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Antifungal potential of the Seed and Leaf *Foeniculum vulgare* Mill essential Oil in liquid and vapor phase against phytopathogenic fungi

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ABSTRACT

Analysis of essential oils obtained from the seeds (SEO) and leaves (LEO) of *Foeniculum vulgare* Mill cultivated in southeast of Morocco revealed that trans-anethole was the major constituent (54.67% of seed oil and 50.60% in leaf oil), followed by estragol (35.33% of seed oil and 30.15% in leaf oil). Other components present in leaf oil included fenchone, linalool and α-pinene, while seed oil contained fenchone, limonene and γ-terpinen. Besides, minor constituents like camphene, camphor, myrcene, pahllandrene, fenchyl acetate and β-bisabolene were obtained from seeds and leaves.

The essential oils of the seeds and leaves of *Foeniculum vulgare* were assayed for their antifungal activity by poison food (PF) technique and the volatile activity assay (VA) against five agricultural pathogenic fungi. The results indicated that the essential oil of *Foeniculum vulgare* inhibited the mycelial growth of all fungal strains tested. VA assay of essential oil was consistently found to be more effective than PF technique.

The mycelium growth was completely inhibited by LEO on *Alternaria* sp., *Fusarium oxysporum* f. sp. *albedinis and Aspergillus brasiliensis* at 0.25 µl mL^{-1} air. *Rhizopus stoloniferaw*as the most sensitive with a minimal inhibitory concentration (MIC) = 0.25 µl mL^{-1} air.

INTRODUCTION

Foeniculum vulgare Mill (Umbelliferaceae). Commonly known as fennel, is a small genus of annual, biennial or perennial herbs. It is widely cultivated for its aromatic fruits, which are used as culinary spices (Rather *et al.,* 2012). Herbal drugs and essential oil of fennel has different pharmacological properties such as anti-spasmodic, anti-allergic, diuretic, anti-inflammatory, analgesic and antioxidant effect (Ebeed *et al.,* 2010, Choi and Hwang, 2004, Misharina and Polshkov, 2005, Parejo *et al.,* 2002, Pradhan *et al.*,2008, Kooti *et al.,* 2015). In addition, the volatile oil showed antimicrobial and hepatoprotective (Sellam *et al.,* 2014, Toma *et al.,* 2008, Özbek *et al.,* 2003), antithrombotic activity (Tognolini *et al.,* 2007), antidiabetic activity (El-Soud *et al.,* 2011), antitumour activity (Pradhan *et al.,* 2008) and acaricidal activity (Lee, 2004).

In this study, the chemical compositions were studied by GC/SM analysis and the antifungal activity was evaluated in vitro by poison food (PF) technique and the volatile activity assay (VA) against fives agricultural pathogenic fungi.

MATERIAL AND METHODS

Plant material

The seeds and leaves of *Foeniculum vulgare* were collected from Tafilalet (southeast of Morocco). The voucher specimens have been deposited at the Biochemistry of Natural Products Laboratory, Department of Biology, Faculty of Sciences & Techniques, Errachidia, Morocco.

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Hydrodistillation apparatus and procedure

The essential oils were obtained by hydrodistillation method from fresh leaves and seeds collected from the plants grown in the Errachidia region during juin 2013.

The yield of essential oil obtained from the seeds (SEO) and leaves (LEO) was found to be 2.8% and 2%. The obtained essential oil was dried over anhydrous sodium sulfate and after filtration, stored at + 4°C until tested and analyzed.

Essential oil analysis

Components were identified on the basis of gas chromatography-mass spectrometry (GC-MS) library and confirmed by calculation of retention indices from GC-FID. GC-MS was performed on a GC 6890 Agilent equipped with an HP-INNOWAX capillary column (50m x 0.2 mm; film thickness 0.5 µm). Carrier gas: Helium 1.6 ml/min, split 1/100; injector temperature: 280°C; oven temperature: 60°C (2 min isothermal) then 3°C/min to 180°C, then 8°C/min to 245°C (10 min isothermal). MS 5973 N Agilent; source temperature: 230°C; mass range: 35 to 350 amu; scan speed: 1 scan/sec. GC-FID: Fast GC HP- 6850 equipped with a DB-WAX capillary column (20 m, 0.1 mm, 0.2 µm). Carrier gas H2 at 0.7 ml/min, split 60 ml/ min. Injector temperature 275°C; Detector temperature 275°C; oven temperature 60°C (2 min isothermal) then 12°C/min to 248°C (5min isothermal). Detected compound concentrations are relative percentages (ISO7609), with a threshold of 0.05%.

