الجمهورية الجزائرية الديمقراطية الشعبية
People's Democratic Republic of Algeria
وزارة التعليم العالي والبحث العلمي
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END OF STUDY MEMORY

With a view to obtaining the Academic Master in Biological Sciences

Speciality: Biodiversity and Environment

THEME

Biological control against *Tribolium castaneum (Herbst, 1797)* using essential oils extracted from some medicinal plants.

Presented by: ASSAS FOUAD

In front of the jury made up of:	**Grade**	**University**
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University year: 2023 - 2024

Acknowledgements

First and foremost, I want to praise and thank Allah Almighty for His abundant blessings upon me. Praise be to Allah and thanks to Allah who made the beginnings easy for me and allowed me to reach the ends. Praise be to Allah who blessed me with health, strength, and determination, and made all my matters easy in all situations. These words of praise are for Allah, who, despite my shortcomings, encompassed my weakness with His mercy. Peace and blessings be upon the noble Messenger of Allah.

Thank you very much to my supervisor, Professor Mouane Aicha, for her encouragement, follow-up, and efforts, and for staying up late to ensure the completion of our thesis in the best possible way.

Thank you very much to my dear family, especially my mother and father, who have been the beating heart and the pump of strength and determination that always pushed me forward. Thank you to my brothers and sisters who have supported me throughout my life, in my work, and in my studies.

Heartfelt thanks to all those people, including administrators, professors, colleagues, and even people I do not know whom Allah made a means of support for me.

Thank you, Douaa, Hajar, Najla, Salima, Mohammed. Thank you, Professor Aliat Sawsan, Gharib Amina, Marabet Soumaya Laouaj and Gharebi Adel. Thank you very much to everyone who lent a helping hand and whom I may have forgotten to mention.

Dedication

Praise be to Allah for providing and easing the means. Praise be to Allah who encompassed my fear and weakness, made the beginnings easy for me, and allowed me to complete this thesis.

To the light of my eyes, to my beating heart, to the one who has accompanied me since my childhood, dressed me, fed me, and tied my shoelaces. To the one who, despite her poverty and weakness, never withheld anything from me. To the one who supported me in my weakness and encouraged me with her tenderness and kind words. To the one who rejoiced in my achievements and success. To the one who always wanted to see me in the highest ranks. To the one who, despite my shortcomings, still shows affection, support, and smiles. To the one who stayed up nights for my sake and checked on me in every situation. To the one who sacrificed her health to care for us.

To my heart, to my dear mother, Louiza Assas, I dedicate this thesis as a token and a humble gift for everything you have done for me. I pray to Allah to heal you, grant you good health, and prolong your life.

To my father, that steadfast and towering mountain, the foundation and support of our home. To the one who dedicated his life to work and sacrificed his health to raise us and provide us with lawful sustenance. To my dear father, who always wishes for our success and high ranks, even if he only shows it when he sees us succeed, his eyes welling up with joy. This thesis is for you, my father, as evidence that, by the grace of Allah, your son has succeeded, with more successes to come, God willing.

To my dear brothers, who have been like a wall I could lean on in times of weakness, who have never withheld anything from me since childhood. To my brothers Zidan, Omar, Walid, and Saleh, thank you for accompanying me throughout my life and for your continuous support. Not forgetting your kind wives, who have fulfilled their duties and more, treating me as if I were their own brother.

To my second mother, my caregiver, and my first teacher, my sister N. To the compassionate one who always rejoiced in my happiness and grieved in my sadness, my sister M. To my kind sister H, who helped me with my studies. To my sister and childhood friend, Z, a special dedication to you for always being attached to me and constantly waiting for my success. I inform you, my sister, that by the grace of Allah, I have succeeded.

To the little ones in the household, the young children, I hope you reach higher and achieve more than I have.

To all my teachers at all levels: Professor Warda Ghannam, Siham, Boukhrufa, Mubarak, Camelia, Mouwaledi, Latifa, Merabet, Linda, Wahida, Souad, Baragdi, Jamila, Nabil, Amin, Anissa, Bassa, Laouj, Mouane, Aliat, Gharib Amina, Khashkhash, Khazzani, Merabet Soumaya, and to everyone whose name my memory may have lost but whose impact remains in my heart.

Summary

The work we conducted involves studying the effect of essential oil from two medicinal plants, *Mentha aquatica* and *Peganum harmala L*, collected from the Reguiba region, El Oued province, on the stored food pest, *Tribolium castaneum*. The essential oil from *Mentha aquatica* was extracted from the aerial parts using a Clevenger apparatus, yielding 2.3%. For *Peganum harmala L*, the fixed oil was extracted from the seeds using a Soxhlet apparatus, yielding 8.13%.

The study was carried out using two methods: direct and fumigation, applying three different concentrations (2.5, 5, and 10µl/ml) with three repetitions for the direct method and two repetitions for the fumigation method. A 100% mortality rate of the insects was recorded for all concentrations and both methods at different times for each studied plant.

For the direct method, a 100% mortality rate was recorded with *Mentha aquatica* oil at concentrations of 2.5µl/ml and 5 after 72 hours, and at a concentration of 10µl/ml after 54 hours. In the same experiment, a 100% mortality rate was also recorded with *Peganum harmala L* oil at 24 hours for concentrations of 2.5 and 5µl/ml, and at 12 hours for the concentration of 10µl/ml.

In the fumigation experiment, a 100% mortality rate was recorded at concentrations of 2.5 and 5µl/ml after 48 hours and at a concentration of 10µl/ml after 36 hours for *Mentha aquatica*. For *Peganum harmala L*, a 100% mortality rate was recorded at 48 hours for the concentration of 5µl/ml and at 36 hours for the concentration of 10µl/ml.

We also calculated the concentration needed to kill 50% of the insects (LD50). For the direct method, we obtained an LD50 of 6.56µl/ml at 18 hours for *Peganum harmala L* and an LD50 of 3.24µl/ml at 54 hours for *Mentha aquatica*. For the fumigation method, the LD50 was 7.33µl/ml at 12 hours for *Peganum harmala L* and 2.45µl/ml at 24 hours for *Mentha aquatica*.

Through this study, we demonstrated the efficacy and toxicity of *Mentha aquatica* and *Peganum harmala* oils against the insect *Tribolium castaneum*, showing their potential as natural pesticides.

Keywords: Essential oil, *Mentha aquatica*, *Peganum harmala*, *Tribolium castaneum*, LD50.

Résumé

Le travail que nous avons effectué consiste à étudier l'effet de l'huile essentielle de deux plantes médicinales, *Mentha aquatica* et *Peganum harmala L*, recueillies dans la région de Reguiba, wilaya d'El Oued, sur l'insecte des denrées stockées, *Tribolium castaneum*. L'huile essentielle de *Mentha aquatica* a été extraite des parties aériennes à l'aide de l'appareil Clevenger, avec un rendement de 2,3%. Quant à *Peganum harmala L* , l'huile fixe a été extraite des graines à l'aide de l'appareil Soxhlet, avec un rendement de 8,13%.

L'étude a été réalisée par deux méthodes : directe et par fumigation, en utilisant trois concentrations différentes (2,5, 5 et 10µl/ml) avec trois répétitions pour la méthode directe et deux répétitions pour la méthode de fumigation. Un taux de mortalité de 100% des insectes a été enregistré pour toutes les concentrations et les deux méthodes à des temps différents pour chaque plante étudiée.

Pour l'expérience directe, un taux de mortalité de 100% a été enregistré avec l'huile de *Mentha aquatica* à des concentrations de 2,5 et 5µl/ml au bout de 72 heures, et à la concentration de 10 au bout de 54 heures. Dans la même expérience, un taux de mortalité de 100% a été également enregistré avec l'huile de *Peganum harmala L* à 24 heures pour les concentrations de 2,5 et 5µl/ml, et à 12 heures pour la concentration de 10µl/ml.

Concernant l'expérience de fumigation, un taux de mortalité de 100% a été enregistré à des concentrations de 2,5 et 5 µl/ml au bout de 48 heures et à la concentration de 10µl/ml au bout de 36 heures pour *Mentha aquatica*. Pour *Peganum harmala L*, un taux de mortalité de 100% a été enregistré à 48 heures pour la concentration de 5µl/ml et à 36 heures pour la concentration de 10µl/ml.

Nous avons également calculé la concentration nécessaire pour tuer 50% des insectes (LD50). Pour l'expérience directe, nous avons obtenu un LD50 de 6,56µl/ml à 18 heures pour *Peganum harmala L* et un LD50 de 3,24µl/ml à 54 heures pour *Mentha aquatica*. Pour l'expérience de fumigation, le LD50 était de 7,33µl/ml à 12 heures pour *Peganum harmala L* et de 2,45µl/ml à 24 heures pour *Mentha aquatica*.

À travers cette étude, nous avons prouvé l'efficacité et la toxicité des huiles de *Mentha aquatica* et de *Peganum harmala* contre l'insecte *Tribolium castaneum*, démontrant leur potentiel en tant que pesticides naturels.

Mots-clés : Huile essentielle, *Mentha aquatica*, *Peganum harmala L*, *Tribolium castaneum*, LD50.

الملخص

يتمثل العمل الذي قمنا به في دراسة تأثير الزيت الأساسي لنبتتين طبيتين من نوع *Mentha aquatica* و *Peganum harmala L* اللتين تم التقاطهما من منطقة الرقيبة، ولاية الوادي، على حشرة تصيب المواد الغذائية المخزنة *Tribolium castaneum*. حيث تم استخراج زيت أساسي من نبتة النعناع المائي *Mentha aquatica* انطلاقاً من الأجزاء الهوائية باستخدام جهاز Clevenger ، وتحصلنا على مردود 2.3%. أما بالنسبة لنبتة الحرمل *Peganum harmala L* فقد تم استخراج الزيت الثابت انطلاقاً من البذور بواسطة جهاز Soxhlet ، حيث تحصلنا على مردود يساوي 8.13%.

تمت الدراسة بواسطة طريقتين: مباشرة وطريقة الشم، حيث قمنا باستعمال 3 تراكيز مختلفة (2.5، 5، و10µl/ml) وبتكرار 3 مرات بالنسبة للطريقة المباشرة وتكرارين بالنسبة لطريقة الشم وتم تسجيل نسبة وفاة 100% افراد الحشرة في الطريقتين لجميع التراكيز في أوقات مختلفة لكل من النبتتين المدروستين .

بالنسبة للتجربة المباشرة، تم تسجيل عدد الوفيات بنسبة 100% عند إضافة زيت نبات *Mentha aquatica* عند التركيز 2.5 و5 µl/ml في الساعة 72، وعند التركيز 10µl/ml في الساعة 54. وفي نفس التجربة، فقد تم أيضاً تسجيل نسبة وفاة 100% عند إضافة زيت *Peganum harmala L* في الساعة 24 عند التركيز 2.5 و5µl/ml وفي الساعة 12 عند التركيز 10µl/ml .

وفيما يخص تجربة الشم، فقد تم تسجيل نسبة 100% في التركيز 2.5 و5ملل عند الساعة 48 وفي التركيز 10ملل عند الساعة 36 لنبات *Mentha aquatica*، ويقابله تسجيل 100% ل *Peganum harmala L* في الساعة 48 عند التركيز 5µl/ml وفي الساعة 36 عند التركيز 10µl/ml .

كما قمنا أيضاً بحساب التركيز اللازم لقتل 50% من افراد الحشرات، حيث تحصلنا في التجربة المباشرة على LD50 يساوي 6.56 µl/ml عند الساعة 18 بالنسبة لنبات *Peganum harmala L* وLD50 يساوي 3.24 µl/ml عند الساعة 54 لنبات *Mentha aquatica*. أما فيما يخص تجربة الشم، فتحصلنا على LD50 يساوي 7.33 µl/ml عند ساعة 12 لنبات *Peganum harmala L* وLD50 يساوي 2.45 µl/ml عند ساعة 24لنبات *Mentha aquatica*.

ومن خلال دراستنا هذه، فقد تم إثبات فعالية وسمية زيت *نبات Mentha aquatica* و *Peganum harmala* ضد حشرة *Tribolium castaneum* وإمكانية استخدامهما كمبيدات حشرية طبيعية.

الكلمات المفتاحية : زيت أساسي , *Mentha aquatica, Peganum harmala L ,Tribolium castaneum*, LD50

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List of abbreviations

- **%: Percentage**
- **°C: Degree Celsius**
- **µl: Microliter**
- **cm: Centimeter**
- **DL50%: Lethal dose 50%**
- **FAO: Food and Agriculture Organization**
- **g: Gram**
- **h: Hour**
- **d: Day**
- **Kg: kilogram**
- **M: Mass of the plant in grams**
- **Mhe: Mass of essential oil in grams**
- **M0%: Observed mortality after spraying**
- **Mc%: Corrected mortality**
- **Min: minute**
- **ml: milliliter**
- **mm: millimeter**
- **Rdt (%): Yield of essential oil in (ml/g)**
- **RHE: Essential oil yield**
- **T: Temperature**
- **EO: Essential oil**
- **G: gram**
- **ml: milliliter**
- **°C: degrees Celsius**
- **pH: Hydrogen potential**
- **DL: Lethal doses**
- **DL50%: Lethal concentration that kills 50%**
- **WHO:World Health Organization**

Introduction

Introduction

Cereals occupy the first place in terms of agricultural land exploitation, as they are considered a staple food for a large part of the world population, In Algeria and throughout North Africa, These crops are the main source of agricultural production and attract many processing activities in the milling, baking and food industries. They are also the basis of the diet and play a major role in the eating habits of populations (Boulal et al., 2007).

However, the cultivation of cereals is threatened by various factors, both biotic and abiotic, such as pests, which, in addition to their destructive effect, are also capable of carrying pathogens (Aymen , 2005).

Due to their effectiveness and ease of application, chemical pesticides have been the most common solution to deal with this problem. However, the intensive and unregulated use of these pesticides has resulted in considerable pollution of the food chain. In addition, the widespread diffusion of these substances has led to the emergence of resistant insect strains (Leonard and Ngamo, 2004).

Faced with the growing health and environmental risks associated with chemical pesticides, the World Health Organization (W.H.O) has been forced to prohibit some of these products due to the rate of losses resulting from their use, about 35% of the world's cereal production. As a result, most countries have adopted new, more environmentally friendly methods to limit the use of chemicals. In this context, much recent research has focused on the discovery of substances with insecticidal properties while being safe for human health and the environment.

