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Morpho anatomical characterization and essential oils of *Tagetes terniflora* and *Tagetes minuta* (Asteraceae) growing in Tucumán (Argentina)

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Summary: The essential oil composition and morpho-anatomy of *Tagetes minuta*, collected at "El Mollar" and two different populations of *Tagetes terniflora* collected in Tafí del Valle (Tucumán, Argentina) were studied. The essential oil of aerial parts (flowers and leaves) from both species was analyzed by gas chromatography-mass spectrometry. Qualitative and quantitative differences were observed in the composition of the essential oil from both species. Oxygenated monoterpenes were the dominant compounds in the essential oil from both collections of *T. terniflora* where the main component was *cis*-tagetone (33.6% and 58.4% respectively) accompanied by significant amounts of the monotepene hydrocarbon *cis*-*β*-ocimene (17.1% and 17.4% respectively). *cis*-Tagetone (53.2%) was also a dominant component in *T. minuta* along with dihydrotagetone (10.4%) and *cis*-*β*-ocimene (19.9%). *cis*- and *trans*-ocimenone were distinctive components of *T. terniflora* which were not detected in *T. minuta*. Anatomically stands out the presence of secretory cavities and glandular trichomes in foliar blades and secretory ducts in stems and petiole of both species.

Key words: *Tagetes terniflora*, *Tagetes minuta*, essential oil, secretory tissues, morpho-anatomy.

Resumen: Caracterización morfo-anatomía y aceites esenciales de *Tagetes terniflora* y *Tagetes minuta* (Asteraceae) de Tucumán (Argentina). Se estudió la composición del aceite esencial y morfo-anatomía de *Tagetes minuta* coleccionada en "El Mollar" y de dos poblaciones diferentes de *Tagetes terniflora* de Tafí del Valle (Tucumán, Argentina). El aceite esencial de las partes aéreas (flores y hojas) de ambas especies se analizó por cromatografía gaseosa-espectrometría de masa. Se observaron diferencias cuali- y cuantitativas en la composición del aceite esencial de ambas especies. En las dos colecciones de *T. terniflora* los compuestos dominantes del aceite esencial fueron monoterpenos oxigenados donde el componente principal fue la *cis*-tagetona (33.6% y 58.4% respectivamente) acompañado por cantidades significativas del hidrocarburo monoterpénico *cis*-*β*-ocimeno (17.1% y 17.4% respectivamente). La *cis*-tagetona (53.2%) fue también el componente dominante en *T. minuta* junto con dihidrotagetona (10.4%) y *cis*-*β*-ocimeno (19.9%). La *cis*- y *trans*-ocimenona fueron componentes característicos de *T. terniflora* que no fueron detectados en *T. minuta*. Anatómicamente se destaca la presencia de cavidades secretoras y tricomas glandulares secretores en los bordes foliares y conductos secretores en tallos y pecíolo de ambas especies.

Palabras clave: *Tagetes terniflora*, *Tagetes minuta*, aceite esencial, tejidos secretores, morfo-anatomía.

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Introduction

Tagetes L. (Asteraceae, tribe Heliantheae) is a genus of about 43 species originating in Central and South America (Kaplan, 1958). Their natural range extends from the south of the United States and México to Argentinean Patagonia. The genus comprises ornamental, aromatic herbs and shrubs popularly known as marigolds. In Argentina is represented by 23 species, among which, *Tagetes terniflora* Khunt "wild tagetes" is native from Ecuador to the north of Argentina, and introduced in South Africa, Australia and the South of Europe; and *T. minuta* L. "chinchilla", "mexican marigold" is distributed from the South of the United States to the Argentinean Patagonia, and introduced in Europe, Asia, Africa, Madagascar, India, Australia, Japan, Croatia and Hawaii (Soulé, 1993; Flora Argentina, 2016).

Several species of *Tagetes* are extensively used as condiment, ornamentals, medicinals, and ritual plants (Soulé, 1996; Barboza *et al*., 2009), being *T. minuta*, *T. erecta* and *T. patula* the most studied (Vasudevan *et al*., 1997).

T. terniflora essential oil showed bactericidal (Tereschuk *et al*., 2003) and insecticidal activities (Stefanazzi *et al*., 2006), while *T. minuta* is an economically important worldwide resource due to the agrochemical, phytotoxic, fungicidal, insecticidal and pharmacological properties of its essential oil (Maradufu *et al*., 1978; Perich *et al*., 1995; Macedo *et al*., 1997; Bii *et al*., 2000; López *et al*., 2009; Karimian *et al*., 2014; Shirazi *et al*., 2014). The industry produces "tagetes oil" which is marketed for its various medicinal properties, health benefits and uses (Martijema *et al*., 1998; Sadia *et al*., 2013). *T. minuta* and *T. teniflora* essential oils are rich in monoterpenes, sequiterpenes, flavonoids, thiophenes and aromatics compounds. The major components reported for *T. minuta* were *cis*-tagetone, *trans*-tagetone, *cis*-*β*-ocimene, dihydrotagetone, *cis*-ocimenone, *trans*-ocimenone and the sesquiterpene alcohol spathulenol (López *et al*., 2009; Karimian *et al*., 2014.) while *cis* and *trans*-tagetone were the main components of *T. terniflora* (Stefanazzi *et al*., 2006). Studies related to differences in oil composition and activity among species, different populations of a same species, throughout the life cycle, and even between different organs in the same species have been reported (Zygadlo *et al*., 1990, 1993; Bii *et al*., 2000; López *et al*., 2009).

Tagetes are characterized by the presence of oil glands (= secretory cavities), ducts and secretory cells that contain volatiles constituents (Metcalfe & Chalk, 1950; Lawrence, 1985; Simon *et al*., 2002). These secretory structures have been analyzed in *T. minuta* (Del Fueyo, 1986; Simon *et al*., 2002; López *et al*., 2009), but they are not described for *T. terniflora.*

In the province of Tucumán *T. minuta* and *T. terniflora* overlap in their distribution range being often misidentified by local villagers. *Tagetes minuta* is easily distinguishable from *T. terniflora* by capitula features (Fig. 1 A-B) (Cabrera, 1978; Flora Argentina, 2016), however, the identification of both species becomes difficult in vegetative state. Thus, the aim of this paper is to characterize the essential oil composition and vegetative organs morpho-anatomy of both *Tagetes* species, collected in different places of Tucumán province.

