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Composition and yield variation of essential oils from French marigold (*Tagetes patula* L.) genotypes using GC-MS

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ABSTRACT

Essential oil content in flowers from different genotypes of marigold belonging to the species *Tagetes patula* were estimated by hydro-distillation and examined by gas chromatography (GC) and GC-mass spectrometry (GC-MS). Essential oil content in floret tissue ranged from 0.02 to 0.09% on fresh weight basis. The maximum essential oil content based on 50 g fresh weight was found in genotype Orange Winner followed by Bolero Red, Boy-o-Boy, and Pusa Arpita, whereas, minimum essential oil content was in F/R-2. The essential oil showed higher concentration of components like β-caryophyllene, terpinolene, caryophyllene oxide, (Z)-β-ocimene, piperitenone, piperitone, (E)-ocimenone, (Z)-ocimenone, limonene and germacrene-D. The results clearly showed that the genotypes had wide variations for essential oil content, quite similar qualitative oil composition but differed for their relative oil percentages. Therefore, thorough screening of these genotypes for their phyto-constituents and further crop improvement programme will be beneficial for pharmaceutical and cosmetic industries.

Key words: Essential oil, flowers, GC-MS, genotypes, *Tagetes patula* L.

INTRODUCTION

The genus *Tagetes* spp. popularly, known as French marigold belongs to the Asteraceae family is a annual flower crop, native of Mexico. In India, it ranks first in area and production among loose flowers crops, *i.e.* it occupies an area of 55,890 hectares with production of 5,11,310 metric tonnes loose flower (Anon, 2). The major marigold growing states are Karnataka, Gujarat, Maharashtra, Haryana, Andhra Pradesh, Uttar Pradesh, Chhattisgarh, Odisha, Jammu & Kashmir, Puducherry, Andaman & Nicobar Islands, Arunachal Pradesh, West Bengal, Tamil Nadu, *etc.* There are about 36 species listed under genus *Tagetes*; out of which two species, namely, *T. erecta* (African marigold) and *T. patula* (French marigold) are most popular as loose flower crops (Nehar, 1968). Besides ornamental value, marigold has a long history of human use for its aromatic essential oils as well as for its medicinal and nematicidal property (Olabiye and Oyedunmade, 9). The essential oil extracted from the flower contains antioxidants as reported by (Priyanka *et al.*, 12) and also found that it can be used an antiseptic, a fly repellent and as hair lotion modifier (Piccaglia *et al.*, 20). Some typical constituents of *Tagetes* spp. oil are reported to have specific properties such as ocimenones, which show antifungal and larvicidal activity besides dihydrotagetones, tagetones, ocimenones, metyl chavicol, *etc*. which possess pleasant scent and currently employed in flavour and fragrance industries (Marotti *et al.*, 6). The objectives of present study were to study the essential oil variation and its components in flowers of different genotypes of *T. patula*, which will be beneficial for further crop improvement programme.

MATERIALS AND METHODS

The experimental material comprised of 11 French marigold (*Tagetes patula* L.) genotypes, namely, F/R-8, F/R-2, Dainty Marietta, Boy o Boy, Gulzafri Orange, Gulzafri Yellow, Bolero Red, Queen Sohpia, Pusa Arpita, Orange Winner and Red Brocade. The field experiment was carried out at the research farm of IARI, New Delhi. The seeds of all the genotypes were sown in the protrays with soilless mixture comprising of cocopeat:perlite: vermiculite (3:1:1) inside a polyhouse during rainy season, *i.e.* June during 2014. The seedlings were transplanted at spacing of 45 cm × 45 cm and the standard cultural practices like hoeing, irrigation, weeding, staking and fertilizer application were followed as per the requirement of the crop.

The flower samples (1 kg) were harvested early morning, chopped and dried under shade. Harvesting was done at full growth stage before the initiation of flower buds. The extraction of essential oil was undertaken at ICAR-NBPGR, New Delhi. The essential oils were isolated by hydro-distillation for 3 h using a

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Clevenger-type of apparatus. The oil content (% v/w) was calculated on fresh weight basis. All the samples so obtained from flowers were dried over anhydrous sodium sulphate (Na_2SO_4) and stored in amber coloured glass bottles at refrigerated conditions prior to GC-FID and GC/ MS analyses.

