	90	100
R2	10	35	50	75	90	100
R3	15	30	50	75	95	100
m±SD	**11.66±2.88**	**35±5**	**55±8.66**	**75±0**	**91.66±2.88**	**100±0**

Table08: Effect of the mixture 50% and 50%of *R. graveolens* and *R. montana*EOs (µL/mL) in *Culiseta longiareolata* larvae. Analysis of variance with one factor after transformation analysis of recorded mortalities (%).

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment	17407	5	3481	F (5, 12) = 179	P<0,0001
Residual	233,3	12	19,44		
Total	17640	17			

The mixture 50% and 50%of *R. graveolens* and *R. montana*EOs 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 1.69 µl/ml of the interval (1.36-2.01); 2.60from the range 2.29-2.90; and 6.13 from the interval 4.96-7.89 with a Hill Slope of 2.55 and R^2 of 0.98.

Table09: Effect of the mixture 50% and 50% of *R. graveolens* and *R. Montana* EOs (µL/ mL) in L4 stage larvae of *Culiseta longiareolata at* lethal concentrations, LC_{25}, LC_{50}, and LC_{90}.

LC_{25}	LC_{50}	LC_{90}	Slope	R^2

IC	IC	IC		
1.69	**2.60**	**6.13**	**2.55**	**0.98**
1.36-2.01	2.29-2.90	4.96-7.89		

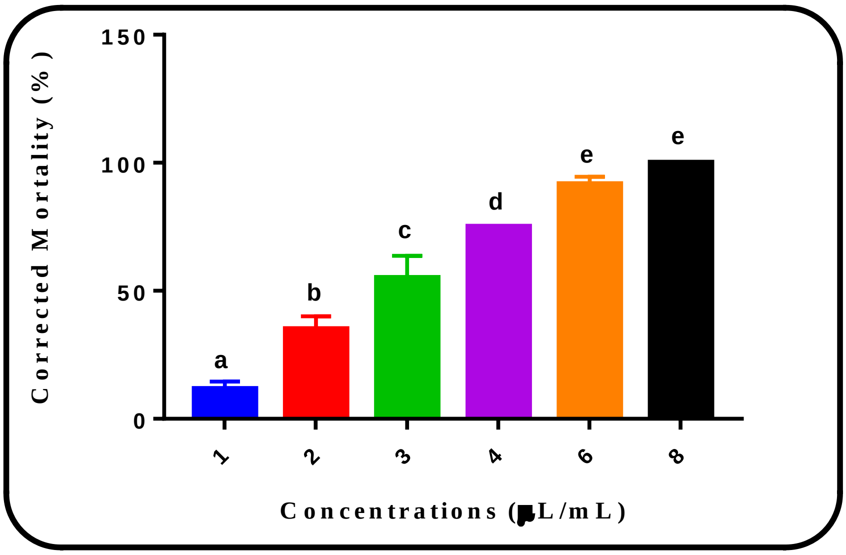

Figure22: Bar chart presenting the mortality percentages of newly exuviated L4 stage *Culiseta longiareolata* larvae treated with different concentrations of the mixture 50% and 50%of *R. graveolens* and *R. Montana* EOs : Corrected mortality (%) (m±SD, n = 3 repetitions each comprising 20 individuals). Different letters indicate a significant difference between concentrations.

II.4. Combined effect of *Ruta graveolens* and *Ruta montana* essential oilswith the percentage 25% and 75% respectively against *Culiseta longiareolata*

Our toxicological studies made it possible to determine the effectiveness of the mixture 25% and 75%respectively of *R. graveolens* and *R. montana*EOs on the L4 larvae of *Cs longiareolata* evaluated from the mortality recorded in the target individuals with a direct effect.

Different concentrations of the mixture 25% and 75%respectively of *R. graveolensand R. Montana* EOs were applied to the fourth instar larvae of *Cs longiareolata* (2, 4, 6, 8 and 10 µL/mL) for up to 24 hours. The observed mortality is corrected from natural mortality. It is mentioned in table 10.

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 11.

Table10: Toxicity of the mixture 25% and 75%respectively of *R. graveolens* and *R. Montana* Eos applied to *Cs longiareolata* L4 larvae: Corrected mortality (%) (m ± SD, n = 3 repetitions each comprising 20 individuals).

Concentrations (µL/mL)	2	4	6	8	10
R1	10	35	50	80	100
R2	5	35	40	65	95
R3	5	30	45	70	95
m±SD	**6.66±2.88**	**33.33±2.88**	**45±5**	**71.66±7.63**	**96.66±2.88**

Table11: Effect of the mixture 25% and 75%respectively of *R. graveolensand R. Montana* EOs(µL/mL) in *Culiseta longiareolata* larvae. Analysis of variance with one factor after transformation analysis of recorded mortalities (%).

