Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(1): 896-903
Received: 21-11-2017
Accepted: 22-12-2017

A Kaur
Department of Chemistry, Punjab Agricultural University, Ludhiana, Punjab, India

KK Chahal
Department of Chemistry, Punjab Agricultural University, Ludhiana, Punjab, India

D Kataria
Department of Chemistry, Punjab Agricultural University, Ludhiana, Punjab, India

A Kumar
Department of Chemistry, Punjab Agricultural University, Ludhiana, Punjab, India

Urvashi
Department of Chemistry, Punjab Agricultural University, Ludhiana, Punjab, India

Correspondence
KK Chahal
Department of Chemistry, Punjab Agricultural University, Ludhiana, Punjab, India

Assessment of carrot seed essential oil and its chemical constituents against *Meloidogyne incognita*

A Kaur, KK Chahal, D Kataria, A Kumar and Urvashi

Abstract
Carrot seed essential oil, its fractions, isolated and derivatised compounds were screened for their nematicidal activity against plant parasite root knot nematode *Meloidogyne incognita*. GC-MS analysis showed the presence of 51 compounds in carrot seed essential oil. Sesquiterpenes are the main constituents of carrot seed essential oil out of which carotol, daucol and daucene were the major compounds whereas β-Cubenene and β- Farnesene were the minor compounds present. Carotol, daucol, daucene, polar and nonpolar fractions of carrot seed oil were isolated by column chromatography. Daucol and carotol were chemically derivatized. The isolated and derivatized compounds were characterized using FTIR, ^{1}H NMR and ^{13}C NMR spectroscopy. All these components *viz*. carrot seed essential oil, its fractions, daucene, carotol, daucol, daucone, daucol acetate, 4-Chloro carotol and 3-Bromo carotol were evaluated for egg hatch inhibition and mortality of second stage juveniles (J_2) of *Meloidogyne incognita* at different concentrations. The nematicidal activity of carrot seed essential oil, carotol and polar fraction were found to be more effective than other components tested. Nematicidal activity was found to be concentration and time dependent.

Keywords: carotol, daucene, daucol, *Daucus carota*, essential oil, nematicidal activity

1. Introduction
Nematodes attack a wide range of economically important horticultural, field crops and forest systems (Inam-ul-Haq and Shahina 2005) [6]. *Meloidogyne incognita* is widely distributed and most destructive nematode found throughout the world causing 100$ billion annual losses globally (Oka *et al*. 2000) [14]. Root-knot nematodes spend part of their life in soil either as eggs or as second-stage larvae, then enter the roots and establish feeding sites in susceptible hosts, inducing root swelling with a characteristic knotty appearance. Root galling can significantly limit water, nutrient uptake leading to malnutrition, chlorosis, causing considerable quantitative and qualitative losses in several crop plants. At present, the major control method for nematodes is based on the use of chemical nematicides, but alternative management strategies like natural plant nematicides must be adopted due to the ban on soil fumigants, environmental and human health concerns and development of resistance to chemicals (Hallmann *et al*. 2009) [5]. Many plant constituents and metabolites including essential oils and monoterpenoids have been investigated for activity against plant parasitic nematode (Li *et al*. 2013, Chahal *et al*. 2016) [13, 3].
Carrot (*Daucus carota*) is a herbaceous, biennial flowering plant belonging to family Apiaceae and genus *Daucus* comprises of about 60 species of weedy plants, widely distributed and commonly cultivated for their fleshy edible roots (Ahmed *et al*. 2004) [1]. The carrot seed essential oil possesses various biological activities (Kilibarda *et al*. 1996, Kaur *et al*. 2016) [10, 9]. Carotol, daucol and daucene are the major compounds present in carrot seed essential oil (Jasicka-Misiak *et al*. 2004) [7]. In continuation with our earlier studies on carrot seed oil and its constituents (Kaur *et al*. 2016, Kataria *et al*. 2016) [9, 8] the present study was undertaken to evaluate nematicidal activity of carrot seed essential oil, its fractions, compounds isolated and derivatized.

2. Materials and Methods
2.1 Experimental plant material
For the extraction of essential oil, carrot seeds were purchased from Director Seeds, Punjab Agricultural University, Ludhiana.

2.2 Extraction of essential oil
The dried seeds were powdered and subjected to hydro-distillation using Clevenger apparatus for 10 h.

The pale yellowish brown essential oil possessing woody, earthy smell was collected, dried over anhydrous sodium sulfate and stored in refrigerator until analyzed. The refractive index, optical rotation, pH and density of carrot seed oil were 1.43, +18˚, 7.4 and 0.987 gm cm^{-3} respectively.

2.3 GC-MS Analysis

The chemical composition of the essential oil was carried out by gas chromatography-mass spectrometry (GC-MS) (Kaur *et al*. 2016) [9]. GCMS analysis showed the presence of sesquiterpene alcohols and hydrocarbons using the NIST, WILEY MS library search. Carotol, daucol and daucene were the major whereas (E)-β-Farnesene, β-Cubebene, longifolenaldehyde, β-Elemene, (E)-Caryophyllene and β-Bisabolene were the minor compounds present (Table 1).

Table 1. GC-MS data of carrot seed essential oil (Kataria *et al*. 2016) [8]

