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Chemical profile and antifungal potential of essential oils from leaves and flowers of *Salvia algeriensis* (Desf.): A comparative study

Fatiha Medjahed[1], Abdelaziz Merouane[2*], Abdelkader Saadi[1], Ammar Bader[3], Pier Luigi Cioni[4], and Guido Flamini[4]

ABSTRACT

Salvia is a plant genus widely used in folk medicine in the Mediterranean area since antiquity. A large number of *Salvia* essential oils have been reported against diverse microorganisms. In the current study, chemical composition of essential oils from leaves and flowers of *Salvia algeriensis* (Desf.) was determined using gas chromatography-electron impact mass spectrometry (GC-EIMS) as well as their antifungal activity against phytopathogenic fungi *Alternaria solani* and *Fusarium oxysporum* exploring disk method. The GC-EIMS analysis identified 59 compounds (84.8%) in the essential oil obtained from leaves of *S. algeriensis*. Its major constituents were benzaldehyde (9.7%), eugenol (8.7%) and phenylethyl alcohol (8.4%). In flowers oil, 34 compounds (92.8%) were detected. The main ones were viridiflorol (71.1%) and globulol (8.6%). The essential oil obtained from leaves exhibited the highest antifungal activity, where the effective dose inhibiting 50% of mycelial fungal (ED_{50}) against *A. solani* was 0.90 µL mL^{-1} with minimum inhibitory concentration (MIC) equal to 2 µL mL^{-1}, whereas the ED_{50} and MIC in *F. oxysporum* culture was 1.84 µL mL^{-1} and 3 µL mL^{-1} respectively. The mycelial inhibition by flowers oil varies from 1.77 µL mL^{-1} (ED_{50}) with *A. solani* culture (MIC 6.5 µL mL^{-1}) to the lowest effect recorded (ED_{50} 3.00 µL mL^{-1} and MIC 9.33 µL mL^{-1}) against *F. oxysporum*. To our best knowledge, this is the first report on *S. algeriensis*, their leaves oil can constitute an alternative biocontrol against phytopathogenic fungi commonly controlled by chemical fungicides.

Key words: *Alternaria solani*, *Fusarium oxysporum*, GC-EIMS, natural fungicide.

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INTRODUCTION

Plants belongings *Lamiaceae* family (Mint family) includes herbaceous plants and shrubs, annual or multiannual, of 264 genera and 6990 species (Gurcharan, 2010). *Salvia* is one of the largest and fascinate genus of *Lamiaceae* family, which includes approximately 900 species throughout the world (Kivrak et al., 2009). Different *Salvia* species are used in food as spice and in cosmetic, perfumery and pharmaceutical industries for its powerful and camphoraceous odor (Bagci and Kokak, 2008). Some members of this genus have been used by ancient civilizations to perfume bodies and temples (Arslan and Celik, 2008).

Particular interest has been shown in the members of the genus *Salvia* due to a wide range of biological activities such as antifungal (Fraternale et al., 2005; Dellavalle et al., 2011), antibacterial, antioxidant (Kivrak et al., 2009; Ben Farhat et al., 2013; Babak Bahadori et al., 2015), antitumor (Russo et al., 2013), antiviral and cytotoxic activities (Babak Bahadori et al., 2015); furthermore, it seems to be useful for the treatment of cardiovascular and brain diseases, arthritis, diabetes, cancer and immune system decline consequently to their richness in bioactive molecules (Sepahvand et al., 2014).

The antifungal activities of essential oils against human and/or animal pathogens fungi have been widely reported (Dulger and Hacioglu, 2008; Taran et al., 2011). Unfortunately, there is a little evidence on their antifungal action against phytopathogenic fungi, which can cause alterations during different developmental stages of plants.

The *Alternaria* species are cosmopolitan and can survive as saprophytes as well as pathogens parasites, most of them are considered as secondary invaders with the ability to cause pre- and postharvest damage of the plant fruits or kernels by producing a mixture of potential mycotoxins (Logrieco et al., 2009). *Alternaria solani*, agent of early blight, is considered as one of the most devastator fungi of potato together *A. alternata* (Leiminger et al., 2014).

Fusarium oxysporum is a genetically and phenotypically complex species with ability of living as pathogen on more than 120 agriculturally and horticulturally important plants (Michielse and Rep, 2009), their mycotoxins constitute an immense contaminant in different agricultural sectors (Antonissen et al., 2014). The international community of fungal pathologists, in a recent survey, classed *Fusarium oxysporum* the fifth from top 10 fungal plant pathogens based on scientific and economic importance (Dean et al., 2012).

