



Gas chromatography-Mass spectrometry analysis for profiling of essential oil constituents derived from leaves of French marigold (*Tagete patula*)

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ABSTRACT

Different genotypes of marigold (*Tagetes* sp) belonging to *T. patula* were investigated for essential oil content in leaves by hydro distillation and examined by gas chromatography (GC) and GC-mass spectrometry (GC-MS). Results indicated that there were wide variations in genotypes for essential oil yield and chemical composition. Essential oil content in leaves of *T. patula* ranged from 0.08 to 0.15% on fresh weight basis. The maximum essential oil content in leaves was found in cultivar Boy-o-Boy followed by Dainty Marietta, Gulzafri Orange, Bolero Red, Gulzafri Yellow. The essential oil of leaves contained higher amounts of terpinolene, (*Z*)- β -ocimene, piperitone, piperitone, limonene, (*E*)-ocimenoxide, β -caryophyllene, (*E*)- β -ocimene, (*Z*)-ocimenone and (*E*)-tagetone. The results of our study clearly showed the presence of wide variations in essential oil yield content in the leaves. The genotypes showed very similar qualitative oil composition and differing only in their relative essential oil percentages. Therefore, thorough screening of genotypes for its phytoconstituents will be beneficial for further crop improvement programme.

Key words: Essential oil, GC-MS, Hydrodistillation, Leaves, *Tagetes patula*

The genus *Tagetes* (marigold) belongs to the Asteraceae family and is native of Central and South America, especially Mexico. It is mostly grown as ornamental crop worldwide due to their wider adaptability to grow in different types of soils under diverse agroclimatic conditions. The genus comprises of 36 species worldwide, out of which, five species have been introduced into the India were *Tagetes erecta* L. (African marigold), *Tagete sminuta* L. (wild marigold), *Tagete spatula* L. (French marigold), *Tagetes lucida* Cav. (sweet scented marigold), *Tagetes tenuifolia* (striped marigold) as reported by Rydberg (1915). Most of these species produce essential oils and compounds of unique biological activities for use in agro chemicals, perfumery, cosmetics, foods, colourants, nutritional supplements and in pharmaceutical applications (Anonymous 1976). *Tagetes patula* and *Tagetes erecta* were mainly utilized for floriculture purposes and raw material for commercial production of several kinds of useful compounds (Singh *et al.* 2016). In India, marigold occupies an area of 55 890

ha with production of 511 310 metric tonnes loose flower (Anonymous 2014). It is grown as a major commercial loose flower crop and being used in religious and social ceremonies. Besides, it is traditionally utilized for human use as beverages, condiments and in folk medicines. First time chemistry of *T. patula* essential oil was studied by Igolen (1936). The oils of *T. erecta* and *T. patula* were found to contain limonene, α -terpinolene, piperitone and β -caryophyllene components (Hethelyi *et al.* 1998). Besides, Machado *et al.* (1994) had identified β -caryophyllene, limonene, piperitone and piperitinone as main constituents in *T. patula* oil. The essential oil of the leaves, flowers and stems of *T. patula* was reported to contain ocimene, limonene, linalol, linayl acetate and tagetone (Dhingra and Dhingra 1956). In the present research, the essential oil yield and chemical composition of 11 genotypes of *T. patula* genotypes leaves have been assessed.

MATERIALS AND METHODS

The field experiment was carried out at the research farm of Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, New Delhi, at 77° 12' E longitude 28°40'N latitude and at an altitude of 228.16m amsl. The experimental design was a randomized block with three replications. The materials utilized consisted of eleven genotypes of French marigold (*Tagetes patula*), namely F/R-8, F/R-2, Dainty Marietta, Boy-o-Boy, Gulzafri Orange, Gulzafri Yellow, Bolero Red, Queen Sophia,

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PusaArpita, Orange Winner and Red Brocade. The seeds of all the genotypes were sown in the pro-trays with soilless mixtures comprising of cocopeat:perlite:vermiculite in ratio of 3:1:1 and planted in polyhouse during rainy season, i.e. June during 2014. Irrigation was given daily with rosecan so as to maintain proper moisture. Drenching was done with 19:19:19 @ 0.5% (5g/l) at 15 days after sowing. Transplanting of these seedlings was carried when plants reached four to five leaf stage after sowing. The seedlings were then transplanted at spacing of 45×45 cm and the standard cultural practices like hoeing, irrigation, weeding, staking and fertilizer application were followed as per the requirement of the crop.

