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Evaluation of the insecticidal effect of *Eucalyptus globulus* and *Juniperus communis* on *Blattella germanica*

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Abstract

Several investigations have assessed the efficacy of essential oils as potential replacements for synthetic insecticides, with the aim of addressing insect resistance to conventional pesticides. *Blattella germanica*, commonly known as the German cockroach, is regarded as a significant pest and disease vector, exhibiting considerable resistance to chemical insecticides. Eucalyptus and juniper, both medicinal plants prevalent in Algeria. The objective of this review was to evaluate the toxicological effects of essential oils derived from these two plants on adult male and female *Blattella germanica*. The review focused on studies that extracted essential oils from the aerial parts of the plants. The methodologies examined involved the application of juniper essential oil, eucalyptus essential oil, and a combination thereof to adult cockroaches via inhalation for a duration not exceeding six days, utilizing three distinct concentrations (100%, 50%, and 25%).

The experimental outcomes revealed promising results against *Blattella germanica*. The efficacy of eucalyptus essential oil on male specimens was observed during the initial three days, while its effect on females manifested after six days. Juniper essential oil demonstrated comparable effectiveness for both sexes. The combination of both essential oils exhibited enhanced efficacy at higher concentrations compared to lower ones. These findings suggest that the essential oils derived from eucalyptus and juniper possess potent insecticidal properties against German cockroaches, potentially serving as natural alternatives to synthetic pesticides.

Keywords: *Blattella germanica*; *Eucalyptus globulus*; Essential oil; *Juniperus communis*.

Résumé

Plusieurs études ont évalué l’efficacité des huiles essentielles comme substituts potentiels aux insecticides synthétiques, dans le but de lutter contre la résistance des insectes aux pesticides conventionnels. Blattella germanica, est considérée comme un important vecteur de ravageurs et de maladies, présentant une résistance considérable aux insecticides chimiques. Eucalyptus et genévrier, deux plantes médicinales répandues en Algérie. L'objectif de cette revue était d'évaluer les effets toxicologiques des huiles essentielles dérivées de ces deux plantes sur Blattella germanica mâle et femelle adulte. L'examen s'est concentré sur les études qui ont extrait les huiles essentielles des parties aériennes des plantes. Les méthodologies examinées impliquaient l'application d'huile essentielle de genévrier, d'huile essentielle d'eucalyptus et d'une combinaison de celles-ci sur des blattes adultes par inhalation pendant une durée n'excédant pas six jours, en utilisant trois concentrations distinctes (100 %, 50 % et 25 %).

Les résultats expérimentaux ont révélé des résultats prometteurs contre Blattella germanica. L’efficacité de l’huile essentielle d’eucalyptus sur les spécimens mâles a été observée au cours des trois premiers jours, tandis que son effet sur les femelles s’est manifesté après six jours. L'huile essentielle de genévrier a démontré une efficacité comparable pour les deux sexes. La combinaison des deux huiles essentielles a montré une efficacité accrue à des concentrations plus élevées par rapport à des concentrations plus faibles. Ces résultats suggèrent que les huiles essentielles dérivées d'eucalyptus et de genévrier possèdent de puissantes propriétés insecticides contre les blattes germaniques, servant potentiellement d'alternatives naturelles aux pesticides synthétiques.

Mots-clés : *Blattella germanica*; *Eucalyptus globulus*; *Juniperus communis*; huile essentielle

ملخص

قامت العديد من التحقيقات بتقييم فعالية الزيوت العطرية كبدائل محتملة للمبيدات الحشرية الاصطناعية، بهدف معالجة مقاومة الحشرات للمبيدات الحشرية التقليدية. يعتبر *Blattella germanica*، المعروف باسم الصرصور الألماني، من أهم ناقلات الآفات والأمراض، ويظهر مقاومة كبيرة للمبيدات الحشرية الكيميائية. الكينا والعرعر، وكلاهما من النباتات الطبية المنتشرة في الجزائر. كان الهدف من هذه المراجعة هو تقييم التأثيرات السمية للزيوت العطرية المشتقة من هذين النباتين على الذكور والإناث *Blattella germanica* البالغين. ركزت المراجعة على الدراسات التي استخرجت الزيوت العطرية من الأجزاء الهوائية للنباتات. تضمنت المنهجيات التي تم فحصها تطبيق زيت العرعر الأساسي وزيت الكينا العطري ومزيج منهما على الصراصير البالغة عن طريق الاستنشاق لمدة لا تتجاوز ستة أيام، وذلك باستخدام ثلاثة تركيزات متميزة (100٪، 50٪، و 25٪).

أظهرت النتائج التجريبية نتائج واعدة ضد *Blattella germanica*. وقد لوحظت فعالية زيت الكينا الأساسي على العينات الذكور خلال الأيام الثلاثة الأولى، بينما ظهر تأثيره على الإناث بعد ستة أيام. أظهر زيت العرعر الأساسي فعالية مماثلة لكلا الجنسين. أظهر مزيج الزيوت العطرية فعالية معززة بتركيزات أعلى مقارنة بالتركيزات الأقل. وتشير هذه النتائج إلى أن الزيوت الأساسية المشتقة من الكينا والعرعر تمتلك خصائص مبيدات حشرية قوية ضد الصراصير الألمانية، ومن المحتمل أن تكون بمثابة بدائل طبيعية للمبيدات الحشرية الاصطناعية

.الكلمات المفتاحية: الصراصيرالألمانية؛ الكينا؛ العرعر؛ الزيوت الاساسية.

Dedication

The journey was not short, the dream was not close, nor was the road fraught with ease, but I did it. I am very proud of myself.

To everyone who contributed to this work from near or far, especially my family, as they are always my first supporter.

First and foremost, I am deeply indebted to my parents. To Allah's paradise above earth, to the one whose heart embraced me in her arms, to the caring heart, to the light that lit the way for me, to the secret of my strength and success, to my encouragement and support, to my mother, no matter how you describe it, I will never do her justice.

To the one whose name I carry with pride, to my support and the source of my hopes... my father.

In the same way, I owe my thanks to my aunts Bella and Hakima and my uncle Omar for all the support they have provided.

In the same way, I owe my thanks to my aunts Bella and Hakima and my uncle Omar for all the support they have provided. *Thank you to my sisters Khawla and Bahia for their continuous encouragement and patience.*

Thank you for always being by my side! Expressing my affection and appreciation in words will never do justice to how deep my feelings are for them.

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I am deeply grateful to the Allah for granting me the privilege of reaching this stage of scholarly pursuit. My heartfelt appreciation extends to the divine bestowal of fortitude and perseverance, which empowered me to execute this modest contribution.

The culmination of this endeavor is the culmination of years of dedicated study. For this achievement, I would like to extend my sincere gratitude firstly to my esteemed teachers, whose tutelage shaped my academic journey. Moreover, I am profoundly indebted to my supervisor, Dr. SAIDA MALIKA, whose unwavering guidance, mentorship, and oversight proved invaluable in bringing this project to fruition.

Moreover, I extend my heartfelt gratitude to the distinguished members of the jury, my esteemed President ELAFRI ALI and my Examiner MAAMAR HICHEM, for their invaluable commitment to meticulously evaluating and providing their expert appraisal of this culminating dissertation.

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Introduction

Insects, particularly resilient pests, present a significant challenge in the realm of pest management. Their rapid reproduction rates, adaptability to various environments, and potential to transmit diseases make them a significant concern for both public health and sanitation **(Lee *et al.*, 2021).** Specifically, cockroaches also known as *Blattella germanica*, belonging to the order Blattodea and family Ectobiidae, is a ubiquitous nuisance pest found infesting man-made structures across the globe **(McPherson *et al.*, 2021)**. These insects exemplify one of the most evolutionary enduring insect lineages, retaining virtually the same appearance over the staggering timespan of nearly 400 million years (**Ebeling *et al.*, 1995).** *B. germanica* is highly adapted to different environments and easily reproduces in human places such as homes, hospitals, kitchens, bakeries, and restaurants **(Fazeli-Dinan *et al.*, 2022).** It has the ability to consume a wide range of organic materials, including human food, pet food, and livestock feed. This voracious behavior makes them potential carriers of various human pathogens, such as bacteria, viruses, fungi, and parasites, serving as efficient vectors to mechanically transfer these pathogens to humans **(Abbar & Wang, 2021).** Therefore, it is crucial to consistently monitor and implement control measures against *B. germanica* infestations **(Fazeli-Dinan *et al.*, 2022).**

Additionally, *B. germanica* is regarded as a significant pest from medical and economic standpoints due to its rapid reproductive cycle and high fecundity rates compared to other cockroach species. **(Morakchi *et al.*, 2010).** Conventional synthetic insecticides like pyrethroids, carbamates, and organophosphates are widely employed for cockroach management. However, these chemical pesticides pose risks of acute or chronic toxic effects to humans and animals. Furthermore, their environmental persistence can lead to bioaccumulation and disruption throughout food chains **(Saenmanot *et al.*, 2018).** Moreover, cockroach populations have developed resistance to every class of synthetic insecticide that has been introduced for their control since the early 1950s **(Fardisi *et al.*, 2019).**

In contrast, plant extracts offer a safer, eco-friendly alternative to synthetic insecticides. **(Saenmanot *et al.*, 2018)**. Plant-derived oils are gaining recognition as effective pest control agents for public health applications. These natural substances offer advantages such as low mammalian toxicity, rapid environmental degradation, and minimal ecological impact when used judiciously. **(Zhu *et al.*, 2012).**

For instance, eucalyptus, particularly its essential oil, is recognized as an effective natural insecticide. The oil, extracted from eucalyptus leaves, contains compounds like cineole, which have insecticidal and repellent properties **(Filomeno *et al.*, 2017)**. These compounds disrupt insects' nervous systems and mask attractant scents, effectively targeting pests. Eucalyptus oil offers a safer alternative to synthetic chemicals, reducing environmental contamination and health risks. Additionally, eucalyptus-based insecticides are biodegradable, making them suitable for organic farming and household pest management, thus promoting a more sustainable approach to insect control **(Batish *et al.*, 2008).**

Similarly, juniperus commonly known as juniper, is gaining attention for its potential as a natural insecticide **(Wedge *et al.*, 2009).** The essential oil derived from juniper berries and needles contains active compounds such as alpha-pinene and limonene, which exhibit insecticidal and repellent properties. These compounds interfere with the nervous systems of insects, leading to their immobilization or death. **(Höferl *et al.*, 2014)**. As a natural alternative to synthetic insecticides, juniper oil offers the benefits of reduced environmental impact and lower health risks for humans and animals. Its biodegradability and compatibility with organic farming practices make it an attractive option for sustainable pest management solutions **(Kun, 2012)**.

In line with the exploration of natural insecticidal alternatives, this study aims to evaluate the insecticidal activity for controlling *B. germanica* populations. Additionally, our objective is to investigate the toxicity of eucalyptus and juniper essential oils on adult male and female cockroaches.

This manuscript is organized into three main chapters, beginning with a characterization of *Blattella germanica*, followed by discussions on Eucalyptus and Juniperus respectively. The methodology employed to achieve the study's objectives is then detailed, succeeded by a presentation of the results. The work concludes with a summary of findings and proposes potential avenues for future research in this field.

Chapter 01

Blattella germanica

1. History

The insect *Blattella germanica*, commonly referred to in casual language as the German cockroach. The designation of this species was made by **Linnaeus (1758)**, attributing it to the geographic origin of his initial samples. *B. germanica* exhibit a generally widespread distribution across various global locales, it is present in many different regions worldwide **(Tang *et al*., 2018).** They represent one of the most ancient insect groups found in the fossil record, with specimens preserved in sediments from the Carboniferous geological period. This makes them among the oldest fossilized insect species discovered thus far **(Bezerra *et al*., 2020)**.

