



الجمهورية الجزائرية الديمقراطية الشعبية  
DEMOCRATIC AND POPULAR ALGERIAN REPUBLIC  
وزارة التعليم العالي والبحث العلمي  
MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH  
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DEPARTMENT OF AGRICULTURAL SCIENCES

## *Master's Thesis*

In order to obtain a diploma of an Academic Master In Agricultural Sciences  
Specialty: Agronomy

## *Them*

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# **Comparative Insecticidal Activity of *Artemisia herba-alba* Essential Oil from Batna and Khenchela Against *Callosobruchus maculatus* F in Stored Chickpeas.**

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## *Acknowledgements*

*First, I would like to thank Almighty God for His countless blessings and great grace, which enabled me to complete this work. I ask Him to accept this humble effort sincerely for His sake and to make it beneficial.*

*I extend my sincere thanks and gratitude to the members of the discussion committee, the chair and the examiner, for their valuable time, insightful comments, and constructive criticism that greatly contributed to improving and enriching this work.*

*I also express my deep appreciation and gratitude to my esteemed supervisor, Professor Mouane Aisha, and the assistant supervising professor, for their valuable guidance and constructive feedback, which had a significant impact on the development of this research, as well as their continuous support throughout the completion period. Special thanks go to everyone who helped me, including colleagues, professors, and friends, during the various stages of this scientific project, especially the head of the laboratory, Omar Khanoufa, Engineer Ali Taleba, and Dr. Yahya Khalaf.*

## إهداء

إلى من كانا ضياءً طريقي، ونبع عطائي، ومصدر قوتي،

إلى من حملاني حبًا، ورّيباني صبرًا،

إلى والديّ العزيزين

لا كلمات توفيكما حقكما، ولا جهد يرقى لمقامكما،

أهدي إليكما ثمرة سنين من الكفاح

إلى إخوتي الأعزاء،

أنتم النبض الذي يبعث في قلبي الطمأنينة،

والظل الذي احتميت به في لحظات التعب

كنتم دائمًا العون والسند

فلكم من القلب كل الامتنان

إلى أساتذتي الكرام

من غرستم في دربي بذور المعرفة وسقيتموها بعلمكم وتوجيهاتكم،

كنتم مشاعل نور في مسيرتي،

لكم أرفع أسمى عبارات التقدير والاحترام

كـهـ هـارون عائشة

## الإهداء

وأخر دعواتهم أن الحمد لله رب العالمين الحمد لله عند البدء وعند الختام  
لقد كانت طريقا طويلة مليئة بالإخفاقات والنجاحات فخورين بكفاحنا لتحقيق أحلامنا,  
لحظة لظالمات انتظرتنا وحلمت بها في حكاية اكتملت فصولها  
أهدي هذا التخرج لمن علمني أن أعطي ولمن أحمل اسمه بفخر وأتمنى أن يطيل الله حياتك  
حتى ترى ثمارا حان وقت حصادها بعد إنتظار طويل " والدي العزيز "  
وإلى ملاكي في الحياة وإلى الإحساس بالحب والحنان والإخلاص وإلى إبتسامة سر الوجود,  
وإلى التي كان دعاؤها سر نجاحي حبيبي العزيزة " أمي الحبيبة "  
وإلى شريكي حياتي, زوجي الغالي شكرا لوقوفك بجاني ولتفهمك وصبرك ودعمك المستمر  
أنت النور الذي يضيء دربي ويمنحني الأمل في مواصلة المسيرة  
لى اخي العزيز وزوج أختي الكريم، أهدي هذا الإنجاز المتواضع تعبيراً عن امتناني العميق لما  
قدمتماه لي من دعم ومساندة, فكنتما خير عون في الأوقات الصعبة  
شكرا من القلب, وبارك الله فيكما وجزاكما عني كل خير  
إلى أهلي الأعزاء كل فرد منكم له بصمة في هذا النجاح فأنتم دائما الملاذ الأمن أهديكم  
جميعاً هذه اللحظة فهي ثمرة جهودكم وحب

كلمة أميمة مستور

## **Abstract**

This study aims to evaluate the insecticidal efficacy of *Artemisia herba-alba* essential oil, extracted from two Algerian regions: Batna and Khenchela, against *Callosobruchus maculatus* F, a major pest that infests stored chickpea seeds. The essential oils were obtained through hydrodistillation and tested using two bioassay methods: direct contact and fumigation. The results revealed strong toxic effects, with high mortality rates recorded after short exposure periods. The essential oil from Batna showed higher effectiveness via direct contact, while the oil from Khenchela was more potent in fumigation tests. These differences are attributed to the variation in chemical composition of the oils due to environmental and climatic conditions in each region. Lethal concentrations (DL10, DL50, DL90) were also calculated using statistical analysis. These findings support the potential of essential oils as eco-friendly and effective alternatives to synthetic insecticides and highlight their role in integrated pest management strategies, particularly in the protection of stored grain products and environmental safety.

**Keywords :** *Artemisia herba-alba* , *Callosobruchus maculatus* F, stored pests , chickpea.

تهدف هذه الدراسة إلى تقييم الفعالية الحشرية لزيت الشيح الأبيض (*Artemisia herba-alba*) المستخلص من منطقتي باتنة وخنشلة ضد حشرة *Callosobruchus maculatus* F، وهي من الآفات الرئيسية التي تصيب بذور الحمص أثناء التخزين. تم استخراج الزيوت الأساسية بطريقة التقطير بالبخار، وتقييم تأثيرها باستخدام طريقتين بيولوجيتين هما: التلامس المباشر والتبخير. أظهرت النتائج أن الزيوت العطرية تمتلك خصائص سامة قوية ضد الحشرة، حيث سجلت نسب وفيات مرتفعة خلال فترات قصيرة من التعرض. تفوق زيت الشيح من منطقة باتنة في طريقة التلامس المباشر، في حين أظهر زيت خنشلة فعالية أكبر عند استخدامه بالتبخير، ويُعزى ذلك إلى اختلاف التركيب الكيميائي لكل زيت تبعًا للظروف المناخية والبيئية لكل منطقة. كما تم تحديد الجرعات القاتلة (DL10، DL50، DL90) لكل زيت باستخدام التحليل الإحصائي. تدعم هذه النتائج اعتماد الزيوت الأساسية كبديل طبيعي وأمنة للمبيدات الكيميائية، وتُبرز دورها المهم في برامج مكافحة المتكاملة للآفات المخزنية، بما يعزز من الحفاظ على جودة الحبوب المخزنة وسلامة البيئة.

**الكلمات المفتاحية:** *Artemisia herba-alba* ، *Callosobruchus maculatus* F ، الشيح

الأبيض، الآفات المخزنية ، الحمص.

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# Introduction

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## Introduction

Legumes are an essential component of the human diet, especially in low-income countries, where they serve as a major source of protein and energy. Their high nutritional value, particularly in terms of protein content and amino acid composition, makes them a critical dietary element in regions with limited access to animal protein. However, post-harvest storage remains a major challenge, as physicochemical and biological factors contribute to significant quality deterioration and economic losses.

Legumes such as beans, peas, and chickpeas typically contain between 20% and 40% protein, a figure that far exceeds the average protein content found in cereals, which ranges between 10% and 12% (Tomić, 2023). They are particularly rich in essential amino acids like lysine, which is often lacking in cereal-based diets. This amino acid profile makes legumes an ideal complementary food source when combined with cereals, helping to achieve a balanced protein intake (Rockland & Radke, 1981). In low-income regions, legumes contribute approximately 10% to the total dietary protein intake and about 5% to the overall energy supply (Kellouche et al., 2010).

Despite their nutritional advantages, legumes are highly sensitive to storage conditions. Factors such as high temperature and relative humidity can lead to biochemical changes that reduce the content of key amino acids, including lysine and methionine, over time (Reddy & Pushpamma, 1986). In addition to these abiotic factors, biological agents like insects pose a severe threat during storage. Infestations not only result in physical damage to the seeds but also lead to further degradation of nutritional quality, compounding the losses caused by environmental conditions (Reddy & Pushpamma, 1986).

*Callosobruchus maculatus*, commonly known as the cowpea weevil, is a serious pest affecting stored legumes, especially in developing countries. This insect poses a direct threat to food security due to the damage it causes to protein-rich crops. Because of the health and environmental risks associated with chemical pesticides, many studies have focused on finding safe and natural alternatives. Among these, essential oils from aromatic plants such as *Ocimum canum* and *Hyptis spicigera* have shown high effectiveness against *C. maculatus* in both field and laboratory studies (Sanon et al., 2018). Results indicate that efficacy increases with higher doses. Extracts of *Piper nigrum* have also proven effective when applied through steaming, with mortality rates

rising as exposure time increases, especially at higher ethyl alcohol concentrations (Almeida et al., 2006). Additionally, some traditional farmers use crushed leaves of *Cassia occidentalis* to repel the pest from storage areas. These botanical methods offer a promising alternative to chemical pesticides. They help protect health and the environment while reducing food losses. Therefore, plant-based pest control represents a sustainable strategy to enhance food security.

As part of the search for Algerian plants with insecticidal properties, we tested the insecticidal activity of essential oils extracted from *Artemisia herba alba* against the insect *Callosobruchus maculatus*, a pest that attacks stored food products.

Our manuscript is divided into two parts: The first part consists of two chapters. The first chapter is dedicated to general information about medicinal plants. The second chapter focuses on the insect *Callosobruchus maculatus*. The second part, the experimental section, describes the materials and methods used in various procedures and the protocols applied during the tests. It also presents the main results obtained, followed by a discussion. Finally, a general conclusion, based on a careful review of the results and perspectives offering a set of reflections, completes this work.

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## Theorecal part

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**Chapter I : General information  
on medicinal plants and  
*Artemisia herba-alba***

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## **1. Medicinal plants**

### **1.1. Medicinal plants between past and present**

Medicinal plants have been discovered since prehistoric times and used in traditional medicine. Hundreds of chemical compounds have been synthesized from plants to combat insects, fungi and diseases. Pharmaceutical research has relied on folk botany to discover medicinally active plants, which has led to the discovery of hundreds of useful compounds including aspirin, digoxin, quinine and opium. These compounds are found in a variety of plants, but most belong to four main biochemical classes: alkaloids, glycosides, polyphenols and terpenes. Many countries have some form of regulation of traditional medicine, but the World Health Organization organizes a network that promotes the safe and rational use of these plants. Medicinal plants face global threats such as climate change and environmental destruction, and specific threats such as overharvesting to meet the needs .

