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Theme

Evaluation of the toxicity of a Bioinsecticide Formulation based on Essential Oils of Artemisia Absinthium L. and Artemisia Herba Alba on the Beetle Pest ''Tribolium Castaneum''

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Dedications

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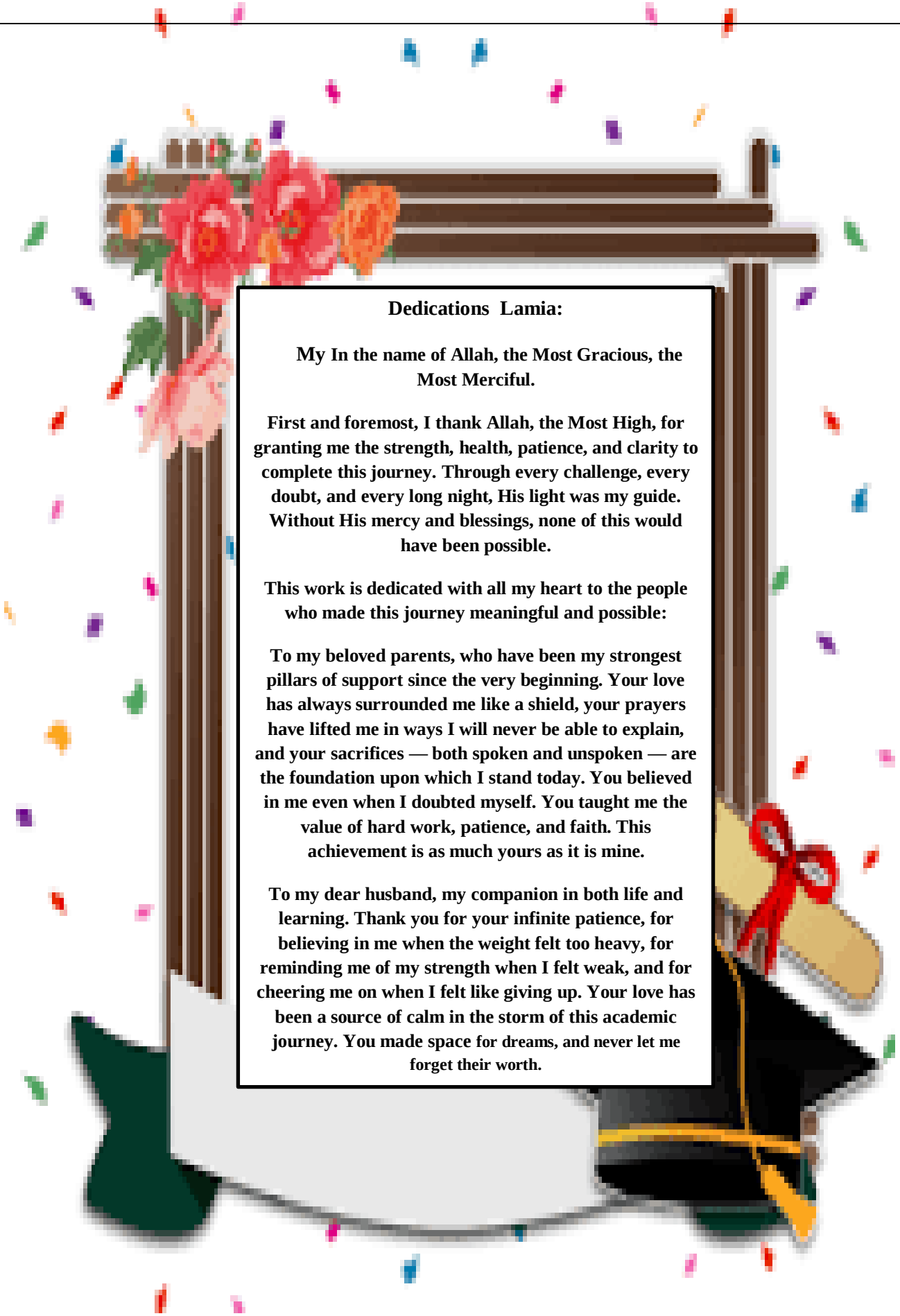
Use me through it, open the doors of good to me and make it a reason to satisfy You. To the one who has supported me at every stage of my life, To the one who has taught me that perseverance makes the impossible possible, To my dear father, the source of my strength and pride, I dedicate this graduation to you as a sign of gratitude and gratitude, For your words that have encouraged me and for your presence that has reassured me, My success today Dedicated to my mother - May God have mercy on you To her who has left this world, but whose presence will never be erased from my heart... To my mother, eternal light, and to the prayers that continue to surround me even after your departure. May God have mercy on you as much as you have wished me happiness and success, and may he place this accomplishment among your good deeds.



Dedications Lamia:

My In the name of Allah, the Most Gracious, the Most Merciful.

First and foremost, I thank Allah, the Most High, for granting me the strength, health, patience, and clarity to complete this journey. Through every challenge, every doubt, and every long night, His light was my guide. Without His mercy and blessings, none of this would have been possible.

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Dedications

*Thank you, **Allah**, first and last to rephrase to my knowledge,*

*To my dream, to my source of passion, optimism and hope to your pure heart; "my dear **Mother**"* Peace to his soul .

*To who drank the empty glass to water me with love, to who worked very hard to give me a little joy, to direct me towards the path of knowledge, to my big heart; "my dear **Father**"*

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*To the very pure heart my brother "**SOFIANE**".*

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*To all my P**aternal** and **Maternal family**.*

CHAFIKA

Abstract:

The aim of this study was to investigate the chemical composition and evaluate the insecticidal formulation of essential oils from ***Artemisia herba-alba*** and ***Artemisia Absinthium*** (aerial parts) in Algeria using the microencapsulation technique. **Fumigation** toxicities were assessed against a major stored-product beetle: *Tribolium castoreum*. The chemical composition of both oils was characterized by qualitative and quantitative differences. The main common compounds of *Artemisia. Herba Alba* were camphor, 1,8-cineole (eucalyptol), camphene, α-thujone, β-thujone, and chrysanthemum, while β-thujone was the characteristic component of *A. Absinthium* oil.

The results indicated that both oils exhibited potential fumigant toxicity. Fumigation bioassays showed that the essential oil of *A. herba-alba* was more toxic against *T. castoreum*. The LC_{50} and LC_{95} values revealed that the essential oil of *Artemisia Herba-Alba* was more effective than that of *Artemisia A absinthium*.

Our work highlights the importance of using microencapsulated formulations of *Artemisia* essential oils in Algeria, both as fumigants and as bioinsecticides against economically important stored-product pests.

Résume :

L'objectif de cette recherche était d'étudier la composition chimique et d'évaluer la formulation insecticide des huiles essentielles d'Artemisia herba-alba et d'*Artemisia Absinthium* (parties aériennes) en Algérie par la technique de microencapsulassions. Les toxicités par fumigation ont été évaluées pour un principal coléoptère des denrées stockées : *Tribolium castaneum.* La composition chimique des deux huiles était caractérisée par des différences qualitatives et quantitatives. Les principaux composés communs étaient **camphre, 1,8-cinéole (eucalyptol), camphène, α-thuyone et β-thuyone, chrysanthénone,** tandis que la **bêta-thuyone** était le composant caractéristique de l'huile d'*A. Absinthium*. Les résultats ont indiqué que les deux huiles présentaient un potentiel de toxicité par fumigation. Les essais biologiques par fumigation ont montré que l'huile essentielle d'*A. herba-alba* était plus toxique et celle de *Tribolium castaneum*. Les valeurs de CL50 et CL95 indique l'huile essentielle d'*Artemisia Herba Alba* que celle d'*Artemisia Absinthium*. Nos travaux révèlent l'importance de l'utilisation de la formulation par micro encapsulation des huiles essentielles d'Artemisia en Algérie, à la fois comme fumigant et comme bio insecticide contre les ravageurs des denrées stockées d'importance économique.

ملخص

هدف هذه الدراسة هو دراسة التركيب الكيميائي وتقييم فعالية التركيبة الحشرية للزيوت الأساسية لنباتي الأجزاء الهوائية التغليف الدقيق *Artemisia herba -alba* و*Artemisia absentium* في الجزائر باستخدام تقنية

تم تقييم السمية بالتبخير ضد احد الخنافس الرئيسية التي تصيب المواد المخزية وقد تميز *Tribolium Castenium* التركيف الكيميائي لكلا الكيميائي لكلا الزيتين باختلافات نوعية و كمية ومن بين المركبات المشتركة الرئيسية : الكافور سينول -1،8،

(الاوكاليبتول والكامفين ،والافاثوجين ،والبيتاثوجين، والكريسانثينون بينما كانت البيتاثوجون هي المركب المميز في الزيت

أظهرت النتائج ان كلا الزيتين يمتلكان قدرة محتملة على السمية بالتبخير وقد أظهرت التجارب الحيوية ان كما أظهرت قيم *Tribolium Castaneum* A. herba -alba كان أكثر سمية ضد

Artemisia absentium أكثر فعالية من زيت Artemisia herba -alba زيت الأساسي لنبات ال

ان تكشف نتائجنا عن أهمية استخدام تقنية التغليف الدقيق للزيوت الأساسية لنبات كمواد مبخرة او كمبيدات حيوية ضد افات المواد الغذائية المخزنة ذات الأهمية الاقتصادية

Summary

BIBLIOGRAPHY

ABREVIATION LIST:

EO: Essential Oil

NPs: Pest Essential Oil

PEOs: Ribonucleic acid

SEM: Scanning electron microscope

T. Castenium: Tribolium Castenium

T. Confusum: Tribolium Confusum

EOs: Essential oil

C10: Carbone 10

SCFE: Supercritical fluid extraction

MAF: Micro-Assisted extraction

UAE: Ultra Energy extraction

AChE: Acetyl cholinesterase

GABA: Gamma aminobutyric acid

O/W: oil-in water

PH or HP: Hydrogene Potential

List of tables:

LISTES OF FIGURES :

Figure13	Photograph of *Artemisia herba-alba* **(N. Feinbrun -Dothan, 2010) .**

Figure 30	Presents the evolution of the corrected mortality of *Tribolium castaneum* in response to exposure to *Artemisia absinthium* essential oil. The results reveal a clear dependence on both exposure time and applied dose.
Figure 31	Percentage of corrected mortality of *Tribolium castaneum* exposed to different doses of *Artemisia absinthium* essential oil as a function of exposure time (24h to 264h).
Figure 32	LC50 and LC95 values (µL/L air) of *Artemisia herba-alba* essential oil against *Tribolium Castaneum* adults after fumigant bioassay at different exposure times.
Figure 33	LT50 and LT95 values (in hours) of *Artemisia herba-alba* essential oil against *Tribolium Castenium* adults at different fumigant concentrations.
Figure 34	LC50 and LC95 values (µL/L air) of *Artemisia absinthium* essential oil against *Tribolium Castaneum* adults after fumigant bioassay at different exposure times.
Figure 35	LT50 and LT95 values (in hours) of *Artemisia absinthium* essential oil against *Tribolium Castaneum* adults at different fumigant concentrations

Introduction

In many countries, most of the food grains produced is stored for contingency and regular supply. These stored grains, in general, are directly or indirectly infested by insects, resulting in severe grain damages and storage losses, thus, causing a threat to food safety and security (**P. N. Guru et al ., 2022) .** The internal-feeding insects have been referred to historically as primary pests, while those feeding outside the kernels on broken and fine material have been referred to as secondary pests (**Getachew Bezabih et al ., 2022**) **.** Secondary pests include Tribolium Castaneum and Tribolium Confusum (**Jianxiu Yao et al ., 2019**) . The red flour beetle, *T. castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a cosmopolitan insect pest and can be found in flour mills, store grain warehouses and grocery shops (**Misha Khalil et al ., 2024).** The control of this pest relies heavily on the use of chemical insecticides/pesticides and several classes of insecticides are used, including pyrethroids, organophosphates, organochlorides, carbamates and abamectin .However, the continuous application of pesticides in the field and granaries has accelerated the development of resistance in this agricultural pest to diverse insecticides from several classes **(Muhammad M. Mukhtar et al ., 2023)** . The fight against insect pests primarily relies on the utilization of synthetic insecticides. However, improper application of these chemicals can lead to detrimental effects on both the environment and human health, as well as foster the development of insect resistance. Consequently, novel strategies must be implemented to address the challenges stemming from the prolonged use of synthetic insecticides in agricultural and public health environments. Certain strategies involve the combination of crop protectants, which not only enhance insecticidal effectiveness but also reduce application rates. Plant-based natural products emerge as promising alternatives for insect management. Monoterpenes, which are abundant plant compounds produced through the activation of various enzymes, have attracted significant attention for their effectiveness in insect control . Notably, they are prolific in fragrance-producing plants (**Muhammad Qasim et al ., 2024).**Plants such as : The Artemisia genus that is one of the most diversified among the Asteraceae family, which contains more than 500 species including a wide number of aromatic species (**Ikbal Chaieb et al ., 2018**) . The first species is *Artemisia herba-alba* Asso, also known as desert wormwood, is a resilient, perennial dwarf shrub belonging to the Asteraceae family. It grows in arid and semi-arid areas of Spain, North Africa and the Middle East . It plays an important role in the traditional medicine of various cultures due to

its numerous therapeutic properties: antidiabetic, antihypertensive, antioxidant, antifungal, antimalarial, antispasmodic (**Beáta Baranová et al ., 2025)** and insecticidal **(Nesrine Benkhaira et al ., 2022**) .The second is Artemisia absinthium is native of temperate regions of Eurasia, Northern Africa and naturalized in Canada and the northern United States. Its medicinal effects include antimicrobial, antifungal, neuroprotective, hepatoprotective but also antioxidant and anti-inflammatory **(Abdulmonem Awwad et al ., 2020)** . Owing to these two plants, the essential oil can be extracted mainly by the hydrodistillation method . Entrapment of EOs by encapsulation is a way to protect them from the mentioned factors (**Jina Yammine ., 2023) .**Microencapsulation is a technology based on the coating of solid, liquid, or gaseous particles through an encapsulating agent that acts as a barrier, completely isolating the core material from the external environment. Microencapsulation of EOs methods includes spray drying, spray chilling or spray cooling, extrusion coating, fluidized bed coating, liposome entrapment, coacervation, inclusion complexation, centrifugal extrusion, and rotational suspension separation**(Amal E. Abd El Kader , H.M. Abu Hashish ., 2020)** .

The present study aimed to determine the chemical composition of the essential oils and evaluate the repellent effects and toxicity of powders and essential oils from Two aromatic and medicinal plants: *Artemisia Herba Alba* , *Artemisia Absinthium* and formulation based of microencapsulation about EOs of this plants against Tribolium castaneum adults .

I-1- Damage caused by tribolium castaneum :

The particular insect pest species present in the storage depends on several factors, can be classified into two main categories: The abiotic factors are non-living environmental conditions, including grain moisture, storage temperature, and humidity, as well as poor storage facilities. Among all the biotic factors, insects and pests are considered the most important and cause huge grain losses during storage (30–40%) (**Kumar, D.; Kalita, 2017**). The internal-feeding insects have been referred to historically as primary pests, while those feeding outside the kernels on broken and fine material have been referred to as secondary pests. On the other hand, secondary storage pests such as red flour beetle T. castaneum Herbst (Coleoptera: Tenebrionidae), potato tuber moth Phthorimaea operculella and the rice moth Corcyra Cephalonia attack the grains that have already been damaged by primary pests or other factors. Hence, pests under this category thrive on broken kernels, grain dust, and moldy grains, further contributing to the deterioration of damaged stored products ((**Arthur F.H., 2019**), (**Srivastava C., et al., 2016**), (**Bell C.H., 2014**)).

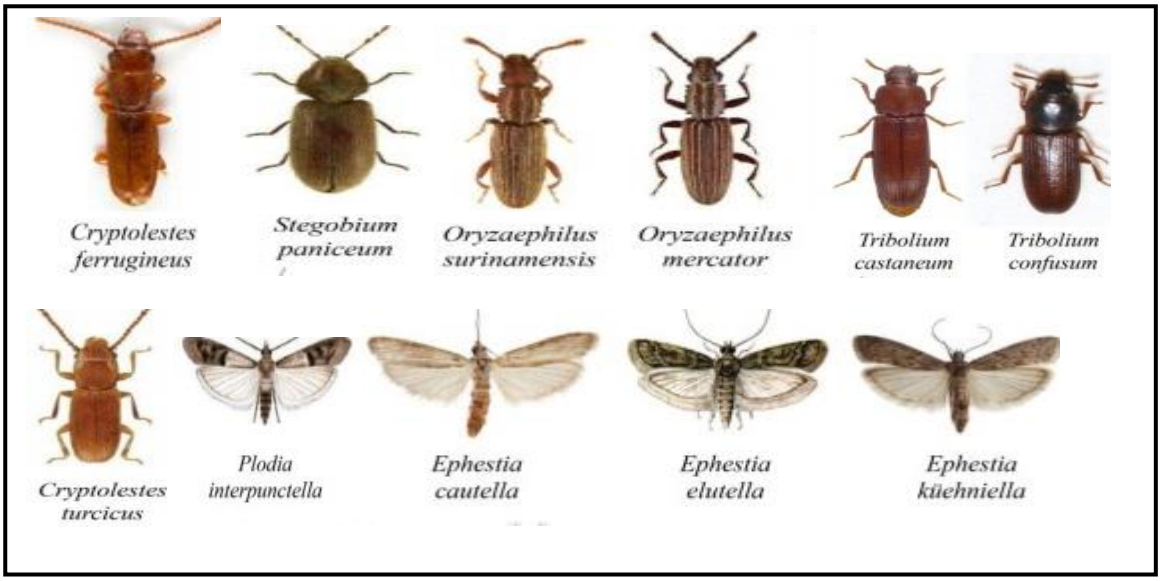


Figure 1: Secondary Pests on stored foods (Lijun Zhu et al., 2022)

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is one of the most destructive secondary pests of cereal grains (**Balakrishnan et al., 2016**). This particular species negatively impacts various crops such as rice, maize, wheat, beans, and

oilseeds, flour, millet, potatoes, sweet potatoes, dried fruit, nut, and sorghum leading to substantial food weight reduction and causing severe economic losses (**LUDJI PANTJA ASTUTI et al., 2020**. Nayak **et al., 2014**). The infestation of this insect causes unpleasant smell due to the benzoquinone secretion from its abdominal gland (**Oaya, C. S. & Barde, R., 2024**).

I-2- Systemic position Of *Tribolium Castaneum*:

Domain:	Eukaryota
Kingdom:	Animalia
Phylum:	Arthropoda
Class:	Insecta
Order:	Coleoptera
Suborder:	Polyphaga
Infraorder:	Cucujiformia
Family:	Tenebrionidae
Genus:	Tribolium
Species:	T. castaneum

Binomial name: *Tribolium castoreum* (Herbst, 1797) (**ITIS, 2019**)

I-3- Morphology and Biology:

Adults of Tribolium Castaneum first emerge creamy yellow and then change into rust in color within 2–4 days. Size3.8±0.03 mm and 0.55±0.02 mm in length and width, respectively, in both wheat and rice media (**Ranjeet Kumar Issrani et al., 2024**). The red flour beetle is reddish-brown in color and its antennae end in a three-segmented club. Whereas the confused flour beetle is the same color but its antennae end is gradually club-like, the "club" consisting of four segments (**Baldwin and Thomas.Fasulo.,2020).**

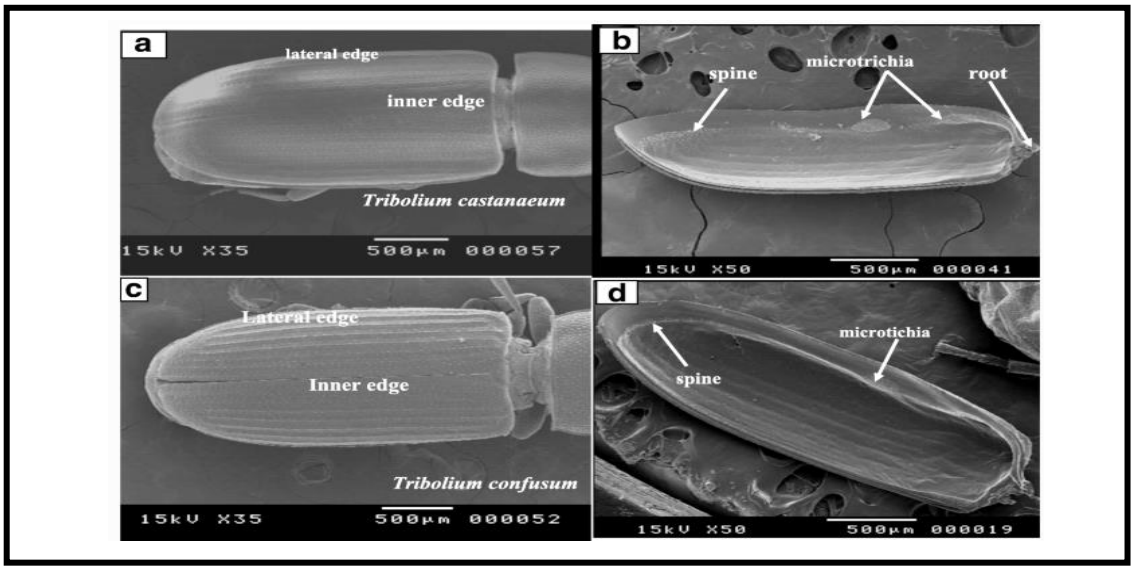


Figure 2: Scanning electron microscope (SEM) images of Tribolium elytral morphology. a dorsal view and b ventral view Tribolium castanaeum. c dorsal view and d ventral view Tribolium confusum (Zohry, N.M et al., 2019).

I-4- Life Cycle:

As a typical holometabolous insect, T. castaneum develops through several larval stages (usually 7, but 5 or 6 when starved (**Chafino S et al., 2019**), and up to 11 instars based on some anecdotal accounts) followed by metamorphosis (Fig.). Its embryonic developmental comfort zone is between 22 and 32 °C, lasting 7 days at 25 °C and 3 days at 32 °C Likewise, development from egg to adult speeds up with temperature from 74 days at 22.5 °C to about 23 days at 32 °C, which is short enough for large scale genetic experiments.

I-4-1-Egg:

Eggs are microscopic, oval in shape and pale white in color, small in size, with 0.6±0.03mm and 0.4±0.02mm in length and width. Flour particles often stick to their surfaceas they are sticky when laid. This makes it more difficult to identify even under the microscope.

I-4-2- Larva:

A total of seven instars were recorded. They are campodeiform, slender in shape. Their dorsal surface is covered with fine bristles and the last abdominal segment is demarcated by the presence of anal cerci. Each larval stage is smaller in size from its succeeding stage. The 1st instar larva, emerged post incubation, is very tiny and hence very difficult to see it with the naked eye. They are ivory white in color and measures about 0.87±0.04mm in length and 0.096±0.005 mm in breadth. 2ndinstar is mobile, linen white in color, and measured about 1.78±0.04 mm in length and 0.28±0.02 mm in breadth. The 3rd instar is thread like, light yellowish in color. They measure about 2.06±0.05mm in length and 0.376±0.03 mm in breadth. The4th instar is light brownish in color & measured about 2.852±0.06 mm in length and 0.45±0.05 mm in breadth. The next one is tortilla colored, 5th instar, immature, and measured about 3.84±0.2 mm in length and 0.67±0.03 mm in breadth. The 6th instaris large and bulgy in appearance. They are light brown and measured about 4.97±0.04 mm in length and 0.79±0.02 mm in breadth. The 7th instar is highly mobile, heavy, and tawny in color and measured by about 5.95±0.05 mm in length and 0.972±0.03 mm in breadth.

I-4-3-Pupa:

The prepupal stage is light yellowish, smaller in size. The post pupal stages are demarcated by the presence of fully developed eyes, hind limbs, and dark brownish coloration. Moreover, papillae in the case of females are longer and reach the length of theriomorphic whereas in males it is small and restricted to the last abdominal segment. Pupa measured about 3.88±0.04 mm in length and0.96±0.05 mm in breadth.

I-4-4- Adult:

Adults are dark brownish in color. They have capitate type of antennae which is very prominent. They measure about 3.96±0.05 mm in (**Mamata Deb and Dolly Kumar , 2021) .**

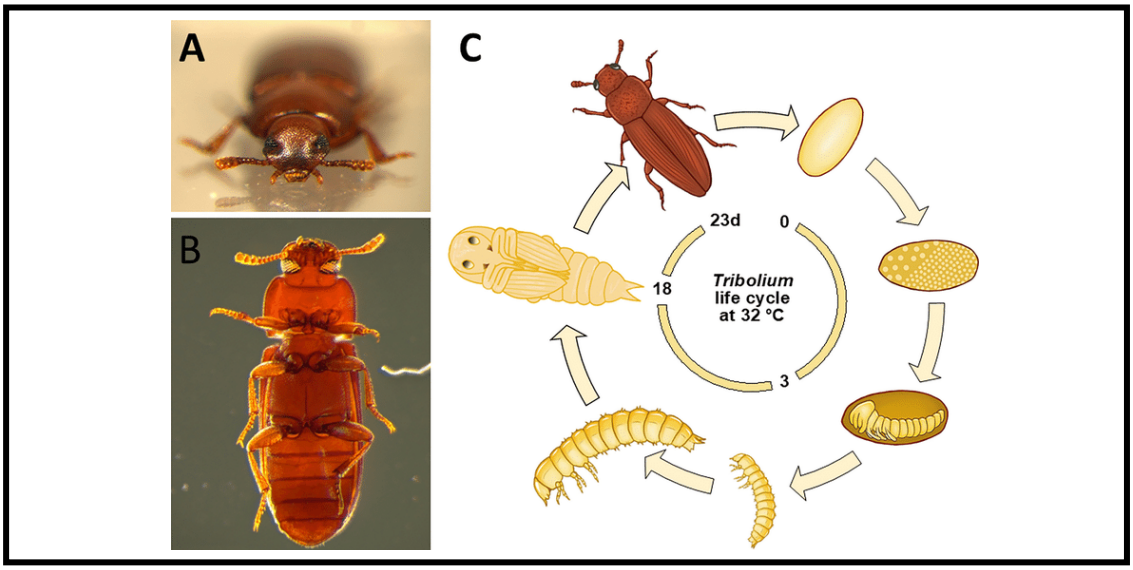


Figure 3: The life cycle of T. castaneum. A Face-to-face with a wild type Tribolium castaneum. B Ventral view of a male under dark field illumination. White eyes (vermilion mutant) allow for sensitive detection of transgene eye markers. The size of an adult beetle is 3.5 mm (length). C Life cycle of Tribolium: eggs are laid into the substrate (flour) and embryonic development takes 3 days at 32 °C. Only two out of a variable number of larval stages (ca. 7) are depicted. The pupa libera allows visual inspection of external structures .facilitating phenotypic studies of metamorphosis. Female beetles need a few days after hatching until they start laying eggs, which they continue for 3–4 months (drawings not to scale). Life cycle sketch by Benjamin Schwarz (Martin Klingler & Gregor Bucher ., 2022) .

