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Tagetes minuta L.in Southern Brazil: A broad overview of research at Cascata Experimental Station















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Tagetes minuta L. in Southern Brazil: A broad overview of research at Cascata Experimental Station

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Foreword

The search for complementary income sources for family farming represents a challenge for research, but it can also be seen as an opportunity. Bioactive plants, considered medicinal, aromatic, and culinary herbs and spices, along with the direct commercialisation of raw plant material to industry, enable farming families to add value through the extraction of essential oils on a small to medium scale at the farm level.

Although the topic is not new, species selection is generally limited to more traditional ones, such as mint, rosemary, citronella, or eucalyptus, which are more readily absorbed by the market. However, the competition and scale of production required often render family-based ventures unfeasible.

Many species with market potential could be successfully explored, and *Tagetes minuta* L. is not an obvious choice. However, considering the increasing number of scientific studies on the biological properties of its essential oil, the multiple applications in different fields of knowledge, and,

above all, the fact that it is a naturalised species in Brazil and well adapted to the Southern Region, its potential as a sustainable crop in Brazil is promising and should not be overlooked.

This document systematises the research results of the organic cultivation of *T. minuta* carried out at the Cascata Experimental Station, Embrapa Temperate Agriculture, in recent years. It discusses aspects ranging from transplanting time to essential oil composition. When possible, comparisons are made with results obtained in countries with greater expertise in *T. minuta* cultivation to identify similarities, competitive opportunities, and research needs. In this way, this document also aims to contribute to the achievement of the Sustainable Development Goals (SDGs) proposed by the UN 2030 Agenda, particularly in alignment with the promotion of sustainable agriculture (SDG 2).

Leonardo Ferreira Dutra
Head of Embrapa Temperate Agriculture

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Introduction

In Brazil, as in many other countries, Tagetes minuta L. is invariably reported as an invasive species in fallow lands or areas prepared for the cultivation of economically important crops. However, its origin is linked to the Andean peoples' ancestral traditions (Hastorf; Bruno, 2020), especially in Peru, where its leaves are used as a condiment for meats in a traditional ceremony known as Pachamanca (Pacha = earth, manka = pot, cooking vessel) (Arellano; Arroyo, 2019; Chávez-Núñez et al., 2021). Currently, the revival of this ancestral gastronomy is also seen in academia and in the business world as a market opportunity, with several studies exploring the use of T. minuta in the development of beverages, sauces, and mixtures for meats and cheeses (Arturo, 2021; Cortez, 2025; Medina; Meza, 2018; Mena, 2022).

Among the countries cultivating T. minuta, India plays a prominent role. In 1999, the Central Institute of Medicinal & Aromatic Plants (CIMAP), in Lucknow, registered the variety Vanphool (Kumar et al., 1999). Two years later, on National Science Day, the Institute of Himalayan Bioresource Technology (IHBT), in Palampur, released the commercial variety Him Gold (Singh et al., 2001), followed by the array Him Swarnima in 2019 (Kumar; Singh, 2020), both from breeding projects. According to IHBT technical reports, *T. minuta* cultivation in India covers approximately 400 ha and involves over 500 farming families, who obtain returns two to three times higher than from traditional crops such as wheat or rice (CSIR-IHBT, 2019, 2020). In 2018, the price of T. minuta essential oil ranged from USD 100/kg to USD 140/kg, with net returns to farmers between USD 1,7/ha and USD 2,1/ha (Jyoti, 2018). South Africa also has a significant production of *T. minuta* essential oil, although it is primarily concentrated in private companies, and little information is available on this topic.

In Argentina, research on the selection and characterization of *T. minuta* genotypes, aiming at improved agronomic performance, has been ongoing for over a decade (Massuh, 2014; Massuh et al., 2017a, 2017b). In 2014, the National University of Córdoba (UNC) registered the cultivar Don Monje,

and the cultivars Serrano and Aromisky are currently undergoing registration (Martinez et al., 2020).

Information about *T. minuta* essential oil market is scarce because the estimated global demand of 12 tons per year (CBI, 2015) is relatively low compared to essential oils of other species such as *Citrus sinensis* (50,000–55,000 tons) or *Mentha arvensis* (25,000–40,000 tons) (Bizzo; Rezende, 2022). Nevertheless, due to its limited supply, between 2013 and 2014, *T. minuta* essential oil of lower quality was traded at prices ranging from € 70/kg to € 80/kg, while high-quality oil reached values between € 150/kg and € 190/kg (CBI, 2015).

Based on current research trends on the species in some countries, its market potential, and its suitability as an alternative crop in the state of Rio Grande do Sul, southern Brazil, this publication seeks to systematize cultivation data on *Tagetes minuta* L. at Cascata Experimental Station, Embrapa Temperate Agriculture. The research carried out between 2015 and 2019 addressed aspects such as the phenology and growth cycle of the species, transplanting date, biomass production, essential oil yield and productivity, and the chemical composition of the essential oil according to harvest time.

Botany and ecology

Tagetes minuta L. is a species of the Asteraceae, tribe Tageteae, native to South America and naturalized in Brazil, where it is found virtually in all regions, especially in the South, Southeast, and Central-West (Carneiro; Ritter, 2018). Synonyms include *T. glandulifera* Schrank, *T. glandulosa* Link, *T. porophyllum* Vell., and *T. bonariensis* Persoon (Carneiro; Rebouças, 2025; Schiavinato et al., 2017).

It is an annual herb with erect growth habit, ranging from 0.4 to 2.0 m in height, with a well-developed taproot system with extensive branching. The leaves are simple, opposite, sessile, with an acute apex and translucent oil glands that appear as elongated punctate structures along the margins (Carneiro; Ritter, 2018).

The inflorescences are radiate capitula (bearing both ray and disc florets) and heterogamous (with flowers exhibiting distinct sexual arrangements), numerous, and arranged in dense corymbs. The involucre is cylindrical, slightly pubescent at the margins and glabrous on the remaining surface, with linear or elliptical oil glands. The ray florets are pistillate (possessing only a gynoecium) and have a white limb with two or three lobes, while the disc florets are perfect (with both androecium and gynoecium) and have a yellowish pubescent tubular corolla (Gutiérrez; Stampacchio, 2015).

The fruits are classified as cypselae – elongated, fusiform, and dark-colored – with a pappus composed of one or, rarely, two long awns (Carneiro; Ritter, 2018). The pappus is an apical tuft of trichomes or bristles that facilitates fruit dispersal and, due to its high morphological variability, it plays a significant role in species identification within the Asteraceae family (Roque; Bautista, 2008).

T. minuta has capitula with distinct floral arrangements, presenting pistillate and perfect hermaphroditic florets (Gutiérrez; Stampacchio, which enable both allogamous and 2015), autogamous pollination processes. According to Massuh et al. (2017b), the multiple cross-pollination pathways contribute to significant intra- and interpopulational variability. Kumar et al. (2020) reported a high frequency of bee activity during flowering, suggesting that insect-mediated cross-pollination is the primary mechanism. They also found that the seed set was significantly higher under cross-pollination conditions (94%) compared to self-pollination (46%). However, Bandana and Raina (2017) reported that seed production, seed size, and seed weight were not affected by the type of pollination. Nevertheless, seeds from cross-pollination showed greater vigour and resulted in taller seedlings with higher fresh and dry biomass. The thousand-seed weight (TSW) of the Him Gold variety is approximately 0.465 g, with an average germination rate of 87% under conditions of 25 °C of temperature and 85% of humidity (Pal et al., 2023).

Like other species in the genus, *T. minuta* has essential oil-secreting structures (Carneiro; Ritter, 2018). These secretory structures are distributed throughout nearly all plant organs, though they differ in form, size, and distribution. There are three types of structures: secretory ducts, which occur more frequently in supporting tissues such as roots, stems, petioles, and veins; secretory cavities, which occur in the leaf blade and involucral bracts of the floral capitula; and glandular trichomes, present on all organs except the roots (Simon et al., 2002).