#### **Prehistory:**

Many plants were used for medicine in prehistory, including many herbs and spices that are used today without necessarily being proven effective. Spices were used to combat food-spoiling bacteria, especially in hot climates and with quickly spoiling meat dishes. Angiosperms (flowering plants) were the original source of most medicinal plants, and human settlements often settled near growing medicinal plants such as nettles and hops .

#### **Ancient Period:**

In ancient Sumer, hundreds of medicinal plants such as myrrh and opium were found mentioned on clay tablets dating back to around 3,000 BC. More than 800 medicinal plants were listed in the Ebers Papyrus in ancient Egypt, including aloe, Indian hemp, castor, garlic, and juniper. In the 4th century BC, Aristotle's student Theophrastus wrote the first systematic text on botany, *The Treatise on Plants*, and around 60 AD, the Greek physician Dioscorides of the Roman army documented more than 1,000 prescriptions based on more than 600 medicinal plants in his *Pentatonic Book*, which remained the standard reference on medicinal herbs for more than 1,500 years, until the 17th century AD .

#### **Middle ages :**

In the early Middle Ages, herbalism flourished in the Islamic world, especially in Baghdad and Andalusia. The Cordovan physician Al-Zahrawi (936-1013) wrote the *Book of*

Simple Compounds, while Ibn al-Baytar (1197-1248) listed hundreds of medicinal herbs, such as aconite (crown of kings), vomiting nut, and tamarind in his Collection of Simples. Ibn Sina's Canon of Medicine of 1025 AD included many medicinal plants, and further pharmacopoeia was written by Abu Rayhan al-Biruni, Ibn Zuhr, Peter of Spain, and John of Saint-Amand .

### **Early Modern Times :**

The early modern period saw a flourishing of herbal plant depictions across Europe, beginning with the Great Herbal Book of 1526. John Gerard wrote the Herbal or General History of Plants in 1597 based on information from the physician Rembert Dodoyens, while Nicholas Culpeper published his Enlarged English Pharmacopoeia .

### **19th and 20th centuries :**

The importance of plants in medicine changed dramatically in the 19th century with the introduction of chemical analysis techniques, Alkaloids were isolated from medicinal plant species, beginning with morphine from the poppy in 1806, followed by ipecac and striction in 1817, and quinine from cinchona and later from many other plants, New classes of pharmacologically active substances were discovered in medicinal plants with the development of chemistry, and the commercial extraction of alkaloids (including morphine) began at the Merck Group in 1826, The first synthesis of the drug discovered in medicinal plants from salicylic acid began in 1853, By the end of the 19th century, pharmacology began to oppose the use of medicinal plants because enzymes often modify the activity of the drug components when dried, and moved towards the use of alkaloids and glycosides extracted from plant material. Drugs discovered from medicinal plants have maintained their importance throughout the 20th and 21st centuries, and important anti-cancer drugs have been discovered from the yew tree and the Madagascar periwinkle (Khalil, 2022) .

## **1.2. Definition of medicinal plants**

Anything of plant origin that is used medicinally is a medicinal plant.” A medicinal plant is defined as a plant that contains a medicinal substance or substances capable of treating a specific disease or reducing the incidence of it, or that contains the raw materials used in preparing medicinal substances (Ibrahim, 2005) .

Today, the World Health Organization (WHO) estimates that about 80 percent of the world's population still uses traditional remedies, including medicinal plants (Figure 1), as

primary health care tools , There are hundreds of common herbs, flowers, fruits and medicinal plants that serve all sorts of important medical and health purposes that may surprise you: they are anti-inflammatory, antifungal, antibacterial, insect repellent, antiseptic, expectorant, detoxifier, fever reducer, antihistamine and pain reliever (Youssef, 2018) .



**Figure 1.** medicinal plants (Mathur, 2023) .

### **1.3. Source of medicinal plants**

#### **1.3.1. Wild plant**

These are plants that grow naturally in their areas without human intervention, which leads to the collection of other plants similar to them but different from the plant intended for cultivation. The collection of these plants depends largely on the type of plant. Foreign plants are undesirable, which reduces the medical and commercial value of the desired plant. And other plants that may grow and mix with it. Not knowing the shape and types of the plant to be collected leads to the crop being different from what is required or to a certain extent from the medical and experience of the people who collect the samples, and the areas of plant growth by specialists in the field of plant division and classification for the purpose of diagnosing and separating it. Therefore, wild medicinal plants are not considered a reliable source in the medical field except after identifying what may be mixed with it from other plants (Hassan, 2022) .

#### **1.3.2. Cultivated medicinal plants**

Pharmaceutical companies or investment institutions establish private farms to produce specific varieties and types that the local or international market needs in specific quantities (Bouhezza & Bou El-kandoul, 2020).

#### **1.4. Factors limiting the production of medicinal plants**

There are several factors that affect and even determine the production of medicinal plants in terms of quantity and quality, the most important of which are the following :

##### **1.4.1. Soil quality**

The quality of the soil determines the success of growing a type of medicinal plant. For example, it is not recommended to grow digitalis (flax flower) and pine in calcareous soils. In sandy soils, it is good to grow colocynth, licorice, aloe vera, and squill onions, while belladonna and mushroom grains are preferred in light, mixed soils (Yousef, 2011) .

##### **1.4.2. Soil and water salinity**

Some medicinal plants tolerate salinity and water to some extent, such as chamomile and coriander, and some are very sensitive to salinity, such as mint and basi .

##### **1.4.3. Temperature**

The volatile oils in many aromatic plants increase with the rise in air temperature, while fixed oils such as linseed, castor and sunflower oils increase in plants at low temperatures, and most of the fatty acids in them are unsaturated at high temperatures (Altaee, 2020) .

##### **1.4.4. Light**

Some plants give an active substance when exposed to a long period of light, such as Datura, which gives a higher percentage of the alkaloid Hyoscine in the period before and during flowering, and the Ain Al-Bazun plant gives a high percentage of the anti-cancer alkaloids Vincristine and Vinblastine, and mint gives a higher amount of Menthone and its derivatives, which are converted into Menthol, especially in the young leaves before flowering. However, in the shade or a short day of light, most of the oil content is the inactive substance Menthofuran (Altaee, 2020) .

##### **1.4.5. Geographical location**

Some plants thrive in hot regions, such as vanilla, cola, and eucalyptus, while others thrive in cold regions, such as dinar, saffron, and pine (Yousef, 2011) .

### **1.5. Dates for collecting plant parts of medicinal plants**

The person collecting must be familiar with the characteristics of the required plant, and the places where it is found naturally or where it has been cultivated, according to the following principles :

The whole plant, or its leaves and flowers, is collected in the middle of the day, while the roots are collected from the moist soil for easy uprooting .

The plant or its parts are collected in the season, or at the appropriate time when its active components are at their peak concentrations, which are linked to a specific time in the plant's life cycle. For example: if the flowers are what is required, they are picked during the peak of flowering, while the leaves are picked at the beginning of flowering, and the peels are collected during the spring .

Take great care to keep the plant pickings clean during collection from soil, dirt, weeds, insects, pesticides, etc (Al-Aghwani, 2024) .

### **1.6. Drying and preservati on of medicinal plants**

#### **1.6.1. Drying**

Drying is one of the most important processes that helps preserve the active ingredient in the drug and prevent it from being damaged by fungal growth, plant rot, or increased enzyme activity and accompanying decomposition processes that often lead to undesirable changes in the active components of the plant. Drying quickly stops the activity of enzymes, facilitates the crushing of plants before the extraction process with solvents as well, and reduces the weight of the plants, thus facilitating the packaging, transportation and storage processes (ACSAD, 2012) .

The purpose of the drying process :

- Water activates plant enzymes that degrade (break down) active compounds, so drying preserves them.
- Drying plant parts helps in grinding them in preparation for extracting them with organic solutions or water and preparing various usable forms. Good drying also provides protection from the appearance and growth of fungi and rotting during storage, There are many drying methods depending on the type of plant, its components, the nature of its tissues, and the percentage of water in it (Al-Aghwani, 2024) .

(According to Dr. Eyyat, 2020) mentioned three drying methods which are :

#### **1.6.1.1. Natural drying**

It depends on using natural energy sources (sun and air) to evaporate moisture from plants such as :

- Drying by direct sunlight .
- Drying in the shade (air drying) .

#### **1.6.1.2. Industrial drying**

It depends on the use of industrial energy sources (such as burning fuel or electricity) to evaporate moisture from Air speed and relative humidity in proportion to the nature of the materials being dried, including :

- Cabinet dryers .
- B-Drying tunnels .
- C-Conveyor belt dryers .

#### **1.6.1.3. Solar drying**

This method depends on absorbing and collecting sunlight by absorbing cells or surfaces, then heating the air that is used to dry the plant .

### **1.7. Save (storage)**

The storage process begins after drying and is considered the last stage of medicinal plant production. It must be taken care of, as untreated storage may result in the loss of the plant's real estate value (Hamza, 2006) .

After using the above-mentioned drying methods for medicinal plants and in order to preserve them for the longest possible period while maintaining their medicinal effect, they must be stored and packaged in appropriate ways. This can be done either by grinding the plant part and storing it in special containers after writing the collection date and type of plant on the package, or by storing the plant part in its natural state, i.e. without grinding, and placing it in nylon bags or special containers and storing it in a good place free of insects, diseases and humidity, because such factors expose the plant to the following conditions :

- Oxidation by air.
- B- Side reactions that may change the nature of the active substance present in the plant .
- C- Activating some enzymes and carrying out hydrolysis processes, which leads to a change in the active components of the plant .
- Evaporation of some volatile compounds, especially volatile oils and some aromatic compounds, which are one of the active components in medicinal plants (Altaee, 2020) .