Alternaria and *Fusarium* genus belong genera producing mycotoxins (Mata et al., 2015). One of the most effective measures to control their diseases is the use of synthetic fungicides known by their negative effects on health and environment (Ramaiah and Garampalli, 2015). Thus, scientific community and agro-industry are directed towards biocontrols particularly herbal extracts in order to satisfy the increase demand, pathogens resistant and requirements on the application of agrochemicals and environment protection. The use of plant extracts is being encouraged because of their availability, biodegradability, fewer side effects and less toxicity.

Alternaria solani and *Fusarium oxysporum* are the commonest pathogens of potato fields in the region of Chlef (northwestern of Algeria). Farmers apply massive quantities of fungicides to control or prevent their cultures. As a part of research of biocontrols resolving or minimizing this serious problem, our study was invested on *Salvia algeriensis* (Desf.) as local bioresource, this species is an annual sage native to northwest Algeria, growing up to 600 m. It is about 1 m in height, with bright green 8 × 8 cm oval leaves; each plant produces three or four inflorescences with pale violet flowers having violet specks on the lower lip; the plant has a light scent when crushed (Surhone et al., 2010). To the best of our knowledge, no previous scientific studies have been published on *S. algeriensis* (Desf.)

Therefore, the aim of this investigation is to determine chemical composition of essential oils obtained from leaves and flowers of *Salvia algeriensis* (Desf.) and to test their antifungal activity against *Alternaria solani* and *Fusarium oxysporum* considered as the commonest fungal pathogens of potato culture in the region of Chlef, northwestern Algeria.

MATERIALS AND METHODS

Plant material

The aerial parts (leaves and flowers) of *S. algeriensis* were collected in Sobha (36°38'59" N, 01°08'28" E, 134 m a.s.l.), within the region of Chlef, northwestern Algeria. This species was identified by Pr. Saadi Abdelkader at the Department of Biology, University of Hassiba Benbouali, Chlef (UHBC). Leaves and flowers were separately air dried in the shadow at room temperature (20-25 °C), and the essential oils were isolated by steam distillation. The oils were dried over anhydrous sodium sulfate and then stored in glass vials covered with aluminum foil at 4 °C until use.

Determination of oil yield

The essential oil yield (w/w) was calculated in percent according to the following formula:

$$\text{Yield } (\%) = (W1 \times 100)/W2$$

where *W1* is the weight of the essential oil and *W2* is the weight of the plant part extracted.

GC analysis

The GC analyses were performed with a gas chromatograph (HP-5890 Series II, Hewlett-Packard Company, Wilmington, Delaware, USA) equipped with DB-WAX and DB-5 capillary columns (30 m × 0.25 mm, 0.25 µm film thickness), working with the following temperature program: 60 °C to 240 °C at 3 °C min^{-1}; injector and detector temperatures 220 °C; carrier gas nitrogen (2 mL min^{-1}); detector dual FID; split ratio 1:30; injection of 0.5 µL of 10% hexane solution. The identification of the components was performed, for both columns, by comparison of their retention times with those of pure authentic samples and by mean of their linear retention indices (LRI) relative to the series of *n*-hydrocarbons.

GC–EIMS analysis

Gas chromatography-electron impact mass spectrometry (GC-EIMS) analyses were performed with a gas-chromatograph (CP-3800, Varian Inc., Walnut Creek, California, USA) equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220 and 240 °C, respectively; oven temperature programmed from 60 °C to 240 °C at 3 °C min^{-1}; carrier gas helium at 1 mL min^{-1}; injection of 0.2 µL (10% hexane solution); split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and home-made library mass spectra (MS) built up from pure substances and components of known oils and MS literature data (Adams, 1995). Moreover, the molecular weights of all the identified substances were confirmed by GC-chemical ionization mass spectrometry (CIMS), using MeOH as ionizing gas.

Strains and growth conditions

Fungal isolates of *A. solani* and *F. oxysporum* tested in our study were previously isolated from potato culture ('Spunta', The Netherlands) none treated with pesticides. *Fusarium oxysporum* was authenticated at the laboratory of phytopathology of the Institute of Agricultural Sciences, UHBC, whereas the authentication of *A. solani* was guaranteed at regional section of the Institut National de Protection des Végétaux (INPV, Algeria). The fungal strains were cultured and maintained on potato dextrose agar medium (PDA) at 27 ± 2 °C.