I-5- Economic and Health impact of *Tribolium Castaneum*:

It infests usually those grains that had already been damaged by other pests. The affected grains and products become contaminated promting fungal growth. Economic losses include unpleasant smell, reduced weight, reduced trade and nutritional value. Presence of this insect pest may also cause allergic responses. Quinones secreted from thoracic and abdominal glands deteriorate grain and product quality to worst. T. castaneum can spread infection of *Aspergillus flavus, Aspergillus fumigatus, Cladosporium herbarum, Enterococcus faecalis, Penicillium citinum and P. purpurogenum* (**Channaiah et al., 2010; Yun et al., 2018**).

I -6- Control Methods:

In modern storage technologies, controlling insects is managed by:

I -6-1-Chemical Insecticides:

Including both fumigants and contact insecticides, which present serious threat to human health and environment, leave residues and enhance insect resistance. Besides, the high cost of the treatment requires new alternatives for insect control. Fumigation is still among the most effective and widespread techniques for the control of stored product.

Methyl bromide and phosphine are the two most common and widely used fumigants. Carbon dioxide and sulfuryl fluoride are also used for fumigation of stored grain as alternatives to phosphine (**Ikbal Chaieb et al., 2018**).

I-6-2 -Biological Control:

Biological agents such as natural predators, parasitoids, and microbial agents for pest control. For instance, parasitoid *Trichogramma* spp (**Wang Y et al.,2024**). and predatory beetles (Coccinellidae) (**Atrchian H et al., 2024**) are used effectively in these strategies. Microbial controls using *Bacillus thuringiensis* (Bit) and *Beauveria bassiana* (**Atrchian H et al., 2024**), botanical insecticides like neem extracts (Azadirachtin) and pyrethrum (**Peter M.J et al., 2024**), and pheromones or semi chemicals (**Audley J.P et al., 2024**).

I-6-3 -Physical Control:

Recent studies have suggested some kind of NPs in various forms, including a nano formulation (an NPs-based formulation for enhanced delivery) **(Iqbal Het al., 2024)**, a nano emulsion (a dispersion of NPs in liquids), or a nanosuspension (a colloidal suspension of NPs) (**Adak T et al.,2020**), as emerging and promising alternatives for managing stored-product insect pests (**Jasrotia P et al., 2022**). The use of NPs has the potential to overcome the constraints associated with the conventional pesticides by boosting insecticide action, improving the stability of active components, lowering the dose of the required insecticide and conserving agronomic inputs **(Riyaz M et al., 2022**, **Li N et al., 2024, Mittal D et al., 2020).**

I-6-4- Molecular control:

The RNAi technique is a new eco-friendly method of insect pest management. In this technique, synthetic RNA are used to target and inhibit specific genes within the insect species. This approach has been applied and found effective against T. castaneum (**Tang et al., 2016; Ga o e tal.,2017**).

I-7- PEOS:

The main approach to managing these stored-product pests is through the use of chemical control tools. However, this approach has proven to be problematic due to issues such as environmental pollution, insect resistance, and recurring populations. As a result, there is a pressing need to develop low-cost, effective, specific, and environmentally safe alternatives for the ecosystem, especially considering the problem of resistance to synthetic insecticides. Consequently, research efforts have shifted towards the development of products derived from plants, particularly those sourced from EOs, which have now become a primary focus in this field of study **(Lirui Zhang et al., 2023).** They are composed essentially of terpenoids, represented by monoterpenes (C10) and sesquiterpenes (C15) and a minority of aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketones that can attribute to the aromatic profile of the plant. The chemicals in essential oil play a crucial function in plant defense against fungal and insecticidal attacks. Botanicals are considered safe to humans due to their relatively high median lethal dose (LD50) values to mammals so they have an important role in natural control strategies (**Ikbal Chaieb et al., 2018**).

II-1-Definition and properties:

EOs are mixtures of volatile lipophilic constituents generally procedure by specialized metabolite of aromatic and medicinal plants from a wide range of botanical families (lamiaceae, Myrtaceae, Rutaceae, Apiaceae, and other) as responsible for their distinctive odor, flavor or scent, though also present in non-vascular plants such as some liverworts (**Franz C, Novak J.2010).**

EOs are stored in plant secretory epithelial cells, forming structures of various kinds such as glandular trichomes or excretory idioblasts. **(Lange B.M.2015),** An essential oil is a concentrated hydrophobic liquid containing volatile chemical compounds such as terpenes (monoterpenes and sesquerpenes), **(Alzogary, R. A et al 2018).** plant material, such as leaves, flowers, peels, barks, stems, buds and seeds, can be used to extract volatile odoriferous essential oils **(Raveau, R et al 2020)**

II-2- Bioactive Compounds:

The chemical constituents of EOs can be classified into four groups: terpenes, terpenoids, phenylpropanoids, and other constituents **(Hyldgaard et al 2012, Pandy et al 2017)**

II-2-1- Terpenes:

Terpenes or isoprenoide are the major constituents found in EOs with molecular structures containing carbon backbone **(Hyldgaard et al., 2012). The** number of isoprene units are primarily responsible for structural of terpenes. Hemiterpenes are formed by one isoprene unit (C5), monoterpene (C10), sesquiterpenes (C15), disterpene(C20), triterpnes (C30), and tetraterpenes (C40) **(Bhavaniramya, et al 2019)**

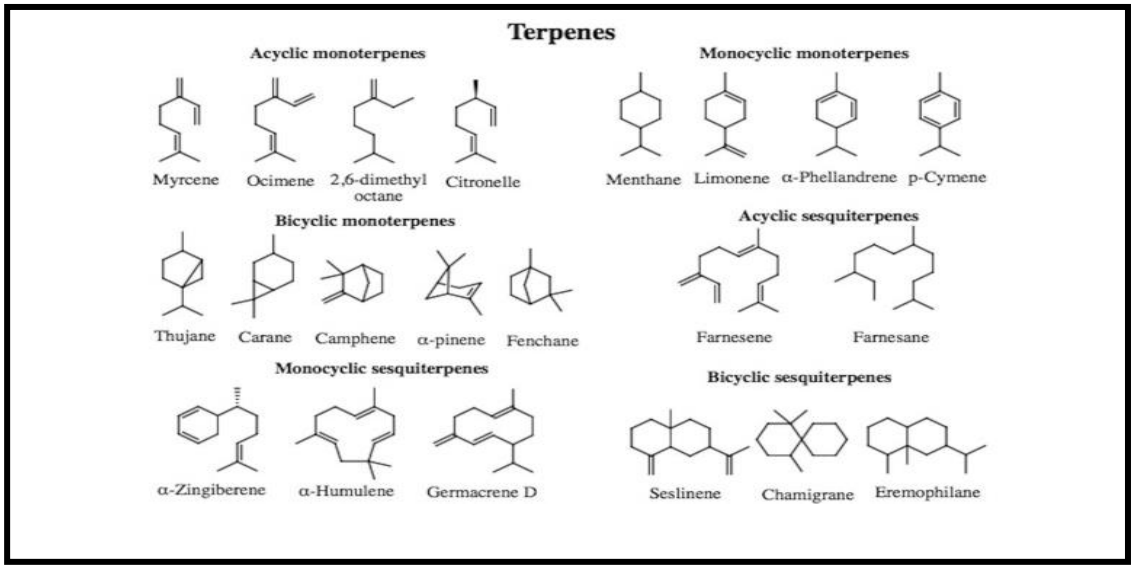


Figure 4: Represented the bioactive compounds of EO Terpenes (Ayu Masyita et al ., 2022)

II-2-2-Terpenoids:

Tepenoids are another type of terpenes countaining oxygene molecules that are constructed via biochemical modification (removal or addition of group) **(Pandey et al 2017).** Terpenoids can be divides into alcohol, aldehydes, ester, ether, epoxides, ketones and phenol. Example of terpenoids is: carvacrol, citronella, geraniol, linalool, linalyl acetate, piperidone, menthol and thymol **(Hyldgaard et al.,2012).**

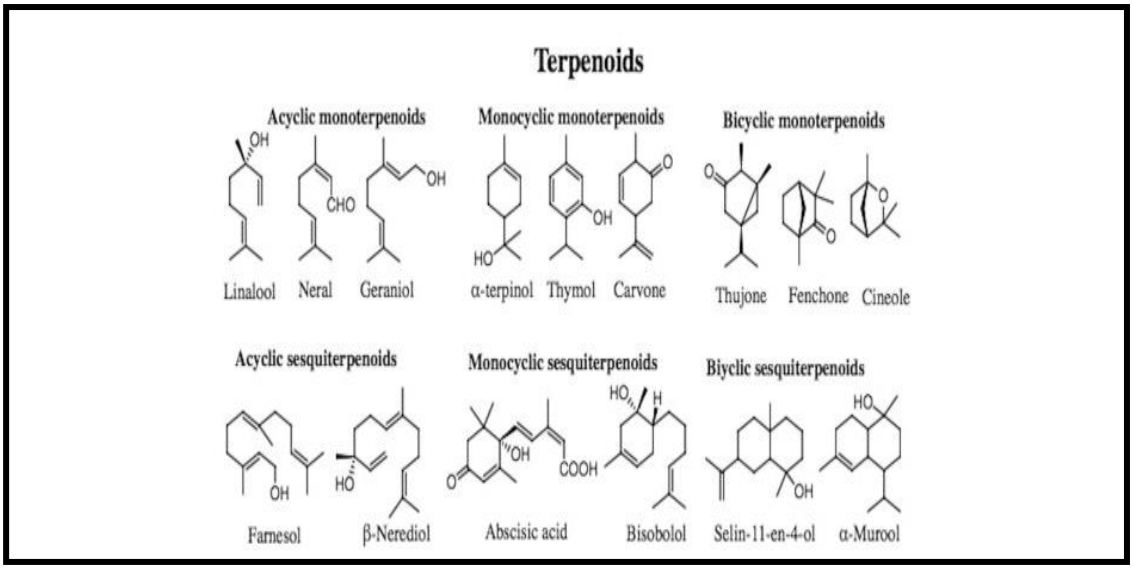


Figure 5: Presented Terpenoids (Ayu Masyita et al ., 2022)

II-2-3-Phenylpropanoids:

Phenylpropanoids are synthesized by the shikimic -acid pathway and their basic structure from the six-carbon aromatic phenol group linked usually to the three -carbon propene tail of cinnamic -acid, oxygenated in the third /fourth /fifth position frequently possess a carbon-carbon double bond **(Stevanovic et al ,2020). Example** of phenylpropanoids such as, cinnamaldehyde, eugenol, isoeugenol, myristicin, safrol, and vanillin **(Contant et al ,2021)**

II-2-4-Other constituants:

EOs contain several derivatives of amino acids such as alanine, isoleucine, valine and methionine. Polyketides, lipides and sulfate -derivative are rarely found in EOs such as jasmonic -acid, methy-jasmonate, cis-jasmone, (Z)-3hexenal, allicin **(Pandey et al.,2017, Stevanovic et al.,2020).**

II-3- EXTRACTION METHODE:

Aromatic herbe or parts thereof, such as leaves, flowers, bark, seeds, and fruits, are subjected to extraction processes after being collected at specific stages of maturity and stored under controlled condition (light, temperature and humidity (Stratakos et al ,2016)

II-3-1-Hydrodistillation:

This methode is characterized by direct contact between the solvent and plant material, that is, the raw material is submerged in boiling **(Silva, M. G.F 2011).**

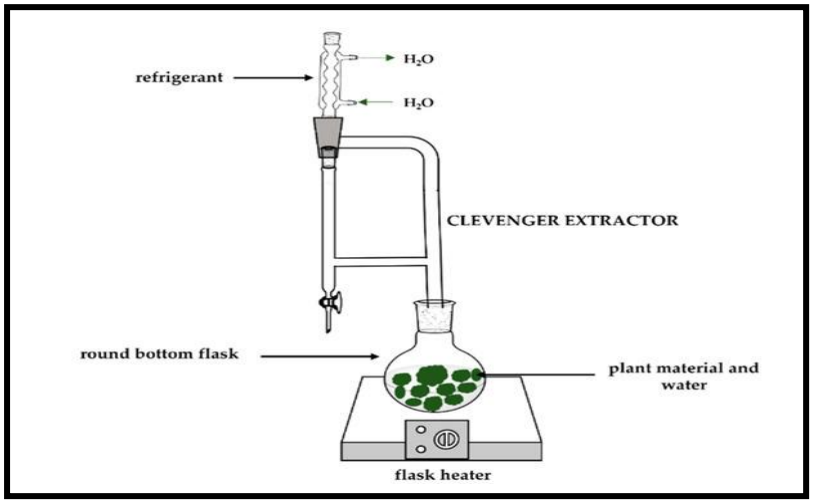


Figure 6: Schematic representation of hydro distillation (Sousa, V.I et al ., 2022)

In this procedure, the cell walls are broken, and the oil evaporates together with the water and then condensed into a mixture of water vapour volatile compounds of vegetable raw. However, these two phases (volatile compounds and water) are immiscible rendering possible an additional separation to the difference in density **(Sartor et al 2009)**

This technique is inexpensive, but at the same it is not selective because of the waste of large amounts in the solvent (part of the extract can be lost in the aqueous phase) and can provide low yields **(Masango et al ,2005; Luque et al 1998)**

Despite being the oldest method, hydro distillation is still used to day for extracted oils from different matrices.

II-3-2-Stream distillation:

The extraction procedure is based on the same principale as hydro distillation. The difference essentially lies in the absence of contact between the substrate to be extracted and water, which causes a reduction in the extraction time

The sample is placed in a column where the bottom part is connected to a flask with water under heating **(Next figure)**. The top part is connected to a condenser, where the steam produced passes through the sample, talking essential oils to the condenser. The process causes the condensation of the water -oil mixture, and this mixture can be separated by liquid -liquid extraction **(Fitriady, et al 2017).**

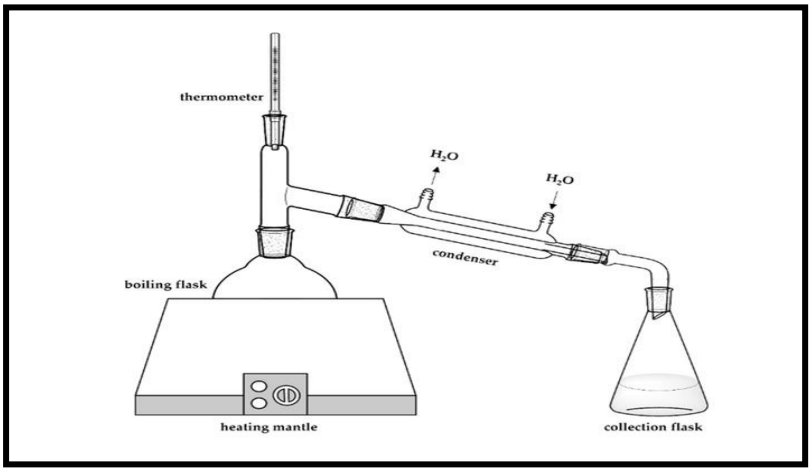


Figure 7: Experimental setup used in stream distillation (Sousa, V.I et al ., 2022)

II-3-3-Organic Solvent Extraction:

In organic solvent extraction ,the sample is placed in contact with the organic solvent (which can be hexane ,benzene ,toluene ,or petroleum ether ,among other) for a period that allows the transfer of the soluble content of the sample .The extracted matrix is concentrated by evaporating the solvent present in the liquid phase .This method allows the sample to be permanently in contact with a quality of fresh solvent and at the end of the process ,it is not necessary to carry out filtration ,as long as there are high yields **(Luque de Castro 1998).**

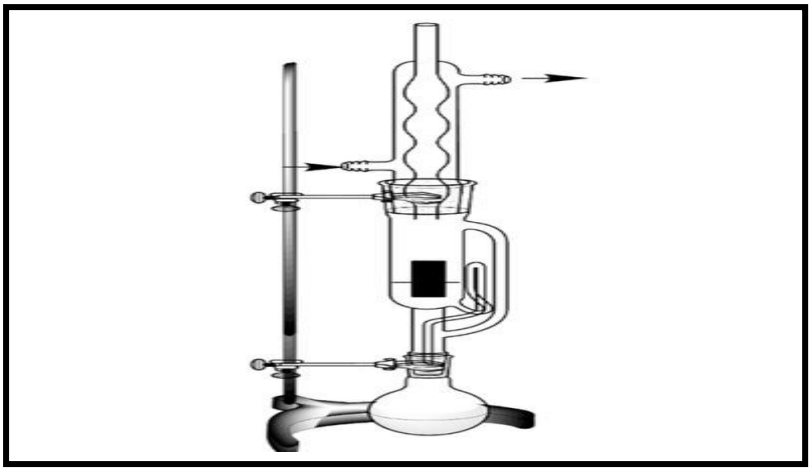


Figure8: Schematic representation of organic solvent extraction using the Soxhlet Methode (Sousa, V.I et al ., 2022)

II-3-4-Cold Pressing:

Essential oil IS mechanically removed by cold pressing, where the oil glands are broken and volatile oils are released. In this process an aqueous emulsion is formed, where the oil present can be obtained through centrifugation, decantation or fractional distillation **(Dima, C; Dima S .2015)**

II-3-5- Supercritical Fluid Extraction (SCFE):

Supercritical fluid extraction is an efficient environmentally friendly and clean technique for isolating EOs. In this technique SCFE are used as extraction agents due to the supercritical state of fluids, conferring characteristics for the extraction process, such as low viscosity high density (close to that of a liquid) and high diffusivity (high penetration power)

Several substances can be used as supercritical solvent, such as water, carbon dioxide (CO2),methane ,ethylene and ethene .However C02 is the most-used solvent due to its critical point being easily reached (low temperature and pressure 31.2° and 72.9° atm respectively)low toxicity and reactivity low cost and non -flammability .After selecting the ideal temperature and pressure for extraction fluid passes through the sample and the oils are dissolved and extracted .Subsequently ,the extraction solution is maintained at a pressure below the critical point and as the pressure decreases ,the supercritical fluid passe to the gaseous state and loses its solvating capacity being recycled **(Youcefi ,2019)**

II-3-6-Microwave -Assisted Extraction (MAE)

Microwave -Assisted Extraction is an eco-friendly and energy-efficient alternative to conventional extraction methods. In MAE, the sample is exposed to microwave radiation without added solvents. The microwaves generate heat by evaporating the moisture within the sample which raises internal temperature and pressure. This pressure causes cellular structures such as glands to rupture releasing essential oils **(Martinez -Abad et al.;2020).**

II-3-7-Ultrasound -Assisted Extraction (UAE):

Ultrasound energy allows the intensification of EO extraction **(Sandhu et al 2021) .**It is often combined with other extraction technique to accelerate the process and improve mass transfer efficiency .In this method ,the sample is immersed in a solvent and exposed to ultrasound waves .These waves generate rapide solvent movement ,which induce mechanical vibrations

in the cell walls and membranes of the sample ,leading to the release of essential oils .In certain secretors such as the medical and food industries ,This technique is already being applied on a large scale .It helps improve the quality of the extracted compounds ,reduces processing time and increases overall yield **(Zheng,2021)**

II-4-Insecticidal Activity of Essential oils:

II-4-1-Essential oil AchE :

EOs inhibit the activity of acetylcholinesterase (AChE)(e.g.,**(Ingkaninan ,k et al ,2003),**which is one of the most important enzymes in neuro-neuronal and neuromuscular junction in both insects and mammals **(Gnagey ,A.L,1987 ,Bourguet D et al 1987 ,Marcel .Vet al 1998).**Since the insect AChE differs from the mammalian one by a single residue ,known as the insect-specific cysteine residue, can be an insect -selective target for the newly developed insecticides ,safe for non -target vertebrates **(Kim,J.I.; et al 2006 ,Pang ,Y Pet al 2012)**

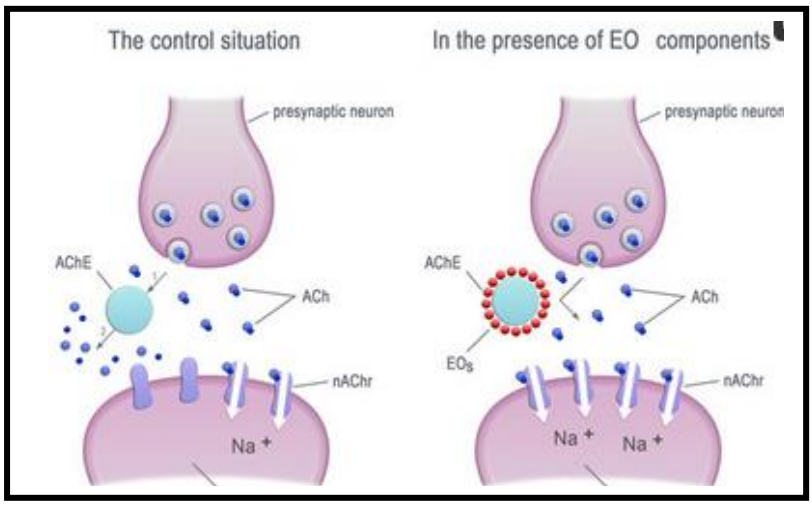


Figure 9: The EO components inhibit the acetylcholinesterase (AChE) activity. ACh-acetylcholinesterase, nAChr -nicotinic acetylcholine receptors, EOs -essential oil components (Jankowska, M et al ., 2018)

II-4-2-Essential Oil -Modifier of GABAA Receptor's:

Essential oils (EOs) have shown insecticidal properties in many studies but their effects on insect GABA receptors (GABA rs) are not well understood. Research on RDL receptors indicates that thymol strongly enhances GABA-induced chloride currents and can also induce a small current on its own. Thymol, carvacrol, and pulegone have been found to enhance GABA activity by increasing (3H)-TBOB **(Tong, F.;2010)** binding and GABA-induced chloride uptake, suggesting they act as positive allosteric modulators of insect GABArs. Supporting this, thymol reduced flight muscle activity in *Phaenica sericata*, *mimicking* GABA 's effects. However, more research is needed to fully understand how EOs interact with insect GABArs. **(Priestley, C.M 2018) .**

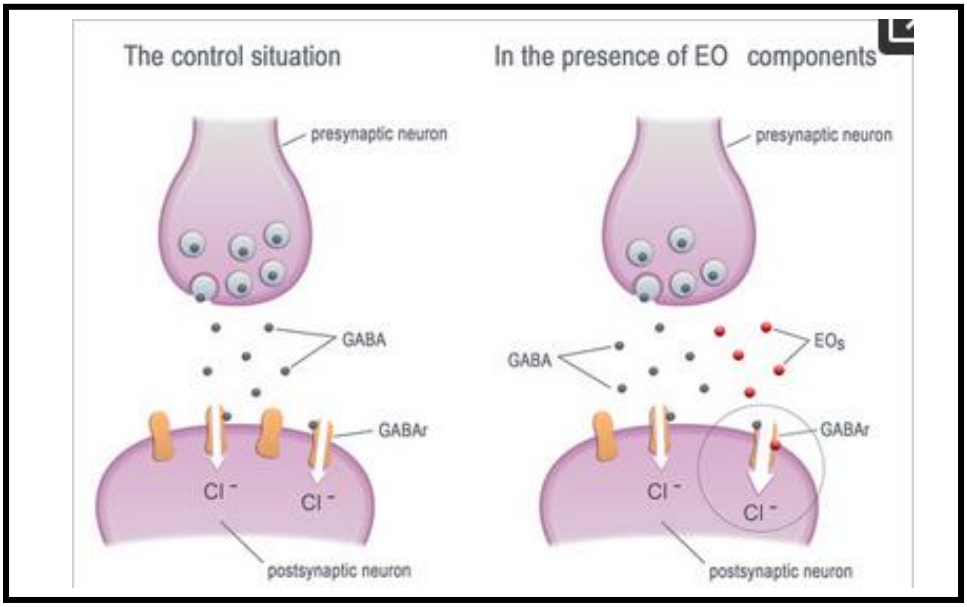


Figure 10: The EO components increase the choride by allosteric modulation of the GABA receptors GABA-Ɣ-aminobutyric acid, GABAr—GABA receptors, EOs—essential oil components (Jankowska, M et al ., 2018).

II-4-3- Essential Oils-Ligands of Octopamine Receptors:

Octopamine (OA) is an invertebrate multifunctional molecule structurally and physiologically retard to vertebrates' noradrenaline. It has been found that it can act as a neurotransmitter as a neurohormone and as a neuromodulator **(Nathanson, J.A. Orchard et al 2018).** OA is

presente in the involved in the regulation of different forms of insect activity e.g. arousal level. It also plays an essential role in the insect stress response, aggressive and social behavior **(Davenport, et al 2018)**

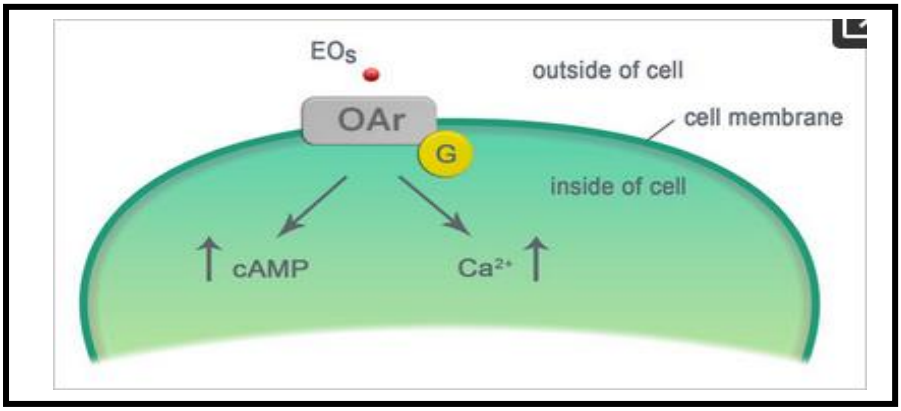


Figure 11: The EO components activate the octopamine receptors. EOs-essential oil components, Oar- octopamine receptor, G-protein G, cAMP -cyclic adenosine monophosphate, Ca^{2+} --calcium ions, -increase in the molecule leve (Jankowska, M et al ., 2018).

