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Larvicidal activity of medicinal plants against mosquito species.

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بسم الله الرحمن الرحيم

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

۞ إِنَّ ٱللَّهَ لَا يَسۡتَحۡيِۦٓ أَن يَضۡرِبَ مَثَلٗا مَّا بَعُوضَةٗ فَمَا
فَوۡقَهَاۚ فَأَمَّا ٱلَّذِينَ ءَامَنُواْ فَيَعۡلَمُونَ أَنَّهُ ٱلۡحَقُّ مِن
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﴿ سورة البقرة الآية 26. ﴾

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Dedication

The journey was not short, nor should it be. The dream was not close, nor was the road easy, but I did it and followed it. Praise be to God for love, thanks and gratitude, thanks to which I am here today looking at a long-awaited dream that has come true. I'm really proud of it.

To my pure angel, my strength after God, my first and eternal support. "**My dear mother**," I dedicate this achievement to you, which without your sacrifices would not have existed.

To the deceased who remains in my heart, to **"my father"**, may God have mercy on him.

To those among whom it was said : "سَنَشُدُّ عَضُدَكَ بِأَخِيك"

To my only brother **Ramzi**.

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Malak.

Dedication

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ABSTRACTS

ملخص

هذه الدراسة تهدف إلى تقييم الأنشطة البيولوجية والسمية لمستخلصات نبات *إكليل الجبل والدفلة* من منطقة ترعي باينان ولاية ميلة على يرقات *culex pipiens*. حيث أظهرت مستخلصات أوراق نبات *إكليل الجبل والدفلة* التي تم الحصول عليها عن طريق الغلي والنقع وجود الفلافونويد ومركبات فينولية، بحيث مستخلصات النقع والغلي لنبات *إكليل الجبل* بها مستويات أعلى من المركبات الفينولية والفلافونويد مقارنة بمستخلصات الغلي والنقع لنبات *الدفلة*. وأظهرت نتائج اختبار سمية مستخلصات نبات *إكليل الجبل والدفلة* على يرقات *culex* أن تأثير معدل موت اليرقات يعتمد على تركيز المستخلصات، وأن وقت التعرض فعال في زيادة معدل موت اليرقات. وقد أظهر منقوع *إكليل الجبل* أعلى معدل موت لليرقات، يليه التسريب ثم مغلي نبات *الدفلة*.

الكلمات المفتاحية: معدل موت اليرقات، *الدفلى، إكليل الجبل*، النقع، الغلي culicidea, culex pipiens

Abstracts

This study aims to evaluate the biological and toxicological activities of rosemary and *N. oleander* plant extracts from the Terrai Bainen area of Mila region ,on *Culex* larvae.The extracts of *rosemary* and *N.oleander* leaves obtained by decoction and infusion showed the presence of flavonoids and phenolic compounds, so that the decoction and infusion extracts of the *rosemary* plant had higher levels of phenolic compounds and flavonoids compared to the decoction and infusion extracts of the *N.oleander* plant. The results of testing the toxicity of *rosemary* and *N.oleander* plant extracts on *Culex* larvae showed that the effect of larval mortality rate depends on the concentration of the extracts, and that exposure time is effective in increasing he larval mortality rate. The *rosemary* infusion showed the highest larval mortality rate, followed by infusion and *N. oleander* decoction.

Keywords: Larvicidale activity, culicidea, *culex pipiens*, *Nerium oleander*, *Rosmarinus officinalis*, infusion, decoction.

Résumé

Cette étude vise à évaluer les activités biologiques et toxicologiques des extraits de plantes de *romarin* et de *laurier rose* de la zone Terrai Bainen de la région de Mila, sur les larves de *Culex*. Les extraits de feuilles de *romarin* et de *laurier rose* obtenus par décoction et infusion ont montré la présence de flavonoïdes et des composés phénoliques, de sorte que les extraits de décoction et d'infusion de la plante de *romarin* présentaient des niveaux plus élevés de composés phénoliques et de flavonoïdes par rapport aux extraits de décoction et d'infusion de la plante *laurier rose*. Les résultats des tests de toxicité des extraits de plantes de *romarin* et de *laurier rose* sur les larves de *Culex* ont montré que l'effet du taux de mortalité larvaire dépend de la concentration des extraits et que le temps d'exposition est efficace pour augmenter le taux de mortalité des larves. L'infusion de *romarin* a montré l'activité larvicide la plus élevée, suivie de l'infusion et de la décoction de *laurier rose*.

Mots clés : Activité larvicidale, culicidea, *culex pipiens, Nerium oleander, Rosmarinus officinalis*, infusion, décoction.

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Abreviations

Abbreviations

Alcl3: Aluminum chloride

mg GAE/g: Milligram gallic acid equivalent per gram

mg QE/g: Milligram quercetin equivalent per gram

NaOH: Sodium hydroxide

TFC: Total flavonoids contents

TPC: Total phenolic contents

N. oleander*: Nerium oleander*

R. officinalis*: Rosmarinus* officinalis

Cx. Pipiens: Culex pipiens

WHO: World Health Organization.

%: percentage.

g/l: gram per liter.

h: time.

ml: milliliter.

T: Temperature.

g: gram.

mm: millimeter

µl: micro liter

v: volume

min: minute

mg: milligram

nm: nano meter

L1, L2, L3 and **L4**: Larvae 1st, 2nd, 3rd and 4th instars.

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Introduction

Introduction

Mosquitoes are a major focus of entomological research worldwide due to their role as vectors for numerous viral and parasitic diseases that affect both humans and animals (Baker *et al*., 2010). According to the World Health Organization (WHO), over 80% of the world's population lives in areas at risk of at least one vector borne disease, leading to more than 700,000 deaths annually (WHO, 2017).

The Culicidae family, commonly known as mosquitoes, includes more than 3,300 species grouped into 37 genus. They are globally distributed, occurring in both tropical and temperate regions (Carnevale & Robert, 2009). In addition to being harmful insects, mosquitoes are responsible for transmitting diseases such as malaria, dengue fever, lymphatic filariasis, chikungunya, yellow fever, and Japanese encephalitis (Goddard, 2007). In recent years, these diseases, previously confined to tropical and subtropical areas, have now emerged in temperate regions due to climate change, making these areas more susceptible to the emergence and re-emergence of these diseases (Linthicum *et al*., 2010; Ogden, 2017).

Controlling mosquito vectors using traditional synthetic insecticides such as organophosphates, carbamates, and pyrethroids has led to the development of resistance among target mosquito populations, poisoning of non-target organisms, and environmental and health side effects on human health (Cuervo-Parra *et al*., 2016). These drawbacks have prompted researchers to explore alternative methods for controlling disease vectors, focusing increasingly on developing new selective insect products with low toxicity risks (Thomas *et al*., 2014).

In Algeria, numerous studies have been conducted on mosquito control by evaluating new, reliable, and less polluting insecticides. These studies included chitin synthesis inhibitors (Rahim & Soltani, 1999; Soltani *et al*., 1999; Bouaziz *et al*., 2011; Djegedour *et al*., 2013), and ecdysteroid agonists (Boudjelida *et al*., 2005). Current research trends towards the use of biologically active natural compounds of plant origin have garnered significant attention due to their insecticidal properties, biodegradability, and non-toxicity to non-target organisms (Amer & Mehlhorn, 2006).

The Algerian flora, which includes 3000 species belonging to different plant families (Quezel & Santa, 1963), remains largely unexplored pharmacologically and phytochemically. In this context, our selection focused on two plants, rosemary (*Rosmarinus officinalis*) and oleander (*Nerium Oleander*), since several pharmacological activities have been reported for both extracts and essential oils of these species. These activities are largely due to the presence of phenolic compounds (Jerridan *et al*., 2007).

Rosmarinus officinalis is an aromatic plant belonging to the *Lamiaceae* family, native to the temperate regions of the Mediterranean. It is widely spread in Algeria and is used in traditional medicine to treat many diseases. Rosemary and its components have been the subject of significant research interest due to their potential antioxidant and anti-insect activities (Singletary, 2016).

Nerium oleander L (Apocynaceae) is a traditional medicinal plant that belongs to the Apocynaceae family. In traditional medicine, the roots are used to treat headaches and colds (El-Seedi *et al*, 2013). Decoctions of the leaves are used for skin diseases and against paralysis and pain in extremilies (Kuele, 2014) Besides these, the bark, stem, leaves, flowers and roots of *N. oleander* possess antibacterial (Hussain and Gorsi, 2004), anticancer (Rashan *et al*., 2011), antidiabetic (Dey *et al*., 2015) and larvicidal (El-Akhal *et al*., 2015) activities.

Thus, we aimed to determine through research to the potential larvicidal activity of phenolic extracts from *Rosmarinus officinalis* and *Nerium oleander* on the larvae of the mosquito *Culex pipiens*, the most abondant mosquito species in the Mila region.

Chapter one:

Culicidea family and control method.

I. Culicidae family

I.1. Generality

Mosquitoes, belonging to the suborder Nematocera within the order Diptera, are holometabolous insects and arthropods classified in the Animal kingdom. Mosquitoes are widespread globally, withthe exception of permanently frozen regions. The Culicidae family is divided into three subfamilies: Toxorhynchitinae, Anophelinae, and Culicinae. These insects are notorious for their significant role as disease vectors and sources of annoyance.

Throughout their development, mosquitoes exhibit a rich array of anatomical and morphological features, with adultsand larvae typically providing the most valuable systematic characteristics for identifying species and genera. In contrast, eggs are seldom utilized for this purpose. They are known for their distinctive biting and sucking mouthparts and are members of the Culicidae family, which is recognized for its extensive diversity and uniformity.

They are distinguished by various morphological criteria observed across different developmental stages. In Algeria, six genera are classified under the Anophelinae and Culicinae subfamilies (Figure 1), with no representation of the Toxorhynchitinae subfamily (Matoug, 2018).

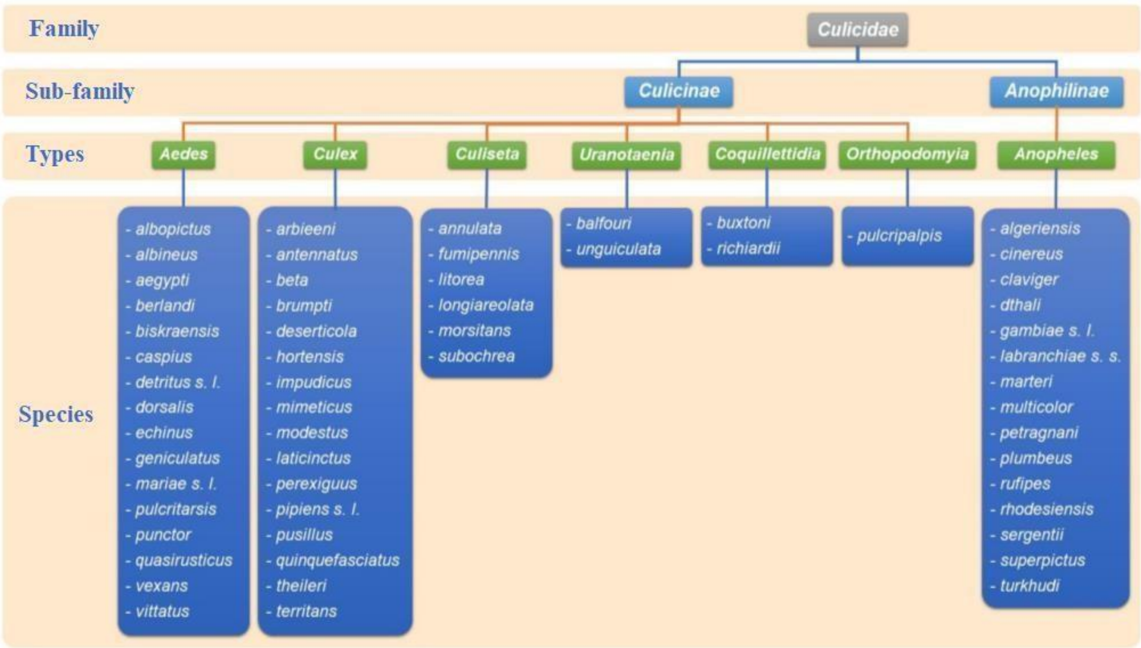

Figure 1: General systematics of the Culicidae of Algeria (Amara Korba, 2016).

I.2. Systematic position

Including to Matoug 2018, the systematic position of Culicidae is a follow:

Table 1: The systematic position of mosquitoes (Matoug, 2018).

Kingdom	Animalia
Subregnum	Metaoa
Branching	Arthpoda
Sub-branch	Hexapoda
Class	Insecta
Subclass	Pteregota
Order	Diptera
Sub-order	Nematocera
Family	Culicidae

I.3. Morphology

Mosquitoes, being holometabolous insects, undergo complete metamorphosis, resulting in distinct morphological differences (Figure 2) among their life stages: adult, larva, and pupa. These differences are crucial for identifying subfamilies, genera, and species (Chahed, 2022).