### **1.8. Areas of use of medicinal plants**

- Preparing some medicines such as medicines for joint pain and rheumatic inflammation, medicines for high blood pressure and arteriosclerosis, and as an antiseptic .
- Production of fixed oils, as the seeds of some of these plants contain fixed oils that are used in the composition of some medical preparations .
- Prepare foods for the treatment of atherosclerosis and angina, such as jojoba seed oil, sunflower, flax, and castor oil (Ibrahim, 2005) .
- It is used in the manufacture of insecticides based on the toxins present in it and used to exterminate insects, fungi, rodents, or nematodes, The most important of these are lemon grass, white and red squill onions, and henna.
- Preparation of cosmetics, dyes, creams, hair dyes, toothpastes and facial soaps
- Plants used as spices, seasonings, beverages, food additives, flavourings, fragrances or colouring materials .
- It is used in the manufacture of aromatic scents such as rose, jasmine, basil and cloves (Yousef, 2011) .

### **1.9. Essential oils**

#### **1.9.1. Definition of essential oils**

They are oils extracted from certain plants, or from certain parts of them. Essential oils are characterized by their strong smell and complete evaporation. They also do not contain fatty substances; therefore, they dissolve in water. When added to it, we find them floating on its surface in the form of spots (Wep 1).

### 1.9.2. How to extract essential oils

Essential oils can be extracted from several plants with different parts by various extraction methods, The manufacturing of essential oils, and the method used for essential oil extraction are normally dependent on botanical material used, State and form of material is another factor used for consideration, Extraction method is one of prime factors that determine, the quality of essential oil. Inappropriate extraction procedure can lead to the damage or alter action of chemical signature of essential oil. This results in the loss in bioactivity and natural characteristics, For severe case, discoloration, off-odor/ flavor as well as physical change such as the increased viscosity can occur, Those changes in extracted essential oil must be avoided .

The extraction process is done in several ways :

- Solvent extraction – Solvent
- Supercritical CO<sub>2</sub>
- Subcritical water
- Distillation – Steam
- Hydrodiffusion Solvent-free microwave
- Combination methods - Solvent + Steam (Tongsuthep ; Benjakul, 2014) .

### 1.9.3. Types of essential oils

• **Lavender Oil** : Helps relieve headaches, relax and sleep. Applied topically, it reduces itching and swelling caused by insect bites .

• **Chamomile Oil** : Helps to calm the mind and relax, and is also beneficial for the skin to treat inflammation and eczema .

• **Rose Oil** : Helps relieve anxiety, has antioxidant properties, treats acne and improves skin for a more youthful appearance .

• **Hyssop Oil** : is an earthy, herbal essential oil used to reduce scars and

• **Ylang Ylang Oil** : It has a spicy yet sweet scent, helps in relaxation, promotes hair growth and is also used as an insect repellent (Kamal, 2021).

## **2. *Artemisia herba-alba***

### **2.1. General**

It is a perennial plant belonging to the Asteraceae family, known by various names, such as wormwood and wormwood ,These species are grown in various regions around the world and are used in other cultures for medicinal and culinary purposes. Wormwood is characterized by its pungent aroma and bitter taste, making it a common ingredient in some dishes and beverages (Wep 2) .

It is a perennial plant, its lifespan reaches 3-4 years, and its length reaches about 60 cm. Its leaves have a strong, pleasant aromatic smell and a bitter taste. They are compound, successive, and numerous pinnate. Some of its species have leaves covered with white fluff. Its flowers are yellow bracts called bracts, and their number ranges between 14-20 bracts. Its seeds are small, 0.6-1 mm long, cylindrical or flat, gray-brown in color, crowned with hairs that help them disperse easily. One plant can produce 100,000 seeds (Shehab, 2021) .

### **2.2. Origin**

Artemisia is a large plant genera in the Asteraceae family, comprising more than 280 species native to the arid regions of Asia, North and South America, and North and South Africa. It is a drought-tolerant plant species typical of the arid continental environment. This plant is highly specialized in the types of soil it grows in and their ability to retain water. Artemisia is found on dry, sandy soils with moderate permeability and grows in floodplains and valleys where heavy soils with good moisture-retention capabilities are available. Artemisia cannot tolerate high soil salinity, and its germination rate is affected by high salinity (Shehab, 2021) .



**Figure 2.** *Artemisia herba-alba* (Esmail *et al.*, 2015) .

### 2.3. Scientific classification

There are several classification that have been adopted (Gacem *et al.*, 2020).

<b>Kingdom</b>	<i>Plantae</i>
<b>Division</b>	<i>Magnoliophyta</i>
<b>Class</b>	<i>Magnoliopsida</i>
<b>Order</b>	<i>Asterales</i>
<b>Family</b>	<i>Asteraceae</i>
<b>Genus</b>	<i>Artemisia</i>
<b>Species</b>	<i>Artemisia herba-alba</i>

### 2.4. Chemical composition

According to an article (Wep 2) wormwood contains a group of active ingredients :

#### 2.4.1. Active ingredients :

- **Santonin** : It is one of the active compounds in wormwood and is used to expel intestinal worms, as it works by affecting the parasites' nervous system
- **Flavonoids** : These compounds are powerful antioxidants that help reduce inflammation and promote cardiovascular health. Flavonoids are useful in protecting cells from damage caused by free radicals .
- **Artemisinin** : It is extracted from some types of wormwood and is used to treat malaria, making it one of the most powerful drugs available against this disease. It is said to have powerful effects on parasites, making it an important option in traditional medicine .

#### 2.4.2. Essential oils :

Camphor (39.5%)

1,8-cineole (8,6%)

$\alpha$ -thujone (7,03%)

Borneol (3,35%)

Bornyl acetate (2,52%) (Amor *et al.*, 2019) .

## **2.5. Biological activity of plant**

### **2.5.1. Antibacterial activity**

A study showed that methanolic extracts of white wormwood possess antibacterial activity, showing efficacy against *Staphylococcus aureus* and some Gram-negative bacterial strains. Disk diffusion and microdilution techniques were used to determine the antibacterial activity, and the results showed that white wormwood extracts possess antibacterial properties, which enhances their potential use in controlling plant pathogenic bacteria (Al-Lafi, 2009) .

### **2.5.2. Antifungal activity**

Studies have shown that white wormwood has antifungal properties. In a study published in the MDPI journal, essential oils were extracted from the white wormwood plant in Morocco and their chemical composition was analyzed using GC-MS. The results showed that these oils contain active compounds with antifungal properties, suggesting their potential as a natural fungicide (Houti *et al.*, 2023) .

### **2.5.3. Activity as an insecticide**

Research has shown that *Artemisia herba-alba* has effective properties in controlling some types of insects. In a study published in the Arab Journal of Plant Protection, the effect of aqueous extracts of several plants, including *Artemisia herba-alba*, on the pea beetle (*Bruchus pisorum*) was evaluated. The results showed that the *Artemisia* extract resulted in a mortality rate of 35.5% in different stages of the insect, indicating its effectiveness as a natural insecticide (Halak, 2013) .

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**Chapter II : General  
information about chickpeas and  
*Callosobruchus maculatus* F**

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## 1. Chickpeas

### 1.1. The origin chickpeas

Chickpea, scientifically known as *Cicer arietinum L.*, is a member of the Cicereae tribe within the Fabaceae family, specifically belonging to the Papilionaceae subfamily. This ancient pulse crop holds significant importance as one of the world's most vital legume crops. Across various cultures and languages, chickpea is embraced with a plethora of names, such as garbanzo in Spanish, pois chiche in French, kichar or chicher in German, chana in Hindi, and gram or Bengal gram in English (Zhang et al., 2024). A staple of Middle Eastern, African and Indian cuisines, the chickpea is the world's second most widely grown legume after the soybean, and one of the eight founder crops of the origins of agriculture on our planet. Chickpeas store really well and are high in nutritive value, although they are not very disease resistant, compared to other legumes (Hirst, 2019).

### 1.2. Definition of chickpeas

Chickpea *Cicer arietinum L.* is an important pulse crop grown and consumed all over the world, especially in the Afro-Asian countries. It is a good source of carbohydrates and protein, and protein quality is considered to be better than other pulses (Jukanti et al., 2012). Moreover, chickpea cultivation has low water requirement and, as other legumes, improves soil characteristics being recommendable for a sustainable agriculture (Millàn et al., 2015).

Chickpea is used predominantly for human food products but also can be used in animal feed. Chickpeas have a wide variety of food uses, including salads, hummus and cooked in stews or curry, Chickpea flour is used as batter for deep-fried meats and vegetables or an ingredient to make flat breads and desserts (Keene et al., 2020).



**Figure 3.** Chickpeas (Wep 3)

### 1.3. Scientific classification chickpeas

According to the classification (Perumal *et al.*, 2021 ).

<b>Kingdom</b>	plantae
<b>Phylum</b>	Tracheophyta
<b>Class &amp; order</b>	Magnoliposida & Fabales
<b>Family</b>	<i>Leguminosae</i>
<b>Subfamily</b>	Papilionoidae
<b>Genus</b>	<i>Cicer</i>
<b>Section &amp; Species</b>	<i>Monocicer &amp; arietinum L</i>

### 1.4. Chemical composition

According to (Hevryk *et al.*, 2020) The chemical components of chickpeas are presented in Table 1.

**Table 1** . Chemical Composition of chickpeas .

<b>Nutrients</b>	<b>Average value of indicators (g/100 g)</b>
Protein	19.5–21.6
Total fat :	6.7–6.8
– saturated	0.66–7.0
– unsaturated	2.60–3.00
– monounsaturated	1.40–1.50
Carbohydrates	50–60
Food fiber	18–26
– soluble	4–8
– insoluble	10–18
Starch	28–29
Sugar :	5.4–10.7
– restoring	1.5–3.1
– not restoring	3.5–6.8
Minerals (ash)	2.7–3.6
General composition of polyphenols	1300–1500**
Flavonoids	400–450**
Anti-food substances-solutions	–
– Phytic acid	230–265**
– Tannins	460–480**

## **2. *Callosobruchus maculatus* F**

### **2.1. Definition of pest**

Is an important pest of legume seeds both in the field and in storage (Figure 4) , the infestation of the crops starts in the field while most damage occurs during storage (Garima *et al.*, 2021) , This pest is native to Africa, but today it is distributed all over the world, especially in tropics and subtropics, which causes significant damage to storage products (Hamzei *et al.*, 2023) .

The primary source of infestation starts in the field where adult females lay their eggs on the pods, causing minor damage. The larvae penetrate the pods and remain hidden inside the developing seeds as latent infestations. The damage caused by the larvae results in the

hollowing out of the seeds, which in turn renders them unfit for human consumption and bud production. The presence of rounded exit holes on the surface of the seeds indicates seed infestation. Therefore, for its management, an understanding of its biology becomes essential (Mutalikdesai *et al.*, 2023) .



