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Liquid and vapour-phase antifungal activities of essential oil of *Salvia aucheri* Boiss. var. *mesatlantica* Maire. (endemic from Morocco) against fungi commonly causing deterioration of apple

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ABSTRACT

The essential oils of Salvia aucheri var. mesatlantica, an endemic medicinal plant from Morocco, have been studied using gas chromatography (GC) and GC-mass spectrometry (GC-MS). 38 compounds were identified and the major components were camphor (49.0%), 1,8-cineole (9.5%), viridiflorol (8.8%), camphene (7.8%), α-pinene (2.9%) and p-cymene (1.5%). The antifungal activity of the essential oil was evaluated in vitro by poison food (PF) technique and the volatile activity assay (VA) against three phytopathogenic causing the deterioration for apple such as Alternaria sp., Penicillium expansum and Rhizopus stolonifer. The results indicated that the essential oil of S. aucheri mesatlantica inhibited significatively the mycelial growth of all fungal strains tested ($p<0.05$). VA assay of essential oil was consistently found to be more effective than PF technique. The MIC (minimum inhibitory concentration) was 2 µL/mL air, whereas >2 µL/mL in PF technique for all strains tested. Fungal sporulation was also completely inhibited at 2 µL/mL air for three pathogens. The overall results suggest that S. aucheri mesatlantica essential oil have a potential as antifungal preservatives for the control of postharvest diseases of apple.

Keywords: Antifungal activity, *Salvia aucheri* var. *Mesatlantica*, Essential oil, GC-MS analysis, Apple

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INTRODUCTION

The apple (*Malus domestica*) is the third most cultivated fruit crop in the world (5280 Mha) and the third in production (59 059 Mt) after *Citrus sinensis* (orange) and *Musa paradisiaca* (banana) in 2004, according to Food and Agricultural Organization statistics [1]. However, the quality of apple deteriorates because of postharvest diseases, such as blue mold caused by *Penicillium expansum*, Bull's-eye rot caused by *Alternaria* species and Rhizopus soft rot caused by *Rhizopus stolonifer* [2]. The survival of these pathogenic fungi in food is a serious problem in developing countries which can lead to spoilage and deteriorate the quality of food products. These fungal agents produce also mycotoxins that can be mutagenic, teratogenic, carcinogenic causing feed refusal and emesis in humans or animals [3]. Recently, many researchers have shown that natural sources such as essential oils could develop as a promising alternative to synthetic fungicides because of their low mammalian toxicity, less environmental effects and wide public acceptance [4].

Salvia aucheri, species of *Lamiaceae* family, is a sub-shrub hermaphrodite and small woody herbaceous perennial often purplish with a rod, right from 75 to 100 cm in length. Its leaves are simple and opposite, mostly basal and often grayish green. The flowers, blue or pink, bloomed from August to October. Is a species native to the Mediterranean, growing on rocky slopes, crevices of limestone rocks and mountains. The variety *Salvia aucheri* var.

mesatlantica is a spontaneous shrub and endemic to the middle and high Atlas of Morocco. The leaves and stems of this plant in decoction are used by the local population as herbal tea and against stomach ailments, rheumatism and digestive disorder. GC and GC-MS analysis of essential oils of *Salvia aucheri* variety has been also reported. These studies indicate that camphor and/or 1,8-cineole are the main constituents of *Salvia aucheri* oils [5-11]. Recently, we have reported the first studied of the chemical composition of essential oil of *Salvia aucheri* var. *mesatlantica* [12]. Also, previous reports revealed that the *Salvia aucheri* variety had various biological activities such as antioxidant [10] and antimicrobial and antimycobacterial [11]. However, to our best knowledge, the biological activities of essential oil from *Salvia aucheri* var. *mesatlantica* have never been studied. Therefore, the aim of this paper was to investigate the chemical composition of *Salvia aucheri* var. *mesatlantica* essential oil and to evaluate their antifungal properties against phytopathogens causing severe diseases in apples, such as *Alternaria* sp., *Penicillium expansum* and *Rhizopus stolonifer*.

