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Table: 3, Figs : 3

Chemical composition of essential oils and their effects on biochemical parameters in seeds and seedlings of caraway (*Carum carvi* L.)

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ABSTRACT

We studied the composition of caraway, fennel (*Foeniculum vulgare* Mill.) and marjoram (*Origanum majorana* L.) oils and their effects on the biochemical response of caraway (*Carum carvi* L.). Essential oils obtained by steam distillation of their seeds were analyzed using gas chromatography-mass spectrometry. The main components of caraway, fennel and marjoram oils were : carvone, (E)-anethole and γ-terpinene, respectively. Caraway seeds and seedlings were subjected to continuous influence of each essential oils present in the air at different concentrations (0.625%, 1.25%, 2.5% and 5% v/v). The contents of proline and total polyphenols were determined in caraway seeds and seedlings after 48 and 240 h, respectively. Additionally, contents of pigments (chlorophyll *a*, chlorophyll *b* and carotenoids) were measured in caraway seedlings (240 h after sowing). The proline and polyphenol contents increased in both seeds and seedlings. However, essential oils (except caraway oil) decreased the pigment contents in seedlings. The observed effects increased with the concentration of essential oils. The fennel oil had the strongest allelopathic properties.

Key words: Allelopathic effect, caraway, *Carum carvi*, essential oils, fennel, GCMS, germination, marjoram, photosynthetic pigments, polyphenols, proline, seeds, seedling growth, volatiles.

INTRODUCTION

Essential oils are the products of secondary metabolism of plants. Their major components are group of organic substances like terpenes. These compounds are isoprene oligomers and occurs naturally. Chemically, essential oils are complex and multicomponent mixtures of monoterpene and sesquiterpene compounds and their derivatives, including aromatic derivatives. They may include alcohols, ketones, aldehydes, esters and ethers (8) as well as chemical compounds containing nitrogen and sulfur. One of their main features is that single oil may contain numerous compounds of different concentration and properties (31).

In the last few decades, essential oils have also been explored in agriculture as source of natural pesticides [fungicides (26) and insecticides (1,23)]. Phytotoxic potential of essential oils has gained more attention recently (41). Amri *et al*. (5) listed more than 80 essential oils and over 50 constituents with phytotoxicity. Due to the complexity and structural diversity of their constituents, essential oils affect many physiological and biochemical processes in treated plants. Essential oils cause, inter alia, allelochemical stress (4,34). Allelopathic interaction is a process in which a plant (donor) secretes

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chemical substances in to the environment to modify growth and development of other plants (acceptors). The interactions may result in inhibition or stimulation, however, the most common effect of interactions described in the literature is inhibition, which mainly causes a negative influence on seed germination and seedlings growth (20,47). In acceptor plants, the essential oils cause pronounced physiological response: (i). inhibits the seed germination and root growth in seedlings (2,16,30,36,38,41,51), (ii). influences the photosynthetic pigments synthesis (10) and (iii). thereby reduces the photosynthetic activity (21) and (iv). may induce oxidative stress. Monoterpenes in essential oils blocks the mitosis and inhibits the cell elongation, while cineol causes deformation of plant cells (46,51). Both essential oils and their individual components affects the level of enzymatic and non-enzymatic antioxidants (18). The proline accumulates during the exposure of plants to adverse environmental conditions. Proline metabolism may also contribute to reactive oxygen species formation in mitochondria, which plays role in hypersensitive response in plants (35). Therefore, this study aimed to investigate the chemical composition and phytotoxic activity of caraway, fennel and marjoram essential oils on seeds germination and seedlings growth of caraway.

MATERIALS AND METHODS

I. Extraction of essential oils: The seeds and aerial parts of caraway, fennel and marjoram were dried at 30 °C in laboratory dryer at constant air flow rate of 0.5 m/s for 48 h. The thickness of the dried raw material layer was 1 cm (43). Then the dried materials were powdered and subjected to water distillation using a Clevenger-type apparatus (15). The obtained oils were dried over anhydrous sodium sulfate and after filtration, stored at +4 °C until tested and analyzed.

