RESEARCH ARTICLE

Pollen Morphology of Some Taxa of Aromatic Genus *Tanacetum* L. (Asteraceae)

Edibe OZMEN*°, Ilginc KIZILPINAR*, Barıs OZUDOGRU*, Cahit DOGAN*, Sadik ERIK*

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Summary

In this research, pollen morphology of four taxa, T. corymbosum subsp. corymbosum, T. armenum, T. nitens and T. haussknechtii, belonging to the aromatic genus Tanacetum L. (Asteraceae), which has wide medicinal uses, were examined with light microscope (LM) and scanning electron microscope (SEM). According to the investigation, pollen grains of these four taxa are radially symmetrical, isopolar, spheroidal, tricolporate and echinate-perforate. In our opinion, the palynological features of these taxa might be helpful to investigate the taxa in both taxonomical and pharmaceutical researches.

Key Words: Tanacetum, Asteraceae, Pollen morphology, medicinal, aromatic

Aromatik Tanacetum L. (Asteraceae) Cinsine Ait Bazı Taksonlarının Polen Morfolojisi

Özet

Bu araştırmada, geniş medikal kullanımı olan aromatik Tanacetum L. (Asteraceae) cinsine ait T. corymbosum subsp. corymbosum, T. armenum, T. nitens ve T. haussknechtii olmak üzere dört taksonun polen morfolojileri ışık mikroskobu (LM) ve taramalı elektron mikroskobu (SEM) ile incelenmiştir. Yapılan incelemelere göre, bu 4 taksona ait polenler radyal simetrik, izopolar, siferoid, trikolporat, ekinat-perforat özellik göstermektedir. Bu taksonlara ait palinolojik özelliklerin hem taksonomik çalışmalarda hem de farmasötik botanik çalışmalarında taksonların daha doğru teşhis edilmesine yardımcı olacağını düşünmekteyiz.

Anahtar Kelimeler: Tanacetum, Asteraceae, Polen morfolojisi, tibbi, aromatik.

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INTRODUCTION

Tanacetum L. is an aromatic, herbaceous and perennial plant (1). It was previously considered to be a subgenus of the genus *Chrysanthemum*, which is native to several parts of Asia, including China, Japan, and the Mediterranean region (2). *Tanacetum* has been separated from the genus *Chrysanthemum* and it currently consists of about 150 species (3).

Many species of genus *Tanacetum* have a wide range of use in pharmaceutical researches and ethnobotanical uses all around the world. The taxa of this genus are rich in essential oils, bitter substances and sesquiterpene lactones and they are used for their antihistaminic, anti-inflammatory and insecticidal effects (4). *T. polycephalum* Schultz-Bip. is used in folk medicine to treat many disorders (5). Oil of *T. vulgare* L. rubbed on skin is supposed to repel insects (6). In moderate doses, the plant and its essential oil are stomachic and cordial (6). Also, they are used as a food additive (6). Mikulášová et al. (7) have reported that essential oils from *T. vulgare* possess antibacterial and anti-yeast activity. The roots and rhizomes of *T. parthenium* (L.) Schulz. Bip. have been used in the Iranian traditional

^{*} Hacettepe University, Faculty of Science, Department of Biology, Beytepe-Ankara-Turkey

[°] Corresponding author e-mail: edibeozm@hacettepe.edu.tr



Figure 1. Distribution of the four investigated *Tanacetum* taxa in Turkey. $\star = T$. *corymbosum* subsp. *corymbosum*, $\bigcirc = T$. *armenum*, $\blacksquare = T$. *nitens*, $\triangle = T$. *haussknechtii*.

medicine as digestive and stomachic tonic (8). Aerial parts (especially the leaves) of *T. parthenium* are eaten or used as infusions in conditions like arthritis, migraine and asthma. It has been also claimed to be useful for treating conditions like tinnitus, vertigo, fever, menstrual disorders, difficulty in labour, stomachache, toothache and insect bites (9-12). Petrovic et al. (13) reported that *T. larvatum* (Griseb.) Kanitz may be used as an alternative or supplementary herbal remedy for the treatment of inflammatory diseases. Because of its anti-inflammatory as well as gastroprotective effects, some taxa, especially *T. larvatum*, may have beneficial effects when used together with drugs known for a strong anti-inflammatory activity as well

as for ulcerogenic side effects, such as NSAIDs (13). *T. balsamita* L. is an aromatic species which grows widely in Azerbaijan provinces (14). It has been used in the Iranian folk medicine as a tranquilizer and cardiac tonic (8).