In order to reduce dependence on chemical pesticides, which have been proven to be dangerous for both humans and the environment, modern technology has turned to biological pest control, which combines efficiency and selectivity (Azaizeh et al., 2002). Many recent studies have focused on the isolation or identification of secondary compounds extracted from plants with insect activity (Ndomo et al., 2009). Several researchers (Regnault-Roger et al., 2008; Glitho et al., 2008; Arnason et al., 2008) demonstrated that plant extracts have many properties that make them suitable for alternative strategies to reduce the use of synthetic pesticides in agriculture and eliminate pests.

Plants naturally produce active substances such as essential oils, which protect them from insects, diseases and external attacks. Currently, essential oils play a crucial role in control systems, their importance in plant protection research is widely recognized in several countries of the world. Naturally occurring substances, including essential oils, are therefore a promising alternative for pest control (Lahlou, 2004).

This study focuses on evaluating the effect of the essential oils of two medicinal plants, *Peganum harmala* and Mentha aquatica, on the insect *Tribolium castaneum*.

Our manuscript is divided into two parts:

- The first part consists of two chapters. The first chapter on a general introduction to medicinal plants, essential oils and studied plants (*Peganum harmala* L and *Mentha aquatica*). The second chapter is devoted to the insect *Tribolium castaneum*.

- The second part includes a first chapter that explains the materials and methods used in the different experiments and protocols applied during the tests, and a second chapter containing the main results obtained and their discussion. Finally, the study concludes with a general conclusion.

Theorecal part

Chapter 1.-

General Informations

Chapter 1.- General informations

1.1.- Medicinal plants

A medicinal plant is any plant with properties that act on the human or animal organism in a beneficial way. Medicinal plants are used in natural medicine. Generally, only part of the plant is used, whether the bulb, roots, leaves, seeds, fruits or flowers. The branch of medicine that uses medicinal plants is called herbal medicine. Some of the most common active ingredients in medicinal plants include polyphenols, terpenes, steroids and alkaloids (Gineste, 2010).

For thousands of years, humans have used plants found in nature to treat and cure diseases (Sanagor, 2006).

According to the World Health Organization (W.H.O) (2003), about 65-80% of the population uses traditional medicine (Mabry and Ulubelen, 1980).

Currently, medicinal plants remain the first reservoir of new drugs. (Maiza et al.,1993; Maurice Nicole, 1997**).**

1.2.- Peganum harmala L

Peganum harmala L belongs to the family Zygophyllaceae. The plants belonging to this family, are very recognizable by the appearance of its grasses, shrubs, or trees. In the classification of Cronquist (1981), these plants constitute a family with about 285 species, which are subdivided into five subfamilies and 27 genera. They are widely distributed in arid, semi-arid regions, salt fields, and desert pastures (Asgarpanah and Ramezanloo, 2012). The genus Peganum takes its name from the Greek and is attributed to street species, while the name of the species harmala derives from that of the Lebanese city Hermel (Mars, 2009).

Peganum harmala L is a herbaceous plant, perennial, glabrous, bushy, 30 to 100 cm high, with thick rhizome, its strong, unpleasant smell reminiscent of that of the Street (Iserin, 2001).

Figure 1. Picture of *Peganum harmala L* (original,2024).

1.3.- Classification system

According to Cronquist (1981), the taxonomic position of the Harmel is in (Tab.1) as follows:

Table 1. Classification system of *Peganum harmala L* (Cronquist , 1981).

Kingdom	Plants
Subkingdom	**Tracheophyta**
Division	**Magnoliophyta**
Class	**Magnoliopsida**
Subclass	**Rosids**
Order	**Sapindales**
Family	**Zygophyllaceae**
Genus	**Peganum**
Species	***Peganum harmala . L., 1753***

1.4.- Répartition géographique

This plant is widely distributed throughout the world. It is prevalent in arid and dry Mediterranean areas in sandy and slightly nitrated soils. (Iserin, 2001).

• In Europe it is very common in dry areas, from Spain to Hungary to the steppes of southern Russia.

• In Asia, it is widespread in the steps of Iran and Turkestan to Tibet.

• In the United States it is found in Arizona and Texas or called "Mexican Street".

•In Africa, it is abundant in the arid Mediterranean areas of the Middle East to the north of Africa (Tunisia, northern and central Sahara in altitude, Algeria's Upper Plateaux and Orania, eastern Morocco) (Hammiche et al.,2013).

• In Algeria, *Peganum harmala L* Is common in the high plateaux, northern and southern Sahara, and in the mountains of the central Sahara. *Peganum harmala L*. Is renowned for sandy terrain, in oak beds and inside agglomerations (Ozenda, 1991).

1.5.- Botanical description of the plant Peganum harmala L

Peganum harmala L. Is a 30-80 cm tall herbaceous plant with thick rhizome, their roots are short creeping. This plant has round seeds (Mouloudizargari et al.,2013) and yellowish white flowers (Fig.2) (Tahri et al.,2004), its strong, unpleasant smell seems to be that of the street (Ghaouas, 2014).

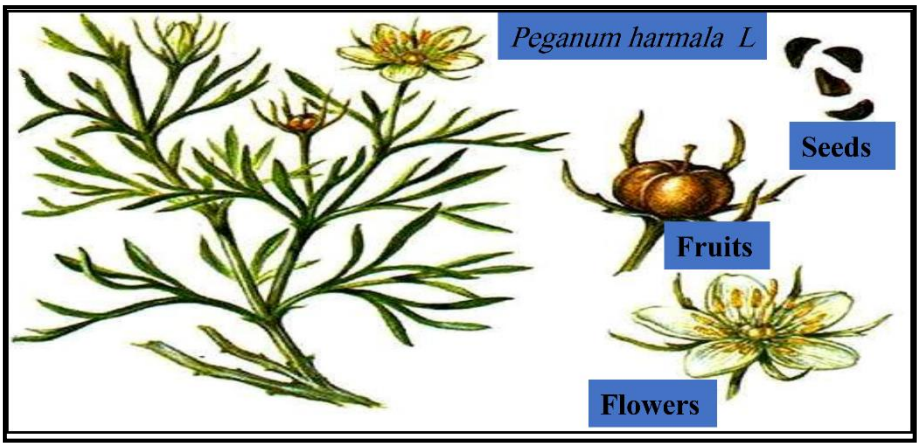


Figure 2. Parts of the species *Peganum harmala L* (Preedy et al., 2011).

1.5.1.- The roots

Are oblong, hard and rich in fiber, there are two branches: main central root and secondary roots (Doumi, 1993).

1.5.2.- Stems

Erect and very rowdy,with alternating leaves in small strips (Fig.3) (Hammiche and Merad, 1990).

Figure 3. The stems of *Peganum harmala L* (Weckesser, 2013).

1.5.3.- The leaves

Very thin, can reach 5×5 cm lengthy with glauque green coloration (Fig.4) (Dahel and Massaoudi, 2019) .

Figure 4. The leaves of *Peganum harmala L*(Weckesser, 2013).

1.5.4.- Flowers

Large solitary flowers from 25 to 30 mm (Fig.5) (Tahri et al., 2004) . According to Ozenda (1958), they are formed by: Five green and linear sepals that exceed the corolla, five oval petals and ten to fifteen stamens. They have globular fruits that consist of round seed capsules containing more than 50 seeds (Mouloudizargari et al.,2013).

Figure 5. The flower of *Peganum harmala L* (Asgarpanah and Ramezanloo, 2012).

1.5.5.- Fruits

Spherical capsules with 3 valves,with a diameter of 6 to 10 mm (Fig.6) (Yousefi et al.,2009). The fruits move away from the release of triangular seeds (Dahel and Messaoudi, 2019).

Figure 6. The fruits of *Peganum harmala L* (Asgarpanah and Ramezanloo, 2012).

1.5.6.- Seeds

Dark brown, small in size and subtriangular in shape with a diameter of 3 to 4 mm 2 mm (Tahri et al.,2004). The seeds are harvested especially in summer, their capsules contain a reddish pigment called Turkey red (Iserin, 2001).

Figure 7. The seeds of *Peganum harmala L* (Asgarpanah et al.,2012).

1.6.- Uses of *Peganum harmala L*

Peganum harmala L, commonly known as Harmel, has a rich history of traditional use in various cultures for its medicinal and cultural significance. Among its medicinal applications, it has been noted for its potential in treating a range of conditions, including Parkinson's disease, diabetes, high blood pressure, and intestinal infections. These therapeutic properties have been documented in several studies, highlighting its importance in folk medicine (Leporatti, 2009; Hammiche et al.,2013).

In addition to its medicinal value, Harmel serves culinary and cultural purposes. It is used as an analgesic and as a flavoring agent in gastronomy, adding its distinctive taste and aroma to dishes (Mina et al, 2015; Bakiri et al.,2016). Furthermore, its role in traditional practices

extends to the management of childhood convulsions through fumigation and the treatment of rheumatism and fever with external ointments derived from the plant (Aouadhi, 2010).

Culturally, *Peganum harmala* holds significance in Saharan regions, where it is revered as a symbol of good fortune. Bouquets made from its flowers adorn the thresholds of houses, believed to bring luck and ward off evil spirits (Hammiche et al.,2013). This cultural association reflects the deep-rooted beliefs and customs surrounding the plant in these communities.

Beyond its medicinal and cultural uses, *Peganum harmala* also finds application in crafts and industries. The red dye extracted from its seeds is prized for its vibrant color and is used in the dyeing of textiles, particularly in Turkey and Iran, where it adds a distinctive hue to carpets and fabrics (Baytop, 1999).

In summary, *Peganum harmala L* exemplifies the intricate interplay between medicinal, culinary, and cultural practices, underscoring its multifaceted significance in various aspects of human life and society.

1.7.- *Mentha aquatica L* (Water mint)

It is a moisture-loving perennial aquatic plant that thrives in the rich soils along rivers, streams, and other water sources. Its lush green foliage forms dense mats on the water's surface, providing habitat and shelter for various aquatic organisms (Fig.8). This plant plays a crucial role in stabilizing riverbanks and preventing erosion. However, its rapid growth can lead to ecological imbalances, requiring careful management strategies. Efforts are underway to control its spread while preserving its ecological benefits. (Schanzer et al., 2012).

Figure 8. Picture of espèce *Mentha aquatica* (Mozaffariane, 1996).

1.8.- Botanical description

It is a perennial plant with long rhizome, crawling, tracing, hairy. The stem, from 50 to 80 centimeters, erect or ascending, divides into opposite branches. Its leaves are 4 to 10 cm long, they are oval, opposite, short petiolated, lanceolate, acute, toothed, are of a very beautiful green and are tinted with reddish shades in the sun and copper red in the shade, they are covered with large, rounded secretory hairs in which volatile odorous substances accumulate (Benayad, 2008).

The flowers, purplish, form very short, ovoid spikes at the end of the twigs. The fruit, divided into four parts, is surrounded by a persistent calyx. It has a powerful, pungent and refreshing flavour (Jahandiez et al., 1934).

1.9.- Classification

The classical classification of the plant *Mentha aquatica*, is presented in (Tab.2)

Table 2. Botanical classification of *Mentha aquatica* (Benayad,2008).

Kingdom	**Plants**
Subkingdom	**Plantae**
Division	**Spermatophytes**
Class	**Angiosperms**
Subclass	**Magnoliopsida**
Order	**Gamopetals**
Family	**Lamiales**
Genus	**Lamiaceae**
Species	**Mentha**
Kingdom	***Mentha aquatica L, 1753***

1.10.- Geographical distribution

Most mints originate from Europe, Asia and North Africa. However, by following migration flows, mints are present on the quasitotality of continents (Fig.9)(Tucker and Naczi,2007) .

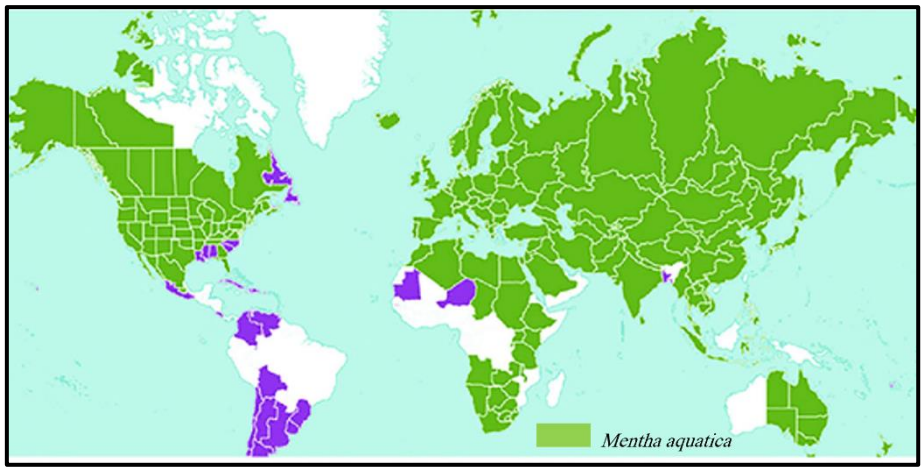


Figure 9. Geographical distribution of *Mentha aquatica* (Tucker and Naczi ,2007).

1.11.- Chemical composition

The analysis of Andro et al. (2013) of plants harvested in the southwest of Romania identified 41 substances in varying proportion according to the phase of the cycle: vegetative, flowering or senescence, this composition is represented in (Tab.3).

Table 3. Composition of the essential oil of *Mentha aquatica* according to vegetative phasesand flowering (Andro et al., 2013).

Phase	Composé	Pourcentage (%)
Vegetative	Menthofuran	58.59
	Limonene	9.91
	Trans-β-Ocimene	5.59
	β-Caryophyllene	3.55
	Ledol	3.29
Flowering	Menthofuran	51.26
	Limonene	12.06
	Trans-β-Ocimene	8.10
	β-Caryophyllene	2.92
	Ledol	3.01

Several other analyses of aquatic mint in other locations have shown similar patterns, generally with the majority of menthofuran. However, other chemotypes have been observed such as the one analyzed by Akbar et al. (2006).