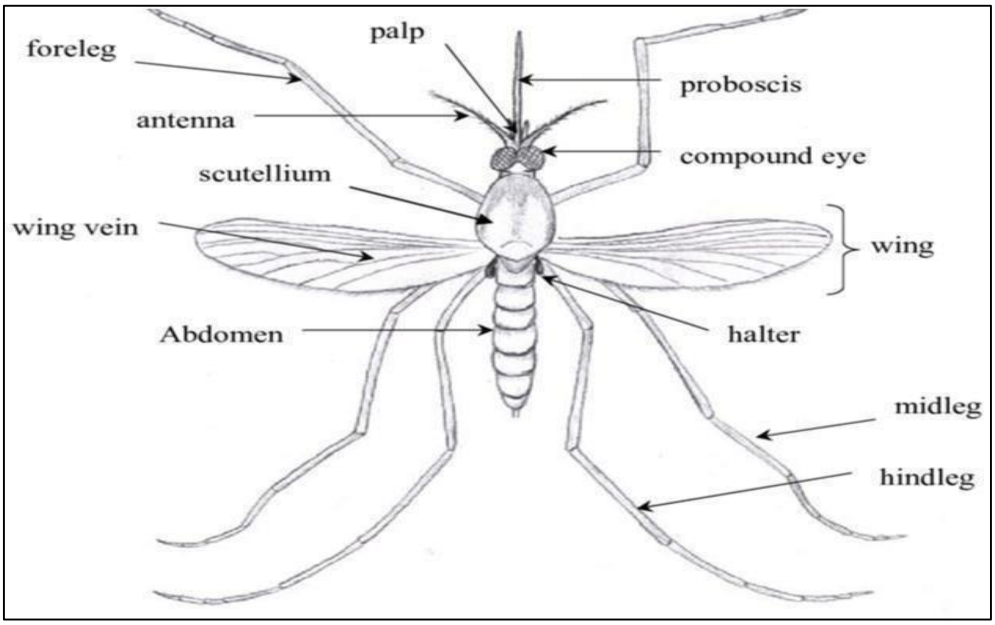

Figure 2: General schematic morphology of an adult mosquito (Beaulah, 2021).

I.3.1. Eggs

The egg of Culicidae is typically fusiform in shape and measures approximately 1 mm in length. Initially whitish during oviposition, it undergoes a color transformation to gray or jet - black within a few hours due to the oxidation of specific chemical components in the theca upon contact with water or air. Enclosed within a dense shell, it features a micropyle at its anterior pole. Traditionally, the Culicidae egg consists of, from innermost to outermost layers: the embryo, the translucent vitelline membrane, the thick endochorion, and the more or less pigmented, raised, or patterned exochorion. The (Figure 3) illustrate some eggs of mosquito (Merabti, 2016).

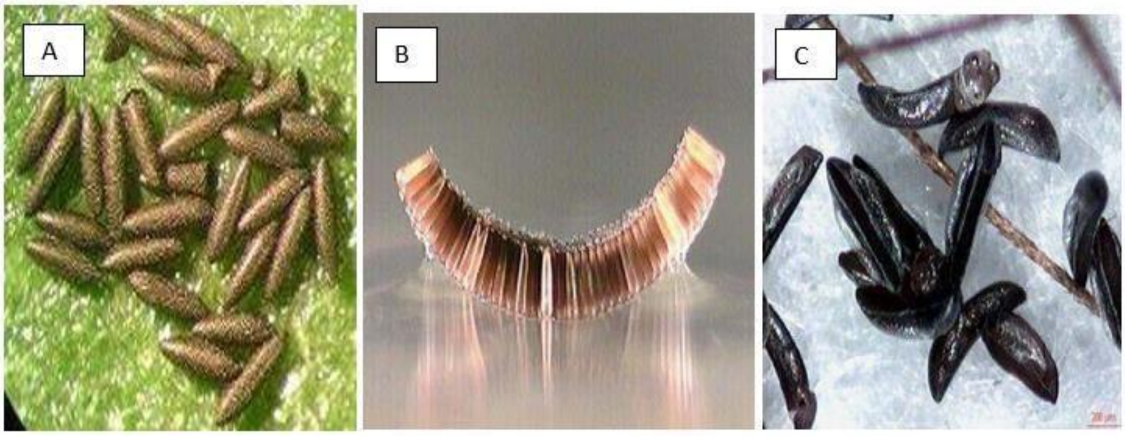

Figure 3: Some photos of mosquito eggs:
(A): eggs of *Aedes*; (B): eggs of *culex*; **(C)**: eggs of *Anopheles* (Messai, 2017).

I.3.2. Larva

Mosquito larvae stand out from most other aquatic insects due to their distinctively shaped head, thorax, and abdomen. They rely on water for their development, unable to endure dry conditions. While most larvae feed on detritus, some exhibit predatory or even cannibalistic behavior. Their movement is characterized by jerky motions, and they typically feed through filtration, whether near the water's surface or at the bottom of their nest. Feeding involves the larva vigorously and regularly stirring its mandibular brushes, known as rotatory palps, which form small tufts(Merabti, 2016). There are indeed four post-embryonic stages (L1, L2, L3, and L4), all of which are aquatic. The larva typically ranges in size from approximately 2 to 12 mm (Hamaidia, 2020).

Larvae exhibit small size and a pale to brown coloration. Their head is wider than it is long, featuring mouthparts, a pair of compound eyes, and antennae. The larval mouthparts consist of lateral brushes, which serve to filter organic materials from the water. Antennae are shorter than the head in length (Alomar *et al*., 2021). The abdomen is cylindrical and comprises eight distinct segments (Figure 4).

Figure 4 : Culicidae larva (Dahmana et Medianniko, 2020).

I.3.2.1. Head

The head structure comprises three chitinous plates: one dorsal, which is odd -numbered and median, known as the fronto-clypeus, and the other two lateral plates are symmetrical. Encased within a hardened capsule made of sclerotin, the head houses the mouthparts of the crusher type, featuring transversally movable mandibles composed of long, curved bristles that serve a prehensile function. The antennae, inserted on the sides, are generally long and specular, bearing a tuft of bristles widely used in systematics (figure 5) (Kharoubi, 2021).

I.3.2.2. Thorax

The thorax, stout in appearance and lacking appendages, is theoretically divided into three sections: the prothorax, mesothorax, and metathorax. It presents as a spherical mass with slight dorsoventral flattening (Kharoubi, 2021). Larvae exhibit several groups of fan-shaped setae along their sides, distinguished through chetotaxy. At the antero-dorsal angle, one may observe a pair of transparent lobes, sometimes referred to as "notched organs" in English, or retractable bilobed organs, although they may not be visible on all specimens (Figure 5) (Dahchar, 2017).

I.3.2.3. Abdomen

The abdomen of Culicidae larvae is elongated and sub-cylindrical, comprising nine segments, each adorned differently. While the first seven segments exhibit similar morphology, the 8th and 9th segments stand out significantly: they constitute the anal segment rather than a continuation of the body. This anal segment features a saddle (sclerotized plate), varying in size across species, and numerous bristles crucial for systematic classification. One of these bristles is modified into a ventral brush, facilitating larval movement, while two pairs of anal papillae encircle the anus, aiding in osmotic balance. The respiratory siphon is adorned with spines resembling a siphon comb and additional bristles, their shape and arrangement utilized in species-specific diagnoses (Figure 5) (Chahed, 2022).

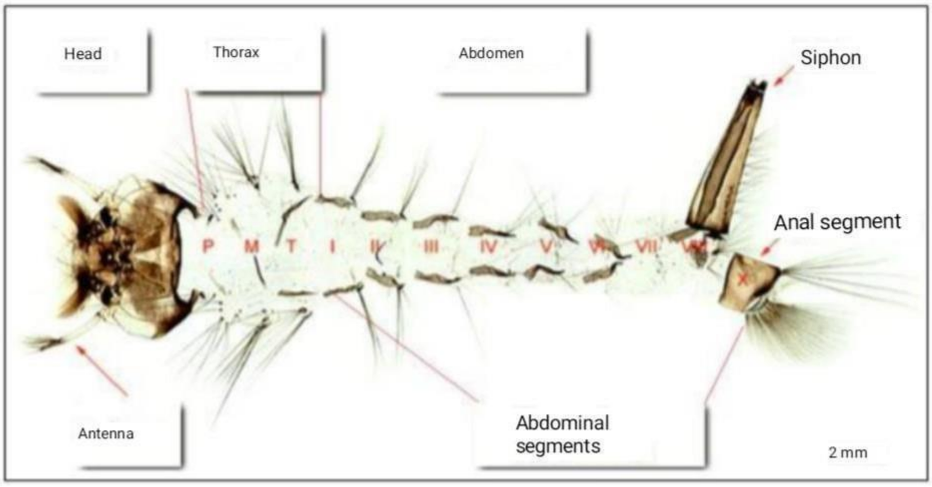

Figure 5: General morphology of a Culicidae larva (Chahed, 2022).

I.3.3. Nymphs

The Culicidae nymphs is characterized by a head and thorax joined into a single globular mass, the cephalothorax, and a tapered, curved posterior part forming the abdomen; this gives the nymph an overall comma-like shape (Figure 6) (Hamaidia, 2020). The cephalothorax encompasses the outlines of the eyes and various appendages such as antennae, proboscis, legs, and wings. Additionally, it contains two prothoracic respiratory trumpets, which exhibit highly variable shapes and serve as the physiological equivalent of the larva's respiratory siphon. These trumpets are remarkably sensitive and prompt the nymph to dive into the water at the slightest perception of movement.

This nymphal stage lasts from 24 to 48 hours, during which the nymph does not feed, relying on reserves accumulated during the larval stage. However, significant morphological and physiological transformations occur during this period. Ultimately, the nymph metamorphoses into an adult, either male or female (Dahchar, 2017).

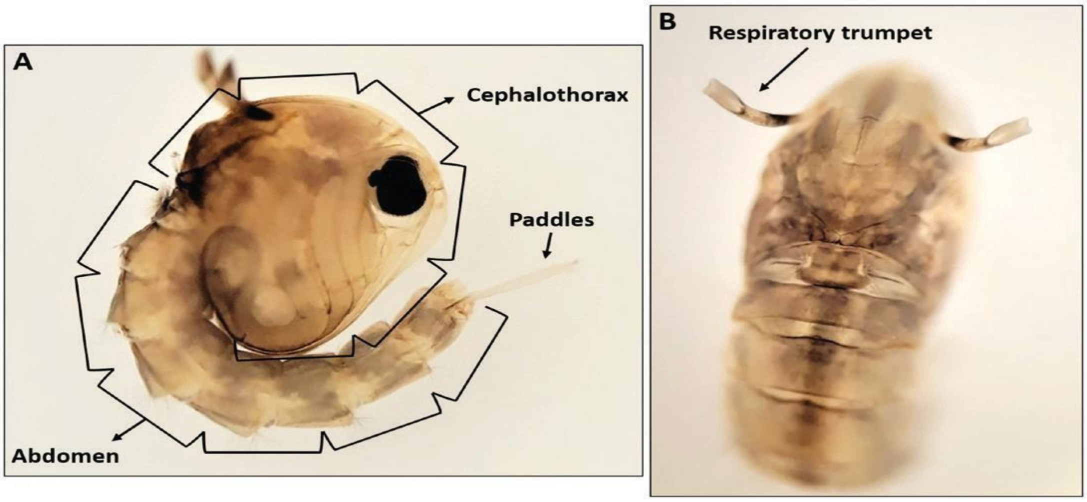

Figure 6: Nymphe representation of the Culicidae family (Alomar *et al*., 2021).

I.3.4. Adult

Male mosquitoes primarily feed on plant juices and nectar, and their lifespan typically ends shortly after mating. In contrast, females can live anywhere from 3 weeks to 3 months, influenced by factors like temperature and nest quality. They also consume plant juices but additionally require blood for egg development.

After emerging, adult mosquitoes typically remain close to their larval habitat, generally within a 3-kilometer radius, unless strong winds disperse them further. Mating occurs within 48 hours of female emergence and prior to the first blood meal. Females typically mate only once during their flight, covering a wide area, indicating a eurygamous species. Males are attracted to female mosquitoes through sound frequencies and pheromones. Following mating, females seek a host for a blood meal necessary for egg maturation, usually around 5 days after their last meal.

As autumn sets in, females prepare for diapause, a period of dormancy where they seek shelter and refrain from feeding forseveral months. During this time, they rely on lipid reserves acquired from plant juices for survival. They emerge from diapause in spring, resuming their blood-feeding behavior (Benserradj, 2014). The Figure 7 illustrate the general morphology of Culicidae.