The genus *Tagetes* was described by Carl von Linné in 1737. In 1753, Linné described the species as "*Tagetes* with a simple and straight stem, with scaly multiflorous peduncles" and incorporated the description by Johann Jakob Dillenius published in 1732, *minuto flore albicante*, a Latin expression for "with small whitish flowers". Thus, the specific epithet refers to the small, whitish flowers of the species.

The most widespread explanation for the origin of the genus name is associated with the myth of the Etruscan deity Tages (Quattrocchi, 2000), who is said to have emerged around 900 BC in the present-day region of Tuscany, Italy. According to this legend, a farmer ploughing the soil witnessed the appearance of a wise child from a deep furrow, who revealed various rules for interpreting the will of the gods. These teachings quickly spread throughout the region and formed the basis of the Etruscan religion (Grummond, 2006). However, Kaplan (1960) disagrees with this hypothesis and proposes an alternative one based on the works of Leonard Fuchs from 1542 and Matthiae de Lobel in 1576, suggesting that Tagetes derives from Tagum, the Latin name for the Tagus River, along the banks of which T. erecta and T. patula occurred spontaneously.

Nevertheless, the ecology and physiology of many Asteraceae species share similarities with the Tages myth, which could have influenced Linné in his nomenclatural choice. Environmental conditions strongly influence seed germination in Asteraceae, particularly about light availability. Many species are positively photoblastic or exhibit weak photoinhibition (Ferreira et al., 2001; Kumar; Sharma, 2012). In the case of T. minuta, some studies indicate evident positive photoblastism (Felippe; Polo, 1983; Forsyth; Van Staden, 1983; Karlsson et al., 2008). Thus, soil disturbance can expose seeds to light, promoting germination. Furthermore, T. minuta seeds do not exhibit dormancy (Martinez-Ghersa et al., 2000) and, under optimal light and temperature conditions, they can achieve germination rates exceedind 70% within just 4 to 6 days (Forsyth; Van Staden, 1983; Kumar et al., 2022b).

Like the myth of Tages, many Asteraceae species such as *Taraxacum officinale*, *Sonchus oleraceus*, *Bidens pilosa*, and *T. minuta*, exhibit high dispersal capacity. The presence of a pappus on the fruit facilitates dispersal by wind (anemochory) or enables adherence to the surface of animals (epizoochory) (Cortés-Flores et al., 2013; Roque; Bautista, 2008). Human activities also favour the spread of *T. minuta*, as its fruits easily adhere to clothing and the fur or

feathers of domestic and farm animals. This fact is supported by Wang et al. (2023), who documented occurrences of *T. minuta* in anthropogenic areas, including residential backyards, abandoned lots, riverbanks, roadsides, and sites with livestock activity. Consequently, the high environmental adaptability and invasive potential of *T. minuta* have raised concerns in several countries (Ngondya; Munishi, 2021; Qi et al., 2022).

Pre-Columbian peoples of the Americas have long utilised *Tagetes* species in religious rituals, traditional medicine, and culinary practices (Neher, 1968). As a result of historical and cultural interactions between these native populations and Spanish and Portuguese colonisers, a wide variety of vernacular terms have been used referring to *T. minuta* (Table 1).

Table 1. Vernacular names for *Tagetes minuta* in different languages.

Language	Vernacular names	Reference
Quechua	Wakátay (Huacatay); Chinchu.	Aparco et al. (2022); Vita (2009); Weber et al. (2008).
Spanish	Chinchilla; Chilche; Chilchita; Chil-chil; Huacatay; Suico; Sueco; Suique; Suiquillo; Flor amar; Manzanilla de la Sierra; Margarita; Picón del rey; Quenchihué.	Gutiérrez; Stampacchio (2015); Martinez et al. (2020); Vita (2009).
Portuguese	Chinchilho; Chinchila; Cinchilho; Rabo-de-rojão; Vara-de-foguete; Vareta-de-rojão; Guizo-de- cascavel; Mata-pulgas; Cravo-de-defunto; Cravo-do-mato.	Carneiro e Ritter (2018); Carneiro e Rebouças (2025).
English	Mexican marigold; Muster John Henry; Stinking-Roger; Southern marigold; Aztec marigold.	Wiersema (2019).

Although many cultures across Central and South America may have their vernacular names for T. minuta, the Quechua language is one of the most significant languages, encompassing various ethnic groups, especially from Andean countries such as Peru, Bolivia, Chile, Colombia, and Ecuador. In the Southern Region of Brazil, the most well-known vernacular name is "chinchilho", which closely resembles the term "chinchilla" used in neighbouring Argentina. The Portuguese vernacular name "cravode-defunto" is perhaps the least appropriate to refer to T. minuta in Brazil, since T. patula and T. erecta - exotic species with ornamental appeal - are also widely known by that name, despite being visibly distinct in terms of growth habit, capitulum size, and colouration (Carneiro; Ritter, 2018).

In Quechua dictionaries, "wakatay" or "huacatay" is the most frequently cited vernacular name for *T. minuta* (Gobierno Regional Cusco, 2005; Pérez, 2022b). However, some sources also reference the term "chinchu" (Vita, 2009; Weber et al., 1998), which could be the linguistic root of "chinchilho" or "chinchilla". In Spanish, the verb "chinchar" means to disturb or annoy someone, to be irritating (Pérez, 2022a).

By linking the etymology of the scientific name, the vernacular name "chincho", and the botanical characteristics of the species, it is possible to interpret "chinchilho" as "the herb with small white flowers, whose fruits cling to clothing and are bothersome".

Biological properties and uses

Ancient Andean peoples utilised *T. minuta* for multiple purposes, including culinary use, traditional medicine, and pest control (De La Cruz et al., 2014; Hastorf; Bruno, 2020; Vasquéz; Peláez, 2014). Its biological properties have been extensively investigated and systematised in comprehensive literature reviews, highlighting its antibacterial, antifungal, antioxidant, insecticidal, acaricidal, nematicidal, and larvicidal potential, among others (Bandana et al., 2018; Joshi; Barbalho, 2022; Santos et al., 2017; Verma et al., 2024; Walia; Kumar, 2020).

In the industry, its use primarily occurs in fragrances, flavourings, cosmetic products, and food and beverage manufacturing (CBI, 2015; Wang et al., 2023; Singh et al., 2003; Walia; Kumar, 2020). Pigments from the aerial parts of *T. minuta* can also be used as natural dyes for textiles such as silk, wool, and cotton (Kumar et al., 2014).

Despite its various applications, recent studies have highlighted the essential oil of T. minuta as a promising basis for developing innovative products to control high-priority public health agents. Bordón et al. (2025) demonstrated that the oil exhibits rapid and effective bactericidal action against Escherichia coli and Staphylococcus aureus, including resistant strains, with a mechanism of action associated with cell membrane destabilisation, suggesting a mode of action distinct from that of conventional antibiotics. Additionally, Sartor et al. (2025) reported high larvicidal toxicity against Aedes aegypti, the primary vector of dengue, Zika, and chikungunya viruses, with median lethal concentration (LC₅₀) values indicative of strong efficacy. The relevance of these findings is further enhanced by the fact that T. minuta offers a high essential oil yield and demonstrates excellent agronomic adaptability to Brazilian growing conditions, providing economic, ecological, and logistical advantages for large-scale application.

Research study conditions at Cascata Experimental Station (EEC)

The Cascata Experimental Station (EEC), Embrapa Temperate Agriculture, is located in the municipality of Pelotas, Rio Grande do Sul State (RS), Brazil, at a latitude of -31°37′24″ South and a longitude of -52°31′37″ West, at an average altitude of 180 meters. Cultivation results from the 2015/2016, 2016/2017, and 2018/2019 crop seasons were systematised.

Seedlings were produced in a greenhouse using expanded polystyrene trays with 128 cells, each cell being pyramidal with a volume of 29.67 cm³. The substrate consisted of a mixture of earthworm humus and carbonised rice husk in a 2:1 (v:v) ratio. Variations in transplanting time and harvest date were implemented across different crop seasons (Table 2).

Table 2. Crop season and date of sowing, transplanting, and harvest of *Tagetes minuta* L. at Cascata Experimental Station (EEC), Embrapa Temperate Agriculture, Pelotas, RS. Brazil.

