2025, 10(63s)

e-ISSN: 2468-4376

https://jisem-journal.com/

**Research Article** 

# Integration of a Natural Biopesticide into Grain Storage Systems: Insecticidal Toxicity and Antifungal Activity of Mentha Pulegium Essential Oil

Wahiba Aous<sup>1</sup>, Samia Sadaoui Yahia Cherif<sup>2</sup>, Nadia Boughelit<sup>3</sup>, Fadila Neffah<sup>4</sup>, Karima Abdellaoui<sup>5</sup>, Hinda Hacib<sup>6</sup>, Naima Hadjadj<sup>7</sup>, Mohamed Hazzit<sup>8</sup>

¹Laboratory of Valorisation and Conservation of Biological Resources, Department of Agronomic Sciences, University of M'hamed Bougara, Boumerdes, Algeria. w.aous@univ-boumerdes.dz 2Laboratory of Biological Sciences, Dynamics and Biodiversity, University of Science and Technology, Houari Boumediene, BP39 El Alia, Algiers, Algeria. samia.sadaoui\_fsb@usthb.edu.dz 3Laboratory of Soft Technologies, Valorisation, and Physico-Chemistry of Biological Materials, Department of Agronomic Sciences, University of M'hamed Bougara, Boumerdes, Algeria. n.boughelit@univ-boumerdes.dz

4Laboratory of Soft Technologies, Valorisation, and Physico-Chemistry of Biological Materials, Department of Agronomic Sciences, University of M'hamed Bougara, Boumerdes, Algeria. fadhilabaziz@yahoo.fr

- <sup>5</sup> Laboratory of Valorisation and Conservation of Biological Resources, Department of Agronomic Sciences, University of M'hamed Bougara, Boumerdes, BP, Algeria; karimaviro@yahoo.fr
- <sup>6</sup> Ethnobotany and Natural Substances Laboratory, Bachir El Ibrahimi Higher Normal School, Kouba, Algiers. hinda.hacib@g.ens-kouba.dz
  - <sup>7</sup>Department of Food Sciences, Faculty of Natural and Life Sciences, University of Blida 1, Algeria. hadjadj\_naima@univ-blida.dz
  - <sup>8</sup>Department of Food Technology and Human Nutrition, National High School of Agronomy, El-Harrach (ENSA), Algiers, Algeria. mohazzit@gmail.com

#### **ARTICLE INFO**

#### ABSTRACT

Received: 17 Aug 2025 Revised: 28 Oct 2025

Accepted: 04 Nov 2025

Post-harvest losses of stored cereals are largely due to insects and phytopathogenic fungi, particularly in developing countries where storage conditions remain precarious. In the context of reducing the use of synthetic insecticides, essential oils are a promising alternative. This study evaluates (i) the insecticidal activity of Mentha pulegium L. (pennyroyal) essential oil against two major pests of stored commodities, Sitophilus oryzae L. and Tribolium castaneum Herbst, by inhalation and ingestion, and (ii) its in vitro antifungal activity against three phytopathogenic fungi, Rhizoctonia spp., Fusarium spp. and Alternaria spp.

The essential oil was extracted by hydrodistillation using a Clevenger apparatus and then characterised by GC-MS. Insecticidal bioassays were performed on adults fed or exposed to different doses of the essential oil; mortalities were corrected using the Abbott formula and the  $LC_{50}/LD_{50}$  estimated by probit analysis. Antifungal activity was studied by direct comparison on PDA medium supplemented with 0.25 and 0.5% (v/v) essential oil.

The essential oil of M. pulegium is dominated by pulegone ( $\approx$  60%) and menthone ( $\approx$  7.5%). When inhaled, it induces up to 100% mortality in both insect species at high doses, with a lower LC50 in S. oryzae (54.20  $\mu$ L/L of air) than in T. castaneum (93.31  $\mu$ L/L). When ingested, the LD50 is lower in T. castaneum (46.01  $\mu$ L/mg of grain) than in S. oryzae (66.02  $\mu$ L/mg).

2025, 10(63s) e-ISSN: 2468-4376

https://jisem-journal.com/

#### **Research Article**

In vitro, M. pulegium strongly inhibits the mycelial growth of Rhizoctonia spp. ( $\approx$  90%), Alternaria spp. ( $\approx$  85%) and, to a lesser extent, Fusarium spp. (54-66%).

These results confirm the potential of M. pulegium as a natural biopesticide with dual insecticidal and antifungal action for the protection of stored commodities.

**Keywords:** Mentha pulegium, essential oils, biopesticide, Sitophilus oryzae, Tribolium castaneum, phytopathogenic fungi.

#### 1. Introduction

Stored cereals provide a particularly favourable environment for the development of insects and microorganisms due to the simultaneous availability of food, oxygen and water (Groot, 2004). Infestations result in significant quantitative losses, but also qualitative deterioration (decrease in specific weight, germination capacity, sanitary quality), which can exceed 20% in developing countries (Phillips & Throne, 2010; Banga et al., 2018). These losses are exacerbated when storage conditions are poor (Abukar et al., 1986; Ratnadass & Sauphanor, 1989; Lavigne, 1991).

