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Amit Tiwari
CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Pantnagar, P.O.- Dairy Farm Nagla, Udham Singh Nagar, Uttarakhand, India

Prakash Goswami
CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Pantnagar, P.O.- Dairy Farm Nagla, Udham Singh Nagar, Uttarakhand, India

Bharat S Bisht
CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Pantnagar, P.O.- Dairy Farm Nagla, Udham Singh Nagar, Uttarakhand, India

Amit Chauhan
CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Pantnagar, P.O.- Dairy Farm Nagla, Udham Singh Nagar, Uttarakhand, India

Ram S Verma
CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Pantnagar, P.O.- Dairy Farm Nagla, Udham Singh Nagar, Uttarakhand, India

Rajendra C Padalia
CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Pantnagar, P.O.- Dairy Farm Nagla, Udham Singh Nagar, Uttarakhand, India

Correspondence:
Rajendra C Padalia
CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Pantnagar, P.O.- Dairy Farm Nagla, Udham Singh Nagar, Uttarakhand, India

Essential oil composition of African marigold (*Tagetes minuta* L.) harvested at different growth stages in foothills agroclimatic conditions of North India

Amit Tiwari, Prakash Goswami, Bharat S Bisht, Amit Chauhan, Ram S Verma and Rajendra C Padalia

Abstract
Tagetes minuta L., commonly known as African marigold, is reputed as a source of 'Tagetes oil' of trade that finds an extensive use in food, flavoring, pharmaceutical, perfumery and cosmetic industry. In present study, variation in the essential oil content and composition of *T. minuta* grown in foot hills agroclimatic conditions of northern India and harvested in flower initiation, full flowering, late flowering and seed setting stages were compared and analysed using GC-FID and GC-MS. Essential oil content was found to vary from 0.52 to 0.78% in different growth stages of the crop. Altogether, 19 constituents, representing 81.2-93.9% of the total oil composition were identified. The essential oil composition was mainly dominated by monoterpenoids (80.5-92.9%) represented by (*E*)-ocimenone (31.8-42.2%), (*Z*)-β-ocimene (22.9-32.7%), (*Z*)-tagetone (8.0-11.0%), and (*Z*)-ocimenone (6.0-10.3%), dihydrotagetone (1.7-3.7%), (*E*)-tagetone (1.0-2.5%) and limonene (1.0-1.5%). The essential oil of *T. minuta* from foothills of northern India consist the constituents in entirely different quantitative composition.

Keywords: *Tagetes minuta*; essential oil; (*Z*)-β-ocimene, tagetone, ocimenone

1. Introduction
The genus *Tagetes* (Asteraceae), comprising more than 30 species, is native to the central and southern part of America, Argentina and Mexico. Most members of this genus are annual and perennial, branched herbs or shrubs known for horticultural and essential oil-yielding purpose [1-3]. Members of the genus *Tagetes* have a long history of human use as beverages, condiments, ornamentals, and medicinal purpose such as analgesics, antiseptics, carminative, diuretic, antispasmodic, anthelmintic, stimulants, vermin repellents, and for treatment of stomach and intestinal diseases [3-5]. *Tagetes minuta* L., commonly known as African marigold, is a highly aromatic annual perennial herb growing naturally as weed and/or cultivated for 'Tagetes oil' of trade [6]. *T. minuta* used in indigenous medicines as a natural source of raw material due to its anti-microbial, anti-inflammatory, anti-fungal and insecticidal and acaricidal activities [7-9]. Moreover, the essential oil of *T. minuta* finds an extensive use in food, flavoring, pharmaceutical, perfumery and cosmetic industry [10]. Tagetes oil commercially produced mainly in Argentina, Australia, Brazil, France, Spain, Venezuela, Iran and other countries [3]. Tagetes oil and its terpene constituents has been reported to possess antibacterial, anti-inflammatory, hypotensive, larvicidal, insecticidal, aphicidal activities [9-13]. To restrict its illicit collection from wild and to meet out the industrial demand of Tagetes oil, CSIR-Central Institute of Medicinal and Aromatic Plants has developed the cultivation practices of *T. minuta* in northern and southern part of India [10, 14-18]. The chemical composition of the essential oil of *T. minuta* has been studied previously from different countries. Monoterpenoids, viz. dihydrotagetone, (*Z*)-β-ocimene, (*Z*)-tagetone, (*E*)-tagetone, (*Z*)-ocimenone, (*E*)-ocimenone, α-terpineol and limonene were the prevalent constituents distributed in the essential oil of *T. minuta* from different origin [3,19-22]. However, the content of essential oils and its constituents reported to vary significantly depending upon variations in climatic and agricultural conditions, growing season, growth/harvesting stage, plant parts, cultivation practices and origin [6, 14-16, 23-26]. Considering the huge potential of Tagetes oil and the conducive agroclimatic conditions for its cultivation in foot hills of north India, the present study was carried out to investigate variations in essential oil yield and composition of *T. minuta* crop in different growth stages, viz. flowering initiation, full flowering, late flowering and seed setting

stage using gas chromatographic retention index (RI) and mass spectral data.