MATERIALS AND METHODS

Plant material

The aerial parts of *Salvia aucheri* var. *mesatlantica* were harvested in May 2009 in the wild in the mountain Assoul located at the south-east of Errachidia (Morocco) at an altitude of 2000 m. Identification of the species was confirmed by biology unity and voucher specimens were deposited in the herbarium of Faculty of Sciences and Technology of Errachidia (Marocco).

Essential oil isolation

The dried vegetal material (100 g) were water-distillated (3 h) using a Clevenger-type apparatus. The essential oil obtained was dried under anhydrous sodium sulfate and stored at 4°C in the dark before analysis. The essential oils average yield was approx 2.0%.

Gas chromatography (GC) analysis

GC analysis was carried out using a Perkin-ElmerAutosystem XL GC apparatus (Waltham, MA, USA) equipped with a dual flame ionization detection (FID) system and the fused-silica capillary columns (60 m ×0.22 mm I.D., film thickness 0.25 µm) Rtx-1 (polydimethylsiloxane) and Rtx-wax (polyethyleneglycol). The oven temperature was programmed from 60°C to 230°C at 2°C/min and then held isothermally at 230°C for 35 min. Injector and detector temperatures were maintained at 280°C. Samples were injected in the split mode (1/50) using helium as a carrier gas (1 mL/min) and a 0.2 µL injection volume of pure oil.

GC-MS analysis

Samples were analyzed with a Perkin-Elmer turbo mass detector (quadrupole) coupled to a Perkin-Elmer Autosystem XL equipped with the fused-silica capillary columns Rtx-1 and Rtx-wax. Carrier gas: helium (1 mL/min), ion source temperature: 150°C, oven temperature programmed from 60°C to 230°C at 2°C/min and then held isothermally at 230°C (35 min), injector temperature: 280°C, energy ionization: 70 eV, electron ionization mass spectra were acquired over the mass range 35-350Da, split: 1/80, injection volume: 0.2 µL of pure oil.

Components identification

The identification of the components was based on a comparison: (i) between the calculated retention indices on the polar (RI *p*) and apolar (RI *a*) columns with those of pure standard authentic compounds and literature data [13]; and (ii) of the mass spectra with those of our own library of authentic compounds and with those of a commercial library [14]. Relative amounts of individual components were calculated on the basis of their GC peak areas on the two capillary Rtx-1 and Rtx-Wax columns, without FID response factor correction.

Fungal strains isolation

Three fungal isolates causing apples rot: *Alternaria* sp., *Penicillium expansum* and *Rhizopus stolonifer* were isolated directly from rooted apples collected from different rooms in Midelt station (Morocco). All isolated fungal species were transferred to sterilized three replicates 9 cm Petri dishes containing fresh Potato Dextrose agar (PDA) medium in the presence of a quantity of streptomycin to stop the growth of bacteria. The plates were incubated at 25±2°C for 7 days and darkness. The developing fungal colonies were purified and identified up to the species level by microscopic examination through the help of the following references [15]. The isolates collected were maintained on PDA at 4°C.

Antifungal activity assay

The antifungal activity of the essential oil of *Salvia aucheri* var. *mesatlantica* against mycelial growth of fungi isolated was undertaken using poisoned food technique (PF) [16] and volatile activity assay (VA) [17] with some modifications.

In PF, the essential oil were dispersed as an emulsion in sterile agar suspension (0.2%) 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 0.125 to 2 µL/mL. 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., and *Penicillium expansum* at 25±2°C.

In VA assay, the Petri dishes (90×20 mm) 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: 0.125, 0.25, 0.5, 1 and 2 µL/mL air are deposited on the inverted lid and incubated for 3 days for *Rhizopus stolonifer* and 6 days for *Alternaria* sp., and *Penicillium expansum* at 25±2°C. For each corresponding control equal amount of water was poured on the sterilized paper filter.