The research work was carried out at ICAR-National Bureau of Plant Genetic Resources, New Delhi. Fresh leaves of *T. patula* genotypes were collected separately early morning around 5 to 7 AM at full growth stage before the initiation of flower buds. Harvested fresh leaves weighing one kilogram were cut into small pieces and immediately subjected to hydrodistillation for 3 hr using a Clevenger-type of apparatus. The extracted oils from leaves were dried over anhydrous sodium sulphate (Na₂SO₄) and kept in brown glass bottles at refrigerated conditions prior to GC-FID and GC/MS analysis.

Capillary gas chromatography (GC) was carried out using an 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 (30x 0.25 mm, 0.25 µm film thickness). Helium was used as the carrier gas at the flow rate of 1 ml/minute. The oven temperature was programmed from 60° to 240°C at a rate of 3°C /minute 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 were carried out on an Agilent GC/MS equipped with a MSD detector 5975C and a HP-5MS capillary column (30 m length/0.25mm 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 m/z. 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) 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 2001, Davies 1990). The relative amounts of individual components were calculated based on GC peak area (FID response) without using a correction factor.

RESULTS AND DISCUSSION

Marigold (*T. patula*) genotypes leaves collected were

evaluated for essential oil content and oil quality. Results showed wide variation in essential oil content among genotypes ranging from 0.08 to 0.15% on fresh weight basis. The Boy-o-Boy (0.15%) was found to be superior for high essential oil content followed by Dainty Marietta (0.13%), Gulzafri Orange (0.13%), Bolero Red (0.13%), Gulzafri Yellow (0.13%), Pusa Arpita (0.12%), Red Brocade (0.12%) and lowest in Queen Sophia (0.08 %) and F/R-2 (0.08 %). The colour obtained from essential oil varied from green to greenish yellow. Igolen (1936) also reported essential oil content (0.3%) by steam distillation in *T. patula*. Rondon *et al.* (2006) reported essential oil colour variation from greenish yellow to yellow in *T. patula* and essential oil content (0.17%).

Thirty-three aroma compounds were identified in the leaves oil of *T. patula* by GC and GC/MS analysis representing 65% to 99% of total oil (Table 1). The leaves oil contain large amounts of oxygenated monoterpenes ranged (75.54-84.66%) followed by monoterpenes hydrocarbons (5.50-10.85%), sesquiterpenes hydrocarbons (4.11-11.12%) and oxygenated sesquiterpenes (0.72-2.16%). The majority of them were monoterpenes with high percentage being oxygenated compounds. Marotti *et al.* (2004) evaluated six species of *Tagetes* viz., *T. patula*, *T. erecta*, *T. tenuifolia*, *T. lucida*, *T. filifolia* and *T. minuta* and identified 35 compounds which were mostly monoterpenes with high percentage being oxygenated compounds and among sesquiterpenes β-caryophyllene was found in abundance.

The essential oils from leaves showed higher amounts of terpinolene ranged from (13.72 to 21.79%) followed by (*Z*)-β-ocimene (4.09 to 19.53%), piperitone (2.46 to 19.46%), piperitone (2.46 to 19.46%), limonene (4.89 to 8.83%) and (*E*)-ocimenoxide (2.21 to 8.08%), β-caryophyllene (2.23-6.65%), (*E*)-β-ocimene (0.79-10.56%), (*Z*)-ocimenone (0.20-9.42%) and (*E*)-tagetone (0.52-7.74%). The result is in close confirmation by Szarka *et al.* (2006), oils from *T. patula* leaves contained high concentration terpinolene (21.1%). Besides, Machado *et al.* (1994) had identified β-caryophyllene, limonene, piperitone and piperitinone as main constituents in *T. patula* oil. The essential oil of the leaves, flowers and stems of *T. patula* was reported to contain ocimene, limonene, linalool, linayl acetate and tagetone (Dhingra and Dhingra 1956).