Capillary gas chromatography (GC) was carried out on gas chromatograph (Agilent gas chromatograph 7890 A), equipped with a flame ionization detector (FID) and a non-polar HP-5MS capillary column made up of 5% phenyl methyl silicone, 95% dimethylpolysiloxane (30 × 0.25 mm, 0.25 µm film thickness). Helium was used as the carrier gas at the flow rate of 1 ml/min. The oven temperature was programmed from 60° to 240°C at a rate of 3°C /min. with initial hold of 10 min. at 60°C and final hold of 10 min. at 240°C. The injector and detector temperatures were maintained at 220° and 250°C, respectively. The sample (0.1 µl) was injected neat in a split ratio (1:40) at 220°C. Area percentage reports obtained by GC-FID were used for quantification purposes. GC/ MS analysis was carried out on an Agilent GC/MS equipped with a MSD detector 5975C and a HP-5MS capillary column (30 cm length/ 0.25 mm internal diameter: 0.25 µm film coating) under similar chromatographic conditions as mentioned above. Helium was used as the carrier gas. The mass unit conditions were ion source 250°C, ionization energy 70 eV. The acquisition mass range used was 40-400 mHz. The volatile constituents were identified by comparing the retention indices determined with reference to a homologous series of n-alkanes under identical experimental condition, co-injection with that of authentic compounds (Sigma grade) and matching mass spectral data of the peaks with mass spectra with those stored in NIST/ Wiley and Adams mass spectral libraries and literature values (Adams, 1). The relative amounts of individual components were calculated based on GC peak area (FID response) without using a correction factor.

RESULTS AND DISCUSSION

Essential oils yield ranged from (0.02 to 0.09%) on fresh weight basis, respectively. The maximum oil yield was found in genotype Orange Winner (0.12%) followed by Bolero Red (0.09%) , Boy-o-Boy (0.09%), Pusa Arpita (0.09%), Gulzafri Yellow (0.08%), Dainty Marietta (0.07%), Queen Sophia (0.07%), F/R-8 (0.07%) and lowest in F/R-2 (0.02%). The essential oil yield of different genotypes are presented in Fig. 1. Earlier, Garg *et al*. (4) have isolated the essential oil (0.09%) from capitula, while Negi *et al.* (7) reported 0.18% volatile crop oil and Rondon *et al*. (14) found that aerial parts yielded 0.17% oil.

Gas chromatography (GC) and GC-mass spectrometry (GC-MS) were analysed and the components are listed in order of the retention index of the composition (Table 1). Essential oil composition showed 33 components of which oxygenated monoterpenes constituted the major part ranged from

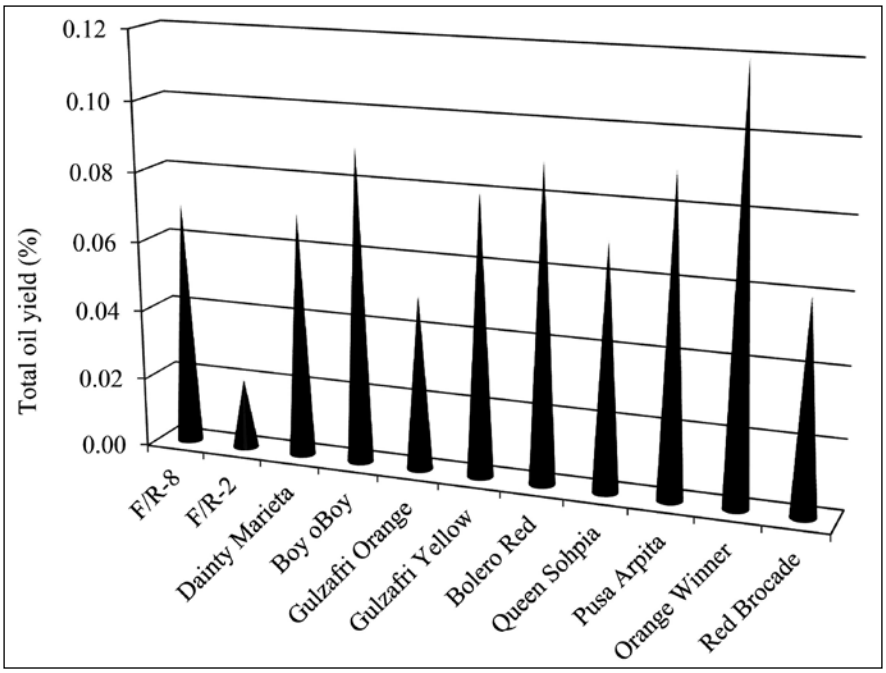

Fig. 1. Total essential oil yield from flowers of *T. patula* genotypes.

