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Inhibitory effect of essential oils from *Pulicaria mauritanica* and *Micromeria debilis* on growth of *Alternaria* spp., the causal agent of tomato early blight

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Abstract

The aim of the present study was the evaluation of antifungal activity of essential oil (EO) extracted from two medicinal and aromatic plants (*Pulicaria mauritanica* and *Micromeria debilis*) as biological treatment for *Alternaria* spp., a pathogenic mould that causes early blight on tomato plants. EO was extracted by hydrodistillation and antifungal activity was analyzed *in vitro* using direct contact on agar against ten isolates of *Alternaria* spp. Extraction yield was 0.30 and 0.045% for *P. mauritanica* and *M. debilis*, respectively. Both EOs had inhibitory effect against mycelial growth of all studied isolates. However, the antifungal effect of EO isolated from *P. mauritanica* was stronger than for that from *M. debilis*, with 100% inhibition against *Alternaria tenuissema* (isolates A18 and A57), *Alternaria alternata* (isolate AT4), *Alternaria solani* (A37) and *Alternaria tomatophila* (AT01), when used at 1.1 µL mL^{-1} concentration. EO from *M. debilis* had 100% inhibition against isolates A42 and A57 of *A. solani* and *A. tenuissema* when used at 1.1 µL mL^{-1} concentration. The antifungal activity of the EOs might due to the synergic effects of their chemical compounds, mainly monoterpenes and sesquiterpenes.

Key words: *Alternaria* spp., antifungal activity, essential oils, medicinal and aromatic plants, *Micromeria debilis, Pulicaria mauritanica*.
Abbreviations: EO, essential oil; PDA, Potato Dextrose Agar.

Introduction

In Algeria, similar to other countries, tomato (*Solanum lycopersicum* L.) is the second most consumed vegetable after potatoes, revealing its economic importance (Foolad 2007). Tomato crops are challenged by several diseases, among which early blight, a fungal disease caused by species of the genus *Alternaria* and destroying plant foliage and fruit, is one of the most common and costly diseases, decreasing tomato fruit size and yield by up to 78% (Rotem 1994; Chohan et al. 2015). The disease is more abundant in areas with high dew incidence, abundant precipitation and high relative humidity (Roy et al. 2019).

The fungi of the genus *Alternaria* belong to the phylum Ascomycota, order Pleosporales and family Pleosporaceae (Simmons 2007). *Alternaria* spp. with imperfect forms belongs to the division Deuteromycota. The genus is characterized by production of dark-coloured conidia with transverse and longitudinal septa (phaeodictyospore; Pryor, Gilbertson 2000). While some *Alternaria* spp. are saprophytes, many of them are highly pathogenic for several animals and plants (Rotem 1994; De Hoog, Horre 2002; Lawrence et al. 2013).

Chemical fungicides are the main method used to control *Alternaria* in tomato fields, but their use needs to be restricted due to their toxicity, environmental pollution and negative impacts on human health (Bernard et al. 1997). Therefore, it is important to look for alternatives to control the fungal agent of early blight of tomato. The safest approach is using vegetable-originated products, since they are biodegradable and not toxic for both human and environment. Previous studies revealed antimicrobial activity of some samples of essential oil (EO) extracted from medicinal and aromatic plants (Soliman, Badeaa 2002; Bourkhiss et al. 2007; Jazet-Dongmo et al. 2009; Magina et al. 2009). In particular, many types of EO have good antifungal activity (Hu et al. 2007; Bakkali et al. 2008; Lang, Buchbauer 2012)

Flora of aromatic plants is highly diverse and abundant in Algeria, and many species are used as therapeutic plants. The genus *Pulicaria* belongs to the Asteraceae family and contains more than 100 species, distributed in Europe, North Africa, the Mediterranean basin and Asia (Williams et al. 2003). Many *Pulicaria* species have antibacterial and antifungal activity (Hanbali et al. 2005) because of their high level of phytochemical active components (Fahmi

et al. 2018). *Pulicaria mauritanica* Coss., locally known under the vernacular of Mariwa Sfayrya (Gherib 2014), is an aromatic and endemic plant that grows in Algeria and Morocco (Xu et al. 2015; Hamdouch et al. 2018).

