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**Thème**

# Comparative Efficacy of Essential Oils Extracted from the Fruits and Leaves of *Eucalyptus globulus* in the Control of a Stored-Product Pest.

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**Note :**…… **Mention :**……..

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# INTRODUCTION

## INTRODUCTION

Cereals form the backbone of global food security, supplying nearly 50% of the world's caloric intake while serving as a key source of essential macronutrients and micronutrients (Sabagh et al., 2024). As a dietary staple for billions and a critical component of livestock feed, their production and availability directly influence socioeconomic stability and nutritional outcomes worldwide. However, rising climatic uncertainties, resource constraints, and geopolitical disruptions pose growing threats to cereal supply chains, exacerbating food insecurity in vulnerable regions (Sabah et Rabie, 2024). In Algeria, cereals dominate agricultural land use, covering approximately 40% of the country's arable area, underscoring their strategic importance in both food provision and economic sustainability (Sabah et Rabie, 2024). Wheat, in particular, holds a central position in the Algerian diet, acting as the primary source of protein and carbohydrates. Despite its agricultural significance, domestic production remains insufficient to meet demand, forcing the country to rely heavily on durum wheat imports—a dependency that exposes Algeria to market volatility and supply chain risks (Kourat, Smadhi, et Madani, 2022).

Cereals are a cornerstone of global and national food security, necessitating effective post-harvest management to preserve their nutritional and economic value. As primary sources of dietary energy and protein, grains like wheat require proper storage, transportation, and distribution systems to minimize losses. However, inadequate storage conditions, coupled with rising demand and population growth, contribute to the loss of approximately 20% of stored grains (Pekmez, 2016). These losses are driven by complex natural and biological factors, including microbial contamination and infestation by birds, rodents, and particularly insects. Insect pests are the most destructive biological threat, accounting for nearly 70% of storage-related damage—mainly from Coleoptera (60%) and Lepidoptera (10%) (Tripathi, 2018). Among them, Rhyzopertha dominica is especially harmful due to its ability to infest intact kernels, leading to significant quantitative and qualitative deterioration of stored grains. This degradation includes reduced protein content and compromised grain integrity, which collectively threaten food quality and security (FAO).

Chemical fertilizers and pesticides are extensively used in modern agriculture to enhance productivity, however, their long-term application has led to significant environmental consequences, including soil degradation and loss of biodiversity. To address these issues, integrated pest management (IPM) has emerged as a more sustainable approach, combining chemical, biological, and cultural methods to minimize dependence on synthetic inputs (Koshariya et al., 2023). Pesticides defined as substances used to kill, repel, or control pests have played a key role in increasing agricultural yields, particularly in response to global population growth since the early 20th century (AL-Ahmadi, 2019; Blair et al., 2015). Nevertheless, their widespread use poses serious risks to human health and the environment. Studies have linked pesticide exposure to cancer, neurological disorders, and mutagenic effects in plants, while also raising concerns about environmental contamination through air, water, and soil (Pandya, 2018). In response to these concerns, the World Health Organization (WHO) has banned several chemical insecticides and supports the adoption of safer alternatives, such as biopesticides and scientifically informed IPM strategies.

The growing demand for safer pest control strategies has driven increasing interest in natural alternatives, particularly those derived from plants. Plant-based products are gaining global attention as effective, environmentally friendly insecticides and pesticides, offering safe, cost-effective, and preventive solutions against a range of pests (Nile, Kwon, & Nile, 2019). Several plant-derived substances, such as clove essential oil (Syzygium aromaticum), Chenopodium ambrosioides terpenes, Ginkgo biloba and Psoralea corylifolia extracts, and Tephrosia candida extract, have been approved for use in biological control in various countries, including the EU, US, China, and Brazil (Souto et al., 2021). Essential oils, in particular, have become prominent in stored-product pest management due to their natural origin, broad-spectrum activity, and multiple modes of action—ranging from repellency and anti-feeding effects to reproduction inhibition. Despite their effectiveness, their high volatility and environmental sensitivity can limit their long-term persistence. Notably, their insecticidal action often targets the octopaminergic system, a pathway present in insects but absent in vertebrates, which enhances their safety for humans and non-target organisms (Chaubey, 2019).

Given the increasing scientific interest in essential oils as environmentally friendly pest control agents, this study investigates their practical application in managing stored-product

insects. ***The first objective*** of the research involves extracting essential oils from the leaves and seeds of Eucalyptus globulus using hydrodistillation. The yields from each plant part are quantified and compared to identify the most efficient botanical source.

***The second objective*** of the study evaluates the toxicity of these essential oils against *Rhyzopertha dominica,* a major pest of stored grains. This includes fumigation and contact toxicity assays, as well as the repellent activity, to comprehensively determine their potential as alternative pest control agents.

# MATERIALS AND METHODS

## II. MATERIALS AND METHODS

### 2.1. Presentation of the insects studied

#### 2.1.1. Rhyzopertha dominica (Fabricius, 1792)

*Rhyzopertha dominica (F.)* is a major cosmopolitan pest of stored cereals such as wheat, rice, and maize. Both adults and larvae contribute to post-harvest losses by feeding on grain kernels, resulting in weight loss and reduced commercial value (Aulicky et al., 2022). Adults, measuring 2–3 mm, are dark brown, cylindrical, and distinguished by a hood-like pronotum that conceals the head, aiding movement through grain (Sakka et al., 2024). Females can lay up to 500 eggs among or within grains (Adarkwah et al., 2019), and the larvae develop entirely inside the kernels, creating hollow grains filled with frass and often escaping early detection (Shahid et al., 2020).

Under favorable conditions (32–35 °C; 65–75% RH), the complete life cycle spans 25–30 days, comprising egg (5–8 days), larva (12–15 days), pupa (5–7 days), and adult stages, with development and emergence occurring inside the grain (Sakka et al., 2024; Wang et al., 2025). Environmental factors like temperature and humidity significantly influence developmental timing (Aulicky et al., 2022).

The systematic position of *Rhyzopertha dominica*:

- **Kingdom :** Animalia
- **Phylum :** Arthropoda
- **Subphylum :** Hexapoda
- **Class :** Insecta
- **Order :** Coleoptera
- **Family :** Bostrichidae
- **Genus :** Rhyzopertha
- **Species :** *Rhyzopertha dominica* (Fabricius, 1792).



**Figure 1**. R. dominica (lateral side, ventral side) (Gherissi & tartar , 2025).

### 2.2 Collection and breeding

Adult *Rhyzopertha dominica* were collected in February 2025 from storage facility in Aouinat (Algeria). Due to poor sample quality in Tébessa, live insects were successfully collected from Aouinat. Insects were separated from infested wheat using a 2 mm mesh sieve over white filter paper to improve visibility. Live adults were then transferred to 2 L plastic containers filled with clean wheat and maintained at 25 ± 2 °C under ambient humidity and natural light. This setup ensured continuous rearing of healthy specimens for bioassays.