**Figure 4.** picture of *Callosobruchus maculatus* F (Garima *et al.*, 2021) .

## 2.2. Scientific classification

According to the classification (Bousquet *et al.*, 2013)

<b>Kingdom</b>	Animalia
<b>Phylum</b>	Arthropoda
<b>Class</b>	Insecta
<b>Order</b>	Coleoptera
<b>Family</b>	Chrysomelidae
<b>Genus</b>	Callosobruchus

## 2.3. Morphological, reproductive and developmental traits of the pest

**Table 2.** Morphological traits .

Stage	Sex	Size	Colour	Abdomen	Antenna	Antenna size	Mobility
Adults	Male	Smaller	Brownish	Obtuse	Pectinate	Larger	Hyper
	Female	Blackish	Blackish	Pointed	Serrate	Shorter	Hypo

**Table 3.** Reproductive traits .

<b>Mating after emergence</b>	<b>Mating duration</b>	<b>Oviposition after mating</b>	<b>Egg laying potential</b>	<b>Choice of egg laying</b>
40-60 min	30-40 min	45-60 min	24h>48h>72h	1 egg in 1 seed and more than 1 when seeds are inadequate

**Table 4.** Developmental (egg-adult) traits .

<b>Incubation (Days)</b>	<b>Larval stage (Days)</b>				<b>Pupal stage (Days)</b>		<b>1st Emergence (Day/s)</b>	<b>Longevity (Days)</b>
Egg	Instar				Pre-pupa	Pupa	Adult (♂ & ♀)	♂ 6.5±1.5 ♀ 10.5±3.5
	1st	2 <sup>nd</sup>	3rd	4th				
1-3	4-9	10-12	13-16	17-20	21	22-26	27	
3	6	3	4	4	1	5	1	

## 2.4. Life cycle

### 2.4.1. Eggs :

A single female *Callosobruchus maculatus* lays more than 100 eggs during its lifetime and deposits one egg per seed. Eggs are transparent, oval, or spindle-shaped and glued to the seed surface . These are small, 0.74 mm in length, and 0.38 mm in width at 50X. suggested that the female beetle's life cycle duration increases if the female lays multiple eggs on each seed .

### 2.4.2. Larva :

The egg hatches and the larva bores inside the seed. Once the larva gets inside, the eggshell turns opaque as it gets filled with the larval frass . It has four larval instars before turning into the pupa .

### 2.4.3. Pupa :

The pupa is whitish, about 3.87 mm long and 1.76 mm wide at 50X. When the larva starts to pupate, the seed's shell starts turning thinner.

#### 2.4.4. Adult :

The adult *Callosobruchus maculatus* chews the seed coat and emerges out of the seed, adult is metallic in color with some pale spots/stripe. The head of the male and female is black, and the wings are brown with black patches. The male and female can be distinguished by the plate's color at the abdomen's end. In the female, the plate is large and colored black on the sides with a white longitudinal line, while in the male, it is smaller and lacks strips. The adults become sexually mature after 24- 48 hours of emergence. The adult lives for two-three weeks .

Male *Callosobruchus maculatus* beetles have spines in genital organs, and they tend to puncture females' genital tract during copulation. Males and females are both polygamous. Female *C. maculatus* mating with multiple virgin males live longer, lay more and larger sized eggs throughout life than females mating only once. The number of eggs laid by the female depends on the hosts' availability to decrease the larval competition (Garima *et al.*, 2021) .



**Figure 5.** Life cycle of *C. maculatus* (Mssillou *et al.*, 2022 ) .

#### 2.5. Damage caused by the pest :

When seeds are damaged, they lose weight, germination capacity, and market value. Damaged seeds can also lead to significant economic losses (Umeannaeto *et al.*, 2020).

#### 2.6. Control methods :

In the present time, bruchids cause enormous loss to pulses and a severe issue of concern. Several strategies have been developing for the prevention of vibrations from insect

infestation especially *C. maculatus* , There are many methods and control measures taken against storage beetles are (Kalpna *et al.*, 2022) .



**Figure 6.** Pest management practices for stored grain pests (Kalpna *et al.*, 2022)..

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**The applied part**

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## Chapter III: Materiels and Methodes

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## 1. Matérials and Méthods

This work was conducted in the laboratory of the Faculty of Natural and Life Sciences, university of El Oued . It consists of highlight the insecticidal potential of the essential oil of the plan *Artemisia herba-alba* In this study, we used the hydro-distillation method to extract the essential oil from the plant *Artemisia herba-alba* which we tested on the chickpea pest *Callosobruchus maculatus* F.

To assess the insecticidal effect of oil *Artemisia herba-alba* ,we estimated the mortality rate of adults using two methods: contact and inhalation.

Our research was conducted on the aerial parts of *Artemisia herba-alba* which are widely used by most humans. Samples were collected from the Batna and Khenchela regions , The sample collection process was carried out using the means available to us .

### 1.1. Presentation of the study area

#### 1.1.1. Batna region

The city of Batna, capital of the Aurès, chief town of wilaya, is located in the Eastern Highlands region, in the middle of the Aurès massif in a val ley at the junction of two Atlas Mountain ranges (Tellian and Saharan), this city is located 400 km east of the capital, Algiers, and rises to > 900 m a.s.l. It is bounded in the north, by the wilaya of Mila; In the north east, by the wilaya of Oum-El-Bouaghi; in the east, by the wilaya of Khenchela, in the south, by the wilaya of Biskra, in the west, by the wilaya of M'Sila; in the northwest, by the wilaya of Sétif. In the WGS 84 coordinate system used by the Global Positioning Satellite, the willaya of Batna is located between the following geographic co-ordinates: 35°33'21"N, 6°10'26"E (Fekkous *et al.*, 2022) .

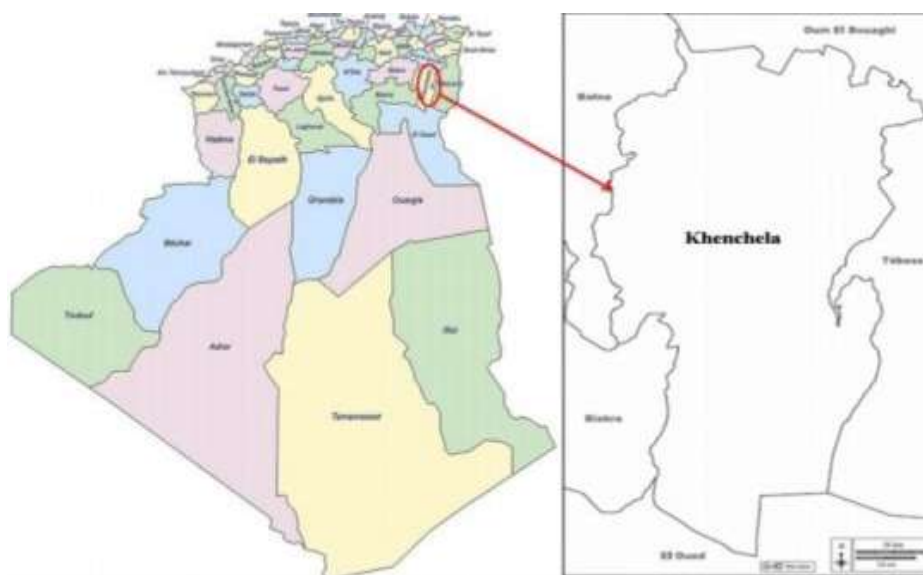


**Figure 7.** Geographical location of Batna region

### 1.1.2. Khenchela region

The region of Khenchela is located 7°34' East longitude and between 35°7' m), The city is bordered by the province of in the northeast of Algeria (6°32' and and 35°38' North latitude, altitude of 1200 Oum El Bouaghi in the north, the province of El Oued and Biskra in the south, the province of Tebessa in the east and the average minimum

temperature of 1.85°C province of Batna in the west (Figure 8).July, and a very cold winter with an a hot and dry summer recording a The region of Khenchelais characterized by a semi-arid climate, with maximum temperature of 34.9°C during in January. The annual average rainfall is about 508.83 mm (Halimi *et al.*, 2022) .



**Figure 8.** Geographical location of Khenchela region (Algeria) (Halimi *et al.*, 2022) .

## 1.2. Materials used

### 1.2.1. Plant materials

#### ❖ *Artemisia herba-alba*

The plant *Artemisia herba-alba* used in this study was taken by taking random samples from two regions, Batna and Khenchela. They were collected and then dried naturally away from light to avoid changing the active ingredients due to light. After drying , they were cut into small pieces and stored at room temperature in the laboratory away from sunlight .



**Figure 9.** *A. herba-alba* of Khenchela .



**Figure 10.** *A. herba-alba* of Batna

The part of the plant used is the dried leaves of the plant .

### 1.2.2. Animal material

#### ❖ *Callosobruchus maculatus*

*C. maculatus* was chosen because of the significant damage this pest causes to economically important stored goods. Furthermore, it can be easily propagated in the laboratory .

### 1.2.3. laboratory equipment



**Figure 11.** Equipment used in the laboratory .

1 : Test tube s / 2 : Micropipettes / 3 : Pipette tip ( yellow,blue,white ) / 4 : Small bottle of distilled water / 5 : Electronic scale / 6 : UV spectrometer / 7 : laboratory oven / 8 : Rotary evaporator / 9 : Clevenger / 10 : Inflate ballon .

### 1.3. Method

#### 1.3.1. Method of breeding bruchus

Individuals of *Callosobruchus maculatus* are maintained under laboratory conditions of temperature: 25-35°C and a relative humidity ranging from (65 to 70%), in glass jars containing chickpea seeds as a food substrate, To separate the insects, we used 0.5 mm diameter pieces of cloth. Then, using tweezers, we placed the adult insects in separate compartments inside 9 cm diameter Petri dishes, Note that these insects were brought to a room set at 35°C to accelerate their life cycle for use in our experiments .