Primary insects, such as the rice weevil *Sitophilus oryzae*, the grain weevil *Rhyzopertha dominica* and the grain moth *Sitotroga cerealella*, develop inside the grain and pave the way for secondary insects, notably *Tribolium castaneum*, which proliferate in flour and broken grains (Fleurat-Lessard, 2011). In addition to directly consuming the substrate, these pests contaminate foodstuffs with their exuviae, excrement, secretions and carcasses, which can cause allergic reactions in consumers and promote the growth of toxigenic fungi (Stejskal et al., 2003). According to the FAO, losses due to pests can reach nearly 35% of global agricultural production.

Chemical control, mainly based on the use of synthetic insecticides and fumigants, is now increasingly being questioned due to its impact on human health, the environment and the emergence of resistance. In this context, plant extracts, particularly essential oils, are attracting growing interest as environmentally friendly alternatives. Many aromatic species from the Apiaceae, Lamiaceae, Lauraceae and Myrtaceae families have demonstrated antimicrobial (Erhan et al., 2012; Cherrat et al., 2014; Abdelli et al., 2016), antioxidant (Khalife et al., 2013; Carrasco et al., 2015; Salhi et al., 2017), anti-inflammatory (Khlifi et al., 2013), nematicidal (Ortu et al., 2016) and insecticidal (Ben Slimane et al., 2015; Abdelli et al., 2016; Tampe et al., 2016; Ben Chaaban et al., 2019).

Mentha pulegium L. (pennyroyal, Lamiaceae) is an aromatic plant widely used in traditional medicine and described for its antimicrobial, antioxidant and insecticidal properties. Its essential oil, rich in pulegone, has shown strong activity against several storage pests and various phytopathogenic fungi (Hajlaoui et al., 2009; Abou & Fareh, 2017; Baali et al., 2019).

The objectives of this study are: to characterise the chemical composition of *M. pulegium* essential oil harvested in Algeria; to evaluate its insecticidal activity by inhalation and ingestion against *S. oryzae* and *T. castaneum*; and to determine its antifungal activity against *Rhizoctonia spp.*, *Fusarium spp.* and *Alternaria spp.* 

2025, 10(63s) e-ISSN: 2468-4376

https://jisem-journal.com/

**Research Article** 

#### 2. Experiment

#### 2.1. Plant material and isolation of essential oil

The aerial parts of *Mentha pulegium* L. were collected at the flowering stage in a preserved natural site, free from major sources of pollution. After harvesting, the plant material was carefully cleaned of impurities and then slowly dried away from light in a ventilated room in order to best preserve the volatile compounds. Once completely dry, the dried leaves and stems were manually fragmented to facilitate the subsequent extraction of the essential oil.

The essential oil (EO) was isolated from the dried aerial parts of the plant by hydrodistillation of a 100 g sample using a Clevenger apparatus for 3 hours following the protocol of the European Pharmacopoeia. The recovered EO was dried using anhydrous sodium sulphate and then stored at 4°C for further analysis.

#### 2.2.GC-MS analysis and identification of essential oil components

Gas chromatography-mass spectrometry (GC-MS) analysis was performed using a Hewlett-Packard computerised system comprising a 6890 gas chromatograph coupled to a 5973A mass spectrometer, equipped with a non-polar HP-5 MS capillary column (30 m  $\times$  0.25 mm i.d., 0.25  $\mu$ m film thickness). Helium (He) served as the carrier gas at a flow rate of 0.5 mL/min, operating in split mode (1:25), with an injection volume of 0.2  $\mu$ L (diluted 1:10 in hexane, v/v). The column temperature was initially held at 60°C for 8 minutes then ramped at a rate of 2°C/min to reach 280°C, where it was maintained for 15 minutes. Electron ionisation at 70 eV was employed with a scan range of 30–550 atomic mass units.

# 2.3. Identification of volatile compounds

The identification of the essential oil constituents was based on the simultaneous comparison of mass spectra and experimental retention indices. The retention indices were calculated using the Van den Dool and Kratz method and then compared with values published in the literature, in particular those compiled by Adams. The NIST and Wiley databases were used as references for the mass spectra. This dual approach (spectral and chromatographic) enabled rigorous identification of the major and minor compounds in the essential oil.

#### 2.4. Insecticidal activity

The activity of the essential oil against adults of *S. oryzae and T. castaneum* was tested using two modes of action: fumigation and contact/ingestion.

#### 2.4.1. Insect rearing

Entomological bioassays were performed on two stored product pests: *Sitophilus oryzae* and *Tribolium castaneum*. The breeding colonies were kept in glass jars containing healthy soft wheat. The containers were covered with a fine cloth to allow sufficient ventilation while preventing escape. The breeding was carried out at  $25 \pm 2$  °C and  $70 \pm 5\%$  relative humidity, in darkness, which are optimal conditions for the continuous reproduction of both species. The individuals used for the tests came from homogeneous populations a few days old.

