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First report on the antifungal potential of *Pistacia atlantica* essential oil against *Fusarium oxysporum* f. sp. *albedinis*

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The aim of this study was to investigate the antifungal activity of *Pistacia atlantica* essential oil against *Fusarium oxysporum* f. sp. *albedinis* (Foa) using the direct contact method. The essential oils were extracted from the gall part of the plant in two regions (Ain-Oussera and Laghouat). The extracts were analysed using GC and GC-MS techniques to determine their chemical composition. The results showed that all tested essential oils possess inhibitory effects on Foa, with varying degrees of effectiveness based on concentration. The percentages of inhibition of essential oils from two regions reveals that the essential oil from Ain-Oussera is more active than that from Laghouat, particularly at the concentration range of 0.3 to 4.0 µL/mL. Moreover, no significant differences were noted in mycelial growth inhibition between the two regions within the concentrations range of 10 to 20 µL/mL. These results indicate that the *P. atlantica* essential oil may be a promising alternative or supplementary treatment in phytopathogen management strategies.

Keywords: *Pistacia atlantica*; *Fusarium oxysporum* f. sp. *albedinis*; essential oil; GC analysis; GC-MS analysis.

Introduction

Fusarium oxysporum f. sp. *albedinis* (Foa) is a pathogenic fungus that specifically infects date palm trees (*Phoenix dactylifera*). It causes a vascular wilt disease known as Bayoud disease, which can lead to severe economic losses in date palm cultivation (Khoulassa et al., 2022). Understanding the biology and pathogenicity of Foa is crucial for the development of effective strategies to manage Bayoud disease and protect date palm crops (Meliani et al., 2022). The infection process of *F. oxysporum*, typically enters the host plant through natural openings, such as stomata or wounds. The pathogen can also penetrate the root system directly, especially in stressed or damaged plants. Once inside, the fungus colonizes the vascular tissues (xylem), which are crucial for water and nutrient transport. This colonization leads to wilting and eventual death of the plant (Srivastava et al., 2024). The enzymatic activity of Foa plays a critical role in its pathogenicity by facilitating the degradation of plant cell walls and promoting infection. The pathogen produces a variety of enzymes, such as cellulases, pectinases, and xylanases, which degrade the plant cell walls. This breakdown facilitates the invasion of the fungus into plant tissues. The proteases can also degrade host proteins, weakening plant defences and allowing further fungal growth. Some studies have indicated that *Fusarium* species can produce lipases that degrade lipids, which may play a role in altering plant membrane integrity and facilitating infection (Kikot et al., 2009; Kubicek et al., 2014).

Essential oils have gained attention for their potential antifungal properties, and research has explored their efficacy against various fungal species, including phytopathogenic fungi (Tian et al., 2022). The study of the antifungal activity of essential oils is of great importance in various fields, including food safety and healthcare. Essential oils have been widely recognized for their potential antimicrobial properties, making them valuable in combating infectious diseases caused by fungi (Elhouiti et al., 2022). One essential oil that has been extensively studied for its antifungal activity is *P. atlantica* essential oil. Several research studies have investigated its composition, antioxidant capacity, and antibacterial effects (Sifi et al., 2015, 2020), also a antidiabetic activity (Sifi et al., 2024). *P. atlantica*, also known as "Boutma" in Algeria, is a tree belonging to the Anacardiaceae family. It has been used in traditional medicine for its medicinal properties (Sifi et al., 2022). Essential oils are one of the main components reported from different parts of *Pistacia* species, including leaves, resin, ripe and unripe fruits, galls, leaf-buds, twigs, and flowers. The analysis of essential oils is commonly performed using gas-chromatography (GC) based techniques (Mecherara-Idjeri et al., 2008; Gourine et al., 2011; Sifi et al., 2015). The content of essential oils can vary qualitatively and quantitatively due to various factors such as plant species and part, sex of cultivars, harvesting time, geographical origin, and climatic conditions (Chelghoum et al., 2021).

Some essential oils have demonstrated antifungal activity, making them interesting for applications in agriculture, food preservation, and medicine. The aim of this study is to investigate the antifungal activity of the essential oil from the gall part of *P. atlantica* against *Fusarium oxysporum* f. sp. *albedinis* (Foa).