Antifungal activity

Fungal strains

Fives agricultural pathogenic fungi were selected for their implication in the contamination and the deterioration of the foodstuffs and production of mycotoxins. The fungal species used in the experiments are *Alternaria sp*, *Pencillium expansum*, *Rhizopus stolonifer*, *Fusarium oxysporum* f. sp. *albedinis*and *Aspergillus brasiliensis* ATCC 16404. These fungi are obtained from the culture collection at Faculty of Sciences & Technology, Errachidia.

Antifungal activity assay

The antifungal activity of the SEO and LEO of *Foeniculum vulgare*against mycelial growth of fungi was tested following poisoned food technique (PF) (Perrucci *et al.*, 1994) and volatile activity assay (VA) (Soylu *et al.*, 2010) with some modifications.

Poison food (PF) technique

The essential oil was dispersed as an emulsion in sterile agar suspension (0.2%) (Remmal *et al.*, 1993) and added to PDA immediately before it was emptied into the glass Petri dishes (90×20 mm in diameter) at a temperature of 40–45°C.

The concentrations tested were 0.062 to 1µl mL^{-1}. The controls received the same quantity of sterile agar suspension (0.2%) mixed with PDA. The tested fungi were inoculated with 6 mm mycelial plugs from 7-days-old cultures cut with a sterile cork and incubated for 3 days for *Rhizopus stolonifer* and 6 days for *Alternaria sp*, *Pencillium expansum*, *Fusarium oxysporum* f. sp. *albedinis*and *Aspergillus brasiliensis* at 25±2°C.

Volatile activity assay

The Petri dishes were filled with 20 mL of potato dextrose agar (PDA) medium and then seeded with a mycelial disc (6 mm diameter), cut from the periphery of 7-days--old mycelium culture of the tested fungi. The Petri dishes (90×20 mm, which offer 80 mL air spaces after addition of 20 mL agar media), were inverted and sterile filter paper discs (9 mm in diameter) impregnated with different concentrations of essential oil: 0.062, 0.125, 0.25, 0.5 and 1µl mL^{-1} air are deposited on the inverted lid and incubated for 3 days for *Rhizopus stolonifer* and 6 days for *Alternaria sp*, *Pencillium expansum*, *Fusarium oxysporum* f. sp. *albedinis*and *Aspergillus brasiliensis* at 25±2°C.

In both types of experiments, three replicate plates were inoculated for each treatment and the radial growth was recorded for each plate by calculating the average of two perpendicular diameters. Fungitoxicity of essential oil was expressed in terms of percentage of mycelial growth inhibition (I %) and calculated following the formula of Pandey *et al.* (Pandey *et al.*, 1982):

$$\text{Percentage of mycelial growth inhibition (IP)} = \left(1 - \left(\frac{Dc}{Dt}\right)\right) \times 100$$

Where:

Dc: Average diameter (in mm) of mycelial in control,

Dt: Average diameter (in mm) of mycelial in treatment.

The fungistatic–fungicidal nature of essential oil was tested by observing revival of growth of the inhibited mycelial disc following its transfer to non-treated PDA. A fungicidal effect was where there was no growth, whereas a fungistatic effect was where temporary inhibition of microbial growth occurred.

Statistical analysis

Results are presented as mean ± SD of three independent tests. All tests were carried out in an identical condition.

RESULTS AND DISCUSSION

Chemical Composition of the Essential Oil

The yellowish oils isolated by hydrodistillation from the leaves and the seeds of *F. vulgare* was obtained in a yield of 2% and 2.8% (w/w) respectively. The essential oil was analyzed by means of GC-MS. The components of the oil, the retention times (RT) the percentage constituent (%) are summarized in Table 1. The number of identified compounds was 24 in the seeds and 26 in the leaves, representing 97.05% and 98.16% of the total composition of the two organs.

Trans anethole (50.60%), estragole (30.15%), fenchone (4.32%), linalool (2.83%), α-pinene (2.56%) and γ-terpinene (2.02%) were the main components for the leaf oil, comprising 92.48% of the oil. Trans anethole (54.67%), estragole (35.33%) and α-pinene (2.01%) were the main components for the seed oil, comprising 92.01% of the oil.

