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COMPARATIVE SEM ANALYSIS OF LEAF SURFACES IN TWO FORMS OF *TANACETUM BALSAMITA* L.

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Abstract: The leaf surfaces of two *Tanacetum balsamita* forms: *Chrysanthemum balsamita* f. *balsamita* and *C. balsamita* f. *tanacetoides* were examined by scanning electron microscopy (SEM) in order to identify similarities and differences. The two infraspecific taxa show differences in the morphology and structure of their inflorescences, chromosome number, and the chemical profiles of their essential oils synthesized and accumulated in the secretory structures of the epidermis. SEM images revealed that the two *C. balsamita* forms share similar epidermal structures in both non-glandular and glandular hairs, yet differ in the density of glandular secretory hairs between forms and between the adaxial and abaxial leaf surfaces. The highest mean density of glandular secretory hairs was observed in *Tanacetum balsamita* f. *balsamita,* on the abaxial leaf surface.

Key words: *Tanacetum balsamita,* costmary, SEM, infraspecific taxa

Introduction

Costmary, also known as alecost or bible leaf, is an aromatic perennial herb of the *Asteraceae* native to southwestern temperate Asia and widely introduced into Europe and North America [41]. Although listed as *Tanacetum balsamita* L., World Flora Online [41] alone cites at least 20 synonyms for this species, including *Chrysanthemum balsamita* Baill., *Balsamita major* Desf., *Balsamita vulgaris* Willd., *Pyrethrum majus* (Desf.) Tzvelev, *Pyrethrum tanacetum* DC., *Pyrethrum balsamita* Willd., etc., along with several varietal names (e.g., *B. major* var. *tanacetoides* (Boiss.) Moldenke, *C. balsamita* var. *tanacetoides* Boiss., *P. balsamita* var. *tanacetoides* Boiss, *Tanacetum balsamita* var. *balsamita*) and even subspecies (e.g., *Tanacetum balsamita* subsp. *balsamita*, *Balsamita major* subsp. *major*).

The Flora RPR [32] cites two forms: *C. balsamita* f. *balsamita*, characterized by capitula with yellow central tubular florets and white ray ligulate florets, and *C. balsamita* f. *balsamitellum* (syn. *C. balsamita* f. *tanacetoides* (Boiss.) B. Bovin), which has capitula lacking white ray florets, consisting only of yellow tubular florets. Flora Europaea [40] places costmary in the genus *Balsamita*, species *Balsamita major* Desf., with synonyms *Chrysanthemum balsamita* (L.) Baillon and *Pyrethrum majus* (Desf.) Tzelev, noting that the infraspecific taxa with white ray florets in the inflorescences is *more* common. Recent synthetic works on the flora of Romania [3, 7, 33] do not mention the two forms cited in Flora RPR [32], referring only to differences in chromosome number within the species.

In Romania, costmary is commonly grown in gardens and cemeteries as an aromatic, medicinal, and ritual plant [1, 2, 7, 26, 32]. Since 1987, the two forms of *T. balsamita* (f. *balsamita* and f. *tanacetoides*) have been cultivated at the Bod Station, owned by SC Nivea Brașov, and their extracts and essential oils have been used in the development of hygienic, cosmetic, and medicinal products, most of them patented and registered with the Ministry of Health, the most well-known being those from the *Tonorelaxin* range [27, 29]. In traditional Romanian medicine, as in other countries, costmary was used for wound healing, as an antihemorrhagic agent in uterine disorders, and in the treatment of hepatic and neurological conditions [1, 2, 9, 34].

Hiller and Melzig [17] reported two chemical types for Balsamitae herba: one containing essential oil with 72-83% camphor as the main component, and a carvone type containing 55-60% L-carvone as the main component of the essential oil. These two infraspecific taxa are also mentioned by Muntean et al. [26]. Previous studies have examined the histological structure of costmary leaves [27], the chromosome number of its two forms [36], the chemical composition of the species [12, 13-15, 21, 22, 28, 38], the antimicrobial and antifungal activities of its essential oils [35], and, in particular, the hepatoprotective effects of costmary extracts [5, 11, 29-31].

