Journal of Food Safety and Food Quality 67, Heft 3 (2016), Seiten 61–92

Arch Lebensmittelhyg 67,
72–78 (2016)
DOI 10.2376/0003-925X-67-72

ISSN 0003-925X

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Antifungal activities of different essential oils against anise seeds mycopopulations

Fungizide Aktivität verschiedener ätherischer Öle gegen Anissamen-Pilzpopulationen

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Summary

The aim of this study was to investigate the possibility of biological control of fungal species isolated from anise seeds using essential oils from medicinal plants: mint (*Mentha spicata* L.), sage (*Salvia fruticosa* L.), rosemary (*Rosmarinus officinalis* L.), anise (*Pimpinella anisum* L.), bitter fennel (*Foeniculum vulgare* spp. *piperituum* L.) and myrtle (*Myrtus communis* L.). Ten fungal species isolated from anise seeds: *Bipolaris/Drechslera sorociniana, Fusarium subglutinans, F. vertricilioides, F. oxysporum, F. tricinctum, F. sporotrichioides, F. equiseti, F. incarnatum, F. proliferatum* and *Macrophomina phaseolina,* were used in this experiment. The minimum inhibitory concentrations (MIC) were determined by micro-dilution method using selected essential oils (EOs). A qualitative and quantitative chemical analyses of EOs were carried out. All EOs exhibited a significant antifungal activity against all tested fungal isolates. The myrtle EO proved to be the most potent one (MIC 0.0003–3.25 mg/mL, then mint 0.0003–7.75 mg/mL and sage 0.0003–10 mg/mL). All tested fungi were observed to have a susceptibility to all selected essential oils. These results suggest the possibility for application of the EOs in biological control of anise production.

Keywords: Medicinal plants, fungi, essential oil, biological control, minimum inhibitory concentration

Zusammenfassung

Das Ziel dieser Studie war es, ätherische Öle als biologische Pflanzenschutzmittel gegen Schimmelpilze, isoliert aus Anissamen, einzusetzen und deren Wirkung zu untersuchen. Die ätherischen Öle wurden aus verschiedenen Heilpflanzen gewonnen: Minze(Mentha spicata L.), Salbei (Salvia fruticosa L.), Rosmarin (Rosmarinus officinalis L.), Anis (Pimpinella anisum L.), Bitterfenchel (Foeniculum vulgare ssp. piperitum L.) und Myrte (Myrtus communis L.). Zehn Pilzarten wurden aus Anissamen isoliert und für die Untersuchungen herangezogen: Bipolaris / Drechslera sorokiniana, Fusarium subglutinans, F. verticillioides, F. oxysporum, F. tricinctum, F. sporotrichioides, F. equiseti, F. incarnatum, F. Proliferatum und Macrophomina phaseolina. Die minimalen Hemmkonzentrationen (MIC) wurden mittels Mikrodilutionsverfahren der ausgewählten ätherischen Öle bestimmt. Qualitative und quantitative chemische Analysen der ätherischen Öle wurden durchgeführt. Alle ätherischen Öle zeigten eine signifikante antimykotische Aktivität gegenüber allen getesteten Schimmelpilzarten. Das ätherische Öl der Myrte erwies sich als am wirkungsvollsten (MIC 0,0003-3,25 mg /ml) gefolgt von Minze (MIC 0,0003-7,75 mg /ml) und Salbei(MIC 0,0003-10 mg / ml). Alle untersuchten Pilze zeigten eine Anfälligkeit gegenüber den ausgewählten ätherischen Ölen. Diese Ergebnisse legen nahe, das die Anwendung ätherischen Öle als Pflanzenschutzmittel für Anis eine Möglichkeit darstellen.