The genus *Tanacetum* is represented by 59 taxa in Flora of Turkey (15). After the revision of the genus, a new species has been added and the total number has reached to 60 taxa (16). 27 taxa are endemic to Turkey and the rate of endemism is 45% (17).

In this study, 4 taxa belonging to the genus *Tanacetum*, *T. corymbosum* (DC.) Schultz. subsp. *corymbosum*,

Таха	Locality and date	Voucher numbers
<i>Tanacetum corymbosum</i> (L.) Schultz. subsp. <i>corymbosum</i>	B6 Sivas: Gemerek, Karababa Mountain, South-east slopes of Karasivri Hill, calcerous rocks, 39° 28' 23.9" N, 36° 05' 05.1" E, 2000-2050 m, 04/07/2007.	B. Özüdoğru, 1342
<i>Tanacetum armenum</i> (DC.) Schultz.	B6 Sivas: Gemerek, Karababa Mountain, South-east slopes of Karasivri Hill, opens <i>Juniperus excelsa</i> , 39° 27' 859'' N, 36° 06' 918'' E, 1700-1900 m, 20/05/2007.	B. Özüdoğru, 1101
<i>Tanacetum haussknechtii</i> (Bornm.) Grierson	B6 Sivas: Gemerek, Karababa Mountain, East slopes of Karasivri Hill, calcerous rocks, 39° 28′ 23.4″ N, 36° 06′ 30.7″ E, 1900-2000 m, 09/06/2007.	B. Özüdoğru, 1193
<i>Tanacetum nitens</i> (Boiss. & Noë) Grierson	B6 Sivas: Gemerek, Karababa Mountain, South-east slopes of Karasivri Hill, calcerous rocks, 39° 28' 23.9" N, 36° 05' 05.1" E, 2000-2050 m, 04/07/2007.	B. Özüdoğru, 1331

Table 1. Voucher specimens

T. armenum (DC.) Schultz, *T. nitens* (Boiss. & Noë) Grierson. and *T. hausknechtii* (Bornm.) Grierson, were investigated.

T. nitens is a widely distributed endemic species and its thread category is "**LC**". *T. hausknechtii* is a local endemic species, the distribution area of which is Sivas province and its thread category is "**VU**" according to Red Data Book (17). The other two are widely distributed, nonendemic taxa. The distribution map of the taxa is given in Figure 1.

The family Asteraceae is a typical example of eupalynous group and most of its genera possess trizonocolporate pollen (18). The pollen grains of Asteraceae have been characterized as basically helianthoid, spherical or slightly flattened, tricolporate and echinate (19-22). Since there are no reports on the pollen morphology of these taxa, the present report gives an account of the palynological characters in order to strengthen the recognition of 4 species of *Tanacetum* from Karababa Mountain (Sivas-Turkey).

MATERIAL AND METHODS

Materials of this study were collected in 2007 from the Sivas-Yozgat city border. Localities and voucher numbers are given in Table 1. The samples have been dried and prepared as herbarium specimens to deposit at HUB. The light microscopy (LM) observations with their measurements were made on pollen from mature anthers, which have been prepared according to the acetolysis method as described by Erdtman (22). The measurements of the pollen grains were taken on 50 pollen grains per species by an immersion object-lens (x100) and a scale ocular (10x). In addition, the ornamentation and the structure were established. All the statistical analyses of the palynological characters were made by the SPSS package program. In the SEM studies, the pollen grains taken from the anther were placed directly on the stubs, and were covered with gold. The detailed surface ornamentation and the aperture characteristics were examined under the Jeol JSM-6060 scanning electron microscope, and the microphotographs were taken. The terminology used is of Erdtman (23) and Punt et al. (24).

