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Thème:

Assessment of toxicological activities of various active substances extracted from *Lavandula angustifolia* against the pine processionary caterpillar (*Thaumetopoea pityocampa Schiff*) under laboratory conditions.

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Dedication

الحمدُ لله الذِي هيأ البدْء ويسَر اليُسر وطَيبَ المُنْتهى الحَمد لله الذِي أغْرقنَي سرورًا وجعل طرِيقي يَسيرا تم بحمد الله إكمال هذه المذكرة

First and foremost, I extend my greetings to myself for standing strong through every hardship. I salute you for completing your studies despite all the challenges and illness. Not even the nights in the hospital stopped you from reaching this day. I am proud of myself.

To my mother ***Zineb,*** the light of my life, always selfless Your eldest daughter has graduated. What a blessing it is to have you.

May you remain my support forever. May Allah protect you for us. To my beloved father ***Houssine*** **,**

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To my entire family, to my uncle ***Rabeh*** and his wife ***Zineb*** my grandmother the second family Thank you for being there.

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And thank you all for bearing with my endless graduation post we made it to the end . To those who transcended limits, Who believed that raising the banner of truth is worth the pain, Who knew that the ceiling of possibility is astonishing, And that the impossible is nothing but a bendable obstacle.

This work is for you just as much as it is for me.

Oumeyma

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List of abreviations

°C: Degree Celsius

ABCD: Advisory Board on Cat Diseases

ACL: Association for the Conservation of Lepidoptera

FAO: Food and Agriculture Organization

GDF: General Directorate of Forests

INRA: National Institute for Agronomic Research

IPCC: Intergovernmental Panel on Climate Change

IUCN: International Union for Conservation of Nature

NFI: National Forest Inventory

ONS: National Office of Statistics

PPM: Pine Processionary Moth

Pa·s: Pascal-Second (Viscosity unity)

T: Temperature

USEPA: United States Environmental Protection Agency

WWF: World Wide Fund for Nature

Résumé

Cette étude évalue l'efficacité des molécules bioactives extraites de *Lavandula angustifolia* (lavande) contre la chenille processionnaire du pin (*Thaumetopoea pityocampa*), un ravageur majeur des forêts algériennes. Trois formulations ont été testées : une huile essentielle préparée en laboratoire, une huile végétale et une huile essentielle commerciale. Les résultats montrent que l'huile essentielle fraîchement distillée présente la meilleure activité larvicide, avec un taux de mortalité atteignant 99,99% à 3% de concentration après 8 jours, grâce à des composés actifs comme le linalol et l'acétate de linalyle. L'huile végétale a montré une efficacité modérée, tandis que l'huile commerciale a été moins performante, probablement en raison de sa dégradation.

Mots-clés : Lavandula angustifolia , Thaumetopoea pityocampa , huile essentielle, huile végétale, lutte biologique, mortalité larvaire, forêt de pin d'Alep, molécules bioactives.

ملخص

تهدف هذه الدراسة إلى تقييم فعالية الجزيئات النشطة المستخلصة من نبات الخزامى ضيق الأوراق في مكافحة اليرقة الجوالة للصنوبر، وهي آفة خطيرة تهدد الغابات الجزائرية. تم اختبار ثلاث تركيبات: زيت عطري مُحضر معملياً، زيت نباتي، وزيت عطري تجاري. أظهرت النتائج أن الزيت العطري الطازج كان الأكثر فعالية، حيث حقق نسبة نفوق 99.99% عند تركيز 3% بعد 8 أيام، وذلك بفضل مركبات مثل اللينالول وأسيتات اللينااليل. بينما كان الزيت النباتي أقل فعالية، والزيت التجاري أظهر نتائج متباينة ربما بسبب التخزين .

الكلمات المفتاحية: خزامى ضيقة الأوراق(Lavandula angustifolia) ، اليرقة الجوالة للصنوبر (Thaumetopoea pityocampa)، الزيت العطري، الزيت النباتي، المكافحة الحيوية، معدل نفوق اليرقات، غابات الصنوبر الحلبي، الجزيئات النشطة حيوياً .

Summary

This study evaluates the efficacy of bioactive molecules extracted from *Lavandula angustifolia* (lavender) against the pine processionary moth (*Thaumetopoea pityocampa*), a major pest in Algerian forests. Three formulations were tested: laboratory-prepared essential oil, vegetable oil, and commercial essential oil. Results showed that freshly distilled essential oil had the strongest larvicidal effect, achieving 99.99% mortality at 3% concentration after 8 days, due to active compounds like linalool and linalyl acetate. Vegetable oil showed moderate efficacy, while commercial oil performed less effectively, likely due to degradation.

Keywords: *Lavandula angustifolia, Thaumetopoea pityocampa*, essential oil, vegetable oil, biological control, larval mortality, Aleppo pine forest, bioactive molecules.

INTRODUCTION

I-Introduction:

Forests represent the most vital ecosystems on Earth, covering approximately 31% of the global land area **(FAO, 2022)**, they play a critical role in maintaining ecological balance by regulating climate, storing carbon, preserving biodiversity, and supporting livelihoods **(IPCC, 2019).** As the planet's lungs, forests absorb carbon dioxide and produce oxygen through photosynthesis, making them indispensable in mitigating climate change **(WWF, 2023).** Beyond their environmental functions, forests provide essential resources, including timber, medicinal plants, and food, sustaining over 1.6 billion people **(World Bank, 2021).** They also act as natural water filters and prevent soil erosion, safeguarding agricultural productivity **(IUCN, 2020).**

Algeria's forests cover approximately 4,1 million hectares, representing 1,7% of the country's total area **(FAO, 2020)**. These forests are primarily concentrated in the northern regions, including the Atlas Mountains and coastal areas, where the Mediterranean climate supports diverse ecosystems. However, Algeria faces significant challenges such as deforestation, wildfires, and desertification, with an estimated 17,000 hectares of forest lost annually due to human activities and climate change **(World Bank, 2021).**

Algerian forests host a variety of endemic and adapted species, including, Cork oak (*Quercus suber*), A keystone species in the Tell Atlas region, vital for biodiversity and cork production **(Médail and Quézel, 2022).** Aleppo pine (*Pinus halepensis*) which dominates semi-arid zones and provides resilience against wildfires **(Belhadj-Khedher et *al.,* 2021)** and Atlas cedar (*Cedrus atlantica*) endangered species due to overexploitation and climate stress, and only protected in national parks **(IUCN, 2023).**

Aleppo pine (*Pinus halepensis*) forests currently cover approximately 1,2 million hectares in Algeria, representing about 29% of the country's total forest area **(NFI, 2023).** Aleppo pine is an important species in Mediterranean ecosystems, providing critical ecological services as Soil Conservation. In fact, its deep root system stabilizes soils, reducing erosion by up to 60% in mountainous areas **(Moya et *al.,* 2021)**. In addition, the tree plays a principal role in climate resilience exceptionally in drought-tolerance, surviving with <300mm annual rainfall **(Boudiaf et**

***al.,* 2023).** Aleppo pine is a biodiversity support species for many hosts (27 bird species and 14 mammal species, including the endangered Barbary macaque) **(Belhabib et *al.,* 2022).**

Algeria's Aleppo pine forests face compounding threats from both natural and anthropogenic sources. Wildfires have caused a 38% loss of original forest cover since 2010, with 72% of fires originating from human activities like agricultural burns and negligence **(GDF, 2023).** Simultaneously, pest infestations are intensifying, particularly from the pine processionary moth *(Thaumetopoea pityocampa)* which causes important annual defoliations in the country **(Belhadj -Kheder et *al.*,2018).**

The pine processionary moth (PPM) (*Thaumetopoea pityocampa*) is a significant defoliator native to southern Europe, North Africa, and parts of the Mediterranean. Its larvae form communal silk nests on pine and cedar trees and are known for their "processions" when moving **(Battisti et *al.,* 2015)**. The species larvae develop during winter, making pine forests vulnerable to defoliation when trees are less capable of regeneration **(Robinet et *al.,* 2007)**. Infestations can significantly reduce tree growth and leave trees more susceptible to drought and secondary pests **(Hódar et *al.,* 2003)**. Climate change has facilitated the moth's northward spread in recent decades, bringing ecological and public health challenges to new areas **(Roques et *al.,* 2015)**.

The presence of the PPM larvae causes significant ecological, economic, and health-related damage in affected regions. When larvae feed voraciously on the needles of pine and cedar trees, they cause huge defoliation that reduces photosynthesis, weakens trees, and makes them more vulnerable to drought, pathogens, and secondary insect attacks (**Hódar et *al.,* 2003)**. In young or already stressed trees, repeated defoliation can cause growth loss or even mortality. The damage is not limited to forests urban green spaces, parks, and ornamental trees are also frequently affected, decreasing their aesthetic and ecological value **(Battisti et *al.,* 2015).**

In addition to its negative impact on vegetation, the moth poses serious health risks. In fact, the larvae are covered with microscopic, urticating hairs that can become airborne and cause skin irritation, allergic reactions, or respiratory issues in humans and animals **(Martínez et *al.,* 2005)**. These health hazards often lead to restricted access to infested areas and can require medical intervention.

Several methods are used to control the pine processionary moth (*Thaumetopoea pityocampa*), depending on the scale of infestation, location, and environmental impact. One of the most common techniques is the use of biological control agents, such as natural predators and parasitoids especially parasitoid wasps that target the moth's eggs and larvae **(Georgiev et *al.,* 2020)**. Bacillus thuringiensis (Bt), a naturally occurring bacterium, is also applied as a biopesticide during the early larval stages and has proven effective in forested and urban settings **(Battisti et *al.,* 2015)**. Mechanical methods, including the removal and destruction of silk nests in winter, help reduce populations but are labor-intensive and best suited for smaller areas. Physical barriers**,** such as sticky bands or trap collars placed around tree trunks, intercept larvae as they descend to pupate in the soil **(Roques et *al.,* 2015)**.

Pheromone traps are widely used for monitoring adult moth populations and, in some cases, for mass trapping to reduce mating success **(Robinet et *al.,* 2007)**. In urban areas, chemical insecticides may be used under strict regulation, though environmental concerns have limited their application. An integrated pest management approach combining multiple strategies is often the most effective and sustainable way to control this pest.

The use of chemical insecticides to control pest populations can have several negative effects on the environment, human health, and ecosystem balance. This practice poses serious threats of contaminating soil and water (**USEPA, 2023).** One major concern is the non-selective nature of many chemical pesticides, which can harm beneficial insects such as pollinators, natural predators, and parasitoids, thereby disrupting ecological relationships **(Desneux et *al.,* 2007)**. Overuse or misuse of these chemicals can also lead to resistance development in target pests, reducing the effectiveness of treatments over time and necessitating higher doses or new formulations **(Whalon et *al.*, 2008)**. Additionally, chemical residues may contaminate ecosystems, posing risks to wildlife and potentially entering the food chain **(Aktar et *al.,* 2009)**. As a result, there is growing emphasis on reducing reliance on chemical control in favor of more sustainable and targeted pest management approaches.

Switching from chemical treatments to biological alternatives, such as bioactive molecules extracted from plants, is increasingly recognized as a crucial step toward sustainable pest management. Unlike synthetic pesticides, plant-derived compounds are typically biodegradable**,** pose less risk to non-target organisms, and have minimal environmental persistence **(Regnault-**

Roger et *al.,* 2012; Isman, 2023). This makes them safer for ecosystems, agricultural workers, and consumers. Bioactive plant molecules; such as essential oils, alkaloids, and phenolics can act as insect repellents, growth inhibitors, or toxins specific to pests, offering effective control while reducing the likelihood of resistance development due to their complex chemical structures **(Isman, 2006)**.

Furthermore, the use of these natural products supports integrated pest management strategies, which aim to minimize ecological disruption. Transitioning to such biologically based treatments not only helps preserve biodiversity but also aligns with public demand for eco-friendly and health-conscious solutions in agriculture and forestry. This shift is essential for maintaining long-term pest control efficacy while protecting both human and environmental health (**Isman, 2023)**

These natural compounds possess insecticidal, repellent, antifeedant, or growth-inhibiting properties that can effectively disrupt the life cycle of various pest species. Moreover, the complex chemical structures of many plant extracts make it harder for insects to develop resistance, enhancing their long-term efficacy **(Miresmailli and Isman, 2014)**. These natural compounds can be applied as sprays, soil treatments, or integrated into crop management systems, contributing to sustainable agriculture and forest protection. Research continues to explore the use of bioactive plant molecules as part of integrated pest management strategies, offering a safer, more sustainable approach to pest control.

The effect of bioactive molecules can vary significantly depending on the extraction technique employed in the laboratory, as different methods can influence the yield, purity, and structural integrity of these compounds. For instance, traditional solvent extraction may result in the degradation of thermolabile bioactive molecules, whereas advanced methods like supercritical fluid extraction can preserve their bioactivity by operating under milder conditions **(Herrero et *al.,* 2010)**. Additionally, ultrasound-assisted extraction has been shown to enhance the release of bioactives from plant matrices without significant thermal damage, thus improving their biological efficacy **(Chemat et *al.,* 2017)**. Microwave-assisted extraction, on the other hand, can increase extraction efficiency but may also alter the chemical composition of sensitive molecules due to localized heating effects **(Mandavgane and Pandharipande, 2014)**. Therefore, the choice of extraction method plays a critical role in determining the functional potential of bioactive molecules in both research and therapeutic applications.

In our study, we evaluated the efficacy of different lavender-derived formulations, including laboratory prepared essential oils, vegetable oil, and commercial essential oil against fourth-instar larvae of the pine processionary caterpillar (*Thaumetopoea pityocampa*) using concentrations of 1, 2, and 3 g/l for each formulation under laboratory conditions. We determined regression equations and calculated lethal doses (lD50% and lD90%) for each treatment after 4 and 8 days of exposure to assess their insecticidal potential.

BIBLIOGRAPHIC STUDY

II-BIBLIOGRAPHIC STUDY:

Chapter 01: General Overview of the Pine processionary Caterpillar:

1. Historical context and knowledge about the pine processionary caterpillar:

1.1. First discovery and scientific description:

Thaumetopea pityocampa was first described in 1775 by the Austrian naturalists "Michael Denis" and "Ignaz Schiffermüller", as part of their work on butterflies in the Vienna area. The scientific name reflects the surprising behavior of the species, especially the characteristic larval procession of caterpillars **(Denis and Schiffermüller, 1775) (Fig01).**

Figure 1. The pine processionary caterpillar (***Thaumetopoea pityocampa***) **(Pablo and Franco, 2013)**

1.2 Scientific interest in the nineteenth century:

During the nineteenth century, European scientists began to take an increased interest in forest insect pests, including the caterpillars of *Thaumetopea pityocampa*. Naturalist works from this period document this species as a potential threat to pine forests, although knowledge of its life history is still limited **(Guérin, 1844).**

1.3 Recognition of the PPM as a forest pest in the twentieth century:

At the beginning of the twentieth century, *Thaumetopea pityocampa* was recognized as a major pest of pine forests in southern Europe, particularly in France, Spain and Italy. Between the 1950s and 1970s, monitoring and chemical control campaigns multiplied to deal with its presence. At the same time, research is being carried out on its health effects, due to its stinging hairs **(Demolin, 1969; Demolin and Prévost, 1975).**

2.Taxonomy of species of the genus Thaumetopoea:

The pine processionary caterpillar (*Thaumetopoea pityocampa*) is a moth of the Thaumetopoeidae family (Notodontidae) **(Fagrell, 2008).** The pine processionary caterpillar, known for its single-file movement and whose larvae feed on the needles of various pine species, cause significant weakening of trees and allergies to people exposed to caterpillar bristles **(Robinet, 2006; Hodat et *al.,* 2012).** The pine processionary caterpillar is the main defoliating pest of pine and cedar forests throughout the Mediterranean basin. The insect is present in parts of Europe, North Africa and the Middle East. There are three main species of the genus Thaumetopoea named:

- *Thaumetopoea pinivora*: Predominant in northern regions.
- *Thaumetopoea processionea*: Found in central regions.
- *Thaumetopoea pityocampa*: Localized in Mediterranean areas **(Fagrell, 2008).**

2.1. The pine processionary caterpillar *Thaumetopoea pityocampa*:

Thaumetopoea pityocampa, **(Fig 02)** is a native species of very short-lived moth whose larvae are called pine processionary caterpillars. The insect takes this name from its characteristic behavior: the caterpillars move in single file, forming a procession. These insects are feared not only for the damage they cause to trees, devouring their needles, thus weakening their health, but also for the health risks they represent.

Their stinging hairs, released when threatened, can cause severe allergic reactions in humans and animals, including skin, eye or respiratory irritation**,** present mainly in southern Europe, this species tends to move northwards with global warming **(Martin et *al,* 2016).**

Figure 02. The PPM *Thaumetopoea pityocampa* **(Original picture)**

2.2. Systematic position of the pine processionary caterpillar:

According to Denis and Schiffermüller **(1775),** the pine processionary caterpillar belongs to:

Kingdom: Animalia.

Embranchement : Arthropoda.

Class: Insecta.

Superorder: Endopterygota.

Ordre: Lepidoptera.

Family: Notodontidae.

Subfamily: Thaumetopoeinae.

Genus: *Thaumetopoea*.

Species: *pityocampa*

Binomial name: *Thaumetopoea pityocampa*

Synonymes : Traumatocamp

2.3. Life cycle of the processionary caterpillar:

The life cycle of *Thaumetopoea pityocampa* is generally annual, but can sometimes extend over several years depending on climatic conditions (temperature, humidity, sunshine, etc.). It is divided into two main stages: an aerial phase from egg-laying to the end of larval development and an underground phase from pupation to the adult stage **(Fig 03) (Battisti et *al*. 2017)**

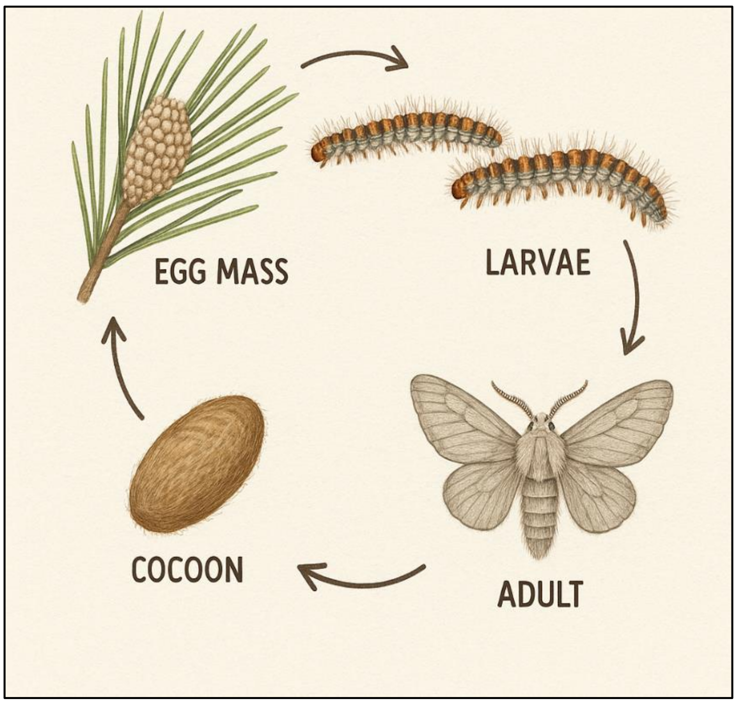

Figure 03. Annual life cycle of the pine processionary caterpillar **(Webography 01)**

2.3.1. Sequential steps of the PPM life cycle:

2.3.1 .1. Aerial phase:

- **Butterflies:**

Butterflies emerge from the ground during the month of June to July, depending on the climate conditopns. Females emit a sex pheromone in an effort to attract males for mating **(Brinquin 2017).** A few hours after copulation, the male dies and the female flies away in search of an ideal egg-laying site. It lays about 200 eggs around two pine needles **(Paiva et *al.*, 2011) (Fig04).**

Figure 04. Male and female pine processionary caterpillar butterflies **(INRA, 2017)**

❖ **Eggs laying:**

One or two days after mating, the males die and the females fly away and lay between 70 and 300 eggs on the pine needles, before dying in turn. About 30 to 45 days after laying, the caterpillars hatch and begin feeding on the pine needles. They remain connected to each other by a silk thread. As they grow, the caterpillars change color and gradually become covered with an increasing number of hairs, up to a million **(Messaadia et *al.,* 2021) (Fig05).**

Figure 05. Sleeve bearing the eggs of the pine processionary caterpillar **(Webography 02)**

❖ **Larval development:**

The larval phase consists of five well-differentiated caterpillar stages. After eggs hatching, the caterpillars of the primary stage (L1) will begin to feed at the expense of the needles near the egg-laying, then the group of caterpillars will move according to the nutritional needs of the larvae, which increases from one stage to the next. Each movement gives rise to the development of a new winter nest made up of the caterpillars of a simple silky network and serving as a refuge during the cold season.

The pine processionary caterpillar (*Thaumetopoea pityocampa*) **(Fig 06)** undergoes five larval stages before pupation. At the L1 stage (3-5 mm) just after hatching, the gregarious larvae begin to consume the pine needles and weave a silky pre-nest. In **L2-L3** (6-20 mm), they develop stinging hairs and migrate to denser winter nests, often exposed to the sun. The L4 stage (20-30 mm) sees the appearance of dark lines on the back and an increase in their aggressiveness. Finally, at the L5 stage (30-40 mm), the caterpillars leave the tree in a characteristic procession to burrow into the soil, where they individually spin a cocoon before pupating **(Battisti et *al.*, 2017).**

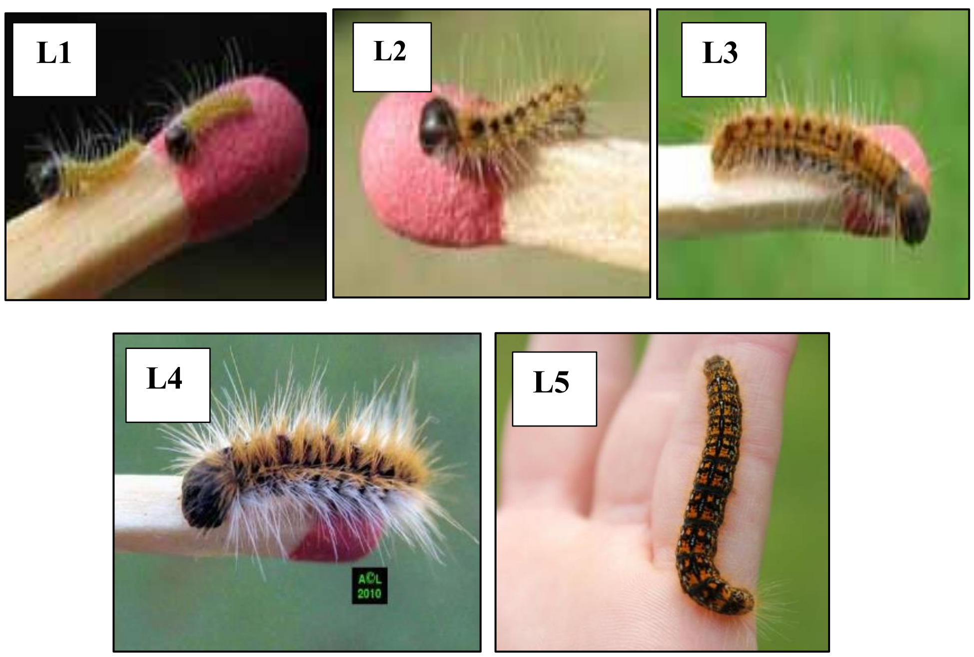

Figure 06. Evolution of the larval stages of the pine processionary caterpillar, from L1 to L5 **(ACL 2010)**

❖ **Winter nests:**

The winter nest **(Fig 07)** is a shelter that protects the caterpillars from the cold and infrared rays **(Martin and Bonnet 2008).** During their larval development phase, from early August to mid-February, all caterpillars from the same clutch remain grouped together in the tree where they hatched.