Table 1. Chemical composition (%) of essential oil extracted from flowers of French marigold genotypes.

Sl. No.	Component	Genotype code*														
		RRI cal	RRI lit	1	2	3	4	5	6	7	8	9	10	11	Range	Mean
1.	α-Pinene	931	939	1.52	0.99	0.23	0.34	-	-	0.12	0.15	0.24	0.36	0.23	0.12-1.52	0.46
2.	Sabinene	976	976	0.52	0.42	0.23	0.73	0.28	0.43	0.34	0.51	0.57	0.65	0.44	0.23-0.73	0.47
3.	Myrcene	986	991	4.23	2.65	0.22	0.26	-	0.27	0.20	0.23	0.17	0.65	-	0.17-4.23	0.99
4.	α-phellandrene	1004	1005	0.35	0.24	0.55	0.56	0.33	0.16	0.12	0.25	0.57	-	0.40	0.12-0.57	0.35
5.	Limonene	1022	1031	6.11	0.90	3.01	5.13	4.89	5.93	3.52	4.33	6.26	7.76	5.94	0.90-7.76	4.89
6.	(Z)-(β)-ocimene	1033	1040	9.74	3.55	14.66	7.23	10.87	6.43	15.94	16.82	12.88	11.23	8.71	3.55-16.82	10.93
7.	(E)-(β)-ocimene	1043	1050	1.08	-	2.83	1.65	1.38	4.65	2.36	3.39	1.54	1.28	1.13	1.08-4.65	2.13
8.	Dihydrotagetone	1056	1054	0.78	0.83	1.17	1.08	0.86	2.85	0.90	1.81	0.59	0.44	2.29	0.44-2.85	1.24
9.	Terpinolene	1088	1088	18.55	0.77	6.77	10.32	16.59	12.11	9.38	12.58	11.61	25.50	16.76	0.77-25.5	12.81
10.	Linalool	1098	1098	0.35	-	0.29	0.54	0.33	0.61	0.29	0.47	0.34	0.19	0.45	0.19-0.61	0.39
11.	P-mentha-1,3,8-triene	1112	1111	0.56	0.39	-	0.34	0.67	0.28	-	0.73	0.11	0.16	0.31	0.11-0.73	0.39
12.	Allo-ocimene	1129	1129	0.21	-	0.31	0.99	0.27	0.22	0.63	0.66	0.40	0.38	0.33	0.21-0.99	0.44
13.	(Z)-ocimenoxide	-	-	1.83	0.33	0.77	1.21	1.64	0.59	1.37	0.46	3.79	0.68	0.72	0.33-3.79	1.22
14.	(E)-ocimenoxide	-	-	0.99	0.51	1.38	2.12	0.70	1.56	1.37	0.63	2.03	0.41	0.60	0.41-2.12	1.12
15.	(E)-tagetone	1147	1146	3.26	1.48	1.29	2.89	3.56	2.62	2.46	4.38	3.53	2.06	2.29	1.29-4.38	2.71
16.	(Z)-tagetone	1151	1153	4.63	0.81	1.30	4.32	6.15	3.13	2.94	4.54	0.20	4.38	3.59	0.20-6.15	3.27
17.	Borneol	1168	1165	0.21	-	-	0.23	0.21	0.25	0.44	-	0.17	0.35	0.36	0.17-0.44	0.28
18.	Terpinen-4-ol	1177	1177	0.70	0.65	0.39	0.42	0.60	0.59	0.34	0.50	0.84	-	0.79	0.34-0.84	0.58
19.	p-cymen-8-ol	1185	1183	0.65	0.78	0.52	0.56	0.34	1.37	0.42	0.51	0.82	0.56	1.19	0.34-1.37	0.70
20.	α- terpineol	1189	1176	0.48	-	0.34	0.73	0.94	0.75	0.29	0.57	0.78	0.87	0.52	0.29-0.94	0.57
21.	(Z)-ocimenone	1231	1231	7.27	2.81	4.00	4.32	6.23	3.56	6.31	5.43	6.29	4.09	3.01	2.81-7.27	4.93
22.	(E)-ocimenone	1240	1239	2.46	-	2.53	6.23	5.23	2.85	4.48	2.89	6.38	-	3.41	2.46-7.34	4.05
23.	Piperitone	1257	1252	3.67	0.44	4.87	1.23	3.35	6.23	1.22	1.69	5.98	5.19	10.20	0.44-14.90	4.0
24.	Thymol	1288	1289	0.40	2.97	-	2.12	0.32	0.80	1.10	1.12	0.11	-	1.10	0.11-2.97	1.12
25.	Piperitenone	1346	-	13.82	3.04	5.56	8.43	8.96	14.75	5.36	4.18	12.77	12.96	12.47	3.04-14.76	9.86
26.	Piperitenone oxide	1369	-	0.56	-	0.65	0.76	0.22	0.15	0.16	-	0.55	-	0.57	0.15-0.76	0.45
27.	β-caryophyllene	1418	1418	7.34	42.76	31.13	21.20	15.15	5.75	24.49	20.26	6.36	3.92	8.36	3.92-42.76	16.90
28.	Germacrene-D	1480	1480	2.56	2.13	6.72	4.50	3.40	2.27	5.00	4.66	1.48	2.46	2.37	1.48-6.72	3.41