II. GCMS analysis of essential oils: The essential oils were analyzed using Varian Chrompack CP-3800 GC equipped with mass-selective detector 4000 GC/MS/MS and flame ionization detector (FID). The separation was achieved by using capillary column, VF-5ms (30 m × 0.25 mm, film thickness 0.25 μm). The column temperature was kept at 50 °C for 1 min and programmed to 250 °C at a rate of 4 °C/min. Helium was the carrier gas, at a flow rate of 0.5 ml/min. A sample of 0.1 μl of essential oil was injected manually (in split ratio 1:100). The temperature of injector was 250 °C. Kovats retention indices (non-isothermal Kovat's retention indices) were determined based on series of alkanes C10-C40.

III. Bioassay : Caraway seeds (*Carum carvi* L.) were placed in 5 cm petri dishes in 5 l phytotrons [photoperiod 12 h light/12 h dark, 20 °C, photosynthetically active radiation (PAR) : 700 $\mu mol \cdot m^{-2}$ and relative humidity (RH): 70%]. To differentiate the concs of oils in the environment of plants, oils were dissolved in non-volatile silicone oil and the concentrations (% v/v) were prepared based on the geometric sequence with common ratio of 2. A blank test with silicone oil was done to find out that it was neutral to the tested plants. Glass volumetric flasks with the oil solution in 20 ml silicone oil were placed in each of the chambers excluding control one. The amounts of oil were as under: 0.125 ml, 0.250 ml, 0.500 ml, 1 ml to obtain the 0.625, 1.25, 2.5 and 5.0 % (v/v) concentrations, respectively. The seeds were kept under the constant influence of oil in the atmosphere for 14 days. The tests were done thrice after 48 h for seeds and 240 h for seedlings.

IV. Estimation of proline content : Proline concentration in seeds and seedlings was measured as per Bates *et al*. (9). Plant tissue was homogenized in 10 ml of aqueous 3 % sulfosalicylic acid. The homogenate was filtered through Whatman No. 2 filter paper. A 2 ml filtrate was pipetted into a test tube with 2 ml of glacial acetic acid and 2 ml of ninhydrin solution (1.25 g of ninhydrin dissolved in 30 ml of glacial acidic acid and 20 ml of 6 M phosphoric acid). The tubes were then placed in a water bath (100 °C) for 1 h. The reaction was stopped by cooling the tubes to room temperature and adding 4 ml toluene. Aqueous and toluene phases were separated after vigorous shaking. The absorbance of toluene phase was measured spectrophotometrically at 520 nm using toluene as a blank with UV-1601 PC (Shimadzu). The proline concn was calculated from a standard curve.

V. Total polyphenol content : One g of seeds or seedlings were treated with 10 cm^3 methanol at room temperature with stirring to extract the total polyphenols. This procedure was repeated at least five times until the extraction solvent became colourless. The obtained extracts were filtered over Whatman No. 1 and dissolved in methanol in 50 ml volumetric flask (27). The total polyphenol content of seed and seedling extracts was determined using Folin-Ciocalteu reagent (48). The extracts (0.1 ml) were mixed with 0.5 ml of the Folin-Ciocalteu reagent and 1.5 ml of 20 % sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml using distilled water. Then the absorbance was determined at 765 nm using UV-1800 spectrophotometer (Shimadzu). These data were used to estimate the total polyphenol contents for a standard curve obtained from various concentrations of gallic acid equivalents (GAE).

VI. Chlorophyll and carotenoid content : Chlorophyll and carotenoid contents were determined according to Arnon *et al*. (6) with modification from Lichtenthaler and Welburn (28). About 500 mg of caraway seedlings from all treatments were extracted with 80 % aqueous acetone in a mortar using a pestle and the suspension was filtered through a Whatman No. 2. Chlorophylls *a* and *b* and carotenoid contents were determined spectrophotometrically using UV-1800 spectrophotometer (Shimadzu) at three wavelengths: 663 nm for chlorophyll *a*, 645 nm for chlorophyll *b* and 440 nm for carotenoids.

VII. Statistical analysis : The proline, total polyphenols and photosynthetic pigments concentration were analyzed statistically using Statistica 13.1 (StatSoft Inc.) for seeds and seedlings separately. Two-way analysis of variance was performed for oil as a first factor and concentration as a second factor. The differences among the means were determined for significance at $p < 0.05$ using Tukey's test. The obtained $LSD_{0.05}$ was shown on the graphs.