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment	14477	4	3619	F (4, 10) = 167	P<0,0001
Residual	216,7	10	21,67		
Total	14693	14			

The mixture 25% and 75%respectively of *R. graveolensand R. Montana* Eos 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 3.88µl/ml of the interval (1.9-5.85); 5.61from the range 3.93-7.28; and 11.72 from the interval 7.60-32.64 with a Hill Slope of 2.98 and R^2 of 0.93.

Table12: Effect of the mixture 25% and 75% respectively of *R. graveolensand R. Montana* EOs (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
3.88	**5.61**	**11.72**	**2.98**	**0.93**
1.9-5.85	3.93-7.28	7.60-32.64		

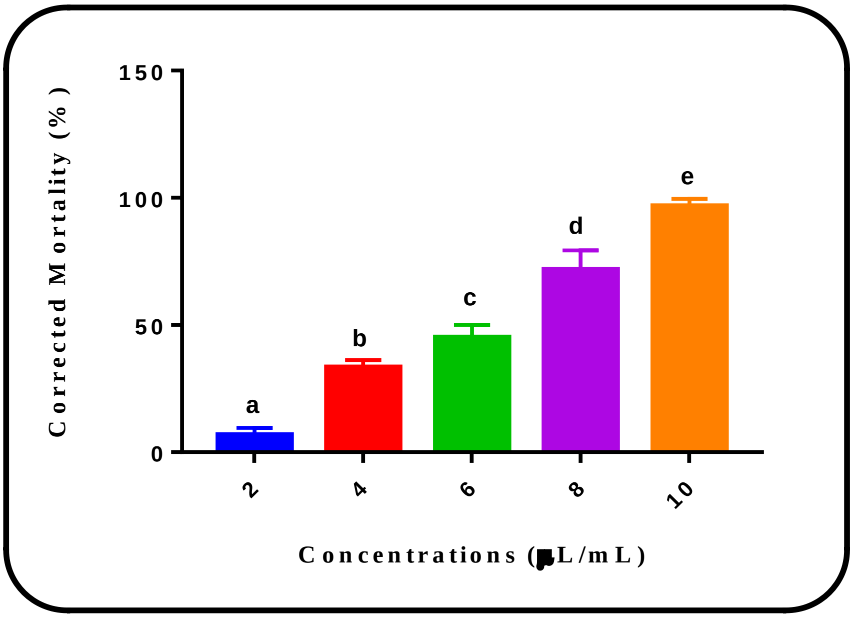

Figure23: Bar chart presenting the mortality percentages of newly exuviated L4 stage *Culiseta longiareolata* larvae treated with different concentrations of the mixture 25% and 75% respectively of *R. graveolens* and *R. Montana* EOs : Corrected mortality (%) (m±SD, n = 3 repetitions each comprising 20 individuals). Different letters indicate a significant difference between concentrations.

DISCUSSION

IV.Discussion :

IV.1 Essential oil yield of *Rutamontana* and *Rutagraveolens*

Extraction yieldof essential oil from the aerial parts of *Ruta montana* and *Ruta graveolens* was made by hydrodistilation are0.55 and 0.48 of the dry matter of the plant respectively.

Kambouche *etal*., (2008) found thatthe hydrodistillationof *Rutamontana*, coming from the Oranregion, gave a return in 1.63% essential oil. In addition, a low yield of about 0.4% has beenobtained by **Soleimani *et al*.,(2009)** using *Ruta graveolens L*. and using the same extraction technique. In addition, essential oil yield of the aerial parts of *Ruta chelepensis* was 0.27%**(Dob*et al*., 2008)** and compared to the sheets dry *Ruta graveolens* **(bouabida&Dris, 2020)** obtained essential oil content1.78 0.07%. EO yields vary from plant to plant and the high rate is reported in *O. basilicum* with an average of 1.56 0.15%, followed by *M. piperita* (1.46 0.04%) and *L. dentate* with the lowest yield (1.18 0.05%) **(Dris, 2018).Attia*et al*.,(2018)** obtained a yield of 0.36 and 0.21% for leaves and flowers, respectively for samples of the same species *(Ruta graveolens*) submitted to a hydrodistillation using a Clevenger type apparatus. Furthermore, a high yield of 1.29% was obtained by**(Reddy&Al- Rajab, 2016),** using aerial parts of the same species, and using the same extraction technique.**FrançaOrlanda & Nascimento (2015),** report that the essential oil obtained by hydrodistillation of a sample of fresh leaves of *R. graveolens* L. has a green colorwith a yield of 1.29%. **Semerdjieva *et al.*(2019)** obtained a yield in EO of dried fruits of *Rutagraveolens*0.39%, and they noted that the majority of the amount of EO (31% ofthe total amount recovered) is obtained during the 30 to 60 minute period ofdistillation.**Boughendjioua(2019)** processed dried leaf samples of *Ruta chalpensisL*. collected from the Souk-Ahras area and obtained a return in EO of the orderby 0.65%.