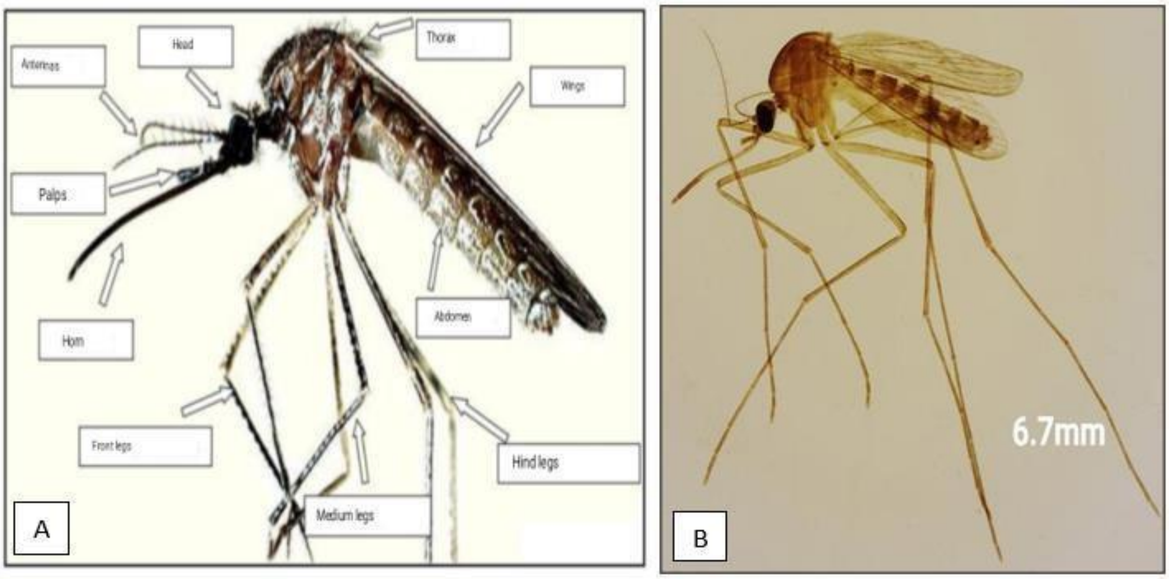

Figure 7: *Culex* adult representation. A: General morphology; B: Adult *culex* mosquito measured 6.7 mm (Dahchar, 2017; Beaulah, 2021).

The three basic parts of the mosquito's body are quite distinct:

I.3.4.1. Head

Generally globular in shape, it features large, faceted eyes that are nearly contiguous, separated by a narrow frontal strip, often metallic blue or green in color. It also has a pair of antennae with fifteen segments, feathery in males and nearly smooth in females. Additionally, it possesses piercing-sucking mouthparts (Aouati, 2016).

I.3.4.2. Thorax

The thorax results from the fusion of three rigid segments: the prothorax, mesothorax, and metathorax. It is adorned with elongated hairs. The second segment, being the most pronounced, carries a pair of wings covered in numerous scales, exhibiting relatively simple venation. Additionally, on this segment, there are small membranous scales, thick at the edges, referred to as wing dependencies. The third segment, although less conspicuous, lacks wings but is endowed with a complex nervous network (Merabti, 2016).

I.3.4.3. Abdomen

The abdomen of the Culicidae is elongated and much narrower than the thorax. It consists of ten segments, but only the first eight are differentiated and externally visible, each made up of a dorsal chitinous plate, the tergite, and a ventral plate, or sternite. The arrangement and placement of scales on the abdominal tergites allow for species identification. The last two segments are modified for reproductive functions (Hamaidia, 2020).

I.4. Development cycle

The mosquito development cycle spans approximately twelve (12) to twenty (20) days and consists of four (4) stages: egg, larva, pupa, and adult. This metamorphosis unfolds in two (2) phases.

I.4.1. Aerial phase

Both male and female mosquitoes mate either in flight or on vegetation (Keffous, 2023). Typically, within a flight distance of one to two kilometers. Males utilize the long, erect hairs on their antennae to detect the buzzing sound produced by the rapid wing beats of approaching females during their nuptial flight. At this point, the male fertilizes the female, leaving her a supply of his own semen. After fertilization, females seek out a blood meal to obtain the necessary proteins and amino acids for egg maturation. Once pregnant, females embark on a quest to find suitable aquatic sites for laying their eggs, ensuring the development of their larvae (Dahchar, 2017).

I.4.2. Aquatic phase

Egg-laying typically ranges from 100 to 400 eggs, with an incubation period lasting two (2) to three (3) days, influenced by various factors such as environmental temperature, water pH, the presence of aquatic vegetation, and the abundance of associated fauna (Matoug, 2018). Upon hatching, the eggs give rise to stage 1 larvae, measuring 1 to 2 mm in length. These larvae continue to develop through stage 4, reaching lengths of up to 1.5 cm (Dahchar, 2017).

Typically, mosquito larvae feed by filtering fine organic particles and other microorganisms (bacteria, protists, micro-metazoans) from their environment (Belkhiri, 2022). Despite their aquatic habitat, mosquito larvae rely on air for respiration.

After six (6) to ten (10) days or more, depending on water temperature and food availability, the fourth molt gives rise to a nymph; this is pupation (Merabti, 2016). In the last of the nymphal stage, the nymph stretches, and its exoskeleton splits dorsally.Slowly, the adult mosquito emerges from the exoskeleton in a process called emergence (Figure 8).

This process lasts approximately fifteen (15) minutes, during which the insect is exposed and vulnerable to various surface predators (Dahchar, 2017).

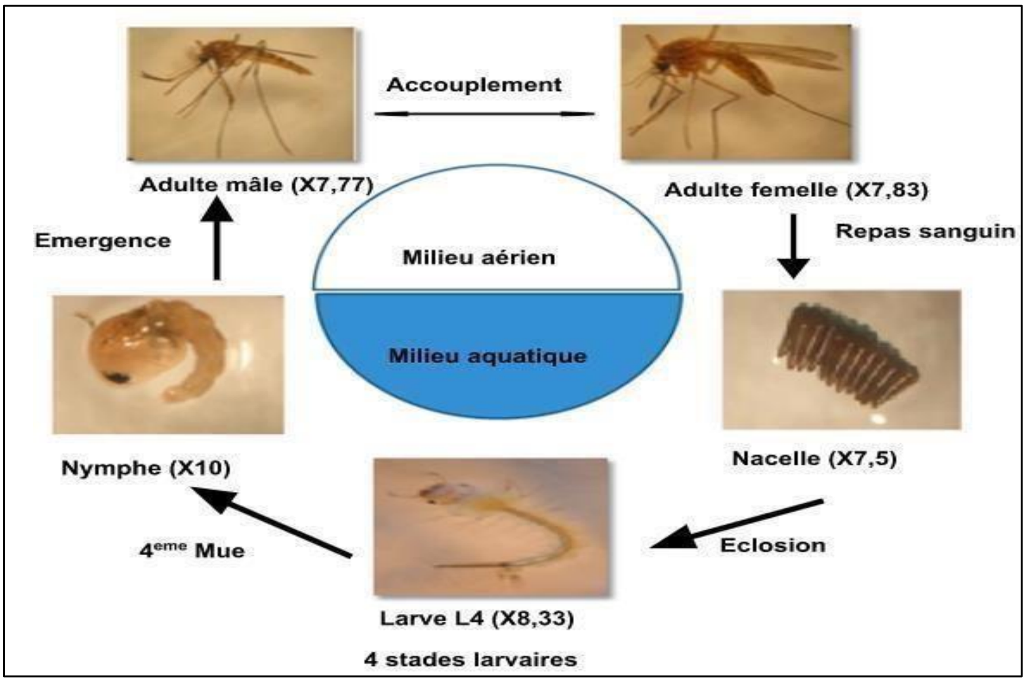

Figure 8: Mosquito development cycle (Keffous, 2023).

I.5. Ecosystem interests

Mosquitoes play a crucial role in the dynamics of aquatic ecosystems, serving as a vital link in the trophic chain. Their abundance translates into a significant biomass, which serves as a primary food source for various organisms. As a result, they contribute to the intricate web of interactions within wetland ecosystems (Benserradj, 2014).

Culicidae larvae play an important role in trophic chains, feeding on microorganisms, algae, protozoa, invertebrates, and detritus. This highly varied diet enables them to participate in the self-purification process, eliminating organic matter accumulated at the bottom of aquatic environments. They are considered essential prey for several species of fish and amphibians, underscoring their significant role in trophic chains (Chahed, 2022).

I.6. Nuisance

Mosquitoes are a significant public health concern for both humans and animals due to their role as vectors for some of the world's most endemic diseases. Within the Culicinae subfamily, the genera Culex, Aedes, and Anopheles are particularly notable as carriers of pathogens. The stinging behavior of adult female mosquitoes, necessary for egg maturation, poses more than just a temporary inconvenience associated with blood feeding. This direct uptake of blood provides a pathway for various pathogens, including viruses, protozoa, worms, and nematodes, to exploit mosquitoes as vectors for transmission to hosts. Protozoan pathogens, such as Plasmodium falciparum, responsible for malaria, use mosquitoes to complete their life cycles, ultimately infecting humans and causing numerous diseases. This intricate relationship underscores the critical role of mosquitoes in disease transmission and highlights the importance of vector control measures in public health efforts. (Benserradj, 2014).

I.7. Control methods

For a long time, plant-based insecticides such as rotenone and nicotine were the only substances used by man to defend himself against insect pests. Today, the use of insecticides such as pesticides and fertilizers in agriculture, creates new ecological situations, favorable to mosquito outbreaks, when this use is not reasoned (Duvallet et Chabasse, 2020).

Duvallet et Chabasse, (2020) have reported that the main objective of control methods is to reduce nuisance and to limit pathogen transmission (Duvallet et Chabasse, 2020). A number of effective methods are used in mosquito control and depend upon the targeted mosquito species. These approaches are classified into five classes: chemical control, genetic control, biological control mechanical control and physical control (Toqeer *et al*., 2019).

I.7.1. Chemical control

To control the major genera of mosquitoes such as *Anopheles*, *Aedes,* and *Culex* many chemical insecticides are being used for many years (Chaudhry *et al*., 2019). Insecticides utilized against mosquitoes encompass various molecules from different chemical families, including organophosphates, carbamates, pyrethrinoids, among others, each with distinct modes of action (Kharoubi, 2021).

The use of conventional chemical pesticides comes with significant drawbacks. These pesticides can harm beneficial organisms, non-target plants, pollute air, water, and soil, posing environmental risks threats to human health (Keffous, 2023). The resistance most often evolves through one or more of four different types of adaptations (Meier *et al*., 2022):

- Increased metabolic elimination or sequestration of the insecticide,
- A change in target site structure that prevents insecticide binding,
- A change in the insect cuticle to limit the entry of the insecticide into the body,
- Behavioral modification that results in the avoidance of the insecticide (Figure 9).

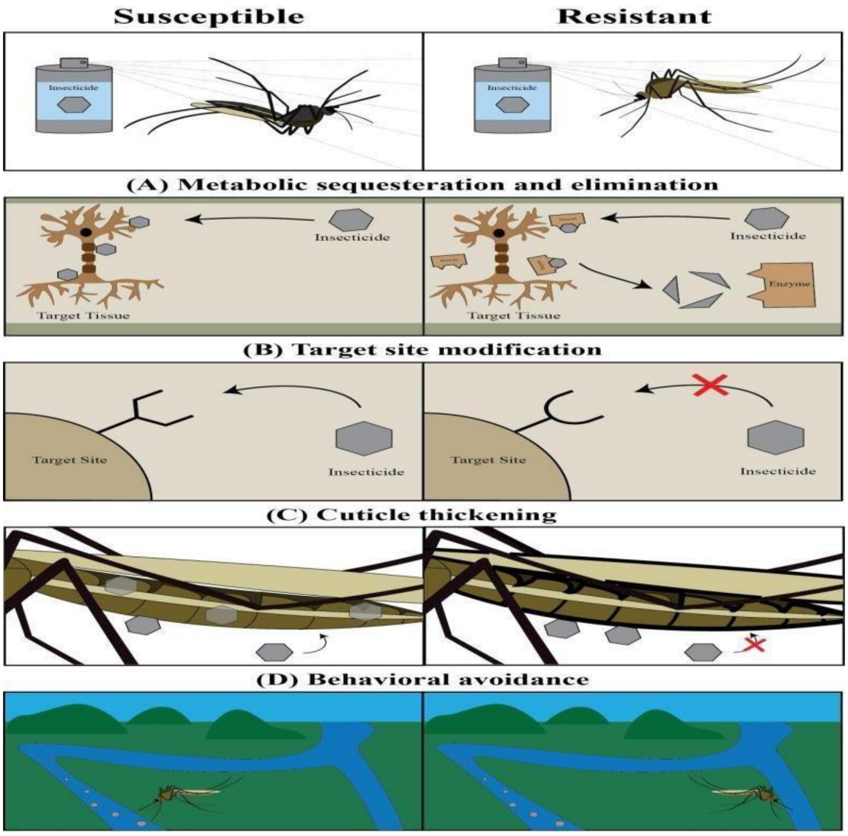

Figure: Resistance strategies of mosquito against chemical insecticide (Meier *et al*., 2022).