RESULTS AND DISCUSSION

I. Analysis of essential oils : Quantitative composition of examined oils varied little in fennel oil. Its predominant ingredient was E-anethol [70 % of total (Table 2)]. In caraway oil, its two main components : Carvone 51.13 % and limonene 38.60 % had major share (Table 1). The most diverse was marjoram oil, which contained 18 % of terpinen-4-ol, 12 % of ɣ-terpinene, while all others < 10 % (Table 3). Similar results of caraway, fennel and marjoram oils compositions were reported earlier (4,17,40,42).

Table 1. Qualitative and quantitative analysis of caraway oil (n=3)

Compounds	GC/MS			GC/FID		
	RI	Area [%]	SD	RI	Area [%]	SD
α-Pinene	931	Tr	0.00	(-)	Tr	0.00
Sabinene	971	Tr	0.00	(-)	Tr	0.00
β-Pinene	976	Tr	0.00	(-)	Tr	0.00
Myrcene	988	0.06	0.00	(-)	Tr	0.00
N.I.	1004	0.07	0.01	(-)	Tr	0.00
o-Cymene	1021	0.11	0.00	(-)	Tr	0.00
Limonene	1027	38.60	0.82	1016	33.21	0.08
γ-Terpinene	1976	Tr	0.01	(-)	Tr	0.00
p-Cymene	1081	Tr	0.01	(-)	Tr	0.00
Linalool	1089	Tr	0.00	(-)	Tr	0.00
N.I.	1102	Tr	0.00	(-)	Tr	0.00
trans-*p*-menth-2,8-dien-1-ol	1112	0.42	0.01	1106	0.11	0.01
cis-Limonene oxide	1124	1.09	0.01	1120	0.50	0.01
trans-Limonene oxide	1129	1.10	0.01	1124	0.31	0.02
cis-β-Terpineol	1143	Tr	0.00	(-)	Tr	0.00
N.I.	1165	0.07	0.00	(-)	Tr	0.00
β-Pinene oxide	1176	0.07	0.00	(-)	Tr	0.00
trans-*p*-metnha-1,7(8)-dien-2-ol	1187	0.11	0.00	(-)	Tr	0.00
N.I.	1196	0.31	0.00	1185	0.53	0.00
cis-Dihydrocarvone	1199	1.	0.01	1189	0.15	0.01
trans-Dihydro Carvone	1205	0.28	0.00	1193	0.12	0.00
trans-Carveol	1222	0.78	0.04	1208	0.25	0.02
cis-*p*-metnh-1,7(8)-dien-2-ol	1234	0.05	0.01	-	Tr	0.00
cis-Carveol	1237	0.35	0.01	1221	0.11	0.02
Carvone	1251	51.13	0.55	1236	64.07	0.00
N.I.	1264	0.07	0.00	(-)	Tr	0.00
cis-oxide-Carvone	1266	Tr	0.00	(-)	Tr	0.00
N.I.	1272	0.05	0.00	(-)	Tr	0.00
trans-Carvone oxide	1280	0.48	0.01	1270	0.19	0.01
cis-Verbenyl acetate	1286	Tr	0.00	(-)	Tr	0.00
(E)-Anethole	1289	0.05	0.00	(-)	Tr	0.00
Limonen-10-ol	1293	0.10	0.00	(-)	Tr	0.00
N.I.	1301	0.07	0.00	(-)	Tr	0.00
Perilla alcohol	1303	0.10	0.01	(-)	Tr	0.00
(Z)-patchenol	1310	0.22	0.02	1316	0.11	0.03
N.I.	1323	0.19	0.01	(-)	Tr	0.00
(E)-Patchenol	1334	0.12	0.00	1333	0.07	0.14
N.I.	1348	0.39	0.01	(-)	Tr	0.00
N.I.	1361	0.22	0.02	(-)	Tr	0.00
N.I.	1375	0.60	0.01	1368	0.19	0.01
N.I.	1378	0.11	0.00	(-)	Tr	0.00
N.I.	1383	0.13	0.01	1374	0.09	0.00
N.I.	1410	0.27	0.03	(-)	Tr	0.00
N.I.	1414	0.23	0.02	(-)	Tr	0.00
N.I.	1478	0.06	0.00	(-)	Tr	0.00
N.I.	1525	0.10	0.00	(-)	Tr	0.00
Caryophyllene oxide	1584	Tr	0.00	(-)	Tr	0.00
Total	-	99.71	-	-	100.00	-

