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Composition and Antifungal Activity of Essential Oil of *Artemisia sieberi* Bess. on Soil-Borne Phytopathogens

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Abstract: Aerial parts of *Artemisia sieberi* Bess. were collected at flowering stage. The essential oil obtained by means of hydro-distillation and their chemical components were identified by GC-MS. The major components of the oil of *A. sieberi* found to be β-thujone (19.79%), α-thujone (19.55%), camphor (19.55%), verbenol (9.69%), *p*-mentha-1,5-dien-8-ol (6.39) and davanone (5.79%). The antifungal activity of the essential oil was evaluated *in vitro* against four soilborn phytopathogenic fungi. The oil was slightly effective against *Tiarosporella phaseolina*, *Fusarium moniliforme* and *Fusarium solani* whereas against *Rhizoctonia solani* exhibited high antifungal activity.

Key words: *Artemisia sieberi*, essential oil components, antifungal activity, soil-borne phytopathogens

INTRODUCTION

The genus *Artemisia* is one of the largest and widely distributed genus of the family *Asteraceae*. Thirty-four species of this genus are found wild all over Iran. Among them, *Artemisia sieberi* Bess. is widely distributed in desert area of Iran (Mozafarian, 1988, 1996). In Iranian folk medicine, some *Artemisia* species are used for their various medicinal properties. Local people used aerial parts of *A. sieberi* for their antiviral and spasmolytic effects (Zargari, 1996; Ramezani *et al*., 2005). Essential oil of *A. sieberi* from Semnan province of Iran have been studied previously and the main components found to be comphor (49.3%), 1,8-cineole(11.1%) and bornyl acetate (5.8%) (Sefidkon *et al*., 2002). Camphor (44%), 1, 8-cineole (19%) and camphene (5%) were the main components of the oil of *A. sieberi* from north of Tehran (Weyerstahl *et al*., 1993). Studies on the antifungal activity of essential oils and plant extracts to the development of new agents for pest and disease control in agriculture have been reported by several authores (Juteau *et al*., 2002a, b; Shimoni *et al*., 1993; Muller-Riebau *et al*., 1995 ; Zambonelli *et al*., 1996; Carta *et al*., 1996; Regnault-Roger and Hamraoui, 1994). Many *Artemisia* species are rich sources of various types of biologically active compounds and possess antifungal, antibacterial and antioxidant activities (Dikshit and Husian, 1984; Wang *et al*., 1990; Swiader and Lamer-Zarawska, 1996; Burits *et al*., 2001; Juteau, 2002a, b; Vajs *et al*., 2004; Ramezani *et al*., 2005). The aim of this investigation was to study the composition of essential oil of *A. sieberi* from south of Khorasan province of Iran and its biological activity on some soil-borne pathogens.

MATERIALS AND METHODS

Plant material: Aerial parts of *A. sieberi* were collected on 30 October 2005 at full flowering stage from south of Khorasan province of Iran, Bajestan at an altitude of 1300 m. Plant materials were dried at ambient temperature and shade condition. Voucher specimen have been deposited in herbarium of the Faculty of Horticulture and Plant Protection, University of Tehran, Karaj, Iran.

Oil isolation procedure: The essential oil of air-dried samples (100 g) of each species was isolated by hydrodistillation for 3 h, using a Clevenger-type apparatus. The distillated oils were dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4°C until the analysis and tests.

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GC analysis: GC analysis was performed by using a Thermoquest gas chromatograph with a Flame Ionization Detector (FID). The analysis was carried out using fused silica capillary DB-1 column (60 m×0.25 mm i.d.; film thickness 0.25 μm). The operating conditions were as follows: Injector and detector temperatures were 250 and 300°C, respectively. Nitrogen was used as carrier gas at a flow rate of 1 mL min; oven temperature programme, 60-250°C at the rate of 5°C min^{-1} and finally held isothermally for 10 min.

