الجمهورية الجزائرية الديمقراطية الشعبية
PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA
وزارة التعليم العالي والبحث العلمي
MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH
جـامعـة 20 أوت 1955 –سكيكدة-
UNIVERSITY 20 AUGUST 1955- SKIKDA

Faculty of Sciences
Department of Ecology and Environment

Thesis Submitted for the Purpose of Obtaining the Master's Degree

Field: Ecology and Environment

Specialization: Ecosystem Protection

Theme :

Study of the bio-insecticide, antibacterial and antioxidant activities of some plants of the genus *Salvia*

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University Year : 2023-2024

Acknowledgments

FIRST AND FOREMOST, WE EXPRESS OUR DEEPEST AND HEARTFELT GRATITUDE TO ALLAH (SWT). HIS INFINITE WISDOM, BOUNDLESS MERCY, AND UNWAVERING GUIDANCE HAVE BEEN THE CORNERSTONE OF OUR STRENGTH THROUGHOUT THIS JOURNEY. WITHOUT HIS DIVINE ASSISTANCE, GRACE, AND BLESSINGS, WE WOULD NOT HAVE HAD THE FORTITUDE TO COMPLETE THIS THESIS. EVERY STEP TAKEN AND EVERY ACHIEVEMENT MADE IS A TESTAMENT TO HIS ENDURING SUPPORT AND BENEVOLENCE.

WE EXTEND OUR HEARTFELT THANKS TO OUR ESTEEMED SUPERVISOR, DR. SOUILAH NABILA. HER INVALUABLE GUIDANCE, INSIGHTFUL FEEDBACK, AND UNWAVERING SUPPORT HAVE BEEN A BEACON OF LIGHT THROUGHOUT THE COURSE OF THIS RESEARCH. HER WISDOM AND EXPERTISE HAVE SIGNIFICANTLY ENRICHED THE QUALITY OF THIS WORK, AND FOR THAT, WE ARE PROFOUNDLY GRATEFUL.

WE ALSO WISH TO EXPRESS OUR SINCERE APPRECIATION TO THE PRESIDENT OF THE EXAMINER JURY, HAFSI ZAKARIA, AND OUR EXAMINER, LARIT SABAH, FOR TAKING THE TIME TO REVIEW OUR THESIS. YOUR CONSTRUCTIVE CRITICISM AND THOUGHTFUL SUGGESTIONS WILL BE INSTRUMENTAL IN REFINING AND ENHANCING THIS RESEARCH.

OUR DEEPEST GRATITUDE GOES TO OUR FAMILIES, WHOSE LOVE, ENCOURAGEMENT, AND UNWAVERING SUPPORT HAVE BEEN OUR FOUNDATION. TO OUR FRIENDS, COLLEAGUES, AND TEACHERS, YOUR COMPANIONSHIP AND SUPPORT HAVE MADE THIS JOURNEY NOT ONLY POSSIBLE BUT ALSO MEMORABLE AND ENRICHING.

LASTLY, WE ACKNOWLEDGE WITH GRATITUDE THE SUPPORT AND RESOURCES PROVIDED BY THE DEPARTMENT OF SCIENCE AND THE CHEMISTRY OF SOIL LAB AT THE DEPARTMENT OF AGRICULTURAL SCIENCES.

THANK YOU ALL.

Dedication

YOUR MEMORY IS A GUIDING LIGHT THAT CONTINUES TO INSPIRE ME. YOUR WISDOM AND LOVE WILL FOREVER REMAIN A PART OF ME FOR LIFE, , TO MY DEAR GRANDPA .

THANK YOU FOR YOUR UNWAVERING SUPPORT AND ENDLESS LOVE. YOU HAVE BEEN MY LIFE'S PILLAR, SUPPORTING ME THROUGH EVERY CHALLENGE AND CELEBRATING EVERY TRIUMPH. YOUR SACRIFICES AND DEDICATION HAVE SHAPED WHO I AM TODAY.
TO MY DEAR PARENTS

OUR SHARED EXPERIENCES AND UNCONDITIONAL SUPPORT HAVE BEEN A CONSTANT SOURCE OF STRENGTH ALWAYS GRATEFUL TO HAVE YOU BY MY SIDE, TO MY LOVELY SIBLINGS : NESRINE , RAHMAN , DOUDOU , JOUJOU , RAHIM AND WAHIB .

TO MY CHOSEN FAMILY THAT HAVE BEEN THERE THROUGH THE HIGHS AND LOWS, OUR BOND IS ONE I TREASURE DEEPLY , ESPECIALLY MY BEST FRIEND RANIA B , RANIA M , MALAK , RIHAB , ANFEL , FATIMA .

TO MY ONLINE FRIENDS , DESPITE THE DISTANCES YOU HAVE BEEN THERE FOR ME : HANA , ANAÏS , AL ZUMMURD , SHEEMY , MAHA , ALL ARABIC JOON & SUGA TEAM .

TO ALL MY FAMILY, AUNTIES & UNCLES AND COUSINS , THE LOVE AND JOY WE SHARE IN FAMILY GATHERINGS CREATE CHERISHED MEMORIES THAT I HOLD CLOSE TO MY HEART , ESPECIALLY MY COUSIN FERIAL WHO WAS MY ROOMMATE AND MY CHILDHOOD FRIEND.

TO MY PARTNER IN RESEARCH, LINA , I AM GRATEFUL TO SHARE THIS EXPERIENCE WITH YOU , I'M SO GLAD THAT I WON A SISTER FOR LIFE .

TO ALL MY TEACHERS, COLLEAGUES THROUGHOUT MY JOURNEY I AM DEEPLY THANKFUL FOR GUIDANCE AND CONTRIBUTION IN MY GROWTH AND MATURITY.

TO MY BANGTAN BOYS , WHO HAS BEEN A SOURCE OF INSPIRATION AND COMFORT.

THIS DEDICATION IS A TRIBUTE TO ALL OF YOU WHO HAVE TOUCHED MY LIFE IN MEANINGFUL WAYS. YOUR INFLUENCE, LOVE, AND SUPPORT HAVE BEEN THE FOUNDATION OF MY JOURNEY, AND I AM FOREVER GRATEFUL.

IMI

DEDICATION

This thesis is dedicated to the memory of my beloved mother, [ZOHRA], whose love, wisdom, and unwavering support continue to inspire me every day. Although she is no longer with us, her memory lives on in my heart and guides me through life's challenges.

Her strength, kindness, and endless encouragement have been my driving force, and I hope this work honors her legacy.

May she rest in peace, knowing her spirit and love have profoundly shaped the person I am today and the work I present here.

With deepest love and gratitude,

LINA

العنوان: دراسة الأنشطة الحشرية الحيوية والمضادة للبكتيريا والمضادة للأكسدة لبعض نباتات لنوع *Salvia*

الملخص: يهدف عملنا إلى تقييم الزيوت الأساسية لثلاثة نباتات طبية من أصول مختلفة من جنس المريمية من عائلة الشفوية: المريمية الفرنسية *Salvia sclarea*، والمريمية الطبية من الجزائر *Salvia officinalis*، والمريمية الطبية من لبنان *Salvia officinalis*. الهدف الرئيسي من هذه الدراسة هو استكشاف الخصائص النباتية لهذه النباتات من خلال: استخراج الزيوت الأساسية بالتقطير المائي باستخدام جهاز تقطير كليفنجر، دراسة نشاط مضادات الأكسدة (DPPH) والسعة المضادة للأكسدة الكلية(CAT) ، اختبار الحشرات الحيوي على آفة المنتجات المخزنة، حشرة إفيستيا كونييلا *Ephestia kuhenilla*، دراسة النشاط المضاد للبكتيريا ضد ثلاثة سلالات بكتيرية سالبة الجرام (كليبسيلا نيومونيا، إيشيريشيا كولاي، وسلمونيلا تيفيموريوم). تظهر النتائج أن مردود الزيت الأساسي لنوعي المريمية الطبية هو 0.6% للجزائر و1.1% للبنان، مع خصائص حسية مشابهة لتلك الخاصة بـ :(1999) AFNOR بالنسبة للمريمية الطبية الجزائرية، نلاحظ مظهر سائل متحرك، عديم اللون، برائحة الكافور الطازج وإكليل الجبل؛ وبالنسبة للمريمية الطبية اللبنانية، مظهر سائل متحرك، ذو لون أصفر فاتح، برائحة الصنوبر الكافورية الطازجة وقاعدة من الثوجون المنثولية والعشبية. تظهر اختبارات CAT و DPPH للزيوت الأساسية للمريمية الفرنسية، والمريمية الطبية الجزائرية، والمريمية الطبية اللبنانية نشاطًا مضادًا للأكسدة جيدًا مع قيم CAT تبلغ 291.57 ± 0.73، 36.32 ± 1.40، و 35.52 ± 1.57 مجم/مل على التوالي، وقيم DPPH تبلغ 0.38 ± 0.02، 0.15 ± 0.01، و 0.17 ± 0.01 مجم/مل على التوالي. تقييم النشاط الحشري الحيوي على حشرة إفيستيا كونييلا مع تركيزات مختلفة من الزيوت الأساسية للمريمية الفرنسية (0.1، 0.2، 0.3، 0.5، و1.5 ميكرولتر)، المريمية الطبية الجزائرية (0.5، 0.8، 1.0، 1.5، 3، و3.5 ميكرولتر)، والمريمية الطبية اللبنانية (2، 2.5، 3، و3.5 ميكرولتر) يظهر الجرعات القاتلة التالية: بالنسبة للزيت الأساسي للمريمية الفرنسية، LD_{10} = 0.0782436 ميكرولتر، LD_{25} = 0.1388 ميكرولتر، LD_{50} = 0.2461ميكرولتر، و LD_{90} = 0.7744 ميكرولتر. ومع ذلك، لا تظهر النوعان من المريمية الطبية نشاطًا حشريًا جيدًا ضد حشرة إفيستيا كونييلا. بالنسبة للنشاط المضاد للبكتيريا، تظهر الزيوت الأساسية للنباتات الثلاثة مقاومة ضد السلالات البكتيرية الثلاثة التي تمت دراستها: إيشيريشيا كولاي (مقاومة - ؛ بين 0.8 مم للمريمية الفرنسية والمريمية الطبية الجزائرية و 1.27 مم للمريمية الطبية اللبنانية)، سلمونيلا تيفيموريوم (مقاومة - ؛ 0.6 مم، 1.5 مم، و 3.3 مم للمريمية الطبية الجزائرية، المريمية الطبية اللبنانية، والمريمية الفرنسية على التوالي)، وكليبسيلا نيومونيا (مقاومة - ؛ مع 1.2 مم للمريمية الطبية الجزائرية والمريمية الطبية اللبنانية و 3.3 مم للمريمية الفرنسية). في النهاية، تشير هذه النتائج إلى أن الزيوت الأساسية للأنواع الثلاثة من المريمية يمكن استخدامها كبديل طبيعي وفعال للمواد الكيميائية المستخدمة عادة في الزراعة لمكافحة آفات المنتجات المخزنة، وكذلك كمضادات أكسدة صناعية. تفتح هذه الدراسة الطريق لمزيد من الأبحاث لتحسين استخدام هذا النبات في مختلف المجالات.