**Figure 12.** Chickpeas infected with *C. maculatus* .

#### 1.3.2. *Artemisia herba-alba* essential oil Method extraction

Essential oil is extracted using the hydro-distillation method. In a bottle, 750 ml of distilled water is placed and 100 grams of *Artemisia herba-alba* are added. The contents of the bottle are boiled at a high temperature for 3 hours, The vapors loaded with oil pass through the radiator, and then the oil separates from the water due to the difference in density, The oil is kept in small glass bottles at a low temperature .



**Figure 13.** Clevenger device for extracting *Artemisia herba-alba* essential oil .

There are some basic precautions to take when storing essential oils, That's why we keep them at a temperature close to 4° .

### 1.3.3. Bioprocessing Application

To evaluate the insecticidal effect of the essential oil extracted from *Artemisia herba-alba* from Batna and kenchela and to determine and verify the level of effectiveness of this evaluation, we conducted several mortality tests :

- Direct contact test, also called corrected mortality test
- Fumigation test, also called inhalation test

#### 1.3.3.1. By direct contact

To perform this test, an Acetone solution is prepared consisting of 5% Acetone and 95% distilled water, then volumes of 0.357, 0.75 and 1.5 microliters of essential oil (Batna and Khenchela) are dissolved in 18 ml of Acetone solution

We prepare 18 sterile Petri dishes (9 dishes for the first wormwood and 9 dishes for the second wormwood) and cut circles of Watman paper with the same diameter as the dishes and place them at the bottom of the dishes, 10 adult insects of the type *Callosobruchus maculatus* *F* are added to each one, 3 repetitions are carried out for all sizes and then placed at a medium temperature. Finally, we note the number of dead insects after 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes .



**Figure 14.** Contact toxicity test of *Artemisia herba-alba* oil (Batna and Khenchela) to *C. maculatus* .

### 1.3.3.2. By fumigation

Three doses of the essential oils of Batna and Khenchela corresponding to concentrations of 0.35, 0.75 and 1.5  $\mu\text{l}$  are used in a single application, Toxicity tests are performed by vaporization in a glass container, To do this, place a quantity of the essential oil on a piece of cotton and tie it with a thread, Then, observe its evaporation in a glass container, Then, place groups of ten (10) adult insects of the *C. maculatus*, place a cotton ball soaked in the essential oil and close it in the container, Each test is repeated three times. The number of insects in each batch is counted, We note the death of the insects after 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes and 60 minutes .



**Figure 15.** Fumigation test of *Artemisia herba-alba* oil (Batna and Khenchela) on *C. maculatus* .

### 1.3.3.3. Data analysis

### 1.3.3.3.1. Enumeration

The mortality rate of adults with *C. maculatus* is estimated as a function of exposure time and different doses applied D1, D2, D3 compared to the control. However, verification of the mortality of adults is carried out using a clamp .

### 1.3.3.3.2. Estimation of observed mortality

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

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

### 1.3.3.3.3. 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 a toxicant is not the actual number of individuals killed by that toxicant, There is, in fact, in any treated population a natural mortality which is added to the mortality caused by the toxicant, For this, the mortality percentages must be corrected by the formula of Abbott (1925), which is as follows :

$$M_c = (M_0 - M_t / 100 - M_t) \times 100$$

**M<sub>c</sub>** : Mortality corrected in percentage

**M<sub>t</sub>** : Mortality in treated lot

**M<sub>0</sub>** : Mortality in untreated control lot

### 1.3.3.3.4. 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 Cavlier (1976), These probits are graphically represented as a function of the nepberian 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 DL50 .

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## **Chapter IV: Results**

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## 1. Results

### 1.1. Oil performance calculation

#### 1.1.1. *Artemisia herba-alba* from Batna

The organic matter obtained after extraction is an oily substance with a very strong odor characteristic of *Artemisia herba-alba* and a light green color, with a yield of 0.927 % .

#### 1.1.2. *Artemisia herba-alba* from Khenchela

The organic matter obtained after extraction is an oily substance with a very strong odor characteristic of *Artemisia herba-alba* and a light green color, with a yield of 1.59 % .



**Figure 16.** The amount of oil extracted from *Artemisia herba-alba* .

## 1.2. Evaluation of the insecticidal effect of different concentrations of essential oil of *Artemisia herba-alba* on *C. maculatus*

### 1.2.1. Direct contact processing

#### 1.2.1.1. *Artemisia herba-alba* of Batna

According to Figure 17, compared to the control group, we see that all treatments have a toxic effect over time on *C. maculatus* individuals with (C3 = 1.5  $\mu$ L) toxicity being superior compared to other doses tested .

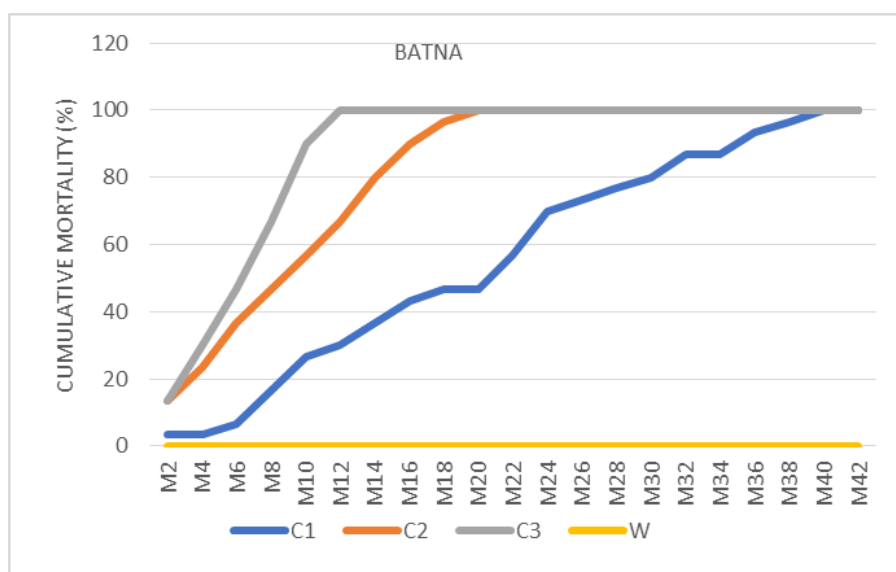
We see that the mortality rate of individuals appears from the first minutes of the experiment, The mortality rate of *C. maculatus* individuals varies according to the doses tested, The higher the HE dose the higher the mortality rate .

From Figure 17, we note that the lowest concentration of *Artemisia herba-alba* oil (0.35  $\mu$ l) begins to affect adult *C. maculatus*, from the second minute of the test, with a mortality rate of 3.33%, This rate gradually increases to 46.66% after 20 minutes, It then gradually increases to its highest rate of 93.34% at minute 36, and at 38 minutes, it reaches its maximum mortality rate of 100% .

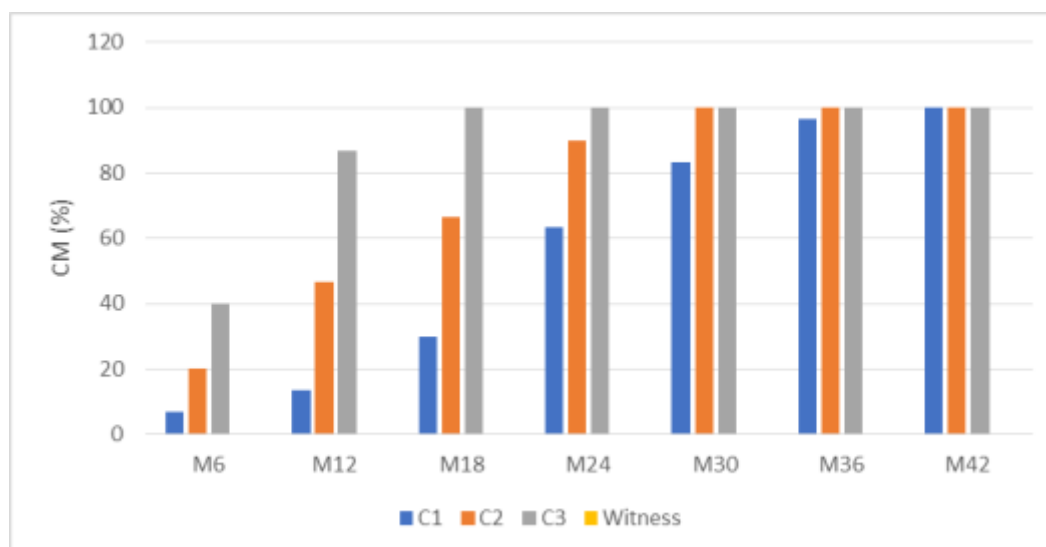
As for the (C2 =0.75  $\mu$ L) the effect begins as early as the second minute with a mortality rate of 13.33%, this rate then gradually increases until reaching 46.66% at minute 8, It then continues to gradually increase until reaching a rate of 80% at minute 14 and continues to increase until its maximum of 96.66% at minute 18. It then reaches the maximum mortality rate at 20 minutes with a mortality rate of 100% .

Finally, the highest (C3 = 1.5  $\mu$ L) began to take effect from the second minute, with a mortality rate of 13.33%. This rate then increased rapidly, reaching a peak of 90% at the tenth minute. It then gradually increased until it reached its maximum rate of 100% at the twelfth minute.

We did not observe any deaths in the control groups .



**Figure 17.** Time course of cumulative mortality of *C. maculatus* treated with *Artemisia herba-alba* oil of Batna at three concentrations for 42 minutes via direct contact .



**Figure 18.** Insecticidal effect of *Artemisia herba-alba* oil of Batna on *C. maculatus* by direct contact .

Within 6 minutes, we found that at a concentration of (C1= 0.35 $\mu$ l), it caused a cumulative mortality rate of 6.66%. While we recorded 70% at 24 minutes, resulting in a reduction of more than half the number of insects, the final time (42 minutes) saw complete exclusion of insects compared to the controls .

For the second concentration (C2= 0.75  $\mu$ L), he observed a cumulative mortality rate of 36.66% for the first 6 minutes, after 18 minutes, he observed an increase in insect sensitivity of 96.66%, and over another 42 minutes the insects were eliminated .

For the third concentration (C3= 1.5  $\mu$ l), a rapid increase in mortality is observed during the first six minutes, with a mortality rate of 46.66%, but after 12 minutes, a reduction of half the number of insects is observed, or a final mortality rate of 100% .