SD: Standard deviation, Tr : Content below 0.05%, (-) : Value not calculated and not data, N.I. : Not identified , RI : Kovats retention index

Table 2. Qualitative and quantitative analysis of fennel oil (n=3)

Compounds	GC/MS			GC/FID		
	RI	Area [%]	SD	RI	Area [%]	SD
Tricyclene	921	Tr	0.00	(-)	Tr	0.00
α-Thujone	924	Tr	0.00	(-)	Tr	0.00
α-Pinene	931	4.10	0.05	929	3.25	0.02
Camphene	948	0.12	0.00	943	0.08	0.00
Thuja-2,4(10)-diene	951	Tr	0.00	(-)	Tr	0.00
Sabinene	971	0.05	0.00	(-)	Tr	0.00
β-Pinene	976	0.11	0.00	968	0.07	0.00
Myrcene	988	0.28	0.00	978	0.20	0.00
α-Phellandrene	1005	0.27	0.00	982	0.14	0.00
α-Terpinene	1014	Tr	0.00	(-)	Tr	0.00
p-Cymene	1021	0.60	0.01	1010	0.29	0.00
Limonene	1025	2.62	0.02	1015	2.31	0.01
β-Phellandrene	1031	Tr	0.00	1036	Tr	0.00
ɣ-Terpinene	1051	0.28	0.00	1044	0.15	0.00
cis-sabinene hydrate	1062	Tr	0.00	(-)	Tr	0.00
Terpinolene	1076	Tr	0.00	(-)	Tr	0.00
Fenchone	1080	9.04	0.05	1075	12.69	0.07
α-Pinene oxide	1089	Tr	0.00	(-)	Tr	0.00
Fenchol-endo	1109	Tr	0.00	(-)	Tr	0.00
Fenchol-exo	1114	Tr	0.00	(-)	Tr	0.00
trans-Pinene hydrate	1117	Tr	0.00	(-)	Tr	0.00
trans-*p*-metnh-2,4(8)-diene-1-ol	1129	Tr	0.00	(-)	Tr	0.00
trans-Pincarvacol	1133	Tr	0.00	(-)	Tr	0.00
Iso-3-thujanol	1135	Tr	0.00	(-)	Tr	0.00
Camphor	1141	0.19	0.00	1132	0.11	0.00
Terpinen-4-ol	1178	Tr	0.01	1265	Tr	0.00
Cryptone	1186	Tr	0.01	(-)	Tr	0.00
α-Terpineol	1195	Tr	0.00	(-)	Tr	0.00
Methyl chavicol	1199	2.99	0.03	1186	1.70	0.02
Verbenone	1208	Tr	0.00	(-)	Tr	0.00
trans-carveol	1220	Tr	0.00	(-)	Tr	0.00
Fenchyl acetate	1232	Tr	0.00	(-)	Tr	0.00
Carvone	1246	0.17	0.00	1234	0.09	0.00
Arvotanacetone	1251	Tr	0.00	(-)	Tr	0.00
(Z)-Anethole	1255	0.19	0.01	(-)	Tr	0.00
p-Anisaldehyde	1259	1.78	0.00	1244	1.05	0.00
(E)-Anethole	1295	74.13	0.00	1280	78.71	0.11
Carvacrol	1303	Tr	0.00	(-)	Tr	0.00
N.I.	1361	0.24	0.02	1363	0.11	0.00
α-Copaene	1378	Tr	0.00	(-)	Tr	0.00
N.I.	1381	0.82	0.11	1375	0.07	0.01
N.I.	1384	1.59	0.07	1378	0.97	0.01
(E)-Caryophyllene	1422	Tr	0.00	(-)	Tr	0.00
α-Trans-bergamotene	1437	Tr	0.00	(-)	Tr	0.00
Myrysticin	1531	Tr	0.00	(-)	Tr	0.00
total	-	99.57	-	-	100.00	-

SD : Standard deviation, Tr : Content below 0.05%, (-) : Value not calculated and not data, N.I. : Not identified , RI : Kovats retention index

Table 3. Qualitative and quantitative analysis of marjoram oil (n=3)