Contd...

Table 1 Contd...

Sl. No.	Component	RRI cal	RRI lit	Genotype code* 1	2	3	4	5	6	7	8	9	10	11	Range	Mean
29.	Bicyclogermacrene	1505	1508	1.23	1.27	3.12	2.10	2.03	1.34	2.19	1.66	1.02	1.51	1.26	1.02-3.12	1.70
30.	(E)-β-farnesene	1459	1458	0.27	0.85	0.51	0.31	0.39	0.33	0.43	0.48	0.27	0.20	0.10	0.10-0.85	0.38
31.	(E)-nerolidol	1586	1580	0.33	0.69	0.25	0.76	0.46	0.57	0.21	0.25	0.30	0.51	0.63	0.21-0.76	0.45
32.	Spathulenol	1580	1577	0.45	4.29	1.21	1.21	0.45	0.92	0.53	0.55	0.86	0.46	1.35	0.45-4.29	1.12
33.	Caryophyllene oxide	1590	1581	1.36	15.23	2.13	2.17	0.47	1.59	1.45	1.25	-	0.68	3.15	0.47-15.23	2.9
	Monoterpenes hydrocarbons			24.16	11.72	22.04	21.23	18.34	18.89	24.33	27.46	22.74	22.31	18.28	11.72-27.46	21.22
	Oxygenated monoterpenes			64.75	15.81	32.14	51.51	60.19	55.27	39.46	43.15	57.29	58.22	60.96	15.81-64.75	51.93
	Sesquiterpenes hydrocarbons			13.25	47.01	41.48	28.11	20.97	9.69	32.11	27.06	9.13	8.09	12.09	8.09-47.01	21.26
	Oxygenated sesquiterpenes			2.14	29.28	3.59	4.14	1.38	3.08	2.19	2.05	1.16	1.65	5.13	1.16-29.28	5.43

t = trace (<0.1%); RRI cal, relative retention indices calculated; RRI lit, relative retention indices reported in literature; *Genotypes code: 1 = F/R-8, 2 = F/R-2, 3 = Dainty Marieta, 4 = Boy o Boy, 5 = Gulzafri Orange, 6 = Gulzafri Yellow, 7 = Bolero Red, 8 = Queen Sohpia, 9 = Pusa Arpita, 10 = Orange Winner, 11 = Red Brocade

(15.81-64.75%) followed by monoterpens hydrocarbons (11.72-27.46%), sesquiterpenes hydrocarbons (8.09-47.01%) and oxygenated sesquiterpenes (1.16-29.28%), respectively. The essential oil had higher concentration of β-caryophyllene ranged from (3.92-42.76%), terpinolene (0.77-25.5%), caryophyllene oxide (0.47-24.3%), (Z)-β-ocimene (3.55-16.82%), piperitenone (3.04-14.76%), piperitone (0.44-14.90%)%),(E)-ocimenone (2.53-7.34%), limonene (0.90-7.76%), (Z)-ocimenone (2.81-7.27%), and germacrene-D (1.48-6.72%). The result are in close confirmation by Szarka *et al.* (15), oils from flower heads were rich in β-caryophyllene (53.5%) and Marotti *et al.* (6) experiment showed among sesquiterpenes, β-caryophyllene (18.2%) was the most abundant responsible for woody note and the lasting aroma of the essential oils in flower. The major terpenes, *viz*. piperitone (24.74%), piperitenone (22.93%), terpinolene (7.8%), dihydro tagetone (4.91%), cis-tagetone (4.62%), limonene (4.52%), and allo-ocimene (3.66%) were the major essential oil compounds obtained from capitula (Romagnoli *et al.*, 13). Recently, Prakash *et al.* (11) experiment conducted on the chemical compositions from the capitula result in identification of (Z) -α-ocimene, (E) -β-ocimene, terpinolene, (Z)-ocimenone and (E)-ocimenone. The essential oil of the leaves, flowers and stems were reported to contain ocimene, limonene, linalool, linayl acetate and tagetone (Dhingra and Dhingra, 3).

