

Effectiveness of Essential Oils from Three Medicinal Plants Against Bayoud Disease (*Fusarium oxysporum* f. sp. *albedinis*) of Date Palm (*Phoenix dactylifera* L.)

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Abstract

Plant extracts and essential oils are promising new sources of non-toxic alternatives to chemicals substances. They are used for their antimicrobial properties against plant diseases of fungal origin, against bacterial, and those of virus origin. This study was carried out to study the antifungal activity of *Artemisia herba-alba*, *Foeniculum vulgare* and *Citrus sinensis* essential oils against *Fusarium oxysporum* f. sp. *albedinis*. Essential oil was extracted by steam distillation. Antifungal activity of essential oil was investigated by macro-broth method of dilution by a minimal inhibitory concentration (MIC) assay against this pathogen. The yield of essential oil obtained by steam distillation of *Foeniculum vulgare* samples was 2.31% greater than that obtained from *Citrus sinensis* sample which was 1.8%, followed by *Artemisia herba alba* samples 1.22%. Regarding antifungal activity, the results revealed a better inhibitory activity of *Artemisia herba alba* against the tested strains at the lowest LC₅₀ values (0.1 µl/ml). On the other hand, *Foeniculum vulgare*, *Artemisia herba alba* and *Citrus sinensis* essential oils show similar MICs of mycelial growth against this pathogen. The value of the MIC and CMF is greater than 50 µl/ml for the three essential oils.

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Introduction

The date palm (*Phoenix dactylifera* L.) is considered one of the main perennial crops in the oasis ecosystem of several countries around the world (Fernández-López *et al.*, 2022). The date palm is, first of all, a fruit tree and therefore the origin of a foodstuff, the date, which is fundamental for Saharan agricultural societies. It represents an ecological importance in the

control against desertification (Jonoobi *et al.*, 2019). The Algerian date palm sector has been showing signs of crisis for decades, with a duality between a traditional system that is not very competitive due to geomorphological conditions (in the Sahara zones) and a modern system, intended mainly for export. Despite this importance, the date palm has suffered from a major biotic constraint

called, Bayoud, in Algeria, Mauritania and Morocco (Bouguedoura *et al.*, 2015).

Bayoud is a destructive and fatal vascular disease caused by the soil fungus *Fusarium oxysporum* f. sp. *albedinis* (Foa) (Benzohra *et al.*, 2017). The fungus lives in the soil and attacks palm trees of all ages through the roots. After a period the heart of the tree ends up giving way causing its death. This disease has killed in the last fifty years more than 10 million palm trees in Morocco and three million in Algeria (Bouguedoura *et al.*, 2015). Efforts to control this epidemic have focused on several methods such as prophylactic measures, varietal selection and biological control, in order to limit the damage caused by this epidemic. In addition to these measures, the control against the bayoud agent has been highlighted in previous works (Belaidi, 2022a; Belaidi, 2022b).

Due to the problems caused by chemical substances, the development of alternative control measures is of great importance. Biological control has been considered as a desirable and realistic alternative. Numerous studies have demonstrated the ability of several essential oils to possess antibacterial, antifungal, antiviral, antioxidant activities and play an important role in the protection of the plants and the human **body** against plant pathogens both *in vitro* and *in vivo* (Mutlu-Ingoket *et al.*, 2020; Marin-Tinoco *et al.*, 2021). Note that essential oils can be an effective solution, as their toxicity is much lower, better specificity of action, biodegradable and environmentally friendly (Campos *et al.*, 2019). It has been reported that essential oils possess various actions on pathogenic fungi, such as disruption of the cytoplasmic membrane, disruption of the proton driving force, electron leakage and coagulation of the protein content of cells, acidification from inside the cell, blocking the production of cellular energy and the synthesis of structural components. The essential oil may inhibit the cell growth and proliferation by interrupting ergosterol

biosynthesis (Gao *et al.*, 2016). Essential oil enables us to integrate into the lipids of the cell membrane, increasing permeability occur as a due of loss of ions and reduction of membrane potential, collapse of the proton pump and depletion of the ATP pool, which eventually lead to leaking of intracellular constituents, coagulation of cell contents, lysis and cell death (Turgis *et al.*, 2012). The aim of this research is to evaluate the antifungal activity of extracts (natural substances) of three locally available plants against *Fusarium oxysporum* f. sp. *albedinis*.