Among all insect species, *B. germanica* possess the lengthiest historical existence on earth, characterized by exceptional resilience and the ability to adapt to diverse ecological conditions **(Gondhalekar *et al*., 2021)**. Certain cockroach varieties exhibit a synanthropic ecological pattern, meaning they live in close proximity to human habitats and food sources. They are often collectively termed 'synanthrope' and accordingly display an extensive global distribution intertwined with human, animal, and food item transport **(Abudin *et al*., 2023).** *B. germanica* have coexisted and interacted with human civilizations for hundreds of years. They originated in Africa and eventually spread to Europe through ancient trade networks, establishing an enduring interconnection with human populations via their movements along routes facilitated by human activity and commerce over the centuries **(Tang *et al*., 2018).**

During the age of exploration, the unintentional transportation of *B. germanica* aboard ships contributed to their widespread distribution across various continents. As international travel and commerce expanded in the 19th century, the prevalence of *B. germanica* became global **(Wang *et al*., 2021).**

The ability of these insects to adapt to diverse environments, thrive, and reproduce at high rates has been instrumental in their persistence and infestation of urban areas **(Johnson & Munshi-South, 2017).**

2. Origine and geographic distribution

Some species of *B. germanica*, originally from tropical regions, have been accidentally brought into close association with human settlements, adapting to become fixtures of urban environments. Now ubiquitous inhabitants occupying all major world cities, these omnivorous *B.*

germanica species have achieved a global cosmopolitan distribution intertwined with human activity **(Tang *et al*., 2018).**

B. germanica have acclimated to diverse human living spaces, and have proven able to thrive across the full spectrum of human dwelling places, including private residences like houses, apartments, and hotels, as well as healthcare facilities such as hospitals. Additionally, these insects infest commercial establishments such as restaurants, farms, animal housing, storage sites, and mobile containers used for transport **(Tang *et al*., 2016).**

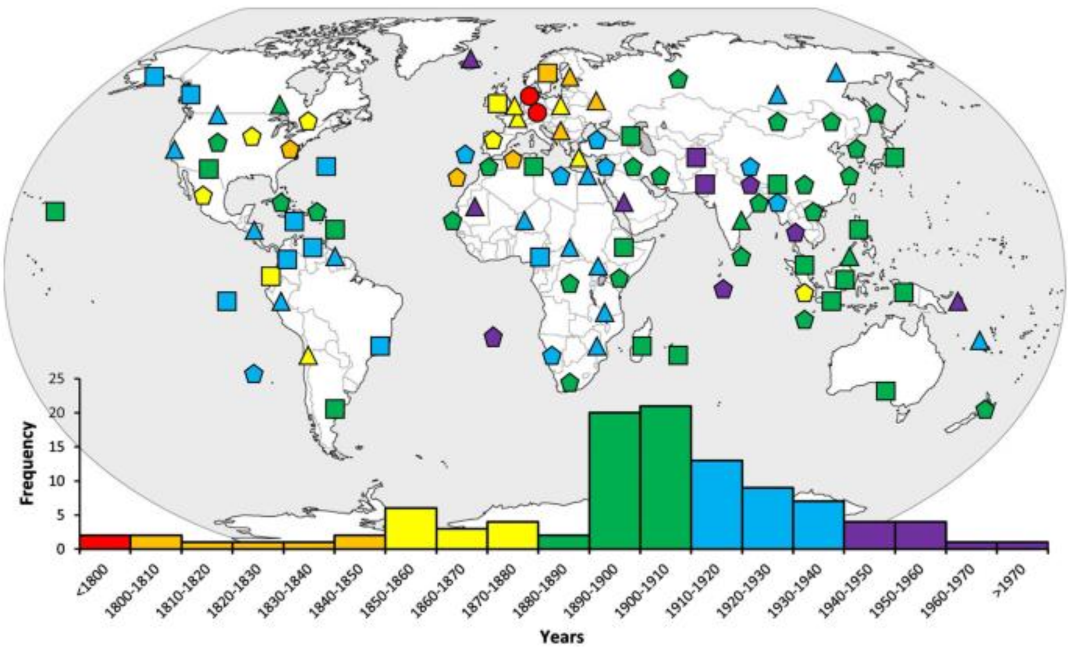

Figure 1. Map summarizing the first reports of *B. germanica* around the world **(Tang *et al.*, 2018)** Each dot indicates the first occurrence of *B. germanica* in a specific region. The colors represent 30-year periods, with the first decade represented by triangles, the second decade by rectangles, and the third decade by pentagons. The histogram shows the number of reports for each 10-year interval.

3. Taxonomy

B. germanica belong to the Pterygota subclass of insects which have wings present in the mature, adult stage of their life cycle **(Agnès, 2014).**

McKittrick (1964) classified *B. germanica* primarily based on their physical characteristics and egg-laying behavior, resulting in the subdivision of these insects into two major superfamilies: Blaberoidea and Blattoidae. The Blaberoidea superfamily consists of three families: Blaberoidae, Blattellidae, and Polyphagidae, while the Blattoidae superfamily consists of two families: Blattidae and Cryptocercidae. This classification remains in use today, supported by studies in morphology, physiology, and behavior.

Descendants of *B. germanica* belong to the class Apterygota, order Dictyoptera, and family Blattellidae. The systematic position of this insect, according to **Linnaeus (1767)**, is as follows:

Régne: Animalia

Embranchement: *Arthropoda*

Sous embranchement: *Mandibulata*

Classe: *Insecta*

Sous classe: *Pterygota*

Ordre: *Dictyoptera*

Sous ordre: *Blattaria*

Super famille: *Blaberoidae*

Famille: *Blattellidae*

Sous famille: *Blattellinae*

Genre: *Blattella*

Espèce: *Blattella germanica*

4. Description

4.1 Eggs

B. germanica eggs have an oval shape and range in color from light to dark brown. Females deposit the egg capsules, called oothecae, in hidden, sheltered sites. A mature female transports each oblong, shiny brown ootheca **(Noda, 2020),** measuring approximately 7mm long by 3mm wide, attached to her hind region. The egg case's slimy, pliable texture helps protect the 30 to 40 eggs inside from physical damage. A female *B. germanica* carries the ootheca with her until the eggs are ready to hatch. Over her lifetime, an individual female *B. germanica* produces about eight oothecae. The eggs require 17 days of incubation before they emerge as nymphs **(Gemeno *et al*., 2011).**

Figure 2. Oothecae of *B. germanica* **(Agnès, 2014)**

4.2 Larva

The immature nymphal stage lasts around 3 months, during which German cockroach young progress through 6 to 7 molts transitioning between instars. Given suitable environmental conditions of moderate warmth, 25 to 33°C, and humidity, 60 to 80% relative humidity **(Agnès, 2014).** The larvae resemble adults but are wingless, the main changes in larval development occur in size and pigmentation **(Ghermoul, 2020).**

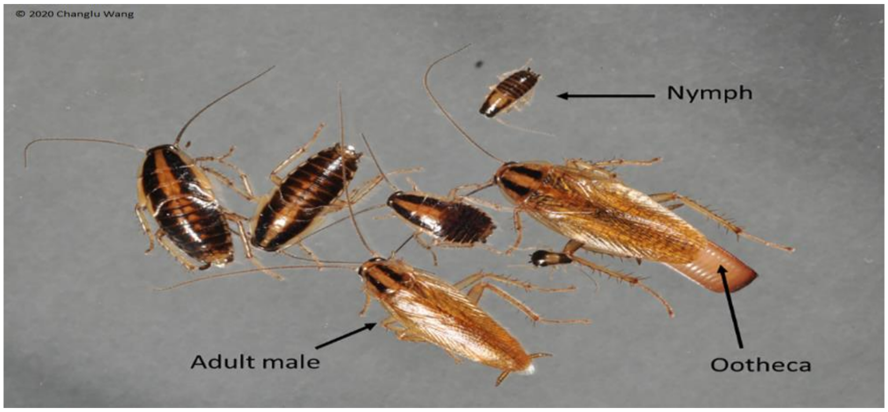

Figure 3. Larva (juvenile) of *B. germanica*, approximately 5 mm **(Wang, 2020).**

4.3 Adult

The general shape of *B. germanica* is dorsoventrally flattened and oval, and their coloring is light brownish-yellow **(Wilson *et al.*, 2007).** Their size varies, ranging from 10 to 15 mm in length, with a highly mobile head that is mostly hidden beneath a shield-shaped portion of the thorax known as the pronotum, a shield-shaped plate covering the head. They possess two slender, mobile, and flexible antennae. Their mouthparts are of the conventional grinding type, positioned hypognathically **(Pai *et al*., 2003).**

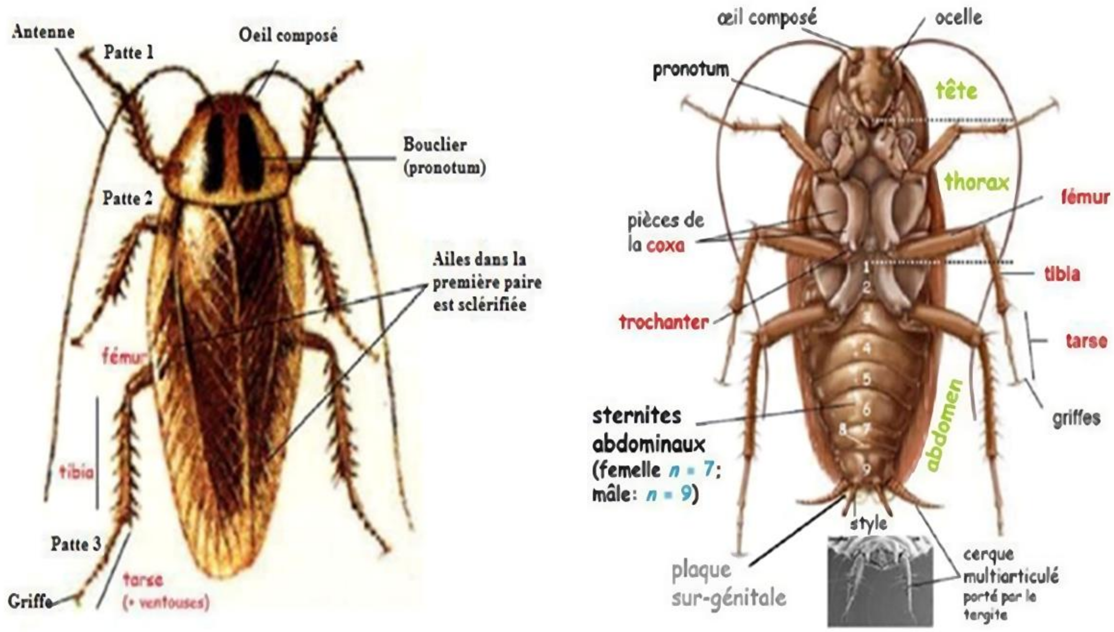

Figure 4. Dorsal and ventral view of an adult male *B. germanica* **(Ghermoul, 2020)**

5. Biological cycle

5.1 Reproduction

Throughout the breeding season, female *B. germanica* release pheromones to attract males. In certain species, males participate in a courtship ritual characterized by intricate movements of their appendages and the production of sounds through stridulation. During mating, the male and female adopt an inverted position, facilitating direct contact between their genitalia **(Wada-Katsumata & Schal, 2019).** As demonstrated by **Woodruff (1938)**, a single mating event may provide enough sperm to fertilize all the eggs a female *B. germanica* produces as an adult. Around 8 days after molting into the sexually mature imago form, a female begins forming 6 to 8 ootheca egg capsules - each 8 mm long and containing 30 to 40 eggs, during successive gonadotropin-driven reproductive cycles**.** *B. germanica* is an insect that undergoes heterometabolous development, also known as incomplete metamorphosis, which is characterized by two distinct post-embryonic forms, larvae and adults **(Cruz *et al*., 2023)**.