Determination of antifungal activity

Antifungal activity of essential oils was tested using the modified method of Tian et al. (2011). Aliquots of essential oils dissolved separately in 0.5 mL of 5% (v/v) Tween 80 were added aseptically onto petri dishes (ø: 9 cm) containing 9.5 mL liquid PDA medium culture at 45-50 °C in order to

obtain different concentrations (1, 2, 3, and 5 µL mL^{-1}). A fungal disk (5 mm) of mycelium obtained from fresh fungal culture (120 h at 27 ± 2 °C) was placed in the center of agar surface. Plates containing PDA without essential oil and receiving fungal disk were used as negative control. All plates were incubated for 8 d at 27 ± 2 °C. All experiments were carried out in triplicate. Antifungal activity was expressed as percentage of mycelial inhibition, compared with the negative control considered as 100% growth, via the following formula:

Percentage of mycelial inhibition = [(dc – dt)/dc] × 100

where *dc* is the mean mycelial growth diameter for the negative control and *dt* is the mean mycelial growth diameter for the test oil. The minimum inhibitory concentration (MIC) was determined as the lowest concentration that completely inhibited the growth of the fungus. Supplement concentrations were tested if necessary.

Statistical analysis

All tests were carried out in triplicate and results were presented as mean value ± standard deviation of three replicates. Results of mycelial inhibition were subjected to one-way ANOVA, and means comparisons were made using Duncan's multiple range tests. The effective dose for 50% (ED_{50}) and 95% (ED_{95}) inhibition was calculated using probit analysis. The statistical analysis was accomplished using SPSS 16.0 software for Windows (SPSS Inc., Chicago, Illinois, USA). Differences at $P \leq 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Oils yield and composition of essential oils

The steam distillation extraction afforded a yellow oil from leaves where yield is 0.83% w/w, and a pale yellow one from flowers (1.53% w/w), both with a very strong and persistent sage aroma. A wide range of essential oil yields among *Salvia* species show their dependence on the extraction method and plant parts distilled. According to Kivrak et al. (2009), the yield of *S. potetillifolia* was 0.55% (w/w) using steam distillation while the hydrodistillation method furnished 0.88% (w/w).

The composition of oils is given in Table 1. The components are listed in order of their retention indices on the HP-5MS column. GC-EIMS analysis permitted to identify 59 (84.80%) and 34 (92.80%) compounds in leaves and flowers oils, respectively. The major constituents in leaves oil were benzaldehyde (9.7%), eugenol (8.9%) and phenylethyl alcohol (8.4%); in the case of flowers oil, they were viridiflorol (71.1%), globulol (8.6%), and α-cadinene (2.6%). Flowers oil was found to be richer in oxygenated sesquiterpenes than leaves oil (82.8% *vs*.11.3%) (Table 1). On the other hand, non-terpene constituents represent the main fraction (44.6%) of the oil obtained from the leaves compared to flowers oil (3.3%).

To the best of our knowledge, there are many reports on the chemical composition of the essential oils isolated from

Table 1. GC-EIMS of essential oils of *Salvia algeriensis* (Desf.)

