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Achene mucilage content of six Turkish endemic *Tripleurospermum* (Asteraceae) taxa and its ecological significance

Türkiye endemiği altı Tripleurospermum (Asteraceae) taksonunun aken müsilaj içeriği ve ekolojik önemi

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Abstract
In this study, the achene mucilage content of six Turkish endemic *Tripleurospermum* taxa, namely *T. baytopianum* E. Hossain*, T. conoclinium* (Boiss. & Bal.) Hayek*, T. fissurale* (Sosn.) E. Hossain*, T. hygrophilum* (Bornm.) Bornm.*, T. rosellum* (Boiss. & Orph.) Hayek var. *album* E. Hossain and *T. ziganaense* Inceer & Hayirlioglu-Ayaz was determined by micro-staining reactions. Within the examined taxa, the mucilage cells on the achene surface are capable of forming a musilage envelope resembling a gel during hydration. The mucilage is heterogeneous and chemically contains pectin and cellulose. There are differences in the capacities of mucilage forming as well as adhesive to soil of the achenes in the studied taxa. The mucilage could have great importance in distribution and adaptation of *Tripleurospermum* taxa to diverse environments.

Özet
Bu çalışmada, Türkiye endemiği altı *Tripleurospermum* taksonunun, *T. baytopianum* E. Hossain, *T. conoclinium* (Boiss. & Bal.) Hayek, *T. fissurale* (Sosn.) E. Hossain, *T. hygrophilum* (Bornm.) Bornm., *T. rosellum* (Boiss. & Orph.) Hayek var. *album* E. Hossain ve *T. ziganaense* Inceer & Hayirlioğlu-Ayaz, aken musilaj içeriği mikroboyama ile belirlenmiştir. İncelenen taksonlarda, musilaj hücreleri perikarp yüzeyinde bulunur ve hidrasyon sırasında müsilaj zarf oluşumu ile karakterize edilir. Bu taksonlardaki müsilaj kimyasal olarak pektin ve selüloz yapısındadır. İncelenen taksonlarda müsilaj oluşturma kapasitelerinin yanı sıra akenlerin toprağa yapışma kapasiteleri arasında da farklılıklar vardır. Müsilaj, *Tripleurospermum* taksonlarının farklı ortamlara dağılımında ve uyumunda büyük öneme sahip olabilir.

INTRODUCTION

Mucilage, or slime, is common in the fruit (achene-cypsela) of the family Asteraceae (Grubert 1974, Kreitschitz et al. 2009, Inceer 2011). It produced by epidermal cells in seeds and fruits of some species mainly consists of pectin and hemisellulose (Western 2012, Gorai et al. 2014).

The mucilage has been proven to have a significant effect on many events such as maturation, germination, seed dispersal and compete with pathogens, facilitating the adaptation of plants to diverse environments (Kreitschitz et al. 2009, Western 2012, Gorai et al. 2014).

Tripleurospermum Sch. Bip. is a member of the tribe Anthemideae of the family Asteraceae. It is widespread in both northern and southern hemispheres and comprises *ca*. 40 (Oberprieler et al. 2007). *Tripleurospermum* taxa can grow in various habitats such as disturbed meadows, vacant lots, areas along roads, waste and dry areas. The variety of habitats and wide geographical distribution may lead to different adaptations to diverse environments.

The genus is characterized by the achenes that are triquetrous with one adaxially and two laterally arranged ribs (Oberprieler et al. 2007). In the pericarp of the achenes in some taxa, musilage, or slime, secreting cells which is also called as myxogenic cells are found. The mucilage envelope formation is thought to be an important taxonomic character in the diagnosis of *Tripleurospermum* species (Enayet Hossain 1975). However, no research has been conducted on the achene mucilage content and its function in the

Tripleurospermum genus. In Turkey, this genus is represented by 32 taxa and 16 of them are endemic (Ozbek and Onaylı 2020). Thirteen taxa, of which eight are endemic, have mucilaginous achenes. The main aims of this study: to determine the mucilage content of achene belonging to six endemic *Tripleurospermum* taxa and to determine their ability to adhere to the soil by forming mucilage, thus contributing to ecological knowledge of the genus.

