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Taxonomic Evaluations of the Achene Fatty Acid Composition of Three Morphologically Similar *Tripleurospermum* (Asteraceae) Species

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ABSTRACT

Tripleurospermum tempskyanum, *T. disciforme* and *T. decipiens* (Asteraceae) are species with similar morphological characteristics that can therefore be easily confused with one another. In this study, achene fatty acid content of *T. tempskyanum* and *T. disciforme* from Türkiye was analysed using gas chromatography (GC) for the first time. The fatty acid data for these species together with the fatty acid data in the literature of *T. decipiens* were evaluated in terms of taxonomy using cluster analysis and principal components analysis. Eleven fatty acids were determined in the achenes of *T. tempskyanum* and *T. disciforme*. Additionally, linoleic acid (C18:2n6c), palmitic acid (C16) and α-linolenic acid (C18:3n3c) were detected as major fatty acids. The results of cluster analysis and principal components analysis indicated that achene fatty acids may be used as a chemotaxonomic marker to support the morphological separation of these species.

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Morfolojik Olarak Birbirine Benzeyen Üç *Tripleurospermum* (Asteraceae) Türünün Aken Yağ Asidi İçeriğinin Taksonomik Değerlendirmeleri

ÖZET

Tripleurospermum tempskyanum, *T. disciforme* ve *T. decipiens* (Asteraceae) morfolojik özellikleri bakımından birbirlerine benzeyen türlerdir ve bu yüzden birbirleri ile karıştırılabilirler. Bu çalışmada, Türkiye'den *T. tempskyanum* ve *T. disciforme*'nin aken yağ asidi içeriği ilk kez gaz kromatografisi (GC) kullanılarak analiz edildi. *T. decipiens*'in literatürdeki yağ asidi verileri ile birlikte bu türlerin yağ asidi verileri, kümeleme analizi ve temel bileşenler analizi kullanılarak taksonomik açıdan değerlendirildi. *T. tempskyanum* ve *T. disciforme*'nin akenlerinde 11 adet yağ asidi tespit edildi. Ayrıca, sırasıyla linoleik asit (C18:2n6c), palmitik asit (C16) ve α-linolenik asit (C18:3n3c)'lerin, major yağ asitleri olduğu belirlendi. Kümeleme analizi ve temel bileşenler analiz sonuçları, yağ asidi içeriğinin, bu türlerin morfolojik ayrımını desteklemek için kemotaksonomik bir belirteç olarak kullanılabileceğini gösterdi.

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INTRODUCTION

Tripleurospermum Sch.Bip., with c. 41 species, is one of the small genera of Asteraceae (Oberprieler et al., 2007). Most *Tripleurospermum* species are distributed in Türkiye with c. 33 taxa, the main center of its diversity (Inceer, 2021a; Inceer & Ozcan, 2021; Teksen et al., 2022). The endemism rate in the genus is above fifty percent with 17 endemic taxa in Türkiye.

Tripleurospermum tempskyanum (Freyn & Sint.) Hayek, *T. disciforme* (C.A.Mey.) Sch.Bip. and *T. decipiens* (Fisch. & Mey.) Bornm. are similar to one another in terms of their morphological traits and can therefore be easily confused with one another (Inceer, 2021b, Figure 1). *T. tempskyanum* is separated from *T. disciforme* by possessing non-mucilaginous achenes and from *T. decipiens* by having perennial habits and a hemispherical receptacle (Inceer, 2021b). *T. disciforme* differs from *T. decipiens* by possessing

ecoranate achenes and an ovoid-oblong receptacle (Enayet Hossain, 1975). *T. disciforme* and *T. decipiens* share similar anatomical traits in the leaves and achenes, while *T. tempskyanum* differs from these species to some degree in terms of various anatomical traits of the leaves and achenes (Inceer & Ozcan, 2021).

