https://doi.org/10.26651/2017-42-1-1110 0971-4693/94 Euro 20.00
Allelopathy Journal 42 (1): 123-134 (September, 2017) *International Allelopathy Foundation* 2017
Table: 4, Figs : 4

Chemical characterization of essential oil and extracts of *Daucus carota* seeds and their antifungal activity against wheat fungi

D. Kataria, K.K. Chahal*, A. Kumar and R. Singh
Department of Chemistry, Punjab Agricultural University, Ludhiana 141004, India
e-mail: drkkchahal@pau.edu

(Received in revised form: July 14, 2017)

ABSTRACT

We assessed the antifungal efficacy of the essential oil and extracts of *Daucus carota* seeds against *Alternaria triticina, Bipolaris sorokiniana* and *Ustilago segetum* var. *tritici.* The essential oil of carrot seeds was obtained by hydrodistillation of hexane, dichloromethane and methanol by Soxhlet extraction. The major and minor compounds identified in carrot seed oil were carotol (52.73), daucol (5.10), daucene (5.68), (E)-β-farnesene (5.40), β-cubebene (3.19), longifolenaldehyde (3.23), β-elimene (3.23), (E)-caryophyllene (1.22), β-bisabolene (2.95) etc. The chemical composition of hexane, dichloromethane and methanol extracts was different. Carotol was the common compound in carrot seed essential oil and extracts. Major compounds were isolated from the carrot seed essential oil by column chromatography. Carrot seed essential oil, isolated compounds and extracts had strong inhibitory effects on spore germination of all tested plant pathogens at 100 to 1000 mg/ml concentrations.

Key words: *Alternaria triticina*, antifungal, *Bipolaris sorokiniana*, carotol, carrot seed oil, daucene, daucol, *Daucus carota***,** extracts, GCMS, spore germination, *Ustilago segetum*.

INTRODUCTION

The essential oils are secondary metabolites produced by plants for their own self defence from pests. They are complex mixtures of 20-60 organic compounds that provide the characteristic odour and flavour to leaves, flowers, fruits, seeds, barks and rhizomes (4). Essential oils are widely used for their pharmacological activities (27). Essential oils from *Daucus* genus (family Apiaceae) possess antioxidant (6), antifungal (35) and medicinal properties (37).

Plants are constantly threatened by numerous pathogenic microorganisms present in their environment and the diseases caused by plant pathogenic fungi causes great losses in crop yield worldwide (25,22). Phytopathogens viz., *Alternaria triticina* (leaf blight of wheat), *Bipolaris sorokiniana* (Spot blotch of wheat) and *Ustilago segetum* var. *tritici* (loose smut of wheat) reduces quality and yields of wheat. Widespread use of pesticides has significant drawbacks like cost, handling hazards, pesticide residues, threats to human health and the environment (29). Now there are > 113 commercial fungicides worldwide (18) but the fungi have developed resistance (7). Public awareness of these factors has increased interest in finding safer alternative protectants to replace synthetic chemical pesticides by natural product or green pesticides, which are biodegradable and efficient in pest management (10,21,22).

*Corresponding author

Essential oil from carrot seeds possesses strong antimicrobial activity against fungi (*Candida albicans* and *Alternaria alternate*) and bacteria *i.e. Staphylococcus aureus (*14,15, 20). Medicated soap using carrot seeds oil is successful treat the fungal infestations caused by *Trichophyton rubrum* (1). This study aimed to identify compounds present in essential oil of *D. carota* and their antifungal potential.

MATERIALS AND METHODS

Carrot seeds (var. PC-34) were purchased from Directorate of seeds of our University. The fresh seeds of carrot were dried, powdered and dipped in water overnight. The essential oil was extracted using Dean and Stark apparatus by refluxing the contents for 15 h. The carrot seed essential oil layers were pooled in a conical flask and partitioned thrice using diethyl ether. The diethyl ether layer (upper layer) containing carrot seed essential oil was stored over anhydrous sodium sulphate to remove traces of moisture present, if any.

Soxhlet extraction

The Powdered solid material was packed in a thimble and placed in the Soxhlet extractor. An organic solvent (hexane/dichloromethane /methanol separately) in the round bottomed flask was heated and refluxed, which passed through thimble. As it boiled its vapour moved up and vapours were condensed in a condenser. The condensed solvent then filled the thimble. After it was filled with enough solvent it automatically siphoned back down into the flask of organic solvent. This process was repeated again and again until all the material to be extracted from the solid powdered material passes into the organic solvent. The organic solvent was filtered and the filtrate was concentrated to a minimum volume by distillation. The organic layer was dried over anhydrous sodium sulphate to get crude extract. The process was repeated to get enough material for further analysis and evaluation.

Chemical analysis of essential oil

Analysis of carrot seed essential oil for identification of compounds present in the essential oil was carried out using GC-MS (QP2010 Plus, Shimadzu, Japan), equipped with an Rtx-5 MS capillary column (30.0 m × 0.20 mm i.d., 0.25 μm film thickness) to separate the components of carrot seed essential oil. The injector was maintained at 250 ºC and operated in split injection mode with the split valve closed for 1 min. Helium gas was used as the carrier gas at a constant pressure of 69 kPa. The column oven was initially maintained at 50 ºC for 2 min, raised to 180 ºC at 3 ºC/min, then to 280 ºC at 10 ºC/min. The interface temperature was 260 ºC and the ionization mode was electron impact (70eV). The mass selective detector was operated in the scan mode between 40 and 600 m/z. Data acquisition was started 3.0 min after injection. MS parameters used were; Ionization Voltage (EI) 70 eV, peak width 2 s, mass range 40-600 amu and detector voltage 1.5 V. Peak identification was carried out by comparison of the mass spectra with mass spectra data available on database of NIST08, WILEY8, Perfumery and Flavor and Fragrance libraries. The composition of carrot seed oil is tabulated (17,18).