Schlüsselwörter: Heilpflanzen, Schimmelpilze, ätherisches Öl, biologische Pflanzenschutzmittel, minimale Hemmkonzentration

Introduction

In recent years, essential oils have received renewed attention due to their wide spectrum of biological activities against several pests such as microorganisms (Gayoso et al. 2005; Maksimovic et al. 2005; Cosic et al. 2010; Istianto and Emilda, 2011; Vitoratos et al. 2013; Mahilrajan et al. 2014;). Yegen et al. (1992) reported that the essential oils of several aromatic plants exhibited fungicidal toxicity against soil-borne phytopathogenic fungi *in vitro*. The essential oils and other products of plants have a wide application in folk remedies, fragrance industry, food flavoring and preservation, but only in recent years they have started to be recognized for their potential antimicrobial activity (Girish and Satish, 2008; Mousavi et al. 2009; Mousavi and Raftos, 2012). Numerous studies have documented the antifungal properties of plant products (Bouchra et al. 2003; Carmo et al. 2008; Tawassoli et al. 2011). Several essential oils have shown promising medicinal use due to their antimocrobial properties (Soliman and Badeaa, 2002; Sökmen et al. 2004; Sitara et al. 2008). The cultivation of medicinal plants is affected by many plant diseases mainly caused by phytopathological fungi. The seeds are often infected and they germinate into the diseased seedlings. The presence of the fungi in medicinal plants reduces their quality and usefulness (Essono et al. 2007). The fungi produce mycotoxins which can be very dangerous for the people who consume medicinal plants in different forms. Mycotoxins are thermo-stable and cannot be destroyed by cooking. At the same time the medicinal plants present a very rich source of a biologically active compounds including antifungal activity (Douk et al. 1995; Kumar et al. 2007; Carmo et al. 2008; Arrebola et al. 2010; Zomorodian et al. 2011; Bouzenna ans Krichen, 2013; Stević et al. 2014). The antimicrobial activity of selected species has already been demonstrated (Maksimovic et al. 2005; Carmo et al. 2008; Cosic et al. 2010; Tawassoli et al. 2011; Istianto and Emilda, 2011; Vitoratos et al. 2013; Mahilrajan et al. 2014).

One of the important means of the disease transmission is through seeds. Planting infected seeds may result in a widespread distribution of disease within the crop, and an increased number of initial infection sites from which the disease can spread. As the fungi are the largest group of pathogens, it is almost impossible to keep the seeds completely healthy. The only solution is biological protection of seeds from phytopatogenic mycopopulation. In this work we assess the possibility of using EOs as antifungal agents to prevent the development of fungal diseases on anise seeds.

Material and methods

Essential oils

In this study, mint (*Mentha spicata* L.), sage (*Salvia fruticosa* L.), rosemary (*Rosmarinus officinalis* L.), anise (*Pimpinella anisum* L.), bitter fennel (*Foeniculum vulgare* spp. *piperituum* L.) and myrtle (*Myrtus communis* L.) were obtained from Mersin (Turkey). A voucher specimen is kept in the herbarium of the Department of Food Engineering, Faculty of Agriculture, University of Selçuk, and identified by Dr. Bagcı. The voucher specimen'cod numbers are MS334, SF534;RO278; PA333,FAV879 and MC207 for mint, sage, rosemary, anise and bitter fennel and myrtle, respectively.

Essential oils of selected plants including mint, sage, rosemary, anise, bitter fennel and myrtle were obtained by hydro-distillation in a Clevenger-type apparatus. The obtained essential oils were stored in sealed glass bottles, protected from the light by wrapping in aluminium foil and storing at –18 °C.

Essential oils analysis

Analytical gas chromatography (GCIFID)

GC/FID analysis of tested essential oils was carried out on an Agilent Technologies gas chromatograph, model 7890A (Agilent Technologies, China), equipped with a split-splitless injector and automatic liquid sampler (ALS), attached to HP-5 column (30 m · 0.25 mm, 0.25 µm film thickness) and fitted to a flame ionization detector (FID). Carrier gas flow rate (H2) was 1 ml/min, injector temperature was 250 °C, detector temperature 260 °C, while column temperature was linearly programmed from 40–260 °C (at rate of 4 °/min), and held isothermaly at 260 °C for the next 5 minutes. Sample solutions in ethanol (15 µl/ml) were consecutively injected by ALS (1 µl, split mode, 1:30).