Phytochemically, the results obtained by Benomari (2014) show a rich and varied composition of secondary metabolites, surprising to show that:

• The plant has a high content of sterols and steroids, tannins and coumarins.

• Anthocyanins also marked their presence in the two extracts tested (ethanolic and aqueous).

• The use of hexanic extract in these tests showed the presence of fatty acids and a residue indicating the presence of volatile molecules.

• Alkaloids and flavonoids particularize the aqueous extract at a modest rate.

The results of the analysis of the determination of the percentage of water revealed a rate of 79.39%, which gives the plant a very powerful wet character (Benomari, 2014).

1.12.- Uses of Mentha aquatica

Aquatic mint leaves are analgesic, antiseptic, antispasmodic, astringent, carminative, choleretic, sudoriparous, anti-vomiting (Benin and Redouani ,2019)

it is made into tea in traditional leaf medicine and is used to treat fever, headaches, digestive disorders and minor nutritional disorders (Benomari,2014).

It is used as a mouthwash and can be gargled to treat throat infections, canker sores and bad breath (Batsatsashvili et al., 2017).

Mentha aquatica leaves are harvested before and during the flowering process, dried and stored for future use. (Bown, 1995).

Leaf essential oil is generally considered an antiseptic, and the latter becomes toxic if taken in high doses (Foster and Duke, 1999).

In South Africa, the dried leaves of *Mentha aquatica* are burned with Tagetes minuta and the smoke is inhaled to treat mental illnesses by the Venda people (Arnold et al.,1984).

This plant is also used in traditional medicine in South Africa to treat colds, respiratory problems and to protect against bad spirits (Pooley, 2005).

1.13.- General information on essential oils

1.13.1.- Definition

Essential oils are mixtures of many compounds that are not complex molecules such as terpenes, phenols, methyl ethers, oxides, esters, ketones, etc. They are produced by plants as a defence against plant pests (Taleb-Toudert , 2015).

According to Bouchikhi-Tani (2011), essential oils, also called essences, are mixtures of aromatic substances produced by many plants.

The term "oils" comes from their property of being soluble in fats, while the term "essential" refers to the odor released by the producing plant (Bouyahya et al.,2018).

The EO have a significant antiseptic activity since antiquity, they are used in many fields: pharmaceutical, cosmetic, and food (Sadou et al.,2015).

A EO is defined as the product obtained from a plant or parts thereof by hydrodistillation, steam distillation, dry distillation or by an appropriate mechanical process without heating (for example, citrus) (Labiod, 2016), and are extracted from aromatic plants by different techniques (Bouhdid et al., 2012).

EOs are liquids, volatile, clear and colored, they are soluble in lipids and organic solvents that have a lower density than water. And are usually stored by the plant in secretory cells, cavities, canals, glandular trichomes or epidermal cells (Bouyahya et al., 2017), and characterized by a strong odor (Labiod, 2016).

1.13.2.- Distribution and location

Essential oils exist almost exclusively in higher plants. The genera capable of developing these volatile principles are spread over almost sixty families. Lauraceae, Apiaceae, Lamiaceae, Astéraceae are among the most important vegetable families producing essential oil (Zeghib, 2013).

These species can be located in all plant organs (Liolios and Col, 2009), in the same plants, these oils can exist both in different organs (flowers, leaves, barks, woods, roots and rhizomes), where the chemical composition may vary from one organ to another. These aromatic essences are developed by secretory glands that are found on almost the entire part of the plant (Bouchikhi-Tani, 2011).

Table 4. Location of essential oils in plant organs (Samate,2002).

Organ	Genus	Species
Root	**Vetiver**	***Vetiveria zizanioides Stapf***
Rhizome	**Ginger**	***Zingiber officinalis Roscoe***
Bark	**Cinnamon**	***Cinnamomum zeylanicum Blume***
Wood	**Camphor tree**	***Cinnamomum camphora Sieb***
Stem	**Rosemary**	***Rosmarinus officinalis L***
Leaf	**Mint**	***Mentha piperita L***
Needle	**Pine**	***Pinus sylvestris L***
Flower	**Clove**	***Syzygium aromaticum L***
Seeds	**Nutmeg**	***Myristica fragrans Houtt***
Fruit	**Bitter orange**	***Citrus aurantium L***

1.13.3.- Physicochemical properties of essential oils

1.13.3.1.- Chemical properties

Essential oils are complex and highly variable mixtures of constituents that belong to two groups characterized by distinct biogenetic origins: the terpenenoid group on the one hand and the aromatic compounds derived from phenylpropane group, much less common on the other hand (Labiod, 2016).

1.13.3.2.- Physical properties

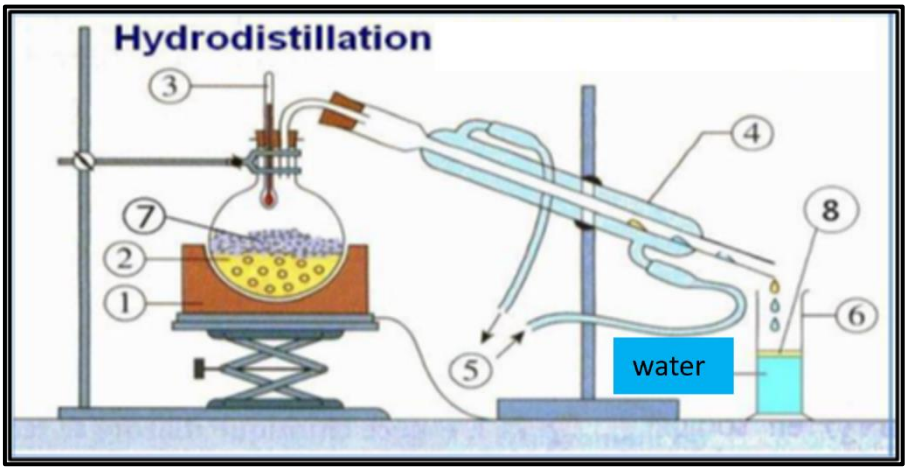
Liquids at room temperature, essential oils are volatile, which differentiates them from fixed oils. Their density is generally lower than that of water. They have a high refractive index and most deflect polarized light. Soluble in the usual organic solvents, they are liposoluble. They can be driven by water vapour and are very poorly soluble in water (Labiod, 2016).

Their colors are varied, many are yellowish or colorless but the colors can range from yellow green to brown red. They are very fragrant (Chabert, 2013).

1.14.- Essential oil extraction techniques

1.14.1.1.- Extraction by hydro-distillation

This is the most widely used method of extracting essential oils. The plant is brought into contact with water in a flask and brought to a boil. The vapours are condensed in a refrigerant and the oils separate from the water by difference in density (Bouhaddouda , 2016).



1: Hot Water Tank; 2:Boiling Water; 3:Thermometer; 4:Water Cooler; 5:Cold Water Inlet and Cool Water Outlet; 6:Essencier; 7:Vegetable; 8:Essential Oil.

Figure 10. Hydro distillation (Jouault, 2012).

1.14.1.2.- Steam drive

Steam training is a more recent variant of distillation, in which there is no direct contact between plant matter and water. The water vapour is produced in a separate boiler and then injected into the base of the still in which the plant is located. The steam rises in the still and passes through the plant. The water vapor thus discharged of gasoline returns to the liquid state by condensation. As a result, the product of distillationseparates into two distinct phases: oil and condensed water, known as eauflorale or hydrolisa (Lucchesi, 2005).

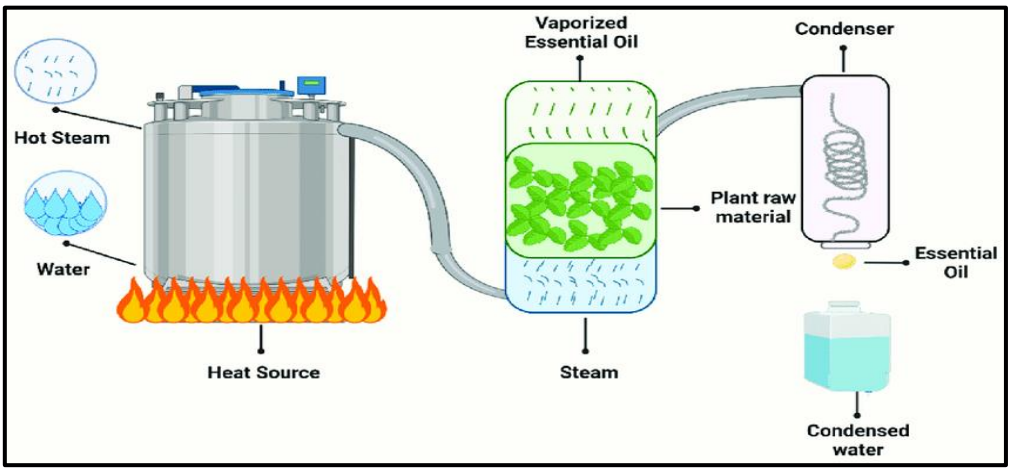


Figure 11. Mechanism of steam-driven distillation (Kilani, 2019).

1.14.1.3.- Extraction by volatile organic solvents

The most commonly used solvents are aliphatic carbides (pentane, hexane) or aromatic carbides (benzene). It is usually operated at the usual temperature and is also used in the perfume industry (Djahra , 2010).

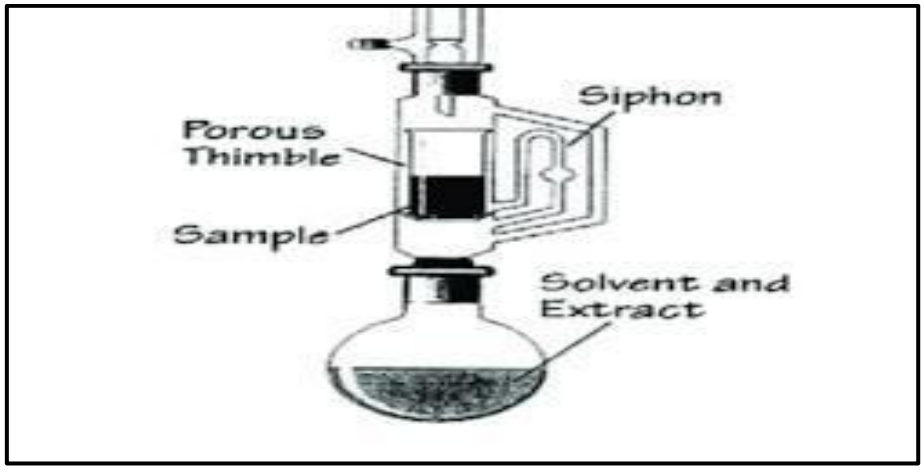


Figure 12. Extraction by volatile solvent (Jerry Louis, 2020).

1.14.2.- Biological activities of essential oils

1.14.3.- Antioxidant activity

The antioxidant power of essential oils is developed as a substitute in the food preservation. Phenols and polyphenols are mainly responsible for this power (Laib,2011).

1.14.3.1.- Antibacterial activity

The action of EOs on microbial proliferation is done through the alteration of the membrane permeability of bacteria by disrupting ion transport systems; electron transport and energy production. EO's mode of action depends on the type of microorganism (Saimi, 2014). In general GRAM- bacteria are more resistant than GRAM + thanks to the structure of their internal membrane (Ouis, 2015)

1.14.4.- Antifungal activity

The antifungal power of aromatic plants EOs against mold allergens and against pathogenic and opportunistic fungi such as Candida albicans (Saimi, 2014).

1.14.4.1.- Antiviral activity

Viruses are generally highly sensitive to aromatic molecules of EOs tell than monoterpenols and monoterpenals (Saimi ,2014). Many severe viral pathologies treated with EOs (Zenasni, 2014)

1.14.4.2.- Insecticide activity

Plants can provide potential alternatives to commercial insect control agents because they are a rich source of bioactive chemicals such as polyphenolic compounds, phenolic acids and flavonoids that cause disturbance of the natural mortality of the insect, the toxicity of polyphenols is positively correlated with the attractiveness of these compounds (Regnault-Roger and Hamraoui (1995).

The insecticide effect of EOs by contact, ingestion and fumigation has been well demonstrated against predators of stored foods (Isman, 2000).

The insecticidal activity of some EOs such as mint, thyme, sage, rosemary.. etc. These essential oils are very effective against a few insects including: *Rhyzopertha domonica* ; *Sitophylus oryzae* and *Tribolium castaneum* (Benazzeddine , 2010). The latter is a pest of ground cereal products such as flour and cereals (Shaaya and Kostyukovysky, 2006).

1.14.5.- Toxicity of essential oils

Essential oils contain thousands of components they are very effective, but also very dangerous (Kesbi , 2011). While it is important to emphasize that frequent and abusive self-medication especially with regard to dosage and duration as well as the mode of internal or

external application is harmful (Ouis, 2015). From the skin, toxicity affects different physiological systems of the body varies from acute to chronic in animals.

Common use EOs have low or very low oral toxicity with LD50 greater than 50g/Kg . With respect to Savory and Oregano. Toxicity is slightly higher around 1.4g/Kg (observed in animals) (Heni, 2016). The toxic effects are very variable from one essential oil to another and depend a lot on the sensitivity of consumers (Labiod, 2016).

Thymus L and *Lavandula angustifolia* EOs are cytotoxic to Chinese hamster cells, and *Origanum vulgare* EOs have shown strong cytotoxicity on human cancer-derived cells (Heni, 2016).

1.14.6.- Preservation of essential oils

Essential oils are very delicate substances and are easily altered, making their preservation difficult. The risks of degradation are multiple: photoisomerization, photocyclization, oxidative cutting of propenylphenols, carbide peroxidation and decomposition into ketones and alcohols (limonene) (Bruneton, 1999). These degradations can change their properties if they are not enclosed in clean, dry aluminum, stainless steel or tinted glass vials, away from light and heat (Bruneton, 1999).

Chapter 2.-

General informations on

Tribolium castaneum

Chapter 2.- General information on *Tribolium castaneum*

2.1.- General description

It is an insect belonging to the family Tenebrionidae. The adult measures from 3 to 4 mm, uniformly reddish brown in color. It is narrow, elongated, with parallel edges, with pronotum almost as wide as the elytra and not previously edged. The last 3 antenna items are significantly larger than the others. The larva measures about 6mm, 8 times longer than wide, of a very pale yellow at maturity, with laterally a few short yellow bristles (Fig. 13). The cephalic capsule and dorsal surface are slightly reddish (Camara, 2009).