Materials and Methods

Sample collection

Aerial parts of *T. minuta* were collected at "El Mollar" Prov. Tucumán, Dpto. Tafí del Valle, 26º 55' S 65º 41' O (1906 masl); and *T. terniflora* from two population, from Tafi del Valle, Dpto. Tafí del Valle, Prov. Tucumán, Argentina, Route 307, km 43 and km 77 (1988 masl and ca. 2700 masl). Each collection was named as **Tm** and **Tt** (**CI** and **CII** respectively). Voucher specimens of each collection were deposited at the Herbarium of Fundación Miguel Lillo (LIL).

Extraction of essential oil

Fresh aerial parts of vegetal material (100g) of each collection were hydrodistilled in a Clevenger's type apparatus for 2 hours in accordance with European Pharmacopeia's procedure (Clevenger European Pharmacopeia, 1983). The essential oils obtained were dried over anhydrous sodium sulfate to remove traces of moisture, flushed with argon and stored in a refrigerator in the dark at 4°C in a well-sealed bottle until analysis. All samples were analyzed within 72 hs of obtaining them. The essential oils of both *T. minuta* and *T. terniflora* deteriorate rapidly (oxidation, polymerization) in contact with air and light.

Fig. 1. General Aspect. A: *Tagetes minuta*. B: *Tagetes terniflora*.

Analysis

The chemical analysis was undertaken by gas chromatography-mass spectroscopy (GC-MS) techniques using a Hewlett Packard 6890 gas chromatograph coupled to a Hewlett-Packard 5973 quadrupole mass spectrometer equipped with a Perkin-Elmer Elite-5MS capillary column (5% phenyl methyl siloxane, length= 30 m, inner diameter= 0.25 mm, film thickness= 0.25 mm) and a selective mass detector was used for GC-MS detection; an electron ionization system with ionization energy of 70 eV was used. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. Injector and ion source temperature were set at 250 and 300 ºC respectively. Injection volume was 1 mL with a split ratio 80:1 and the oven temperature was programmed as follows: 60 ºC (5 min), 60-180 ºC (3.0 ºC/min), 180 ºC (10min), 180-250 ºC (7 °C/min), 250 ºC (3 min).

Identification of components

The components percentage reported in Table 1 was taken from capillary GC traces with FID (Flame Ionization Detector) using an integrator HP 3395 without FID response factor correction. The identification of the individual components was based on (a) computer matching with commercial mass spectra libraries (NBS75K, NIST 98, WILEY275) and published data (Adams, 2007); (b) comparison with spectra available in our files; (c) comparison of their GC arithmetic index (AI) on a HP-5 column. The arithmetic indices were calculated using a homologous series of n-alkanes C_8-C_{18} (Adams, 2007).

Microscopy

For histological preparations samples were fixed in FAA (formalin, ethanol, acetic acid, water, 100:500:50:350 mL). Sections of 3-4 mm, of the midregion of leaves (petiole and apical segment of fully expanded leaves), roots (first lateral root, next to the soil surface) and stems (third internode from the apex) were placed between two plates of dental wax and sectioned. Sections 15-20 µm were obtained with a rotation microtome MICROM HM 315, cleared with sodium hypochlorite (50% commercial bleach) and washed with distilled water. They were then stained with astra blue-safranin and mounted in 50% glycerol (Zarlavsky, 2014).

Whole foliar segments (third pair of leaves from the apex) and stems (third-fourth internode from the apex) were cleared according to Dizeo de Strittmatter (1973) and stained with cresyl violet 1% in distilled water. Leaf architecture was described using the terminology proposed by

Table 1. Chemical composition of the essential oils from *Tagetes minuta* and *Tagetes terniflora* collected in Tucumán.

Compound	Tm %	Tt		AI	AI_{calc}	Identification
		CI %	CII %			
Ethyl 2-methyl-butanoate	0.1	0.1	0.1	840	842	MS, AI
Ethyl 3-methyl-butanoate	-	0.4	0.3	849	845	MS, AI
2-Methylbutyl acetate	trace	-	-	871	868	MS, AI
Sabinene	0.2	-	trace	966	963	MS, AI, Co-GC
Myrcene	0.1	-	-	988	985	MS, AI, Co-GC
Octanal	0.1	-	-	998	996	MS, AI
Hexyl acetate	-	-	0.1	1007	1003	MS, AI
p-Cymene	0.1	-	-	1020	1016	MS, AI, Co-GC
Limonene	2.4	-	-	1024	1022	MS, AI, Co-GC
cis-*β*-Ocimene	19.9	17.1	17.4	1032	1034	MS, AI
trans-*β*-Ocimene	0.2	0.1	0.1	1044	1041	MS, AI
Dihydrotagetone	10.4	2.8	0.8	1046	1044	MS, AI
Unidentified oxygenated monoterpene I $C_{10}H_{16}O$	-	1.1	0.1	-	1072	MS
Unidentified oxygenated monoterpene II $C_{10}H_{16}O$	-	0.9	1.5	-	1085	MS
Allo-ocimene	0.2	0.2	-	1128	1122	MS, AI
cis-Epoxyocimene	trace	-	-	1128	1124	MS, AI
Dill ether stereoisomer	-	-	1.3	-	1127	MS
trans-Tagetone	3.0	17.0	2.2	1139	1143	MS, AI
cis-Tagetone	53.2	33.6	58.4	1148	1150	MS, AI
cis-Ocimenone (= *cis*-tagete- none)	-	8.0	0.1	1226	1221	MS, AI
trans-Ocimenone (= *trans*- tagetenone)	-	8.2	10.2	1235	1232	MS, AI
Thymol	-	0.4	-	1289	1288	MS, AI, Co-GC
p-Cymen-7-ol	-	-	0.3	1289	1271	MS, AI
β-Caryophyllene	0.7	0.4	-	1408	1396	MS, AI, Co-GC
α-Humulene	0.7	0.1	-	1452	1539	MS, AI
Germacrene-D	0.2	0.1	-	1484	1470	MS, AI
Bicyclogermacrene	0.5	-	-	1500	1496	MS, AI, Co-GC
Spathulenol	0.7	-	-	1577	1571	MS, AI, Co-GC
Caryophyllene oxide	trace	0.2	-	1582	1569	MS, AI
Total identified (%)	92.7	90.7	92.9			
Monoterpene hydrocarbons	23.1	17.4	17.5			
Oxygenated monoterpenes	66.6	72.0	74.9			
Sesquiterpene hydrocarbons	2.1	0.6	-			
Oxygenated sesquiterpenes	0.7	0.2	-			
Others	0.2	0.5	0.5			