Isolation of compounds

Carrot seed essential oil was subjected to column chromatography to isolate the sesquiterpenes and sesquiterpenoids. The column was packed with silica gel of 60 ×120 mesh size activated at 110ºC for 1h. Carrot seed essential oil was dissolved in hexane, was adsorbed on silica gel for 5 min. Column was eluted with petroleum ether and polarity was increased with dichloromethane. Daucene (**I**) in petroleum ether, carotol (**II**) in petroleum ether: dichloromethane (5:1 v/v) and daucol (**III**) in dichloromethane were collected as fractions, monitored by thin layer chromatography (TLC). The compounds isolated were characterised by FTIR, H^1NMR and C^{13} NMR spectroscopic techniques.

Fungal cultures

The phytopathogenic fungi used in this study, *Alternaria triticina*, *Bipolaris sorokiniana* and *Ustilago segetum* var. *tritici* were isolated from wheat plant. Single spore strains of these fungi were maintained on Potato Dextrose Agar (PDA) plates. A one week old culture of each fungus was used to inoculate the agar plates. Fungal spores were harvested by flooding PDA plates with 5 mL of sterile distilled water containing 0.05% (*v*/v) Tween 80, and passing the suspension through two layers of sterile cheese cloth to remove hyphal fragments. The spore concentration was determined by haemocytometer and adjusted with sterile distilled water. Different concentrations (100 to 1000 ugmL^{-1}) of essential oils, isolated compounds and extracts were prepared and added at 0.02 mL to depression slide. Tween 80 was used as emulsifier for extracts and compounds. The spore suspensions at 0.02 mL were individually added to each depression slides. Inoculated slides were placed on moist filter paper in Petri plates, sealed with parafilm to avoid evaporation and then incubated at 25 ºC for 24 h. A spore was scored as germinated, if the germ tube length was equal or superior to the length of the spore body at least. In control, equal amounts of sterilized water and Tween 80 was used. The results were expressed as percent spore germination inhibition and calculated as under:

$$Sporegermination(\%) = \frac{Sporegerminationincontrol - sporegerminationintested}{Sporegerminationincontrol} \times 100$$

Statistical analysis

All data are presented as mean values ± Standard Deviation. Significance differences for multiple comparisons were determined by one way analysis of variance (ANOVA) followed by Tukey test at a level of significance of $p<0.05$ using SPSS 16.0 statistical software.

RESULTS AND DISCUSSION

Chemical composition of the essential oil and extracts

We studied the chemical composition, antifungal activities of carrot seed oil, isolated compounds and extracts. Carrot seed essential oil was yellowish brown liquid with strong and pleasant odour having refractive index and density of 1.45 and 0.987 g cm^{-3}, respectively. The essential oil was soluble in organic solvents like acetone, benzene, methanol, dichloromethane but insoluble in water. The analysis of the chemical

Table 1. GC-MS data of carrot seed essential oil

Sr. No.	Name	Retention Time (min)	Area (%)
1	α-Pinene	7.732	0.22
2	β-Pinene	9.354	0.76
3	Myrcene	9.971	0.36
4	Limonene	11.524	0.90
5	Phenylacetaldehyde	12.190	0.07
6	Trans- Linalool oxide	14.170	0.09
7	Linalool	14.715	0.87
8	Trans-Pinocarveol	16.420	0.18
9	Trans-Verbenol	16.724	0.18
10	Non -(2E)-enal	17.426	0.11
11	3-Cyclohexen-1-ol	18.204	0.06
12	3-Cyclohexene-1-methanol	18.832	0.11
13	Myrtenol	19.105	0.19
14	Verbenone	19.672	0.10
15	Trans-Carveol	20.132	0.14
16	Carvone	21.249	0.11
17	Bornyl acetate	23.149	0.08
18	α-Terpinyl acetate	25.926	0.22
19	Daucene	27.283	5.68
20	γ-Cadinene	27.466	1.46
21	α-Cis-Bergamotene	28.714	0.18
22	(E)-Caryophyllene	28.878	1.22
23	α-Trans-Bergamotene	29.579	1.82
24	β-Santalene	29.909	0.35
25	(E)- β- Farnesene	30.507	5.40
26	β-Cubebene	31.021	3.19
27	α-Curcumene	31.495	0.16
28	β-Elemene	32.154	3.23
29	β-Bisabolene	32.565	2.95
30	Sesquisabinene	33.138	0.67
31	α-Chamigren	33.312	0.27
32	Salvial-4(14)-en-1-one	33.522	0.17
33	Longifolenaldehyde	34.588	3.23
34	(*E*)-Farnesene epoxide	35.209	0.33
35	Caryophyllene oxide	35.442	0.09
36	Carotol	36.660	52.73
37	β -caryophyllene 4,5 α-oxide	36.996	0.38
38	Caryophylla-3(15),7(14)-dien-6-ol	37.233	0.86
39	Alloaromadendrenoxide-(1)	37.433	0.84
40	Daucol	37.763	5.10
41	Eudesm-4(14)-en-11-ol	38.632	0.96
42	2,6,10-trimethylundecan-(5E)-2,5,9-trien-4-one	38.802	0.24
43	α-Cedrane	38.981	0.59
44	α-Bisabolol	39.202	0.09
45	1-Heptatriacotanol	39.498	0.19
46	Juniper camphor	39.672	0.08
47	14- β-Pregna	40.075	0.08
48	Farnesol	40.567	0.08
49	Dihydrojasmone	42.156	0.29
50	Phytone	44.763	0.14
51	9-(*Z*)-9-octadecenoic acid	48.371	0.08

Source: 17

composition of the essential oil was done by gas chromatography- mass spectrometry (GC-MS) which showed the presence of sesquiterpene alcohols and hydrocarbons. A total of 51 compounds were identified in carrot seed essential oil. Carotol (52.73%) was the major,

while the daucol (5.10%), daucene (5.68%), (E)-β-farnesene (5.40%), β-cubebene (3.19%), longifolenaldehyde (3.23%), β-elimene (3.23%), (E)-caryophyllene (1.22%), β-bisabolene (2.95%)etc (Table 1) were minor compounds (18).