Tămaș et al. [37] identified two infraspecific chemical taxa of *Tanacetum balsamita* based on CSS and GC analyses of its essential oil. These two taxa correspond to the two morphological forms of the species. In essential oil isolated from *T. balsamita* f. *tanacetoides*, the main component is carvone (55-60%), whereas in *T. balsamita* f. *balsamita*, the main component is camphor (85-90%). Consequently, the two infraspecific chemical taxa were proposed as *T. balsamita* chemovar. carvone and *T. balsamita* chemovar. camphora, in accordance with infraspecific chemical nomenclature [38].

Regarding the karyotype of plants cultivated in Cluj-Napoca, it was previously shown that *T. balsamita* chemovar. camphora has $2n = 2x = 18$, while *T. balsamita* chemovar. carvone has $2n = 6x = 54$, the first being diploid and the second hexaploid [36].

Given the morphological differences in the anthodia of the two taxa, their different chromosome numbers, and the major differences in the main components of their essential oils, we set out to analyze their leaf surfaces, particularly the secretory structures, using scanning electron microscopy (SEM) to detect possible similarities and differences at this level. We examined the shape, size, and density of secretory hairs on both leaf epidermises.

Material and Methods

Fresh samples of leaves of *Tanacetum balsamita* forms were collected from plants cultivated in Cluj-Napoca. Three mature leaves were collected from each infraspecific taxa on the same day, from the middle section of the stem and with the same orientation (south-west), sampling three different stems from each clump. The voucher specimens were identified and deposited in the herbarium of "Alexandru Borza" Botanical Garden, Cluj-Napoca, under the numbers: 674532 for *C. balsamita* f. *tanacetoides* and 674533 for *C. balsamita* f. *balsamita*.

SEM analyses of the leaf surfaces were performed on both sides of the leaves of the two forms, in the median region of the leaf blade, on both sides of the midrib. The protocol for scanning electron microscopy (SEM) was previously described by Ciorîță et al. [4]. For SEM examination, leaves were prefixed immediately after dissection by immersion in 2.7% glutaraldehyde solution (GTA) in 0.1 M PBS (phosphate-buffered saline), pH 7.2. After one hour, the glutaraldehyde solution was removed and the samples were rinsed four times with 0.1 M PBS. The samples were then cut and adjusted depending on what part is examined and further dehydrated using increasing concentrations of acetone: 30%, 50%, 70% (30 min each step), 80%, 90%, 4X 100% (one hour each step). Subsequently, the samples were placed in hexamethyldisilazane:acetone solution (1:2, 1:1, 2:1, 1:0 for 1 h each). All steps were conducted at 4°C and the reagents were acquired from Sigma Aldrich (Merck, Bucharest, Romania). For examination, SEM HITACHI SU8230 (Hitachi, Tokyo, Japan) was used at an acceleration voltage of 30 kV after the samples were covered with a 9-nm- thick layer of gold, using the Quorum Q150T ES turbomolecular pumped coater (Quorum Technologies, London, UK).

Results and Discussions

From our observations on costmary cultivation in Cluj-Napoca, we found major differences between the two forms regarding flowering phenophases. In *T. balsamita* f. *balsamita*, flowering occurs in June-July, after which the flowering stems dry out and numerous leaves sprout from the rhizomes, forming compact tufts in August (Fig. 1 A, B). By contrast, in *T. balsamita* f. *tanacetoides*, flowering occurs only in September (Fig. 1 C).

The leaves of the two chemical taxa also differ in colour: those of *T. balsamita* f. *tanacetoides* (Fig. 2 A - left) are yellowish-green, whereas those of *T. balsamita* f. *balsamita* (Fig. 2 A - right) show a bluish-green shade. Leaf morphology also differs, with *T. balsamita* f. *balsamita* having more acutely pointed leaf tips, and, naturally, the leaves differ in scent due to the two main oil components (Fig. 2 C).