Essential oils contained more or less the same compounds differing only in the relative percentages. Among the 11 genotypes, β-caryophyllene was found highest in flowers of F/R-2 (42.76%) followed by Dainty Marietta (31.13%), Bolero Red (24.49%), Boy-o-Boy (21.20%), Queen Sophia (20.26%), Gulzafri Orange (15.15%), F/R-8 (9.19%) and lowest in Orange Winner (3.92%). Flower of Orange Winner (25.5%) recorded highest terpinolene content followed by F/R-8 (18.55%), Red Brocade (16.76%), Gulzafri Orange (16.59%), Queen Sophia (12.58%), Gulzafri Yellow (12.11%), Pusa Arpita (11.61%), Bolero Red (9.38 %) and minimum in F/R-2 (0.77 %). Caryophyllene oxide content was found highest in F/R-2 (24.30%) followed by Red Brocade (3.15%), Boy-o-Boy (2.17 %), Dainty Marietta (2.13%) and minimum in Orange Winner (0.68%). (Z)-β-ocimene content was the highest in Queen Sophia (16.82%) followed by Bolero Red (15.94%), Dainty Marietta (14.66%), Pusa Arpita (12.88%), Orange Winner (11.23%), Gulzafri Orange (10.87%) and lowest in F/R-2 (3.55%). Maximum piperitenone content was recorded in Gulzafri Yellow (14.75%) followed by F/R-8 (13.82%), Orange Winner (12.96%), Pusa Arpita (12.77%), Red Brocade (12.47%) and minimum in F/R-2 (3.04%). Red Brocade (10.20%)

recorded the maximum content of piperitone followed by Gulzafri Yellow (6.23%), Pusa Arpita (5.98%), Orange Winner (5.19%), Dainty Marietta (4.87%), Boy-o-Boy (3.90%) and minimum content was recorded in F/R-2 (0.44%). (E)-ocimenone content was found highest in Gulzafri Orange (7.34%) followed by Pusa Arpita (6.38%), Boy-o-Boy (6.23%), F/R-8 (5.83%), Bolero Red (4.48%), Red Brocade (3.41%) and lowest was recorded in Dainty Marietta (2.53%). F/R-8 (7.27%) content maximum (Z)-ocimenone followed by Gulzafri Orange (7.14%), Bolero Red (6.31%), Pusa Arpita (6.29%), Queen Sophia (5.43%), Boy-o-Boy (4.32%), Orange Winner (4.09%) and minimum content was found in F/R-2 (2.81%). Limonene content was recorded highest in Orange Winner (7.76%), Pusa Arpita (6.26%), F/R-8 (6.11%), Red Brocade (5.94%), Gulzafri Yellow (5.93%), Boy-o-Boy (5.13%), whereas, the lowest content was observed in F/R-2 (0.90%). Germacrene-D content found maximum in Dainty Marietta (6.72%) followed by Bolero Red (5.00%), Queen Sophia (4.66%), Boy-o-Boy (4.50%), Gulzafri Orange (3.40%), F/R-8 (2.56%) and minimum content was found in Pusa Arpita (1.48%). The essential oil of the flowering shoots was reported to contain (Z)-ocimenone, (E)-ocimenone, limonene, terpinolene, piperitenone and Caryophyllene (Lawrence *et al.,* 5). β-ocimene, α-terpinolene, *trans*-caryophyllene, Z-ocimenone, dl-limonene, piperitenone, α-pinene and car -3-en-2-one are predominant components in flowers oil (Negi *et al*., 7) crop.

