



## Bioaggressors of the olive tree in a semi-arid region: identification, characterization, and biocontrol alternatives using essential oils of *Rosmarinus officinalis* and *Cupressus sempervirens*

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### Abstract:

The olive tree represents a major arboricultural resource in Algeria; however, its cultivation is threatened by various bioaggressors. This study aims to inventory fungal infestations and olive pests in the Laghouat region and to evaluate the effectiveness of natural alternatives to chemical pesticides. The methodology is based on three main approaches: (i) identification of pathogenic fungi sampled from different parts of the olive tree and the surrounding soil; (ii) extraction by hydrodistillation of essential oils (EOs) from *Rosmarinus officinalis* and *Cupressus sempervirens*; (iii) evaluation of their in vitro antifungal activity through direct confrontation against phytopathogenic fungi, combined with an inventory of entomofauna using various trapping techniques. The results led to the identification of five fungal strains (*Fusarium solani*, *Fusarium* sp., *Penicillium* sp., *Colletotrichum* sp., and *Alternaria* sp.), with extraction yields of 0.51% for *R. officinalis* and 0.39% for *C. sempervirens*. Antifungal tests revealed that pure rosemary essential oil exerted total inhibition (100%) against *F. solani* and significant inhibition (80%) against *Fusarium* sp., whereas cypress essential oil showed inhibition rates of 66.67% and 71.43%, respectively. Even at reduced concentrations (25% and 50%), both essential oils maintained inhibitory activity exceeding 50%. The entomological survey identified 11 species, including 5 natural predators, with a predominance of Hymenoptera (29%). These results confirm the potential of essential oils as promising biopesticides for the sustainable management of olive tree bioaggressors.

## 1. Introduction

An emblematic crop of the Mediterranean basin, the olive tree (*Olea europaea* L.) covered more than 10 million hectares worldwide in 2021 (International Olive Council, 2024) and accounts for nearly 70% of global olive oil production (FAO, 2023). In Algeria, this sector has experienced sustained expansion, particularly in arid and steppe regions, with a cultivated area exceeding 430,000

ha in 2017 (MADRP, 2018). Beyond its economic importance (Amrouni Sais et al., 2021), olive growing plays a major social and ecological role in rural areas, contributing to territorial stabilization and the preservation of agroecosystems.

However, olive cultivation remains highly exposed to numerous bioaggressors, particularly phytopathogenic fungi and insect pests. This vulnerability is intensified by the expansion of olive growing into arid and semi-arid environments, as

well as by the effects of climate change, which increase parasitic pressure.

Diseases such as verticillium wilt (*Verticillium dahliae*) and anthracnose, along with insects such as *Bactrocera oleae* and *Prays oleae*, are responsible for considerable economic losses and deterioration in production quality.

To date, conventional chemical control has been the main strategy for managing olive bioaggressors. However, repeated applications of fungicides and insecticides have promoted the gradual selection of resistant populations, thereby reducing treatment effectiveness (FAO, 2012). Excessive pesticide use has also led to significant adverse effects, notably the development of resistance in certain pathogens (Leroux, 2003), resulting in a vicious cycle characterized by increased application frequency and dosage. This situation exacerbates negative impacts on the environment and human health (Agarwal & Pandey, 2017).

In this context, essential oils extracted from aromatic plants appear as an ecological and sustainable alternative to synthetic pesticides. Rich in bioactive compounds, they offer significant potential for integrated pest management. Accordingly, the present study aims to:

- (i) identify fungal pathogens and harmful insects present at the study site in the Laghouat region (a province located approximately 400 km south of Algiers);

- (ii) extract and characterize essential oils from *Rosmarinus officinalis* and *Cupressus sempervirens*;

- (iii) evaluate their antifungal effectiveness against the isolated strains.