GC-MS analysis: GC-MS analysis was performed by using Thermoquest-Finnigan gas chromatograph equipped with above mentioned column and coupled to a TRACE mass quadrapole detector. Helium was used as carrier gas with ionization voltage of 70 ev. Ion source and interface temperatures were 200 and 250°C, respectively. Mass range was from m/z 43-456. Gas chromatographic conditions were as given for GC.

Identification of compounds: The constituents of the essential oil were identified by calculation of their retention indices under temperature-programmed conditions for *n-alkanes* (C_6-C_{24}) and the oil on a DB-1 column under the same chromatographic conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature (Vajs *et al*., 2004). For quantification purpose, relative area percentages obtained by FID were used without the use of correction factors.

Fungal strains and media: Strains of the phytopathogenic fungi *Rhizoctonia solani* Kuehn., *Tiarosporella phaseolina* (Tassi)Aa., *Fusarium moniliforme* Sheld., *Fusarium solani* (Mart.) Sacc. Cultures of the phytopathogenic organisms were maintained on Potato Dextrose Agar (PDA) medium.

Antifungal assays: The antifungal tests were carried out *in vitro* according to the method described by Pitarokili *et al.* (2003) using Petri dishes 8 cm in diameter, containing PDA. The concentrations tested were 150, 250, 500, 1000, 1500 and 2000 μL L^{-1}. Mean mycelial growth were calculated from five replicates of each fungal species every 24 h until fungi in control filled the Petri dishes completely. The measurements were used to determine the Minimum Inhibitory Concentration (MIC) and the EC50 values (concentration causing 50% inhibition of mycelial growth on control media). Fungi toxicity was expressed in term of percentage of mycelial growth inhibition. EC50 values were calculated from the data subjected to probit analysis (statistical software SPSS 10.0 Inc., Chicago, IL). To ascertain if the essential oils showed a fungicidal or fungistatic activity, fungal disks from plates without mycotic growth were transferred into new PDA plates; no fungal growth after an incubation of 10 days was indicative of fungicidal activity.

RESULTS

Identification of compounds: The oil yield of *A. sieberi* obtained 1.4% (w/w). This oil had a pale yellow color and thirty one compounds were identified making a total of 94.8% of the oil. Oxygenated monoterpenes (78.2%) were found to be present in high concentration. β-thujone (19.8%), camphor (19.5%), α-thujone (10.6%), verbenol (9.7%), *p*-mentha-1,5-dien-8-ol(6.4%) and 1,8-cineole (5.7%) were the major identified compounds (Table 1).

Table 1: Percentage of essential oil compositions of *A. sieberi* from Iran

Compound	RI	*A. sieberi*
Tricyclene	927	0.2
α-Pinene	936	2.5
Camphene	950	3.6
Sabinene	971	0.4
β-Pinene	978	0.3
Myrcene	985	0.8
α-Phellandrene	1001	0.6
α-Terpinene	1014	0.3
p-Cymene	1017	1.2
1,8-Cineol	1027	5.7
γ-Terpinene	1053	0.7
trans-Linalool oxide	1076	0.8
Linalool	1089	0.5
Hotrienol	1091	0.2
α-Thujone	1096	10.6
β-Thujone	1108	19.8
Camphor	1133	19.5
Verbenol	1139	9.7
p-Mentha-1,5-dien-8-ol	1154	6.4
p-Cymene-8-ol	1167	0.2
4-Terpineol	1170	0.9
α-Terpineol	1180	1.0
Myrtenol	1188	0.2
Nordavanone	1211	0.4
Carvone	1224	0.3
cis-Chrysanthenyl acetate	1250	0.7
α-Terpinenyl acetate	1338	0.5
(*E*)-Jasmone	1365	t
(*Z*)-Jasmone	1377	0.2
Zingiberene	1490	0.3
Davanone	1569	5.8
(*E*)-Sesquilavandulol	1617	0.5
Monoterpene hydrocarbons		9.9
Oxygenated monoterpenes		78.1
Sesquiterpene hydrocarbons		0.5
Oxygenated sesquiterpenes		6.3
Other		-
Total		94.8