When the first cold weather arrives, a much larger "winter nest" is built to allow the group to survive. By capturing the sun's rays, the latter functions as a real thermal radiator **(Genoux, 2019).**

Figure 07. Winter nest of the pine processionary caterpillar **(Original picture).**

- **The procession:**

The procession corresponds to the moment when the caterpillars leave their winter nest, and head towards the land in single file (like a "procession" in search of loose and sunny ground to bury themselves and finish their development under the ground **(Genox, 2019).**

This procession **(Fig 08)** is the result of social and survival behavior, where caterpillars follow an olfactory trail left by their conspecifics to move in groups, in order to reduce the risk of predation **(Fitzgerald, 2003).**

Figure 08. Procession of the pine processionary caterpillars
(**Webography 03)**

2.3.1.2. Underground phase:

- **Chrysalis and Diapause:**

After a fortnight of procession, the buried caterpillars enter a state of slowed life called pupation, marked by the diapause of caterpillars surrounded by pupae **(Huchon and Démolin, 1970).** Each caterpillar will weave an individual cocoon in which the transformation into a chrysalis **(Fig09)** and then a butterfly will take place.

Development is then interrupted for a variable period of time, during a diapause, which will last up to a month before the locally favourable date for the adults to be discharged. In case of temperatures that are too low or too high at the time of morphogenesis, diapause can last several years (2 to 4 years) **(Sarl, 2006).**

Figure 09: Male (left) and female (right) chrysalises extracted from their cocoons **(Boutchiche and Boutrigue 2016).**

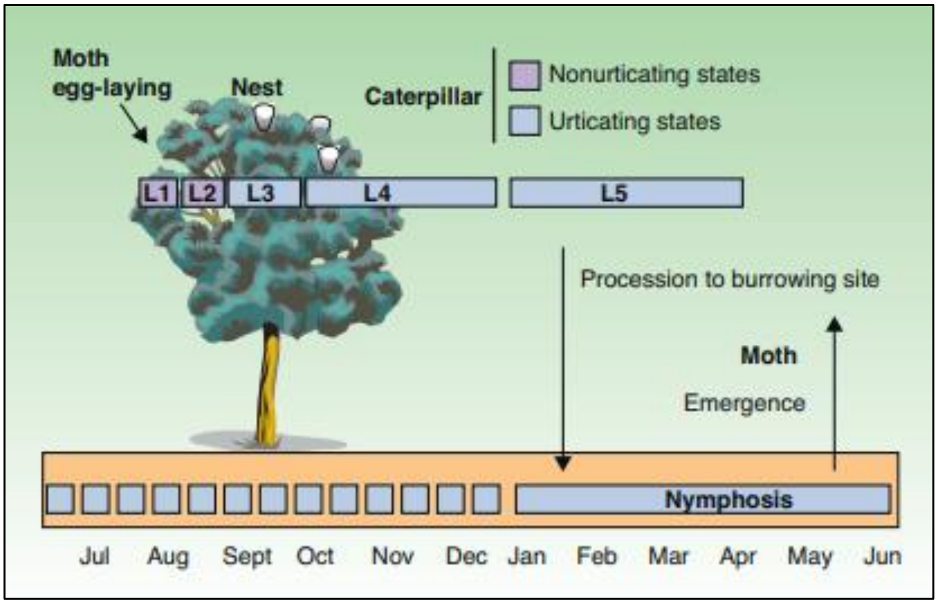

Figure 10. Summary diagram of the life cycle of the pine processionary caterpillar **(Battisti et *al.*, 2011)**

3. Geographical distribution of the pine processionary caterpillar:

3.1 Worldwide:

Globally **(Fig 11),** the pine processionary caterpillar (*Thaumetopea pityocampa*) is mainly found in southern Europe, North Africa and parts of the Middle East. It is widely distributed in Mediterranean countries, with the notable exception of Egypt. It is found in France, Spain, Italy, Greece, Croatia, Albania, Turkey, Lebanon, Switzerland, Yugoslavia (present-day regions such as Serbia, Bosnia, etc.), Algeria, Morocco and Tunisia. Although its origin is Eurafrican, some observations also mention its presence in the United States, where it tends to develop in environments similar to Mediterranean climates. This distribution is constantly changing, in particular due to global warming, which allows the species to move up to northern Europe **(Turpin, 2006).** The subfamilies Thaumetopoeinae include about 100 species, divided into 23 genera. These species are widely distributed across Africa, the Mediterranean region, southern Europe, the Middle East, as well as parts of Australia and New Caledonia **(Basso et *al.,* 2017)**

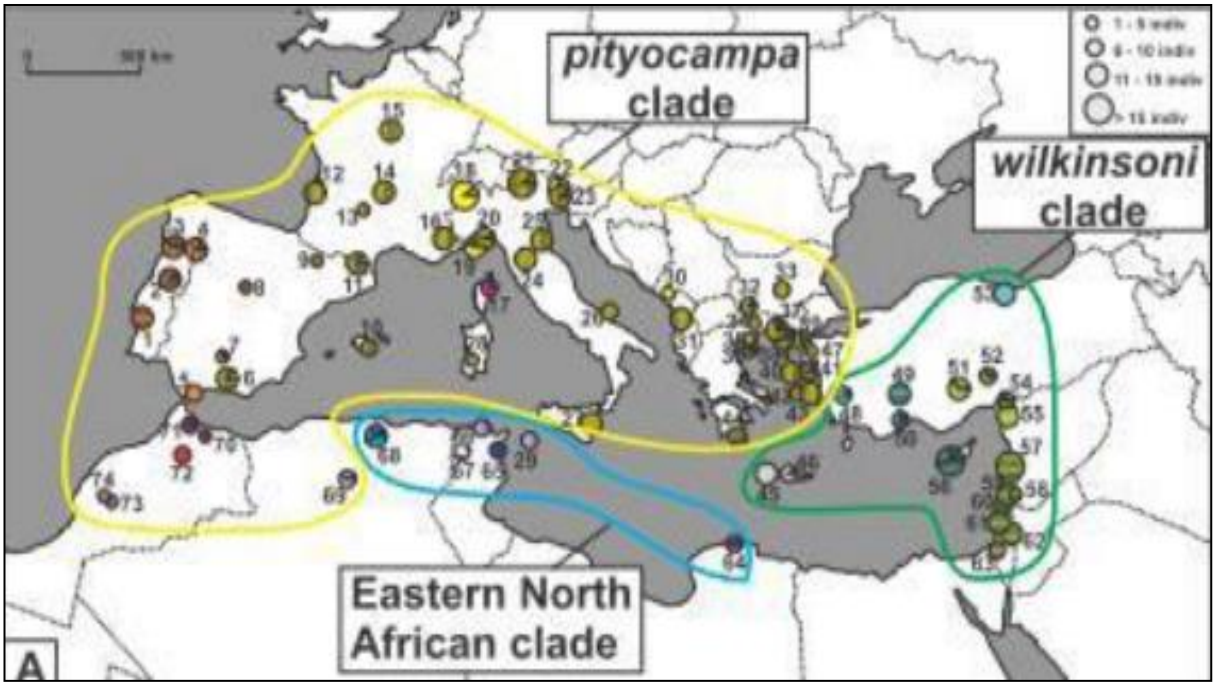

Figure 11: Geographical distribution of pine processionary caterpillar in the world **(Kerdelhué et *al.*,2009).**

3.2 In Algeria:

The processionary caterpillar populations are found in Algeria **(Fig 12)** in the range of its host species, mainly in the reforestation of the green dam project to its southern limit **(Bouchou and Chakali, 2014)** and continue to rise in altitude as reported by Sebti and Chakali **(2014)**. Indeed, on the Chréa massif, the spatial and temporal distribution of nests as well as their abundance are significantly greater at high altitudes (1200-1400 m) compared to lower altitudes (1000-1200 m), which reflects the dynamic potential of the pine processionary caterpillar.

In Algeria, the insect was first reported in 1982 in the forest of Bélezma, in the wilaya of Batna. Since then, its presence has been confirmed in several pine forests of the country **(Kerris, 2002).**

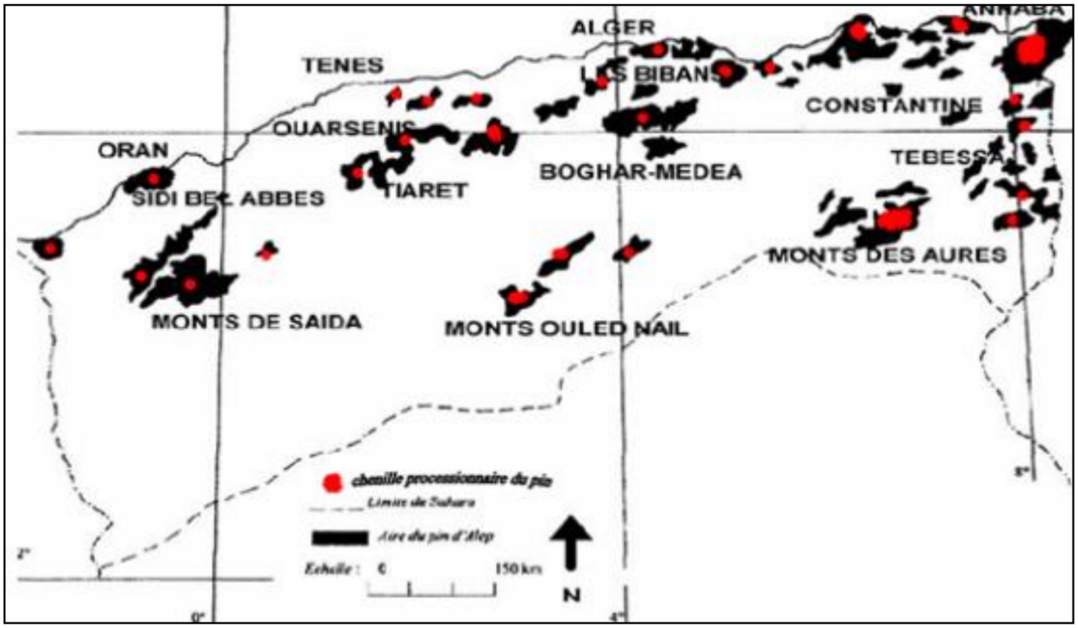

Figure 12: Geographical distribution of the pine processionary caterpillar in Algeria. **(Zamoum, 1986).**

4. Environmental consequences and damage caused by the PPM:

Damage caused by the pine processionary caterpillar has economic, ecological and health repercussions. Defoliation phenomena are becoming more and more visible, especially in large forest areas as well as in young pine and cedar plantations **(Jactel et *al.*, 2012).**

Defoliation **(Fig13)** caused by processionary caterpillars reduces the growth of affected trees by an average of 43%. The data indicate a direct correlation between the intensity of defoliation and the extent of growth loss: the latter reaches about 20% for light defoliation (between 5% and 24%), and around 50% in the case of severe defoliation (greater than 50%). However, beyond this threshold, the impact on growth tends to stabilize **(Fredon, 2024).**

Processionary caterpillars feed on tree needles, weakening their host. A massive infestation can lead to:

- A loss of vigour of the tree.
- Increased susceptibility to diseases and attacks by other pests **(Trouillet, 2024).**

Figure 13. Damage caused by the PPM in Ferdoua forest **(Original picture)**

These dangerous effects can be separated into several levels of consequences:

- **Economic consequences:**

The economic consequences are mainly linked to growth losses in production forests, due to massive defoliation by pine processionary caterpillars, which can have repercussions over several years. Thus, according to Morel **(Morel, 2008),** a year of high proliferation will lead to an economic loss of a full year of wood production, spread over the 3 years following the attack

- **Health consequences:**

According to the ANSES report "Human exposure to caterpillars emitting stinging hairs", there were about 1300 symptomatic cases of exposure to processionary caterpillars recorded by the Poison Control Centres (CAP) between 2012 and 2019.

Caterpillars also cause health problems due to the release of highly allergenic stinging hairs into the air that can cause skin damage (itching that can take up to two weeks to disappear, oedema, etc.), eye damage (glaucoma, cataracts, etc.) or respiratory damage (asthma attack, etc.). **(Brinquin et Martin, 2017).** The following table follows various symptoms depending on the location of the disorder:

Table 1: Various symptoms depending on the location of the disorder **(Genoux, 2025)**

Location of the disorder	Symptoms
Skin involvement	Itching (pruritus) and/or lesions and/or sweating patches mainly in areas close to the ground (head, neck, limbs, abdomen)
Eye damage	Glaucoma, cataract... if the hair is not removed quickly
Oral involvement	Hypersalivation (ptyalism), mouth ulcers, inflammation of the tongue sometimes evolving into oedema (or even partial necrosis in the most severe cases)
Damage to the tracks Respiratory	Inflammation or even obstruction of the airways following edema of the laryngeal region causing breathing difficulties.
System Impairments digestive and/or reproductive	Colic... The ingestion of stinging hairs could also, in some cases, lead to the abortion of pregnant mares. By anchoring themselves in the mucous membrane of the digestive system, the stinging hairs would cause lesions that would allow bacteria to invade the mare's circulatory system and contaminate the fetus.

The processionary caterpillar has thousands of stinging harpoon-shaped hairs that will be released in case of danger. They are the cause of more or less severe allergic reactions that range from simple urtication to anaphylactic shock **(González-Moreno and Sánchez-Sánchez, 2011).**

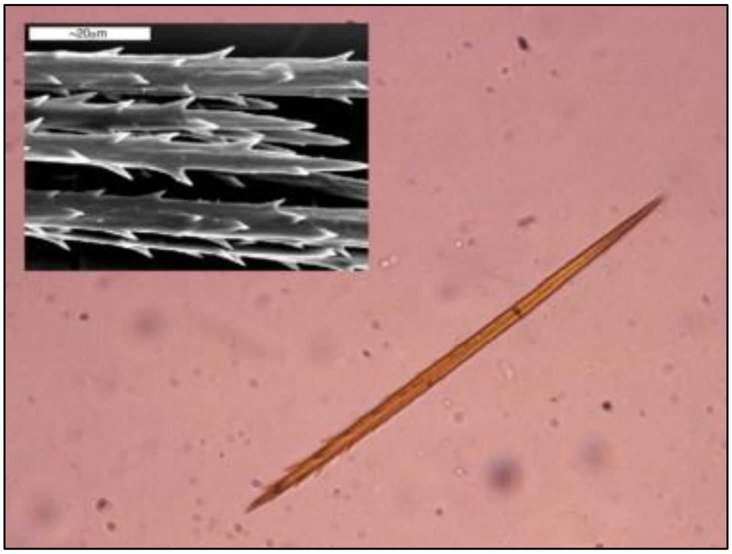

Figure 14. Stinging hair of the processionary caterpillar under electron microscope **(Gx40).**
(Vega *et al*., 2011)

Figure 15. (A) Papulo-erythematous rash and **(B)** intense contact with edema due to exposure to pine processionary caterpillars **(Vega et *al*., 2011)**

Animal health:

Dogs and cats are highly vulnerable to Thaumetopoea pityocampa caterpillars. Contact (sniffing/licking) can cause:

✓ Tongue necrosis (often requiring partial amputation) due to proteolytic enzymes **(Domingo et *al*., 2020)**

✓ Vomiting and severe edema from histamine release **(ABCD, 2023)**

✓ Fatal respiratory distress via IgE-mediated anaphylaxis **(Pérez et *al.*, 2021).** Mortality exceeds 50% without immediate steroids/antihistamines.

Figure 16. Signs of necrosis due to exposure to pine processionary caterpillars **(Vega et *al*., 2011)**

5. Dynamics and rate of evolution of pine processionary caterpillar populations:

In recent decades, the pine processionary caterpillar (*Thaumetopoea pityocampa*) has significantly expanded its range, mainly as a result of climate change. Initially confined to Mediterranean regions, the species progresses northwards and to higher altitudes, taking advantage of milder winters that improve larval survival **(Battisti et *al.,* 2005).** In France, its northern limit has shifted significantly, now reaching the Paris region, an advance of nearly 87 km between 1972 and 2004 **(Robinet et *al.,* 2007).** This dynamic is also visible in the Italian Alps, where the species now colonizes areas up to 230 meters higher than its previous limit **(Battisti et *al.,* 2006).**

Extreme weather events, such as the 2003 heatwave, have also accelerated this expansion, allowing adult females to fly farther and lay eggs in previously inaccessible areas **(Battisti et *al.,* 2005).** In North Africa, particularly in Algeria, the caterpillar has been observed at higher altitudes, colonizing species such as the Atlas cedar, which testifies to increased ecological plasticity **(Imbert, 2014).** In addition to this natural dynamic, there are anthropogenic factors, such as the transport of infested plants or wood, which can facilitate the introduction of the insect into new areas **(Roques et *al.,* 2015).**

6. Fight methods against the pine processionary caterpillar:

The pine processionary caterpillar (*Thaumetopoea pityocampa*) is a major pest of pine forests in the Mediterranean region and other temperate zones of Europe. Due to its deleterious effects on tree health, as well as on public and animal health because of its stinging hairs, several control methods have been developed to limit its impact. These strategies are part of an integrated management approach and include mechanical, biological, biotechnological and chemical approaches, each with specific advantages and limitations **(Battisti et *al.,* 2015; Roques et *al.,* 2016).**

The choice of method depends on many factors such as the stage of development of the insect, the type of habitat, environmental constraints and management objectives **(Jacquet et *al.,* 2012).**

The control means against the pine processionary caterpillar are based on a combination of complementary methods, adapted to ecological and human contexts. Among them, we can mention:

6.1. Mechanical control method:

Mechanical control mainly consists of manually removing the silky nests formed in winter in pines. This method is particularly suitable for urban areas or sensitive sites (schools, parks). The nests are cut and then incinerated to prevent any dissemination of stinging caterpillars **(Démolin, 1969).** Another technique consists of installing trap collars at the base of the trunks, which capture the caterpillars during their descent in procession for pupation **(INRA, 2012).**

Figure 17. **(A, B)** Mechanical Control Techniques **(INRA, 2012)**

6.2. Biological control method:

6.2.1. Use of entomopathogenic bacteria:

Bacillus thuringiensis kurstaki (Btk) **(Fig18)** is a bacterium widely used against caterpillars. It produces specific toxins that perforate the larvae's intestines after ingestion. Efficacy is highest when sprays are made at an early larval stage **(Battisti et *al.,* 2015)**

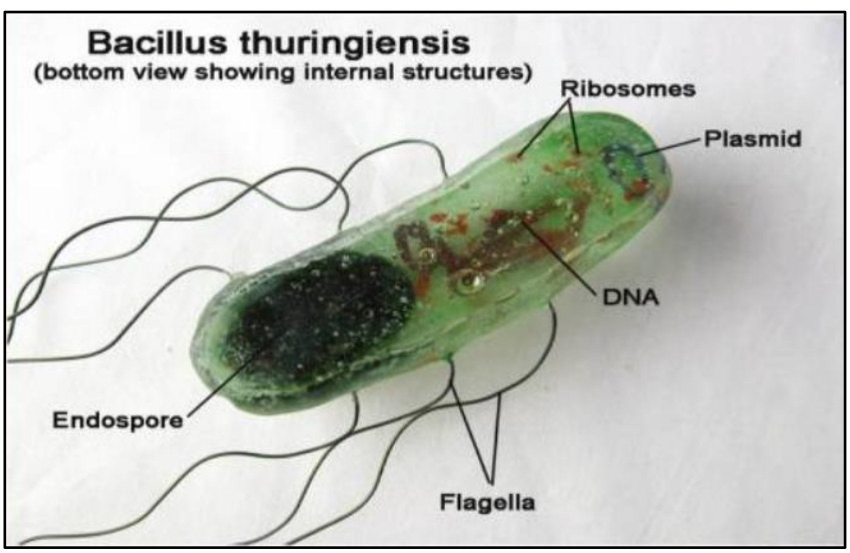

Figure 18. *Bacillus thuringiensis* under the microscope **(Webography 04)**

6.2.2. Favoring natural enemies:

Chickadees (*Parus major*) are effective predators of caterpillars. Locating nest boxes near infested areas can increase predatory pressure **(Barbaro et *al.*, 2008).**
Natural enemies **(Fig19)** are positioned around the life cycle of processionaries according to the stages they impact **(Zamoum et *al.*, 2017).**

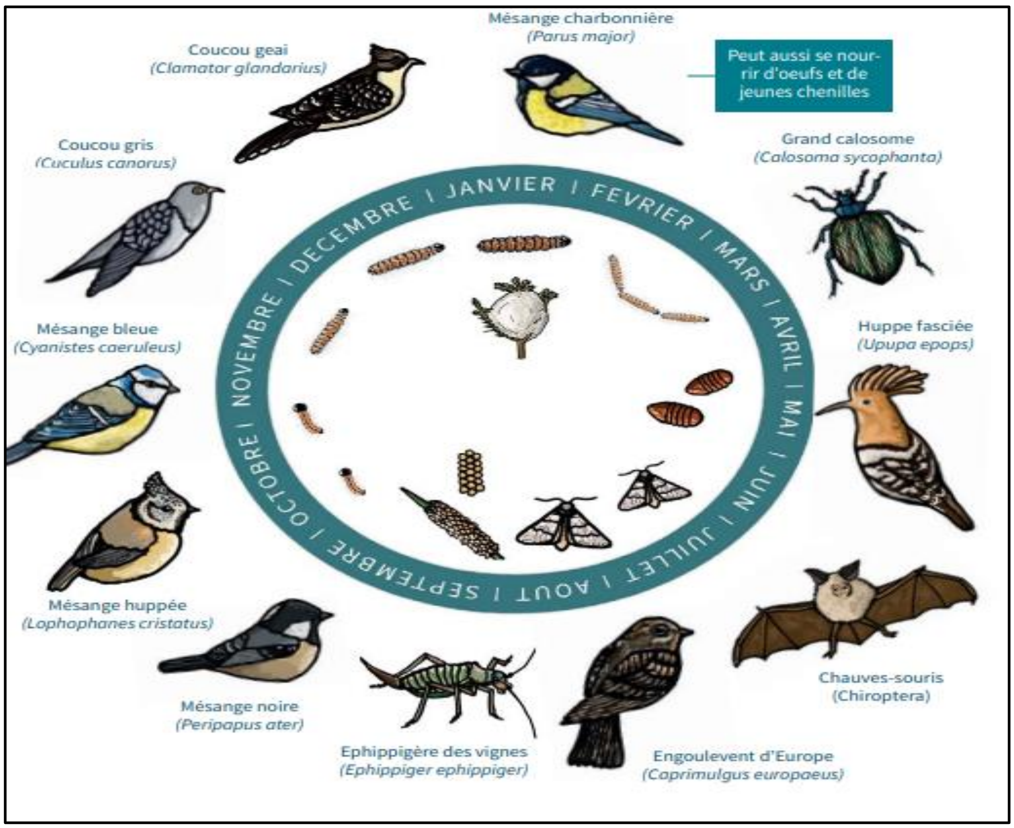

Figure 19. Natural enemies of the pine processionary **(Martin,2014).**

6.3. Biotechnological control method (pheromonal trapping):

The use of sex pheromone traps **(Fig20)** makes it possible to capture adult males to monitor populations or reduce reproduction. This technique is mainly used for monitoring or integrated pest management but is not sufficient on its own to eradicate the species **(Roques et *al.*, 2016).**

Figure 20. Sex Pheromone Trap **(Webography 05).**

6.4. Chemical control:

Chemical treatments **(Fig21)**, although increasingly regulated or even banned in some areas, can still be used in forests with high economic value. They consist of spraying synthetic insecticides on the canopy (pyrethroids or systemic insecticides). However, their use is controversial due to their impact on biodiversity and pollinators **(Jacquet et *al.*, 2012).**