Crop season	Sowing	Transplant	Harvest
			19/April/2016
2015/2016	20/Santambar/2015	1/December/2015 -	27/April/2016
2015/2016	30/September/2015	1/December/2015 —	05/May/2016
		_	12/May/2016
			04/May/2017
		_	11/May/2017
	21/Ostabor/2010	20/December/2016	17/May/2017
	31/Octobe1/2016	04/May/2017 11/May/2017 17/May/2017 24/May/2017 31/May/2017 07/June/2017 04/May/2017 11/May/2017 11/May/2017 17/May/2017	24/May/2017
2046/2047			
2016/2017			27/April/2016 05/May/2016 12/May/2016 04/May/2017 11/May/2017 17/May/2017 24/May/2017 31/May/2017 07/June/2017 04/May/2017 11/May/2017
		04/May/2017 11/May/2017 17/May/2017 24/May/2017 31/May/2017 07/June/2017 04/May/2017 11/May/2017 17/May/2017 17/May/2017	
	07/10	- 1011	17/May/2017
	07/December/2016	18/January/201 <i>7</i> –	24/May/2017
		_	31/May/2017
		_	07/June/2017

Table 2. Continued.

Crop season	Sowing	Transplant	Harvest
			11/May/2017
			17/May/2017
2046/2047	27/December/2016	13/February/2017	24/May/2017
2016/2017		_	31/May/2017
	07/June/2017		
	17/January/2017	07/March/2017	07/June/2017
0040/0040	44/D	20/1/0040	17/May/2019
2018/2019	11/December/2018	30/January/2019 -	30/May/2019

Soil variables

The cultivation areas were prepared using a disk harrow, with no lime application or fertilisation carried out. The soil in the area is classified as an Argisol (IBGE, 2023). In the 2015/2016 and 2016/2017 growing seasons, cultivation was carried out in a continuous area, where the soil presented the following chemical characteristics: pH in water of 5.1; organic matter 2.07%; phosphorus 65.6 mg dm⁻³; potassium 116 mg dm⁻³; calcium 3.2 cmol dm⁻³; magnesium 1.0 cmol dm⁻³; and base saturation of 51%. The adopted spacing was 0.25 m between rows and 0.20 m between plants, representing a density of 200,000 plants per hectare. Manual irrigation was applied during the first 15 days to ensure the establishment of seedlings. Manual weeding with a hoe was carried out between 20 and 25 days after transplanting. No insect or disease control products were used, nor topdressing fertilisation were applied to the experimental plots.

Climate and crop climatic variables

The climate of the region is classified as humid subtropical, characterised by a lack of a dry season, but with hot summers (Cfa), according to the Köppen classification (Alvares et al., 2013).

Weather variables were monitored using an automatic data acquisition system (datalogger), Campbell, model CR800. Daily air temperature (°C), relative air humidity (%), photosynthetically active radiation (MJ m⁻²), and daily rainfall (mm) Were recorded. Using the average, maximum, and minimum air temperature values, groWing degree days (°C day⁻¹) Were calculated for the period

from transplanting to harvest, folloWing the method described by Ometto (1981) and validated by Renato et al. (2013). A lower base temperature of 10°C was adopted (Kumar et al., 2010). Due to the lack of specific data on the upper base temperature for *T. minuta*, a threshold of 35°C was used, the same temperature at which development is impaired in sunflower, another species within the Asteraceae family (Castro; Farias, 2005).

The earliest transplanting of *T. minuta* in the experiments conducted at the EEC occurred on December 2, 2015, and the latest harvest on June 7, 2017. The climatic variables during the different cultivation periods are presented in Table 3.

As stated above, Tagetes minuta is native to South America and can be found from the southeastern United States to northern Patagonia in Argentina, and is now also found in several countries across Europe, Asia, Africa, and Australia (Gutiérrez; Stampacchio, 2015). One of the leading producers of T. minuta essential oil is India, where it is cultivated in regions with highly distinct climatic characteristics, at altitudes ranging from 490 m to over 2600 m above sea level (Walia et al., 2020b). In Brazil, studies on yield and the characterization of the essential oil have been carried out using plants collected from spontaneous populations or cultivated plots in the states of Bahia and Pernambuco (Craveiro et al., 1988), Ceará (Furtado et al., 2005; Macedo et al., 2013), the Federal District (Koketsu et al., 1976), Mato Grosso do Sul (Garcia et al., 2012), Paraná (Cepeda et al., 2023; Zimmermann et al., 2021), Santa Catarina (Sperandio et al., 2019), São Paulo (Albuquerque, 2018), and Rio Grande do Sul (Cunha et al., 2016; Fonseca, 2018; Gomes, 2017; Moreira, 2021; Oliveira et al., 2019; Rostignoli, 2019; Santos et al., 2021; Schiedeck, 2023; Siqueira et al., 1982). All these studies highlight the species' adaptive plasticity to different environments.

Table 3. Climatic variables according to the transplanting date and harvest period of Tagetes minuta in the 2015/2016, 2016/2017, and 2018/2019 growing seasons. Cascata Experimental Station (EEC), Embrapa Temperate Agriculture, Pelotas, RS. Brazil

Transplant	Harvest	Harvests	temp	Mean air temperature (°C)	(0,)	Relative humidity (%)	Degree-day (Σ °C day)	PAR ⁽¹⁾ (MJ m ⁻²)	Photoperiod (hours) ⁽²⁾	eriod	Rainfall (mm)
day	season		Maximum	Mean	Minimum	Mean	Mean	Mean	Transplant Harvest	Harvest	Amount
02/December/2015	19/April to 12/May	4	27.27	21.35	16.60	90.27	1833.39	6.28	14.05	10.86	467.11
20/December/2016	04/May to 07/June	9	27.05	20.76	16.58	90.92	1825.25	5.80	14.22	10.42	1159.12
18/January/2017	04/May to 07/June	9	26.45	20.19	15.96	90.70	1406.71	5.61	13.91	10.42	956.96
30/January/2019	17/May to 30/May	2	24.96	19.70	14.99	85.73	1147.55	4.37	13.65	10.36	435.40
13/February/2017	11/May to 07/June	5	25.57	19.38	15.23	91.62	1076.25	4.90	13.24	10.35	841.52
07/March/2017	07/June	_	23.10	17.48	13.65	92.28	812.80	3.72	12.55	10.15	615.04
Mean			25.73	19.81	15.50	90.25	1350.33	5.11	13.60	10.47	740.86

(1) Photosynthetically active radiation, in megajoules per square meter.
(2) Calculated for the coordinates latitude -31°37'14.39" South and longitude -52°31'19.02" West

As stated above, Tagetes minuta is native to South America and can be found from the southeastern United States to northern Patagonia in Argentina, and is now also found in several countries across Europe, Asia, Africa, and Australia (Gutiérrez; Stampacchio, 2015). One of the leading producers of T. minuta essential oil is India, where it is cultivated in regions with highly distinct climatic characteristics, at altitudes ranging from 490 m to over 2600 m above sea level (Walia et al., 2020b). In Brazil, studies on yield and the characterization of the essential oil have been carried out using plants collected from spontaneous populations or cultivated plots in the states of Bahia and Pernambuco (Craveiro et al., 1988), Ceará (Furtado et al., 2005; Macedo et al., 2013), the Federal District (Koketsu et al., 1976), Mato Grosso do Sul (Garcia et al., 2012), Paraná (Cepeda et al., 2023; Zimmermann et al., 2021), Santa Catarina (Sperandio et al., 2019), São Paulo (Albuquerque, 2018), and Rio Grande do Sul (Cunha et al., 2016; Fonseca, 2018; Gomes, 2017; Moreira, 2021; Oliveira et al., 2019; Rostignoli, 2019; Santos et al., 2021; Schiedeck, 2023; Siqueira et al., 1982). All these studies highlight the species' adaptive plasticity to different environments.

Earlier transplanting dates expose the plants to higher average temperatures, solar radiation, and longer photoperiods. Studies conducted at EEC revealed that the absolute maximum temperature reached 36.5°C in January 2016, and the absolute minimum was recorded at 2.6°C on May 1st of the same year. In India, crops are grown in temperatures ranging from 41°C to 2.6°C (Sharma et al., 2017; Sood et al., 2020). However, according to Singh et al. (2001), oil quality is better when air temperature remains between 12°C and 30°C during reproductive stages.