I.7.2. Genetic control

Genetic control methods offer a more sophisticated approach. The two most important genetic control methods include the use of the refractory gene and the introduction of lethal gene in the mosquitoes to suppress the mosquito population. One of the most widely studied methods is *Wolbachia* bacteria mediated infection to control mosquitoes' population. More than 70% of insects are infected with these endosymbiotic bacteria (Chaudhry *et al*., 2019).

Use of *Wolbachia* bacteria for the introduction of a refractory gene has also been proposed. These genetic alterations can also extend to plants like algae, which proliferate in mosquito breeding habitats. By integrating bacterial toxin genes into the genetic makeup of algae, these modified organisms can target mosquito larvae (Kharoubi, 2021).

I.7.3. Mechanical control

The objective is to mitigate the spread of vector-borne diseases and minimize human-mosquito interactions. This goal is accomplished by eradicating potential mosquito breeding grounds near human habitation, which includes actions like draining and filling stagnant water sources, and managing habitats appropriately. Additionally, the use of insecticide-treated mosquito nets serves as a protective measure against mosquito bites (Kharoubi, 2021).

I.7.4. Physical control

This practice involves deliberately altering the biotope to diminish or eradicate surface water bodies where mosquitoes breed, employing physical methods. These methods encompass various activities such as drainage, excavation, redirecting water sources, filling in stagnant areas, and planting trees to alter the environment. Physical interventions typically involve implementing measures to regulate water flow, enhance drainage systems, or modify the physical landscape through other means (Merabti, 2016).

I.7.5. Biological control

There is an urgent need to reduce reliance on conventional insecticides and explore alternative approaches (Keffous, 2023). Biological control approaches have been found to have an important influence on mosquito population reduction. The strategy is to introduce natural enemies into mosquito habitats. Some of these are: plants products, Predators, insecticidal bacterial spp and fungi (Alshaimaa *et al*., 2022). These various biocontrol strategies target different stages of the mosquito lifecycle (Figure 10).

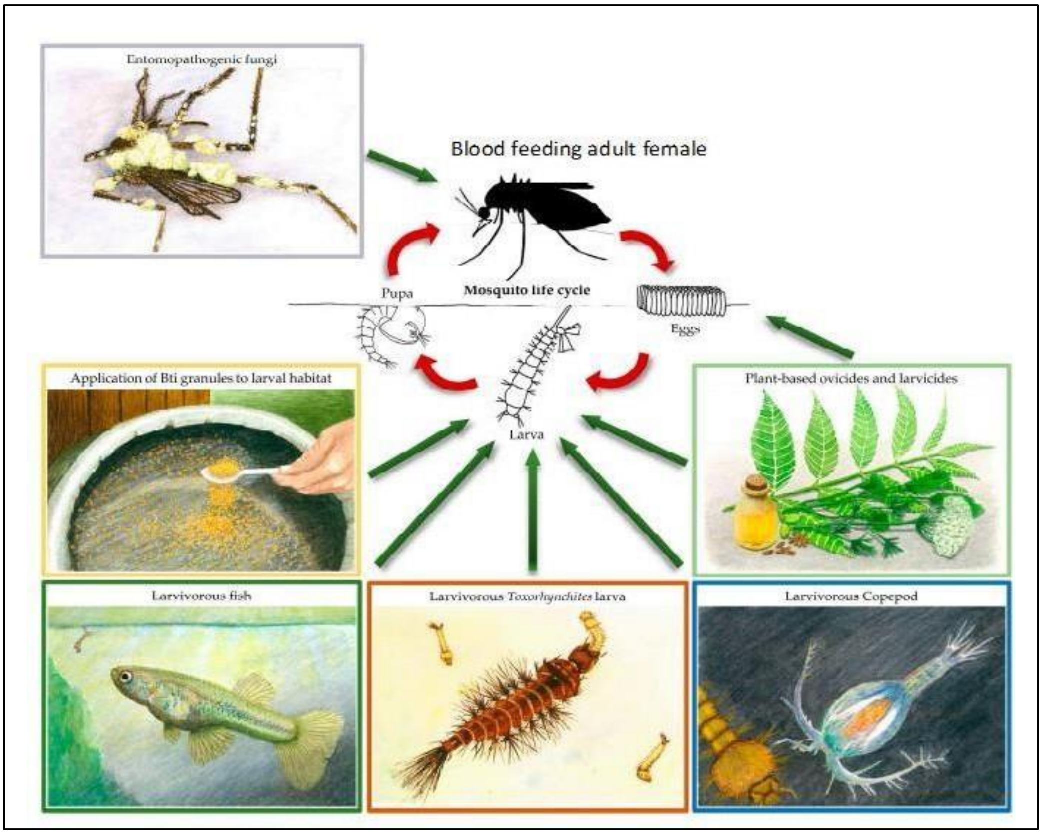

Figure 10: Biocontrol methods of mosquitoes (Benelli et *al*., 2016).

Bti: *Bacillus thuringiensis* var. *israelensis.*

I.7.5.1. Naturel predator

Invertebrates which are the natural predators of mosquitoes and feed on them provide another biological control remedy. These invertebrates include the elephant mosquito *Toxorynchites spp.* and freshwater crustacean copepods. *Toxorynchites splendens* is the most effective and widely reported species among the other mosquito predator species. They naturally feed on the larvae of other mosquito species including that of *Culex, Aedes,* and *Anopheles* which are the major disease transmitter genera of mosquitoes (Chaudhry *et al*., 2019). The release of native larvivorous fish into a lake or pond is one of the most cost effective mosquito vector control strategies, resulting in a long-term reduction in mosquito vectors (Das *et al.*, 2018).

I.7.5.2. Insecticidal bacterial spp

One suitable option for controlling the mosquito vectors is the use of bacteria which are pathogenic to the mosquito species (Chaudhry *et al*., 2019). *Bacillus* bacteria, known to cause mortality in mosquito larvae of the *Culex* and *Anopheles* genera. (Merabti, 2016). The *Bacillus sphaericus* produces a protein which, upon ingestion by the larvae, gets hydrolyzed in the stomach by the action of proteases. This solubilized protein damages both the nervous and digestive system and often lead to kill the mosquito larvae that have ingested this toxic protein (Chaudhry *et al*., 2019).

Bacillus thuringiensis var. *israelensis* is currently the most common mosquito larvicide employed in European countries, that releases insecticidal toxins and virulence factors that selectively target the larval stages of insects (Benelli *et al.*, 2016).

I.7.5.3. Entomopathogenic fungi

Insect fungi are numerous and widespread, and they can lead to many problems in the mosquito vectors population (Alshaimaa *et al*., 2022). Entomopathogenic fungi produce infective spores (conidia) that attach to and penetrate the cuticle of mosquitoes, releasing toxins that result in mosquito death (Benelli *et al.*, 2016).

I.7.5.4. Plant products

Insecticides of botanical origin received much attention, recently, due to their safe, cheap and environmentally friendly nature (Toqeer *et al*., 2019). These insecticides act as a larvicidal, pupicide, repellent, oviposition deterrent, or fumigants to control the mosquito population (Rawani, 2023). So, Plants have a diversity of phytochemicals used for their defence purposes, that can be applied to protect against microbial parasites and insect outbreaks such as phenols, alkaloids, sterols, terpenes, carotenoids…etc. (Toqeer *et al*., 2019).

However, recent years have seen a resurgence of interest in botanical pesticides at different life stages, such the use of plant- based essential oils (Chaudhry *et al*., 2019). Additionally, plants may exhibit a juvenoid mimetic action of the juvenile hormone, prolonging larval life and inhibiting pupation. This can lead to the emergence of abnormal pupae and mortality during pupation and metamorphosis (Kharoubi, 2021).

In this context, we decided to conduct a scientific study on the insecticidal activity of plant extracts of *Rosmarinus officinalis* and *Nerium olea*

Chapter II

Medicinal plants.

II. Medicinal plants

II.1. Rosemary

II.1.1. Origin of the name

Rosemary is a shrub whose name is derived from the Latin words “ros,” meaning dew, and “marinus,” meaning sea. Rosemary is a plant that thrives in areas where sea dew spreads at dawn (Figure 11). (Lepat, 2017).

Figure 11: Original photo of Rosemary (Original, 2024).

II.1.2. Botanical description

The rosemary plant is a fragrant, woody perennial shrub. Belonging to the *Lamiaceae* family, it typically grows upright and can reach heights of up to 2 meters. Its leaves are evergreen, measuring 2-4 cm long and 2-5 mm wide, with a green upper surface and a white underside covered in dense, short, woolly hairs (Figure 12). Varieties of rosemary exhibit differences in leaf size, branch growth, and flower color (Dejene *et al*., 2024).

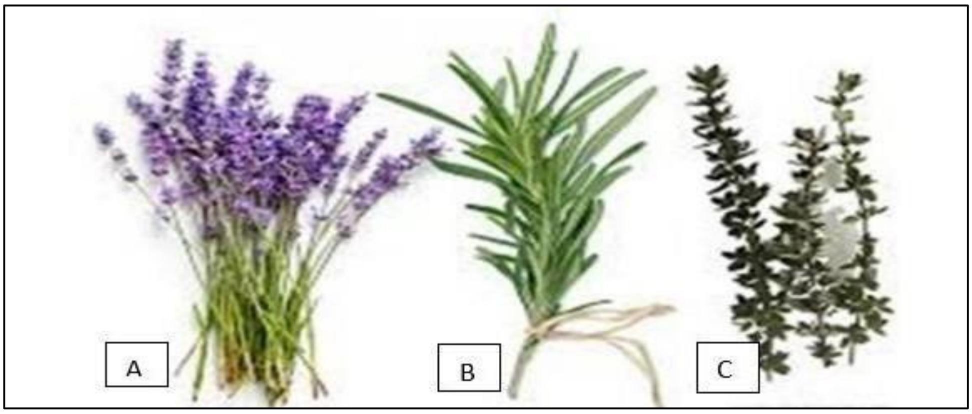

Figure 12: Rosemary Plant.

(**A):** Flower; (**B)**: Roots; (**B, C)**: Leaves (Chatterjee *et al*., 2022).

II. 1. 3. Taxonomical classification

According to (Chatterjee *et al*., 2022), *Rosmarinus officinalis* is classifed as follow:

Kingdom: Plantae

Sub-kingdom: Tracheobionta

Super division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Sub class: Asteridae

Clade: Tracheophytes

Order: Lamiales

Family: Lamiaceae

Genus: *Rosmarinus*

Species: *Rosmarinus officinalis*

II.1. 4. Geographical Distribution

Rosemary, as thrives across the Mediterranean Sea and throughout Europe, earning its moniker "rose of the sea". In France, it flourishes on limestone-rich soils in the southern regions, notably along the Mediterranean coast and extending to the Massif Central (Provence, Roussillon, Languedoc), while also being cultivated extensively in countries like Spain, Italy, Tunisia, and Morocco. In Algeria, rosemary is widespread, with sightings in various terrains including the steppe at Sid Djilali in the Sid El Makhfi region, along the coast at Béni Saf in the Sidi Safi area, and even at different altitudes such as Tlemcen at lala Setti, standing at 1025 meters (Mouas, 2018).

II.1.5. Chemical composition

This highly polymorphic species comes in several varieties. But instead of this morphological differentiation, many botanists prefer to rely on the chemical composition of the essential oil to list four dominant compounds: rosemary cineole, rosemary verbenone verbenone, rosemary camphor/ borneol, and sometimes rosemary myrcene (Mouas, 2018). The compound found in Rosmarinus officinalis can be classified in Table 2.

Table 2: Chemical composition of *R. officinalis* **(**Mouas, 2018).