Gas chromatography/mass spectrometry (GC/MS)

The same analytical conditions as those mentioned for GC/FID were employed for GC/MS analysis, along with column HP-5MS (30 m · 0.25 mm, 0.25 µm film thickness), using HP G 1800C Series II GCD system [Hewlett-Packard, Palo Alto, CA (USA)].

Instead of hydrogen, helium was used as carrier gas. Detector was heated at 260 °C. Mass spectra were acquired in EI mode (70 eV), in m/z range 40-450. Sample solutions in ethanol (15 µl/ml) were injected by ALS (1 µl, split mode, 1:30).

The constituents were identified by comparison of their mass spectra to those from Wiley275 and NIST/NBS libraries, using different search engines (NIST and PBM). The experimental values for retention indices were determined by the use of calibrated Automated Mass Spectral Deconvolution and Identification System software (AMDIS ver.2.1.), compared to those from available literature (Adams, 2007), and used as additional tool to approve MS findings. Area percent reports, obtained as result of standard processing of chromatograms recorded by FID were used as base for the quantification purposes.

Isolations and identification of fungal species from anise seeds

The anise seeds were collected in three localities in the province of Vojvodina (Mošorin, Veliki Radinci and Ostojićevo). Four hundred seeds from each locality were sterilized with NaOCl for 3 minutes, rinsed with sterile water and transferred to the filter paper on Petri dishes, 10 cm in diameter. Fifty seeds from each locality were transferred to the PDA medium following the seed surface sterilization. After the eight-day incubation at 25 °C, parts of the mycelia taken from well-developed colonies were transferred to the PDA in order to be further examined (ISTA, 2003). All fungi isolates were subcultured on PDA and the *Fusarium* isolates on carnation leaf agar (CLA). Fungal development from seeds were estimated and identified based on their morphology and cultural characteristics according to different protocols for fungal identification.

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TABLE 1: *Percentage composition of tested mint, sage, rosemary, anise, bitter fennel and myrtle oils.*

Constituents	KIE*	%					
		M. communis	*M. spicata*	*S. fructicosa*	*R. officinalis*	*P. anisum*	*F. vulgare*
1 Isobutyl isobutyrate	916.3	0.4	/	/	/	/	/
2 Tricyclene	918.6	/	/	0.1	/	/	/
3 α-Pinene	930.1	6.0	0.3	7.0	7.5	0.1	0.6
4 Camphene	944.6	/	trace**	4.4	1.9	trace	0.1
5 Sabinene	971.1	/	0.1	/	/	trace	0.1
6 β-Pinene	972.8	/	0.3	2.1	0.3	/	trace
7 1-Octen-3-ol	986.5	/	trace	/	0.4	/	/
8 Myrcene	991.4	/	0.2	2.4	1.9	/	0.5
9 Dehydro-*trans*-linalool oxide	992.9	0.3	/	/	/	/	/
10 3-Octanol	1001.1	/	0.2	/	/	/	/
11 α-Phellandrene	1003.2	/	/	trace	0.1	/	0.1
12 α-Terpinene	1014.7	/	trace	0.1	0.3	/	/
13 p-Cymene	1023.0	0.2	0.9	0.2	0.8	trace	0.8
14 Limonene	1026.2	/	2.7	0.6	/	trace	7.1
15 1,8-Cineole	1028.5	22.0	3.1	53.9	70.3	0.5	0.5
16 γ-Terpinene	1056.3	/	0.2	trace	0.2	0.0	0.2
17 *cis*-Sabinene hydrate	1067.0	0.8	1.0	trace	/	/	trace
18 Fenchone	1085.4	/	trace	/	/	0.2	/
19 Terpinolene	1086.1	/	/	0.2	0.1	/	/
20 *trans*-Linalool oxide (furanoid)	1088.1	0.9	/	/	/	/	/
21 Fenchone	1089.7	/	/	/	/	/	25.7
22 *trans*-Sabinene hydrate	1098.5	/	0.3	/	/	/	/
23 Isopentyl 2-methyl butanoate	1100.3	/	/	/	/	/	trace
24 Linalool	1102.5	35.7	/	1.4	0.5	/	/
25 *cis*-Thujone	1104.0	/	/	1.0	/	trace	/
26 α-Pinene oxide	1109.7	0.7	/	/	/	/	/
27 *trans*-Thujone	1115.0		/	0.8	/	/	/
28 Fenchol	1115.7	0.1	/	/	/	/	trace
29 *trans*-p-Mentha-2,8-dien-1-ol	1121.6	/	/	/	/	/	0.2
30 3-Octanol acetate	1124.5	0.1	0.1	/	/	/	/
31 α-Campholenal	1125.4	0.2	/	/	/	/	/
32 Camphor	1140.8	0.2	trace	15.6	4.6	trace	0.8
33 *trans*-Verbenol	1145.5	0.7	/	/	/	/	/
34 δ-Terpineol	1167.9	0.3	0.3	0.9	/	/	/
35 Terpinen-4-ol	1175.9	0.4	1.0	0.5	0.7	/	trace
36 α-Terpineol	1192.3	8.3	/	2.6	3.0	/	/
37 *cis*-Dihydro carvone	1195.9	/	5.0	/	/	/	/
38 *trans*-2-Hexenyl butanoate	1198.1	0.6	/	/	/	/	/
39 Methyl chavicol	1198.3	/	/	/	/	3.2	61.7
40 Dihydro carveol	1202.7	/	5.0	/	/	/	/
41 Verbenone	1208.6	0.3	/	/	/	/	/
42 *endo*-Fenchyl acetate	1218.9	/	/	/	/	/	0.1
43 3α-Hydroxy-1,8-cineole	1225.3	0.2	/	/	/	/	/
44 Nerol	1230.9	0.6	/	/	/	/	/
45 *cis*-Carveol	1231.2	/	1.3	/	/	/	/
46 *exo*-Fenchyl acetate	1231.4	/	/	/	/	/	0.6
47 Pulegone	1238.0	/	0.1	/	/	/	/
48 Carvone	1244.2	0.1	66.1	trace	/	trace	trace
49 *cis*-Anethole	1254.6	0.3	/	/	/	/	/