Photosynthetically active radiation (PAR) is directly related to plant growth and development; however, few studies have addressed this factor in *T. minuta*. For crops grown at EEC, average daily PAR in December was 1.7 times higher than in June, and this difference was reflected across all biomass parameters measured. Kumar et al. (2014) found that reduced light intensity in *T. minuta* can affect biomass accumulation, yield, productivity, and the composition of essential oil.

In the studies conducted at EEC, the average photoperiod in June was approximately 3 hours and 8 minutes shorter than that in December. The harvest in all three growing seasons occurred when the average photoperiod was 10 hours and 28 minutes. Luciani-Gresta (1975) observed that *T. minuta* flowers under a photoperiod between 10 and

13 hours, which is also influenced by light intensity and temperature. Evaluating transplant times, Kumar et al. (2012) observed the onset of flowering when day length ranged between 12 and 13 hours and harvested when it was between 11 and 12 hours. Similar results were reported by Ramesh and Singh (2008).

The total accumulated rainfall in the EEC experiments ranged from 435 to 1159 mm, depending on the year and growing season. In the study by Ramesh and Singh (2008), rainfall varied from 610 to 1407 mm across different growing seasons. In contrast, Walia et al. (2020b) cultivated *T. minuta* in areas with accumulated precipitation ranging from 204 to 2665 mm.

According to Singh et al. (2003), *T. minuta* requires a minimum of 500 mm of rainfall, well distributed throughout the cycle, and preferably in well-drained soils. Low oxygen availability to the roots in waterlogged soils is the leading cause of yield loss in *T. minuta* (Kumar et al., 2022a).

Harvesting

Plant growth and development were monitored on a weekly basis. The harvest point was defined as the stage when the cypselae (achenes) were released after manual shaking of the stems. In the 2015/2016 and 2016/2017 seasons, after the first harvest, weekly harvests were conducted until no more plants remained in the plots or the plants had reached advanced senescence, with aerial parts completely dry and unsuitable for essential oil extraction or measurement of leaf area.

At harvest, stem diameter was measured at the stem base using a digital calliper, and plant height was measured with a ruler from the stem base to the apex of the youngest shoot.

After harvest, the plants were manually separated into flowers, leaves, branches, and stem, and each fraction was weighed per plant. The leaf area of each plant was measured using a Li-COR LI-3100C area meter. For dry mass determination, the fresh fractions were placed in a forced-air circulation oven at 105°C for approximately 72 hours and weighed again once constant weight was achieved.

The phenological scale used to monitor the growth and development of *T. minuta* was proposed by Fonseca (2018), based on the phenological growth stage codes described by Hack et al. (1992).

Essential oil extraction

The essential oil was extracted from fresh flowers and leaves by hydrodistillation using a modified Clevenger apparatus. A total of 300 g of fresh biomass was used, maintaining the proportion between flowers and leaves as determined in the biomass fractioning at each harvest time. The approximate distillation time was 2 hours and 45 minutes, with the process being completed when no further increase in essential oil volume was observed in the separation burette. The oil from each sample was transferred to glass vials and stored in a freezer until the compounds were identified and characterised.

Essential oil yield (%) was calculated as the ratio between the extracted volume (mL) and the fresh mass of flowers and leaves (g). The essential oil productivity (kg ha⁻¹) was determined by multiplying the oil content of the distilled flower and leaf biomass by the biomass of flowers and leaves harvested from one hectare. To this end, the oil volume in millilitres obtained from distillation was converted into grams using the approximate density of 0.9 g cm⁻³ for *T. minuta* essential oil (Kumar et al., 2014).

The essential oil was analysed at the Laboratory of Natural Products Chemistry at Embrapa Agroindustry following the previously described by Castro et al (2019). Gas chromatography coupled to mass spectrometry (GC-MS) analysis was carried out on an Agilent 7890B GC/5977A MSD chromatograph, operating with electron impact ionization at 70 eV, split injection mode (1:30), carrier gas flow at 1.50 mL min⁻¹, injector temperature at 250°C, and transfer line temperature at 250°C. The oven temperature program started at 70°C With a ramp of 4°C min⁻¹ up to 180°C held for 27.5 minutes, folloWed by a ramp of 10°C min⁻¹ up to 250°C, ending the run at 34.5 minutes.

Compound identification was carried out by analysing the fragmentation patterns in the mass spectra with those from the equipment's database (NIST version 2.0), as well as comparing their retention indices with those of known compounds obtained by injecting a mixture of standards and literature data (Adams, 1995). Quantification was carried out by normalization of the relative peak areas, obtained by gas chromatography coupled with a flame ionization detector (GC-FID). The equipment

used was a Shimadzu GC-2010 Plus fitted with a VF-5MS methylpolysiloxane column (30 m × 0.25 mm × 0.25 µm, Varian), operating in split injection mode (1:30), using nitrogen as the carrier gas at a flow rate of 1.00 mL min $^{-1}$, With injector temperature at 250°C and detector temperature at 280°C. The oven temperature program Was as folloWs: initial temperature of 70°C With a ramp of 4°C min $^{-1}$ to 180°C held for 27.5 minutes, folloWed by a ramp of 10°C min $^{-1}$ to 250°C, ending the run at 34.5 minutes. Compound characterization Was based on retention index determination using a homologous series of n-alkanes (C7–C30).

Agronomic performance

Crop cycle

T. minuta can be propagated either by direct seeding, like broadcast sowing or in rows, or by seedling production (Pal et al., 2023; Sood et al.,

2020). At the EEC, seedling production was chosen as it allows field preparation just a few days before transplanting. In the three crop seasons evaluated, seedlings were transplanted at ages ranging from 42 to 62 days and had an average height of approximately 10 cm. Although this is considered a relatively long duration compared to other species, it is a typical age for transplanting *T. minuta*. Different authors report transplanting seedlings at 30 days (Pandey et al., 2015), 45 days (Bandana et al., 2018; Ramesh; Singh, 2008), or even 60 days (Rathore et al., 2018; Walia et al., 2021).

The average crop cycle duration between transplanting and harvest in the cultivations carried out at the EEC is presented in Figure 1.

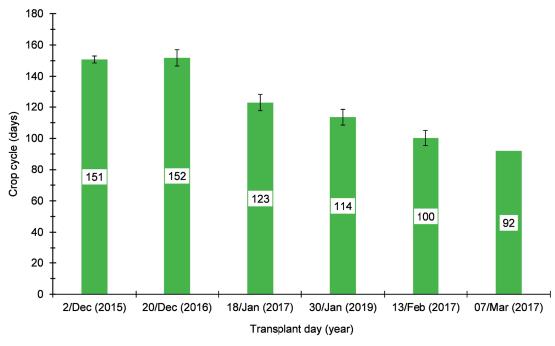


Figure 1. Average crop cycle of *Tagetes minuta* from transplanting to harvest. Data arranged in ascending order by month and day of transplanting. Vertical bars above the columns indicate the mean standard error. Cascata Experimental Station (EEC), Embrapa Temperate Agriculture, Pelotas, RS. Brazil.

The crop cycle of *T. minuta* was reduced by the delay in transplanting. Plants transplanted in December exhibited a cycle 30 to 50 days longer than those transplanted in January and February, and up to 60 days longer than those transplanted in March. This behaviour was also reported by Kumar et al. (2012) in their study on the effect of transplanting date on the cycle of *T. minuta*. According to the authors, the species is a short-day plant, and by delaying transplanting, the plants are exposed to a shorter photoperiod, which stimulates flowering and, consequently, shortens the cycle.

In India, cultivation of *T. minuta* when transplanting was carried out in June resulted in an average cycle of 100 to 120 days until harvest (Kumar et al., 2012; Syamasundar; Rao, 2013; Walia et al., 2021). This cycle is similar to that observed at the EEC when transplanting is carried out between January and February.

The relationship between transplanting date and crop cycle duration could be calculated by converting transplanting dates into Julian days. The Julian day is a sequential method of counting dates that allows their use in regression models (Figure 2).