Secondary metabolites	***Compounds***
Essential oil	1,8-cineole, camphor, a-pinene, other monoterpenes (borneol, limonene, camphene, a-terpineol).
Tricyclic phenolic diterpenes	Carnosolic acid, Carnosol, Rosmanol, Epirosmanol, Isorosmanol, Rosmaridiphenol, rosmari-quinone, Rosmadial.
Phenol acids	Caffeic acid, Chlorogenic acid, Rosmarinic acid
Methylated flavones	Genkwanine, Luteolein, Diosmetin
Triterpenes and steroids	Oleanolic acid, Ursolic acid derivatives, a- and amyrins
Other components	Acid polysaccharides, Traces of salicylates

II.1.6. Traditional use

The alcoholic extract derived from rosemary buds is utilized for treating sprains, strains, contusions, and torticollis. Meanwhile, the aqueous decoction serves as a gargle for angina and a mouthwash for mouth ulcers, and can also be added to invigorating baths. Rosemary essential oil aids in relieving rheumatic and circulatory disorders, promoting blood circulation, healing wounds, alleviating headaches, enhancing concentration and memory, combating stress and fatigue, and reducing inflammation in the respiratory. Additionally, rosemary essential oil facilitates hair growth by stimulating scalp irrigation through a simple application to the head. Rosemary possesses stimulating, antispasmodic, and cholagogue properties. It is recommended for conditions such as weak digestion, intestinal fermentations, as well as influenza (Mouas, 2018).

II.1.7. Pharmacological properties and biological activity

II.1.7.1. Antioxidant activity

The antioxidant activity of *R. officinalis* is well-documented in the literature. For example, rosemary essential oil and extract have been shown to destroy free radicals, to prevent lipid peroxidation and to reduce the reactive species amount in the body (Hamidpour *et al*., 2017). Rosemary extracts have shown efficacy in shielding the skin from damage caused by free radicals (Bousbia, 2011).

II.1.7.2. Antimicrobial activity

Rosemary has been shown to inhibit the growth of bacteria such as *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus*. According to one study, rosemary has the potential to inhibit the drug resistance of some bacteria by reducing the impermeability of these bacteria's membranes. The plant's essential oil has been shown to inhibit the adhesion of *Candida albicans* by denaturing cellular structures and altering membrane permeability (Jordan *et al*., 2013).

II.1.7.3. Anticancer activity

Many studies have reported on the anticancer mechanisms of *R. officinalis*. Rosemary has displayed significant anti proliferative activities against several human cancer cell lines. Carnosic acid appears to be the strongest promoter of apoptosis. In one study, the extract was found to strongly inhibit skin tumorigenesis in mice by preventing carcinogens from binding to epidermal DNA (Hamidpour *et al*., 2017).

II.1.7.4. Antidiabetic Activity

R. officinalis as a promising anti diabetic agent. Rosemary's antioxidant properties execute several anti-diabetic and hypoglycemic mechanisms. In one study, rosemary extract lowered blood glucose levels in normoglycemic, hyperglycemic, and diabetic rabbits. By inhibiting lipid peroxidation and activating antioxidant enzymes, the extract also promoted insulin secretion (Hamidpour *et al*., 2017).

II.1.7.5. Anti inflammatory activity

R. officinalis displayed potent anti-inflammatory mechanisms in several of the reviewed studies. Rosemary essential oil and extract were found to significantly inhibit leukocyte migration *in vivo*. This reduced the number of leukocytes at the site of inflammation, resulting an anti-inflammatory response (Yu *et al*., 2013).

II.1.8. Other utilizations

II.1.8.1. Agriculture

The high steppe plains are currently experiencing severe degradation biological potential and the disruption of ecological and socio-economic balances. To preserve the soil in these regions, the development of spontaneous species known for their tolerance to drought and salinity, such as rosemary, is essential (Mouas, 2018).

II.1.8.2. Food industry

Rosemary finds extensive application in the food industry for preserving foodstuffs by preventing both oxidative and microbial degradation. It serves as a natural substitute for chemical additives in various culinary preparations including poultry seafood sausages, and salads. Additionally, it adds flavor as a spice in potato chips (Bousbia, 2011).

II.2. Oleander

II.2.1. Origin of the name

Nerium oleander, commonly known as oleander (locally called Defla), is the sole species classified in the Nerium genus, belonging to the Apocynaceae family (Aruna *et al*., 2020). The Latin term Nerium originates from the Greek word nerion, meaning "wet," reflecting the plant's preference for wetlands. The specific name oleander is derived from the Italian "oleandro," which comes from the Latin "olea," referring to the olive tree (Figure 13) due to the ressemblance of its foliage to that of an olive tree (Sinha et Biswas, 2016).

Figure 13: Original photo of *Nerium oleander* (Original, 2024).

II.2.2. Botanical description

Nerium oleander L. is a small evergreen shrub or densely branched tree reaching heights of 2-5 meters **(**Farooqui et Tyagi, 2018). It features flexible branches with smooth, pale-green to light-gray bark that emits milky sap when cut. Each stem node typically bears two or three narrow, elliptic leaves with smooth margins on short petioles. (Garima et Amla, 2010). The leaves, arranged oppositely or in whorls, range from 5 to 20 cm in length, are tough, with numerous secondary veins. The flowers are approximately 5 cm in diameter, with five petals displaying various colors such as lilac, carmine and pink, they bloom from June to September. The fruit consists of a narrow follicle, 5-23 cm long, which splits open when ripe, releasing numerous downy seeds (Figure 14). The fluffy seeds are topped with a sessile egret that aids in their dispersal (Hussain et Gorsi, 2004).

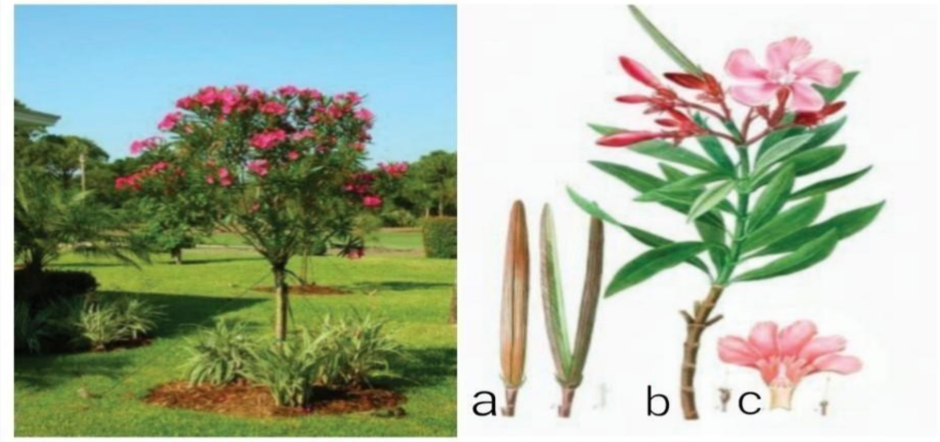

Figure 14: Nerium oleander:

(a): Seeds; **(b)**: Leg; **(c)**: Flower (Ayouaz *et al*., 2023).

II.2.3. Taxonomical classification

According to (Nia, 2018), *Nerium oleander* is classifed as follow:

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida

Order: Gentianales

Family: Apocynaceae

Genus: Nerium

Species: *Nerium oleander*.

II.2.4. Geographical distribution

N. oleander has been distributed across various continents and countries including Africa (Algeria, Libya, Morocco, Tunisia, Mauritania, and Niger), Asia (United Arab Emirates, Afghanistan, Cyprus, Iran, Iraq, Palestine, Jordan, Lebanon, Syria, Turkey, China, India, Nepal, Pakistan), and Europe (Albania, Croatia, Greece, Italy, Malta, France, Portugal, Spain). It is extensively cultivated in wide areas. Its presence is notable in Algeria, especially on alluvial and rocky terrain. It spreads along the wadis in the Northern Sahara and thrives in the Tassili and Hoggar mountains (Farooqui et Tyagi, 2018).

II.2.5. Chemical composition

The initial phytochemical screening revealed the presence of alkaloids, flavonoids, carbohydrates, tannins, phenolics, saponins, cardenolides, cardiac glycosides, triterpenes, and steroids in the plant (Balkan *et al*., 2018). The ethanolic extract of *N. oleander* leaves, revealing the presence of pentacyclic triterpenes, including oleander ocinoic acid, as well as flavonoid glycosides such as quercetin-5-O-[α-L-rhamnopyranosyl-(1→6)]-β-D glucopyranoside and kaempferol-5-O-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside (Santhi *et al*., 2011).

Approximately, Thiry cardenolides were isolated or identified, with oleandrin or 3-o-α-Loleadrosyl-16 acetylgitoxigenine, being the most prevalent among them (Chaudhary *et al*., 2015). The highest concentration of oleandrin was found in the roots, followed by leaves, stems, and flowers. (Tayoub *et al*., 2014). Approximately 1.5% of the leaf composition comprises cardenolides, with oleandrin, accounting for 0.1% of this content (Balkan *et al*., 2018). The seeds contain glucosides like oleandrine, odorosides, and adigoside. Additionally, the bark contains glucosides such as rosaginoside, nerioside, and corteneroside (Farkhondeh *et al*., 2020).

II.2.6. Traditional use

Despite its known toxicity, *N. oleander* continues to be employed in traditional medicine for treating various diseases and features prominently in numerous regional pharmacopoeias (Adome *et al*., 2003). The traditional applications of different parts of *N. oleander* across various nations are detailed in (Table 3).

Table 3: Traditional applications of different parts of *N. oleander* (Ayouaz *et al*., 2023).

Parts used	Country	Indications	Instruction of use
Fresh or dried leaves	South Africa	Abortifacient	*
	Algeria	Cleaning and softening of the feet (skin), against cavities and dental rages	Decoction
	Iran	Cardiotonic and diuretic	Infusion
	Marocco	Antidiabetic, abortifacient, itching, head male, antigale, against hair loss and eczema	Decoction infusion, maceration
	Turkey	Antibacterial	Decoction
Flowers	*	Emetic, cardiotonic, diaphoretic expectorant, sternutatory, diuretic, anticancer, antibacterial	*
Root	*	Gynaecological disorders treatment, adjunctive treatment, rheumatic pain, haemorrhoids and ulcers, venereal diseases. Oil of root bark gave good results in leprosy and it is applied to tumours	Fumigations stickers, decoction powder
Bark	*	Expectorant, heart tonic, diuretic, emetic, diaphoretic cathartic, febrifuge and intermittent fever. Oil prepared from the root bark is used in the treatment of leprosy and skin diseases of a scaly nature	Maceration, decoction
Seeds	*	Purgative in dropsy and rheumatism	*
Different parts	Cuba	Folklore medicine	*
	India	Ulcers on the penis, antibacterial	*

II.2.7. Pharmacological properties and biological activities

According to (Ayouaz *et al*., 2023), The Pharmacological properties and biological activities of *Nerium oleander* are summarized in the following (Table 4).

Table 4: Biological activities of *N. oleander* (Ayouaz *et al*., 2023).

Parts Used	Biological Activities
Leaves	Cardiotonic CNS depressant effect, anticancer, antimicrobial, antifungal, Larvicidal activity, antihyperglycaemic, antioxydant, anti-inflammatory activities, antiviral activity, immunomodulating activity antiproliferative activity, antidiabetic activity
Flowers	Cardiotonic, root CNS-active and spasmolytic activity antifungal antimicrobial, Larvicidal activity, Antioxidant activity, anti-inflammatory activity, anticancer activity, hepatoprotective activity
Roots	Anticancer, anti-leprosy, anti-ulcer, antibactériens, cardiotonic, antioxydant, larvicidal, antifungal activities
Barks	antimicrobial, antifungal, larvicidal activities
Stems	Antioxdant, larvicidal antifungal, anticacer activities

II.2.8. Toxic properties

Various studies have suggested that *N. oleander* may employed as insecticides, pesticides and rodenticides. Ingesting just five *N. oleander* leaves can be fatally poisonous, with even one leaf causing severe toxicity in children. The severity of *N. oleander* toxicity depends on factors such as the toxin concentration in the ingested part of the plant, the age, and the health condition of the individual who consumed it (Farkhondeh *et al*., 2020).

Materials and methods

I. Materials

I.1. Animal material

Mosquito larvae of *Culex pipiens* were collected from an untreated tank water in the Thenia, Mila region. The larvae were transported to the laboratory by plastic bottles containing dechlorinated water. *Cx. pipiens* (Figure 15) is the most abundant mosquito species in urban and rural area. These larvae were used to evaluate the larvicidal properties of *N. oleander* and *R. Officinalis* extracts.

Figure 15 : *Cx. pipiens* larvae (original 2024).

I.2. Plant material

The leaves of *N. oleander* and *R. officinalis* were collected in late February and early March of 2024 from the Terrai Bainen area of Mila region. The drying time took about 30 days. Once the leaves are dried, they are ground into a fine powder using an electric grinder (Figure 16).

Figure 16: Plant material.

(A): *N. oleander* Leaves; **(B)**: *R. Officinalis* leaves (original, 2024).

II. Methods

II.1. Study area

The wilaya of Mila (Figure 17) is located in the North-East of Algeria. It is limited to the north by the wilaya of Jijel, To the North-East by the wilaya of Skikda, to the East by the wilaya of Constantine, to the West by the wilaya of Setif, and to the south by the wilaya of Oum el Bouaghi (Bounemeur, 2024). Mosquito larvae of Culex pipiens and material plant were collected in the Thenia, Terrai Bainen, Mila region, respectively.