## 2. Olive Tree Bioaggressors and Chemical Control

### 2.1. Impact of Bioaggressors on the Olive Tree

The olive tree is exposed to numerous phytopathological threats, particularly of fungal origin. Among the most damaging diseases are anthracnose, verticillium wilt (*Verticillium dahliae*), and peacock eye disease (*Spilocaea oleagina*), which severely affect yield and production quality (Jiménez-Díaz et al., 2022; Fernandez et al., 2023). The genus *Fusarium*, responsible for root rots, is also experiencing a worrying expansion in olive groves (AE Hassan et al., 2009; Chliyah et al., 2017). In addition, more than 200 harmful organisms have been recorded on olive trees, including major insect pests such as *Bactrocera oleae*, *Prays oleae*, and *Euphyllura olivina*. These pests can cause losses of up to 80% of production and significantly alter oil quality

(Benhadi-Marín et al., 2021; Karimi et al., 2023; Bouteldja & Kourgli, 2020). The intensification of cultivation systems—characterized by high planting densities, irrigation, and fertilization—favors the proliferation of these bioaggressors (Alarcón Roldán et al., 2020).

### 2.2. Limitations of Chemical Control and Interest in Natural Alternatives

Chemical control, long dominant in olive tree protection, now shows its limitations. The development of resistance—particularly in certain strains of *V. dahliae* in Algeria (Boumaaza et al., 2024)—as well as the presence of pesticide residues in olive oil, constitute major constraints, especially for export markets (International Olive Council, 2019). Added to this are significant environmental impacts, such as soil and groundwater contamination and ecosystem imbalance (Rezgui et al., 2021). In response to these limitations, interest has grown in alternative, sustainable, and environmentally friendly strategies, particularly those based on the use of natural compounds such as essential oils (Olive Oil Producer's Guide, 2007).

### 2.3. Bioactive Properties of Essential Oils

Essential oils are rich in secondary metabolites, notably terpenes, ketones, and phenolic compounds, which are recognized for their antifungal, insecticidal, and repellent activities (Regnault-Roger et al., 2012). Their multiple modes of action limit the risk of resistance development in bioaggressors (Trombetta et al., 2005; Nazzaro et al., 2013). Several studies conducted in the Mediterranean region have demonstrated the antifungal effectiveness of essential oils derived from the Lamiaceae and Cupressaceae families (Boufares, 2020; Boussalem, 2022; Dekiche & Benzaid, 2023). Rosemary essential oil has proven particularly active against genera such as *Fusarium* and *Alternaria* (Boubekri et al., 2021), while cypress essential oil, rich in  $\alpha$ -pinene, shows notable antifungal potential (de Barros, 2022; Galovičová et al., 2023). However, their effectiveness against olive-specific bioaggressors under semi-arid conditions remains poorly documented, which justifies the need for localized studies.

## 3. Materials and Methods

### 3.1. Study Area

The study was conducted in an olive orchard located in Hamda (Laghout), within an arid region with cool winters (33°51'47"N, 2°51'20"E, 788 m). The climate is characterized by a mean annual rainfall of 141.9 mm and summer temperatures exceeding 32 °C. The Emberger pluviothermic quotient ( $Q_2 = 15.65$ ) confirms the aridity of the site. The chemically untreated orchard comprises 320 olive trees, 8 years old, of the Sigoise and Chemlal varieties, covering an area of 1.51 ha. No phytosanitary treatments were applied in this orchard.

## 3.2. Isolation and Identification of Pathogenic Fungi

### 3.2.1. Sampling and Isolation

Fungal isolation was carried out from different parts of the olive tree (twigs, leaves, roots, and fruits) showing symptoms of infection, as well as from soil samples collected around the trees. The technique used was that of Rappily (1968), involving the following steps:

- Sampling of symptomatic plant tissue fragments;
- Surface disinfection with sodium hypochlorite (2–3 min);
- Rinsing with sterile distilled water;
- Inoculation onto PDA medium (Potato Dextrose Agar).

For soil samples, the successive dilution method was applied according to the protocol of Davet and Rouxel (1997). Inoculated Petri dishes were incubated at 25 °C for 6–7 days for plant samples and at 30 °C for 7 days for soil samples.

### 3.2.2. Purification and Identification

The fungal isolates obtained were purified through successive subcultures and monospore culture (Buxton, 1954) until pure cultures were obtained. Strain identification was performed by combining:

- macroscopic observation: colony appearance, color, and texture;
- microscopic observation: conidial and mycelial morphology.

The identification keys used were those of Barnett and Hunter (1972), Botton et al. (1990), and Lepoivre (2003).