The components present in the essential oil obtained from seeds and leaves of fennel cultivated Tafilalet region are similar to those reported for sweet fennel but the relative percentage of compounds such as anethole, estragole and fenchone differed (Chowdhury *et al.*, 2009, Ebeed *et al.*, 2010, Raal *et al.*, 2012, Fratini *et al.*, 2014, Diao *et al.*, 2014). It may be attributed to different factors such as geographical environment, growth season and physiological age of the plant besides the method of oil isolation (Díaz-Maroto *et al.*, 2005).

Table 1: Essential oil Composition of *Foeniculum vulgare* Mill cultivated in southeast of Morocco.

Rt [a]	Compounds [b]	Leaf oil (LEO)	Seed oil (SEO)
4,08	α-thujone	0.21	0.21
8,49	**α-pinene**	**2.56**	**2.01**
9,95	camphene	0.2	0.32
10,56	sabinene	0.29	0.29
10,95	β-pinene	0.3	-
11,68	mycerene	0.03	0.06
11,82	Δ-3-carene	0.61	0.61
12,25	α-terpinene	0.4	0.06
12,92	p-cymene	0.22	0.23
13,04	**limonene**	**1.2**	**0.4**
13,84	**γ-terpinene**	**2.02**	**1.2**
13,95	**fenchone**	**4.32**	**0.06**
14,46	**linalool**	**2.83**	**1.09**
15,9	camphor	0.27	0.98
16,04	terpinen-4-ol	0.3	0.03
16,84	methyl chavicol	0.02	0.27
17,15	fenchyl acetate	0.13	0.03
18,01	**estragole**	**30.15**	**35.33**
21,05	**trans anethole**	**50.6**	**54.67**
23,83	thymol	0.02	0.03
24,81	α-copaene	0.21	0.05
27,09	β-caryophyllene	0.06	0.16
27,71	α-phellandrene	0.07	-
28,36	β-bisabolene	0.03	0.07
	Total	**97.05%**	**98.16%**

[a] Compounds listed in order of elution.
[b] Retention time (as minutes).

Antifungal activity

The antifungal activity was obtained using the PF technique with different concentrations of SEO and LEO of *Foeniculum vulgare* is reported in Figures1 and 2, respectively.

The results (Fig.1) showed that *Alternaria sp.* and *F. oxysporumalbedinis*, was found to be The fungal susceptible to the LEO of *Foeniculum vulgare* followed by *P. expansum* with the percentages of inhibition (IP) are 100±0.00% and 81.00±2.42% at 1µl mL^{-1}, respectively. Conversely, *A. brasiliensis* had a high resistance to this essential oil with IP equal to 70.80±0.67 at 1µl mL^{-1}. The IP against *Alternaria sp.*, *P. expansum* and *F. Oxysporumobedience* were moderates (more than 30 %) at 0.0625 µl mL^{-1}.

The results (Fig.2) showed that the SEO of *Foeniculum vulgare* is less active against fives phytopathogens tested. The IP were moderately at 0.25µl mL^{-1} (ranged from 22.09±1.16% to 43.82±1.17%) and relatively effective at 1µl mL^{-1}. The IP was low at small concentrations (0.0625µl mL^{-1}) (13.90±2.32% for *Alternaria* sp and 03.10±0.67% for *A. brasiliensis*).

Table 2: Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) values in µl / mL air.

	SEO		LEO	
Strains	**MIC**	**MFC**	**MIC**	**MFC**
Alternaria sp.	0.25	1	0.25	0.5
P. expansum	0.5	1	0.5	1
F. oxysporumalbedinis	0.5	1	0.25	0.5
A. brasiliensis (ATCC 16404)	0.5	1	0.25	0.5
R. stolonifer	0.5	1	0.125	0.5

Using VA assay, the results showed that the activity of the vapor of the *Foeniculum vulgare* essential oil was more pronounced for all strains tested (Figures3 and 4). The mycelium growth was totally inhibited (100±0.00%) at 0.25µl mL^{-1} air by LEO for *Alternaria sp.*, *A. brasiliensis* and *F. oxysporum albedinis*. Moreover, the mycelium growth of *P. Expansum* and was only partially inhibited at the same concentration for LEO. Conversely, *R. stolonifer* had a high sensitivity to the LEO with the CMI was 1.125 µl mL^{-1} air.

It is interesting to know the fungitoxic nature of this vapor oil against all fungal strains tested. Indeed, the transfer of mycelial discs where growth inhibition was complete by *Foeniculum vulgare* vapor into the PDA medium without this oil.