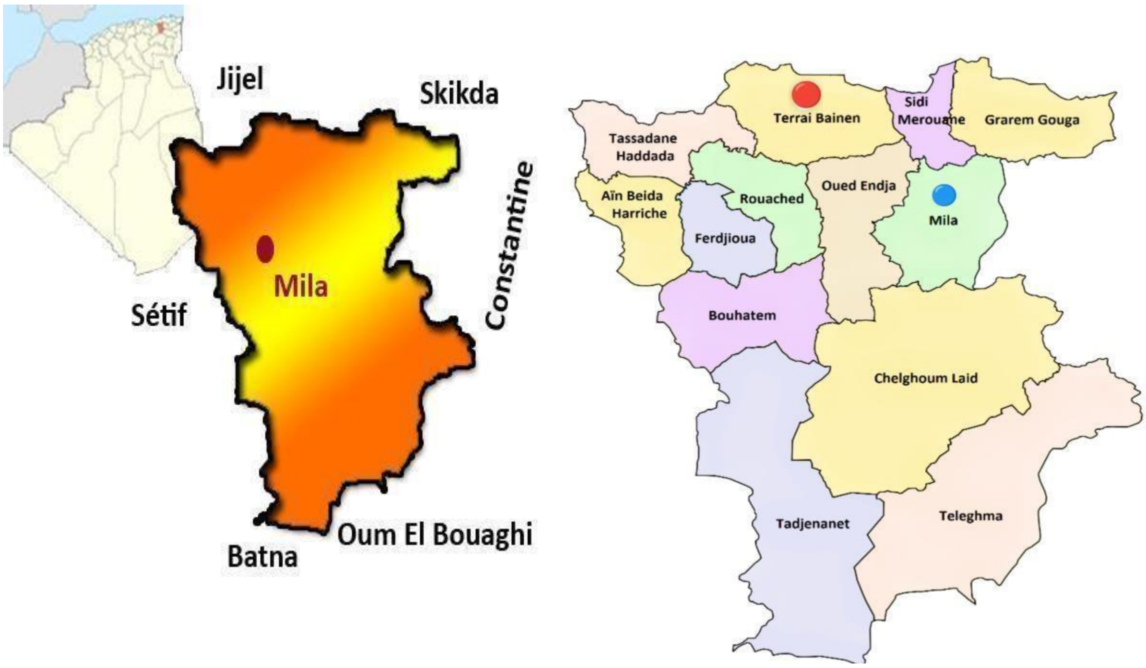

Figure 17: Geographical location of the area study (Bounemeur, 2024).

II.2. Extraction

II.2.1. Preparation of the aqueous extract by decoction

The preparation of plant extracts was carried out according to the method of (Martins *et al.*, 2014). Decoction extract was prepared by boiling 20 g of dried Leaves in 200 mL of distilled water at heating plate, for 5 min. Then the mixture was filtered through filter paper and dried to obtain a dry extract (Figure 17).

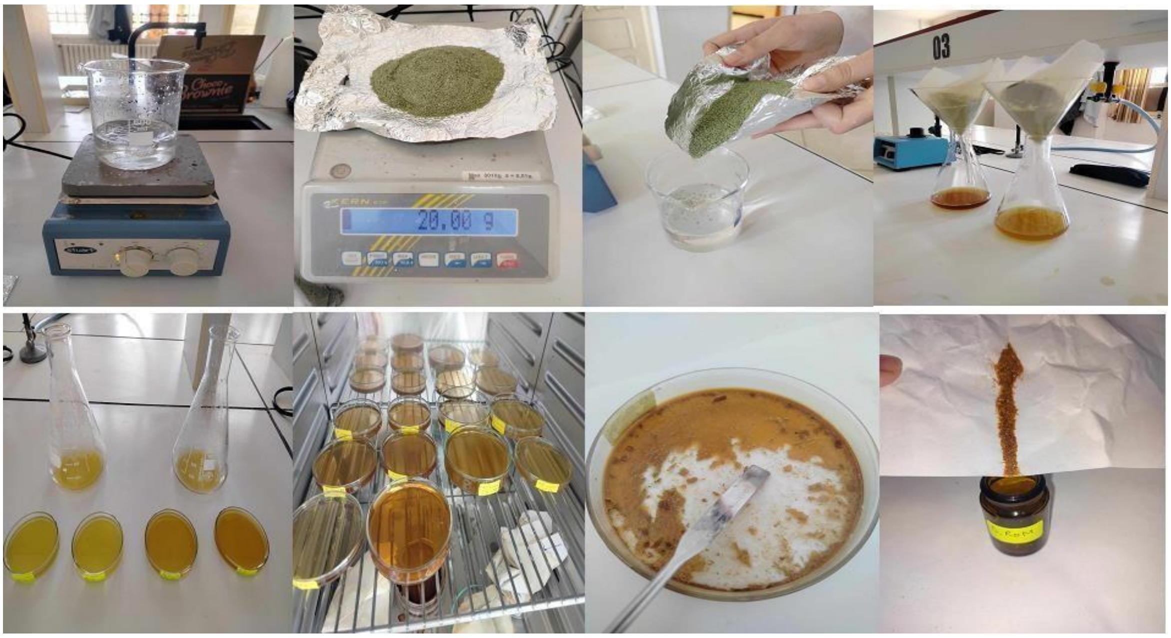

Figure 17: Steps of the decoction method (Original, 2024).

II.2.2. Preparation of the aqueous extract by infusion

The preparation of the infusion extract was carried out according to the method of (Martins *et al*., 2014). Infusion extract was prepared by mixing 20 g of dry sample in 200mL of boiled distilled water and left to stand at room temperature during 5 min. Then the mixture was filtered and dried to obtain a dry extract (Figure 18).

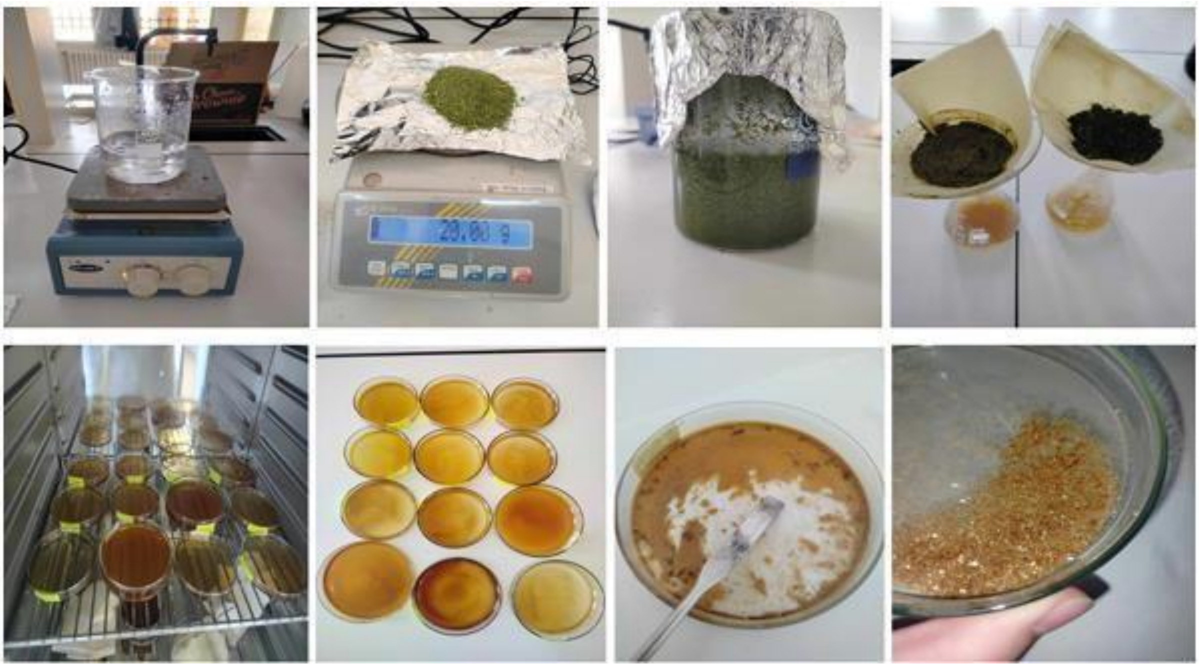

Figure 18: Steps of the infusion method (Original, 2024).

II.2.3. Determination of extraction yield

The percentage of extraction yield for each extract was calculated by the following formula as the ratio of the mass of the dried extract to the mass of the ground plant sample.

% Yield= Weight of extract obtained/ Total weight of the sample×100

II.3. Quantitative phytochemical analysis

II.3.1. Determination of phenolic compounds

The total phenolic in extracts is estimated using the method of Folin-Ciocalteu method (Karbab *et al*., 2020). In tubes, a volume of 100 µL of each extract was added to the500 µL of Folin-Ciocalteu reagent for 4min, 400 µL of a 7.5 % sodium carbonate solution was added. Then, the tubes are shaked and incubated for 2h. The absorbance is determined at 765 nm (Figure 19).

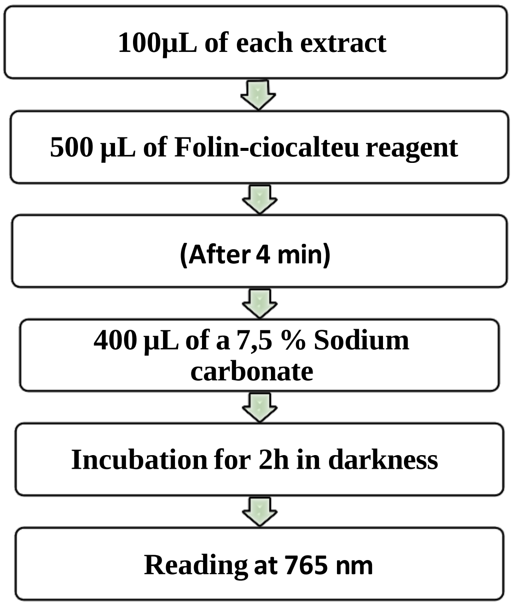

Figure 19: Total polyphenols protocol (Karbab *et al*., 2020)**.**

Polyphenolic content was expressed as µg gallic acid equivalent (GA Eq)/mg dried extract. The number of total polyphenols in different extracts was determined from a standard curve of gallic acid ranging from 10 to 160 µg/mL (Figure 20).

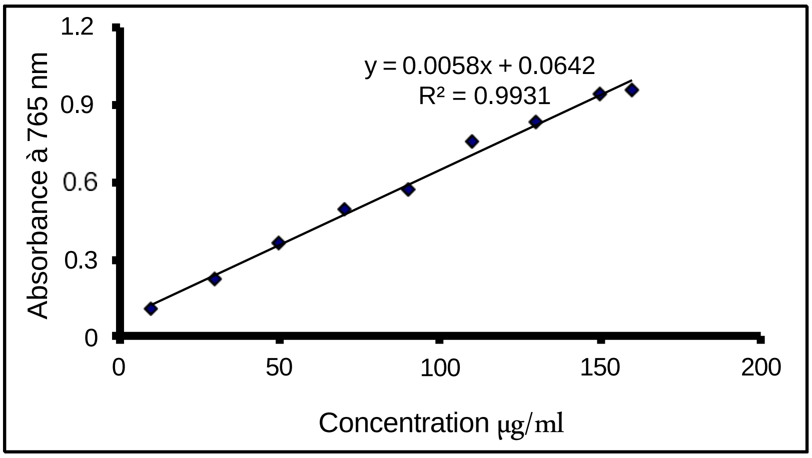

Figure 20: Standard curve of gallic acid.

II.3.2. Determination of flavonoids

The total flavonoids content was evaluated by the method of aluminum chloride ($AlCl_3$) (Karbab *et al*., 2020)**.** A 500 µL of each extract was added to 500 µL of the solution of $AlCl_3$ (2% in ethanol). After 10 min of incubation, at room temperature, the absorbances are measured at 430 nm (Figure 21).

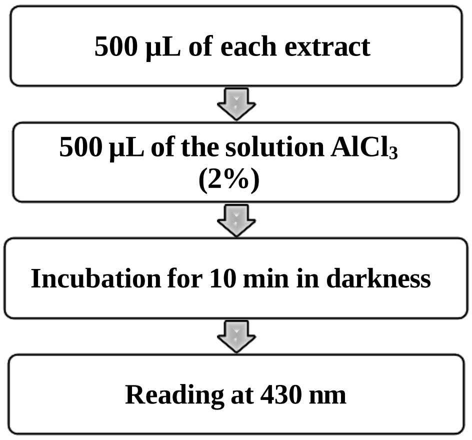

Figure 21 : Total flavonoids dosage protocol (Karbab *et al*., 2020)**.**

Total flavonoids were reported as µg of quercetin equivalent (Q Eq)/mg dried extract. The number of total flavonoids in different extracts was determined from a standard curve of quercetin ranging from 2.5 to 40 µg/mL (Figure 22).