Micromeria debilis Pomel (Lamiaceae), formerly known as Satureja briquetii Maire, is an endemic plant that grows in Algeria and Morocco (Gherib et al. 2016). Species of genus *Micromeria* have been used by Mediterranean indigenes for thousands of years as medication (Azab 2016). Diverse biological properties of the EO and other biological extracts of *Micromeria* have been reported, mainly pharmacological (Gulluce et al. 2004), antioxidant (Öztürk et al. 2009; Šamec et al. 2015), insecticidal, antibacterial and antifungal activity (Öztürk et al. 2011).

The aim of the present study was to evaluate antifungal activity of EO extracted from the two aromatic and medicinal plants, *Pulicaria mauritanica* and *Micromeria debilis*, against ten isolates of *Alternaria* spp., the etiological agent of early blight of tomato, *in vitro*.

Materials and methods

Fungal material

Alternaria spp. isolates were collected from tomato plants growing at different places of Algeria (Oran, Mostaganem and Ain Temouchent). Fungal isolation was performed from leaves, stems and fruits of tomato showing *Alternaria* blight symptoms (Rapilly 1968; Table 1). The obtained isolates were then purified by monosporic culture (Rapilly 1968) and identified by morphological characterization (Simmons 2007).

Plant material

Plant samples of *P. mauritanica* and *M. debilis* were collected in Nâama province (southwest of Algeria) in the Ain Sefra region (Djbel Mekter for *P. mauritanica* and Mograr for *M. debilis*) during February 2019 and November 2018, respectively. Plant identification was confirmed by Prof. A. Marouf (University of Nâama, Algeria).

Extraction of EO

Plants were dried in the laboratory at room temperature for eight days and EO was extracted from 50 g of dried aerial parts by hydrodistillation for 2 to 3 h in a Clevenger apparatus. The collected EO samples were stored at 4 °C in opac sealed tubes until use (Msaada et al. 2012).

In vitro antifungal activity of EO

The antifungal activity of EOs was evaluated on the mycelial growth of highly pathogens *Alternaria* spp. using the method of direct contact on agar (Fandoham 2004), with minor modifications. Briefly, different concentrations (0.95 or 1.1 µL mL^{-1}) were obtained by adding 14.25 and 16.5 µL of EO samples to 15 mL of supercooled Potato Dextrose Agar medium (PDA) at 45 °C containing Tween 20 (Sigma 0.5%, v/v). The mixture was homogenized by vortexing and poured into Petri dishes (90 mm). Mycelial discs (6 mm) were taken from the periphery of the 10-day-old fungal colonies and aseptically deposited at the middle of the Petri dishes containing PDA medium mixed with different concentrations of EO. The experiment was conducted in triplicate. Petri dishes containing 15 mL PDA medium supplemented with Tween 20 without EO were used as a positive control. Petri dishes were incubated in dark at 25 ± 2 °C for 10 days. Mycelial growth was measured daily during incubation, starting from day 3 by measuring two perpendicular diameters of the colonies. The antifungal activity of EO isolated from *P. mauritanica* and *M. debilis* was determined by calculating the inhibition rate of mycelial growth (Kasmi et al 2018):

$$\text{Percentage of mycelial growth inhibition (\%)} = [(DC - DT) / DC] \times 100,$$

where DC is an average diameter (mm) of the fungal colony in the control and DT is the average diameter (mm) of the fungal colony in the treatment.

Statistical analysis

All measurements were conducted in triplicate. Comparison of inhibitory rates of mycelial growth of *Alternaria* spp. by EO was performed by an ANOVA test, using Excel 2010 software. Results were considered significant at $P < 0.05$.

Table 1. Origin and taxonomic identity of *Alternaria* spp. isolates used in this study

Isolate code	Species	Geographic origin	Date	Organ of tomato plant
A1	*A. tenuissema*	Mostaganem (Stidia)	18.03.2015	Fruit
A10	*A. alternata*	Mostaganem (Ouriah)	18.03.2015	Fruit
A18	*A. tenuissema*	Mostaganem (Ouriah)	18.03.2015	Fruit
A37	*A. solani*	Oran (Sidi Maarouf)	25.03.2015	Leaves
A42	*A. tomatophila*	Oran (Sidi Maarouf)	25.03.2015	Leaves
A57	*A. tenuissema*	Oran (Bousfer)	24.04.2015	Leaves
AT01	*A. tomatophila*	Mostaganem (Stidia)	18.03.2015	Stem
AT04	*A. alternata*	Mostaganem(Ouriah)	18.03.2015	Stem
A51	*A. solani*	Ain Temouchent	10.04.2015	Leaves
A45	*A. tomatophila*	Oran (Bousfer)	24.04.2015	Leaves

Results

Yield and characterization of EO

EO extracted from dried aerial parts of both plants had amber yellow color in the case of *P. mauritanica* and light green color for *M. debilis*. Both EOs had a strong odor.