RI, retention indices relative to C_6-C_{24} *n*-alkanes on the DB-1 column; t, trace <0.1%

Table 2: Inhibition of mycelial growth (Percent of control) at different concentrations of the essential oil of *A. sieberi*

	150	250	500	1000	1500	2000
Funguse	(μL L^{-1})					
Tp*	13.61	23.21	73.45	83.29	97.94	100
Rs	21.61	49.86	100	100	100	100
Fm	19.95	36.96	65.72	85.24	100	100
Fs	4.02	19.99	87.30	89.18	100	100

*Tp = *Tiarosporella phaseolina*, Rs = *Rhizoctonia solani*, Fm = *Fusarium moniliforme*, Fs = *Fusarium solani*

Table 3: Minimum Inhibitory Concentration (MIC) values (μL L^{-1}) of the oil of *A. sieberi*

	Tiarosporella phaseolina	*Rhizoctonia solani*	*Fusarium moniliforme*	*Fusarium solani*
A. sieberi	2000	500	1500	1500

Table 4: EC50 values (μL L^{-1}) of the oil of the *A. sieberi* on fungal species tested

	Tiarosporella phaseolina	*Rhizoctonia solani*
A. sieberi	406.839 (291.368-546.304)*	243.596 (228.030-261.484)
	Fusarium moniliforme	*Fusarium solani*
A. sieberi	422.144 (242.318-657.712)	374.268 (241.396-548.184)

*Numbers in parantheses indicate 95% confidence limits determined by probit analysis

Antifungal activity: The antifungal activities of the essential oil of *A. sieberi* was studied against *R. solani*, *T. phaseolina*, *F. moniliforme* and *F. solani*. A dose-dependent inhibition was observed by the oil for all examined phytopathogenic fungi (Table 2 and 3). Mycelial growth of *R. solani*, which found to be the most sensitive fungus, was completely inhibited at a concentration of 500 μL L^{-1} (Table 2). The oil of *A. sieberi* showed fungistatic activity against *T. phaseolina* (MIC = 2000, EC50 = 406.839), *F. moniliforme* (MIC = 1500, EC50 = 422.144) and *F. solani* (MIC = 1500, EC50 = 374.268), whereas against *R. solani* (MIC = 500, EC50 = 243.596) exhibited high fungicidal activity (Table 3 and 4).

DISCUSSION

β-thujone, camphor, α-thujone, verbenol and 1, 8-cineol were previously reported as predominant components in the oil of other *Artemisia* from Iran (Khazraei-Alizadeh and Rustaiyan, 2001; Sefidkon *et al*., 2002; Mohammadpoor *et al*., 2002). There are considerable differences between oil compositions of *A. sieberi* in this study, collected from desert area of Khorasan province and those of previously reported from different parts of Iran (Sefidkon *et al*., 2002; Weyerstahl *et al*., 1993). This variation could be due to genetic and environmental factors and development of different chemotypes within natural populations of *A. sieberi* in Iran. Therefore a comprehensive study is necessary for identifying of these chemotypes.

Antifungal and antibacterial activity of β-thujone, camphor, α-thujone, davanone and 1, 8-cineole as major components of *A. sieberi* were reported in different studies (Carson and Riley, 1995; Pattnaik *et al*., 1997; Sivropoulou *et al*., 1997; Griffin *et al*., 1999; Saikia *et al*., 2001; Kalemba *et al*., 2002; Ramezani *et al*., 2005; Shafi *et al*., 2004). Therefore antifungal activity of essential oil of *A. sieberi*, observed in this study, can be attributed to the presence of these compounds in the oil and their synergistic effects. This idea can examine by antifungal evaluation of different fractions of the oil especially against *R. solani* as the most sensetive fungi. Further *in vivo* experiments is also necessary for testing the antifungal activity of the oil of *A. sieberi* against *R. solani*.

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