In both types of experiments, three replicate plates were inoculated for each treatment (fungi/amount) and the experiment was conducted three times and the mycelial growth was followed by measuring the diameter following two perpendicular lines passing by the centre of the dish. Fungitoxicity of essential oil was expressed in terms of percentage of mycelial growth inhibition (I %) and calculated following the formula of Pandey et *al*. [18].

$$I(\%) = \frac{D_t - D_i}{D_t} x100$$

where D_t and D_i is represent mycelial growth diameter in control and treated Petri plates, respectively.

The measurements were used to determine the Minimum Inhibitory Concentration (MIC) (lowest concentration of the essential oil that will inhibit the visible growth of a microorganism after overnight incubation) and The EC50 values (concentration causing 50% inhibition of mycelial growth on control media). The EC50 value was calculated according to the relationship of essential oil concentrations and percentage inhibition of mycelial growth. 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.

Spore production assay

Fungal spore production was tested using the modified method of Tzortzakis and Economakis [19].The spores of the previously exposed colonies by essential oil vapour were collected by adding 5 mL sterile water containing 0.1% Tween-20 to each Petri dishes and rubbing the surface three times with the sterile L-shaped spreader to free spores. The spore suspensions obtained were filtered through sterilize cheesecloth into a sterile 50 mL glass beaker and homogenized by manual shaking. Spore concentration was estimated using a haemocytometer slide (depth 0.1 mm, 0.0025 mm^2). The percentage reduction of spore production was computed by the following equation:

$$I(\%) = \frac{N_t - N_i}{N_t} x100$$

where N_t and N_i represent the number of spore in control and treated Petri plates, respectively.

Data analysis

The inhibitory effect of essential oil on mycelial growth was analyzed by an analysis of variance (ANOVA). Mean and standard error of data were calculated using SAS software (SAS for Windows. version 9.0). The separation of means was done by using the Least Significant Difference (LSD) test at $p<0.05$.

RESULTS

Essential oil composition

The analysis of essential oil composition of *S. aucheri* var. *mesatlantica* was carried out using GC and GC-MS according to the methodology described in experimental part. Thus, 38 components accounting for 95.4% of the total oil were identified by comparison of their EI-mass spectra and their retention indices with those of our own authentic compound library (Table 1).

Table 1 Chemical composition of essential oil from *S. aucheri* var. *mesatlnatica* from Morocco [12]

N°[a]	Components	RI a[b]	RI p[c]	%[d]
1	Tricyclene	921	995	0.3
2	α-Pinene	930	1007	2.9
3	Camphene	943	1046	7.8
4	β-Pinene	967	1088	1.2
5	Myrcene	976	1132	0.2
6	p-Cymene	1007	1229	1.5
7	1,8-Cineol*	1016	1183	9.5
8	Limonene*	1016	1167	1.9
9	Camphenilone	1051	1407	0.2
10	Linalool	1078	1498	0.2
11	α-Campholenal	1096	1436	0.1
12	Camphor*	1119	1467	49.8
13	*trans*-Pinocarveol*	1119	1599	1.0
14	*cis*-Verbenol	1122	1626	0.4
15	Pinocarvone	1132	1511	0.4
16	Borneol	1143	1646	1.7
17	p-Cymen-8-ol	1154	1789	0.5
18	Terpinen-4-ol	1155	1551	0.4
19	Myrtenal	1163	1570	0.5
20	α-Terpineol	1166	1643	0.4
21	Myrtenol	1174	1734	0.3
22	trans-Carveol	1192	1777	0.3
23	Carvone	1210	1673	0.2
24	Bornyl acetate	1265	1529	1.0
25	Carvacrol	1275	2135	0.5
26	α-Terpinyl acetate	1329	1643	0.4
27	Geranyl acetate	1358	1706	0.1
28	γ-Cadinene	1506	1706	0.3
29	*trans*-Calamenene	1509	1777	0.1
30	Caryophyllene oxyde	1569	1919	0.3
31	Globulol	1575	1994	0.2
32	Viridiflorol	1584	2021	8.8
33	Epoxyde d'Humulene II	1594	1973	0.3
34	Caryophylla-4(14),8(15)-dien-5α-ol	1621	2220	0.3
35	τ-Cadinol	1627	2102	0.5
36	β-Eudesmol	1636	2157	0.3
37	α-Cadinol	1640	2161	0.3
38	Cadalene	1657	2140	0.3
	Total identified			**95.40**
	Monoterpene Hydrocarbons			15.8
	Oxygenated Monoterpenes			67.9
	Sesquiterpene Hydrocarbons			0.7
	Oxygenated Sesquiterpenes			11.0