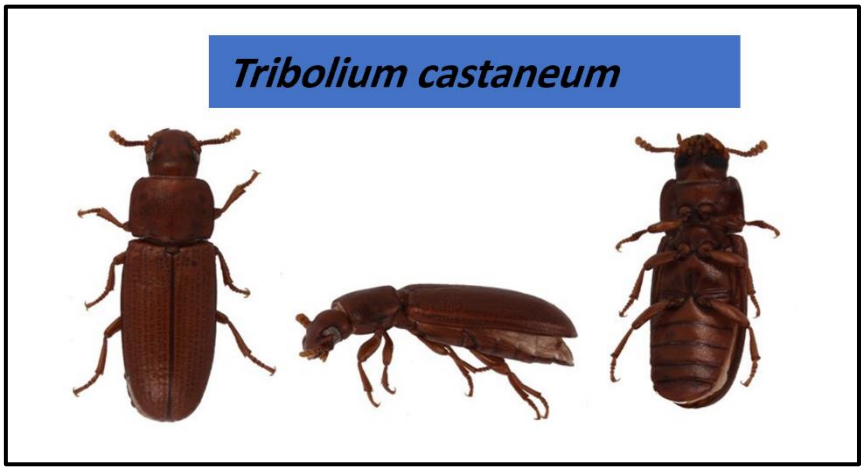


Figure 13. Picture of *Tribolium castaneum* (Loboda, 2012).

2.2.- General characters of the *Tribolium castaneum*

According to Telli and Bengaurina (2021), Tenebrionidae are one of the largest families of Coleoptera that contain the most described species. The larvae are cylindrical in shape and the adults, which are usually dark in color, have a wide variety of aspects. They are generally classified as the species most harmful to cereal stocks. The diet of Tenebrionidae is above all saprophage, some of the species mentioned as harmful to stored products. Among these insects the genus Triboluim includes two main cosmopolitan and harmful species: *Tribolium castaneum* and *Tribolium comfusum* (Delobel and Tran, 1993).

2.3.- Description of the different stages of development

2.3.1.- Eggs

The eggs are whitish or colorless and their size is about 5mm, with food particles adhering to the surface (Fig. 14) (Godon and Wilim, 1998).

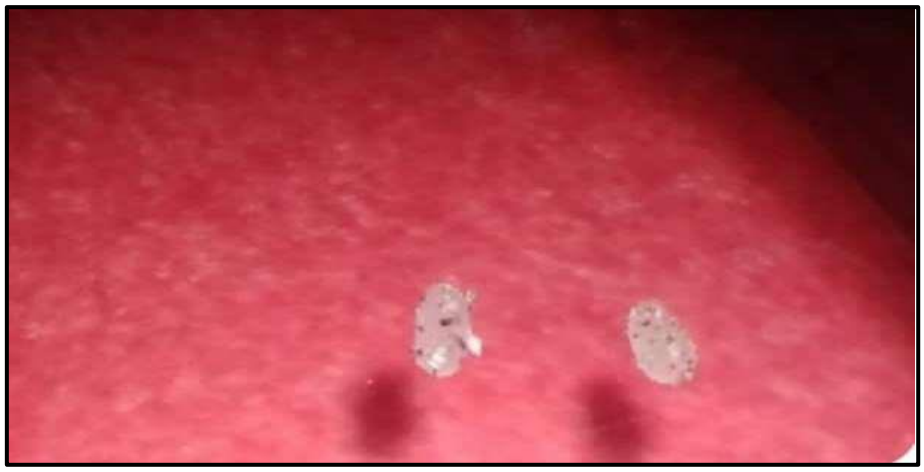


Figure 14. Eggs of *Tribolium castaneum* (Godon and Wilim, 1998).

2.3.2.- Larve

The larvae are vermiform and have legs at the end of the last abdominal segment and a pair of short appendages, the «Urogomphs». The larvae are 6 mm, about 8 times longer than wide, very pale yellow at maturity, with a few short yellow bristles laterall (Fig. 15) , The cephalic capsule and dorsal face are slightly reddish (Godon and Willm, 1998).

Figure 15. The larval stage of *Tribolium castaneum* (Knorr et al.,2009).

2.3.3.- Nymph

The nymph has a cylindrical shape. It is whitish in colour turning yellow (Fig.16).The terminal abdomen has two spines (Christine, 2001).

Figure 16. The evolution of nymphs of *Tribolium castaneum* according to (Knorr et al.,2009).

2.3.4.- Grown-up

According to Christine (2001), the adult description of *Tribolium castaneum* (Fig.17) is:

The length is from 3mm to 4mm. Colour: uniformly reddish brown. It is narrow, elongated, with parallel edges.

The head and upper part of the chest are covered with tiny punctures. Wings and elytra are striated along their entire length. The anterior and middle tarsi have 5 joints, while the posterior tarsi have only four. The integuments are almost always very robust and dark. The eyes of red color. The terminal abdomen has two spines (Christine, 2001).

Figure 17. Adult of *Tribolium castaneum* (Loboda, 2012).

2.4.- Taxonomic Classification

According to (Haines, 1991; Bolev, 2014; Myers et al., 2016) *Tribolium castaneum* classification (Tab.5) is as follows:

Table 5. The *Tribolium castaneum* Classification (Haines, 1991 ; Bolev, 2014; Myers et al.,2016).

Kingdom	**Animalia**
Phylum	**Arthropoda**
Subphylum	**Hexapoda**
Class	**Insecta**
Order	**Coleoptera**
Superfamily	**Tenebrionoidea**
Family	**Tenebrionidae**
Subfamily	**Tenebrioninae**
Genus	**Tribolium**
Species	***Tribolium castaneum* (Herbst, 1797)**

2.5.- Origin and geographical distribution

Tribolium castaneum is found in all parts of the world (Cosmopolitan). It exists where stored cereals exist in the form of grains or wheat flour. It is very abundant in tropical regions. In cold climates, it is present only in high temperature storage (Christine,2001).

2.6.- Life cycle

Larvae and adults feed on broken pits. The development of the egg to the adult ends in 28 days, when the conditions of temperature and humidity are optimal. At low humidity (8%), development develops more slowly (Dave et al.,2001). From the age of three days, females lay about ten eggs a day, which hatch at about 30°C after five days. Eggs are laid freely on the seeds and are difficult to detect. Larvae circulate freely in infested food and without nymphs. At 30°C, larval life lasts about three weeks and the adult leaves the fairy six days after its formation (Kassimi, 2014). The average adult millet egg development time is 37 days at 25°C, 26 days at 28°C, 23 days at 35°C and 38°C . Depending on the diet, the cycle time can be up to 120 days at temperatures between 35°C and 38°C . The average shelf life is 250 days at 25°C, 200 days at 30°C, 2 to 3 months at 35°C for wheat grains, more than one year for flour

(maximum observed: 4 years) (Delobel and Trane, 1993). Females lay between 500 and 800 eggs (Kassimi, 2014).

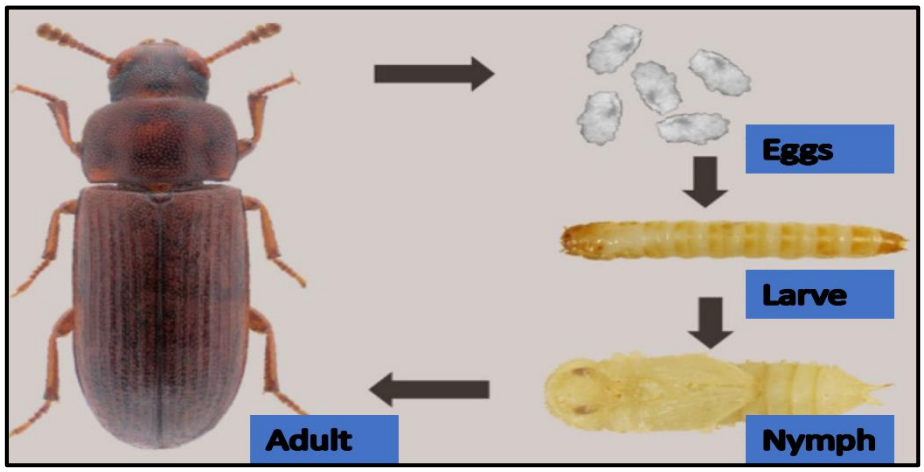


Figure 18.*Tribolium castaneum* life cycle (Chenni, 2016).

2.7.- Damage and Distribution

Tribolium mainly seeks starchy powdery foods such as cereal flours, rice etc Fig xx. (Lepesm,1944). Adults and larvae feed primarily on broken kernels, attack damaged kernels, and thrive on seed germs (Robiche et al.,2002).According to Campbell and Hagstrum (2002), Its movement and dispersion in nature are favoured by several factors such as in (Tab.6):

Table 6. several dispersion factors (Campbell and Hagstrum ,2002)

Insect density	Nutritional quality
The age of insects	Heritability (genetic factors) of dispersion

The response to volatile food substances and aggregation pheromones. Feeding of granivorous insects at the expense of grains causes quantitative damage such as loss of appreciable dry matter, and alterations in the stock that cause qualitative damage such as loss of nutrients, aesthetic value, increased levels of releases to grain mass and loss of industrial value (Fleurat-Lessard, 2003).

Figure 19. Damage caused by *Tribolium castaneum* (Original, 2024).

2.8.- Methods of control

Many of the methods are used against stored food pests and especially *Tribolium castaneum*.

2.8.1.- Preventive struggle

According to Taruvinga et al, (2014), the following procedures are adopted:

- Cleaning and drying of grains and warehouse facilities.
- Temperature and humidity control before and after grain storage.
- Bulk grain storage.
- Damage to grains is monitored regularly

2.8.2.- Chemical control

Chemical control remains the most effective means of protection against insect pests in stocks with advantages and disadvantages (Hall, 1970; Haubruge et al., 1988; Relinger et al., 1988 and Arab, 2018).

For the protection of stored foodstuffs, the pesticides frequently used are organophosphates, carbamates and synthetic pyrethroids (Isman, 2006).

In Algeria, among the pesticides authorized against insects of stored foodstuffs are Deltamethrin, alpha cypermethrin and aluminum phosphorus. These substances are registered and registered on the phytosanitary products index. According to Cruz et al. (1988) two types of treatment are generally used, contact and fumigation treatment.

2.8.2.1.- Contact processing

Contact treatment consists of covering the grains, packaging and storage rooms with an insecticide film that acts on the depredators, whose effect is more or less rapid with a longer persistence of action.

2.8.2.2.- Fumigation treatment

Fumigation treatment consists of treating the grains with a toxic gas called fumigant, the major interest of fumigation is to facilitate the penetration of gasses inside the grain in order to destroy the eggs, larvae and nymphs that develop there.

All these phytosanitary products have one characteristic in common. They are neurotoxic and pesticide residues have been detected in many sectors of the food chain (Regnault-Roger et al., 2002).

2.8.3.- Physical struggle

The use of physical control methods must be part of an integrated pest management approach. This mode of physical struggle can use several technologies, some of which use active methods (Vinncent, 2001) .

2.8.3.1.- Thermal control

The use of high temperatures is to increase the internal temperature of pests from 50°C to 60°C to reduce food intake, reproduction and survival, similarly a decrease in the temperature of these organisms below freezing results in a similar situation (Vinncent,2001).

2.8.3.2.- Electromagnetic control

Balachowsky (1963), indicates that in the physical fight, several avenues of application of electromagnetic radiation have been explored as a tool to fight against insects stocks such as ultraviolet radiation, infrared, non-ionizing electromagnetic radiation, radio frequencies and microwaves. Fleurat-Lessard (2015), adds that other actions enter into physical struggle such as; drying, separation of impurities, blowing and vacuuming, ventilation by cooling stocks to ambient air after harvest and the use of seed transfers periodically from cell to cell to reduce moisture.

2.8.4.- Biological control

All possible levers that can help protect cereal stocks from insect attacks need to be activated in a modern approach of integrated production applied to the preservation of cereal stocks after harvest in order to minimize losses and use of plant protection products (Fouar Belaif, 2015).

2.8.4.1.- Auxiliary struggle

Hymenopteran bugs and various anthocorid bugs are the most frequently used predators to control insect pests at the deposition level mainly against beetles and lepidoptera.

2.8.4.2.- Microbiological control

Entomopathogenic microorganisms, bacteria (*Bacillus thuringiensis*), fungi (*Beauveria bassiana*), nematodes, protozoa and viruses can be used against these insects.

2.8.4.3.- Control by botanical biopesticides

According to Bernard (2006), some plant species have insecticidal molecules in the composition of their plant extracts (aqueous extracts and essential oils). They act mainly by inhalation, contact and ingestion on eggs, larvae and adults of pests.

Expérimentale

Parte

Chapter 3.-

Materiels and Methodes

Chapter 3.- Materiels and Methodes

This work was carried out in the laboratory of the Faculty of Natural Sciences and Life at the University of El Oued. It consists of highlighting the potential of the essential oil of two medicinal plants, *Peganum harmala L*. and *Mentha aquatica*. In this study, we used the hydrodistillation method using Hydrodistillateur, type Clevenger for the extraction of essential oil from these two plants, which we tested on a cereal pest insect, *Tribolium castaneum*. To evaluate the effect of the essential oil of *Peganum harmala* and *Mentha aquatica*, we estimated the mortality rate of adults using two methods: contact and fumigation.

Our research was conducted on the aerial parts of the *Peganum harmala* and *Mentha aquatica* plants, which are widely used by most of humanity. The sample was collected during the month of March 2024 in the Reguiba region, El Oued province, located in southeastern Algeria (Fig). The choice of sampling station, the place where the studied species were collected, is based on the presence and accessibility of this species and according to the means at our disposal.

3.1.- Presentation of the study area

3.1.1.- The region of El-Oued

The region of El-Oued, located in the south-east of Algeria (33° to 34° N; 6° to 8° E), is situated at an altitude of 70 meter above sea level (Voisin, 2004).

The wilaya of El Oued (Fig. 20) covers an area of 44.585 km^2 with a population of 990,000 inhabitants, resulting in a density of 12 inhabitants/km2. The study area consists of 18 municipalities, covering an area of approximately 14,518.33 km^2 (Voisin, 2004).