From the figure 1:

- **1 g du sol** → 1 g of soil
- **Solution mère** → Stock solution
- **1 g du sol + 9 ml d'eau distillée stérile** → 1 g of soil + 9 mL of sterile distilled water
- **1 ml** → 1 mL
- **$10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$**  → Serial dilutions ( $10^{-1}$  to  $10^{-5}$ )
- **Chaque concentration est étalée dans des boîtes de Pétri** → Each dilution is spread onto Petri dishes
- **Boîtes de Pétri** → Petri dishes
- **Incubation à 25 °C pendant 07 jours** → Incubation at 25 °C for 7 days

## 3.3. Essential Oil Extraction Protocol

The aerial parts (leaves and twigs) of *Rosmarinus officinalis* and *Cupressus sempervirens* were air-dried at room temperature, protected from light, for 10 days. Extraction was carried out by hydrodistillation using a Clevenger-type apparatus (Figure 3).

- 200 g of plant material were immersed in a 2-L flask filled with distilled water.
- Distillation was maintained for 2 hours.
- The essential oils were collected and stored in amber glass vials under refrigeration.

The extraction yield was calculated using the following formula:

$$R (\%) = (MEO / Ms) \times 100,$$

where *MEO* is the mass of essential oil obtained and *Ms* is the mass of plant material used (in g).

## 2.4. Antifungal Activity Tests

Antifungal activity was evaluated by direct confrontation, following an adaptation of the Kirby and Bauer (1966) protocol.

### Protocol

- Essential oils were tested at 100%, 50%, and 25% (diluted in DMSO).
- A mycelial plug was placed at the periphery of a PDA plate.
- A filter paper disc impregnated with 10 µL of essential oil was placed opposite the plug.
- After 3 days of incubation at 25 °C, growth inhibition was measured.

The inhibition rate (I%) was calculated as:

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

where  $dC$  represents the colony diameter in control plates and  $dE$  the colony diameter in plates containing essential oil.

### 3.5. Entomofauna Sampling

Entomofauna sampling was based on the use of three trapping methods (see Figure 5):

- Suspended baited traps containing a sugar solution;
- Barber traps (pitfall traps), consisting of buried containers filled with salted water;
- Colored traps using yellow sticky cards.

Identification was performed under a stereomicroscope using the keys of Borror and White (1970), Balachowsky (1962), and Dajoz (2007).

The centesimal frequency or relative abundance (F%) of the different insect orders was calculated using the formula:

$$F(\%) = (N_i \times 100) / N,$$

where  $N_i$  is the number of individuals of a given order and  $N$  is the total number of individuals of all orders combined.

## 4. Results

### 4.1. Identified Pathogenic Fungi

Five fungal genera were isolated (Table 1): *Fusarium solani*, *Fusarium* sp., *Penicillium* sp., *Colletotrichum* sp., and *Alternaria* sp.

- *F. solani* is characterized by rapid growth and white, cottony colonies.
- *Penicillium* sp. forms a fast-growing green thallus, commonly found in the environment.
- *Colletotrichum* sp., the causal agent of anthracnose, is responsible for diseases causing significant economic damage.
- *Alternaria* sp. produces thick, dense, blackish-green colonies.

### 4.2. Essential Oil Yield

As shown in Table 2 below, the yields obtained were 0.51% for *Rosmarinus officinalis* and 0.39% for *Cupressus sempervirens*.

### 4.3. Antifungal Activity of Essential Oils

In vitro tests showed (Table 3):

- Total inhibition (100%) of *F. solani* by pure rosemary essential oil;
- An inhibition rate of 80% against *Fusarium* sp. at the same concentration.

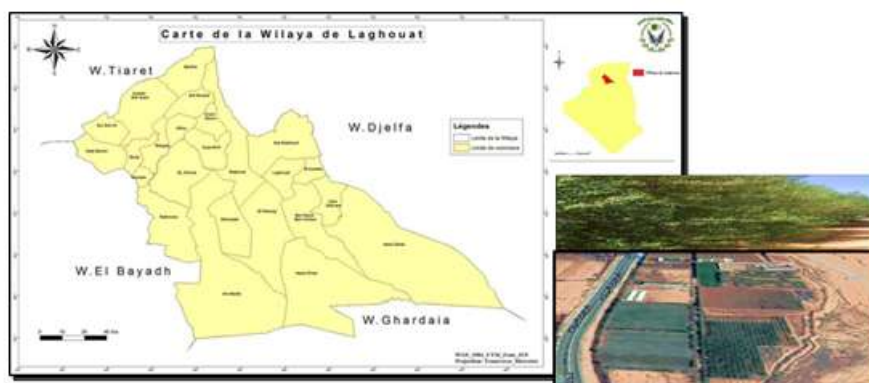
Green cypress induced inhibition rates of 66.67% (*F. solani*) and 71.43% (*Fusarium* sp.). Even when diluted to 25%, both essential oils maintained antifungal activity greater than 50%. Rosemary essential oil remained the most active, particularly against *F. solani*.