الكلمات المفتاحية: نوع *Salvia*، الزيت الأساسي، النشاط المضاد للأكسدة، النشاط المضاد للبكتيريا، الحشرات الحيوية.

Title: Study of the Bio-insecticide, Antibacterial, and Antioxidant Activities of Some Plants of the Genus Salvia

Abstract: Our work aims to valorize the essential oils of three medicinal plants of different origins from the genus *Salvia* of the Lamiaceae family: *Salvia sclarea* from France, *Salvia officinalis* from Algeria, and *Salvia officinalis* from Lebanon. The main objective of this study is to explore the phytochemical properties of these plants through: The extraction of essential oils by hydrodistillation using a Clevenger-type apparatus, an antioxidant activity study (DPPH and Total Antioxidant Capacity - CAT), a bio-insecticide assay on the stored product pest *Ephestia kuehniella*, an antibacterial activity study against three Gram-negative bacterial strains (*Klebsiella pneumoniae, Escherichia coli*, and *Salmonella typhimurium*). The results show that the essential oil yield of the two *S. officinalis* species is 0.6% for Algeria and 1.1% for Lebanon, with organoleptic characteristics similar to those of AFNOR (1999): for *Salvia officinalis* from Algeria, a mobile liquid aspect, colorless, with a fresh camphor and rosemary odor; and for *Salvia officinalis* from Lebanon, a mobile liquid aspect, light yellow in color, with a fresh camphor pine odor and a mentholated and herbaceous thujone base note. The CAT and DPPH tests of the essential oils of *S. sclarea* from France, *S. officinalis* from Algeria, and *S. officinalis* from Lebanon show good antioxidant activity with CAT values of 291.57 ± 0.73, 36.32 ± 1.40, and 35.52 ± 1.57 mg/ml respectively, and DPPH values of 0.38 ± 0.02, 0.15 ± 0.01, and 0.17 ± 0.01 mg/ml, respectively. The evaluation of bio-insecticide activity on *Ephestia kuehniella* with different concentrations of essential oils of *S. sclarea* from France (0.1, 0.2, 0.3, 0.5, and 1.5 µL), *S. officinalis* from Algeria (0.5, 0.8, 1.0, 1.5, 3, and 3.5 µL), and *S. officinalis* from Lebanon (2, 2.5, 3, and 3.5 µL) shows the following lethal doses: for the essential oil of *S. sclarea* from France, LD10 = 0.0782436 µL, LD25 = 0.1388 µL, LD50 = 0.2461 µL, and LD90 = 0.7744 µL. However, the two species of *S. officinalis* do not show good insecticidal activity against *Ephestia kuehniella*. Regarding antibacterial activity, the essential oils of the three plants show resistance to the three bacterial strains studied: *Escherichia coli* (resistant -; between 0.8 mm for *S. sclarea* and *S. officinalis* from Algeria and 1.27 mm for *S. officinalis* from Lebanon), *Salmonella typhimurium* (resistant -; 0.6 mm, 1.5 mm, and 3.3 mm for *S. officinalis* from Algeria, *S. officinalis* from Lebanon, and *S. sclarea* from France respectively), and *Klebsiella pneumoniae* (resistant -; with 1.2 mm for *S. officinalis* from Algeria and *S. officinalis* from Lebanon, and 3.3 mm for *S. sclarea* from France). Ultimately, these results suggest that the essential oils of the three Salvia species could be used as a natural and effective alternative to the chemicals commonly used in agriculture for the control of stored product pests, as well as synthetic antioxidants. This study paves the way for future research to optimize the use of this plant in various fields.

Keywords: Genus *Salvia*, essential oil, antioxidant activity, antibacterial, bio-insecticide.

Titre : Étude des activités bio-insecticides, antibactériennes et antioxydantes de quelques plantes du genre *Salvia*

Résumé : Notre travail vise à valoriser les huiles essentielles de trois plantes médicinales d'origine différente du genre *Salvia* de la famille des Lamiaceae : *Salvia sclarea* de France, *Salvia officinalis* d'Algérie et *Salvia officinalis* du Liban. L'objectif principal de cette étude est d'explorer les propriétés phytochimiques de ces plantes par : L'extraction des huiles essentielles par hydrodistillation à l'aide d'un hydro-distillateur de type Clevenger, une étude d'activité antioxydante (DPPH et Capacité Antioxydante Totale - CAT), un bio-essai insecticide sur le ravageur des denrées stockées, Ephestia kuehniella, une étude d'activité antibactérienne contre trois souches bactériennes de Gram négatif (*Klebsiella pneumoniae, Escherichia coli* et *Salmonella typhimurium*). Les résultats obtenus montrent que le rendement en huile essentielle des deux *S. officinalis* est de 0,6% pour l'Algérie et de 1,1% pour le Liban, avec des caractères organoleptiques similaires à ceux de l'AFNOR (1999) : pour *Salvia officinalis* d'Algérie, on note un aspect liquide mobile, sans couleur, avec une odeur de camphre frais et de romarin, et pour *Salvia officinalis* du Liban, un aspect liquide mobile, de couleur jaune clair, avec une odeur de pin camphré fraîche et une note de fond de thuyone mentholée et herbacée. Les tests de CAT et de DPPH des huiles essentielles de *S. sclarea* de France, de *S. officinalis* d'Algérie et de *S. officinalis* du Liban montrent une bonne activité antioxydante avec des valeurs de CAT de 291,57 ± 0,73, 36,32 ± 1,40 et 35,52 ± 1,57 mg/ml respectivement, et des valeurs de DPPH de 0,38 ± 0,02, 0,15 ± 0,01 et 0,17 ± 0,01 mg/ml, respectivement. L'évaluation de l'activité bio-insecticide sur *Ephestia kuehniella* avec différentes concentrations d'huiles essentielles de *S. sclarea* de France (0.1, 0.2, 0.3, 0.5 et 1.5 µL), de *S. officinalis* d'Algérie (0.5, 0.8, 1, 1.5, 3 et 3.5 µL) et de *S. officinalis* du Liban (2, 2.5, 3 et 3.5 µL) montre les doses létales suivantes : pour l'huile essentielle de *S. sclarea* de France, DL_{10} = 0.0782436 µL, DL_{25} = 0.1388 µL, DL_{50} = 0.2461 µL et DL_{90} = 0.7744 µL. En revanche, les deux espèces de *S. officinalis* ne montrent pas une bonne activité insecticide contre *Ephestia kuehniella*. Concernant l'activité antibactérienne, les huiles essentielles des trois plantes montrent une résistance sur les trois souches bactériennes étudiées : *Escherichia coli* (résistante - ; entre 0,8 mm chez *S. sclarea* et *S. officinalis* d'Algérie et 1,27 mm chez *S. officinalis* du Liban), *Salmonella typhimurium* (résistante - ; 0,6 mm, 1,5 mm et 3,3 mm chez *S. officinalis* d'Algérie, *S. officinalis* du Liban et *S. sclarea* de France respectivement) et *Klebsiella pneumoniae* (résistante - ; avec 1,2 mm chez *S. officinalis* d'Algérie et *S. officinalis* du Liban et 3,3 mm chez *S. sclarea* de France). Finalement, ces résultats suggèrent que les huiles essentielles des trois plantes du genre Salvia pourraient être utilisées comme une alternative naturelle et efficace aux produits chimiques couramment utilisés en agriculture pour le contrôle des ravageurs des denrées stockées, ainsi qu'aux antioxydants synthétiques. Cette étude ouvre la voie à de futures recherches pour optimiser l'utilisation de cette plante dans divers domaines.

Mots clés : Genre *Salvia*, huile essentielle, activité antioxydante, antibactérienne, bio-insecticide.

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FAO :	Food and Agriculture Organization
AGRODIV :	Le groupe AGRO-INDUSTRIE
EO :	Essential oil
NCBI:	National Center for Biotechnology Information
USDA:	United States Department of Agriculture
AFNOR:	Association française de normalisation
YEO:	Essential oil yield expressed as a percentage (%).
MEO:	Mass of the essential oil expressed in grams (g).
MMP:	Mass of dry plant matter of the plant used expressed in grams (g).
DPPH:	2,2-diphenyl-1-picryl-hydrazyl-hydrate
TAC:	Total Antioxidant Content
AAR:	The anti-radical activity
PPM:	PhosphoMolybdate
Tukey's HSD test:	Honestly significant difference
BHIB:	Bouillon cœur-cervelle

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Summary

I. Introduction

I. Introduction

The Union Nation Environment Programme's Food Waste Index Report 2024 highlights that latest data from 2022 shows 1.05 billion tonnes of food went to waste, some 19% of food available to consumers was lost overall at retail, food service, and household levels. That is in addition to around 13% of food lost in the supply chain, as estimated by the Union Nation Food and Agriculture Organization **(FAO, 2024)**, from post-harvest up to the point of sales **(UN, 2024)** the storage of grains, particularly wheat, is crucial for global food security.

Wheat is a staple food for over 2.5 billion people worldwide, and disruptions to wheat supplies can have severe consequences for food insecurity **(Shiferaw *et al.*, 2013)** this paradox highlights the urgent need to improve grain storage and reduce food waste. Grain reserves can help stabilize prices and ensure availability during emergencies, but measuring and managing these stocks remains a challenge **(Galtier, 2013).** While the use of chemical insecticides can help prevent food from spoiling, this practice poses significant risks to human health and the environment.

Insecticides often contain toxic substances that can accumulate in the food chain and have detrimental effects on consumers **(Nicolopoulou-Stamati *et al.*, 2016)**. Exposure to these chemicals has been linked to a range of adverse health outcomes, including neurological disorders, endocrine disruption, and increased cancer risk **(Mostafalou & Abdollahi, 2017)**. Moreover, the overuse of insecticides can lead to the development of resistant pest populations, rendering the chemicals less effective and necessitating the use of even stronger, more hazardous compounds **(Sparks & Nauen, 2015)**.

The environmental impact of insecticide use is also concerning, as these chemicals can contaminate soil, water, and air, harming wildlife and disrupting delicate ecosystems **(Aktar *et al.*, 2009)**. Sustainable alternatives, such as integrated pest management and the use of natural, biodegradable compounds, offer a safer and more environmentally friendly approach to preserving food quality and reducing waste **(Isman, 2006)**.