Table 2. GC-MS data of carrot seed extracts

Sr. No.	Name	Retention Time (min)	Carrot Seed extracts(percent area)		
			Hexane	Dichloro methane	Methanol
1.	Undecane	4.197	0.55	0.72	-
2.	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	5.00	-	-	6.87
3.	1-deoxy-d-arabitol	6.379	-	-	15.23
4.	5-(hydroxymethyl)-2-furaldehyde	6.525	-	-	10.64
5.	2-butoxy-acetate	7.749	-	-	5.29
6.	2-methoxy-4-vinylphenol	8.670	-	-	2.17
7.	Murolene-14-Hyroxy-alpha	14.198	1.40	1.76	1.21
8.	Isoshyobunone	15.838	1.22	1.55	-
9.	2-dodecenal	15.899	0.87	1.67	-
10.	4,6,10,10-tetramethyl-5-oxa-tricy	16.201	0.33	0.55	-
11.	Carotol	16.944	10.89	2.09	8.15
12.	Neoclovenoxid- alcohol	17.546	1.66	2.12	-
13.	Pregen-4-ene-3,20-dione	17.884	1.21	1.78	-
14.	1H-3α,6-epoxyazulen-7-ol	18.074	4.22	4.98	3.54
15.	Spiro[4.5)dec-6-en-8-one	18.993	0.38	0.52	-
16.	9H-cycloisolongifolene,8-oxo	19.479	1.07	1.69	-
17.	1H-indene,1-hexadecyloctahyd	19.573	0.69	0.90	-
18.	3-buten-2-one,4-(2,6,6-trimethyl-1 cyclohene- 1 yl)	20.201	2.04	2.47	-
19.	Isoaromadendrene epoxide	21.186	7.91	9.96	11.47
20.	Octadecenoic acid	21.281	1.26	1.52	-
21.	6-(5-methoxy-2-furyl)-6-methyl-2-heptanone	21.893	2.48	3.22	-
22.	Shyobunone	22.692	1.64	1.78	-
23.	2,6,10,15,19,23-hexamethyl-tertacosa	23.025	2.53	4.29	-
24.	Murolan-3,9(11)-diene-10-peroxy	25.941	7.94	11.34	3.44
25.	[dodecanoyl(methyl)amino)acetate	26.537	-	-	1.92
26.	1-(+)ascorbic acid 2,6-dihexadecanoate	26.627	5.44	6.18	-
27.	Phthalic acid	26.652	-	-	1.21
28.	9-octzdecadienoic acid,methyl ester	29.987	1.75	-	-
29.	9,12-octadecadienoic acid(Z,Z)	30.753	2.20	-	-
30.	Octadec-9-enoic acid	31.033	15.96	13.40	9.84
31.	Octadecanoic acid	31.470	0.98	-	-
32.	10,18-Bisnorabieta-8,11,13-triene	32.334	-	-	5.63
33.	Benzyl beta-d-glucoside	33.126	-	-	1.75
34.	5 α -methyl-3,8-dimethylene-2-oxo	37.760	1.98	1.75	-
35.	Hexatriacontane	38.809	0.61		-
36.	1,5-heptadien-4-on,3,3,6-trimethyl	39.195	4.03	2.30	-
37.	Hexatriacontane	43.924	3.76	2.61	-

The previously reported GC-MS analysis of seed essential oil (27,32) identified the sesquiterpenoids namely carotol, daucol, daucene, (Z,Z)-α-farnesene, germacrene D, trans-α- bergamotene, β-selinen, β- bisabolene, bicyclogermacrene, β-caryophyllene, β-caryophyllene, copaenol, α-pinene, elemicin, trans-dauca-8,11-diene, acora-4,9-diene,

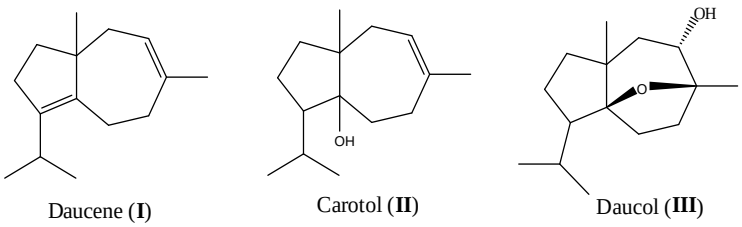
acora-4,10-diene, (E)-β-10,11-dihydro-10,11-epoxyfarnesene and (E)-methylisoeugenol, α- terpinolene, β-caryophyllene, α-pinene, myrcene, α- terpinene and limonene (11,33). In wild carrot, GC-MS analysis of seeds essential oil showed that sabinene (40.9%), α-pinene (30.1%), β-bisabolene (6.2%), β-pinene (5.7%) and trans-caryophyllene (5.3%), were the dominant compounds. While, the major constituents of essential oil from seeds of cultivated carrot were carotol (22.0%), sabinene (19.6%) and α-pinene (13.2%) (24).