The essential oil yield of the two plants was expressed as a mass percentage by weight relative to the dry matter. The extraction yields were 0.30 and 0.045% for *P. mauritanica* and *M. debilis*, respectively.

In vitro evaluation of antifungal activity of EO

Antifungal activity of EO from *P. mauritanica* and *M. debilis* against the mycelial growth of the phytopathogenic *Alternaria* spp. are summarized in Table 2 and Fig. 1.

Both EOs had inhibitory effect against all *Alternaria* strains, however, the antifungal activity varied between isolates. Increasing EO concentration resulted in increased inhibition of mycelial growth and consequently a decrease in the colony diameter on the Petri dish.

EO from *P. mauritanica* had stronger antifungal activity compared to that from *M. debilis* and resulted in a total inhibition (100%) of the mycelial growth of *A. tenuissema* (isolates A18 and A57), *A. alternata* (isolate AT4), *A. solani* (A37) and *A. tomatophila* (AT01) when used at highest concentration (1.1 μL mL^{-1}). These results suggest that this EO has significant activity and inhibits mycelial growth of all *Alternaria* spp. isolates at different concentrations (P = 0.000317). Each isolate was influenced differently (P = 0.027384). When used at a concentration between 0.95 and 1.1 μL mL^{-1}, EO from *P. mauritanica* EO not fully inhibited mycelial growth of *Alternaria* isolates.

Isolates of *Alternaria* spp. had similar sensitivity to EO from *M. debilis* at 0.95 or 1.1 μl mL^{-1} concentration, and the difference between the two concentrations was not significant (P = 0.063141). However, there was a significant difference of the inhibitory effect of *M. debilis* EO between the isolates (P = 0.043158). Mycelial growth of isolate A42 from *A. solani* and isolate A57 from *A. tenuissema* were highly inhibited at 1.1 μL mL^{-1} by EO from *M. debilis*.

The two EOs significantly inhibited growth of Alternaria isolates at both concentrations (P = 0.000897) and each isolate was influenced differently (P = 0.015405). *A. tenuissema* (A57 isolate) was the most inhibited by both EOs while *A. solani* strain (A51 isolate) were the least inhibited.

Discussion

The present study confirmed antifungal activity of EO samples extracted from *P. mauritanica* and *M. debilis* against *Alternaria* isolates, agents of early blight of tomato. While both EOs were effective at low concentrations, the antifungal activity differed between pathogenic species, depending on their virulence level and the composition of the EO.

Table 2. Growth inhibition effect of essential oils from *Pulicaria mauritanica* and *Mecromiria debilis* on *Alternaria* isolates. Each value is presented as mean ± standard deviation (n = 3)

Isolate	Control	*Pulicaria mauritanica*				*Mecromiria debilis*			
	0	0.95		1.1		0.95		1.1	
	Colony diameter (mm)	Colony diameter (mm)	Growth inhibition (%)	Colony diameter (mm)	Growth inhibition (%)	Colony diameter (mm)	Growth inhibition (%)	Colony diameter (mm)	Growth inhibition (%)
A1	65.33 ± 2.52	21.00 ± 3.50	74.72 ± 5.9	19.33 ± 1.53	77.53 ± 2.57	24.5 ± 0.50	68.82 ± 0.84	18.33 ± 1.53	79.21 ± 2.57
A10	87.17 ± 1.04	47.00 ± 1.73	49.49 ± 2.13	33.50 ± 8.19	66.12 ± 10.08	54.50 ± 1.32	40.25 ± 1.63	42.50 ± 9.12	55.03 ± 11.24
A18	79.83 ± 0.76	28.50 ± 6.38	69.53 ± 8.65	6.00 ± 0.00	100 ± 0	31.00 ± 2.29	66.14 ± 3.10	27.33 ± 2.25	71.11 ± 3.05
A37	82.17 ± 0.76	35.33 ± 11.03	61.49 ± 14.48	6.00 ± 0.00	100 ± 0	53.33 ± 2.08	37.86 ± 2.73	43.50 ± 1.50	50.77 ± 1.97
A42	79.00 ±1.50	40.83 ±3.51	52.28 ±4.81	28.00 ±6.95	69.86 ± 9.52	52.67 ±1.26	36.07 ±1.72	6.00 ±0.00	100 ± 0
A45	83.00 ± 1.32	40.50 ± 6.76	55.19 ± 8.78	31.00 ± 3.00	67.53 ± 3.90	32.33 ± 1.76	65.8 ± 2.28	29.83 ± 1.26	69.05 ± 1.63
A51	76.33 ± 2.52	44.33 ± 1.61	45.50 ± 2.29	37.50 ± 3.28	55.21 ± 4.66	43.00 ± 4.50	47.39 ± 6.40	42.83 ± 4.54	47.63 ± 6.45
A57	78.50 ± 5.50	32.83 ± 1.89	62.99 ± 2.61	6.00 ± 0.00	100 ± 0	6.00 ± 0.00	100 ± 0	6.00 ± 0.00	100 ± 0
AT1	82.83 ± 0.76	35.33 ± 4.16	61.82 ± 5.42	6.00 ± 0.00	100 ± 0	48.33 ± 2.93	44.90 ± 3.81	43.00 ± 4.00	51.84 ± 5.21
AT4	82.67 ± 2.08	28.83 ± 4.25	70.22 ± 5.55	6.00 ± 0.00	100 ± 0	42.33 ± 1.26	52.61 ± 1.64	36.17 ± 2.25	60.65 ± 2.94

