Antioxidant and antifungal activities of essential oils from Algerian spontaneous plants against five strains of *Fusarium* spp.

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Summary The present study evaluates the antioxidant and antifungal effects of essential oils (EOs) from *Thymus vulgaris*, *Thymus algeriensis*, *Mentha piperita*, *Mentha pulegium*, *Artemisia herba-alba* and *Artemisia campestris*, on five strains of *Fusarium*. The chemical composition of EOs of *T. vulgaris*, *T. algeriensis*, *A. herba-alba*, *M. piperita* and *M. pulegium* is characterized by a dominance of the family of oxygenated monoterpenes with 73.85%, 59.41%, 70.01%, 60.01% and 87.2%, respectively. On the other hand, the EO of *A. campestris* showed a diverse composition by similar percentages between all families. The two strains BD17 and INRA 349 were found to be resistant to low concentrations of EOs from *T. algeriensis*, *A. campestris* and *A. herba-alba*, sensitive to EOs of *M. pulegium* and *M. piperita* and very sensitive to *T. vulgaris* EO (0.25 µl/ml). A high antioxidant effect was recorded by *T. vulgaris* EO in BCB assay with an effective concentration (0.5 mg/ml) 3 to 60 times higher compared to the other EOs tested. This antioxidant capacity of *T. vulgaris* EO was also recorded with DPPH assay at an EC₅₀=1.41 mg/ml.

Additional keywords: *Thymus*, *Mentha*, *Artemisia*, antifungal activity, antioxidant activity, *Fusarium*

Introduction

The dangers of intensive use of chemical pesticides in agriculture have forced the search for new natural alternatives based on secondary metabolites of plant origin which have shown significant protective power of crops with no side effects or toxic residues in treated products and the environment by their slow degradation (Kordali *et al.*, 2009; Sumalan *et al.*, 2013; Gordon, 2017). This has led to the emergence of new eco-based products consisting mainly of terpenoids such as DMC Base Natural, Eugenol-Tween®, Protecta, Herbalox® and Pycnogenol® (Prakash and Kiran, 2016). Furthermore, the use of EOs from spontaneous or cultivated plants for their antifungal and antioxidant properties in tests against phytopathogenic agents has been the objective of several studies (Luchesi *et al.*, 2020; Chibane *et al.*, 2020). These natural extracts have shown significant capaci-

ty to fight against spoilage and pathogenic microorganisms which gives them an interest with regard to their possible use as alternatives to the food preservatives currently used (Velluti *et al.*, 2004).

Fusarium wilt is a disease that affects crops before harvest, reducing their quality and yield, while it can also develop after harvest on seeds, causing quality degradation and contamination by mycotoxins dangerous for humans and animals (Gordon, 2017). Several studies have focused on the capacity of some food-grade antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), trihydroxybutyrophenone (THBP) and propylparaben (PP) to inhibit fungal growth and production of mycotoxins in an alternative and corrective strategy, the results of which have showed potential for controlling germination and growth of mycotoxigenic strains of *Fusarium* and *Penicillium* (Etcheverry *et al.*, 2002; Torres *et al.*, 2003). The EOs of six plants (*Melissa officinalis*, *Salvia officinalis*, *Coriandrum sativum*, *Thymus vulgaris*, *Mentha piperita* and *Cinnamomum zeylanicum*) at a range of 500 to 2000 ppm, have shown a protective power of wheat seeds against *Fusarium* and *Asper-*

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gillus fungi after five days of treatment. On the other hand, the antioxidant powers have negatively correlated with seed contamination index (SCI) suggesting that essential oils with high antioxidant characteristics induce low fungal contamination and cannot have a crucial role in the expression of the antimycotoxin effect (Sumalan *et al.*, 2013).