Essential oils derived from various plants offer a promising alternative to synthetic insecticides, providing a more environmentally friendly and sustainable approach to pest control. Many essential oils have been found to possess insecticidal properties due to their complex mixtures of volatile compounds **(Isman, 2000)**. Unlike conventional insecticides, essential oils are biodegradable, have low mammalian toxicity, and do not persist in the environment **(Bakkali *et***

***al.*, 2008)**. Furthermore, the use of essential oils aligns with the principles of integrated pest management, as they can be combined with other non-chemical control methods to enhance their efficacy and reduce the risk of resistance development **(Pavela, 2016)**.

As consumer demand for organic and sustainably produced food continues to grow, the use of essential oils as biofriendly insecticides offers a promising solution for reducing the environmental impact of agriculture while maintaining crop yields and quality **(Isman & Grieneisen, 2014)**.

Human interest in medicinal and aromatic plants began with its creation and existence. Since Antiquity, man has relied on nature to provide his basic needs such as food, housing, clothing, healthcare, etc. Therefore, we see that the use of medicinal plants by humans was and still is important. Aromatic plants represent an inexhaustible source of traditional and effective remedies thanks to the active ingredients they contain (alkaloids, flavonoids, vitamins, etc.) **(Koualdi & Boughrara, 2018)**. Essential oils contain insecticidal, antioxidant, bacterial and antifungal properties, they are used in many medical, pharmaceutical and cosmetic fields…etc. **(Khamouli & Grazza, 2007)**.

Our research study focuses on the valorisation of three promising medicinal plants from the genus of *Salvia*: the first one from Algeria named *Salvia officinalis* L., the second from Lebanon named *Salvia officinalis* L., and the third from France named *Salvia sclarea* L. These plants possess significant biological properties that can be harnessed to develop effective natural alternatives. The aim of our research is to evaluate the following biological activities: bio-insecticidal, antibacterial, and antioxidant properties, and to assess their potential use as natural sources with therapeutic and bio-insecticidal capabilities. This includes applications in the medical field and the preservation of stored food products against a crucial pest, *Ephestia kuehniella*, which causes significant damage in Algeria and in the world.

II. Materials and Methodes

II.1. Biological material

II.1.1. Presentation of flour

Flour is a product prepared from grain of soft wheat (*Triticum aestivum* L.*)* by grinding or milling processes in which the bran is essentially removed and the remainder is comminuted to a suitable degree of fineness. **(FAO, 1985)**, the importance of wheat flour in Algeria's food security is further highlighted by the government's plans to increase grain storage capacities to 9 million tonnes by building 350 local cereal storage centers and rehabilitating 16 existing centers in 2024 **(USDA, 2023)**. We carefully gathered flour at the AGRODIV Mill in El Harrouche, paying close attention to every corner of the warehouse. Our efforts resulted in the collection of 10 kilograms of spoiled flour in total **(Figure 1).**

Figure 1. Flour contaminated with *Ephestia kuehniella* **(Personal photo)**

II.1.2. Presentation of *Ephestia kuehniella*

II.1.2.1. Biology and classification

Ephestia kuehniella **(Zeller, 1879)**, commonly known as the Mediterranean flour moth, is a species of moth in the family Pyralidae **(Sharpe & DeMichele, 1977)**, the larvae of this species are known to feed on a wide range of food sources, including grains, cereals, and other plant-based materials **(Schoolfield & Mutti, 1981)**.

Figure 2. *Ephestia kuhniella* (Zeller, 1879) (**Personal photo**)

According to the National Center for Biotechnology Information **(NCBI, 2018)**, *Ephestia kuhniella* **(Zeller, 1879)** belongs to the following classification:

- **Kingdom:** Animalia
- **Subkingdom:** Metazoa
- **Phylum:** Arthropoda
- **Subphylum:** Hexapoda
- **Class:** Insecta
- **Subclass:** Pterygota
- **Order:** Lepidoptera
- **Family:** Pyralidae
- **Genus:** *Ephestia*
- **Species:** *Ephestia kuhniella* **(Zeller, 1879)**

II.1.2.2. Biological cycle

- **The egg:** Is whitish and more ovoid, with average dimensions of 0.30 mm long and 0.20 mm wide **(Danysz, 1893**), it is laid in cereals by adult moths, in which the caterpillars develop **(Zekri, 2016).**

- **Larvae**: They are whitish or pinkish, measuring 1 to 1.5mm, accompanied by silken webs in which they live. After six larval molts, they complete their growth. They are entirely brown and measure between 10 to 20 mm in the final stage and can travel up to 400 mm **(Bouzerra, 2014).**

- **Chrysalis**: Formed after the final larval molt, the pupa does not feed. In some species, it is enclosed in a cocoon woven by the larva. During its pupal life, the insect undergoes a complete internal and external metamorphosis that leads to the adult stage **(Zekri, 2016)**.

- **Adult:** The moth measures 10mm in length and has a wingspan of 1.25cm **(Mason, 2003)**. It is characterized by two pairs of wings; the scales on the apical half of the forewing are brown, while those on the basal half are copper-colored. Additionally, the hindwings exhibit scales of grayish color. Its longevity is 5 to 7 days **(Campos-Figueroa, 2009)**.

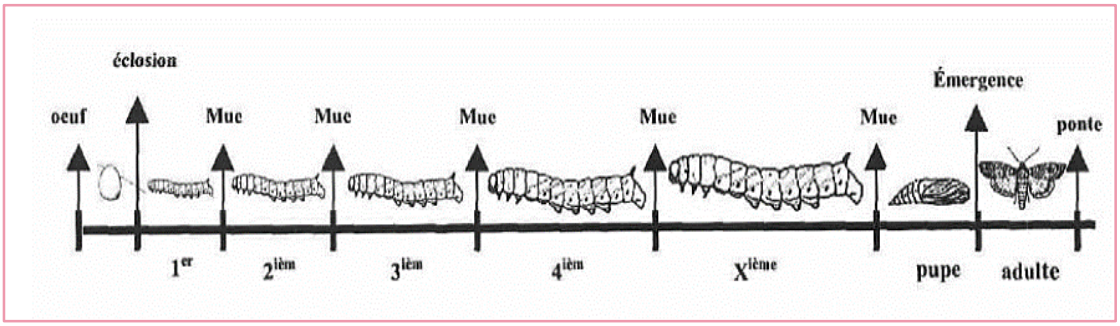


Figure 3. Schematic development of Lepidoptera (**Frisco, 2006)**

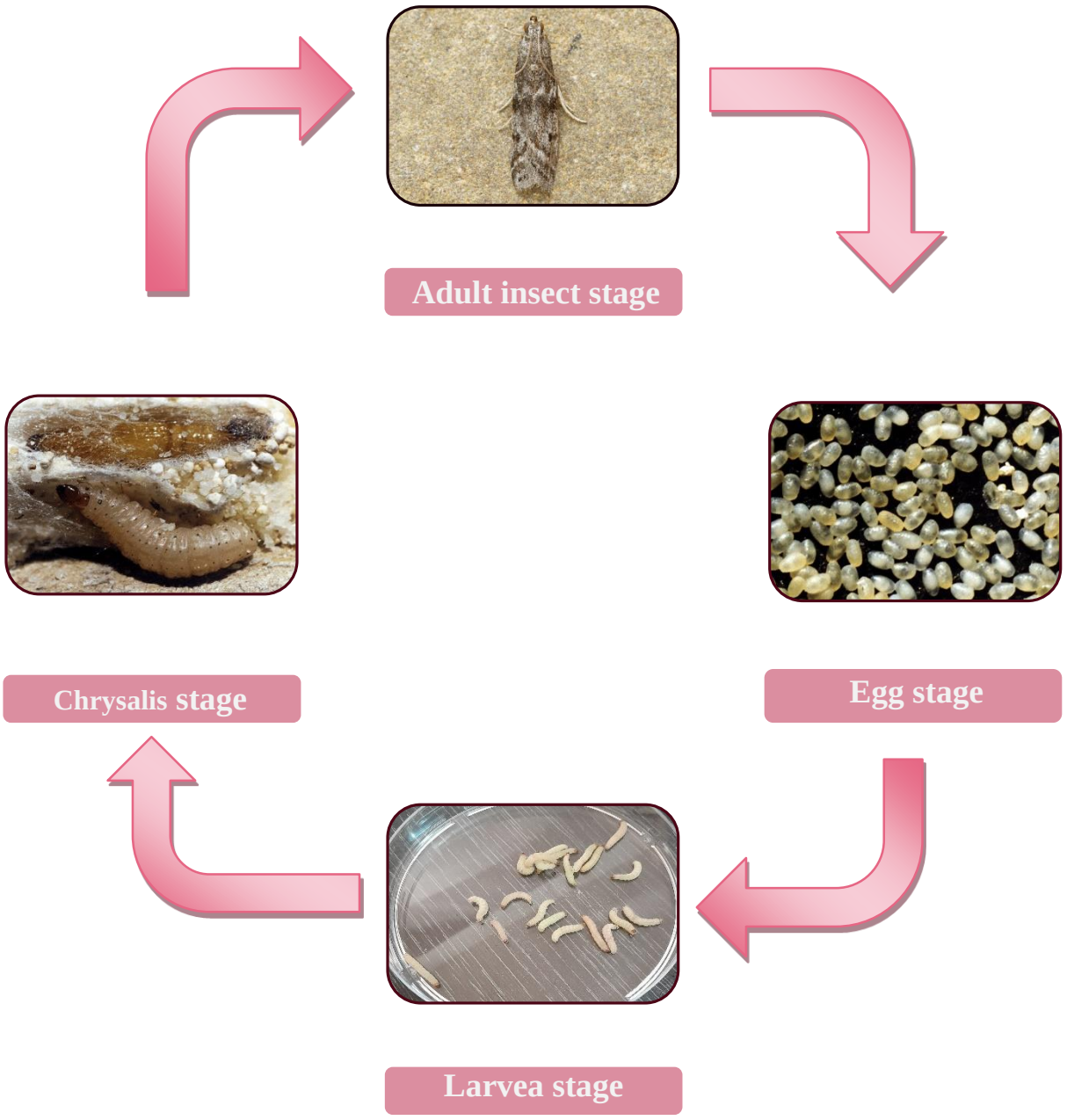


Figure 4. Illustrates the developmental cycle of *E. kuehniella* at 27°C.

II.1.3. Presentation of plants

II.1.3.1. *Salvia sclarea* L.