Ref. Tm: *Tagetes minuta*; **Tt**: *Tagetes terniflora*; **CI**, **CII**: collection site I and II respectively; **AI** = arithmetic index reported in Adams (2007) for a HP-5 column. **AI_{calc}**: arithmetic index obtained in this work on the same column. **MS**: identification based on comparison of mass spectra with published data (Adams 2007), computer matching with WILEY 275 and NIST 3.0 (National Institute of Standards and Technology) libraries provided with the computer controlling the GC-MS system. **Co-GC**: co-injection with an authentic standard; trace were considered < 0.05%.

Hickey (1979) and Ellis *et al*. (2009). Stomata types were described according to Dilcher (1974). For light microscopy a Zeiss Axiolab optic microscope equipped with a Zeiss Axiocam ERc 5s digital camera and AxioVision Rel.4.3 acquisition software was used.

For scanning electron microscopy (SEM) samples were fixed in glutaraldehyde phosphate 5% buffered with 0.1 M sodium cocadylate at pH 7, and postfixed in 1.5% osmium tetroxide buffered with 0.1 M sodium cocadylate at pH 7.2. Leaf slices were dehydrated in a graded acetone solutions series, and coated with gold (Fine Coat Ion Sputter JEOL JFC-1100). Scanning electron microscopy (SEM) of gold coated samples was performed using a ZEISS SUPRA-55 VP field emission scanning electron microscope at Centro Integral de Microscopía Electrónica (CIME), CONICET-UNT.

Statistical analyses

Epidermal characteristics, stomatal sizes and stomatal densities were compared within and among species by analyses of variance (ANOVA) and differences among means were tested by the Tukey (InfoStat, 2002).

Results

Essential oil

The hydrodistillation of fresh aerial parts of each collection yielded yellowish oils, 0.91% for **Tm** and 2.4% and 2.8% for **Tt**, **CI** and **CII** respectively. For **Tm** 20 compounds were identified accounting for 92.7% of total, while for **Tt** 17 compounds were identified in **CI** and 14 compounds in **CII** constituting 90.7% and 92.9% of the oil respectively. The components identified in each collection and their relative percentages are listed in Table 1.

Tm was characterized by high percentages of oxigenated monoterpenes, *i.e.*, dihydrotagetone (10.4 %), *cis*- and *trans*-tagetone (53.2% and 3.0% resp.) accompanied by lower amounts of monoterpene hydrocarbons the most relevant being *cis*-*β*-ocimene (19.9%) and limonene (2.4 %). Only minor amounts of sesquiterpenoids (2.8%) were detected in the oil: *β*-caryophyllene (0.7%), *α*-humulene (0.7%), germacrene-D (0.2%), bicyclogermacrene (0.5%), spathulenol (0.7%) and caryophyllene oxide (trace); ocimenones (= tagetenones) were not detected (Table 1).

The most distinctive difference between the oils from **Tm** and **Tt** was the presence of ocimenones in the latter. Both collections of **Tt** showed a high percentage of oxigenated moneterpenes with tagetones (50.6% and 60.6% in **CI** and **CII** resp.) and ocimenones (16.2% and 10.3% in **CI** and **CII** resp.). *cis*-Tagetone was the dominant component in both collections (33.6% and 58.4% respectively). It is noteworthy the variation in the relationship of *cis*- and *trans*-tagetone between collections, *ca.* 2:1 for **CI** and *ca.* 26:1 for **CII**. A quantitative difference was also found for dihydrotagetone: 2.8% in **CI** and 0.8% in **CII**. *cis*-*β*-Ocimene was the main monoterpene hydrocarbon with almost the same percentage in both collections (17.1% and 17.4% resp.). Also significant differences were also observed in the content of sesquiterpenoids. Significant differences were also observed in the content of sesquiterpenoids. Thus, the essential oil of **CI** contains small amounts of *β*-caryophyllene, *α*-humulene, germacrene-D and caryophyllene oxide while none of these were detected in **CII** (see Table 1).

Foliar morphology architecture and anatomy

The two species of *Tagetes* studied in this work showed green yellowish, elliptic, pinnatisect noto-mesophyll leaves (4-20 cm long. x 3-8.5 cm lat. in *T. minuta* and 5-15 cm long. x 3-9 cm lat. in *T. terniflora*); constituted by 4-10 (2.5-8 cm long., 1-3 cm lat.) segments in *T. minuta* and 3-8 segments (1.2-0.50 cm long. x 0.2-1.2 cm lat.) in *T. terniflora*. Segments are opposite in the base to alternate in the apex, elliptic-lanceolate, with acute apex, lineal raquis and serrate-dentate margin, sometimes with a fully developed second order teeth (apical side straight, basal side convex to flexuous with angular sinus) (Fig. 2 A-B). Oil glands appeared macroscopically as translucent dots on the surface of the leaves. In *Tagetes minuta* conspicuous rounded-elliptic, pellucid glands (446.3 ± 32.7 μm long. x 153.3 ± 19.0 μm lat.), occur in the basal region of the teeth, and less frequently smaller rounded glands (124.7 ± 47.3 μm long. x 123.1 ± 24.5 μm lat.) occur along leaf blade in the proximity of the midvein, 10-15 per segment (Fig. 2 A) while in *T. terniflora* glands were slightly elliptic or rounded (210.7 ± 55.8 μm long. x 148.9 ± 22.9 μm lat.) distributed the largest ones in the basal region of the teeth and the smallest in the foliar lamina (Fig. 2 B). Short petiole with stipules surrounds the steam.