Both the adaxial (upper) and abaxial (lower) leaf surfaces of the two forms were examined by SEM, revealing T-shaped also known as „kompass needle" non-glandular hairs and glandular secretory hairs distributed across the epidermal surfaces. The T-shaped hairs possess equal, opposite arms, which are generally oriented parallel to those of adjacent glandular hairs (Fig. 3 A-D).

Regarding the glandular secretory hairs, these appear laterally compressed and exhibit a small depression corresponding to the two rows of secretory cells, being anchored in a slight invagination between epidermal cells in both forms (Fig. 4 A-F). When viewed from above, their maximum length and width reached 101.28 × 56.79 µm in *T. balsamita f. tanacetoides* (Fig. 4 A; Table 1) and 70.44 × 41.38 µm in *T. balsamita* f. *balsamita* (Fig. 4 D; Table 1).

Fig. 1: *T. balsamita* forms: A) f. *balsamita* at anthesis; **B)** f. *balsamita* post-anthesis; **C)** f. *tanacetoides* at anthesis.

Fig. 2: Morphological characteristics in leaves of *T. balsamita* forms: A) close-up image f. *tanacetoides* (left) vs. f. *balsamita* (right); **B)** f. *tanacetoides*; **C)** f. *balsamita*.

We further observed that the mean longitudinal axis of glandular secretory hairs was larger in *T. balsamita* f. *tanacetoides* compared to *T. balsamita* f. *balsamita* (96.63 vs. 67.51 µm) (Table 1).

Table 1: Height and width (µm) of glandular secretory hairs - surface view.

***Tanacetum balsamita* forms**	**Height (µm)**	**Mean**	**Width (µm)**	**Mean**
Tb. 1 (ad)	**101.28**/98.22/97.63/96.24/94.25/92.27	**96.63**	**56.79**/54.72/54.57/54.08/54.03/53.23	**54.3**
Tb. 2 (ad)	**70.44**/70.44/68.46/67.76/64.49/63.5	**67.51**	**41.38**/41.21/41.18/40.38/39.21/38.92	**40.79**

Tb. 1 = *Tanacetum balsamita* f. *tanacetoides*; Tb. 2 = *T. balsamita* f. *balsamita*; (ad) = adaxial epidermis.

We determined the mean density of glandular secretory hairs (Fig. 3 A-D) for both *T. balsamita* forms on the adaxial and abaxial epidermis, using surface areas of 1 mm² (Table 2).