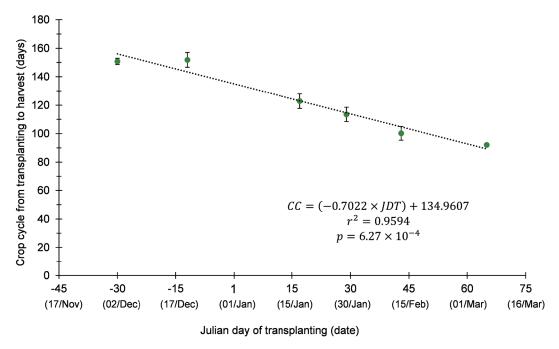


Figure 2. Estimated crop cycle (CC) from transplanting to harvest based on the transplanting date in Julian days (JDT). Corresponding calendar dates are shown in parentheses. Dates before January 1st were considered negative Julian days, while dates after were considered positive. Vertical bars over the points indicate the mean standard error. Cascata Experimental Station (EEC), Embrapa Temperate Agriculture, Pelotas, RS. Brazil.

The Julian day of transplanting explained more than 95% of the variation in the duration of the *T. minuta* cycle. Thus, future cultivations will be able to predict the approximate harvest date rapidly and straightforwardly.

The study conducted by Fonseca (2018) at the EEC monitored the development cycle of *T. minuta* at various transplanting and harvesting dates. Based on these observations, a phenological scale was developed, from sowing to plant senescence (Figure 3).

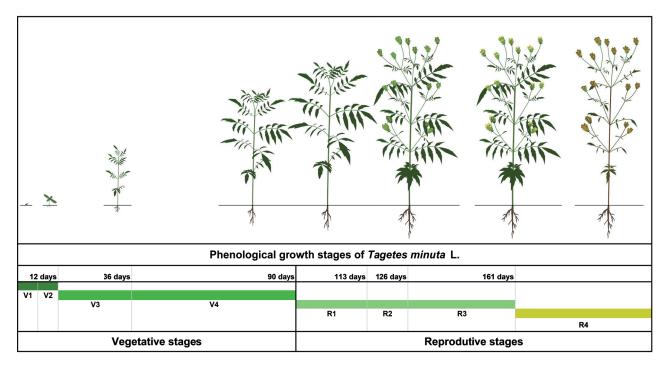


Figure 3. Proposed phenological scale of *T. minuta* based on sowing on December 27 and transplanting on February 13. V1 – Germination, V2 – Emergence, V3 – True leaves, V4 – Leaf and stem growth, R1 – Onset of floral head development (bud onset), R2 – Full flowering, R3 – Fruit dispersal, R4 – Senescence. Source: adapted from Fonseca (2018).

Illustration: Larissa Fonseca Silveira.

The duration of *T. minuta* phenophases is strongly related to the growing season (Kumar et al., 2010; Ramesh; Singh, 2008). Fonseca (2018) carried out the transplant 48 days after sowing, during stage V4. Considering the periods after transplanting, the onset of floral head development (R1) occurred at 65 days, full flowering (R2) at 78 days, and the beginning of fruit dispersal (R3) at 113 days. According to the author, the optimal harvest period for maximising essential oil yield and productivity is at the R2 and R3 phenological stages. Harvesting at these stages agree with the findings of other authors (Gupta; Khajuria, 2007; Singh et al., 2006; Singh et al., 2003).

Biomass production

The transplanting day influenced the growth and development of *T. minuta*. Except for the transplant carried out on December 2, 2015, during the 2015/2016 season, earlier transplants resulted in greater plant biomass accumulation, leaf area index, plant height, and stem diameter (Table 4). The biomass of the plants transplanted in early December 2015 may have been negatively affected by the low volume and uneven distribution of rainfall throughout the growth cycle. Approximately 80% of the total rainfall volume occurred in the last 36 days of cultivation, which also impacted the photosynthetically active solar radiation, with a daily average of only 2.42 MJ m⁻² during this period.

Tage 4. Agronomic performance of Tagetes minuta at different harvest periods during the 2015/2016, 2016/2017, and 2018/2019 crop seasons. Cascata Experimental Station EEC), Embrapa Temperate Agriculture, Pelotas, RS. Brazil

Transplant day	Harvest Season	Number of	Cycle¹ (days)	rs)	Total dry mass (t ha ⁻¹)	dry ha⁻¹)	Flowers dry mass (t ha ⁻¹)	s dry t ha⁻¹)	Leaves dry mass (t ha¹¹)	s dry t ha ⁻¹)	ΓΨ	<u>-</u> 2	Plant height (m)³	int t (m)³	Stem diameter (cm)	n r (cm)
		Samples	Mean	se	Mean	se	Mean	Se	Mean	se	Mean	se	Mean	se	Mean	Se
02/December/2015	02/December/2015 19/April to 12/May	16	150.75	2.22	17.91	1.52	2.69	0.22	1.73	0.17	2.27	0.17				
20/December/2016	20/December/2016 04/May to 07/June	9	151.83	5.17	20.89	2.00	4.52	0.58	3.45	0.25	3.45	0.26	2.16	90:0	16.62	0.80
18/January/2017	04/May to 07/June	9	122.83	5.17	18.11	1.22	4.15	0.43	3.34	0.18	3.08	0.16	2.11	0.04	14.53	0.55
30/January/2019	17/May to 30/May	7	113.50	5.03	13.24	2.06	4.65	1.01	1.95	0.51	1.89	99.0	,			
13/February/2017	11/May to 07/June	5	100.20	4.81	5.85	69.0	1.78	0.28	1.35	0.11	1.91	0.20	1.23	0.04	8.97	0.41
07/March/2017	07/June	-	92.00		4.27		1.45		1.14		1.40		0.48	0.04	5.16	0.38

(1) From transplanting to harvest.

(2) Leaf area index.

Plant height and stem diameter were estimated based on the average of 24 plants. The 'se' indicates the mean standard error.

Higher total biomass production, as well as increased accumulation of flowers and leaves in early-season crops, has also been reported in other studies (Kumar et al., 2012; Ramesh; Singh, 2008). Plants that are transplanted later remain in the field for a shorter period because flowering, the optimal time for harvesting, is stimulated by the reduction in photoperiod. Additionally, late crops accumulate fewer growing degree days and are exposed to fewer hours of sunlight, which negatively affects their growth (Kumar et al., 2010).

Plants transplanted at the EEC during the second half of December were harvested when they were over 2 m tall, while those transplanted in March reached the harvest point when they were less than 50 cm tall. Kumar et al. (2012) found that early-transplanted plants were harvested at a height of 2.26 m, while those transplanted 74 days later reached only 1.16 meters.

Plant height and biomass productivity are also affected by planting density. At the EEC, crops were carried out with a spacing of 25 cm between rows and 20 cm between seedlings, representing a planting density of 200,000 plants per hectare. In India, denser plantings are typically carried out with a spacing of 30 cm by 30 cm, or approximately 111,000 plants per hectare (Kumar et al., 2012; Singh et al., 2008). Some studies suggest that denser plantings tend to produce taller plants, although biomass accumulation increases only up to a specific limit (Pal et al., 2023; Walia; Kumar, 2021a).

Comparison biomass productivity across different studies can be challenging due to the lack of information regarding the calculation basis (fresh or dry mass) or the biomass fraction considered (total, useful, with or without stems or branches). One way to minimise this issue is through regression equations, as presented in Figure 4.

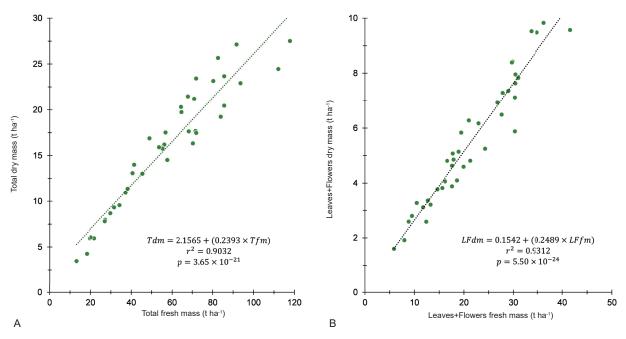


Figure 4. Estimation of total dry mass (Tdm) and leaves and flowers dry mass (LFdm) from total fresh mass (Tfm) and leaves and flowers fresh mass (LFfm) of *Tagetes minuta* L. Cascata Experimental Station (EEC), Embrapa Temperate Agriculture, Pelotas, RS. Brazil.