The venation pattern was similar in both species. Terminal leaf segment presented primary vein pinnate, massive with straight course. Major secondary veins were decurrent, alternated, eucamptodromous becoming reticulodromous distally, sometimes, with irregular spacing gradually increasing proximally, forming straight to acute angles with the main vein. In *T. minuta* they form an intramarginal sencondary vein. Minor secondary veins were semicraspedodromous terminating at the apex of the teeth or becoming reticulodromous. In both species intersecondary veins were rare, parallel to major secondaries with a ramifying distal course, some times basiflex. Intercostal and epimedial terciary veins were irregular reticulated, forming areoles sometimes with one or two freely ending veinlets, often with one or two branchs. In *T. terniflora* tertiary exterior veins form looped marginal ultimate venation and teeth vascularization (Fig. 2 B).

In frontal view *Tagetes* species showed amphistomatic leaves, striated cuticle (Fig. 3 E) and anomocytic stomata (Fig. 3 A-D). Stomata lengths were similar in both species (Table 2), significant differences were found only for the stomatal density (Table 2), been mayor for the adaxial suface in *T. minuta* and for the abaxial surface in *T. terniflora*.

Both species presented polygonal adaxial and abaxial epidermal cells, isodiametric with sinuous-undulating anticlinal walls (Fig. 3 A-D), uniseriate non glandular trichomes and uni to biseriate capitate glandular trichomes. Non glandular trichomes showed 5-8 cells with rounded hyaline apex (164.3 ± 54.8 μm long.), were located along principal veins and clustered in the sinus of some teeth on both epidermal surfaces (Fig. 3 F-G). Glandular trichomes present a stalk, with 3-6 pairs of cells distributed symmetrically or asymmetrically, and a head formed by an apical pair of secretory cells (108.1 ± 16.2 μm long.), they were found in the proximity of the main veins (Fig. 3 H).

Table 2 In transverse section, the midvein at the medium third part of the leaf blade had a uniseriate epidermis. Sub epidermically, two to three layers of angular collenchymas and a collateral vascular bundle immersed in a fundamental parenchyma (Fig. 4 A-B).

Secondary veins presented collateral vascular bundles with collenchymatous cap on the phloem side, surrounded by a parenchymatous sheath transcurrent to both epidermises. Minor order veins presented a one layer parenchymatous sheath (Fig. 4 C-D).

The apical segment of the lamina presented dorsiventral mesophyll, with one layer of palisade parenchyma and four to seven layers of compact spongy parenchyma. Along the blade margin next to the teeth basis on the proximal flank, large oil glands occupied the entire mesophyll (Fig. 4 F). The secretory cavity surrounded by a parenchymatic sheath showed a multilayered epithelium with a large light 170-300 μm partially fill with dense lipidic material (Fig. 4 E-F). Smaller oil gland distributed on the lamina (more frequents in *T. terniflora*), presented the same characteristics but light of the secretory cavity measured 66-150 μm and they only occupied part of the mesophyll, leaving one layer of palisade parenchyma between the adaxial epidermis and the gland sheath (Fig. 4 G).

In cross section the petioles of both species, were half-oval (near to the stem) to winged shaped (near to the blade) (Fig. 5 A-C). Trichomes were abundant in the adaxial surface with similar features to those previously described for the leaf. Sections showed epidermis uniseriate, one or two layers of angular collenchyma adjacent to the epidermis near the mid vein and margin. The wing mesophyll was dorsiventral formed by one layer of palisade parenchyma and four to seven layers of compact spongy parenchyma (Fig. 5 D). Seven to eight collateral vascular bundles arranged in a straight line or forming a semi circle open to the adaxial surface, were immersed in fundamental parenchyma, one large and central with scleremchymatous caps at phloem and xylem poles, two laterals half as large as the latter and two or four small marginal bundles which extend toward the wing. Three to four schizogenous ducts (23.4 ± 5.8 μm) with uni, rare incomplete biseriate ephitelium, were located in the ground parenchyma, abaxially between the vascular bundles at the phloem level (Fig. 5 E-F).

Stem anatomy

In both species caulinar axis were ramified, green, erect to decumbent. In face view, the epidermis presented anomocytic stomata (30.8 ± 4.0 long. x 20.3 ± 2,7 lat. μm) even or slightly raised regarding the other epidermal cells (Fig. 6 A), polygonal quadrangular