Materials and methods

The plant material utilized in this study consisted of the gall part of *P. atlantica*, collected in September 2018 from two distinct locations (Fig. 1): Ain-Oussera (35°20’55.6’’ N, 2°57’5.1’’ E) and Laghouat (33°31’56.6’’ N, 3°1’47.0’’ E). After collection, the galls were air-dried in shaded conditions at room temperature. A voucher specimen (PAUG-52S/08/10) was deposited in the herbarium of the Biology Laboratory at the University of Laghouat, Algeria. The essential oil was obtained by hydro-distillation (100 g of sample in 1 L of distilled water), using a Clevenger-type apparatus, for 3 hours. The resulting essential oil was treated with filtered anhydrous sodium sulfate and stored in sealed glass vials at +4 °C until analysis.

The GC/MS analysis was conducted using an Agilent 6890 gas chromatograph coupled with a 5973-mass selective detector, equipped with an HP5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness) and a 70 eV EI quadrupole detector. Helium served as the carrier gas at a flow rate of 1 mL/min. The injector temperature was set to 250 °C, while the mass spectrometer transfer line was maintained at 220 °C. The column temperature program began at 60 °C with a 2-minute hold, followed by an increase to 125 °C at a rate of 2 °C/min (held for 2 minutes), and then further ramped to 220 °C at 5 °C/min, with an additional hold of 2 minutes. A sample volume of 1 µL, diluted in ethanol at a ratio of 1:100 (v/v), was manually injected in split-less mode. Linear retention indices were determined using a homologous series of n-alkanes (C_8–C_{40}). Component identification was achieved by comparing mass spectra with those in the Wiley and NIST libraries.

The antifungal activity of essential oils was evaluated using the direct contact method as reported by Elhouiti et al. (2022). *F. ox-*

ysporum was obtained from the culture collection of the National Higher Agronomic School El-Harach (Algeria) by Dr. Touati-Hattab Sihem. The inoculum was in the form of a fungal disc with a diameter of 6 mm, obtained from a 7-day culture on PDA medium (in Petri dishes with a diameter of 90 mm, incubation temperature 25 ± 2 °C). Dilutions are prepared in agar solution 0.2%. In test tubes, each containing 13.5 mL of PDA medium, sterilized by autoclaving (120 °C for 20 min) and cooled to 45 °C, a 1.5 mL was added of each dilution to complete final concentrations of 20, 10, 4, 2, 1, 0.5, and 0.3 µL/mL. The controls, containing only the culture medium and 0.2% agar solution, were also prepared. A mycelia disk of 6 mm in diameter, of a 7-day-old culture, was inoculated in the centre of each PDA plate (90 mm diameter) and then incubated at 25 ± 2 °C for 7 days. Each experiment was repeated three times. Diameter measurements of the inhibition zones were taken after the incubation period for each concentration. The % inhibition of radial growth was calculated using the following formula:

$$\% \text{ Inhibition} = \left(1 - \frac{D_s}{D_c}\right) \times 100$$

where: DS – diameter (mm) of sample proliferation with essential oil and DC – diameter (mm) of control proliferation without essential oil.

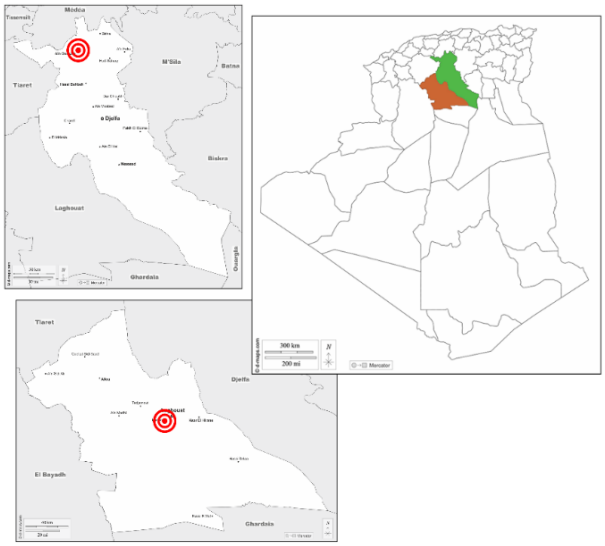

Fig. 1. Map indicating the collection sites of the gall part of *P. atlantica*

The results of the data are shown as mean value ± standard deviation (SD), which were calculated from three replicates. A probability value of $P < 0.05$ was considered statistically significant.