III - Artemisia species:

The genus *Artemisia* is one of the largest and most widely distributed genera of the family Asteraceae (Compositae). It is a heterogenous genus, consisting over 500 diverse species distributed mainly in the temperate zones of Europe, Asia and North America. These species are perennial, biennial and annual herbs or small shrubs **(Watson et al., 2002**; **Mehrdad et al., 2007).**

General morphological features of the genus *Artemisia* is described as leaves alternate, capitula small, usually racemouse, paniculate or capitate, inflorescence, rarely solitary; involucral bracts in few rows, receptacle flat to hemispherical, without scales and sometimes hirsute; florets all tubular, achenes obovoid, pappus absent or sometimes a small scarious ring **(Heywood & Humphries, 1997; Mucciarelli & Maffel, 2002; Polyakov & Shishkin, 1995).**

III -1- Diversity of Artemisia:

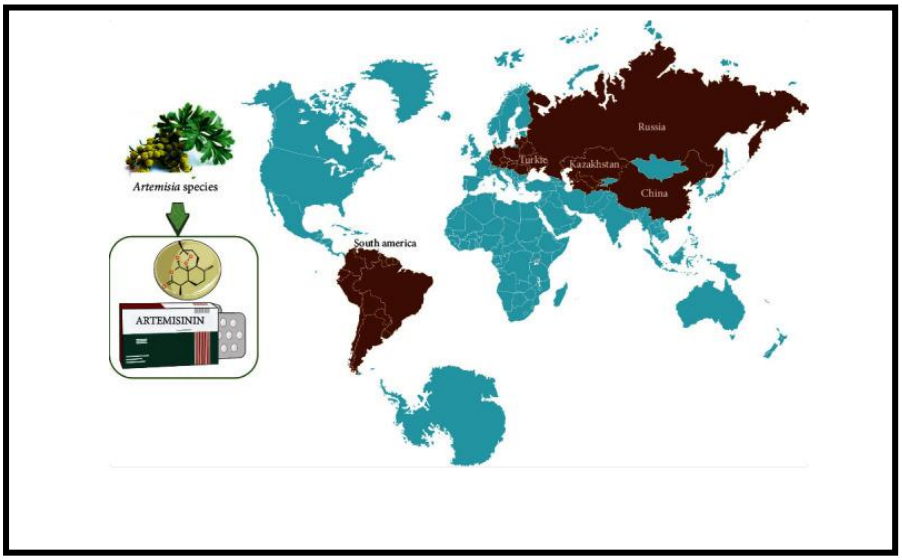


Figure 12: Geographical distribution of Artemisia species (Javad Sharifi-Rad's et al ., 2022) .

III -2- Artemisia Herba -alba:

III-2- Botanical aspects:

III -2-1- Morphology:

Artemisia herba alba is a green -silver perennial herba growth 20-40 cm in height, it is a chamerophyte (i, e the buds giving rise to new grouth each year are borne close to the ground, them stems are rigid and erect. The grey leaves of sterile shoots are petiolate, ovate to orbicular in outline whereas leaves of flowering stems are much smaller. The flowering heads are sessile oblong and tapering at base. The plants flower from September to December. Plants are oblong and tapering at base Plante are found on the steppes of the Middle east and north Africa where they common and sometimes stand forming **(N. Feinbrun Dothan 2010)**

III -2-2- Distribution:

The genus A. herba -alba is a medicinal and aromatic dwarf shrub that grows wild in arid areas of the Mediterranean basin extending into northwestem Himalayas this plant is abundant in the Iberian Peninsula and reaches highest population in the center of Spain spreading over the eastern southeastern and Southam Spain. Plants are found on the steppes of the Middle East and North Africa where they are common and sometimes stand-forming (**Figure)** (N. Feinbrun-Dothan, 1978) This taxon grows wild on nitrophilous and gypsum -rich substrata **(Salido, L.R et al 2010)** .

Figure 13: Photographe of Artemisia herba -alba (N. Feinbrun -Dothan, 2010)

III -2-3- Taxonomy: Arthemisia herba -alba is classified kingdom:

Plantae: Subkingdom, Tracheoionta, Superdivision, Spermatophyta.

Division: Magnoliophyta.

Class: Asterales.

Order: Asteroidease.

Family: Asteraceae.

Subfamily: Artemisinin.

Tribe: Anthemideae.

Subtribe: Artemisia.

Genus: Artemisia.

Subgenus: Seriphidium and species: Artemisia herbe -alba **(L, Boulos, J. Valles, M et al 2010**

III -2-4- Chemical composition:

Table 1: Essential oil composition of the Artemisia genu from different geographical region

Plant species	**Chemical composition**	**Region /country**	**References**
Artemisia herba alba	camphor, cmazulene, bornyl acetate and myrcene	Tunisia	**Ikbal Chaieb[1,2], Amel Ben et al ,2017**
Artemisia herba alba	Camphor, hamphillin, terpinene -4-ol; α-santonin; α-thujone; β-thujone ;2,5 bornane Dione	Morocco	**Amkiss S.; et al 2021**
Artemisia Herba alba	Camphor,1,8cineol, camphene and borneol	Tunisia	**Olfa Bachrouch et al. 2014)**

III -3- Artemisia absinthium

III -3-1-Botanical Description:

Arthemisia absinthium L, commonly known as wormwood is an important perennial shrubby medicinal plant native to Asia, Middle East, Europe and North Africa **(Sharopov F.S et al 2012)**, Arthemisia is one of the most predominant and widely distribution genus in Asteraceae

family that is composed of more than 500 different species classified as annual perennial and biennial natural plants or small shrubs **(El Gaber S.B et al 2019)**

Absinthium root is perennial with a firm prolonged woody and leaf stem and has a warm and aromatic taste. The stem is about 2 -2,5 feet tall white in color and almost covered with fine silky hairs. The leaves are white on both sides 3, inches long and 1,5 wide with slender and unshaped segments and the leaf-stalks are slightly winged at the margin and the leaves are reduced to three or even one linear subdivision on the flower-stalks. Flowering takes place from early summer to early autumn. **(Nin S., Arfaioli P., et al 2020)**

The flower heads are short nearly and hang in an erect leafy panicle and the little flowers with a bitter with a distinctive aroma resembling that of thujone.

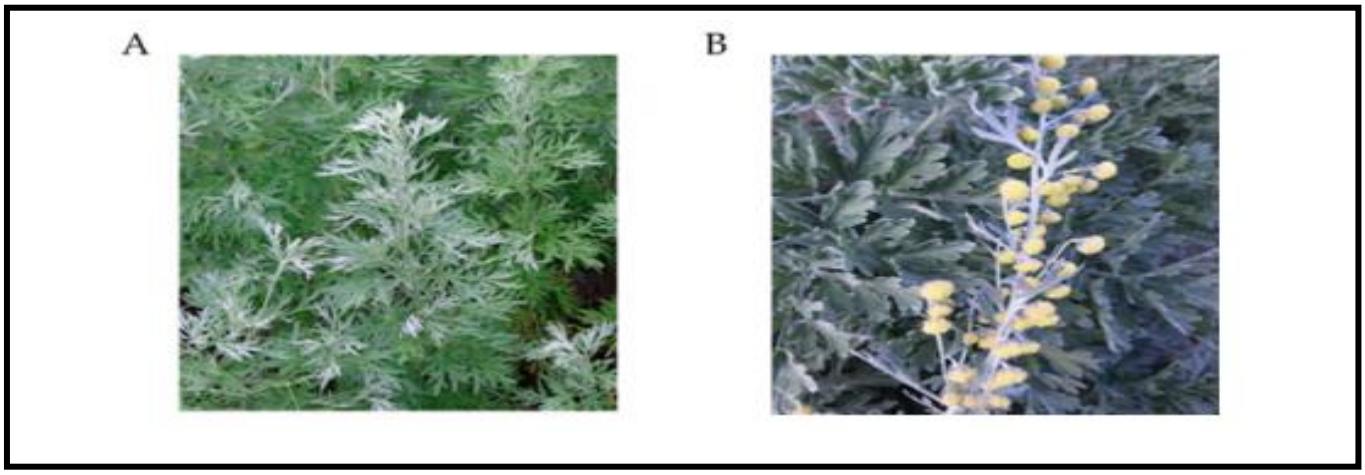


Figure 14: Aerial parts (A) and flower (B) of Artemisia absinthium (Batiha, G.E.-S et al ., 2020).

III -3-2-Taxonomy (EL Gaber, S.B 2020)

Scientific classification of Athemisia absinthium:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Asterales

Family: Asteraceae

Genus: Artemisia L, sagebrush

Species: Absinthium

III -3-3- Chemical composition:

Table 2: Essential oil composition of the Arthemisia genus from different geographical region (2017-2021)

Plante species	**Chemical composition**	**Region /country**	**References**
Arthemisia absinthium L	Camphor-caryophyllene; eucalyptol; germacrene D; α-cardinal	Brazil	**(Viera T.M et al 2017)**
Arthemisia absinthium L	Artemisinin; α-thujone; bornylacetate camphene; chamazulene; cadinene; myrcene; guaiazulene; linalool; γ terpinene	Egypt	**(BatihaG.E .S et al 2020)**
Arthemisia absinthium	Thuyone principe compose, camphre ,1,8 _cineol, le camphène et le bornéol	Tunisies	**Olfa Bachrouch et al . 2014)**

IV - Application Methods:

IV -1-Principles of Microencapsulation:

Microencapsulation is the protection of small solid, liquid or gaseous particles through a coating system wall can be a natural, synthetic, or semi -synthetic polymeric coating in this technology, microparticles are formed, which can be classified in relation to their size and morphology, according to the encapsulating agent and microencapsulation method used.

Microparticles can be distinguished according to their form: they are classified as a reservoir -type system microcapsules, when the core is concern -traded in the central region, coated by a cautious wall material: or a monolithic system, microspheres when the active agent is dispersed in a matrix system. In general, on the surface of the microparticle.

The physicochemical characteristics of the microcapsule are defined by the encapsulating agent and the active agent. The wall material must form a cohesive film that bonds with the encapsulated materiel. Several material can be used for the coating, with proteins ,carbohydrates, and lipids being frequently used .Furthermore ,the materials must e chemically compatible and the encapsulating agent chemically inert, so as not to react with the core .Microencapsulation technologies achieve several objectives and they are particularly used to protect the core active agent's sensitivity to oxygen ,light , and moisture or to prevent interaction with other compounds .However ,the most important reason for encapsulating an active agent is to a controlled release .

The process of defining a microencapsulation system is mainly dependent on the purpose of the microcapsules. Characteristics such as shape, size, permeability, biodegrade -ability, or biocompatible, or biocompatibility are defined depending on the application of this material.

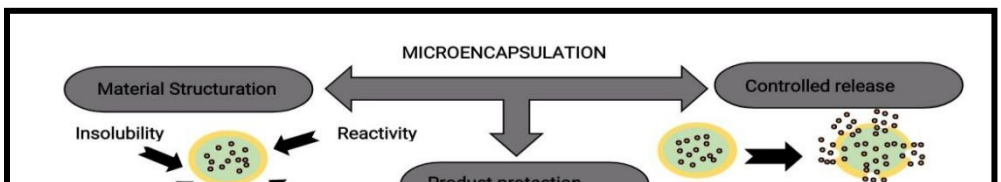


Figure15: Objectives of microencapsulation (Petrusic, S; koncar, V ., 2016)

IV -2- Advantage of microencapsulation:

One of the great advantages of microencapsulation is the mechanism of the controlled, sustained, or targeted release of the active agent. This release can occur at a certain defined time or not, though a mechanism of diffusion though or rupture of the wall. The release can be activated though temperature variation, solubility, Ph changes, or even the biodegradability of the wall material **(Sousa, V.I et al ., 2022) . .**

IV -3- Techniques of Microencapsulation:

IV -3- 1-Emulsification:

Emulsification is a key step in the oils, used in the food and pharmaceutical industries. It encapsulated bioactive substances in aqueous solutions, which can be used in liquid or dried form (by spray drying or freeze- frying)

An emulsion is composed of at least two immiscible liquid, one dispersed as spherical droplet within the other. Four types of emulsions exist: oil-in water (O/W), water -in -oil(W/O), oil-in-water-in-oil (O/W/O), and water -in -oil-in-water (W/O/W). The droplets measure between 0,1and 100µm.

The O/W emulsion is the most common, easy to prepare and inexpensive but suffers from limited physical stability. Adding modified emulsifiers such as Maillard reaction products can improve encapsulation and protect oils from oxidation.

Finally, another method is not evaporating the obtain small droplets, which is useful for encapsulating liquid or solid material. However, this method is expensive inefficient and solvent residue.

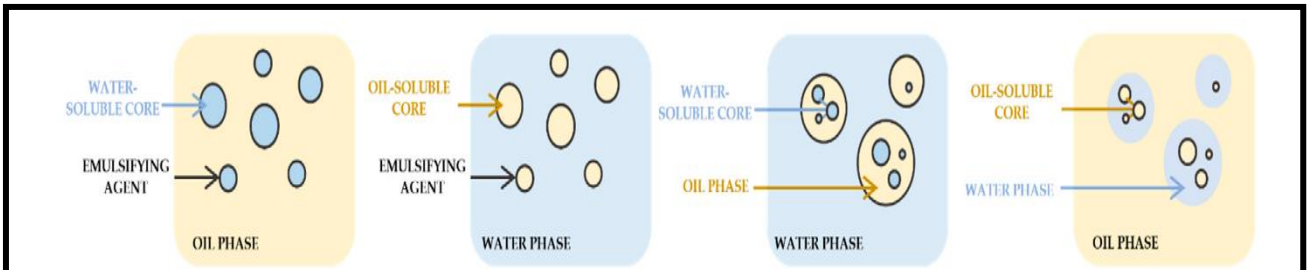


Figure16: Illustration of emulsion systems (Sousa, V.I et al ., 2022) .

IV -3- 2-Coacervation:

Coacervation is a commonly used technique for microencapsulation. It relies on the interaction between oppositely charged polyelectrolyte polymers that form a wall around the active agent. There are two types: **simple coacervation** (a single polymer) and **complex coacervation** (two or more polymers)

IV -3- 3-In Situ Polymerization:

In situ polymerization is a commonly used method for manufacturing microcapsules and functional fibers. It involves forming a wall around a core via a chemical reaction occurring in the continuous phase, often using an oil-in-water emulsion containing a melamine - formaldehyde resin. Adjusting the PH and stirring promote of shells around the oil droplets.

This technique is used in various field (perfume, food packaging, repellents, footwear) and produces microcapsules with a smooth thermally resistant surface and capable of controlled release of their contents. The polymers used provide strength and stability and the use of copolymers reduces the toxicity of formaldehyde.

However, the methods have limitations: it is control requires a large amount of solvent and uses monomers that are often non -biocompatible. Complex coacervation involves phase separation caused by neutralization of two opposing colloids often a polysaccharide and (engeletin/ gum Arabic gelatin/ alginate; etc.) The process involves emulsification of the active agent in an aqueous solution containing the polymers, followed by phase separation due to electrostatic interaction, A wall forms around the hydrophobic particles upon cooling, the solidifies using a crosslinking agent.

Simple coacervation is more economical (using inexpensive inorganic salts) and less sensitive to PH. However, complex coacervation remains an attractive industrial method: simple, scalable, inexpensive, reproducible and solvent-free.

IV -3- 4-Spray Drying:

Spray drying is the most-used technology in the microencapsulation of essential oils. It is mainly used on an industrial scale, as it allows simple, reproducible, continuous, and low-cost production. Being used more frequently in the food industry, this process is also utilized in the cosmetics, pesticides, and pharmaceutical industries **(Estevinho B.N., et al 2013),** This technique allows encapsulated and powdered Eos to be obtained due to the ability to dry them in just one operation, in this process, the atomisation of emulsions occurs in a drying chamber with relatively high temperature. Where the evaporation of the solvent takes place and, consequently, microcapsules are formed **(Gharsallaoui, A; et 2007).**

The spray-drying technique involves four steps where the preparation of dispersion fist occurs, I, e, the wall materials are dissolved in water with agitation and controlled temperature, still in the same step, the addition of the EOs follows and, if necessary, the emulsifier can be added, Afterwards, the dispersion is homogenized to be injected into the equipe though an atomising nozzle ,where small droplets enter the drying chamber with a flow of hot air present .Finally the dehydration of the atomized microparticle is done though the evaporation of the solvent ,with dries the microparticles wiche can them be recovered in the form of powder in a collector or filter **(Mohammed ,N.K.; et al 2020).** The main limitation of this technique is related to the wall material, which must have good water solubility and to the number of encapsulating agents available. In addition, some materials may be sensive to be sensitive to the high temperatures introduced in the atomisation process. In addition, the production of microcapsules in fine powder form can cause agglomeration and an additional process may be required **(Bakry, A.M; et al 2016)**

IV -3- 5-Freezy Drying:

Freeze drying also known as lyophilization is a simple process that is used to dehydrate most materials sensitive to heat and aromas such as oils, Sublimation is the major principale involved in the drying process where water passes directly from a solid state to a vapour state without the liquid state. Before starting this process, the oil is dissolved in water and frozen **(Hazarika, U.; Gosztola, B; 2020)** Afterwards, the pressure is reduced and heat is added to allow the frozen water to sublimate the material directly from the solid phase to the gas phase.

Freeze -dried materials appear to have the maximum retention of volatile compounds some oils, with high yields **(Gardeli, C et al 2010)** This method helps to better preserve the EO content in many types of herbs and spices compared with other preservation techniques **(Vujanovic, M.D.; et al 2021)**. Lyophilization is simple and easy to operate **s**howing that lyophilization samples are more resistant to oxidation and less efficient in microencapsulation **(Arujo, J.S.F., et al 2020).**The process disadvantages include high energy use long processing time and production costs **(Sebaaly, C et al 2016)**

IV -3- 6- Supercritical Fluid (SCF)Technology:

Many pharmaceutical cosmetic and food industries use supercritical fluid technology to form the microcapsules of essential oils due to their inherent advantage. The use of a wide variety of materials that produce controlled particle sizes and morphologies, the easy solvent removal, the non-degradation of the product and being a non -degradation of the product and being a non-toxic method are some of the many advantages of the SCF technology.

The methods used for supercritical fluids are the precipitation of gas anti-solvent particles of saturated gas solution, the extraction of fluid emulsion and the rapid expan -sion of supercritical solution **(Cocero, M.J.et al 2010).** The supercritical solvent impregnation process has proven to be successful in a wide variety of substances (essential oils, fragrances active pharmaceutical compounds and dyes and matrices (wood polymers cotton and contact lenses).

An alternative to spray drying (that degrades oils at high temperature) is impreg nation with supercritical solvent, as it is an ecological process where supercritical carbon dioxide is usedas

IV -3- 7-Coaxial electrospray System:

The food cosmetic and pharmaceutical industrie use a new technology to encapsulate oils called coaxial electro spraying **(Zhang, S et al 2014).** This system is used in two phases with external and internal solution being sprayed coaxially and simultaneously though two feed channels separated by a nozzle. In the electrospray process, the Taylor cone is composed of a core-shell structure that is formed at the spray nozzle ending up with the polymeric solution encapsulating the internal liquid. This method is distinguished by its ease and efficiency, and the maximum speed of the core material. The coaxial electrospray system provides a uniform size distribution, a high encapsulation efficiency, and an effective protection of bioactivity. However, the encapsulation efficiency and the stability of the microcapsules are affected by

the wall materials **(Koo, S.Y et al 2014).** Furthermore, controlling the process in coaxial electro spraying is difficult to some extent **(Zhang, L et al 2012).**In experimental terms, the reported work on coaxial electrospray is based on individual laboratory experiments, consisting of specific combinations of materials and empirical process parameters. The fabrication of polymeric microparticles and nanoparticles is hampered by the

lack of standard protocols. Regarding the collection of particles, the methodology cannot facilitate the hardening of the shell or maintain the morphology of particle, or even prevent its aggregation. On the theoretical side, many existing process models are empirical or semi-quantitatively empirical. The simulated results are not enough for the quantitative control of the process, as numerical simulations, such as computational fluid dynamics modelling, have been used to simulate the formation of the liquid cone and atomisation in a single axial electrospray process **(Lastow, O et al 2006).** In summary, more experimental and theoretical study is needed to better understand the physical nature of coaxial electrospray and to provide quantitative guidance for process control. A green solvent.

IV -3- 8. Fluidized Bed Coating:

Fluidized bed coating is one of the most efficient coating methods, in which the ingredients can be mixed, granulated, and dried in the same container. Consequently, the handling and processing time of the material is reduced. This approach was recently used to encapsulate fish oil by spraying and coating it **(Anwar, S.H et al, 2011).** Fluidized bed coating is carried out by suspending the solid particles of the core material by an air stream under controlled temperature and humidity and then sprayed, building, over time, a thin layer on the surface of the suspended particles. This material must have an acceptable viscosity for atomization, and the pumping should be able to form an appropriate film and be thermally stable (**Teunou, E.; Poncelet, D., 2005)**

There are several methods used in fluidized bed coating, including top spray, bottom spray, and tangential spray methods. In the top spray system, the coating solution is sprayed in the opposite direction with air in the fluid bed. The opposite flows lead to an increase in the efficiency of encapsulation and the prevention of agglomerates formation, achieving microcapsules with a size between 2 and 100 μm. The bottom spray, known as the Wurster system, uses a coating chamber that has a cylindrical steel nozzle (used to spray the coating material) and a cribriform bottom plate, coating small particles (100 μm). This multilayer coating procedure helps to reduce particle defects, although it is a time-consuming process. On the other hand, tangential spray consists of a coating chamber with a rotating bottom of

the same diameter as the chamber. During the process, the drum is raised to create a space between the edge of the chamber and the drum. A tangential. nozzle is placed above the rotating drum, where the coating material is released. Then, the particles move through the space into the spray zone and are finally

Encapsulated **(Desai, K.G.H.; Jin Park, H. 2005).** During this process, there are three mechanical forces, namely, centrifugal force, lifting force, and gravity.

The particles to be coated must be spherical and dense, and must have a narrow size distribution and perfect fluidity, with the non-spherical particles having the largest possible surface area and requiring more coating material.

This technique has a low operating cost and a high thermal efficiency process, al-lowing total temperature control. However, it can be time consuming, which becomes a disadvantage **(Lam, P.L.; Gambari, R. 2014)**

IV –4- Microencapsulation of Essential Oils:

Microencapsulation is an alternative that can be utilized to overcome several limitations in the application of essential oils. This application is profoundly affected by the high volatility and chemically unstable nature of EOs **(Aguiar, M.C.S et al. 2020,).** In addition, EOs are compounds that can be easily degraded due to interactions with other chemical components and exposure to several factors such as light, temperature, and oxygen **(Bakry, A.M et al, 2016)**

Essential oils can be "trapped" in microcapsules, which act as micro-reservoirs, ensur ing excellent protection **(Martins, I.M et al. 2014)** The encapsulation process, where small particles are enclosed in solid carriers to increase their protection, has the ability to reduce evaporation, promote easier handling, and control the release of essential oils during storage and application [199]. Furthermore, through microencapsulation, it is possible to change the appearance of EOs (which behave like a powder), without changing their structure and properties **(Mohammed, N.K et al. 2020)**

In EO microencapsulation, the first step is often to emulsify or disperse the essential oils in an aqueous solution of a wall material, which also acts as an emulsifier. This process happens because the EOs exist in liquid form at room temperature. Then, the resulting mi-crocapsules must be dried under controlled conditions, so that the loss of the encapsulated material by volatilization is reduced **(Mohammed, N.K et al. 2020)** One of the areas that has also aroused interest in the microencapsulation of EOs is in the agrochemical industry. Yang et al. prepared and characterized microcapsules based on polyurea, containing essential oils as an

active agent for possible applications in the controlled release of agrochemical compounds **(Scarfato, P et al, 2007).** The microcapsules were synthesized by O/W emulsion interfacial polymerization and the syn-thetic conditions that showed the best results were used to encapsulate four essential oils (lemongrass, lavender, sage, and thyme), capable of interfering with the seed germination and root elongation of some plants. In cases of pest control, biological pesticides must be more effective than synthetic pesticides.

Practical part

Materials and Methods

2.1. Material Plan :

2.1.1 Artemisia absinthium:

The required quantity of Artemisia absinthium used in this study was harvested on November 10, 2024, from the Quatre Fermes nursery, located in the commune of Guerouaou, within the forest district of the wilaya of Blida. After harvesting, the plants were carefully cleaned to remove debris and then transported to the Bio.Extrapamal laboratory, located in Oued Alleug (Blida), for the extraction of their essential oil (**Figue 17**).

Figue17: Harvesting of Artemisia absinthium in the "Quatre Fermes" nursery (Guerouaou municipality, Blida province) (original photo).

2.1.2 Artemisia herba-alba

2.2. Essential oil extraction and chemical analysis

2.2.1 Essential oil extraction

2.2.1.1 Extraction of Artemisia absinthium essential oil

The essential oil extraction was carried out on November 18, 2024, at the Bio.Extrapamal laboratory, located in Oued Alleug (Blida), using a hydro distillation device. The Artemisia absinthium plant, previously cut into small fragments, underwent a 3-hour aqueous distillation using a 50-liter still.

For this operation, 10 kg of fresh plant material were placed in the apparatus's cucurbit, then topped up with 20 liters of tap water, a volume of water equivalent to twice the plant's weight. Under the influence of the heat source, the water contained in the cucurbit boils, generating steam that carries away the volatile compounds present in the plant. The steam-water-oil mixture then passes through the top before reaching the coil condenser, which is continuously cooled by circulating water. At this point, the vapors condense upon contact with the cold walls of the coil.

The resulting condensate flows into the Florentine vase, where the essential oil, less dense than water (hydrosol), naturally separates through decantation.

The recovered essential oil is then transferred to an amber glass bottle, hermetically sealed with an aluminum lid, and stored in the refrigerator until analysis.

Figue18: Alembic-type apparatus with a 50-liter capacity (original photo)

2.2.1.2 Extraction of Artemisia herba-alba essential oil

The essential oils obtained were dried using anhydrous sodium sulfate, then transferred to tightly sealed glass vials and stored in a refrigerator at 4°C until analysis.

To estimate essential oil yields, four separate experiments were conducted. Yields were calculated based on the weight of the plant material used.

2.2.2 Determination of essential oil yield

The yield is generally expressed as a percentage (m/m), i.e., the percentage of oil obtained relative to the dry mass of the plant used.