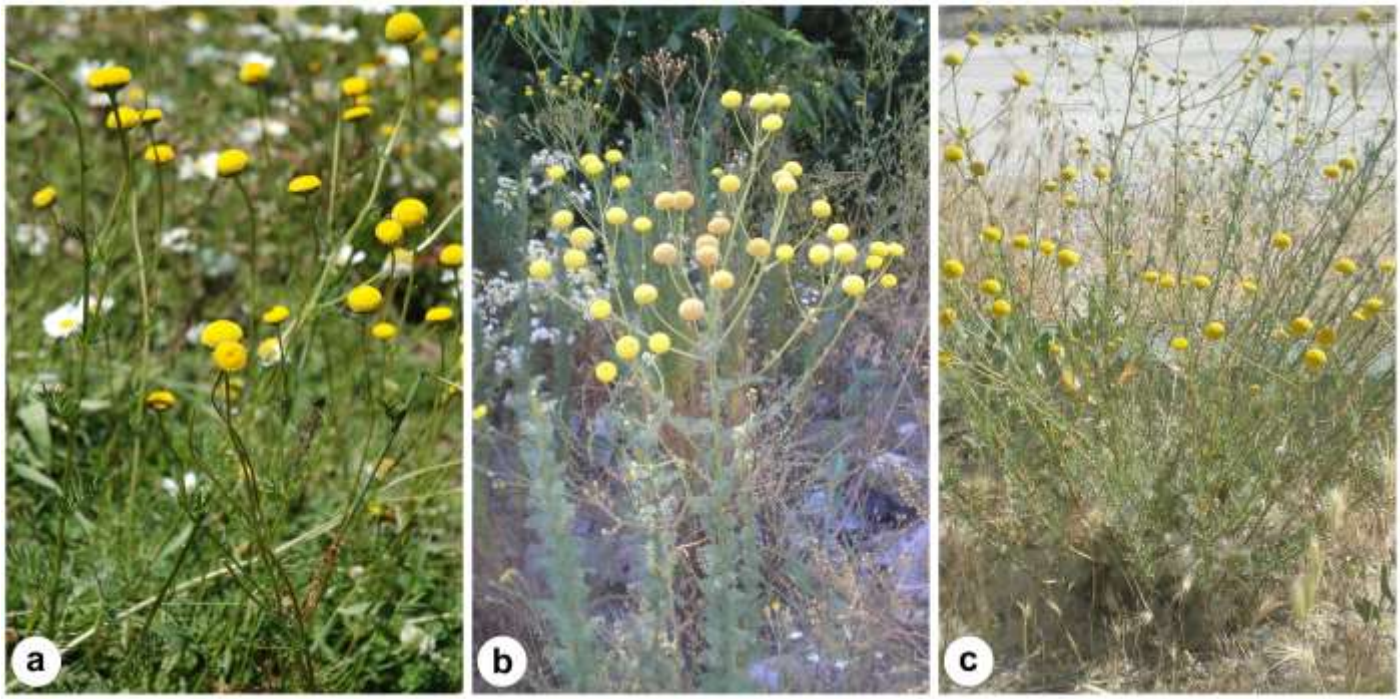

Figure 1. Habit of the studied species; a: *T. tempskyanum*, b: *T. disciforme*, c: *T. decipiens*
Şekil 1. Çalışılan türlerinin genel görünüşü; a: *T. tempskyanum*, b: *T. disciforme*, c: *T. decipiens*

In Türkiye, *T. tempskyanum* mainly grows in wet environments in Uludağ National Park in the province of Bursa (Inceer, 2021b). *T. disciforme* grows in damp places, such as meadows, fields and river beds (Enayet Hossain, 1975), while *T. decipiens* grows on steppe, rocky slopes and cultivated and fallow fields (Enayet Hossain, 1975).

Fatty acids (FAs) are found in all organs of plants. Additionally, FAs in the seeds can be good chemotaxonomic markers in certain plant groups, such as *Achemilla* L., *Carex* L., *Micromeria* Bentham, *Satureja* L. and *Thymus* L. (Marin et al., 1991; Ayaz et al., 1999; Ayaz & Olgun, 2000). Likewise, the FAs in the fruit (achene/cypsela) are of chemotaxonomic significance in some members of Asteraceae, such as *Centaurea*, L. *Matricaria* L., and *Tripleurospermum* (Ayaz et al., 2016; 2017; Janaćković et al., 2017). The achene FA profile of *T. decipiens* was previously reported by Ayaz et al. (2016). However, no FA analysis of the achenes in *T. tempskyanum* and *T. disciforme* has been conducted to date. The aims of the present work are to fill the gaps in the existing literature and to investigate FA variation among *T. tempskyanum*, *T. disciforme* and *T. decipiens* using cluster analysis and principal components analysis.