The different genotypes of *T. patula* were evaluated for individual constituents of the essential oil content in leaves. It was found that terpinolene content was highest in genotype Red Brocade (21.79%) followed by Pusa Arpita (19.92%), F/R-8 (19.75%), Bolero Red (19.59%), Gulzafri Yellow (18.14%) whereas lowest was found in Dainty Marietta (13.72%). Maximum (*z*)-β-ocimene content was found in F/R-2 (19.53%) followed by F/R-8 (19.10%), Bolero Red (15.63%), Gulzafri Yellow (11.23%), Dainty Marietta (15.50%) and lowest was in Boy-o-Boy (4.09%). Red Brocade (18.30%) recorded highest piperitone content followed by Pusa Arpita (18.24%), Boy-o-Boy (16.48%), Gulzafri Orange (15.16%), Queen Sophia (14.04%) and minimum in F/R-2 (6.25%). The piperitone

Table 1 Chemical composition (%) of essential oil extracted from leaves of *Tagetes patula* genotypes

Components	RRI cal	RRI lit	Genotypes code *											Range	Mean		
			1	2	3	4	5	6	7	8	9	10	11				
α -Pinene	931	939	0.33	0.27	0.24	0.37	0.30	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.24-0.37	0.27
Sabinene	976	976	0.7	0.74	0.60	0.55	0.28	0.4	0.74	0.79	0.48	0.62	0.81	0.28-0.81	0.61	0.28-0.81	0.61
Myrcene	986	991	0.24	0.27	0.24	0.48	0.13	0.21	0.26	0.09	0.34	0.09-0.48	0.25	0.09-0.48	0.25	0.09-0.48	0.25
α -Phellandrene	1004	1005	0.45	0.73	0.69	0.45	0.33	0.44	0.48	0.46	0.60	0.34	0.51	0.33-0.73	0.50	0.33-0.73	0.50
Limonene	1022	1031	7.09	8.78	6.76	7.36	4.89	5.39	7.65	8.15	6.81	7.39	8.83	4.89-8.83	7.19	4.89-8.83	7.19
(Z)-(β)-Ocimene	1033	1040	19.10	19.53	15.50	4.09	10.87	11.23	15.63	9.52	9.95	9.46	9.38	4.09-19.53	12.21	4.09-19.53	12.21
(E)-(β)-Ocimene	1043	1050	2.28	2.49	1.41	4.23	1.38	1.45	1.46	2.79	1.32	1.96	0.79	0.79-4.23	1.96	0.79-4.23	1.96
Dihydrotagetone	1056	1054	1.04	2.01	0.23	1.56	0.86	0.99	1.24	3.08	0.75	1.7	0.23-3.08	1.35	0.23-3.08	1.35	
Terpinolene	1088	1088	19.75	20.12	13.72	16.05	16.59	18.14	19.59	17.45	19.92	17.62	21.79	13.72-21.79	18.25	13.72-21.79	18.25
Linalool	1098	1098	0.40	0.45	0.24	0.82	0.33	0.27	0.35	0.36	0.35	0.35	0.27	0.24-0.82	0.38	0.24-0.82	0.38
P-mentha-1,3,8-triene	1112	1111	0.24	0.22	0.25	0.78	0.67	0.14	0.22	0.27	0.64	0.77	0.14-0.78	0.42	0.14-0.78	0.42	
Allo-ocimene	1129	1129	0.33	0.34	0.34	0.10	0.27	0.28	0.23	0.46	0.30	0.10-0.46	0.29	0.10-0.46	0.29		
(Z)-ocimenoxide			0.24	0.18	4.43	0.12	1.64	1.52	1.09	0.70	0.99	0.99	2.06	0.12-4.43	1.27	0.12-4.43	1.27
(E)-ocimenoxide			0.42	0.37	2.21	2.88	0.7	0.52	0.58	0.68	0.42	1.02	0.86	0.37-2.88	0.97	0.37-2.88	0.97
(E)-tagetone	1147	1146	6.09	6.13	3.98	0.52	6.15	6.58	7.74	6.97	4.79	5.57	1.78	0.52-7.74	5.12	0.52-7.74	5.12
(Z)-tagetone	1151	1153	5.26	5.88	2.41	0.7	3.56	4.23	3.9	5.33	2.65	4.3	1.46	0.7-5.88	3.61	0.7-5.88	3.61
Borneol	1168	1165	0.22	0.22	0.08	0.08	0.21	0.16	0.23	0.56	0.56	0.08-0.56	0.24	0.08-0.56	0.24		
Terpinen-4-ol	1177	1177	0.84	0.55	0.63	0.46	0.6	0.33	0.56	1.01	0.61	1.88	0.48	0.33-1.88	0.72	0.33-1.88	0.72
p-cymen-8-ol	1185	1183	0.59	0.67	0.50	0.50	0.34	0.84	0.84	0.89	0.55	0.73	0.84	0.34-0.89	0.66	0.34-0.89	0.66
α -terpineol	1189	1176	0.94	0.81	0.78	0.53	0.94	0.85	0.48	1.08	1.23	1.04	0.48-1.23	0.87	0.48-1.23	0.87	
(Z)-ocimenone	1231	1231	9.42	5.64	6.87	0.20	7.14	7.66	5.63	3.63	6.02	5.77	6.70	0.20-9.42	5.88	0.20-9.42	5.88
(E)-ocimenone	1240	1239	4.81	2.21	7.19	7.12	7.34	8.08	3.88	3.47	5.95	3.73	5.81	2.21-8.08	5.42	2.21-8.08	5.42
Piperitone	1257	1252	2.46	3.36	5.35	19.46	3.35	3.32	6.14	11.28	2.79	6.62	4.47	2.46-19.46	6.24	2.46-19.46	6.24
Thymol	1288	1289	0.26	0.81	0.5	0.52	0.32	0.85	0.19	0.43	0.77	0.61	0.50	0.19-0.85	0.52	0.19-0.85	0.52
Piperitenone	1346		7.33	6.25	13.31	16.48	15.16	12.89	10.83	14.04	18.24	10.89	18.3	6.25-18.3	15.30	6.25-18.3	15.30
Piperitenone oxide	1369		0.22		0.54	0.08	0.22	0.18		1.22	0.81	0.53	0.94	0.08-1.22	0.53	0.08-1.22	0.53
β -Caryophyllene	1418	1418	3.53	2.23	4.00	2.23	4.65	4.39	3.86	2.38	3.61	6.65	6.39	2.23-6.65	4.04	2.23-6.65	4.04