II.1.3.1.1. Biology and classification

"Sage," previously "Salje", derives from the Latin "*Salvia*", which referred to these plants in Latin. It comes from salvo: to heal, due to the plant's medicinal properties **(Couplan, 2012)**. According to **Alloun (2013)**, there are over 600 species of sage worldwide, not all of

which are edible, with many being used as ornamental plants in gardens. Clary sage *(Sage sclarea)* is widely distributed in southern Europe and Central Asia **(Salvatori, 2005)**. Although it is native to Southern Europe, it is now cultivated in France and Russia for its essential oil. It grows on dry, sunny soils and is harvested in summer **(Iserin, 2007).**

S. sclarea is a perennial bearing hairy vesicle, its leaves, 15 to 25 cm long, are oval, oblong, notched, irregularly toothed and puckered. They are medium green, with a cordate or perfoliate base. In spring and summer, terminal panicles or clusters appear, formed of an abundance of flowers 2 to 3 cm long, cream and lilac to pink or blue, with large lilac bracts, whose pink stems bear spikes of pinkish-white flowers **(Figure 5)** **(Brickell & Mioulane, 2004)**.

Figure 5. The plant of *Salvia sclarea* L.

The taxonomic classification of *Salvia sclarea*, commonly referred to as clary sage, follows the system proposed by **Iserin (2001)**. It is structured as follows:

- **Kingdom:** Plantae
- **Subkingdom:** Tracheobionta
- **Division:** Magnoliophyta (Tracheophyta)

- **Class:** Magnoliopsida (Dicotyledonae)
- **Subclass:** Asteridae
- **Order:** Lamiales
- **Family:** Lamiaceae
- **Genus:** *Salvia*
- **Species:** *Salvia sclarea L.*

II.1.3.1.2. Harvest location

The essential oil is purchased from the province of Marseille (South of France) from a pharmacy of Doctor Valnet specializing in medicinal plants and it's extracted by a Clevenger type hydrodistiler (**Figure 6**).

Figure 6. Essential oil of *S. sclarea* L.

II.1.3.2. *Salvia officinalis* L.

II.1.3.2.1. Biology and classification

The officinal sage *(Salvia officinalis* L.*),* which belongs to the Lamiaceae family **(Maatoug, 1990)**, consists of small shrubs with delicate, wind-swept leaves, possessing a characteristic camphor-like scent. It is an aromatic and medicinal plant widely used either in

its natural state or in the form of extracts or essential oil. In addition to its traditional use in home cooking and folk medicine, this plant, especially its essential oils, is utilized by the perfume and cosmetics industries, the food industry, and ultimately, the pharmaceutical industry **(Fellah *et al.*, 2006).**

This perennial plant has a woody base that forms a bush sometimes exceeding 80 cm in height. The stems are greenish-white, the leaves are quite large, thick, greenish-white and opposite. The light blue-violet flowers are arranged in loose terminal spikes, with three to six flowers in each whorl. The calyx is bell-shaped with five long teeth, and the corolla is bilabiate with an upper helmet and a lower trilobed lip. The fruits are in the form of tetraquenes **(Hans, 2007).**

Figure 7. The plant of *Salvia officinalis* L. *(***Mazza, 2022).**

The taxonomic classification of *Salvia officinalis* L., follows the system structured as follows **(Cronquist, 1968 ; Loic. F, 2009)**:

- **Kingdom:** Plantae (Plant)
- **Phylum:** Cormophytes
- **Division:** Magnoliophyta
- **Class:** Magnoliopsida
- **Subclass:** Asteridae
- **Order:** Lamiales
- **Family:** Lamiaceae

- **Genus:** *Salvia*
- **Species:** *Salvia officinalis* L.

II.1.3.2.2. Harvest location

We chose the species *Salvia officinalis* L. collected from two different regions, the first from Algeria (Department of Sétif, Municipality of Aïn Oulmene) (**Figure 8**) and the second from Lebanon (Mountain region purchased from herbalist on Dubai in Union Arab Emirates) (**Figure 9**).

Figure 8. Plant of *S. officinalis* collected from Algeria **(Personal photo)**

Figure 9. Plant of *S. officinalis* collected from Lebanon **(Personal photo)**

II.2. Study methods

II.2.1. Essential oil extraction

Figure 10. Clevenger-type hydro-distillation apparatus **(Personal photo)**.

The aerial parts (leaves, stems) of the species *Salvia officinalis L*. undergo hydro-distillation utilizing a Clevenger extraction apparatus (**Figure 10**) **(Clevenger, 1928),** in accordance with the protocol outlined in the European Pharmacopoeia X edition **(European Directorate for the Quality of Medicines & HealthCare, 2020)**

The extraction process takes place in the Chemistry Laboratory of the Department of Agronomic Sciences at the University of August 20, 1955 in Skikda. Here's how it works:

- First, we put 40 grams of dried and chopped plant material into a one-liter glass flask with 700 ml of distilled water. Then, we heat the mixture to around 40°C using a heating mantle for 3 hours **(Figure 11)**
- As the mixture heats up, the vapor containing the essential oil (EO) travels through a condenser, a part of the apparatus where it cools down and transforms back into liquid form. Due to differences in density between the EO and water, the oil layer naturally rises to the surface, making it easier to separate and retrieve. This phenomenon is a result of the EO being lighter than water, allowing it to float atop the aqueous solution.
- After collecting the oil, we dry it using sodium sulfate to remove any remaining water. The whole process takes about 3 hours.

- Finally, after all the extraction and purification processes, we carefully store the essential oil in a dark bottle. This is crucial because exposure to light can degrade the oil's quality over time. Additionally, we maintain a cool temperature (4°C) to further ensure the stability and longevity of the oil.

Figure 11. The plant material in the glass flask during the heating process **(Personal photo)**.

II.2.2. Calculation of essential oil yield

Expressed as a percentage, the essential oil yield is defined as the ratio between the mass of essential oil obtained after extraction and that of the dry or fresh plant material initially used according to **(AFNOR, 1999)**. It is calculated by the following formula:

$$YEO = (MEO / MMP) * 100$$

YEO: Essential oil yield expressed as a percentage (%).

MEO: mass of the essential oil expressed in grams (g).

MMP: mass of dry plant matter of the plant used expressed in grams (g).

II.2.3. Determination of organoleptic characteristics

The EO is characterized by its organoleptic properties such as smell, appearance and color.

- **Smell**: Smell is a very sensitive chemical sense and the ability of perfumers to classify and characterize chemical substances allows them to dose natural products and their perception can go up to ten millionths of grams per liter of air.
- **Color**: The coloring of an essential oil depends on the products that constitute it. Some solvents have the power to extract a lot of pigment, which intensifies the color of a given oil.
- **Appearance**: The appearance of an extract depends on the products that constitute it, which can appear to us in solid, liquid or solid-liquid form.

II.2.4. Antioxidant activities

Antioxidants can be defined as: "any substance which, when present at low concentrations compared to that of an oxidizable substrate; significantly delays or inhibits the oxidation of this substrate" **Young and Woodside, (2001)**. When added to food products, act as an anti-radical agent, prevent radical oxidation reactions, delay or inhibit the oxidation process and increase shelf life by delaying the lipid peroxidation process **Mital and Sumitra (2012).**

II.2.4.1. DPPH Free Radical Trapping Test

The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an electron transfer-based antioxidant assay that produces a purple solution in ethanol **Mital and Sumitra, (2012).** In the DPPH free radical method, the antioxidant effectiveness is measured at room temperature and therefore eliminates the risk of thermal degradation of the molecules tested. However, the reaction mechanism between the antioxidant and DPPH depends on the structural conformation of the antioxidant **Bondet *et al*. (1997)**.

The reduction of DPPH is monitored to monitor the decrease in its absorbance at a characteristic wavelength during the reaction. In its radical form, DPPH absorbs 515nm, but after reduction by an antioxidant or a radical species, the absorption disappears **Brand *et al.* (1995).**

The anti-radical activity against DPPH was measured by the DPPH test described by **Blois (1958)** with a slight modification. Briefly a solution of 0.1 mM DPPH in methanol was prepared and 4 ml of this solution was added to 1 ml of sample solutions in methanol at different concentrations. After 30 minutes of incubation in the dark at room temperature, the absorbance

is measured at 517 nm. Lower absorbance of the reaction mixture indicated greater free radical scavenging activity.

The antioxidant activity is expressed as a percentage of DPPH radical inhibition, and calculated from the following equation:

$$\% \text{ of inhibition} = (A_{control} - A_{Sample} / A_{control}) * 100$$

The IC50 value, defined as the inhibitory concentration of the extract necessary to reduce the initial concentration of the DPPH radical to 50%, is calculated from the percentage graph of the scavenging effect of the different concentrations of the extract **(Bertoncelj *et al.*, 2007; Marxen *et al.*, 2007; Fabri *et al.*, 2009 and Scherer & Godoy, 2009)**.

We can deduce the anti-radical activity (AAR) of our extracts by calculating the inverse of the IC50 values found (**Maisuthisakul *et al.*, 2007**), using the following formula:

$$AAR = 1/IC_{50}$$

Quercetin, BHT, and α-tocopherol were used as antioxidant standards for activity comparison.

II.2.4.2. Total antioxidant Content (TAC)

The PPM (phosphoMolybdate) test is a variation of the DPPH test. During this test, the hydrogen and the electron are transferred from the reducing compound (extract-antioxidant) to the oxidizing complex (PPM). This transfer depends on the redox potential, the pH of the medium and the structure of the antioxidant compound.

- **Principle:** The test is based on the reduction of molybdenum Mo present in the form of molybdate ion $MoO4^{2-}$ to molybdenum Mo MoO^{2+} in the presence of the extract or an antioxidant agent. This reduction is materialized by the formation of a greenish complex (phosphate/Mo (V) at an acidic pH **(Prieto *et al*, 1999).** The increase in the coloring of the complex is measured molybdenum (VI) in the presence of an antioxidant. Unlike other tests, this test not only makes it possible to quantify the contribution of the antioxidant activity of polyphenols but also of other antioxidant compounds such as vitamins (C, E, etc.).
- **Assay method:** The total antioxidant capacity of the extract was evaluated by the phosphomolybdenum method of **Ramalakshmi *et al.* (2008)**. This technique is based on

the reduction of Mo^{6+} ions into Mo^{5+} ions by the antioxidants contained in the extract. Consequently, there is formation of a greenish-colored phosphate-Mo^{5+} complex, in an acidic environment, the intensity of which is proportional to the concentration of antioxidants **(Veerapur *et al.*, 2009)**.

- It consists of adding 1 ml of the phosphomolybdate reagent (0.6 M Sulfuric Acid, 28 mM Sodium Phosphate and 4 mM Ammonium Molybdate) to 100 μl of each extract. After 90 minutes of incubation in the water bath at 95°C, the absorbance is measured at 695 nm. Total antioxidant capacity is expressed as mg ascorbic acid equivalent/g dry matter (mg AAE/g DM) (**Figure 12**).