Name	Retention Time(min)	Percent Area
α-Pinene	7.732	0.22
β-Pinene	9.354	0.76
Myrcene	9.971	0.36
Limonene	11.524	0.90
Phenylacetaldehyde	12.190	0.07
Trans- Linalool oxide	14.170	0.09
Linalool	14.715	0.87
Trans-Pinocarveol	16.420	0.18
Trans-Verbenol	16.724	0.18
Non -(2E)-enal	17.426	0.11
3-Cyclohexen-1-ol	18.204	0.06
3-Cyclohexene-1-methanol	18.832	0.11
Myrtenol	19.105	0.19
Verbenone	19.672	0.10
Trans-Carveol	20.132	0.14
Carvone	21.249	0.11
Bornyl acetate	23.149	0.08
α-Terpinyl acetate	25.926	0.22
Daucene	27.283	5.68
γ-Cadinene	27.466	1.46
α-Cis-Bergamotene	28.714	0.18
(E)-Caryophyllene	28.878	1.22
α-Trans-Bergamotene	29.579	1.82
β-Santalene	29.909	0.35
(E)- β- Farnesene	30.507	5.40
β-Cubebene	31.021	3.19
α-Curcumene	31.495	0.16
β-Elemene	32.154	3.23
β-Bisabolene	32.565	2.95
Sesquisabinene	33.138	0.67
α-Chamigren	33.312	0.27
Salvial-4(14)-en-1-one	33.522	0.17
Longifolenaldehyde	34.588	3.23
(*E*)-Farnesene epoxide	35.209	0.33
Caryophyllene oxide	35.442	0.09
Carotol	36.660	52.73
β -caryophyllene 4,5 α–oxide	36.996	0.38
Caryophylla-3(15),7(14)-dien-6-ol	37.233	0.86
Alloaromadendrenoxide-(1)	37.433	0.84
Daucol	37.763	5.10
Eudesm-4(14)-en-11-ol	38.632	0.96
2,6,10-trimethylundecan-(5E)-2,5,9-trien4-one	38.802	0.24
α-Cedrane	38.981	0.59
α-Bisabolol	39.202	0.09
1-Heptatriacotanol	39.498	0.19
Juniper camphor	39.672	0.08
14- β–Pregna	40.075	0.08
Farnesol	40.567	0.08
Dihydrojasmone	42.156	0.29
Phytone	44.763	0.14
9-(*Z*)-9-octadecenoic acid	48.371	0.08

2.4 Isolation of major sesquiterpenoids

The carrot seed essential oil (8 g) was chromatographed over silica gel (480 g). The column was eluted with solvents in order of increasing polarity (petroleum ether and dichloromethane). Daucene (1, 0.4g) in petroleum ether, carotol (2, 4.2g) in petroleum ether: dichloromethane (3, 5:1 v/v) and daucol (0.5g) in dichloromethane were collected.

2.5 Characterization of compounds

The purity of the compounds was ascertained by thin layer chromatography on silica gel G in various developing systems using iodine as the visualizing reagent. All the melting points are uncorrected and were determined in open capillaries, on a Buchi B-545 melting point apparatus. The structures of the isolated compounds were confirmed by spectroscopic techniques (Table 2). IR spectra were measured on Perkin Elmer, Model RX-1 FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded with Bruker AC (400 MHz) in $CDCl_3$ using TMS as an internal reference.

Table 2: Spectroscopic data of compounds

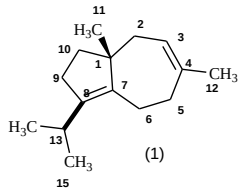
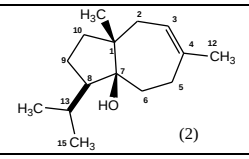
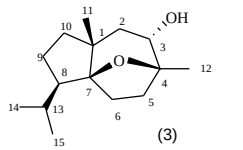
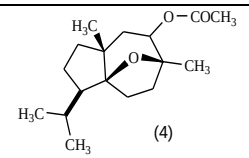
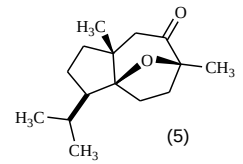
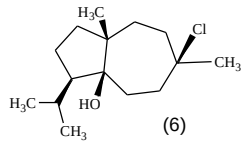
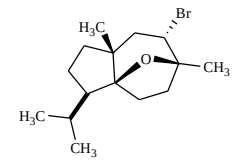
Compounds	IR(cm^{-1})	^{1}H NMR (δ)	^{13}C NMR (δ)
 (1)	1673,1453 and 1372	0.70 (3H, d, J= 8.12, C_{14}), 0.80 (3H, d, J=8.12, C_{15}), 1.46 (3H,s, C_{12})	48.31(C_1), 26.66(C_2),116.75(C_3),142.51 (C_4), 32.88(C_5), 35.19(C_6), 117.94 (C_7), 143.02(C_8),39.86(C_9),32.71(C_{10}),19.42 (C_{11}),20.26(C_{12}),52.84(C_{13}),21.38(C_{14}), 21.41(C_{15}),
 (2)	3520,2953, 2927,1448, 1461and 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})
 (3)	3305,2950, 2931,2872, 1470,1151 and 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})
 (4)	2950,2568, 1148and 1050	0.81 (3H, s, J= 8.12, C_{14}), 1.04(3H, s, J=8.12, C_{15}), 1.11 (3H, s, C_{12}), 2.20 (1H, m, C_{13}), 4.90 (1H, m, C_3), 2.2(m, H, C_{13})	45.45(C_1), 40.75(C_2), 170(C_3), 83.84(C_4), 32.83(C_5), 31.46(C_6), 91.97(C_7), 52.50(C_8), 30.96(C_9),26.34(C_{10}),23.46(C_{11}),22.86(C_{12}) ,40.75(C_{13}),22.86(C_{14}),21.78(C_{15}),21.31(C_{16}),22.04 (C_{17})
 (5)	2953,2871 1721,1661 and 1109	0.99 (3H, d, J= 8.1, C_{14}), 1.1 (3H, d, J=8.12, C_{15}), 1.31(3H, s, C_{12})	48.18 (C_1), 40.09 (C_2), 209.55 (C_3), 86.91 (C_4), 32.17 (C_5), 31.59 (C_6), 91.71(C_7), 52.00 (C_8), 29.68 (C_9), 27.61(C_{10}), 23.26 (C_{11}), 22.66 (C_{12}), 34.59 (C_{13}), 21.43(C_{14}), 18.85(C_{15})
 (6)	3306,2952, 2933, 2869 and 823	1.05 (3H, d, J=6.6 Hz, C_{15}), 0.85 (3H, d, J= 6.4 Hz, C_{14}), 2.13-2.18 (1H, m, C_{13}), 1.32 (3H, s, C_{12}) and 0.99 (3H, s, C_{11}). 1.00 (3H, d, J= 8.12,C_{14}), 1.10 (3H, d, J=8.12, C_{15}), 1.30 (3H,s,	52.51(C_1),25.85 (C_2), 40.85(C_3), 82.66(C_4), 38.53(C_5),33.64(C_6),91.73 (C_7), 33.59 (C_8), 32.26(C_9), 31.54 (C_{10}),26.28(C_{11}), 25.85 (C_{12}),23.63(C_{13}),21.64(C_{14})and 21.82(C_{15}).
 (7)	2933, 1084, 1065and 548	C_{12}), 1.90 (1H,m, C_{13}), 4.21 (1H, m, C_3)	44.35(C_1),44.68(C_2),56.94(C_3),85.90(C_4),32.76(C_5),31.46(C_6),91.90(C_7),52.85(C_8),30.56(C_9),26.15(C_{10}),25.16(C_{11})23.41(C_{12}),40.92(C_{13}),21.83(C_{14}),21.63(C_{15})