### 2.2 Presentation of the plant, *Eucalyptus globulus* ( Labill 1800 ) :

*Eucalyptus globulus*, commonly referred to as the blue gum tree, is a fast-growing evergreen species of the Myrtaceae family that can exceed 60 meters in height (Jugreet et al., 2020). The tree features smooth, grayish bark, aromatic lanceolate leaves (up to 15 cm long) rich in oil glands, and small white to cream flowers (Danna et al., 2024a). It is native to southeastern Australia but widely cultivated in temperate and Mediterranean climates due to its environmental adaptability.

The tree produces woody capsules ("gumnuts") that serve as seed-bearing fruits. These capsules are irregularly shaped, dark brown to black, and measure between 1–3 mm (Silva et al., 2016). Fruiting typically begins in the fourth or fifth year and recurs in 3–5-year cycles. Notably,

fruit morphology including size, texture, and cotyledon shape is genetically inherited and often used for taxonomic classification (Hayat et al., 2015).

From a biochemical perspective, the fruits and leaves of *E. globulus* contain essential oils and phytochemicals such as phenolics, flavonoids, and terpenoids, known for their antimicrobial and insecticidal activities (Yan et al., 2021). The leaf essential oil is especially rich in 1,8-cineole, which can comprise up to 85% of its composition (Hu et al., 2022). This oil is widely used in cosmetics, medicine, and agriculture—particularly in pest management for stored grains (Awadalla et al., 2021; Danna et al., 2024b).

The chemical profile and biological activity of Eucalyptus essential oils are influenced by several factors, including the extraction method, harvest season, and geographic origin (Hu et al., 2022). In addition to insecticidal and repellent properties, *E. globulus* demonstrates antifungal, antibacterial, antioxidant, and fumigant activities (Majeed et al., 2024), making it a valuable resource in pharmaceutical and agro-industrial applications.

**Systematic Classification:**

- **Kingdom :** Plantae
- **Clade :** Tracheophytes
- **Clade :** Angiosperms
- **Clade :** Eudicots
- **Clade :** Rosids
- **Order :** Myrtales
- **Family :** Myrtaceae
- **Genus :** Eucalyptus
- **Species :** *Eucalyptus globulus Labill*., 1800



**Figure 2**. Presentation of *Eucalyptus globulus* (Gherissi & tartar , 2025).

### 2.3. Extraction and Yield of *Eucalyptus globulus* Essential Oil

Leaves and fruits of *Eucalyptus globulus* were collected in February 2025 from Mreij (Tébessa region, Algeria), rinsed to remove debris, and air-dried in the shade. Fifty grams of dried material were mixed with 500 mL of distilled water and subjected to hydrodistillation using a Clevenger-type apparatus for two hours. The process was conducted in a 2-L round-bottomed flask with controlled heating (100–150°C), and the oil was separated by condensation and decantation.

The extracted essential oil was filtered with anhydrous sodium sulfate ($Na_2SO_4$) and stored in amber glass bottles, sealed and wrapped in aluminum foil to prevent photodegradation and preserve its chemical stability (Seven Scents, 2023). The oil yield was measured precisely for further analysis.

The essential oil yield represents the ratio between the weight of the extracted essential oil and the weight of the plant biomass used. It is expressed as a percentage (%) and calculated using the following formula:

$$R\ (\%) = (PB / PA) \times 100$$

Where:

- **R** = Yield of essential oil (%),
- **PB** = Weight of essential oil extracted (g),
- **PA** = Weight of dry plant material used (g).

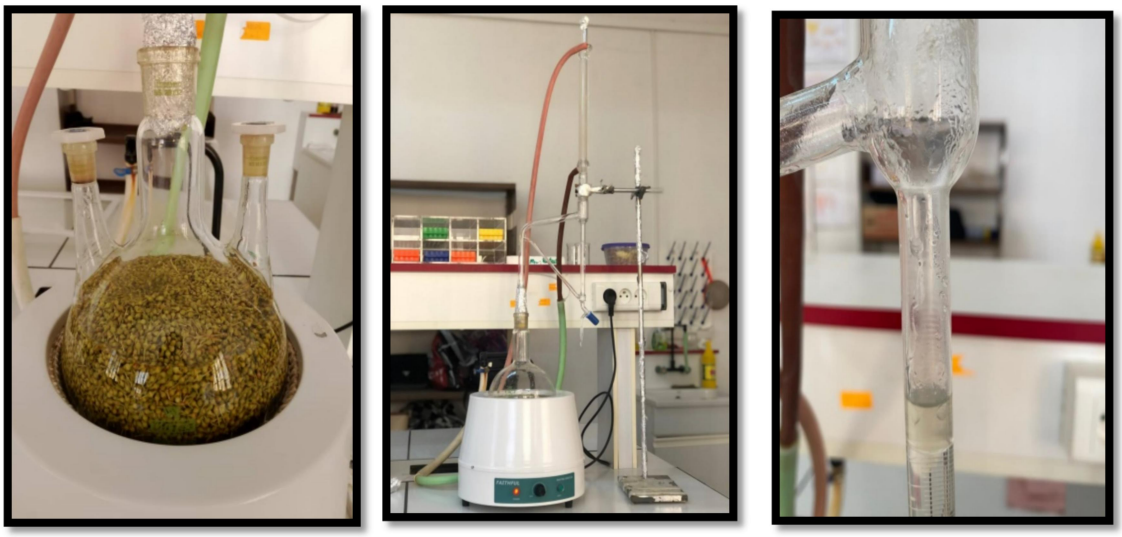


**Figure 3**. *Eucalyptus globulus* extract (Gherissi and Tartar, 2025)

## 2.4. Application of Treatments and Evaluation of Bioactivity

### 2.4.1. By ingestion

Following a preliminary screening, the essential oils extracted from *Eucalyptus globulus* leaves and fruits, dissolved in acetone, were applied at various concentrations (2, 4, 6, and 8 µl/ml) for each leaves and fruits against *Rhyzopertha dominica.* Each concentration was applied to 3 g of healthy wheat grains placed in plastic vials (Fig. 5). After complete evaporation of the solvent (15 minutes), 10 unsexed adults of *R. dominica* were introduced into each vial. The bioassay was conducted with five replicates per dose. A control series was run in parallel, where wheat grains received only the solvent (acetone).

Mortality was recorded at 12, 24, and 48 hours after treatment, and corrected using Abbott's formula (1925) to account for natural mortality. Lethal concentrations (LCs) and their 95% confidence intervals (CLs) were calculated using GraphPad Prism 10 software.

### 2.4.2. by Fumigation

Following a preliminary screening, different concentrations of *Eucalyptus globulus* essential oil were applied by fumigation: 83, 125, 166 and 208 µl/L of air *Rhyzopertha dominica* for both leaves and fruits. The essential oil was applied onto a 2 cm diameter filter paper disc, which was suspended from the inner surface of the plastic vial lid using a thread. The treatment was administered in 60 ml plastic containers containing 3 g of healthy wheat grains. Five replicates of 10 unsexed adults were conducted for each concentration. A control group was run in parallel using untreated filter paper discs.