MATERIALS and METHODS

Plant Material

The achenes of the *Tripleurospermum* taxa were obtained from Dr. H. Inceer's herbarium collections (Table 1) deposited in KTUB (herbarium of Karadeniz Technical University Biology)

Fluorescence Microscopy

The achenes were directly observed with fluorescence microscope. Mucilage cells on the achene surface were photographed with Leica DM 4000B fluorescence microscope using I3 fluorescence filter (BP 450- 490, LP 515 nm).

Mucilage Identification by Chemical Reactions

Mucilage content of the achenes was determined by micro-staining. For hydration, the achenes were first soaked in tap water at room temperature for 1-5 minutes and then stained with methylene blue, safranine and ruthenium red to identify the mucilage type. (Kreitschitz et al. 2009, Inceer 2011). The prepared preparations were photographed with the help of Leica DM 4000B (Leica DFC 490 digital camera attachment) light microscope.

Determination of the Swelling Factor

The swelling factor was estimated according to Grubert (1974), slightly modified: 0.25 g of ripe achene are placed in a 10 ml graduated cylinder of 1 cm diameter inside and 5 ml distilled water added (temperature 22°C). Then the cylinder was vigorously shaked during a period of five minutes. After this time as well as after an additional time 90 minutes the volume occupied by swollen achenes was noted as swelling factor (Grubert 1974).

The Behavior of Mucilaginous Achene on Sandy Soil

A fine grained quartz sand (SiO_2) was used for the behavior of mucilaginous achene on sandy soil. A petri dish was filled up with 20 ml of dry, fine grained quartz sand and distilled water was added to wet the substratum. Then the achenes previously soaked in distilled water were placed on wetted substratum. This petri dish was kept at room-temperature overnight and subsequently placed into a dessicator (50°C) for 6 h. A factor was estimated using initial and final weights of the achenes (Grubert 1974).

Table 1. Localities and voucher numbers of *Tripleurospermum* taxa investigated.

Taxon	Locality	Voucher
T. baytopianum E. Hossain	A1 Çanakkale: Koru Dağı, between Keşan and Evreşe, 70 m	Inceer 327
T. conoclinium (Boiss. & Bal.) Hayek	B2 İzmir: Bozdağ, 1185 m	Inceer 828
T. fissurale (Sosn.) E. Hossain	A8 Artvin: Between İspir-Yusufeli, 653 m	Inceer 533
T. hygrophilum (Bornm.) Bornm.	B1 İzmir: Yamanlar Dağı, 887 m	Inceer 273
T. rosellum (Boiss. & Orph.) Hayek var. *album* E. Hossain	B1 Balıkesir: Edremit Kaz Dağı, 650-700 m	Inceer 721
T. ziganaense Inceer & Hayirlioglu-Ayaz	A7 Gümüşhane: Zigana Dağı, between Zigana Pass and Torul, 1200-1300 m	Inceer 666

RESULTS

All the taxa have mucilage cells on the surface of the achenes (Figure 1). These mucilage cells on the surface of the pericarp are in isolated rows. As a result of mucilage identification studies, it was observed that the achenes kept in water rapidly secrete mucilage. The mucilage is a distinct gel-like envelope, and belongs to cellulosic type which representing a heterogenous system with a pectinous matrix and a cellulosic skeleton surrounding the matrix (Figure 2).

The present results indicate that the slime has different colours on the basis of micro-staining with methylene blue, safranine and ruthenium red. There is a very faint blue mucilage envelope around the achene with micro-staining methylene blue, whereas there are an orange-red and pink of the mucilage envelope around the achenes with micro-staining safranine and ruthenium red, respectively (Figure 2). On the other hand, the micro-staining of pectin and cellulose have almost the same colour. Besides, cellulosic threads or fibrils forming a characteristic radical skeleton around the achene are

clearly visible, whereas pectin colour is spread homogenously within the envelope.