Accumulate sexual attraction pheromones in female antenna, upon a female encountering a male, antennal rubbing between the pair stimulates the courtship sequence. The male raises his wings upward, exposing the tergal gland, then, the female feeds on the gland secretions and, taking advantage of the feeding position, the male accomplishes mating **(Belles *et al*., 2022).**

5.1.1 Egg stage

The process begins with the fertilization of the eggs and ends with their hatching.The eggs are collected within a hardened capsule known as an ootheca, which is formed and extruded during the laying process. The ootheca typically holds around 30 to 40 eggs, aligned perpendicularly in twin vertical columns on either side of a central divider, with individual partitioned pockets each housing a single egg **(Latorre *et al*., 2022).**

Over a typical lifespan, female *B. germanica* lay about 300 eggs in total. The ootheca egg cases are often deposited by the female near food sources just prior to hatching, though a small fraction may remain affixed to the mother's legs for a brief period until the nymphs emerge. The formation of a second ootheca typically takes about two weeks **(Appel, 2021).**

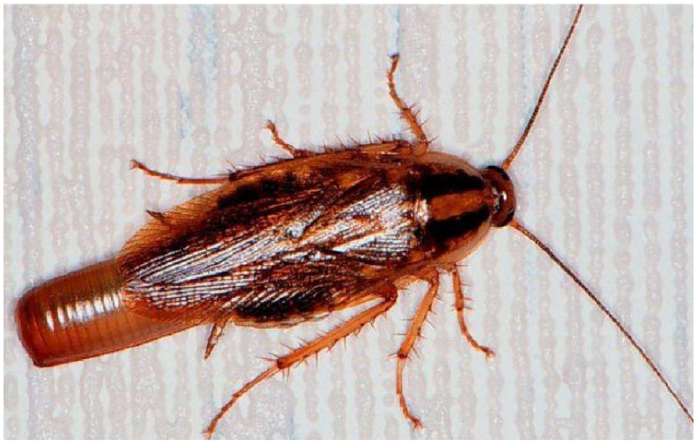

Figure 5. Female *Blattella germanica* carrying her ootheca **(Agnès, 2014).**

5.1.2 Nymph stages

At the onset of their emergence, larvae are grounded, lacking the ability to take flight **(Alzogaray *et al*., 2013).** Over subsequent development at 25°C, spanning approximately 100 days **(Dingha, 2005),** *B. germanica* nymphs undergo six successive molts, transitioning between larval instars. The last nymph stage persists for 40 days in males and 41 days in females before the final imaginal molt produces mature winged adults **(Perkins *et al*., 1961).** Main transformations during larval growth involve increasing body size and progressive pigment changes**.** Older late-instar larvae closely resemble adults morphologically but lack wings, possessing a singular pale stripe along the dorsal midline surface **(Rust *et al.*, 1995).**

5.1.3 Adult stage

The adult phase commences with the imaginal molt, marking day 0 of adulthood. At this stage, the adult possesses two pairs of wings in the case of Diptera, but it does not possess the ability to fly **(Wigglesworth, 1972).** The duration of the adult stage in *B. germanica* varies based on influential external factors like ambient temperature, humidity, food availability, and threats from predators, as well as differences linked to biological sex. On average, adult females exhibit greater longevity compared to their male counterparts **(Gore *et al*., 2007).**

Figure 6. Newly molted adult *B. germanica* **(Ghermoul, 2020)**

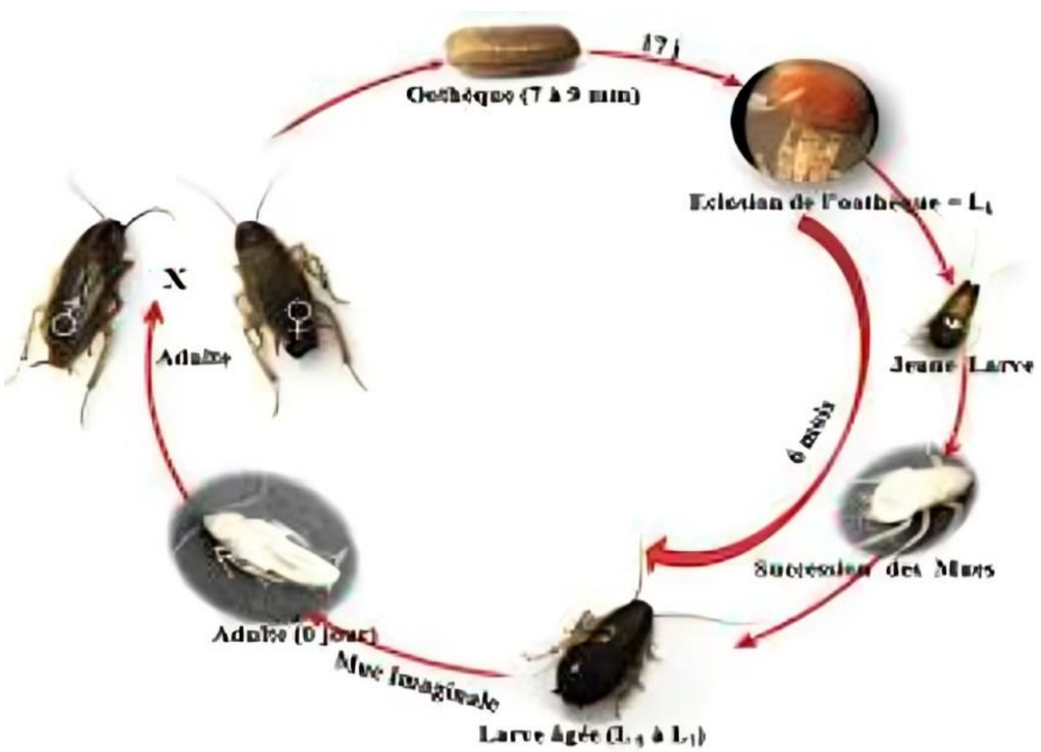

Figure 7. Life cycle of the *B. germanica* **(Ghermoul 2020).**

6. Habitat

B. germanica varieties tend to exhibit a cosmopolitan global distribution, inhabiting most world regions rather than having a restricted endemic range. Originally of tropical lineage, certain *B. germanica* species have been accidentally transported by human activities into close association with anthropogenic habitats **(Bourguignon *et al.*, 2018).** Displaying remarkable adaptability, these insect pests can thrive across diverse climate extremes, from tropical to subtropical, temperate, and desert conditions **(Tang, 2018).** When selecting habitat spaces, *B. germanica* minimize travel distances required to access the resources needed to carry out essential survival functions **(Rivault & Cloarec, 1993).**

7. Communication

Chemical communication plays a vital role in various stages of partner acquisition in *B. germanica* This communication primarily depends on the exocrine glands present in the sensilla of the antennae, which release volatile pheromones **(Habbachi *et al.*, 2009).** These glands include the collar glands, upper and lower hypostomal glands, maxillary glands, hypopharyngeal glands, mandibular glands, tergal glands (found in males), and sternal glands. These glands facilitate long-distance attraction of males towards females **(Habbachi *et al.*, 2009).**

According to **Boutta (2017)** there are three categories of pheromones:

- **Sexual pheromones:** Are emitted by one of the two sexes. In *B. germanica*, the pheromone produced by the female is volatile and it attracts the male from a distance of 2 meters. It is produced by pygidial glands located at the end of the abdomen.
- **Herding pheromones**: The tendency to group together is common in *B. germanica*.

8. Diet

B. germanica with their omnivorous diet, demonstrate remarkable adaptability to a wide array of food sources **(Gordon, 1996).** This trait is particularly evident in species that pose a threat to human habitats. They consume vegetables, fruits, human and animal waste, as well as various types of discarded items **(Mullen *et al.*, 2009).** Domestic *B. germanica* typically gravitate towards areas abundant in food and water. They prefer to consume their food at the site rather than transporting it back to their hiding spots. While they are solitary creatures, males generally require smaller amounts of food compared to females **(Ward *et al.*, 2016).**

9. Impact on human health

B. germanica frequently migrate between buildings and infiltrate homes through gutters, sewers, and latrines. Given their diet, which includes both human waste and food, they can transmit pathogenic bacteria. The texture of their cuticle provides an ideal surface for germs to adhere to **(Tang *et al.*, 2016).** These insects move across surfaces in search of sustenance or warmth. Consequently, upon encountering food, they can directly or indirectly deposit germs, often through contact with excrement **(Mullen *et al.*, 2009).**

9.1 Nuisance

The unhygienic behaviors of *B. germanica* make them a nuisance pest **(Roth & Willis, 1954).** They contaminate stored goods with fecal traces and a foul-smelling blackish vomit residue deposited as they eat, imparting an unpleasant odor to infested areas **(Donkor, 2020).** *B. germanica* salivary and body secretions can alter the flavor of foods prepared with tainted cookware, causing items contacting roaches to retain a persistent, unpalatable stench **(Agnès, 2014).** Their presence becomes associated with unclean conditions, offensive odors, and general disgust **(Roth & Willis, 1954).**

9.2 Disease transmission

B. germanica traverse environments without restrictions, allowing contact with human and animal feces as they undertake exploratory travels routinely utilizing sewer systems, latrines, plumbing conduits and other unsanitary infrastructure networks as thoroughfares **(Tachbele *et al.*, 2006).**

B. germanica can transport pathogenic agents for humans **(Cornwell, 1968).** While they are not the direct cause of specific diseases, they can play an additional role in the mechanical propagation of agents responsible for certain conditions **(Roth & Willis, 1954).** *B. germanica* coming from outside will potentially be able to contaminate food with bacteria, specifically *Salmonella spp*, which is responsible for food poisoning, these bacteria can persist for up to 35 days in *B. germanica* feces **(Tachbele *et al.*, 2006).**

Their role in this regard is suspected or demonstrated in cases of diarrhea, dysentery, cholera, leprosy, plague, typhoid fever, virosis, etc **(Roth & Willis, 1954).** *B. germanica* have the reputation of transmitting organisms such as *Staphylococci*, *Streptococci*, coliform bacteria, and the poliomyelitis virus. A study demonstrated that *Staphylococcus aureus* is found for 14 days in the feces of *B. germanica* **(Tachbele *et al.*, 2006).** These insects also transmit infectious diseases such as hepatitis, cholera and tuberculosis **(Gordon, 1996),** as well as their presence can trigger asthma attacks **(Cipriani *et al.*, 2017).** Studies on bacteria carried by *B. germanica* have revealed their widespread antibiotic resistance, observed in both hospital and urban environments. These microorganisms are disseminated through several ways, primarily by depositing them with their excrement onto human food **(Guzman, 2020).**

9.3 Allergies

B. germanica bodily products like feces, saliva, secretions, and cast exuviae contain potent allergens capable of provoking cutaneous reactions, atopic dermatitis, pruritic responses, eyelid inflammation, rhinitis, and most notably, asthma attacks. As early as the 1940s, researchers linked *B. germanica* antigen exposure to hives, skin rashes and asthmatic episodes in sensitive individuals **(Agnès, 2014).**