Results

The extracted essential oils were analysed using GC and GC-MS techniques to determine their chemical composition. The yield of essential oil from the galls of *P. atlantica* was measured at 0.99 mL for the Ain-Oussera region (A) and 1.44 mL for the Laghouat region (L). The chromatographic analyse resulted in the identification of 21 compounds representing a total of 97.85% for Ain-Oussera essential oil, and 26 compounds representing a total of 97.85% for Laghouat essential oil (Table 1). The chromatographic analysis of essential oils from the galls of *P. atlantica* revealed notable differences in the chemical composition between the Ain-Oussera (A) and Laghouat (L) regions. The major compounds obtained in this study were: α-pinene (63.15%), β-pinene (10.36%), myrcene (9.53%), camphene (5.18%) and limonene (5.06%, Fig. 2).

The antifungal activity against Foa of the essential oils was evaluated using the direct contact method. The results of the antifungal activity of essential oils from the galls of *P. atlantica* for each region (Ain-Oussera and Laghouat) are presented in Table 2. From these results, it appears that all our samples of essential oil have an inhibitory capacity against the growth of the tested strain of Foa. The proliferation diameter of Foa decreases with an increase in essential oil concentration to 20.5 and 30.25 mm, respectively, for the Ain-Oussera and Laghouat stations.

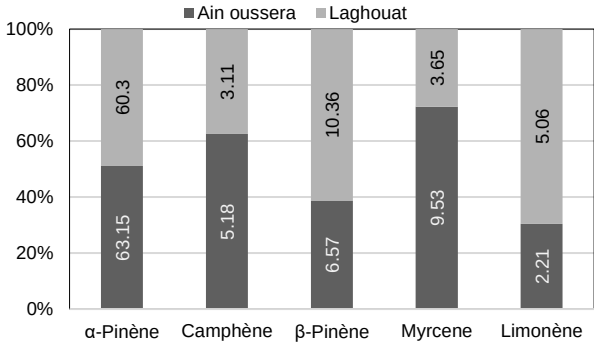

Fig. 2. The major compounds of essential oil from the galls of *P. atlantica*

Table 1
Chemical composition of essential oils from the galls of *P. atlantica*

No.	Constituents	t	LRI	Ain-Oussera	Laghouat	Identification
1	Tricyclene	9.720	1011	1.36	1.10	MS, RI
2	*α*-Pinene	10.053	1025	63.15	60.30	MS, RI
3	Camphène	10.987	1063	5.18	3.11	MS, RI
4	*β*-Pinene	12.062	1106	6.57	10.36	MS, RI
5	Sabinene	12.403	1119	0.81	0.52	MS, RI
6	*δ*-3-Carene	13.205	1147	–	0.97	MS, RI
7	Myrcene	13.683	1165	9.53	3.65	MS, RI, AS
8	*α-Phellandrene*	13.810	1169	–	0.52	MS, RI
9	Limonene	14.866	1205	2.21	5.06	MS, RI, AS
10	*β-Phellandrene*	15.195	1214	0.47	2.25	MS, RI
11	*p*-Cymene	17.433	1274	0.59	1.29	MS, RI
12	*α*-Terpinolene	18.263	1296	–	2.08	MS, RI
13	Unknown 1	21.559	1386	0.23	0.14	MS, RI
14	*β*-Thujone	24.049	1450	–	0.19	MS, RI
15	Camphor	25.165	1477	–	0.12	MS, RI
16	Bornyl acetate	29.440	1591	2.41	1.33	MS, RI, AS
17	Terpinen-4-ol	30.383	1617	0.67	0.37	MS, RI, AS
18	*E*-Pinocarveol	32.369	1670	0.13	0.13	MS, RI
19	Cryptone	33.292	1694	0.30	0.30	MS, RI
20	Unknown 2	33.542	1701	0.42	0.27	MS, RI
21	*α*-Terpineol	33.741	1707	2.10	1.16	MS, RI, AS
22	Unknown 3	37.163	1807	0.11	0.23	MS, RI
23	*p*-Cymen-ol	39.319	1869	0.22	0.73	MS, RI
24	Unknown 4	47.678	2130	–	0.21	MS, RI
25	Spathulenol	48.071	2143	0.79	0.12	MS, RI
26	Myristic acid	63.938	2726	0.46	0.25	MS, RI
27	Palmitic acid	68.735	2912	0.14	–	MS, RI
	Total identified	–	–	97.85	96.76	–
	Monoterpene hydrocarbon	–	–	89.87	91.21	–
	Oxygenated monoterpene	–	–	5.53	4.03	–
	Total monoterpenes	–	–	95.4	95.24	–
	Sesquiterpene hydrocarbon	–	–			–
	Oxygenated sesquiterpene	–	–	0.79	0.12	–
	Total sesquiterpenes	–	–	0.79	0.12	–
	Other compounds	–	–	1.12	1.40	–
	Essential oil yield % (v/w)	–	–	0.99	1.44	