Table 3: Effect of carrot seed essential oil, its fractions, isolated compounds and derivatives on cumulative percent egg hatch inhibition studies of *M. incognita*

% Hatching					
Compounds	**Concentration(ppm)**	**24hr**	**48hr**	**72hr**	**96hr**
Carrot seed essential oil	**250**	35.30(±0.30)a-gr	52.45(±0.55)f-j	72.20(±0.20)n-p	95.00(±1.00)bc
	500	44.80(±0.20)a-f	55.30(±0.30)c-f	70.25(±0.25)p-r	74.90(±0.10)l-m
	1000	59.95(±0.05)ax-z	66.37(±1.37)s-u	75.20(±0.20)k-n	80.67(±0.33)ij
	1500	74.10(±0.10)m-o	77.25(±0.25)kl	81.75(±0.25)hi	85.30(±0.30)fg
	2000	80.82(±0.18)h-j	83.85(±0.15)gh	88.50(±0.50)de	92.50(±0.50)c
	2500	85.80(±0.20)e-g	89.20(±0.20)d	92.95(±0.05)c	96.95(±0.05)ab
Nonpolar fraction	250	9.95(±0.05)a-bx-z	11.85(±0.15)g-m	15.35(±0.35)e-i	24.15(±0.15)ax-z
	500	17.90(±0.10)f-k	23.90(±0.10)l-n	25.50(±0.50)u-x	34.90(±0.10)w-z
	1000	26.25(±0.25)q-v	32.40(±0.40)xy	35.90(±0.10)d-g	42.50(±0.50)v-y