Materials and Methods

Inoculum preparation

The tested *Fusarium oxysporum* f. sp. *albedinis* strain B7b7245 was preserved at the Bioresources Laboratory of the Center for Scientific and Technical Research on Arid Regions, Biskra, Algeria. *Fusarium oxysporum* f. sp. *albedinis* readily produced conidia after 14 days on potato dextrose agar (PDA; potato dextrose agar.SPA CRAPC Algeria) plate at 25°C in the dark. Conidial suspension was obtained by flooding plates with distilled water and rubbing gently with a glass rod, then filtered through sterile cheesecloth. The conidia concentration present in the initial suspension (adjusted to the concentration 1×10^5 conidia.mL⁻¹) was quantified using the hemocytometer.

Extraction of essential oils

The essential oils were extracted from the dry leaves and seeds by steam distillation carried out in a still system (Machado *et al.*, 2022). The plant material was placed in a pressure cooker that was connected to a cooler through a conduit. The whole (pressure cooker, leaves and water separated by a grid) was brought to the boil on an electric stove. Water vapor and condensed aromatic molecules were collected in a separatory funnel. The supernatant oils were recovered by decantation and stored in opaque bottles.

Essential oils yield (%)

The yield (% , w/w) from plants was calculated as: $R (\%) = mH \cdot 100/mS$.

Growth inhibition evaluation

Evaluation the growth inhibition of *Fusarium oxysporum* f. sp. *albedinis* strains, was calculated by the following equation Mohareb *et al.* (2017):

$$L (\%) = [(D1 - D2)/D1] \times 100$$

Where:

L (%): Rate of mycelial growth inhibition

D1: Mycelial growth in control

D2: Mycelial growth in the presence of treatment.

In vitro antifungal assay

Antifungal activity of the essential oil against *Fusarium oxysporum* f. sp. *albedinis* was investigated by macro-broth method of dilution (Balouiri *et al.*, 2016). Then, 100 μ l of the essential oil was poured into the plates. Mycelium growth inhibition was evaluated by placing 30 μ l of the inoculum containing 10^5 spores.mL⁻¹ in the centre of a PDA plate. Cultures were incubated at 25°C in the dark. Colony diameters were measured in two perpendicular directions, after 24 hours and again after 96 hours. Each treatment had five replications. In the control group, the oil was replaced by sterile distilled water.

Statistical analysis

All data were analyzed using ANOVA test. A Comparison of means was performed by test of Kruskal-Wallis at 5% of probability. The software used was STATBOX 6.0.4.

Results and Discussion**Essential oils yield (%)**

The average essential oil yields were estimated using the plant material. The rate obtained from *Foeniculum vulgare* samples was 2.31% greater than that obtained from *Citrus sinensis* sample which was 1.8%, followed by *Artemisia herba alba* samples 1.22% (Table 1). This rate is low when compared to that obtained from *Foeniculum vulgare* from Iran and Turkey, which is 3.77 and 2.67% respectively (Sabzi-Nojadeh *et al.*, 2021). In addition, the essential oil yields for *Citrus sinensis* fresh fruits reported in this study are similar to those reported by Bhandari *et al.* (2021). For the *Artemisia herba alba* species, our yield is higher compared to *Artemisia herba alba* provenances in Morocco (1.18%) (Allali *et al.*, 2022). In Tunisia, the same infraspecific variance in *Artemisia herba alba* yield was varied between 0.68% and 1.93% based on dried weight of samples (Mohsen and Ali, 2009). Plant species, environmental conditions, extraction technique, drying, and cultivation practices, are all aspects that influence the yield, content and chemical composition of essential oils (Rezouki *et al.*, 2021).

Table 1. Essential oils yield (%) and groups of compounds, region of collection

Species	Essential oils yield (%)	Previous studies on the majority compounds (References)
<i>Artemisia herba alba</i>	1.22	Camphor : Algeria (Lakehal <i>et al.</i> , 2016) α -Thujone : Tunisia (Mohsen and Ali, 2009); Morroco (Bencheqroun <i>et al.</i> , 2012) 1,8-cineole Spain (Mohamed <i>et al.</i> , 2010) Verbenol : Morroco (Tilaoui <i>et al.</i> , 2011)

<i>Citrus sinensis</i>	1.8	Flavonoids: Florida (Abbate <i>et al.</i> , 2012) Limonene : Uganda (Njoroge <i>et al.</i> , 2013 ; Kamal <i>et al.</i> , 2011) Linalool ; Egypt (Tisserand <i>et al.</i> , 2014)
<i>Foeniculum vulgare</i>	2.3	Limonene: Morocco (El Ouariachi <i>et al.</i> , 2014) Stigmasterol: Egypt (Nassar <i>et al.</i> , 2010) trans-anethole: Slovakia (Petra <i>et al.</i> , 2021) a-pinene : Iraq (Belaidi <i>et al.</i> , 2020)