50	Linalool acetate	1255.0	9.8	/	0.1	/	/	/
51	Neryl formate	1260.9	/	/	trace	/	0.2	/
52	*trans*-Carvone oxide	1280.0	/	0.1	/	/	/	/
53	Bornyl acetate	1282.4	0.5	/	0.2	1.0	/	0.3
54	Menth-1-en-9-ol	1290.2	0.3	/	/	/	/	/
55	*trans*-Anethole	1294.6	/	/	/	/	93.6	/
56	*cis*-Pinocarvyl acetate	1310.6	0.6	/	/	/	/	/
57	Carvacrol	1314.7	/	/	0.1	/	/	/
58	Myrtenyl acetate	1322.9	0.6	/	/	/	/	/
59	*iso*-Dihydro carveol acetate	1328.5	/	3.8	/	/	/	/
60	*exo*-2-Hydroxycineole acetate	1339.7	0.2	/	/	/	/	/
61	α-Terpinyl acetate	1347.1	1.4	/	0.8	trace	/	/
62	*cis*-Carvyl acetate	1362.9	/	2.7	/	/	/	/
63	Neryl acetate	1363.6	0.6	/	trace	/	/	/
64	(3Z)-Hexenyl hexenoate	1369.9	0.2	/	/	/	/	/
65	β-Bourbonene	1379.3	/	1.1	/	/	/	/
66	Geranyl acetate	1383.4	2.9	/	trace	/	/	/
67	β-Elemene	1387.3	/	0.1	/	/	trace	/
68	*trans*-Sobrerol	1387.5	0.2	/	/	/	/	/
69	*cis*-Jasmone	1399.3	/	0.2	/	trace	/	/
70	Methyl eugenol	1406.2	0.7	/	/	/	/	/
71	*trans*-Caryophyllene	1412.8	/	0.3	0.8	2.1	/	/
72	β-Copaene	1423.0	/	0.1	/	/	/	/
73	*trans*-α-Bergamotene	1430.9	/	0.1	/	/	trace	/
74	8-Hydroxycarvotanacetone	1432.6	0.5	/	/	/	/	/
75	6,9-Guaiadiene	1438.2	/	0.3	/	/	/	/
76	α-Himachalene	1442.5	/	/	0.4	0.3	trace	/
77	*trans*-β-Farnesene	1453.8	/	0.2	/	/	/	/
78	γ-Himachalene	1472.4	/	/	/	/	0.7	
79	Germacrene D	1475.4	/	0.3	/	/	/	trace
80	*trans*-Calamenene	1518.0	/	0.2	trace	/	/	/
81	Spathulenol	1575.0	/	0.1	trace	/	/	/
82	Caryophyllene oxide	1578.1	0.7	0.1	0.4	trace	/	/
83	Viridiflorol	1588.5	/	0.4	0.4	/	/	/
84	Humulene epoxide II	1604.4	0.2	/	0.2	trace	/	/
85	1,10-di-*epi*-Cubenol	1611.3	/	0.1	/	/	/	/
86	Humulene epoxide III	1630.2	/	/	0.2	/	/	/
87	α-Cadinol	1652.7	/	0.1	/	/	/	/
88	Cedr-8(15)-en-10-ol	1656.3	/	/	0.2	/	/	/
89	*trans*-Pseudoisoeugenyl 2-methylbutyrate	1843.2	/	/	/	/	0.5	/
90	Manool	2049.9	/	/	0.1	/	/	/
	Sum of contents (% m/m) =>		**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
	Number of constituents =>		**48**	**55**	**54**	**39**	**28**	**31**