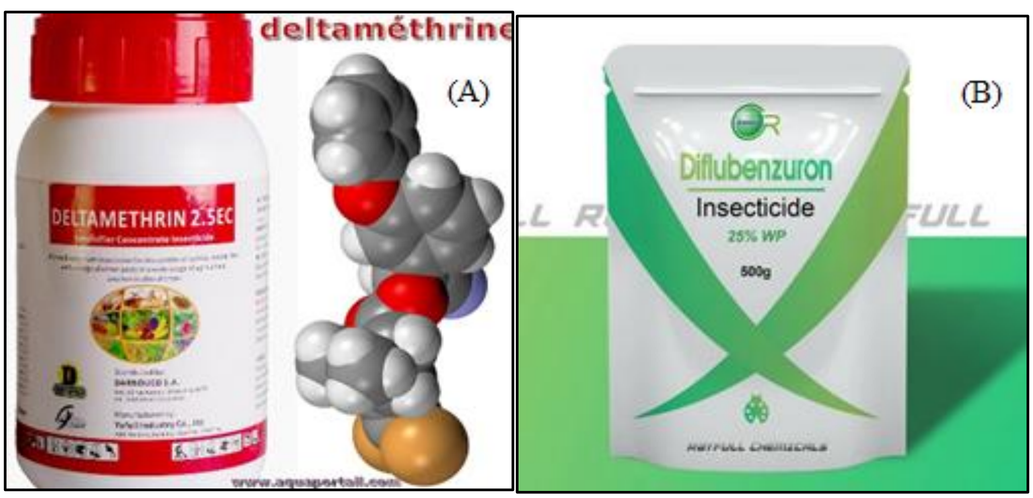

Figure 21. Products for aerial or terrestrial phytosanitary treatment against the PPM **(Webography 06)**

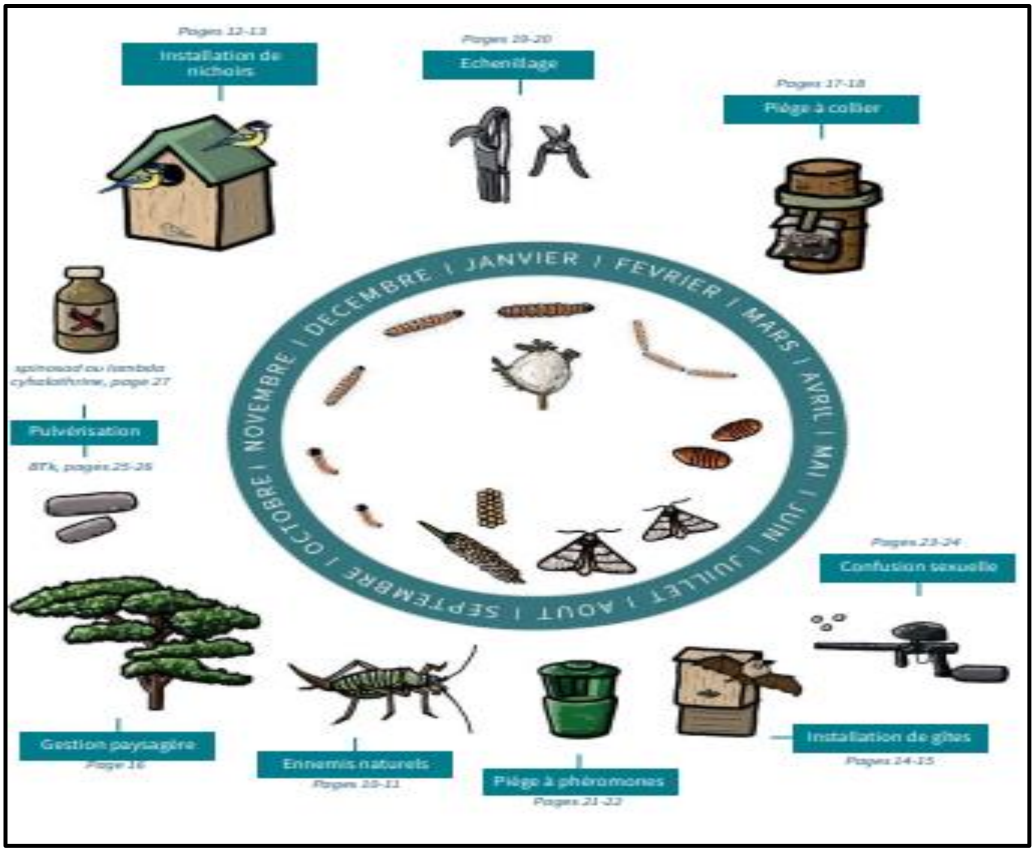

Figure 22. Summary of the different methods of control of the processionary caterpillar according to the Calendar **(Webography 07)**

Chapter 02: Bioactive molecule and extraction methods:

1.Fondamental concepts:

1.1. Definition of extraction:

Extraction as the term indicates is used in the pharmaceutical industry, it involves the separation of the active medicinal molecules of the plant using selective solvents in standard extraction procedures **(Malini et *al.,* 2023).** An extraction is the action of removing a substance, element or information from a larger whole, with a view to isolating or using it **(Han et *al.,* 2011).**

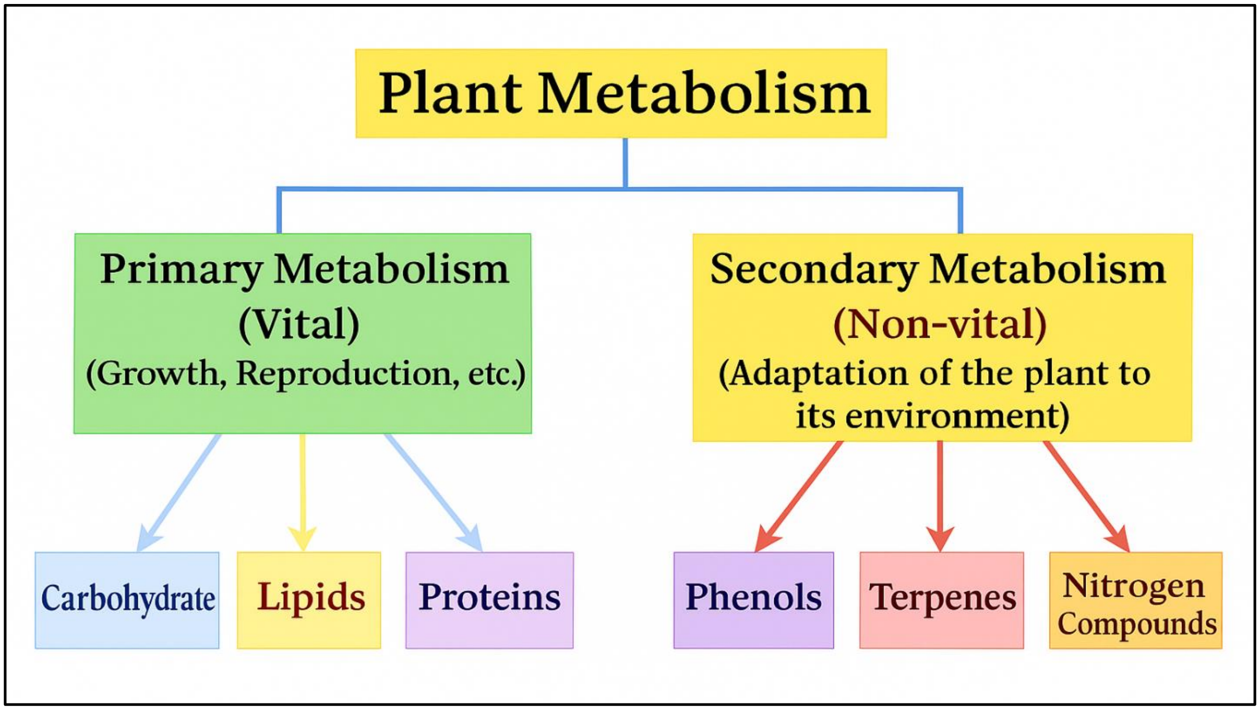

Figure 23. Difference between primary and secondary metabolite of plants **(Labbani, 2022).**

1.2. Definition of bioactive molecules:

Bioactive plant molecules**,** also known as bioactive phytochemicals, are substances naturally occurring in plants that exert biological effects on living organisms, including humans. They are generally not essential for plant growth or basal metabolism (unlike nutrients) but play an important role in plant defense and may have therapeutic, preventive, or functional properties in humans **(Wink, 2015).**

Bioactive components are substances present in small amounts in food, capable of improving health or preventing diseases. Compounds such as bioactive peptides, phytosterols, fibers, fatty acids and vitamins act on various metabolic processes (Neutralization of free radicals, genetic regulation). They are extracted from natural and economic sources (food waste, aquaculture by-products). Before their incorporation into foods, it is crucial to assess their bioavailability, bioaccessibility, and bioactivity **(Sahu and Kumar, 2023).**

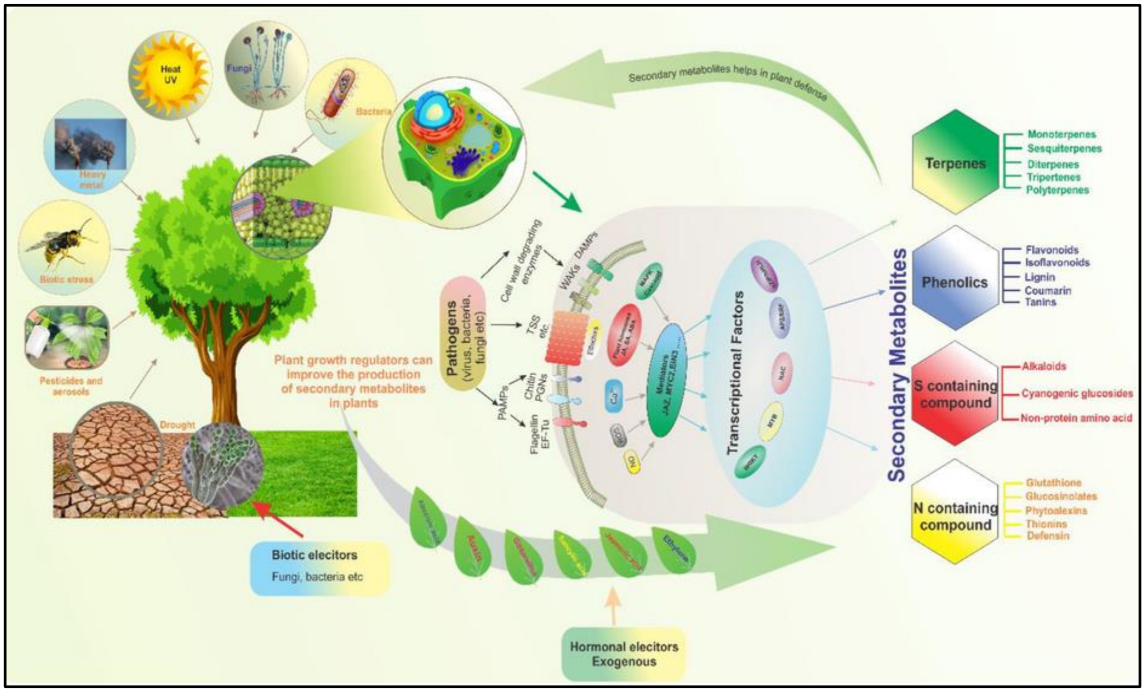

Figure 24. Secondary metabolites of plants **(Webography 08)**

1.2.1. Characteristics of bioactive molecules in plants:

Bioactive molecules of plant origin have a set of characteristics that distinguish them from conventional nutrients and explain their importance in pharmacology, nutrition and phytotherapy. These are mostly secondary metabolites, compounds produced by plants not for their direct growth, but to respond to environmental stresses, defend against herbivores, attract pollinators or inhibit the growth of competing plants **(Wink, 2015).**

These molecules are characterized by a great chemical diversity: they can belong to families such as alkaloids, flavonoids, terpenoids, saponins or tannins, each with various structures and mechanisms of action **(Dewick, 2009).**

They are often lipophilic, allowing them to cross cell membranes and interact with intracellular targets such as enzymes, receptors, or ion channels **(Kumar and Pandey, 2013).** Many of them possess potent pharmacological properties, such as antioxidant, anti-inflammatory, antimicrobial, anticancer, or neuroprotective effects, even at low doses **(Wink, 2015; Kumar and Pandey, 2013).** However, their bioavailability may be limited, depending on factors such as solubility, digestive stability, and interactions with the gut microbiota **(Jaganath and Clifford, 2009).** Some may also be toxic at high doses or poorly tolerated, highlighting the need for rigorous evaluation for therapeutic use **(Dewick, 2009; Wink, 2015) (Fig24).**

1.2.2. Classification of bioactive molecules in plants:

Bioactive molecules from plants are mainly secondary metabolites, produced by plants to ensure their defense, attract pollinators or interact with their environment. They are classified according to their chemical structure and biosynthetic pathway, and are grouped into several main classes:

❖ **Phenolics**

These are molecules containing one or more hydroxylated aromatic rings. They are mainly derived from the shikimate or acetate-malonate pathway. Phenolics have antioxidant, anti-inflammatory, anti-tumor and antimicrobial properties **(Crozier et *al.,* 2006).** They can be:

- **Flavonoids:** Quercetin, catechins, anthocyanins.
- **Phenolic acids:** Caffeic acid, ferulic acid.
- **Tannins:** Condensed tannins (proanthocyanidins), hydrolysable.
- **Coumarins:** Psoralen, osteol.

- **Terpenes and terpenoids**

Terpenes are the largest among the secondary metabolites. They are polymers of isoprene units (C_5H_8), classified according to the number of units: monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), etc. Terpenoids are terpenes modified by functional groups (e.g., hydroxyls). They are often volatile, odorous, and biologically active, playing a role in chemical defense and ecological interactions **(Dewick, 2009; Wink, 2010).** They can be:

- **Monoterpenes:** Limonene, menthol.
- **Sesquiterpenes:** Artemisinin.
- **Diterpenes:** Taxol, ginkgolides.

- **Nitrogenous compounds (alkaloids and glucosinolates)**

These molecules contain nitrogen in their structure, which often makes them very active on the nervous system or enzyme. They have potent effects at low doses, sometimes toxic **(Fahey et *al.*, 2001).** Alkaloids are derived from amino acids that can be:

- **Alkaloids:** Morphine, nicotine, atropine, caffeine.
- **Glucosinolates:** Present in cruciferous vegetables, produce isothiocyanates with anti-cancer effects.

- **Saponins**

They are triterpene or steroid glycosides with surfactant properties (foaming in water). They play a defense role against pathogens and insects, and are often used for their immunomodulatory, cholesterol-lowering and antifungal effects **(Sparg et *al.*, 2004).** Examples**:**

- Diosgenin.
- Glycyrrhizin.
- Quinoa or soy saponins.

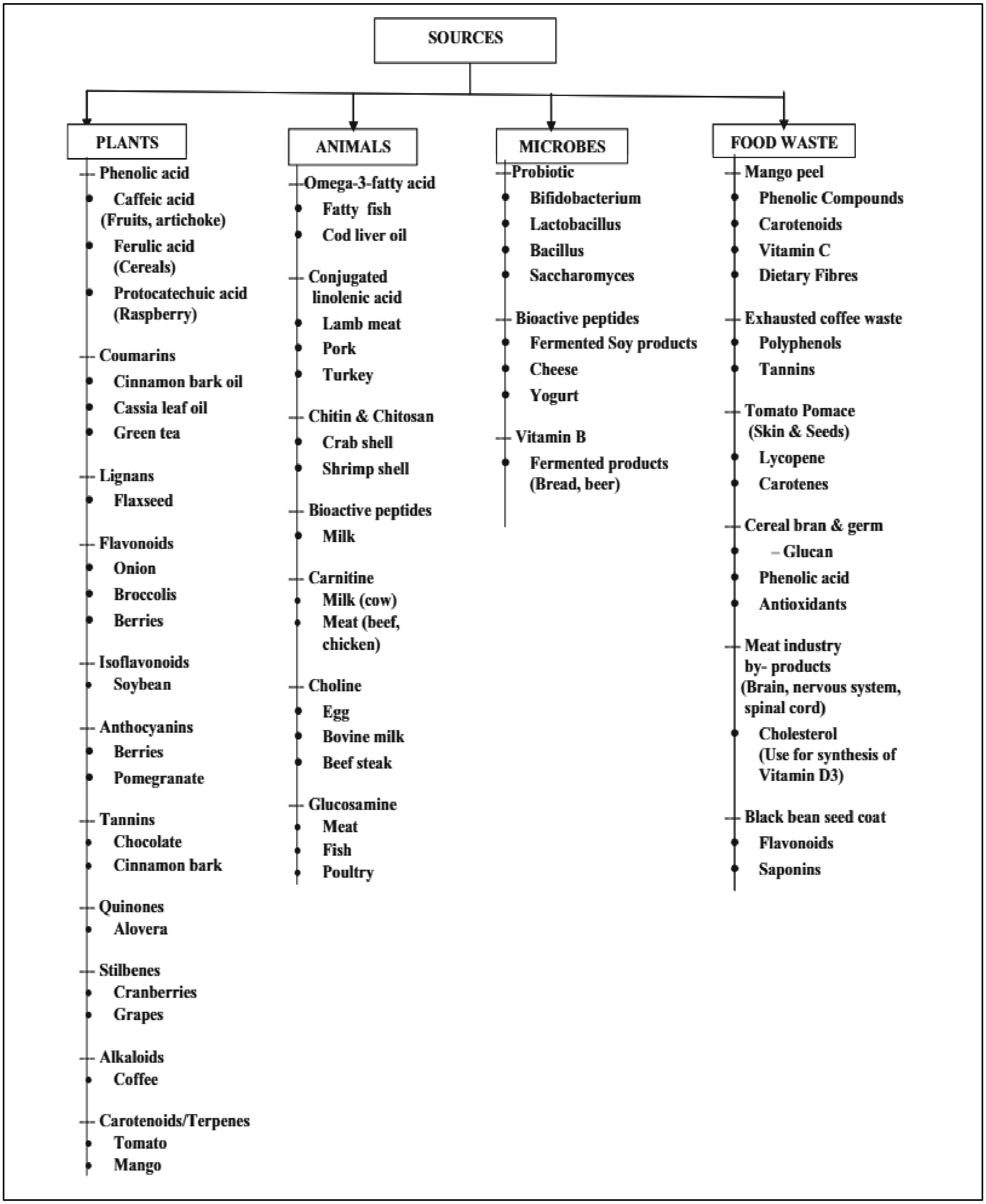

Figure 25. Main sources of bioactive molecules in nature **(Bansal et *al.,* 2023).**

2.Common and unusual applications of bioactive compounds:

Typically, these naturally ubiquitous bioactive compounds are added to staple foods or food products to provide healthy calories and health-promoting nutrients in the consumer's daily diet. These compounds have the potential to synchronize essential metabolic processes in the human body; to name a few: the capture of free radicals, the inhibition or induction of gene expression, and the functioning of receptors and enzymes **(Correia et *al.,* 2012).** Biological activities such as the antimicrobial, anticancer, antimutagenic, antiallergic, antioxidant, and anti-inflammatory properties demonstrated by these compounds reinforce their need to be included in the daily diet **(Ham et *al.,* 2009; Parvathy et *al.,* 2009).** Their addition to foods will help treat and prevent a variety of current and potential lifestyle diseases. After closely observing people's current and future lifestyles, many commercial industries, such as the pharmaceutical, food, and chemical sectors, are getting into the mining of bioactive compounds. In addition to the benefits described above, these compounds can also serve as naturally derived food additives and processing aids for food industries **(Hamzalıoğlu and Gökmen 2016).** Some of these benefits include:

- **Antioxidant:**

Processing and prolonged storage conditions generate free radicals in food systems, which affects food quality. Bioactive compounds acting as antioxidants will solve this problem by eliminating free radicals and singular oxygen molecules, chelating metal ions, breaking the chain reaction (auto-oxidation), reducing the concentration of oxygen in the storage or processing system **(Frankel and Meyer 2000).**

- **Enzyme inhibition or induction**:

These compounds will help during treatment to inhibit or induce a particular type of reaction by controlling the activity of the enzyme **(Correia et *al.,* 2012).**

- **Coloring agent** :

Natural coloring agents such as anthocyanins, carotenoids, turmeric, and squid ink can replace all synthetic dyes that are very stable in food systems, but extremely toxic and non-nutritious at the same time for the masses.

- **Flavoring agents** :

Various natural flavors such as cinnamaldehyde and vanillin are used to flavor sugary foods, chewing gum, and beverages **(Hamzalıoğlu and Gökmen 2016).**

- **Antimicrobial agents** :

Food is the most suitable medium for the growth of millions of harmful microorganisms and foodborne pathogens. Food breakdown agents attack the organoleptic aspects of food by metabolizing various food compounds to produce altered flavors, gases, mucus, etc., and foodborne pathogens make food unsafe to eat. Antimicrobial agents such as phenolic compounds will result in food of intact quality and safe for consumption **(Reineccius 1991; Bhattacharya et *al.,* 2010).**

3. Difference between essential and vegetable oils:

3.1. Definition of essential oils:

Essential oils (EOs) are concentrated, volatile plant extracts obtained through steam distillation, hydrodistillation, or cold pressing. These oils consist of bioactive compounds (Terpenes, phenols, esters), that confer antimicrobial, anti-inflammatory, and aromatic properties. Their applications span pharmaceuticals, aromatherapy, food preservation, and sustainable agriculture, making them a critical focus of modern phytochemical research **(Tariq et *al.,* 2019; Sharifi-Rad et *al.,* 2021).**

3.1.1. Characteristics of essential oils:

- **Physicochemical properties:**

Essential oils are volatile compounds that evaporate at room temperature due to their low molecular weight and hydrocarbon nature **(Tariq et *al.,* 2019).**

They exhibit lower density than water, causing them to float on surfaces, and demonstrate selective solubility - being miscible with oils and alcohol but immiscible with water **(Sharifi-Rad et *al.,* 2021).** Their color and odor profiles vary significantly depending on the plant source.

Chemical composition:

The therapeutic properties derive from complex chemical constituents. Terpenes like limonene contribute to fragrance and biological activity **(Salehi et *al.,* 2019).** Phenolic compounds such as eugenol provide antiseptic properties, while aldehydes offer anti-inflammatory effects **(Isman, 2020).**

Therapeutic properties:

Research has validated several therapeutic applications. Antimicrobial efficacy has been well-documented **(Nazzaro et *al.,* 2022).** Anti-inflammatory properties help reduce swelling, while some oils demonstrate significant antioxidant capacity **(Prakash et *al.,* 2023).**

Toxicology of essential oils:

Essential oils can cause acute toxicity when ingested, inhaled, or applied topically in high concentrations. Symptoms may include: Gastrointestinal distress (nausea, vomiting) Neurological effects (dizziness, seizures) Respiratory depression (camphor, eucalyptus oil) **(Tisserand and Young, 2014).**

Toxicity of essential oils against insects:

Essential oils (EOs) are volatile plant extracts with insecticidal, repellent, and antifeedant properties. They are considered eco-friendly alternatives to synthetic pesticides due to their low mammalian toxicity and biodegradability **(Isman, 2020).** Lavender essential oil is widely studied for its insecticidal, repellent, and larvicidal properties. Its primary bioactive compounds, linalool and linalyl acetate, contribute to its toxicity against various insect pests **(Benelli et *al.,* 2018).**

- **Mechanisms of Action:**

Neurotoxicity: Essential oils (EOs) exert their insecticidal effects primarily by targeting the insect nervous system. They inhibit acetylcholinesterase **(AChE),** the enzyme responsible for breaking down acetylcholine in synaptic clefts, leading to the accumulation of this neurotransmitter and continuous nerve stimulation. Additionally, certain EO components act as modulators or antagonists of gamma-aminobutyric acid receptors **(GABA)**, which are inhibitory neurotransmitter receptors in insects. Disruption of GABA signaling leads to neuronal hyperexcitation, convulsions, paralysis, and eventually death **(Tong and Coats, 2019).**

Respiratory inhibition: In essential oils (EOs), monoterpenes can impair insect respiration by physically blocking the spiracles the openings through which insects breathe—or by interfering with oxygen uptake and gas exchange at the tracheal level. This disruption can lead to hypoxia, reduced metabolic activity, and eventually death **(Pavela and Benelli, 2016).**

Growth Regulation: Certain essential oils (EOs) function as insect growth regulators (IGRs) by interfering with the hormonal processes that control molting and development. These compounds can disrupt the synthesis or action of ecdysteroids and juvenile hormones, leading to abnormal or incomplete molting, developmental delays, or failure to reach adulthood, ultimately reducing insect populations **(Abdelgaleil et *al.,* 2019).**

3.1.2. Practical Applications of essential oils:

Essential oils (EOs) are increasingly used in practical applications as natural insecticides and repellents due to their effectiveness and environmental safety. They control a wide range of pests in agriculture by disrupting insect nervous systems, respiration, and growth, making them valuable in integrated pest management strategies. EOs are also applied to protect stored products from insect infestation, reducing post-harvest losses without harmful chemical residues. Additionally, they serve in veterinary and public health settings to repel or control ectoparasites, improving animal welfare and reducing disease risks. Their antimicrobial properties further support post-harvest treatments to extend the shelf life of fruits and vegetables **(Pavela and Benelli, 2016; Abdelgaleil et *al.,* 2019; Tong and Coats, 2019) (Tab02).**

3.2.Definition of Vegetable oils:

Vegetable oils **(Tab02)** are lipid-based substances extracted from the seeds, fruits, or other parts of plants. They primarily consist of triglycerides (esters of glycerol and fatty acids) and are typically liquid at room temperature. These oils are obtained through mechanical pressing or chemical extraction (Solvent extraction) and are widely used in cooking, food processing, cosmetics, and biofuel production **(Gunstone, 2020).**

3.2.1. Characteristics of vegetable oils:

- **Chemical composition:**

Vegetable oils primarily consist of triacylglycerols (95-98%), with minor components including phospholipids, tocopherols (vitamin E), sterols, and polyphenols **(Gunstone, 2020).** The fatty acid profile varies by source, containing different proportions of saturated, monounsaturated, and polyunsaturated fatty acids **(Dubois et *al.,* 2022).**

- **Physical characteristic:**

The physical properties of oils can vary significantly depending on their composition. Viscosity typically ranges between 30-70 Pa·s at 20°C **(Kochhar, 2021)**. The smoke point also shows considerable variation, with refined oils having a higher smoke point compared to unrefined oils **(Kochhar, 2021)**. Additionally, oxidative stability is influenced by the fatty acid composition of the oil, with different oils exhibiting varying levels of resistance to oxidation **(Krishna et *al.,* 2022)**.

