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Tagetes minuta: An overview

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Abstract
Tagetes minuta, commonly known as wild marigold; is a plant belonging to family Asteraceae. The plant is valued for its essential oil which is present almost in every part of plant with little percentage in stem and roots. The plant is native to South America and has been introduced to many countries since the time of Spanish conquest. The plant occurs in temperate forests and mountain regions of world. Himachal Pradesh, Uttrakhand and Jammu &Kashmir are the main sources of "*Tagetes* oil" in India where it occur at an altitude of 1000 to 2500 m amsl. The plant is drought tolerant and survives easily in poor soils hence it colonize waste ground, roadsides, gardens, orchards and vine yards. The crop lasts for 4 to 6 months depending upon the time of sowing, crop practice followed and the prevalent climatic conditions. The major constituents (90-95%) of *Tagetes minuta* essential oil are Z-β-ocimene, limonene (hydrocarbons) and acyclic unsaturated monoterpenes ketones, dihydrotagetone, tagetones (E, Z) and ocimenones (E, Z). The plant possess pharmacological, phytotoxic effect, antibacterial and antifungal effect, insecticidal properties, nematicidal properties, antiviral and antimicrobial activity and also find use in perfume and flavour industry. Its oil has a great demand, so species holds a lot of potential for undertaking its large scale cultivation.

Keywords: *Tagetes minuta*, marigold, essential oil, plant

Introduction
Plants of *Tagetes minuta* L. are annual aromatic herbs of sunflower family (Asteraceae) which is one of the most abundant plant taxonomical grouping, comprising of about 1,000 genera and over 23,000 species (Sadia *et al*. 2013) [57]. The genus *Tagetes* is composed of about 56 species, out of which 27 are annual and 29 perennial (Soule, 1996) [70]. The genus name *Tagetes* refers to the Latin name for marigold 'Tages', an Etruscan god associated with agriculture (Kumar *et al*. 1999) [34]. The specific epithet of *Tagetes minuta* is from the Latin word '*minutes'* meaning small referring to its small sized capitula (Hulina, 2008) [29]. Many species of genus *Tagetes* are commercially grown in respective agro-ecological regions of different countries as a multipurpose new crop. Of these species, *T*agetes *minuta*, *Tagetes erecta*, *Tagetes patula*, and *Tagetes tenuifolia* are most common with *Tagetes minuta* being the most studied and widely grown species in several countries due to high grade and percentage of essential oil (Chalchat *et al*. 1995; Singh *et al*. 2003) [17, 67].

Taxomomy
Preferred Scientific Name
Tagetes minuta L. Species Plantarum 887. 1753; Synonym: *Tagetes bonariensis* Pers., *Tagetes glandulifera* Schrank, *Tagetes glandulosa* Link, *Tagetes montana* (Hort.) DC. And *Tagetes porophyllum* Vell (Maheshwari, 1972; Anonymous, 2017b) [41, 9]. The taxonomic classification of *Tagetes minuta* L. is given below (Anonymous, 2017a) [8]:

Kingdom	:	Plantae
Subkingdom	:	Viridiplantae
Infrakingdom	:	Streptophyta
Superdivision	:	Embryophyta
Division	:	Tracheophyta
Subdivision	:	Spermatophytina
Class	:	Magnoliopsida
Superorder	:	Asteranae
Order	:	Asterales
Family	:	Asteraceae

Genus	:	*Tagetes* L.
Species	:	*Tagetes minuta* L.

Common Names

The various common as well as local names of *Tagetes minuta* are as below: (Anonymous, 2017b; Qureshi *et al*. 2007) [9, 51].

English:	Mexican marigold, stinking Roger,stinkweed, tall khaki weed, wild marigold, Muster John Henry, khaki bushh, Aztec marigold;
Hindi:	Jungli Gainda;
Spanish:	huacatay, chinchilla, enana;
Angola:	ekaibulo;
Argentina:	chil chil;
Brazil:	chinchilla, coora, cravo de mato, margarita, rabo de foguete, rabo de rajao, suique, voadeira;
Chile:	quinchihue;
Germany:	Wilde Sammetblume;
Kenya:	ang'we, anyach, bhangi, mubangi, muvangi, nyanjaga, nyanjagra, omotioku, omubazi gwemhazi;
Madagascar:	mavoadala;
Malawi:	khaki;
Portugal:	cravo de defunct;
Paraguay:	Agosto, suico.