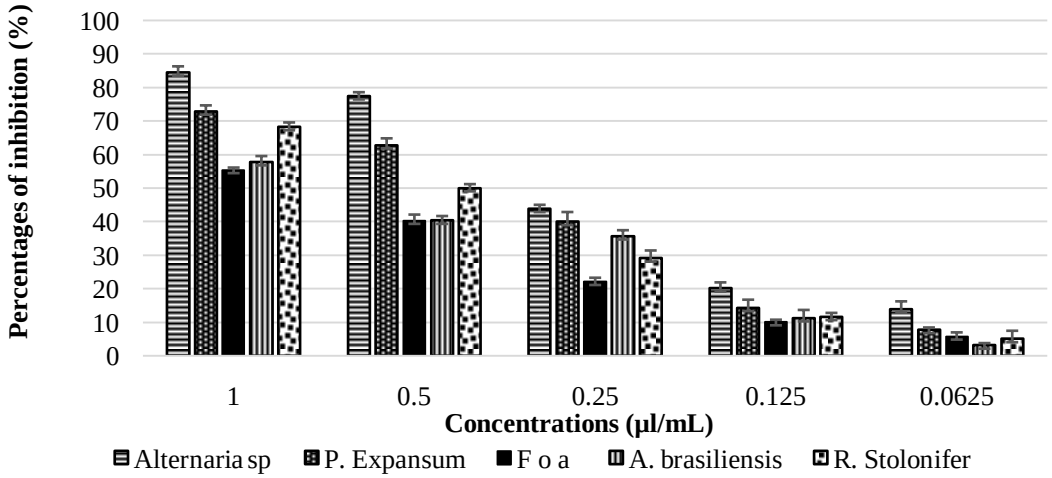

Fig. 1: The effect of different concentrations of *Foeniculum vulgare*SEO using a PF technique against the mycelial growth.

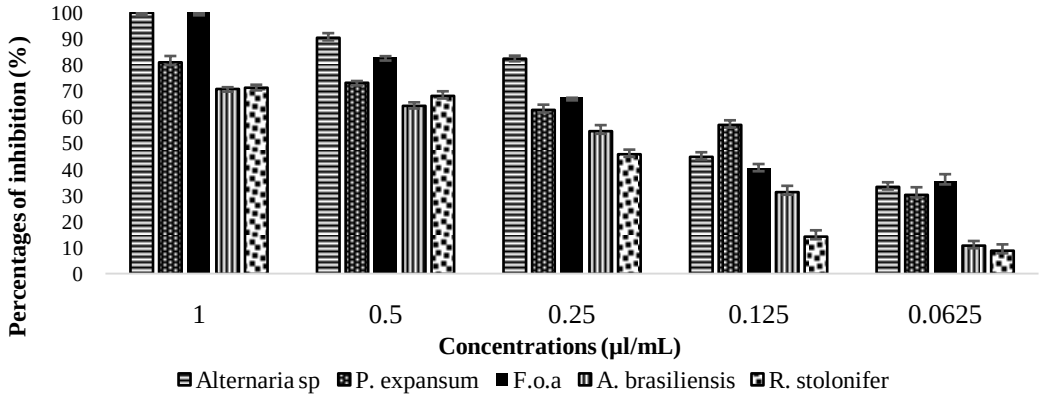

Fig. 2: The effect of different concentrations of *Foeniculum vulgare*LEO using a PF technique against the mycelial growth.