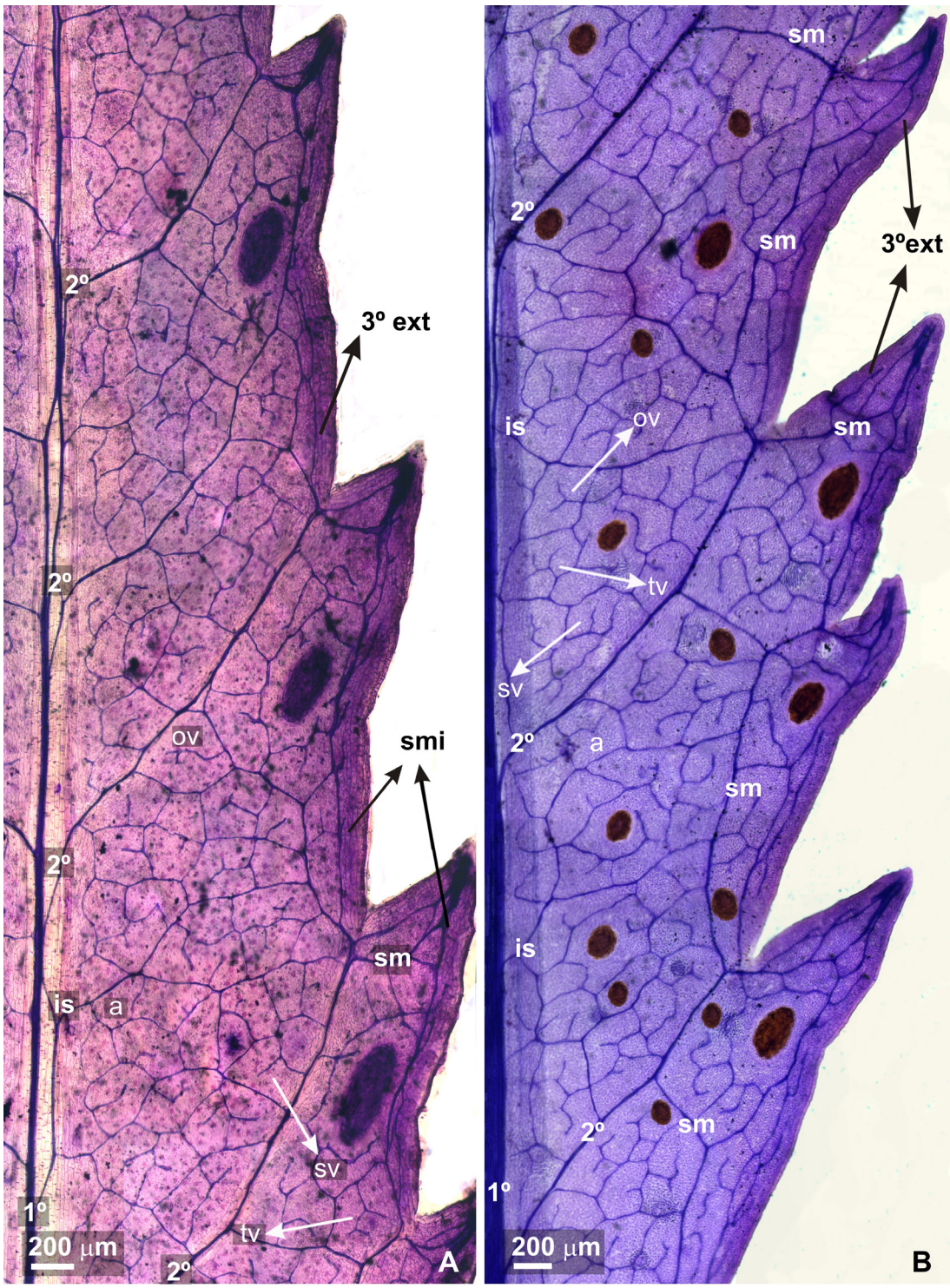

Fig. 2. Leaf architecture. A: *Tagetes minuta*. B: *Tagetes terniflora*. Abbreviations: 1º, primary vein; 2º, secondary vein; 3º ext, exterior tertiary; a, areole; is, intersecondary vein; sm, minor secondary; smi, intramarginal minor secondary; sv, simple veinlet; ov, one brunch veinlet; tv, two brunch veinlet.

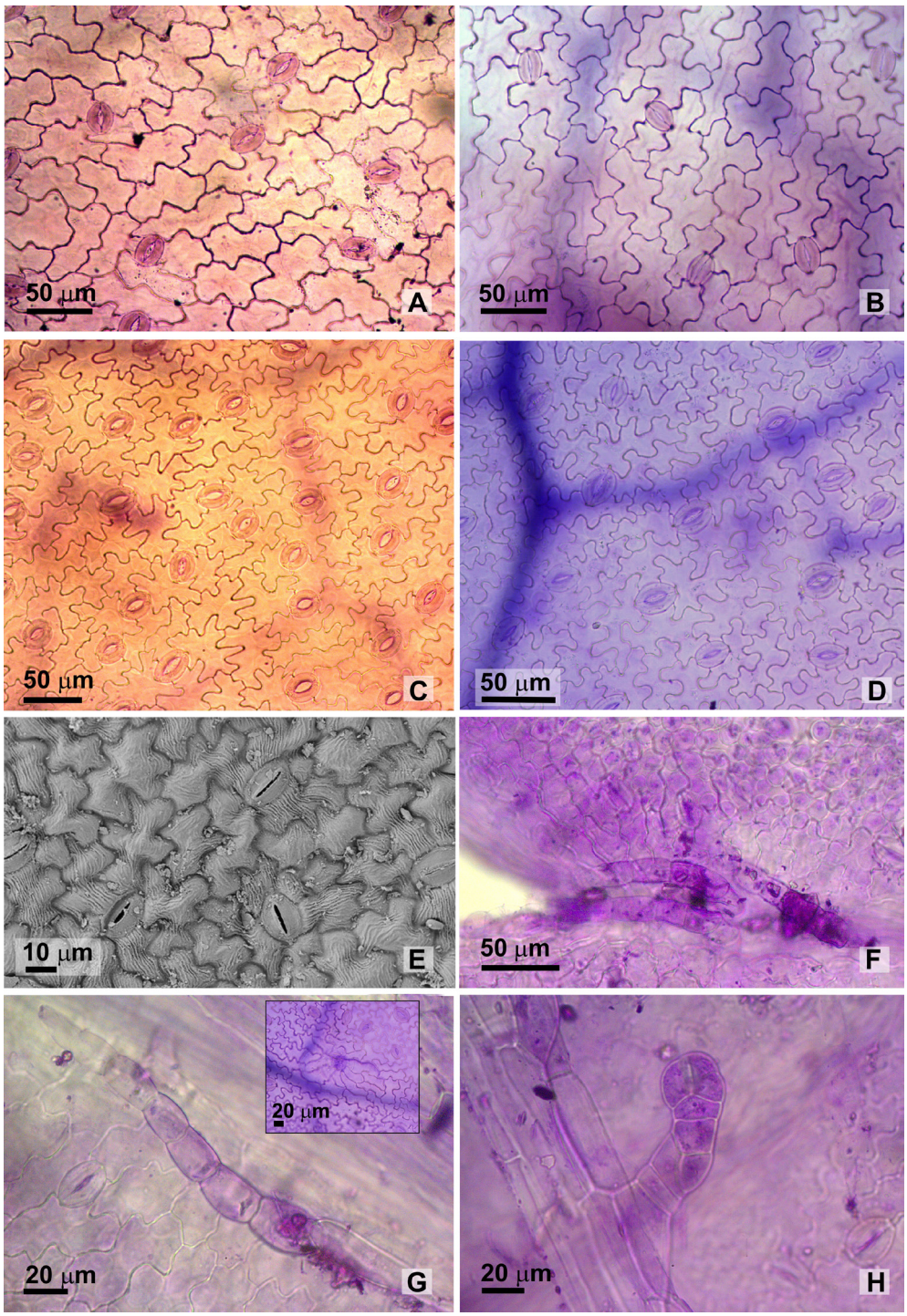

Fig. 3. Leaf paradermal view. A-B: Adaxial epidermis, *Tagetes minuta* and *T. terniflora* respectively. C-D: Abaxial epidermis, *T. minuta* and *T. terniflora* respectively. E: SEM showing abaxial striated cuticle on *T. minuta*. F. Tuft of non glandular trichomes at the tooth sinus. G. Uniseriate non glandular trichome. Detail of the insertion. H. Biseriate capitate glandular trichome.