Mortality was recorded at 24, 48, and 72 hours post-treatment and corrected using Abbott's formula (1925) to account for natural mortality. Lethal concentrations (LCs) and their 95% confidence intervals (CLs) were calculated using GraphPad Prism10.

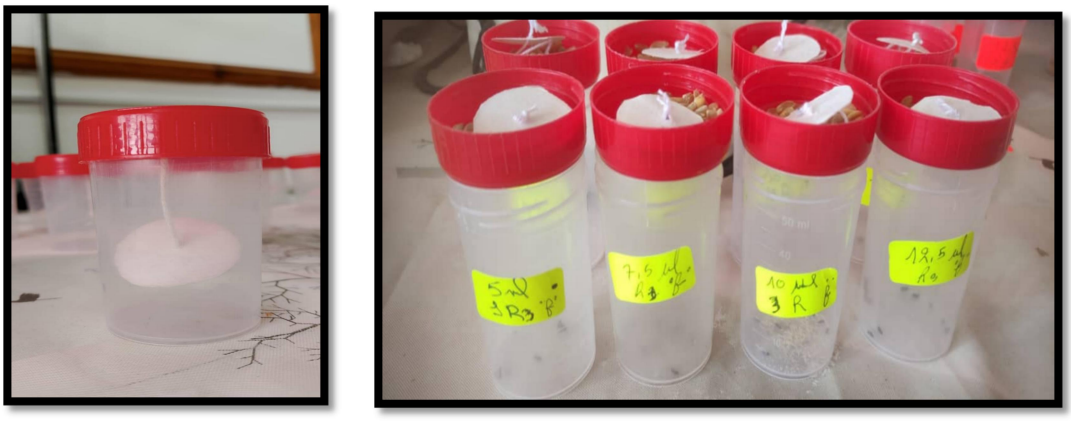


**Figure 4.** Toxicity test by fumigation (Gherissi & tartar , 2025).

## 2.5. Repulsion test

This test was used to evaluate the repellent activity of *Eucalyptus globulus* essential oil against *Rhyzopertha dominica* adults, using the preferential zone method on filter paper as described by McDonald et al. (1970). Filter paper discs with a diameter of 9 cm were cut into two equal halves: one half was treated with the essential oil diluted in acetone, while the other half received acetone only as a control.

Oil doses of 6, 12, and 24 µl were each diluted in 1 ml of acetone to ensure uniform distribution over the treated half of the filter paper. Both treated and control halves were air-dried, reassembled into full discs, and placed in Petri dishes. Ten adult insects were released at the center of each dish, and four replicates were conducted for each dose.

Insect distribution on each half of the disc was recorded after 30 minutes, 1 hour, 3 hours, and 6 hours of exposure. The percentage of repellency (PR) was then calculated using the formula proposed by Nerio et al. (2009) :

$$PR\ (\%) = [(NC - NT) / (NC+NT)] \times 100$$

**NC:** Number of insects present on the half-disc treated with acetone only.

**NT:** Number of insects present on the half-disc treated with oil.

The percentage of repulsion calculated is assigned to one of several classes ranging from 0 to 5. (Mc Donald et al.,1970) (Tableau 1).

**Table 1.** Percentage of repulsion according to the classification of McDonald et al. (1970).

| Classes | Repellency Intervals | Properties |
|---|---|---|
| **0** | PR ≤ 0,01% | Very slightly repellent |
| **I** | 0,1% < PR ≤ 20% | Slightly repellent |
| **II** | 20% < PR ≤ 40% | Moderately repellent |
| **III** | 40% < PR ≤ 60% | Averagely repellent |
| **IV** | 60% < PR ≤ 80% | Repellent |
| **V** | 80% < PR ≤ 100% | Highly repellent |

# RESULTS

## III. RESULTS

### 3.1. Essential Oil Yield and Organoleptic Characteristics

The essential oils were extracted from the leaves and fruits of *Eucalyptus globulus* through hydrodistillation using a Clevenger-type apparatus. The oils obtained from both plant parts appeared as clear, mobile liquids with a characteristic strong and penetrating eucalyptus aroma. No phase separation or turbidity was observed, indicating good stability and purity at room temperature.

The yield of essential oil is an important parameter, reflecting both the efficiency of the extraction method and the oil content of the plant material. In our study, the hydrodistillation of *Eucalyptus globulus l*eaves produced an average yield of 1.8%, while a higher yield of 2.6% was recorded for the fruits. These values were calculated based on the fresh weight of the plant material used for each distillation.

### 3.2. Toxicological Bioassays

The toxicological bioassays were conducted to evaluate the insecticidal efficacy of *Eucalyptus globulus* essential oils extracted from both leaves and fruits. The effectiveness was assessed based on the mortality rates observed in *Rhyzopertha dominica* adults at various time intervals following treatment.

#### 3.2.1. By ingestion

Following a preliminary screening test, various concentrations (2, 4, 6, and 8 µl/ml) of Eucalyptus globulus essential oils extracted from both leaves and fruits were tested for their contact toxicity against *Rhyzopertha dominica* adults.

Corrected mortality rates recorded for *Rhyzopertha dominica* following ingestion of *Eucalyptus globulus* leaves essential oil ranged from approximately 30% to 60% at the lowest concentration (2 µl/ml), and reached up to 100% at the highest concentration (8 µl/ml) after 72 hours (Fig. X). A significant increase in mortality was observed with a dose-response relation. Statistical analysis confirmed that the mortality rates varied significantly according to the applied

concentrations and exposure periods: at 24 h ($F_{3,12}$ =121.3 ; p<0,001), 48 h ($F_{3,12}$ =61.76 ; p<0,001), and 72 h ($_{F3,12}$=88.22; p<0,0001).

Similarly, the essential oil extracted from *Eucalyptus globulus* fruits exhibited a marked toxic effect on *Rhyzopertha dominica* adults when applied by ingestion. As shown in Figure 5, corrected mortality increased progressively with both the concentration and the exposure duration. At the lowest dose (2 µl/ml), mortality remained under 30% across all time points, while the highest dose (8 µl/ml) consistently induced mortality rates approaching or exceeding 95% after 72 hours. Statistical analysis confirmed a highly significant difference among the tested concentrations at each observation period,at 24 h ($F_{3,12}$ =17.96 ; p<0,001), 48 h ($_{F3,12}$ =33.28 ; p<0,001), and 72 h ($F_{3,12}$ =33.20; p<0,0001) supporting a strong dose-response relationship.

The results demonstrate that *Eucalyptus globulus* essential oils, when applied by ingestion, exhibit insecticidal activity against *Rhyzopertha dominica*, following a clear dose-response relationship. The classification of doses using Tukey's HSD test revealed the presence of two distinct statistical groups for the mortality rates observed at 24h, and three groups 48h and 72h post-treatment for leaves and the presence of four groups for the mortality rates observed at 24h and 72h and three groups 48h post-treatment for fruits.