The name "Souf" comes from the Berber language, referring to a river or oued. Originally, the primary occupation of the region's inhabitants was agriculture. Each palm grove was established through significant physical and financial efforts (Voisin, 2004).

3.1.1.1.- The boundaries of the wilaya of El Oued

According to Voisin (2004), the boundaries of El Oued are shown in the table below (Tab.7).**:**

Table 7. El Oued boundaries (Voisin, 2004 ; C. F. O., 2020).

	The administrative boundaries	The natural boundaries
North	Tebessa and Khenchla	Chotts zone (Melghir and Merouane)
East	Tunisia	Tunisian Chott El-Djerid
South	Ouargla	Extension of the Erg Oriental
West	Biskra and Ouargla	Valley of Oued Righ

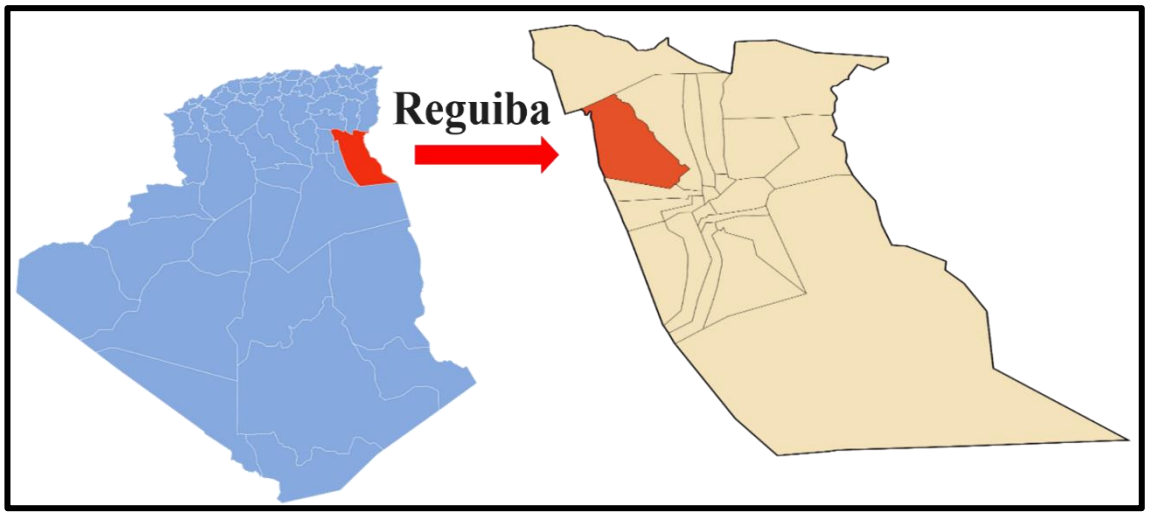


Figure 20 . The Wilaya Of El Oued (Beggas Y, 1992).

3.1.2.- Reguiba zone: A vibrant historical oasis

The Reguiba zone, one of the municipalities of the Algerian province of Oued Souf, is located 30 kilometers north of the provincial capital. It is distinguished by its strategic geographical location, bordered to the north by the municipality of El Hamraia, to the south by the municipality of Teghzout, to the east by the municipalities of Qamar and Sidi Aoun, and to the west by the municipalities of Sidi Khelil, Tendla, and Jamaa.

The area of Reguiba is 144.7 square kilometers and is inhabited by approximately 20,000 people according to the 2014 census. Agriculture is the main economic activity in the region, where grains, legumes, vegetables, fruits, and date palms are cultivated, along with some traditional crafts such as carpet weaving, leatherwork, and pottery (C.F.O., 2020).

The Reguiba is rich in numerous historical landmarks that embody the essence of the past and the civilization of the area. Among the most important are the Reguiba Fortress, the ancient mosque, and the Qadiriya Sufi lodge (Ben Salah M, 2010).

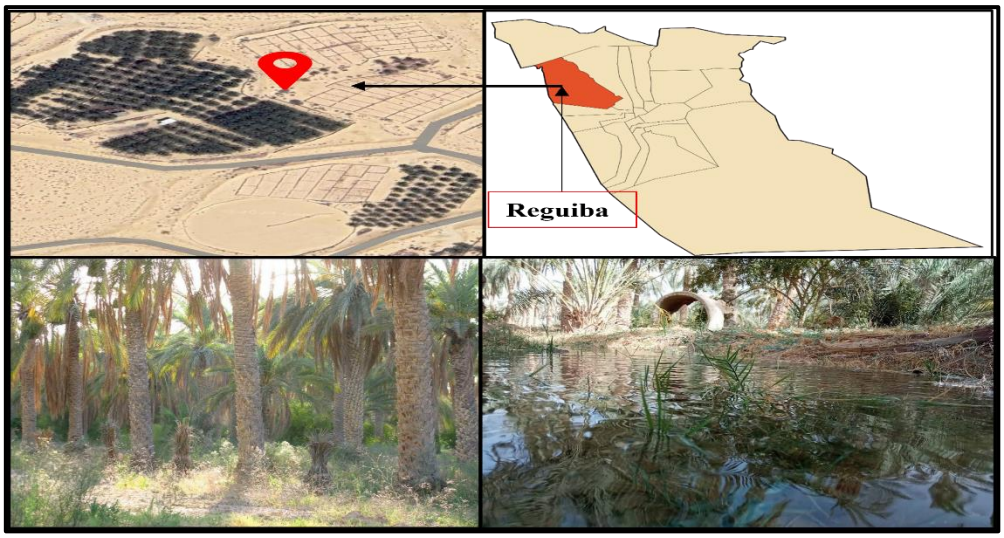


Figure 21. The Reguiba region.

3.2.- Materials

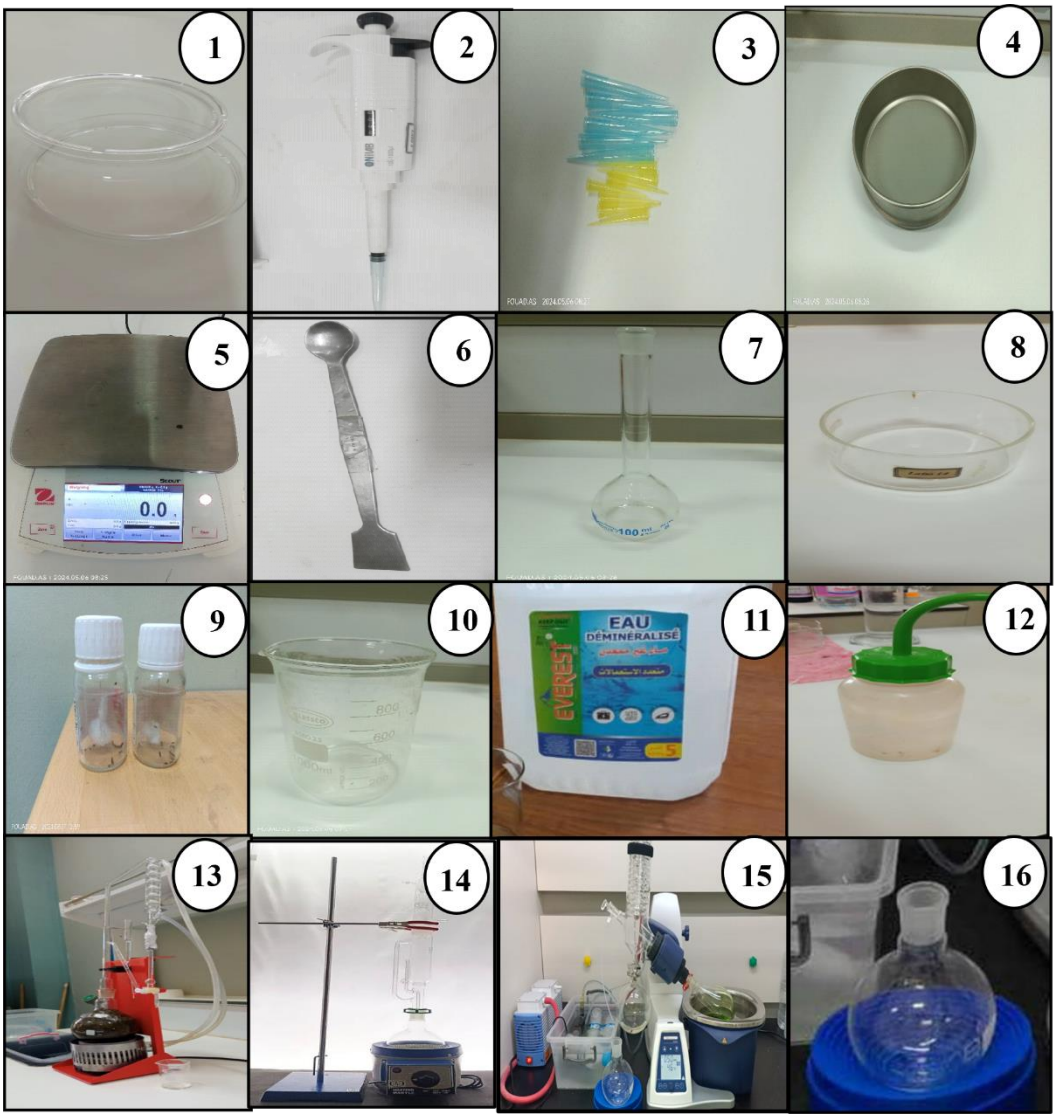
3.2.1.- Animal material

Tribolium castaneum was chosen because of the importance of the damage caused by this pest to stored commodities of economic importance. Additionally, its breeding can be easily carried out in the laboratory.

3.2.2.- Plant material

The plant material used in our experiment is based on two aromatic plants, namely *Peganum harmala* and *aquatic Mentha*. These plants were chosen because of the presence and accessibility of these species, as well as their widespread use by most of humanity.

3.2.3.- Non biological material: laboratory equipment



1: Petri dishes; 2: Micropipettes; 3: Pipette tip, yellow and blue; 4: SIEV; 5: Digital scale ; 6: spatula ;7: Conical Flask ;8: Becher; 9: GLASS BOUTTLES ;10: Beaker ;11: bouttle of distilled water ;12: small Bouttle of distilled water ;13: Clevenger ;14: Soxhlet device ;15: rotary evaporator device; 16:inflate ballon.

Figure 22 . Laboratory equipment(Original,2024).

3.3.- Methodes

3.3.1.- Breeding of the insect

Breeding *Tribolium castaneum* is common in laboratories for biology:

- **Materials Needed**

A: Breeding Containers: Glass jars or plastic boxes with perforated lids for ventilation.

B: Food Substrate: Whole wheat flour mixed with dry yeast for added protein.

C: Heat Source: An incubator to maintain a consistent temperature.

D: Handling Tools: Tweezers, soft brushes, and sieves for managing beetles and cleaning substrates.

- **Breeding Steps**

A: Preparing the Substrate

Mix whole wheat flour with approximately 5% dry yeast; Place the mixture in breeding containers to a depth of about 2-3 cm.

B: Introducing Adults

Place healthy adult beetles into the prepared containers; A ratio of 50-100 adults per liter of substrate is recommended to prevent overcrowding.

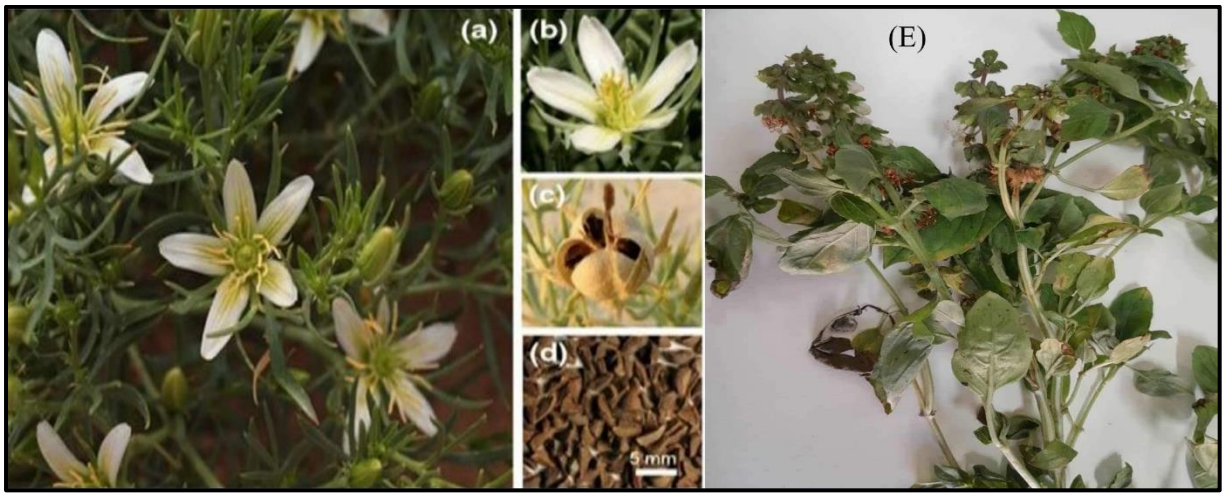
3.3.2.- Collection of plants

Peganum harmala and *Mentha aquatica* were collected during the month of November 2024 in the Reguiba.

3.3.3.- Drying the plants

The *Peganum harmala* seeds were cleaned of all impurities, washed with tap water and dried away from light and humidity for a few days, in order to maintain the quality of the plant material. Once dried, the seeds are ground using an electric mill until a fine powder is obtained from which the fixed oil has been extracted .

The sample of *Mentha aquatica* washed and dried in the open air, away from light and humidity, at room temperature for few dayes. The plant material was cut into small pieces before use (Fig. 21).



(a) Whole plant, (b) Flowers, (c) Fruit capsule and (d) Seeds; (E) : Dryin of *Mentha aquatica*

Figure 23. Photos representing of plants studied.

3.3.4.- Method of extraction

1.3.4.1.- Fixed oil of *Peganum harmala*

The fixed oils are extracted using the Soxhlet device , as showen in figure 22. The crushed plant material is placed in a cartridge and exposed to the extraction solvent (hexane 200ml) at an evaporation temperature of 40°C. After approximately 8 extraction cycles, the cartridge is

removed, and the solvent loaded with plant extract is recovered to be concentrated and dried under vacuum using a rotary evaporator.