* KIE = Kovats (retention) index experimentally determined by calibrated AMDIS (uncorrected values); **-value less than 0.1 %

Antifungal assay *in vitro*

A minimum inhibitory concentration (MIC) was determined by modified micro-dilution method in 96 well microtiter plates (Douk et al. 1995; Nikolić, 2014). The fungal spores were washed from the surface of agar plates with sterile 0.75 % saline containing 0.1 % Tween 80 (vol/vol). The spore suspension was filtered and adjusted with sterile saline to a concentration of approximately $1.0x10^5$–$5.0x10^5$ spores per ml using a hemocytometer. In each well with 90 µL potato dextrose medium with appropriate dilutions of the EO 10 µL fungal inoculum was added. All experiments were performed in duplicates and repeated four times. The microplates were incubated for 72 h at 28 °C. The MIC was defined as the lowest concentration of essential oils (EO) which completely inhibited the visible fungal growth. Fluconazole was used as positive control.

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Statistical analysis

The values of the minimal inhibitory concentrations (MIC) were accomplished by Duncan's multiple range tests. Analysis of the variance was performed on MIC data of eight oils on 10 pathogenic fungi. Significance was evaluated at $p<0.05$ for all tests. Statistical analyses were done by procedures of STATISTICA v.7 (StatSoft, Inc.) and IBM SPSS Statistics v.20 (SPSS, Inc.).

Results and discussion

Chemical composition

The results obtained by chemical analysis of spearmint (*Mentha spicata* L.), Greek sage (*Salva fruticosa* L.), rosemary (*Rosmarinus officinalis* L.), anise (*Pimpinella anisum* L.), bitter fennel (*Foeniculum vulgare* spp. *piperituum* L.) and myrtle (*Myrtus communis* L.) EOs are presented in Table 1. In total of 146 compounds were identified and presented in relative percentages. Ninety-six 96 compounds were present in concentration of 0.1 % and above. The oils of spearmint were characterized by the presence of 55, sage 54, rosemary 39, anise 28, bitter fennel 31 and myrtle 48 constituents. Results showed that 1,8-cineole was present in the major portion of three EOs samples: sage (53.88 %), rosemary (70.31 %) and myrtle (22.01 %). Chemical profiling of *Myrtus communis* oil revealed 48 compounds, with linalool (35.7 %) and 1,8-cineole (22 %) being the major ones, while spearmint oil was found to contain 55 compounds, with the biggest content of carvone (66.14 %). A chemical analysis of bitter fennel oil showed 31 compounds, and the main constituent was estragole (methyl chavicol) (61.75 %) followed by fenchone (25.66 %). This has already been shown by Özcan et al. (2006) from different part of plants, indicated that the oil of Turkish fennel (bitter) belongs to the methyl chavicol (estragole)-rich type. The *Mentha spicata* oil contained a high concentrations of carvone (66.1 %), but lower than *M. spicata* oil from South Africa (87.9 %), which Combrinck et al. (2011) reported. Essentional oil compositions are largely determined by genetic, climatic and geographical factors (Van Vuuren, 2008).