2025, 10(63s)

e-ISSN: 2468-4376

https://jisem-journal.com/

**Research Article** 

#### 2.4.2. Fumigant toxicity

The fumigation tests were carried out in 40 mL airtight bottles. Filter paper discs with a diameter of three centimetres were impregnated with 8, 16, 32 or 64  $\mu$ L of essential oil, corresponding to concentrations of 200, 400, 900 and 1600  $\mu$ L/L of air, respectively. These discs were attached under the caps to prevent direct contact with the insects while allowing for even diffusion of the vapours. Twenty adults of each species were placed in each vial, and three replicates were performed for each dose as well as for the acetone control. Mortality was recorded after 24, 48, 72 and 96 hours. The values observed were corrected using the Abbott formula to take account of spontaneous mortality. Median lethal concentrations (LC50) were calculated using probit analysis.

## 2.4.3. Contact/ingestion toxicity

For the contact/ingestion test, 10 g of soft wheat were sprayed with different doses of the essential oil diluted in acetone. The doses applied were 24  $\mu$ L, 48  $\mu$ L, 96  $\mu$ L and 192  $\mu$ L of diluted essential oil, equivalent to concentrations of 2.40% v/v and its dilutions in the substrate. Wheat grains treated with acetone alone served as a control. Twenty adult individuals were then added per 9 cm diameter Petri dish. Each dose was repeated three times. The mortality rate was calculated at the same intervals as in the inhalation test: 24 hours, 48 hours, 72 hours and 96 hours.

## 2.5. Expression of Insecticide Results

Mortality rates (M) were corrected using Abbott's formula (1925) to obtain corrected mortality percentages (Mc), taking into account natural mortality observed in the control boxes (Mt) according to the following formula:

$$CM\% = (M-Mt*100)/(100-Mt)$$

Where M represents the percentage of deaths in the treated population and Mt represents the percentage of deaths in the control population.

The efficacy of the toxicant was measured by determining the 50% lethal concentrations for inhalation and contact/ingestion using Probit analysis.

#### 2.6. Evaluation of Antifungal Activity (Direct Comparison Method)

The antifungal activity test was performed *in vitro* using the culture medium poisoning method. The essential oil (EO) was added under sterile conditions (using a 0.45 $\mu$ m filter syringe) to 100 mL of Potato Dextrose Agar (PDA) medium just before pouring (at 45°C) to obtain two final concentrations: 0.25% v/v and 0.5% v/v. PDA medium alone served as a negative control. Inoculation consisted of placing a mycelial disc (5 mm in diameter) from a young fungal culture in the centre of each Petri dish. The dishes were incubated at 25°C, with each treatment repeated five times (n=5). Mycelial growth was measured every 24 hours (by calculating the average of two perpendicular diameters). The inhibition rate (IR%) was then calculated after seven days of incubation using the formula:

$$II(\%) = 100 \times (dC - dE) / dC$$

Where dE Diameter represents the diameter of the treated colony and dC represents the diameter of the control colony

2025, 10(63s) e-ISSN: 2468-4376

https://jisem-journal.com/

**Research Article** 

## 2.7. Statistical analysis

Statistical analysis of the data (expressed as Mean  $\pm$  Standard Deviation or SEM) was performed using one-way analysis of variance (ANOVA). The Newman-Keuls test or Tukey's test (depending on the consistency of your results) was used as a post-hoc test for multiple comparisons, with a significance threshold set at P < 0.05. For LC 50 values, a Probit analysis was performed.

#### 3. Results

## 3.1. Chemical composition of the essential oil

GC-MS analysis identified 32 constituents representing 87.7% of the total oil (Table 1). The profile is dominated by pulegone ( $\approx 60\%$ ), followed by menthone (7.5%), isopulegol acetate (5.5%), neoisopulegol and piperitone, accompanied by several oxygenated sesquiterpenes in smaller proportions. This composition is characteristic of the pulegone-rich chemotypes described for *M. pulegium* in the Mediterranean region.

Table 1. Composition (%) of essential oils of spearmint

No.	Component	%	ERI	LRI
1	Ethyl-amyl carbinol	0.3	998	998
2	Limonene	0.35	1022	1022
3	Neoisopulegol	3.8	1146	1146
4	Menthone	7.5	1163	1160
5	Neomenthol	1.15	1160	1161
6	Pulegone	60	1229	1226
7	Piperitone	2.5	1257	1261
8	Isopulegyl acetate	5.5	1279	1281
9	Caryophyllene oxide	1	1572	1574
10	Humulene epoxide II	0.9	1601	1604
11	Oleic acid	0.35	2141	2142
12	Octadecanol acetate	1.5	2209	2208
13	Manool oxide	2.3	2214	2216

2025, 10(63s) e-ISSN: 2468-4376

https://jisem-journal.com/

# **Research Article**

			1	1	
14	Methyl eperuate	0.65	2222	2220	
15	Methyl sandaracopimarate	0.7	2252	2254	
16	Methyl communate	0.45	2257	2259	
17	β-Pinene	0.45	1010	1010	
18	Cineole	0.3	1035	1035	
19	Terpinolene	0.25	1080	1080	
20	Linalool	0.4	1098	1098	
21	Citronellal	0.35	1155	1155	
22	Citronellol	0.3	1165	1165	
23	Geraniol	0.25	1175	1175	
24	Terpineol	0.2	1180	1180	
25	Carvone	0.3	1205	1205	
26	Farnesene	0.2	1450	1450	
27	β-Caryophyllene	0.35	1520	1520	
28	Guaiol	0.25	1570	1570	
29	Selinene	0.15	1585	1585	
30	Bicyclogermacrene	0.2	1605	1605	
31	α-Humulene	0.15	1610	1610	
32	β-Farnesene	0.15	1640	1640	
	Total identified (%)	ı		87.7	
	Oxygen-containing monoterpenes  Esters Oxygen-containing sesquiterpenes Oxygenated diterpenes Monoterpene hydrocarbons				
	Others				
	Oil yield % (v/w)				