Figures 6, 7, and 8 below illustrate the results of the direct confrontation tests of the two essential oils against the two *Fusarium* strains.

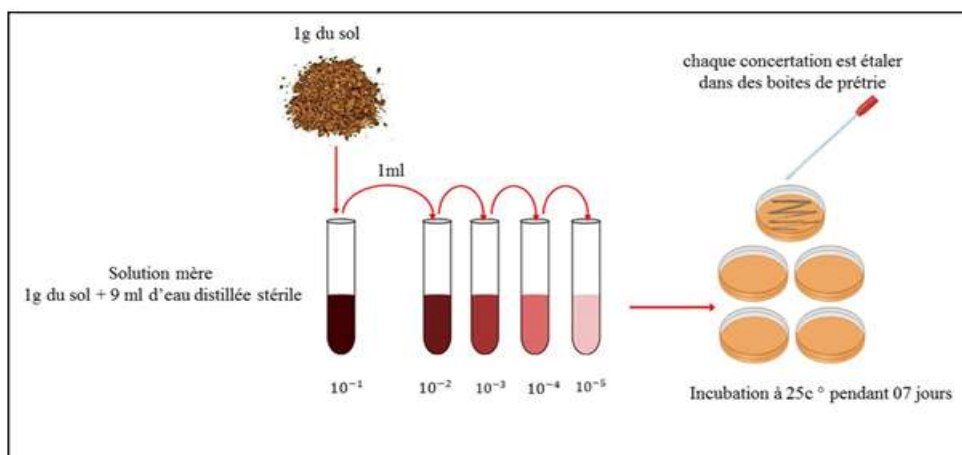
### 4.4. Entomofauna Associated with the Olive Tree

The inventory of entomofauna associated with the olive tree made it possible to identify various species belonging to different insect orders. Analysis of relative abundance shows that Hymenoptera are predominant (29%), followed by Hemiptera (17%) and Diptera (12%). Coleoptera represent 11% (8% + 3%) of the collected insects, while Lepidoptera and Thysanoptera account for 7% and 5% of the entomofauna, respectively.

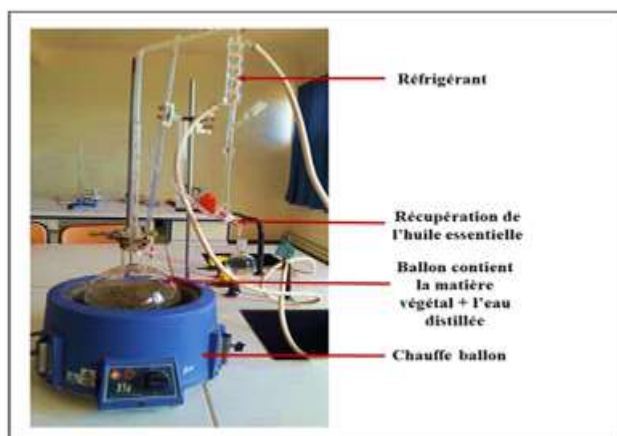
Among the identified species (Table 4) are olive-specific pests such as *Euphyllura olivina* (olive psyllid), *Prays oleae* (olive moth), *Hylesinus oleiperda* and *Phloeotribus scarabaeoides* (xylophagous insects), *Agromyza* sp. (phyllophagous), and *Liothrips oleae*. Sampling also revealed the presence of potentially beneficial predatory species such as *Lasius niger* (black ant), *Heliophanus tribolosus* (spider), Geophilomorpha (centipede), and *Rumina decollata* (predatory snail).