Yield formulation:

$$\textbf{yield}(\%) = \frac{\textbf{mass ofextraction essential oil}(\textbf{g})}{\textbf{masse of plant matter used}(\textbf{g})} \times \textbf{100}$$

2.2.3 Chemical Analysis

2.2.3.1 Chemical Analysis of Artemisia absinthium

The chemical composition of Artemisia absinthium essential oil was determined at the Center for Research and Application of Phytotherapy and Medicinal and Aromatic Plants at Gaziantep University using gas chromatography-mass spectrometry (GC/MS) using a Shimadzu GCMS-QP2020 system (Japan) equipped with an Rxi-5MS capillary column (30 m × 0.25 mm × 0.25 µm; 5% diphenyl / 95% dimethylpolysiloxane).

The carrier gas used was helium (purity ≥ 99.99%) with a flow rate of 1.61 mL/min. The interface, ionization source, and injector temperatures were 250°C, 220°C, and 250°C, respectively. Analysis was performed in Scan mode, with an ionization energy of 70 eV and a detection range from m/z 40 to 400.

The oven temperature program was as follows: initial temperature at 40°C, held for 2 minutes, followed by a temperature increase of 6°C/min to 240°C, then held for 10 minutes. The total analysis time was 45.33 minutes.

Compound identification was performed by comparing the spectra obtained with those of the NIST reference library. Relative quantification was expressed as a percentage of the area of each peak, without correction factors.

Figure19: Shimadzu GCMS-QP2020 instrument (original photo).

2.2.3.2 Chemical Analysis of Artemisia herba-alba

The chemical composition of Artemisia herba-alba essential oil was determined at the CRAPC using gas chromatography-mass spectrometry (GC/MS).

The apparatus used was a Hewlett Packard Agilent 6890 Plus chromatograph coupled to a Hewlett Packard Agilent 5973 mass spectrometer, where 0.2 µL of the substance was injected at a temperature of 250°C using a 1:80 split ratio.

The column used was an HP-5MS column with a length of 30 m, an internal diameter of 0.25 mm, and a film thickness of 0.25 µm, with a stationary phase comprising 5% phenyl and 95% dimethylpolysiloxane. The furnace temperature was maintained at 60°C for 8 minutes and increased at a rate of 2°C/min to 250°C, which was maintained for 10 minutes. Pure helium 6.0 was used as the carrier gas with a flow rate of 0.5 ml/min.

The mass scan time of the solvent in SIM mode was 3.5 minutes. Fragmentation was performed by electron impact under a field of 70 eV, the filament intensity. The total analysis time was 113 minutes.

Figure 20: Hewlett Packard GCMS- Agilent 6890 instrument (original photo X).

2.3. Insect rearing

Breeding was done at the level of the faculty of blida -1- in the pfe laboratory .

Insects used in this study were taken from infested semolina samples.

Figure 21: Infested semolina samples (original photo).

Tribolium castaneum were reared on wheat flour and semolina. The cultures were maintained in the dark in growth chamber set at 32,5 °C±1°C and 65%±5% relative humidity.

Figure 22: *Tribolium castaneum* rearing (original photo).

Adults of *T. castaneum* with same-age were used in fumigant toxicity and persistence bioassays.

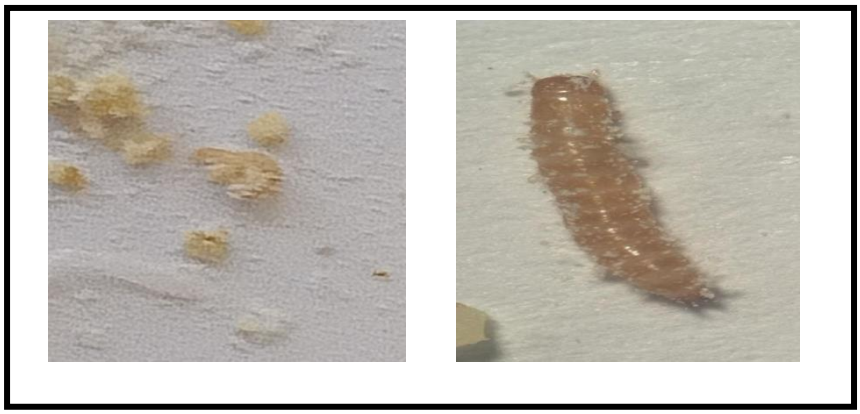


Figure 23: Eggs and Larva of *Tribolium castaneum* observed under a stereomicroscope (original photo).

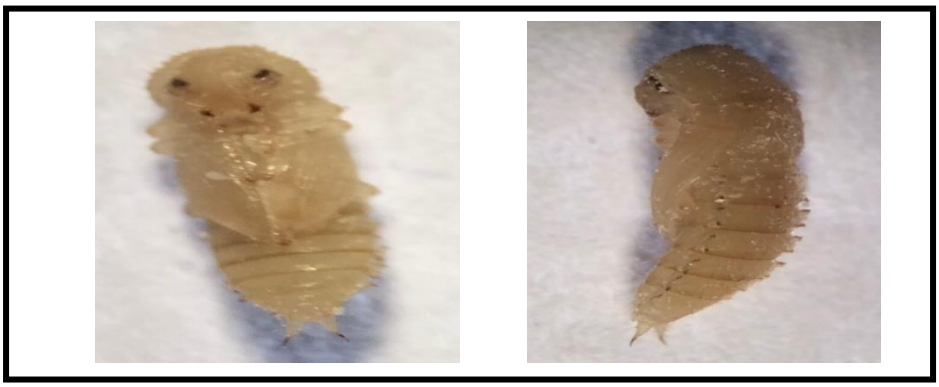


Figure 24: Pupa of *Tribolium castaneum* observed under a stereomicroscope (original photo).

Figure 25: Adult of *Tribolium castaneum* observed under a stereomicroscope (original photo).

2.4. Fumigant Toxicity Bioassays of Non-Formulated Essential Oils of *Artemisia absinthium* and *Artemisia herba-alba* :

A Whatman filter paper (2.0 cm in diameter), impregnated with the essential oil, was affixed and suspended from the screw cap of a 60 mL plastic flask using a 2 cm-long wire holder. The caps were tightly sealed onto the flasks, each containing 10 same-aged adults of *Tribolium castaneum*.

The tested doses of *Artemisia absinthium* essential oil were 60, 80, 100, 140, and 160 µL, corresponding to air concentrations of 1000, 1333.33, 1666.66, 2333.33, and 2666.66 µL/L of air, respectively.

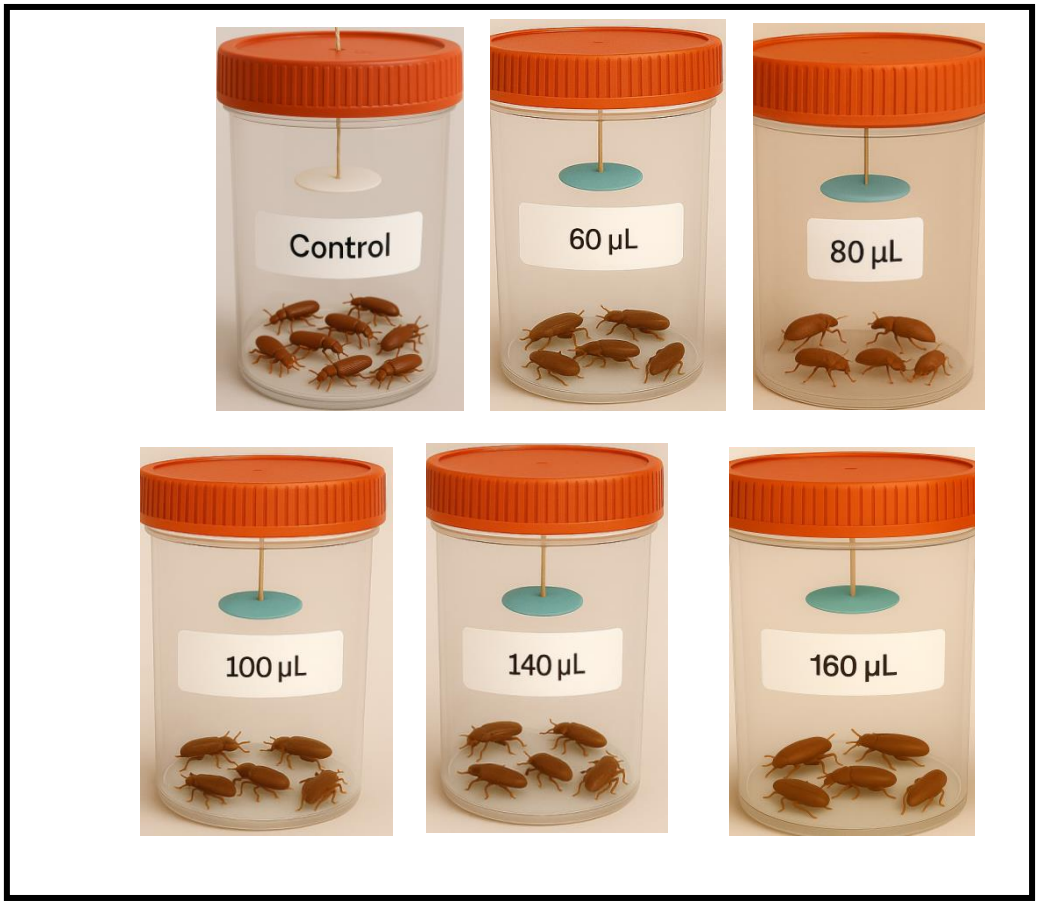


Figure 26: Illustrative representation of the fumigant bioassay setup with *Artemisia absinthium* essential oil

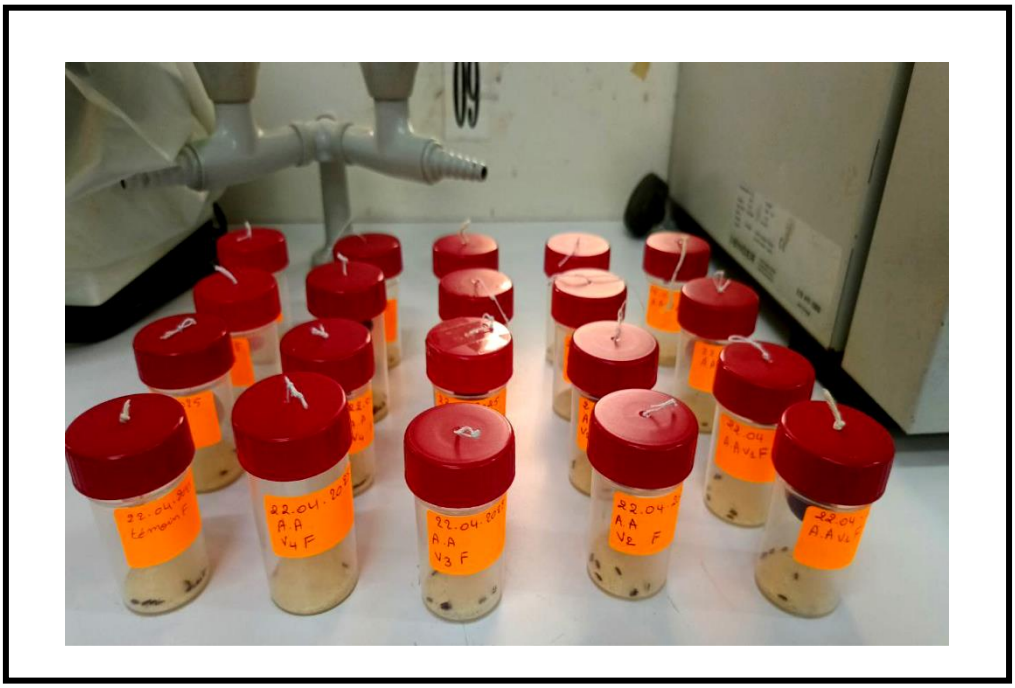


Figure 27: Fumigant toxicity of *Artemisia absinthium* essential oil (original photo).

The tested doses of *Artemisia herba-alba* essential oil were 25, 30, 35, 40, 45, and 50 µL, corresponding to concentrations of 416, 500, 583.33, 666.66, 750, and 833.33 µL/L of air, respectively.

The bioassays were conducted in the dark, at a temperature of 27 ± 3 °C and a relative humidity of 70–75%.

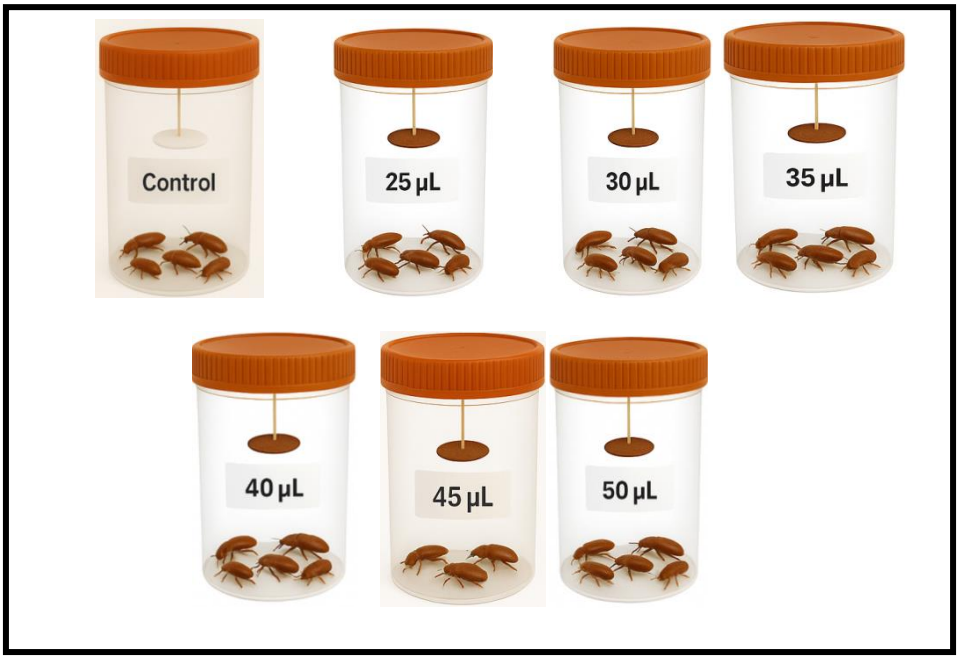


Figure 28: Illustrative representation of the fumigant bioassay setup with *Artemisia herba-alba essential Oil*

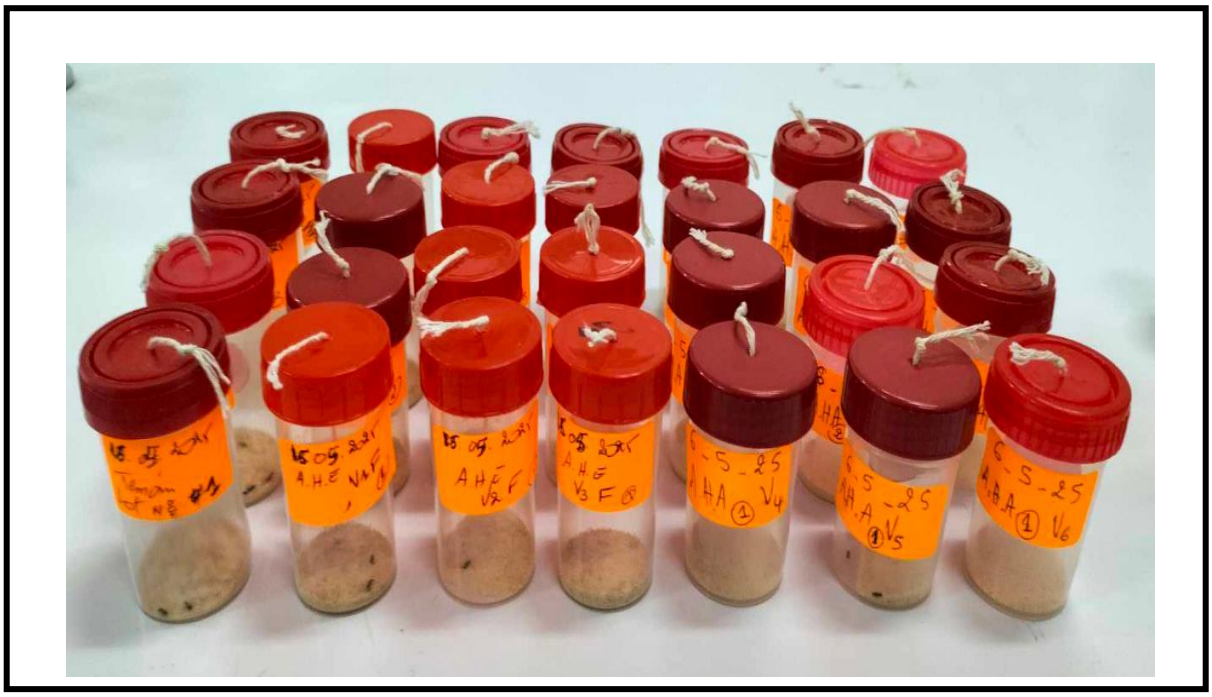


Figure 29: Fumigant toxicity of *Artemisia herba-alba* essential oil (original photo).

2.5. Persistence assays of Non-Formulated Essential Oils of *Artemisia absinthium* and *Artemisia herba-alba*

LC 95 values for *Artemisia absinthium* and *Artemisia herba-alba* essential oils, respectively) obtained from fumigant toxicity bioassay were used to determine the persistence of the oil. From the date of the treatment, every 5 days, 10 adults were inserted to each experimental unit. Then, the mortality rate was determined 72 h after exposure **(Ziaee et al., 2014).** The condition for the persistence experiment was according to fumigant toxicity section.

Each treatment, including the control, was replicated four times. Mortality was recorded hourly.

2.6. Microencapsulation of *Artemisia absinthium* and *Artemisia herba-alba* essential oil

2.6.1. Preparation of encapsulation solution

For the ME process, an initial emulsification step between the core and the wall material was performed, followed by freeze-drying of the obtained emulsion.

In these formulations, maltodextrin (MD) and gelatin (GEL) were employed as wall materials.

Two formulations were prepared: one based on *Artemisia absinthium* essential oil (AAEO) and the other on *Artemisia herba-alba* essential oil (AHAEO). In both cases, the wall materials used were maltodextrin (MD) and gelatin (GEL), combined in a 1:1 ratio.

These formulations had a fixed Artemisia absinthium (AAEO) and/or Artemisia herba-alba essential oil (AHAEO) content (i.e., 10%, w/w).

The microencapsulation (ME) process was carried out according to the steps described below.

2.6.2. Emulsion preparation

Emulsions were prepared by addition of the AAEO or AHAEO in MD/ GEL solutions.

Encapsulating agents were weighed into separate flasks, homogenized in sterilized distilled water (35 °C) up to complete dissolution, followed by addition of Tween 80 as a surfactant [final concentration of 5% (v/w) of the encapsulating agents].

Next, AAEO or AHAEO was slowly added to the system, which was maintained under constant stirring (7000 rpm) for 15 min using a mechanical homogenizer.

The procedure was performed in an ice bath (4 ± 0.5 °C) to avoid AAEO and AHAEO volatilization.

2.6.3. Freeze-drying

The obtained emulsions were transferred to plastic containers, frozen (- 25 °C for 24 h) and subjected to lyophilization under a pressure of 0.11 mbar and temperature of 60 °C for 60 h. During the lyophilization, the drying chamber was covered with aluminum foil to avoid light interference on the materials.

Dried emulsions were macerated using a mortar and pistil up to forming a homogenous powder, which was placed in a plastic container packed with aluminum foil to avoid light interference and stored under refrigeration (7 ± 0.5 °C).

2.7. Characterization of microcapsules:

2.7. 1. Particle size analysis of emulsions and microcapsules:

The volume particle size distribution of emulsions and solid particles were determined by laser diffraction using a Mastersizer 2000 (Malvern instrument). The solutions were suspended in water whereas the solid particles were suspended in an air flow at 0.2 MPa using a Sirocco unit for dry via measurements. The mean particle size of emulsions and solid particles was expressed using the Sauter mean diameter, D32 (μm), and was calculated using the equation 1, where ***mi*** is the volume of particles and ***di*** is the diameter:

(Equation 1)

$$D_{32} = \sum mi.di\text{^}3)/\sum mi.dix\text{^}2$$

The width of particle size distribution was characterized by the Span. The span of a volume-based size distribution is defined according to equation 2, where **dv** (0.1), **dv** (0.5) and **dv** (0.9) are the particle size diameters of 10, 50 and 90 percent of the cumulative 216 distribution curve in volume, respectively:

(Equation 2)

$$Spam = \frac{d(0,9) - d(0,1)}{d(0.5)}$$

2.7. 2. Morphological analysis:

Appearance and size of solid microcapsules containing *Artemisia Absinthium or /and Artemisia Herba-alba essential* oil were examined under a scanning electron microscope (SEM) (KYKY-EM3200 model, KYKY, Beijing, China) using an acceleration voltage of 26.0 kV. The samples were coated with gold using a sputter coater.

2.7. 3. Determination of encapsulation efficiency:

The encapsulation efficiency in the emulsions and microcapsules was determined by distilling 5 g of emulsion or encapsulated powder in a Clevenger-type apparatus for 3 h. The oil volume collected was multiplied by a density factor (i.e. density of the 0.908 g/mL) to calculate the weight of recovered oil **(Baranauskienė et al., 2007).** Determination was carried out in duplicate. The encapsulation efficiency was calculated according to equations 3 and 4, respectively.

(Equation 3)

$$Emulsioio\ encapsulation\ efficiency = \frac{\frac{volum\ of\ oil\ in\ microcapsules\ (ml)}{weig\text{?}t\ of\ emulsion\ (g)}}{\left(\frac{volume\ of\ initial\ oil\ (ml)}{weig\text{?}t\ of\ water\ (g) + \text{maltodextrin (MD) and gelatin (GEL) } (g)\ + weig\text{?}t\ of\ oil\ (g)}\right)}$$

(Equation 4)

$$Dry\ particle\ encapsulation\ efficiency = \frac{Concetration\ of\ oil\ in\ solid\ formulation\ (g)/g\ particles}{\frac{volume\ of\ oil\ in\ microcapsules\ (ml) * Density\ of\ oil}{Weig\text{?}t\ of\ formulation\ (g) * \left(\frac{\text{maltodextrin (MD) and gelatin (GEL) (\%)}}{100} + \frac{OIL\%}{100}\right)}} \times 100$$

The total encapsulation efficiency was calculated using equation 5 taking into account the final concentration of oil in the solid formulation (i.e. microcapsules) and the mass of oil per mass of starch employed in the preparation of the initial emulsion.

(Equation 5)

$$Total\ encapsulation\ efficiency = \frac{\frac{concentration\ of\ oil\ in\ solid\ (g)/g\ particles}{Weig\square t\ of\ initial\ oil\ (g)}}{Weig\square t\ of\ initial\ (g) + Weig\square t\ of\ \text{maltodextrin (MD) and gelatin (GEL)}\ (g)} \times 100$$

2.7. 4. Determination of drying yield:

The drying yield (%) was calculated according to equation 6 as the percentage of the mass of particles recovered in the cyclone respect to the mass of solid material in the volume of emulsion processed.

(Equation 6)

$$Drying\ yield = \frac{weight\ of\ particles\ (g)}{(\frac{Volum\ of\ emulsion\ (ml) \times (weight\ of\ \text{maltodextrin (MD) and gelatin (GEL)}\ (g) + weight\ of\ oil\ (g)}{weight\ of\ water\ (g) + weight\ of\ starch\ (g) + weight\ of\ oil(g)})} \times 100$$

2.7. 5. Determination of emulsion stability:

The stability of the emulsions was calculated by visual determination of the de-emulsified oil after 21 and 50 days of storage at 25±2 ºC in the dark: 7 mL of the emulsion were poured in a vertical glass tube with an inner diameter of 13 mm (height of emulsion: 55mm). The height of visible supernatant oil layer was measured, and the volume of de emulsified oil calculated. The percent of the supernatant oil was calculated using equation 7:

(Equation 7)

$$V\% = \frac{Vt}{V0} \times 100$$

Where ***Vt*** is the volume of de-emulsified oil and ***V0*** is the total volume of oil in the emulsion.

2.7. 6 Controlled release analysis of microcapsules:

For controlled release analysis, 2 g of encapsulated powders from different tests were introduced to Petri dishes (9 cm diameter) and stored at 27±3 ºC and 70–75% relative humidity using sodium chloride salt, in the dark for 15 and 30 days. After these periods, the remained oil was determined by distilling 2 g of encapsulated powder in a Clevenger-type apparatus for 3 h. The percentage of released oil was calculated as ratio of the difference between the initial oil and the remained oil respect to the initial oil amount of oil encapsulated in the powder, multiply by 100.

2.7. 7 Determination of water activity (aw):

The water activity of solid particles was measured using water activity meter (Rotronic probe type HC2-AW- (USB)) after calibration. Spray **dried** samples were kept overnight in a refrigerator (4 °C); after being allowed to come at 25±2 °C, the water activity was measured for about 5 min in a temperature stable area **(Baranauskienė et al., 2007).**

2.8. Fumigant Toxicity Bioassays of Formulated Essential Oils of *Artemisia absinthium* and *Artemisia herba-alba*

For the microencapsulated oil treatments, a mesh fabric was placed under the caps of the flasks, and specific amounts of the essential oil powder (mg) were carefully applied to achieve various **formulated essential oils** concentrations (µl). The flasks were then hermetically sealed to prevent leakage or external contamination.

Empty flasks and microcapsule matrix components maltodextrin (MD) and gelatin (GEL) in a 1:1 ratio without oil were used as controls.

Each treatment, including the control, was replicated four times. Mortality was recorded hourly.

2.9. Persistence assays of Formulated Essential Oils of *Artemisia absinthium* and *Artemisia herba-alba*

LC 95 values for ***Artemisia absinthium*** and ***Artemisia herba-alba*** formulated essential oils, respectively) obtained from fumigant toxicity bioassay were used to determine the persistence of microcapsules.

From the date of the treatment, every 5 days, 10 adults were inserted to each experimental unit. Then, the mortality rate was determined 72 h after exposure **(Ziaee et al., 2014).** The condition for the persistence experiment was according to fumigant toxicity section. Also, four replicates were performed.

2.10. Statistical analysis

Lethal concentrations (LC_{50} and LC_{95}) and lethal times (LT_{50} and LT_{95}) were estimated using probit regression analysis performed with **IBM SPSS Statistics software, version 25.0**, based on observed mortality data at different concentrations and exposure times.

Duncan's multirange test was used to assess the comparison between the mean values at $p<0.05$.