Components	RRI cal	RRI lit	Genotypes code *											Range	Mean
			1	2	3	4	5	6	7	8	9	10	11		
Germacrene-D	1480	1480	1.35	0.98	1.41	0.99	3.40	3.57	1.50	1.11	2.04	2.72	2.09	0.98-3.57	2.01
Bicyclogermacrene	1505	1508	0.89	0.66	1.10	1.68	2.03	1.98	0.73	0.79	1.27	1.32	1.70	0.66-2.03	1.29
(E)- β -farnesene	1459	1458	0.21	0.24	0.20	0.34	0.39	0.34	0.20	0.11	0.27	0.43	0.34	0.11-0.43	0.28
(E)-nerolidol	1586	1580	0.29	0.21	0.20	0.53	0.46	0.35	0.31	0.32	0.45	0.74	0.48	0.2-0.74	0.41
Spathulenol	1580	1577	0.29	0.22	0.42	0.03	0.45	0.21	0.35	0.24	0.34	1.42	0.36	0.03-1.42	0.39
Caryophyllene oxide	1590	1581	0.46	0.29	0.37	0.18	0.47	0.39	0.69	0.39	0.25	0.54	0.54	0.18-0.69	0.40
Monoterpenes hydrocarbons			8.48	10.85	8.56	9.08	5.50	6.36	9.45	9.96	8.3	8.35	10.82	5.50-10.85	8.82
Oxygenated monoterpenes			81.91	78.23	79.89	83.61	78.64	79.67	80.81	84.66	79.61	75.54	76.43	75.54-84.66	82.79
Sesquiterpenes hydrocarbons			5.98	4.11	6.71	6.81	10.47	10.28	6.29	4.39	7.19	11.12	10.52	4.11-11.12	7.62
Oxygenated sesquiterpenes			0.75	0.72	0.99	0.74	1.38	0.95	1.35	0.95	1.04	2.16	1.38	0.72-2.16	1.2