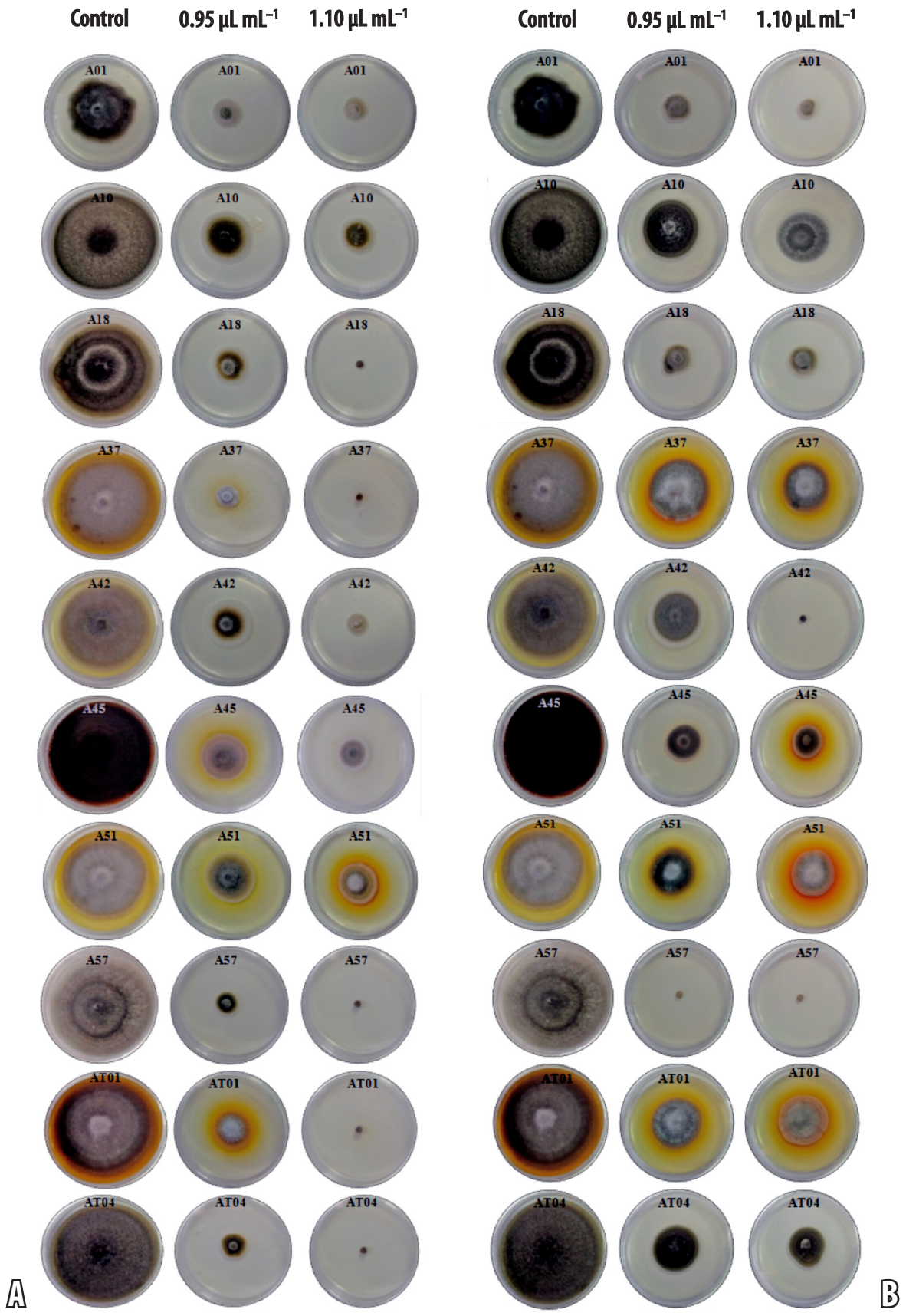

Fig. 1. Effect of different concentration of essential oil from *Pulicaria mauritanica* (A) and *Micromeria debilis* (B) on mycelial growth of Alternaria isolates within 10 days of incubation.

The effectiveness of antifungal activity of both EOs can be linked to differences in their chemical composition, mainly regarding monoterpenes such as carvotanacetone, 2-5-dimethoxy-p-cymene, β-pinene and linalool; and sesquiterpenes such as germacrene D and β-eudesmol (Cowan 1999; Tan et al. 1999; Nakamura et al. 2004). Furthermore, the high level of oxygenated monoterpenes, mainly carvotanacetone, in *P. mauritanica* might explain its high antifungal activity. EOs with high level of oxygenated terpenes are shown to have higher antimicrobial activity as compared to EOs with high level of hydrocarbons (Knobloch et al. 1987). The above can explain why EO from *P. mauritanica* was more effective against *Alternaria* isolates as compared to that from *M. debilis*.

P. mauritanica had an inhibitory effect on mycelial growth of *Alternaria* spp. after seven days of incubation at 25 °C, with a minimum inhibitory concentration of 2 μL mL^{-1} (Gherib et al. 2016a; Znini et al. 2013). A recent study reported that *Alternaria* spp. and *Aspergillus brasiliensis* were totally inhibited by 0.25 μL mL^{-1} of *P. mauritanica* EO after six days of incubation at 25 °C (Bammou et al. 2019).

EO extracted from *Pulicaria jaubertii* has a strong antimicrobial activity, especially on *Candida albicans* (minimum inhibitory concentration 300 μg mL^{-1}), and the inhibitory effect might due to its high level of carvotanacetone (93.5%; Al-Fatimi et al. 2015). Furthermore, strong antimicrobial activity of oxygenated monoterpenes results from their ability to block enzymatic activity of cell wall synthesis, leading to rearrangement of lipids and changes in functions and properties of cell membrane, including permeability (Zambonelli et al. 1996).