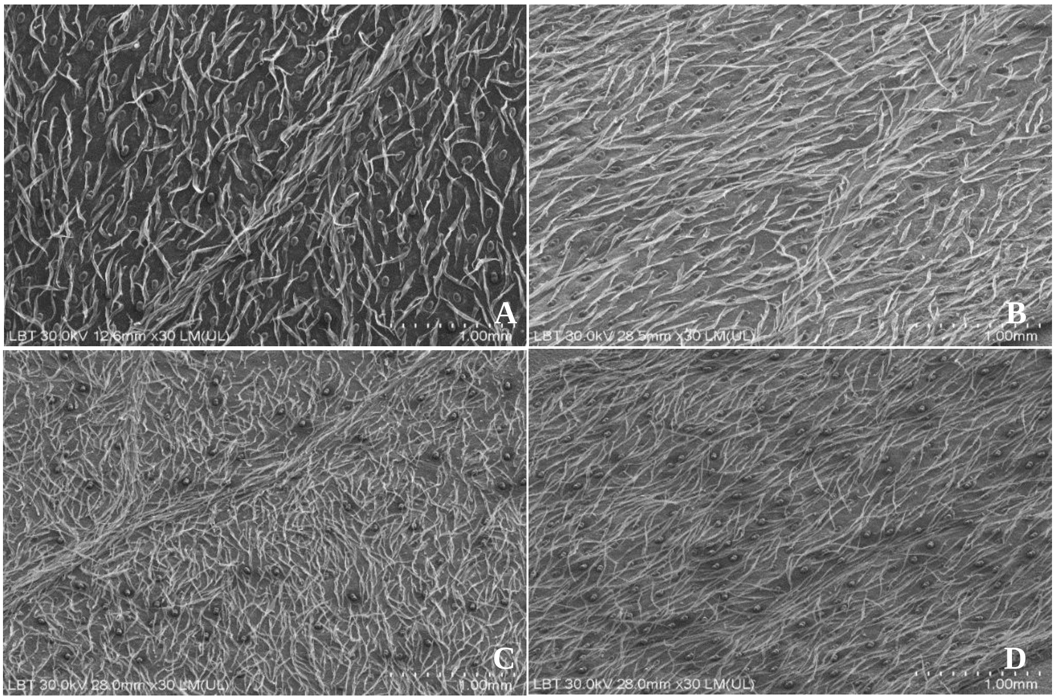

Fig. 3: SEM image of the leaves of *T. balsamita* forms: A) f. *tanacetoides* (adaxial epidermis); **B)** f. *tanacetoides* (abaxial epidermis); **C)** f. *balsamita* (adaxial epidermis); **D)** f. *balsamita* (abaxial epidermis).

Table 2: Glandular secretory hair density (per 1 mm² epidermis).

Tanacetum balsamita forms	**Number of secretory hairs**	**Average**
Tb. 1 (ad)	13 13 11 11 10 10 9	**11,00**
Tb. 1 (ab)	16 15 15 15 14 14 14	**14,71**
Tb. 2 (ad)	12 10 10 10 9 9 9	**9,85**
Tb. 2 (ab)	18 16 16 14 13 13 11	**15,28**

Tb. 1 = *Tanacetum balsamita* f. *tanacetoides*; Tb 2 = *T. balsamita* f. *balsamita*; (ad) = adaxial epidermis; (ab) = abaxial epidermis.

Analysis of glandular hair density showed that the highest values were recorded on the abaxial epidermis of *T. balsamita* f. *balsamita* leaves (average 15.28/mm²), which, however, also exhibited the lowest density on the adaxial epidermis (9.85/mm²) (Fig. 3 C, D). In *T. balsamita f. tanacetoides*, the mean density was 11.00/mm² on the adaxial epidermis and 14.71/mm² on the abaxial epidermis (Fig. 3 A, B).

Following SEM analysis of the leaf surface details in the two varieties, we found that, despite the clear differences in inflorescence morphology and structure, chromosome numbers [24, 26-31, 36], leaf blade coloration, phenophases, and the distinct chemical composition of the essential oils secreted by their leaves, the overall appearance of the leaf surfaces is very similar with respect to both T-shaped trichomes and glandular trichomes. The main difference lies in the density of the secretory structures, both between the two forms and between the adaxial and abaxial leaf surfaces.

In the context of comparative studies on micromorphological and secretory structures in Asteraceae and particularly in the genus *Tanacetum*, several relevant works provide a framework for interpreting our results, while also highlighting existing research gaps.

Dere and Akcin [8] investigated foliar secretory structures of diagnostic value for several Asteraceae species from northern Turkey. Their study emphasized the role of trichome morphology as a taxonomic character, but notably did not include *T. balsamita* among the analyzed taxa. Thus, while their results confirm the systematic importance of micromorphological traits in this family, they leave unaddressed the particular features of *T. balsamita*. Similarly, Kodak et al. [19] examined the leaf micromorphology of eight *Tanacetum* species distributed across Turkey, including two subspecies of *T. balsamita*. Their analysis highlighted the diagnostic value of the distribution of covering trichomes and stomata in differentiating species. However, their focus remained strictly on these epidermal traits, without investigating the secretory structures themselves. Consequently, their study provides important comparative morphological data but leaves open questions regarding the potential taxonomic and ecological roles of secretory tissues.