Compounds	GC/MS			GC/FID		
	RI	Area [%]	SD	RI	Area [%]	SD
α -Thujone	924	0.81	0.00	923	0.35	0.01
α -Pinene	931	1.25	0.00	929	0.59	0.01
Camphene	948	Tr	0.00	(-)	Tr	0.00
Cabinene	971	6.27	0.01	964	4.75	0.03
β-Pinene	976	0.55	0.01	968	0.40	0.00
3-Octanone	984	Tr	0.00	(-)	Tr	0.00
Myrcene	988	1.56	0.01	978	1.33	0.02
Menth-1(7),8-diene	1003	0.07	0.00	(-)	Tr	0.00
α-Phellandrene	1005	0.84	0.00	992	0.62	0.00
α-Terpinene	1015	8.37	0.01	1003	6.86	0.02
p-Cymene	1022	3.40	0.01	1010	2.21	0.00
Sylvestrene	1026	2.31	0.11	(-)	Tr	0.00
δ-3-Caranene	1027	2.34	0.03	1016	4.18	0.03
1,8-Cineole	1028	2.21	0.09	1018	2.15	0.01
(Z)-β-ocimene	1031	0.07	0.00	(-)	Tr	0.00
(E)-β-ocimene	1041	0.10	0.00	1032	0.07	0.00
ɣ-Terpinene	1052	12.15	0.12	1045	12.56	0.07
cis-Sabinene hydrate	1063	3.48	0.01	1053	3.10	0.01
Terpinolene	1076	3.71	0.02	1074	2.67	0.01
p-Cymenene	1081	Tr	0.00	(-)	Tr	0.00
Linalool	1091	3.82	0.31	(-)	Tr	0.00
1-Terpineol	1092	8.47	0.5	1084	12.72	0.03
Trans-*p*-menth-2,8-den-1-ol	1115	1.57	0.01	1084	12.72	0.03
cis-β-Terpineol	1136	1.05	0.01	1108	0.97	0.01
p-Menthone	1151	Tr	0.00	(-)	Tr	0.00
Borneol	1170	0.09	0.02	1126	0.58	0.00
Terpinen-4-ol	1181	18.14	0.07	1167	23.54	0.13
p-Cymen-8-ol	1187	0.08	0.00	(-)	Tr	0.00
α-Terpineol	1196	4.26	0.09	1179	3.25	0.08
Methyl chavicol	1199	0.48	0.07	(-)	Tr	0.00
trans-Dihydrocarvone	1206	0.05	0.01	(-)	Tr	0.00
trans-Piperitol	1210	0.54	0.00	1185	0.57	0.10
trans-Carveol	1220	Tr	0.00	(-)	Tr	0.00
Neciso-dihydrocarveol	1225	0.09	0.01	1197	0.31	0.01
Citronellol	1228	Tr	0.00	(-)	Tr	0.00
Pulegone	1240	0.06	0.00	(-)	Tr	0.00
Carvone	1248	0.44	0.00	1234	0.20	0.00
Linalyl acetate	1251	3.17	0.08	1244	1.49	0.01
Piperitone	1256	Tr	0.00	(-)	Tr	0.00
trans-Ascaridol glycol	1277	0.28	0.00	1261	0.11	0.00
Bornyl acetate	1287	0.07	0.00	(-)	Tr	0.00
(E)-Anethole	1290	0.12	0.00	(-)	Tr	0.00
Menthyl acetate	1292	Tr	0.00	(-)	Tr	0.00
N.I.	1295	0.21	0.00	(-)	Tr	0.00
Terpinen-4-ol acetate	1299	0.27	0.00	1279	0.11	0.01
Carvarcol	1302	0.12	0.00	(-)	Tr	0.00
N.I.	1325	0.29	0.00	1293	0.12	0.01
δ-Elemene	1333	0.07	0.00	1313	0.09	0.00