However the chemical composition of hexane, dichloromethane and methanol extracts was different. The total compounds identified in hexane and dichloromethane extracts were 55, 50 and 30 (Table 2). However some of the compounds in all three extracts were similar. The major compounds detected in the hexane, dichloromethane and methanol extracts were octadec-9-enoic acid (15.96,13.40 and 9.84%), murolan-3,9(11)-diene-10-peroxy (7.94, 11.34 and 3.44%), isoaromadendrene epoxide (7.91,9.96 and 11.47%), carotol (10.89, 2.09 and 8.15%), 1H-3α,6-epoxyazulen-7-ol, (4.22, 4.98 and 3.54%), murolene-14-hydroxy-alpha respectively. (1.40, 1.76 and 1.21%) (Table 2). Octadec-9-enoic acid (15.96%) and carotol (10.89%) octadec-9-enoic acid (13.40%) and murolan-3,9(11)-diene-10-peroxy and murolan-3,9 (11)-diene-10-peroxy (12.44%) were the major compounds present in hexane, dichloromethane and methanol extracts of carrot seed.

The compounds present in ethyl acetate extract of wild carrot seeds were geranyl acetate, β-caryophyllene, oleic acid and linoleic acid with highest concentration. The 1, 1, 2-trichloro-l, 2, 2-trifluoroethane, methylfuran, ethanol and dichloromethane extract of carrot seeds showed geranyl acetate and carotol as the major components (16). In this study, the major compounds detected in 3-extracts were octadec-9-enoic acid, murolan-3, 9(11)-diene-10-peroxy, isoaromadendrene epoxide, 1-octen-3-ol, carotol and 1H-3α,6-epoxyazulen-7-ol and (4.22, 4.98 and 3.54%).

Our results regarding composition were different from the findings of other scientists, except that carotol (52.73%) was the major component present in the carrot seed essential oil. The variations may be due to climatic conditions, the nutritional status plant variety and other factors.

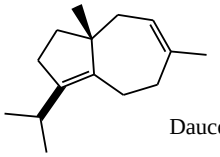
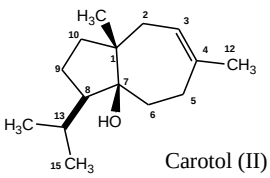
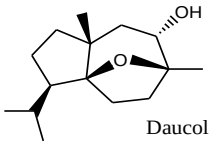
Extensive column chromatography resulted in isolation of daucene (**I**), carotol (**II**) and daucol (**III**) from carrot seed oil (Figure 1.). Purity of the compounds was checked by TLC. The structural elucidation of isolated compounds was done using spectroscopic techniques (Table 3).



Daucene (**I**) Carotol (**II**) Daucol (**III**)

Figure 1. Structures of some isolated compounds.

Table 3. Spectroscopic data of isolated compounds (**I-III**)

Compounds	IR(cm^{-1})	^{1}H NMR (δ)	^{13}C NMR (δ)
 Daucene (I)	1673,1453 and 1372	0.70 (3H, d, J= 8.12, C_{14}), 0.80 (3H, d, J=8.12, C_{15}), 1.46 (3H,s, C_{12})	48.31(C_1),26.66(C_2),116.75 (C_3),142.51(C_4),32.88(C_5),35.1 9(C_6),117.94(C_7),143.02 (C_8),39.86(C_9),32.71(C_{10}),19.42 (C_{11}),20.26(C_{12}), 52.84(C_{13}),21.38(C_{14}),21.41(C_{15})
 Carotol (II)	3520, 2953, 2927, 1448, 1461 and 1374	0.93 (3H, d, J= 8.1, C_{14}), 0.99 (3H, d, J=8.12, C_{15}), 1.03 (3H, s, C_{12}), 1.95 (1H, m, C_{13}), 5.3 (1H, m, C_3)	49.82 (C_1), 25.23 (C_2), 122.11 (C_3), 138.46 (C_4), 27.84 (C_5), 29.40 (C_6), 84.43 (C_7), 39.40 (C_8), 38.59 (C_9), 34.38 (C_{10}), 24.36 (C_{11}), 24.05(C_{12}), 52.48 (C_{13}), 21.44 (C_{14}), 21.38 (C_{15})
 Daucol (III)	3305,2950, 2931, 2872, 1470, 1151, 1053	0.81 (3H, d, J= 8.1, C_{14}), 1.05 (3H, d, J=8.12, C_{15}), 1.33 (3H, s, C_{12}), 2.15 (1H, m, C_{13}), 3.73 (1H, dd, C_3)	45.13 (C_1), 41.07 (C_2), 71.47 (C_3), 85.33 (C_4), 32.96 (C_5), 31.46 (C_6), 91.20 (C_7), 52.42 (C_8), 29.47 (C_9), 26.76 (C_{10}), 23.46 (C_{11}), 22.95 (C_{12}), 40.98 (C_{13}), 22.37 (C_{14}), 21.78(C_{15})

Antifungal Activity

The fungi used in the present study represent the mainly wheat pathogens, responsible for significant economic losses worldwide (12,30,31). The essential oils were reported to be effective against plant pathogenic fungi. In recent years, the use of plant-based essential oils and extracts to control phytopathogens in agriculture increased due to adverse effect of chemical fungicides (3, 9).

Several studies have documented the antifungal activities of essential oils and plant extracts (3,26,36,38). Essential oils are natural antifungal agents with potential to control pathogenic fungi causing severe destruction in crops. Monoterpene and sesquiterpene hydrocarbons and their oxygenated derivatives are the major components of plant essential oils, which exhibit biological activities. The active compounds present in essential oils are terpenes, mainly responsible for their antifungal activity (8,12).