The swelling factor in the achenes of the studied taxa varies from 3.4 in *T. conoclinum* to 4.6 ml in *T. fissurale* (Table 2). Within the studied taxa, *T. hyrophilum* has the highest the adhesive capacity of the slime, whereas *T. ziganaense* has the lowest one (Table 2).

Table 2. Mucilage forming capacity and the behavior on sand of the achenes in the *Tripleurospermum* taxa investigated

Taxon	Volume of dry achenes (ml)	Volume occupied by the swollen achenes after 90 min. (ml)	Weight of dry achene (g)	Weight of achene with adherent sand (g)	Difference between initial and final weight (g)
T. baytopianum	1.1	4.1	0.0045	0.0989	0.09
T. conoclinium	1.5	3.4	0.0128	0.1257	0.11
T. fissurale	1.0	4.6	0.0086	0.1175	0.11
T. hygrophilum	1.7	3.6	0.0094	0.1870	0.18
T. rosellum var. *album*	1.5	4.4	0.0085	0.1833	0.17
T. ziganaense	1.3	3.8	0.0058	0.0882	0.08

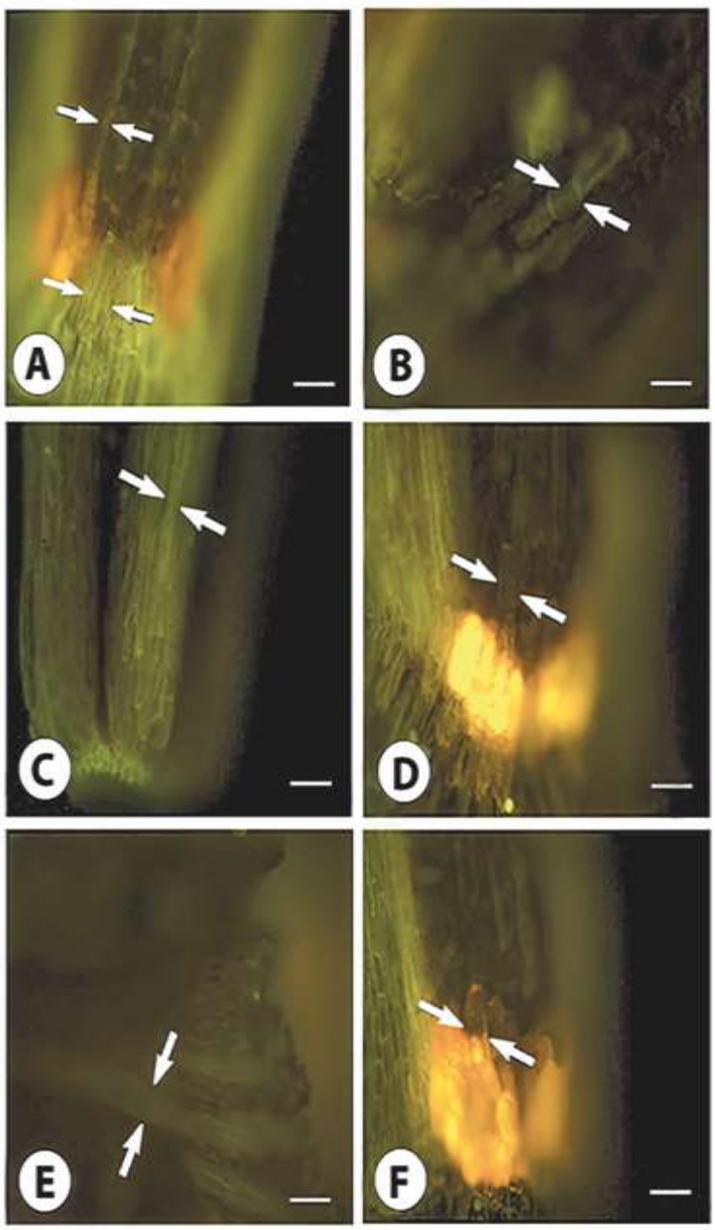

Figure 1. Fluorescence microscopy images of the mucilage cells on the *Tripleurospermum* achenes. A) *T. baytopianum*, B) *T. conoclinium*, C) *T. fissurale*, D) *T. hygrophilum*, E) *T. rosellum* var. *album*, F) *T. ziganaense*. White arrows indicate mucilage cells, scale bars 100 µm