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	Pollen		Polar ê (µm	i i i i i i i i i i i i i i i i i i i	Equator axis (µn	ial Ar	(mu) du) Exin	le (µm)	Sexin	le (µm)	Nexine	(mn)	Lengtl spin	e E	ase of a	spine	Cl.g/	Clg (µm	2	Clt (µm	E C	ال م	g (µm)	[]	t (µm)	•	(und
Таха	shape	P/E	M (Var.)	s	M Var.)	S (Va	r. r	M (Var.)	s	M (Var.)	s	M (Var.)	s	M (Var.)	s	M (Var.)	s	<u>ີ</u> ວີ	M Var.)	<u>ح</u> ا ً	ar.)			s v	M (Var	s (M (Var.	s
T.corymbosum ssp. corymbosum	spheroidal	0.92	21.59 (18.6- ± 23.52)	± 1.19 (1 24	23.32 19.6- ±	1.37 (17. 22.5	28 -6- ± 1.(6.10 09 (4.9- 7.84)	± 0.7	5.06 1 (3.92- 6.87)	± 0.68	1.04 (0.98- 1.96)	± 0.30	2.62 (2.00- ≟ 3.00)	± 0.49	3.10 (2.00- ± 4.00)	± 0.48	2.95 (9	1.95 1.95 1.70) ± 1	4 1.00 (2 5.	.05 .94- ±0 88)	.88 1.2	4.1 23 (2.9 4.9(1 + + 0.5	3.3 4.90	+ ± 0.5	12.3 5 (9.80 13.72	3 ± ± 1.07
Т. аттепит	spheroidal	0.94	27.17 (25.4- ¹ 29.4)	±1.08 (2 30	8.87 27.4- ±i).38)	24.5 1.08 (22. 26.4	30 5- ± 0.5	97 (5.88- 7.84)	+ 0.3	5.91 9 (4.9- 6.86)	± 0.31	1.04 (0.98- 1.96)	± 0.25	2.96 (2.00- ≟ 4.00)	± 0.34 (3.80 (3.00- ≟ 5.00)	± 0.66	1 2.99 (1 16	4.86 2.74- ± 1 5.66)	4 1.03 (3 6.	.96 .92- ±0 86)	.77	2.5 2.7 (3.9) 6.8(5 2- ± 0.5	4.3 (2.9 ² 4.90	7 H ± 0.6	9.17 7 (8.82 11.76	± 0.65
T. nitens	spheroidal	0.92	28.09 (26.4- ₌ 30.38)	±1.13 (2 32	0.44 27.4- ± .	24.0 1.36 (21. 27.4	56 ± 1.5 .5- ± 1.5	7.66 52 (5.88- 8.82)	+ 0.8	7 (4.9- 7.84)	± 0.85	1.07 (0.98- 1.96)	± 0.30	2.84 (2.00- ≟ 3.00)	± 0.37	4.00 (3.00- ≟ 6.00)	± 0.87	1 2.93 (1 11	5.71 3.72- ± 1 7.64)	5 1.13 (3 6.	.35 .92- ±0 86)	.67 1.:	5.7 17 (4.9) 7.84	1)- ± 0.7	4.8 3 (3.9% 5.88	5 ± 0.5	9.3 4 (7.84 14.70	+ 1.42
T. haussknechtii	spheroidal	0.94	27.44 (24.5- ± 31.36)	± 1.94 (2	8.94 26.4- ± 2.34)	1.79 23.5 26.4	58 :5- ±1.4 (6)	49 (6.86- 9.8) (9.8)	- ± 0.8	7 (5.88- 7.79)	± 0.77	1.14 (0.98- 1.96)	± 0.37	3.38 (3.00- ≟ 4.00)	± 0.49	4.06 (3.00- ≟ 6.00)	± 0.91	2.41 (8 11	4.66 ± 1 3.82- ± 1 7.64)	00 1.73 (3	5.07 5.07 5.07 ± 1 84)	.13 1.2	4.9 28 (2.9 6.86	3 4- ± 0.9	3.8 1 (2.94 5.88	5 - ± 0.9	15.4 ⁵ 3 (13.77 17.64	- ± 1.14

Table 2. The palynological measurements and observations of the four investigated Tanacetum taxa. P: polar axis, E: equatorial axis, Amb: diameter of polen at the

polar view, Clg: length of colpus, Clt: latitude of colpus, Plg: length of porus, Plt: latitude of porus, t: distance between colpi ends, M: median, Var.: variation,

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RESULTS

The palynological measurements and observation of each species are given in Table 2. In addition, the boxplot graphs and mean plot graphs of equatorial axis, polar axis and Amb diameter are given in Figure 6 and Figure 7.

The pollen grains of examined *Tanacetum* species are radially symmetric, isopolar, tricolporate, spheroidal and circular in polar view. Colpi are long and with distinct margin. Pori are circular and also with



distinct margin. The porus latitude is smaller than the colpus latitude. Exine is getting thicker from colpus margin to mesocolpium. Sexine is thicker than nexine. Exine ornamentation is echinate-perforate. Spines are concave and their bases are broad.

Tanacetum corymbosum (DC.) Schultz. subsp. *corymbosum*

The pollen grains are radially symmetric, isopolar, tricolporate, spheroidal (Fig. 3). The polar axis is 21.59 µm and equatorial axis 23.32 µm. In polar







Figure 2. Flowers (a) and pollen grains (b, c, d) of *T. corymbosum* subsp. *corymbosum*; equatorial view (b), polar view (c), details of exine ornamentation (d).