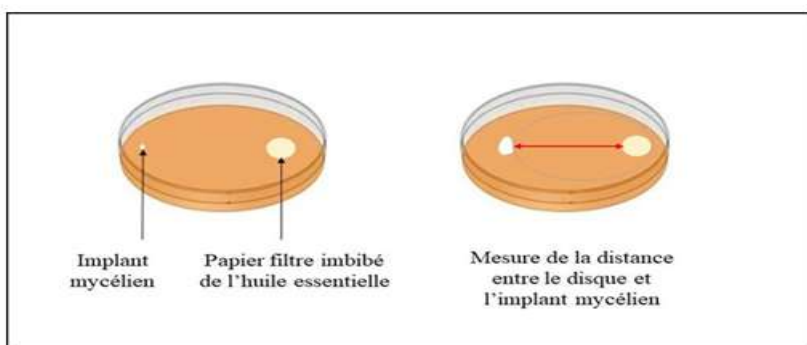
**Figure 1:** Location of the study site: olive orchard of the Hamda station, Laghouat Province.



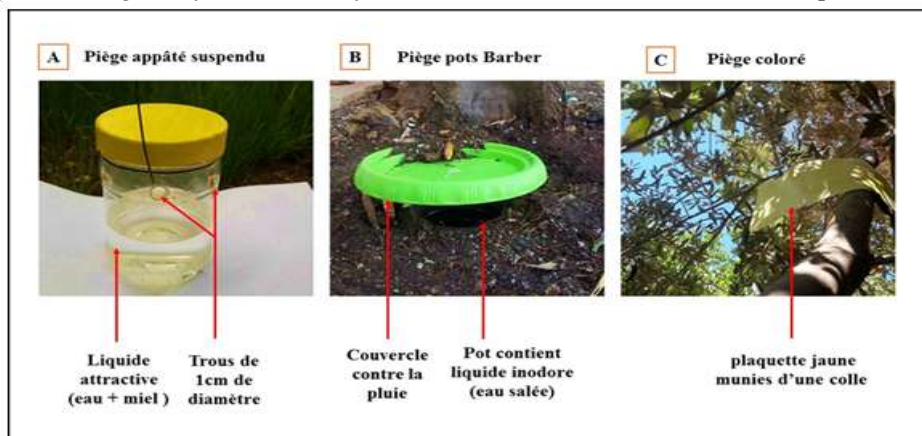
**Figure 2:** Diagram of the procedure followed for the isolation of fungi from soil according to Davet and Rouxel (1997)