In this study, essential oils of six plants which belong to two families; *Lamiaceae* and *Asteraceae* were compared for their fungal growth inhibiting effects on five *Fusarium* strains using concentrations between 0.25 and 4 µl/ml as well as their antioxidant powers. In this respect, this first comparison of local botanical sources of terpenoids in Algeria allows their biochemical valorization for economic and health interests in agriculture and food industry.

Materials and Methods

Plant material and extraction of essential oils

Four species of *Lamiaceae*: *Mentha piperita* L., *Mentha pulegium* L., *Thymus vulgaris* L. and *Thymus algeriensis* Boiss. & Reut. and two species of *Asteraceae*: *Artemisia herba-alba* Asso and *Artemisia campestris* L. were collected in June 2017 from the region of ElHadjeb (Laghouat) and ElGhicha in the south of Djebel Amour in the Algerian hautes plaines. Collected samples were dried at room temperature, away from light and humidity. Essential oils were obtained by hydro-distillation during 2.5 to 3 h, using a Clevenger type apparatus (Clevenger, 1928). The obtained oil was treated by anhydrous sodium sulfate, filtrated then stored at +4°C, until analysis.

Gas chromatography analysis

Chromatographic analyses of volatile compounds were carried out at the Research Laboratory of Fundamental Sciences at the University of Laghouat in Algeria, using a gas chromatograph GC-5400 equipped with a flame ionization detector (FID) and a fused silica capillary column of type DB-5 (30 m × 0.32 mm, film thickness = 0.10 µm). The vec-

tor gas used is hydrogen with a flow rate of 1 ml/min. Temperature of the column is programmed at a rate of 5°C/min from 50°C to 250°C. The temperature of injector and detector was set at 250°C and 280°C, respectively. Essential oil solutions are prepared by dissolving 10 µL of each in 1 ml of pentane organic solvent. Linear retention indices of the constituents are based on a series of alkane (C8–C20) analyzed under the same operating conditions as those of the samples.

Determination *in vitro* of the antifungal activity of the obtained essential oils

The antifungal power of EOs was tested on five strains; INRA 349 of *F. graminearum* from CBS collection 185.32 (Centraalbureau voor Schimmelcultures, Netherlands), BD17 of *F. culmorum* from the collection of Touati-Hattab Sihem (Touati-Hattabet *et al.*, 2016), *Fusarium oxysporum* f.sp. *lycopersici* (FOL) from National Institute of Agronomy (El Harrach), *Fusarium oxysporum* f.sp. *albedinis* (FOA) from Regional Plant Protection Station (SRPV Ghardaïa) and *Fusarium oxysporum* f.sp. *pisi* (FOP) obtained from Mycology laboratory of agronomy department (University of Blida).

The methodology of the bioassays for the evaluation of the effect of EOs on mycelial growth and the determination of inhibition rates in solid culture medium are described in a previous study (Elhouiti *et al.*, 2017). In brief, 1/5th–1/200th dilutions of the EOs were prepared in an agar solution (2%). In tubes, each containing 13.5 ml of sterile PDA medium (45°C), 1.5 ml of each EO dilution was added to obtain final concentrations ranging from 0.5 to 20 µl/ml. The effect of the EOs on mycelial development was evaluated by calculating the inhibition percentage of the mycelium after 7 days of incubation at 25 ± 2°C.

Evaluation of Antioxidant Activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

DPPH radical scavenging assay was carried out according to the method of Brand-

Williams *et al.* (1995). In order to test their antioxidant power, dilutions of essential oils were prepared in absolute ethanol. A volume of 1 ml of the ethanolic solution of DPPH (200 μ M) is added to 1 ml of the solutions of extracts or of the standard antioxidant (BHT, Vit E, Vit C) at different concentrations, the mixture is vigorously stirred. After 30 minutes of incubation in the dark and at room temperature, the absorbances were read at 517 nm by a UV-Vis spectrophotometer (Shimadzu 1601) against a blank. DPPH inhibition in terms of percentage was calculated using the following equation: Percent scavenged (DPPH) = $[(A_0 - A_S)/A_0] \times 100$, where A_0 is the absorbance of DPPH solution without EO and A_S is the absorbance in presence of EO. Antioxidant activity of EOs was expressed as IC₅₀ defined by the concentration (in mg/ml) of tested EOs inhibiting the formation of DPPH radicals by 50%.