t, trace (<0.1%); RRI cal, relative retention indices calculated; RRI lit, relative retention indices literature reported. * Genotypes code: 1- F/R-8, 2- F/R-2, 3- Dainty Marieta, 4- Boy oBoy, 5-Gulzafri Orange, 6- GulzafriYellow, 7- Bolero Red, 8- Queen Sohpie, 9- PusaArpita, 10-Orange Winner, 11- Red Brocade.

content was maximum in Boy-o-Boy (19.46%) followed by Queen Sophia (11.28%), Orange Winner (6.62%), Dainty Marietta (5.35%) and minimum in F/R-8 (2.46%). Limonene content was maximum in Red Brocade (8.83%) followed by F/R-2 (8.78%), Queen Sophia (8.15%), Bolero Red (7.65%), Boy-o-Boy (7.36%) and minimum in Gulzafri Orange (4.89%). (E)-ocimene content was maximum in Gulzafri yellow (8.08%) followed by Gulzafri Orange (7.34%), Dainty Marietta (7.19%), Boy-o-Boy (7.12%), Pusa Arpita (5.95%), Red Brocade (5.81%), F/R-8 (4.81%) and minimum in F/R-2 (2.21%). Orange Winner (6.65%) recorded highest β -caryophyllene content followed by Gulzafri Orange (4.65%), Gulzafri yellow (4.39%), Dainty Marietta (4.00%), Bolero Red (3.86%), F/R-8 (3.53%) and F/R-2 (2.23%). (E)- β -ocimene content was maximum in Boy-o-Boy (10.56%), Queen Sophia (2.79%), F/R-2 (2.49%), F/R-8 (2.28%), Orange Winner (1.96%), Bolero Red (1.46%) and minimum in Red Brocade (0.79%). F/R-8 (9.42%) recorded highest (Z)-ocimene content followed by Gulzafri Yellow (7.66%), Gulzafri Orange (7.14%), Dainty Marietta (6.87%), Red Brocade (6.7%), Pusa Arpita (6.02%) and lowest was observed in Boy-o-Boy (0.2%). (E)-tagetone content was maximum in Bolero Red (7.74%) followed by Queen Sophia (6.97%), Gulzafri yellow (6.58%), Gulzafri Orange (6.15%), F/R-2 (6.13%), F/R-8 (6.09%) and whereas minimum content was recorded in Boy-o-Boy (0.52%).

A representative chromatogram (GC/FID) of major component of 33 compounds identified by GC/MS analysis with percentage of corresponding peak ranging from 60 to 96 % of the total in leaves. The oils of all genotypes of *T. patula* leaves were characterized by the presence of higher amounts of oxygenated monoterpenes viz. dihydrotagetone, terpinolene, piperitenone, (E)-tagetone, (E)-tagetone, followed by monoterpenes hydrocarbon, viz. limonene, (Z)- β -ocimene, thymol and small quantities sesquiterpenes hydrocarbons, viz. β -caryophyllene. Essential oil analysis obtained from leaves of four species including *T. erecta*, *T. minuta*, *T. patula* and *T. tenuifolia* were also reported to possess the compounds, viz. dihydrotagetones, tagetones, ocimenes and piperitone (Marotti *et al.* 2004). Terpenes, α -pinene, diterpenes, menthol and geraniol in the essential oil of the leaves and flowers of *T. erecta* were also reported by Balas and Singh (1980). A comparison between essential oils chromatograms of leaves of *T. patula* genotypes displayed more or less the same compounds differing only in the relative percentages. Out of thirty three compounds major component identified in leaves were piperitenone (27.33%) at retention time (RT) 29.626 followed by terpinolene (19.75%) at 19.512 (RT), (Z)- β -ocimene (19.10%) at 16.979 (RT), limonene (7.19%) at 16.476 (RT), (E)-tagetone (6.09%) at 21.959 (RT), (Z)-tagetone (5.26%) at 21.946 (RT), β -Caryophyllene (3.53%) at 32.029 (RT), piperitone (2.46 %) at 26.501 (RT), dihydrotagetone (1.04 %) at 17.585 (RT) and least in thymol (0.26%) at 27.849 (RT). Forty four components were revealed in the essential oil of the leaves of *T. erecta* including major