The effectiveness of EO from *P. mauritanica* might be the result of the presence of phenolic compounds such as thymol and carvacrol. These phenolic compounds contribute to antifungal activity of EO through synergy with other active compounds (Ultee et al. 2002). It was confirmed that fungi are sensitive to carvacrol and thymol (Kim et al. 1995; Curtis et al. 1996). Thymol is the major component of EO from *Thymus eriocalyx* (63.8%) and *Thymus x-porlock* (31.7%) (Rassoli et al. 2006). The authors correlated the high level of thymol with the morphological alterations of the hyphae observed during the treatment of the *Aspergillus niger* mycelium with the two EO from different thyme species.

Hydrocarbonated monoterpenes (α-pinene and β-pinene) were highly abundant in *M. debilis* EO, representing 21.2% of the total composition (Gherib et al. 2016b). It is possible that antifungal activity of EO from *M. debilis* against *Alternaria* spp. was mainly due to the high level of β-pinene (19.3%). These findings are in agreement with the results that diverse dematiaceous moulds, mainly *Alternaria brassicola*, are sensitive to β-pinene, with minimum inhibitory concentration of 125 μL mL^{-1} (Moreira 2007). Likewise, pinenes have effective antimicobial activity against yeast and pathogenic moistures (Gayoso et al. 2004; Lima et al. 2005). However, β-pinenes are more active on *Candida albicans* than are α-pinènes (Hammer et al. 2003). In addition, EO from *M. debilis* had a wide spectrum of antimicrobial activity, especially against *Candida albicans* with a minimum inhibitory concentration of 2.0 μL mL^{-1} (Gherib et al. 2016b).