A research group in Tunisia conducted a prospective study of 105 randomly selected middle-aged patients aged 1approximately 26 years to investigate connections between *B. germanica* exposure events and associated health impacts. For domestic infestations, the *B. germanica* was identified most frequently, 34% of cases, as the culprit species triggering medical issues **(Ben M'rad *et al*, 2004).** Even low *B. germanica* populations can generate substantial allergen loads; individual adult female *B. germanica* are capable of producing between 25,000 to 50,000 allergenic protein units over their lifespan **(Gore & Schal, 2007).**

10.Control methods

10.1 Biological control

By definition, the term biopesticide denotes a pest management product derived either directly from a living organism or representing substances naturally synthesized by biological systems, as distinguished from synthetic sources **(Koul, 2016).** The definition adopted by the International Organization for Biological Control is the use by humans of natural enemies such as predators, parasitoids or pathogens to control populations of harmful species **(Gaire *et al.*, 2019; Pan & Zhang, 2020).**

10.1.1 Bacteria

The use of entomopathogenic bacteria to control certain crop pests and disease vectors of medical importance has been the subject of several studies, due to their high specific insecticidal activity **(Pan & Zhang, 2020).**

Bacteria of the genera Bacillus, Lysinibacillus, Pseudomonas, Rhizobium, and Serratia are the most common bacterial pathogens, of which Bacillus is the most widely utilized genus. Indeed, *Bacillus sphaericus* and Bacillus cereus were tested in *B. germanica* larvae **(Nishiwaki *et al.*, 2004; Nishiwaki *et al.*, 2007).**

10.1.2 Fungi

Metarhizium anisopliae and *Beauveria bassiana* are two entomopathogenic fungi found naturally in the soil and they are harmless to both humans and pets. Their spores settle on the insect's cuticle, developing into hyphae that penetrate and cross the cuticle, leading to the growth of the fungus inside the insect and ultimately causing its death **(Strasser *et al.*, 2000; Gutierrez *et al.*, 2015; Pan & Zhang, 2020).** Once the infected insect dies, the fungus produces spores which can contaminate other insects. The contaminated insect will also be able to contaminate its fellow insects by self-cleaning behaviors. Water represents the attractant element for *B. germanica*.

10.1.3 Protozoa

The families most used in biological control are the Amoebidae and the Nosematidae **(Kouassi, 2001).** These pathogenic microorganisms have the ability to overcome the defenses of the host insect and infect it; they then multiply there and cause its death in the more or less long term, whether through the emission of toxic substances and/or the destruction of certain tissues **(Bawin *et al.*, 2014; Lepage, 2021).**

10.1.4 Arthropods

In nature, there are many types of natural enemies against insects, including parasitoids and predators **(Grandcolas, 1998).** There are more than 15 species of parasitic enemies, such as wasps from the family Evaniidae that attack oothecae. These wasps lay their eggs inside them and their larvae devour developing *B. germanica* nymphs **(Tatfeng *et al.*, 2005).** Additionally, there are around 18 species of predatory enemies such as spiders, as indicated by **Kassiri *et al.* (2018)** and **Yang *et al.* (2019).**

10.1.5 Phyto-insecticide

The first substances of plant origin, recognized and widely used against pests, can be classified into alkaloids, flavonoids and plant essential oils. These substances are processed from effective chemical constituents extracted from plants, belonging to the families Meliaceae, Compositae, Ephedraceae, Lauraceae, etc. They can control by various means, such as contact toxicity, gastric toxicity, repellent effects and interference in insect development **(Castillo *et al.*,**

2017; Ling *et al.*, 2018). According to **Linnaeus (1767) and Corbett *et al*. (1984),** nicotine is the primary alkaloid extracted from tobacco, a plant in the Solanaceae family. Nicotine functions as a smoke-producing insecticide that impacts the synapses of insects' central nervous systems. These compounds act through contact and ingestion, blocking the absorption of oxygen by cells. Similarly, Pyrethrum, extracted from Chrysanthene flowers belonging to the Asteraceae family, functions by contact on the nervous system of insects, causing loss of balance, convulsive phenomena, paralysis and ultimately death **(Gaudin, 1937).**

Essential oils are among the most effective biopesticides, often constituting the bioactive fraction of plants **(Boné *et al.*, 2020).** Their toxicity is linked to the presence of certain oxygenated functional sites and the complex, variable chemical composition of essential oil constituents revealed **(Viaud, 1993; Chaaban *et al.*, 2019; Oladipupo *et al.*, 2020).** Indeed, several essential oils extracted from different plants, *Angelica sinensis, Curuma aeruginosa, Cyperus rotundus, Eucalyptus robusta, Illicium verum, Lindera aggregate, Ocimum basilicum* and *Zanthoxylum bungeanum* were tested in *B. germanica* **(Liu *et al.*, 2015).**

10.2 Chemical control

Historically, *B. germanica* control has primarily relied on chemical insecticides, which pose toxicity risks to humans. These insecticides consist of various compounds with differing physical properties and modes of toxicological action **(Saipollizan *et al.*, 2021).**

The initially employed compounds against pests were relatively simple first-generation pesticides. These pesticides are characterized by their relatively high toxicity for non-target organisms, especially their persistence, or slow decomposition in the environment. Subsequently, second-generation synthetic compounds, namely organochlorines, organophosphorus compounds, pyrethroids, and carbamates, were introduced **(Djouhri *et al*,. 2022).** They directly target the central nervous system of insects **(Gouaidia & Boudeguig, 2020).** Pesticide exposure may adversely impact human health, with possible links to neurological dysfunctions, metabolic disorders, and certain cancer types according to research **(Bortoli & Coumoul, 2018).**

10.3 Physical control

It involves the use of glue traps **(Kim *et al.*, 2000).** Baits have long been used extensively against *B. germanica* and can take several forms (see Figure 9) including pastes, granules, gels, or powders **(Pachamuthu *et al.*, 1999; Montalva *et al.*, 2016).** Typically, these baits are based with long-acting insecticide, it takes at least 7 days to produce effect **(Durier & Rivault, 2000).**

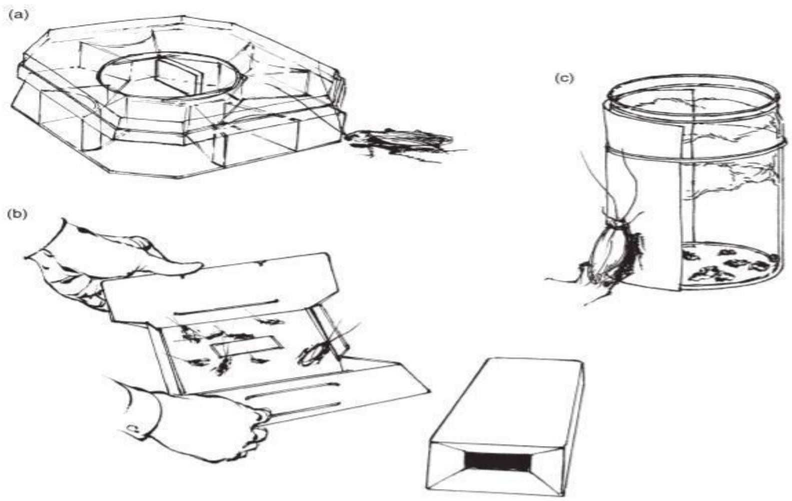

Figure 8. Some trap models **(Ghermoul, 2020)**

(a): Elaborate mechanical trap containing an attractive food. (b): Sticky paper covered with trapped *B. germanica*: the trap contains an attractive chemical. (c): Trap consisting of a simple jar garnished with raisins as bait: a sheet of paper facilitates the entry of *B. germanica*

Chapter 02

Eucalyptus

1. Origine and history of *Eucalyptus*

Eucalyptus is a tree species that belongs to the Myrtaceae family **(Hayat *et al.*, 2015).** It is also widely known by the common name "Tasmanian blue gum", it is originating from the southeastern region of Australia, where it is indigenous **(Cerasoli *et al.*, 2016).** The eucalyptus genus encompasses over 700 different species within it, a medium to large evergreen tree with broad leaves, capable of reaching towering heights up to 70 meters tall and having a trunk diameter ranging from 4 to 7 feet **(Hayat *et al.*, 2015).** The Eucalyptus species was first identified and named by the naturalist Labillardiere in 1791 **(Boukhalfoun, 2012).** Over the past two centuries, Eucalyptus trees have been extensively cultivated and established in regions far beyond their original native habitat **(Badalamenti *et al.*, 2018).** In many areas, Eucalyptus species have been intentionally planted and cultivated for more than 150 years **(Yost *et al.*, 2021**). Eucalyptus was initially brought to Europe in approximately 1804, marking its introduction to the continent, subsequently, in 1856, Eucalyptus was introduced to Algeria by Ramel, who imported it from Australia with the intention of utilizing the tree's affinity for humid environments to help drain marshy areas. Following its introduction, Eucalyptus has become widespread along the Algerian coastal regions **(Boukhalfoun, 2012).** In modern times, the cultivation of Eucalyptus has become extensive globally due to several favorable factors, including its ability to readily adapt to various environmental conditions, ease of cultivation, rapid growth rate, and substantial production of woody biomass **(Shala & Gururani, 2021).**

2. Botanical description of Eucalyptus

The taxonomic classification of Eucalyptus is as follows according to **Quezel and Santa (1963):**

Kingdom:	Plantae
Clade:	Rosids
Order:	Myrtales
Family:	Myrtaceae
Genus:	Eucalyptus

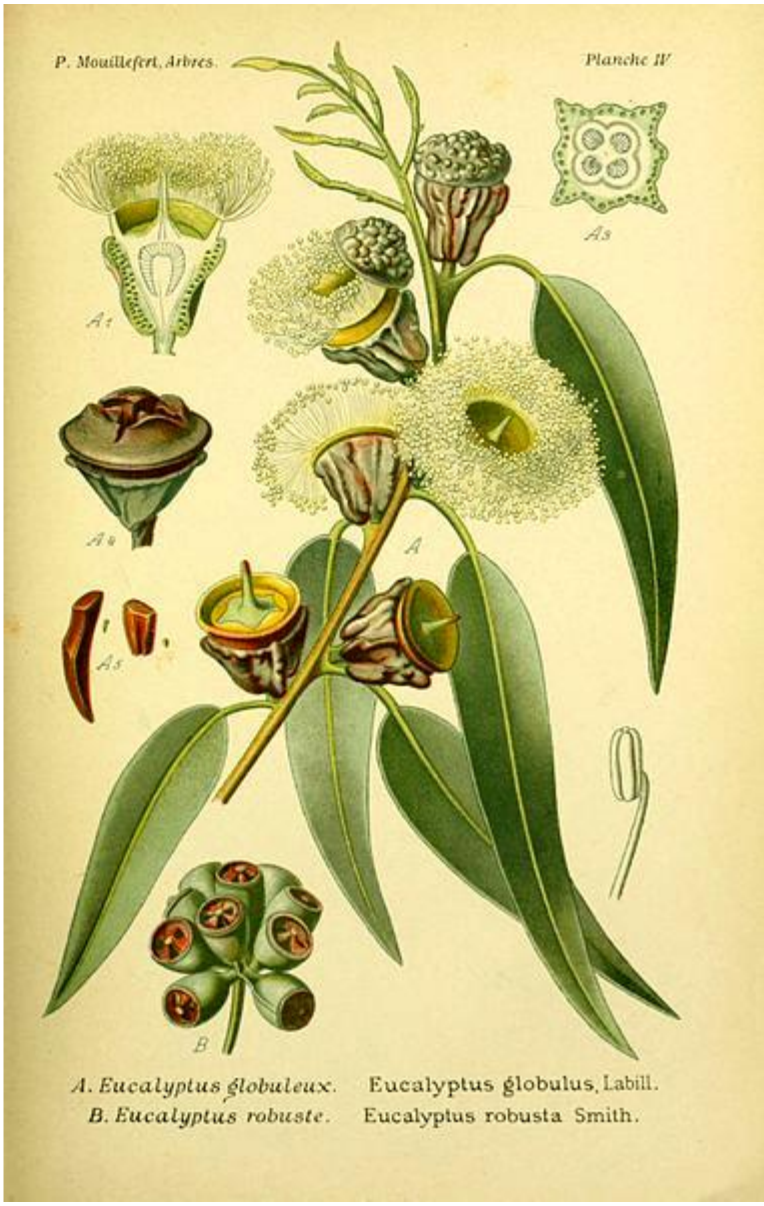

Figure 9. Flowers, leaves, and fruits of *Eucalyptus globulus* **(Pauline, 2019)**