This difference could be due to several factors Geoclimatic factors (the nature of the soil, thetemperature), harvest period, drying time, extraction mode and partsof the plant used **(Ndomo*et al.*,2009 ;Fadil*et al*.,2015).**

IV.2. Antioxydant activity test of *R. graveolens* and *R.montana* essentiel oils

The percentage of oxidative activity inhibition by *R. graveolens* EO ranges from 20.26% (at 0.1 mg/mL) to 37.01% (at 1.6 mg/mL). *R. Montana* EO exhibits a range of oxidative activity inhibitions, from 18.49% at 0.1 mg/mL to 43.65% at 1.6 mg/mL.

On the other hand, antioxidant activity of *R.montana* essentil oil was assessed using DPPH and reducing power assays in a different study conducted by (**Mohammedi *et al*.,**

2018). The maximum antioxidant activity was found in samples from semi-arid regions of Djelfa and M'sila (IC50 = 50.2 ± 3.3 mg/L and 49.6 ± 2.7 mg/L, respectively).

However, the research conducted by (**Drioiche *et al*., 2020)** The 2,2-diphenyl-1-picrylhydrazyl test (DPPH), which measures antioxidant activity, revealed that *R.montana* essential oil has a high potential for antioxidants, with an IC50 of 548.5± 27.4 µg/ml.

In the test conducted by (**Merghem &Dahamna., 2020),** Following methanol, aqueous, and chloroform extract, ethyl acetate extract demonstrated the highest scavenging capacity in the DPPH experiment (IC50 = 0.044 ± 0.001 mg/ml). assess the antioxidant activity of *Ruta montana L*. extracts in vitro.

In contrast, another study by **(Poonkod *et al*. , 2017)** found that methanol extracts of dry *R. graveolens* had the highest inhibitory concentration for DPPH (19.50±1.25 µg/mL).

Also, The results of the antioxidant tests showed that the extracts of the three species had varied antioxidant capacity: in particular, the *R. chalepensis* extract exhibited the best radical scavenging activity (IC_{50} = 1.665 ± 0.009 mg/mL), while the *R. graveolens* extract displayed the highest chelating property (IC_{50} = 0.671 ± 0.013 mg/mL). Finally, all the extracts showed good activity against *Staphylococcus aureus* with MIC values of 250 µg/mL for the *R. corsica* extract and 500 µg/mL for both *R. graveolens* and *R. chalepensis* extracts. Test done by **(Szewczyk *et al*., 2022).**

In contrast,(**Althaher's *et al*., 2020**) study The antioxidant activities of *Ruta chalepensis L*. essential oil were evaluated utilizing DPPH radical scavenging assay (IC50 = 35.27 µg/ml) and reducing power ability (EC50 = 20110 µg/ml).

IV.3.Combined toxic effect of *Ruta graveolens* and *Ruta montana* essential oils on *culiseta longiareolata* larvae in three different mitures.

In this investigation Combined effect of *Ruta graveolens* and *Ruta montana* essential oils were tested for their toxicity to *Cs longiareolata*larvae. Our results clearly indicated that the combined effect essenail oils of two plants possess insecticidal activity against mosquitolarvae. Our results clearly indicated that the mixture 75% and 25% respectively of *R. graveolens* and *R. montana* EOs 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 2.78µl/ml of the interval (2.33-3.19); 3.69from the range 3.31-4.09 ; and 9.51 from the interval 5.26-8.19 with a Hill Slope of 3.87 and R^2 of 0.98. The mixture 50% and 50% of *R. graveolens* and *R. montana* EOs 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 1.69 µl/ml of the interval (1.36-2.01); 2.60from the range 2.29-2.90 ; and 6.13 from the interval 4.96-7.89 with a Hill Slope of 2.55 and R^2 of 0.98.The mixture 25% and 75% respectively of *R. graveolens* and *R. montana* EOs 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 3.88µl/ml of the interval (1.9-5.85); 5.61from the range 3.93-7.28 ; and 11.72 from the interval 7.60-32.64 with a Hill Slope of 2.98 and R^2 of 0.93.