2025, 10(63s)

e-ISSN: 2468-4376

https://jisem-journal.com/

#### **Research Article**

<sup>a</sup>Components quantified on the HP 5MS capillary column and listed in order of their elution.

ERI and LRI: Experimental and Literature retention indices relative to n-alkanes C<sub>7</sub>-C<sub>17</sub>on non-polar column HP 5MS.

Main compounds (≥5%) marked in bold.

## 3.2. Insecticidal activity of Mentha pulegium essential oil

The insecticidal efficacy of the essential oil was evaluated against Sitophilus oryzae and Tribolium castaneum by inhalation and ingestion.

#### 3.2.1. Insecticidal activity by fumigation

## 3.2.1.1. Test on Sitophilus oryzae

The corrected mortality rates of *S. oryzae* increased significantly with dose and exposure time. At doses C3 (900  $\mu$ L/L) and C4 (1600  $\mu$ L/L), mortality reached 99.66% and 100% after 72 and 96 hours respectively, while at doses C1 and C2, mortality remained lower, particularly at 24 hours (< 50%) (Figure 1).

ANOVA shows a highly significant effect of dose on mortality (p < 0.001). The LC50 by fumigation is estimated at 54.20  $\mu$ L/L of air.

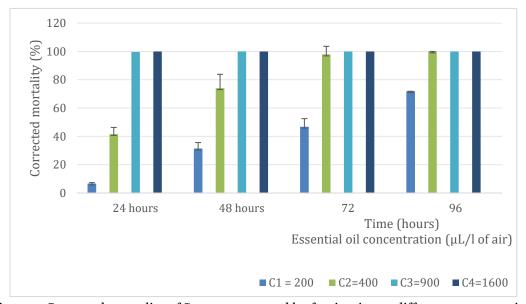


Figure 1. Corrected mortality of S. oryzae exposed by fumigation to different concentrations of M. pulegium essential oil as a function of time.

## 3.2.1.2. Test on Tribolium castaneum

In *T. castaneum*, the increase in corrected mortality is also dose-dependent. Doses C3 and C4 induce mortality close to 100% after 72–96 hours, while C1 and C2 show more moderate effects at short times

2025, 10(63s)

e-ISSN: 2468-4376

https://jisem-journal.com/

# **Research Article**

(Figure 2). The calculated LC50 is 93.31  $\mu$ L/L of air, revealing a lower sensitivity of the red flour beetle compared to the weevil.

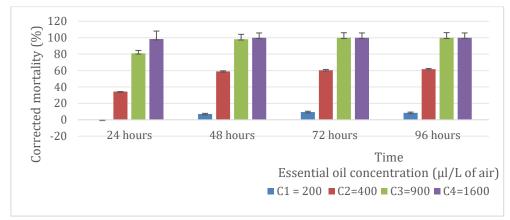


Figure 2. Corrected mortality of T. castaneum exposed by fumigation to different concentrations of M. pulegium essential oil as a function of time.

## 3.2.2. Insecticidal activity by ingestion

The insecticidal activity of the essential oil tested was evaluated by the mortality of adults of *S. oryzae* and *Tribolium castaneum* obtained by contact/ingestion. Their efficacy was determined by LC50 values.

## **3.2.2.1.** Test on *S. oryzae*

The cumulative and corrected mortality percentages of adult *S. oryzae* as a function of time and essential oil dose are shown in Figure 3.

The insecticidal activity of the essential oil by ingestion against S. oryzae was initially low for the first two doses, but a gradual increase was observed at concentration 3 (96  $\mu$ l).

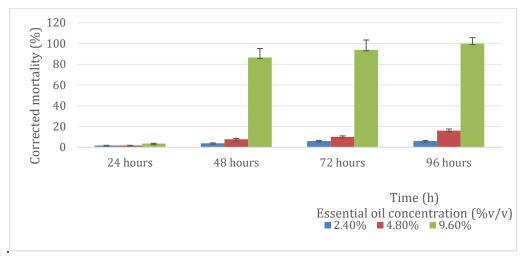


Figure 3. Corrected mortality by ingestion in S. oryzae as a function of M. pulegium essential oil doses and exposure time.

2025, 10(63s)

e-ISSN: 2468-4376

https://jisem-journal.com/ Research Article

#### 3.2.2.2.Test on T. castaneum

The cumulative and corrected mortality percentages of *T. castaneum* adults as a function of time and essential oil concentration are shown in Figure 4.

For *T. castaneum* by contact/ingestion, low mortality was observed after 24 hours for all doses. Activity only reached 100% on the third and fourth days (concentrations 3 and 4).