Studies carried out at the EEC revealed the highest average values of leaf and flower fresh mass harvested, at 30.4 t ha⁻¹ and 7.7 t ha⁻¹ on a dry basis, respectively, When transplanting occurred betWeen December 20 and January 18. Using 30 cm × 30 cm spacing, Kumar et al. (2012) obtained 18.5 t ha⁻¹ of leaf and floWer fresh mass, While Singh et al. (2008) achieved 8.15 t ha⁻¹ on a dry basis. When soWing Was done broadcast or in roWs spaced 60 cm apart, With a final stand of 200,000 plants per hectare, Pal et al. (2023) obtained 28.1 t ha⁻¹ of fresh mass of *T. minuta*.

Certain cultivation practices also affect the performance of the species. Weed density has a negative correlation with T. minuta biomass, particularly in the early stages of development, due to competition for nutrients, water, and light. Weeding at 25 and 50 days after transplanting can increase biomass by about 2.5 times compared to no control measures (Walia et al., 2021). At the EEC, only one manual weeding was carried out between 20 and 25 days after transplanting. Manual weeding is a labour-intensive activity and may make it infeasible to cultivate crops in larger areas. For weeding with motorised implements to be feasible, the spacing between cultivation rows must be increased to at least 50 cm, which would reduce plant population per hectare and, consequently, biomass production. Therefore, further studies are needed to assess whether the lower plant density could be offset by a greater number and frequency of weeding with motorized implements.

Regarding soil conditions, Singh et al. (2001) state that *T. minuta* prefers slightly acidic soils, with a pH between 5.5 and 7.0. Although no specific studies on *T. minuta* exist in Brazil, inferences can be made based on information available for *Calendula officinalis* (Asteraceae), for which a pH of 5.5 is recommended in the state of Rio Grande do Sul (Sociedade Brasileira de Ciência do Solo, 2016).

Soil fertilisation is a widespread practice in countries that commercially cultivate T. minuta. Several authors report positive responses to fertilisation, especially with nitrogen, on plant height and total biomass (Pandey et al., 2015; Walia; Kumar, 2021b), flower productivity (Sharma et al., 2017), and essential oil yield and productivity (Omidbaigi et al., 2008; Pandey et al., 2015; Rao et al., 2006). In general, organic fertilisation is performed before cultivation with 15 to 30 t ha⁻¹ of cattle manure (Singh et al., 2003; Walia; Kumar, 2021b) or 5 t ha⁻¹ of poultry manure (Pandey et al., 2015). Nitrogen fertilisation is traditionally carried out using urea, With 30 to 40 kg ha⁻¹ at planting and 60 to 80 kg ha⁻¹ as a top-dressing, divided into tWo applications. Phosphorus and potassium are supplied at planting With 60 kg ha⁻¹ of P₂O₅ (single superphosphate) and 40 kg ha⁻¹ of K₂O (potassium chloride) (Upadhyay et al., 2021; Walia et al., 2021).

The production of secondary metabolites by plants is an evolutionary response to survival and adaptation to the environment conditions, and biotic or abiotic stress factors often act as inducers of their synthesis (Alami et al., 2024). In the case of using highly soluble nitrogen fertilizers, it tends to increase plant biomass production; however, beyond a certain threshold, they may reduce the production of essential compounds (Hao et al., 2024; Qiao et al., 2018; Song et al., 2023; Sun et al., 2020).

In *T. minuta*, the results regarding the effects of fertilisation on essential oil composition are still conflicting. While some studies report no significant effect of nutrient sources or levels (Pandey et al., 2015; Singh; Rao, 2005), others show that nitrogen applications ranging from 120 to 150 kg ha⁻¹ tend to reduce the content of Dihydrotagetone and increase the content of cis-beta-Ocimene in the essential

oil (Graven et al., 1991; Singh et al., 2008; Walia; Kumar, 2021b).

The *T. minuta* crops at the EEC were planted in areas to take advantage of the residual effect of organic fertilisation carried out in previous crops. Thus, further studies could be set to evaluate adjustments in the organic production system for the plants to express their full productive potential and assess the impact on essential oil quality.

Yield and productivity of essential oil

During the crop seasons conducted at the EEC, the yield and productivity of essential oil varied according to the transplanting and harvesting times of the plants (Table 5).

Table 5. Yield (%) and productivity (kg ha⁻¹) of essential oil from *Tagetes minuta* at different transplanting dates and harvest periods. Cascata Experimental Station (EEC), Embrapa Temperate Agriculture, Pelotas, RS. Brazil.

					Essen	tial oil		
Turnantant	Harvest	Number		Yield (v/w, %)		Producti	vity (kg/
Transplant	period	of samples	Fre	sh	Dı	y	ha) ⁽¹⁾
			Mean	se	Mean	se	Mean	se
02/December/2015	19/April to 12/May	16	0.53	0.07	2.03	0.21	75.30	7.65
20/December/2016	04/May to 07/June	6	0.94	0.09	3.55	0.29	249.69	22.97
18/January/2017	04/May to 07/June	6	0.85	0.09	3.09	0.34	204.89	20.77
30/January/2019	17/May to 30/May	2	0.90	0.05	3.53	0.01	158.75	37.90
13/February/2017	11/May to 07/June	5	0.87	0.11	3.41	0.36	98.35	17.41
07/March/2017	07/June	1	1.00	_	4.80	-	111.71	_

^{*}The volumes in milliliters obtained through distillation were converted into grams considering the approximate density of *T. minuta* essential oil as 0.9 g cm⁻³.

The data collected at the EEC enabled the development of tools that may be useful in future studies. Considering the hydrodistillation of only flowers and leaves in a Clevenger apparatus, it is possible to estimate essential oil yield on a dry basis using the following linear equation ($r^2 = 0.90$, $p = 2.61 \times 10^{-12}$):

$$Y_D = 0.22 + (3.56 \times Y_E)$$

where Y_D is the essential oil yield on a dry basis (%) and Y_F is the yield on a fresh basis (%). It is also possible to estimate essential oil productivity with the following multiple linear equation ($r^2 = 0.95$, $p = 2.51 \times 10^{-23}$):

$$P_{adj}$$
= -158,39 + (7,01 × LF_{fm}) + (199,34 × Y_{F})

where P_{adj} is the essential oil productivity (kg ha⁻¹) adjusted by the average oil density (0.9 g cm⁻³), LF_{fm} is the fresh mass of leaves and flowers (t ha⁻¹), and Y_{F} is the essential oil yield on a fresh basis (%) obtained through hydrodistillation using a Clevenger apparatus.

However, comparing essential oil yield and productivity between studies is often difficult due to a lack of details regarding the type and preparation of the distilled plant material and the distillation method. Walia and Kumar (2020) compiled production information from various studies and observed

essential oil yield variation between 0.25% and 0.78%, with productivity ranging from 29.52 kg ha⁻¹ to 68 kg ha⁻¹, depending on the cultivation season, spacing, and groWing method (direct seeding or transplanting). Kumar et al. (2012) achieved higher results when using 30 cm \times 30 cm spacing and applying 30 t ha⁻¹ of cattle manure to the soil: the yield ranged betWeen 1.19% and 1.58% on a fresh basis, and the essential oil productivity ranged from 110 kg ha⁻¹ to 180 kg ha⁻¹.

Nevertheless, the cultivation system developed at the EEC has proven to be quite competitive compared to those recommended in other countries. Seedlings transplanted between December 20 and January 18 and plants harvested between May 4 and June 7 resulted in an average essential oil yield of 0.85±0.07% on a fresh basis and 3.40±0.36% on a dry basis. During this period, the average productivity was 227.29±16.23 kg ha⁻¹.