Table 2. Stomatal characteristics in *Tagetes sp*.

	Stomatal	T. minuta	T. terniflora	
			CI	CII
Adaxial	Longitude (μm)	26.8 ± 2.0[a]	26.5 ± 2.0[a]	26.1 ± 2.0[a]
	Latitude (μm)	18.7 ± 1.1[a]	18.2 ± 2.0[a]	16.8 ± 2.0[a]
	Density	138 ± 10[a]	53 ± 12[b]	50 ± 10[b]
Abaxial	Longitude (μm)	27.0 ± 1.6[a]	27.9 ± 3.5[a]	28.0 ± 2.6[a]
	Latitude (μm)	20.8 ± 1.6[a]	20.2 ± 2.7[a]	21.0 ± 1.0[a]
	Density	225 ± 25[a]	339 ± 72[b]	353 ± 69[b]

Ref. Density was measure as number of stomata x mm^{-1}; within series, means (± SE) different letters indicate significant differences (Tuckey $p < 0.05$)

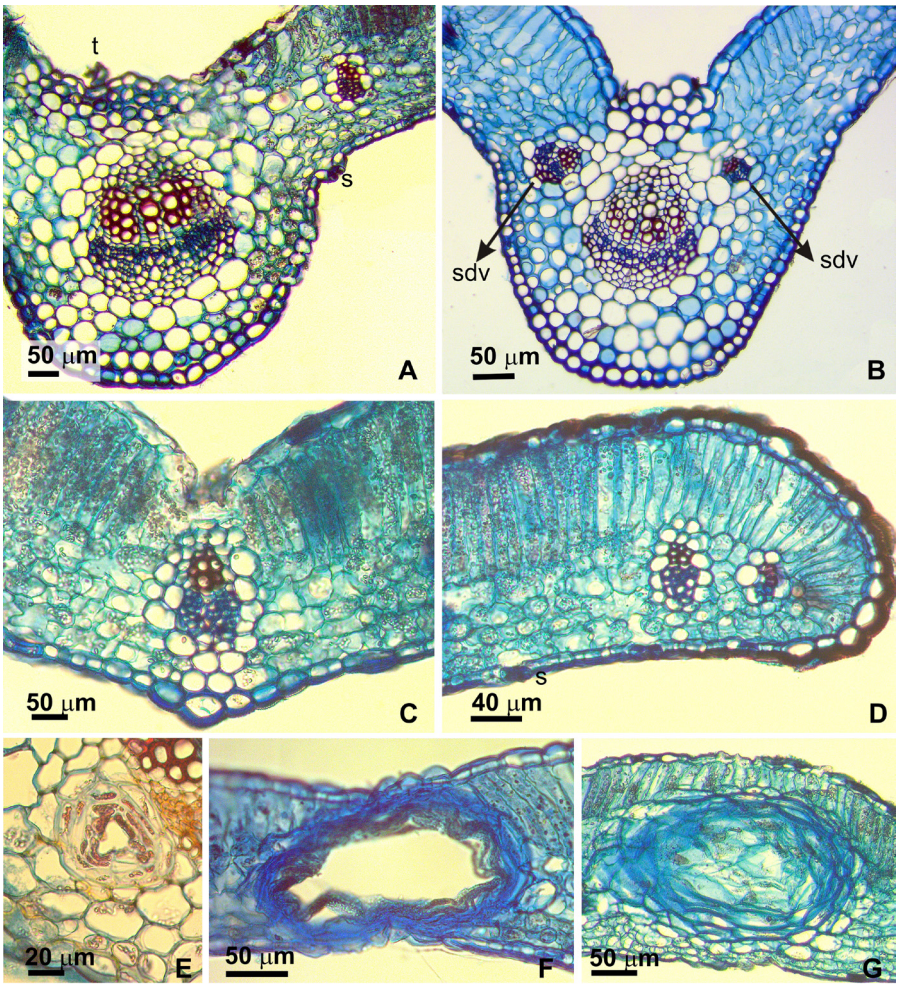

Fig. 4. Leaf transection. A-B: Mid vein, *Tagetes minuta* and *T. terniflora* respectively. **C:** Secondary veins, vascular bundle with collenchymatous cap on the phloem and surrounded by a parenchymatous sheath. D. Marginal minor order vein with parenchymatous sheath. E. Secretory cavity with multilayered epithelium surrounded by a parenchymatous sheath. F. Marginal large oil gland, occupied the entire mesophyll G. Smaller oil gland distributed on the lamina, occupied only part of the mesophyll leaving one layer of palisade parenchyma between the adaxial epidermis and the gland sheath. Abbreviations: t, trichome; s, stomata; sdv; secondary decurrent vein.