X2,500

B

10 Mm

view, the pollen grains are circular, amb diameter is 20.28 μ m. The apocolpial area is wide. The distance between colpi ends is 12.38 μ m.

The colpi are long and with distinct margin; length of colpus (Clg) 11.95 μ m, latitude of colpus (Clt) 4.05 μ m. The pori are circular and with distinct margin; length of porus (Plg) 4.11 μ m, latitude of porus (Plt) 3.33 μ m.

The exine is $6.10 \,\mu\text{m}$ thick (sexine $5.06 \,\mu\text{m}$ and nexine $1.04 \,\mu\text{m}$). Sexine is thicker than nexine. The exine ornamentation is echinate-perforate. Spines are



concave and their bases are broad. Spine length is $2.62 \ \mu m$ and spine base is $3.10 \ \mu m$.

Tanacetum armenum (DC.) Schultz

The pollen grains are radially symmetric, isopolar, tricolporate, spheroidal (Fig. 2). The polar axis is 27.17 μ m and equatorial axis 28.87 μ m. In polar view, the pollen grains are circular, amb diameter is 24.30 μ m. The apocolpial area is wide. The distance between colpi ends is 9.17 μ m.

The colpi are long and with distinct margin; Clg 14.86μ m, Clt 4.96μ m. The pori are circular and with



Figure 3. Flowers (a) and pollen grains (b, c, d) of *T. armenum*; equatorial view (b), polar view (c), details of aperture and exine ornamentation (d).

distinct margin; Plg 5.55 µm, Plt 4.87 µm.

The exine is 6.95 μ m thick (sexine 5.91 μ m and nexine 1.04 μ m). Sexine is thicker than nexine. Exine ornamentation is echinate-perforate. Spines are concave and their bases are broad. Spine length is 2.96 μ m and spine base is 3.80 μ m.

Tanacetum nitens (Boiss. & Noë) Grierson.

The pollen grains are radially symmetric, isopolar, tricolporate, spheroidal (Fig. 4). The polar axis is 28.09 μ m and equatorial axis 30.44 μ m. In polar view, the pollen grains are circular, amb diameter is 24.66 μ m. The apocolpial area is wide. The distance between colpi ends is 9.30 μ m.

The colpi are long and with distinct margin; Clg 15.71 μ m, Clt 5.35 μ m. The pori are circular and with distinct margin; Plg 5.71 μ m, Plt 4.86 μ m.

The exine is 7.66 μ m thick (sexine 6.59 μ m and nexine 1.07 μ m). Sexine is thicker than nexine. The exine ornamentation is echinate-perforate. The spines are concave and their bases are broad. Spine length is 2.84 μ m and spine base is 4.0 μ m.

Tanacetum haussknechtii (Bornm.) Grierson

The pollen grains are radially symmetric, isopolar, tricolporate, spheroidal (Fig. 5). The polar axis is 27.44 µm and equatorial axis 28.94 µm. In polar view, the pollen grains are circular, amb diameter is



Figure 4. Flowers (a) and pollen grains (b, c, d) of *T. nitens*; equatorial view (b), polar view (c) and details of exine ornamentation (d).

 $23.58~\mu m.$ The apocolpial area is wide. The distance between colpi ends is $15.45~\mu m.$

The colpi are long and with distinct margin; Clg 14.66 μ m, Clt 6.07 μ m. The pori are circular and with distinct margin; Plg 4.93 μ m, Plt 3.85 μ m.

Exine is 7.93 μ m thick (sexine 6.79 μ m and nexine 1.14 μ m). Sexine is thicker than nexine. Exine ornamentation is echinate-perforate. Spines are concave and their bases are broad. Spine length is 3.38 μ m and spine base is 4.06 μ m.

DISCUSSION

Asteraceae is a eurypalynous family (23) and most of its genera possess zonocolporate pollen (18). The

characteristic of pollen spine is its significance in evolution and at specific and generic levels in the classification of this family (25). Similarly, Pinar & Oybak Donmez (26) reported that spine cavities of pollen exine can be utilized as diagnostic characters in the genera of Asteraceae. In addition, the pollen morphology of different Asteraceae taxa studied by several researchers has reported that the exine feature has an importace in taxonomy and phylogenetic classification (21, 27-28). Therefore, ornamentation and spine characteristics of *T. corymbosum* subsp. *corymbosum*, *T. armenum*, *T. nitens* and *T. haussknechtii* have been examined in detail in our study.