α-Terpinnyl acetate	1348	Tr	0.00	(-)	Tr	0.00
Neryl acetate	1360	0.07	0.00	(-)	Tr	0.00
Geranyl acetate	1379	0.12	0.00	(-)	Tr	0.00
β-Elemene	1389	Tr	0.00	(-)	Tr	0.00
Methyl eugenol	1401	Tr	0.00	(-)	Tr	0.00
(Z)-Caroyphyllene	1404	Tr	0.00	(-)	Tr	0.00
α-Gurjunene	1408	Tr	0.00	(-)	Tr	0.00
(E)-Caroyphyllene	1422	3.28	0.02	1421	1.39	0.00
α-Trans-bergamotene	1437	Tr	0.00	(-)	Tr	0.00
Aromadendrene	1444	0.06	0.00	(-)	Tr	0.00
α-Himachalene	1452	Tr	0.00	(-)	Tr	0.00
α-Humulene	1463	0.16	0.00	(-)	Tr	0.00
Allo-Aromadandrene	1468	Tr	0.00	(-)	Tr	0.00
β-Acoradiene	1483	Tr	0.00	(-)	Tr	0.00
Viridiflorene	1503	0.23	0.01	(-)	Tr	0.00
Bicyclogermacrene	1508	1.28	0.00	(-)	Tr	0.00
(E,E)-α-Farnesene	1515	0.07	0.00	(-)	Tr	0.00
ɣ-Cadinene	1523	Tr	0.00	(-)	Tr	0.00
δ-Cadinene	1528	Tr	0.01	(-)	Tr	0.00
Spathulenol	1580	0.23	0.01	(-)	Tr	0.00
Caryphyllene oxide	1584	0.16	0.02	(-)	Tr	0.00
Globulol	1587	Tr	0.00	(-)	Tr	0.00
Viridiflorol	1595	Tr	0.01	(-)	Tr	0.00
(B)-Altantol	1611	0.05	0.00	(-)	Tr	0.00
Selina-3,11-dien-6-α-ol	1640	0.33	0.00	(-)	Tr	0.00
Caryophylla-4(14),8(15)-dien-5-α-ol	1645	Tr	0.00	(-)	Tr	0.00
14-Hydroxy-9-epi-(E)-caryophyllene	1667	Tr	0.01	(-)	Tr	0.00
Glusia-3,10(14)-dien-11-ol	1678	Tr	0.00	(-)	Tr	0.00
Khusinol	1684	Tr	0.00	(-)	Tr	0.00
8-Cedren-13-ol	1689	Tr	0.00	(-)	Tr	0.00
8-α-11-Elemodiol	1732	0.06	0.00	(-)	Tr	0.00
Cembrene	1951	Tr	0.00	(-)	Tr	0.00
Total	-	99.35	-	-	100.00	-

SD : Standard deviation, Tr. : Content below 0.05%, (-) : Value not calculated and not data, N.I. : Not identified , RI : Kovats retention index

II. Proline content: The concentrations of proline in untreated seeds and seedlings of caraway were 0.53 and 0.71 µg/g FM (Fresh matter), respectively. Treatment with essential oils increased the proline content both in seeds and seedlings. The observed effects increased with the increase in essential oil concentration. The highest increase in proline content was observed with application of 5.0 % v/v of essential oils, which for caraway, fennel and marjoram oils was about 82 %, 261 % and 149 % in seeds and 128 %, 286 % and 127 % in seedlings, respectively (Figure 1). These results showed that fennel oil has the largest influence on the proline concentration.

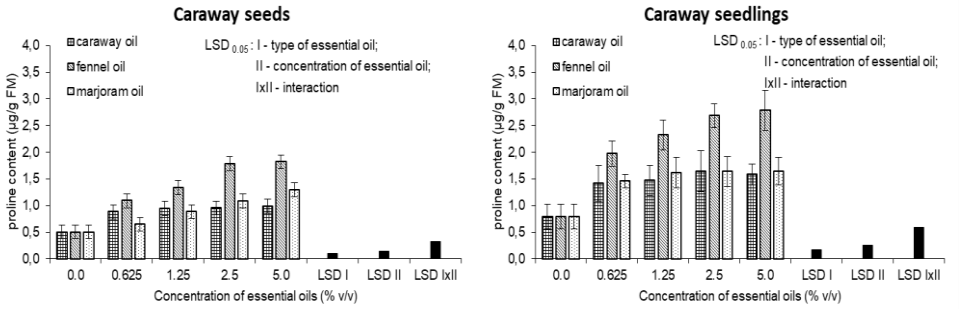

Figure 1. Effects of essential oils on proline content in caraway seeds and seedlings

Zhu *et al*. (50) argued that proline is good indicator of the intensity of stress. Proline is involved in the stabilization of membranes and as carbon and nitrogen source in cell (37,45). Free proline and protein-bound proline is one of the solid components of plant cells. Proteins rich in proline and hydroxyproline are important structural compounds of cell walls in higher plants (13).