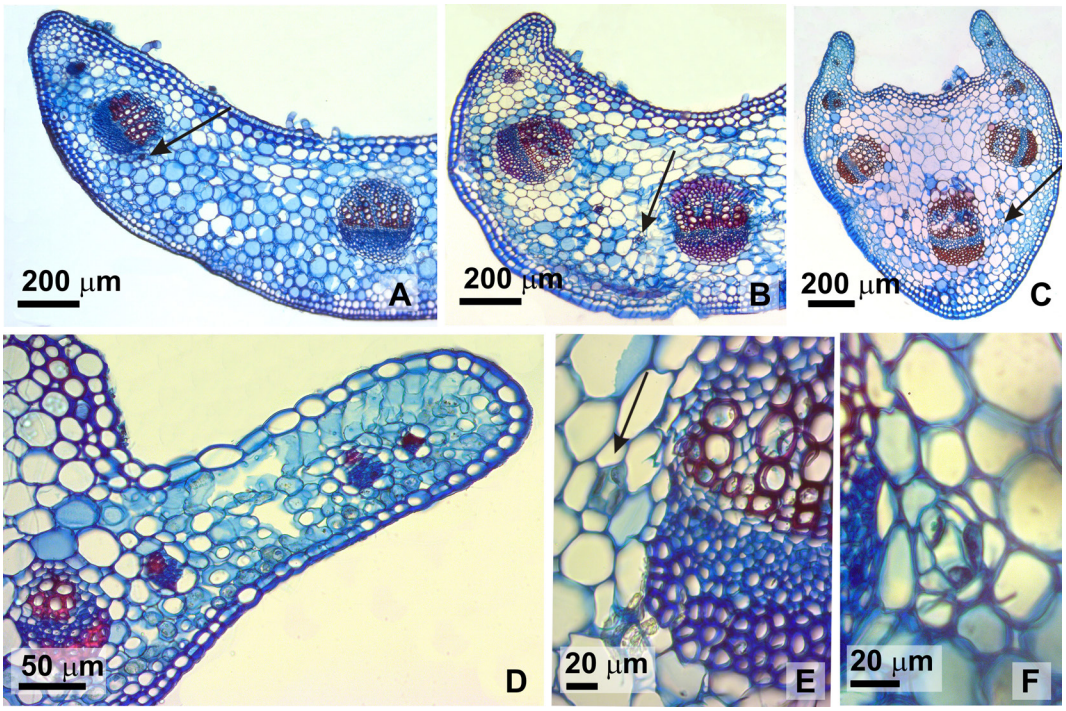

Fig. 5. Petiole transection. A: Half-oval shape near to the stem. B: Half-oval shape middle part of the organ. C: Winged shaped near to the blade. D: Dorsiventral wing. E: Schizogenous duct with uniseriate ephitelium. F: Schizogenous duct with incomplete biseriate ephitelium. Arrows indicate schizogenous ducts.

cells with thick straight anticlinal cell walls, coated by a thick cuticle. Pluricellular uniseriate non-glandular trichomes (Fig. 6 B) and biseriate (rare uniseriate) glandular trichomes (Fig. 6 C), sparse, similar to the trichomes previously observed at the leaf.

Transections were quadrangular with evident ribs in *T. minuta* (Fig. 6 D) to circular in *T. terniflora* (Fig. 6 E), revealing insipient secondary growth at the stem level analyzed. Epidermis uniseriate, 1-4 layers of sub epidermal angular collenchyma, reinforced at the ribs level in *T. minuta*, and 1-2 layers of discontinuous angular to laminar collenchyma in *T. terniflora*. Two to five layers of thin walled parenchymatic cortex. In the cortical parenchyma, alternating with the vascular bundles, secretory ducts (20 ± 5 μm), with uniseriate epithelium surrounded by a parenchymatous sheath, were found. Collateral open vascular bundles form a eustele, with perivascular fibres which may adjoin the bundles and isolate the phloem. The pith, which tends to be collapsed, was formed by thick walled parenchymatic cells, larger than cortex cells.

Root anatomy

Transection of the root at the level analyzed reveals secondary growth in both species. Uniseriate rizodermis, 3-4 layers of thin-walled cortical parenchyma, endodermis with evident casparian band, one layer pericycle, secondary phloem well developed, xylem with diffuse porosity showed abundant fibers. Secretory ducts in the cortex and the phloem were not observed (Fig. 7 A-B).

Discussion

Tagetes minuta for Tucumán province was characterized by high amounts of *cis*-tagetone. Collections of **Tm** from San Juan, Mendoza and Rio Negro showed dihydrotagetone as major constituent (Gil *et al*., 2000). Plants coming from Buenos Aires showed *cis*-tagetone, dihydrotagetone and *cis*-*β*-ocimene approximately 20% each; plants obtained from Jujuy showed significant amounts