Notes: LRI – linear retention indices relative to homologous n-alkanes C_8-C_{40} obtained on UB-Wax column; RI – identification relative to linear retention indices; MS – identification relative mass spectra; AS – identification relative to retention indices of pure authentic samples.

The study used a direct contact method to measure the efficacy of essential oils from two geographic regions, Ain-Oussera and Laghouat. The results indicate that all tested essential oils possess inhibitory effects on Foa, with varying degrees of effectiveness based on concentration. The data illustrated in Table 2 has a clear trend as the concentration of essential oils increases, the proliferation diameter of Foa decreases, indicating enhanced antifungal activity (Fig. 3). The percentages of *Fusarium* growth inhibition by the essential oils of galls

of *P. atlantica* from the two regions (Ain-Oussera, Laghouat) are represented in Figure 4. Comparing the percentages of inhibition of essential oils from the two regions reveals that the essential oil of Ain-Oussera is more active than that of Laghouat. But we cannot distinguish any major difference in the inhibition of mycelial growth during treatment with essential oils from the Ain-Oussera and Laghouat region in the concentration range from 10 to 20 µL/mL. The results suggest that varying concentrations of the essential oils have a significant impact on inhibiting the growth of Foa, which is crucial for managing plant diseases caused by this pathogen.

Table 2
The proliferation diameter (mm)
of *Fusarium oxysporium* f. sp. *albedinis* (Foa)

Concentration, µL/mL	The proliferation diameter of Foa, mm	
	Ain-Oussera	Laghouat
20	20.50 ± 6.36	20.75 ± 1.35
10	34.75 ± 5.95	32.50 ± 1.71
4	58.00 ± 2.12	54.50 ± 6.63
2	61.00 ± 2.83	66.75 ± 3.18
1	64.00 ± 1.41	69.75 ± 3.89
0.5	67.00 ± 2.83	71.50 ± 2.12
0.3	67.00 ± 2.38	72.00 ± 1.41

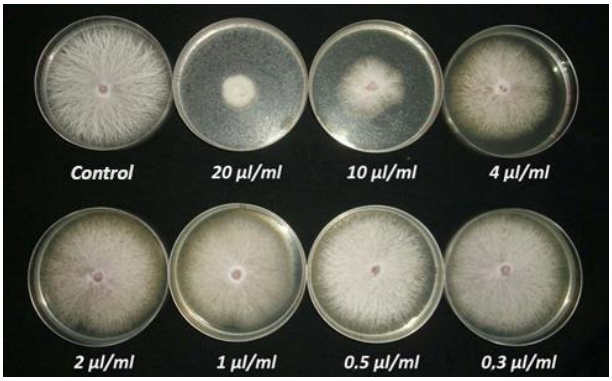

Fig. 3. Photo shows the antifungal activity of the EOs of the galls of *P. atlantica* (Ain-Oussera)