A: Soxhlet device; B rotary evaporator device

Figure 24. Photos represent the devices of extraction of *Peganum harmala* oil and solvent recovery(Original, 2024).

1.3.4.2.- Essential oils of *aquatic Mentha*

The extraction of essential oils was done using the hydrodistillation method using Hydrodistillator type Clevenger. In a flask containing 600 ml of distilled water, 50g of *aquatic Mentha*. The contents of the flask are brought to a boil at 100°C. The vapors are collected for 3 hours. The vapors, which are loaded with essential oil, pass through the refrigerant and condense before flowing into a decantation bulb. Then, the oil separates from the water due to the difference in density.

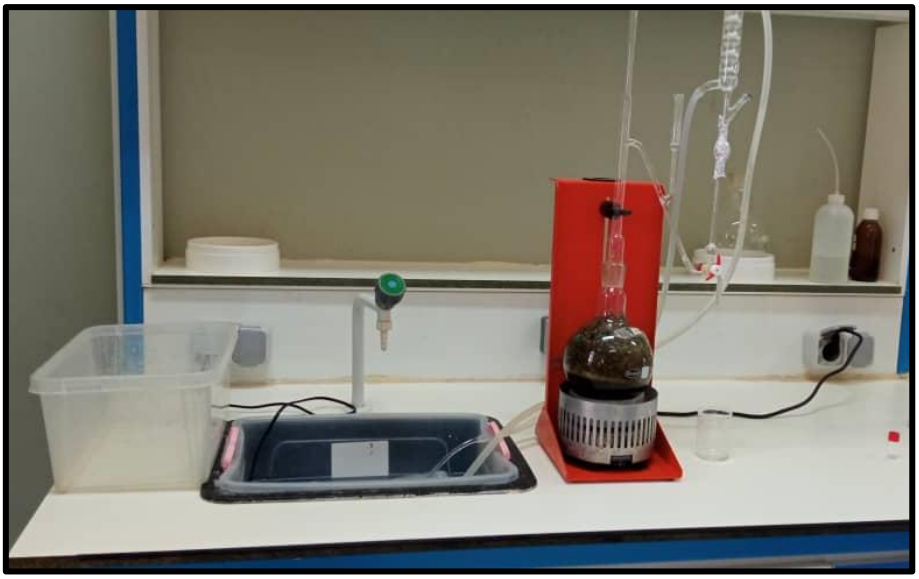


Figure 25. Photo represent the extraction device type Clevenger for Essential oil of *aquatic Mentha* (Original, 2024).

According to Afnor (1986), the essential oil yield is defined as the ratio of the mass of essential oil obtained after extraction to the mass of the dry plant material used. It is expressed as a percentage and calculated using the following formula:

$$Y\,he = \frac{M\,he}{M\,p} \times 100$$

- **Y he**: Essential oil yield in %;
- **M he**: Essential oil mass recovered in grams;
- **M p**: Dry plant mass in grams.

3.3.5.- Preservation of essential oils

There are some essential precautions that must be taken when storing EOs. That's why we kept them at close to 4°C.

Figure 26. Conservation of the oil (Original, 2024).

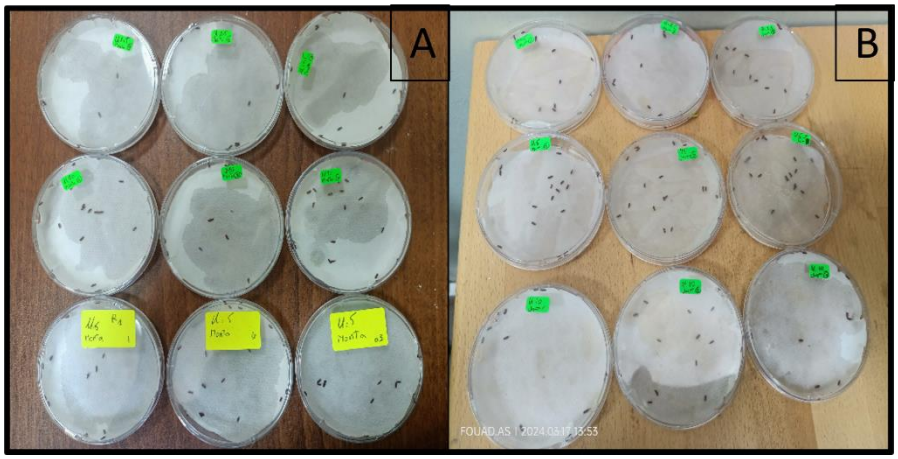
3.4.- Evaluation of the activity of fixed oils of *Peganum harmala* and essential oils of *aquatic Mentha*

To separate the insects, we used a 0.5mm diameter sieve. Then, using a brush, we take the adult individuals and place them separately in plastic petri dishes with an 8cm diameter.

3.4.1.- By direct contact

In Petri dishes, 20 adults of *Tribolium castaneum* were placed. These were treated with three doses of essential oil prepared as previously mentioned, by dissolving volumes of 2.5, 5, and 10 µl/ml of essential oil in 0.2 ml of tween. After 5 minutes of stirring for homogenization and complete evaporation of tween, three repetitions were performed for all doses. All Petri dishes were placed in an enclosure at an average temperature of 26.25 ± 1.5°C and a relative humidity of 53 ± 1.41%.

Tribolium castaneum were counted every 1 hour for 72 hours regarding the eo of *aquatic Mentha* and for 24h for *Peganum harmala* . After enumeration, the mortality percentage was estimated based on the concentration of the tested essential oil.



A: By *Mentha aquatica* B: By *Peganum harmala*

Figure 27. Tests for direct contact toxicity of *Peganum harmala* and *Mentha aquatica* essential oils on adults *Tribolium castaneum* (Original,2024).

3.4.2.- By fumigation

Three doses of essential oils corresponding to the following concentrations (2.5, 5, and 10 µl/ml air), calculated in relation to the volume of air in the jars (µl/ml), are used in a single application. Toxicity tests are carried out by fumigation in glass jars by depositing an essential oil charge on a disc of filter paper. Subsequently, lots of twenty (20) adult insects of *Tribolium castaneum* are introduced into the jars. Each test is repeated twice (2 repetitions). The number of dead insects in each batch is counted every 1 hour of exposure and for 2 days (48h) for both the eo of two plants *aquatic Mentha* and *Peganum harmala*.

Figure 28. Tests toxicity by fumigation of essential oils of *Peganum harmala* and *Mentha aquatica* on adults *Tribolium castaneum* (Original, 2024).

3.4.3.- Data analysis

3.4.3.1.- Enumeration

The mortality rate of adults of *Tribolium castaneum* is estimated as a function of exposure time and different doses applied D1, D2, D3 compared to the control, however, the verification of the mortality of adults of individuals is carried out using a clamp.

3.4.3.2.- Estimation of observed mortality

The percentage of mortality observed in control and treated individuals is estimated by the following formula:

$$\text{Observed Mortality} = \frac{\text{Number of dead individuals}}{\text{Individuals total number}} \times 100$$

3.4.3.3.- Estimation of corrected mortality

The effectiveness of a product is evaluated by the mortality of the target organism. However, the number of individuals counted dead in a population treated with toxic is not the actual number of individuals killed by this toxic. There is in fact in any treated population a natural mortality which is added to the mortality caused by the toxic, For this the mortality percentages must be corrected by the formula of Abbott (1925) which is as follows:

$$Mc = \frac{(Mo - Mt)}{(100 - Mt)} \times 100$$

Mc: mortality corrected in percentage

Mt: mortality in treated lot

Mo: mortality in untreated control lot

3.4.3.4.- Calculation of lethal doses 50

The effectiveness of a toxic product is measured by the LD50 which represents the amount of toxic substance that causes the death of 50% of individuals of the same batch. It is deduced from the drawing of a regression line, taking into account the problems of the values of the mortalities corrected in ordinates through the table of problems and the decimal logs of the doses in abscissa. The percentages of corrected mortality are transformed into probits according to the table of Bliss cavelier(1976). These probits are graphically represented as a function of the neperian logarithm in order to evaluate the lethal dose 50 (LD50) which is determined from the equation of the regression line

obtained using the Excel software: Y = ax + b, Y being the problem of the corrected mortality value, x the decimal logarithm of the dose, and the slope of the equation of the regression line, the dose corresponding to a 50% mortality will be determined, hence the LD50.

Chapter 4.-

Results and Discussion

Chapter 4.- Results And Discussion

4.1.- Oil Performance Calculation

4.1.1.- Plant of *Mentha aquatica*

Mentha aquatica essential oil yield obtained by a Clevenger type hydro distiller, is a viscous liquid, clear of a yellowish green coloration and with a strong smell characteristic of Mentha aquatica, with a yield of 2.3%.

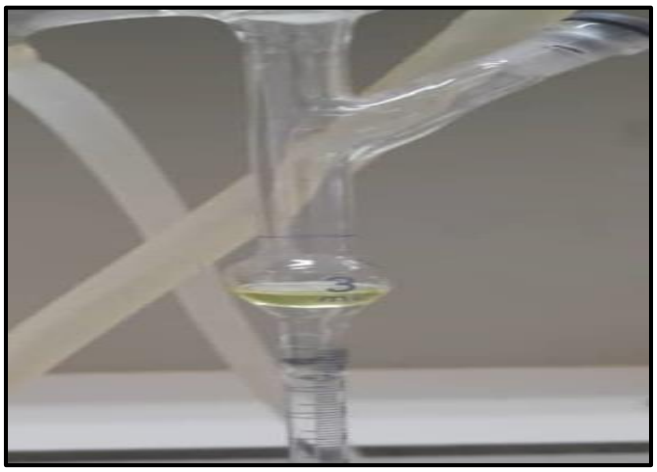


Figure 29. *Mentha aquatica* oil exctructed.

4.1.2.- Plant of *Peganum harmala*

The extraction of seed oil was carried out by Soxhlet using hexane as a solvent. After extraction and removal of traces of solvent, the yield obtained is 8.13%.

Figure 30.*Peganum harmala* oil exctructed.

4.2.- Evaluation of the effect of different concentrations of *Peganum harmala* and *Mentha aquatica* essential oil on *Tribolium castaneum*

4.2.1.- Plant of *Peganum harmala*

4.2.1.1.- Direct contact processing

The number of dead insects under the effect of essential oil of *Peganum harmala* were followed, during a period of 24 hour 6,12,18 and 24 h. The results of the insect treatment test *Tribolium castaneum* with essential oil of *Peganum harmala* is shown in the following figure:

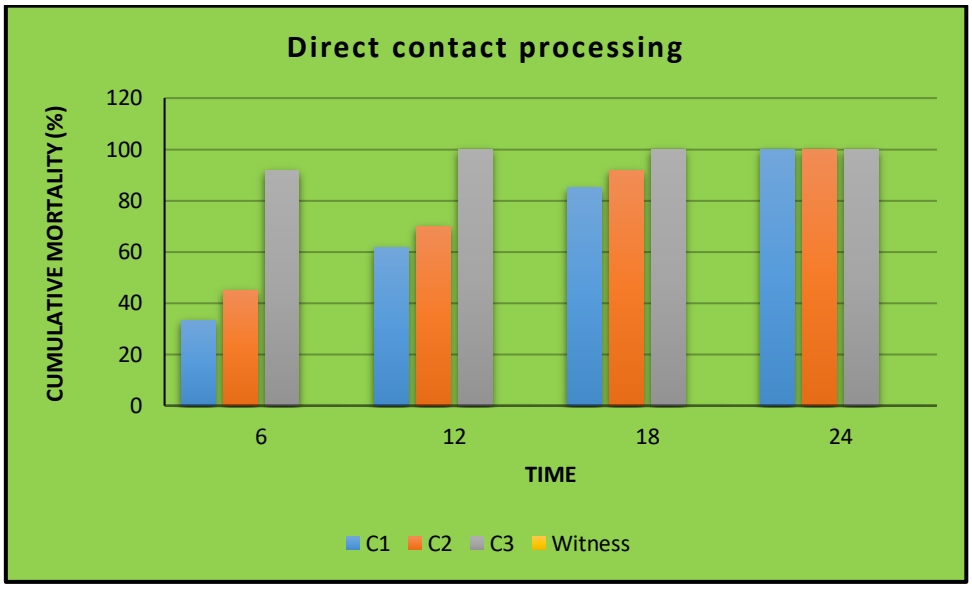


Figure 31. Effect of *Peganum harmala* essential oil on *Tribolium castaneum* by Direct contact processing.

The bar graph represents cumulative mortality rates (%) at different time points (H6, H12, H18, 24 hours) for various concentrations labeled as C1, C2, C3, and a control group (witness). When compared to the witness, all treatments have a toxic effect over time on individuals of *Tribolium castaneum*, with C3 showing the highest toxicity compared to the other tested doses.

Based on figure 29, we observed that the lowest concentration of *Peganum harmala* fixed oil tested (2.5µl/l) starts to affect *Tribolium castaneum* adults within the first hour of the test, with a mortality rate of 11.67%. This rate increases to 33.3% by the sixth hour, and gradually rises to its peak of 85% at the 18th hour. The maximum mortality of 100% is achieved at the 24th hour.

Regarding the concentration (5 µl/l), the effect begins in the 1st hour with a mortality rate of 16.67%. Then, this rate gradually increases to reach 45% at the 6th hour. It continues to gradually increase to reach a rate of 70% at the 12th hour and further increases to a maximum of 91.67% at the 18th hour, where it reaches maximum mortality. The mortality rate remains at 100% at the 24th hour.

While the effect of the third concentration (10µl/l) begins at the first hour with a mortality rate of 36.67%, it increases significantly to reach 91.67% at the 6th hour and reaches its maximum mortality at the 9th hour when it reaches 100% (Fig.29). Regarding the Witness, we noticed that there were no deaths during the study phases estimated at twenty-four hours.