3. Phytochemical characteristics of Eucalyptus

3.1 Essential oil of Eucalyptus

Eucalyptus trees are aromatic plants valued for medicinal applications. The essential oils extracted from various eucalyptus species exhibit antioxidant and antimicrobial properties, rendering them useful commodities for pharmaceutical and cosmetic industrial products **(Čmiková *et al*., 2013).** Scientific investigations into the *Eucalyptus* essential oil **(**EEO**)** began in the first half of the 20th century **(Lmloul & Bouridah, 2021).** Medicinal EEO is produced via water distillation processes using fresh eucalyptus foliage as the raw botanical source material **(Kesharwani *et al*., 2018).** EEO rank among the top 18 most extensively traded essential oil products globally. Consequently, there is growing research interest and commercial focus on harnessing the beneficial properties of eucalyptus oils as versatile raw materials suitable for applications across the food, pharmaceutical, and cosmetic industries from both academic and industrial sectors **(Shala & Gururani, 2021).** Due to its effective effect on humans, EEO has found therapeutic uses in treating conditions such as rhinitis, and respiratory infections that include the bronchi, in addition to reducing high body temperatures associated with fever **(Čmiková *et al*., 2013).** EEO widespread use across modern cosmetic, food, and pharmaceutical manufacturing sectors, biologically active properties including anti-inflammatory, analgesic, antiviral, antimicrobial, antioxidant, and insecticidal effects **(Atmani *et al*., 2018).** The key constituent present in eucalyptus essential oil is eucalyptol, comprising up to 70% of the oil's volume in the species Eucalyptus. Other major components include oxygenated monoterpenes, regular monoterpenes, and oxygenated sesquiterpenes. Specifically, the primary oxygenated monoterpenes are 1,8-cineole (72.71%), α-terpineol (2.54%), terpinen-4-ol (0.34%), and linalool (0.24%). The main sesquiterpene constituents are α-eudesmol (0.39%), globulol (2.77%), and epi-globulol (0.44%). Several other notable compounds present are α-terpineol acetate (3.1%), geranyl acetate (0.71%), L-pinocarveol (0.36%), β-sabinene (0.25%), and terpinolene (0.19%). A small portion (0.26%) of the total oil constituents remains unidentified **(Kesharwani *et al*., 2018).**

4. Utilization of *Eucalyptus*

Eucalyptus serves a multitude of purposes in the household domain. According to farmers, some of the primary advantages of this species for domestic use include its utility in constructing homes, fashioning household utensils, providing fuel for cooking and heating, and crafting handles for agricultural implements. Notably, the revenue generated from Eucalyptus cultivation significantly exceeds the income derived from cereal crop production **(Bekele, 2015).**

Additionally, Eucalyptus has a traditional use in treating sore throats, aches and pains, headaches, neuralgic pain, otitis, sinusitis, asthma, bronchitis, skin infections, and urinary tract infections. Its leaves are commonly utilized in pharmacy and also serve as a repellent against biting insects **(Salehi *et al*., 2019).**

5. Toxicity of Eucalyptus

The toxicity of Eucalyptus can vary depending on the method and dosage of exposure. While the essential oil of Eucalyptus is widely used in aromatherapy and traditional medicine for its therapeutic properties, it can be toxic if ingested in large quantities. Ingestion of eucalyptus oil can lead to symptoms such as nausea, vomiting, diarrhea, and abdominal pain, **(Ray *et al*., 2015)**. In severe cases, it may cause central nervous system depression and respiratory distress. Topical application of diluted eucalyptus oil is generally safe for adults, but it can cause skin irritation in some individuals. As with any essential oil, it is important to use caution and follow recommended guidelines for safe use **(Kesharwani *et al*., 2018).**

6. Biological control and bio-insecticidal activity

The biological control and bio-insecticidal activity of eucalyptus exemplify nature's capacity to offer sustainable solutions to pest management challenges **(Russo *et al*., 2018).** Eucalyptus trees, renowned for their aromatic foliage and medicinal properties, also possess natural defenses against insect pests. Essential oils extracted from eucalyptus leaves contain bioactive compounds such as 1,8-cineole, which exhibit insecticidal and repellent properties **(Salehi *et al*., 2019).** When applied as bio-insecticides, eucalyptus oil can effectively control pest populations while minimizing harm to beneficial insects and the environment. By harnessing the biological control and bio-insecticidal activity of eucalyptus, farmers and gardeners can achieve sustainable pest management outcomes, reducing reliance on synthetic pesticides and promoting ecological balance in agricultural and urban landscapes **(Russo *et al*., 2018).**

Chapter 03

Juniperus

1. Origin end history of juniper

Juniper is a dioecious, evergreen woody plant that can grow as either a shrub or a small tree **(Rabska *et al.*, 2021).** It is distinguished by its characteristic berry-like cones. Remarkably long-lived, this species can persist for up to 600 years **(Judžentienė, 2019).** Considered a pioneer species, the common juniper is among the first plants to colonize and establish itself in new areas, playing a vital role in facilitating the natural succession and development of forest ecosystems over time **(Rabska *et al.*, 2021).**

The juniper is a plant species found growing naturally in the wild or purposefully cultivated. The genus Juniperus, comprising approximately 70 different species, belongs to the cypress family Cupressaceae **(Judžentienė, 2019).** Native to Europe, South Asia, and North America **(Bais *et al.*, 2014)**, it stands out as the sole species within the Juniperus genus that has a natural distribution spanning both the Northern and Southern Hemispheres **(Elmastaş *et al.*, 2006).**

Juniper relies on wind pollination for reproduction, and it exhibits an extremely slow growth rate, typically achieving only around 20 centimeters (roughly 8 inches) in height over the course of five years **(Judžentienė, 2019).**

2. Botanical description of Juniper

Junipers belong to the following systematics **(Roudane, 2021).**

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Gymnospermae
Class:	Pinopsida
Order:	Cupressales
Family:	Cupressaceae
Genus:	*Juniperus*

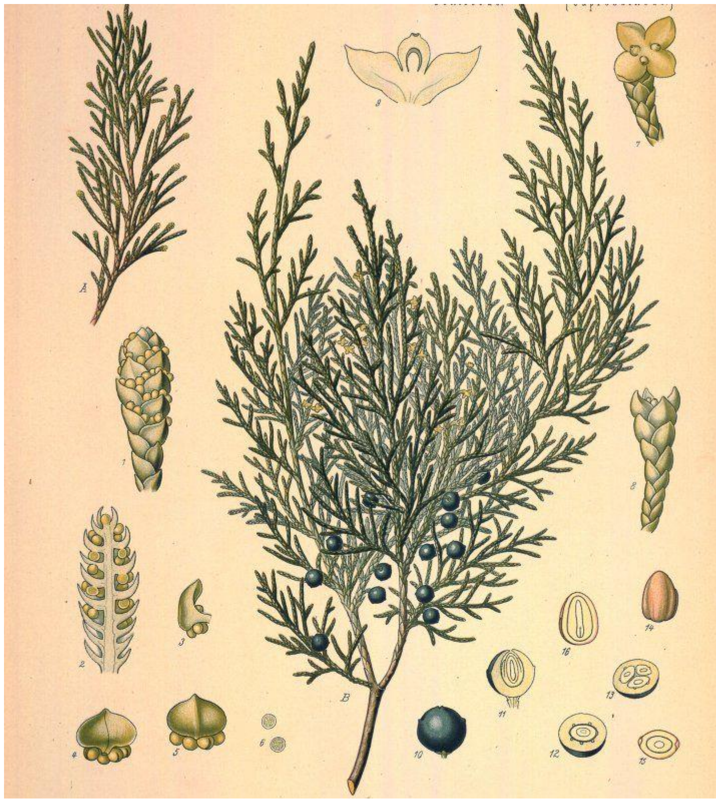

Figure 10. Flowers, leaves, and fruits of Juniper **(Wilhelm *et al*., 1914)**

2.1 Flowering

The flowering of juniper, is a subtle yet crucial phase in the plant's life cycle. Junipers are dioecious, with separate male and female plants. Male junipers produce small, yellowish pollen cones, which release pollen in spring **(Gruwez *et al.*, 2014).** Female junipers bear tiny cones that, upon fertilization, develop into blue or purple berry-like structures called juniper berries. This flowering process, though not visually striking, is essential for reproduction and genetic diversity **(Dimitri, 2017).**

2.2 Fruits and Seeds

The fruits of this plant are cone-like fruits that have a berry-like appearance. Initially green in color, these fruits undergo an 18-month ripening process, during which they turn a deep purple-black hue and develop a bluish waxy coating on their surface. These berry-like cones are spherical in shape, measuring between 4 and 12 millimeters in diameter. They typically consist of three fused, fleshy scales, although occasionally they may have six scales. Each individual scale contains a single seed within it **(Raina *et al.*, 2019).**

The seeds lack wings and are elongated with an ovoid three-cornered structure. They possess grooves and resin pockets covering their entire surface. Each cone scale contains a single seed, and a complete cone holds six seeds in total. These seeds measure approximately 4-5 mm in length and are embedded within a resinous, mealy pulp inside the cone. Unlike some other conifers, the juniper seeds remain within the cone during dispersal. The female cones take two to three years to fully mature and ripen before they can release their seeds **(Thomas *et al.*, 2007).**

3. Phytochemical Characteristics of Juniper

3.1 Essential oil of Juniper

Juniper, a type of coniferous plant, is best known as an aromatic tree **(Raina *et al.*, 2019).** The Juniper plant produces a diverse array of volatile organic compounds, varying in both quantity and composition, contingent upon factors such as its geographic origin, the specific plant organ, and the stage of development. These essential oils are extracted from various parts of the plant through specialized processes among the various parts of the Juniper plant, the berries or fruit are considered highly prized, as they are abundantly endowed with essential oil, rendering them the most valuable component of this species **(Judžentienė, 2019).** The essential oil extracted from juniper manifests as a pale green or yellowish liquid with a distinct, terpene-rich aroma. This aromatic oil finds numerous applications in the realm of medicine, serving various therapeutic purposes **(Gari *et al.*, 2020).**

4. Utilization of Juniper

The juniper plant has held a prominent place in human history since ancient times, primarily utilized for its medicinal properties. Owing to its potent and disinfecting aroma, this plant has found diverse applications for protective purposes, such as safeguarding fields and crops from pests, shielding animals, and serving as a defense against epidemic diseases that afflicted human populations **(Judžentienė, 2019).**

In both traditional and modern medicine, Juniper demonstrates a broad spectrum of pharmacological effects. These include antifertility, hypoglycemic, antidiabetic, diuretic, antiseptic, carminative, stomachic, and anti-rheumatic properties **(Dahmane *et al.*, 2016)** in addition to its various applications, the essential oil derived from Juniper is occasionally employed as a topical stimulant, harnessing its invigorating properties when applied locally to the skin or affected areas **(Gari *et al.*, 2020).**