[a] *Order of elution are given on apolar column (Rtx-1)*
[b] *RI a = retention indices on the apolar column (Rtx-1)*
[c] *RI p = retention indices on the polar column (Rtx-Wax)*
[d] *% = relative percentages of components are given on the apolar column except for components with an asterisk (*) (percentages are given on the polar column)*

The essential oil of *S. aucheri* var. *mesatlantica* from Morocco was dominated by oxygenated monoterpenic compounds (67.9%) with camphor **12** (49.8%) followed by 1,8-cineol **7** (9.5%) as the major components. The aerial parts were also characterized in having a high content of monoterpene hydrocarbons (15.8%) with camphene **3** (7.8%) and α-pinene **2** (2.9%) as main compounds. Eight oxygenated sesquiterpenes and three sesquiterpene hydrocarbons were also identified in essential oil, amounting to 11.0% and 0.7%, respectively. Viridiflorol **32** (8.80%) was the major oxygenated sesquiterpene. Moreover, some other minor components were also detected such as limonene **8** (1.9%), borneol **16** (1.9%), p-cymene **6** (1.5%), β-pinene **4** (1.2%) and bornyl acetate **24** (1.0%) (Fig. 1).

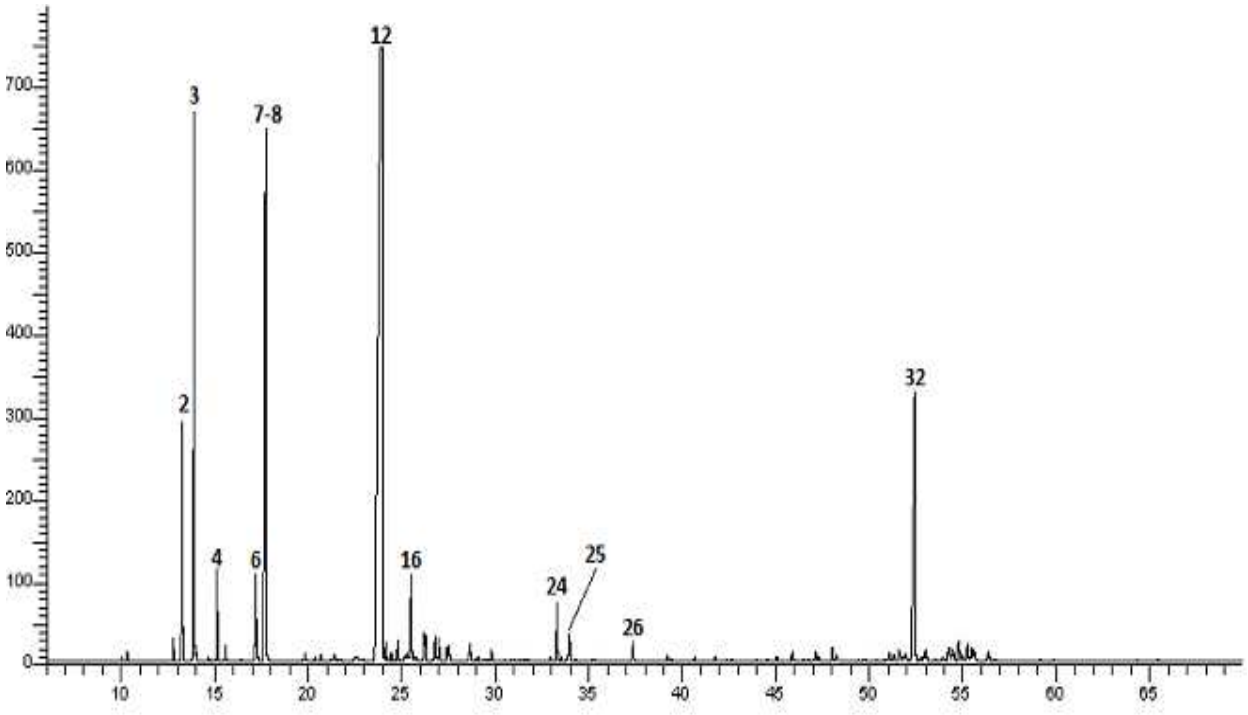

Fig. 1. Chromatogram on apolar column (Rtx-1) of essential oil of aerial parts of *S. aucheri* var. *mesatlantica* from Morocco

In vitro Antifungal activity

The antifungal activity obtained by PF and VA techniques with different concentrations of essential oil of *S. aucheri* var. *mesatlantica* is reported in Tables 2 and 3, respectively. In both techniques, the results indicate that the inhibition of the mycelial growth of each strain was significantly influenced by the essential oil concentration (p<0.05). Also, the percentage inhibition of mycelial growth increased with increasing amounts of *S. aucheri* var. *mesatlantica* oil for all the strains tested suggesting that this essential oil inhibited the growth of all the strains in a dose-dependent manner.