β -Carotene Bleaching (BCB) Assay

A modified method of β -carotene bleaching (BCB) assay described by Marco (1968) and Miller (1971). 1 mg of β -carotene is dissolved in 1 ml of chloroform. 1 ml of this solution (1 mg/ml) is mixed with 200 mg of Tween 20 and 20 mg of linoleic acid. Then chloroform is evaporated under vacuum at 45°C for 3 minutes, and then 75ml of distilled water was added to the emulsion to form the final reagent.

For each experiment a fresh solution of the reagent was prepared. A volume of 0.2 ml of each extract and of the synthetic antioxidant BHT (at different concentrations) is added to a volume of 2 ml of the emulsion of β -carotene/linoleic acid. The absorbance of the extracts is measured at 470 nm after incubation at 50°C for 120 min. The percentage of antioxidant activity of EOs was calculated with the following equation: $I(\%) = [(A_E - A_C)/(A_0 - A_C)] \times 100$. Where A_E is the absorbance in presence of EOs at $t = 120$ min, A_C is the absorbance of control at $t = 120$ min and A_0 is the absorbance of control at t_0 .

Statistical analysis

All experiences were performed in trip-

licate. Data processing was carried out with Origin b9.6.5.169. The determination of IC₅₀ and MIC was performed with dose-response curve with variable Hill slope at error probability ≤ 0.05 .

Results and discussion

Chemical composition analysis

The essential oil yields of *Thymus vulgaris*, *Thymus algeriensis*, *Mentha piperita*, *Mentha pulegium*, *Artemisia herba-alba* and *Artemisia campestris* were 2.38%, 0.72%, 2%, 1.3%, 1.02% and 0.2% (w/w) respectively. The chemical composition of essential oils of *T. vulgaris*, *T. algeriensis*, *A. herba-alba*, *M. piperita* and *M. pulegium* is characterized by a dominance of the family of oxygenated monoterpenes with 73.85%, 59.41%, 70.01%, 60.01% and 87.2% respectively (Fig. 1). EO of *A. campestris* showed a diverse composition by similar percentages between all families. The elemental composition of EOs of the genus *Thymus* revealed high percentages of Carvacrol (63.42%), γ -Terpinene (9.10%) and *p*-Cimene (6.73%) in *T. vulgaris* EO and Limonene (11.49%), α -Pinene (9.26%) and Carvacrol acetate (14.16%) in *T. algeriensis* EO. On the other hand, high percentages of Carvone (70.8%), Piperitenone (4.94%), and Menthofurane (6.8%) have characterized *M. pulegium* EO, contrary to *M. piperita* EO which is dominated by Piperitone (57.51%) and Limonene (28.29%). *A. herba-alba* EO contained high percentages of Camphor (36.48%), Chrysanthenone (13.99%) and Limonene (10.44%), while the composition of *A. campestris* EO did not show high percentages of distinct compounds but Sabinene (5.71%), γ -Muurolene (5.08%), Bicyclogermacrene (16.8%) and (Z)-3-Hexenyl benzoate (25.72%) generally characterized its composition.

The composition of *T. vulgaris* EO suggests that it belongs to Carvacrol chemotype (Iten *et al.*, 2009) which has shown significant antiinflammatory and insecticidal potential (Fachini-Queiroz *et al.*, 2012; Szczepanik *et al.*, 2012). The variability of EOs chemical composition in the populations of

Figure 1. Chemical compositions by families of terpenoids in essential oils of *Thymus vulgaris*, *Thymus algeriensis*, *Mentha piperita*, *Mentha pulegium*, *Artemisia herba-alba* and *Artemisia campestris*.