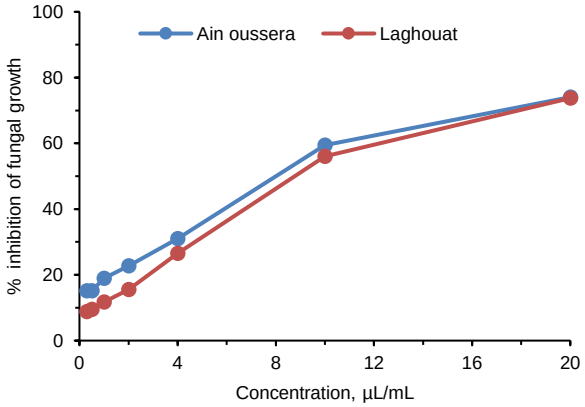

Fig. 4. The percentage inhibition of Foa growth by *P. atlantica* essential oil

Discussion

The yield from Laghouat region is significantly higher than that from Ain-Oussera, with a difference of 0.45 mL. This suggests that environmental factors in Laghouat may be more conducive to essential oil production. Possible factors contributing to this difference could include variations in soil composition, climate conditions (such as temperature and humidity), and the specific ecological interactions present in each region (Chelghoum et al., 2021; Qian et al., 2024). The observed difference in essential oil yield between the Ain-Oussera and Laghouat regions highlights the importance of environmental factors in influencing plant secondary metabolite production. The chemical compositions of essential oils from the galls of *P. atlantica* in the Ain-oussera (A) and Laghouat (L) regions can be compared based on various factors, including the types of compounds present and their relative abundances. The essential oils of *P. atlantica* typically contain a variety of monoterpenes and sesquiterpenes. Key components often include α-pinene, β-pinene, and terpinen-4-ol, which are known for their aromatic properties and potential biological activities (Mahjoub et al., 2018; Sifi et al., 2015). In particular, α-pinene is frequently reported as a major constituent, contributing to the characteristic scent and potential therapeutic effects of the oil (Sifi et al., 2022).

The essential oil from Ain-Oussera revealed greater antifungal activity than that from Laghouat, particularly at the concentration range of 0.3 to 4.0 µL/mL However, no significant differences were noted in mycelial growth inhibition between the two regions within the concentrations range of 10 and 20 µL/mL. This observation aligns with findings from other studies that indicate varying effectiveness of essential oils based on their geographic origin and chemical composition, which can be influenced by environmental factors such as soil type and climate conditions (Sharma et al., 2017; Moutassem et al., 2019). Similar studies have shown that other essential oils, such as clove and eucalyptus, also exhibit significant antifungal activities against *Fusarium* species, often demonstrating dose-dependent effects (Sharma et al., 2017; Chacón et al., 2021). Clove oil, for instance, has been noted for its complete inhibition of mycelial growth at certain concentrations (Sharma et al., 2017).

The essential oil of *P. atlantica* is primarily composed of monoterpenes, with significant amounts of α-pinene, β-pinene, and myrcene. These compounds are known for their antimicrobial properties (Salehi et al., 2019). The presence of monoterpenes like α-pinene and β-pinene is also noted in other essential oils with antifungal properties, such as clove and oregano oils, which contain high levels of oxygenated compounds like eugenol and carvacrol (Cárdenas-Laverde et al., 2021). These compounds contribute significantly to the antifungal activity observed against various fungal pathogens (Chacón et al., 2021). The antifungal action is likely due to the ability of these monoterpenes to disorder cellular membranes or interfere with metabolic processes in fungi, leading to cell death. This mechanism is supported by studies showing that essential oils can alter membrane permeability and inhibit spore germination in fungal pathogens (Roselló et al., 2015).

The mode of action of essential oils in exerting their antimicrobial activity has been investigated by various authors. It is believed that terpenes in the oils interact with enzymatic systems involved in energy production and synthesis of structural components within microbial cells. Additionally, they may cross the cell membrane and interact with critical intracellular sites. Furthermore, it has been proposed that essential oils can affect not only permeability but also other functions of cell membranes (Li et al., 2022). The antimicrobial activity of monoterpenes, such as those found in the essential oil of *P. atlantica*, is known to inhibit the growth of pathogens by damaging cell membrane structures (Zhou et al., 2023). However, the antifungal activity of the oil is not solely attributed to these monoterpenes, as there are other major or trace components in the oil that could contribute to its antifungal effects. It has been suggested that these components may interact synergistically or antagonistically with each other (Duru et al., 2003).

Conclusion

In conclusion, the evaluation of the antifungal activity of *P. atlantica* essential oil against *Fusarium oxysporum* using the direct contact method revealed significant antifungal activity. The oil's composition, rich in monoterpene hydrocarbons such as α-pinene, β-terpinene, and limonene, may contribute to its antifungal effects. This positions *P. atlantica* essential oils as a promising alternative or supplementary treatment in phytopathogen management strategies. Further research is needed to fully understand the mode of action and potential applications of *P. atlantica* essential oil as an antifungal agent.

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