It also seems that the high level of linalool (6.5%), a monoterpene alcohol, might increase the antifungal activity of EO from *M. debilis* (Gherib et al. 2016b). The antimicrobial activity of monoterpene alcohols has been attributed to their high affinity for cell membranes and strong bounding properties with molecular structures such as proteins or glycoproteins and altering cell permeability leading to leakage of cytoplasmic material (Hemaiswarya et al. 2009; Wang et al. 2012).

These findings provide an alternative solution to more safe and ecologically-friendly ways of control of *Alternaria* blight in tomato plants. Validating our results *in vivo* would be the next step to determine the host-pathogen-essential oil interaction.

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References

Al-Fatimi M., Awadh N.A.A., Wurster M., Al-Sokari S.S., Lindequist U., Setzer W.N. 2015. Chemical composition, antimicrobial and antioxidant Activity of the essential oil of *Pulicaria jaubertii* from south Yemen. *World J. Pharm. Res.* 4: 1–9.

Azab A. 2016. *Micromeria*: chemistry and medicinal activities. *Eur. Chem. Bull.* 5: 300–307.

Bakkali F., Averbeck S., Averbeck D., Idaomar M. 2008. Biological effects of essential oils – A review. *Food Chem. Toxicol.* 46: 446–475.

Bammou M., Bouhlali E.D.T., Sellam K., Ramchoun M., McDonald A.G., Ouzidan Y., Ibijbijen J., El Rhaffari L., Nassiri L. 2019. Investigation on chemical composition, antioxidant and antimicrobial potential of *Pulicaria mauritanica* essential oil applied by direct addition or vapor contact. *Am. J. Essent. Oil Nat. Prod.* 7: 7–13.

Barnard C., Daberkow S., Padgitt M., Smith M.E., Uri N.D. 1997. Alternative measures of pesticide use. *Sci. Total Environ.* 203: 229–244.

Bourkhiss M., Hnach M., Bourkhiss B., Ouhssine M., Chaouch A. 2007. Chemical composition and antimicrobial properties of the essential oil extracted from the leaves of *Tetraclinis articulata* (Vahl) of Morocco. *Afr. Sci. Rev. Int. Sci. Technol.* 3: 232–242.

Chohan S., Perveen R., Mehmood M.A., Naz S., Akram N. 2015. Morpho-physiological studies, management and screening of tomato germplasm against *Alternaria solani*, the causal agent of tomato early blight. *Int. J. Agric. Biol.* 17: 111–118.

Cowan M. 1999. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 12: 564–582.

Curtis O.F., Shetty K., Cassagnol G., Peleg M. 1996. Comparison of

the inhibitory and lethal effects of synthetic versions of plant metabolites (anethole, eugenol, carvacrol and thymol) on a food spoilage yeast (*Debaromyces hansenei*). *Food Biotechnol.* 10: 55–73.

De Hoog G.S., Horre R. 2002. Molecular taxonomy of the *Alternaria* and *Ulocladium* species from humans and their identification in the routine laboratory. *Mycoses* 45: 259–276.

Fahmi A.A., Abdur-Rahman M., Aboul-Naser A.F., Hamed M.A., Abd-Alla H.I., Nasr M.I. 2019. *Pulicaria crispa* mitigates gastric ulcer induced by ethanol in rats: Role of treatment and auto healing. *Biomarkers* 24: 286–294.

Fandohan P., Gbenou J.D., Gnonlonfin B., Hell K., Marasas W.F.O., Wingfield M.J. 2004. Effect of essential oils on the growth of *Fusarium verticillioides* and fumonisin contamination in corn. *J. Agric. Food Chem.* 52 : 6824–6829.

Foolad M. 2007. Genome mapping and molecular breeding of tomato. *Int. J. Plant Genom.* 2007: 64358.

Gayoso C.W., Lima E.O, Souza E.L .2004. Inhibitory effect of *Cinnamomum zeylanicum* Blume essential oil, α-pinene and β-pinene on fungi isolated from onychomycoses. *Braz. J. Phytomed.* 1: 25–29.

Gherib M., Bekhechi C., Atik-Bekkara F., Bighelli A., Casanova J., Tomi F. 2016a. Chemical composition and antimicrobial activity of the essential oil from aerial parts of Algerian *Pulicaria mauritanica*. *Nat. Prod. Commun.* 11: 109–112.