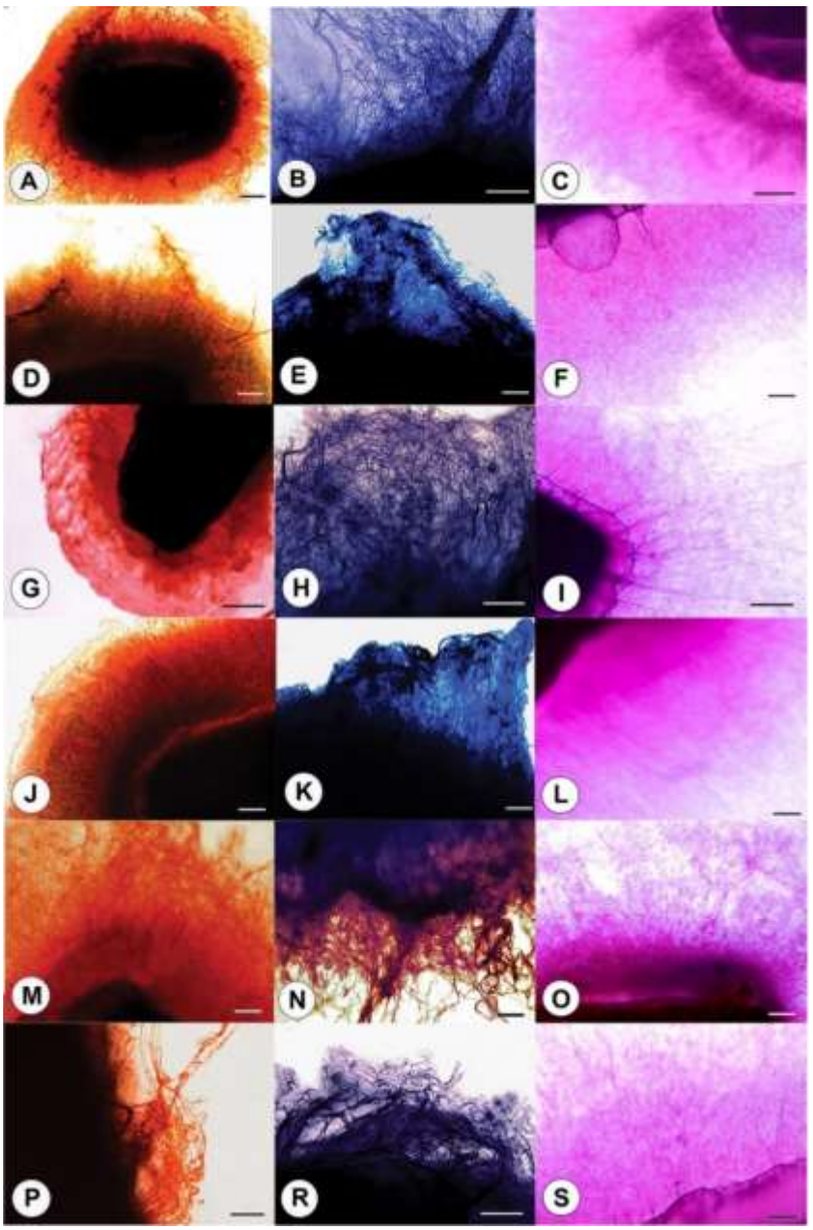

Figure 2. Mucilage envelope in the *Tripleurospermum* achenes. A) *T. baytopianum* (safranine), B) *T. baytopianum* (methylene blue), C) *T. baytopianum* (ruthenium red), D) *T. conoclinium* (safranine), E) *T. conoclinium* (methylene blue), F) *T. conoclinium* (ruthenium red), G) *T. fissurale* (safranine), H) *T. fissurale* (methylene blue), I) *T. fissurale* (ruthenium red), J) *T. hygrophilum* (safranine), K) *T. hygrophilum* (methylene blue), L) *T. hygrophilum* (ruthenium red), M) *T. rosellum* var. *album* (safranine), N) *T. rosellum* var. *album* (methylene blue), O) *T. rosellum* var. *album* (ruthenium red), P) *T. ziganaense* (safranine), R) *T. ziganaense* (methylene blue), S) *T. ziganaense* (ruthenium red), scale bars 100 µm