Using PF technique, the results (Tables 2) showed that *Alternaria* sp., was found to be the fungal pathogen susceptible to the *S. aucheri* var. *mesatlantica* essential oil followed by *P. expansum* with the EC_{50} are 0.32 and 0.54 µL/mL, respectively. The percentages of inhibition against both phytopathogens were moderately at 0.125 µL/mL **(**34.13±3.14% and 27.57±1.14%) and relatively effective at 2 µL/mL (83.34±1.67% and 72.60±3.17%), respectively. Conversely, *R. stolonifer* had a high resistance to the essential oil with the EC_{50} was 1.29 µL/mL. The percentage of inhibition was low at small concentrations (06.24±1.11% at 0.125 µL/mL and 13.90±0.64% at 0.25 µL/mL) whereas the action of the oil was moderate (58.44±3.26%) from 2 µL/mL. These results obtained indicate that the MIC against all strains was > 2 µL/mL.

Table 2 The effect of different concentrations of *S. aucheri* var. *mesatlantica* essential oil using PF technique against the mycelial growth of *Alternaria* sp., *P. expansum* and *R. stolonifer*

Strain	*Alternaria sp.,*	*P. expansum*	*R. stolonifer*
Incubation time	*6 days*	*6 days*	*3 days*
Concentrations (µL/mL)	*25±2°C*	*25±2°C*	*25±2°C*
2	83.34±1.67[1. a]	72.60±3.17[1. b]	58.44±3.26[1. c]
1	72.04±2.74[2. a]	60.50±0.72[2. b]	48.70±1.20[2. c]
0.5	56.70±1.51[3. a]	47.21±0.[653]. b	26.30±1.91[3. c]
0.25	44.00±5.59[4. a]	35.38±1.29[4. b]	13.90±0.64[4. c]
0.125	34.13±3.14[5. a]	27.57±1.14[5. b]	6.24±1.11[5. c]

Mean values (± standard deviation) followed by different numbers and letters in each row (line and column) indicate significant differences (p<0.05) by least significant difference test (LSD)

Using VA assay, the results (Tables 3) showed that the activity of the vapour of the *S. aucheri* var. *mesatlantica* essential oil was more pronounced for all strains tested with the EC_{50} are 0.25 µL/mL air for *Alternaria* sp., 0.35 µL/mL air for *P. expansum* and 0.44 µL/mL air for *R. stolonifer*, and where their mycelium growth was totally

inhibited (100.00±0.00%) at 2 µL/mL air indicating that this latter was the MIC of *S. aucheri* var. *mesatlantica* oil against all fungus tested. Moreover, the mycelium growth of the same microorganisms was only partially inhibited at other concentration used in the tests. In fact, the mycelial growth inhibition ranged from 75.87±2.20 to 19.63±1.89% by application of oil vapour at the concentration range of 1 to 0.125 µL/mL air.