The fruits of Juniper are a rich source of antioxidants and have been used as a treatment for rheumatic disorders such as rheumatism, arthritis, and gout, capitalizing on their healing properties to alleviate the symptoms associated with these conditions **(Fernandez & Cock, 2016)**

5. Toxicity of Juniper

The toxicity of juniper is an important consideration for both humans and animals. Certain species of juniper contain compounds like thujone and other essential oils that can be toxic if ingested in large quantities **(Pal & Dey, 2023).** Symptoms of juniper poisoning in humans include gastrointestinal distress, such as vomiting and diarrhea, as well as potential kidney damage **(Muyassarovich, 2024).** For animals, particularly livestock like cattle and horses, consuming significant amounts of juniper foliage can lead to severe digestive issues and even death **(Boyle & Dearing, 2003).**

6. Biological control

Juniper plants have been found to possess natural compounds with antimicrobial properties that can reduce populations of bacteria, fungi, and insects **(Moein *et al.*, 2010).** The essential oils and other chemical constituents in juniper have demonstrated efficacy in inhibiting the growth of various microbial pathogens, including bacteria and fungi, making them useful in natural plant protection strategies **(Ghasemnezhad *et al.*, 2020)**. For instance, juniper extracts can be used as natural pesticides and fungicides, reducing the need for synthetic chemicals in agriculture. Moreover, these antimicrobial properties can deter insect pests, protecting both juniper and neighboring plants from infestations **(Moein *et al.*, 2010).** The antimicrobial potential of Juniper extends to a wide array of microorganisms, demonstrating activity against 40 different species of fungi, viruses, and bacteria, including some clinical strains known for their resistance **(Mahmutović *et al*., 2017).**

By leveraging the natural defenses of juniper, it is possible to manage harmful microorganisms and insects in an environmentally friendly manner, promoting healthier plant communities and reducing reliance on chemical treatments **(Elshafie *et al.*, 2020).**

Chapter 4

Materials and Methods

Materials and Methods

1. Animal material

The collection of cockroaches was conducted at various locations within the city (hospitals, commercial structures, university residences, and housing). Cockroach capture was done manually.

The rearing of cockroaches was conducted in transparent ventilated plastic container (20 cm in length by 9 cm in height) without exposure to any insecticide for six months. Further, each container was provided with paper egg cartons as shelter. Cockroaches were fed dog kibble and provided water through cotton soaked in a plastic test tube filled with water. The rearing conditions were maintained at an ambient temperature, and a photoperiod of 12 hours at Biotechnology, Environment, and Health Laboratory at the Faculty of Natural and Life Sciences, University Abbes Laghrour of Khenchela.

Figure 11. Rearing of *Blattella germanica* (personal photo, 2024).

2. Plant material

The aerial part of the eucalyptus and juniper were manually collected during the months of November and December (2024) from Kenchela respectively, located in the province of Khenchela, Algeria. The plants were dried for a period of two weeks in the shade (at room temperature).

a b

Figure12. Dried aerial parts of plants, a. Juniperus aerial parts, b. Eucalyptus aerial parts (personal photo, 2024).

3. Extraction of essential oils

Essential oils were isolated through steam distillation of 3000 g of dried plants material for 3 h using a Hydro Steam Distillation Apparatus. Separatory funnel was used to separate water and oil after extraction. The oils were stored in sealed glass vials at 4–5°C prior to the bioassay. The yield of the extraction was calculated based on the dry weight of each sample.

a b

Figure 13. Extraction equipment, a: Hydro steam distillation apparatus, b: Separatory funnel (personal photo, 2024).

4. Bioassay

Different concentrations were prepared to evaluate insect mortality of adult insects after an initial dose-setting experiment. Bioassay experiments utilized three increasing concentrations of essential oils: 25%, 50%, and 100% from both plants, dissolved in dimethyl sulfoxide (DMSO). Each concentration, at a volume of 0.1 ml, was injected into a small cottons ball and subsequently placed in Petri dishes. The control insects were kept under the same conditions without any essential oil. Each concentration was replicated three times each comprising 10 insects. The number of live insects for each essential oil was counted daily until the end of exposure period.

5. Statistical analysis

5.1 Calculating plant yield post-extraction

In order to ascertain the yield of essential oil from a plant, our procedure commences with the measurement of the raw material, representing the quantity of plant material utilized for extraction, measured in grams. Subsequently, the extraction process is executed to procure the essential oil. Upon completion of the extraction, the extracted essential oil is weighed in grams. Ultimately, the yield is determined by applying the following formula to calculate the percentage:

Yield (%) = [Weight of extracted essential oil/ Weight of raw plant material used] × 100

5.2 Calculation of corrected mortality

The XLSTAT software was used to perform the statistics. The percentage of observed mortality is corrected using **Abbott's formula (1925),** which allows for the elimination of natural mortality. The formula for calculating corrected mortality is:

Corrected Mortality (%) = [Number of survivors in the control group - Number of survivors in the treated group / Number of survivors in the control group] * 100

The normalized data are subjected to one-way analysis of variance (ANOVA), followed by dose ranking using the Tukey test.

Chapter 5

Results

Results

1. Yield of essential oils

1.1 Yield of Eucalyptus

The essential oil was extracted from the aerial parts of Eucalyptus using a Hydro Steam Distillation Apparatus. The process produced an orange oil with a pleasant fresh scent. A total of 4 ml of essential oil was collected, achieving a 0.1% yield.

1.2 Yield of Juniper

The essential oil was extracted from the aerial parts of Juniper using a Hydro Steam Distillation Apparatus. The process produced an orange oil with a pleasant, fresh scent. A total of 6 ml of essential oil was collected, achieving a 0,2% yield.

2. Effect of Eucalyptus essential oils on *B. germanica*

2.1 Male mortality rate

During the exposure of male cockroaches to D3, D2, and D1 concentrations of Eucalyptus essential oil mortality rates increased significantly. The data indicated that after 3 days of treatment, all three concentrations resulted in a 100% mortality rate (see Fig. 14).

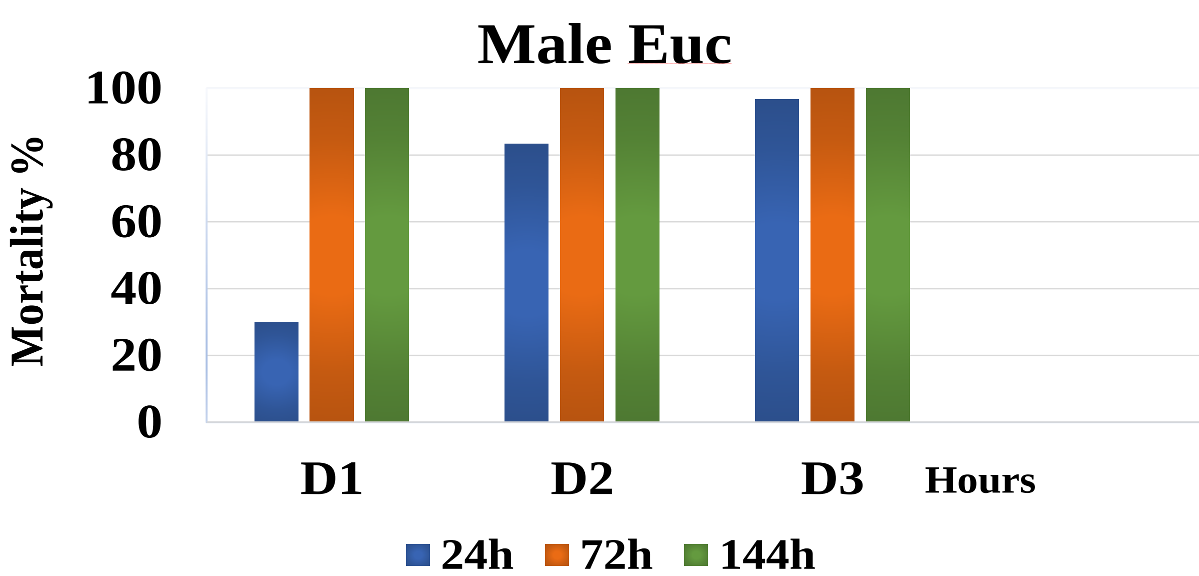

Figure 14. Mortality rate of adult male *B. germanica* exposed to Eucalyptus essential oil extract

D1, D2, D3 represent doses of 100%, 50%, and 25% respectively.

The statistical analysis of the results (Table 1) shows that there is a highly significant difference in mortality recorded for the three concentrations at the end of 72h and 144h, but there is no significant difference at 24h and 72h.

Table 1. Comparison of mortality rates of males treated with eucalyptus essential oil over time

	F_{obs}	*P*
24 hours	1.72	0.26
72 hours	16	0.004**
144 hours	16	0.004**

[* : *significant difference*]

The comparison of the variances in males' mortality (Table 2) illustrates that there is a significant difference for the 50% concentration.

Table 2. Comparison of mortality in males treated with eucalyptus essential oil based on concentrations

	F_{obs}	*P*
25%	2.88	0.13
50%	6.46	0.03*
100%	4.55	0.06

[* : *significant difference*]

2.2 Female mortality rate

Findings indicate that at concentrations of D3 and D1, complete mortality occurred after 6 days of treatment (see Fig. 15). Conversely, at the concentration of D2, complete mortality was observed after 3 days of treatment. This was observed throughout the exposure of female cockroaches to various concentrations of Eucalyptus essential oil.

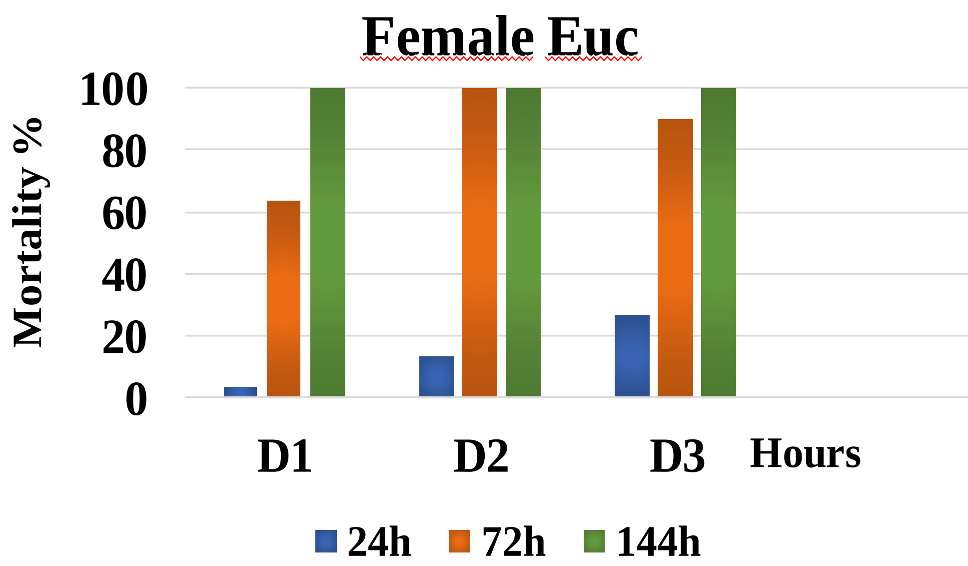

Figure 15. Mortality rate of adult female *B. germanica* exposed to Eucalyptus essential oil extract

D1, D2, D3 represent doses of 100%, 50%, and 25% respectively.