#### 1.2.1.2. *Artemisia herba-alba* of Khenchela

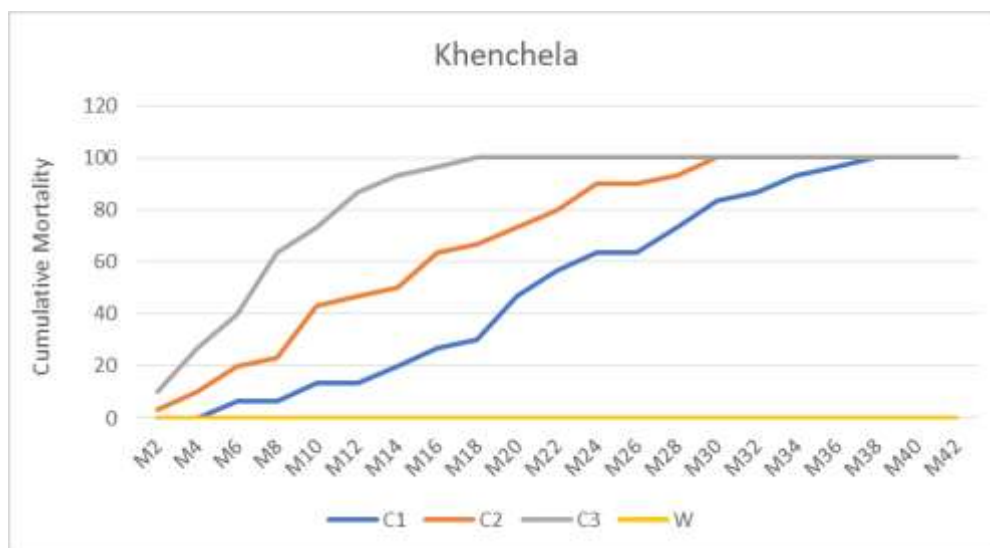
According to Figure 19, we note that the mortality rate of *Callosobruchus maculatus* F individuals appears from the first minutes of the experiment, The mortality rate of *Callosobruchus maculatus* F individuals varies depending on the doses tested, The higher the HE dose, the higher the mortality rate, This means that all treatments have a toxic effect over time on *C. maculatus* individuals, with the (C3 =1.5  $\mu$ L) toxicity being higher than other doses, such as *Artemisia herba-alba* of Batna .

From Figure 19, we note that the lowest concentration of white artemisia oil (0.35  $\mu$ L) begins to affect *C. maculatus* adults starting from the sixth minute of the experiment, with a

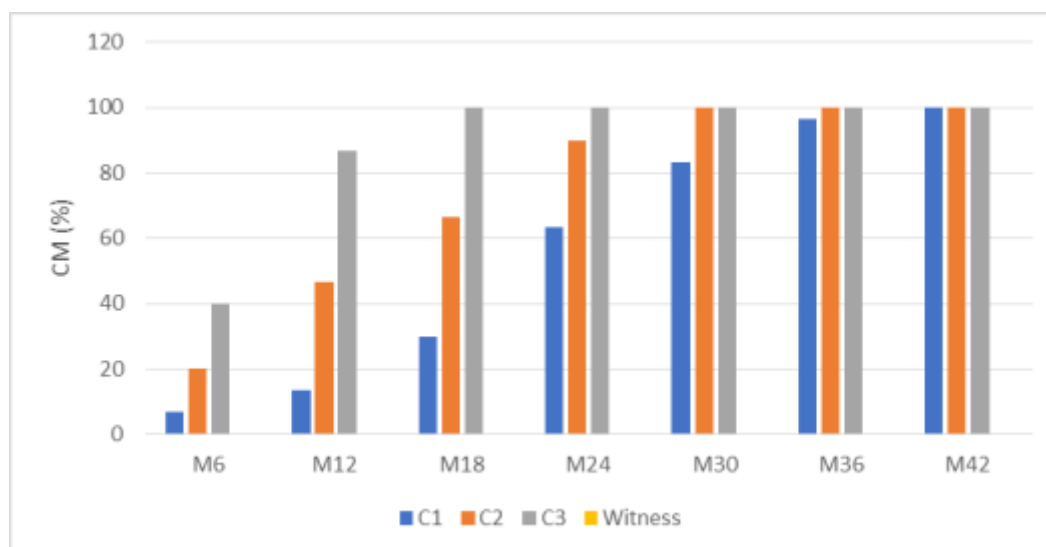
mortality rate of 6.67%, This rate gradually increases to 63.34% after 24 minutes, then gradually increases to its highest rate of 96.34% at the 36th minute, and reaches its highest mortality rate of 100% at the 38th minute .

At a (C2 = 0.75  $\mu$ L), the effect began at the second minute with a mortality rate of 3.33%, This rate then gradually increased to 73.33% at the 20th minute. It then continued to increase gradually until it reached 90% at the 24th minute, and continued to increase until it reached a peak of 93.33% at the 28th minute, The highest mortality rate was reached at 30 minutes with a mortality rate of 100% .

Finally, the highest (C3 = 1.5  $\mu$ L) began to affect the second minute with a mortality rate of 10%, This rate then rapidly increased, reaching a peak of 96.67% at the 16th minute, and then gradually increased until it reached its highest rate of 100% at the 18th minute .



**Figure 19.** Time course of cumulative mortality of *C. maculatus* treated with *Artemisia herba-alba* oil of Khenchela at three concentrations for 42 minutes via direct contact .



**Figure 20.** Insecticidal effect of *Artemisia herba-alba* oil of Khenchela on *C. maculatus* by direct contact .

Within 6 minutes, we found that at a (C1 = 0.35  $\mu$ L), it caused a cumulative mortality rate of 6.67%, while at 24 minutes we recorded a cumulative mortality rate of 63.34%, resulting in a reduction of more than half the number of insects. The final time (42 minutes) saw complete exclusion of insects compared to the control group .

For the second concentration (C2 = 0.75  $\mu$ L), we observed a cumulative mortality rate of 20% within the first 6 minutes, After 18 minutes, we observed an increase in insect sensitivity to 66.66%, and over another 30 minutes the insects were eliminated .

For the third concentration (C3 = 1.5  $\mu$ L), a rapid increase in mortality was observed within the first 6 minutes, with a mortality rate of 46.66%. However, after 12 minutes, we observed a reduction in insect numbers by half, or a final mortality rate of 100% .

## 1.2.2. Fumigation processing

### 1.2.2.1. *Artemisia herba-alba* of Batna

When using *Artemisia herba-alba* oil by fumigation we recorded a mortality rate over time on *C. maculatus*, the toxicity of (C3= 1.5 $\mu$ L) was superior compared to other doses tested, as shown in Figure 18 .

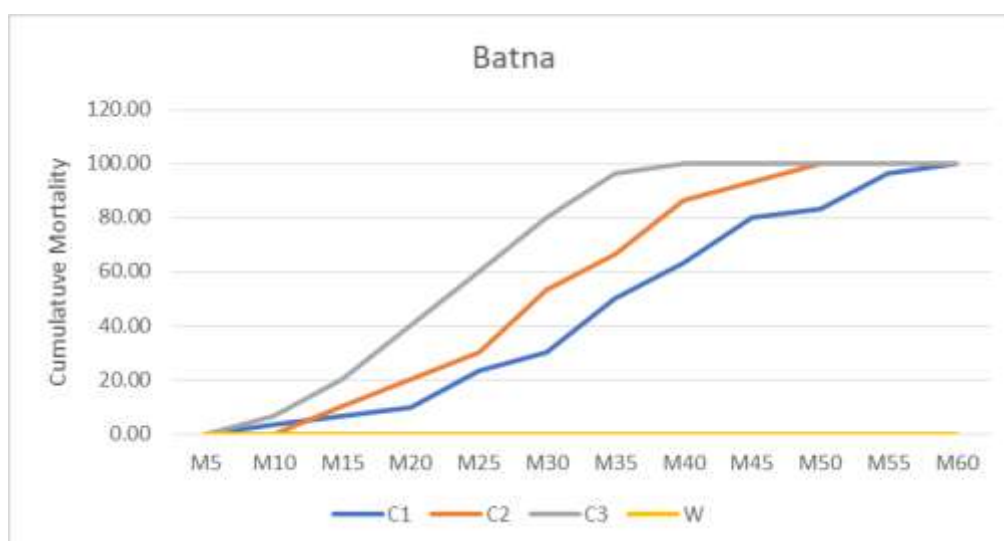
We note that the lowest concentration of *Artemisia herba-alba* oil (0.35  $\mu$ L) begins to affect *C. maculatus*, adults starting at the 10th minute of the experiment, with a mortality rate of 3.33%, This rate gradually increases to 23.33% at 25 minutes, then gradually increases to

its highest rate of 96.66% at the 55th minute, and reaches its highest mortality rate of 100% at the 60th minute .

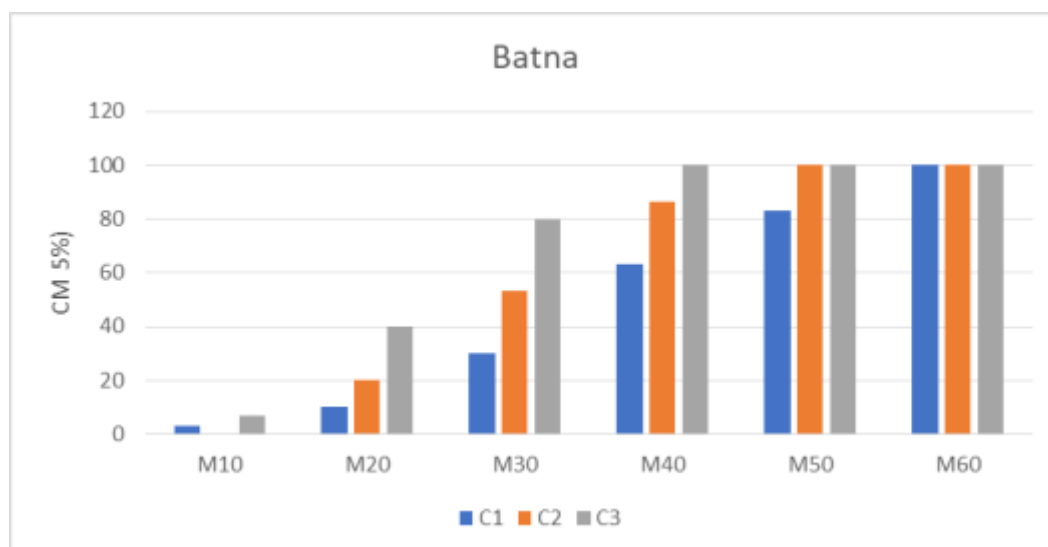
For (C2 = 0.75  $\mu$ L) the effect began at minute 15 with a mortality rate of 10%. This rate then gradually increased to 53.33% at minute 30, then continued to increase gradually to 86.67% at minute 40, and continued to increase until it reached its peak of 93.33% at minute 45, It then reached its highest mortality rate at minute 50 with a mortality rate of 100 % .