4.2.1.2.- Fumigation processing

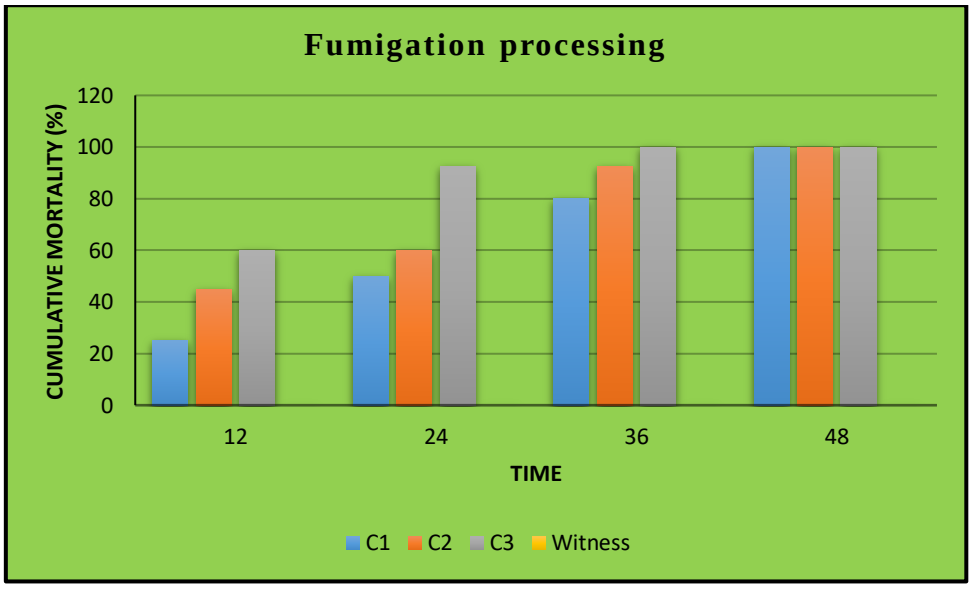


Figure 32. Effect of *P. harmala* essential oil on *Tribolium castaneum* by fumigation processing.

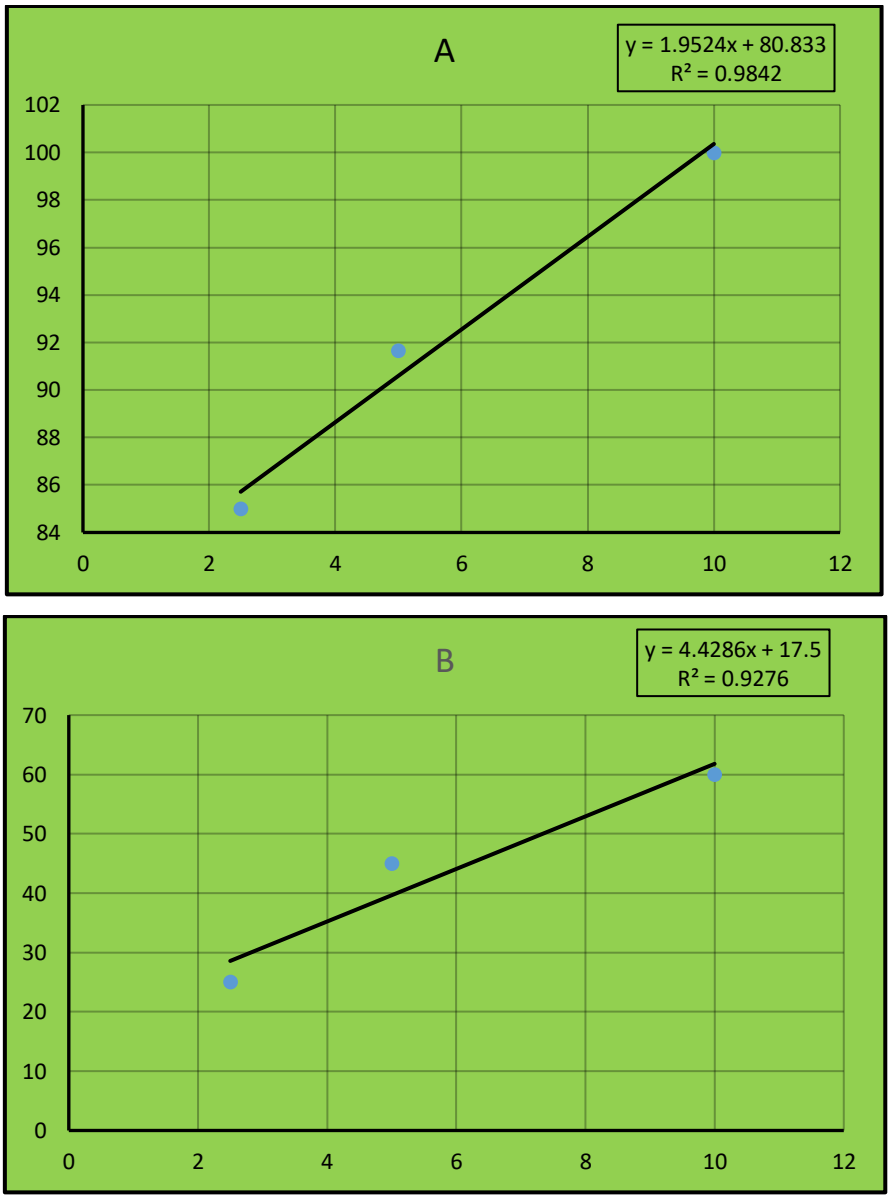
According to figure (30), and compared to the control, all treatments have a toxic effect over time on individuals of *Tribolium castaneum* with a supremacy of toxicity of C3 compared to other doses tested.

At each time point, mortality rates were assessed as follows:

- At 12 hours (H12), C1 exhibited a mortality rate of approximately 22%, while C2 showed a slightly higher rate at around 41%. C3 significantly increased to 60%, whereas the witness had the lowest mortality rate, close to 0%.
- At 24 hours (H24), the mortality rate for C1 increased to 50%, whereas both C2 and C3 demonstrated a substantial increase, with C2 reaching 60% and C3 reaching about 90%. At 36 hours (H36), the mortality rate for C1 increased to 80%, whereas both C2 and C3 demonstrated a substantial increase, with C2 reching to around 90 % and C3 reaching 100%.
- At 36 hours (H36), the mortality rate for C1 increased to 80%, whereas both C2 and C3 demonstrated a substantial increase, with C2 reaching around 90% and C3 reaching 100%.
- At 48 hours, all groups (C1, C2, C3) displayed high mortality rates while witness remains much lower than the treated groups. All had a mortality rate of 100%.

4.2.1.3.- Estimation of DL50

It corresponds to the lethal dose which gives a mortality of 50%). It is derived graphically from the regression line of corresponding to the percentages of cumulative mortality as a function of logarithms of treatment doses which is representative of the function Y= f(x).



A : Direcet Contact (18h) ; B : Fumigation (12h)

Figure 33.LD50 essential oil mortality curve in relation to concentrations.

The results obtained made it possible to determine from the method, the LD50 values of the different modes of exposure of the essential oil of *Peganum harmala.* The results show that the essential oil tested is toxic on the populations of *Tribolium castaneum*.

The examination of the LD50 values after 18 hours of exposure of adult individuals for the direct contact mode and after 12 hours for the fumigation mode allows us to deduce that the

applied essential oil has variable toxicity vis-à-vis *T. Castaneum* adults and mortality depends on the applied dose.

According to the results, the essential oil of *P. harmala* showed insecticidal activity with 7.33 µl/ml for fumigation and 6.56 µl/ml for direct contact.

Based on the LD50 values the order of toxicity in relation to the mode of exposure is as follows:

Table 8. DL50 of *Peganum harmala* essential oil in two modes.

C	***DL50***	
	Direct Contact (18h)	**Fumigation (12h)**
	6.56	**7.33**

4.2.2.- Plant of *Mentha aquatica*

4.2.2.1.- Direct contact processing

The number of dead insects under the effect of essential oil of *Mentha aquatica*, were followed, during a period of 72h 18,36,54 and 72h. The results of the insect treatment test *Tribolium castaneum* with essential oil of *Mentha aquatica* by direct contact processing is shown in the following figure.

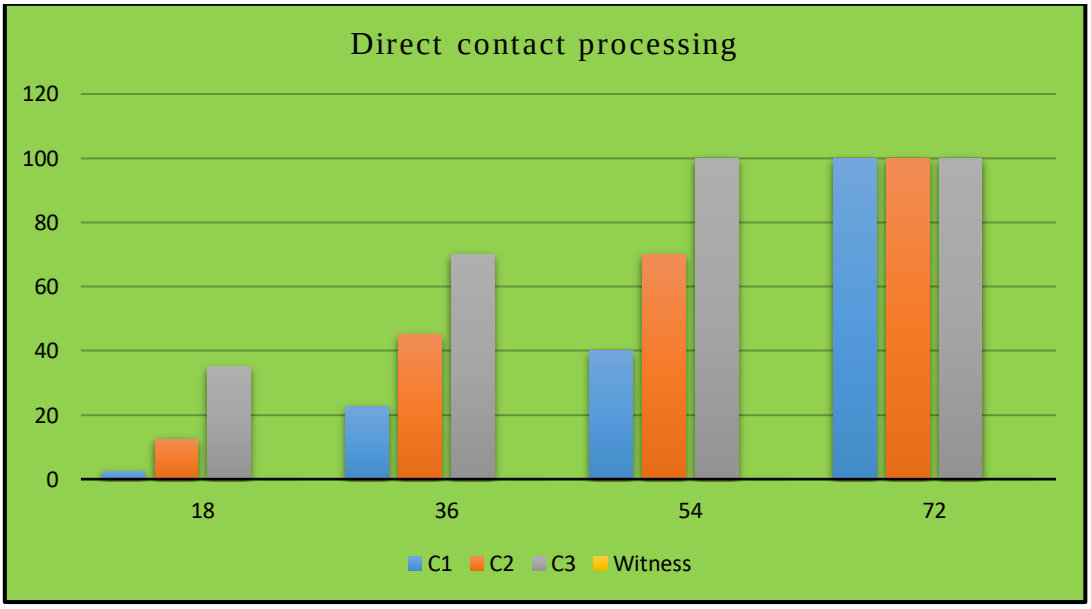


Figure 34. Effect of *Mentha aquatica* essential oil on *Tribolium castaneum* by Direct contact processing.

Over time, all treatments have a toxic effect on individuals of *Tribolium castaneum* , with C3 having a greater toxicity effect than other doses tested.

❖ Mortality Rates Analysis:

- At 18 hours (H18):

- C1 shows a low mortality rate of 2.5%.
- C2 has a higher rate at around 12.5%.
- C3 is shows 35 % of mortality rate.
- Witness has the lowest, close to 0%.

- At 36 hours (H36):

- C1 increases to 22.5%.
- C2 shows 45% of mortality rate and C3 reaching 70%.
- Witness remains much lower.

- At 54 hours (H54):

- C1 continues to rise to about 40%.
- C2 is slightly higher than C1 and reaching around 70%.
- C3 remains the highest, maintaining 100%.
- Witness remains significantly lower than the treated groups.

- At 72hours (H72):

- All treated groups (C1, C2, C3) Reach 100% of mortality rates
- Witness remains much lower than the treated groups.

4.2.2.2.- Fumigation processing

Fumigation processing was followed for a 48-hour period (12,24,36 and 48h) to determine the number of dead insects caused by *Mentha aquatica* essential oil. The results of the insect treatment test *Tribolium castaneum* with essential oil of *Mentha aquatica* is shown in the following figure:

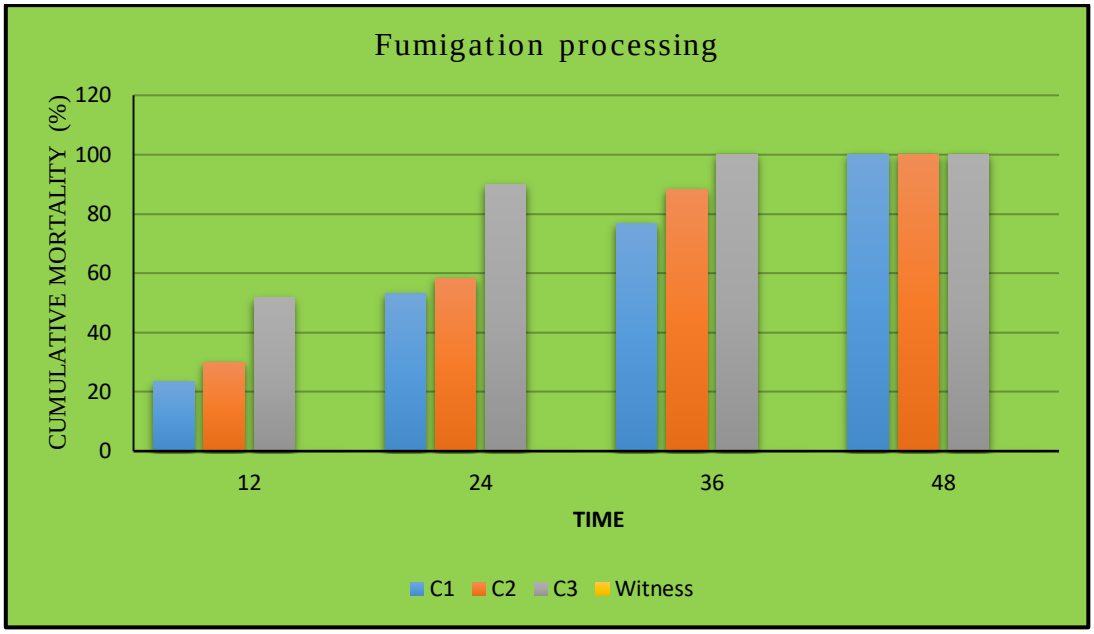


Figure 35. Effect of *Mentha aquatica* essential oil on *Tribolium castaneum* by fumigation processing.

Tribolium castaneum individuals experience a toxic effect from all treatments over time, with D3 being the most toxic compared to other doses tested.

The bar graph illustrates cumulative mortality rates (%) observed at different time intervals (H12, H24, H36, 48 hours) for varying concentrations denoted as C1, C2, C3, along with a control group (Witness).

❖ Mortality Rates Analysis:

- At 12 hours (H12):

- C1 shows a mortality rate of approximately 20%.
- C2 has a slightly higher rate at around 30%.
- C3 is shows 51.33% of mortality rate.
- Witness has the lowest, close to 0%.

- At 24hours (H24):

- C1 increases to 53.33%.
- C2 shows 58.33% of mortality rate and C3 reaching 90%.
- Witness remains much lower.