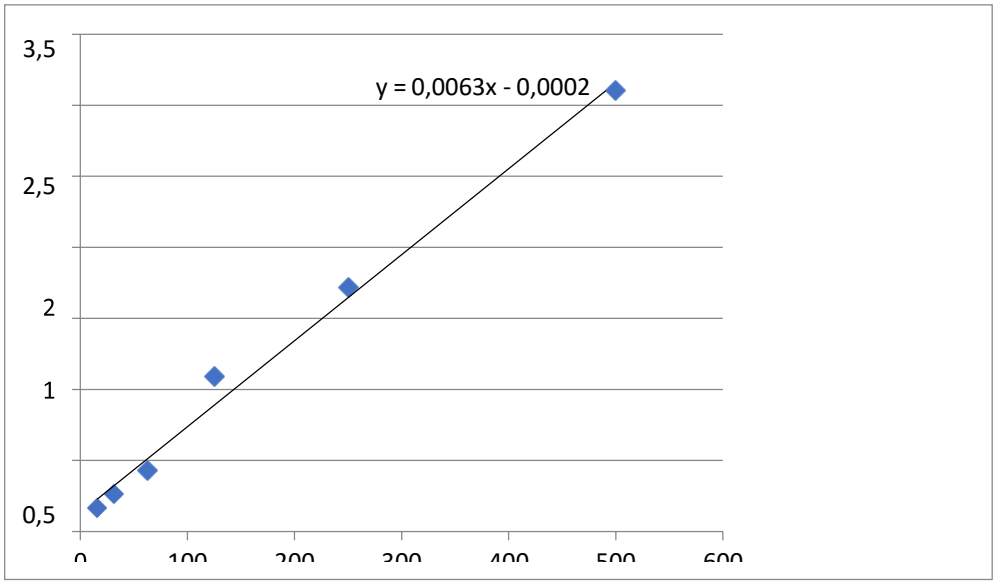


Figure 12. Ascorbic acid calibration curve for TAC

II.2.5. Breeding *Ephestia kuehniella* in the laboratory

Ephestia kuehniella requires specific temperature and humidity conditions for optimal growth. the temperature should be maintained at 25°C ± 1°C, (**Figure 13)** and the humidity should be kept at 70% ± 10%, as recommended by **Payne (1966).**

Figure 13. The temperature of oven that was maintained at 25°C for optimal growth of *Ephestia kuehniella* **(Personal photo)**.

The final instar larvae are carefully collected, sorted, and placed into plastic containers with a thin layer of flour and folded paper to facilitate pupation, as depicted in **Figure 14 & 15**. The breeding progress is meticulously monitored on a daily basis to ensure optimal conditions for larval development. Furthermore, newly molted pupae, aged at (0 days), are selected for experimental purposes, reflecting their pristine state and suitability for subsequent investigations.

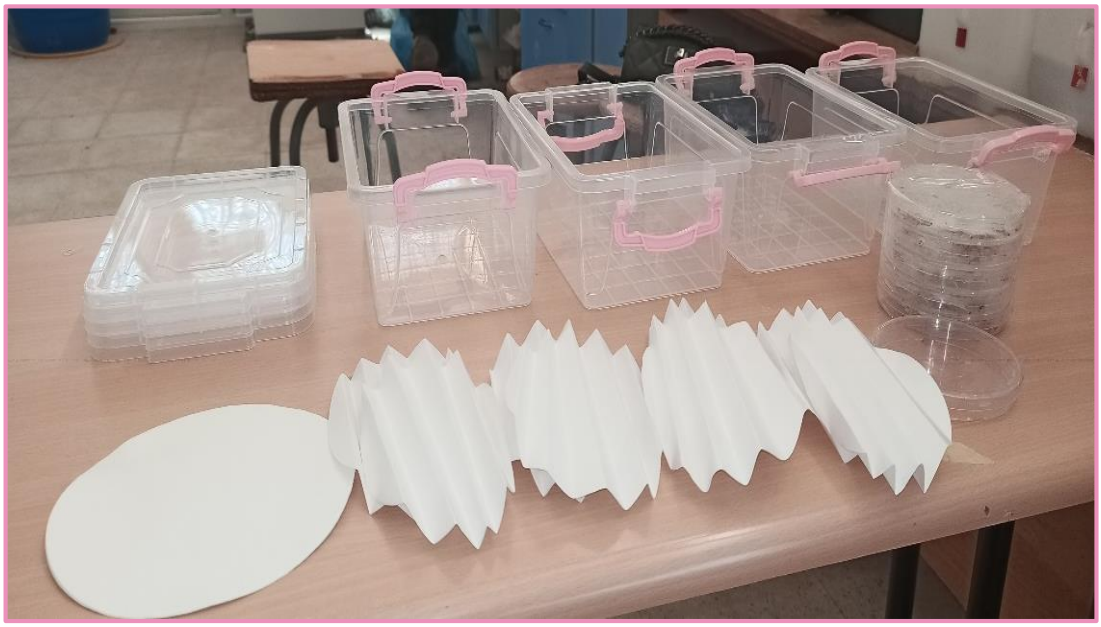


Figure 14. The preparation process of breeding *Ephestia kuehniella* **(Personal photo)**.

Figure 15. Larvae of *Ephestia kuehniella* in folded paper **(Personal photo)**.

II.2.6. Bioassay of insecticidal activity by fumigation

The evaluation of essential oil toxicity via fumigation involves saturating the environment with newly exuviated adult of *Ephestia kuehniella*. Different doses were considered: for *S. sclarea* with 0.1, 0.2, 0.3, 0.4 and 1.5 µl, for Algerian *S. officinalis* with 0.5, 0.8, 1, 1.5, 3 and 3.5 µl, and for Lebanese *S. officinalis* with 2, 2.5, 3 and 3.5 µl. The treatment was administered in plastic pill boxes with a capacity of 60 ml, each containing 10 adults. Three replicates of 10 individuals were carried out for each concentration. A control series was conducted in parallel with untreated disks (**Figure 16**). Mortality was recorded at 2, 4, 6, and 8 hours after treatment.

Figure 16. Distribution of 10 insects in plastic pill boxes according to doses **(Personal photo)**.

Ten specimens are placed within vials, and various doses of essential oil are sprayed onto filter paper discs of 5 mm diameter. These treated papers are then affixed to the inner

surface of the plastic vial lids **(Figures 17 & 18)**. The countification of death insects is conducted at intervals of 2, 4, 6, and 8 hours.

Figure 17. Spraying the essential oil doses into the filter paper **(Personal photo)**.

Figure 18. The plastic pill boxes after the process **(Personal photo)**.

The percentages of inhibition observed in the different series were determined and then corrected according to the **Abbott (1925)** formula to eliminate natural mortality. The percentages of corrected inhibitions underwent an angular transformation according to the tables of **Bliss (1938)**, cited by Fisher and **Yates (1957)**, and were subjected to an analysis of variance with a classification criterion allowing the classification of doses by Tukey's HSD test to evaluate the effect of EO. Finally, non-linear regression expressing the percentage of corrected inhibition as a function of the logarithm of the dose was used to estimate, for the EO of the three plants studied, the inhibition doses for the adult insect. ID_{25} and ID_{50} (doses causing

inhibition of adult emergence in 25% and 50% of treated insects, respectively) were calculated with their confidence intervals (95% FL) and Hill slope.

II.2.7. Antibacterial activity

Bacteria are responsible for various infections in living organisms. Researchers hoped to eradicate certain diseases with the discovery of antibiotics. Unfortunately, the widespread use of these drugs has led to increasing bacterial resistance to antibiotics. In response, there has been great interest in searching for new biologically active and effective substances as alternatives from natural resources. In particular, medicinal plants constitute a potential source of antimicrobial compounds and/or inhibitors of antibiotic resistance mechanisms **(Fettah, 2019).**

The antibacterial activity of plant oils has formed the basis of numerous applications, including pharmaceuticals, medicine, and natural therapy **(Sagdic *et al.*, 2002)**.

II.2.7.1. Bacterial strains studied

To evaluate the antibacterial activity of essential oils, we tested three Gram-negative strains:

- ***Escherichia coli***

  This species is naturally present in the intestinal flora, but certain strains can cause more or less serious intestinal infections. This potentially fatal bacterium inhabits the intestines of warm-blooded organisms and humans, which explains why it is mainly responsible for food poisoning **(Pierre, 2003).**

- **Klebsiella pneumoniae**

  Belonging to the "*Klebsiella, Enterobacter*, and *Serratia*" group **(Ferron, 1976; Bereche et al., 1989; Monteil & Avril, 1992)**, this group includes bacterial species long considered commensal but currently implicated in a large number of infectious complications in hospitals **(Ferron, 1976)**. They are often multi-resistant to antibiotics.

- ***Salmonella typhimurium***

  This bacterium is a virulent *enterobacterium* with a digestive tropism that is pathogenic for humans and animals. It is one of the main causes of food poisoning **(Le Minor *et al.*, 1982)**.

II.2.7.2. Antibacterial activity essay

Antibacterial activity is a method used to determine the antagonistic and inhibitory effects of certain biomolecules (or other compounds) on the growth of target microorganisms. This method, inspired by the antibiogram, involves impregnating paper discs with a solution of the antagonist to be tested (in our case, essential oils) and placing them on the surface of an appropriate agar that has already been inoculated with the microbial agent. This is called the "disk diffusion method." Antimicrobial activity is then evaluated by measuring the diameter of the inhibition zones around the discs **(Tyagi & Malik, 2011).**

The antimicrobial activity was evaluated using the diffusion method on agar medium described by Benjelali et al. (1986). This involves measuring the inhibition zones resulting from the effect of our samples on the reference strains tested.

- ***Sterilization of the equipment:*** The physiological water, culture media, test tubes used in the preparation of the bacterial solutions, and Whatman paper discs (6 mm in diameter) were sterilized in an autoclave at 121°C for 3 hours.
- ***Revivification of strains:*** To allow stressed bacteria to recover their potential, 1 ml of the bacterial suspension was added to 9 ml of BHIB. The tubes were incubated for 24 hours at 37°C.
- ***Substitution of the strains:*** The bacteria were seeded using a loop on nutrient agar by making tight streaks, then incubated at 37°C.
- ***Standardization:*** Using a platinum loop, a few well-isolated and identical colonies of each of the bacterial strains to be tested were taken. The bacterial suspension was homogenized to an opacity of 0.5 McFarland, equivalent to an OD of 0.08 to 0.10 at 625 nm.
- ***Seeding and depositing the discs***: The tested strains were seeded by swabbing. The discs impregnated with our samples (the essential oils) were delicately placed on the surface of the pre-inoculated agar using sterile forceps. The petri dishes were incubated for 18 to 24 hours at 37°C.
- ***Reading of the results***: Results were read by measuring the diameters of the inhibition zones around the discs, and sensitivity of the strains to our samples was indicated by signs based on the measured diameters **(Ponce et al., 2003**

  - Non-susceptible (-) or resistant strain: diameter ≤ 8 mm
  - Sensitive strain (+): diameter between 9 and 14 mm

- Very sensitive strain (++): diameter between 15 and 19 mm
- Extremely sensitive strain (+++): diameter ≥ 20 mm.