- **Nutritional value:**

Oils provide essential fatty acids that must be obtained through the diet, as the body cannot produce them. They also offer varying amounts of vitamin E, an antioxidant that supports immune function and protects cells. Additionally, oils contain phytosterols, which can help reduce cholesterol absorption and contribute to heart health **(Trautwein et *al.,* 2020)**.

- **Toxicology of vegetable oils Against Insects:**

Vegetable oils have gained attention as eco-friendly insecticides due to their low mammalian toxicity and biodegradability. Their insecticidal properties stem from physical and biochemical mechanisms, including suffocation, repellency, and disruption of insect metabolism. Below is a synthesis of recent findings on their efficacy and modes of action.

- **Mechanisms of Insect Toxicity**

Vegetable oils exhibit insecticidal toxicity through multiple mechanisms. Physically, they coat insects and block their spiracles (The respiratory openings), leading to suffocation and hypoxia **(Nerio et *al.,* 2020)**. Thin films of these oils also disrupt gas exchange in insect eggs, increasing egg mortality **(Pavela et *al.,* 2021)**. On a biochemical level, compounds such as linoleic acid found in vegetable oils inhibit acetylcholinesterase (AChE), causing neurotoxic effects that impair nerve function **(Isman, 2020)**. Additionally, vegetable oils interfere with molting hormones in larvae, disrupting growth and development **(Campolo et *al.,* 2019)**.

Table 02. Comparison between essential oil and vegetable oil

Component	Essential Oil	Vegetable Oil
Source	Extracted from plants, flowers, leaves, or bark	Extracted from seeds, nuts or fruits of plants
Main Constituents	Volatile compounds (terpenes, phenols)	High fat content, primarily triglycerides
Fat Content	0% to very low	High fat content
Aroma	Strong and aromatic	Mild, usually flavorless or neutral
Vitamins & Nutrients	Minimal or none; may contain trace nutrients (eucalyptol)	Rich in Vitamins (Vitamin E, Vitamin K)
Oxidative Stability	Generally unstable; prone to rapid oxidation	More stable, especially if refined (Vitamin E)
Processing	Typically steam distilled	Usually cold-pressed

Essential oils are extracted from plants, flowers, or leaves and mainly consist of volatile compounds like terpenes and phenols, giving them a strong aroma **(Baser and Buchbauer, 2015).** They have little to no fat content and are used for fragrance or medicinal purposes, but not for cooking **(Sadeghi et *al.,* 2020)**. In contrast, vegetable oils come from seeds, nuts, or fruits and are rich in fatty acids, such as oleic acid and linoleic acid, making them suitable for cooking (Kochhar, 2021). They also contain vitamins like Vitamin E and K, and have higher oxidative stability

(Trautwein et *al.,* 2020). Vegetable oils are processed through cold-pressing or refining and are commonly used in cooking, while essential oils are usually steam-distilled and not used in cooking **(Baser and Buchbauer, 2015; Kochhar, 2021) (Tab02).**

4.Methods of extracting bioactive molecules from plants:

4.1. Extraction by maceration:

Maceration **(Fig 26)**, is a simple method of solid-liquid extraction, where dry or fresh herbs are immersed in a liquid for a period of time. This technique, particularly suitable for plants rich in essential oils, allows you to take full advantage of the vitamins and minerals they contain. The vegetable powder is left in prolonged contact with a solvent, such as dichloromethane, methanol, or an ethanol/water mixture (50%, 50%), thus preserving the integrity of the molecules at room temperature. In the extractive protocol, the plant material is macerated in each solvent for 24 hours, filtered, and the process is repeated twice for each solvent. The extracts obtained are then combined to form the crude extract **(Benhamza Louiza, 2008).**

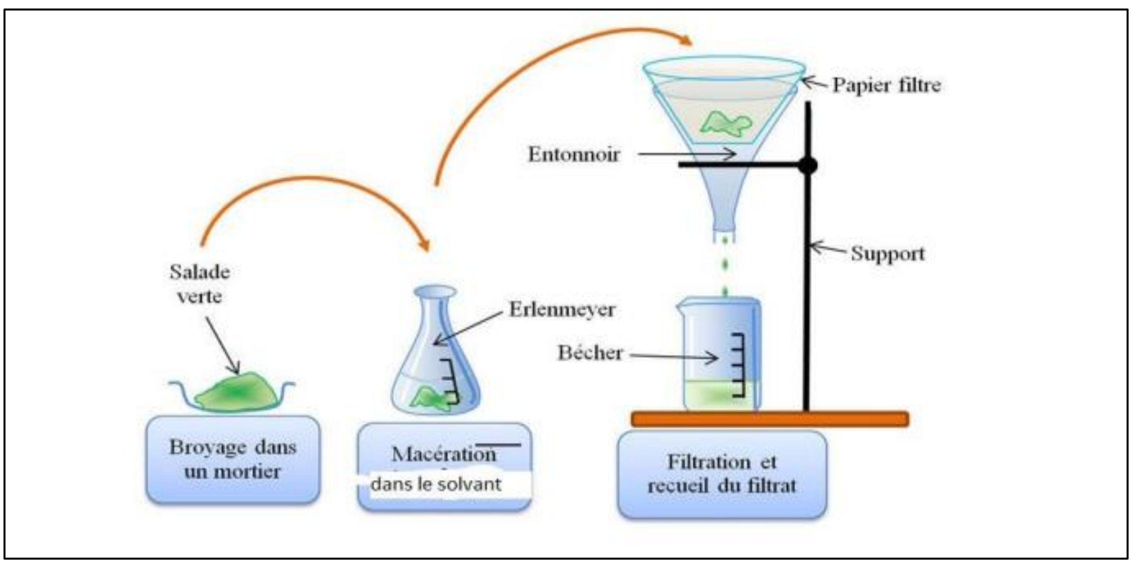

Figure 26. Cold maceration assembly **(Webography 09)**

4.2. Extractions by soxhlet:

Soxhlet extraction **(Fig 27)**, is a method of hot organic solvent extraction, where plants are immersed in a heated volatile organic solvent. This technique is used to obtain products that cannot be extracted by other methods or to increase yields (vegetable oils). In this apparatus, the heated solvent evaporates in the flask, passes through the material to be extracted, condenses and returns to the body of the extractor. Extraction continues until the solid material is exhausted **(Marrouf and Tremblin, 2007).**

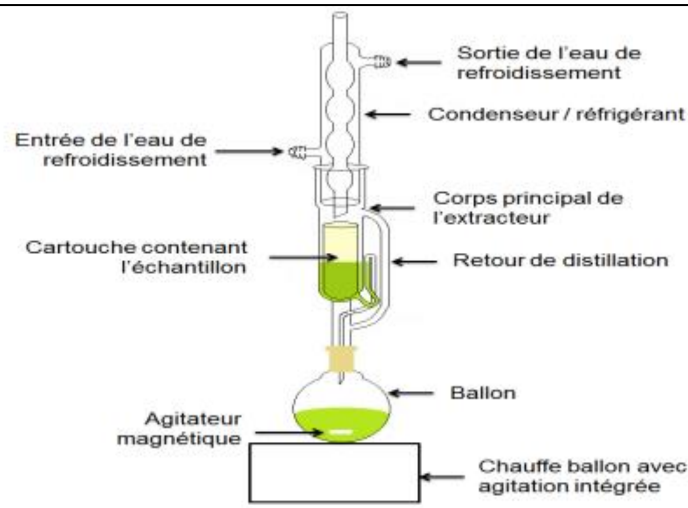

Figure 27. Extraction assembly by Soxhlet **(Bouarab, 2021).**

4.3. Hydrodistillation :

This is the standard method for extracting essential oil from plants. In this technique, plant matter is immersed in water and brought to a boil, with the water acting as a barrier against overheating. The volatile compounds are then carried away by water vapour, and the essential oil is recovered by condensation and liquid-liquid extraction or simple decantation. Although hydrodistillation is economical, it can degrade some aromatic compounds. Steam entrainment extraction is preferred for a higher yield, improving that of hydrodistillation by 33%. Hydrodistillation is carried out using a Clevenger-type device **(Fig 28)** , The essential oil differs from the hydrosol (aromatic water) by its difference in density and colour. It is separated from the latter by decantation **(Collin, 2003).**

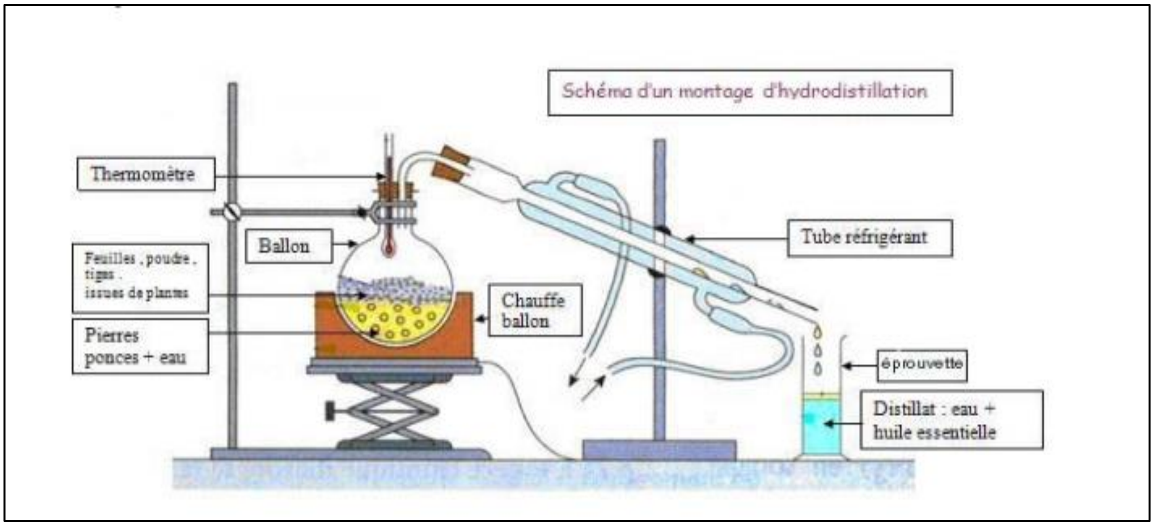

Figure 28. Hydrodistillation extraction setup **(Bouarab, 2021).**

4.4.Steam Drive:

This is the most common way to extract volatile molecules. The plant material is not in contact with water, but the water vapour produced by a boiler is injected and passes through the plant material from bottom to top, bursting the cells and carrying away the volatile molecules. The steam **(Fig 29)** loaded with essential oil is condensed by cooling in a condenser before being recovered in an essencier. The hydrosol and the essential oil, of different densities, separate naturally in the essencier **(Bouarab, 2021).**

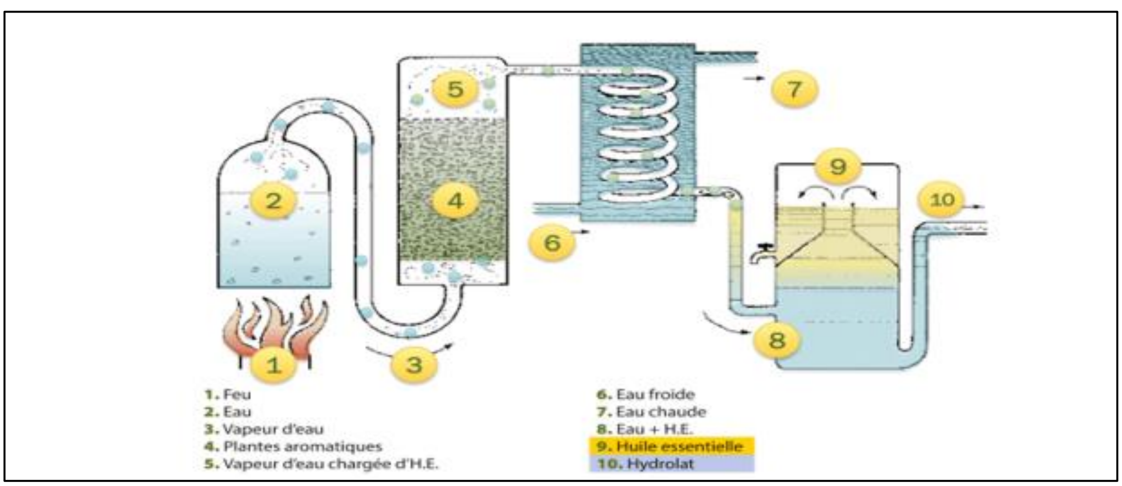

Figure 29. Steam distillation extraction setup **(Webography 10)**

4.5.Microwave extraction:

Microwaves are electromagnetic waves that are located in the electromagnetic spectrum in the frequency range 300 MHz to 300 GHz between radio and infrared waves **(Chemat, 2011).** However, only a few frequencies are authorized for industrial, scientific, medicinal uses in order to avoid interference with radiocommunications, and in general 0.195 to 2.45 GHz are the most widely used in the world **(Chemat et *al.*, 2012).** In microwave-assisted extraction, plant material is extracted in a microwave reactor with or without organic solvents or in water, under different conditions depending on the experimental protocol **(Chenni, 2015).** Microwave-assisted extraction (MAE) corresponds to a submission of the solvent/matrix mixture to an electromagnetic field of 2.45 GHz, under the influence of alternating current, molecules with a dipole moment will change orientation 4.9,109 times per second. The transfer of energy to the mixture is done through the rotation of the dipoles and ion conduction. In the case of a hydro-alcoholic mixture combining two polar solvents, the mixture will heat up very quickly. The MAE thus increases the penetration of the solvent into the matrix. In the case of fresh plant material, the water intrinsic to the plant has a dipole moment (1.85 D) and can also play a role as a solvent **(Gourguillon et *al.*, 2016) (Fig 30).**

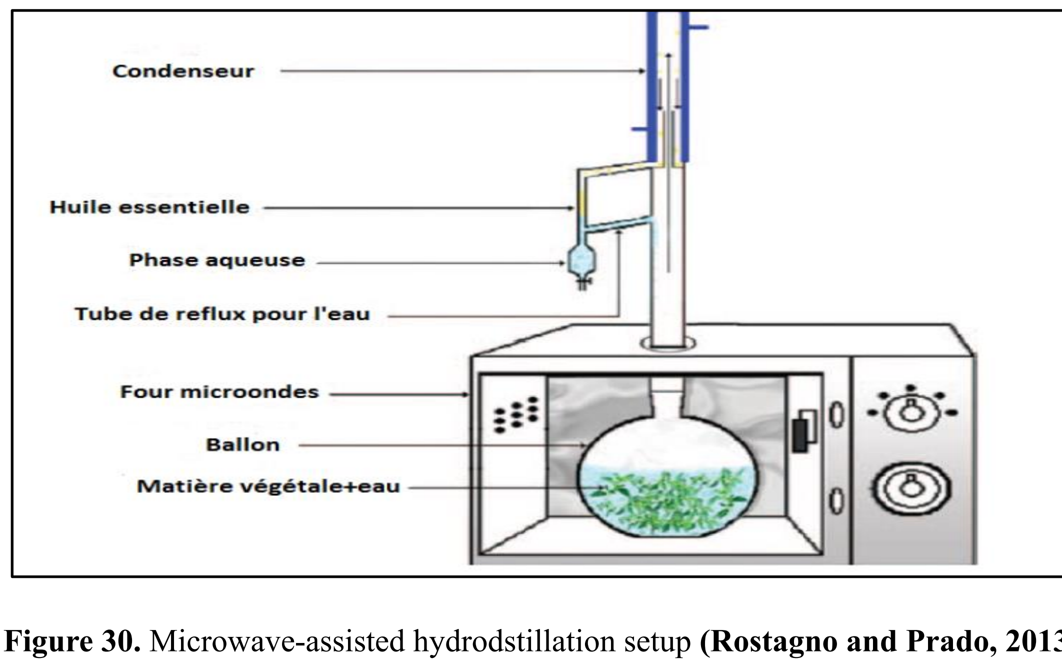

Figure 30. Microwave-assisted hydrodstillation setup **(Rostagno and Prado, 2013)**

MATERIAL AND METHODS

III-Materials and Methods:

1. Sampling Site:

1.1. Overview of Mila Region:

1.1.1. Geographic and Morphological Setting:

Mila **(Fig31)** is a wilaya (province) located in northeastern Algeria, Coordinates: 36°27′N 6°16′E, approximately 350 km east of Algiers with its area is estimated at 3,407 km². It is bordered by the wilayas of Jijel, Constantine, Oum El Bouaghi, Batna, and Sétif. The region features diverse terrain, including mountains (such as Mount Mégris), fertile plains, and rivers like the Rhumel wadi **(ONS, 2023).** The Wilaya of Mila is renowned for the purity of its soil and its excellent climate. Since the earliest centuries of its existence, the region has witnessed significant progress in various fields most notably in the agricultural sector, thanks to the abundant availability of water and the vast expanses of cultivable land. It is a region that is essentially agricultural in nature, distinguished by its remarkable biological and ecological diversity, as well as its lush greenery that endures throughout the year. The most important component of its forested areas is made up of natural forests, which include species such as cork oak**,** beech**,** and wild mares, alongside artificial forests comprising mountain pine woodlands and cypress groves or jungles**.** These forests contribute greatly to the ecological richness and environmental balance of the region **(FGD Mila, 2016).**

Figure 31. Geographical Location of Mila Province **(Webography 11)**

1.1.2. Climatic Setting:

As per the meteorological records of Ain Tin station, the provided data presents10-year climatological overview (2014–2024) of Mila region. Key findings reveal distinct seasonal patterns:

- **Temperature Trends:**

The area exhibits a typical Mediterranean climate with distinct seasonal variations. Average monthly temperatures (Moy, Temp, Ordi) range from 8.9°C in January to 28.6°C in July, peaking in summer and dropping in winter. The Moy, Temp, Max (average maximum temperatures) show July and August as the hottest months (34.8°C and 34.5°C, respectively), while Moy, Temp, Mini (average minimums) highlight January and February as the coldest (5.0°C and 4.7°C). Notably, ground temperatures (Temp, Mini, Sol and Temp, Max, Sol) reflect more extreme variations, with soil temperatures reaching 51.9°C in July but dropping to 3.0°C in January, indicating significant surface heating and cooling **(Fig32).**

Table 03. Climatological Data Summary for Mila Region **(from 2014 to 2024)**: Monthly Averages of Temperature, Precipitation, Wind, Humidity, and Solar Radiation **(Ain Tin Meteorological, 2025)**

Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec	Parametres
8.9	9.0	11.3	14.5	17.7	23.4	28.6	26.3	22.3	18.7	13.2	9.7	**Temp(°C)**
13.5	13.8	16.2	20.3	24.1	30.7	34.8	34.5	29.3	23.9	17.4	13.6	**Max T Air (°C)**
5.0	4.7	6.5	8.8	11.7	16.3	20.6	20.2	15.8	13.6	9.4	5.8	**Min T Air (°C)**
83	99	96	55	51	25	7	24	32	58	70	73	**Precip (mm)**
21	22	18	18	17	16	17	21	22	20	23	20	**Wind (m/s)**
74	76	72	70	63	54	43	49	64	66	73	76	**Humidity (%)**
155.4	175.0	236.4	252.2	284.9	313.2	357.3	330.1	238.5	227.6	164.5	145.2	**Sunshine (dm/mol)**
3.0	0.8	4.2	3.4	7.0	8.5	13.4	14.3	23.0	14.8	5.1	1.0	**MinT Groud (°C)**
22.0	24.3	27.9	37.4	41.1	48.6	51.9	50.4	41.6	36.6	26.5	21.8	**Max T Ground (°C)**

- **Precipitation and Humidity:**

Rainfall (Precipitation) follows a Mediterranean pattern, with the wettest months being February (99 mm) and November (70 mm), and the driest period occurring in July (7 mm). Humidity is highest in winter (76% in February and December) and lowest in summer (43% in July), correlating inversely with temperature trends **(Fig32).**

- **Wind and Insolation:**

Wind speeds (Vent) remain relatively stable year-round, averaging 17–23 m/s, with slight increases in winter. Insolation (Insolation) peaks in summer (357.3 hours in July), reflecting long daylight hours, and drops sharply in winter (145.2 hours in December) **(Fig32).**

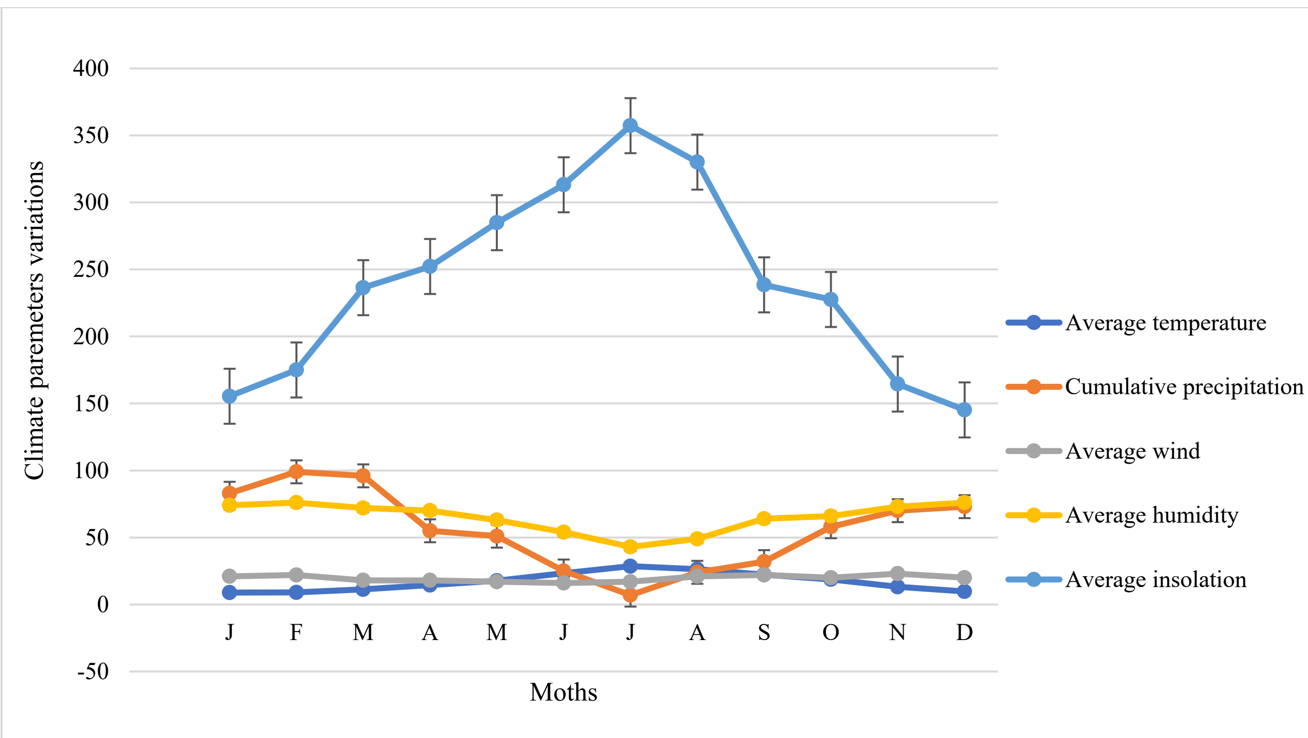

Figure 32. Average values of meteorological parameters for Mila region between **2004** and **2024.**

1.2. Sampling Municipality:

1.2.1. The Municipality of Sidi Merouane:

Sidi Merouane **(Fig33)** is one of the thirty-two municipalities of Mila Province in Algeria, located 12 km northeast of the provincial capital. The municipality of Sidi Merouane sits in an area of rugged terrain with a steep south-north slope. It is bordered to the north in a semicircular arc by the Beni Haroun Dam, which gives it a peninsula-like appearance. Furthermore, the municipality of Sidi Merouane is equipped with a wastewater treatment plant that plays a crucial role in protecting the Beni Haroun Dam and preserving the environment and ecology **(FGD Mila, 2016).**

Figure 33. Geographical Position of Sidi Merouane Municipality **(Webography 11)**

1.2.2. Presentation of the Sampling Region:

1.2.3. "Fardoua" Forest:

The "Fardoua Forest" in the municipality of Sidi Merouane is located in Mila Province. It is a young reforestation area of Aleppo pine (*Pinus halepensis*) and cypress trees, dating back to 2006.The forest covers an area of 150 hectares, with the average height of its trees ranging between 3 to 6 meters **(FGD, Grarem 2025) (Fig34).**

Figure 34. Ferdoua forest **(Original picture)**

The "Fardoua" pine forest is located on the upper slope of the Ben Haroun dam and in close proximity to the municipality of Sidi Merouane. The Fardoua Forest is bounded:

- To the north by the Ben Haroun Dam,
- To the east by the municipality of Sidi Merouane,
- To the west and south by the city of Mila.