Distribution

Tagetes minuta has been widely cultivated around the world due to its agrochemical and pharmacological properties (Hulina, 2008) [29]. Native to South America including countries like Argentina, Chile, Bolivia, Peru and Paraguay (Reiche, 1903; Perkins, 1912) [55, 50] and severely noxious weed in over 35 countries (Holm *et al*. 1997) [28], it has been introduced in Europe (Jordano and Ocano, 1955) [33]; Asia (Cherpanov, 1981) [20]; Africa (Hillard, 1977) [27]; Madagascar (Humbert, 1923) [30]; India (Rao *et al*. 1988) [53]; Australia (Webb, 1948) [80] and Hawaii (Hasaka and Thistle, 1954) since the time of Spanish conquest. It has been deliberately distributed across the tropics, subtropics and several temperate countries as an ornamental, medicinal or perfume plant as well as accidentally as a weed (Stadler *et al*. 1998) [71]. According to Maheshwari (1972) [41], its plants have naturalized itself in Himalayan and sub Himalayan regions up to altitudes of 2000 m in waste places, roadsides, rocky hill slopes and cultivated fields of Uttar Pradesh, Himachal Pradesh, Sikkim, Arunachal Pradesh, Nagaland and Meghalaya. According to Thappa *et al*. (1993) [74], the plant is found in western Himalayas of India between altitudes of 1000 to 2500 m. Himachal Pradesh, Jammu & Kashmir and hills of Uttar Pradesh are the main growing regions where it occurs in natural habitat. Wild growth of *Tagetes minuta* in these regions forms the most important source of '*Tagetes oil*' in India (Singh *et al*. 1995) [69].

Ecology and habitat

The plant grows in moist and dry areas from sea level to reasonable altitudes (3000 m) in the tropics and subtropics with soil p^H ranging from 4.3 to 6.6 (Holm *et al*. 1997) [28]. Wild marigold is a problematic weed of pastures and numerous crops (Hulina, 2008) [29] as it may colonize waste ground, roadsides, gardens, orchards and vine yards. This affinity for disturbed sites has allowed the species to colonize many areas around the world (Meshkatalsadat *et al*. 2010) [44]. The plant is often found growing in areas having loose soil. A minimum of 50 cm of rainfall, spread over the season or its equivalent of irrigation water is sufficient for its good growth (Singh *et al*, 2003) [67]. Associate species of *Tagetes minuta* are usually ruderal broad-leaved species such as *Bidens pilosa* L. and *Bidens subalternans* DC. (Campos, 1997) [15]. the best growth and quality of oil is produced in the temperate climate where fall in the night temperature is favourable for the plant. For production of high grade oil, temperature between 12-30ºC is desirable during the reproductive phase of its plants. Mostly acidic soils are preferred by the crop. The seeds cling to the hair of animal and human clothes once in contact and are therefore dispersed by human, domesticated by wild animals (Singh *et al*. 2003) [67].