Identification of fungal species from anise seeds

Ten different fungal species were identified, according to the morphological and molecular examination, in the collected anise seeds mycopopulation. The fungal species used in this study were specified in Table 2.

TABLE 2: *The fungal species and their concentration of spores used in antifungal assay.*

No.	Species	Locality	Concentrations of spores	Accession No.
1	*Bipolaris/Drechslera sorociniana*	Mošorin	1.0×10^5	KR866080
2	*Fusarium subglutinans*	Mošorin	2.0×10^5	KP126606
3	*F. vertricilioides*	Veliki Radinci	3.0×10^5	KP126613
4	*F. oxysporum*	Mošorin	4.0×10^5	KP126610
5	*F. tricinctum*	Veliki Radinci	5.0×10^5	KP126607
6	*F. sporotrichioides*	Veliki Radinci	5.0×10^5	KP126611
7	*F. equiseti*	Ostojićevo	4.0×10^5	KP126609
8	*F. incarnatum*	Ostojićevo	2.0×10^5	KP126612
9	*Macrophomina phaseolina*	Mošorin	1.0×10^5	KP281809
10	*F. proliferatum*	Mošorin	3.0×10^5	KP126608

Antifungal assay *in vitro*

In this paper we examined the antifungal activity of EOs from spearmint (*Mentha spicata* L.), Greek sage (*Salva fruticosa* L.), rosemary (*Rosmarinus officinalis* L.), anise (*Pimpinella anisum* L.), bitter fennel (*Foeniculum vulgare* spp. *piperituum* L.) and myrtle (*Myrtus communis* L.). The inhibitory activity of these oils on the growth of fungi was tested on the following species: *Bipolaris/Drechslera sorociniana, Fusarium subglutinans, F. vertricilioides, F. oxysporum, F. tricinctum, F. sporotrichioides, F. equiseti, F. incarnatum, Macrophomina phaseolina* and *F. proliferatum.*

Results are shown on Table 3., Fig.1 and 2. The essential oils exerted varying levels of antifungal effects against fungal pathogens. Essential oils MIC values were in the range of 0.0003–10 mg/mL. Among the oils tested, myrtle EO proved to be the best inhibitor of all tested fungal isolates in concentrations between 0.0003 and 3.25 mg/mL, followed by spearmint (0.003–7.25 mg/mL) and Greek sage (0.003–10 mg/mL) which manifested a similar effect on all fungal species, although somewhat lower towards *F. verticilioides.*

The antifungal potential of oil tested can be presented as: *Myrtus communis* > *Mentha spicata* > *Salva fruticosa* > *Pimpinella anisum* > *Foeniculum vulgare* spp. *piperituum* > *Rosmarinus officinalis. F. vertricilioides* (MIC: 0.325–10 mg/mL) was the most resistant to the tested EOs, whilst the most sensitive was *Bipolaris/Drechslera sorociniana* (MIC: 0.000303–0.325 mg/mL).

According to the data from Jayant and Sankunny (2014) essential oil from *Rosmarinus officinalis, Salvia fruticosa, S.*

TABLE 3: *Antifungal activity of the essential oils is expressed through the minimal inhibitory concentrations (mg/mL).*