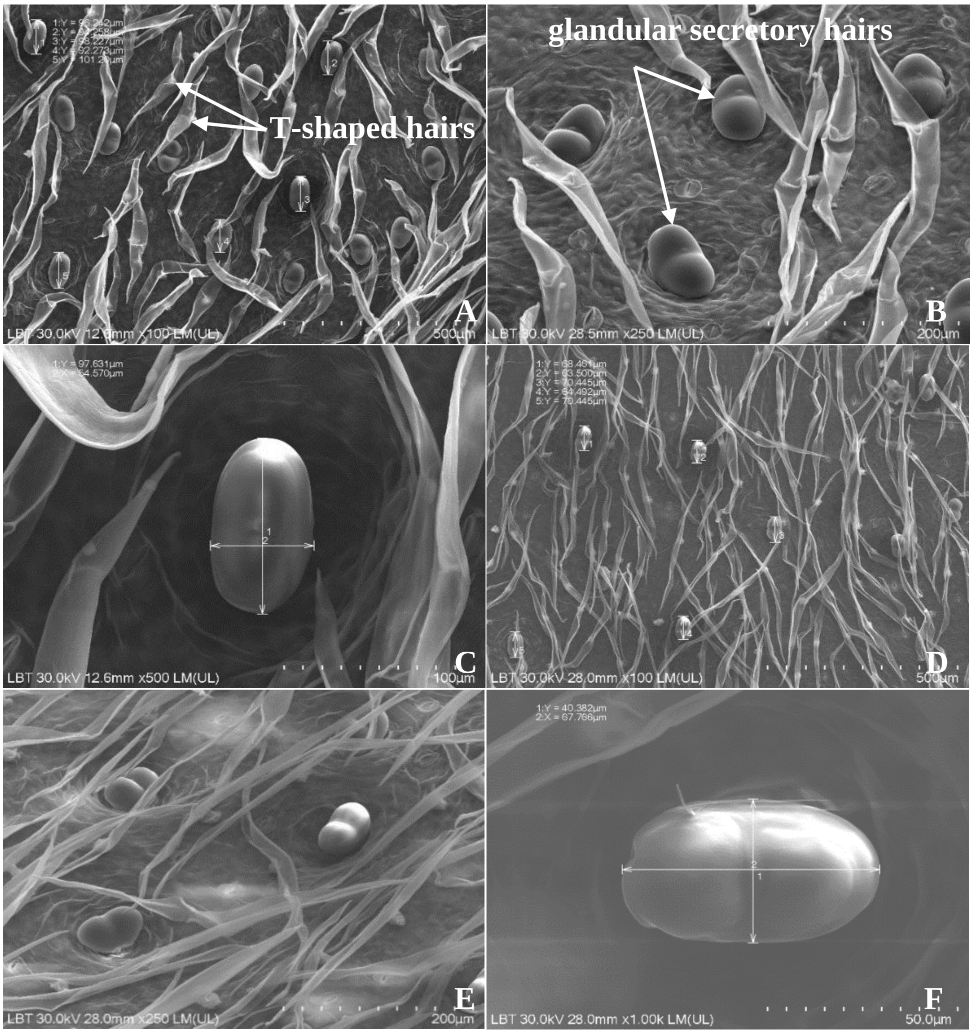

Fig. 4: SEM image of the leaves of *T. balsamita* forms showing the size of glandular secretory hairs: A-C) f. *tanacetoides*; **D-F)** f. *balsamita*.

A more integrative approach was presented by Giuliani et al. [10], who studied three *Tanacetum* species (*T. vulgare* L., *T. parthenium* (L.) Sch.Bip., and *T. corymbosum* (L.) Sch.Bip.) cultivated in the Botanical Garden of the University of Milan. Their work successfully linked the presence and density of secretory structures with the chemical composition of essential oils, thereby demonstrating the functional significance of micromorphological traits. Although this study did not include *T. balsamita*, it provides a methodological and conceptual parallel to our own approach, underscoring the relevance of coupling anatomical investigations with phytochemical analyses. The similarities between their integrative framework and our current research indicate that our work aligns with contemporary trends in systematic and functional plant anatomy. By contrast, Hoang et al. [18] examined ornamental *Tanacetum* species from Korea, emphasizing their morphological and karyological characteristics. Although this study contributes to the broader understanding of the tribe Anthemideae, it does not encompass *T. balsamita*. Finally, Moț and Madoşa [25] concentrated on the morphological characteristics of *T. balsamita* leaves in local populations from Banat. Their work is valuable for documenting intraspecific variation in Romanian populations but remains limited to external morphology.