Morphology

Tagetes minuta is an annual herb characterized by an erect stem of about 1-2m height (Wang and Chen, 2006) [77]. Its stems are typically woody, grooved or ridged, initially green but often maturing to brownish or reddish; usually branched only in upper part, unless broken or cut off near the base (Anonymous, 2003) [4]. The leaves are pinnately compound, stalked, opposite, slightly glossy green, 7-15 cm long, pinnately dissected into 4-6 pairs of pinnae. The leaflets are lanceolate in shape with finely serrate margins (Singh, 2003; Hulina, 2008; Anonymous, 2003; Ofori *et al*. 2013) [29, 4, 49]. The undersurface of the leaves bears number of small, punctuate, multicellular glands which exude liquorice like aroma when ruptured (Singh *et al*. 2003) [67]. Flower heads are numerous, yellowish green, usually in flat topped cymes; involucres cylindrical, 8-14 mm high, 2-3mm wide, apex 3-5 toothed; ray florets usually 3 per head; rays 1-2mm long; disc florets usually 3-5 per head, corollas white, approximately 2.5 mm long; pappus consists of 1-2 unequal awn like scales, 2-3 mm long, 3-5 ovate to lanceolate scales, 0.5 -1 mm long (Wang and Chen, 2006) [77]. Flowers are arranged in solitary clustered panicled branches. Four or five fused involucre bracts surround each head (Wanzala *et al*. 2012) [78]. Achenes are brown, 10-12 mm long, with a pappus of 1-4 tiny scales and 0-2 retrosely serrulate, awns 1-3 mm long. Weight of 1000 achenes varies from 0.9 g to 1.1 g (Singh *et al*. 2003) [67]. The taproots are usually short surrounded by fibrous lateral roots, which form mycorrhizal associations (Anonymous, 2003) [4]. Chromosome number of the species is 2n=48 chromosomes (Gupta and Gill, 1988) [26].

Phenology

The flowering occurs in the month of September (Maheshwari, 1972) [41]. The crop is harvested in the month of Oct-Nov when it blooms fully and seeds are harvested in the end of November. Removal of apical meristem is considered beneficial in mid-August in order to check the erect growth and more lateral branches (Singh *et al*. 2003) [67].

Agro-Practices

The plant is propagated by seed either by direct sowing or by transplanting nursery raised plants in main field. Studies conducted by Kumar and Singh (2008) [65] at Palampur, H.P. have revealed that 30th and 31st MSW (Meteorological Standard Week) that corresponds to 23rd July to 5th August period is the most suitable time period for transplantation in the main field as this gives maximum leaf and flower biomass yield. Kumar and Singh (2008) [65] have also opined that 45 days old seedlings are suitable for transplantation and

accordingly seed sowing in the 2nd week of June should be undertaken.

The crop lasts for 4 to 6 months depending upon the time of sowing, crop practice followed and the prevalent climatic conditions. The practice of nursery raising is better than direct sowing for maintaining desired crop geometry. Medium light to medium heavy soil, free from all weeds is ideal for getting optimum herb and oil yield. The crop requires 120 kg N, 60 kg P and 40 kg K per hectare in addition to 20-30 tons ha^{-1} farm yard manure applied at the time of preparation of land. Good soil moisture is required throughout the crop season for better growth and yield. The standard crop practice most suitable for western Himalayas is from Mid-June to Mid Nov (Singh *et al*. 2003) [67]. Different spacings of 30x30cm (Rao *et al*. 2000) [52]; 45x45 cm (Kumar and Singh, 2008) and 30x45cm (Singh *et al*. 2003) [67] have been evaluated with 30x45 cm being the most optimum. Cappellari *et al*. (2013) [16] observed a promotory effect of soil inoculation by plant growth promoting rhizobacteria like *Pseudomonas fluorescens, Azospirillum brasilense* on plant growth, essential oil composition and phenolic composition of this species. Singh *et al*. (2008) [65] observed the influence of row spacing and nitrogen levels on herb, essential oil production and oil quality of *Tagetes minuta* L. and suggested that *Tagetes minuta* should be planted at 30 cm row spacing and fertilized with 150 kg N ha^{-1} or more to obtain higher oil yield in sub-tropical climate. Rao *et al*. (2000) [52] studied the effect of nitrogen and harvest stage on the yield as well as oil quality of *Tagetes minuta* L. in tropical India and found that application of 100 kg nitrogen per hectare significantly increased the yields of both the main and ratoon crops.

Elite varieties

An improved variety named "*Vanphool"* derived from an open population collected from the Almorah hills has been released for North India by selection method having high oil yield (61 kg ha^{-1}), dihydrotagetone (32%) and (Z)-tagetone (16.7%) by Central Insitute of Medicinal and Aromatic Plants (CIMAP) (Kumar *et al*. 1999) [34]. "*Himgold",* an improved cultivar has been developed by Institute of Himalayan Bioresource Technology (IHBT), Palampur (Anonymous, 2001) [3].