Activity of essential oil on mycelial growth

Artemisia herba alba essential oil was evaluated at concentrations of 0.1, 1, 10, 30, and 50 $\mu\text{l.ml}^{-1}$, which resulted in inhibition rates of 51.9, 61.5, 76.2, 76.4, and 82.6%, respectively (Table 2). Indeed, the concentration equal to or greater than 0.1 $\mu\text{l.ml}^{-1}$ of essential oil inhibits 50% the growth of *Fusarium oxysporum* f. sp. *albedinis*. *Fusarium oxysporum* f. sp. *albedinis*'s growth is inhibited by 50% at concentrations equivalent to or greater than 0.1 $\mu\text{l.ml}^{-1}$. In terms of kinetics of action,

24 hours of treatment is enough to limit 50% mycelial growth. Similarly, Janačković *et al.* (2015) shown that *Artemisia herba alba* essential oil showed better antifungal activities against eight fungal species; *A.niger*, *A. ochraceus*, *A. versicolor*, *A. fumigatus*, *Penicillium ochrochloron*, *P. funiculosum*, *Trichoderma viride* and *Candida albicans*. According to earlier results, oxygenated monoterpenes, particularly thujone, have a stronger antifungal potential (Sokovi *et al.*, 2010).

Table 2. Antifungal activity of *Artemisia herba alba* essential oil against fungal strain. Values are the means of the inhibition rates \pm SD

Time	Essential oil concentrations ($\mu\text{l.ml}^{-1}$)				
	0.1	1	10	30	50
1st day	54.7 \pm 4.2	58.1 \pm 3.6	62.8 \pm 6.4	73.6 \pm 3.7	77.6 \pm 1.7
2nd day	40.4 \pm 5	50.5 \pm 10.3	65.4 \pm 4.5	76.3 \pm 2.17	78.1 \pm 1.44
3rd day	47.9 \pm 2.3	59.5 \pm 4.92	72.1 \pm 4.6	79.2 \pm 1.7	81.2 \pm 2.2
4th day	51.9 \pm 3.3	61.5 \pm 4.8	76.2 \pm 4.0	76.4 \pm 1.8	82.6 \pm 3.81

The results of antifungal activity assays showed that the 0.1, 1, 10, 30 and 5 $\mu\text{l.ml}^{-1}$ *Foeniculum vulgare* essential oil produce an inhibition of 35.9, 36.7, 37.5, 38.4 and 50.4% respectively the mycelium growth of *Fusarium oxysporum* f. sp. *albedinis* after 24 hours of incubation at 25°C compared to untreated (Table 3). According to these results, fennel essential oil inhibits growth at a rate of more than 50% in Petri dishes containing 50 $\mu\text{l.ml}^{-1}$ concentrations of the oil. According to Garzoli *et al.* (2018), the essential oil of *Foeniculum vulgare* has an inhibitory effect on the growth of the genus

Candida due to the higher constituents of estragole, limonene, and fenchone. Some authors have demonstrated that trans-anethole is the primary active ingredient responsible for fennel oil's antimicrobial activity (Petra *et al.*, 2021).

Table 3. Antifungal activity of *Foeniculum vulgare* essential oil against fungal strain. Values are the means of the inhibition rates ± SD

Time	Essential oil concentrations (µl.ml ⁻¹)				
	0.1	1	10	30	50
1st day	50.1±12.7	54.5±3.6	55.6±4.8	55.5±2.8	61.4±3.4
2nd day	35.9±2.1	36.7±2.0	37.5±3.3	38.4±3.0	50.4±3.7
3rd day	35.5±3.8	36.6±1.5	40.9±5.8	42.7±3.1	45.2±2.3
4th day	37.9±3.6	44.2±6.6	47.9±5.1	48.7±2.5	50.1±2.8

The *Citrus sinensis* essential oil reached the inhibition rate of 23.3, 25.9, 28.4, 28.4 and 34.6% for concentrations 0.1, 1, 10, 30 and 50 µl.ml⁻¹, respectively (Table 4). In fact, citrus essential oils did, have a very weak activity when applied to the tested strain of *Fusarium oxysporum* f. sp. *albedinis*. Sweet *C. lemon*, *C. aurantifolia*, *C. maxima*, and *Citrus sinensis*

essential oil were reported to inhibit the growth of several fungal species, such as fungal species, such as *R.solani*, *S.rolfsii*, *F.solani*, *F.oxysporum*, *F.semtectium*, *B.cinerea*, and *A. alternata* (Sedeek *et al.*, 2021). Their high content of monoterpenes, particularly limonene, may be responsible for their powerful antifungal effect (Singh *et al.*, 2010).