Constituents	LRI	L (%)	F (%)
(*E*)-3-Hexen-1-ol	851	3.0	-
(*E*)-2-Hexen-1-ol	866	0.4	-
(*Z*)-4-Heptenal	900	tr	-
Heptanal	902	0.1	-
2-Acetyl furan	912	tr	-
2,5-Dimethyl pyrazine	912	tr	-
Benzaldehyde	963	**9.7**	-
5-Methylfurfural	964	tr	-
1-Heptanol	970	tr	-
endo-2-Norborneol	981	1.9	-
6-Methyl-5-hepten-2-one	986	tr	-
trans-Dehydroxy-linalool oxide	991	0.1	-
octanal	1002	0.7	-
cis-Dehydroxy-linalool oxide	1011	0.9	-
β-Phellandrene	1032	0.7	-
Benzyl alcohol	1034	1.6	-
Phenylacetaldehyde	1044	0.6	-
2-Acetyl pyrrole	1061	0.6	-
Acetophenone	1066	0.3	-
1-Octanol	1071	2.6	-
Fenchone	1089	-	0.3
ortho-Guaiacol	1089	0.6	-
Linalool	1101	1.5	-
Nonanal	1104	**6.5**	0.3
Phenylethyl alcohol	1111	**8.4**	-
Isophorone	1120	0.4	-
4-Ketoisophorone	1144	0.9	-
(*E,Z*)-2,6-Nonadienal	1156	0.3	-
Ethyl benzoate	1171	0.4	-
2-Phenylethyl formate	1176	1.9	-
4-Terpineol	1178	-	tr
cis-Pinocarveol	1183	0.4	-
p-Methylacetophenone	1184	0.3	-
α-Terpineol	1191	0.3	-
Safranal	1198	3.2	-
n-Dodecane	1200	-	tr
Decanal	1205	0.6	-
β-Cyclocitral	1221	1.9	-
Carvone	1244	tr	-
Indole	1290	0.9	-
2-Undecanone	1293	0.7	-
n-Tridecane	1300	-	tr
Carvacrol	1301	0.6	-
Eugenol	1358	**8.9**	-
(*E*)-β-Damascenone	1383	tr	tr
n-Tetradecane	1400	-	tr
Methyl eugenol	1403	0.3	-
p-Cimen-7-ol acetate	1422	tr	-
(*E*)-α-Ionone	1428	tr	-
Aromadendrene	1441	-	tr
cis-Muurola-3,5-diene	1448	-	0.7
(*E*)-Isoeugenol	1449	0.3	-
(*E*)-Geranylacetone	1455	0.6	-
Alloaromadendrene	1462	-	0.4
α-Acoradiene	1464	2.3	-
γ-Himachalene	1478	2.8	-
γ-Curcumene	1481	-	0.4
(*E*)-β-Ionone	1487	1.6	-
cis-β-Guaiene	1491	-	0.5
trans-β-Guaiene	1499	-	0.4
n-Pentadecane	1500	-	0.4
Silphiperfolan-6-a-ol	1507	0.4	-
δ-Amorphene	1508	-	0.4
δ-Cadinene	1524	tr	0.5
β-Thujaplicinol	1536	1.2	-
α-Cadinene	1538	0.7	2.6
Selina-3,7(11)-diene	1544	-	0.5
Presilphiperfolan-8-ol	1584	**7.4**	-
Globulol	1585	-	**8.6**
Viridiflorol	1591	-	**71.1**
Guaiol	1598	3.5	-
1,10-di-*epi*-Cubenol	1616	-	tr
10-*epi*-γ-Eudesmol	1620	-	tr

Table 1 (continued).

Constituents	LRI	L (%)	F (%)
t-Cadinol	1641	-	tr
3-*iso*-Thujopsanone	1642	-	1.1
α-Cadinol	1655	-	1.5
γ-Dodecalactone	1676	-	0.7
(*E*)-Asarone	1678	0.9	-
Junipercamphor	1693	-	0.5
n-Heptadecane	1700	0.3	tr
n-Octadecane	1800	-	tr
Hexahydrofarnesylacetone	1845	-	tr
n-Nonadecane	1900	-	tr
n-Heneicosane	2100	0.3	tr
n-Docosane	2200	0.3	0.7
n-Tricosane	2300	-	1.2
Monoterpene hydrocarbons		**0.7**	**-**
Oxygenated monoterpenes		**5.7**	**0.3**
Sesquiterpene hydrocarbons		**5.8**	**6.4**
Oxygenated sesquiterpenes		**11.3**	**82.8**
Apocarotenoids		**7.6**	**-**
Phenylpropanoids		**9.1**	**-**
Non-terpene derivatives		**44.6**	**3.3**
Total identified (%)		**84.8**	**92.8**

LRI: Linear retention index; L: leaves essential oil; F: flowers essential oil; tr: trace (≤ 0.1%).
The components are listed in order of their retention indices on HP-5MS column.

various species of *Salvia* genus, some of them indicate that 1,8-cineole (eucalyptol) and borneol are the main and/or characteristic constituents of *Salvia* oils (Sengul et al., 2007; Kivrak et al., 2009). Our findings are not in accordance with these studies. On other hand, the main component of flowers oil, viridiflorol, was reported as major constituent in essential oils obtained from two populations of *S. argentea* L. oil with 18.75% and 26.93% (Ben Farhat et al., 2013).