Secretory glands

Simon *et al*. (2002) [64] studied the occurrence, structure and distribution of secretory structures in the different organs of *Tagetes minuta*. The secretory structures of essential oils collapse during leaf senescence (Croteau, 1986; Sharma *et al*. 2003) [22, 61]. Lopez *et al*. (2009) [39] studied the seceretory cavities in the leaf and reproductive structure of *Tagetes minuta* and found that the cavities located in leaf blade half were comparatively larger (150-200µm) than those close to midvein (70µm).

Chemistry

The primary importance of *Tagetes minuta* is due to its essential oil which is present in almost all parts of its plants except stem, with best quality oil present in its flowers (Singh *et al*. 2003) [67]. *Tagetes minuta* is rich in many secondary metabolite compounds including acyclic, monocyclic and bicyclic monoterpenes, sesquiterpenes, flavonoids, thiophenes and aromatics (Brene *et al*. 2009; Lawrence, 1996; Bansal *et al*. 1999) [13, 36, 11]. The major constituent (90-95%) of *Tagetes minuta* essential oil are Z-β-ocimene, limonene (hydrocarbons) and acyclic unsaturated monoterpenes ketones, dihydrotagetone, tagetones (E, Z) and ocimenones (E, Z) (Thappa *et al*, 1993; Lawrence, 1996; Bansal *et al*. 1999, British Pharmacopeia, 1988) [74, 36, 11, 14]. Lohani *et al*. (2012) [38] studied the chemical constituents of volatile oil of *Tagetes minuta* L. cultivated in Uttarakhand Himalayas and found that oil content varied between 0.2-0.4 percent. Tiwari *et al*. (2016) [76] studied variation in essential oil content and composition of *Tagetes minuta* grown in foot hill agroclimatic conditions of northern India harvested at flower initiation, full flowering, late flowering and seed setting stages 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 and composition was mainly dominated by monoterpenoids (80.5-92.9%).

Significant differences in chemical profile of essential oil of *Tagetes minuta* have been attributed to anthropogenic and environmental factors. Variation in chemical composition is attributed to (i) the harvesting method (ii) geographical location of the target plant (Senatore *et al*. 2004) [59] (iii) growth stage at harvest (Moghaddam *et al*. 2007) [45], (iv) plant parts used (Chalchat *et al*. 1995, Chamorro *et al*. 2008) [17, 18] and (v) the climatic conditions under which the plant grows (Mohamed *et al*. 2002) [46]. Variations in the chemical composition of essential oil from the same plant species resulted in chemotypes, which is due to genotypic × environment interactions (Rohloff, 2003) [56] implying the possibility of chemical composition of same species of plant to vary. Hydrodistillation of *Tagetes minuta* leaves is mostly done for 3 hours in Clevenger type apparatus and oil can be stored in closed vials (Tankeu *et al*. 2013) [73]. The identification of *Tagetes* oil components is usually carried out by Gas Chromatography -Mass spectroscopy (Chamorro *et al*. 2008) [18]. Wild marigold oil is a pale yellow to dark yellow coloured liquid and is dried immediately after distillation (Singh *et al*. 2003) [67]. The crop is harvested at full bloom stage for maximum herb yield and oil quality (Singh *et al*. 1995) [69].

Molecular studies

Shahzadi *et al.* (2010) [60] obtained good quality DNA from fresh leaf tissues with a modified cetyltrimethylammonium bromide buffer protocol method. A relatively large amount of DNA was also extracted from the sun- and shade-dried tissues, but its quality was not as good as that from seeds. The DNA extracted from seeds and fresh leaves was successfully amplified by PCR using arbitrary RAPD primers.

Bioactive properties

Tagetes minuta is an aromatic plant with a broad spectrum of biological activities among which are medicinal, antioxidant and antibacterial properties. *Tagetes minuta* not only exhibits very good medicinal properties but also has strong nematicidal, insecticidal and antimicrobial activity (Daizy *et al*. 2007) [23].