The statistical analysis of the results (Table 3) shows that there is a significant difference in mortality recorded for the three concentrations at the end of 144h, but there is no significant difference at 24h and 72h.

Table 3. Comparison of mortality rates of female treated with eucalyptus essential oil over time

	F_{obs}	*P*
24 hours	1.90	0.23
72 hours	4.75	0.06
144 hours	12.90	0.01*

[* : *significant difference*]

The comparison of the variances in female mortality (Table 4) illustrates that there is a significant difference between all the exposure concentrations.

Table 4. Comparison of mortality in female treated with eucalyptus essential oil based on concentrations

	F_{obs}	*P*
25%	7.08	0.02*
50%	4.95	0.05*
100%	5.15	0.05*

[* : *significant difference*]

3. Effect of Juniper essential oils on *B. germanica*

3.1 Male mortality rate

Throughout the exposure period of male cockroaches to various concentrations (D3, D2, and D1) of Juniperus essential oil, there was a consistent increase in mortality rates. According to the data (Fig. 16), at a D2 concentration, mortality rates increased to 90% within 3 days, reaching 100% by the end of the treatment period. Similarly, at a concentration of D1, mortality rates achieved 100% within 3 days. Conversely, when exposed to a D3 concentration, the mortality rate reached 70% after 3 days and remained constant until the end of the experiment.

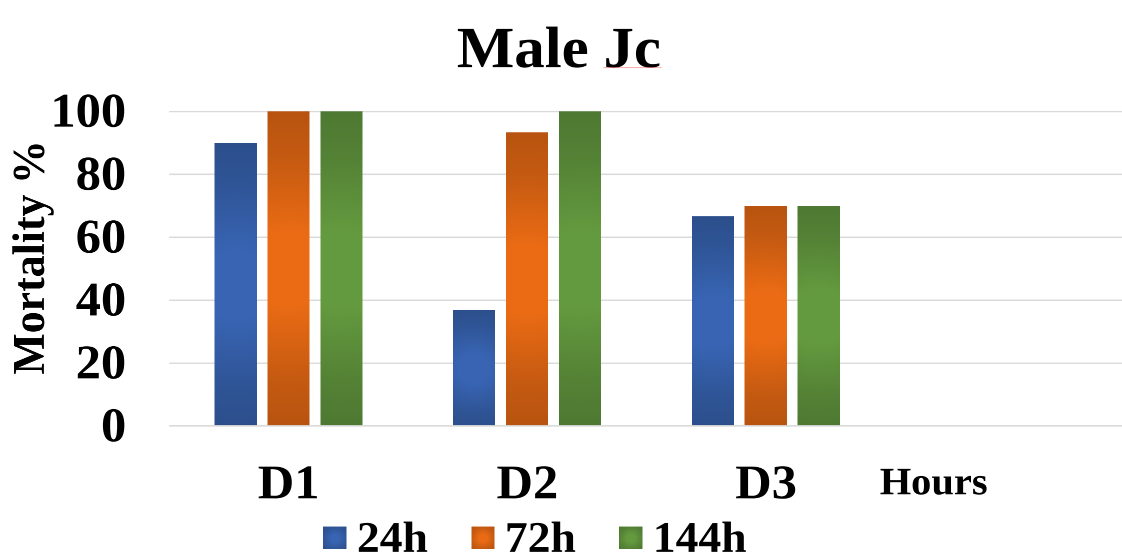

Figure 16. Mortality rate of adult Male *B. germanica* exposed to Juniper essential oil extract

D1, D2, D3 represent doses of 100%, 50%, and 25% respectively.

The statistical analysis of the results (Table 5) shows that there is a significant difference in mortality recorded for the three concentrations at the end of 144h, but there is no significant difference at 24h and 72h.

Table 5. Comparison of mortality rates of males treated with Juniper essential oil over time

	F_{obs}	*P*
24 hours	3.74	0.1
72 hours	4.15	0.1
144 hours	10.09	0.01*

[* : *significant difference]*

The comparison of the variances in males' mortality (Table 6) illustrates that there is a significant difference between exposure times for the 50% and 100% concentration.

Table 6. Comparison of mortality in males treated with Juniper essential oil based on concentrations

	F_{obs}	*P*
25%	0.02	0.98
50%	8.62	0.02*
100%	6.17	0.04*

[* : *significant difference*]

3.2 Female mortality rate

The exposure of female cockroaches to various concentrations of Jenupur essential oil reveals that at a concentration of D2, complete mortality was recorded after 3 days of treatment. Conversely, at a concentration of D3, a mortality rate of 90% was observed after 6 days of treatment. Notably, at a concentration of D1, a mortality rate of 98% was observed after 3 days (refer to Fig. 17).

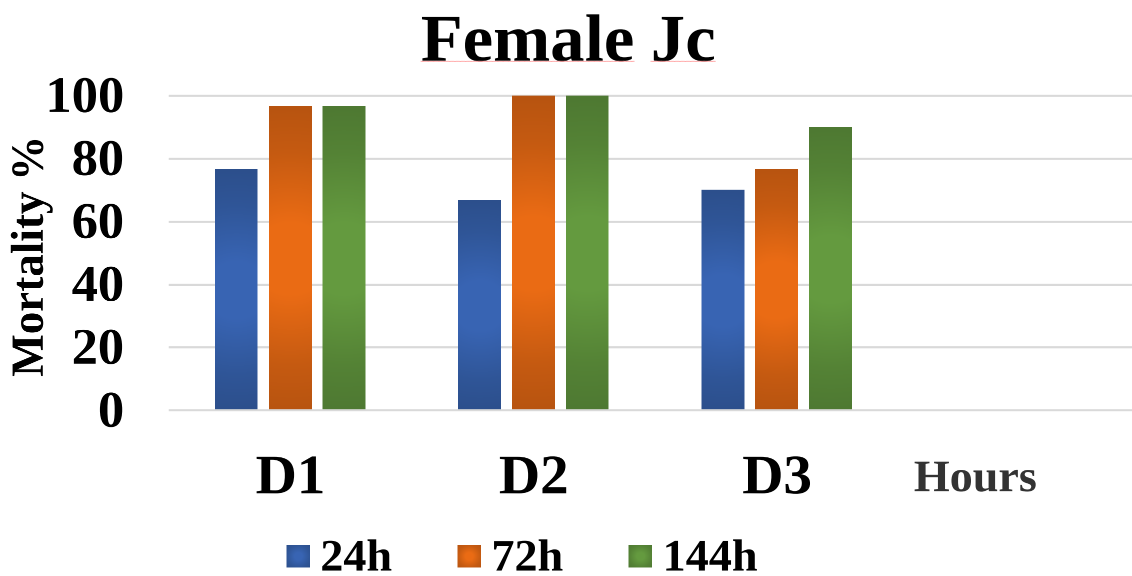

Figure 17. Mortality rate of adult female *B. germanica* exposed to Juniper essential oil extract D1, D2, D3 represent doses of 100%, 50%, and 25% respectively.

The statistical analysis of the results (Table 7) shows that there is a significant difference in mortality recorded for the three concentrations at the end of 24h and 72h, also there is a highly significant difference at 144h.

Table 7. Comparison of mortality rates of female treated with Juniper essential oil over time

	F_{obs}	*P*
24 hours	7.60	0.02*
72 hours	9.77	0.01*
144 hours	16	0.004**

[* : *significant difference*]

The comparison of the variances in female mortality (Table 8) illustrates that there is a significant difference between exposure times for the 25% concentration and highly significant for the 50% concentration.

Table 8. Comparison of mortality in female treated with Juniper essential oil based on concentrations

	F_{obs}	*P*
25%	8.51	0.02*
50%	16	0.004**
100%	2.99	0.13

[* : *significant difference*]

4. The effect of mixing Eucalyptus and Juniper essential oils on *B. germanica*

4.1 Male mortality rate

After administering a combined essential oil treatment of both Juniper and Eucalyptus oils to male *B. germanica* at varying concentrations, we observed complete mortality of the tested populations within 3 days at D1 concentration. At D3 concentration, the mortality rate occurred within 6 days. However, the D2 concentration resulted in a lower mortality rate of only 50% after the 6-day exposure period, as depicted in Figure 18.

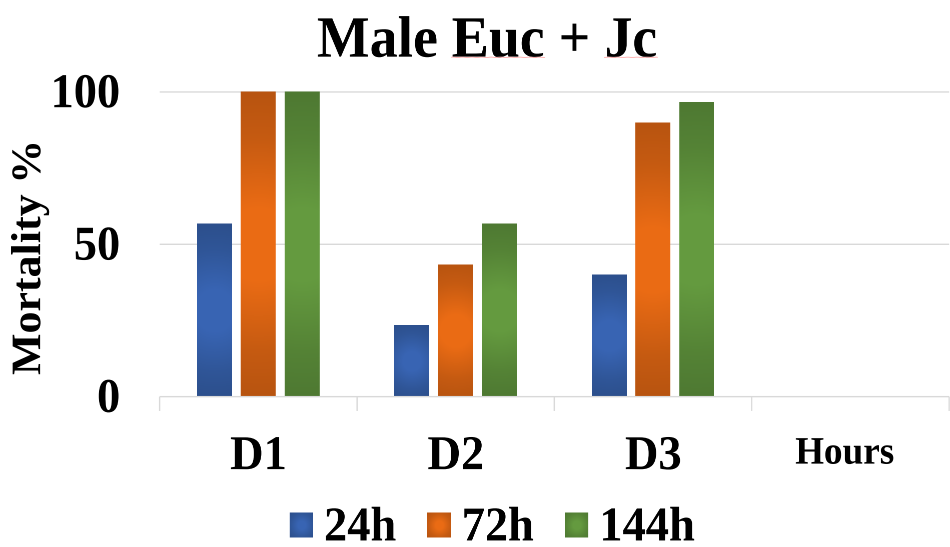

Figure 18. Mortality rate of adult male *B. germanica* exposed to the mixture of Juniper and Eucalyptus essential oil extracts

D1, D2, D3 represent doses of 100%, 50%, and 25% respectively.

The statistical analysis of the results (Table 9) shows that there is no significant difference in mortality recorded for the three concentrations.

Table 9. Comparison of mortality rates of males treated with the mixture of Juniper and Eucalyptus essential oil over time

	F_{obs}	***P***
24 hours	3.15	0.11
72 hours	3.36	0.10
144 hours	4.87	0.06

[* : *significant difference*]

The comparison of the variances in males' mortality (Table 10) illustrates that there is a highly significant difference for 100% concentration.

Table 10. Comparison of mortality in males treated with the mixture of Juniper and Eucalyptus essential oil based on concentrations

	F_{obs}	***P***
25%	2.29	0.18
50%	1.02	0.41
100%	16	0.004**

[* : *significant difference*]

4.2 Female mortality rate

The findings reveal that when female German cockroaches were exposed to a combined essential oil treatment consisting of Eucalyptus and juniper oils, at D2 concentrations, complete 100% mortality was achieved within 3 days, as depicted in Figure 19. At D3 concentrations, complete 100% mortality was achieved within 6 days. However, the undiluted D1 concentration did not induce mortality exceeding 80% in the test population even after the 6-day exposure period.