Similarly, representative chromatogram (GC/FID) of major component of thirty three compounds identified by GC/MS analysis with percentage of corresponding peak ranging from 60 to 96% of the total in flowers is presented in (Fig. 2). Among 33 compounds identified majority of them were monoterpenes with a high percentages of oxygenated monoterpenes, *viz*. dihydrotagetone, terpinolene, piperitenone, (E)-tagetone, (E)-tagetone, followed by monoterpenes hydrocarbon, *viz*. limonene, (Z)-β-ocimene, thymol and β-caryophyllene. The most abundant among sesquiterpenes was β-caryophyllene followed by germacrene-D. The oil of the flowers showed higher concentration of β-caryophyllene (20.06%) at rentention time (RT) 32.029, (Z)-β-ocimene (16.82%) at 16.979 (RT), terpinolene (12.58%) at 19.512 (RT), germacrene-D (4.66%) at 33.951 (RT), piperitenone (4.18%) at 29.626 (RT), (Z)-tagetone (4.38%) at 21.946 (RT), (E)-tagetone (4.54%) at 21.959 (RT), piperitone (1.69%) at 26.502 (RT), dihydrotagetone (1.81%) at 17.585 (RT), thymol (1.12%) at 27.849 (RT), and limonene (0.47%) at 16.476 (RT), which resemblance the composition reported by Marotti *et al.* (6) made on *T. patula* flowers had high β-caryophyllene (18.2%) content among sesquiterpenes. The oil of flowers had high concentration of (Z)-β-ocimene, linalool, dihydrotagetones, piperitenone, β-caryophyllene and piperitone oxide by Romagnoli *et al.* (13) also reported that the composition of a steam-distilled oil from the capitula of *T. patula*, which shows low levels of tagetone (dihydrotagetone 4.91%) and higher levels of piperitone (24.74%) and piperitenone (22.93%).

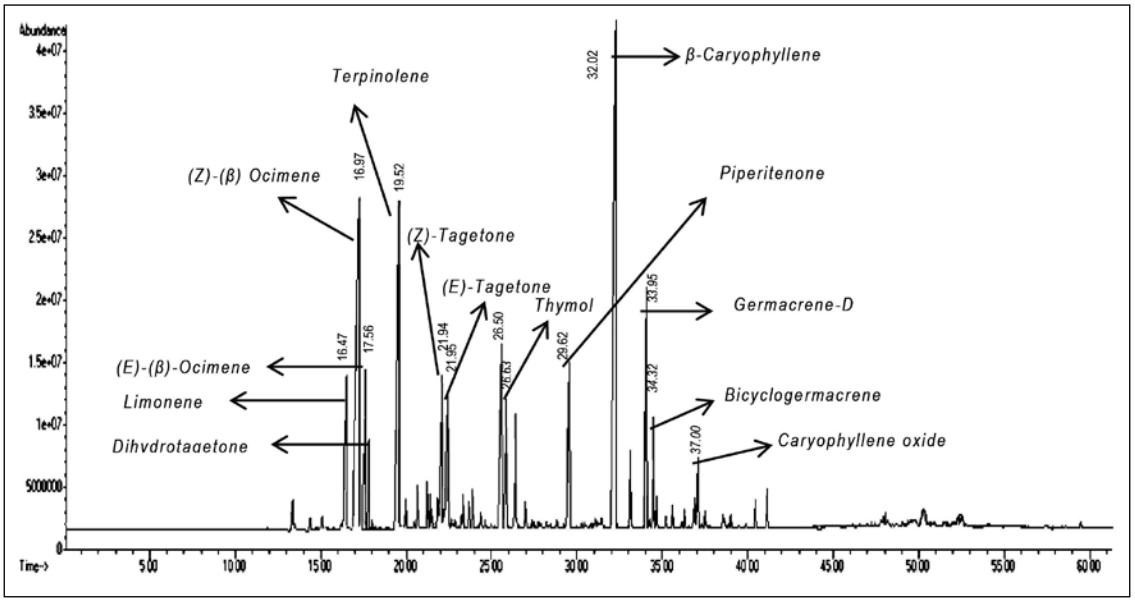

Fig. 2. Total-ion chromatograms of major essential oil components extracted from flower in *T. patula* genotype Queen Sophia.

Our results revealed wide variation for essential oils yield in flowers of *T. patula* genotypes. Although the genotypes showed very similar qualitative oil composition and differed only in the relative percentages. Terpinolene, piperitenone, (z)-β-ocimene, piperitone, limonene and β-caryophyllene were major constituents of essential oils identified in flowers. Genotypes such as Boy-o-Boy, Dainty Marietta, Gulzafri Orange, Bolero Red, Gulzafri Yellow of *T. patula* besides ornamental value has potential source for commercial exploitation of various compounds. Therefore, thorough screening of genotypes for their phytoconstituents will be beneficial for further crop improvement programme of marigold.

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