The lethal concentration values of *Eucalyptus globulus* essential oils from leaves and fruits, along with their fiducial limits, are presented in Table 2. Based on the calculated lethal concentrations, the leaves essential oil exhibited higher toxicity against *Rhyzopertha dominica* compared to the fruit essential oil, indicating that *R. dominica* is more susceptible to the leaves-derived treatment.

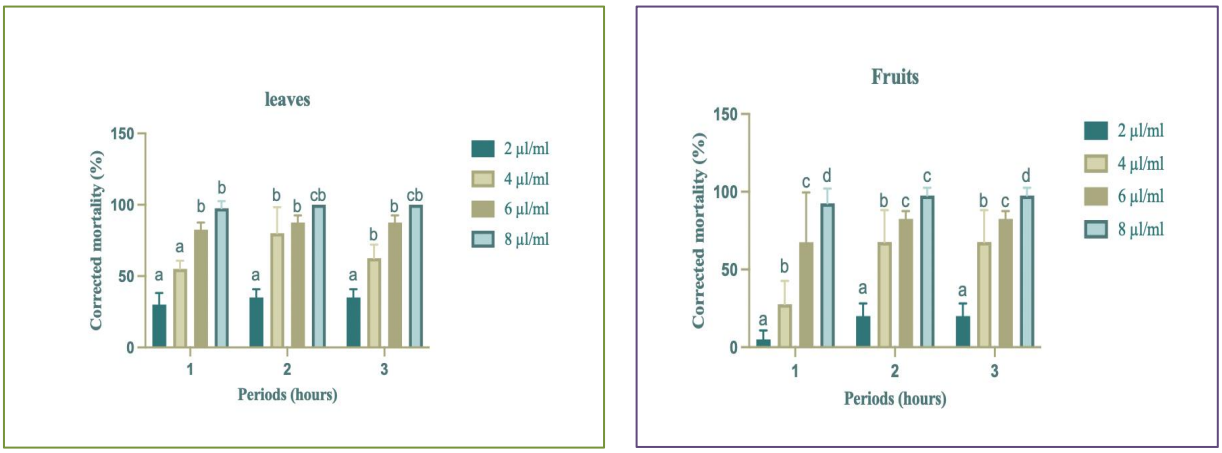



**Figure 5.** Toxicity of *Eucalyptus globuls* fruits EO leaves and fruits applied by ingestion (µl/ml) on adults of *Rhyzopertha dominica* at different exposure times. Corrected mortality (%) (mean ± SEM, n = 5 replicates of 10 individuals each); Tukey's HSD test.

**Table 2.** Probit analysis of the ingestion toxicity of *Eucalyptus globulus* essential oils (leaves and fruits) on adults of *Rhyzopertha dominica.*

| Treatment | Time | $LC_{25}$ (CI95%) | $LC_{50}$ (CI95%) | $LC_{90}$ (CI95%) |
|---|---|---|---|---|
| Leaves | 24h | 1.975 [0.1444-3.853] | 3.213 [1.045-4.962] | 8.504 [4.045-113.1] |
| | 48h | 1.736 [0.1568-3.452] | 2.786 [0.8802-4.284] | 7.181 [3.188-50.46] |
| | 72h | 1.579 [0.004971-3.20] | 2.525 [0.04407-4.240] | 6.450 [2.298-725.0] |
| Fruits | 24h | 3.213 [1.045-4.962] | 9.387 [5.236-153.7] | 4.137 [1.048-6.470] |
| | 48h | 2.786 [0.8802-4.284] | 6.729 [4.858-10.31] | 2.190 [1.518-2.903] |
| | 72h | 2.525 [0.04407-4.240] | 4.610 [4.236-5.010] | 2.144 [2.018-2.279] |

### 3.2.2. By fumigation

Following a preliminary screening, different concentrations of *Eucalyptus globulus* leaves essential oil (83, 125, 166, and 208 µl/L of air) were applied by fumigation against *Rhyzopertha dominica* adults. No mortality was observed in the control series.

As shown in Figure 6, the corrected mortality of *R. dominica* after fumigation with *E. globulus* leaves oil increased significantly with both the concentration and exposure time. For the lowest concentration (83 µl/L of air), the corrected mortality ranged from approximately 20% at 24 h to 30% at 72 h. For the highest dose (208 µl/L of air), mortality reached up to nearly 100% as early as 24 h and was maintained over the 72-hour period.

Statistical analysis revealed a significant dose-dependent and time-dependent effect on insect mortality, with ANOVA results indicating strong significance at 24 h ($F_{3,12}$ =92.48; p<0,001), 48 h ($F_{3,12}$ =45.58; p<0,001), and 72 h ($F_{3,12}$ =67.07; p<0,0001).

The fumigant activity of *Eucalyptus globulus* fruit essential oil against *Rhyzopertha dominica* adults was assessed using four concentrations: 83, 125, 166, and 208 µl/L of air. Mortality was recorded at 24, 48, and 72 hours. As with the leaf oil, no mortality was observed in the control groups exposed to air alone.

The corrected mortality rates increased significantly with both concentration and exposure time (Figure X). At the lowest concentration (83 µl/L), mortality rates were relatively low, averaging around 17.5%, 22.5%, and 27.5% at 24, 48, and 72 hours, respectively. Increasing the concentration to 125 µl/L improved efficacy, reaching approximately 57.5% mortality at 72 hours. More pronounced effects were observed at 166 µl/L, with mortality exceeding 90% by 24 hours and remaining stable thereafter. At the highest concentration (208 µl/L), mortality consistently approached or exceeded 95% across all time points.

Statistical analysis confirmed a significant effect of dose and time on mortality. ANOVA revealed highly significant differences at 24 hours ($F_{3,12}$ =206.6; p<0,001), 48 h ($F_{3,12}$ =136.6; p<0,001), and 72 h ($F_{3,12}$ =233.7; p<0,0001). Tukey's HSD test distinguished three mortality groups at each time point for leaves and four groups at 24h with three groups at 48h and 72h.

The lethal concentration values of *Eucalyptus globulus* essential oils from leaves and fruits by fumigation, along with their fiducial limits, are presented in Table 3. Based on the calculated LCs values, the essential oil derived from the leaves demonstrated greater fumigant toxicity against *Rhyzopertha dominica* than the oil obtained from the fruits. This suggests that *R. dominica* is more sensitive to fumigant exposure from leaf essential oil.