RESULTATS AND DISCUSSION

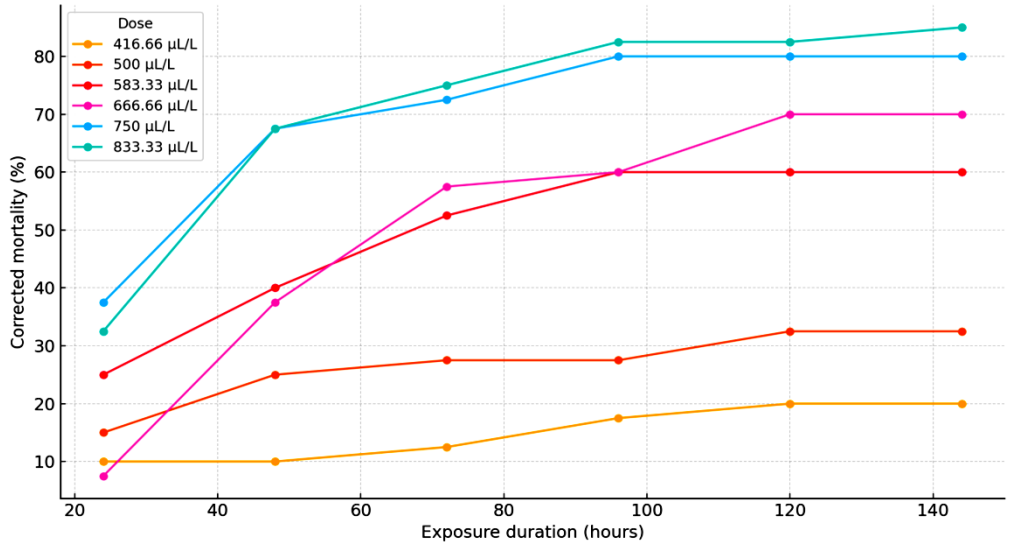
1. Percentage of corrected mortality of *Tribolium castaneum* exposed *to different doses of Artemisia Herba-alba* essential oil for various durations *(24h to 144h).*

Figure 30: Illustrates the evolution of the corrected mortality percentage of *Tribolium castaneum* adults exposed to various concentrations of *Artemisia herba-alba* essential oil (from 416.66 to 833.33 µL/L of air) over a period of 24 to 144 hours.

The results show a positive relationship between the applied dose and the exposure time on insect mortality. At lower doses (416.66 and 500 µL/L), mortality remains low and increases slowly over time, reaching a maximum of 32.5% after 144 hours. In contrast, starting from 583.33 µL/L, a marked increase in mortality is observed, reaching 60% as early as 96 hours.

The highest doses (750 and 833.33 µL/L) cause rapid and significant mortality: up to 85% after 144 hours at 833.33 µL/L. The peak mortality is generally observed after 96 hours, suggesting a cumulative effect of exposure to the essential oil.

These results indicate an increasing insecticidal efficacy of *Artemisia herba-alba* essential oil as a function of both concentration and exposure duration, confirming its potential as a biocontrol agent against stored product pests.



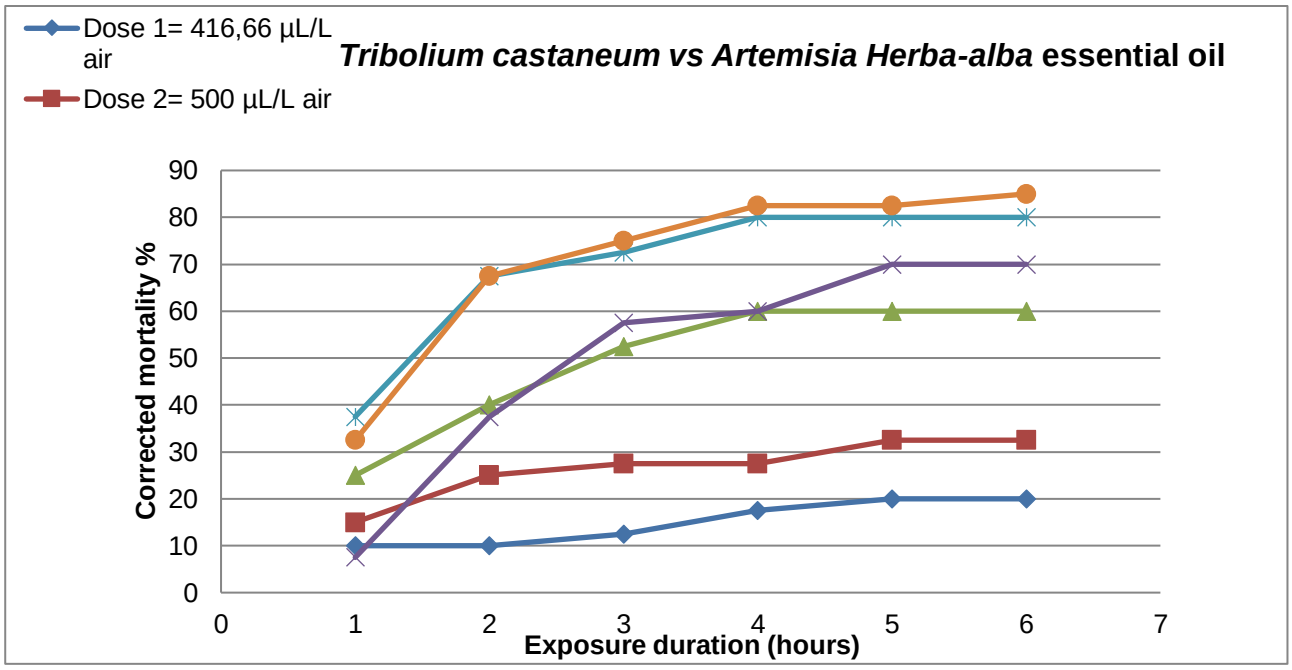


Fig. 30. Percentage of corrected mortality of *Tribolium castaneum* exposed *to different doses of Artemisia Herba-alba* essential oil for various durations *(24h to 144h).*

1. ***Percentage of corrected mortality of Tribolium castaneum exposed to different doses of Artemisia absinthium essential oil as a function of exposure time (24h to 264h).***

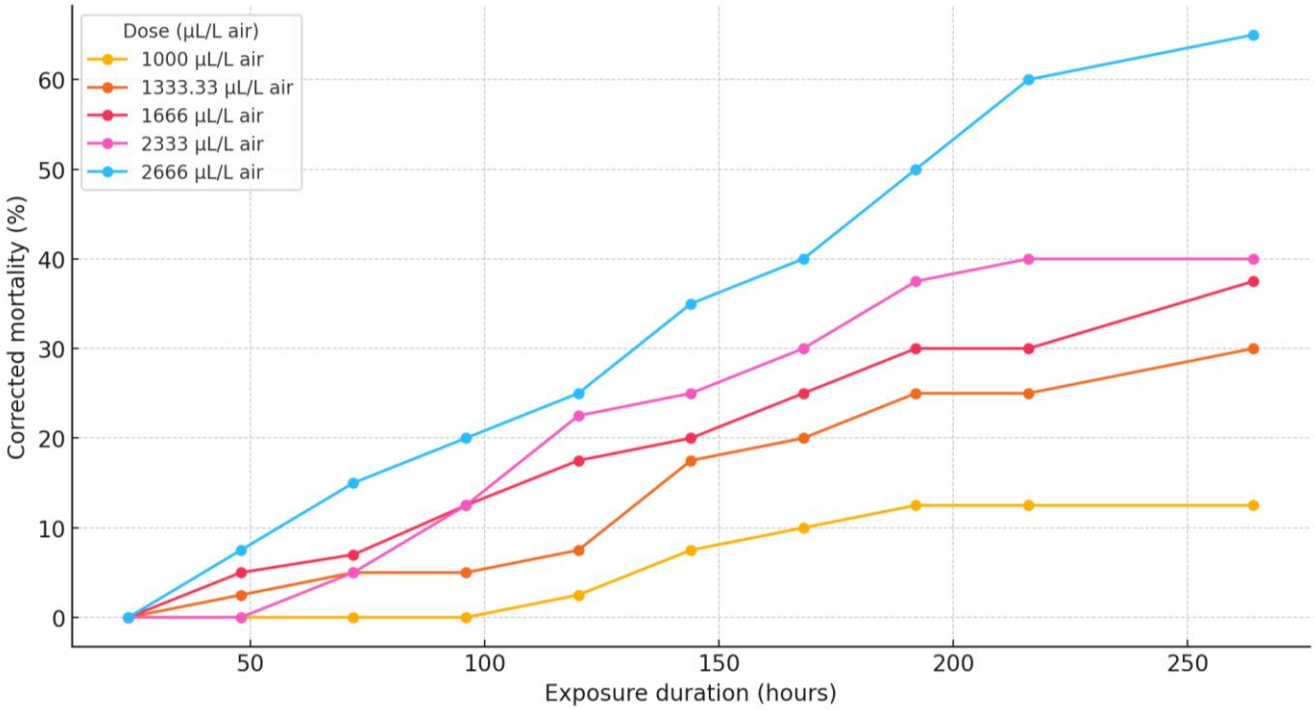
Fig. 31 : presents the evolution of the corrected mortality of *Tribolium castaneum* in response to exposure to *Artemisia absinthium* essential oil. The results reveal a clear dependence on both exposure time and applied dose.

At all tested concentrations, mortality remains null or negligible during the first 48 hours, indicating a relatively slow insecticidal action of this essential oil. However, from 72 hours onward, a progressive increase in mortality is observed, more pronounced at higher doses.

The lowest dose (1000 µL/L of air) shows a maximum mortality of 12.5% after 264 hours, suggesting limited efficacy. In contrast, the highest tested dose (2666 µL/L of air) induces significant mortality, reaching 65% at 264 hours, with a rapid increase starting at 48 hours

of exposure. Intermediate doses (1333, 1666, and 2333 µL/L) produce proportional effects, reflecting a clear dose–response relationship.

These results indicate that *A. absinthium* essential oil exhibits increasing insecticidal activity with longer exposure times and higher concentrations. However, even at the highest dose, the insecticidal efficacy remains partial, which may suggest either a moderate tolerance of the target species or the need to optimize application conditions (e.g., formulation, exposure duration, or synergistic combination).



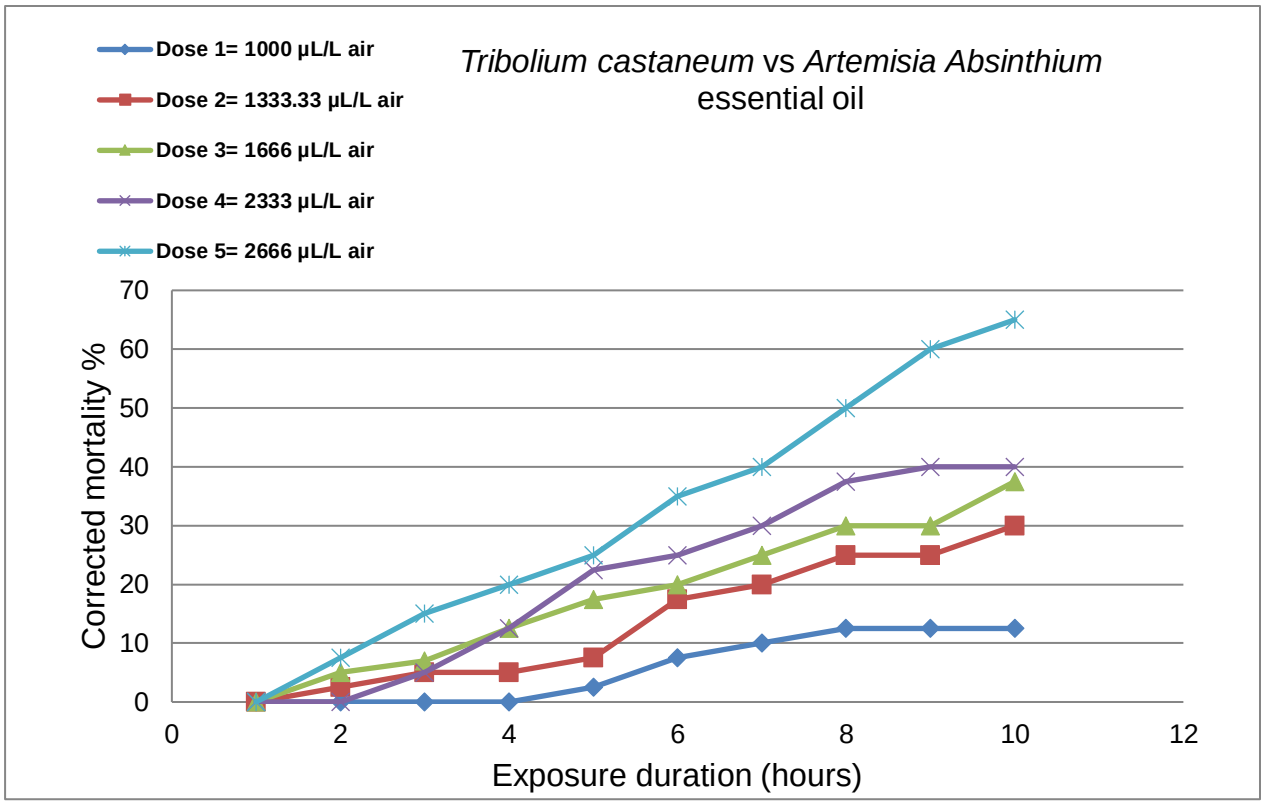


Fig. 31. ***Percentage of corrected mortality of Tribolium castaneum exposed to different doses of Artemisia absinthium essential oil as a function of exposure time (24h to 264h).***

2. LC_{50} and LC_{95} values (µL/L air) of *Artemisia herba-alba* essential oil against *Tribolium Castaneum* adults after fumigant bioassay at different exposure times.

The LC_{50} and LC_{95} values of *A. herba-alba* essential oil decreased with increasing exposure time, indicating a time-dependent increase in toxicity. At 24 hours, the LC_{50} was 1090.09 µL/L air, which progressively declined to 562.44 µL/L at 120 hours. A similar trend was observed for LC_{95} values. This suggests that prolonged exposure enhances the fumigant efficacy of *A. herba-alba* oil against *T. Castaneum* adults.

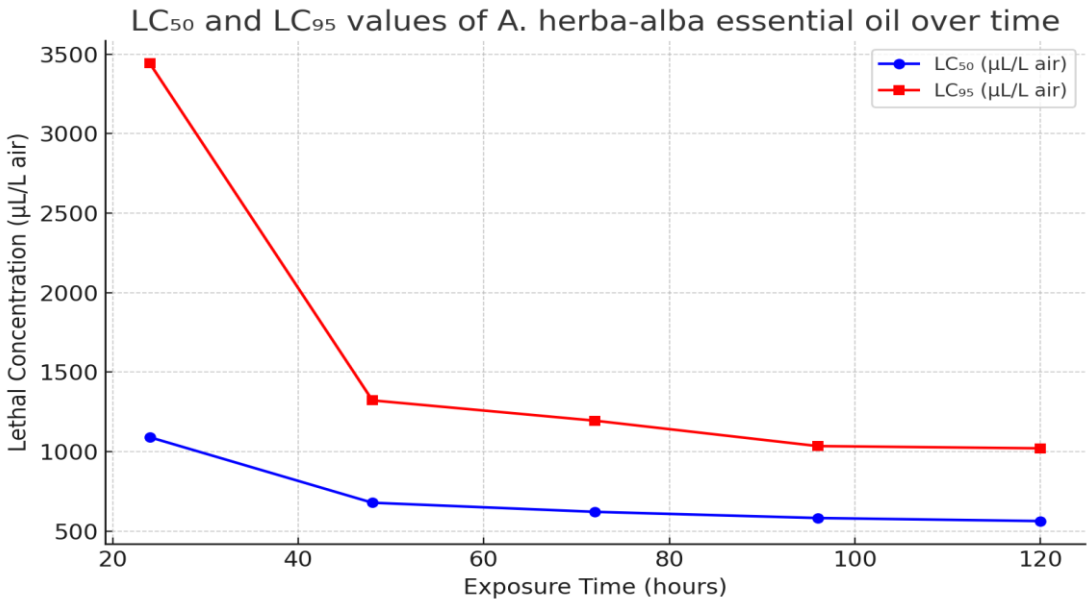


Fig. 32. LC_{50} and LC_{95} values (µL/L air) of *Artemisia herba-alba* essential oil against *Tribolium Castaneum* adults after fumigant bioassay at different exposure times.

3. LT_{50} and LT_{95} values (in hours) of *Artemisia herba-alba* essential oil against *Tribolium Castaneum* adults at different fumigant concentrations.

The lethal time values (LT_{50} and LT_{95}) decreased significantly with increasing concentration, showing a clear dose-dependent response. At low concentration (416.66 µL/L), the LT_{95} was extremely high (over 611,000 h), indicating very weak effectiveness. At higher doses (750 and 833.33 µL/L), LT_{50} values dropped below 40 hours, demonstrating rapid insecticidal action.

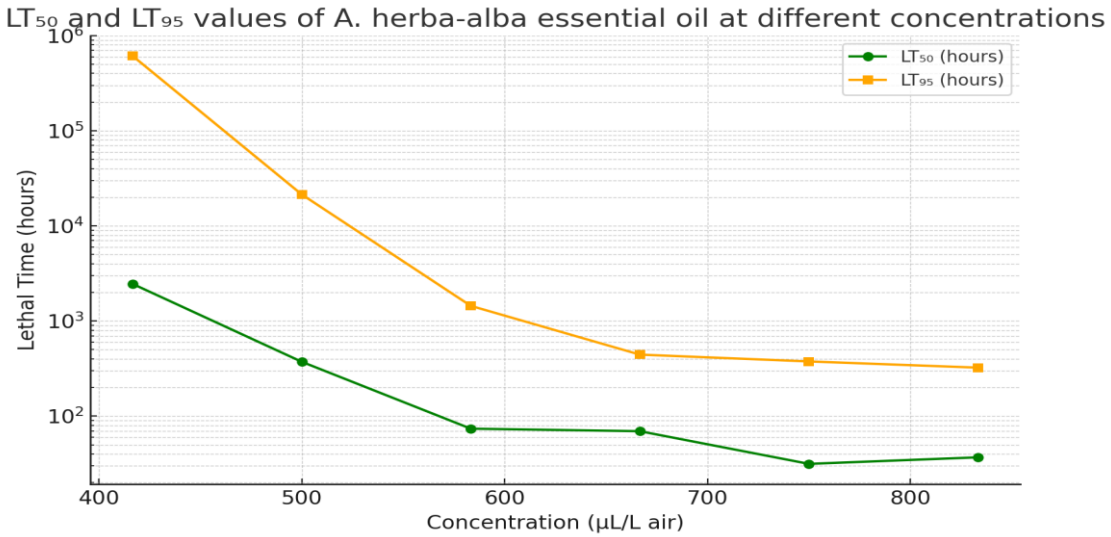


Fig. 33: LT_{50} and LT_{95} values (in hours) of *Artemisia herba-alba* essential oil against *Tribolium Castaneum* adults at different fumigant concentrations.

4. **LC_{50} and LC_{95} values (µL/L air) of *Artemisia absinthium* essential oil against *Tribolium Castaneum* adults after fumigant bioassay at different exposure times.**

The median lethal concentration (LC_{50}) and 95% lethal concentration (LC_{95}) values obtained after exposing *Tribolium Castaneum* adults to *Artemisia absinthium* essential oil by fumigation are presented in Table 5. These data highlight an inverse relationship between exposure duration and lethal concentrations, underscoring the cumulative effect of the essential oil over time.

At 48 hours of exposure, the concentrations required to reach 50% and 95% mortality were high (LC_{50} = 24,278.29 µL/L air; LC_{95} = 230,512.44 µL/L), indicating low short-term efficacy. This trend is even more pronounced at 72 hours, with an LC_{50} of 55,837.27 µL/L and an LC_{95} exceeding 1,880,000 µL/L, suggesting a high variability in individual response and considerable resistance during this early phase.

However, starting at 96 hours, a significant decrease in LC_{50} and LC_{95} values is observed, reflecting a marked improvement in insecticidal efficacy. This trend continues steadily, with both LC_{50} and LC_{95} gradually decreasing up to 264 hours (LC_{50} = 2,268.76 µL/L; LC_{95} = 8,458.54 µL/L), where the essential oil appears to reach its optimal activity.

The analysis also reveals a narrowing of the LC_{50}–LC_{95} interval over time, suggesting that variability in individual sensitivity decreases as exposure duration increases. This may reflect a more homogeneous action of the essential oil within the exposed population, likely due to the progressive accumulation or physiologically induced degradation caused by the active compounds.

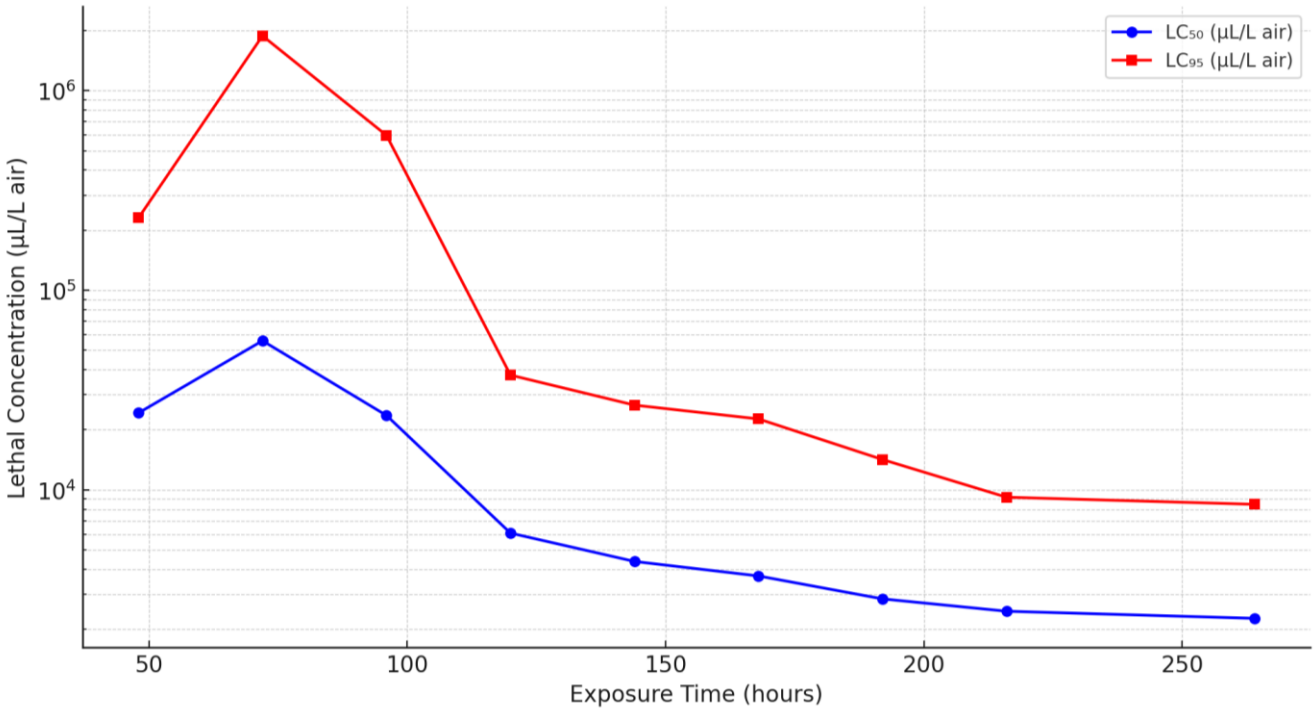


Fig. 34: LC_{50} and LC_{95} values (µL/L air) of *Artemisia absinthium* essential oil against *Tribolium Castaneum* adults after fumigant bioassay at different exposure times.

5. LT_{50} and LT_{95} values (in hours) of *Artemisia absinthium* essential oil against *Tribolium Castaneum* adults at different fumigant concentrations.

Figure 35 : presents the mean lethal times (LT_{50}) and the times to 95% lethality (LT_{95}), expressed in hours, for different concentrations of *Artemisia absinthium* essential oil applied via fumigation against adult *Tribolium Castaneum* .

The results reveal an inverse dose–time relationship, typical of a temporal toxic response: the higher the concentration, the shorter the time required to induce mortality. At the lowest tested concentration (1000 µL/L of air), LT_{50} is estimated at 646.51 h and LT_{95} at 2548.14 h, indicating very limited and slow efficacy at this dose.

As the concentration increases, both LT_{50} and LT_{95} values markedly decrease. At 1333.33 µL/L, LT_{50} drops to 430.34 h, then to 341.42 h at 1666 µL/L, demonstrating increasing but still relatively slow efficacy. The most notable reductions are observed at the highest concentrations:

At 2333 µL/L, LT_{50} is 201.87 h and LT_{95} drops to 548.21 h, indicating rapid mortality in the exposed population.

At 2666 µL/L, although LT_{50} is slightly higher (210.33 h), LT_{95} is also higher (999.50 h), suggesting increased heterogeneity within the population or a plateau effect at high dose.

Overall, the lethal time values indicate that the efficacy of the essential oil is strongly influenced by the applied concentration, with optimal effects starting at 2333 µL/L. However, the relatively high LT_{95} values even at high concentrations reveal that some tolerant individuals persist, possibly justifying the use of prolonged or combined-action formulations.

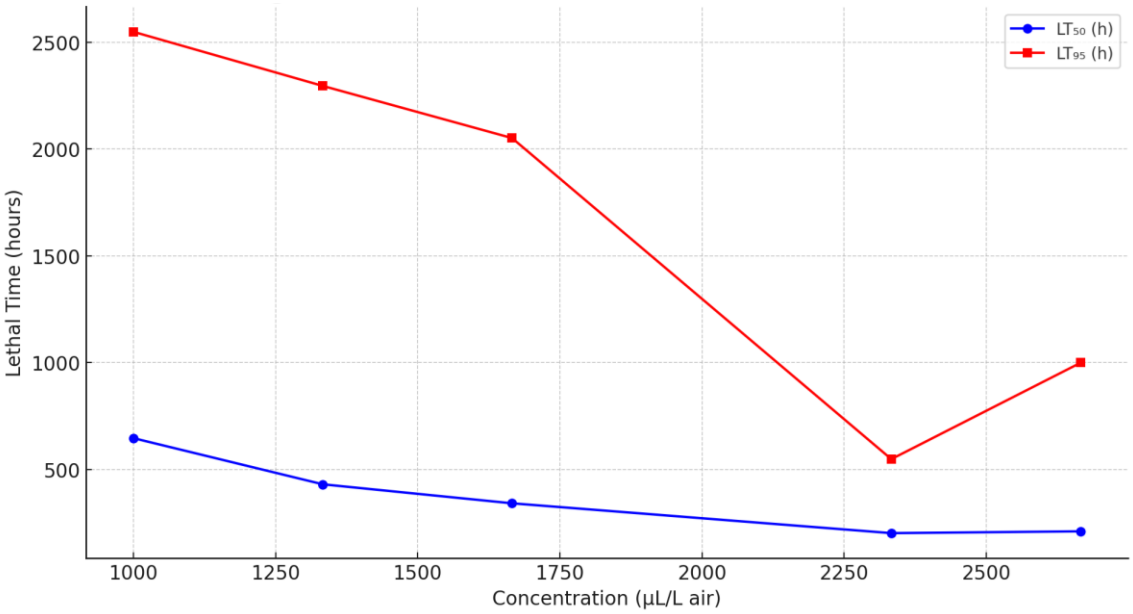


Fig. 35: LT_{50} and LT_{95} values (in hours) of *Artemisia absinthium* essential oil against *Tribolium Castaneum* adults at different fumigant concentrations.