DISCUSSION AND CONCLUSIONS

The achene mucilage content in *Tripleurospermum* is presented here for the first time in detail. The mucilage cells on the epicarp surface of the achenes in the studied taxa are characterized by forming a mucilage envelope resembling a transparent gel after hydration. It has been determined that this mucilage is a cellulosic type with having pectin and cellulose after micro-staining with ruthenium red, methylene blue and safranine (Figure 2). In addition, it has been observed that the individual cellulose threads are interconnected by a large number of trabeculae, and thus forming a net-like structure. These findings show that the achene mucilage in the studied taxa have a heterogenous structure. Similar results were reported in *Matricaria* (Inceer 2011).

The micro-staining results obtained from with ruthenium red, methylene blue and safranine revealed different colours viz., pink, carmine-red, red, violet-blue, blue, orange-red, orange, of the mucilage based its structure in the studied taxa (Figure 2). These findings are in

agreement with previous reports in other genera of Anthemideae (Kreitschitz and Vallès 2007, Inceer 2011)

According to Grant et al. (1969), the cellulosic mucilage usually originates from pectins. This mucilage can facilitate the attachment of achenes to the soil surface. On the other hand, cellulose treads may strengthen the attachment and keep the achene in the soil. Therefore, the cellulosic mucilage can play an important role in adaptation of the *Tripleurospermum* taxa to diverse environments, which is in line with previous studies (O'Brien and Mccully 1981, Kreitschitz and Vallès 2007, Kreitschitz et al. 2009, Inceer 2011).

The present results show that all taxa examined have mucilage cells being isolated rows on both surfaces of the achene. These structural characteristics of the mucilage cells agree with what has previously been reported by Inceer et al. (2012) for *Tripleurospermum*. Similar results have been also reported in other Anthemideae genera (Grubert 1974, Inceer 2011). Many studies have revealed the ecological importance of the mucilage produced in seeds and/or fruits (Kreitschitz and Vallès 2007 and references therein). The studied *Tripleurospermum* taxa grow in diverse habitats such as rocky places, near fields and damp areas. The presence of a mucilage envelope facilitates a quick colonization of such places in the taxa. In addition, the dispersal of the achenes is possible by attaching to animals' fur (epizoochory) by means of cellulose threads. Similar results were reported in *Matricaria* (Inceer 2011).

Furthermore, our results show that all studied taxa have a different production in the achenes (Table 2). The mucilage production in the achenes of the taxa can vary depending on their habitats, which is in line with previous reports in other members of the tribe Anthemideae (Kreitschitz and Vallès 2007, Inceer 2011). Within the studied taxa, *T. fissurale* has the highest mucilage production (Table 2). This species grows in particularly dry habitats such as rocky places, rocky slopes and crevices. The achenes of this species in the dry areas can need much producing mucilage for facilitating the adhesive capacity as well as much mucilage production for stimulating the germination. On the other hand, the adhesive capacity on the sand of mucilaginous achenes after being wetted are higher in *T. hygrophilum* than other taxa (Table 2). This species mainly grows in wet environments such as montane meadows and damp subalpine regions. The ability to form high adhesive capacity of the achenes may be advantageous adaptive feature adhering to wet grounds as well as facilitating germination. These ecological functions of the mucilage agree with what has previously been reported in some species of *Artemisia* (Huang and Gutterman 1999, Huang et al. 2000), *Matricaria* (Inceer 2011) and *Henophyton* (Gorai et al. 2014).

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