2. Materials and methods

2.1 Plant material and essential oil isolation

The fresh aerial parts of *T. minuta* var. Vanphool were harvested in four different growth stages, viz. flower initiation, full flowering, late flowering and seed setting stages from the winter crop raised at experimental field of CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Pantnagar. The experimental site is located at latitude of 29° N, longitude of 79.38° E and at an altitude of 243.84 MSL at foothills of Uttarakhand, India. The soil of the experimental site was sandy-loam in texture, with neutral pH. The maximum temperature ranges between 35-45 °C, and minimum between 2-5 °C. Samples of fresh plant materials in different growth stages were submitted to hydrodistillation process for three hours, in a Clevenger-type apparatus. Essential oil was measured directly in the extraction burette and content (%) was calculated as volume (mL) of essential oil per 100 g of fresh plant material. The essential oils collected were subsequently dried over anhydrous sodium sulfate (Na_2SO_4) and kept refrigerated until be analyzed.

2.2 Gas Chromatography (GC) analysis

Gas chromatography analysis of the essential oil samples were carried out on a Varian-3900 Gas Chromatograph equipped with flame ionization detector (FID) and a DB-5 (5% phenyl polysiloxane, 60 m × 0.32 mm; 0.25-µm film coating) fused silica capillary column. The oven column temperature ranged from 70-250 °C, programmed at 3 °C min^{-1}, using N_2 as carrier gas at 1.0 mL min^{-1}. Injector and detector temperatures were 230 °C and 250 °C, respectively. Injection size was 0.02 µL neat (syringe: Hamilton 1.0 µL capacity, Alltech USA) with a split ratio was 1: 40.

2.3 Gas Chromatography-Mass Spectrometry (GC–MS) analysis

GC-MS analyses of the essential oils were performed with a Perkin-Elmer Turbomass Quadrupole Mass spectrometer (Perkin-Elmer, Shelton, USA) fitted with Equity-5 fused silica capillary column (60 m × 0.32 mm; 0.25 µm film thickness; Supeleco Bellefonte, PA, USA). The column temperature was programmed 70 °C, initial hold time of 2 min, to 250 °C at 3 °C min^{-1} with final hold time of 3 min, using helium as carrier gas at a flow rate of 1.0 mL min^{-1}. The injector, ion source and transfer line temperatures were 250 °C. The injection volume was 0.04 µL neat with split ratio 1:30, electron impact ionization mode (EI), with ionization energy 70 eV and mass scan range of 40–400 amu.

2.4 Identification of Constituents

The constituents were identified based on their retention time, retention index (RI; determined using a homologous series of *n*-alkanes (C_8-C_{24}) under the same temperature-programmed conditions), coinjection with standards (Aldrich and Fluka), MS Library search (NIST and WILEY), and by comparing with the mass spectral literature data [27]. The GC-FID chromatogram was used to determine the relative concentrations using peak area calculation by electronic integration without response factor correction.