It has an average altitude ranging between 320 and 340 meters **(Fig35).**

Figure 35. Geographical Location of ‘Frdoua’ Forest **(Webography 12).**

1.2.2. Climatic characteristics for Sidi Merouane municipality:

- **Climate**

Climate plays a fundamental role in the distribution and life of living beings in their environment. Climatic factors include a set of energy-related factors consisting of light and temperature, the hydrological factor represented by precipitation and air humidity, and mechanical factors including wind and snow cover **(FGD Mila, 2016).**

- **Precipitation**

The study area is characterized by a semi-humid climate with a semi-cold and rainy winter and a hot dry summer. Annual precipitation ranges from 600 mm to 800 mm, with irregular rainfall occurring from early autumn to late spring, along with thunderstorms and heavy rains that affect the region and consequently impact ecological diversity, leading to erosion **(FGD Mila, 2016).**

- **Temperature**

The region is characterized by high temperatures with dry summers. The average annual temperature in the region is approximately 16°C, as shown in temperature degree tables that display the maximum, minimum, and average annual temperatures **(FGD Mila, 2016).**

- **Wind**

The study area is influenced by prevailing northern winds, including northwesterly winds, which blow on certain days of the year at light intensities that cause no damage. The most common wind type in the Alsiroko region is shown in the following table, along with the average number of days affected by seasonal winds **(FGD Mila, 2016).**

3.2. Characteristics of *Lavandula angustifolia*:

- **Botanical Characteristics:**

Lavandula angustifolia is a perennial, evergreen shrub native to the Mediterranean region. It typically grows to a height of 30–90 cm and features narrow, gray-green leaves. The plant produces spikes of fragrant purple to blue-violet flowers, which bloom from late spring to early summer. It thrives in well-drained, alkaline to neutral soils **(Fig 36) (Barut, 2022).**

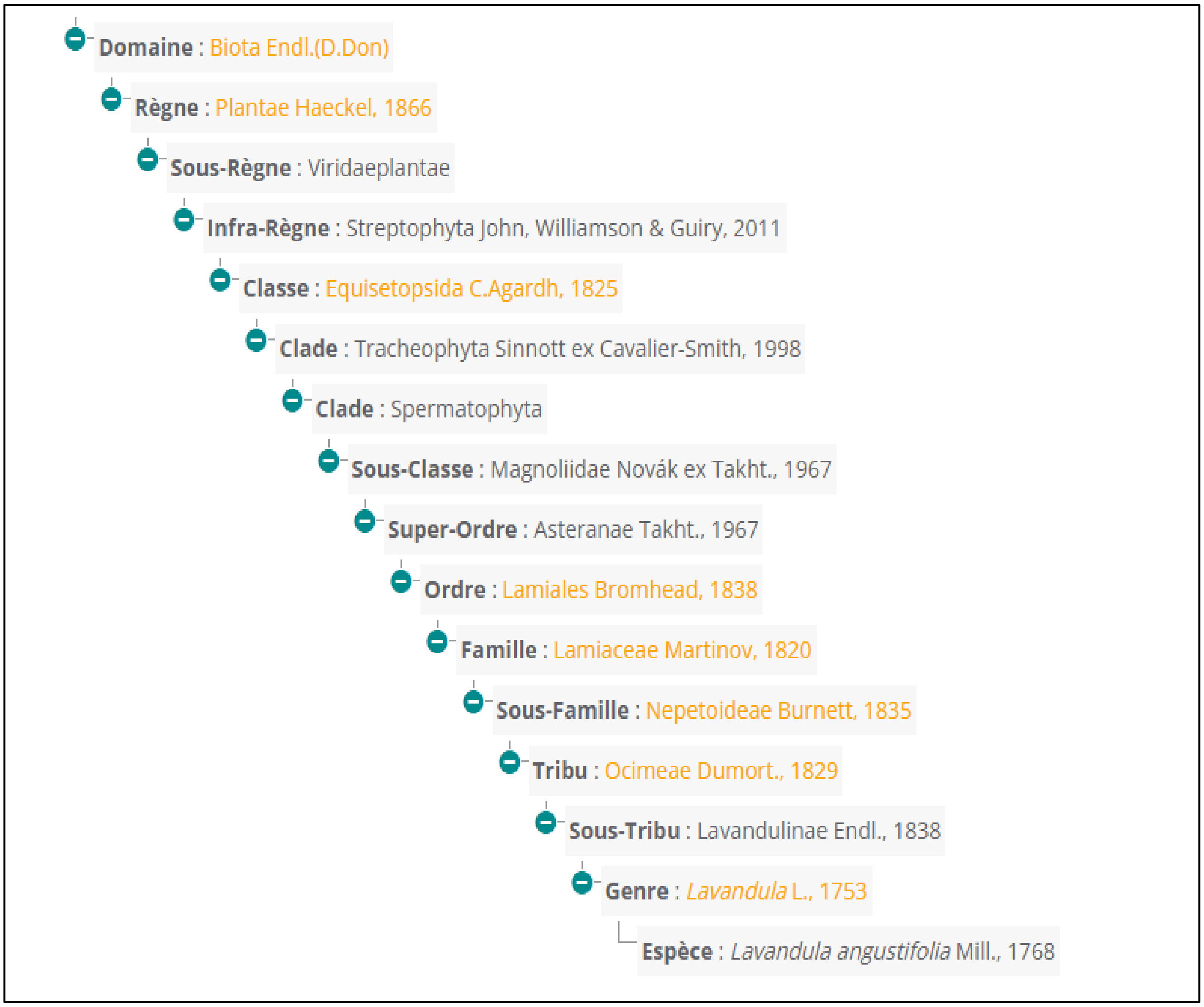

Figure 36. Hierarchical classification of Lavandula angustifolia **(Curcuma-longa, 1753)**

❖ **Ecological Traits of *Lavandula angustifolia*:**

- **Drought Adaptation:** Exhibits xeromorphic leaf structures (thick cuticles, sunken stomata) enabling survival at 40% soil water deficit **(Gori et *al.*, 2022).**
- **Pollinator Attraction:** UV-reflective floral patterns increase bee visitation rates by 62% compared to other Lavandula species **(Jentsch et *al.*, 2021) (Fig37).**

Figure 37. *Lavandula angustifolia* **(Webography 13)**

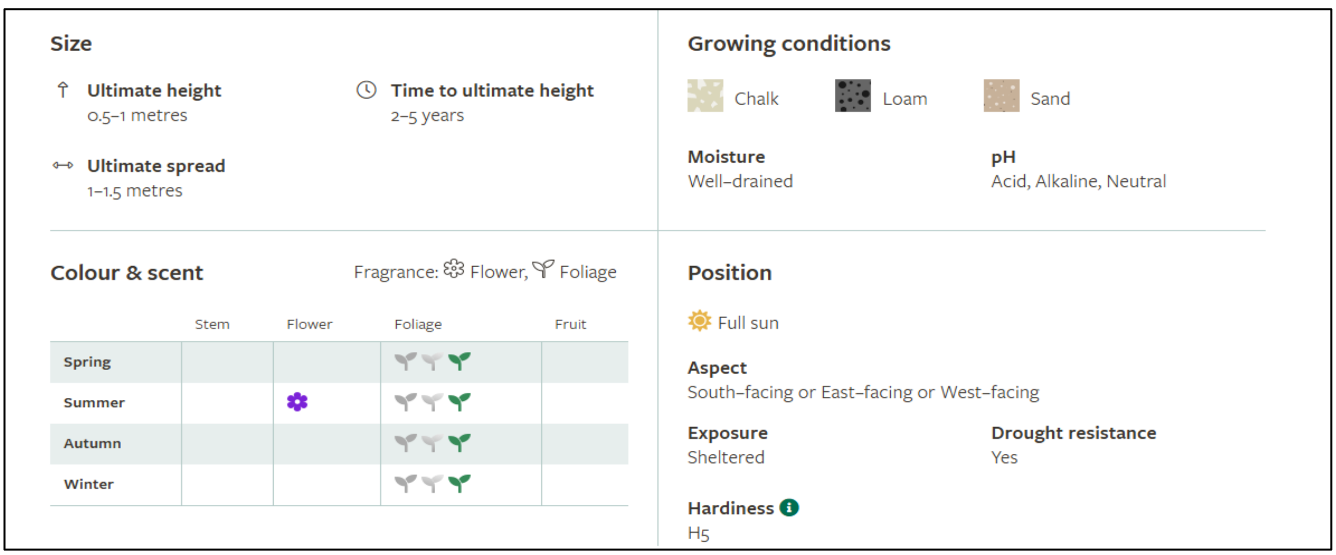

Figure 38. Botanical Characteristics **(The Royal Horticultural Society 2025)**

❖ Chemotype of *Lavandula angustifolia* essential Oil:

The chemical composition of *Lavandula angustifolia* essential oil extracted in our laboratory is summarized by the different analyses of the essential oil collected from different localities and regions of Eastern Algeria, as shown in the following table **(Tab04).**

Table 04. Chemotypes of Lavandula angustifolia essential oil

Region	Major Compounds (% by GC-MS)	Reference
Blida	Linalool (41.25%), Camphor (14.47%), 1,8-Cineole (9.94%), Terpinen-4-ol (9.90%)	**Latreche et *al.*, 2022**
Chlef	Linalool (41.71%), Camphor (12.54%), 1,8-Cineole (7.13%), Terpinen-4-ol (7.25%)	**Latreche et *al.*, 2022**
Laghouat	Linalool (35.8%), Linalyl Acetate (21%), Camphor (7.2%), α-Terpineol (5.2%)	**Benabdallah et al., 2023**
Batna	1,8-Cineole (29.4%), Camphor (24.6%)	**Zighmi et *al.*, 2014**
Skikda	Linalyl Acetate (32.98%), Linalool (28.92%), β-Caryophyllene (4.62%), Lavandulyl Acetate (4.52%)	**Boughendjioua, 2017**
Annaba (Seraïdi)	Linalool (31.27%), Camphor (16.21%), Linalool Oxide (11.98%), Linalyl Acetate (11.93%)	**Bensalem et *al.*, 2022**

The composition of *Lavandula angustifolia* essential oil varies depending on the region and present the most important compounds as:

- **Linalool:**

Linalool is a monoterpene alcohol with well-documented anxiolytic, sedative, anti-inflammatory, and antimicrobial effects. It plays a central role in the relaxing and calming properties associated with lavender essential oil and contributes significantly to its pleasant floral aroma **(Linck et *al.*, 2009).**

- **Linalyl Acetate**

Linalyl acetate is an ester with notable calming, antispasmodic, and anti-inflamatory actions. It works synergistically with linalool to enhance the sedative and stress-relieving effects of lavender oil. Although this sample shows a lower concentration than typical high-grade lavender oils, its presence still supports relaxation-based applications **(Cavanagh and Wilkinson, 2002).**

Camphor

Camphor is a monoterpene ketone known for its stimulant, analgesic, and antimicrobial properties. While camphor is useful in respiratory and muscular treatments, its high concentration in this sample (L. *angustifolia* contains <1%) suggests possible environmental factors, early harvest, or hybridization with lavandin **(Reichling et *al.*, 2009)**

1,8-Cineole

Also known as eucalyptol, 1,8-cineole exhibits **e**xpectorant, bronchodilator, and anti-inflammatory properties. Its presence strengthens the oil's potential for respiratory therapy, especially in steam inhalations and decongestant blends **(Juergens et *al.*, 1998)**

❖ Key Compounds with Insecticidal Activity:

The essential oil of *Lavandula angustifolia* contains several compounds with demonstrated insecticidal

Table 05. Key Compounds with Insecticidal Activity (**Sánchez-Vidaña et *al.* (2023); Tariq et *al.*, 2024)**)

Active Compound	Concentration (%)	Mode of Action Against Larvae
Linalool	24-51%	- Neurotoxic (acetylcholinesterase inhibition) - Disrupts molting (ecdysone inhibition)
Camphor	0.5-3%	- Potent repellent - Blocks larval olfactory receptors
1,8-Cineole	2-5%	- Inhibits digestion (midgut enzyme disruption) - Fumigant effect
β-Caryophyllene	1.8-3.2%	- Disrupts chemical communication (pheromones) - Antifeedant

2. Experimentation:

Our experimentation is divided into two principal axes (**(Fig39)**:

- The 1st axe represents an estimation of the pine processionary moth population abundance in Ferdoua forest, by counting the winter nests.
- The 2end axe includes toxicological treatments using three bioactive molecules extracted from *Lavandula angustifolia.*

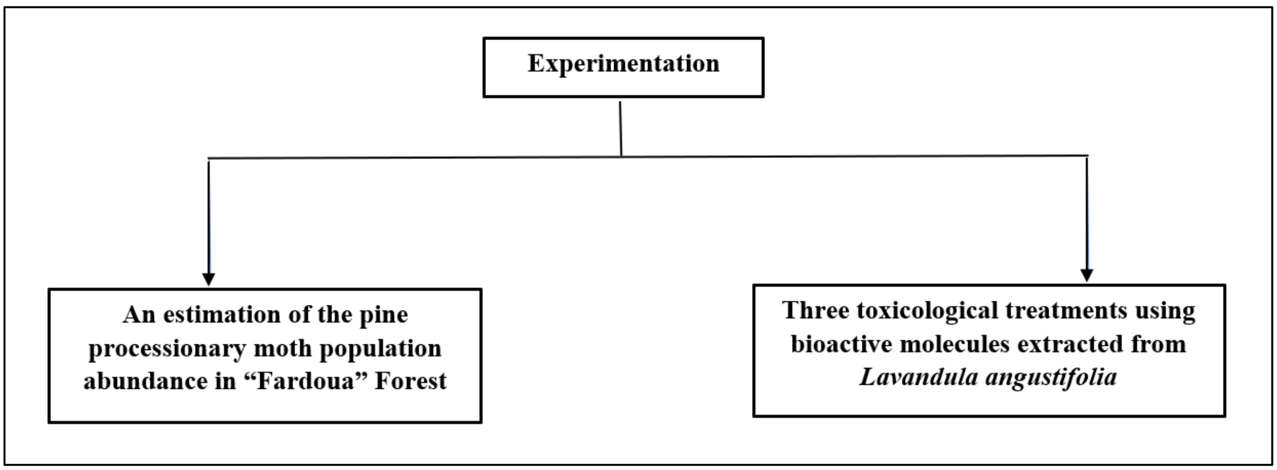

Figure 39. Principal axes of our experimentation **(Original representation)**

3.1. Estimation of the pine processionary moth abundance in Fardoua Forest:

We carried out a survey of the winter nests of the pine processionary caterpillar in "**Ferdoua"** Forest**.** The counting was conducted toward the end of April on 100 Aleppo pine trees, considering every other row within the same forest corridor **(Sebti and Chakali 2014)**. Following this, various ecological parameters and indices were developed in order to estimate the overall population abundance during this season (2024/2025) **(Fig40).**

Figure 40. Winter nest of the PPM in Ferdoua forest **(Original picture)**

2.2. Toxicological treatments:

2.2.1. Biological material sampling:

Lavandula angustifolia was collected from Mila region during the flowering season. It was identified according to the university bibliography of plant inventories of the region. The identification was also confirmed according to the global shape of the flower (Sepals and petals) and most of the areal parts. The plant was washed, dried, then ground using a blender and stored for extraction.

2.2.2. Target Organism:

Caterpillars from the third-fourth larval instar (L3/L4) of *Thaumetopoea pityocampa* were manually collected from the Aleppo pine trees in "Ferdoua" forest by the end of February 2025. Nests were cut using sterile forceps, with strict adherence to biosafety protocols to prevent urticating hair exposure.

4. Biomolecules extraction:

4.1. Essential oil extraction;

4.1.1. Hydrodistillation using the Clevenger Method:

To extract essential oil from *Lavandula angustifolia*, 50 g of dried plant material was placed into a 1 l round-bottom flask along with 400 mL of distilled water. The hydrodistillation was performed using a standard Clevenger apparatus **(Clevenger 1982)** connected to a water condenser and placed over a controlled heat source. As the water reached boiling, steam carried the volatile compounds from the lavender through the apparatus. The resulting vapor condensed back into liquid in the condenser, allowing the essential oil being less dense than water to accumulate and separate in the calibrated Clevenger trap. The extraction was carried out over a period of 1 hour and 30 minutes, after which the essential oil was collected using a glass pipette **(Fig41).**

Materials Used:

- *Lavandula angustifolia* 50 g (dried).
- Distilled Water: 400 ml.
- Round-bottom flask (1 L).
- Clevenger Apparatus (essential oil separator).
- Water condenser.
- Heat source (water bath or heating mantle).
- Glass pipette or tube for essential oil collection.

Figure 41. Hydrodistillation protocol using the Clevenger Apparatus Method **(Original picture)**

The collected essential oil was dried over Anhydrous Sodium Sulfate (Na_2SO_4), then stored in dark glass bottles in a refrigerator at 4ºC for further analysis. The yield (%) of essential oils was determined by the following formula **Afnor (1986)**:

$$Yield\ (\%) = \frac{\text{Weight of refined oil (g)}}{\text{Weight of botanical material (g)}} \times 100\%$$

4.2. Vegetable oil extraction:

❖ **Soxhlet extraction:**

20 g of the areal part of lavender was weighed and placed in an extraction thimble. The Soxhlet Apparatus is then assembled by attaching a boiling flask containing 1 l of Ethanol to the extractor, placing the thimble with lavender in the extraction chamber, and connecting a condenser to the top, ensuring continuous water flow. The heating mantle was adjusted to maintain the ethanol at its boiling point (65–70°C). The extraction process begins with an initial phase lasting approximately 10 minutes, during which the solvent boils, vapor rises, condenses, and drips onto the plant material. This is followed by a cyclic phase where the solvent siphons back into the boiling flask every 15-20 minutes. The cycle is repeated continuously for a total duration of 4

hours. Throughout the process, the temperature is carefully monitored to avoid overheating. The initial phase lasts for 10 minutes and 31 seconds, during which the solvent starts boiling, vapor rises, condenses, and drips onto the lavender. During the cyclic phase, the solvent siphons back every 15–20 minutes, and temperature control is maintained throughout to prevent overheating **(Fig42).**

Materials used:

- Soxhlet extractor apparatus (including: boiling flask, extraction chamber, condenser)
- Heating mantle (compatible with ROTA-VAPEUR device)
- Ethanol (1l)
- Dried lavender flowers (20 g)
- Extraction thimble.
- Timer To track extraction time
- Rotary evaporator (Repeat this part following the essential oil part)

Figure 42. Vegetable oil extraction using Soxhlet **(Original picture)**

The vegetable oil extracted was then collected from the boiling flask **(Fig43).**

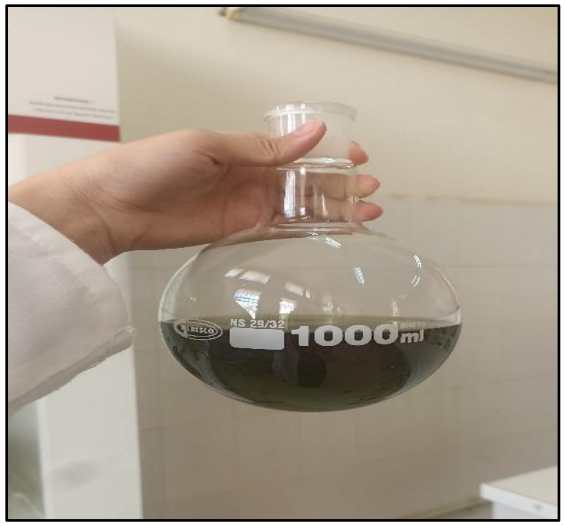

Figure 43. The extract collected from the boiling flask **(Original picture)**

❖ **Rota-Vapor processing:**

The last form of the vegetable oil was then prepared using a Rota-VAapor **(Fig 44)**, which use the rotation speed and the heat power (40°c) to separate evaporated ethanol from the aqueous extract of lavender.

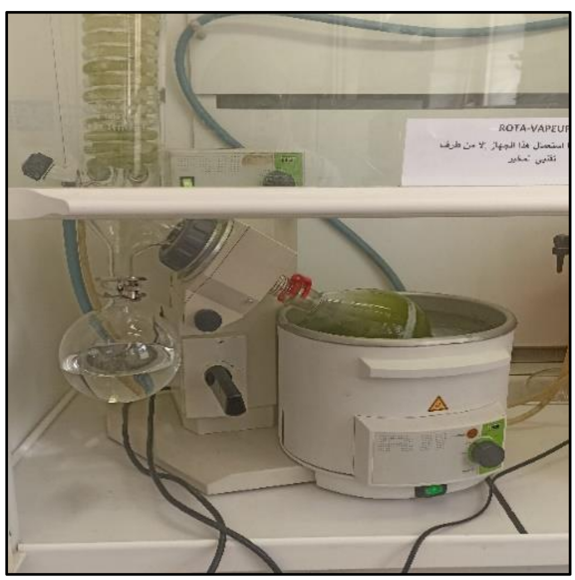

Figure 44. Rotary evaporator processing on Lavander vegetable oil **(Original picture)**

The paw recovered from the rotation procedure is then weighed and kept in a petri dish **(Fig 45)**.

Figure 45. Vegetable Oil weighted and conserved **(Original picture)**

4.3. Commercial oil origin:

Commercial oil **(Fig46)** of lavender was obtained through pharmaceutical means from Heva-Pharm laboratories. The natural oil dilutions were prepared according to the European pharmacological guidelines for commercial essential oils using the following formula:

1 ml = 0.9 g = 35 to 40 drops from the bottle

Figure 46. Commercial oil of *Lavandula angustifolia* **(Original picture)**

Table 05. Technical details about *Lavandula angustifolia* commercial essential oil **(Hava-Pharm laboratory 2025)**

Characteristics	Details
Extraction Process	**Stim distillation**
Chemotype composition	**Acétate of linalyle (35%) + Linalool (25%)**
Aspects	**Fluid**
Aroma	**Characteristic**
Color	**Crystalline**
Density	**0,891**

5. Biomolecules concentration:

3 grams of each primary molecule are mixed with 100 grams of distilled water using Tween 80 (0,3%) to obtain a dose of 3%. From this solution, preliminary tests developed the concentrations of 2% and 1%.