Quality characters of wheat seeds (seed germination, moisture content, seed discolouration and seed-borne fungal prevalence) are influenced by various factors. Under warm and humid conditions wheat crop is vulnerable to infection with the spot blotch caused by *Bipolaris sorokiniana*. The host range of *B. sorokiniana* among monocotyledonous plants, cereals like *Triticum aestivum, Hordeum vulgare, Avena sativa, Sorghum bicolor* and many grasses. Several plant species other than monocotyledons including *Brassica compestris, Glycine max, Lens culinaris, Vignaradiata, Sesamum indicum, Vigna mungo* and *Pennisetum amaricanum* are the host of *B. sorokiniana* (13). Disease can be seed borne, soil borne and

airborne. The soil-borne conidia are considered the main source of inoculums, causing secondary infection resulting in severe foliar disease and yield loss (5). *Alternaria* leaf blight causes major yield and quality loss in wheat crop. *U. segetum* var. *tritici*, the causal agent of loose smut of wheat belongs to family *Ustilaginaceae.* Loose smut is major seed-borne disease of wheat worldwide, particularly in humid areas (37).

Table 4. Antifungal activity of essential oil, extracts and isolated compounds against 3 phytopathogenic fungi of wheat.

Components	Inhibition (%) in spore germination at various conc. (mg/mL)				Mean	ED_{50}
	0.1	0.25	0.5	1.0		
Alternaria triticina						
Carrot seed essential oil	50.60±2.67	70.83±2.12	93.65±0.95	100.00	78.77^{f}	0.10
Hexane extract	41.99±4.65	55.76±3.75	76.17±4.22	100.00	68.48^{d}	0.21
Dichloromethane extract	52.13±3.01	72.29±1.23	87.40±1.22	100.00	77.95^{f}	0.10
Methanol extract	42.67±0.24	61.63±2.30	85.59±3.41	100.00	72.47^{e}	0.15
Daucene	18.58±0.84	32.27±1.13	44.75±2.47	71.12±2.52	41.68^{a}	0.60
Carotol	31.34±2.24	44.29±0.58	69.49±1.29	91.13±1.93	59.06^{c}	0.34
Daucol	26.81±1.41	37.95±0.60	55.80±1.54	82.95±1.48	50.87^{b}	1.09
Carbendazim (Bavistin)	100.00±0	100.00±0	100.00±0	100.00±0	100.00^{g}	0.06
Bipolaris sorokiniana						
Carrot seed essential oil	35.30±2.78	55.36±1.30	74.72±2.40	100.00	66.34^{c}	0.22
Hexane extract	44.22±2.98	61.53±1.43	81.87±1.48	100.00	71.90^{d}	0.18
Dichloromethane extract	49.73±1.47	68.00±0.46	90.46±2.88	100.00	74.81^{e}	0.11
Methanol extract	53.41±1.53	75.99±3.61	100.00	100.00	82.35^{f}	0.10
Daucene	26.52±1.68	31.51±1.31	43.43±1.56	70. 36±1.36	42.95^{a}	0.63
Carotol	31.12±0.43	38.56±0.96	63.22±0.77	87.46±1.06	55.09^{b}	0.39
Daucol	22.71±0.54	33.87±2.54	47.89±1.42	76.60±0.38	45.27^{a}	0.51
Carbendazim (Bavistin)	100.00±0	100.00±0	100.00±0	100.00±0	100.00^{g}	0.07
U. segetum var. *tritici*						
Carrot seed essential oil	40.85±0.91	53.36±1.37	69.98±1.41	100.00	66.04^{d}	1.24
Hexane extract	31.06±1.66	42.21±1.38	70.38±1.93	100.00	61.66^{c}	0.35
Dichloromethane extract	39.22±1.69	50.71±2.40	77.66±1.90	100.00	66.89^{d}	0.28
Methanol extract	42.76±1.75	64.89±2.56	91.07±2.88	100.00	74.68^{e}	0.16
Daucene	13.11±1.11	20.44±0.79	33.02±0.49	47.16±0.91	28.43^{a}	1.15
Carotol	16.04±1.01	24.95±0.10	39.14±1.04	53.46±1.93	33.40^{b}	0.90
Daucol	16.87±0.49	21.41±1.53	33.14±1.78	47.79±1.43	29.80^{a}	1.09
Carbendazim (Bavistin)	97.93±0.79	100.00	100.00±0	100.00±0	100.00^{g}	0.07

Values are given as mean ± S.D. of three experiments.
All values followed by same small letter are non-significant ($p>0.05$) according to Tukey multiple range test

The *in vitro* screening of antifungal activity of carrot seed oil, isolated compounds and extracts completely inhibited the spore germination of the three fungal pathogens at 1.0 mg/mL concentration (Table 4). The 500 mg/mL concentration of the carrot seed oil showed potent inhibitory effect on the growth of *A. triticina* (92.98%), *B.sorokiniana* (76.42%) and *U. segetum* var. *tritici* (70.98%) (Figures 2-4). The hexane extract of carrot seed at 500 mg/mL concentration inhibited the spore germination of *A. triticina* (79.16%), *B.sorokiniana* (80.82%) and *U. segetum* var. *tritici* (71.75%). The dichloromethane extract of carrot seed also showed similar activity against *A. triticina* (88.27%) and *B. sorokiniana* (88.42%), but was less effective against *U. segetum* var. *tritici* (76.32%) than *A. triticina*

(88.27%) and *B. sorokiniana* (88.42%). The methanol extract at 500 mg/mL concentration caused complete inhibition of *B. sorokiniana*. Carotol was most effective against all tested fungi, amongst the isolated compounds.