Table 3 The effect of different concentrations of *S. aucheri* var. *mesatlantica* essential oil vapour using VA assay against the mycelial growth of *Alternaria* sp., *P. expansum* and *R. stolonifer*

Strain	*Alternaria sp.,*	*P. expansum*	*R. stolonifer*
Incubation time	*6 days*	*6 days*	*3 days*
Concentrations (µL/mL air)	*25±2°C*	*25±2°C*	*25±2°C*
2	100.00±0.00$^{1.\ a}$	100.00±0.00$^{1.\ a}$	100.00±0.00$^{1.\ a}$
1	75.87±2.20$^{2.\ a}$	72.96±3.15$^{2.\ ab}$	69.11±1.06$^{2.\ b}$
0.5	59.74±2.54$^{3.\ a}$	52.74±1.40$^{3.\ b}$	48.85±1.00$^{3.\ b}$
0.25	48.19±1.41$^{4.\ a}$	37.02±1.41$^{4.\ b}$	29.04±1.24$^{4.\ c}$
0.125	38.55±2.90$^{5.\ a}$	31.81±1.74$^{5.\ b}$	19.63±1.89$^{5.\ c}$

Mean values (± standard deviation) followed by different numbers and letters in each row (line and column) indicate significant differences ($p<0.05$) by least significant difference test (LSD)

Therefore, it is important to know the fungitoxic nature of this vapour oil at 2 µL/mL air against all fungal strains tested. Indeed, the transfer of mycelial discs where growth inhibition was complete by *S. aucheri* var. *mesatlantica* vapour into PDA medium without this oil, showed mycelial growth after some days of incubation, indicating a fungistatic effect for this vapour oil on all strains tested at 2 µL/mL air concentration.

The both experimental results indicated that *R. stolonifer* was found to be most resistant strain among the pathogens tested, while *Alternaria* sp., was the most sensitive. It is important to note also that the inhibitory effect of the VA assay of *S. aucheri* var. *mesatlantica* oil on mycelial growth (Table 2) was greater than the inhibitory effect exerted during PF technique (Table 3). In this latter assay of the essential oil, relatively higher concentrations were required to inhibit mycelial growth.

Spore production assay

The effects of the *S. aucheri* var. *mesatlantica* essential oil on spore production of each fungi are shown tested in Fig. 2 and Fig 3. The results showed that spore production was significantly ($p<0.05$) inhibited by different concentrations of essential oil. As observed in mycelial growth inhibition experiments, the VA assay of essential oil vapour was found to be more effective on spore production inhibition than the PF technique with complete inhibition was observed at 2 µL/mL air. However, essential oil also exhibited a moderate to potent inhibitory effect on spore production of *Alternaria* sp., *P. expansum* and *R. stolonifer* in the range of 15.48-87.84% at concentration ranging from 0.125 to 2 µL/mL by PF technique.