**Figure 3:** Extraction of essential oils by hydrodistillation










**Figure 4:** Diagram of the direct confrontation test between essential oils and aqueous extract



**Figure 5:** Different trapping techniques used for sampling olive tree entomofauna



**Table 1: Macroscopic appearance of the obtained fungal strains**

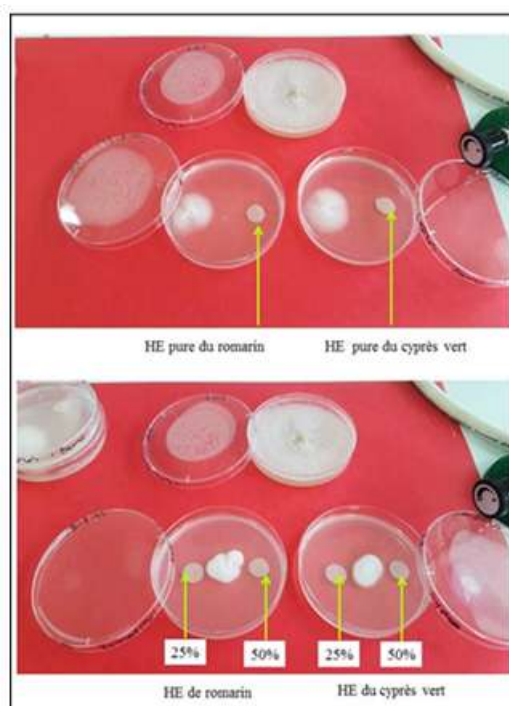
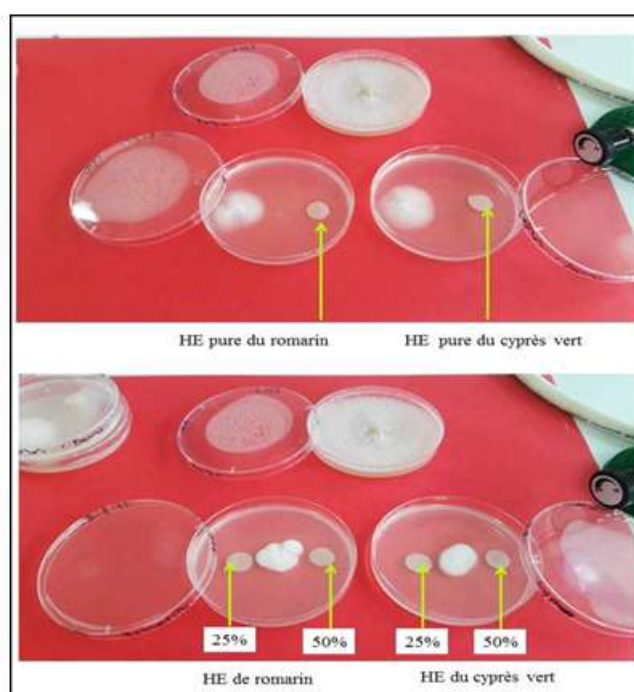
Fungus	Macroscopic observation		Description
	Lower surface (reverse side)	Upper surface (obverse side)	
<i>Fusarium solani</i>			Rapid growth; fluffy to powdery white colony; reverse: colorless
<i>Fusarium spp.</i>			Woolly texture, initially white then yellow; reverse: brown; rapid growth
<i>Penicillium sp.</i>			Rapid growth; green thallus; reverse: yellowish-green
<i>Colletotrichum sp.</i>			Blackish-gray colony with rapid growth; reverse: black
<i>Alternaria sp.</i>			Colony initially white then blackish-green, fluffy, with thick and dense texture; reverse: black

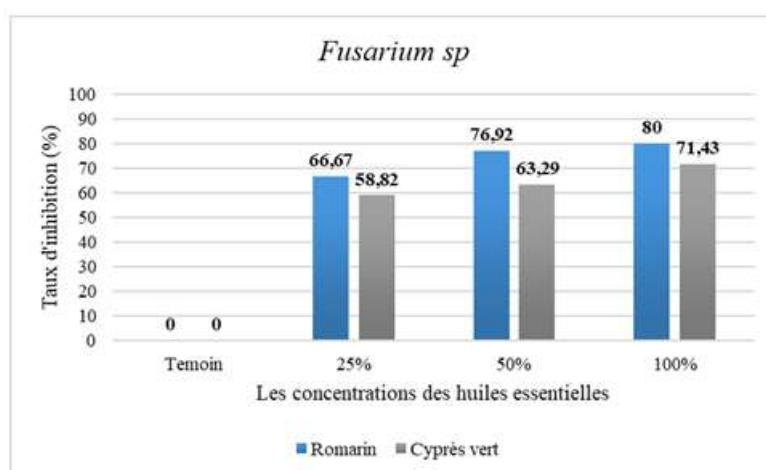
**Table 2: Essential oil yields of the plants**

Plant	Mass of plant material (g)	Yield (%)
<i>Rosmarinus officinalis</i>	200 g	0.51%
<i>Cupressus sempervirens</i>	200 g	0.39%

**Table 3:** Antifungal activity tests of rosemary and green cypress essential oils

Strain	Concentration of EO	<i>Rosmarinus officinalis</i>	<i>Cupressus sempervirens</i>
<i>Fusarium solani</i>	Pure oil	100% inhibition	68.77% inhibition
	50%	63.25%	52.71%
	25%	52.63%	50%
<i>Fusarium</i> sp.	Pure oil	80% inhibition	71.43% inhibition
	50%	76.92%	63.29%
	25%	66.67%	58.82%


**Figure 6:** Effect of essential oils on the growth of the *Fusarium* sp. strain: (A) pure essential oil, (B) ...

**Figure 7:** Effect of essential oils on the growth of the *Fusarium solani* strain: (A) pure essential oils, (B) diluted essential oils