Fungal species	Mint	Sage	Rosemary	Anise	Bitter Fennel	Myrtle
Bipolaris/Drechslera sorociniana	0.003025^{d*}	0.003025^{d}	0.03025^{d}	0.325^{d}	0.000303^{d}	0.000303^{d}
Fusarium subglutinans	0.05275^{d}	2.8^{cd}	3.25^{cd}	0.0775^{d}	1^{d}	0.00325^{d}
Fusarium vertricillioides	7.75^{ab}	10^{a}	0.325^{d}	0.325^{d}	10^{a}	3.25^{cd}
Fusarium oxysporum	0.0775^{d}	0.0775^{d}	7.75^{ab}	0.55^{d}	10^{a}	0.55^{d}
Fusarium tricinctum	0.055^{d}	0.055^{d}	10^{a}	0.325^{d}	0.0325^{d}	0.00055^{d}
F. sporotrichioides	0.0055^{d}	0.0055^{d}	0.0505^{d}	0.055^{d}	0.01^{d}	0.001^{d}
F. equiseti	0.001^{d}	0.001^{d}	0.55^{d}	5.5^{bc}	0.775^{d}	0.0575^{d}
F. incarnatum	0.55^{d}	0.55^{d}	7.75^{ab}	7.75^{ab}	7.75^{ab}	0.055^{d}
Macrophomina phaseolina	0.05275^{d}	0.05275^{d}	7.75^{ab}	0.325^{d}	0.775^{d}	0.0775^{d}
F. proliferatum	0.325^{d}	0.325^{d}	10^{a}	7.75^{ab}	3.25^{cd}	0.3025^{d}

*Values of MIC, followed by the same letter are nost significantly different ($p < 0,05$), according to Duncan's multiple range test.

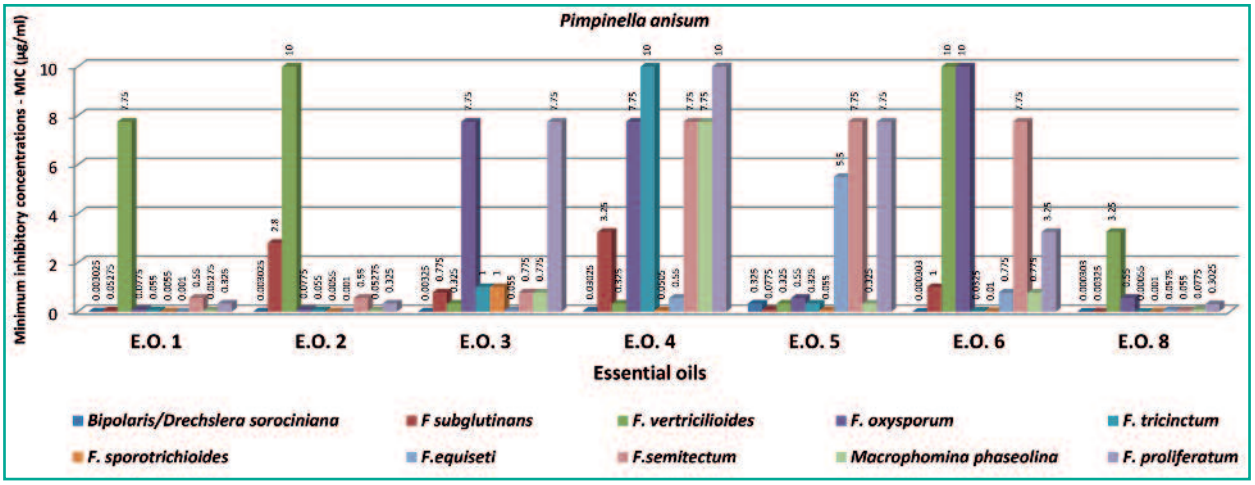

FIGURE 1: *Antifungal activity of the essential oils is expressed through the minimal inhibitory concentrations (mg/mL) to the investigation fungi*

officinalis and *S. rosifolia* have all demonstrated bioactive properties, especially antifungal activity to the *Fusarium oxysporum, F. moniliforme, F. solani* and *F. proliferatum.* Özcan and Chalchat (2008) reported that EO from rosemary *(Rosmarinus officinalis)* have inhibitory activity to the *F. oxysporum.* Results from Ibrahim and Ebady (2014) studies mark the MIC values of rosemary EO as 36.8 mg/ml for *Fusarium* spp., which is up to 5 times more than MIC values in our study to the same genus of fungi.