	1500	33.35(±0.35)a-dx-z	38.70(±0.30)a-d	46.95(±0.05)t-w	53.95(±0.05)jk
	2000	44.95(±0.95)u-x	48.80(±0.20)f-j	52.50(±0.50)n-p	60.10(±0.10)d-f
	2500	53.50(±0.50)f-j	57.95(±0.05)t-v	58.50(±0.50)e-g	63.60(±0.40)c
Polar fraction	250	7.61(±0.39)t-y	16.11(±0.11)q-v	33.5(±0.50)j-o	38.10(±0.10)s-y
	500	17.47(±0.47)e-j	31.45(±0.45)t-z	40.5(±0.50)r-v	44.10(±0.10)c-h
	1000	25.73(±0.27)q-u	38.78(±0.23)f-l	54.5(±0.50)a-ey	62.45(±0.45)s-w
	1500	37.90(±0.10)e-j	45.10(±0.10)xy	65.00(±1.00)m-p	78.20(±0.20)d-g
	2000	40.50(±0.50)q-t	52.30(±0.30)k-n	72.25(±0.25)f-j	88.10(±0.10)x-z
	2500	52.40(±0.40)e-i	65.50(±0.50)a-cz	86.00(±0.00)abz	93.45(±0.55)u-w
Daucene	250	5.40(±0.40)a-c	7.15(±0.15)a-byz	9.30(±0.30)t-z	12.30(±0.30)o-t
	500	12.10(±0.10)p-v	14.00(±0.00)m-p	18.95(±0.05)c-i	23.45(±0.55)av-z
	1000	16.95(±0.05)g-m	20.15(±0.15)b-f	25.40(±0.40)r-v	30.20(±0.20)j-o
	1500	22.10(±0.10)a-cw-z	26.85(±0.15)p-t	32.35(±0.35)f-l	35.10(±0.10)b-h
	2000	31.35(±0.35)i-n	34.15(±0.15)e-i	38.00(±0.00)g-o	40.15(±0.15)v-x
	2500	35.50(±0.50)a-fz	42.30(±0.30)t-w	44.30(±0.30)acx-z	46.10(±0.00)n-q
Daucol	250	9.10(±0.10)u-z	11.10(±0.10)r-w	24.75(±0.25)r-x	31.2(±0.2)l-n
	500	12.20(±0.20)a-u	15.20(±0.20)j-p	32.35(±0.35)f-l	45.35(±0.35)o-t
	1000	18.25(±0.25)d-j	21.25(±0.35)a-dyz	40.1(±0.1)wx	53.95(±0.05)d-g
	1500	27.80(±0.20)o-r	29.85(±0.15)k-p	49.5(±0.5)j-m	68.75(±0.25)q-s
	2000	32.25(±0.25)g-l	45.1(±0.1)o-t	64.8(±0.2)t-v	70.1(±0.1)p-r
	2500	39.80(±0.20)wx	55.2(±0.2)c-f	68.9(±0.1)q-s	74.8(±0.2)l-n
Carotol	250	20.25(±0.25)b-f	33.8(±0.2)e-i	54.3(±0.3)d-g	64.4(±0.4)t-w
	500	35.4(±0.4)a-g	43.35(±0.35)q-v	52.5(±0.5)f-j	58.5(±0.5)abz
	1000	56.3(±0.3)c-e	59.5(±0.5)ayz	67.5(±0.5)r-t	71.45(±0.55)o-q
	1500	63.0(±0.0)v-x	70.5(±0.5)p-r	77.5(±0.5)kl	82.00(±1.00)ih
	2000	75.5(±0.5)k-m	81.45(±0.45)hi	88.35(±0.35)d-f	97.1(±0.0e)ab
	2500	83.85(±0.0)gh	93.75(±0.25)c	100(±0.0)a	100.0(±0.0)a
Daucol acetate	250	8.45(±0.45)aw-z	10.25(±0.25)s-y	13.25(±0.25)n-s	20.3(±0.3)af
	500	16.25(±0.25)g-n	19.25(±0.25)c-g	21.95(±0.05)acw-z	25.85(±0.15)u-t
	1000	24.2±(0.2)s-y	28.85(±.15)m-q	34.00(±0.0)e-t	38.00(±0.00)ax-z
	1500	31.25(±0.25)j-nz	35.2(±0.2)b-g	43.3(±0.3)q-v	52.25(±0.25)f-j
	2000	42.55(±0.55)s-w	47.05(±0.05)m-p	50.45(±0.45)j-i	56.00(±0.00)b-e
	2500	51.5(±0.5)g-k	53.85(±0.15)d-l	56.85(±0.15)a-d	62.15(±0.15)w-y
Daucone	250	2.25(±0.25)aA	5.1(±0.1)aB	9.30(±0.3)aC	12.1(±0.1)p-v
	500	6.15(±0.15)bA	10.35(±0.35)bB	14.35(±0.35)bC	15.8(±0.2)i-n
	1000	9.1(±0.1)cA	14.1(±0.1)cB	19.05(±0.25)cC	24.1(±0.1)s-y
	1500	15.2(±0.2)dA	17.2(±0.2)dB	22.1(±0.1)dC	24.75(±0.25)r-w
	2000	18.1(±0.1)eA	25.1(±0.1)eB	23.1(±4.9)eC	31.95(±0.05)h-m
	2500	20.75(±0.25)fA	28.85(±0.15)fB	35.00(±0.00)fC	38.95(±0.05)xy
4-Chloro carotol	250	8.85(±0.21)s-x	11.84(±0.23)q-v	15.85(±0.21)h-n	19.02(±0.56)s-y
	500	11.60(±0.56)e-i	16.77(±0.32)s-y	22.64(±0.51)r-v	28.74(±0.37)a-fz
	1000	13.97(±0.05)e-f	20.72(±0.38)e-j	27.33(±0.32)a-fz	32.74(±0.37)h-l
	1500	18.25(±0.35)p-t	25.95(±0.07)abx-z	34.84(±0.23)o-s	38.60(±0.56)x-z
	2000	21.92(±0.12)e-i	29.54(±0.65)n-q	42.70(±0.42)b-e	45.76(±0.34)u-w
	2500	26.75(±1.06)d-f	38.45(±0.64)abz	47.84(±0.23)w-y	52.60(±0.57)uv
3-Bromo carotol	250	10.51(±.069)v-z	11.99(±0.05)q-v	16.04(±(0.69)i-n	24.20(±0.90)c-h
	500	18.60(±0.56)q-w	24.45(±0.63)g-m	25.52(±0.67)abv-z	35.49(±0.71)n-q
	1000	26.82(±0.26)n-r	32.75(±0.35)a-e	35.49(±0.71)o-s	42.50(±0.70)f-k
	1500	33.75(±0.35)d-j	38.10(±0.14)q-u	45.60(±0.42)d-h	50.74(±0.36)x-z
	2000	43.77(±0.32)a-cn-z	46.34(±0.93)l-p	56.00(±1.41)r-w	60.04(±1.35)n-r
	2500	54.72(±0.39)p-t	58.40(±0.70)ax-z	61.96(±0.05)l-o	63.45(±0.63)f-j

Values in the same column followed by different letter(s) are significantly different according to Duncan's test ($P < 0.05$). Values in parenthesis show ± standard error.

Table 4. Effect of carrot seed essential oil, its fractions, isolated compounds and derivatives on cumulative percent mortality studies of *M. incognita*

% dead J2					
Compounds	**Concentration(ppm)**	**24hr**	**48hr**	**72hr**	**96hr**
Carrot seed essential oil	250	23.03(±0.18)a-fz	31.90(±0.60)e-k	47.75(±0.25)abx-z	51.45(±0.45)s-v
	500	28.75(±0.25)m-s	47.60(±0.40)abx-z	59.75(±0.75)mn	62.50(±0.50)ml
	1000	35.15(±0.65)a-dyz	54.00(±1.00)p-s	66.45(±0.55)kj	68.85(±0.15)ji
	1500	43.93(±0.08)d-k	64.45(±0.45)kl	70.30(±0.30)ih	72.5(±0.50)gh
	2000	50.95(±0.95)u-w	72.75(±0.25)gh	78.70(±0.70)f	82.80(±0.20)ef
	2500	53.55(±1.55)p-t	85.95(±0.05)d	88.30(±0.30)de	90.40(±0.40)cb
Nonpolar fraction	250	3.25(±0.25)sr	12.40(±0.40)a-dx-z	24.45(±0.45)abwz	30.50(±0.50)h-n
	500	10.25(±0.35)b-i	20.60(±0.60)e-k	29.25(±0.25)k-p	32.50(±0.50)d-j
	1000	12.25(±0.25)a-eyz	22.00(±1.00)b-g	34.45(±0.45)fdae	37.40(±0.40)u-z
	1500	15.80(±0.80)p-v	29.25(±0.25)k-q	38.25(±0.25)xwrt	40.40(±0.40)m-t
	2000	20.50(±0.50)e-k	32.50(±0.50)d-j	37.40(±0.40)zvu	44.60(±0.60)d-i