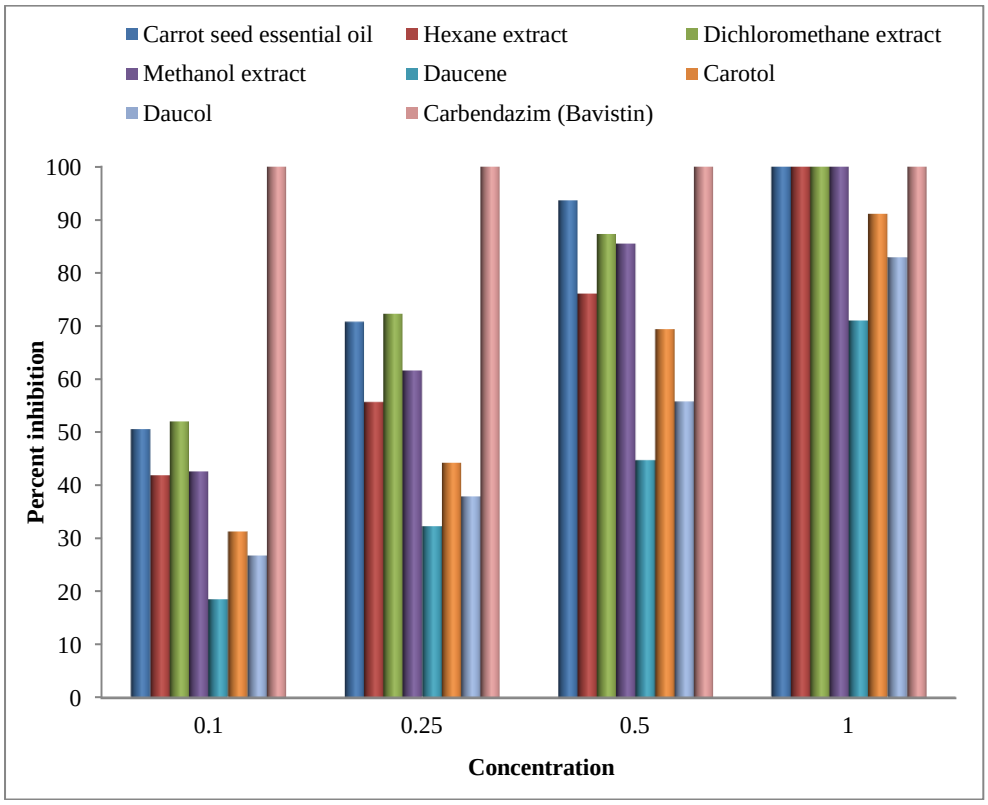


Figure 2. Percent spore germination inhibition of carrot seed essential oil, isolated compounds and extracts against *A. triticina*

The ED_{50} values of three extracts were comparable with standard fungicide carbendazim. Dichloromethane extracts was effective with ED_{50} value of 0.10 mg/mL as compared to hexane and methanol extracts having ED_{50} values 0.21 and 0.15 mg/mL respectively against *A.triticina*. The ED_{50} values of dichloromethane extract against *A.triticina* and that of methanol against *B. sorokiniana* were similar. Methanol extract was more effective than other extracts against *U. segetum* var. *tritici*.

In this study, the essential oils and the different extracts showed varying anti-fungal activity against *Alternaria triticina, Bipolaris sorokiniana* and *U. segetum* var. *tritici* plant pathogenic fungi, which could be attributed to difference in their chemical composition. It was also possible that other components present in essential oils might be involved in some type of synergism with major active compounds leading to biological activities (23).

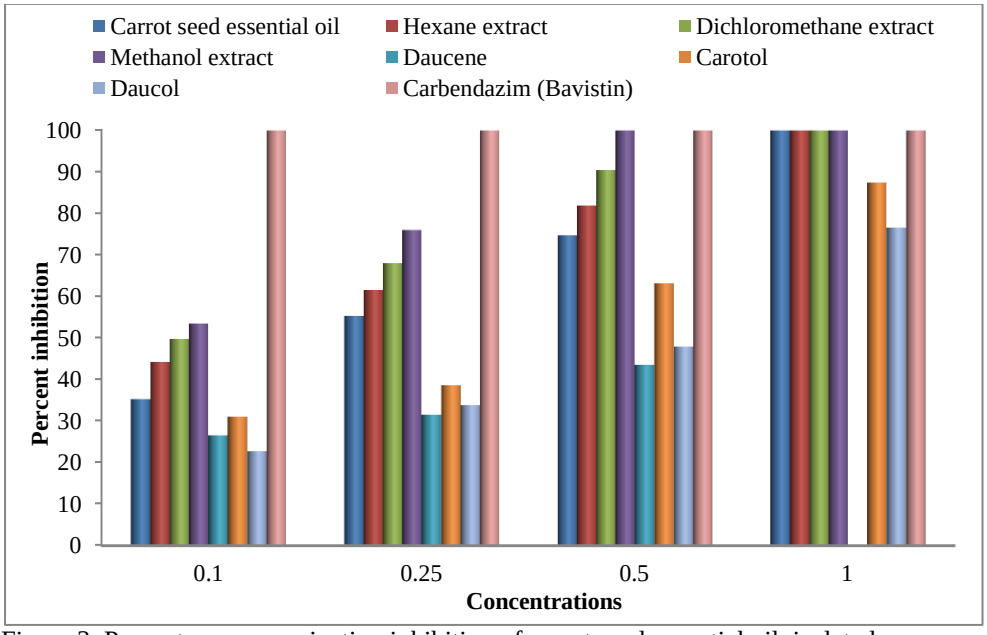


Figure 3. Percent spore germination inhibition of carrot seed essential oil, isolated compounds and extracts against *B. sorokiniana*