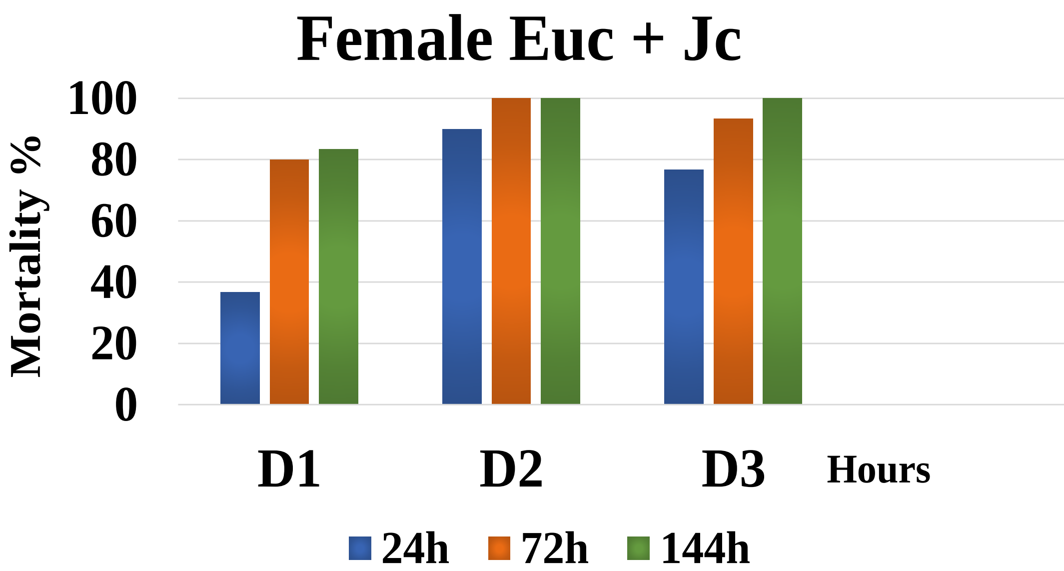

Figure 19. Mortality rate of adult female *B. germanica* exposed to the mixture of Juniper and Eucalyptus essential oil extracts

D1, D2, D3 represent doses of 100%, 50%, and 25% respectively.

The statistical analysis of the results (Table 11) shows that there is a significant difference in mortality recorded for the three concentrations at the end of 144h, but there is no significant difference at 24h and 72h.

Table 11. Comparison of mortality rates of female treated with to the mixture of Juniper and Eucalyptus essential oil over time

	F_{obs}	***P***
24 hours	3.98	0.07
72 hours	4.48	0.07
144 hours	10.59	0.01*

[* : *significant difference*]

The comparison of the variances in female mortality (Table 12) illustrates that there is no significant difference between exposure concentrations.

Table 12. Comparison of mortality in female treated the mixture of Juniper and Eucalyptus essential oil based on concentrations

	F_{obs}	***P***
25%	4.12	0.08
50%	3.22	0.11
100%	0.14	0.87

Discussion

Discussion

B. germanica poses a substantial public health concern and is considered a significant pest The presence of B. germanica is viewed as a critical indicator of unsanitary conditions, as these insects can vector several pathogens to humans, including viruses, bacteria, protozoa, and helminth parasites **(Agnès, 2014).** However, the development of resistance to chemical pesticides by B. germanica has emerged as a significant obstacle in combating this pest species. Efforts are being directed toward environmentally compatible alternative control strategies, with substantial research focusing on aromatic plant substances. Essential oils often contain the primary bioactive components responsible for pesticidal properties **(Ghermoul *et al*., 2020).** In this context, the purpose of this study is to evaluate the toxicity of essential oils from the aromatic plants Eucalyptus and Juniper on adult *B. germanica.*

According to our study results, it was observed that when male *B. germanica* were exposed to Eucalyptus essential oil treatments at D3, D2, and D1 concentrations, substantial increases in mortality rates were documented. Notably, after 3 days of exposure, complete 100% mortality was achieved across all three tested concentration levels. It is also evident in the results for female *B. germanica* subjected to the same Eucalyptus essential oil treatments, the mortality data revealed slightly different trends. At the D3 and D1 concentrations, full mortality required the 6-day exposure period. However, the D2 concentration proved more potent against females, inducing complete mortality within only 3 days of treatment application. This contrasts with the uniform 3-day 100% mortality observed across all concentrations when testing male *B. germanica* populations. These findings demonstrate that Eucalyptus essential oil exhibits significant insecticidal activity against adult *B. germanica*, even at low concentrations. This corroborates the results of **Russo *et al*. (2018)**, who confirmed the efficacy of Eucalyptus essential oil against *Gynaikothrips ficorum.* This study provides additional evidence supporting the potential of Eucalyptus oil as a natural insecticide. Moreover, the effectiveness of essential oils as insecticides is often attributed to their monoterpene content. In the case of Eucalyptus oil, 1,8-cineole constitutes 33.6%, comprising 37.9% of the oil. This high concentration of monoterpenes likely explains its potent insecticidal effects **(Kumar *et al*., 2012).** A similar study on the larval and engorged adult female stages of the *Boophilus microplus* found that 1,8-cineole was primarily responsible for eliminating these ticks at various stages of their lives **(Maciel *et***

***al*,. 2010).** According to **Yang *et al*. (2004)** Eucalyptus essential oil, with its main component, 1,8-cineole, was effective against head lice. Both the complete oil and isolated 1,8-cineole showed strong insecticide activity than commercially available products with delta-phenothrin or pyrethrum. The Eucalyptus oil had a median lethal time (LT50) value of 0.125 mg/cm2, twice as potent as the commercial treatments tested.

In our study, results revealed throughout the exposure trials with male *B. germanica* treated with Juniper essential oil at D3, D2, and D1 concentrations, mortality rates steadily increased over time. The D2 concentration induced 90% mortality within 3 days, eventually reaching complete 100% mortality by the conclusion of the treatment period. Similarly rapid effects were seen at the D1 concentration, achieving 100% mortality within 3 days. In contrast, the D3 treatment resulted in a lower 70% mortality after 3 days, which remained constant until the end of the experiment. Our research has also shown when female *B. germanica* were exposed to the same Juniper essential oil treatments, variations in lethal effects were observed across concentrations. The D2 concentration proved most potent against females, resulting in complete 100% mortality within 3 days. At the D3 level, mortality plateaued at 90% after the full 6-day exposure duration. Notably, the undiluted D1 oil induced 98% mortality in females by the 3-day mark, nearly achieving complete lethality over this shorter timeframe. The increase in the mortality rate could be explained by its insecticidal activity. Previous research has demonstrated that juniper essential oils have primarily been studied for their efficacy in controlling mosquitoes and ticks, with less emphasis on their effectiveness against agricultural pests **(Semerdjieva *et al*., 2021)**. Furthermore, juniper essential oil is mainly composed of monoterpenes like α-pinene (35.4%) and myrcene (15.3%), with smaller amounts of sesquiterpenes and oxygenated terpenoids. In comparison, Estonian juniper oil is higher in α-pinene (47.9%) and has trace amounts of α- and γ-terpinenes **(KraStanov, 2014).** A study by **Carroll *et al*. (2011)** provided evidence validating the insecticidal efficacy of juniper oil. It effectively controlled two pest species: Aedes aegypti mosquitoes and *Amblyomma americanum* lone star ticks. The oil showed a minimum effective dose against mosquitoes and a high 95% effective concentration against lone star ticks. These results underscored the potential of juniper essential oil as an insecticide.

Our findings demonstrate that the combined treatment using a mixture of Eucalyptus and Juniper essential oils resulted in faster and higher mortality rates compared to using each oil individually. **Shalaby *et al*. (2011)** found that Eucalyptus essential oil exhibits insecticidal effects through various mechanisms, disrupting insect metabolism and physiology, ultimately leading to fatal outcomes for exposed insect populations. Essential oils can disrupt insect metabolism by interfering with fatty acids and employing neurotoxic, cytotoxic, and mutagenic mechanisms across various physiological targets in insects. This multifaceted approach reduces the resistance development because insects are less likely to evolve countermeasures against the diverse harmful effects caused by the complex bioactive compounds in essential oils **(Sharaby & El-Dosary, 2016).** A concept supported by **Mills *et al*. (1968),** who proposed that juniper oil disrupts insect physiology by inhibiting protein synthesis in hemolymph and stimulating intestinal protein production, affecting hormonal regulation, metabolism, and development.

Conclusion & Perspectives

In conclusion, this study successfully achieved its dual objectives. We investigated the insecticidal toxicity of essential oils from Eucalyptus and Juniperus against adult *Blattella germanica*, evaluating their effects on both male and female individuals. Our findings highlight the potential of these essential oils as effective and sustainable options for pest management. The results demonstrate that these essential oils exert lethal toxic effects on *Blattella germanica* adults of both sexes under the tested exposure conditions. Consequently, the data supports the potential utilization of these botanically-derived essential oil formulations as effective insecticidal agents for controlling adult *Blattella germanica* infestations. Moving forward, we can develop an eco-friendly natural insecticide derived from these toxic plant sources, presenting a viable and sustainable alternative method for controlling insect pest populations.

To further expand the scope of this research, additional experiments should be conducted, with a particular focus on:

- Evaluating the insecticidal effects on different life stages (eggs, nymphs, etc.).
- Investigating potential resistance development by exposing successive generations of insects to the essential oils.
- Evaluating performance of the essential oils across different environmental conditions (temperatures, humidity, surfaces).
- Trials on semi-field or field scales to assess practical efficacy outside of laboratory conditions.
- Find out what precisely is the compound gift withinside the crucial oil that affected the insect.

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Scientific activities

Part of the results reported in this dissertation gave rise to the communications cited below:

National communications

DEMOCRATIC AND PEOPLE'S REPUBLIC OF ALGERIA.
MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH
UNIVERSITY BATNA 2 MOSTAFA BEN BOULAID
FACULTY OF NATURAL AND LIFE SCIENCES

CERTIFICATE OF PARTICIPATION

The scientific committee of the National Seminar on Biology, Medicinal Plants and Health (SNBPMS-2024), held at the University of Batna 2 on May 8, 2024, certifies that:

SAIDI Malika

has participated with a poster presentation entitled:

The biotoxic effect of *Juniperus communis* essential oil on *Blattella germanica*

Co-authors: TRADI Khouloud, BEKHAKHECHE Manel, BENSIZRARA Djamel

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Attestation de participation

La présidente du colloque national hybride **"Vers une Coexistence Durable : Stratégies intégrées pour la gestion de la pollution, la sécurité alimentaire et le développement Durable"** organisé le 05 et 06 juin 2024 à l'Université Abdelhamid Ibn Badis, Mostaganem, certifie que :

Dr SAIDI Malika, Université Abbes Laghrour- Khenchela

A présenté **en mode virtuel via google meet,** une **communications orale**, intitulée:

«The toxic activity of a mixture of two essential oils - Eucalyptus globulus and Juniperus communis - against adult German cockroaches».

Co-auteurs: TRADI Khouloud , BEKHAKHECHE Manel , BENSIZRARA Djamel

La présente attestation est délivrée pour servir et valoir ce que de droit.

La présidente du colloque
Pre. BENAMAR Nardjess

Le doyen de la Faculté des Sciences de la Nature et de la Vie

عميد كلية علوم الطبيعة والحياة
بجامعة مستغانم
الأستاذ: بن خليفة محمد

Certificate of Achievement

This is to certify that

Saidi Malika

has successfully participated with Poster Presentation titled:

The effectiveness of Eucalyptus globulus essential oil in combating adult German cockroaches' toxicity

Co-authors: TRADI Khouloud, BEKHAKHECHE Manel, BENSIZRARA Djamel

in the 1st National Seminar on Geoscience "WATER-SOIL: Economic and Environmental Impact"organized by the Faculty of Earth Sciences and Architecture, Department of Geology, in collaboration with the Algerian Association of Geosciences and Remote Sensing,held on the 15th and 16th of May, 2024, at the University of Oum El Bouaghi.

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