	2500	25.50(±0.50)u-wx-z	35.35(±0.35)a-dx-z	41.00(±1.00)fn-r	45.50(±1.50)a-dz
Polar fraction	250	5.20(±0.20)o-s	9.60(±0.40)d-j	25.45(±0.45)u-z	30.50(±0.50)h-n
	500	7.70(±0.30)i-o	13.45(±0.55)av-z	28.20(±0.80)n-u	39.25(±0.75)p-u
	1000	10.68(±0.32)a-h	15.45(±0.45)k-p	34.50(±0.50)a-fz	45.85(±0.15)a-e
	1500	17.00(±1.00)m-s	29.45(±0.45)k-p	38.35(±0.35)r-w	40.50(±0.50)m-t
	2000	21.25(±0.25)d-j	32.50(±0.50)d-j	38.00(±1.00)s-y	45.50(±0.50)a-f
	2500	25.00(±0.00)av-y	35.50(±0.50)a-cw-z	40.90(±0.10)l-s	48.15(±0.15)aw-z
Daucene	250	3.25(±0.25)rs	4.25(±0.25)q-s	5.45(±0.25)n-s	7.70(±0.20)j-o
	500	5.90(±0.10)l-q	6.45(±0.25)l-q	7.40(±0.40)j-o	12.75(±0.25)cw-z
	1000	8.45(±0.05)g-m	10.9(±0.10)a-g	11.95(±0.05)a-eyz	14.70(±0.30)r-y
	1500	12.75(±0.25)a-c	13.90(±0.10)t-z	14.50(±0.30)s-y	16.90(±0.10)n-s
	2000	14.25(±0.25)s-z	15.20(±0.20)r-x	16.65(±0.25)f-l	18.70(±0.20)h-p
	2500	16.25(±0.25)o-t	18.50(±0.50)j-o	20.30(±0.30)f-k	21.10(±0.10)d-k
Daucol	250	2.70(±0.30)s	5.61(±0.39)m-s	18.27(±0.27)k-q	19.87(±0.13)g-m
	500	4.73(±0.27)p-s	10.40(±0.50)b-i	25.58(±0.42)u-z	27.48(±0.42)p-v
	1000	6.68(±0.32)k-q	7.93(±0.08)h-o	30.88(±0.13)g-l	37.25(±0.25)au-z
	1500	9.91(±0.10)c-j	20.80(±0.20)e-j	34.73(±0.27)a-ez	45.85(±0.15)a-e
	2000	23.75(±0.21)a-dx-z	32.58(±0.42)d-i	41.94(±0.06)i-q	53.96(±0.46)o-s
	2500	28.57(±0.44)m-t	40.07(±0.07)m-u	53.25(±0.25)q-t	59.42(±0.42)n
Carotol	250	23.30(±0.30)a-eyz	34.30(±0.30)c-f	54.40(±0.40)o-r	60.75(±0.25)mn
	500	28.90(±0.10)l-r	49.40(±0.40)v-z	62.45(±0.45)ml	71.21(±0.21)hi
	1000	35.25(±0.25)a-dyz	54.45(±0.45)o-r	69.50(±0.50)i	80.25(±0.25)d
	1500	44.45(±0.45)c-j	62.35(±0.35)ml	78.25(±0.25)f	91.95(±0.50)b
	2000	49.30(±0.30)v-z	74.80(±0.20)g	88.85(±0.85)cd	99.15(±0.15)a
	2500	56.50(±0.50)o	88.30(±0.30)cd	99.40(±0.40)a	100.00(±0.00)a
Daucol acetate	250	9.40(±0.40)e-k	20.15(±0.15)f-l	36.20(±0.20)abv-z	38.20(±0.20)r-x
	500	19.25(±0.25)g-o	26.50(±0.50)q-x	38.20(±0.20)v-x	40.05(±0.05)m-u
	1000	21.50(±0.50)c-i	32.00(±0.00)e-k	42.20(±0.20)h-o	45.15(±0.15)b-g
	1500	25.30(±0.30)u-z	38.05(±0.05)t-y	45.50(±0.50)a-f	50.00(±1.00)u-x
	2000	31.80(±0.20)f-l	39.95(±0.05)m-u	49.90(±0.10)v-y	51.70(±0.30)r-v
	2500	39.25(±0.25)p-u	45.00(±0.00)b-h	52.50(±0.50)q-u	55.20(±0.20)o-q
Daucone	250	5.30(±0.30)n-s	11.50(±0.50)a-fz	26.45(±0.45)q-x	33.00(±1.00)c-h
	500	8.20(±0.20)g-n	14.50(±0.50)s-y	30.50(±1.50)h-n	35.50(±0.50)aw-z
	1000	10.50(±0.50)b-i	17.45(±0.45)c-f	35.70(±0.70)acw-z	41.50(±0.50)l-r
	1500	12.35(±0.35)a-dx-z	20.50(±0.50)e-k	39.50(±0.50)o-u	42.5(±0.50)g-n
	2000	14.10(±0.10)s-z	24.35(±0.35)a-cw-z	43.50(±0.50)e-l	45.00(±0.00)c-h
	2500	16.65(±0.35)o-t	26.20(±0.20)r-y	45.25(±0.25)a-g	47.75(±0.25)bx-z
4-Chloro carotol	250	5.65(±0.35)q-w	10.77(±0.33)i-p	22.13(±1.31)e-k	16.13(±0.53)s-y
	500	8.75(±0.38)b-h	15.52 (±0.67)t-z	25.76(±0.34)h-o	37.93(±0.10)bd-g
	1000	12.93(±0.10)o-v	19.79(±0.29)j-o	27.76(±0.34)abw-z	40.75(±0.35)h-p
	1500	14.25(±0.35)g-m	20.76(± 0.34)m-u	32.15(±0.21)j-q	45.36(±0.51)a-e
	2000	16.76(±0.33)a-cw-z	25.52(± 0.67)f-m	35.59(±0.57)abx-z	46.99(±0.00)r-v
	2500	18.70(±0.42)n-u	26.75(±0.35)a-cz	39.07(±0.10)q-u	47.75(±0.35)op
3-Bromo carotol	250	15.57(±0.60)m-r	18.65(±0.49)a-h	20.73(±0.38)a-g	25.95(±0.07)j-p
	500	21.60(±0.56)f-l	25.67(±0.46)u-s	30.20(±1.31)t-z	33.72(±0.38)t-y
	1000	27.55(±0.63)abv-z	29.87(±0.46)g-n	35.96(±0.03)g-i	42.12(±1.23)l-t
	1500	30.90(±0.14)s-z	39.97(± 0.03)e-k	41.67(±0.45)e-k	45.72(±0.38)a-g
	2000	35.55(±0.63)s-z	42.76(±0.33)u-z	47.70(±0.42)abw-z	51.62(±0.53)acyz
	2500	39.79(±0.28)h-p	46.92(±0.10)p-w	52.65(±0.49)q-v	56.35(±0.35)ax-z

Values in the same column followed by different letter(s) are significantly different according to Duncan's test ($P < 0.05$).Values in parenthesis show ± standard error.