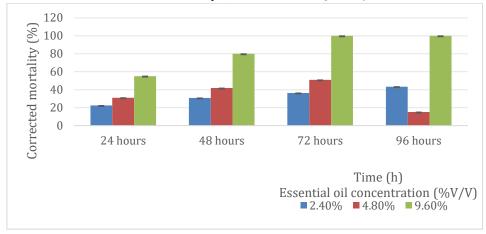


Figure 4. Corrected mortality by ingestion in T. castaneum as a function of M. pulegium essential oil doses and exposure time.

Mentha pulegium essential oil demonstrates variable insecticidal activity, highly dependent on the species and route of exposure. The results of the analysis of variance (ANOVA) confirm a highly significant effect of dose on mortality (p < 0.001), validating the dose-dependent approach. Fumigation is the most effective method, killing 50% of Sitophilus oryzae at a relatively low concentration of LC 50 =  $54.20\mu L/L$ \ of air compared to  $93.31\mu L/L$  of air for Tribolium castaneum, indicating that the weevil is the most sensitive species. However, through contact/ingestion, the efficacy decreases for both species LC50 around 6.7% (v/v) in grains and the difference in sensitivity between pests diminishes (Table 2). In conclusion, the volatile route is the main mode of toxic action, which positions this essential oil as a more promising fumigant than as a direct treatment for grains. Furthermore, the Newman-Keuls test divided the doses into two distinct groups with significantly different averages.

**Table 2.** LC50 and LD50 values of M. pulegium essential oil against S. oryzae and T. castaneum (after 96 hours of exposure).

Insect	Route of exposure	LC50(Fumigation) in μ L/L of air	LC50(Contact/Ingestion) in %(v/v) in grains
S. oryzae	Fumigation	54.2	-
S. oryzae	Contact/Ingestion	-	6.82
T. castaneum	Fumigation	93.31	-
T. castaneum	Contact/Ingestion	-	6.68

2025, 10(63s)

e-ISSN: 2468-4376

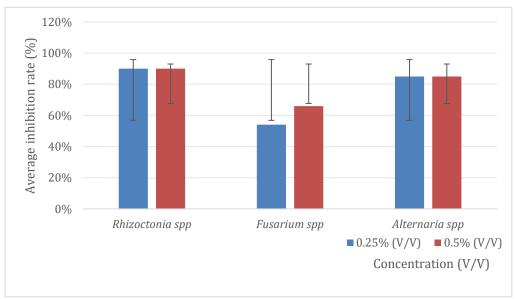
https://jisem-journal.com/

#### **Research Article**

## 3.3. Antifungal activity of Mentha pulegium essential oil

#### 3.3.1. Effect of essential oil on mycelial growth

The antifungal activity of *Mentha pulegium* essential oil was evaluated on three pathogenic fungal strains (*Rhizoctonia spp., Fusarium spp., and Alternaria spp.*) at two different doses (D1 and D2) (Figure 5). The oil's effectiveness varies depending on the fungal strain. The highest inhibition rate was observed on *Fusarium spp.*, reaching an average of 44% at dose D1 and 36% at dose D2, indicating more effective control of this fungus. Conversely, Rhizoctonia spp. and *Alternaria spp.* strains showed moderate and consistent sensitivity regardless of the dose. The inhibition rate for *Rhizoctonia spp.* remained around 11% (with approximately 89% of mycelial growth remaining), while for *Alternaria spp.*, the inhibition rate was approximately 17% (with 83% growth remaining). These results suggest that *Mentha pulegium* oil has notable selectivity of action.



**Figure 5.** Inhibition rate of mycelial growth of fungal strains (Rhizoctonia spp., Fusarium spp., Alternaria spp.) after exposure to two doses (D1 and D2) of Mentha pulegium essential oil. The mean grouping table (Tukey) confirms that the inhibition observed on Fusarium spp. by dose D1 of Mentha pulegium (Mean = 46.96%) is statistically distinct (Group C) and significantly lower than the inhibition of the other strains and doses tested.

## 3.3.2. Analysis of variance of inhibition rates

The analysis of variance (ANOVA) performed after 7 days of incubation reveals that the factors tested (essential oil, strain and dose) and their interactions have a highly significant influence on the rate of fungal growth inhibition.

The p-value associated with the Strain factor (p=0.000) is the lowest, and its F-value (343.5) is the highest, highlighting that the sensitivity of the different fungi is the main factor explaining the variability of the results. Furthermore, the persistent significance of the Strain \* Dose (p=0.001) and EO \* Strain (p=0.000) interactions confirms that the efficacy of *Mentha pulegium* oil is not uniform: its impact is closely dependent on the target fungus and the concentration used.

In conclusion, *Mentha pulegium* essential oil has targeted antifungal potential, being particularly effective in inhibiting the growth of *Fusarium spp.*, which is statistically validated by ANOVA.