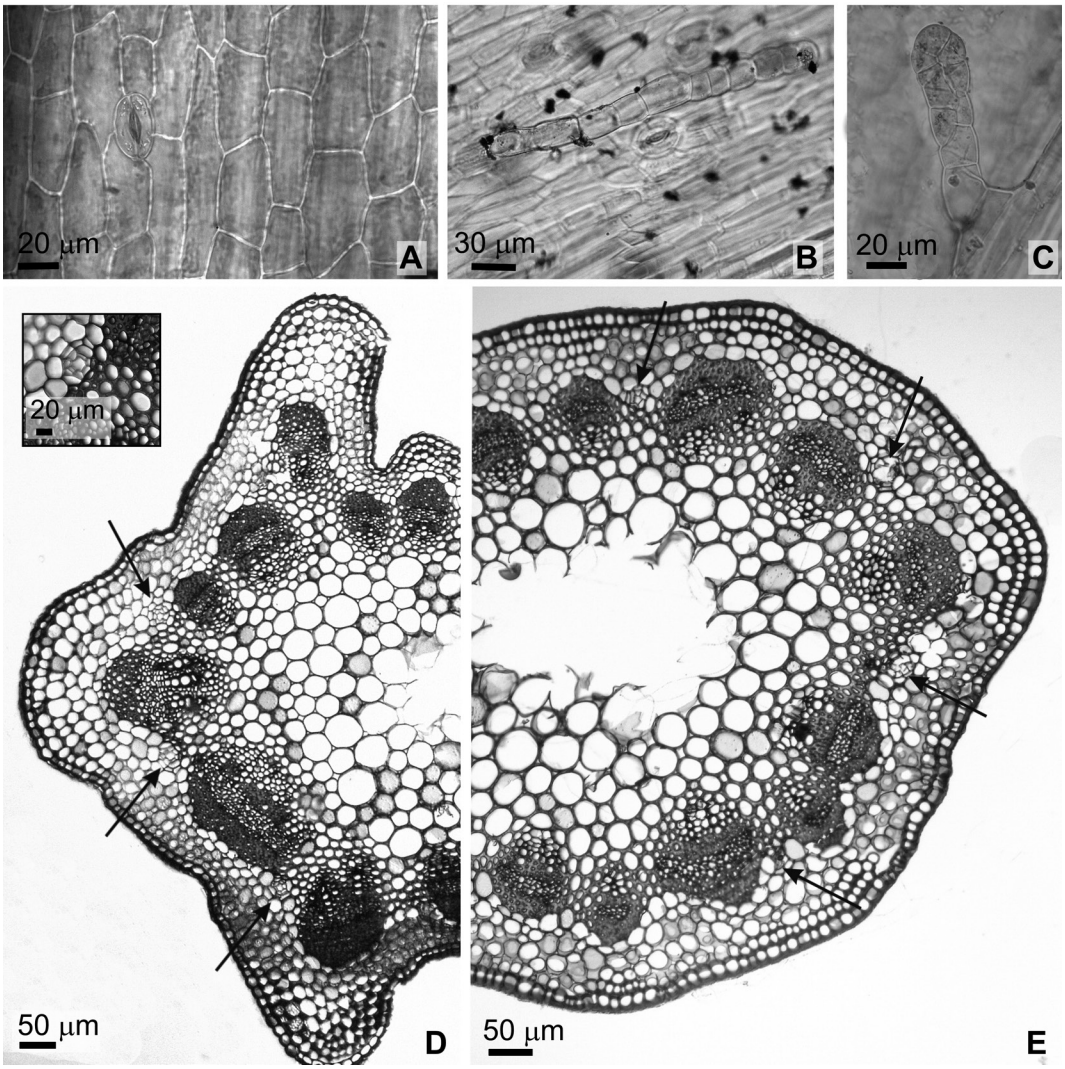

Fig. 6. Stem. A: Paradermal view, polygonal quadrangular epidermal cells with anomocytic stomata. B: Pluricellular uniseriate non-glandular trichome. C: Biseriate capitate glandular trichome. D: *Tagetes minuta* transection with quadrangular ribs. Detail of schizogenous duct. E: *Tagetes terniflora* circular transection. Arrows indicate schizogenous ducts.

of *cis*-*β*-ocimene (26%), *cis*-tagetone (19%), *cis*-tagetenone (17%) and *trans*-tagetenone (12%); and a collection from Salta showed high amounts of *trans*-*β*-ocimene (34%) and *α*-phellandrene (29%) (Gil *et al*., 2000; Vázquez *et al*., 2011). Essential oils of **Tm** collected in various localities of Cordoba province were dominated by *cis* and *trans*-ocimene, *cis* and *trans*-tagetenone, and *cis*- and *trans*-tagetone (Zygadlo *et al*., 1990) while collections from Chaco contain mostly dihydrotagetone accompanied by *β*-ocimene, tagetone and tagetenone (Chamorro *et al*., 2008).

Our two collections of **Tt** from Tucumán province contained high amounts of *cis*-tagetone, *cis*-*β*-ocimene and dihydrotagetone. Our results are only partly in agreement with data previously reported for collections from Argentina and Perú (Zygadlo *et al*., 1993; De Feo *et al*., 2005; López *et al*., 2011). These facts indicate the existence of a high chemical plasticity in *Tagetes* as demonstrated by Zygadlo *et al*. (1990) and Gil *et al*. (2000, 2002).

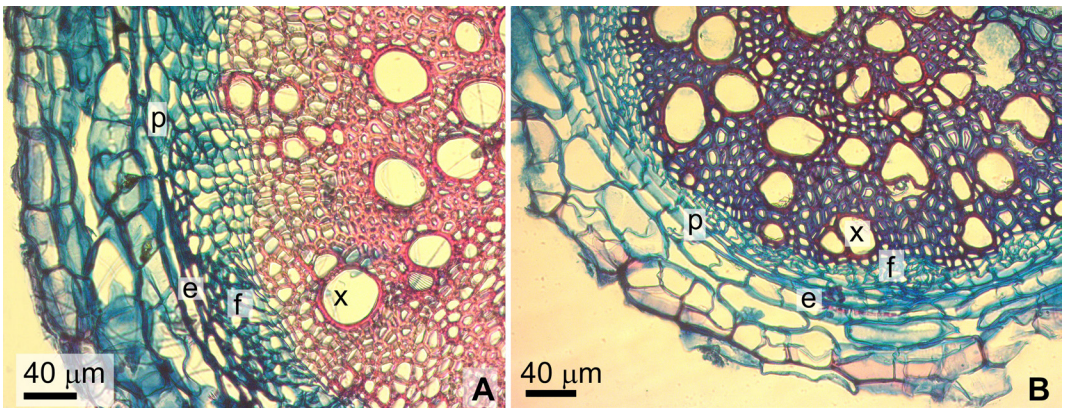

Fig. 7. Root transection at early secondary growth stage. A: *Tagetes minuta*. B: *Tagetes terniflora*. Abbreviations: e, endodermis, p, pericycle; f, phloem; x, xylem.

In general, different growth stages of the plant exhibit variations in their essential oil composition (Chamorro *et al*., 2008). Similarly chemotypes can be found as result of intraspecific variations when the essential oil composition of individual plants of the same population are compare, other differences can arise as a result of the plant response to environmental conditions i.e. growth locations (Zygadlo *et al.*, 1990; Gil *et al.*, 2000). Thus further studies with a larger number of individuals from a same population and from other *Tagetes* populations in different phenological stages are necessary in order to determinate quantitative, qualitative, inter and intraspecific variations in their essential oils composition.

Since the species analyzed in this study do not share the exact same essential oil profile becomes important their correct identification according to the purpose of collection.

Regarding the morpho anatomy, both species were similar. They only showed slight variations in their morphological and anatomical features, such as venation patterns, type and distribution of leaf glands and stem transverse section aspect.

This paper describes the vegetative anatomy of both species, coinciding in the presence of structures associated with the synthesis of essences with those described by Del Fueyo (1986), Simon *et al*. (2002) and Visintin and Bernardello (2005) for *T. minuta*; García-Sánchez *et al*. (2012) and Martínez *et al*. (2013) for *T. lucida*, *T. lunulata* and *T. micrantha*.

In contrast to the observations of Simon *et al.* (2002), no secretory canals were observed at root level, possibly due to the stage of development.

This work describes for the first time the morpho-anatomy of vegetative organs of *T. terniflora* and the venation pattern and presence of non glandular trichomes in the lamina of *T. minuta*.

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