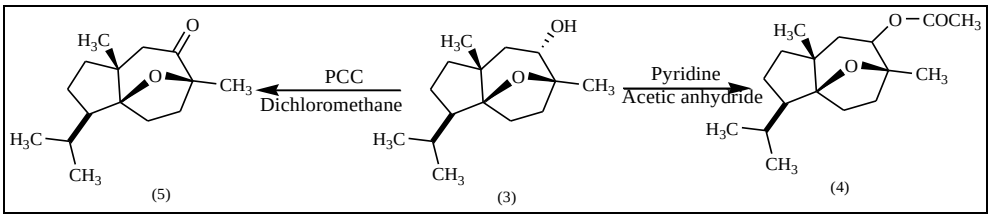
2.6 Reactions of isolated compounds

Daucol with acetic anhydride

A solution of daucol (0.01 M) in pyridine (10 ml) was taken in conical flask and treated with an excess of acetic anhydride at room temperature. After 48 h, the completion of reaction was checked (TLC), the reaction mixture was quenched with water and extracted with diethyl ether (3×50ml). The diethyl ether extract was neutralized with sodium bicarbonate, washed with water and dried over sodium sulfate. Evaporation of solvent afforded a yellowish liquid identified as daucol acetate (4) using spectral data.

Daucol with pyridinium chlorochromate

Daucol (3) was dissolved in dichloromethane taken in conical flask and solution of pyridinium chlorochromate (1.5 mmol) in dichloromethane was added at room temperature. The reaction mixture was stirred for 1 h. The solution was homogeneous with deposition of black insoluble reduced agent. The black reaction mixture was diluted with diethyl ether (3×50 ml) and filtered on silica plug. The filtrate was washed with water thrice, purified and evaporated to yield daucone (5).

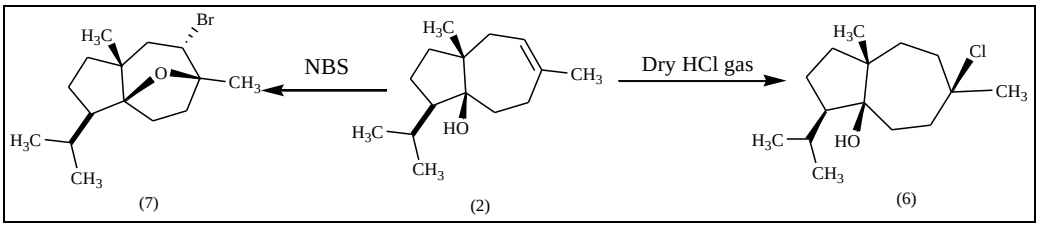


Carotol with hydrochloric gas

A slow stream of hydrochloric acid gas was bubbled through a solution of carotol (1.0 g) in diethyl ether (20 ml) at 0ºC in conical flask till the solution was saturated. The reaction mixture was diluted with water and thoroughly extracted with diethyl ether (3×50 ml). The mixture was finally dried over sodium sulfate. Evaporation of the solvent yielded a thick brownish liquid (6) (Chahal *et al*. 2016) [2].

Carotol with N-bromosuccinimide

Carotol (1.0g), NBS (0.2g) were dissolved in 2 ml of water and mixed in round bottomed flask (100 ml) and silica gel mesh (0.04 g) was added to it. The mixture was stirred at room temperature for 2 h. After the completion of reaction, the mixture was diluted with 50 ml of water and extracted with dichloromethane. The dichloromethane layer was concentrated and pure brown 3-bromo carotol (7) was obtained by column chromatography (Kataria *et al*. 2016) [8].



2.7 Multipication of *M. incognita*

Pure culture of root-knot nematode was raised on brinjal (*Solanum melongena* L.) in pots using single egg mass technique. For maintenance and multiplication of pure culture of *M. incognita* the soil was autoclaved at 15 psi pressure at 120ºC for at least 30 min. The autoclaved soil was filled in the pots. The pots were planted with seedlings of brinjal, a susceptible host for root-knot nematode. Simultaneous inoculations were carried out with freshly hatched second stage juveniles collected from egg masses of pure culture and the culture was multiplied on brinjal plants.

Evaluation of nematicidal activity of carrot seed essential oils, its fractions, isolated and derivatized compounds against *M. incognita* was carried out. Laboratory trials were conducted to test efficacy of carrot seed essential oil, its fractions, isolated and derivatized compounds against *M. incognita*. Nematotoxic activity of all components was evaluated by testing the efficacy on egg hatch and juvenile mortality studies of *M. incognita*.