Gherib M., Bekhechi C., Paoli M., Atik-Bekkara F., Bighelli A., Casanova J., Tomi F. 2016b. Chemical composition and antimicrobial activity of the essential oil from aerial parts of *Micromeria debilis* Pomel from Algeria. *J. Essent. Oil Res.* 28: 383–390.

Gulluce M., Sokmen M., Sahin F., Sokmen A., Adiguzel A., Ozer H. 2004. Biological activities of the essential oil and methanolic extract of *Micromeria fruticosa* (L.) Druce ssp. *serpyllifolia* (Bieb) PH Davis plants from the eastern Anatolia region of Turkey. *J. Sci. Food Agric.* 84:735-741 .

Hamdouch A., Asdadi A., Achemchem F., Chebli B., El Hadek M., Idrissi-Hassani L.M. 2018. Phytochemical analysis, Antifungal, antibacterial and antioxidant properties of *Pulicaria mauritanica* from south-east of Morocco. *J. Mater. Environ. Sci.* 9: 1762–1770.

Hammer K.A., Carson C.F., Riley T.V .2003. Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. *J. Appl. Microbiol.* 95: 853–860.

Hanbali F.E., Akssira M., Ezoubeiri A., Gadhi C.E., Mellouki F., Benherraf A., Blazquez A.M., Boira H. 2005. Chemical composition and antibacterial activity of essential oil of *Pulicaria odora* L. *J. Ethnopharmacol.* 99: 399–401.

Hemaiswarya S., Doble M. 2009. Synergistic interaction of eugenol with antibiotics against Gram negative bacteria. *Phytomedicine* 16: 997–1005.

Hu Y., Zhang J., Kong W., Zhao G., Yang M. 2007. Mechanisms of antifungal and anti-aflatoxigenic properties of essential oil derived from turmeric (*Curcuma longa* L.) on *Aspergillus flavus*. *Food Chem.* 220: 1–8.

Jazet Dongmo P.M., Tatsadjieu L.N., Tchinda Sonwa E., Kuate J., Amvam Zollo P.H., Menut C. 2009. Essential oils of *Citrus aurantifolia* from Cameroon and their antifungal activity against *Phaeoramularia angolensis*. *Afr. J. Agric. Res.* 4: 354–358.

Kasmi M., Aourach M., El Boukari M., Barrijal S., Essalmani H. 2018. Effectiveness of aqueous extracts of aromatic and medicinal plants against gray mold of tomatoes in Morocco. *C. R . Biol.* 340: 386–393.

Kim J., Marshall M.R, Wei C.I. 1995. Antibacterial activity of some essential oil components against five foodborne pathogens. *J. Agric. Food Chem.* 43: 2839–2845.

Knobloch E., Pauli A., Iberl B., Wies N., Weigand H. 1987. Mode of action of essential oil components on whole cells of bacteria and fungi in plate tests. *Bioflavour* 287–299.

Lang G., Buchbauer G. 2012. A review on recent research results (2008–2010) on essential oils as antimicrobials and antifungals. A review. *Flavour Fragr. J.* 27: 13–39.

Lawrence D.P., Gannibal P.B., Peever T.L., Pryor B.M. 2013. The sections of *Alternaria*: formalizing species-group concepts. *Mycologia* 105: 530–546.

Lima I.O., Oliveira R.A.G., Lima E.O., Souza E.L, Farias N.P., Navarro D.F. 2005. Inhibitory action of some phytochemicals on yeasts potentially causing of opportunistic infections. *Braz. J. Pharm. Sci.* 41: 199–203.

Magina M.D.A., Dalmarco E.M., Wisniewski A., Simionatto E.L., Dalmarco J.B., Pizzolatti M.G., Brighente I.M.C. 2009. Chemical composition and antibacterial activity of essential oils of *Eugenia* species. *J. Nat. Med.* 63: 345–350.

Moreira A.C.P., Lima E.O., Souza E.L, Van Dingenen M.A., Trajano V.N. 2007. Inhibitory effect of *Cinnamomum zeylanicum* Blume (Lauraceae) essential oil and β-pinene on the growth of dematiaceous moulds. *Braz. J. Microbiol.* 38: 33–38.

Msaada K., Salem N., Tammar S., Hammami M., Jamal Saharkhiz M., Debiche N., Limam F., Marzouk B. 2012. Essential oil composition of *Lavandula dentata* L. stoechas and *L. multifida* cultivated in Tunisia. *J. Essent. Oil Bear. Plants* 15: 1030–1039.