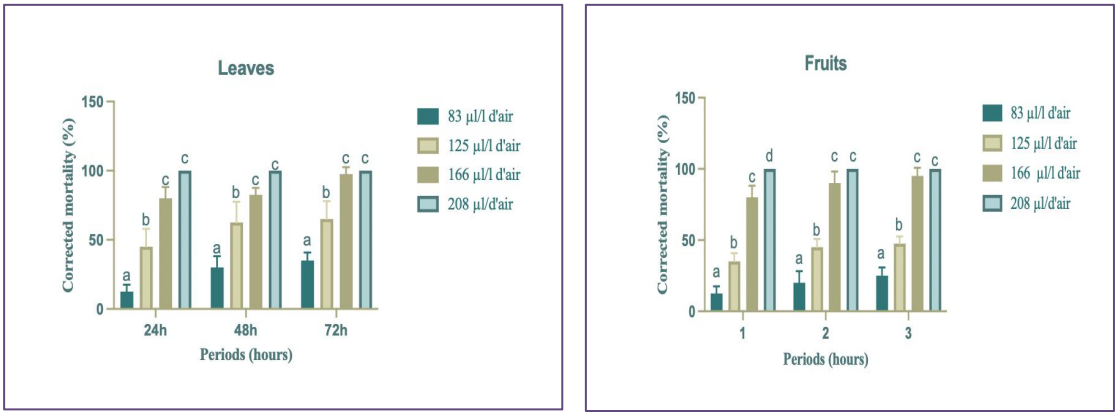



**Figure 6.** Toxicity of *Eucalyptus globuls* fruits EO leaves and fruits applied by fumigation (µl/l of air) on adults of *Rhyzopertha dominica* at different exposure times. Corrected mortality (%) (mean ± SEM, n = 5 replicates of 10 individuals each); Tukey's HSD test.

**Table 3.** Probit analysis of the ingestion toxicity of *Eucalyptus globulus* essential oils (leaves and fruits) on adults of *Rhyzopertha dominica.*

| Treatment | Time | $LC_{25}$ (CI95%) | $LC_{50}$ (CI95%) | $LC_{90}$ (CI95%) |
|---|---|---|---|---|
| Leaves | 24h | 104.9 [77,84-126.5] | 127.7 [107.8-146.2] | 189.4 [79.8-272.7] |
| | 48h | 79.66 [46.33-104.3] | 106.2 [81.00-128.0] | 188.8 [136.1-329.0] |
| | 72h | 76.64 [20.05-103.3] | 99.25 [50.58-129.5] | 166.4 [274.2-467.1] |
| Fruits | 24h | 114.6 [74.17-143.3] | 135.0 [107.4-158.4] | 187.2 [60.0-322.1] |
| | 48h | 100.0 [34.43-142.0] | 122.4 [74.94-156.9] | 183.3 [124.1-594.4] |
| | 72h | 92.99 [31.55-322.1] | 116.3 [124.1-592.4] | 181.9 [50.0-16832] |

### 3.3. Repellent Activity

The repellency results of *Eucalyptus globulus* leaves essential oil against *Rhyzopertha dominica* are presented in Table 4. The percentage of repellency increased with higher concentrations and varied over time. At the lowest tested concentration (6 µL/mL), moderate repellency rates were observed, with values ranging from 40% to 80% and repellency classes of III to IV. The concentration of 12 µL/mL resulted in consistent repellency values ranging from 60% to 80%, corresponding mostly to class IV. At the highest concentration (24 µL/mL), repellency percentages ranged from 65% to 85%, with the highest value recorded at 12 hours, placing it in repellency class V. These results confirm that repellency increases with the concentration of the essential oil, while the effect tends to stabilize or slightly decrease over time at certain doses. Moreover, based on the repellency classes, *R. dominica* exposed to the highest concentration of leaf essential oil was placed in class V, indicating a strong repellent effect.

The repellent activity of *Eucalyptus globulus* fruit essential oil against *Rhyzopertha dominica* is summarized in Table 4. Unlike the leaf oil, the fruit oil exhibited an earlier onset of strong repellency, particularly at intermediate and high concentrations. At 6 µL/mL, the repulsion percentage ranged from 40% to 70%, corresponding to repellency classes III and IV. With the application of 12 µL/mL, the oil demonstrated enhanced efficacy, with repellency values rising between 60% and 95%, and several observations falling into class V, indicating a very strong repellent effect. At the highest concentration tested (24 µL/mL), repellency remained consistently high across all time intervals, ranging from 65% to 95%, and reaching class V at most time points. The maximum repellency (95%) was recorded at both 12 µL/mL and 24 µL/mL after 12 hours of exposure.

These findings confirm that the repellent efficacy of the fruit-derived oil increases with both concentration and exposure time, and in general, the fruit oil showed slightly higher repellent performance than the leaf oil under similar test conditions. This highlights its promising potential for use in integrated pest management strategies targeting R. dominica.

**Table 4.** Repellent percent (RP) and Repellent class (RC) of different concentrations of *Eucalyptus globulus* leaves and fruits EO against adults of *R. dominica*.

| | | Leaves | | Fruits | |
|---|---|---|---|---|---|
| Concentrations | Periods | RP % | RC | RP % | RC |
| 6 µL/mL | ½ h | 40 | III | 40 | III |
| | 1 h | 70 | IV | 65 | IV |
| | 3 h | 80 | IV | 70 | IV |
| | 6 h | 40 | III | 60 | III |
| | 12 h | 45 | III | 65 | IV |
| 12 µL/mL | ½ h | 60 | III | 60 | III |
| | 1 h | 80 | IV | 95 | V |
| | 3 h | 75 | IV | 80 | IV |
| | 6 h | 80 | IV | 85 | V |
| | 12 h | 80 | IV | 85 | V |
| 24 µL/mL | ½ h | 65 | IV | 65 | IV |
| | 1 h | 80 | IV | 85 | V |
| | 3 h | 75 | IV | 90 | V |
| | 6 h | 80 | IV | 85 | V |
| | 12 h | 85 | V | 95 | V |

# Discussion

## IV. Discussion

Essential oils also known as extracts are fragrant and volatile compounds obtained from plant sources through various extraction techniques (Vora et al., 2024). In the present study, hydrodistillation of *Eucalyptus globulus* leaves and fruits resulted in essential oil yields of 1.8% and 2.9%, respectively. Fruits exhibited a substantial yield, likely due to their greater density of secretory glands compared to leaves. These findings align with existing literature identifying fruits as the primary reservoir of essential oils in many Eucalyptus species (Boulekbache-Makhlouf et al., 2016, Shiferaw et al., 2019).

Essential oil content within the Eucalyptus genus varies considerably due to species differences, environmental conditions, plant maturity, and extraction methods. Boulekbache-Makhlouf et al. (2016) reported a yield of 2.53% from leaves and 1.87% from fruits of *Eucalyptus globulus*, confirming the predominance of leaves. Conversely, Marzoug et al. (2011) found higher essential oil content in fruits than leaves in *Eucalyptus oleosa*, highlighting interspecific variation. Additionally, Shiferaw et al. (2019) demonstrated that tree age significantly affects oil yield in Eucalyptus globulus, with younger trees (3 years) yielding 1.32% compared to 0.95% from older trees (100 years).