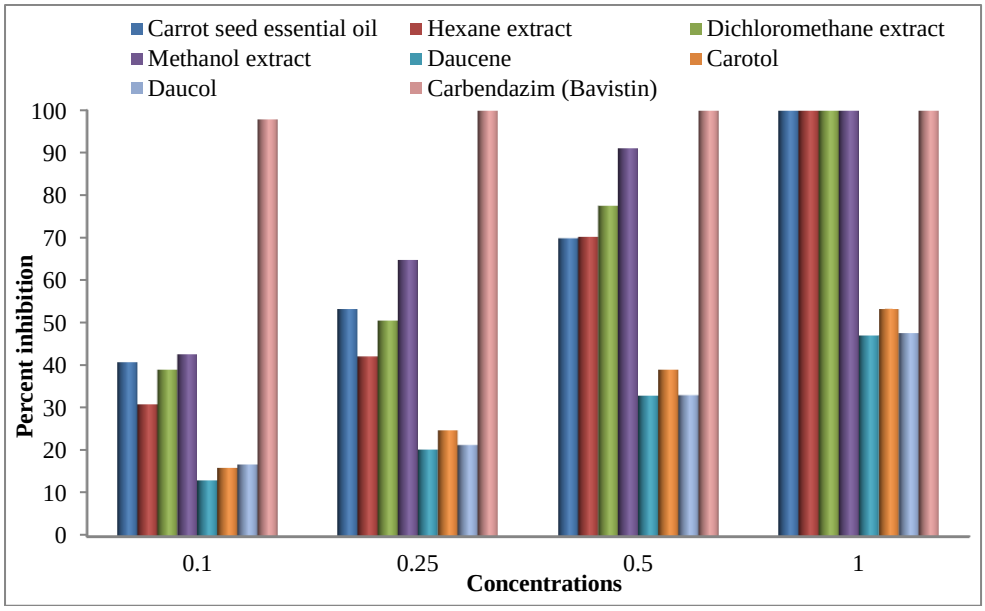


Figure 4. Percent spore germination inhibition of carrot seed essential oil, isolated compounds and extracts against *U. segetum var. tritici*

CONCLUSIONS

The composition analysis indicated that the carrot seed oil was rich in sesquiterpenes, and the main components were carotol, daucol and daucene. The chemical composition of three extracts was different due to the polarity of solvents used with approximately same yield. The major compound in hexane and dichloromethane extracts was octadec-9-enoic acid in 15.96 and 13.40% yield, respectively, whereas in methanol extract murolan-3,9(11)-diene-10-peroxy (12.44%) constituted the main component. All extracts and essential oil showed high antifungal activity. Carotol was most active against all tested plant pathogens as compared to daucene and daucol. Methanol extract at 0.5 mg/mL showed highest antifungal activity and completely inhibited the spore germination of *B. sorokiniana*. Therefore, it would also be interesting to study the effect of essential oil and extracts of carrot seeds against other important fungi for developing new antifungal agents to control serious fungal diseases in valuable crops. Thus, natural compounds from *D. carota* could be an alternative to synthetic fungicides for use in agroindustries and may find use in developing novel selective and natural fungicide.

REFERENCES

1. Abdulrasheed, A., Aroke, U.O. and Sani, I.M. (2015). Parametric studies of carrot seed oil extract for the production of medicated soap. *International Journal of Recent Development in Engineering and Technology* **4**: 1-5.
2. Amini, J., Farhang, V., Javadi, T. and Nazemi, J. (2016). Antifungal effects of plant essential oils on controlling *Phytophthora* species. *Plant Pathology Journal* **32**: 16-24.
3. Bajpai, V.K., Rahman, A. and Kang, S.C. (2007). Chemical composition and antifungal properties of the essential oil and crude extracts of *Metasequoia glyptostroboides* Miki ex Hu. *Industrial Crops and Products* **26**: 28-35.
4. Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. (2008). Biological effects of essential oils: A review. *Food and Chemical Toxicology* **46**: 446-75.
5. Bello, G.M.D., Sistern, M.N. and Monaco, C.I. (2003). Antagonistic effect of soil rhizosphere microorganisms on *Bipolaris sorokiniana*, the causal agent of wheat seedling blight. *International Journal of Pest Management* **49**: 313-317.
6. Bendiabdellah, A., El Amine Dib, M., Djabou, N., Hassani, F., Paolini, H., Tabti, B., Costa, J. and Muselli, A. (2014). *Daucus carota* ssp. *Hispanicus* Gouan. essential oil: Chemical variability and fungitoxic activity. *Journal of Essential Oil Res*earch **26**:55-59.
7. Brent, K.J. and Hollomon, D.W. (1998). *Fungicide Resistance: The Assessment of Risk*. FRAC, Global Crop Protection Federation, Brussels, pp.1-48.
8. Cakir, A., Kordali, S., Zengin, H., Izumi, S. and Hirata, T. (2004). Composition and antifungal activity of essential oils isolated from *Hypericum hyssopifolium* and *Hypericum heterophyllum*. *Flavour Fragrance Journal* **19**: 62-68.
9. Costa, T.R., Fernandes, O.L.F., Santos, S.C., Oliveria, C.M.A., Liao, L.M., Ferri, P.H., Paulo, J.R., Ferreira, H.D., Sales, B.H and Silva, M.R. (2000). Antifungal activity of volatile constituents of *Eugenia dysenterica* leaf oil. *Journal of Ethnopharmcology* **72** : 111-117.
10. Dhaliwal, G.S. and Koul, O. (2007). *Biopesticides and Pest Management Conventional and Biotechnological Approaches*. Kalyani Publishers, New Delhi.
11. Gonny, M., Bradesi, P. and Casanova. (2004). Identification of components of the essential oil from wild Corsican *Daucus carota* L. using ^{13}C-NMR spectroscopy *Flavour and Fragrance Journal* **19**: 424-433.
12. Gudzic, B., Djokovic, D., Vajs, V., Palic, R. and Stojanovic, G. (2002). Composition and antimicrobial activity of the essential oil of *Hypericum maculatum* Crantz. *Flavour Fragrance Journal* **17** :392-394
13. Iftikhar, S., Asad, S., Munir, A., Sultan, A. and Ahmad, I. (2009). Hosts of *Bipolaris sorokiniana*, the major pathogen of spot blotch of wheat in Pakistan. *Pakistan Journal of Botany* **41**: 1433-1436.