III. Total polyphenols contents: The total polyphenol content in caraway seeds without essential oil treatment was 0.72 mg/g FM. It was difficult to clearly evaluate, whether the concentration was high or low because of divergent results in the literature about total polyphenol content in caraway (25,39,44,49). No statistically significant changes were observed in the total polyphenol content in the treatment with caraway or marjoram oils applied in all concentrations, while fennel oil decreased the total polyphenol content, inversely proportional to the concentration of oil. Application of fennel oil at 5.0 % v/v caused 28 % decrease in total polyphenol content than control. A significantly higher concentration of this group of compounds was noted in caraway seedlings, compared to 30 mg/g FM in control.

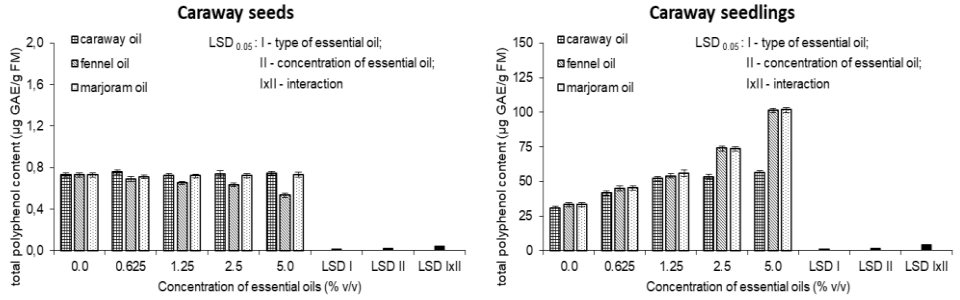

Figure 2. Effects of essential oils on total polyphenol contents in caraway seeds and seedlings

Such high increase in polyphenol content in relation to seeds was because these compounds protects the leaves from UV radiation and photosynthetic apparatus from negative influence of light in the range of visible spectrum. They are also substrates in biosynthesis reactions (for example, caffeic acid is precursor in the biosynthesis of lignin) to protects the plant from the harmful effects of UV (12). According to Cirak *et al*. (14), polyphenols protect the plant cells and its enzymatic systems and increases the plant

resistance to stress, mechanical damage and infections. The total polyphenol content increased with treatment of caraway and fennel oil. This effects increased with the increase in concentration at 5 % v/v dose, it was the highest for the caraway (83 %) and fennel oils (200 %), compared with control. But, application of marjoram oil at 5 % v/v dose statistically increased the total polyphenol content in seeds by 38 % compared to control.

IV. Photosynthetic pigments contents: The concentrations of chlorophyll *a*, *b* and carotenoids in caraway seedlings not treated with essential oils were 490 µg/g, 255 µg/g and 1300 µg/g FM, respectively. The application of caraway oil significantly increased the photosynthetic pigments content at all concentrations (except for chlorophyll *b* at a