Variations in essential oil yield have also been observed across different geographic locations. For example, Aloui (2024) reported a yield of $2.23 \pm 0.14\%$ in the town of Bir El Ater (Tébessa) from dry flowers. A notably high yield of 3.40% was recorded in Tébessa city (Brahmi & Yousfi, 2021). In the sub-humid region of Seraïdi (Annaba), Moussa et al. (2024) recorded a yield of 1.58% from dried leaves. In arid and semi-arid regions of Algeria, hydrodistillation of leaves yielded 2.64% in Saïda (Benabdesslem & Mébarki, 2020), while in Mascara it was $0.36 \pm 0.03\%$ (Bourakna et al., 2022), in M'Sila 0.43% (Lamara et al., 2022) and in Béchar, 0.23% (Djellouli et al., 2024a).

In Morocco, yields ranged from 0.25% to 2.80% depending on environmental factors and extraction methods (El Guerrouj & Taibi, 2023). In Tunisia, yield was 1.25% (Slimane et al., 2014). In France, it ranged between 1.5% and 3.5% ("Assessment report on *Eucalyptus globulus*

Labill.,, 2010). In Portugal, approximately 1.7% was observed (Oliveira et al., 2023), while in Spain, yields ranged between 1.59% and 1.93% (Mediavilla et al., 2021).

These results underscore the impact of geographic origin, climate, plant organ, and cultivation practices on the essential oil yield of *Eucalyptus globulus*. Several factors contribute to this variability, including climatic parameters (temperature, humidity), soil type and quality, and the specific plant organ used, as essential oil content differs significantly between leaves, flowers, roots, and other organs. Moreover, harvesting period, post-harvest handling (such as drying and storage conditions), and extraction technique significantly influence both yield and chemical composition. Collectively, these factors complicate efforts to standardize essential oil production and explain observed variability in composition and biological activity (Véchambre, 2019; Djerbal, 2023).

### 4.2 Toxicity of Treatments

Recent scientific advancements, combined with increasing awareness of the detrimental environmental impacts caused by synthetic pesticides, have driven significant research efforts toward identifying safer and more sustainable alternatives. Among these, essential oils have emerged as promising natural biopesticides due to their diverse bioactive properties and lower ecological risks. This growing interest highlights the potential of essential oils to contribute to integrated pest management strategies while minimizing the negative effects on ecosystems (Popescu, Gostin, and Blidar, 2024).

They exhibit a broad spectrum of biological activities against insects, including toxicity, repellency, feeding deterrence, inhibition of oviposition, and suppression of growth and reproductive capacity. Moreover, essential oils impact insects by disrupting their nervous system through inhibition of acetylcholinesterase and interference with neurotransmitter receptors. Furthermore, they alter feeding and host selection behaviors, reducing food intake and growth. Additionally, essential oils inhibit digestive enzymes, impair nutrient absorption, induce oxidative stress, and disrupt hormonal balance, affecting insect development and reproduction (Czerniewicz, Sytykiewicz, and Chrzanowski, 2024).

Essential oils are biodegradable and exhibit low toxicity toward non-target organisms, making them environmentally friendly alternatives to synthetic pesticides. However, their practical

application faces challenges related to stability and efficacy under varying environmental conditions (Assadpour, Can Karaça, and Fasamanesh, 2023; Rubika et al., 2024). Essential oils, extracted from plants, encompass a vast array of over 3,000 compounds, predominantly terpenes, phenols, and other volatile aromatic constituents. This chemical diversity underpins their wide-ranging biological activities (Yeshi and Wangchuk, 2022).

Insecticide resistance involves a range of physiological, structural, and molecular adaptations that reduce the efficacy of chemical control agents. Penetration resistance is associated with structural and compositional modifications of the cuticle such as thickening and altered hydrocarbon synthesis mediated by the overexpression of proteins and enzymes like cytochrome P450 (e.g., CYP4G16), which reduce insecticide absorption and enhance detoxification (Balabanidou, Grigoraki, and Vontas, 2018). Additionally, certain insecticides can bypass the hemolymph by spreading through the cuticle or diffusing via the tracheal system, enabling rapid distribution along the body surface through capillary forces (Yasuharu Takaku, 2023). At the molecular level, insecticides exert their toxic effects by targeting membrane receptors and ion channels, including voltage-gated sodium channels, GABA receptors, and nicotinic acetylcholine receptors, thereby disrupting neural transmission and causing paralysis or death (Casida and Durkin, 2013).

Our study aimed to evaluate the toxicity of essential oils extracted from the leaves and fruits of *Eucalyptus globulus* against adult *Rhyzopertha dominica* using two different application methods: fumigation and ingestion. Both methods demonstrated insecticidal efficacy, but the degree of toxicity and mode of action varied.

### 4.2.1 Ingestion Toxicity

The ingestion method, which involves mixing essential oil with food substrates, proved to be the more effective application route. Toxicological tests revealed a clear dose-response relationship, where higher concentrations of *E. globulus* essential oil caused significantly increased mortality rates. Ingestion caused marked disruptions in feeding behavior, likely due to both repellent and toxic effects after consumption. Essential oil constituents interfere with digestive enzymes, nutrient absorption, and hormonal regulation ultimately leading to developmental arrest and death (Czerniewicz, Sytykiewicz, and Chrzanowski, 2024).

In this study, ingestion of essential oil from *E. globulus* leaves and fruits resulted in lower lethal

concentration values compared to fumigation, confirming greater efficacy through oral exposure. The potent effect is attributed mainly to the high concentration of 1,8-cineole (eucalyptol), which acts as a neurotoxin and disrupts essential physiological functions through internal absorption mechanisms (Bachheti, 2015; Čmiková et al., 2023; Djellouli, Ziane, and Elhendi, 2024b).

Several recent studies reinforce the highly effective insecticidal action of Eucalyptus essential oils against *Rhyzopertha dominica,* aligning closely with our findings on both leaves and fruits via ingestion. Tewari (2008) evaluated the fumigant toxicity of 16 essential oils including *Eucalyptus globulus* against *R. dominica,* reporting complete inhibition of progeny at 0.4% oil concentration. Notably, *Eucalyptus globulus* displayed strong fumigant activity and long-term population suppression. While that study focused on fumigation, it confirms the rapid onset of toxicity that we also observed through ingestion. More directly relevant, Filomeno et al. (2022) reviewed essential oil efficacy against *R. dominica* and emphasized that oils rich in 1,8-cineole such as those from *E. globulus* exhibit potent fumigant and contact toxicity, achieving $LC_{50}$ values as low as 25 µL/L of air.

The results of Aiboud (2012) showed that the application of essential oils from myrtle, eucalyptus, oregano, and clove via inhalation induced significant toxicity against *Callosobruchus maculatus larvae.* According to Taleb-Toudert (2015), *Eucalyptus globulus* essential oil caused 100% mortality in *C. maculatus* adults after 96 hours of exposure to a dose of 4 µL/L of air. Several studies have also demonstrated the toxicity of *Eucalyptus globulus* oil against various stored-product insect species, such as *Tribolium castaneum* (Reguibi, 2021), *Rhyzopertha dominica* (Belhocine and Hammana, 2017), *Trogoderma granarium* (Brahmi and Yousfi, 2021), and *Acanthoscelides obtectus* (Regnault-Roger and Hamraoui, 1993; Taib, 2015). Their insecticidal property is attributed to the presence of volatile compounds, including eucalyptol or 1,8-cineole (Lee et al., 2004), citronellal, p-cymene, eucamalol, limonene, linalool, α-pinene, α-terpineol, and allocimene (Choi et al., 2002).