II.8. Statistical analysis

Values were expressed as mean ± standard deviation (Mean ± SD).

III. Results and Discussion

III.1. Oil essential

III.1.1. Calculated of yield

The essential oil (EO) extracted by hydro-distillation using a Clevenger apparatus from two plants of *Salvia officinalis* from Algeria and Lebanon is important for many therapeutic and culinary applications. The yield is calculated by the formula mentioned in the materials and methods.

- **Yield of essential oil of Algerian *Salvia officinalis* L.**

- Mass of the plant material used: 200 g

- Volume of EO obtained: 1.2 mL

- Yield of essential oil (%): 0.6%

- **Yield of essential oil of Lebanese *Salvia officinalis* L.**

- Mass of the plant material used: 200 g

- Volume of EO obtained: 2.2 mL

- Yield of essential oil (%): 1.1%

Comparing the results of our work with those of other studies and the literature (using similar methods of obtaining essential oils) shows that our yields are not satisfactory. The yield of essential oil obtained from Algerian (0.6%) and Lebanese *Salvia officinalis* species in our study shows lower performance compared to the work of **Maache *et al.* (2023)** on *Salvia officinalis* from the Morocco region, which had a yield of 1.37%, and on the species of *Salvia lavandulifolia* subsp. *mesatlantica* from the region of Morocco, which had a yield of 0.86%. However, our yields are slightly higher compared to the yield reported by **Nguyen *et al.* (2024)** in the region of Vietnam, which was 0.3%.

This difference could be explained, according to **Kelen and Tepe (2008)**, by the choice of the harvest period, as it is essential in terms of yield and quality of the EO. Factors such as climate, the area's geography, the genetics of the plant, the organ of the plant used, its degree of freshness, the drying period, and the extraction method employed can have a direct impact on EO yields.

III.1.2. Determination of the characters organoleptic

The organoleptic parameters of Algerian *S. officinalis*, Lebanese *S. officinalis*, and French *S. sclarea* essential oils are consistent with those listed in the **AFNOR** standards **(1999).** The organoleptic characteristics of our essential oils, such as asspect, color, and odor, are summarized in Table 1 below:

Table 1. Characters organoleptic of the EO of different species studied

Origin of plants	Characteristic of our plants
Algerian *S. officinalis*	- ***Aspect*** : Liquid and mobile - ***Color*** : Colorless (**Figure 19**) - ***Odor*** : Fresh camphoraceous and rosemary.
Lebanese *S. officinalis*	- ***Aspect*** : Liquid and mobile - ***Color*** : Yellow clear (**Figure 19**) - ***Odor*** : Fresh camphoraceous and rosemary pine like top note and herbal minty thujone base notes.

Figure 19. Essential oil of Algerian *S. officinalis* (at left) and Lebanese *S. officinalis* (at right) **(Personal photo)**

III.2. Antioxidants activities

The results of antioxidant activities, measured by the DPPH free radical scavenging assay and the total antioxidant capacity (TAC), are listed in the following table:

Table 2. Results of DPPH and TAC of the essential oils (EO)

	DPPH IC 50 (mg/mL)	TAC IC 50 (mg/mL)
Algerian *S. officinalis*	0.15 ± 0.01	36.32 ± 1.40
Lebanese *S. officinalis*	0.17 ± 0.01	35.52 ± 1.57
French *S. sclarea*	0.38 ± 0.02	291.57 ± 0.73
α-Tocopherol	7.31 ± 0.17	/
Quercetin	2.07 ± 0.10	250.09 ±0.87
Acid ascorbic	N.T.	7936.48 0.07

III.2.1. Test of trapping of the radical free DPPH

Figure 20. Test trapping of the free radicals DPPH **(Personal photo)**

According to **Table 2**, the essential oils (EO) of Algerian *S. officinalis*, Lebanese *S. officinalis*, and French *S. sclarea* have very significant antioxidant activities with IC50 values of 0.15±0.01, 0.17±0.01, and 0.38±0.02 mg/mL, respectively, compared to the two standards used: quercetin with 2.07 mg/mL and α-tocopherol with 7.31 mg/mL.

According to **Maache *et al.* (2023)**, the antiradical activity of essential oil obtained from

Moroccan species of *Salvia* was significantly higher in *S. lavandulifolia* compared to *S. officinalis*, with IC50 values of 34.55 mg/mL and 40.72 mg/mL, respectively. Additionally, **Boussada *et al.* (2008)** showed that *S. officinalis* from Tunisia exhibited good antioxidant power with an IC50 of 6.7 mg/mL.

Meanwhile, the work of **Karneeb *et al.* (2023)** on the essential oil from Lebanese *Salvia fruticosa* displayed weaker antioxidant activity in the DPPH assay, with an IC50 value higher than 60 mg/mL. On the other hand, the team of **Bouderies *et al.* (2017)** found that the essential oil of *S. chudaei* from the Hoggar region (Algeria) exhibited significantly lower antioxidant activity in preventing linoleic acid oxidation and reducing DPPH radicals, with an IC50 of 3250±8.12 µg/mL.

III.2.2. Total antioxidant content (TAC)

The essential oils (EO) of Algerian *S. officinalis*, Lebanese *S. officinalis*, and French *S. sclarea* exhibit very significant antioxidant activities, as measured by total antioxidant capacity (TAC), compared to ascorbic acid (7936.48 ± 0.07 mg/mL). Their IC50 values are 36.32 ± 1.40, 35.52 ± 1.57, and 291.57 ± 0.73 mg/mL, respectively. The results of previous work by **Maache *et al.* (2023)** recorded the highest total antioxidant capacity in *S. lavandulifolia* (49.941 mg EAA/g DW) compared to *S. officinalis* (36.349 mg EAA/g DW).

III.3. Breeding of *Ephestia kuehniella* in the laboratory

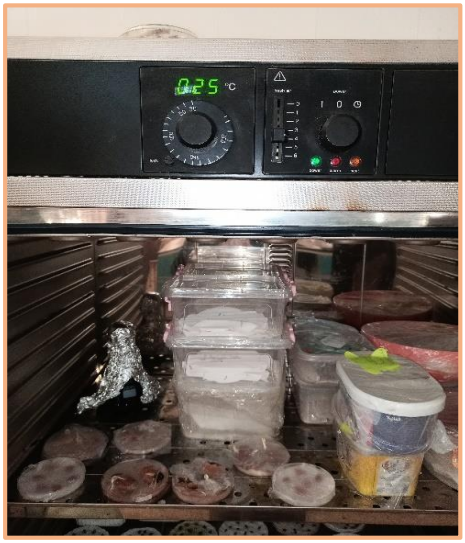


Figure 21. Breeding of *Ephestia kuehniella* (**Personal photo**)

The insects used in this study were collected from Moulin El Harrouch Agro Div. They were bred in the laboratory in an incubator where optimal development conditions were maintained: a temperature of 27°C, a relative humidity of approximately 80%, and total darkness. The adults were placed in glass or plastic jars containing flour, covered with a piece of tulle held in place by an elastic band, allowing the larvae to move on to the pupation stage.

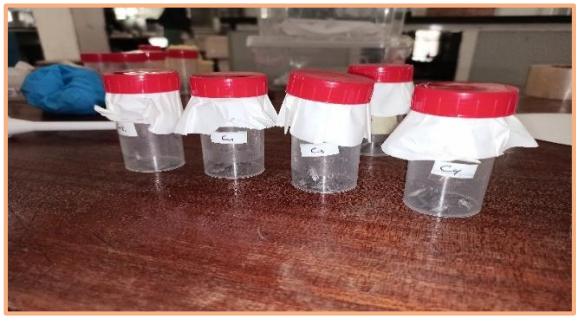
We collected approximately 1.200 worm specimens, which we then subjected to controlled incubation. After a period of 8 days, we successfully obtained a population of approximately 800 adults (Figure 21).

III.4. Bioassay of insecticidal activity by fumigation

- **French *S. sclarea***

The efficacy of the essential oil (EO) of French *S. sclarea* was tested by fumigation on newly emerged adults of *Ephestia kuehniella* Zeller. Mortality was recorded, and the doses corresponding to 10% and 90% adult mortality (LD_{10} and LD_{90}), characterizing the toxicity of the EO, were determined. EO was applied by fumigation at different doses (0.1, 0.2, 0.3, 0.5, and 1.5 µL) on the day of adult emergence of *E. kuehniella*, inducing adult mortality percentages as specified in **Table 3.**

Figure 22. Application of doses of raw extracts on adults of *Ephestia kuehniella* (**Personal photo)**



Figures 23. No mortality was recorded among the controls (natural death), while mortality increased significantly in the treated series, showing a dose-response relationship **(Personal photo)**.

The results reveal that the corrected mortality percentage of the adults ranged from 20±0.00% for the lowest dose (0.1 µL) and increased gradually to 100±0.00% for the highest tested doses (0.5 and 1.5 µL).

Table 3. Effects of OE French *S. sclarea*, applied by fumigation at different doses (µL) on the day of emergence of *E. kuehniella* adults: observed mortality (%) of adults (mean ± SD; n = 3 replicates of 10 individuals each).

Repetition	0.1 µL	0.2 µL	0.3 µL	0.5 µL	1.5 µL	Witness
R1	20	30	60	100	100	0
R2	20	40	60	100	100	0
R3	20	30	70	100	100	0
m±SD	20± 00	33.33±3.00	63.33±3.3	100±00	100±00	0

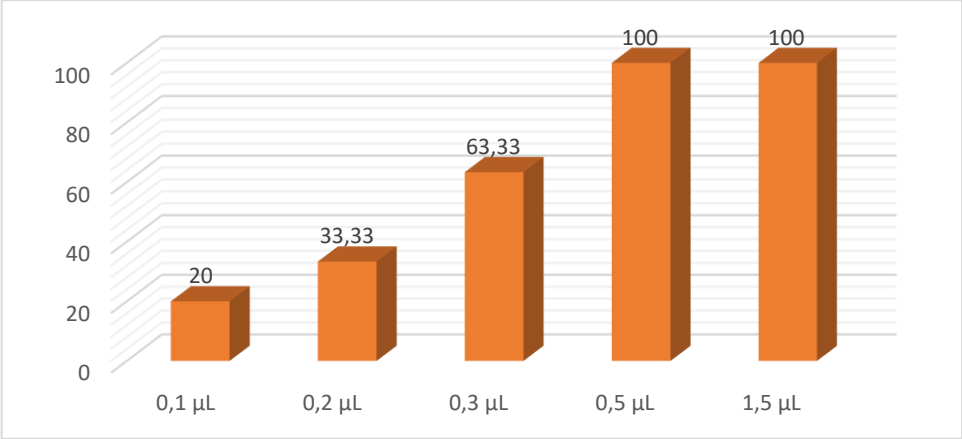


Figure 24. Effect of French *S. sclarea* EO applied by fumigation to newly grown adults emerged *Ephestia kuehniella* on adult mortality (%) (m ± SD; n = 3 replicates each containing 10 adults; values indicated by different letters are significantly different by the HSD test at p <0.0001).