6. Comparison of the fumigant insecticidal activity of essential oils of *Artemisia herba-alba* and *Artemisia absinthium* against *Tribolium castaneum*

The evaluation of the insecticidal activity of *Artemisia herba-alba* and *Artemisia absinthium* essential oils through fumigant bioassays enabled the determination of LC_{50} values at different exposure durations, ranging from 24 to 264 hours (Table 7).

The essential oil of *A. herba-alba* demonstrated marked efficacy as early as 24 hours, with an LC_{50} of 1090.09 µL/L of air. These values progressively decreased over time, reaching an LC_{50} of 560.81 µL/L after 144 hours of exposure. This trend reflects a significant increase in insecticidal efficacy over time, showing that *A. herba-alba* possesses a rapid and sustained fumigant action.

In contrast, *A. absinthium* essential oil initially showed very high LC_{50} values, particularly at 48 hours (LC_{50} = 24,278.29 µL/L; LC_{95} = 230,512.44 µL/L), indicating low short-term efficacy. However, a gradual decrease in these values was observed as the exposure time increased. At 264 hours, the LC_{50} dropped to 2268.76 µL/L, indicating a notable improvement in toxic effect over the long term.

The comparison between the two oils reveals that *A. herba-alba* is significantly more active than *A. absinthium* at short and intermediate time points, with much lower LC_{50} values. This early efficacy may be attributed to the richness of the essential oil in rapidly acting volatile bioactive compounds. On the other hand, the delayed activity of *A. absinthium* suggests a slower release of its components or a cumulative effect requiring prolonged exposure.

These findings confirm the insecticidal potential of both *Artemisia* species, while highlighting their differences in terms of action kinetics. They are promising for the development of natural insecticidal formulations, whether for rapid action or prolonged release, depending on the desired profile.

Table 3: LC50 values (µL/L air) of fumigant bioassay with *A. herba-alba* and *A. absinthium* essential oils.

Time	Artemisia oils	
	A. herba-alba **CL50 (µL/L air)**	***A. absinthium*** **CL50 (µL/L air)**
24h	1090.092	**/**
48h	677.821	24278.288
72h	620.476	55837.266
96h	581.641	23596.428713
120h	562.443	6068.855132
144h	560.808	4375.671088
168h	**/**	3700.174735
192h	**/**	2839.922036
216h	**/**	2463.228776
264h	**/**	2268.755290

Lethal times (LT_{50}) were calculated for different concentrations of *Artemisia herba-alba* and *A. absinthium* essential oils (Table 8) to assess the speed of the toxic effect of each treatment.

For *A. herba-alba*, a clear and consistent decrease in LT_{50} values was observed with increasing concentration. At the lowest dose (416.66 µL/L), the LT_{50} reached 2456.93 hours, indicating very low efficacy at this level. However, starting from 583.33 µL/L, lethal times dropped considerably (LT_{50} = 73.94 h), reaching a maximum effect at 750 µL/L, where LT_{50} was reduced to 31.55 hours. These data confirm the increasing efficacy of *A. herba-alba* with dose and its ability to induce rapid mortality from intermediate concentrations onward.

As for *A. absinthium*, although the tested concentrations were higher (1000 to 2666 µL/L), the LT_{50} values remained generally higher than those of *A. herba-alba* at equivalent doses. At 1000 µL/L, LT_{50} was 646.51 hours, and although this time decreased with increasing doses, it remained high: 210.33 hours at 2666 µL/L compared to 31.55 hours for *A. herba-alba* at 750 µL/L.

These results confirm that *A. absinthium* exhibits slower insecticidal activity, requiring higher concentrations and longer exposures to achieve significant mortality.

Thus, both species' essential oils show a clear dose–time relationship: the higher the concentration, the shorter the time needed to induce mortality. However, *A. herba-alba* stands out for its significantly greater speed of action, making it a more promising candidate for applications requiring rapid results.

Table 4: LT50 (h) values of *A. herba-alba* and *A. absinthium* essential oils calculated at different fumigant concentrations.

***Artemisia* oils**			
Concentration (µL/L air)	***A. herba-alba* LT50 (hours)**	**Concentration (µL/L air)**	***A. absinthium* LT50 (hours)**
Dose 1= 416,66	2456.927	**Dose 1= 1000**	646.505819
Dose 2= 500	373.263	**Dose 2= 1333.33**	430.337431
Dose 3= 583,33	73.942	**Dose 3= 1666**	341.420241
Dose 4= 666,66	69.539	**Dose 4= 2333**	201.866593
Dose 5= 750	31.548	**Dose 5= 2666**	210.328963
Dose 6= 833,33	36.954	**/**	**/**

Analyze chromatography _mass spectrography (GC – MS) :

The Yield of *A. Absinthium was 2,9 %*. The Chemical Composition of *Artemisia herba-alba* Essential Oil, Gas chromatography–mass spectrometry (GC-MS) analysis of the *Artemisia herba-alba* essential oil revealed a chemically diverse profile predominantly composed of monoterpenes, with oxygenated monoterpenes and monoterpene hydrocarbons being the major classes. A total of 28 constituents were identified, contributing significantly to the biological activities and characteristic aroma of the oil. The Major Constituents essential oil is dominated by ca**mphor** (32.59%), The second most abundant compound is **1,8-cineole (eucalyptol)** (15.63%), Other notable monoterpenes include **camphene** (7.93%), as well as **α-thujone** (6.96%) and **β-thujone** (5.53%), **Chrysanthenone** (2.80%) represents a less commonly reported oxygenated monoterpene. Collectively, these six compounds account for over **71% of the total oil composition**, suggesting that this essential oil belongs to a **camphor–1,8-cineole–thujone chemotype**. The essential oil of *Artemisia absinthium*, analyzed by gas chromatography–mass spectrometry (GC-MS), exhibited a chemically diverse profile predominantly composed of **monoterpenes**, including both **oxygenated monoterpenes** and **monoterpene hydrocarbons**.

Chemical Composition of *Artemisia absinthium* **Essential** Oil, the essential oil of *Artemisia absinthium*, analyzed by gas chromatography–mass spectrometry (GC-MS), exhibited a chemically diverse profile predominantly composed of **monoterpenes**, including both **oxygenated monoterpenes** and **monoterpene hydrocarbons**, a total of 19 major constituents were identified, underscoring the biochemical complexity and the potential biological activity of this species. **Main Constituents:**

The dominant compound was **camphor** (23.49%). The second most abundant compound, **3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-** (11.59%), is a monoterpenic alcohol.

Other major constituents include :

- **B-Myrcene** (7.55%).
- **Thujone** (6.90%).
- **γ-Terpinene** (6.08%) and **α-Pinene** (6.04%).
- **Camphene** (5.60%) and **α-Terpinene** (3.74%).

The chemical analysis of *A. absinthium* aerial parts revealed 14 components, corresponding to 98.89% of the total EO composition.

- The yield of EOs (w/w) of absinthium, were 1.32%

- The chemical analysis of *A. absinthium* aerial parts revealed 14 components, corresponding to 98.89% of the total EO composition

The major components were **Camphor (36.22%)** and **α-thujone (30.28%),** followed by **Chamazulene (8.02%), Arborescin (6.82%), Terpinen-4-Ol (6.80%),** and **Sabinene (5.81%),** while, the minor components were reported, with relative peak areas ranging from **0.35% to 1.18%.** (**Naimi et al., 2022)** The results obtained by **Wani et al. (2014)** showed that the major components of the EO of *A. absinthium* from **India** were **β-pinene (42.15%)** and **Chrysanthenyl acetate (49.15%).** According to the results of **Bouchenak et al. (2018)**, the major components of the EOs of *A. absinthium* from **Algeria** were **ß-thujone (60.82%)** and **Chamazulene (16.65%).** GC and GC–MS analysis of *A. herba-alba* and *A. absinthium* essential oils showed that 22 and 31 compounds were identified, which represent respectively **49.03%** and **56.26%** of total constituents.

Camphor (11.48–16.71%); 1,8 cineol (19.59–5.47%); camphene (2.63–2.37%), and **borneol (2.29–1.77%)** were the common major compounds for both oils, whereas **β-thujone (22.72%)** was the major component of *A. absinthium* essential oil **(Bachrouch et al., 2015).**

A. herba-alba essential oil composition investigated using both GC and GC–MS techniques showed that **1,8 cineol (19.59%), camphor (11.48%), α-pinene (5.4%), camphene (2.63%), borneol (2.29%),ß-pinene (1.63%), and α-terpineol (1.09%)** are considered as major compounds **(Bachrouch et al., 2015),** and being reported in previous studies on Tunisian A. herba-alba oils **(Haouari and Ferchichi, 2009; Mighri et al., 2010).**

Moreover, our results were in accordance with those of **(Feuersteinet al. 1988); Salido et al.** (**2004); Bakkali et al. (2008)** reporting **1,8 cineol, camphor, α-terpineol** and borneol as major constituents of A. herba-alba essential oil.

Nevertheless, changes in the composition of desert wormwood from different regions and bioclimatic floors have been widely **s**tudied **(Segal et al., 1987; Salido et al., 2004; Bellata et al., 2014).**

On the other hand, our results showed that A. absinthium essential oil was rich on **β-thujone (22.72%), camphor (16.71%), 1,8 cineole (5.47%),** and **camphene (2.37%).** Previous studies revealed that A. absinthium essential oils from Tunisia were characterized by chamazulene, β-thujone, bornan-2-one, bornyl acetate, 1-terpinen-4-ol, p-cymene, and β-myrcene (**Riahi et al., 2013**). Recently**, (Dhen et al. 2014**) reported that chamazulene,

camphor, bornyl-acetate, myrcene, 1-4 terpineol, camphene, gamma-terpinene, and α-terpinene were the major compounds of Tunisian wormwood oil.

Chemical composition of A. absinthium essential oil was widely investigated. In this context, Lawrence, (**1992**) reported that wormwood essential oil extracted from plants grown in USA showed β-thujone and cis-sabinyl acetate as the main components. Besides, the Algerian oil was characterized by the predominance of camphor and borneol (**Erdogan-Orhan et al., 2010).** Moreover, Orav et al. (2006) reported that four chemotype of A. absinthium essential oil were identified from different geographical areas in Europe: sabinene and myrcene rich oil, α- and β-thujone rich oil, /break epoxyocimene rich oil, and (E)-sabinyl acetate rich oil. Investigations on Artemisia species also revealed that A. absinthium oil from western Canada was distinguished by high amounts of myrcene, trans-thujone and trans-sabinyl acetate (**Lopez-Lutz et al., 2008**).

In addition, β-pinene and β-thujone were the main compounds of Iranian wormwood essential oil (**Khangholi and Rezaeinodehi, 2008**).

The above results clearly demonstrated that both essential oils content bioactive insecticidal coompouds **(Bachrouch et al., 2015)**.

Our data indicate that essential oil yields varied within *Artemisia* species. The highest yield (in %) was recorded in leaves of *A. herba-alba* (0.27 %) and *A. absinthium* (0.16 %) **(Chaieb et al., 2018)** ; data not shown. In total, 48 and 51 components were identified representing 100, and 95.54 % *A. absinthium*, and *A. herba-alba* oil, respectively.

Common major compounds in **all oils (in %) were:** camphor 31.3, β-pinene 14.9, γ-terpinene 14.06 and germacrene 12.15, whereas the major components of ***A. absinthium*** essential oil (in %) were: camphor 24.81, chamazulene 13.71, bornyl acetate 5.89 and myrcene 5.83;

The dominant components detected in ***A. herba-alba*** essential oil were (in %): β-thujone 12.5, α-thujone 8.78, sabinyl acetate 8.56 and terpinene-4-ol 8.51.

The type and proportion of various monoterpenoids in the oil are characteristic of the genus and species. The composition of essential oils obtained from the tested *Artemisia* species in the current investigation showed a significant similarity to previous reports **(*Msaada* K et al., 2015. Haouari M, Ferchichi A et al., 2009., Neffati A et al., 2008. Titouhi F et al., 2017. Younsi F et al., 2016. Bellili S et al., 2017.)** :

The major component of the Tunisian *A. absinthium* was chamazulene, thujones are similar components from the essential oil of *A. herba-alba* and β-pinene was found in *A. campestris*

collected from different geographic localities of Tunisia (**Haouari M, Ferchichi A et al., 2009. Neffati A et al., 2008. Titouhi F et al., 2017**).

LC_{50} and LC_{95} values (µL/L air) of *Artemisia herba-alba* essential oil against *Tribolium castaneum* adults after fumigant bioassay at different exposure times. The LC_{50} and LC_{95} values of *A. herba-alba* essential oil decreased with increasing exposure time, indicating a time-dependent increase in toxicity. At 24 hours, the LC_{50} was 1090.09 µL/L air, which progressively declined to 562.44 µL/L at 120 hours. A similar trend was observed for LC_{95} values. This suggests that prolonged exposure enhances the fumigant efficacy of *A. herba-alba* oil against *T. castaneum* adults.

LC_{50} and LC_{95} values (µL/L air) of *Artemisia absinthium* essential oil against *Tribolium castaneum* adults after fumigant bioassay at different exposure times. The median lethal concentration (LC_{50}) and 95% lethal concentration (LC_{95}) values obtained after exposing *Tribolium castaneum* adults to *Artemisia absinthium* essential oil by fumigation are presented in Table 5. These data highlight an inverse relationship between exposure duration and lethal concentrations, underscoring the cumulative effect of the essential oil over time.

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However, starting at 96 hours, a significant decrease in LC_{50} and LC_{95} values is observed, reflecting a marked improvement in insecticidal efficacy. This trend continues steadily, with both LC_{50} and LC_{95} gradually decreasing up to 264 hours (LC_{50} = 2,268.76 µL/L; LC_{95} = 8,458.54 µL/L), where the essential oil appears to reach its optimal activity.The analysis also reveals a narrowing of the LC_{50}–LC_{95} interval over time, suggesting that variability in individual sensitivity decreases as exposure duration increases. This may reflect a more homogeneous action of the essential oil within the exposed population, likely due to the progressive accumulation or physiologically induced degradation caused by the active compounds.

The corresponding LC50 and LC95 for A. herba alba were respectively 5.22 and 45.18 L/L air for T. castaneum. A. absinthium, the corresponding LC50 and LC95 were respectively 35.18 and 59.87 L/L air for T. castaneum. **(Bachrouch et al., 2015)**.

Results of the present study indicated that Tunisian A. herba-alba and A. absinthium essential oils have fumigant and contact toxicity proprieties against adults of T. castaneum with A. herba-alba oil being more toxic in fumigant assay against T. castaneum with median lethal concentration of 278.66 L/L air (**(Bachrouch et al., 2015)**. Such activity seems to be moderate compared to Moroccan oils: LC50 values of 17.4 and 43.3 L/L air (**Abbad et al., 2014; Moumni et al., 2014**). While, fumigant activity of A. herba-alba seems to be stronger compared with the Iranian oil: LC50 = 564.4 L/L air (**Sharifian et al., 2012**).

The results of fumigation bioassay are shown at the two lowest volume fractions; the Artemisia sp. did not show statistical differences. At 100 µL/L, essential oil from A. herba-alba was more toxic than the other Artemisia sp. after 24 h of exposure. At the highest volume fraction (200 µL/L), all Artemisia sp. reached the highest toxic effect. The fumigant toxicity test showed that A. herba-alba was more toxic than the other Artemisia species. These results were confirmed by LC50 values shown in a fumigant test, a dosage of 142.8 µL/L of A. herba-alba was sufficient to kill 50 % of insects after 24 h of treatment, followed by A. absinthium with LC50 of 147.6 respectively. These results show clearly the effectiveness of A. herba-alba in comparison with the other two Artemisia species. In a previous study of Titouhi et al. (***Titouhi* F et al., 2017.**) A. herba-alba exhibited the best insecticidal effect against the two stored grain insects, Callosobruchus maculatus and Bruchus rufimanus, with LC50 of 7.7 and 8.3 µL/L, respectively **Chaieb et al., 2018)**.

LT_{50} and LT_{95} values (in hours) of *Artemisia herba-alba* essential oil against *Tribolium castaneum* adults at different fumigant concentrations. The lethal time values (LT_{50} and LT_{95}) decreased significantly with increasing concentration, showing a clear dose-dependent response. At low concentration (416.66 µL/L), the LT_{95} was extremely high (over 611,000 h), indicating very weak effectiveness. At higher doses (750 and 833.33 µL/L), LT_{50} values dropped below 40 hours, demonstrating rapid insecticidal action.

LT_{50} and LT_{95} values (in hours) of *Artemisia absinthium* essential oil against *Tribolium castaneum* adults at different fumigant concentrations. The results reveal an inverse dose–time relationship, typical of a temporal toxic response: the higher the concentration, the shorter the time required to induce mortality. At the lowest tested concentration (1000 µL/L of air), LT_{50} is estimated at 646.51 h and LT_{95} at 2548.14 h, indicating very limited and slow efficacy at this dose. As the concentration increases, both LT_{50} and LT_{95} values markedly decrease. At 1333.33 µL/L, LT_{50} drops to 430.34 h, then to 341.42 h at 1666 µL/L,

demonstrating increasing but still relatively slow efficacy. The most notable reductions are observed at the highest concentrations:

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Overall, the lethal time values indicate that the efficacy of the essential oil is strongly influenced by the applied concentration, with optimal effects starting at 2333 µL/L. However, the relatively high LT_{95} values even at high concentrations reveal that some tolerant individuals persist, possibly justifying the use of prolonged or combined-action formulations.

Comparison of the fumigant insecticidal activity of essential oils of *Artemisia herba-alba* and *Artemisia absinthium* against *Tribolium castaneum.* The evaluation of the insecticidal activity of *Artemisia herba-alba* and *Artemisia absinthium* essential oils through fumigant bioassays enabled the determination of LC_{50} values at different exposure durations, ranging from 24 to 264 hours.

The essential oil of *A. herba-alba* demonstrated marked efficacy as early as 24 hours, with an LC_{50} of 1090.09 µL/L of air. These values progressively decreased over time, reaching an LC_{50} of 560.81 µL/L after 144 hours of exposure. This trend reflects a significant increase in insecticidal efficacy over time, showing that *A. herba-alba* possesses a rapid and sustained fumigant action. In contrast, *A. absinthium* essential oil initially showed very high LC_{50} values, particularly at 48 hours (LC_{50} = 24,278.29 µL/L; LC_{95} = 230,512.44 µL/L), indicating low short-term efficacy. However, a gradual decrease in these values was observed as the exposure time increased. At 264 hours, the LC_{50} dropped to 2268.76 µL/L, indicating a notable improvement in toxic effect over the long term.

The comparison between the two oils reveals that *A. herba-alba* is significantly more active than *A. absinthium* at short and intermediate time points, with much lower LC_{50} values. This early efficacy may be attributed to the richness of the essential oil in rapidly acting volatile bioactive compounds. On the other hand, the delayed activity of *A. absinthium* suggests a slower release of its components or a cumulative effect requiring prolonged exposure.

These findings confirm the insecticidal potential of both *Artemisia* species, while highlighting their differences in terms of action kinetics. They are promising for the development of natural insecticidal formulations, whether for rapid action or prolonged release, depending on the desired profile.

The LT50 and LT95 respectively was 17.308 and 35.57 h for A. Herba Alba values. The LT50 and LT95 values 75.576 and 98.167 h, respectively, regarding A. absinthium essential

oils for T. castaneum. This led to that fumigant activity changed with insect strain, plant species, oil concentration, and exposure duration **(Bachrouch et al., 2015)**.

Discussion :

The gas chromatography–mass spectrometry (GC-MS) analysis of *Artemisia herba-alba* essential oil in our study revealed a rich profile of monoterpenes, particularly oxygenated monoterpenes, with camphor (32.59%) as the dominant compound, followed by 1,8-cineole (15.63%), camphene (7.93%), α-thujone (6.96%), and β-thujone (5.53%). These six compounds accounted for over 71% of the total composition, suggesting a camphor–1,8-cineole–thujone chemotype.

These results are partially consistent with those reported by Bachrouch et al. (2015), who identified 1,8-cineole (19.59%), camphor (11.48%), and α-pinene (5.4%) as major constituents in *A. herba-alba*. Similarly, previous studies by Haouari and Ferchichi (2009), Mighri et al. (2010), and Salido et al. (2004) confirmed the recurrent presence of camphor, 1,8-cineole, borneol, and α-terpineol as dominant components in this species.

However, noticeable differences appear in the relative proportions of these compounds depending on geographical origin. For instance, Bachrouch et al. (2015) reported lower camphor content (11.48–16.71%) compared to our result (32.59%), which may be attributed to ecogeographical, seasonal, and genetic factors, as highlighted by Barra (2009).

Regarding *Artemisia absinthium*, our analysis identified camphor (23.49%) as the dominant component, followed by the monoterpene alcohol 3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (11.59%), β-myrcene (7.55%), thujone (6.90%), γ-terpinene (6.08%), and α-pinene (6.04%). This profile highlights a high content of monoterpenes with potential bio insecticidal activity.

Our findings differ from those reported by Naimi et al. (2022), who found higher concentrations of camphor (36.22%) and α-thujone (30.28%), as well as significant amounts of chamazulene (8.02%). Variability in chemical composition is also evident in studies by Wani et al. (2014), who identified β-pinene (42.15%) and chrysanthenyl acetate (49.15%) as major constituents in Indian *A. absinthium*, and Bochenek et al. (2018), who found β-thujone (60.82%) to be the dominant compound in Algerian oils.

Bachrouch et al. (2015) also observed compositional variability, reporting β-thujone (22.72%) as the main component of *A. absinthium* oil, followed by camphor (16.71%), 1,8-cineole (5.47%), and camphene (2.37%). Other studies such as those by Riahi et al. (2013), Dhen et al. (2014), and Lopez-Lutz et al. (2008) confirm the chemical diversity of this species, attributed to soil conditions, climate, plant part used, and phenological stage.

Overall, the variability in essential oil composition of *Artemisia* spp. is well documented. The marked differences between our results and those of other studies may be explained by local pedoclimatic conditions, soil type, altitude, harvest time, and extraction methods.

Our results contribute to the existing body of knowledge by providing original data on the camphor–1,8-cineole–thujone chemotype of *A. herba-alba* and the camphor–myrcene–thujone profile of *A. absinthium* from our study region. These profiles may be correlated with enhanced biological efficacy, especially in insecticidal applications, which warrants further investigation in future studies.

The fumigant toxicity evaluation of *Artemisia herba-alba* and *A. absinthium* essential oils against *Tribolium Castaneum* adults revealed distinct insecticidal profiles and kinetics of action. In our study, *A. herba-alba* demonstrated a rapid and potent fumigant effect, with LC_{50} values decreasing significantly over time—from 1090.09 µL/L at 24 h to 560.81 µL/L at 144 h indicating sustained activity even at lower concentrations. In contrast, *A. absinthium* exhibited a much slower insecticidal response, with a high LC_{50} at 48 h (24,278.29 µL/L) that gradually decreased to 2268.76 µL/L after 264 h of exposure, pointing to a delayed mode of action.

These results are consistent with findings by **Bachrouch et al. (2015),** who reported that *A. herba-alba* essential oil was more toxic to *T. castaneum* adults than *A. absinthium*, with an LC_{50} of 278.66 µL/L for *A. herba-alba*. However, other studies such as **Chaieb et al. (2018)** showed even lower LC_{50} values (142.8 µL/L) for Tunisian *A. herba-alba*, which may be attributed to differences in chemical composition, geographic origin, and test conditions.

The differences in insecticidal efficacy between the two oils in our study are further supported by LT_{50} values. For *A. herba-alba*, a marked decrease in lethal times was observed with increasing doses, from 2456.93 h at 416.66 µL/L to 31.55 h at 750 µL/L. This dose-dependent acceleration of mortality highlights its potential for quick action. In contrast, *A. absinthium* required higher concentrations to achieve comparable mortality, with LT_{50} values remaining high even at 2666 µL/L (210.33 h), confirming its slower efficacy.

The higher fumigant potency of *A. herba-alba* can be attributed to its chemical richness in monoterpenoids such as camphor, 1,8-cineole, camphene, and borneol compounds known for their rapid volatility and lipophilic properties that allow them to penetrate the insect cuticle and disrupt physiological processes quickly (**Isman, 2000; Bakkali et al., 2008; Coloma et al., 2010**). These compounds have been previously associated with insecticidal activity in

multiple studies **(Salido et al., 2004; Paolini et al., 2010; Liu et al., 2014).** Our findings are thus in agreement with those of **(Chaieb et al. 2018),** who associated the strong fumigant effect of *A. herba-alba* with its high ketone content (e.g., chrysanthenone, 2-undecanone) and menthane-type monoterpenoids, including terpinen-4-ol.

On the other hand, the delayed insecticidal action of *A. absinthium* observed in our study may be due to a slower release or bioavailability of active constituents, or possibly due to a cumulative toxic effect. This hypothesis is supported by the work of **Chaieb et al. (2018),** who found *A. absinthium* to be more effective as a **repellent** than as a fast-acting insecticide. They reported ≥80% repellency at 0.08 µL/cm² within 1 h of exposure, likely due to its richness in bicyclic monoterpenes and cycloalkenes.

Moreover, the **synergistic interactions** among various components of the oils may explain the differences in toxic potential. Although camphor and thujone are often cited as the main active compounds, several authors (Coloma et al., 2010; Zhang et al., 2014) have emphasized that the overall insecticidal effect results from the combined action of both major and minor constituents.