6. Toxicological bioassays:

Larvicidal assays were carried out according to the method described by Semiz et *al.,* **(2006)** where all treatments were achieved in freezing perforated small plastic boxes (10x10 cm), with 10 larvae of the same age placed in each one.

Three doses (3%,2% and 1%) (**Fig47)** were used for every molecule, with three replications for each one. A last box containing 10 untreated larvae was used as a witness test to compare natural mortality to the essential oils' activities.

This box was tested using first distilled water (+ 0,3% tween 80) for essential oils tests, then ethanol (+0,3%) for vegetable oil.

Figure 47. Prepared dilutions **(Original picture)**

Toxicological tests were applied by spraying the pine fresh needles with 2 ml of the diluted oil. Treatments using the different essential oils were carried out on the 4^{th} larval stages of the pine processionary moth in laboratory conditions.

Larvicidal mortality was assessed by counting the dead larvae in each box after 24 until the end of tests. The operation was daily repeated and needles were replaced after each observation until the end of the tests **(Fig 48)**.

Figure 48: Application steps for the prepared dilutions **(Original picture)**

7. Data analysis:

Ecological indices for winter nests abundance on Ferdoua trees were estimated using **Past3** program. Larvae mortality was evaluated after 4 and 8 days. It was expressed as mean (percentage) ± standard error. Significant differences between the obtained results ($p < 0.05$) were evaluated by (ANOVA) and Duncan tests using **Minitab 2015**. Lethal doses (lD_{50}) (lD_{90}) were estimated and confirmed by probit program using **IBM-SPSS 29 Software (2024)**. Mortality curves are developed according to the following steps:

- **Observed Mortality:** The percentage of mortality observed in *Thaumetopoea pityocampa* caterpillars treated with different essential oils at different concentrations as well as in controls is determined according to the following formula:

Observed Mortality = Number of dead larvae / Number of treated larvae X 100

- **Corrected Mortality:**

The percentage of observed mortality is corrected by the Abbott formula **(1925)** which allows natural mortality to be eliminated:

Corrected Mortality = Mortality in larvae treated – Mortality in larvae non treated / 100 – Mortality in larvae non treated X 100

The corrected mortality percentages are angularly transformed according to **(Bliss 1938 in Fisher and Yates 1957)**. The data thus normalized are subjected to a one-criterion analysis of variance followed by the classification of concentrations by the Tukey Test.

- **Probits Analysis:**

Lethal concentrations are inferred from the plot of regression lines **(Finney 1971).** For this purpose, the corrected mortality percentages are transformed into probits **(Fisher and Yates 1957).**

- **Regression curves :**

Regression equations are estimated for each oil, during the 3rd-4th larval stages of the pine processionary caterpillar, after 4 and 8 days of treatment on (Excel 2010) then confirmed directly on IBM SPSS software (2024).

RESULTS

VI-RESULTS:

1. Estimation of Lavender hydrodistillation yield:

$$Yield\ (\%) = \frac{\text{Weight of refined oil (g)}}{\text{Weight of botanical material (g)}} \times 100\%$$

- Weight of dried lavender = **50,00 g**
- Weight of extracted oil = **1,45 g**

The hydrodistillation process yielded **2,90 %** for *Lavandula angustifolia* essential oil.

2. Ecological Indices of PPM population in Ferdoua Forest:

In Ferdoua Forest, a total of 191 winter nests of the pine processionary moth (PPM) *Thaumetopea pityocampa* were recorded. The observed nest density averaged 1,91 nests per tree, with a standard deviation of 1,61. These values reflect a variable distribution of nests across the surveyed trees, indicating differing levels of infestation within the forest **(Tab 06).**

Table 6. Ecological Indices for PPM population in Ferdoua Forest

Total Abundance	Equitability	Average Abundance	Infestation rate
191	**0,96**	**1,91±1,61**	**78%**

Equitability index of the winter nests distribution in Fardoua forest recoded a value of 0,96 According to our counting protocol, among the 100 trees sampled, 78 trees were infested by the pin processionary moth winter nests while 22% were non infested **(Tab 07).**

Table 7. PPM winter nests infestation rate in the Ferdoua forest

Ferdoua forest	Infested trees	Non-infested trees
Winter nest infestation	**78%**	**22%**

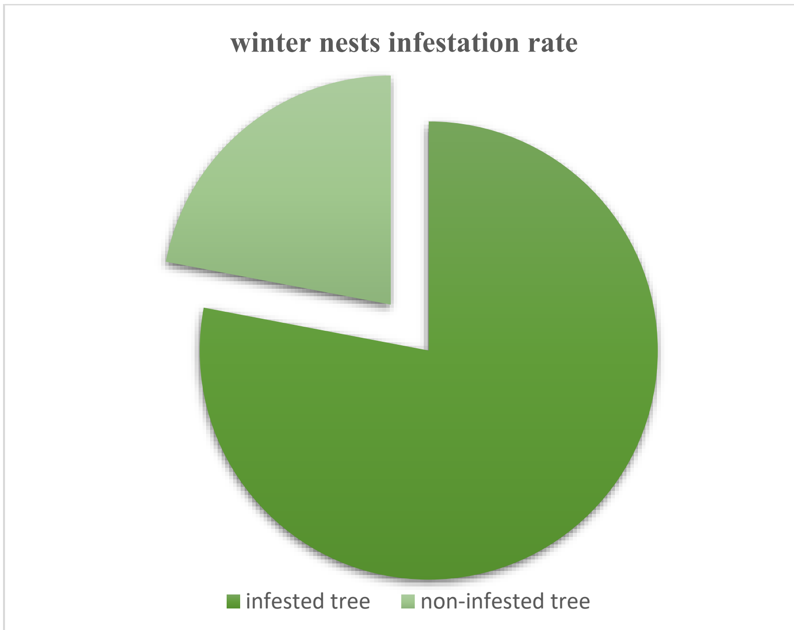

Figure 49: Winter nests infestation rate in Ferdoua forest

3. Toxicological bioassays:

3.1. Toxicological effect of *Lavandula angustifolia* vegetable oil on PPM larvae:

3.1.1. Toxicological results after 4 days of treatment:

The vegetable oil of *Lavandula angustifolia* shows increasing biological activity on caterpillars of the pine processionary moth after 4 days of treatment. Mortality rates rise with higher concentrations: 14, 81% at 1%; 18,51% at 2%, and 22, 22% at 3%, indicating a dose-dependent effect. However, some variation was observed between repetitions, especially at the 2% dose where the standard deviation recorded higher value (12,82) **(Tab08).**

Table 8. Toxicological effect of Lavandula angustifolia vegetable oil on PPM larvae after 4 days

Repetitions \ Doses	1%	2%	3%
R1	11,11	11,11	11,11
R2	11,11	11,11	22,22
R3	22,22	33,33	33,33
Average	**14,81±6,41**	**18,51±12,82**	**22,22±11,11**

R1, R2, R3: Repetitions for mortality rates

3.1.1.1. Variance analyses for mortality levels using the vegetable oil after 4 days of treatment:

Anova test for mortality levels using the vegetable oil of *Lavandula angustifolia* marked a variance F-value of 0, 83, indicating that between-group, variance is similar to within-group variance. There are no significant differences between the mortality levels using the different doses (P> 0,05). The R^2 (11,11%) **(Tab09).**

Table 9. Anova test for mortality levels after 4 days of treatment using thevegetable oil

Source	DF	Adj SS	Adj MS	F-Value	P-Value	R^2
Factor	2	82	41	0,83	0,702	11,11%
Error	6	658	110			
Total	8	741				

SS: Sum of Squares - ADJ DF: Degrees of Freedom - ADJ MS: Mean Square - F: Observed Variance - P: Significance Level - R^2: Coefficient of Determination.

3.1.2. Toxicological results after 8 days of treatment:

At the 8th day of PPM larvae treatment using *Lavandula angustifolia* vegetable oil, the mortality levels demonstrated a clear dose-dependent mortality, with averages increasing from 37,03% (1%) to 66, 66% (3%). While results were relatively consistent at 1% concentration, higher variability appeared at concentrations of 2% and 3%, reflecting higher mortality rates (66,66% and 88,88%) compared to other replicates **(Tab10).**

Table 10. Toxicological effect of Lavandula angustifolia vegetable oil on PPM larvae after 08 days.

Repetitions \ Doses	1%	2%	3%
R1	33,33	33,33	55,55
R2	44,44	44,44	55,55
R3	33,33	66,66	88,88
Average	**37,03±6,41**	**48,14±16,97**	**66,66±19,24**

R1, R2, R3: Repetitions for mortality rates

3.1.2.1. Variance analyses for mortality levels using the vegetable oil after 8 days of treatment:

The F-value resulting for Anova test of mortality levels after 8 days of treatment of PPM larva using *Lavandula angustifolia* vegetable oil (2,88) reflected moderate variance between-group variation that is not statistically conclusive. There are no significant differences between the mortality levels using the different doses (P> 0,05). The R^2 value of 49% indicates that oil concentrations explain nearly half of the observed variance in mortality levels. **(Tab11).**

Table 11. Anova test for mortality levels after 8 days of treatment using the vegetable oil

Source	DF	Adj SS	Adj MS	F-Value	P-Value	R^2
Factor	2	1344	672	2,88	0,133	49%
Error	6	1399	233			
Total	8	2743				

SS: Sum of Squares - ADJ DF: Degrees of Freedom - ADJ MS: Mean Square - F: Observed Variance - P: Significance Level - R^2: Coefficient of Determination.

4.2. Toxicological effect of *Lavandula angustifolia* prepared essential oil on PPM larvae.

4.2.1. Toxicological results after 4 days of treatment:

At the 4th day of PPM larvae treatment using *Lavandula angustifolia* prepared essential oil**,** mortality levels demonstrated an emerging dose-dependent mortality with an average increasing from 40% (1%) to 60% (3%). At higher concentrations (3%) results showed the most important rates (50-80%,). Toxicological tests using *Lavandula angustifolia* prepared essential oil indicated the most larvicidal potency even after 4 days of treatment **(Tab12).**

Table 12. Toxicological effect of Lavandula angustifolia prepared essential oil on PPM larvae after 04 days.

Doses / Repetitions	1%	2%	3%
R1	50	60	50
R2	40	50	50
R3	30	30	80
Average	**40±10**	**46,66±15,27**	**60±17,32**

R1, R2, R3: Repeats for mortality rates

4.2.1.1. Variance analyses for mortality levels using the prepared oil after 4 days of treatment:

Anova test for mortality levels using the prepared essential oil of *Lavandula angustifolia* recorded a variance F-value of 1, 47 and P-value of 0,302), indicating no significant differences between the mortality results using the different doses ($P > 0,05$). The R^2 value of 32,94% indicates that approximately one-third of mortality variance is explained by dose concentrations, while the remaining 67,06% is attributable to other factors **(Tab13).**

Table 13. Anova test for mortality levels after 4 days of treatment using the prepared essential oil.

Source	DF	Adj SS	Adj MS	F-Value	P-Value	R^2
Factor	2	622	311	1,47	0,302	32,94%
Error	6	1267	211			
Total	8	1889				

SS: Sum of Squares - ADJ DF: Degrees of Freedom - ADJ MS: Mean Square - F: Observed Variance - P: Significance Level - R^2: Coefficient of Determination

4.2.2. Toxicological results after 8 days of treatment:

At the 8^{th} day of treatment under *Lavandula angustifolia* prepared essential oil effect. Data revealed a definitive dose-dependent response, with mortality rates escalating from 55,55% (1%) to 99,99% (3%). The highest concentration presented the mortality of the entire population tested after 8 days of treatment. While lower doses showed important larvicidal activity reaching half of the population tested. **(Tab14).**

Table 14. Toxicological effect of Lavandula angustifolia prepared essential oil on PPM larvae after 08 days.

Doses / Repetitions	1%	2%	3%
R1	66,66	66,66	99,99
R2	55,55	77,77	99,99
R3	44,44	66,66	99,99
Average	**55,55±11,11**	**70,36±6,41**	**99,99±0**

R1, R2, R3: Repetition for mortality rates

4.2.2.1. Variance analyses for mortality levels using the prepared oil after 8 days of treatment:

The F-value resulting for Anova test of mortality levels after 8 days of treatment on PPM larva using *Lavandula angustifolia* prepared essential oil indicated a value of 28 and a P-value of 0,001 demonstrating highly significant differences between the mortality levels using the different doses ($P \leq 0,01$). The coefficient of determination R^2 value (90, 32%) shows that doses explain over 90% of mortality level variance **(Tab15).**

Table 15. Anova test for mortality levels after 8 days of treatment using the prepared essential oil.

Source	DF	Adj SS	Adj MS	F-Value	P-Value	R^2
Factor	2	3072,1	1536	28	0,001	90,32%
Error	6	329,2	54,9			
Total	8	3401,2				

SS: Sum of Squares - ADJ DF: Degrees of Freedom - ADJ MS: Mean Square - F: Observed Variance - P: Significance Level - R^2: Coefficient of Determination

5.3. Toxicological effect of *Lavandula angustifolia* commercial essential oil on PPM larvae.

5.3.1. Toxicological results after 4 days of treatment:

Tests results on PPM larvae using commercial *Lavandula angustifolia* oil showed a dose-dependent mortality with averages increasing from 13,33% (1%) to 43, 33% (3%). The 2% concentration exhibited higher variability, while 1% and 3% concentration recorded close values between repetitions **(Tab16).**

Tableau 16. Toxicological effect of *Lavandula angustifolia* commercial essential oil on PPM larvae.

Repetitions \ Doses	1%	2%	3%
R1	10	40	50
R2	10	10	40
R3	20	20	40
Average	**13,33± 5,77**	**23,33 ±15,27**	**43,33± 5,77**

R1, R2, R3: Repetitions for mortality rates

5.3.1.1. Variance analyses for mortality levels using the commercial oil after 4 days of treatment:

Anova results for mortality levels under treatment of PPM larvae using the commercial oil of *Lavandula angustifolia* resulted in a P- value of 0, 027 and an F-value of 7, which indicated that there are significant differences between the mortality levels using the different doses (P ≤0, 05). The R^2 value (70%) **(Tab17).**

Table 17. Anova test for mortality levels after 4 days of treatment using the commercial essential oil

Source	DF	Adj SS	Adj MS	F-Value	P-Value	R^2
Factor	2	1400	700	7	0,027	70%
Error	6	600	100			
Total	8	2000				

SS: Sum of Squares - ADJ DF: Degrees of Freedom - ADJ MS: Mean Square - F: Observed Variance - P: Significance Level - R^2: Coefficient of Determination

5.3.2. Toxicological results after 08 days of treatment:

After 8 days of toxicological tests using the commercial *Lavandula angustifolia* oil. Mortality levels revealed important larvicidal effect with dose-dependent mortality pattern, with averages increasing from 22,22% (1%) to 59,25% (3%). While the concentration of 1% demonstrated the same value (22,22) across replicates, the concentration of 2% showed high variability between repetitions. Compared to the 4th day results, the mortality profile doubled efficacy at the highest concentration (43,33%-59,25%) **(Tab18).**

Table 18. Toxicological effect of Lavandula angustifolia commercial essential oil on PPM larvae.

Repetitions \ Doses	1%	2%	3%
R1	22,22	44,44	55,55
R2	22,22	11,11	66,66
R3	22,22	33,33	55,55
Average	**22,22± 0**	**29,62±16,97**	**59,25± 6,41**

R1, R2, R3: Repetitions for mortality rates

5.3.2.1. Variance analyses for mortality levels using the commercial oil after 8 days of treatment:

The 8th day of PPM larvae treatment using the commercial *Lavandula angustifolia* essential oil recorded an F-value of 10,50 and a P-value of 0,11, which marked significant differences between the mortality levels using the different doses ($P \leq 0,05$). The determination coefficient R^2 (77,78%) shows doses explain majority of mortality variation. **(Tab19).**

Table 19. Anova test for mortality levels after 8 days of treatment using the commercial essential oil

Source	DF	Adj SS	Adj MS	F-Value	P-Value	R^2
Factor	2	2304	1152	10,50	0,11	77,78%
Error	6	658	110			
Total	8	2962				

SS: Sum of Squares - ADJ DF: Degrees of Freedom - ADJ MS: Mean Square - F: Observed Variance - P: Significance Level - R^2: Coefficient of Determination

6. Comparative analysis for different molecules activity on PPM larvae:

6.1. Under concentration of 1%:

The experimental results demonstrated a distinct hierarchy in larvicidal efficacy among the different tests using the variables active molecules of lavender against PPM larvae. At concentration of 1%; bioassay tests results showed that the prepared essential oil was the most effective, causing the highest larval mortality after both 4 and 8 days. At this level the vegetable oil had better activity comparing to the Commercial essential oil. All oils became more effective over time, with the greatest impact seen by day 8 **(Fig 50).**

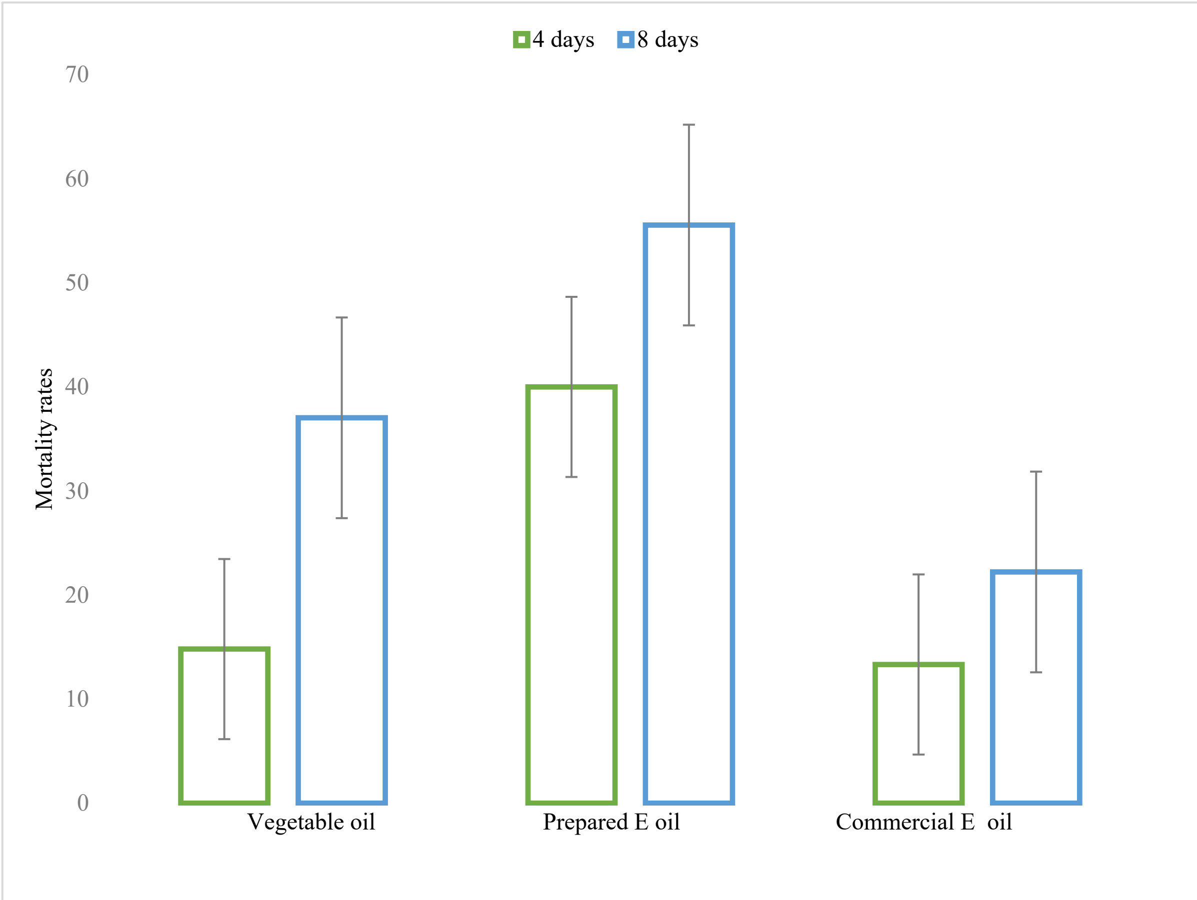

Figure 50. Comparative analysis for different molecules activity on PPM larvae under concentration of 1%.

6.2. Under concentration of 2%:

The comparative study evaluating the three lavender molecules activity at the concentration of 2% over the 4th and the 8th day periods demonstrated that the prepared essential oil exhibited the strongest larvicidal effect with important mortality level (70%) comparing to the vegetable and the commercial oils. The vegetable oil showed moderate efficacy (50%) comparing to the commercial oil which displayed the weakest impact (30%) and this after 8 days of treatment. All toxicological tests showed gradually increased effectiveness from the 4th to the 8th day proving more potency comparing to the previous tests (using 1% of concentration) **(Fig 51).**

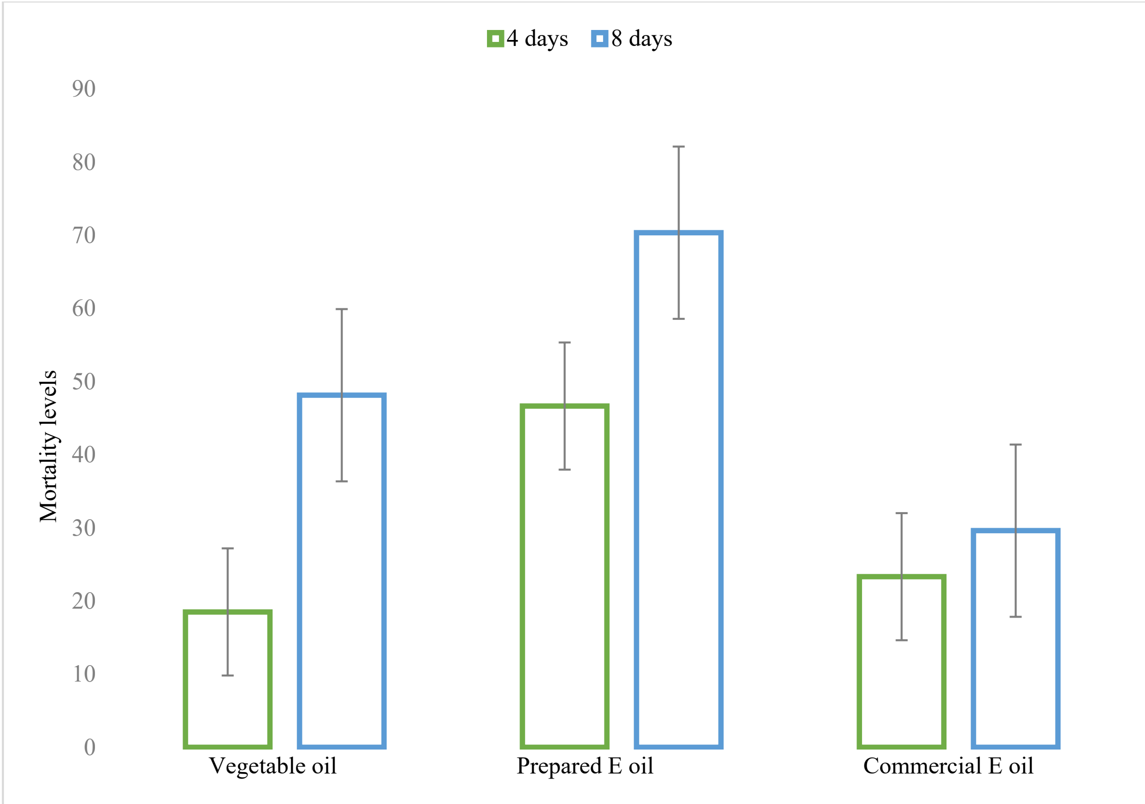

Figure 51. Comparative analysis for different molecules activity on PPM larvae under concentration of 2%.