Taken together, these studies underline that while micromorphological and chemical studies on *Tanacetum* taxa are accumulating, the secretory structures of *T. balsamita* were insufficiently characterized. Our study fills this gap by providing new micromorphological data on foliar secretory surfaces of Romanian populations of *T. balsamita*. To our knowledge, this is the first SEM analysis of leaf surfaces in these two infraspecific taxa.

Our studies further suggest that, as well as being separable by their capitula and chemistry, the two variants of *Tanacetum balsamita* L. differ in chromosome number, the leaf shape, colour and indumentum, and flowering times. The two are therefore probably best treated as two taxonomic varieties, as adopted by several previous authors across the different genera in which the species has been placed since it was named by Linnaeus 1753.

The infraspecific nomenclature has apparently been confused, but the two varieties are:

Florets all tubular, yellow. **var. *balsamita*** (syn. *T. balsamita* L. var. *tanacetoides* Boiss.; *T. balsamita* f. *tanacetoides* sensu auct. Rom.). A.G.C. Grierson, *Flora of Turkey*, 5, 264–268 (1975), who treats these two varieties at subspecific rank, notes that the Linnean type specimen lacks ligules and was of garden origin; also that the capitula are generally more numerous and slightly smaller.

Outer florets ligulate, the ligules white; inner florets tubular, yellow. **var. *balsamitoides*** (Sch. Bip.) P.D. Sell, *Flora of Great Britain and Ireland*, 4, 556 (2006) (syn. T. *balsamitoides* Sch. Bip., *T. balsamita* L. subsp. *balsamitoides* (Sch. Bip.) Grierson; *T. balsamita* f. *balsamita* sensu auct. Rom.). This is the widespread variant in cultivation, presumably favoured for its more showy flowers.

Conclusions

This study provides the first SEM images of the leaf surfaces of two forms of costmary *T. balsamita* f. *balsmita* and *T. balsamita* f. *tanacetoides* widely cultivated in Romania as aromatic, medicinal, and ritual species.

The leaf surfaces of the two forms, as examined by SEM, are very similar, differing only in the density and size of glandular secretory hairs.

The highest mean density of glandular secretory hairs was recorded in *T. balsamita* f. *balsamita* on the abaxial epidermis (15.28/mm²), which, however, also showed the lowest mean density on the adaxial epidermis (9.85/mm²).

The glandular secretory hairs are larger in *T. balsamita* f. *tanacetoides* (96.63/54.30 µm) compared with *T. balsamita* f. *balsamita* (67.51/40.79 µm).

The results presented here contribute to the morphological characterization of costmary, providing further details on the differences between its two forms, which are distinct enough to deserve varietal status.

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STUDII SEM COMPARATIVE ALE SUPRAFEȚELOR FOLIARE LA DOUĂ FORME DE *TANACETUM BALSAMITA* L.

(Rezumat)

Au fost analizate comparativ, prin microscopie electronică de baleiaj (SEM), suprafețele foliare de la două forme de calapăr *Tanacetum balsamita* f. *balsamita* şi *Tanacetum balsamita* f. *tanacetoides*, în vederea stabilirii asemănărilor și deosebirilor între acestea. Cei doi taxoni infraspecifici diferă prin morfologia și structura inflorescențelor, prin numărul de cromozomi și compoziția uleiului volatil secretat și acumulat în formațiunile secretoare ale epidermelor. În urma analizei imaginilor SEM, s-a constatat că formațiunile epidermice ale celor două forme de calapăr sunt asemănătoare atât în privința perilor tectori cât și a perilor secretori glandulari, dar diferă în ceea ce privește densitatea perilor secretori glandulari atât între cele două forme cât și topografic între epiderma adaxială şi abaxială a laminei frunzei. Cea mai mare densitate medie a perilor secretori glandulari se înregistrază la *Tanacetum balsamita* f. *balsamita*, pe epiderma inferioară.

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