Tunisian *T. algeriensis* has shown the presence of five different chemical groups (Ben El Hadj Ali *et al.*, 2012) and this high variation was also shown in the composition of the samples analyzed in other studies (Dob *et al.*, 2006, Amarti *et al.*, 2010). In the composition of *M. pulegium* EO, the high percentage of Carvone also characterized Tunisian samples from semi-arid bioclimatic stage and may represent Carvone-pulegone chemotype (Mkaddem *et al.*, 2007). The high percentage of Piperitone in the composition of *M. piperita* EO has not been reported previously, but the form D of this ketone was isolated from essential oils of the genus *Mentha* and played a role in the biosynthesis cycle of (-)-menthol (Bergman *et al.*, 2019). A high percentage of oxygenated monoterpenes and the percentage of Camphor also characterized the composition of *A. herba-alba*

EO in other studies (Dob and Benabdelkader, 2006; Mohsen and Ali, 2009), unlike *A. campestris* EO which showed high levels of hydrocarbon monoterpenes (Akrouit *et al.*, 2001, 2003).

Antifungal activity

Kinetics of fungal growth of *Fusarium* strains in the presence of different concentrations of the tested plant EOs showed a remarkable sensitivity of the strains of *F. oxysporum*. The growth inhibition curves are shown in Figure 2. The two strains BD17 and INRA 349 were found to be resistant to low concentrations of EOs from *T. algeriensis*, *A. campestris* and *A. herba-alba*, sensitive to EOs of *M. pulegium* and *M. piperita*, very sensitive to the EO of *T. vulgaris*. At the minimum concentration tested 0.25 µl/ml of *T. vulgaris* EO, the growth of strains FOA, FOL and FOP

of *F. oxysporum* was completely inhibited.

In Figure 3, the IC₅₀ and MIC values show very high bioactivity of *T. vulgaris* EO against all strains. In general, IC₅₀ values of the other EOs are between 0.01 and 0.6 µl/

ml against the strains of *F. oxysporum* which are more sensitive to *T. vulgaris* and *A. campestris* EOs, on the other hand, the strains of *F. culmorum* and *F. graminearum* are only sensitive in the presence of *T. vulgaris* EO. A

Figure 2. Growth inhibition of the strains BD17, INRA 349, FOA, FOL and FOP by different concentrations of tested essential oils.

weak inhibitory effect of EOs of *A. campestris* and *A. herba-alba* compared to other oils resulted in high MIC values against the strains BD17, INRA 349, FOA and FOP. Potency and efficiency of EOs of *M. pulegium* and *M. piperita* were important especially against the strains of *F. oxysporum*.

Against strains BD17 and INRA 349, IC₅₀ and MIC values recorded by these tested EOs are more effective than the EO of *Rhanterium adpressum* (0.7 and 1.54 for IC₅₀, 12.81 and 12.73 µl/ml for MIC for the two strains respectively), an endemic Algerian plant that was the subject of a previous study (Elhouiti *et al.*, 2017), and even more effective against FOA than the EO of this same plant (2 µl/ml for IC₅₀ and 10 µl/ml for MIC) (Elhouiti *et al.*, 2016). MIC value of *A. campestris* EO against the growth of INRA 349 is similar to that recorded by Houicher *et al.* (2016) of 1.25 µl/ml against the strain MUCL 53452. Moreover, at

1 µl/ml, growth inhibition of the strains Fc107 and Fc289 of *F. culmorum* was complete (100%) with *T. vulgaris* EO mainly constituted by p-Cymene (21.94%), γ-terpinene (7.8%) and Thymol (44.6%) (Matusinsky *et al.*, 2015). On the other hand, the EO of *M. piperita* from two different regions also showed a strong inhibition of *F. graminearum* in the presence of MIC>0.32 µl/ml (Rekanović *et al.*, 2012).