The high yield obtained in the latest transplanting and harvesting period was due to the plants having a dry mass ratio of flowers to leaves of 2.4:1. In contrast, in the other periods the average was 1.3:1. Since the oil content is generally higher in flowers than in leaves (Kumar et al., 2012, 2014), a greater proportion of flowers in the distilled material tends to increase the yield. This value is similar to that reported by Sartor et al. (2025), who collected *T. minuta* flowers in June and obtained a yield of 4.9% on a dry weight basis.

Chemical composition of essential oil

The essential oil of *T. minuta* obtained at the EEC is rich in cis-Tagetone, cis-beta-Ocimene, and Dihydrotagetone; however, the proportion between these compounds changes according to the harvest time (Table 6 and Figure 5).

Table 6. Mean composition (%) and standard error (se) of *Tagetes minuta* essential oil obtained by hydrodistillation of flowers and leaves harvested between April and June. Cascata Experimental Station (EEC), Embrapa Temperate Agriculture, Pelotas, RS. Brazil.

Company	Molecular	Rical ⁽¹⁾	Apri	I	Ma	у	Jun	9
Compound	formula	Rical	Mean (%)	se	Mean (%)	se	Mean (%)	se
Monoterpenes								
Sabinene	C ₁₀ H ₁₆	975	0.81	0.03	0.41	0.04	0.23	0.04
Limonene	$C_{10}H_{16}$	1029	8.16	0.67	3.68	0.35	2.16	0.36
cis-beta-Ocimene	C ₁₀ H ₁₆	1037	12.21	1.96	23.88	0.67	26.71	1.49
Oxygenated monoterpenes								
Dihydrotagetone	C ₁₀ H ₁₈ O	1052	41.48	3.88	18.55	1.65	12.52	2.30
Linalool	C ₁₀ H ₁₈ O	1096	0.15	0.04	0.10	0.01	_	-
cis-beta-Ocimene epoxide	C ₁₀ H ₁₆ O	1132	0.25	0.02	0.18	0.01	-	-
trans-Tagetone	C ₁₀ H ₁₆ O	1144	5.90	0.25	5.32	0.19	4.91	0.31
cis-Tagetone	C ₁₀ H ₁₆ O	1152	27.40	3.04	45.25	1.66	49.37	2.38
Elsholtzione	C ₁₀ H ₁₄ O ₂	1202	0.14	0.03	_	_	_	-
Sesquiterpenes								
Caryophyllene	C ₁₅ H ₂₄	1419	0.40	0.05	0.24	0.02	0.22	0.03
Humulene	C ₁₅ H ₂₄	1454	0.42	0.06	0.21	0.02	0.15	0.02
Bicyclogermacrene	C ₁₅ H ₂₄	1500	0.48	0.04	0.33	0.03	0.40	0.02

Continued...

Table 6. Continued.

Compound	Molecular formula	Rical ⁽¹⁾	April		Мау		June	
			Mean (%)	se	Mean (%)	se	Mean (%)	se
Oxygenated sesquiterpenes								
Spathulenol	$C_{15}H_{24}O$	1578	0.26	0.07	0.08	0.01	_	_
Caryophyllene oxide	C ₁₅ H ₂₄ O	1583	0.20	0.04	0.18	0.02	0.15	0.04
Monoterpenes	_	_	21.18	_	27.97	_	29.10	_
Oxygenated monoterpenes	-	_	75.34	_	69.41	-	66.81	-
Sesquiterpenes	-	_	1.30	_	0.79	_	0.77	_
Oxygenated sesquiterpenes	-	_	0.47	_	0.26	-	0.15	_
Total	_	_	98.29	_	98.42	_	96.83	_

⁽¹⁾ Retention index calculated

⁽⁻⁾ Information not available or not applicable.

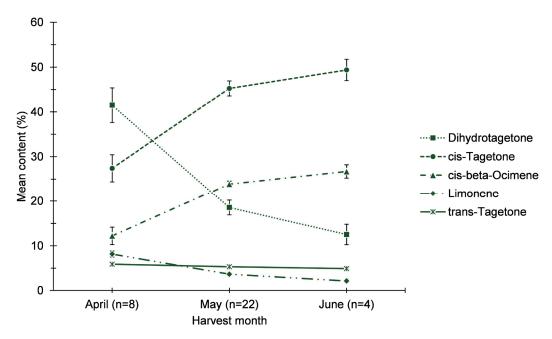


Figure 5. Seasonal variation of major compounds in the *Tagetes minuta* essential oil obtained by hydrodistillation of flowers and leaves harvested between April and June. Vertical bars over the points indicate the mean standard error. Cascata Experimental Station, Embrapa Temperate Climate, Pelotas, RS. Cascata Experimental Station (EEC), Embrapa Temperate Agriculture, Pelotas, RS. Brazil.

The composition of essential oils is affected by biotic and abiotic factors (Khare et al., 2020; Kumar et al., 2022a). Studies have shown that transplanting time (Kumar et al., 2012), plant pinching (Rathore et al., 2018), fertilisation (Walia; Kumar, 2021b), harvest stage (Kumar et al., 2020; Tiwari et al., 2016), post-harvest drying of plant material (Walia et al., 2020a), as well as the distillation method (Babu; Kaul, 2007; Rao et al., 2006) can all influence the chemical composition of *T. minuta*

essential oil. Furthermore, comparisons of essential oil compositions between studies must consider variations in the precision of analytical equipment and methodologies used for analysis and compound identification (Rubiolo et al., 2010; Sadgrove et al., 2022; Syafri et al., 2022).

Another important aspect to consider is the occurrence of chemotypes. In South Africa, Tankeu et al. (2013) identified two chemotypes for *T. minuta* essential oil, whereas in Argentina, Gil et al. (2000)

identified three distinct chemotypes. Chemotypes result from evolutionary adaptation processes in which diversification of biosynthetic pathways occurs (Sadgrove; Jones, 2015). Thus, within the same species, some populations may exhibit very distinct chemical profiles compared to others, including variations in the concentrations of major and minor compounds, as well as the presence or absence of specific compounds. Environmental and genetic factors can stimulate these changes and confer different biological properties to the essential oil (Benomari et al., 2023).

More than 95% of the essential oil composition of *T. minuta* produced at the EEC is composed of five compounds, which on average follow this descending order: cis-Tagetone > Dihydrotagetone > cis-beta-Ocimene > trans-Tagetone > Limonene. A similar result was reported by Lizarraga et al. (2017) in Argentina, analysing essential oil from wild populations in the province of Tucumán.

It is noteworthy that the proportion between the main compounds of the essential oil changes drastically with the phenological evolution of the plants. Between April and June, the Dihydrotagetone content was reduced by 3.3 times, and the cis-Tagetone and cis-beta-Ocimene contents were increased by 1.8 times and 2.2 times, respectively. Harvests carried out in April showed an approximate ratio of 2:1:3 between cis-Tagetone, cis-beta-Ocimene, and Dihydrotagetone. In contrast, the ratio shifted to 4:2:1 in June.

This shift in the ratio of major constituents is also reported in other studies (Chamorro et al., 2008; Kumar et al., 2020) and is linked to the composition of essential oils in different plant parts. According to Kumar et al. (2020), leaves are richer in Dihydrotagetone, while the flowers are richer in cis-Tagetone and cis-beta-Ocimene. Therefore, when harvesting occurs at the beginning of flowering, the Dihydrotagetone present in the leaves tends to predominate in the composition, whereas during full bloom, cis-Tagetone and cis-beta-Ocimene become predominant.

Several abiotic factors also influence the essential oil composition of *T. minuta* (Kumar et al., 2022a). Plants exposed to higher levels of water stress tend to produce more oxygenated monoterpenes (Babaei et al., 2021). Shading reduces the levels of cis-beta-Ocimene and Dihydrotagetone, while increasing those of Tagetones and Ocimenones,

with little effect on Limonene content (Kumar et al., 2014). Intercropping *T. minuta* with maize increased the levels of cis-beta-Ocimene, cis- and trans-Tagetone, and cis- and trans-Ocimenone compared to monocropping (Walia; Kumar, 2021a).