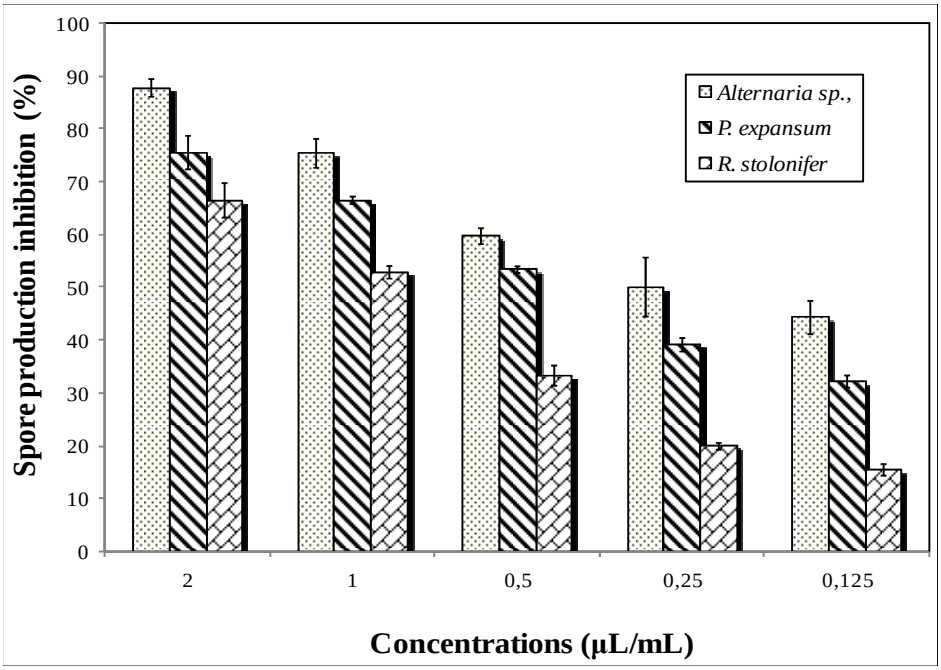

Fig. 2. Effects of different concentrations of *S. aucheri* var. *mesatlantica* essential oil on the spore production of the three fungal strains
Values are means (n=3) ± standard deviations.

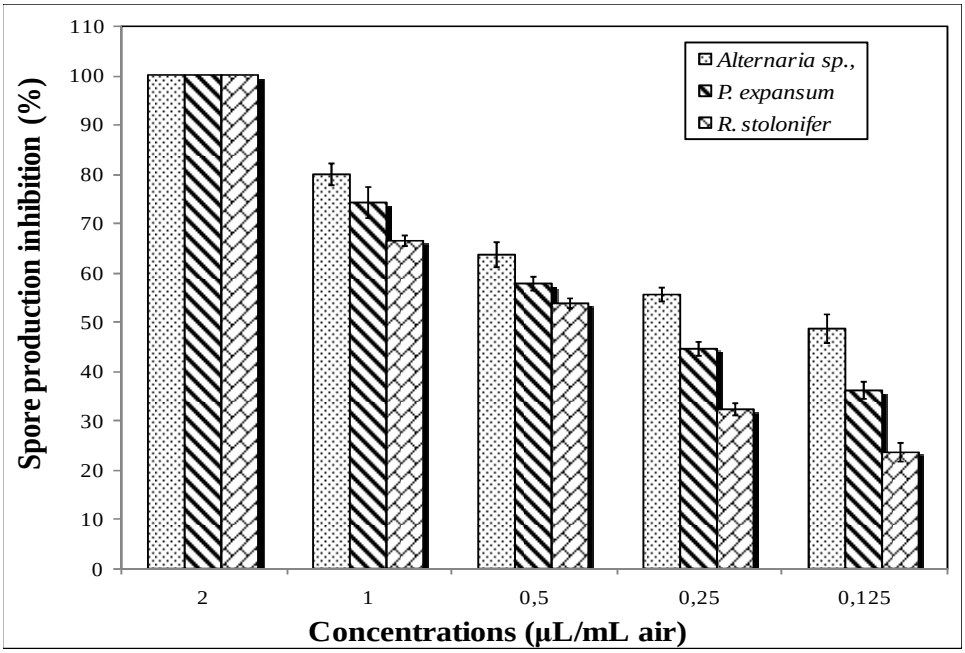

Fig. 3. Effects of different concentrations of *S. aucheri* var. *mesatlantica* essential oil vapour on the spore production of the three fungal strains

Values are means (n=3) ± standard deviations.

DISCUSSION

According to the GC/MS analysis in this study, thirty eight compounds, accounting for 95.40% of the total oil, were identified. Compared with the chemical composition of the essential oils isolated from the some varieties of *Salvia aucheri* (cited previously), our results show a quasi-similarity but our sample is clearly distinguished by the presence of camphor with a fairly high amount (49.8%).