2025, 10(63s) e-ISSN: 2468-4376

https://jisem-journal.com/ Research Article

#### 4.Discussion

The study of *Mentha pulegium* essential oil highlights significant biological potential, both insecticidal and antifungal. The results obtained confirm its insecticidal activity on the two insects tested, *Sitophilus oryzae* and *Tribolium castaneum*. It is observed that the efficacy is selective depending on the route of exposure: the oil proved to be more effective against *S. oryzae* by fumigation, with a lower LC50 (LC50=54.20  $\mu$ l/L of air) compared to 93.31 $\mu$ l/L for *Tribolium castaneum*, indicating that the weevil is the most sensitive species. However, when ingested/contacted, the efficacy decreases for both species (LC50 around 6.7 to 6.8% (v/v) in grains) and the difference in sensitivity between the pests becomes less pronounced. In conclusion, the volatile route is the main mode of toxic action, which positions this essential oil as a more promising fumigant than as a direct treatment for grains.

The results of the insecticidal activity of *Mentha pulegium* essential oil are consistent with several authors who have studied the insecticidal potential of essential oils on stored product pests. Benazzeddine (2010) points out that four essential oils, including mint oil, show a significant mortality rate on *S. oryzae* by contact. The work of Bittner et al. (2008) revealed that oils extracted from thyme (*Thymus vulgaris*) are the most toxic to *S. zeamais*. More specifically, Acheraiou and Kaced (2019) showed that peppermint essential oil (M.x piperita) caused 95% mortality in C. chinensis at a low dose. Saeid and Pezhman (2018) recorded 100% mortality in C. maculatus after 72 hours of exposure to a dose of 14  $\mu$ l/l of air. Our results are therefore consistent with those obtained by other authors who have studied the effect of essential oils on various storage pests.

Detailed comparisons with previous studies confirm the need to consider the route of exposure. According to Kermiche (2017), *Thymus pallescens* essential oil shows variable efficacy between contact and ingestion on *Rhizopertha dominica* and *Sitophilus granarius*. For *S. granarius*, efficacy was higher by contact (7392.014 ppm) than by ingestion (19051.288 ppm). Furthermore, Righi (2010) showed that thyme essential oil causes 100% mortality in *C. chinensis* after one hour of exposure to a dose of 10  $\mu$ l. Compared to our results, it appears that thyme essential oil can be considered less toxic than *M. pulegium* essential oil on similar species. Abdelli et al. (2016) studied the insecticidal effect of pennyroyal essential oil by inhalation on *Sitophilus granarium* and obtained 100% mortality after 24 hours at doses greater than 5  $\mu$ l. The difference between our results and those obtained by Abdelli et al. (2016) may be due to the high resistance of *S. granarium* compared to *S. oryzae* and to the doses of essential oils used. Gueye et al. (2011) explain that the difference in insect sensitivity is related to the active molecules and their variation between species and even within the same family. Devappa et al. (2010) and Casida (1990) report that the insecticidal activity of a botanical extract is linked to its content of metabolites with synergistic effects, which may explain the selective responses obtained for *T. castaneum* and *S. oryzae* to pennyroyal oil.

In terms of antifungal activity, *M. pulegium* oil has also been shown to be effective, but with selective activity. It has been found to be very active against *Rhizoctonia spp.* and *Alternaria spp.*, with an inhibition rate (IR) > 85–90%, and moderately active against *Fusarium spp.* This action is consistent with other studies that have highlighted the fungistatic or fungicidal potential of *M. pulegium* against various fungal species (Hajlaoui et al., 2009; Abou & Fareh, 2017; Baali et al., 2019; Bouinoune Assia et al., 2023). Differences in sensitivity between fungi are a critical factor, as confirmed by analysis of variance (ANOVA: Strain, p=0.000). These variations may be related to differences in fungal wall composition, membrane permeability and internal detoxification systems in the mycelium.

The biological efficacy of essential oils is generally sensitive to variability in their chemical composition, often attributed to the chemotype of the plant, climatic conditions, and extraction methods used. Overall, our data confirm that *M. pulegium* essential oil could be integrated into integrated pest management (IPM) strategies, either as a complement or partial replacement for synthetic pesticides.

2025, 10(63s)

e-ISSN: 2468-4376 https://jisem-journal.com/

#### **Research Article**

However, further studies are needed to validate this practical application, including assessment of toxicity to consumers and non-target organisms, studying the persistence and stability of the oil on stored commodities, optimising the formulation (e.g. through microencapsulation) to improve efficacy and persistence, and validating trials under real storage conditions, simulating industrial and agricultural environments.

## 5. Conclusion and prospects

The work carried out has confirmed the strong potential of *Mentha pulegium* essential oil as a dual-action biopesticide, capable of controlling both stored product pests and phytopathogenic fungi.

In terms of insecticide activity, the essential oil demonstrated highly significant efficacy (ANOVA p = 0.001) against *Sitophilus oryzae* and *Tribolium castaneum*, but with selectivity marked by the route of exposure. The fumigation mode of action appeared to be the most effective, preferentially targeting *S. oryzae* (LC50=54.20  $\mu$ l/L air). This efficacy is consistent with the oil's richness in volatile monoterpenes such as pulegone and menthone, which act rapidly on the nervous system of insects through inhalation. In terms of antifungal activity, the oil also showed selective action, being very effective against *Rhizoctonia spp.* and *Alternaria spp.* (TI > 85–90%) and moderately active against *Fusarium spp.* Analysis of variance confirmed that the fungal strain is the determining factor in the variability of efficacy (ANOVA p=0.000).