Regarding the effects of fertilisation on essential oil composition, studies show conflicting results. Omidbaigi et al. (2008) applied nitrogen doses between 50 and 200 kg ha⁻¹ and found an increase in trans-Tagetone, but no clear trend in Limonene, cis-Tagetone, or Dihydrotagetone levels. In *T. terniflora*, nitrogen fertilisation did not affect essential oil composition (Cruz et al., 2014). Conversely, Upadhyay et al. (2021) reported that increasing nitrogen doses reduced cis-Tagetone content, tended to increase the Dihydrotagetone and cis-beta-Ocimene, and had no effect on Limonene.

According to Pandey et al. (2015), fertilisation with different organic sources did not change the composition of *T. minuta* oil. In that study, supplementation with mineral fertiliser did not affect Dihydrotagetone levels, but increased the concentrations of Limonene, cis-beta-Ocimene, and cis-Tagetone.

In another study, mineral fertilisation with 120 kg ha⁻¹ of nitrogen and 60 kg ha⁻¹ of sulphur resulted in the highest levels of cis-beta-Ocimene compared to unfertilised control plants (Walia; Kumar, 2021b). It Was also found that the inoculation of rhizobacteria can increase the concentration of cis-beta-Ocimene, Limonene, and cis- and trans-Tagetone in the essential oil of *T. minuta* (Santoro et al., 2015).

Additionally, the extraction method can also affect the composition of essential oils. Due to differences in solubility, steam distillation tends to lose significant fractions of oxygenated monoterpenes to the hydrolate. In turn, hydrodistillation extracts higher concentrations of sesquiterpenes and phenolic compounds than steam distillation (Rao et al., 2006).

Understanding this dynamic is important for targeting the appropriate markets for the oil (Tankeu et al., 2013). In general, the five major constituents of the essential oil obtained at the EEC have applications in the flavor and fragrance industry (Breme et al., 2009; EPA, 2025; NCBI, 2025a). Some authors note that concentrations between 35% and 50% of cis-beta-Ocimene are more valued in international markets (Walia; Kumar, 2020, 2021c).

Studies on the use of essential oil as a botanical pesticide show that certain fractions of *T. minuta* oil

are biologically more effective than others. In a survey on aphids, Tomova et al. (2005) found that oxygenated monoterpenes and sesquiterpenes were more effective in reducing reproduction than monoterpenes. According to the authors, when comparing isolated

compounds, Caryophyllene had a greater effect than Limonene or Ocimene. Table 7 presents some of the biological effects already proven in scientific literature for the compounds present in the composition of *T. minuta* essential oil obtained at the EEC.

Table 7. Biological properties and uses of the compounds present in the composition of the essential oil of *T. minuta* obtained by hydrodistillation of flowers and leaves harvested at the Cascata Experimental Station (EEC).

Classes	Compounds	Biological properties and uses	References	
Hydro	Sabinene	Medicine: antifungal, anti-inflammatory Industry: fragrances, flavourings, fine chemicals.	Cao et al. (2018).	
	Limonene	Medicine: antimicrobial, antiviral, antioxidant, anti-inflammatory, antitumor, antidepressant, neuroprotective, anthelmintic. Agriculture: pesticide, herbicide. Industry: fragrances, flavourings, cosmetic products, food manufacturing.	Wang et al. (2024); Lin et al. (2024); Rutnik et al. (2022); Sharmeen et al. (2021); Pavela (2016); Gupta et al. (2023); NCBI (2025b).	
	cis-beta-Ocimene	Public health: vector insect larvicide. Agriculture: attractive to pollinators, nematicidal. Industry: fragrances, flavourings.	Farré-Armengol et al. (2017); Govindarajan; Benelli (2016b); Adekunle et al. (2007); NCBI (2025c).	
	Caryophyllene	Medicine: anti-inflammatory, antimicrobial, antioxidant, analgesic, anticonvulsant, anti-anxiety, antidepressant, anticancer, gastroprotective, neuroprotective, cardioprotective. Agriculture: pesticide, insect attractant. Industry: fragrances, flavourings.	Rutnik et al. (2022); Janadri et al. (2025); Bahi et al. (2014); Tomova et al. (2005); Lee; Ko (2021); Duarte et al. (2024); NCBI (2025d).	
	Medicine: anticancer, anti-inflammatory, anti-allergic, Humulene antidepressant, anti-anxiety, antioxidant. Industry: fragrances, flavourings.		Rutnik et al. (2022); Duarte et al. (2024); Ben Miri (2025); NCBI (2025e).	
	Bicyclo germacrene	Public health: vector insect larvicide.	Govindarajan; Benelli (2016a).	
Alcohols	Linalool	Medicine: anti-inflammatory, antioxidant, antimicrobial, anti-anxiety, neuroprotective, anticancer, sedative, analgesic, anticonvulsant. Agriculture: attractive to pollinators, pesticide, repellent, fumigant. Industry: fragrances, flavourings, cosmetic products.	Rutnik et al. (2022); Aprotosoaie et al. (2014); Sharmeen et al. (2021); Kamatou; Viljoen (2008); Campos et al. (2019); Ben Miri (2025); NCBI (2025f).	
	Spathulenol	Medicine: anaesthetic, vasodilator agent. Agriculture: pesticide.	Benelli et al. (2020); El- Solimany et al. (2024); NCBI (2025g).	
Oxides	cis-beta-Ocimene epoxide	Industry: fragrances, flavourings.	Agrebi et al. (2012).	
	Caryophyllene oxide	Medicine: anti-inflammatory, anticancer, analgesic, antioxidant, chemopreventive. Agriculture: pesticide. Industry: fragrances, flavourings.	Rutnik et al. (2022); Duarte et al. (2024); Di Sotto et al. (2020); El-Solimany et al. (2024); Singh et al. (2014); Tung et al. (2008); NCBI (2025h).	

Table 7. Continued.

Classes	Compounds	Biological properties and uses	References	
Ketones	Dihydrotagetone	Agriculture: nematicidal.	Adekunle et al. (2007); Joshi et al. (2005); Cruz Flores et al. (2021); NCBI (2025i).	
	trans-Tagetone	Medicine: antifungal. Industry: fragrances.	Joshi et al. (2005); Oliveira et al. (2019); Cruz Flores et al. (2021).	
	cis-Tagetone	Industry: fragrances.	Joshi et al. (2005); Cruz Flores et al. (2021).	
	Elsholtzione	Agriculture: fumigant, pesticide.	Li et al. (2024).	

Despite the individual properties of each compound, there is substantial evidence that the biological effects of the whole mixture of essential oil are more significant than the effects of the isolated compounds, whether in medicinal or agricultural applications. This is due to synergistic interactions among major, minor, and even those considered inert constituents (Bunse et al., 2022; Chen et al., 2021; Jiang et al., 2009; Ntalli et al., 2010). Often, the compounds of the essential oil fulfil different roles within a product. For example, while one compound may exert a biocidal effect, another may enhance its cuticular penetration, thereby amplifying its efficacy (Tak; Isman, 2017).

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Final remarks

Brazil has not been producing essential oil from *T. minuta* on a large commercial scale, as stated over 35 years ago by Craveiro et al. (1988). This incorrect statement has recently been clarified (Schiedeck, 2024). However, the research conducted at the Cascata Experimental Station (EEC) demonstrates that the cultivation of this species has a productive potential equivalent to or even greater than that observed in countries with more established expertise.

In India, cultivation practices such as fertilisation, irrigation, and pinching have been recommended to enhance the performance of the species and, consequently, improve the economic outcome (Singh et al., 2003). None of these practices has yet been tested at the EEC, suggesting significant potential for further development. Nevertheless, India and Argentina currently maintain a technological advantage over Brazil by already having genotype selection programs, which focus on higher

productivity and identified chemotypes. In Brazil, seed use is still based on spontaneous populations, which exhibit morphological variability and lack standardised chemical profiles.

Despite its limited presence in the global market, *T. minuta* essential oil is gaining increasing attention, driven by research highlighting its potential in various industrial segments. In this context, the findings from the work carried out at the EEC provide a valuable contribution to the diversification of the essential oil production chain, pointing toward emerging market opportunities and future demands.

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