The statistical analysis reveals a significant dose effect ($_{F4, 10}$) = 609.6; p < 0.0001), and Tukey's HSD test showed a significant increase in mortality with increasing doses. Different lowercase letters indicate a significant difference between corrected mortality rates (p < 0.0001). The lethal doses (LD) and the Hill slope, recorded with their 95% confidence intervals, are listed in **Table 4.**

Table 4. Effects of HE of French *S. sclarea* applied by fumigation to adults newly emergedof *Ephestia kuehniella* at different doses (µL): Determination of molt inhibition doses adult (DI in µL) and their intervals of trust has 95%.

Dose Lethal	Value (µl)	Intervalles de confiance (95%)	R^2
DL_{10}	0.0782436	[0.0424 – 0.1443]	0.97
DL_{25}	0.1388	[0.09602 – 0.2006]	
DL_{50}	0.2461	[0.1967 – 0.3080]	
DL_{90}	0.7744	[0.4318– 1.389]	
Hill Slope	0.2916	[0.9899110 – 2.845]	

▪ Algerian *S. officinalis*

The efficacy of the essential oil (EO) of Algerian *S. officinalis* was tested by fumigation on newly emerged adults of *Ephestia kuehniella* Zeller. Mortality was recorded, and the doses corresponding to 10% and 90% adult mortality (LD_{10} and LD_{90}), characterizing the toxicity of the EO, were determined. EO was applied by fumigation at different doses (0.5, 0.8, 1, 1.5, 3 and 3.5 µl) on the day of adult emergence of *E. kuehniella*, inducing adult mortality percentages as specified in **Table 5** and **Figure 25**. The results reveal that the corrected mortality percentage of the adults ranged from 26.67±3.00% for the lowest dose (0.5 µL) and increased to 33.33±3.00% for the highest tested doses (0.5 and 3.5 µL). After our trials with these doses of Algerian *S. officinalis*, we decided to stop the experiment at this level because reaching the lethal dose of 100% would require more time and more essential oil.

Table 5. Effects of O.E Algerian *S. officinalis*, applied by fumigation at different doses (µL) on the day of emergence of *E. kuehniella* adults: observed mortality (%) of adults (mean ± SD; n = 3 replicates of 10 individuals each).

Repetition	0.5 µL	0.8 µL	1 µL	1.5 µL	3 µL	3.5 µL	Witness
R1	30	30	40	30	30	30	0
R2	20	30	30	40	30	30	0
R3	30	20	30	30	40	40	0
m±SD	26.67± 3.00	26.67±3.00	33.33±3.00	33.33±3.00	33.33±3.00	33.33±3.00	0

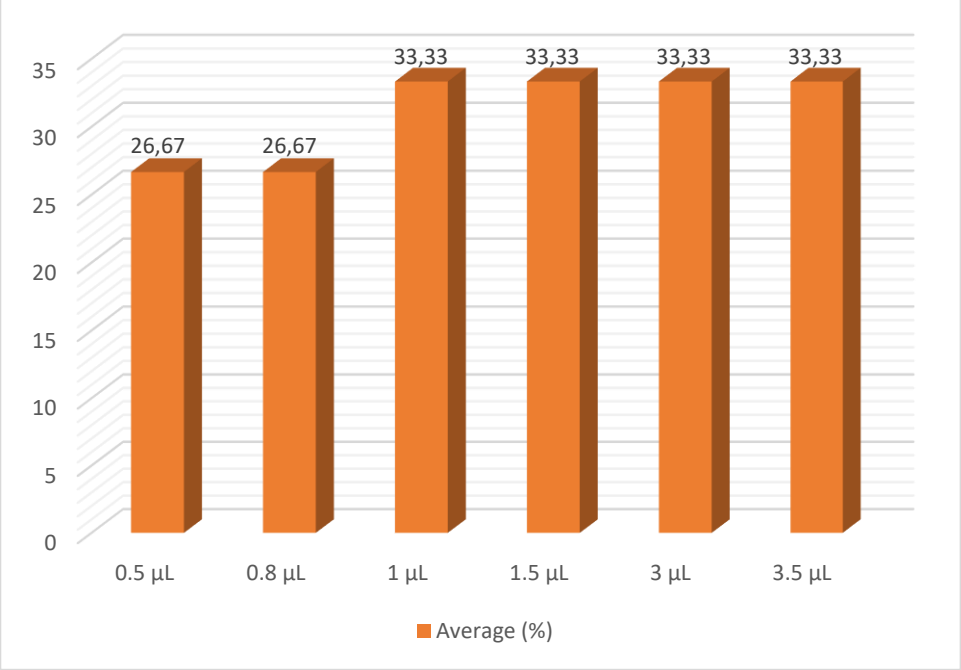


Figure 25. Effect of Algerian *S. officinalis* EO applied by fumigation to newly grown adults emerged *Ephestia kuehniella* on adult mortality (%) (m ± SD; n = 3 replicates each containing 10 adults; values indicated by different letters are significantly different by the HSD test at p <0.0001).

▪ Lebanese *S. officinalis*

The efficacy of the essential oil (EO) of Lebanese *S. officinalis* was tested by fumigation on newly emerged adults of *Ephestia kuehniella* Zeller. Mortality was recorded, and the doses corresponding to 10% and 90% adult mortality (LD_{10} and LD_{90}), characterizing the toxicity of the EO, were determined. EO was applied by fumigation at different doses (2, 2.5, 3 and 3.5 µl) on the day of adult emergence of *E. kuehniella*, inducing adult mortality percentages as specified in **Table 6** and **Figure 26**.

The results reveal that the corrected mortality percentage of the adults ranged from 20.00±00% for the lowest dose (0.5 µL) and gradually increased to 36.67±3.3% for the highest tested doses (0.5 and 3.5 µL). After our trials with these doses of Lebanese *S. officinalis*, we decided to stop the experiment at this level because reaching the lethal dose of 100% would require more time and more essential oil.

Table 6. Effects of OE Lebanese *S. officinalis*, applied by fumigation at different doses (µL) on the day of emergence of *E. kuehniella* adults: observed mortality (%) of adults (mean ± SD; n = 3 replicates of 10 individuals each).

Repetition	2 µL	2.5 µL	3 µL	3.5 µL	Witness
R1	20	20	30	30	0
R2	20	20	30	40	0
R3	20	20	40	40	0
m±SD	20± 00	20± 00	33.33±3.00	36.67±3.3	0

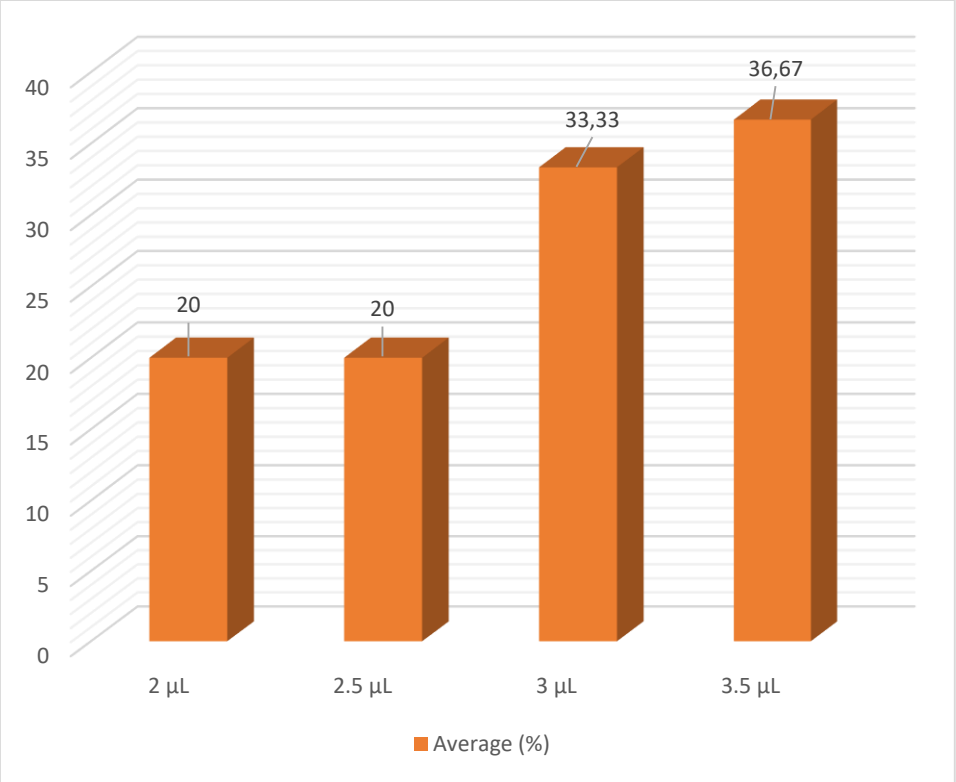


Figure 26. Effect of Lebanese *S. officinalis* EO applied by fumigation to newly grown adults emerged *Ephestia kuehniella* on adult mortality (%) (m ± SD; n = 3 replicates each containing 10 adults; values indicated by different letters are significantly different by the HSD test at p <0.0001).

III.5. Antibacterial activity

The antibacterial activity of the essential oils of the three *Salvia* species was determined by measuring the diameter of the inhibition zones for the three bacterial strains tested, as shown in the table below:

Table 7. Effect of the oils essential of the three *Salvia* species (French *S. sclarea* and Algerian and Lebanese *S. officinalis*) on the strains bacteria tested.