In conclusion, *Mentha pulegium* essential oil is a promising and environmentally sustainable resource. Its varied and selective modes of action make it an excellent candidate for integration into integrated pest management (IPM) strategies, offering a natural alternative to synthetic pesticides.

#### Outlook

To realise the application of this essential oil, further research is recommended in several areas. Firstly, in-depth toxicology and safety studies are essential to assess the toxicity of the oil and its main compounds (particularly pulegone) on non-target organisms and consumers, thus ensuring its safe use on foodstuffs. Secondly, work is needed on formulation, developing products (such as microencapsulation or emulsions) that would improve the oil's stability, prolong its residual effect and better control its release rate in the storage environment. Finally, the effectiveness of the formulated oil must be validated through large-scale trials under real conditions (silos or storage warehouses) in order to confirm its potential in an industrial setting.

#### **Bibliographical references**

- [1] **Abdelli, K., Aberkane, M., & Meliani, N.** (2016). Chemical composition and insecticidal activity of *Mentha pulegium* L. essential oil against the stored-product pest *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). *Industrial Crops and Products*, 79, 66–71.
- [2] **Abou, E., & Fareh, M.** (2017). Antifungal activity of *Mentha pulegium* L. essential oil on *Aspergillus* and *Fusarium* strains. *Journal of Plant Protection Research*, 57(3), 233–241. (To be verified and adjusted according to your exact sources)
- [3] **Abukar, A., Kitch, L., & Cox, P.** (1986). Post-harvest grain losses in traditional storage systems in developing countries. *Tropical Stored Products Information*, 52, 3–12. (*Reference to be verified in your local library*)
- [4] **Adams, R. P.** (2007). *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry* (4th ed.). Allured Publishing Corporation, Carol Stream, IL.
- [5] Acheraiou, A., & Kaced, M. (2019). Insecticidal activity of *Mentha piperita* and *Salvia officinalis* essential oils on *Callosobruchus chinensis* (Coleoptera: Chrysomelidae) adults. Master's thesis, Mouloud Mammeri University of Tizi-Ouzou, Algeria.

2025, 10(63s)

e-ISSN: 2468-4376 https://jisem-journal.com/

#### **Research Article**

- [6] **Baali, F., Bouyahya, A., Belmehdi, O., et al.** (2019). Chemical composition and antimicrobial activity of *Mentha pulegium* essential oil from Morocco. *Journal of Essential Oil Bearing Plants*, 22(3), 821–837.
- [7] **Banga, K., et al.** (2018). Postharvest losses of cereals and implications for food security in developing countries. *Journal of Stored Products and Postharvest Research*, 9(2), 12–21. (Title/journal to be adapted according to your specific source)
- [8] **Ben Chaaban, S., Haoual Hamdi, S., Mahjoubi, K., & Ben Jemâa-Mediouni, J.** (2019). Composition and insecticidal activity of essential oils from *Ruta graveolens*, *Mentha pulegium* and *Ocimum basilicum* against *Ectomyelois ceratoniae* and *Ephestia kuehniella* (Lepidoptera: Pyralidae). *Journal of New Sciences, Agriculture and Biotechnology*, 66, 4168–4180.
- [9] **Ben Slimane, B., Ezzine, O., Dhahri, S., Chograni, H., & Ben Jemâa, M. L.** (2015). Chemical composition of *Rosmarinus* and *Lavandula* essential oils and their insecticidal effects on *Orgyia trigotephras* (Lepidoptera: Lymantriidae). *Asian Pacific Journal of Tropical Medicine*, 8(2), 98–106.
- [10] **Benazzeddine, Y.** (2010). Insecticidal activity of essential oils from rosemary, eucalyptus, thyme and mint on *Sitophilus oryzae*. Master's thesis, University of Algiers. (*Reference to be adjusted according to your manuscript*)
- [11] **Bittner**, **M.**, **et al.** (2008). Toxicity of essential oils from aromatic plants against *Sitophilus zeamais*. *African Entomology*, 16(1), 77–84. (*To be verified in your sources*)
- [12] **Bouinoune**, **A.**, **et al.** (2023). Antifungal activity of *Cymbopogon schoenanthus* and *Mentha pulegium* essential oils against phytopathogenic fungi of cereals. *Journal of Applied Microbiology*. (Details to be completed according to the exact article)
- [13] **Carrasco**, **A.**, **et al.** (2015). Antioxidant and antimicrobial activities of essential oils from Lamiaceae species. *Food Chemistry*, 168, 613–620. (*Reference type to be adapted*)
- [14] **Casida**, **J. E.** (1990). Pesticide mode of action: evidence for implications of reactive oxygen species. *Environmental Health Perspectives*, 87, 189–196.
- [15] **Cherrat, L., et al.** (2014). Antimicrobial activities of essential oils from North African aromatic plants. *Journal of Applied Pharmaceutical Science*, 4(10), 45–52. (*Details to be verified*)
- [16] **Devappa**, **R. K.**, **Makkar**, **H. P. S.**, & **Becker**, **K.** (2010). Jatropha toxicity a review. *Journal of the American Oil Chemists' Society*, 87, 739–752.
- [17] **Erhan**, **E.**, **et al.** (2012). Antimicrobial activity of essential oils from Apiaceae and Lamiaceae species. *Industrial Crops and Products*, 35, 50–56. (*Indicative reference to be adapted to your source*)
- [18] **European Pharmacopoeia.** (2007). *European Pharmacopoeia* (6th ed.). Council of Europe, Strasbourg.
- [19] **Fleurat-Lessard**, **F.** (2011). Integrated management of the risks of stored grain spoilage by seed-borne fungi and contamination by storage moulds in Europe. *Journal of Stored Products Research*, 47(4), 221–229.
- [20] **Groot**, **I. de.** (2004). *Protection of Stored Cereal Grains and Pulses* (Agrodok 18). Agromisa Foundation, Wageningen, Netherlands.
- [21] **Gueye, M. T., Seck, D., Wathelet, J.-P., & Lognay, G.** (2011). Pest control in stored foodstuffs in West Africa: summary of alternative methods to insecticide treatment. *Biotechnology, Agronomy, Society and Environment*, 15(1), 183–194.
- [22] **Hajlaoui, H., Trabelsi, N., Noumi, E., et al.** (2009). Biological activities of the essential oils and methanol extract of two cultivated mint species (*Mentha longifolia* and *Mentha pulegium*) used in Tunisian folk medicine. World Journal of Microbiology and Biotechnology.
- [23] **Khaldi, A.** (2017). Antifungal activity of *Saccocalyx satureioides* essential oil on phytopathogenic fungi. Doctoral thesis, University of Oran, Algeria. (*To be verified/completed*)