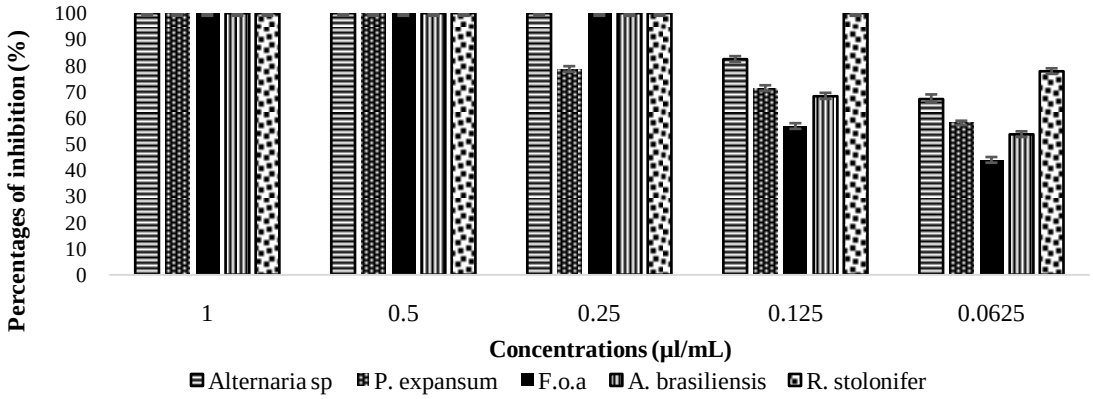

Fig. 3: The effect of different concentrations of *Foeniculum vulgare*LEO using a VA assay against the mycelia.

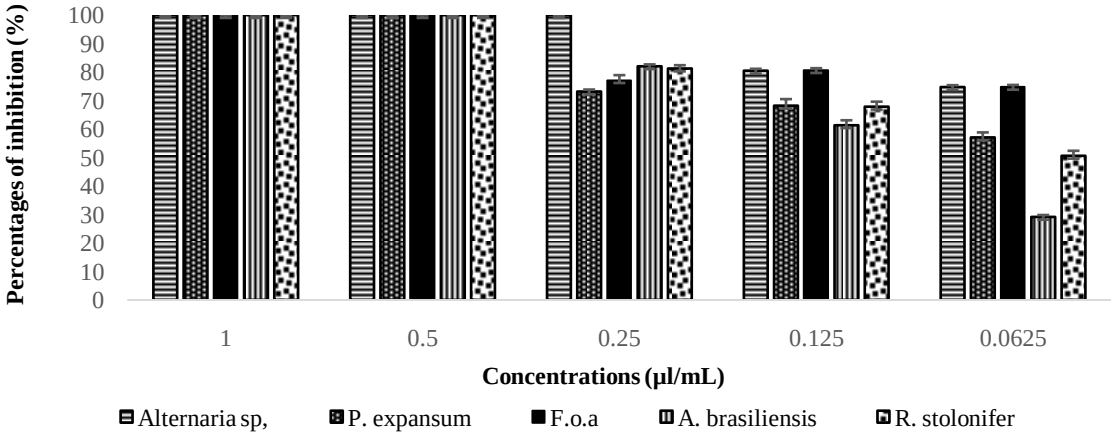

Fig. 4: The effect of different concentrations of *Foeniculum vulgare*SEO using a VA assay against the mycelia.

The results (Table 2) showed no growth over an incubation period, suggesting the fungicidal effect of the LEO on all strains tested at 0.5µl mL^{-1} air, except *P. expansum* with the MFC was 1 µl mL^{-1} air. On the contrary, at same concentration the mycelial growth after some days of incubation was observed, indicating a fungistatic effect for SEO on all strains. The antifungal activity of *Foeniculum vulgare* is probably related to the high content of trans anethole (50.6% from LEO and 54.67% from SEO). The importance of this compound was demonstrated by (Singh *et al.*, 2006) which reported that trans-anethole had strong antifungal activity against various fungi. According to the results obtained in this study, vapor from *Foeniculum vulgare* oil has better antifungal activity against the pathogens tested than that observed in the PF technique. Previous antifungal studies have indicated that VA assay is more effective than the PF method. The volatile fractions of *Rosmarinus officinalis* and *Eucalyptus globulus* strongly inhibited the mycelial growth of all fungi tested (Surviliené *et al.*, 2009).A vapor activity assay of *Citrus sinensis*

essential oil showed that the oil was fungicidal for *Penicillium expansum*, *Ulocladium chartarum* and *Alternaria mali* from apples (Sharma and Tripathi, 2006).

CONCLUSIONS

Concluding these results, we can say that *F. vulgare* volatile oil, which is rich in trans-anethole (54.67% in seed oil and 50.60% in leaf oil) and estragol (35.33% in seed oil and 30.15% in leaf oil), possesses good antifungal activity against *Alternaria sp*, *Pencillium expansum*, *Rhizopus stolonifer*, *Fusarium oxysporum* f. sp. *albedinis*and *Aspergillus brasiliensis* ATCC 16404.

The use of *F. vulgare* oil in vapor phase may be considered as a potential alternative to synthetic fungicides for the phytopathogenic fungi. A further study *in vivo* condition is warranted to confirm the antifungal activity of *F. vulgare* oil, which may be used for preservation and/or extend the shelf life of raw and processed food.

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