Anise oil was found to inhibit the growth of 8 *Fusarium* species in concentrations between 0.7 and 2.2 mg/mL (Stevic et al., 2014), while we found inhibitory concentrations against 7 *Fusarium* species between 0.05–7.75 mg/mL. Bitter fennel EO has already been reported to express a high inhibitory effect against *F. oxysporum* (Özcan et al. 2006). Combrinck et al. (2011) proved high inhibitory role of *Mentha spicata* oil against numerous fungal pathogens.

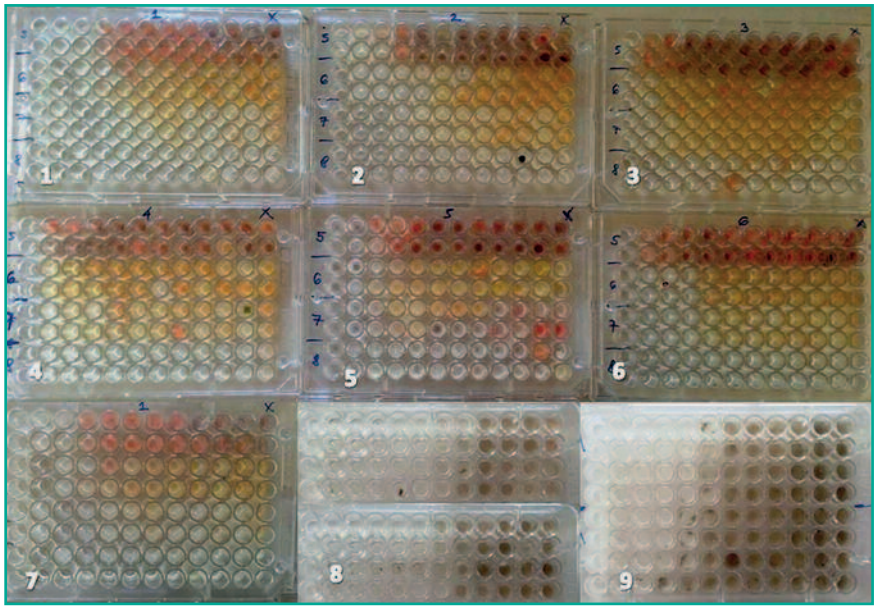

FIGURE 2: *Micro-dilution method: antifungal effect of investigated essential oils (1) F.equiseti (2) F. incarnatum (3) F. verticilioides (4) F. subglutinans (5) F. proliferatum (6) F.oxysporum (7) F. sporotrichoides (8) Bipolaris/Drechslera sorociniana (9) Macrophomina phaseolina.*

The antifungal activity of the EOs from this study can be explained by the high presence of the major oil constituents: 1,8-cineole, linalool, estragole, carvone and anethole as already mention Özcan et al. (2006), Özcan and Chalchat, (2008) and Nikolic et al. (2014). Hung et al. (2010) and Stevic et al. (2014) explained the high activity of anise oil with significant presence of *trans*-anethole. Anise seed oil, sage and fennel are some of the examples of important EOs as already showed by Hussain et al. (2008) and Hammer and Carson (2011).

The results obtained *in vitro* could be useful from the practical point of view. An opportunity to test these results *in vivo,* by seed dressing with EOs investigated here, can lead to better monitoring of seedlings' health.

Conclusions

The selected essential oils exhibited valuable antifungal activity against all tested organisms that are known as food pathogens. In accordance with the earlier reports the results of the present work suggested that some essential oils can be applied as mould inhibitors to prevent growth of toxigenic fungi. A food product requires a very low initial microbial load and inhibition during the production period for an adequate shelf-life. Additionally, a combination of essential oils may provide an effective mean for the inactivation of pathogenic and spoilage microorganisms. These results suggest the potential use of some essential oils as antifungal agents. Further studies on the combined effects of many local plant essential oils is required.

Conflict of interest

The authors have no conflict of interest and confirm that all the information is true and correct.

Acknowledgements

The authors extend their appreciation to the International Scientific Partnership Program ISPP at King Saud University for funding this research work through ISPP# 0015.

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