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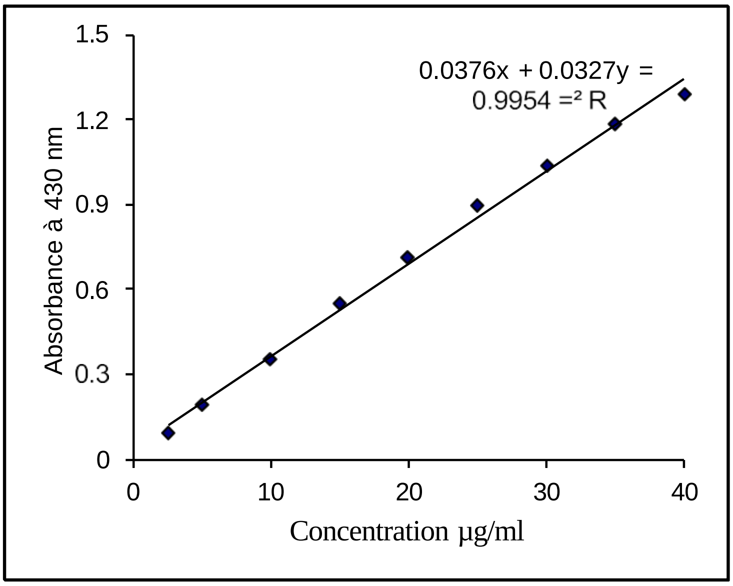


Figure 22: Standard curve of quercetin**.**

II.4. Larvicidal activity

II.4.1. Sampling and rearing of *Cx. pipiens* larvae

The mosquito larvae were placed into clear plastic cups containing dechlorinated water. They were fed were a finely powdered mixture of biscuits and dried yeast, at a ratio of 3:1. yeast provides proteins, carbohydrates, and vitamin B (Rehimi & Soltani, 1999)**.** Care was taken to change the water and add food every two to three days. They were put in cages covered with tulle, leaving one side tightly covered with plastic to facilitate the insertion and removal of the hand.

Once the larvae reach the pupal stage, food is provided for them. Raisins are hung for the males, and a blood meal is provided for the females, approximately 5 ml of blood mixed with heparin to prevent coagulation. The blood is placed in a small glass petri dish covered with adhesive plastic film and then placed on top of the cage upside-down. This process enables the females to pierce the membrane in order to feed on it, a technique described by Enserink (2006). A bowl of water is then placed in the cages to allow the females to lay eggs (Figure 23).

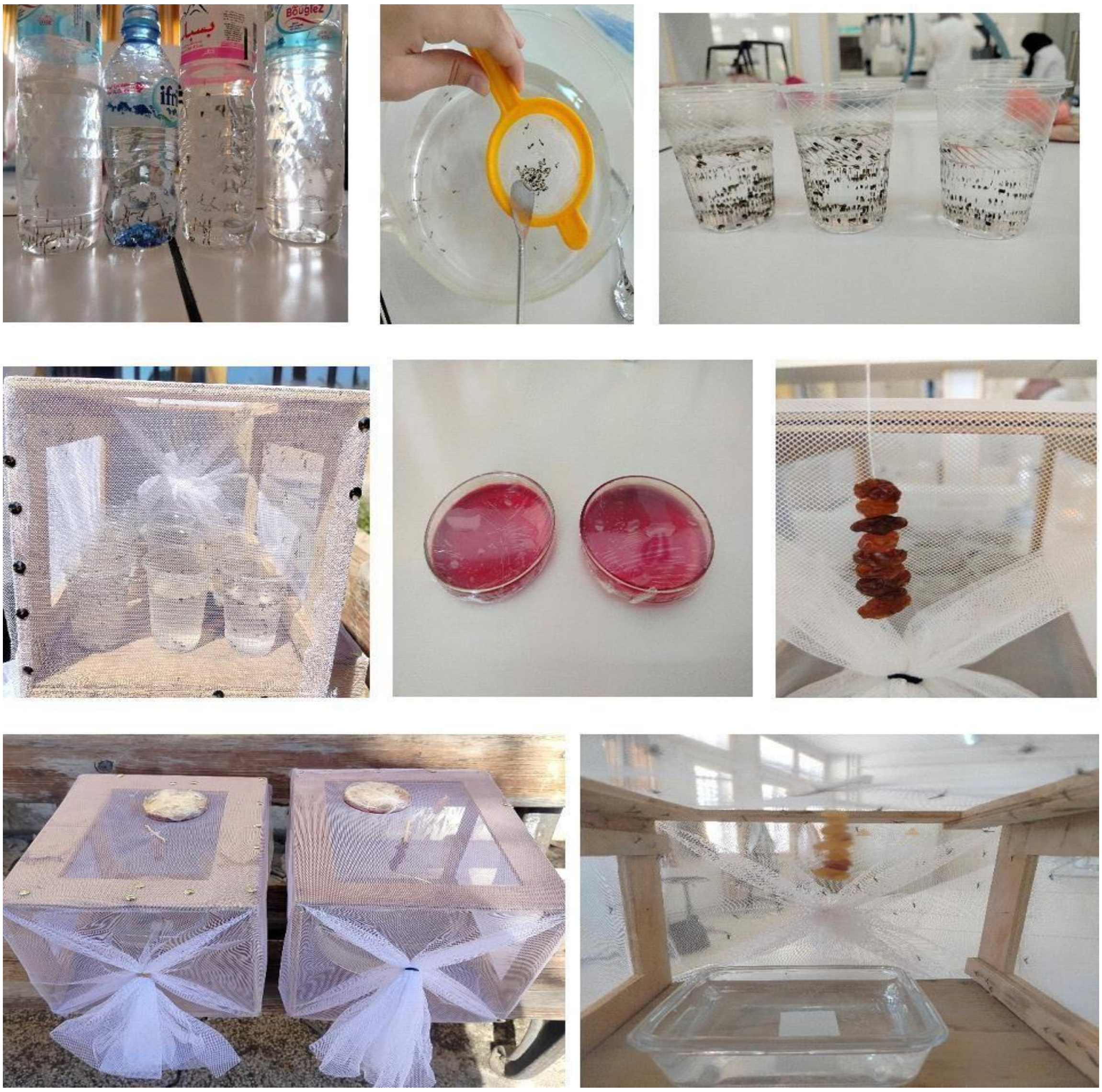


Figure 23: The breeding stages of *Cx.pipiens* larvae (Original, 2024).

II.4.2. Toxicity test

These tests involved assessing the mortality of *Cx. pipiens* larvae in the presence of diluted solutions of plant extracts, using a methodology inspired by the World Health Organization protocol. In fact, 10 *Cx. pipiens* larvae were collected with a spatula and placed in transparent plastic cups, each containing 99 ml of demineralized tap water. Preliminary experiments enabled a range of concentrations to be selected for the tests themselves; from a stock solution of extracts, concentrations of 100, 50, 25 and 12.5 mg/mL were prepared. One milliliter of each diluted solution was introduced into the previously prepared beakers, and 2 replicates were performed for each dilution. Negative control cups were also prepared under identical conditions to the test cups. The negative control contained only water (100ml), with no trace of extracts (Figure 24).

During the experiment, unfortunately, we were not able to obtain the mosquito eggs that the female lays after she gets a blood meal, so we had to use the larvae directly to complete the experiment.

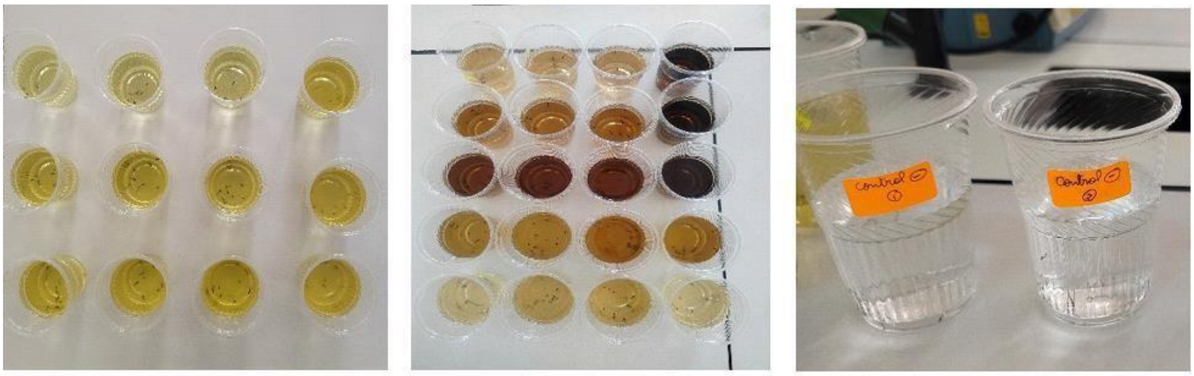
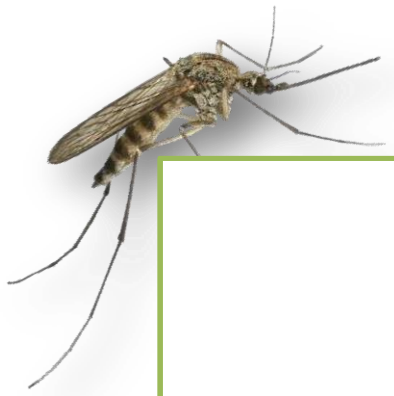


Figure 24: Toxicity tests on *Cx.pipiens* larvae (original, 2024).

Percentage mortality was noted at 24, 48 and 72h after extract inoculation.

% Mortality= (Numbers of dead larvae/numbers of exposed larvae) × 100



Results And discussion

Results and discussion

1. Extraction yield

The table represents the extraction yield percentage for *N. oleander* and *R. officinalis* plants by two extraction methods. The highest yield was noted by decoction (20.65%), followed by infusion extract (17.95%) in oleander plant. These values are lowest to those obtained by the previous scientific report of kgosana. (2019); the yield of decoction and infusion extracts was estimated at value of 22.6 and 43.1%, respectively. Although infusion and decoction extracts of rosemary leaves displayed a lower yield with range from 8.25 to 12.35, respectively. The scientific work of Mutalib. (2015), conducted the lowest extraction yield values ranges from 3.8 to 4.63 % for decoction and infusion extracts of *N. oleander*.

Table 5: Extraction yield of oleander and rosemary by two types of extractions.

	Extracts	**Decoction**	**Infusion**
N. Oleander	Weight(g)	20	20
	Yield (%)	20.65	17.95
R. officinalis	Weight(g)	20	20
	Yield (%)	8.25	12.35

2. Quantification of phytochemicals compounds

The results reported in Figures and show that the distribution of total phenolic compounds (TPC) and total flavonoids (TFC) varied according to plant species and extraction methods.

2.1. Quantification of total phenolic compounds

Figure represents the content of total phenolic compounds (TPC) in the plants of *N. oleander* and *R. officinalis*. The large amount of phenolic compound was obtained by *R. officinalis* extracts (decoction and infusion) compared to those of *N. oleander.* The TPC in leaves extracts from rosemary was the highest, with 46.35 and 44.74 (µg GAE/mg E) for decoction and infusion extracts, respectively, while leaves extracts from oleander exhibited the lowest values: 12.83 (µg GAE/mg E) for infusion extract and 17.2 (µg GAE/mg E) for decoction extract. The value obtained by Redha work on 2020 in leaves extract of oleander was lower to that of present study with TPC of 4.44 (µg GAE/mg E). This difference may be due to the extraction method employed, solvent, and temperatures used. Bianchin and his collaborators (2020) were found *R. officinalis* extract have high total phenolic compounds contents especially in leaves with value of 46.48(µg GAE/mg E). this result is in a good agreement with the present study.