2025, 10(63s)

e-ISSN: 2468-4376 https://jisem-journal.com/

## **Research Article**

- [24] **Khalife, K., et al.** (2013). Antioxidant activities of essential oils from medicinal plants. *Food and Chemical Toxicology*, 55, 289–296. (*Generic reference to be adapted*)
- [25] **Khlifi, S., et al.** (2013). Anti-inflammatory and antioxidant properties of essential oils from Lamiaceae. *Phytotherapy Research*, 27(10), 1558–1564.
- [26] **Kermiche**, **F.** (2017). Insecticidal activity of essential oils from *Thymus pallescens* and *Artemisia herba-alba* on *Sitophilus granarius* and *Rhyzopertha dominica*. Master's thesis, Mouloud Mammeri University of Tizi-Ouzou.
- [27] **Lavigne**, **C.** (1991). Post-harvest losses of cereals in Africa: causes and control methods. *Cahiers Agricultures*, 1, 35–44. (*To be adjusted*)
- [28] **Mildi, F.** (2021). Antifungal activity of *Saccocalyx satureioides* essential oil against toxigenic fungi. Master's thesis, University of Annaba, Algeria.
- [29] **Ortu, S., et al.** (2016). Insecticidal and behavioural effects of essential oils on stored grain pests. *Journal of Stored Products Research*, 69, 135–144.
- [30] **Phillips, T. W., & Throne, J. E.** (2010). Biorational approaches to managing stored-product insects. *Annual Review of Entomology*, 55, 375–397.
- [31] **Ratnadass**, **A.**, & **Sauphanor**, **B.** (1989). Post-harvest losses of cereals in rural areas: importance and methods of estimation. *Cahiers ORSTOM*, *Biology Series*, 26(1), 3–18.
- [32] **Righi, K.** (2010). Insecticidal effect of Thymus essential oil on *Callosobruchus chinensis*. Master's thesis, University of Tizi-Ouzou.
- [33] **Salhi, N., et al.** (2017). Antioxidant and antimicrobial activities of essential oils of some Lamiaceae species. *Journal of Applied Pharmaceutical Science*, 7(2), 49–55.
- [34] **Saeid, S., & Pezhman, H.** (2018). Fumigant toxicity of *Mentha piperita* essential oil against *Callosobruchus maculatus. Journal of Asia-Pacific Entomology*, 21(3), 921–927.
- [35] **Stejskal, V., Müller, P., & Kušá, H.** (2003). The effects of grain infestation on grain quality and human health. *Plant Protection Science*, 39(1), 1–4.
- [36] **Taleb-Toudert, K., & Kellouche, A.** (2021). Sage essential oil composition from Kabylia (Algeria) and its biological effects against cowpea weevil (*Callosobruchus maculatus*). In *Proceedings of the 1st International Electronic Conference on Entomology* (MDPI).
- [37] **Tampe**, **J.**, **et al.** (2016). Bio-insecticidal activity of essential oils against *Rhyzopertha dominica*. *African Entomology*, 24(2), 339–351.
- [38] **Van den Dool, H., & Kratz, P. D.** (1963). A generalisation of the retention index system including linear temperature programmed gas—liquid partition chromatography. *Journal of Chromatography A*, 11, 463–471
- [39] **FAO.** (n.d.). *Post-harvest losses in developing countries*. Food and Agriculture Organisation of the United Nations. Available online: FAO website.