6.3. Under Concentration of 3%:

At the highest concentration, the different molecules used for toxicological treatments against PPM larvae at 3% of concentration revealed the most important mortality levels (20%; 40% and 60%) after 4 days, and (55%; 60% and 100%) after 8 days of treatment. The strongest larvicidal effects was marked for the prepared essential oil which recoded complete effectiveness agents the population tested (99,99%). The commercial and the vegetable oils indicated the same effectiveness (60%) at the and the tests and lower efficacity comparing to the prepared molecule **(Fig 52).**

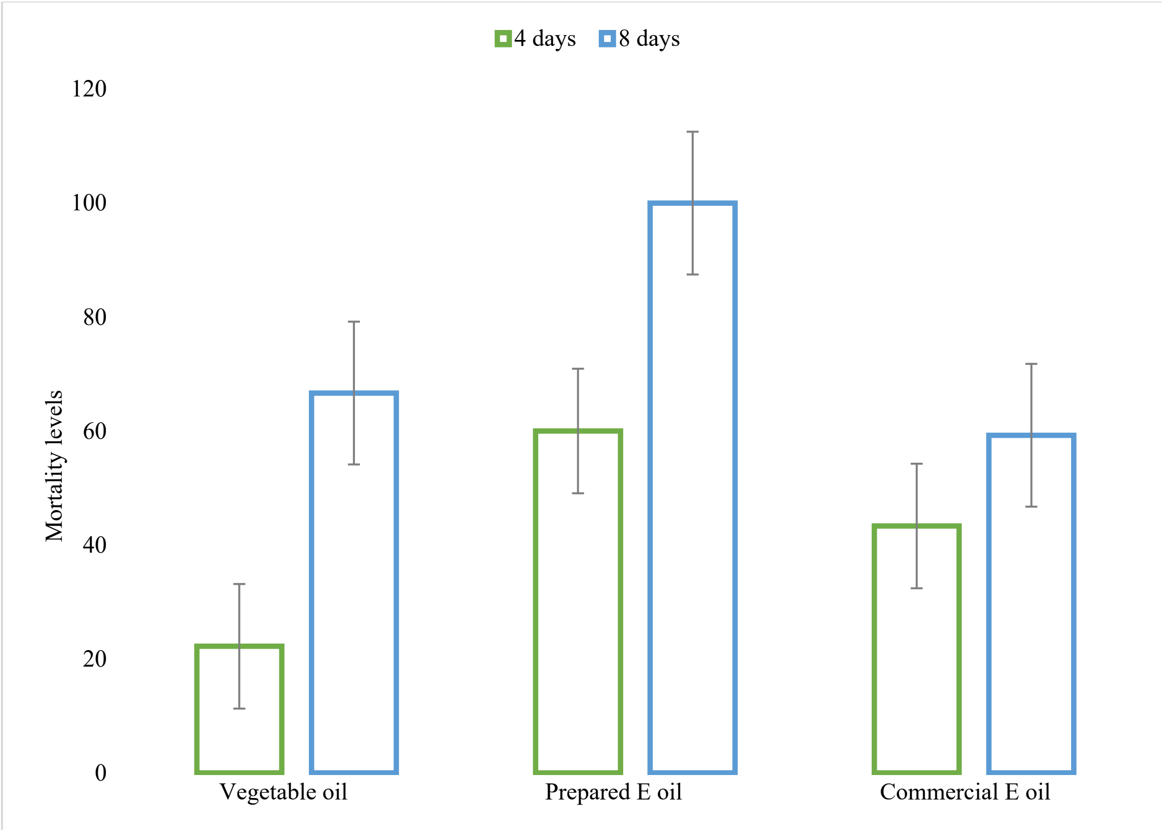

Figure 52. Comparative analysis for different molecules activity on PPM larvae under concentration of 3%.

7.Mortality curve and lethal doses for each treatment:

7.1. For vegetable oil:

7.1.1. After 4 days of treatment:

The mortality curve (Fig 53) after 4 days of PPM larvae treatment using the vegetable oil of *Lavandula angustifolia* showed a clear positive linear relationship between the log dose and probit mortality, as represented by the regression equation(y= 0,60x + 3,94)with a very high coefficient of determination (R^2 = 0,98) and lethal doses of lD_{50} = 58,88 % and lD_{90} = 7943,28 % **(Tab 20).**

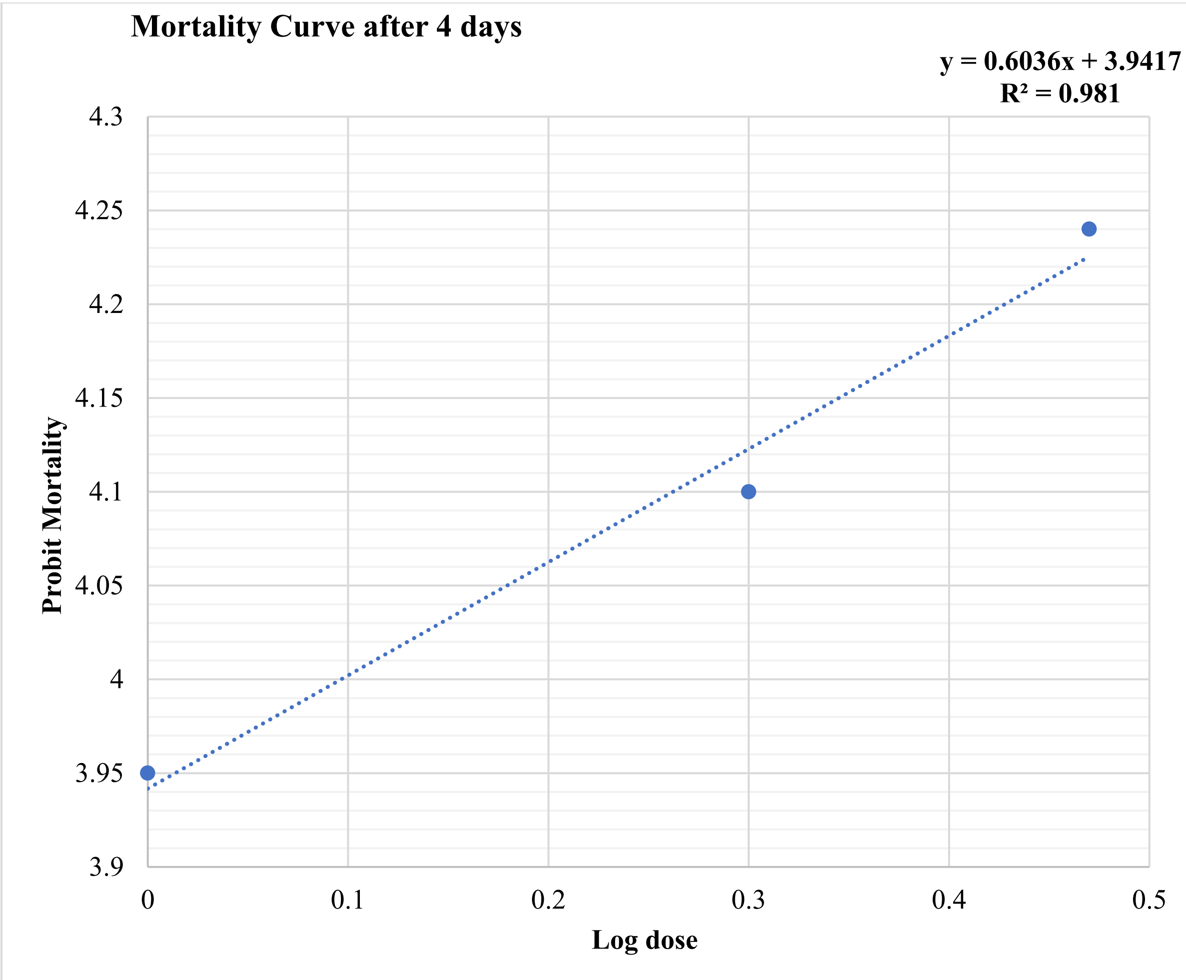

Figure 53. Mortality curve for Vegetable oil after 04 days.

7.1.2. After 8 days of treatment:

The mortality curve **(Fig 54)** after 8 days of treatment of PPM larvae using vegetable oil of *Lavandula angustifolia* indicated a positive linear relationship between the log dose and probit mortality, resulted in the following regression equation (y = 1,53x +4,62) with a very high coefficient of determination (R^2 = 0,90) and important lethal doses of lD_{50}= 1,77 %, and lD_{90} = 12,02 % **(Tab 20).**

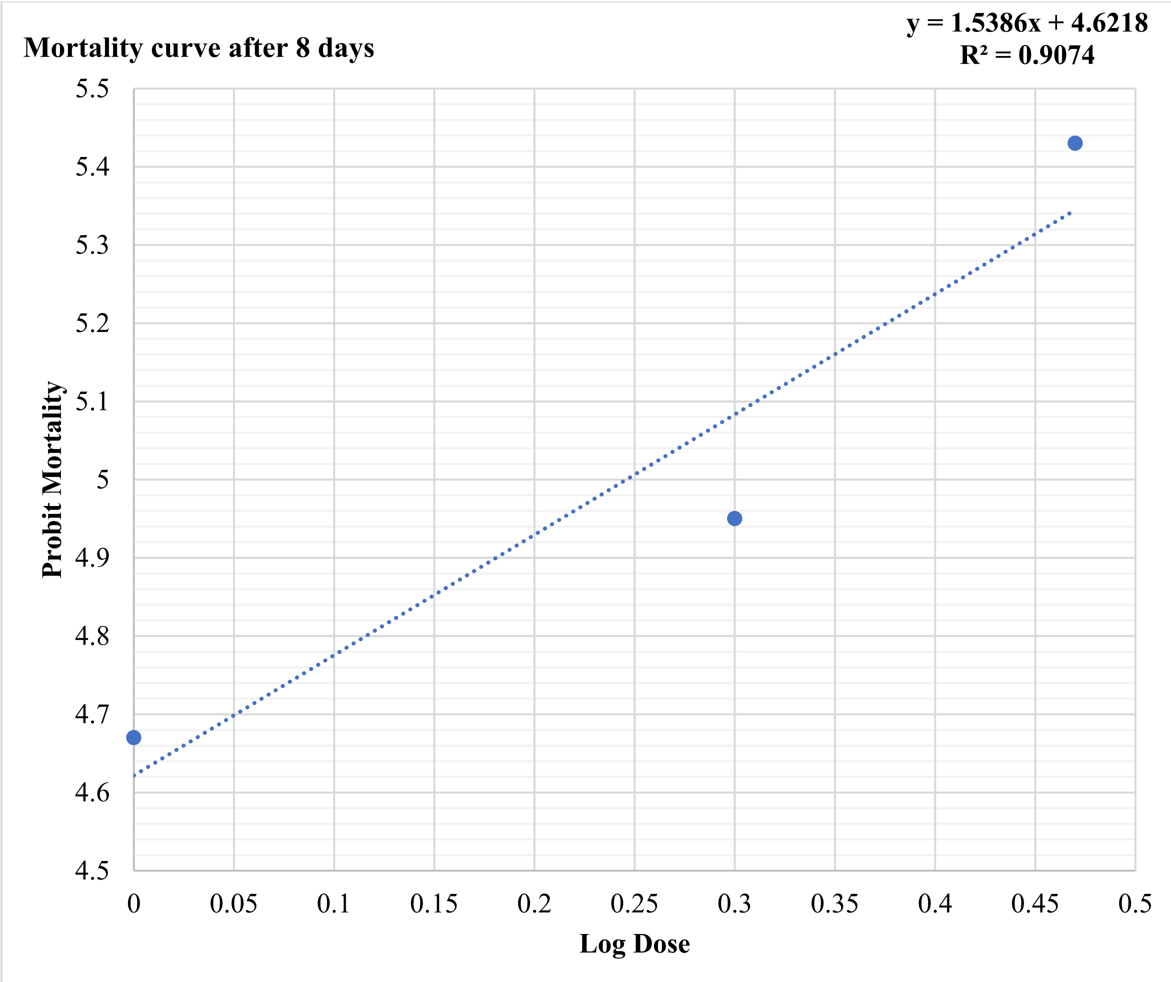

Figure 54. Mortality curve for Vegetable oil after 08 days.

8.2. For prepared essential oil:

8.2.1. After 4 days of treatment:

The image below **(Fig 55)** presents a mortality curve resulted of PPM larvae treatment using the prepared essential oil of *Lavandula angustifolia* after 4 days. The mortality profile showed a clear positive linear relationship between log doses and mortality levels. The regression equation (y =1x +4,71). R^2 (0,87). Lethal doses recorded values of lD_{50}= 1,94 % and lD_{90}= 37,15 % **(Tab 20).**

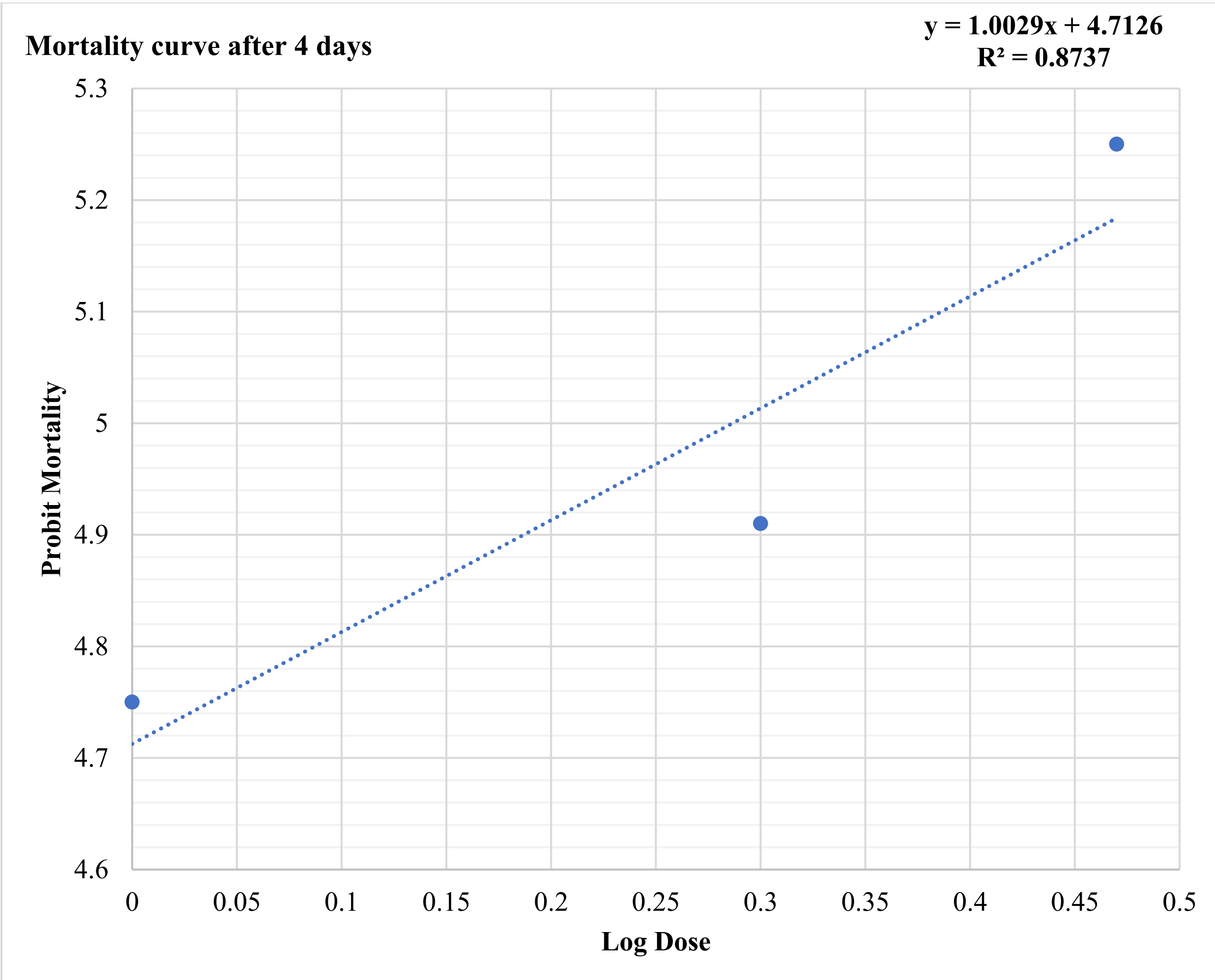

Figure 55. Mortality curve for essential prepared oil after 04 days.

8.2.2. After 8 days of treatment:

The following image **(Fig 56)** illustrates the probit mortality analysis using the different doses of *Lavandula angustifolia* essential prepared oil evaluated on PPM larvae. After 8 days of treatment, the traced curve demonstrated a clear linear correlation between the logarithm of the dose and the observed mortality results. The corresponding regression equation (y=5,70x +4,78), R^2 (0,71), and lethal doses estimated at : lD_{50}= 1,07 % and lD_{90}= 1,81 % **(Tab 20).**

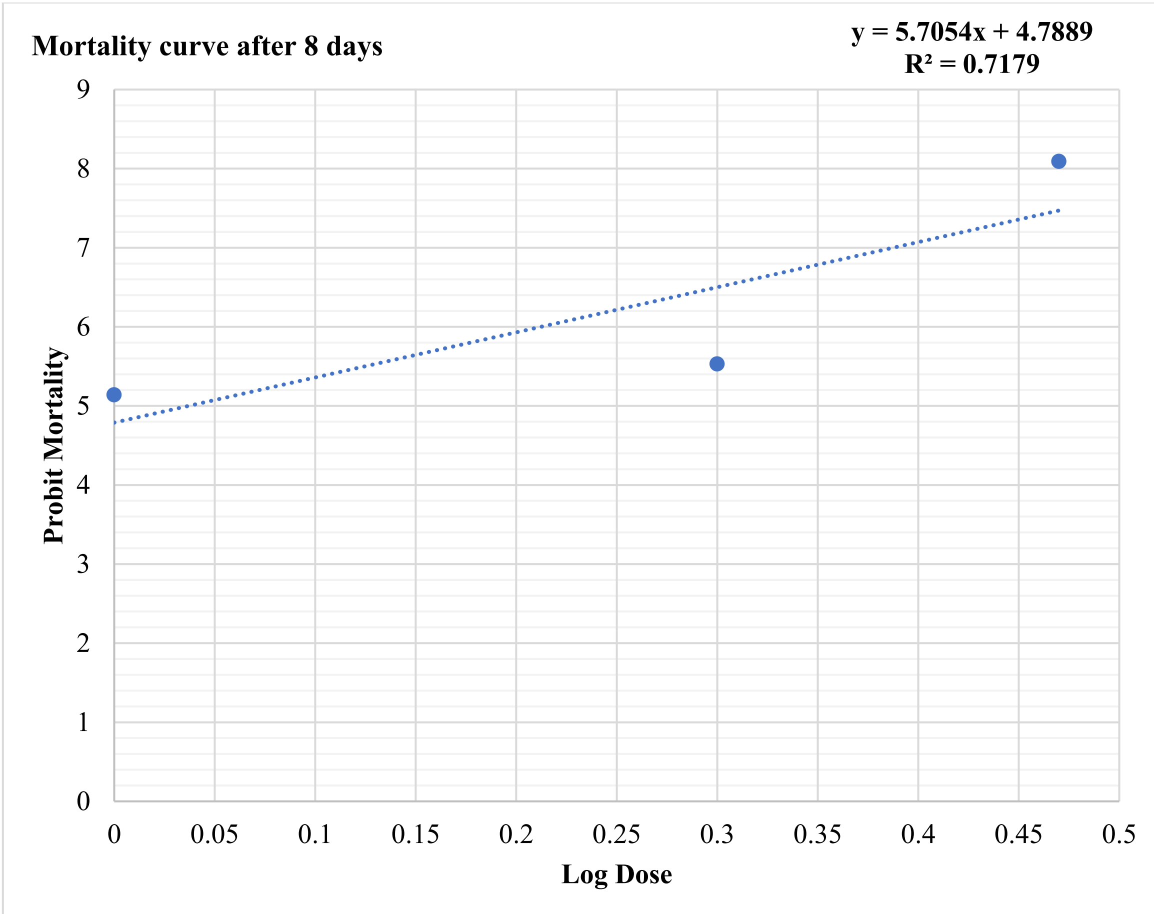

Figure 56. Mortality curve for essential prepared oil after 08 days.

9.3. For commercial essential oil:

9.3.1. After 4 days of treatment:

The probit mortality analysis of PPM larvae treated using the commercial oil of *Lavandula angustifolia* revealed a strong positive dose-response relationship after 4 days, with a regression curve **(Fig 57)** of (y = 1,91x +3,83). An R^2 = 0, 92 and lethal doses of lD_{50}= 4,07 %and lD_{90} =19,05 %[2] **(Tab 20).**

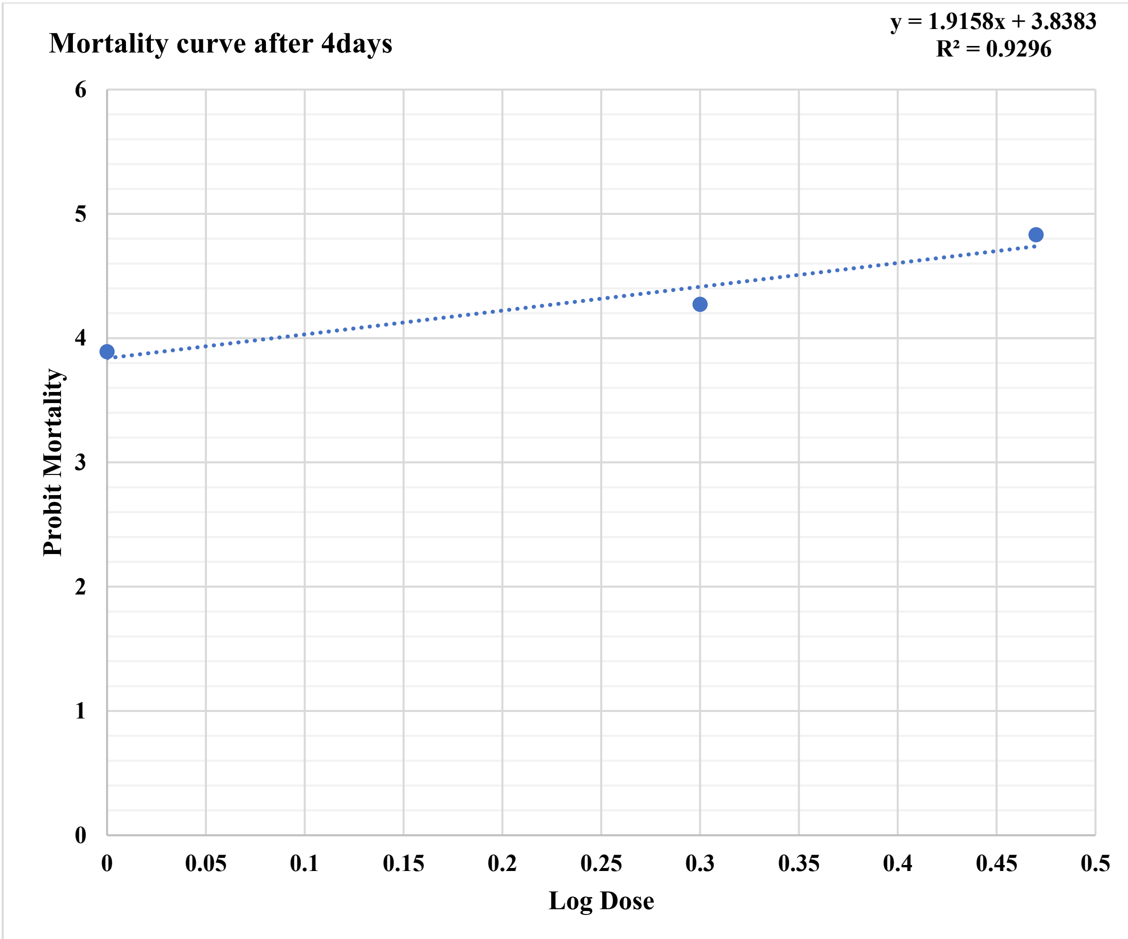

Figure 57. Mortality curve for commercial oil after 04 days.