	Escherichia coli	***Klebsiella pneumoniae***	***Salmonella typhimurium***
Salvia sclarea	Average diameter of inhibition zones =0.8 mm **Resistatnt (-)**	Average diameter of inhibition zones =3.3 mm **Resistatnt (-)**	Average diameter of inhibition zones =3.3 mm **Resistatnt (-)**
Algerian *Salvia officinalis*	Average diameter of inhibition zones =0.8 mm **Resistatnt (-)**	Average diameter of inhibition zones =1.2 mm **Resistatnt (-)**	Average diameter of inhibition zones =0.6 mm **Resistatnt (-)**
Lebanese *Salvia officinalis*	Average diameter of inhibition zones =1.27 mm **Resistatnt (-)**	Average diameter of inhibition zones =1.2 mm **Resistatnt (-)**	Average diameter of inhibition zones =1.5 mm **Resistatnt (-)**

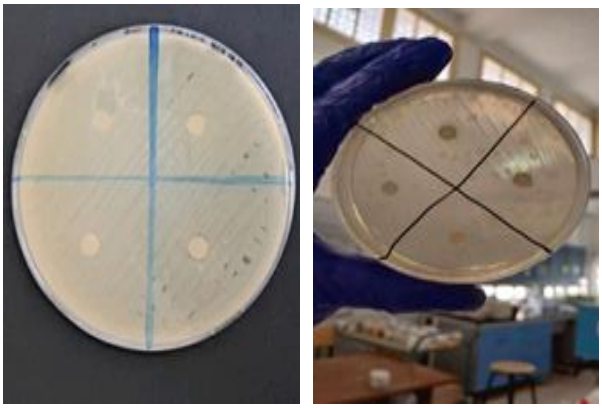


Figure 27. Test of antibacterial activity using the essential oils of Salvia species **(Personal photo).**

We studied the antibacterial power of the essential oils of three *Salvia* species using the disk diffusion method on solid Mueller-Hinton agar for three Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhimurium*) (**Figure 27**). According to **Table 7**, the three *Salvia* species showed no sensitivity for the three bacterial strains tested: French *S. sclarea* had an average inhibition zone diameter ranging from 0.8 mm (*Escherichia coli*) to 3.3 mm (*Klebsiella pneumoniae* and *Salmonella typhimurium*); Algerian *S. officinalis* had average inhibition zone diameters of 0.6 mm (*Salmonella typhimurium*), 0.8 mm (*Escherichia coli*), and 1.2 mm (*Klebsiella pneumoniae*); and Lebanese *S. officinalis* had average inhibition zone diameters of 1.2 mm (*Klebsiella pneumoniae*), 1.27 mm (*Escherichia*

coli), and 1.5 mm (*Salmonella typhimurium*).

These results indicate that *Salvia sclarea* oil was an effective bacterial inhibitor and bactericide with a broad antibacterial spectrum. Our findings are supported by other work carried out by **Cui *et al.* (2015)** on *S. sclarea*, which demonstrated antibacterial effects against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus pumilus* ATCC 27142, *Klebsiella pneumoniae* ATCC 13883, *Bacillus subtilis* IFO 3457, *Salmonella typhimurium* B11, and *Pseudomonas aeruginosa* ATCC 27853.

Sepahvand *et al.* (2014) also demonstrated that *S. sclarea* essential oil had different antibacterial effects, with the magnitude *S. aureus* = *K. pneumoniae* > *P. aeruginosa*. Discrepancies between our observations and previous reports may be due to several factors: (1) differences among species, (2) variability in antibacterial activities of *S. sclarea* essential oil due to different materials and extraction methods, (3) differences between strains of the same origin, resulting from long-term adaptation to ecological environments, artificial selection, and crossbreeding, leading to variable drug resistances, and (4) different experimental methods leading to different results.

Our results for the essential oils of both species of *Salvia officinalis* do not confirm those obtained by **Longaray-Delamare *et al.* (2007)**, who showed that the essential oils of *Salvia officinalis* and *Salvia triloba* exhibited remarkable bacteriostatic and bactericidal activities against *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Aeromonas hydrophila*, *Aeromonas sobria*, and *Klebsiella oxytoca*.

VI. Conclusion

VI. Conclusion

Our work aims to valorize the essential oils of three medicinal plants of different origins from the genus *Salvia* of the Lamiaceae family: *Salvia sclarea* from France, *Salvia officinalis* from Algeria, and *Salvia officinalis* from Lebanon.

The main objective of this study is to explore the phytochemical properties of these plants through: The extraction of essential oils by hydrodistillation using a Clevenger-type apparatus, an antioxidant activity study (DPPH and Total Antioxidant Capacity - CAT), a bio-insecticide assay on the stored product pest *Ephestia kuehniella*, an antibacterial activity study against three Gram-negative bacterial strains (*Klebsiella pneumoniae, Escherichia coli*, and *Salmonella typhimurium*).

The results show that the essential oil yield of the two *S. officinalis* species is 0.6% for Algeria and 1.1% for Lebanon, with organoleptic characteristics similar to those of AFNOR (1999): for *Salvia officinalis* from Algeria, a mobile liquid aspect, colorless, with a fresh camphor and rosemary odor; and for *Salvia officinalis* from Lebanon, a mobile liquid aspect, light yellow in color, with a fresh camphor pine odor and a mentholated and herbaceous thujone base note.

The CAT and DPPH tests of the essential oils of *S. sclarea* from France, *S. officinalis* from Algeria, and *S. officinalis* from Lebanon show good antioxidant activity with CAT values of 291.57 ± 0.73, 36.32 ± 1.40, and 35.52 ± 1.57 mg/ml respectively, and DPPH values of 0.38 ± 0.02, 0.15 ± 0.01, and 0.17 ± 0.01 mg/ml, respectively.

The evaluation of bio-insecticide activity on *Ephestia kuehniella* with different concentrations of essential oils of *S. sclarea* from France (0.1, 0.2, 0.3, 0.5, and 1.5 µL), *S. officinalis* from Algeria (0.5, 0.8, 1.0, 1.5, 3, and 3.5 µL), and *S. officinalis* from Lebanon (2, 2.5, 3, and 3.5 µL) shows the following lethal doses: for the essential oil of *S. sclarea* from France, LD10 = 0.0782436 µL, LD25 = 0.1388 µL, LD50 = 0.2461 µL, and LD90 = 0.7744 µL.

However, the two species of *S. officinalis* do not show good insecticidal activity against *Ephestia kuehniella*. Regarding antibacterial activity, the essential oils of the three plants show resistance to the three bacterial strains studied: *Escherichia coli* (resistant -; between 0.8 mm

for *S. sclarea* and *S. officinalis* from Algeria and 1.27 mm for *S. officinalis* from Lebanon), *Salmonella typhimurium* (resistant -; 0.6 mm, 1.5 mm, and 3.3 mm for *S. officinalis* from Algeria, *S. officinalis* from Lebanon, and *S. sclarea* from France respectively), and *Klebsiella pneumoniae* (resistant -; with 1.2 mm for *S. officinalis* from Algeria and *S. officinalis* from Lebanon, and 3.3 mm for *S. sclarea* from France).

Based on the results obtained in this study, it can be concluded that the essential oils of the studied plant have shown potential antioxidant, bio-insecticidal, and antimicrobial capacity, and could be used as a natural and effective alternative to the chemicals commonly used in agriculture for the control of stored product pests, as well as synthetic antioxidants and specific antibacterials for medical applications.

This study paves the way for future research to optimize the use of this plant in various fields for its potential in combating viruses, fungi, and other pathogens, which could open significant prospects in the medical field, particularly for developing new treatments against emerging or resistant viral infections. At the same time, expanding studies on its antimicrobial spectrum to include evaluation against a greater diversity of pathogenic microorganisms would help determine its versatility and therapeutic potential.

V. References

V. References

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Mrs. BAKRIA Lina

Mrs. ZAROUR Imane

Title: Study of the Bio-insecticide, Antibacterial, and Antioxidant Activities of Some Plants of the Genus Salvia

Abstract: Our work aims to valorize the essential oils of three medicinal plants of different origins from the genus *Salvia* of the Lamiaceae family: *Salvia sclarea* from France, *Salvia officinalis* from Algeria, and *Salvia officinalis* from Lebanon. The main objective of this study is to explore the phytochemical properties of these plants through: The extraction of essential oils by hydrodistillation using a Clevenger-type apparatus, an antioxidant activity study (DPPH and Total Antioxidant Capacity - CAT), a bio-insecticide assay on the stored product pest *Ephestia kuehniella*, an antibacterial activity study against three Gram-negative bacterial strains (*Klebsiella pneumoniae*, *Escherichia coli*, and *Salmonella typhimurium*). The results show that the essential oil yield of the two *S. officinalis* species is 0.6% for Algeria and 1.1% for Lebanon, with organoleptic characteristics similar to those of AFNOR (1999): for *Salvia officinalis* from Algeria, a mobile liquid aspect, colorless, with a fresh camphor and rosemary odor; and for *Salvia officinalis* from Lebanon, a mobile liquid aspect, light yellow in color, with a fresh camphor pine odor and a mentholated and herbaceous thujone base note. The CAT and DPPH tests of the essential oils of *S. sclarea* from France, *S. officinalis* from Algeria, and *S. officinalis* from Lebanon show good antioxidant activity with CAT values of 291.57 ± 0.73, 36.32 ± 1.40, and 35.52 ± 1.57 mg/ml respectively, and DPPH values of 0.38 ± 0.02, 0.15 ± 0.01, and 0.17 ± 0.01 mg/ml, respectively. The evaluation of bio-insecticide activity on *Ephestia kuehniella* with different concentrations of essential oils of *S. sclarea* from France (0.1, 0.2, 0.3, 0.5, and 1.5 µL), *S. officinalis* from Algeria (0.5, 0.8, 1.0, 1.5, 3, and 3.5 µL), and *S. officinalis* from Lebanon (2, 2.5, 3, and 3.5 µL) shows the following lethal doses: for the essential oil of *S. sclarea* from France, LD10 = 0.0782436 µL, LD25 = 0.1388 µL, LD50 = 0.2461 µL, and LD90 = 0.7744 µL. However, the two species of *S. officinalis* do not show good insecticidal activity against *Ephestia kuehniella*. Regarding antibacterial activity, the essential oils of the three plants show resistance to the three bacterial strains studied: *Escherichia coli* (resistant -; between 0.8 mm for *S. sclarea* and *S. officinalis* from Algeria and 1.27 mm for *S. officinalis* from Lebanon), *Salmonella typhimurium* (resistant -; 0.6 mm, 1.5 mm, and 3.3 mm for *S. officinalis* from Algeria, *S. officinalis* from Lebanon, and *S. sclarea* from France respectively), and *Klebsiella pneumoniae* (resistant -; with 1.2 mm for *S. officinalis* from Algeria and *S. officinalis* from Lebanon, and 3.3 mm for *S. sclarea* from France). Ultimately, these results suggest that the essential oils of the three Salvia species could be used as a natural and effective alternative to the chemicals commonly used in agriculture for the control of stored product pests, as well as synthetic antioxidants. This study paves the way for future research to optimize the use of this plant in various fields.

Keywords: Genus *Salvia*, essential oil, antioxidant activity, antibacterial, bio-insecticide.

University Year : 2023-2024