Ingestion bioassays using other Eucalyptus species support this trend. Ebadollahi et al. (2022) showed that ingestion of *Eucalyptus microtheca* essential oil led to $LC_{50}$ values of 25 µL/L air at 24 h, decreasing to 19 µL/L at 72 h. Similarly, the review by Filomeno et al. (2022) reported that cineole-rich Eucalyptus oils consistently produced pronounced toxicity by ingestion.

These findings are consistent with previous research that demonstrated ingestion as an efficient mode of delivery for essential oils and other botanical insecticides against stored-product pests

(Mann and Kaufman, 2012; Ebadollahi et al., 2022). In particular, compounds such as 1,8-cineole, α-pinene, and β-pinene show strong systemic toxicity when ingested, impairing enzyme activity and metabolic function.

### 4.2.2 Fumigation Toxicity

Although ingestion showed higher efficacy, fumigation also exhibited notable insecticidal effects. In our assays, *R. dominica* adults exposed to vapor-phase essential oils experienced increasing mortality with rising concentrations and longer exposure durations. Fumigation allows essential oil volatiles to penetrate the insect's spiracles and tracheal system, inducing neurotoxic effects by interfering with neurotransmission (Casida and Durkin, 2013).

Essential oils used in fumigation act quickly by vapor diffusion, which leads to paralysis and eventual death. The presence of lipophilic monoterpenes such as 1,8-cineole facilitates rapid penetration into the insect's central nervous system. Leaf-derived essential oil produced slightly stronger effects than fruit-derived oil, likely due to compositional differences especially the proportion of cineole and α-pinene.

Several studies have demonstrated the effectiveness of *Eucalyptus globulus* essential oil through fumigation against various stored-product pests, including *Rhyzopertha dominica.* In our study, the fumigation of adult *R. dominica* with *E. globulus* essential oils extracted from leaves and fruits resulted in significant mortality, though slightly less effective than ingestion. These results are consistent with those of Belhocine and Hammana (2017), who reported high toxicity of *E. globulus* EO against *R. dominica* adults through inhalation. Similarly, Taleb-Toudert (2015) observed 100% mortality in *Callosobruchus maculatus* adults after 96 hours of fumigation at a dose of 4 µL/L of air. Aiboud (2012) also reported strong fumigant activity of *E. globulus* and other essential oils (myrtle, oregano, clove) against *C. maculatus larvae*. The fumigant toxicity is largely attributed to volatile constituents such as 1,8-cineole, α-pinene, citronellal, and p-cymene, which interfere with the insect nervous system (Lee et al., 2004; Choi et al., 2002). Additionally, Brahmi and Yousfi (2021) found that *E. globulus* EO exerted lethal effects on *Trogoderma granarium* by vapor-phase exposure, while Reguibi (2021) noted its efficacy against *Tribolium castaneum*. These findings support the fumigant potential of *E. globulus* EO as a natural alternative to synthetic insecticides, though environmental factors such as temperature and exposure time may influence its effectiveness.

The chemical composition of *Eucalyptus globulus* essential oil, especially its richness in 1,8-cineole, α-pinene, α-terpinyl acetate, and globulol, explains its strong insecticidal properties. These compounds are responsible for the interference with neurotransmitter function and the destruction of cellular integrity in insect systems (Bachheti, 2015; Čmiková et al., 2023; Djellouli, Ziane, and Elhendi, 2024b).

### 4.3. Repellent activity

Repellency is a natural physiological response observed in many insect species, acting as a defense mechanism to avoid potentially harmful or toxic substances often emitted by plants (Isman, 2006). In this context, plant-derived essential oils (EOs) have gained increasing attention as environmentally friendly alternatives to synthetic pesticides. Their mode of action often involves the stimulation of olfactory or gustatory receptors in insects, thereby disrupting host-finding behavior and deterring contact with treated surfaces (Talukder, 2006). EOs offer several advantages including biodegradability, relatively low toxicity to non-target organisms, and minimal residual persistence in the environment (Damalas & Eleftherohorinos, 2011; Mossa, 2016). However, their effectiveness is influenced by multiple factors such as the chemical composition of the oil, the volatility and stability of its constituents, the formulation used, the method of application, and prevailing environmental conditions including temperature, humidity, and air flow (Barnard, 2000; Bedini et al., 2024).

Our findings clearly demonstrate the repellent efficacy of *Eucalyptus globulus* essential oils against adult *Rhyzopertha dominica*, with the oils extracted from the fruits exhibiting significantly stronger repellency compared to those derived from the leaves. This difference may be attributed to a higher concentration or different profile of bioactive compounds in the fruits, particularly monoterpenes and oxygenated monoterpenes such as 1,8-cineole (eucalyptol), which is known for its high volatility and potent insect-repellent properties. The prominent activity of the fruit-based oil in our bioassays suggests that the fruit parts of *E. globulus* could serve as a more potent source of repellents than the leaves, aligning with prior research showing variable efficacy of plant organs in EO-based insect control (Tomizawa & Casida, 1999).

Several studies have reported the repellent activity of *E. globulus* and its main constituents against storage pests. Taib (2015) demonstrated a 90% repellency rate of *E. globulus* EO against Acanthoscelides obtectus. Brahmi & Yousfi (2021) observed an 85% repellency rate against *Trogoderma granarium*. Tapondjou et al. (2005) found that *Eucalyptus saligna,* a close relative of *E. globulus,* exhibited a repellency rate as high as 98% against *Tribolium confusum.* Furthermore, 1,8-cineole, the major component of many eucalyptus oils, has shown notable repellent activity against *Sitophilus granarius* (70%), *T. castaneum* (48%), *S. zeamais* (70%), and *Prostephanus truncatus* (50%) at concentrations of 10 µL/mL (Obeng-Ofori et al., 1997). Similar effects have been reported by Aggarwal et al. (2001) against *Callosobruchus maculatus*, *Rhyzopertha dominica,* and *Sitophilus oryzae*. The diversity of responses among insect species is believed to stem from differences in their olfactory receptor sensitivities and the specific interaction between individual EO constituents and insect neurophysiology.

It is also worth noting that in some cases, essential oils may exhibit hormetic effects, where low concentrations can paradoxically attract insects while higher doses elicit repellency (Bedini et al., 2024). This biphasic dose-response underscores the necessity of precise dosing and standardization of bioassay protocols to avoid misleading or inconsistent results when developing plant-based repellent formulations.

Additionally, synergistic effects between different essential oils can enhance repellent efficacy. For instance, Sheikh et al. (2021) demonstrated that a 10% concentration of *E. globulus* EO provided a 77.78% repellency rate against Anopheles stephensi, which increased to 94.44% when combined with *Syzygium aromaticum* EO in equal proportions. This highlights the potential of formulation optimization to improve bioactivity and prolong effectiveness.