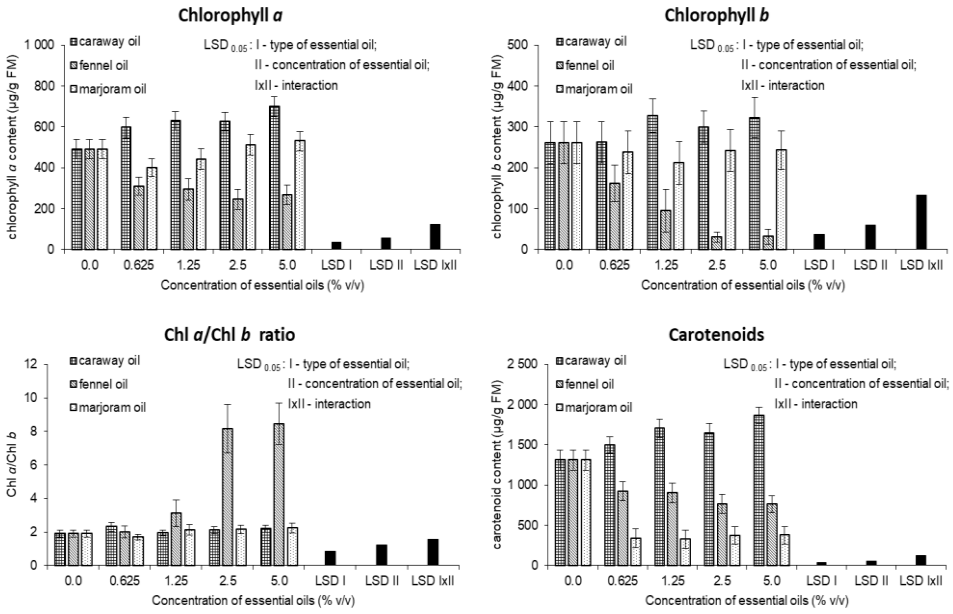

Figure 3. Effects of essential oils on chlorophyll *a* content, chlorophyll *b* content, Chl *a*/Chl *b* ratio and carotenoid content in caraway seedlings

concentration of 0.625 % v/v). At 5.0 % v/v dose, the concentration was highest for chlorophyll *a*, *b* and carotenoids reached 43 %, 24 % and 42 %, respectively, compared to control. An opposite trend was observed after the treatment with fennel oil, which significantly decreased the content of assimilation pigments in caraway seedlings. This effect became more visible with increasing concentration and was 45 %, 88 % and 42 % at 5.0 % v/v dose, for chlorophyll *a*, *b* and carotenoids, respectively, than control. The application of marjoram oils significantly changed the content of chlorophyll *a* only at the concentration of 0.625 % and 1.25 % (stimulation by 18 % and 10 %, respectively, compared to control) and chlorophyll *b* at 1.25 % (inhibition by 19 %, compared to control). On the contrary, the content of carotenoids decreased up to 71-75 % after application of all concentrations of oil. The values of Chl *a*/Chl *b* ratio were very interesting. After the application of caraway and marjoram oils, they were similar to the

control at all concentrations, while fennel oil significantly increased this ratio compared to control. The Chl *a*/Chl *b* ratio in the caraway seedlings for the series with the concentration of 1.25 % was more than 1.5 times higher and for concentrations of 2.5 % and 5 %, it was about 4- times higher than in control seedlings (Figure 3). Changes in Chl *a*/Chl *b* ratio may be due to some disturbances in photosynthesis (11,19,32). Many components of essential oils limits synthesis or degradation of chlorophylls (*a* and *b*) (22).

Our results clearly showed that the caraway, fennel and marjoram oils are allelopathic to seeds and seedlings of caraway. This confirms many studies of phytotoxicity of various essential oils (7,22,29,33,41). Furthermore, the previous research showed that a decrease occurred in the level of two nucleotides (ATP and ADP) and AEC in caraway, affected by increasing concentrations of all essential oils in the atmosphere, whereas catalase and peroxidase activity varied irregularly or was constant (40). Ahuja *et al*. (3) reported that essential oils disrupt both the germination process and growth of seedlings of treated plants. In the seedlings, essential oils or their main compounds, cause oxidative damage mediated by reactive oxygen species (5). The applied essential oils causes visible leaf wilting, within few hours of their application due to membrane disruption (33), decrease in the chlorophyll content and decline in cellular respiration (24).

CONCLUSIONS

The applied essential oils of caraway, fennel and marjoram caused various reactions in seeds and seedling of *Carum carvi* L. The parent oil (i.e., caraway oil) caused intensification of proline synthesis in both seedlings and seeds. This oil slightly changed the total polyphenol content in the seeds, while increased the polyphenols as well as photosynthetic pigments in seedlings. The fennel oil increased the proline content in seedlings but decreased in seeds. In the seedlings, the proline and total polyphenol content increased with the decrease in pigments levels. This effect increased with the increase in the concentration of oil. The marjoram oil increased the proline content and slightly influenced the level of polyphenols in the seeds, while in the seedlings it increased the proline, significantly reduced the carotenoids synthesis and did not influence the level of chlorophyll *a* and *b*. Comparing the impact of individual essential oils on the selected biochemical parameters of caraway seeds and seedlings, the fennel oil had the strongest allelopathic properties.

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