Antioxidant activity

The antioxidant potential of the six EOs was investigated with their capacity to donate hydrogen by DPPH radical scavenging assay and with quenching linoleate free radicals capacity by β-carotene bleaching assay (BCB) was shown by the IC₅₀ values in Table 1. A high antioxidant effect has been recorded by *T. vulgaris* EO with an effective concentration only 5 times more than the concentration of BHT in BCB test, the other oils

Figure 3. IC₅₀ and MIC for essential oils of six plant species (*Thymus vulgaris*, *Thymus algeriensis*, *Mentha piperita*, *Mentha pulegium*, *Artemisia herba-alba* and *Artemisia campestris*) against five strains of *Fusarium*.

Table 1. Antioxidant activity by the BCB and DPPH assays of tested essential oils.

Extracts and references	BCB assay (mg/ml)	DPPH assay (mg/ml)
<i>T. vulgaris</i>	0.5±0.130	1.41±0.012
<i>T. algeriensis</i>	7.68±0.245	8.86±1.13
<i>A. herba-alba</i>	7.36±1.968	50.6±7.9
<i>A. campestris</i>	1.74±1.128	18.45±1.33
<i>M. × piperita</i>	5.52±2.107	40.65±5.44
<i>M. pulegium</i>	30.01±1.903	33.76±1.94
BHT	0.096±0.02	0.086±0.003
VIT C		0.006±0.0004
VIT E		0.009±0.001

showed weak inhibition compared to *T. vulgaris* EO with IC₅₀ 3 to 60 times less. DPPH test showed the weak antioxidant capacity of all oils compared to the standards. However, the capacity of *T. vulgaris* EO is 6 to 35 times higher than the other oils.

Compared to other studies, by BCB test, *T. vulgaris* is more active at a concentration of 0.5 mg/ml than three other species (*T. kotschyanus*, *T. eriocalyx* and *T. daenensis* ssp. *lancifolius*) studied by Amiri (2012), unlike *M. piperita* and *M. pulegium* compared to six species of *Mentha* from Brazil, concentrations of 5.52 and 30.01 mg/ml were required to reach IC₅₀ (de Sousa Barros et al., 2015). As for *A. herba-alba*, the activity revealed a significant effect in comparison with the activity of four Tunisian chemotypes (Mighri et al., 2010). By DPPH test, essential oils of *T. algeriensis* collected from other regions of Algeria showed stronger activities (0.8-1 mg/ml) than in our study (Hazzit et al., 2009). Furthermore, with IC₅₀ of 0.005 mg/ml, *M. piperita* EO from Turkey was more active than that of our study (Kizil et al., 2010). However, the activity of *T. vulgaris* EO is significant compared to other EOs from Morocco (4.57 mg/ml) (Ismaili et al., 2017).

This first report on the comparison of fungal power of some local aromatic plants reveals the significant potential of their essential oils against phytopathogenic strains of *Fusarium*. Our results show that these essential oils exert a significant fungicidal effect starting with low concentrations of 0.25 µl/ml and reaching inhibition rates of 80 to 100%. In addition to this activity, a strong antioxidant capacity has been shown by *T. vulgaris* EO unlike other EOs which have medium or low activity. In this study, *T. vulgaris* EO was identified as a Carvacrol chemotype which may explain its activity by the dominance of this compound and also by its synergistic effect with other components of the EO.

In the study of Saghrouchni et al. (2020), the MIC values for Carvacrol against *F. oxysporum*, *F. nivale* and *F. solani* were 1/1000, 1/2000 and 1/2000 respectively. Moreover, the effect of Carvacrol on topsoil disinfection during 4 weeks of treatment was able

to reduce the fungal load to 0 for 3 weeks with a concentration of 0.8 g/l. However, the use of a single antifungal and antioxidant product in high doses and long term causes health and environmental problems. Thus, natural extracts with multiple antifungal and antioxidant components by their synergistic effect can be used as preservatives and dietary supplements to promote health safety (Weng and Yen, 2015).