components such as limonene, terpinolene, piperitone and piperitenone (Krishna *et al.* 2004). Limonene, (*Z*)- β -ocimene, (*E*)-tagetone, (*Z*)-tagetone, piperitone, and piperitenone as major constituents in leaves were reported by Stojanova *et al.* (2000) and Isiaka (2006) in *T. erecta*.

In conclusion, we observed wide variation of the essential oils yield of leaves of *T. patulagenotypes*. The major constituents of essential oils identified were terpinolene, piperitenone, (*z*)- β -ocimene, piperitone, limonene and β -caryophyllene. Among 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 its phytoconstituents will be beneficial for further crop improvement programme of marigold.

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REFERENCES

- Adams R P. 2001. Identification of essential oil components by gas chromatography/mass spectrometry, carolnstream, IL. Allured Publishing Corporation, USA.
- Annoymus. 1976. *Raw Materials: Wealth of India*. NISCOM, Council of Scientific and Industrial Research, New Delhi.
- Anonymous. 2014. Indian Horticulture Database (NHB). 2015.
- Baslas R K and Singh A K. 1980. Chromatographic separation of essential oil of *Tagete serecta*. *Indian Perfumer* **24**: 163-165.
- Davies N W. 1990. Gas chromatography retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20 m phases. *Journal of Chromatography*. **503**: 1-24.
- Dhingra S N and Dhingra D R. 1956. Essential oil of *Tagetes patula*. *Perfumer, Journal of Essential Oil Research* **47**: 391-4.
- Hethelyi E, Tetenyi P, Kaposi P, Danos B, Kernoczi Z S, Buki G Y and Koczka I. 1988. GC/MS investigation of antimicrobial and repellent compounds, *Herba Hung.* **27**: 89-105.
- Igolen G. 1936. *Tagetes. perfumer* **14**: 6-11.
- Isiaka A, Ogunwande and Nureni O. 2006. The essential oil from the leaves and flowers of African marigold, *Tagetes erecta* L. *Journal of Essential Oil Research* **18**(4): 366-8.
- Krishna A, Kumar S, Mallavarapu G R and Ramesh S. 2004. Composition of the essential oils of the leaves and flowers of *Tagetes erecta* L. *Journal of Essential Oil Research* **16**: 520-2.
- Machado M I L, Silva M G V, Matos F J A, Craveiro A A and Alencar J W. 1994. The presence of Indole as minor constituents of *Tagetes erecta* leaf oil. *Journal of Essential Oil Research* **6**: 203-5.
- Marotti M, Piccaglia R, Biavati B and Marotti I. 2004. Chracterization and yield evaluation of essential oils from different *Tagetes species*. *Journal of Essential Oil Research* **6**: 440-4.
- Rondon M, Velasco J, Morales A, Rojas J, Carmona J, Gualtieri M and Hernández V. 2005. Composition and antibacterial activity of the essential oil of *Salvia leucantha* Cav. cultivated in Venezuela Andes. *Revista Latinoamericana de Química*. **33**: 40-4.
- Rydberg P A. 1915. *Tagetes, North Am. Flora* **34**: 148-59.
- Sigh P, Krishna A, Kumar V, Krishna S, Sigh K, Gupta M and Singh S. 2016. Chemistry and biology of industrial crop *Tagetes species*: A review. *Journal of Essential Oil Research* **28**(1): 1-14.
- SzarkaSz, He E, Thelyi E, Lemberkovics I N, Kuzovkina P Ba and Nyai E. 2006. GC and GC-MS studies on the essential oil and thiophenes from *Tagetes patula* L. *Chromatographia Supplement* **63**.