2.7.1 Hatching test

Five egg masses with an average of 250-300 eggs were collected from pure culture maintained in pot house and placed in 5ml of 250, 500, 1000, 1500, 2000 and 2500 ppm concentrations. These concentrations were prepared by serial dilution of stock solution of 2500 ppm along with 15.00 ml Tween 80 as emulsifier. For each treatment a control in water along with Tween 80 in same amount was taken. Each treatment was replicated thrice. Experiment was conducted at 27º C temperature. Observations on egg hatch were recorded after 24, 48, 72 and 96 h. The percentage of egg hatch inhibition was calculated by the formula.

$$\text{Percent inhibition} = \frac{\text{No. of nematodes in control- No. of nematodes in treatment}}{\text{No. of nematodes in control}} \times 100$$

2.7. 2 Juvenile mortality test

For mortality test, egg masses were collected and kept for hatching of second stage juveniles. Freshly hatched forty juveniles were placed in each concentration of 250, 1000, 1500, 2000 and 2500 ppm and in control. Observations in mortality were recorded after 24, 48, 72 and 96 h. The nematodes were considered dead if found static when probed with fine needle. Revival test was performed. Average of three replications was taken as mortality at a particular concentration.

$$\text{Percent Mortality} = \frac{\text{Total no. of dead nematode juveniles}}{\text{Total no. of nematode juveniles (live + dead)}} \times 100$$

3. Statistical analysis

Percent egg hatch inhibition and per cent mortality data were subjected to statistical analysis using the Duncan's multiple range test on statistical package (SPSS). The interactions of compounds, concentrations and days were tested at P = 0.05 %.

4. Results and Discussion

Carrot seed essential oil was yellowish brown liquid with strong and pleasant odour having refractive index, optical rotation, pH and density of 1.45, +18, 7.8 and 0.987 g cm^{-3} respectively. The essential oil was soluble in dichloromethane and insoluble in water. GC-MS analysis showed the presence of 15 compounds, amounting to 97.8% of the total oil. The analysis showed the presence of sesquiterpene alcohols and hydrocarbons as major components using the NIST, WILEY MS library search. Carotol, daucol and daucene were the major whereas (E)-β-Farnesene, β-Cubebene, longifolenaldehyde, β-Elemene, (E)-Caryophyllene, β-Bisabolene etc were the minor compounds identified. The major compounds daucene, carotol and daucol were isolated by column chromatography. Daucol acetate was prepared by reaction of daucol with acetic anhydride/pyridine. Daucone was prepared by oxidation reaction of daucol with pyridinium chloro chromate/dichloromethane. 4-Chlorocarotol and 3-Bromo carotol were prepared by halogenation of carotol with dry hydrogen chloride gas and N-bromosuccinimide. The derivatives of daucol and carotol were designed to explore the

significance of some structural modifications in relation to their nematicidal activity.

4.1 Egg hatch inhibition studies

The evaluation of carrot seed essential oil against egg hatching of *M. incognita* revealed that at all the concentrations egg hatch count was significantly reduced. Carrot seed essential oil showed 96.95% hatch inhibition at 2500 ppm after duration of 96 h. Polar fraction showed 93.45% inhibition after 96 h at maximum concentration where as non-polar fractions showed only 63.60% inhibition at similar concentration. Among the isolated compounds carotol showed complete egg hatch inhibition at maximum concentration (2500 ppm) and time duration (96 h) whereas for daucol and daucene 74.8 and 46% inhibition was observed under same concentration and time interval. Amongst the derivatized compounds 3- Bromocarotol, daucol acetate, 4-Chlorocarotol and daucone showed 63.45, 62.15, 52.60 and 38.95% inhibition at maximum time interval of 96h and concentration of 2500 ppm. The decreasing order of egg hatch inhibition was as follows

Carotol > Carrot seed essential oil > Polar fraction > Daucol > Non-polar fraction > 3- Bromo carotol > Daucol acetate > 4-Chloro carotol > Daucene > Daucone

Essential oils from *Artemisia absinthium, Artemisia dracunculus, Calamintha citratus, Mentha pulegium, Mentha communis, Origanum vulgare, Salvia officinalis* and *Thymbra mastichina* were previously assessed for nematicidal potential (Kong *et al*. 2006) [11]. Egg hatching tests were more accurate than counting immobile juveniles in a particular population (Oka *et al*. 2000) [14]. The egg hatch inhibition activity tested on egg masses indicated compound ability to penetrate the gelatinous matrix and act on eggs. Essential oils may disrupt the cell membrane of the nematode and change its permeability (Oka *et al*. 2000) [14]. The nematicidal activity of thymol and carvacrol might be mediated through tyramine receptor, as the two compounds were able to trigger a signalling cascade that led to nematode mortality by interacting with a receptor like SER-2 (Lei *et al*. 2010) [12]. Terpenes are known to have the ability to disrupt cell membrane leading to cytoplasmic leakage, cell lysis and cell death (Cox *et al*. 1998) [4]. The isolated compounds in present study *i.e.* daucene, carotol and daucol showed fairly good nematicidal activity. The position of the functional groups and the double bond effect the activity of the monoterpenoids (Park *et al*. 2007) [15].

4.2 Juvenile mortality studies

Carrot seed essential oil showed 90.40% juvenile mortality after 96 h of exposure at maximum concentration of 2500 ppm. The non-polar and polar fractions showed 45.60 and 48.15% mortality after 96 h at maximum concentration of 2500 ppm. From the isolated compounds carotol showed complete mortality after 96 h at maximum concentration whereas for daucene and daucol only 21.10 and 59.42% mortality was recorded under similar conditions. It was found that percent juvenile mortality of carrot seed essential oil, its fractions, compounds isolated and derivatized was both concentration and time dependent. Amongst the derivatives of daucol and carotol, 3-Bromo carotol was more effective than daucol acetate followed by 4-Chloro carotol and daucone. Carotol and daucol were more effective as compared to its derivatives. Thus decreasing order of mortality was as follows:

Carotol > Carrot seed essential oil > Daucol >3-Bromo carotol >Daucol acetate >Polar fraction > 4-Chlorocarotol >Daucone >Nonpolar fraction >Daucene

4.3 Structure activity relationship

It is observed that in egg hatch inhibition and percent juvenile mortality studies carrot seed essential oil was most effective as compared to its fractions. Amongst the fractions of essential oil, polar fraction was more effective than non-polar fraction due to presence of polar compounds like carotol and daucol. Out of three compounds isolated daucene showed minimum egg hatch inhibition and percent juvenile mortality as compared to daucol and carotol. Carotol was highly effective as complete hatching inhibition and juvenile mortality was observed at highest concentration and time interval. It can be inferred that among compounds isolated and derivatised, carotol showed maximum inhibition followed by daucol, daucol acetate, daucene and daucone. The structure activity revealed that maximum activity of carotol might be due to presence of tertiary alcoholic group but when tertiary alcoholic group was converted to secondary alcohol in addition to formation of ether linkage and removal of double bond the nematicidal activity decreased. Thus presence of tertiary alcoholic group might be responsible for higher nematicidal activity whereas secondary alcohol and ether group decreased this activity. In daucene alcoholic group of carotol was replaced by one double bond, which further reduced the activity. Thus presence of double bond decreased the nematicidal activity and this influence of double bond was more than that of secondary and ether group.