Conclusions

The studied EOs have shown significant antifungal activity against *F. oxysporum* strains. The fungal growth of all *Fusarium* strains was strongly inhibited by *T. vulgaris* EO based on its bioactivity in DPPH radical scavenging assay and β-carotene bleaching assay. The EOs of *T. algeriensis*, *A. herba-alba*, *A. campestris*, *M. piperita* and *M. pulegium* exerted a moderate inhibitory activity on the strains of *F. culmorum* and *F. graminearum* with a weak antioxidant power revealed by the two assays. The antifungal and antioxidant activities may be due to monoterpenes which are the major components of the chemical composition of all oils. Overall, the EOs of the investigated Algerian spontaneous plants represent a source of natural antioxidants and antifungals in food industry as good alternative preservatives for foodstuffs.

List of abbreviations:

BCB: β-Carotene Bleaching
 BHA: Butylated HydroxyAnisole
 BHT: Butylated HydroxyToluene
 DB-5: Dimethylsilicone stationary phase with 5% phenyl groups
 DPPH: 2,2-DiPhenyl-1-PicrylHydrazyl
 EO(s): Essential Oil(s)
 FID: Flame Ionization Detector
 Foa: *Fusarium oxysporum* f.sp. *albedinis*
 Fol: *Fusarium oxysporum* f.sp. *lycopersici*
 Fop: *Fusarium oxysporum* f.sp. *pisi*
 GC: Gas Chromatograph
 PDA: Potato Dextrose Agar

PP: PropylParaben
 SCl: Seed Contamination Index
 THBP: TriHydroxyButyroPhenone
 Vit C: Vitamin C
 Vit E: Vitamin E

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Αντιοξειδωτική και αντιμυκητιακή δράση αιθερίων ελαίων από αυτοφυή φυτά της Αλγερίας έναντι πέντε στελεχών *Fusarium* spp.

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Περίληψη Η παρούσα εργασία αξιολογεί την αντιοξειδωτική και αντιμυκητιακή δράση αιθερίων ελαίων από αυτοφυή φυτικά είδη, *Thymus vulgaris*, *Thymus algeriensis*, *Mentha piperita*, *Mentha pulegium*,

Artemisia herba-alba και *Artemisia campestris*, σε πέντε στελέχη *Fusarium*. Στα αιθέρια έλαια των ειδών *T. vulgaris*, *T. algeriensis*, *A. herba-alba*, *M. piperita* και *M. pulegium* κύρια χημική ομάδα είναι τα οξυγονωμένα μονοτερπένια με ποσοστά 73,85%, 59,41%, 70,01%, 60,01% και 87,2 %, αντίστοιχα. Το αιθέριο έλαιο του *A. campestris* παρουσίασε ποικιλομορφία στη χημική του σύσταση, με παρόμοια ποσοστά μεταξύ των χημικών ομάδων. Τα στελέχη BD17 και INRA 349 βρέθηκαν ανθεκτικά σε χαμηλές συγκεντρώσεις αιθερίων ελαίων των φυτικών ειδών *T. algeriensis*, *A. campestris* και *A. herba-alba*, ευαίσθητα στα αιθέρια έλαια των *M. pulegium* και *M. piperita*, και πολύ ευαίσθητα στο αιθέριο έλαιο του *T. vulgaris* (0,25 µl/ml). Σε δοκιμές BCB διαπιστώθηκε υψηλή αντιοξειδωτική δράση του αιθερίου ελαίου του *T. vulgaris*, με αποτελεσματική συγκέντρωση (0,5 mg/ml) 3 έως 60 φορές υψηλότερη σε σύγκριση με τα άλλα αιθέρια έλαια που δοκιμάστηκαν. Ανάλογη αντιοξειδωτική ικανότητα του αιθερίου ελαίου του *T. vulgaris* καταγράφηκε και σε δοκιμές DPPH, με EC50= 1,41 mg/ml.

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