9.3.2. After 8 days of treatment:

The commercial oil of *Lavandula angustifolia* analysis of PPM larvae demonstrated a clear dose-dependent mortality response after 8 days of treatment, with the regression equation of **(Fig 58)** **(** y = 1,99x +4,13) and R^2 value of 0,80. Lethal doses recorded values of lD_{50} = 1,77 and lD_{90} = 12,02**(Tab 20).**

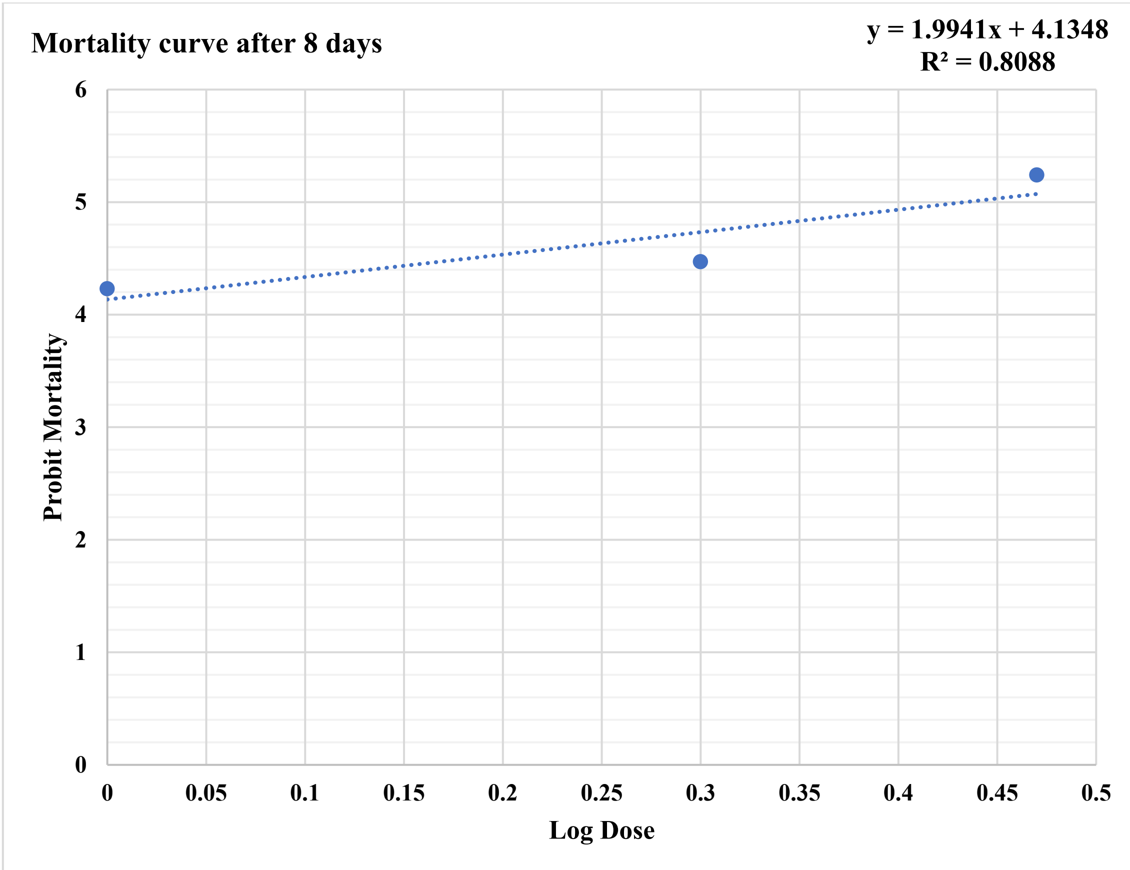

Figure 58. Mortality curve for commercial oil after 08 days.

Lethal concentrations marked lower values for essential oil for *Lavandula angustifolia* and this for both estimation lD50% and lD 90%. This confirms the highest toxicological effect of this bioactive molecule comparing to other substances **(Tab20).**

Table 19: Parameters and lethal doses for toxicological tests

After 4 days of treatment					
Molecules	**Doses**	**Mortality curve**	**lD50%**	**lD 90%**	**R^2**
Vegetable Oil	1% 2% 3%	**y = 0,60x + 3,94**	**58.88%**	**7943.28%**	**0,98**
Prepared E Oil	1% 2% 3%	**y =1x +4,71**	**1.94%**	**37.15%**	**0,87**
Commercial E oil	1% 2% 3%	**y = 1,91x +3,83**	**4.07%**	**19.05%**	**0,92**
After 8 days after treatment					
Molecules	**Doses**	**Mortality curve**	**lD50%**	**lD 90%**	**R^2**
Vegetable Oil	1% 2% 3%	**y = 1,53x +4,62**	**1.77%**	**12.02%**	**0,90**
Prepared E Oil	1% 2% 3%	**y=5,70x +4,78**	**1.07%**	**1.81%**	**0,71**
Commercial E oil	1% 2% 3%	**y = 1,99x +4,13**	**2.69%**	**12.02%**	**0,80**

DISCUSSION

V-Discussion:

The pine processionary caterpillar (*Thaumetopoea pityocampa*) represents a growing multidisciplinary challenge with ecological, health, and socio-economic implications. Originally from the Mediterranean basin, the lepidoptera insect have expanded their range northward and to higher altitudes in response to climate warming, progressing at an estimated rate of 4 km per year in Europe **(Robinet et *al.*, 2022).** Their characteristic silky nests in pine trees release urticating hairs containing a toxin (thaumetopoein) responsible for severe allergic reactions in humans (dermatitis, anaphylactic shock) and domestic animals (lingual necrosis in dogs**) (Vega et *al.*, 2021).**

Ecologically, their cyclic outbreaks cause intense pine defoliation, reducing tree growth and making them vulnerable to secondary pathogens **(Jactel et *al.,* 2023).** Paradoxically, their expansion provides opportunities to study ecosystem adaptation mechanisms, such as the emergence of natural predators (tits, bats) or specific parasitoids **(Branco et *al.*, 2023).**

The estimation of insect population abundance in forests is essential for understanding biodiversity, monitoring ecosystem health, and managing pest outbreaks. Standardized sampling methods such as pitfall traps, light traps, pheromone traps, and sweep netting are commonly used to collect data on insect presence and activity **(Southwood and Henderson, 2000).** For PPM abundance, it can be based on the counting of winter nests, which serve as a reliable indicator of population levels. These silk nests, built at the tips of pine branches during the larval stage, are visible and countable during the winter months when the trees have minimal foliage cover. The number of nests per tree or per hectare can be used as a proxy for larval density and overall population abundance **(Battisti et *al.,* 2015)**. Higher nest densities are often associated with increased defoliation risk and greater threat to both ecological stability and human health due to the urticating hairs of the larvae. Moreover, winter nest abundance provides valuable information for predicting the impact of the moth on forest health and planning targeted control strategies **(Roques et *al.,* 2015)**.

Our estimation for winter nests abundance in Ferdoua forest resulted in a total abundance of 191 winter nests of PPM larvae, with an average of 1,91 nest per tree, an equitability indice of 0,96, and an infestation rate of 78%.

These values are more important comparing to those recorded in 2023 in Ferdoua forest, where a study conducted by Bouketa and Boukhech **(2023)** evaluated the abundance of pine processionary moth population at a total counting of 172 nests, with an average of 1,72 nest par tree and an infestation rate of 75%.

The abundance of the pine processionary moth (*Thaumetopea pityocampa*) in Algerian forests, as estimated by winter nest counts, reveals significant spatial variation influenced by ecological and climatic factors. In Djelfa region, located in the Saharan Atlas, lower nest densities (2.74 ± 2.61 nests/tree) suggest that harsher environmental conditions limit population growth **(Mecheri et *al.,* 2018)**. In contrast, in the El Hamel region (M'Sila), infestation levels were markedly higher in artificial pine plantations (254 nests/100 trees), indicating that monoculture and reforested areas may offer more favorable conditions for moth development **(Derbali and Benzahia, 2021)**. Additionally, studies in northern regions such as Tizi Ouzou and Bordj Bou Arréridj have shown that factors like altitude and canopy orientation affect nest distribution, with higher infestation rates often observed at lower elevations or on sun-exposed slopes **(Habitouche et *al.,* 2017)**.

Facing the growing threat of the PPM, toxicological approaches remain a key control option, despite their environmental challenges. Traditional chemical insecticides remain effective in reducing larval populations but raise serious concerns about their non-target effects on beneficial insects and the global food chain **(Gilioli et *al.*, 2023)**.

Biological pesticides, including vegetable oils and essential oils, are increasingly valued for their role in sustainable agriculture and eco-friendly pest control. Vegetable oils such as neem, olive, and sunflower have demonstrated insecticidal effects by suffocating pests and disrupting oviposition, making them suitable for integrated pest management (**Yessinou et *al.,* 2022)**. Essential oils derived from natural plants like as thyme or eucalyptus, offer a broad spectrum of biological activities, including repellent, ovicidal, larvicidal, and neurotoxic effects against insects **(Regnault-Roger et *al.*, 2012; Kumar et *al*., 2012).** Their variable chemical composition often prevents pests from developing resistance. In addition of their effects, they are rapidly biodegradable and pose minimal toxicity to vertebrates **(Isman, 2020; Nerio et *al*., 2010).**

Several studies have demonstrated the insecticidal potential of bioactive molecules extracted from plants, particularly essential and vegetable oils, against the pine processionary caterpillar

(*Thaumetopoea pityocampa*) **(Pavela and Benelli, 2016; Regnault-Roger et *al.,* 2012).** Essential oils from species like *Thymus vulgaris*, *Origanum onites*, and *Pinus pinaster* have shown high larvicidal activity, with thyme oil achieving up to 100% mortality at 1% concentration due to compounds such as carvacrol, thymol, and γ-terpinene **(Bachrouch et *al.,* 2015; Kordali et *al.,* 2008).** Similarly, essential oils from *Eucalyptus camaldulensis*, *Lavandula stoechas*, and *Cupressus sempervirens* displayed mortality rates ranging from 40% to over 80%, depending on the composition and concentration **(Isman, 2000; Zibaee et al., 2016)**. Forest-derived oils such as wood turpentine and juniper also proved highly toxic to larvae, often outperforming standard commercial oils **(Chiffelle et *al.,* 2013).** Vegetable oils like garlic, sage, and poppy demonstrated considerable effectiveness (95% mortality), although peppermint and lavender oils were less effective **(Mossa, 2016)**. Additionally, endogenous pine volatiles like bornyl acetate were found to increase during infestations, suggesting a natural defense role that could be exploited in biocontrol strategies **(Rossi de Gasperis et *al.,* 2012).**

Lavandula angustifolia essential oil have also shown strong efficacy against *Thaumetopea pityocampa* **(Benelli and Pavela, 2018).** Despite their volatility and short persistence, advancements in nanoformulations and encapsulation technologies are improving their stability and effectiveness in field conditions **(Kumar and Singh, 2019).** *Lavandula angustifolia* is among the most important essential oils due to its insecticidal and repellent properties. A recent comprehensive review found that lavender oil had one of the lowest lethal concentrations (LC_{50}) values against PPM larvae compared to thyme and oregano essential oils **(Tarchoune et *al.*, 2024).**

Under laboratory conditions, *L. angustifolia* essential oil demonstrated significant efficacy against PPM larvae, causing high mortality rates and notable histological damage to the midgut and cuticle **(Zamoum et *al.*, 2020).** More studies have also confirmed its effectiveness against other pests such as the pea leaf weevil, recording 100% mortality at a 1.5% concentration after 72 hours of exposure **(El Fadil et *al.*, 2023).**

Our hydrodistillation process of *Lavandula angustifolia* achieved a yield of 2.90% from 50.00 g of dried plant material, producing 1.45 g of essential oil. Experimental studies on *Lavandula angustifolia* essential oil extraction protocols, showed that the oil yield is influenced by environmental conditions, plant material, and extraction parameters. In **Mississippi, USA,** Zheljazkov et *al.,* **(2013)** found that extending the hydrodistillation time from 20 to 60 minutes increased Lavender oil yield from approximately 0.5% to a maximum of **6.8%**. Danila et al., **(2018)** from **Romania** compared classical hydrodistillation and microwave-assisted hydrodistillation (MAHD), reporting that the traditional method yielded **0.124%,** whereas MAHD improved the yield to **0.6%**. In **South Africa**, semi-industrial scale experiments using the 'Margaret Roberts' variety of *L. angustifolia* reported yields between **4% and 6%,** depending on harvest stage and equipment **(Roberts, 2016).**

Samples of *L. angustifolia* collected from **Batna (Eastern Algeria)**, analysis of *L. angustifolia* essential oil showed important yield and chemical composition **(Bouguezza et *al.,* 2019)**. Studies from **Turkey** and **Iran** have also shown yields between **0.8% and 1.5%,** depending on seasonal and geographic factors **(aydar et *al.,* 2009; Sefidkon et *al.,* 2002).**

More investigations from in Blida (Northern of Algeria) using cultivated *L. angustifolia* and *L. stoechas* reported hydrodistillation yields ranging from **1.17% to 3.9% (Mehaoudi et *al.,* 2021)**. Another study from **Seraïdi** (Northeastern of Algeria) obtained a yield of **1.71%, (Benarfa et *al.,* 2023)**.

The toxicological treatment of pine processionary caterpillars (*Thaumetopea pityocampa*) using three formulations of *Lavandula angustifolia* oil revealed varying mortality levels depending on the oil type, the concentration applied, and the duration of exposure.

Regarding the vegetable oil, the results demonstrated moderate efficacy. After 4 days of treatment, the recorded mortality rates were 14.81% at 1%, 18.51% at 2%, and 22.22% at 3%. These rates increased significantly after 8 days, reaching 37.03%, 48.14%, and 66.66%, respectively, indicating a dose-dependent effect.

In comparison, the prepared essential oil exhibited a much stronger larvicidal activity. After 4 days, the mortality rates were 40% at 1%, 46.66% at 2%, and 60% at 3%. These values significantly increased by the 8th day (55.55%, 70.36%, and 99.99%).

As for the commercial essential oil, larvicidal effects remained modest by comparison. After 4 days, the recorded mortality rates were 13.33% at 1%, 23.33% at 2%, and 43.33% at 3%. By the 8th day, these values increased to 22.22%, 29.62%, and 59.25%. Although a dose-dependent trend was observed, significant differences between doses were only recorded at day 4.

The results of this study demonstrate that the essential oil of *Lavandula angustifolia* obtained by hydrodistillation shows significantly superior larvicidal efficacy against *Thaumetopoea pityocampa* compared to vegetable oil extracted by Soxhlet, achieving mortality rates of up to 99.99% at 3% concentration after 8 days of treatment.

The finding above concluded that all bioactive molecules extracted from *Lavandula angustifolia* using variable protocols possess important larvicidal activities against PPM larvae under laboratory conditions, this can be related to their variable chemical composition, containing for the essential oil of *Lavandula angustifolia* is characterized by high concentrations of monoterpenes, including linalool and linalyl acetate (**Tong et *al*., 2013; Park et *al*., 2016; Benelli et *al*., 2018).**

The prepared essential oil of *Lavandula angustifolia* demonstrated the strongest larvicidal activity against *Thaumetopea pityocampa* (100% of mortality). These results are consistent with previous research highlighting the insecticidal potential of essential oils rich in monoterpenes, which are major components of lavender oil (**Koul et *al*., 2008; Hori, 2004).** More specifically, several studies have evaluated the effects of lavender essential oil on the pine processionary caterpillar. In Algeria, **Saidi et *al*. (2023)** reported significant insecticidal activity of locally distilled lavender oil, while in Tunisia, **Ben Rejeb (2022)** observed mortality rates above 50% after just two days of treatment. In this context Regnault-Roger et *al.,* **(2012)** emphasized that essential oils act quickly through contact or inhalation by disrupting the insect nervous system via neurotoxic mechanisms. **Pavela (2015)** also demonstrated the effectiveness of this oil against lepidopteran species such as *Spodoptera littoralis* and *Cydia pomonella*, with mortality rates exceeding 90% at similar concentrations.

Linalool, often the most abundant component, disrupts the nervous system of insects by inhibiting acetylcholinesterase (AChE), leading to paralysis and death **(Enan, 2001; Regnault-Roger et *al.,* 2012)**. Linalyl acetate enhances the penetration of active compounds through the insect cuticle, increasing their toxic effect **(Bakkali et *al.,* 2008).** Compounds such as 1,8-cineole and camphor contribute additional neurotoxic and repellent activities, often acting synergistically with other constituents **(Koul et *al.,* 2008)**

These chemical compounds have proven their toxicity against many insect pests such as *Aedes aegypti* (mosquito), *Tribolium castaneum* (red flour beetle), *Spodoptera littoralis* (cotton leafworm), and *Sitophilus oryzae* (rice weevil), exhibiting multiple modes of action like neurotoxicity, cuticle disruption, and interference with egg development **(Ben Mbarek et *al.,* 2020; Efil and Kose, 2019; Nerio et *al.,* 2010).**

Numerous global studies have demonstrated a clear difference in the insecticidal efficacy between essential oils and vegetable oils derived from aromatic plants. Our study confirmed this distinction, showing that the essential oil of *Lavandula angustifolia* was significantly more effective than its vegetable oil counterpart in controlling PPM larvae under laboratory conditions.

Our results are conforming to those mentioned by Benhissen et *al.,* (**2020**) which reported that olive essential oil applied to *Spodoptera littoralis* larvae resulted in mortality rates below 30%, reinforcing the view that vegetable oils alone have limited insecticidal potential.

This was also confirmed in the *Eucalyptus globulus* essential oil test, that also show strong dose-dependent activity on *Tribolium castaneum* (Coleoptera) confirming the importance of volatile compounds in insecticidal effects. Although Soxhlet extraction provides higher lipid yields, its inability to extract bioactive terpenes limits its effectiveness **(Kriout et *al.*, 2021).**

Similarly, in mosquito larvicidal tests against *Aedes Aegypti* larvae, essential oils extracted from various natural plants demonstrated LC_{50} values below 50 ppm **(Bakkali et *al.,* 2008)** while vegetable oils often required concentrations above 200 ppm for similar effects **(de Azevido et *al.,* 2021).**

Additionally, Ebadollahi et *al.,* **(2018)** found that freshly extracted essential oils from *Thymus vulgaris* exhibited much stronger toxicity against *Tribolium castaneum* compared to commercial or less concentrated formulations.

This difference is primarily attributed to the chemical composition of essential oils, which are rich in bioactive compounds such as linalool and linalyl acetate, known for their neurotoxic effects on insects **(Regnault-Roger et *al.,* 2012).** In contrast, vegetable oils are composed of fatty acids, particularly linoleic acid (ω-6) and oleic acid (ω-9), as well as γ-tocopherol (0.5-1.2%) **(Sharma et *al.*, 2022; Ngamo et *al.*, 2023; Boukhatem et *al.*, 2020)**, which means that they can induce slower mortality levels through physical mechanisms, limiting their toxicity and effectiveness **(Isman, 2006; Radwan et *al.,* 2016).** Moreover, essential oils retain synergistic interactions among terpenes that enhance toxicity, while vegetable oils lack these interactions due to their simpler composition **(Pavela et *al.,* 2021).**

Whereas the vegetable oil of *Lavandula angustifolia* exhibited moderate larvicidal activity against *Thaumetopea pityocampa*. While the mortality increased in a dose-dependent manner, the overall effectiveness remained significantly lower compared to essential oils. These findings are consistent with previous studies showing that vegetable oils tend to act through physical mechanisms such as blocking the spiracles or disrupting the cuticle rather than inducing neurotoxic effects **(Radwan et *al*., 2016).**

Similarly, research conducted by **Isman (2000)** explained that while some vegetable oils may inhibit feeding or cause suffocation, they typically lack the volatile and bioactive compounds responsible for strong insecticidal action. Therefore, although *L. angustifolia* vegetable oil did demonstrate some toxic effect against *T. pityocampa*, its performance aligns with the broader literature suggesting that vegetable oils are generally less potent and slower-acting than essential oils in pest management strategies.

On the other hand, the freshly prepared essential oil of *Lavandula angustifolia* demonstrated significantly greater larvicidal activity against *Thaumetopea pityocampa* comparing to commercial oil effect. This contrast aligns with previous studies showing that freshly distilled oils retain higher levels of active compounds, which tend to degrade or oxidize during storage and commercial processing **(Pavela, 2015; Regnault-Roger et *al*., 2012)**. Hori **(2004)** further highlighted that fresh lavender oil showed superior insect-repellent properties compared to older batches.

Similar patterns have been observed with other plant species. Ebadollahi et *al.* **(2018)** demonstrated that freshly extracted Thymus vulgaris oil had significantly higher toxicity against *Tribolium castaneum* than commercial thyme oil, due to a greater presence of thymol and carvacrol. Giatropoulos et *al*.**, (2012)** also reported that locally distilled *Origanum vulgare* oil showed stronger larvicidal activity against mosquito larvae than store-bought samples. More recently, **Kaya and Gürer (2024)** found that commercial oregano oils varied greatly in efficacy, often due to dilution or adulteration.

Prepared essential oils extracted in laboratory are often more useful than commercial essential oils in insect control due to their higher purity, freshness, and preserved bioactivity. Laboratory extraction ensures minimal degradation of key insecticidal compounds which are highly effective against insect's pests like **(Isman, 2020; Pavela, 2015; Efil and Kose, 2019).** Unlike commercial oils, which may be diluted, oxidized, or adulterated during processing and storage, freshly prepared oils retain their full chemical complexity, allowing for synergistic interactions between major and minor constituents that enhance their biological and toxicological activity **(Bakkali et *al.,* 2008; Ben Mbarek et *al.,* 2020).**

Essential oils freshly derived from natural plants stand out as the most effective among all extracted bioactive molecules due to their rapid action. Their complex chemical composition rich in monoterpenes and phenolics, allows for multiple modes of action against pests. Compared to other substances, they consistently demonstrate superior efficacy, faster mortality rates, and enhanced potency at lower doses. These characteristics not only make them ideal candidates for environmentally friendly pest control but also reinforce their role as leading natural alternatives to synthetic pesticides.

CONCLUSION

VI-Conclusion:

The study evaluated the efficacy of bioactive molecules extracted from *Lavandula angustifolia* (lavender) against the pine processionary moth *Thaumetopoea pityocampa,* a significant pest affecting Aleppo pine forests in Algeria. The results demonstrated that the freshly prepared essential oil exhibited the strongest larvicidal activity, achieving up to 99.99% mortality at a 3% concentration after 8 days of treatment. This high efficacy is attributed to its rich composition of bioactive compounds, such as linalool and linalyl acetate, which disrupt the nervous system of the larvae. In contrast, the vegetable oil showed moderate effectiveness, while the commercial essential oil displayed variable but generally lower performance, likely due to degradation or adulteration during storage and processing.

The findings highlight the potential of *Lavandula angustifolia* essential oil as a sustainable and eco-friendly alternative to synthetic pesticides for controlling *Thaumetopoea pityocampa* infestations. Its rapid action, high potency, and minimal environmental impact make it a promising tool for integrated pest management strategies. However, further research is needed to optimize application methods, assess field efficacy, and explore synergistic effects with other natural compounds.

This study contributes to the growing body of evidence supporting the use of plant-derived bioactive molecules in pest control, aligning with global efforts to reduce reliance on chemical pesticides and promote ecological balance in forest ecosystems. By leveraging the insecticidal properties of lavender essential oil, forest managers and agricultural stakeholders can mitigate the economic, ecological, and health impacts of the pine processionary moth while preserving biodiversity and environmental health.

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Webography links:

Webography 01: https://www.youtube.com/watch?v=KasORywM7DA.

Webography 02: https://www.monacontureencyclopedia.com/thaumetopea-pitycompa/?lang=en.

Webography 03: www.monaconatureencyclopedia.com.

Webography 04: https://alchetron.com/Bacillus-thuringiensis.

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Webography 10: https://www.pranarom.be/blogs/conseils-experts/tout-savoir-sur-nos-hydrolats-naturels

Webography 11: https://earth.google.com/web/.

Webography 12: https://mapsplatform.google.com/?utm_experiment=13102471.

Webography 13: https://www.pexels.com.