The pollen grains of all four taxa are radially symmetrical, isopolar, tricolporate and spheroidal.



Figure 5. Flowers (a) and pollen grains (b, c, d) of *T. haussknechtii*; equatorial view (b), polar view (c), details of aperture and exine ornamentation (d).

Exine ornamentation of pollen grains belonging to each taxa is echinate-perforate. When these characteristics have been considered, it has been determined that the pollen grains of *T. haussknechtii*, *T. armenum*, *T.*

nitens and *T. corymbosum* subsp. *corymbosum*, cohere to the pollen description of the genus (19, 29-30). Also, Wodehouse (31) has reported that pollen grains of the genus *Tanacetum* have distinct spines. The spin length





Figure 6. Box-plot graphs of equatorial axis (a), polar axis (b) and Amb diameter (c) of the pollen grains belonging to the four investigated *Tanacetum* taxa.

Figure 7. Mean plot graphs of equatorial axis (a), polar axis (b) and Amb diameter (c) of the pollen grains belonging to the four investigated *Tanacetum* taxa.

of the taxa in our research is 2.62-3.38 µm. The spines of the taxa under examination are longer than those many other taxa of Asteraceae (30, 32). When the equatorial axis, the polar axis and the Amb diameter of the pollen grains of the four taxa are compared, it is seen that the smallest pollen grains are found in *T. corymbosum* subsp. *corymbosum* (Table 2, Figure 6-7). Besides, *T. nitens* has the largest pollen grains (Table 2, Figure 6-7).

The One-Sample Kolmogorov-Smirnov test showed that the equatorial axis, the polar axis and the Amb diameter do not distribute normally (Table 3). Therefore, the Post Hoc multiple comparison test, the Dunnet's T3, has been applied to the equatorial axis,

Table 3. One-Sample Kolmogorov-Simirnov test results: E: equatorial axis, P: polar axis, Amb: diameter of polen at the polar view, Asymp. Sig.: *asymptotic significance*. The mean difference is significant at the 0.05 level.

	Ε	Р	Amb
Kolmogorov-Smirnov Z	1,784	2,300	2,178
Asymp. Sig. (2-tailed)	0,003	0,000	0,000

the polar axis and the Amb diameter (Table 4, Figure 7). The significance threshold has been accepted to be p<0.05 for each test. According to the Dunnett's T3 test, the significant differences between taxa are indicated by a star (*) in Table 4. With reference to these results, pollen size can be useful for the identification of these four taxa.

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Table 4. Post Hoc multiple comparison test, Dunnett's T3
results: Sig.: significance threshold. (*) The mean difference
is significant at the 0.05 level.

De- pendent Variable	(I) Taxa	(J) Taxa	Sig.
		T. armenum (*)	0,000
	T. corymbosum	T. nitens (*)	0,000
	bosum	T. haussknechtii (*)	0,000
		T. corymbosum subsp. corymbosum (*)	0,000
	T. armenum	T. nitens (*)	0,000
Б		T. haussknechtii	1,000
L		T. corymbosum subsp. corymbosum (*)	0,000
	T. nitens	T. armenum (*)	0,000
		<u>T. haussknechtii (*)</u>	0,004
		1. corymbosum subsp. corymbosum (*)	0,000
	T. haussknetchtii	T. armenum	1,000
		T. nitens (*)	0,004
	T. corymbosum	T. armenum (*)	0,000
	subsp. corym- bosum	T. nitens (*)	0,000
		T. haussknechtii (*)	0,000
		T. corymbosum subsp. corymbosum (*)	0,000
	T. armenum	T. nitens (*)	0,014
		T. haussknechtii	0,987
Р		T. corymbosum subsp. corymbosum (*)	0,000
	T. nitens	T. armenum (*)	0,014
		T. haussknechtii	0,517
		T. corymbosum subsp. corymbosum (*)	0,000
	T. haussknetchtii	T. armenum	0,987
		T. nitens	0,517
	T. corymbosum	T. armenum (*)	0,000
	subsp. corym-	T. nitens (*)	0,000
	DOSUM	T. haussknechtii (*)	0,000
	T. armenum	T. corymbosum subsp. Corymbosum (*)	0,000
		T. nitens	0,854
		T. haussknechtii	0,176
Amb	T nitens	T. corymbosum subsp. corymbosum (*)	0,000
		T. armenum	0,854
	I	T. haussknechtii (*)	0,045
	T hausskuptchtij	T. corymbosum subsp. corymbosum (*)	0,000
	1. 11113381161011111	T. armenum	0,176
		T. nitens (*)	0,045

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