Finally, the highest rate (C3 = 1.5  $\mu$ L) began to have an effect at minute 10, with a mortality rate of 6.67%, This rate then rapidly increased, reaching its peak of 96.67% at minute 35, It then gradually increased until it reached its highest rate of 100% at minute 40 .

We did not observe any deaths in the control groups .



**Figure 21.** Time course of cumulative mortality of *C. maculatus* treated with *Artemisia herba-alba* oil of Batna at three concentrations for 60 minutes via fumigation .



**Figure 22.** Insecticidal effect of *Artemisia herba-alba* oil of Batna on *C. maculatus F* by fumigation .

At (C1 = 0.35  $\mu$ L), in the first 10 minutes, it caused a cumulative mortality rate of 3.33%, while at 40 minutes, we recorded a cumulative mortality rate of 63.33%, resulting in a reduction of more than half the insect population, At the final time (one hour), the insects were completely eliminated compared to the control group .

For the second concentration (C2 = 0.75  $\mu$ L), we observed a cumulative mortality rate of 20% during the first 20 minutes, After 30 minutes, we observed an increase in insect sensitivity to 53.33%, and over the course of 50 minutes, the insects were eliminated .

For the third concentration (C3 = 1.5  $\mu$ L), a rapid increase in mortality was observed during the first 10 minutes, with a mortality rate of 6.67%, After 30 minutes, we observed a reduction in insect numbers by more than half, and at 40 minutes a final mortality rate of 100% was recorded .

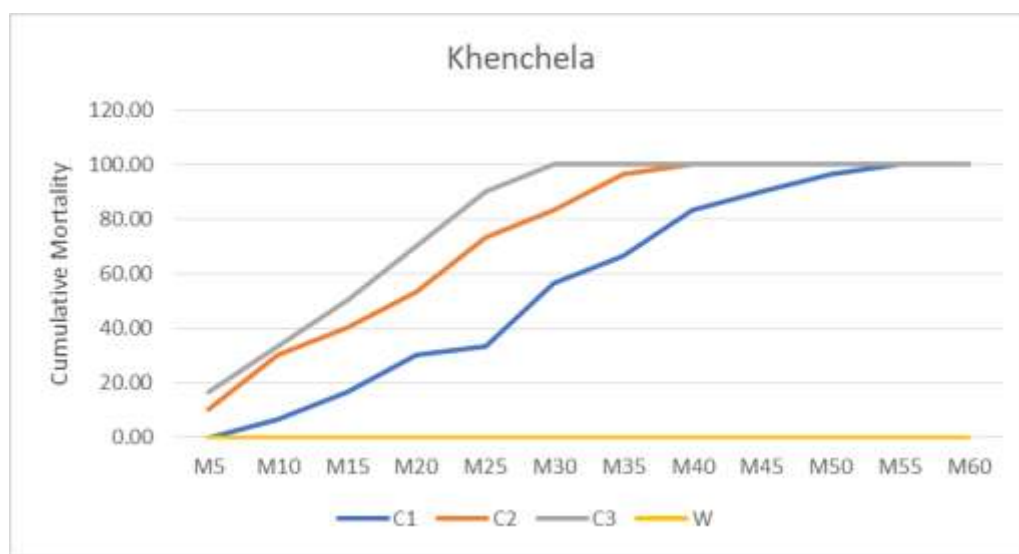
#### 1.2.2.2. *Artemisia herba-alba* of Khenchela

From Figure 23, we note that the lowest concentration of white wormwood oil (0.35  $\mu$ L) begins to affect *C. maculatus F* adults starting at the 10th minute of the experiment, with a mortality rate of 6.67%. This rate gradually increases to 66.67% at 35 minutes, then gradually increases to its highest rate of 96.67% at the 50th minute, reaching its highest mortality rate at the 55th minute, with a mortality rate of 100% .

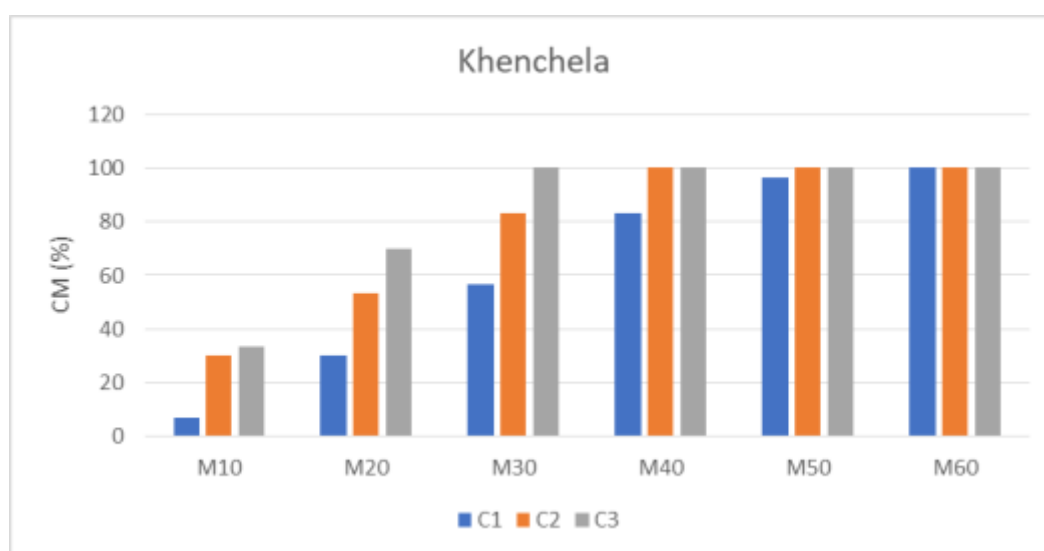
At the concentration of (C2-0.75  $\mu$ L), the effect began at the 5th minute with a mortality rate of 10%, then gradually increased to 73.33% at the 30th minute, It then

continued to increase gradually until it reached its peak of 96.67% at the 35th minute, and reached its highest mortality rate at the 40th minute, with a mortality rate of 100% .

Finally, the highest rate (C3-15  $\mu\text{L}$ ) began to take effect at the fifth minute, with a mortality rate of 16.67%. This rate then increased rapidly, reaching a peak of 90% at the 25th minute, and then gradually increased until it reached its highest rate of 100% at the 30th minute .



**Figure 23.** Time course of cumulative mortality of *C. maculatus F* treated with *Artemisia herba-alba* oil of Khenchela at three concentrations for 60 minutes via fumigation .



**Figure 24.** Insecticidal effect of *Artemisia herba-alba* oil of Khenchela on *C. maculatus* by fumigation.

In the first 10 minutes for the concentration ( $C_3 = 25 \mu\text{L}$ ), we recorded 6.67% mortality, and after 30 minutes we recorded 56.67%, which led to a decrease in the number of insects by more than half, and after 60 minutes 100% mortality was recorded .

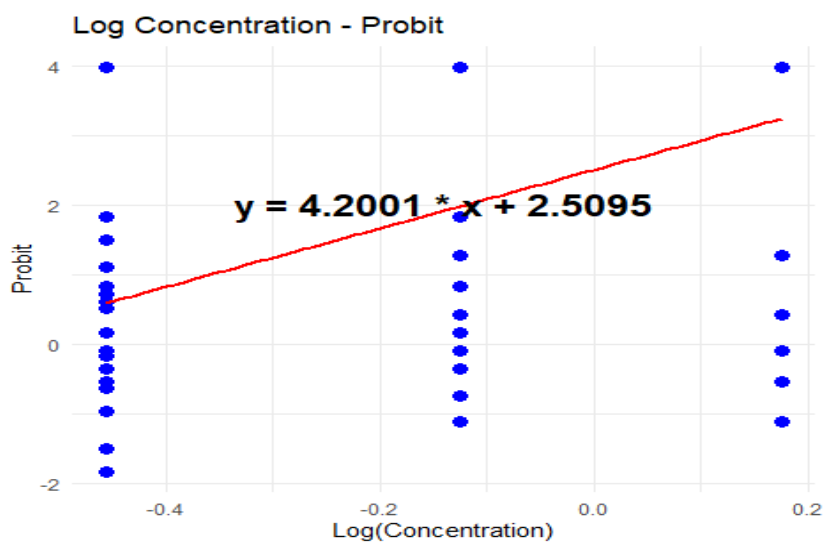
For the second concentration ( $C_2 = 0.75 \mu\text{L}$ ), we observed a cumulative mortality rate of 30% within the first 10 minutes. After 30 minutes, we observed an increase in insect sensitivity to 83.33%, and over the course of 40 minutes, the insects were eliminated.

For the third concentration ( $C_3 = 1.5 \mu\text{L}$ ), a rapid increase in mortality was observed within the first 10 minutes, with a mortality rate of 33.33%. After 20 minutes, we observed a reduction in insect numbers by more than half, and at 30 minutes, a final mortality rate of 100% was recorded.