However, the results obtained and compared to other work of **(Dris *et al.,* 2017),** shows that the essential oil of *Lavandula dentata L*. (*Lamiaceae*) has a larvicide effect for mosquito larvae, the CL50 and CL90 values compared to the larvae of fourth stage were 77.09and 104.45 ppm for *Cs. longiareolata* and 113.38 and 150.38 ppm for Cx. Pipiens. results of (Bouabida & Dris ,2022) indicated that the methanolic extracts of three plants possess insecticidal activity against mosquito larvae. The results revealed a higher larvicidal activity of the extract of *R. graveolens* (LC50 = 43.24 ppm) than that of the extract of *R. montana* (LC50 = 97.74 ppm) and of *A. absinthium* (LC50 = 199.5 ppm). **(Bouabida & Dris, 2020)** shows that the essential oil of *Ruta graveolens* had a LD50 of 10.11ppm (8.64-11.72) ppm and a LD25 of 6.96 ppm (5.33-8.54) ppm a toxic effect against larvae of *Culiseta longiareolata*. The study of (Tabanca et al. 2012) showed that *Ruta graveolens* essential oil had a LD50 of 21.25 (19.6 to 23.7)ppm and a LD90 of 34.35 (30.3 to 41.1) ppm with a toxic effect against *A. aegypti* larvae. Moreover, **(Boutoumi *et al*., 2009)** also showed that the essential oil *Ruta montana* had larvicide activity on the larvae of *Culex pipiens* at a dose of 1.6% of the extract, the percentages of mortality were 99% for the mosquitoes *Culex pipiens* after 30 min. Previous work showed that extracts of the following plants: *Azadirachta indica, Melia azedarach, Calotropis procera, Cionura erecta, Nepeta menthoides, Centaurea bruguierana* had larvicidal effects against the main vector of malaria **(Vatandoost & MoinVaziri, 2004; Hadjiakhoondi *et al*., 2006; Shahi *et al.,* 2010; Khanavi *et al*., 2011; Khanavi et al., 2012, 2013; Mozaffari *et al.,* 2014).** The methanolic extract of unripe avocado fruit peel applied to third instar larvae *Aedes aegypti, Culex quinquefasciatus* and *Anopheles stephensi* shows larvicidal activity with LC50 values 6.65 ppm, 7.12 ppm and 10.78 ppm, respectively **(Louis *et al*., 2020). (Cheng *et al.,* 2013)** examined the larvicidal activity of essential oils of wood and leaves and ethanolic extracts of *Cunninghamia konishii* against *A. aegypti* and *A. albopictus*. The following constituents: myrene, p-cymene, (+) - *limonene* and sabinene showed larvicidal activity with LC50 values of 35.8, 69.4, 71.9 and 74.1 g/ml against *A. aegypti* larvae and 27.0, 68.3, 41.2 and 39.5 g/ml against *A. albopictus larvae*, respectively. The work of **(Sogan *et al.,* 2018)** shows that extracts from leaves and seeds of *R. communis* cause significant mortality against the larvae *Ae. aegypti* and *An.*

culicifacies with the concentrations of 31.25, 62.5, 125, 250, 500 ppm; and 2, 4, 8, 16, 32, 64 ppm, respectively. Similar results were found with extracts of the *Avicennia marina* mangrove against three mosquito larvae. The highest larval mortality was found for the acetone extract of *A. marina against* Culex quinquefasciatus (LC50 = 0.197 mg/ml; LC90 = 1.5011 mg/ml), *Anopheles stephensi* (LC50 = 0.176 mg/ml; LC90 = 3.6290 mg/ml) and *Aedes aegypti* (LC50 = 0.164 mg/ml; LC90 = 4.3554 mg/ml) **(Karthi *et al*., 2020).**

CONCLUSION

Conclusion :

Due to the problems associated with the use of chemical insecticides and their harmful impact on health and the environment, the use of natural alternatives to synthetic insecticides with ecological and economic benefits is necessary.

Essential oils are aromatic substances, with a complex chemical composition, which gives them very interesting insecticidal properties and very slow resistance development, compared to synthetic insecticides

The antioxidant property of essential oils plays an important role in the prevention of free radical formation The evaluation of the antioxidant activity by the free radical trapping method DPPH *R graveolens* EO inhibits oxidative activity with a percentage ranging from 20.26% (at 0.1mg/mL) up to 37.01% (at 1.6mg/mL). *R montana* EO inhibits oxidative activity with a percentage ranging from 18.49% (at 0.1mg/mL) up to 43.65%, from these results , we confirm the antioxidant activities of these essential oils .

Toxicological tests were used to determine the lethal concentrations (CL25 and CL 50and CL 90) the combined oil effect using three different mixtures (75 % *R graveolens* and 25% *R montana*) ,(50 % *R graveolens* and 50% *R montana*) , (25% *R graveolens* and 75 % *R montana*) for *Culiseta longiareolata* larvae,They reveal an insecticidal effect with a dose-response relationship , The most toxic and effective is a mixture,(50 % *R graveolens* and 50% *R montana*)

This indicates a sustainable approach to mosquito control that deserves further investigation and study. By leveraging the natural properties of essential oils, we may be able to develop safer and eco-friendly alternatives to traditional chemical insecticides. This research opens the door to exploring new applications of plant compounds in pest control strategies, and highlights the importance of harnessing nature's resources for a greener future.

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