Together, these results underline the high bio insecticidal potential of *Artemisia* essential oils. The contrasting kinetics observed in our study suggest different application possibilities: *A. herba-alba* may be better suited for rapid-action fumigant treatments, while *A. absinthium* could serve in prolonged-release formulations or as a repellent agent in integrated pest management (IPM) programs.

Conclusion :

The results of this study demonstrate that Artemisia herba-alba and Artemisia absinthium possess significant bio insecticidal properties against the secondary pest Tribolium castaneum, a major threat to stored grain products. Both plant species demonstrated mild fumigation resistance, with A. absinthium exhibiting slightly higher efficacy in terms of adult mortality and repellency over time.

Phytochemicals such as essential oils, particularly thujone and camphor, are likely responsible for the observed insecticidal effects. These results reveal that Artemisia-derived plant insecticides can be environmentally friendly alternatives to synthetic chemical insecticides, thus reducing environmental impact and minimizing the development of resistance within pest populations.

The microencapsulated formulation prolonged the toxic effect and stabilized the essential oil, while reducing its rapid evaporation, thus improving its effectiveness over time. These results suggest that microencapsulation of essential oils represents a promising biocontrol strategy, ecological and suitable for the protection of stored foodstuffs, as an alternative to chemical insecticides.

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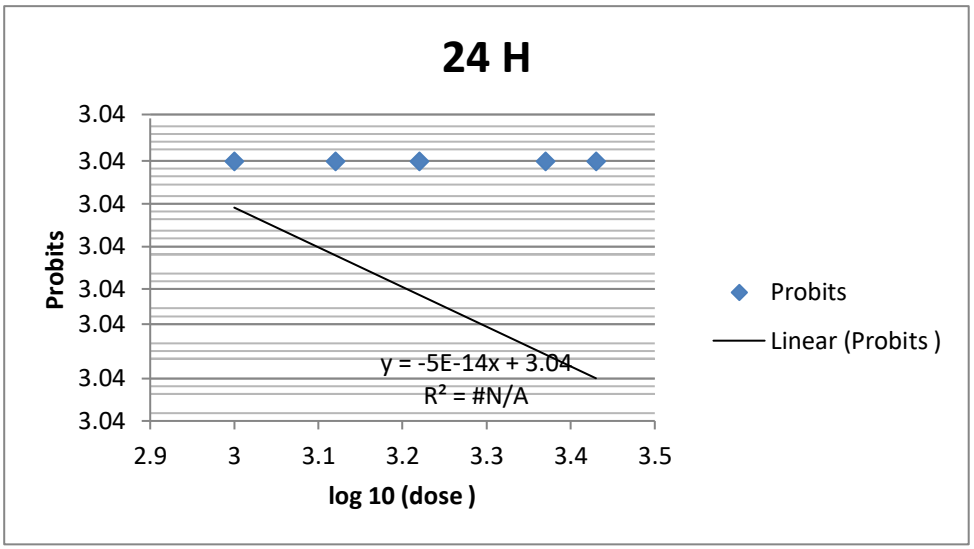
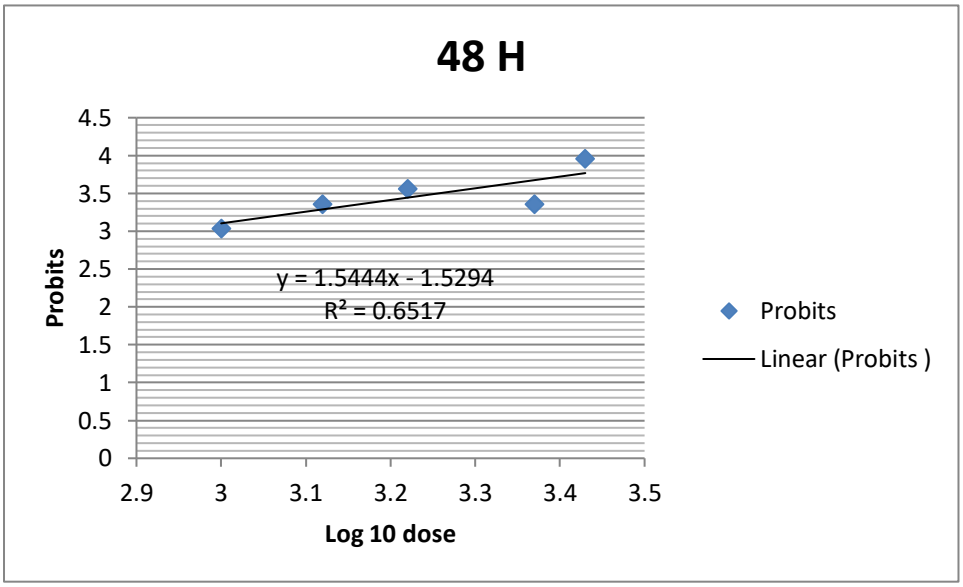
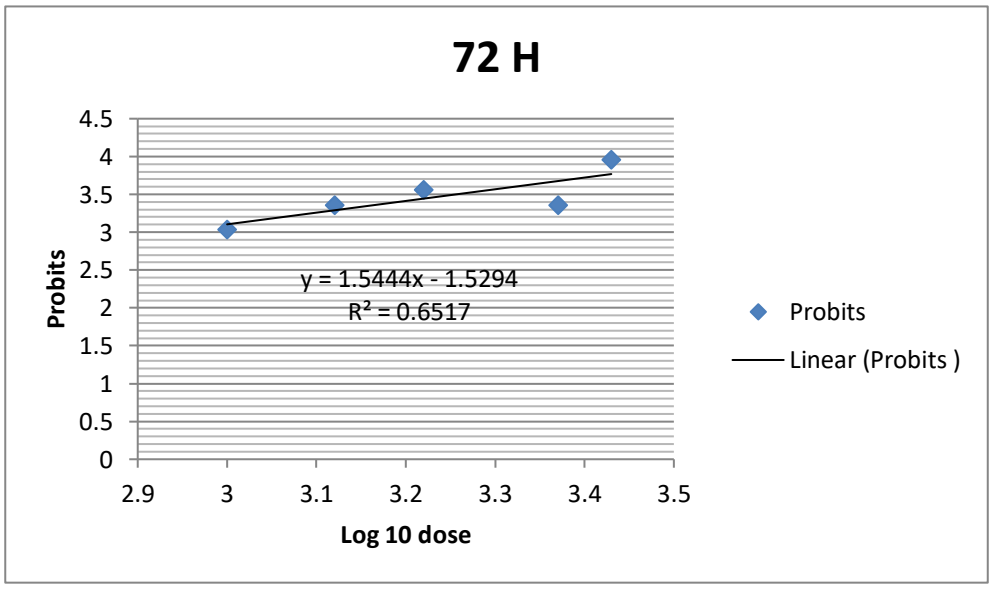
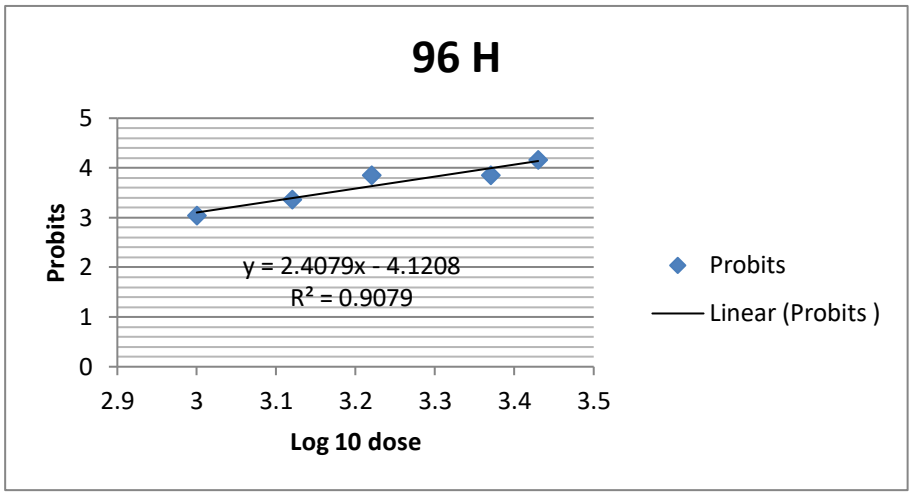
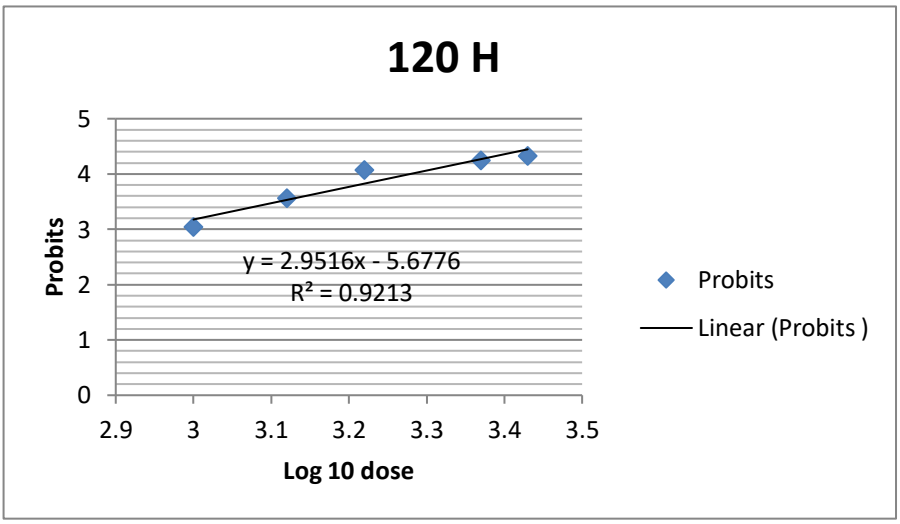
Annexes:

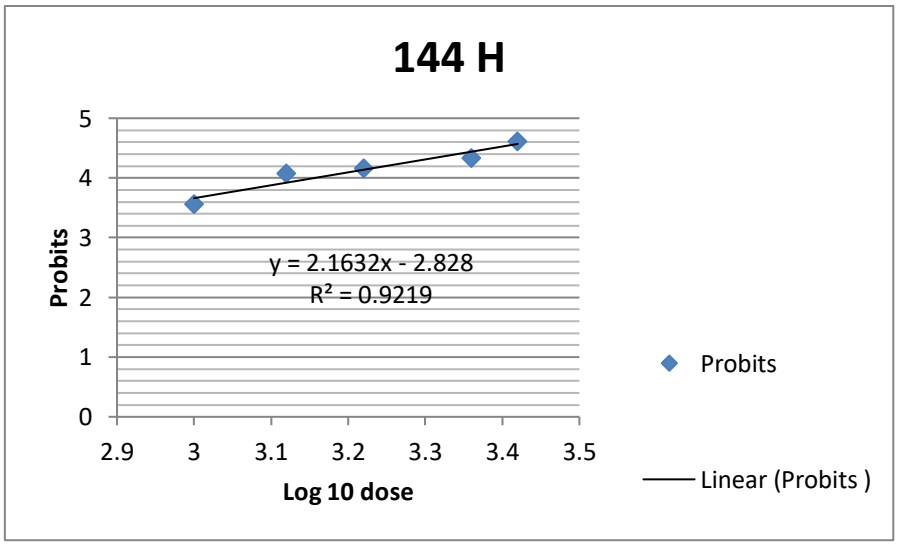

24 H
Probits
3.04
3.04
3.04
3.04
3.04
3.04
3.04
3.04
y = -5E-14x + 3.04
R² = #N/A
2.9
3
3.1
3.2
3.3
3.4
3.5
log 10 (dose)
Probits
Linear (Probits)

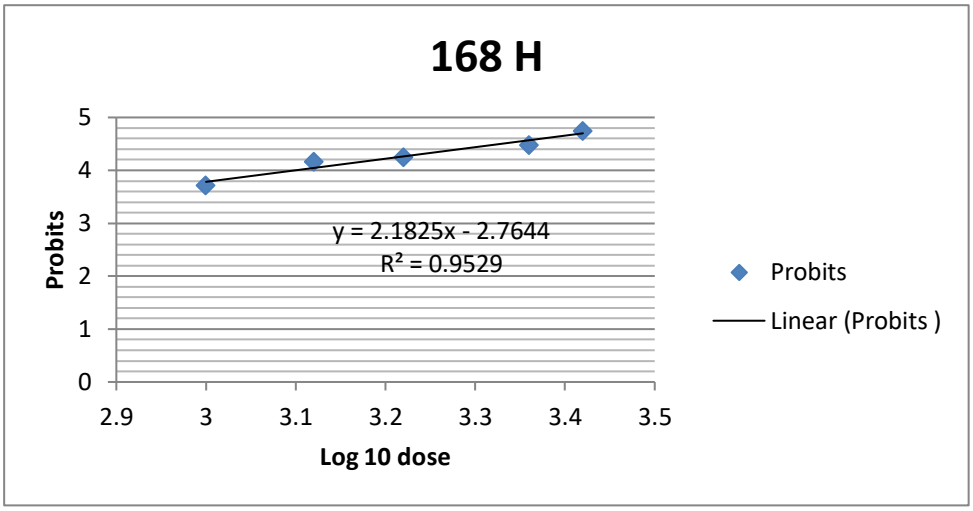

48 H
Probits
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
y = 1.5444x - 1.5294
R² = 0.6517
2.9
3
3.1
3.2
3.3
3.4
3.5
Log 10 dose
Probits
Linear (Probits)

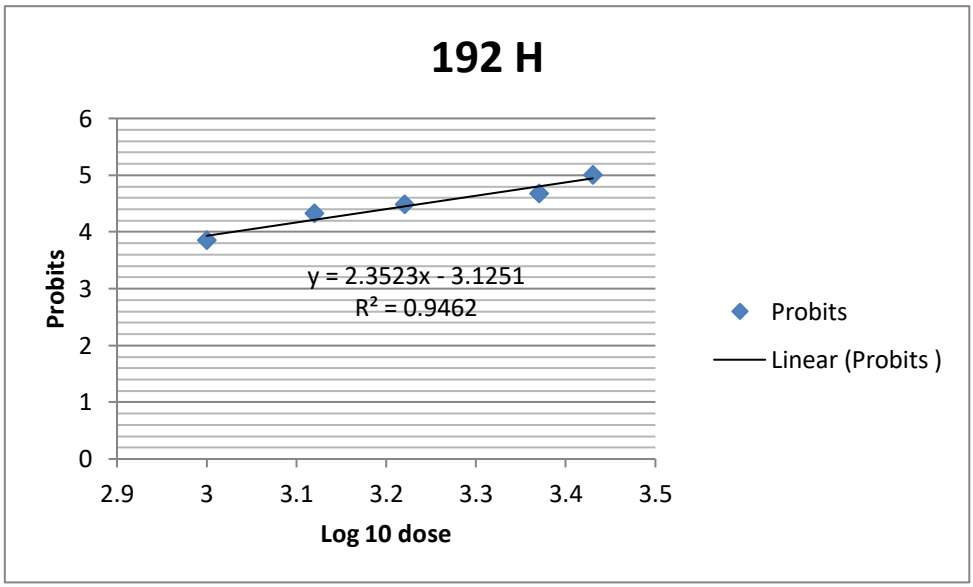

72 H
Probits
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
y = 1.5444x - 1.5294
R² = 0.6517
2.9
3
3.1
3.2
3.3
3.4
3.5
Log 10 dose
Probits
Linear (Probits)

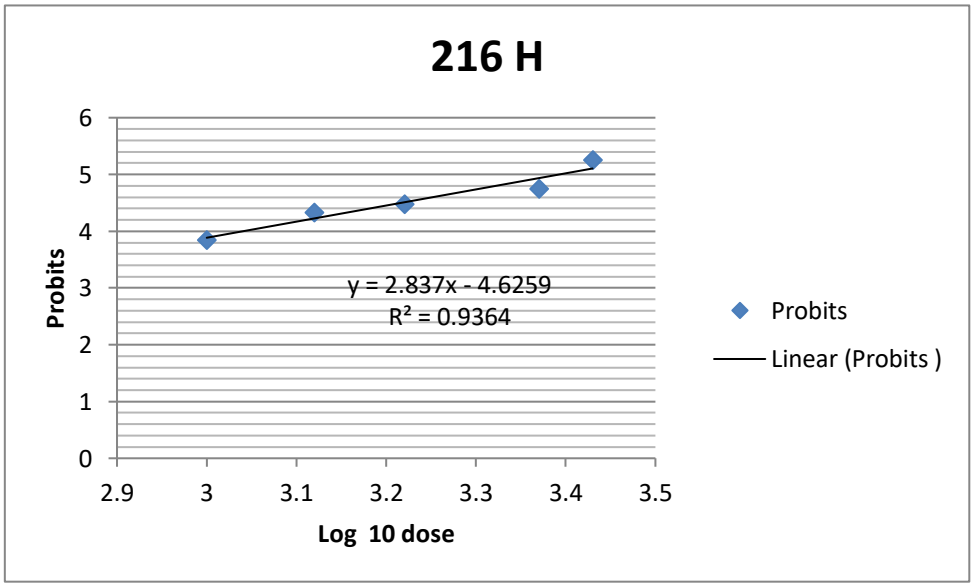

96 H
Probits
y = 2.4079x - 4.1208
R² = 0.9079
Log 10 dose
Probits
Linear (Probits)

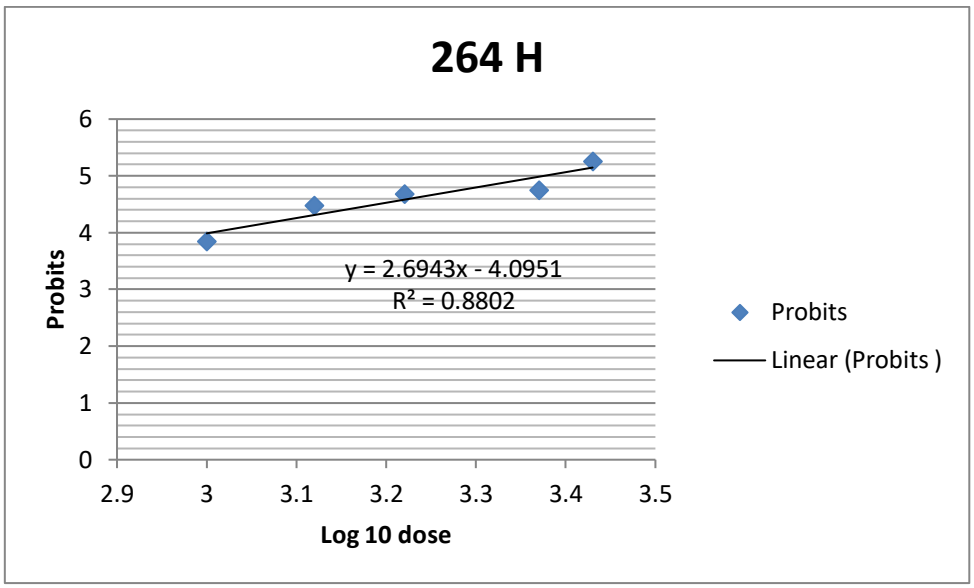

120 H
Probits
y = 2.9516x - 5.6776
R² = 0.9213
Log 10 dose
Probits
Linear (Probits)


144 H
Probits
y = 2.1632x - 2.828
R² = 0.9219
Log 10 dose
Probits
Linear (Probits)

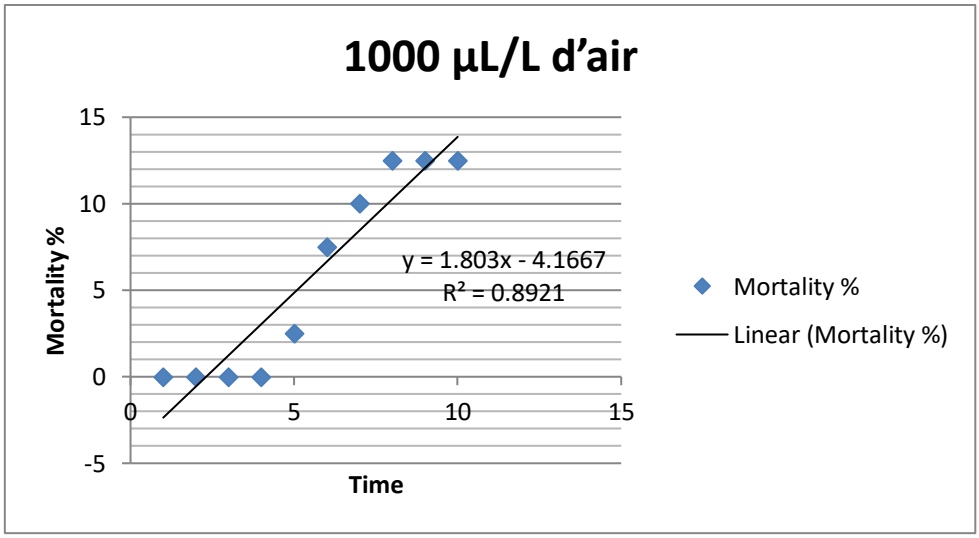

168 H
Probits
Log 10 dose
y = 2.1825x - 2.7644
R² = 0.9529
Probits
Linear (Probits)

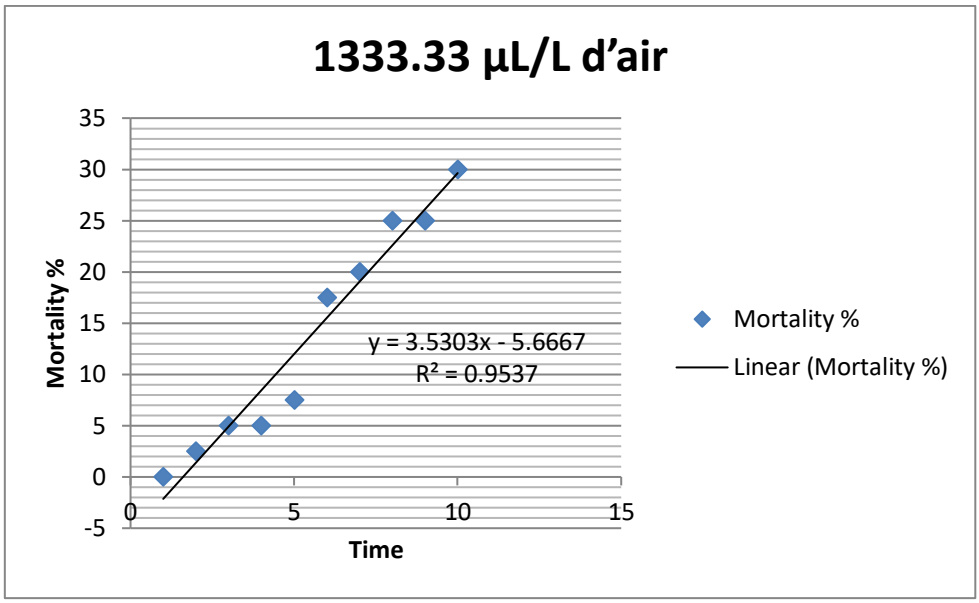

192 H
Probits
Log 10 dose
y = 2.3523x - 3.1251
R² = 0.9462
Probits
Linear (Probits)

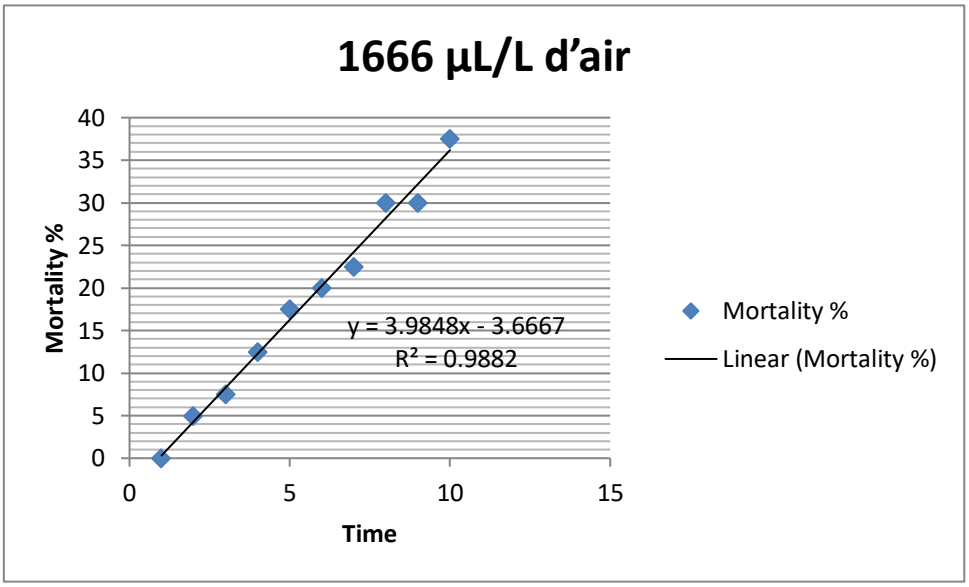

216 H
Probits
Log 10 dose
y = 2.837x - 4.6259
R² = 0.9364
Probits
Linear (Probits)

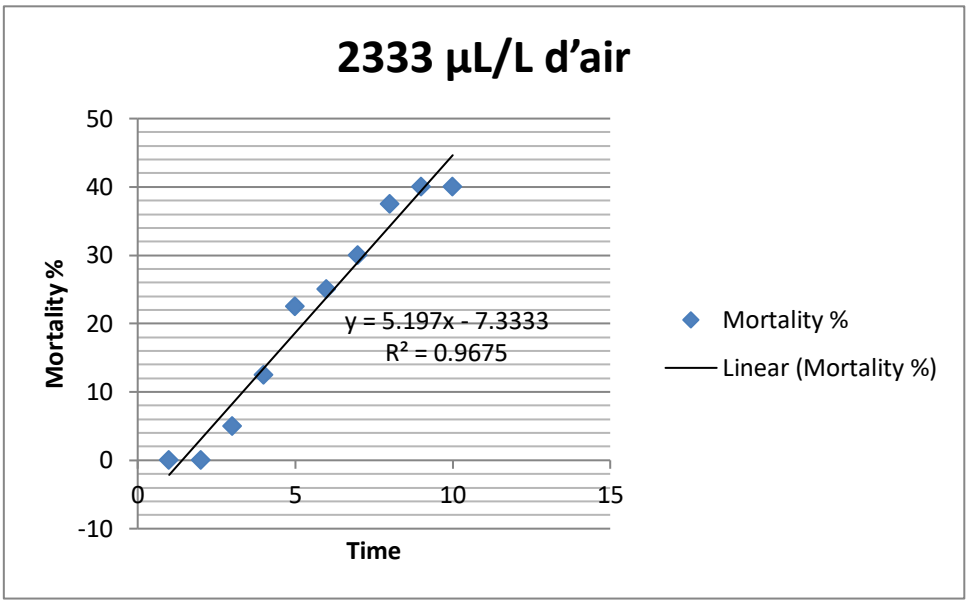


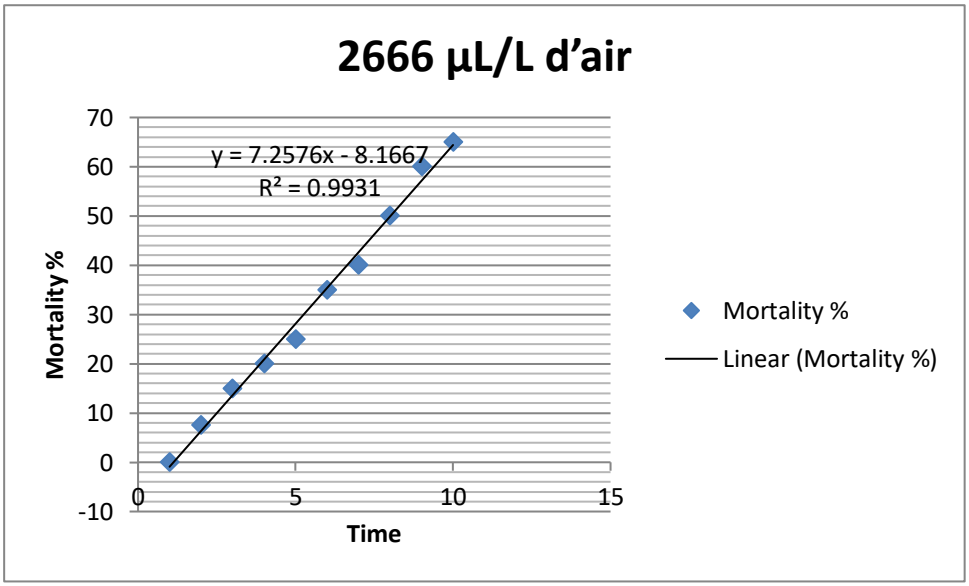
Annexe 1 : LC_{50} and LC_{95} values (µL/L air) of *Artemisia absinthium* essential oil against *Tribolium Castaneum* adults after fumigant bioassay at different exposure times.