- At 36 hours (H36):

- C1 continues to rise to about 76%.
- C2 is slightly higher than C1 and reaching around 88%.
- C3 remains the highest, maintaining 100%.
- Witness remains significantly lower than the treated groups.

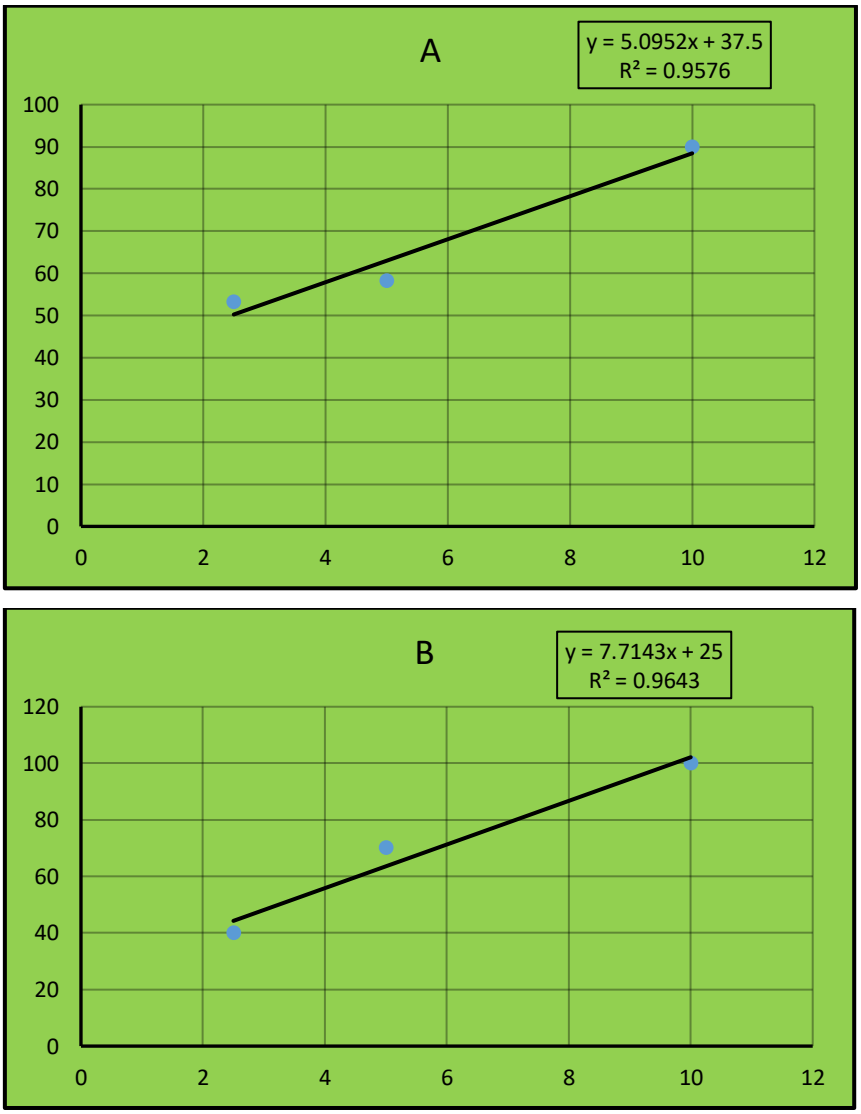
- At 48 hours (H48):

  - All treated groups (C1, C2, C3) Reach 100% Of mortality rates

  - Witness remains much lower than the treated groups.

4.2.2.3.- Estimation of LD50

It corresponds to the lethal dose, which has a mortality rate of 50%. Using graphs, the regression line of Probits is used to derive the percentages of cumulative mortality as a function of the logarithms of treatment doses, represented by the function Y = f (x).



A : Fimigation (24h) ; B : Direcet Contact (54h)

Figure 36. DL50 of Mentha aquatica essential oil on adults of *Tribolium castaneum* in two modes.

By using the Probits method, the results obtained allowed for the determination of the LD50 values for different modes of exposure of the essential oil *Mentha aquatica*. The results show that the essential oil tested is toxic on populations of *Tribolium castaneum*.

The examination of LD50 values after 54 hours of exposure of adult individuals for direct contact modes and after 24h for fumigation mode, allows us to deduce that the essential oil applied has a variable toxicity face to face to *T. Castaneum* adults and mortality depend of the applied dose.

According to the results the essential oil of *Mentha aquatica* showed a high insecticidal activity 2.45 µl/ml for fumigation and 3.24 µl/ml for direct contact.

Based on the LD50 values the order of toxicity in relation to the mode of exposure is as follows:

Table 9. DL50 of *Mentha aquatica* essential oil in two modes.

	DL50	
C	**Direcet Contact (54 h)**	**Fimigation (24h)**
	3.24	**2.45**

4.3.- Discussion

Aromatic and medicinal plants are considered, based on their essential oil constituents, to be a bio-insecticide that controls a variety of insect and pest stocks (Ketho et al., 2004).

In our study on the essential oil extracted from Mentha aquatica, it is a viscous liquid, clear of a yellowish green coloration and with a strong smell, with a yield of 2. 3%.

Ouhaddad and Sidi Salah (2016) obtained a pale-yellow oil with a strong and fresh aromatic smell. The same characteristics were found by Bardeau (2009) and other studies by Sylvain (2010). The essential oil yield of *Mentha aquatica* is 1.01%. This yield is significant compared to that which is exploited industrially, which is 0.51% (Edward, 1987). Montes et al. (1986) report that *Mentha pulegium*, from Chile, has an essential oil yield of 2.3%. Furthermore, Sivropoulou et al. (1995) obtained an essential oil content of *Mentha pulegium* from three stations in Greece, of the order of 1.6 to 2%. In contrast, Teixeira Duarte et al. (2005) argue that the essential oil yield of this same plant species of Brazilian origin is very low, around 0.42%.These values are significantly lower than ours.

The yield obtained from our plant is 2.3%, this yield is higher than those found in other regions, Bejaia with a yield of 1.1% (Brahmi et al., 2016), Setif of 0.9% (Boukhebti et al., 2011) and Saida of 1.3% (Allali et al., 2013).

Our yield is higher than that obtained by Bouhini (2016), who found a yield of 1.56% for the

species *Mentha rotundifolia* harvested in Morocco. At the same time, it is higher than that obtained by Brada et al. (2007) (0.8%), who worked on the same plant harvested in northern Algeria, and that obtained by Talbi (2016) (0.27%), who studied the same species harvested in the western region of Algeria.

However, the study done by Bouyahya et al. (2017) on the aerial part of the *Mentha pouliot* in the Rabat region of Morocco revealed essential oil contents estimated at 5.4%. It appears that it is higher than that obtained in our study in Algeria.

According to the study by Abid and Mordjani (2019), the extraction of EO from *Mentha pulegium* . has a yield of 2.44% almost the same rate as our study.

In another part of our study, After the extraction of oil from the seeds of *Peganum harmal*, the yield obtained is 8.13% lower than that obtained in the study of Dahamna and Chergui (2021) on seeds of the same species and they obtained a yield value equal to 44 . 21% . On the other hand, the fixed oil yield obtained remains quantitatively different from those found by Kiimesnoglu et al. (1995) from seeds of the same species, collected in Turkey, as well as the results of Lalla et al. (1999) from seeds collected in Morocco.

These variations in oil content of our two plants can be attributed to different environmental factors, taking into account that the essential oil is a metabolic product of plant cells and that its quantitative and qualitative composition can be influenced by climatic conditions (including the type of climate, altitude, sun exposure rate), the type of soil, the stage of growth of the plant in question, the choice of the harvest period because it is essential in terms of yield and quality of HE. Geographic area, plant genetics, plant organ used, degree of freshness, drying period, extraction method used, etc. (Benazzouz & Hamdane, 2012) in addition to: ecotype, phenotype, temperature, relative humidity, photoperiod and illumination (Snoussi et al., 2015).

The effect of the essential oils of the two plants studied, *Peganum harmala* and *Mentha aquatica*, was evaluated in terms of adult mortality of *Tribolium castaneum*, obtained using two application modes: direct contact and fumigation. The objective was to determine the different treatments that cause toxicity leading to mortality of *Tribolium castaneum* individuals. Their effectiveness was measured using the LD50, calculated from a dose-dependent regression curve of cumulative mortality, using the method described by Finney (1971).

The EOs we tested appear to both have a toxic effect on adults of *T. castaneum*. We note that the increase in mortality rate is strongly related to the dose of essential oil and time.

In our study, the essential oil of *Peganum harmala* and *Mentha aquatica* gave good results in terms of toxicity on individuals of *Tribolium castaneum*. This efficacy is confirmed by adult mortality of this pest for both application modes. The mortality of all individuals (100%) is

observed with the three doses in the two modes of application, direct contact and fumigation, but at different times. For the direct contact mode, we recorded a LD50 equal to 3.24 µl/ml at hour 54 for the *Mentha aquatica* plant, which is lower than the LD50 obtained for the *Peganum harmala* plant, which is equal to 6.56 µl/ml at hour 18. Regarding the fumigation mode, we calculated a LD50 of 2.45 µl/ml at hour 24 for the *Mentha aquatica* plant, which is also lower than the LD50 observed for the *Peganum harmala* plant, which is equal to 7.33µl/ml at hour 12.

Our results show a 100% mortality with a lower dose (2.5 µl/ml) after 24 hours for our plant *Peganum harmala*. In another study, Sahari (2018) investigated the effectiveness of *M. pulegium* EO on adult mortality of *T. confusum* by conducting a direct contact test. After 48 hours of exposure, the mortality rate reached 96.32% with the lowest dose (5 µl/ml). Therefore, it is concluded that the toxicity of our *Peganum harmala* oil is more important, effective and faster.

Based on the results obtained from the study of Amitouche and Rakem (2017), which was carried out in order to estimate the insecticidal effect of *Pinus pinester* resin essential oil on individuals of *T. confusum*, the test performed is the inhalation test of EO at the dose of 50 µL/ml. After 24 hours, they recorded a mortality of 18% of individuals. Moreover, in our trials, a rate of 92% was recorded for *Peganum harmala* and 90% for *Mentha aquatica* at the very low dose of 5 µL/ml. In terms of efficiency, we can deduce that our essential oil of *Peganum harmala* and *Mentha aquatica* is significantly superior.

Our estimate of the LD50 for *Peganum harmala* oil is 6.56 µl/ml at 18 h in direct contact mode and 7.33 µl/ml at 12 h in fumigation mode. At the same time, we also calculated the LD50 for *Mentha aquatica* oil and found 2.4 µl/ml. These LD50 values are lower than those estimated by Neghaban et al. (2007) for *A. Sieberi* oil, which is 16.76 µL/L versus *T. castaneum* after 24h. Therefore, we conclude that the toxicity of our *Peganum harmala* and *Mentha aquatica* oil is more effective and faster against *T. castaneum*, either by direct contact or by fumigation.

According to Ikhlef et al. (2021), the value of LD50=1.346 µl/ml shows that the essential oil of *Artemisia arborescens* is toxic to insect pests of stored foods.

Aimene (2021) discovered that the essential oil of *Matricaria chamomilla L*. has interesting insecticidal properties against adults of *T. Castaneum*, the pest of stored food. The LD50 value recorded was 2.789 µl/ml after 24 hours.

The study conducted by Wagner et al. (2021) showed that the essential oil of *Asteraceae*, *Lavandula dentata*, had good insecticidal activity on the three insect pests of the stored commodities studied. The LD50 values against *Sitophilus zeamais* (Coleoptera: Curculionidae)

and *T. Castaneum* (Coleoptera: Tenebrionidae) were 26.9 and 11.3 μL/L air (no-food test) and 42.7 and 29.3 μL/L air (no-food test).

The insecticide efficacy of medicinal plants against insects in stored foods has been confirmed by several researchers (Tapondjou et al., 2005). The bioinsecticide effect obtained could be partly explained by the insecticidal effect of the majority of essential oils tested. This is in agreement with the results of several researchers, notably those of Ngamo and Hance (2007), who report that the toxicity of essential oils on insects is induced by the action of their majority compounds. The latter have either singular or synergistic insecticide efficiencies.

Conclusion

4.4.- Conclusion

In fact, the use of chemical insecticides is the most common method of reducing the severity of these pests. However, the use of these synthetic products is not recommended for several reasons: their harm to living organisms, environmental pollution, and especially the development of resistant insects.

The results of our study show that the oils extracted from the two studied plants have a significant yield, with a value of 2.3 for *Mentha aquatica* and 8.14 for *Peganum harmala*. These results indicate the high value of the oils present in the studied plants, which have the ability to fight against insect pests and can be used as a natural and effective alternative to chemical pesticides.

In our study, the essential oils of *Peganum harmala* and *Mentha aquatica* showed good results in terms of toxicity on *Tribolium castaneum*. This efficacy was confirmed by the adult mortality rate of this pest for both application methods, direct contact and fumigation. Total mortality of individuals (100%) was observed at doses of 2.5 ml, 5 ml and 10 ml, but at different times.

The amount of oil required to cause the death of 50% of *Tribolium castaneum* individuals was measured as follows: for the direct contact method, we recorded a LD50 = 6.56 µl/ml for *Peganum harmala* and a LD50 = 3.24 µl/ml for *Mentha aquatica*. Regarding the fumigation method, we obtained a LD50= 7.33 µl/ml of for *Peganum harmala* and a 2.45 µl/ml of for *Mentha aquatica*.

The results of this study allow to conclude that the essential oils of the two studied plants have an important activity and effect on the individuals of *Tribolium castaneum* tested. The interesting results obtained in this work need to be deepened by the study of the active chemical components of these essential oils and their test on other insect species.

At the end of this work, we present some reflections and recommendations in the form of perspectives. It would be interesting:

- To continue studying this essential oil on the main species of grain pests, particularly the rice weevil *Sylophilus oryzae* (Coleoptera) and the western flower thrips *Frankliniella occidentalis* (Thysanoptera).
- To study the chemical composition of this essential oil by identifying its main components and their effects.To recommend and test higher doses for ingestion.
- To study the other activities of the essential oil: antifungal, antiviral, antiparasitic, antioxidant, anti-inflammatory,etc. To determine the mechanism of action of this essential oil.

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