14. Imamu, X., Yili, A., Aisa, H.A., Maksimov, V.V., Veshkurova, O.N. and Salikhov, S. (2007). Chemical composition and antimicrobial activity of essential oil from *Daucus carota* seeds. *Chemistry of Natural Compounds* **43**: 495-496
15. Jasicka-Misiak, I., Wieczorek, P.P. and Kafarski, P. (2005). Crotonic acid as a bioactive factor in carrot seeds (*Daucus carota* L.). *Phytochemistry* **66**:1485-1491.
16. Jian, Q.C., Perineau, F., Delmers, M. and Gaset, A. (1989). Comparison of chemical composition of carrot seed essential oil extracted by different solvents. *Flavour and Fragrance Journal* **4**:225-231.
17. Kataria, D., Chahal, K.K. and Kumar, A. (2016). Chemical transformations of carotol isolated from carrot seed oil. *Asian Journal of Chemistry* **28**: 1790-1792.
18. Kaur, P., Chahal, K.K., Kaur, R. and Kataria, D. (2016). Carrot seed essential oil as stored grain protectant against rust red flour beetle, *Tribolium castaneum. Pesticide Research Journal* **28**(1): 76-83.
19. Knight, S.C., Anthony, V.M., Brady, A.M., Greenland, A.J., Heaney, S.P., Murray, D.C., Powell, K.A., Schulz., M.A., Spinks., C.A., Washington., P.A. and Youle, D. (1997). Rationale and perspectives on the development of fungicides. *Annual Review of Phytopathology* **35**: 349-372.
20. Kolli, El. M., Laouer, H., Kolli, El. H., Akkal, S. and Sahli, F. (2016). Chemical analysis, antimicrobial and anti-oxidative properties of *Daucus gracilis* essential oil and its mechanism of action. *Asian Pacific Journal of Tropical Biomedicine* **6**: 8-15.
21. Koul, O. (2005). *Insect Antifeedants*. CRC Press, Bota Racon, USA.
22. Koul, O., Dhaliwal, G.S., Marwaha, S.S. and Arora, J.K. (2003). Future perspectives in biopesticides. In *Biopesticides and Pest Management* (Eds., O. Koul, Dhaliwal, G.S., Marwaha, S.S. and Arora. J.K.), Vol. 1: 386-388. Campus Books International, New Delhi.
23. Marino, M., Bersani, C. and Comi, G. (2001). Impedance measurements to study the antimicrobial activity of essential oils from *Lamiaceace* and *Compositae*. *International Journal of Food Microbiology* **67**:187-195.
24. Milic, A., Jovana, S., Mirjana, C., Maja, I. and Ljiljana, N. (2016). Chemical characterization of essential oil from seeds of wild and cultivated carrots from Serbia. *Botanica Serbica* **40**: 55-56
25. Montesinos, E. (2007). Antimicrobial peptides and plant disease control. *FEMS Microbiology Letters* **270**: 1-11.
26. Morris, J.A., Khettry, A. and Seitz, E.W. (1979). Antimicrobial activity of aroma chemicals and essential oils. *Journal of the American Oil Chemists' Society* **56**: 595-603.
27. Olle, M. and Bender, I. (2010). The content of oils in *Umbelliferous* crops and its formation. *Agronomy Research* **8:** 687-696.
28. Ozcan, M.M. and Chalchat, J.C. (2007). Chemical composition of carrot seeds (*Daucus carota* L.) cultivated in Turkey: Characterization of the seed oil and essential oil. *Grasas Y Aceites* **58**: 359-367.
29. Paster, N. and Bullerman, L.B. (1988). Mould spoilage and mycotoxin formation in grains as controlled by physical means. *International Journal of Food Microbiology* **7:**257-265.
30. Perello, A.E. and Sisterna, M.N. (2005). Leaf blight of wheat caused by *Alternaria triticina* in Argentina. *New Disease Reports* **11**: 24.
31. Prasada, R. and Prabhu, A. (1962). Leaf blight of wheat caused by a new species of *Alternaria*. *Indian Phytopathology* **15**: 292-293.
32. Savary, S., Teng, P.S., Willocquet, L. and Nutter, F.W. (2006). Quantification and modelling of crop losses: A review of purposes. *Annual Review of Phytopathology* **44**: 89-111.
33. Schaller, R.G. and Schnitzler, W.H. (2000). Nitrogen nutrition and flavour compounds of carrots (*Daucu scarota* L.) cultivated in Mitscherlich pots. *Journal of the Science of Food Agriculture* **80**: 49-56
34. Seifert, R.M. and Buttery, R.G. (1978). Characterization of some previously unidentified sesquiterpenes in carrot roots. *Journal of Agricultural Food Chemistry* **27**: 181-183.
35. Tavares, A.C., Goncalves, M.J., Cavaleiro, C., Cruz, M.T., Lopes, M.C., Canhoto, J. and Salgueiro, L. (2008). Essential oil of *Daucus carota* Sub sp. *halophilus*: Composition, antifungal activity and cytotoxicity. *Journal of Ethnopharmacology* **119**: 129-134.
36. Tawil, M., Bekdash, A., Mroueh, M., Daher, C.F. and Abi-Habib, R.J. (2015). Wild carrot oil extract is selectively cytotoxic to human acute myeloid leukemia cells. *Asian Pacific Journal of Cancer Prevention* **16:** 761-767.
37. Yosep, S.M., Stephen, L.F. and Ronald, E.K. (2004). Inheritance of resistance to loose smut *Ustilago tritici* in three durum wheat lines. *Canadian Journal of Plant Pathology* **26**: 555-562.
38. Yousef, R.T. and Tawil, G.G. (1980). Antimicrobial activity of volatile oils. *Die Pharmazie* **35**: 698-701.