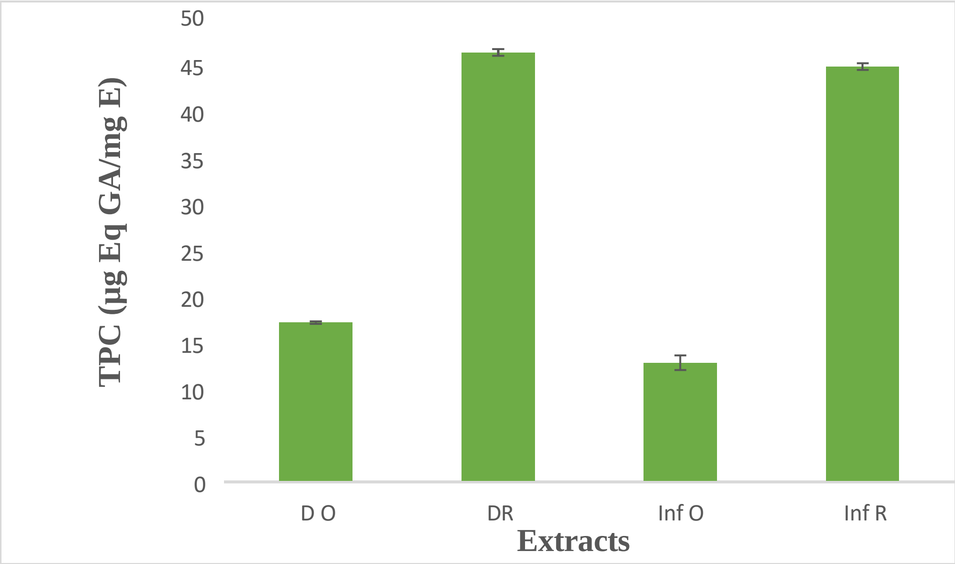


Figure: Total phenolic contents (TPC) of plants leaves extracts.

Each value is represented by the average of three repetitions ± SD.

2.2. Quantification of total flavonoids

The figure represents the total flavonoids content (TFC) in the plants of N. *oleander* and *R. officinalis*. The large amount of phenolic compound was obtained by *R. officinalis* extracts (decoction and infusion) compared to those of *N. oleander*. The TFC in leaves extracts from rosemary was the highest, with 26.83 and 18.96 (µg QE/mg E) for decoction and infusion extracts, respectively, while leaves extracts from oleander exhibited the lowest values: 1.67 (µg QE/mg E) for infusion extract and 22.67 (µg QE/mg E) for decoction extract. Richness in TFC in leaves of oleander in oleander leaves was previously reported by Redha work on 2020 with value of 28.15 (µg QE/mg E). Bianchin and his collaborators (2020) were found *R. officinalis* leaves extract have lowest total flavonoids contents with value of 11.894 (µg QE/mg E).

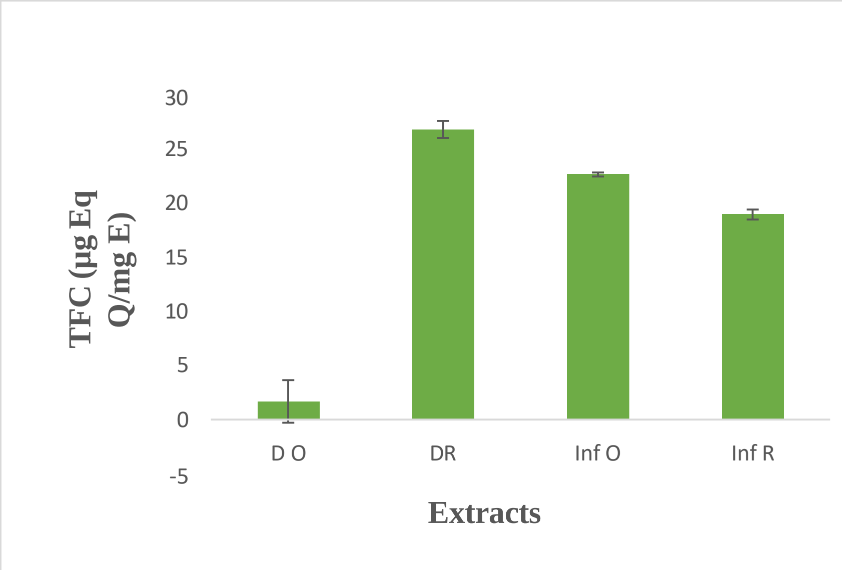


Figure: Total flavonoids contents (TPC) of plants leaves extracts.

Each value is represented by the average of three repetitions ± SD.

3. Evaluation of larvicidal activity

Even though the number of reports on used *N.oleander* and *R. officinalis* in different countries for their larval mosquitocidal activities continues to increase, to the best of our knowledge, few studies have been published the lethal activity of decoction and infusion extracts of these plants against mosquito larvae.

The results of *C.pipiens* larval mortality tested against *N.oleander* and *R. officinalis* decoction and infusion leaves extracts are presented in Table 6. Firstely, the results of the present study revealed the effect of larval mortality was dependent on the concentration of extracts. Secondly, the time of exposure were found to be effective in increasing larvicidal activity. Thirtly, the two extracts proved larvicidal effect, where infusion extract of rosemary revealed the highest larvicidal activity followed by the infusion then the decoction extracts *of* oleander plant at 100 mg/mL, during the 72 h. It was found that there was an overall lethal effect of *N. oleander* extracts and *R. officinalis* against larval mosquitoes (Fakoorziba *et al*., 2015).

Table 6: Percentage mortality of *Culex pipiens* larvae exposed to plants extracts.

		% Mortality		
	Time	**24 h**	**48h**	**72h**
Extracts	**Concentration**			
D O	100	5 ± 7.07	20 ±15.27	**23.33 ± 5.77**
	50	0.00 ± 0.00	5 ± 7.07	10 ± 0.00
	25	0.00 ± 0.00	6.67 ± 5.77	10 ± 0.00
	12,5	0.00 ± 0.00	6.67 ± 5.77	10 ± 0.00
Inf O	100	5 ± 7.07	5 ± 7.07	**25 ± 35.35**
	50	10 ± 0.00	10 ± 0.00	10 ± 0.00
	25	5 ± 7.07	5 ± 7.07	5 ± 7.07
	12,5	15 ± 7.07	15 ± 7.07	15 ± 7.07
DR	100	**15 ± 7.07**	**15 ± 7.07**	**15 ± 7.07**
	50	25 ± 35.35	25 ± 35.35	25 ± 35.35
	25	30 ± 42.42	35 ± 35.35	35 ± 35.35
	12,5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Inf R	100	**35 ± 35.35**	**45 ± 21.21**	**45 ± 21.21**
	50	5 ± 7.07	15 ± 21.21	15 ± 21.21
	25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	12,5	20 ± 14.14	20 ± 14.14	20 ±14.14

All tests were carried out in duplicate and the results are expressed as means ± S

The aqueous extract of *N. oleander* clearly revealed a weaker lethal activity against *Culex* mosquitoes, 25% of mortality was found from the concentration of 10% (100 mg/mL) during the 72-hour exposure of infusion extract. This percentage of mortality is observed for a concentration range between 20 and 40 mg/mL of aqueous extract in 24h (El-Akhal *et al.*, 2015). In another work cited by the previous autour, *N. oleander* did not show any larvae mortality of *Culex sp* in 0.1 mg/mL (100 ppm or 1%) of aqueous extract.

Generally, the active toxic ingredients of plant extracts are secondary metabolites that are evolved to protect them from herbivores. The insects feed on these secondary metabolites potentially encountering toxic sub- stances with relatively non-specific effects on a wide range of molecular targets. These targets range from proteins (enzymes, receptors, signaling molecules, ion-channels and structural proteins), nucleic acids, bio membranes, and other cellular components (Ghosh *et al.,* 2012).

The differential responses induced by these plants against the various species of mosquitoes were influenced by extrinsic and intrinsic factors such as the species of plant, the parts of the plant, age of plant, the solvents used for extractions, the geographical location where the plants were grown and the methods employed for extraction (Rajasingh *et al*., 2014; El-Akhal *et al*., 2015; Fakoorziba *et al*., 2015).

Plants accumulate bioactive chemicals differentially in the various parts of the plant, such as leaves, fruits, flowers, roots and bark and the effectiveness of chemicals derived from specific plant parts often varies with the mosquito species (Ghosh *et al*., 2012). The beneficial potential of plants due to their compounds, such as the presence of saponins, phytosterols, phenols and tannins, including flavonoids in plants having also reported for larvicidal activities against mosquitoes. The presence of flavonoids and tannins in aqueous extracts in plant part had a higher potential against the larval development. Besides quercetin, flavonoids apigenin, vitexin, isovitexin, sitosterol and campesterol was pointed out for positive larvicidal activities against *Culex quinquefasciatus* (Wan *et al*., 2021).

Thus, the larvicidal activity of crude extracts depends upon the mixture of active compound and the specificity of a phytocompound (Rajasingh *et al*., 2014). Moreover, it was pointed out that the active ingredient in the ethanolic extract of *N. oleander* leaves is the glycoside neriifolin (Rajasingh *et al*., 2017). It was thus concluded that there was a significant lethal effect of *N. oleander* leave extracts containing chemicals like oleandrin against larval mosquitoes (Fakoorziba *et al*., 2015).

Some active ingredients of the plant have the ability to penetrate the body of the larva by direct ingestion or enter through the skin which leads to damage to the epithelial lining, causing it to die or change its feeding behavior (Al-Jameeli, 2021). The effect of the extracts on the larvae may occur via ingestion of chemical compounds through these structures, causing asphyxiation. On the other hand, this product can cause cellular disorganization of the gills, contribution to a disorder in the osmotic regulation of the mosquito, which can lead to an imbalance in the absorption of ions from the water (Leandro *et al*., 2021).

The plant extract R. Officinalis showed good lethal activity against Culex mosquitoes compared to that of *N.Oleander*, where 45% mortality was found from 10% concentration (100 mg/ml) during 72- and 48-hour infusion exposure The results showed that the effectiveness of phytochemicals against mosquito larvae may vary greatly depending on the plant species, plant parts employed, age of plant parts (young, mature, or senescent), extraction solvent, and accessible vector species (Anupam *et al*., 2012). We were unable to find previous studies that conducted the same method of extracting rosemary by soaking and boiling on Culex larvae, while some have used essential oils to test their toxicity on Culex larvae (Bianchin *et al.*, 2020; Bosly, 2022).

The efficacy of phytochemicals against mos- quito larvae can vary significantly depend- ing on plant species, plant parts used, age of plant parts (young, mature or senescent), solvent used during extraction as well as upon the available vector species. Sukumar et al have described the existence of varia- tions in the level of effectiveness of phyto- chemical compounds on target mosquito species vis-à-vis plant parts from which these were extracted, responses in species and their developmental stages against the specified extract, solvent of extraction, geo- graphical origin of the plant, photosensitiv- ity of some of the compounds in the extract, effect on growth and reproduction (Alhaithloul *et al., 2023).*

The action of these extracts in all growth stages may be due to the fact that they contain compounds whose effect on mosquito larvae is similar to that of the mode of action of insect growth regulators, such that these compounds interfere with the physiological processes of the insect during its metamorphosis, or there may be an imbalance between stimulating or inhibiting the secretion of Eecdysone hormone or Juvenile hormone. or due to that these compounds can disrupt the work of hormones secreted by the endocrine glands, which leads to a defect in the growth process and death of the insect. These abnormalities arising from this study are similar to those resulting from the effect of growth regulators on mosqu το larvae observed by many researchers (Silva *et* Mendes,2007; Arivoli *et* Tennyson,2011)

Conclusion

Conclusion

To prevent the spread of mosquito-borne diseases and improve environmental quality and public health, mosquito control is essential. Currently, the main tool for mosquito control is the application of synthetic insecticides. However, their use is poorly accepted, due to their harmful effects on human and animal health and other non-target organisms. Added to this are concerns for the environment, given their non-biodegradable nature, and the growing resistance of mosquito populations to these insecticides.

of the most effective alternative approaches in the biological control program is to explore floral biodiversity and promote the use of plant-based insecticides.

Our work has enabled us to evaluate the effect of *N.oleander* and *R.officinalis* decoction and infusion leaves extracts. On Cx. pipiens larval mortality.

We measured the total amount of phenolic compounds for both the oleander and rosemary plants, and we found that the rosemary plant is rich in phenolic compounds, reaching 46.35 (µg GAE/mg E) and 44.74 (µg GAL/mg E) for the decoction and infusion extracts, while we found lower percentages in the oleander plant, as its percentage did not exceed 12.83 (µg GAE/mg E) and 17.2 (µg GAE/mg E) by decoction and infusion.

We also found a large amount of total flavonoids in the oleander plant, amounting to 26.83 (µg QE/mg E) and 18.96 (µg QE/mg E) for the decoction and infusion extracts, while we found small values in the oleander plant that did not exceed 1.67 (µg QE/mg E) in the decoction extract and 22.67 (µg QE/mg E) in the infusion.

The extracts of oleander leaves and rosemary were tested on Culex larvae. We recorded a high mortality rate in the rosemary plant that increased with an increase in the concentration of the extracts, as it was recorded at 45% from the 10% (100 mg/ml.) concentration of the infusion extract, while it did not exceed 25% from the 10% (100 mg/mL) concentration of the infusion extract of the oleander plant.

In the future, it would be interesting to complete this research, but with the use of other solvents that would enable us to estimate the larvicidal effect of this plant, and thus enhance its value.

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