Antibacterial activity

Antibacterial activity of *Tagetes minuta* essential oil has been tested against a range of human, plant and animal pathogenic bacteria. Tahir and Khan (2012) [72] tested the antibacterial potential of crude leaf, fruit and flower extracts against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas auregnosa, Salmonella typhi, Staphylococcus aureus, Streptococcus viridian, Bacillus licheniformis, Bacillus subtilis* and *Pasteurella multocida* and found them effective. Anthony *et al*. (2015) [10] tested the ethanolic extract for its

antibacterial activity against selected microorganisms and found that the growth of these organisms can be controlled with the extract, hence making the extract components potential agents for incorporation into drug production. Plant based antibacterials have huge therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antibacterials (Lwu *et al*. 1999) [40].

Insecticidal activity

It has been known for many years that essential oil of *Tagetes minuta* has both repellent and growth inhibitory properties against insect pests (Jacobson, 1983) [31]. Its oil significantly reduces grain damage due to insect infestation with no adverse effects on seed germination, colour and odour hence can be used as sustainable alternatives to synthetic insecticides in maize storage especially by smallholder farmers (Morgan, 2015) [47]. Nchu *et al*. (2012) [48] has opined that its essential oil may be a potential source of anti-tick agents especially for controlling *Hyalomma rufipes* tick. E-ocimenone is responsible for the larvicidal activity of oil against larvae of *Ades aegypti* (Maradufu *et al*. 1978) [42]. Weaver *et al*. (1994) [79] studied the insecticidal activity of floral, foliar and root extracts of *Tagetes minuta* against adult Mexican bean weevils and found that its flower and leaf extracts can be fast acting insecticides.

Acaricidal activity

Plant essential oils have been studied extensively for their biocidal effect against ticks and mites revealing promising results. The plant essential oils have also been used in the control of *Rhipicephalus microplus* in cattle. *Tagetes minuta* essential oil had significant effect in controlling the spread and reproduction of ticks by affecting their egg production and killing the surviving ones on the bodies of the affected cattle (Andreotti *et al*. 2013) [2].

Antifungal properties

Plant essential oils and extracts in general have been reported to have antifungal activity against a broad range of pathogenic fungi (Grange and Ahmed, 1988) [25]. Of the 49 essential oils analyzed, the most commonly occurring components exhibiting high levels of antifungal activity such as limonene, 1,8-cineole, α-pinene, β-pinene, and camphor (identified as some of the common components of *Tagetes minuta* essential oil) have shown antifungal activity against a wide range of fungi such as *Rhizoctonia solani*, *Fusarium oxysporum*, *Penicillium digitatum*, *Aspergillus niger*, *Verticillium fungicola* and *Trichoderma harzianum* (Matasyoh *et al*. 2007; Saha *et al*. 2012) [43, 58].

Nematicidal properties

Several workers have studied the nematode-suppressant effects of *Tagetes* spp. (Alam *et al*. 1977) [1]. Siddiqui and Alam (1987) [63] while studying the effect of intercropping of tomato, eggplant, cabbage and cauliflower with *Tagetes minuta* (for suppressing root knot nematode) observed significant inhibition of root-knot development caused by Meloidogyne in tomato.

Allelopathic uses

Batish *et al*. (2006) observed that *Tagetes minuta* leaf powder mixed with rice field soil significantly reduced emergence and growth of weed species both in pots under greenhouse conditions as well as rice field. Even its aqueous extract has been found to be inhibitory to seed germination of *Lotus corniculata* and *Lactuca sativa* (Gil *et al*. 2002) [24] as well as callus induction in *Oryza sativa*, *Brassica campestris* ssp.*napus* var. *pekinensis*, *Raphanus sativus* var. *acanthiformis*, and *Sesamum indicum* (Lee *et al*. 2002) [37].