The comparison of results of daucol and its derivatives showed low activity in acetate derivative as compared to parent compound daucol, which revealed that the nematicidal decreased on conversion. Thus the conversion of alcoholic group into acetate decreased the activity. Ketone derivative of daucol was also found to be less effective than parent compound *i.e* daucol. Thus it can be concluded that conversion of daucol into acetate and ketone derivates decreased the nematicidal activity, this decrease was more when daucol was converted into ketone derivative. In order to study the effect of halogen on the nematicidal activity 4-Chlorocarotol and 3-Bromocarotol were prepared. The comparative analysis of these two compounds also showed the decreased nematicidal activity in case of 4-Chlorocarotol. 3-Bromocarotol was more effective as compared to 4-Chlorocarotol (5) which may be due to nucleophilic nature of bromine (Salaun 2000) [16].

Hence we can conclude that carrot seed essential oil was found to be more effective than polar and non-polar fractions. Higher activity of essential oil may be due to synergistic effect. Polar fraction was found to be more effective due to presence of polar compounds in it. Presence of tertiary alcohol increased the nematicidal activity and conversion of tertiary alcohol to secondary decreased the nematicidal activity. Presence of double bond decreased the activity, increase in number of double bonds activity of compound decreased. Presence of ether linkage decreased the activity. Conversion of hydroxyl group to acetate decreased the nematicidal activity and this decrease was more when alcoholic group was converted to ketone group.

4.4 Statistical analysis

Statistically the nematicidal activity of different concentrations differed significantly from each other. Nematotoxicity of carrot seed essential oil, its fractions,

compound isolated and derivatized were also found to be significantly different from each other. This was further confirmed by Duncan multiple range test, which showed that the mean values also varied significantly from each other.

5. References

1. Ahmed A, Ahmed A, Mohktar M, Bishr B, Mohamed A, El-Shanawany C *et al*. Rare trisubstituted sesquiterpenes daucanes from the wild *Daucus carota*. Phytochemistry. 2004; 66:1680-1684.
2. Chahal KK, Kaur P, Kataria D, Kaur R. Carotol: A sesquiterpenoid isolated from carrot seed oil. Asian Journal of Chemistry. 2016; 28(5):1004-1006.
3. Chahal KK, Singh B, Kataria D, Dhillon NK. Nematotoxicity of lemongrass oil, citral and its derivatives against *Meloidogyne incognita*. Allelopathy Journal. 2016; 39(2):219-232.
4. Cox SD, Gustafson JE, Mann CM, Markham JL, Liew YC, Hartland RP, *et al*. Tea oil causes K^+ leakage and inhibits respiration in *Escherichia coli*. Letters in Applied Microbiology. 1998; 26:355-35
5. Hallmann J, Davies KG, Sikora R. Biological control using microbial pathogens, endophytes and antagonists 2009. Perry RN, Moens M, Starr JL Eds, CAB International Publishing, UK, 2009, 89-98.
6. Inam-ul-Haq M, Shahina F. Efficacy of neem (*Azadirachta indica*) formulations on biology of Agrios GN, Plant Pathology Edn 5. Printed in the United States of America, New York, 2005, 948.
7. Jasicka-Misiak I, Lipok J, Nowakowska EM, Wieczorek PP, Młynarz P, Kafarski P. Antifungal activity of the carrot seed oil and its major sesquiterpene compounds. Zeitschrift für Naturforschung. 2004; 59:791-796.
8. Kataria D, Chahal KK, Kumar A. Chemical transformations of carrot isolated from carrot seed oil. Asian Journal of Chemistry. 2016; 28(8):1790-1792.
9. Kaur P, Chahal KK, Kaur R, Kataria D. Carrot seed essential oil as stored grain protectant against rust red flour beetle, *Tribolium castaneum*. Pesticide Research Journal. 2016; 28(1):76-83.
10. Kilibarda N, Nanusevic N, Dogivic N, Ivanic R, Savin K. Content of the essential oil of the carrot and its antibacterial activity. Die Pharmazie. 1996; 51:777-778.
11. Kong JO, Lee SM, Moon YS, Lee GS, Ahn YJ. Nematicidal activity of plant essential oils against *Bursaphelenchus xylophilus* Nematoda: *Aphelenchoididae*. Journal of Asia-Pacific Entomology. 2006; 9:173-178.
12. Lei J, Leser M, Enan E. Nematicidal activity of two monoterpenoids and SER-2 tyramine receptor of *Caenorhabditis elegans*. Biochemical Pharmacology. 2010; 79:1062-1071.
13. Li HQ, Liu QZ, Liu ZL, Du SS, Deng ZW. Chemical composition and nematicidal activity of essential oil of *Agastache rugosa* against *Meloidogyne incognita*. Molecules. 2013; 18:4170-4180.
14. Oka Y, Koltai H, Bar-Eyal M, Mor M, Sharon E, Chet I, *et al*. New strategies for the control of plant parasitic nematodes. Pest Management Science. 2000; 56:983-988.
15. Park I, Kim J, Lee S, Shin S. Nematicidal activity of plant EOs and components from ajowan (*Trachyspermum ammi*), allspice (*Pimenta dioica*) and litsea (*Litsea cubeba*) EOs against pine wood nematode (*Bursaphelenchus xylophilus*). Journal of Nematology. 2007; 39:275-279.
16. Salaun J. Cyclopropane derivatives and their diverse biological activities. In: Topics in current chemistry, Springer-Verlag Berlin Heidelberg, 2000, 1-55.