Nakamura C.V., Ishida K., Faccin L.C., Filho B.P.D., Cortez D.A.G., Rozental S., de Souza W., Ueda-Nakamura T. 2004. *In vitro* activity of essential oil from *Ocimun gratissimun* L. against four *Candida* species. *Microbiol. Res.* 155: 579–586.

Öztürk M., Kolak U., Duru M.E., Harmandar M. 2009. GC–MS analysis of the antioxidant active fractions of *Micromeria juliana* with anticholinesterase activity. *Nat. Prod. Commun.* 4: 1271–1276.

Öztürk M., Kolak U., Topçu G., Öksüz S., Choudhary M.I. 2011. Antioxidant and anticoholinesterase active constituents from *Micromeria cilicica* by radical-scavenging activity-guided fractionation. *Food. Chem.* 126: 31–38.

Pryor B.M., Gilbertson R.L. 2000. Molecular phylogenetic relationships amongst *Alternaria* species and related fungi based upon analysis of nuclear ITS and mt SSU rDNA sequences. *Mycol. Res. 1*04: 1312–1321.

Rapilly F. 1968. *MycologyTechniques in Plant Pathology.* Annals of Epiphyta. Volume 19. Edition INRA, Paris, P. 25-39

Rasooli I., Rezaei M.B., Allameh A. 2006. Growth inhibition and morphological alterations of *Aspergillus niger* by essential oils from *Thymus eriocalyx* and *Thymus x-porlock*. *Food Control* 17: 359–364.

Rotem J. 1994. *The Genus Alternaria: Biology, Epidemiology and Pathogenicity.* APS Press, St. Paul Minnesota, 326 p.

Roy K.C., Akter N., Sarkar M.K.I., Pk M.U., Begum N., Zenat E.A., Jahan M.A.A. 2019. Control of early blight of tomato caused by *Alternaria solani* and screening of tomato varieties against the pathogen. *Open Microbiol. J.* 13: 41–50.

Šamec D., Gruz J., Durgo K., Kremer D., Kosalec I., Valek Žulj L., Martinez S., Salopek-Sondi B., Piljac-Žegarac J. 2015. Molecular and cellular approach in the study of antioxidant/pro-oxidant properties of *Micromeria croatica* (Pers.) Schott. *Nat. Prod. Res.* 29: 1770–1774.

Simmons E.G. 2007. Alternaria. *An identification Manual.* CBS Biodiversity Series No. 6. CBS Fungul Biodiversity Centre, Utrecht,the Netherlands.775 p.

Soliman K.M., Badeaa R.I. 2002. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. *Food Chem. Toxicol.* 40: 1669–1675.

Tan R.X., Lu H., Wolfender J.L., Yu T.T., Zheng W.F., Yang L., Gafner S., Hostettmann K. 1999. Mono- and sesquiterpenes and antifungal constituents from *Artemisia* species. *Planta Med.* 65 : 64–67.

Ultee A., Bennik M.H.J., Moezelaar R. 2002. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus. Appl. Environ. Microbiol.* 68: 1561–1568.

Wang Y.W., Zeng W.C., Xu P.Y., Lan Y.J., Zhu R.X., Zhong K., Huang Y.N., Gao H. 2012. Chemical composition and antimicrobial activity of the essential oil of Kumquat (*Fortunella crassifolia* Swingle). *Int. J. Mol. Sci.* 13: 3382–3393.

Williams C.A., Harborne J.B., Greenham J.R., Grayer R.J., Kite G.C., Eagles J. 2003. Variations in lipophilic and vacuolar flavonoids among European *Pulicaria* species. *Phytochemistry* 64: 275–283.

Xu T., Gherib M., Bekhechi C., Atik-Bekkara F., Casabianca H., Tomi F., Casanova J., Bighelli A. 2015. Thymyl esters derivatives and a new natural product modhephanone from *Pulicaria mauritanica* Coss. (Asteraceae) root oil. *Flavour Fragr. J.* 30: 83–90

Zambonelli A., Zechini d'Aulerio A., Bianchi A., Albasini A. 1996. Effects of essential oil on phytopathogenic fungi. *J. Phytopathol.*144: 491–494.

Znini M., Cristofari G., Majidi L., Paolini J., Desjobert J.M., Costa J. 2013. Essential oil composition and antifungal activity of *Pulicaria mauritanica* Coss., against postharvest phytopathogenic fungi in apples. *Food Sci. Technol.* 54: 564–569.

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