According to Sepahvand et al. (2014), linalool, trans-caryophyllene and β-trans-ocimene are the main components of essential oil isolated from *S. sclareoides*. Whereas γ-muurolene, α-pinene and γ-cadinene were the main ones in *S. ceratophylla* oil (Gürsoy et al., 2012). Therefore, it is apparent that the essential oil composition of different *Salvia* species is quite variable.

Our results show clear quantitative and qualitative differences between the essential oils obtained from leaves and flowers. Many components characterized only one part, such as eugenol (8.9%), safranal (3.2%) and carvacrol (0.6%) in the leaves or viridiflorol (71.1%), globulol (8.6%) and α-cadinol (1.5%) in the flowers. Only seven compounds out of 86 were shared by the two essential oils. Previous published reports by Mohammadhosseini et al. (2008) and Esmaeili et al. (2008) showed qualitative and quantitative variability in essential oil composition between different parts of *Salvia* species.

Antifungal activity

Essential oils can constitute an important source of new antifungal molecules particularly against phytopathogen fungal that cause enormous damage for agricultural sector. In this *in vitro* investigation, essential oils of *S. algeriensis* exhibited different degrees of antifungal activity against *F. oxysporum* and *A. solani* (Figure 1).

The high effectiveness activity was shown with oil isolated from leaves, where the fungal growth was totally inhibited at 2, 3 and 5 μL mL^{-1} in *A. solani* (MIC 2 μL mL^{-1}) and dishes at 3 and 5 μL mL^{-1} for *F. oxysporum* culture (MIC 3 μL mL^{-1}). The leaves oil confirms its strong action by minimizing the mycelial growth of *A. solani* at more than half (55.39 ± 0.85%) with 1 μL mL^{-1} concentration compared with negative control (oil-free medium) growth attained 68 mm diameter. The rate inhibition of leaves oil against *F. oxysporum* was 27.14 ± 2.86% at 1 μL mL^{-1} and 59.52 ± 2.98% at 2 μL mL^{-1} (Figures 1 and 2). The leaves oil action on *A. solani* was significantly higher than *F. oxysporum* at $P \leq 0.05$.

Figure 1. Growth inhibition of *Fusarium oxysporum* by essential oils of *Salvia algeriensis* (Desf.)

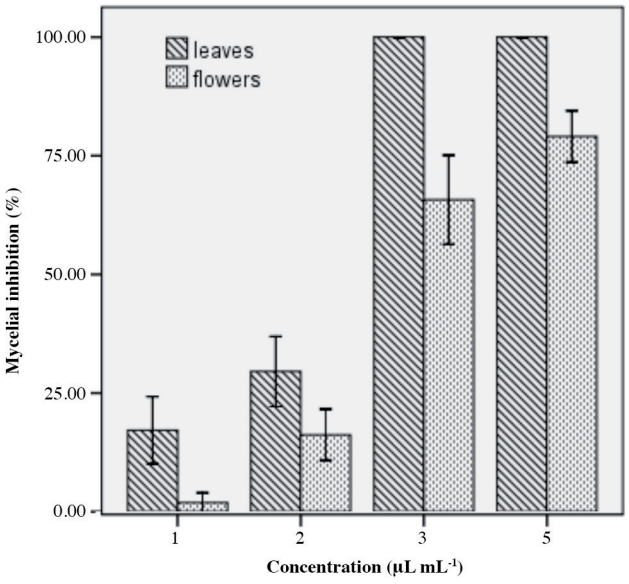
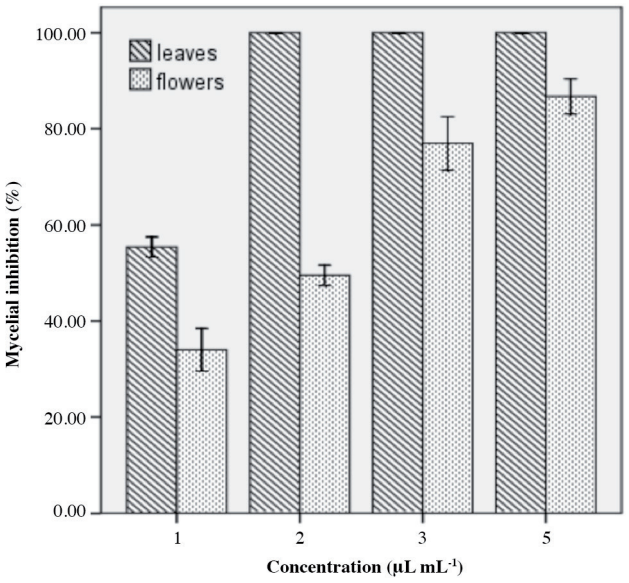


Figure 2. Growth inhibition of *Alternaria solani* by essential oils of *Salvia algeriensis* (Desf.)