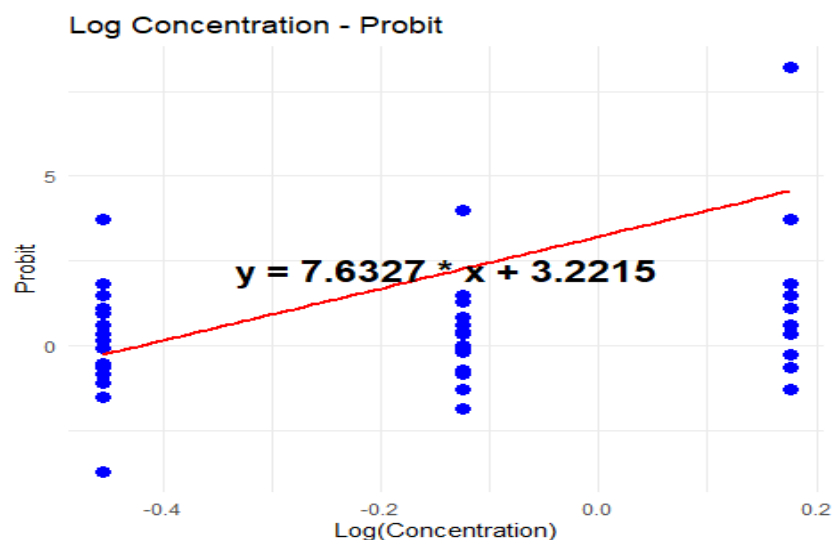
### 1.3. Determining DL 10, DL 50 and DL 90

To determine D 10, DL 50, and DL 90, we used a regression line. The latter represents the logarithm of the tested concentrations and the cumulative mortality rates in the probit .

#### 1.3.1. Direct processing



**Figure 25.** DL 10, DL 50 and DL 90 curves for *Artemisia herba-alba* essential oil from Batna for mortality compared to concentrations after 42 minutes using the direct method .



**Figure 26.** DL 10, DL 50 and DL 90 curves for *Artemisia herba-alba* essential oil from Khenchela for mortality compared to concentrations after 42 minutes using the direct method

**Table 5.** Lethal concentrations (DL10, DL50, DL90) of *Artemisia herba-alba* essential oil extracted from Batna and Khenchela regions were estimated after 42 minutes using the direct application method .

Concentrations	Batna	Khenchela
DL10	= 0.125	= 0.257
DL50	= 0.252	= 0.379
DL90	= 0.510	= 0.558

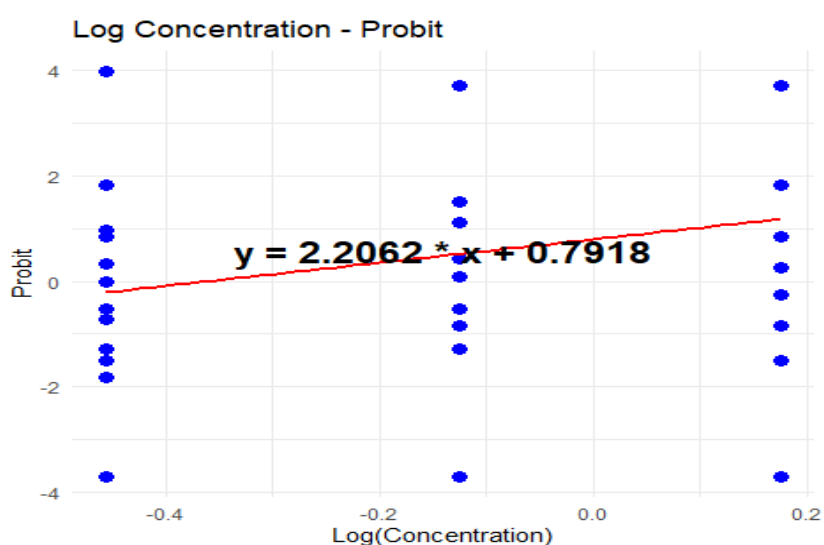
Figures 25 and 26 show the dose-response (Log Concentration-Probit) curves for *Artemisia herba-alba* essential oil extracted from the Batna and Khenchela regions, to assess mortality rates compared to different concentrations after 42 minutes using the direct application method, The regression equation shown in Figure 25 ( $y = 4.2001x + 2.5095$ ) indicates the relationship between the logarithm of the concentration and the corresponding probability of mortality in the Batna sample, Similarly, Figure 26 displays the regression equation for the Khenchela sample ( $y = 7.6327x + 3.2215$ ). In addition, the accompanying table (5) summarizes the lethal concentration values at 10%, 50%, and 90% for both samples. The results show that the Khenchela samples had higher values compared to the Batna samples, indicating a difference in the efficacy of the essential oil between the two regions .

Looking at the lethal concentration values summarized in the accompanying table, differences in the potency of the essential oil between the two regions can be observed using the direct method, For DL 10 (the lethal concentration for 10% of the sample), the estimated value for the samples extracted from Batna was 0.125  $\mu\text{L}$ , while it was higher for the Khenchela samples at 0.257  $\mu\text{L}$ , This indicates that a lower concentration of the essential oil extracted from Batna is sufficient to cause mortality in 10% of the tested organisms compared to the oil extracted from Khenchela .

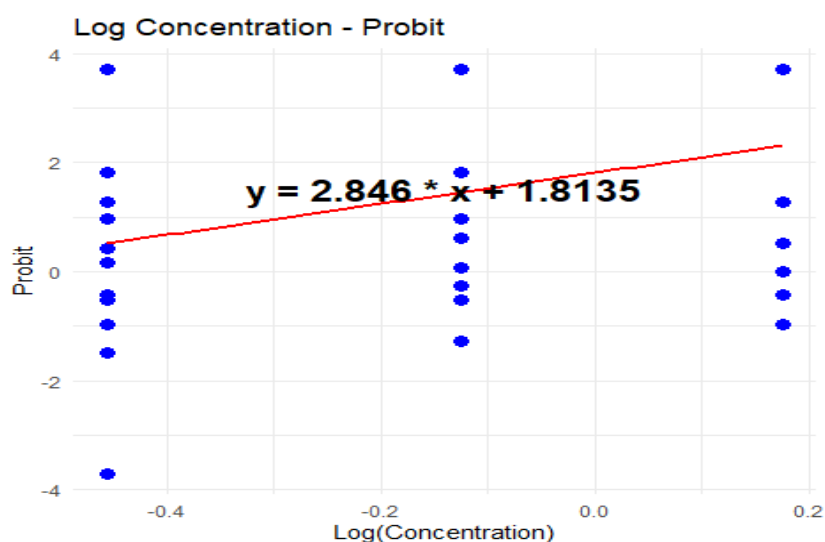
For DL 50 (the lethal concentration for 50% of the sample), which is considered a key measure of toxicity, the estimated value for the Batna samples was 0.252  $\mu\text{L}$ , while it was significantly higher for the Khenchela samples at 0.379  $\mu\text{L}$ , This result indicates that the essential oil extracted from Khenchela is less effective as a pesticide than the oil extracted from Batna, requiring a higher concentration to achieve the same mortality rate in half of the test sample, This difference can be attributed to the variation in the chemical composition of essential oils between the two regions, which may affect their mechanisms of action and interactions with living organisms .

Regarding DL 90 (the concentration that is lethal for 90% of the sample), the value was estimated at 0.510  $\mu\text{L}$  for the Batna samples and 0.558  $\mu\text{L}$  for the Khenchela samples. Although the difference here is less pronounced compared to DL 50, it still indicates that a slightly higher concentration of essential oil extracted from Khenchela is required to eliminate the majority of the test sample .

### 1.3.2. Fumigation processing



**Figure 27.** DL 10, DL 50 and DL 90 curves for *Artemisia herba-alba* essential oil from Batna for mortality compared to concentrations after 60 minutes using the Fumigation method .



**Figure 28.** DL 10, DL 50 and DL 90 curves for *Artemisia herba-alba* essential oil from Khenchela for mortality compared to concentrations after 60 minutes using the fumigation method .

**Table 6.** Lethal concentrations (DL10, DL50, DL90) of *Artemisia herba-alba* essential oil extracted from Batna and Khenchela regions were estimated after 60 minutes using the fumigation method .

Concentrations	Batna	Khenchela
DL10	= 0.114	= 0.0818
DL50	= 0.438	= 0.230
DL90	= 1.672	= 0.652

Figures 27 and 28 show the dose-response (Log Concentration-Probit) curves for *Artemisia herba-alba* essential oil extracted from the Batna and Khenchela regions, to assess mortality rates compared to different concentrations after 60 minutes using the fumigation method, The regression equation shown in Figure 27 ( $y = 2.2062x + 0.7918$ ) indicates the relationship between the logarithm of the concentration and the corresponding probability of mortality in the Batna sample, Similarly, Figure 28 displays the regression equation for the Khenchela sample ( $y = 2.846x + 1.8135$ ). In addition, the accompanying table (6) summarizes the lethal concentration values at 10%, 50%, and 90% for both samples, The results show that the Khenchela samples had higher values compared to the Batna samples, indicating a difference in the efficacy of the essential oil between the two regions .

For Batna oil, the DL 10 value was 0.114  $\mu\text{L}$ , the DL 50 was 0.438  $\mu\text{L}$ , and the DL 90 was 1.672  $\mu\text{L}$ . This means that a significant increase in the amount of vapor from Batna oil was required to increase the killing rate.

Khenchela oil, on the other hand, demonstrated greater effectiveness in vapor form. The DL 10 value was lower 0.0818  $\mu\text{L}$ , the DL 50 value was 0.230  $\mu\text{L}$ , and the DL 90 value was 0.652  $\mu\text{L}$ , which are significantly lower than that of Batna oil. This suggests that the Khenchela oil vapor was more toxic to the organism.

Simplistically, Khenchela oil produces a more effective vapor in killing *C. maculatus* in smaller quantities than Batna oil in fumigation,

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## Conclusion

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### **Conclusion**

The current study evaluated the effect of the essential oil extract of *Artemisia herba-alba* on *Callosobruchus maculatus*, a pest in stored legumes .

In our study, *Artemisia herba-alba* essential oils from Batna and Khenchela, both directly and by vaporization, yielded good results on *Callosobruchus maculatus*. This effectiveness was confirmed by the mortality of adults. Total individual mortality (100%) was observed at doses of 0.35 µl/L, 0.75 µl/L, and 1.5 µl/L, but at different times .

The essential oil contact test was found to be as effective as the fumigation toxicity test, but with differences between *Artemisia herba-alba* from Batna and *Artemisia herba-alba* from Khenchela, with significant mortality recorded in *Callosobruchus maculatus*, which has a long toxicity duration. The amount of extract that causes 50% mortality in *Callosobruchus maculatus* populations is 0.252 for Batna and 0.379 for Khenchela from direct contact tests. The amount of extract for *Artemisia herba-alba* in Batna and Khenchela was 0.438 and 0.230 respectively, respectively, from fumigation tests .

Consequently, the use of essential oils can be proposed as a better alternative to pesticides for preserving stored legumes .

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