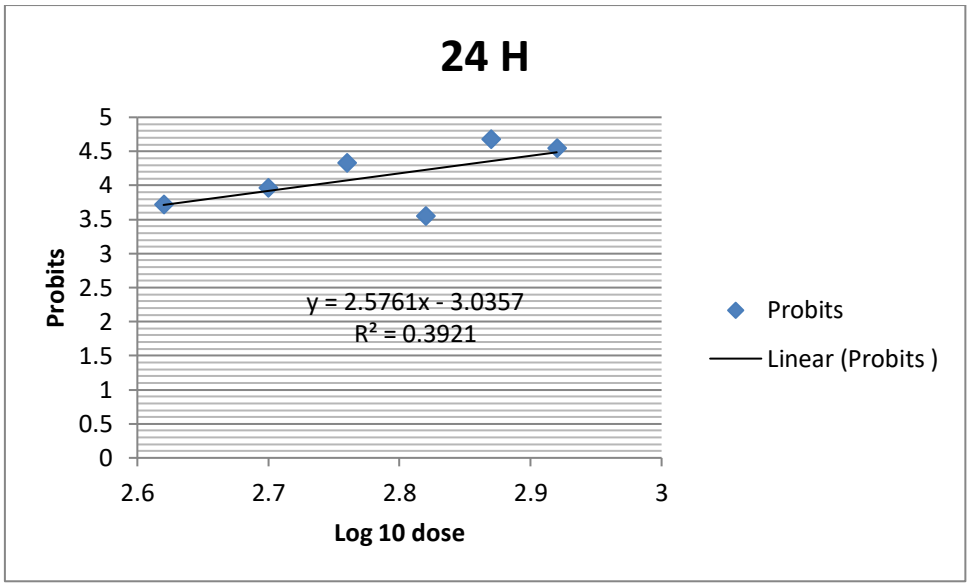

1333.33 µL/L d'air
Mortality %
Time
y = 3.5303x - 5.6667
R² = 0.9537
Mortality %
Linear (Mortality %)

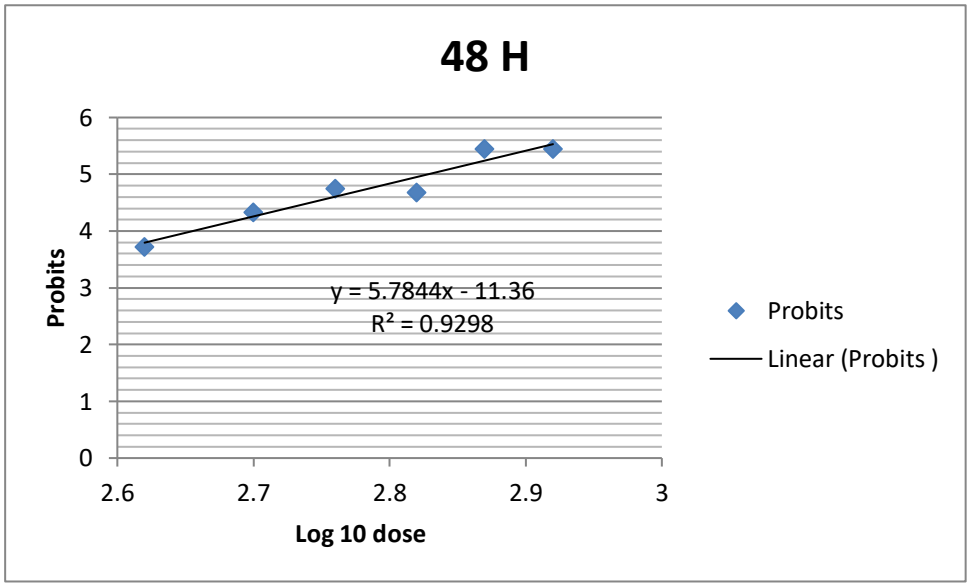

1666 µL/L d'air
Mortality %
Time
y = 3.9848x - 3.6667
R² = 0.9882
Mortality %
Linear (Mortality %)

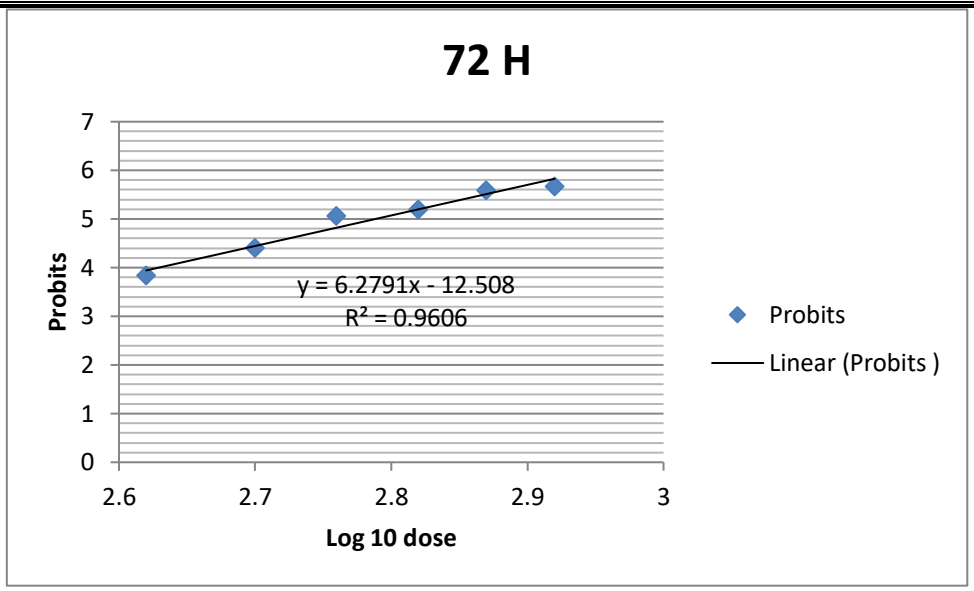


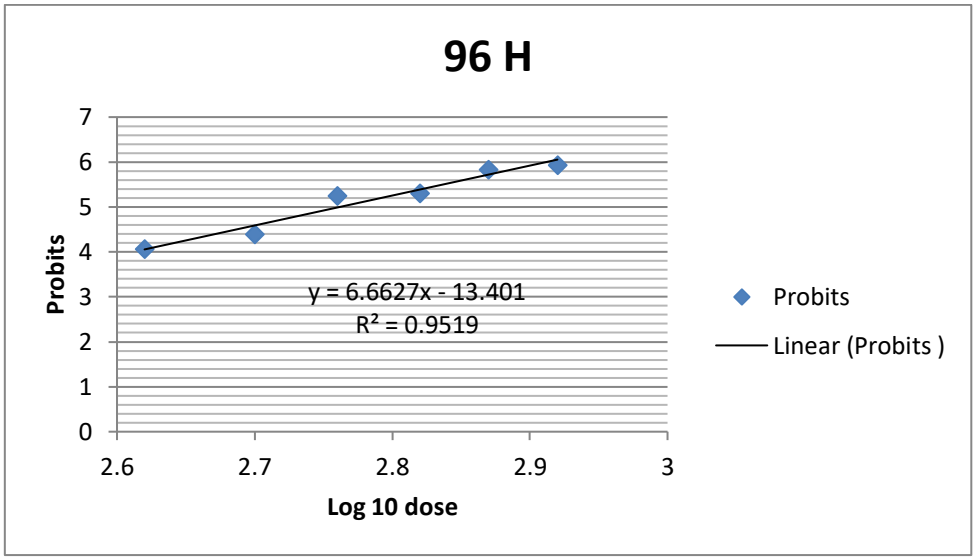


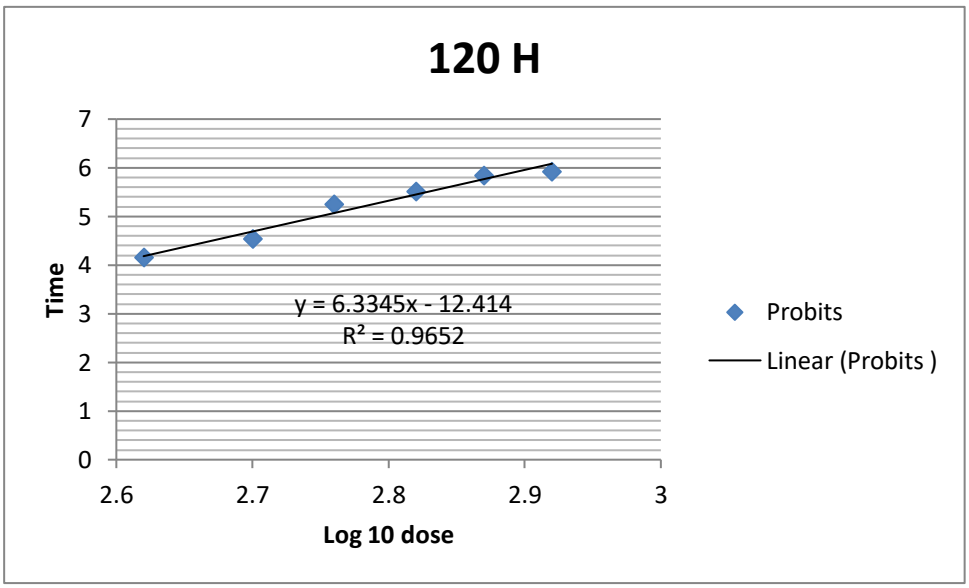
Annexe 2 : LT_{50} and LT_{95} values (in hours) of *Artemisia absinthium* essential oil against *Tribolium Castaneum* adults at different fumigant concentrations.

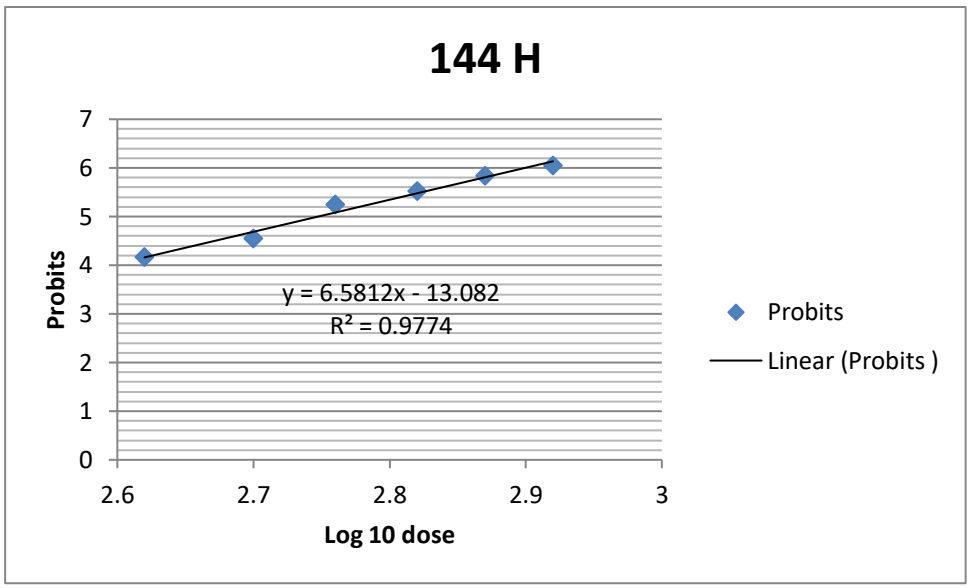

24 H
Probits
Log 10 dose
y = 2.5761x - 3.0357
R² = 0.3921
Probits
Linear (Probits)


48 H
Probits
Log 10 dose
y = 5.7844x - 11.36
R² = 0.9298
Probits
Linear (Probits)

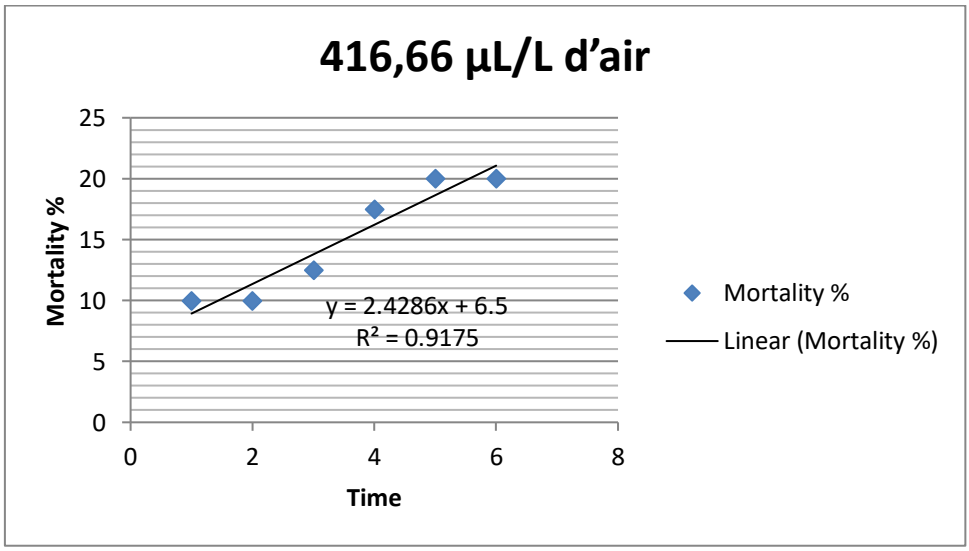

72 H
Probits
y = 6.2791x - 12.508
R² = 0.9606
Probits
Linear (Probits)
Log 10 dose

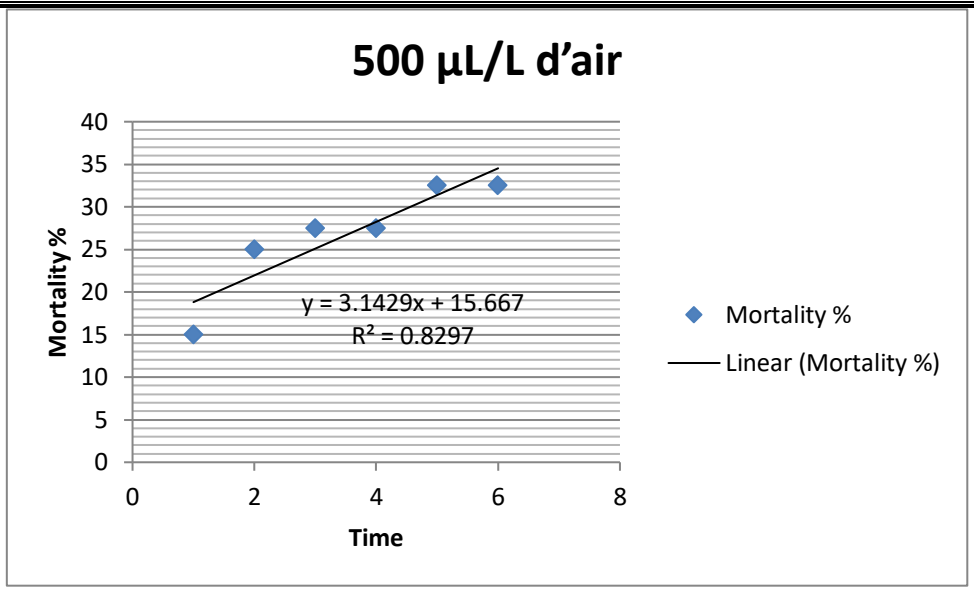

96 H
Probits
y = 6.6627x - 13.401
R² = 0.9519
Probits
Linear (Probits)
Log 10 dose

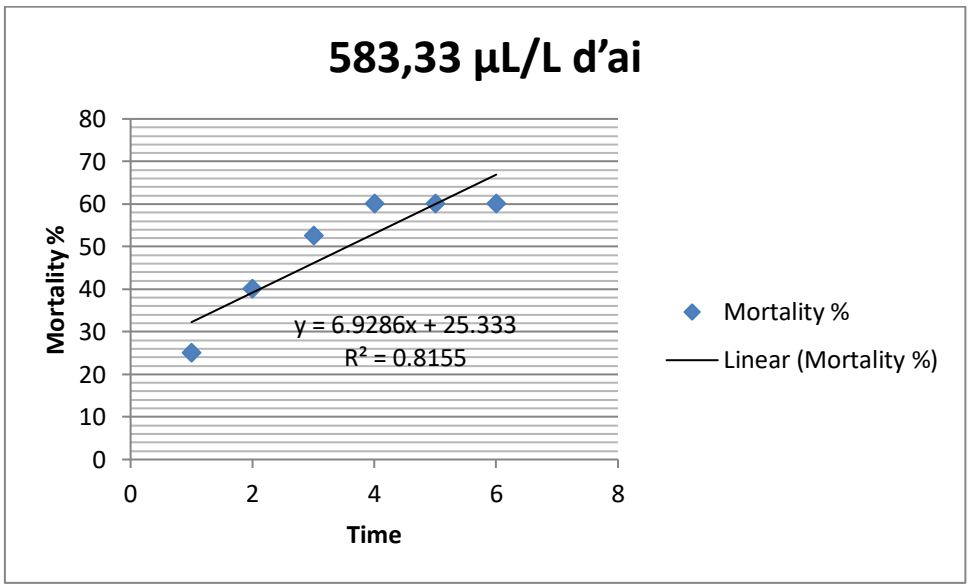

120 H
Time
y = 6.3345x - 12.414
R² = 0.9652
Probits
Linear (Probits)
Log 10 dose

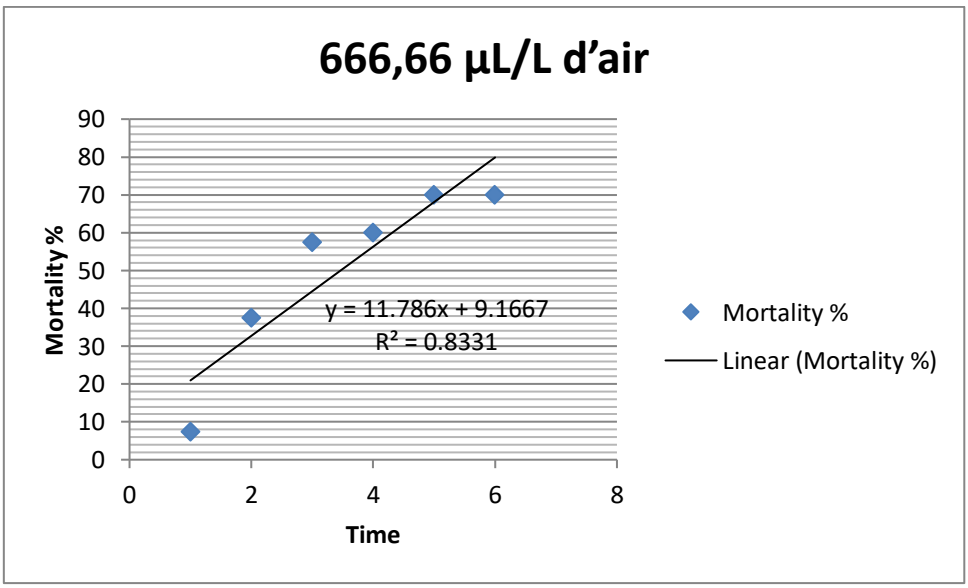


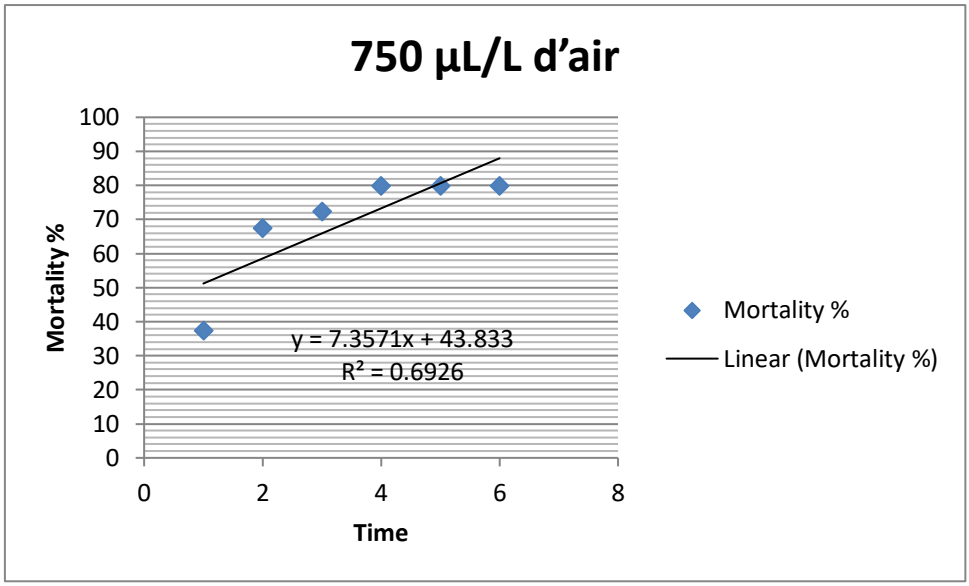
Annexe 3 : LC_{50} and LC_{95} values (µL/L air) of *Artemisia herba-alba* essential oil against *Tribolium Castaneum* adults after fumigant bioassay at different exposure times.

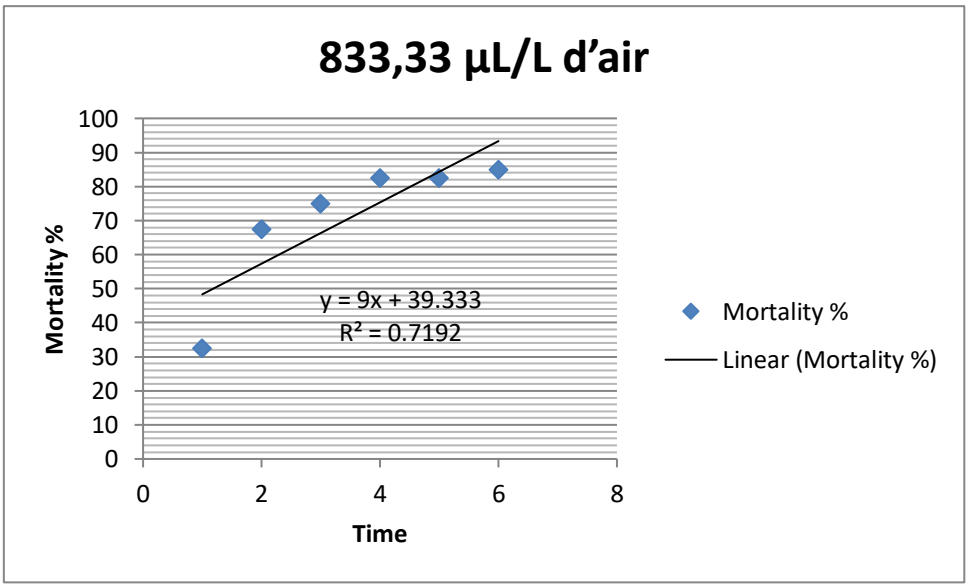



500 µL/L d'air
Mortality %
Time
y = 3.1429x + 15.667
R² = 0.8297
Mortality %
Linear (Mortality %)


583,33 µL/L d'ai
Mortality %
Time
y = 6.9286x + 25.333
R² = 0.8155
Mortality %
Linear (Mortality %)


666,66 µL/L d'air
Mortality %
Time
y = 11.786x + 9.1667
R² = 0.8331
Mortality %
Linear (Mortality %)





Annexe 4 : LT_{50} and LT_{95} values (in hours) of *Artemisia herba-alba* essential oil against *Tribolium Castaneum* adults at different fumigant concentrations.

PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA

MINESTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

UNIVERSITY OF BLIDA -1-

Faculty of nature and life sciences

Department of Biology

Dissertation

Dissertation submitted to the Department of Biology as a Partial Fulfillment for the Requirement for the Degree of Master in the field of nature and life sciences

Branch of Biological sciences

Speciality : Pharmacotoxicology

Theme

Evaluation of the toxicity of a Bioinsecticide Formulation based on Essential Oils of Artemisia Absinthium L. and Artemisia Herba Alba on the Beetle Pest ''Tribolium Castaneum''

Defense Date : 08/07/2025

Presented by :

FARES Noura **ABDALLAH Lamia** **BOUCELHA Chafika**

Jury Members

-Mrs KHALDOUN .H	**Pr**	**USDB-1-**	**Examiner**
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-Mrs HELLAL .N	**MCB**	**USDB -1-**	**Promoter**

Academic Year : 2024/2025