3. Results and discussions

The variation in essential oil content and composition of *T. minuta* harvested in four-growth stages (flower initiation, full flowering, late flowering and seed setting) grown in foot hills agroclimatic conditions of northern India was analysed using gas chromatography-flame ionisation detector (GC-FID) and GC-mass spectrometry (GC-MS). Maximal essential oil content (0.78%) was obtained upon harvesting the crop at seed setting stage followed by late flowering stage (0.72%), full flowering stage (0.66%), and minimal at flower initiation stage (0.52%). These results of oil content variations are in agreement with previous reports reporting maximum oil content in seed setting and late flowering stage from north Indian plains. Altogether, 19 constituents, representing 81.2-93.9% of the total oil composition were identified. The identified constituents with their relative content in essential oils are summerised in Table 1. The essential oil composition was mainly dominated by monoterpenoids (80.5-92.9%) represented by monoterpene hydrocarbons (25.1-35.0%) and oxygenated monoterpenes (55.4-65.1%). The major constituents distributed in essential oils were (*E*)-ocimenone (31.8-42.2%), (*Z*)-β-ocimene (22.9-32.7%), (*Z*)-tagetone (8.0-11.0%), and (*Z*)-ocimenone (6.0-10.3%). Other marker constituents of Tagetes oil identified in significant content were dihydrotagetone (1.7-3.7%), (*E*)-tagetone (1.0-2.5%) and limonene (1.0-1.5%). The content of (*E*)-ocimenone, the major constituents of essential oil in all growth stages, was highest in flower initiation stage (42.2%), and then it decreases towards the crop maturity with following the order as: full flowering (39.5%) > late flowering (34.3%) > seed setting stage (31.8%). However, the content of (*Z*)-β-ocimene, second major constituents of the essential oils, showed a reverse trend with increasing content with crop maturity upto late flowering stage as: flower initiation (25.6%) < full flowering stage (26.5%) < late flowering stage (32.7%), later it is noticed to its minimal in seed setting stage (22.9%). The contents of other oil constituents showed no regular trend based on growth stages. However, constituents in essential oils harvested from different crop maturity revealed minor variations in their quantitative composition (table 1). The chemical composition of the essential oil of *T. minuta* has been studied previously from different origin. Comparison of previous reports on *T. minuta* essential oils revealed that the there is substantial quantitative compositional variability based on plants parts and growth stage, but the prevalent major constituents are consistent at all geographical locations reporting dihydrotagetone, (*Z*)-β-ocimene, (*Z*)-tagetone, (*E*)-tagetone, (*Z*)-ocimenone, (*E*)-ocimenone and limonene as marker constituents. Results of present analysis on essential oil of *T. minuta* from foothills agroclimatic conditions of north India are also in agreement with previous reports in term of the qualitative composition. However, significant quantitative variations were observed in the content of major constituents. In earlier reports, dihydrotagetone was reported as major constituents followed by (*Z*)-β-ocimene/ (*Z*)-tagetone from the Tagetes oil of Rawanda, Zambia, Turkey, southern India and plains of northern India. However, (*Z*)-β-ocimene was reported as the major constituents followed by (*Z*)- & (*E*)-ocimenone from essential oil of *T. minuta* from Brazil, France, Hungary, North America, Bhutan, and Kashmir/ Himachal Pradesh (India). Moreover, the French Tagetes oil consist marker constituents as: (*Z*)-tagetenone > (*Z*)-β-ocimene > (*Z*)-tagetone > dihydrotagetone; and Tagetes oil from Mukoni (Rwanda) consist constituents as: (*E*)-ocimenone > (*Z*)-tagetone > dihydrotagetone, (*E*)-tagetone and (*Z*)-β-ocimene [3]. In contrary to these, In present analysis the essential oil of *T. minuta* from foothills of northern India consist the constituents in entirely different quantitative composition as: (*E*)-ocimenone > (*Z*)-β-ocimene > (*Z*)-tagetone/ (*Z*)-ocimenone > dihydrotagetone /(*E*)-tagetone and limonene in all the growth stages. This quantitative

compositional variability may possibly be due to the influence of various intrinsic and extrinsic factors including climatic and growing location of foothills of northern, India.

Table 1: Essential oil composition of *T. minuta* at harvest stages in foothills of north India

S.No.	Compounds	RI[a]	RI[b]	Content (%)			
				FIS	FFS	LFS	SS
1	Sabinene	972	969	0.1	0.2	0.2	0.1
2	Myrcene	986	988	0.1	0.1	0.1	0.1
3	α-Phellandrene	1002	1002	0.1	0.1	0.1	0.1
4	Limonene	1022	1024	1.2±0.05	1.5±0.11	1.3±0.92	1.0±0.10
5	(*Z*)-β-Ocimene	1034	1032	25.6±0.26	26.5±0.90	32.7±2.02	22.9±0.20
6	(*E*)-β-Ocimene	1040	1044	0.3	0.3	0.3	0.1
7	Dihydrotagetone	1044	1046	3.7±0.20	1.7±0.29	2.8±0.40	3.1±0.40
8	*cis*-Sabinene hydrate	1068	1065	0.3	t	0.5	1.1
9	β-Thujone	1117	1112	0.1	0.1	0.1	0.1
10	allo-Ocimene	1126	1128	0.4	0.3	0.3	0.8
11	(*E*)-Tagetone	1134	1139	1.8±0.15	2.5±0.83	1.2±1.10	1.0±0.25
12	(*Z*)-Tagetone	1146	1148	11.0±0.51	8.9±0.30	10.0±0.57	8.0±1.0
13	(*Z*)-Ocimenone	1224	1226	6.0±0.76	7.5±1.01	6.9±2.45	10.3±0.80
14	(*E*)-Ocimenone	1238	1235	42.2±1.41	39.5±0.90	34.3±1.86	31.8±0.35
15	β-Elemene	1388	1389	0.1	0.1	0.1	0.2
16	β-Caryophyllene	1415	1417	0.2	0.2	0.2	0.1
17	α-Humulene	1454	1452	0.1	0.1	0.1	0.1
18	Germacrene D	1485	1484	0.1	0.1	0.1	0.1
19	δ-Cadinene	1518	1522	0.5	0.4	0.5	0.2
Class composition							
Monoterpene hydrocarbons				27.8	29.0	35.0	25.1
Oxygenated monoterpenes				65.1	60.2	55.8	55.4
Sesquiterpene hydrocarbons				1.0	0.9	1.0	0.7
Total identified				93.9	90.1	91.8	81.2
Essential oil content (%)				0.52±0.03	0.66±0.03	0.72±0.02	0.78±0.03

[a] Retention index (experimental); [b] Retention index (Adams, 2007); t: trace (<0.05%)

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