**Figure 8:** Histogram showing the effect of the two essential oils on the mycelial growth of *Fusarium sp*.

**Table 4:** Olive-associated fauna sampled at the Hamda station

Genus / species	Order	Feeding habit
Porcellio scaber	Isopoda	Decomposer
Geophilomorpha	Acari	Predator
Rumina decollata	Stylommatophora	Predator
Euphyllura olivina	Hemiptera	Phyllophagous
Hylesinus oleiperda	Coleoptera	Xylophagous
Agromyza sp.	Diptera	Phyllophagous
Heliophanus tribolosus	Araneae	Predator
Phloeotribus scarabaeoides	Coleoptera	Xylophagous
Prays oleae	Lepidoptera	Phyllophagous
Lasius niger	Hymenoptera	Predator
Liothrips oleae	Thysanoptera	Phyllophagous

#### 4. Discussion

The results confirm the presence of major phytopathogenic fungi in the olive orchards of Laghouat, particularly those of the genus *Fusarium*, which has already been documented as an agent of root rot (Nucci et al., 2007; Chliyah et al., 2017). The essential oil yields of rosemary and cypress are consistent with data reported in the literature (AFNOR, 1986; Emami et al., 2007), indicating good extraction efficiency under local conditions.

These findings are consistent with several previous studies on the antifungal properties of essential oils. Moreno et al. (2006) and Boubekri et al. (2021) attributed the antifungal effect of rosemary essential oil to rosmarinic acid. Other researchers, such as Cox et al. (2000) and Lima et al. (2005), demonstrated that major terpene compounds of rosemary—particularly 1,8-cineole and borneol—affect fungal cell integrity, inhibit respiration and ion transport processes, and increase the permeability of fungal cell membranes.

Regarding green cypress essential oil, Bouanoun et al. (2007) attributed its antifungal activity mainly to  $\alpha$ -pinene, a major compound of this oil. Giordani et al. (2008), on the other hand, suggested that the

antifungal activity of plant extracts may also result from synergistic effects among different compounds.

In general, the antifungal effectiveness of essential oils depends on their chemical composition, which may vary according to intrinsic factors (species, plant organ, developmental stage) and extrinsic factors (environmental conditions, extraction method). According to Dorman and Deans (2000), Marino et al. (2001), Delaquis et al. (2002), and Nazzaro et al. (2013), the biological activity of essential oils may be related to the functional groups of their components, their proportions, and the interactions between them.

The observed entomological diversity confirms the findings of Harrat (1986) and Gacem and Zerouali (2020), who reported more than 60 insect species associated with olive trees in the Mediterranean region. The predominance of Hymenoptera and Coleoptera is consistent with the results of Deghiche Diab and Deghiche (2013).

Hmimina (2009) emphasized the role of climatic conditions and olive varietal diversity in shaping bioaggressor dynamics. In the present case, the arid climate and the young age of the orchard (8 years) may explain the specific characteristics of the local entomofauna.



## 5. Conclusion

This study enabled the isolation and identification of five genera of pathogenic fungi associated with olive trees in the Laghouat region: *Fusarium solani*, *Fusarium* sp., *Penicillium* sp., *Colletotrichum* sp., and *Alternaria* sp. The extraction of essential oils from *Rosmarinus officinalis* and *Cupressus sempervirens* yielded 0.51% and 0.39%, respectively, in agreement with values reported in the literature.

Antifungal activity tests demonstrated strong efficacy of these oils against the isolated *Fusarium* strains. Rosemary essential oil proved particularly active, completely inhibiting the growth of *Fusarium solani* at the highest tested concentration. Green cypress essential oil also showed significant antifungal activity, although slightly lower than that of rosemary.

The entomofauna inventory revealed a diversity of insects associated with olive trees, including both harmful pests and beneficial predators. Hymenoptera, Hemiptera, and Diptera were the most abundant orders in the studied orchard.

These results highlight the potential of essential oils as ecological alternatives to chemical fungicides, particularly in semi-arid regions where olive cultivation is expanding. However, further studies are needed to (1) identify the active compounds responsible for the observed antifungal effect, (2) evaluate their effectiveness against other olive pathogens, (3) develop formulations suitable for application under field conditions, and (4) analyze interactions between these oils and beneficial entomofauna.

The development of plant protection strategies based on essential oils could thus contribute to more sustainable olive growing, combining productivity with environmental protection.

## Author Statements:

- **Ethical approval:** The conducted research is not related to either human or animal use.
- **Conflict of interest:** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper
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- **Data availability statement:** The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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