Recently, interests have been generated in the development of essential oils to control phytopathogens in food and agriculture. Most of the essential oils have been reported to inhibit postharvest fungi *in vitro* conditions (12,20). Several researchers have reported that the mono- and sesquiterpenes, as the major components of various essential oils, have enormous potential for strongly inhibiting the growth of microbial pathogens. The antifungal activity of these compounds may be attributed to their interference with certain enzymatic reactions evolved in the cell wall synthesis. Indeed, the chemical composition and antifungal activity relationships deduced from our study demonstrates that the antifungal activity of *S aucheri* var. *mesatlantica* is probably related to the high content of camphor (49.8%). The importance of this compound was demonstrated by Kordali et *al*. (21) which reported that camphor had weak antifungal activity against the fungi tested in the present study. In the other hand, the essential oils of various aromatic plants with high relative proportion of camphor showed antifungal activities against various pathogens. Indeed, the essential oils of *Salvia hydrangea* [22], *Chrysanthemum coronarium* [23], *Lavender* oil [17] and *Artemisia sieberi* [24] exhibited considerable antifungal properties against a broad spectrum of fungi. It is also possible that other components such as 1,8-cineole could be contributed to the antifungal activity of the *S. aucheri* var. *mesatlantica* oil [25].

Moreover, According to the results obtained in this study, VA method in disc volatilization has better antifungal activity against the pathogens tested than that in liquid phase observed in the PF assay. This result indicates that the substances in the well diffusion method were less efficient than that in the disc volatilization method. The efficacy of essential oils in vapour state was probably attributable to the direct deposition of essential oils on lipophilic fungal mycelia together with an indirect effect via adsorption through the agar medium [26]. These results are in agreement with those reported in the literature [26,27]. The essential oils are complex mixtures of many different aromatic components with various degrees of lipophilicity and relative hydrophilicity given by the presence of constituents with polar functional groups [28]. When added to a medium, the essential oils distributes more or less into the aqueous phase depending on its relative hydrophilicity. Hence, an essential oils with constituents with low water solubility should dissolve little in aqueous medium, and consequently should show a weak activity. These essential oils, however, showed very good activity when assayed by VA and this might related to their high

volatility [26]. These observations suggest that the physical and chemical properties (solubility and volatility) can have considerable effect on the in vitro antimicrobial activity [29].

The inhibitory effect of the essential oil against sporulation of different fungi has been previously reported [19]. As shown in Fig. 2 and Fig. 3, the percentage of spore production inhibition of *Alternaria* sp., was visibly higher than that of other tested fungi, this can be attributed to greater sensitive of *Alternaria* sp than the other fungi to the essential oil. It has been observed that this essential oil vapour used in this study is more effective on spore production than mycelial growth. This result might reflect the effects of volatile components produced by oil on surface mycelial development and/or perception/transduction of signals involved in the switch from vegetative to reproductive development [19]. In addition, the partial inhibition of spore production could be attributed to mycelial destruction or inhibition of fungal growth [30].

CONCLUSION

In conclusion, thirty eight compounds, accounting for 95.4% of the total of *S. aucheri* var. *mesatlantica* essential oil, were identified by comparison of their EI-mass spectra and their retention indices with those of our own authentic compound library. This essential oil was mainly dominated by camphor (49.8%) and was found to be an effective antifungal against mycelial growth and spore production of *Alternaria* sp., *P. expansum*, and *R. stolonifer*. The results indicated that this essential oil inhibited significatively the mycelial growth of all fungal strains tested ($p<0.05$). The minimum inhibitory concentration (MIC) was 2 µL/mL air in VA assay, whereas >2 µL/mL in PF technique for all strains. In the both experimental results indicated that *R. stolonifer* was found to be most resistant strain among the pathogens tested, while *Alternaria* sp., was the most sensitive.

The use of *S. aucheri* var. *mesatlantica* oil in vapour phase could have additional advantages such as efficacy without requiring direct contact resulting in ease of application and no alteration in organoleptic properties of the edible material/food. With respect to above-mentioned data, our findings demonstrate that *S. aucheri* var. *mesatlantica* oil vapour may be considered as a potential agent for preventing microbial mediated food spoilage. A further study in vivo condition is warranted to confirm the antifungal activity of *S. aucheri* var. *mesatlantica*, which may be used for preservation and/or extension the shelf life of raw and processed food.

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