Uses

Tagetes species have been used as a source of essential oil. The powders and extracts of *Tagetes* are rich in the orange-yellow carotenoid (Zhang *et al*. 2009) [81]; found in the oil of florets of *Tagetes minuta* (petals) and other species of marigolds. This carotenoid has been identified, isolated, and approved by the European Union (INS-Number E161b) for use as food color as well as flavor in various foodstuffs like condiments, pasta, vegetable oil, margarine, mayonnaise, salad dressing, baked goods, confectionery, dairy products, ice cream, yogurt, citrus juice, mustard etc. (Timberlake and Henry, 1986) [75]. Antidepressant activity via negative modulation on GABAergic function, tranquilizing, hypotensive, bronchodilatory, spasmolytic and anti-inflammatory bioactivities (Chandhoke and Ghatak, 1969) [19] as well as antifeedant activities (Reddy *et al*. 2015) [54] are other important attributes of the essential oil of *Tagetes minuta*. It has also several medical benefits such as remedy for colds, respiratory inflammations, stomach problem, anti-spasmodic, anti-parasitic, anti-septic, insecticide and sedative (Shirazi *et al*. 2014) [62].

Trade and market demand

According to Singh *et al*. (2006) [66], about 3t of high quality oil is being produced annually from the state of Himachal Pradesh (India) alone priced at Rs.1935-2160 per kg whereas oil produced from other parts of North India is Rs. 1170-1260 (Jhunjhunwalla, 2004) [32]. As the demand for its oil is increasing, this species holds a lot of potential for undertaking its large scale cultivation. Brazil is one of the major producer of "*Tagetes* Oil" (Craveiro *et al*. 1988) [21]. Worldwide production of the oil was around 1.5 tonnes in 1984 (Lawrence, 1985) [35]. South Africa, India, Zimbabwe, Egypt, France, and Argentina are the major producers of *Tagetes minuta* oil. In 2003, South African *Tagetes* oil production amounted to an estimated 6.5 tonnes. Current production in Zimbabwe and India is estimated at 2 and 4 tonnes, respectively. However, global demand for *Tagetes minuta* oil for all applications is estimated to amount to more than 12 tonnes (Anonymous, 2014) [5]. As the yield of extraction of *Tagetes minuta* oil amounts to only 0.1-0.4%, it is a relatively expensive product. Yield per hectare is around 25 tonnes of raw plant material and between 12.5 and 17.5 kg of *Tagetes minuta* oil. Recently, FOB prices for high-quality oil increased somewhat to around $ 190-250 kg^{-1}, due to decreased availability. Prices for low quality *Tagetes* oil can be as low as $ 90 kg^{-1} (about Rs. 6120) (Anonymous, 2014) [5]. The retail price of one liter of *Tagetes minuta* oil has been quoted at US $177.78 (approximately Rs.12, 119.26) (Anonymous, 2016a) [6].

Imports of *Tagetes* essential oil

India imported "*Tagetes* oil" worth USD 10,028 with total quantity of 42 litres. Switzerland is the largest supplier of "*Tagetes* oil" accounting for imports worth USD 9,037 followed by France and United States which exported "*Tagetes* oil" worth USD 677 and USD 147 respectively. Bombay Air Cargo accounted for 98% of imports followed by Nhava Sheva Sea which account for 2% of imports. Average

price of "*Tagetes* oil" per unit is USD 241.21 and average value per shipment is USD 346 (Anonymous, 2016b) [6].

Exports of *Tagetes* essential oil

The oil is traded under HS Code 3301. India exported "*Tagetes* oil" worth USD 54,572 with total quantity of 475litres. Germany is the largest buyer of "*Tagetes* oil" accounting for exports worth USD 49,580 followed by Taiwan and United States which imported "*Tagetes* oil" worth USD 2,160 and USD 997 respectively. Delhi Air Cargo accounted for 100% of exports followed by Chennai Air Cargo which account for 0% of exports. Average price of "*Tagetes* oil" per unit is USD 114.96 and average value per shipment is USD 1,605 (Anonymous, 2016b) [6].

Conclusion

Tagetes minuta is an aromatic plant which is least impacted by vermins like monkeys etc and also less susceptible to diseases, insects and neamtodes. Although considered as noxious weed in many countries, it is gradually finding a place in farming activities in India due to commercial value of oil.

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