In our case, the superior repellency of *E. globulus* fruit-derived EO against *R. dominica* strongly supports its potential application as a natural insect deterrent in stored grain protection strategies. Given the high economic impact of this pest and the limitations associated with synthetic pesticides such as resistance development, residue accumulation, and environmental hazards the utilization of fruit-derived *E. globulus* EO presents a promising, sustainable alternative. These findings contribute to a growing body of evidence supporting the valorization of aromatic plant

resources in the development of eco-friendly biopesticides and align well with integrated pest management (IPM) principles.

# CONCLUSION AND PERSPECTIVES

## V. CONCLUSION AND PERSPECTIVES

This study provides compelling evidence of the bioinsecticidal potential of essential oils extracted from the leaves and fruits of *Eucalyptus globulus* against *Rhyzopertha dominica*, a key pest of stored cereal grains. Through a comprehensive experimental approach encompassing toxicity (via ingestion and fumigation) and repellency assays, our results highlight the effectiveness of *E. globulus* essential oil as a promising botanical alternative to conventional chemical insecticides.

Toxicological evaluations clearly revealed dose-dependent mortality, with the ingestion pathway proving to be more effective than fumigation in inducing lethal effects. Importantly, between the two plant parts studied, essential oil extracted from the leaves showed the highest toxicity, both in ingestion and fumigation assays. This may be attributed to the qualitative and quantitative differences in bioactive compounds concentrated in the leaves, particularly those with insecticidal properties such as 1,8-cineole.

In contrast, repellency assays conducted using the preferential zone method demonstrated that the oil derived from the fruits of E. globulus exhibited a stronger repellent effect against R. dominica adults than that from the leaves. This suggests a distinct volatile profile in the fruits, potentially containing higher proportions of compounds with olfactory deterrent activity.

To ensure the practical implementation of these findings, further studies are necessary to:

- Target other pest species to broaden the spectrum of action of this essential oil.

- Evaluate the effect of this oil on the reproductive potential of the pests.

- Investigate potential synergistic interactions with other plant-derived extracts or essential oils to enhance bioefficacy of pest control activity.

# ABSTRACT

**Abstract**

This study demonstrates the bioinsecticidal potential of essential oils extracted from the leaves and fruits of *Eucalyptus globulus* against *Rhyzopertha dominica,* a major pest of stored cereals. A series of laboratory bioassays were conducted to evaluate both toxicity (via ingestion and fumigation) and repellent effects of the essential oils. The results demonstrated significant dose-dependent mortality in both ingestion and fumigation assays. Among the tested plant parts, leaves-derived essential oil exhibited the highest toxicity, likely due to a higher concentration of bioactive compounds, particularly 1,8-cineole, known for its strong insecticidal properties.

Repellency tests conducted using the preferential zone method revealed that the oil extracted from the fruits of *E. globulus* displayed superior repellent activity against adult *R. dominica* compared to that of the leaves. This enhanced repellent action is attributed to the distinct volatile profile of the fruits, which may include higher proportions of olfactory-disruptive compounds. These findings underscore the dual mode of action (toxicity and repellency) of *E. globulus* essential oils and support their potential application as eco-friendly alternatives to synthetic insecticides for the protection of stored cereals.

**Keywords**: *Eucalyptus globulus, Rhyzopertha dominica,* essential oil, bioinsecticide, fumigation, ingestion toxicity, repellency.

**Résumé**

Cette étude met en évidence le potentiel bioinsecticide des huiles essentielles extraites des feuilles et des fruits d'Eucalyptus globulus vis-à-vis de Rhyzopertha dominica, un ravageur cosmopolite majeur des céréales stockées. Des bioessais en laboratoire ont été réalisés afin d'évaluer, d'une part, la toxicité des huiles essentielles par ingestion et par fumigation, et d'autre part, leur activité répulsive à l'aide de la méthode des zones préférentielles.

Les résultats ont mis en évidence une mortalité significative et dose-dépendante dans les deux modes d'exposition. L'huile essentielle extraite des feuilles a démontré une toxicité plus marquée que celle des fruits, ce qui pourrait être attribué à une teneur plus élevée en composés bioactifs à propriétés insecticides, notamment le 1,8-cinéole. En revanche, les essais de répulsion ont révélé que l'huile essentielle issue des fruits présentait une activité répulsive supérieure à celle des feuilles vis-à-vis des adultes de R. dominica. Ce pouvoir répulsif accru pourrait résulter d'un profil volatil distinct des fruits, probablement riche en composés perturbant les récepteurs olfactifs des insectes.

Ces résultats mettent en lumière le double mécanisme d'action (toxicité et répulsion) des huiles essentielles d'E. globulus et soutiennent leur intégration potentielle dans des stratégies de lutte alternative, écologiquement responsables, contre les ravageurs des denrées stockées.

**Mots-clés:** *Eucalyptus globulus*, *Rhyzopertha dominica*, huile essentielle, bioinsecticide, fumigation, ingestion, répulsion.

**الملخص**

تُظهر هذه الدراسة الفعالية المبيدة للحشرات للزيوت العطرية المستخلصة من أوراق وثمار *Eucalyptus globulus* ضد حشرة *Rhyzopertha dominica*، وهي من الآفات الرئيسية التي تهاجم الحبوب المخزنة. تم إجراء سلسلة من التجارب المخبرية لتقييم كل من السمية (عن طريق الابتلاع والتدخين) والتأثير الطارد للزيوت العطرية. أظهرت النتائج وجود معدل وفيات مرتفع يعتمد على الجرعة في كلا الاختبارين. ومن بين الأجزاء النباتية المختبرة، أظهرت الزيوت المستخرجة من الأوراق أعلى درجة من السمية، ويُعزى ذلك على الأرجح إلى احتوائها على تركيز عالٍ من المركبات النشطة حيوياً، وخصوصاً 1,8-سينيول المعروف بخصائصه القوية كمبيد حشري.

أما اختبارات الطرد التي أُجريت باستخدام طريقة المنطقة التفضيلية، فقد أظهرت أن الزيت المستخرج من ثمار *E. globulus* يتمتع بفعالية طاردة أعلى ضد البالغات من *R. dominica* مقارنة بزيت الأوراق. ويُعزى هذا التأثير الطارد القوي إلى التركيب الكيميائي الطيار المميز للثمار، الذي قد يحتوي على نسب أعلى من المركبات التي تعيق الإشارات الشمية للحشرات. تؤكد هذه النتائج فعالية الزيوت العطرية لـ *E. globulus* كمبيد طبيعي مزدوج التأثير (سُمي وطارد)، وتدعم استخدامها كبديل صديق للبيئة للمبيدات الكيميائية في حماية الحبوب المخزنة.

**الكلمات المفتاحية**: *Eucalyptus globulus*، *Rhyzopertha dominica*، زيت عطري، مبيد حشري نباتي، التدخين، السمية عبر الابتلاع، الطرد.

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