Table 4. Antifungal activity of *Citrus sinensis* essential oil against fungal strain. Values are the means of the inhibition rates ± SD

Time	Essential oil concentrations (µl.ml ⁻¹)				
	0.1	1	10	30	50
1st day	50.1±5.4	60.9±2.8	61.8±4.8	64.1±3.3	64.9±2.2
2nd day	40.3±4.0	44.9±5.3	53.01±3.1	52.9±1.3	53.4±1.7
3rd day	35.8±6.3	38.2±0.7	38.2±4.1	42.4±5.9	56.5±3.9
4th day	23.3±5.5	25.9±3.6	28.4±4.7	28.4±8.03	34.6±4.6

Comparison between the activities of different EOs on the mycelial growth of *Fusarium oxysporum* f. sp. *albedinis*

The minimum inhibitory concentration (MIC) of essential oils was determined according to the method cited by Mizanur-Rahman *et al.* (2017).

The comparison between the activities of the different essential oils against *Fusarium oxysporum* f. sp. *albedinis*, shows that *Artemisia herba alba* essential oil has a better inhibitory activity of mycelial growth. On the other hand, *Foeniculum vulgare*, *Artemisia herba alba* and *Citrus sinensis* essential oils show similar MICs of mycelial growth against this pathogen (Table 5).

Table 5. Comparison of the Antifungal activity of *A. herba alba*, *Foeniculum vulgare* and *Citrus sinensis* essential oils against fungal strain. Values are the means of the inhibition rates ± SD

Essential oils	Essential oil concentrations µl/ml				
	0.1	1	10	30	50
<i>A. herba alba</i>	51,9±6.2	61,5±8.3	76,2±7.4	76,4±7.7	82,6±11.2
<i>F. vulgare</i>	37,9±3.8	44,2±4.4	47,9±1.9	48,7±2.1	50,1±2.3
<i>C. sinensis</i>	23,3±4.2	25,9±1.2	28,4±1.2	28,4±1.5	34,6±2.01

The value of the MIC and MFC is greater than 50 µl/ml for the three essential oils (Table

6). Previous studies showed that *Artemisia herba alba* essential oil showed fungicidal and

fungistatic effects against *Candida albicans* with MIC (31.25 µl/ml) and MFC (62.5 µl/ml) (Boukhenoufa *et al.*, 2019). Perczak *et al.* (2019) showed that the essential oil of *Foeniculum vulgare* inhibited the growth of *Fusarium culmorum* and the minimum inhibitory concentration (MIC) was >100 µg/cm³. EOs from two medicinal plants (*Citrus*

sinensis and *C. limon*) were investigated for their antifungal activity against *A. flavus* by Ben Miri *et al.* (2018), who reported that the MIC values were 1.75 mg.mL⁻¹ for *C. limon* and 2 mg.mL⁻¹ for *C. sinensis*. The MFC of *Citrus limon* and *Citrus sinensis* occurred at 2 mg.mL⁻¹.

Table 6. Comparison of the Minimum inhibitory concentration (MIC), legal (LC50) and minimum fungicidal concentrations (MFC) of essential oils against fungal strain

	Essential oils		
	<i>A. herba alba</i>	<i>Foeniculum vulgare</i>	<i>C. sinensis</i>
MIC	>50 µl/ml	>50 µl/ml or equal to double CL ₅₀	>50 µl/ml
LC₅₀	0.1 µl/ml	50 µl/ml	>50 µl/ml
MFC	>50 µl/ml	>50 µl/ml	>50 µl/ml

Artemisia herba alba was shown to be the most effective against fungal strain with LC₅₀, values that were lower than those obtained with *Foeniculum vulgare* and *Citrus sinensis* essential oils (LC₅₀=0.1 µl/ml). The lowest LC₅₀ was obtained with *Artemisia herba alba* (0.1 µl/ml), while the highest was obtained with *Foeniculum vulgare* and *Citrus sinensis* essential oils (>50 µl/ml). Theoretically, to produce the fungicidal action with *Artemisia herba alba* and *Foeniculum vulgare* essential oils, the LC₅₀ must be at least doubled. Contrarily, with *Citrus sinensis* essential oil, triple the CL₅₀ is sufficient to produce this effect (MFC).

Conclusion

In summary, the present work confirms the very important of *Artemisia herba alba*, *Foeniculum vulgare* and *Citrus sinensis* essential oils for their possible antifungal activity. Indeed; the agar diffusion method revealed that *Artemisia herba-alba* essential oil exhibited potent antifungal activity against *Fusarium oxysporum* f. sp. *albedinis*. In the same way the lowest LC₅₀ was obtained with *Artemisia herba alba* (0.1 µl/ml), while the highest was obtained with *Foeniculum vulgare* and *Citrus sinensis* essential oils (>50 µl/ml).

Conflict of Interest

The authors declare that they have no conflict of interest.

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