After 8 d of incubation, a mycelial inhibition of *A. solani* ranging from 34.02 ± 1.78% to 86.75 ± 1.48% have been observed in the treatment of flowers essential oil at 1 µL mL^{-1} to 5 µL mL^{-1} respectively with MIC equal 6.5 µL mL^{-1} (Figure 2), where the rate inhibition of *F. oxysporum* culture ranges from 69.05 ± 2.18% (5 µL mL^{-1}) to almost inactive (1.91 ± 0.83%) at 1 µL mL^{-1} (Figure 1) with MIC equal to 9.33 µL mL^{-1}. The antifungal potency of flowers oil on both fungal strains was similar at $P \leq 0.05$. On other hand, there was significant difference among efficiency of part distilled (leaves and flowers) of *S. algeriensis*, where leaves oil showed higher action than flowers oil against both phytopathogenic fungi ($P \leq 0.05$).

The comparison between antifungal effects of *S. algeriensis* oils was further confirmed by comparing their effective concentrations for ED_{50} and ED_{95}. The values of ED_{50} and ED_{95} for leaves oil were 0.90 and 1.76 µL mL^{-1} respectively against *A. solani* which were significantly lower than those of flowers oil exhibiting 1.77 µL mL^{-1} (ED_{50}) and 5.97 µL mL^{-1} (ED_{95}).

The results showed that the least effect was observed for flowers oil contra *F. oxysporum* with significantly higher values of ED_{50} (3.00 µL mL^{-1}) and ED_{95} (10.11 µL mL^{-1}) than those of leaves oil recording 1.84 µL mL^{-1} and 3.58 µL mL^{-1} for ED_{50} and ED_{95} respectively.

Previous studies have been reported the antifungal activity of essential oils and other extracts from *Salvia* species. The essential oil isolated from *S. officinalis* showed significant activity against the phytopathogenic fungi *F. oxysporum*, *Alternaria solani*, *Botritys cinerea*, and *Rhizoctonia solani* (Fraternale et al., 2005). According to Alimpić et al. (2015), essential oil extracted from *S. ringens* Sm. exhibited total mycelial inhibition against seven micromycetes: *Aspergillus glaucus*, *A. fumigates* Fresen., *A. flavus* and *Trichophyton mentagrophytes*, *Candida krusei*, *C. albicans* and *C. parapsilosis* at 0.125 to 3 mg mL^{-1}. In another study, acid extracts obtained from *S. officinalis* and *S. sclarea* were showed total inhibition of *A. solani* growth at concentrations as low as 1/40 dilution (Dellavalle et al., 2011).

It has been clear from comparison between antifungal action of tested oils and previous studies that it depends on their chemical components. Considering the difference observed between part of *S. algeriensis*, the superiority of leaves oil might to be due to the fact of presence of some known antifungal constituents in its chemical profile such as carvacrol (Soylu et al., 2006), eugenol and linalool (Böhme et al., 2014). However, the possible synergistic and modulatory functions of other constituents should not been neglected (Davicino et al., 2007).

The antifungal mechanisms of essential oils depend on their constituents and supported by the lipophilic nature facilitating their absorption. In this regard, volatile components such as carvacrol and thymol disintegrate and emptied the cytoplasmic content of fungal hyphae (Soylu et al., 2006). Moreover, phenolic components may interfere with cell wall enzymes like chitin synthase/chitinase (Camele et al., 2012). In addition, antifungal effect can alter cell permeability by penetrating between the fatty acyl chains making up the membrane lipid bilayers, disrupting lipid packing and changing membrane fluidity (Taweechaisupapong et al., 2012).

CONCLUSION

This study represents the characterization of chemical profiling and biological activities of essential oils from *Salvia algeriensis*. The results showed variable antifungal activity against *Alternaria solani* and *Fusarium oxysporum* and indicates that leaves oil can be exploited as a natural fungicide against these commonest pathogens in local potatoes fields.

Finally, we believe that the study of essential oils as biocontrols should be offered high priority due to their less-harmful effects and environmentally friendly, but their use must be supported by *in vivo* tests besides motivation of farmers to use them as alternative treatment.

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