MATERIALS and METHODS

Plant Material

Achenes from five specimens of *T. tempskyanum* and *T. disciforme* were collected from the Turkish provinces of Bursa and Izmir, respectively. The locality and voucher details are given in Table 1. The vouchers are deposited in the KTUB herbarium.

Table 1. Collection data of *T. tempskyanum* and *T. disciforme*
Çizelge 1 T. tempskyanum ve T. disciforme'nin koleksiyon verileri

Species	Locality	Voucher
T. tempskyanum	A2 Bursa: Uludağ National Park, near hotels, meadows, damp places, 1690 m a.s.l., 27.6. 2007, N40°07′0.4″, E29°06′45.3″	Inceer 354
T. disciforme	B2 Izmir: Boz Dağ, Gölcük plateau, near Gölcük lake, meadows, roadsides, 1057 m a.s.l., 06.7.2008, N38°19′14.26″, E28°01′25.07″	Inceer 593

Fatty Acid Analysis

Total lipids of mature achenes were extracted as described by Folch et al. (1957), with some minor modifications. Pulverized achene samples (0.5 g) were

extracted using chloroform/methanol (2:1, v/v) in triplicate at 4°C for 18 h. The extracted lipids in the chloroform phase were separated by partitioning with one-fourth sodium chloride solution (0.9%, in water, w/v). These were then collected and evaporated using a rotary evaporator (Laborata 4003, Heidolph Instruments, Schwabach, Germany).

Analysis of FA methyl ester (FAME) extracted with *n*-hexane was carried out using an IUPAC (1989) accredited analysis method. A Perkin Elmer Auto System XL gas chromatography (GC) equipped with a flame-ionization detector was used for the FAME analysis. The column characteristics, injector and detector temperatures of the GC running conditions were selected as described elsewhere by Ayaz et al. (2016). The gas carrier was again helium. The FAs in the lipid samples were identified and quantified by comparison with the retention times of a standard FAME, as used earlier by Ayaz et al. (2016).

Multivariate Analyses

The data (12 quantitative characters) for the achene FAs of *T. tempskyanum* and *T. disciforme,* together with additional data for the achene FAs of *T. decipiens* (Ayaz et al., 2016), were evaluated using clustering analysis (UPGMA, dissimilarity, and standardized variable) and principal components analysis (PCA). These multivariate analyses were performed on Statistica version 12 software.

RESULTS and DISCUSSION

Achene Fatty Acids

The results of achene FA analysis are summarized in Table 2. This shows that the FA profiles varied considerably between *T. tempskyanum* and *T. disciforme*. The major FA in these species is linoleic acid, with values of 47.62% and 43.96%, respectively. Table 2 also shows total FA contents (the sum of the individual FAs quantified) in *T. tempskyanum* and *T. disciforme*, 33.43% and 26.13% for SFAs, 4.77% and 10.09% for MUFAs and 61.81% and 63.80% for PUFAs, respectively. These results indicate that PUFA levels are higher than SFAs and MUFAs levels in these species. Similar results have been reported for other species of *Tripleurospermum* (Ayaz et al., 2016). As already noted by Shorland (1963) and Janaćković et al. (2017), Asteraceae species possess rich linoleic acid contents in the achenes.

The present results show that the levels of unsaturated FAs are higher than those of SFAs in both *T. tempskyanum* and *T. disciforme* (Table 2). The concentrations of total unsaturated FAs in *T. tempskyanum* and *T. disciforme* are 66.58% and 73.89%, respectively. These results agree with previous report for a number of other species of the genera *Tripleurospermum*, *Achillea* L., *Anthemis* L., *Matricaria* and *Tanacetum* L. (Ayaz et al., 2016). The present results indicate that palmitic acid is the major SFA in both *T. tempskyanum* and *T. disciforme* (Table 2). As seen in Table 2, palmitic acid levels in *T. tempskyanum* are higher than those of *T. disciforme*. This finding is in agreement with the previous report for *Tripleurospermum* and other Anthemideae (Asteraceae) genera, such as *Anthemis* and *Matricaria* (Ayaz et al., 2016).

The results of this work also show that levels of α-linolenic and oleic acids in both species are higher than those of other FAs, after linoleic and palmitic acids. The results also indicate that these FA levels are higher in *T. disciforme* than in *T. tempskyanum*.

Table 2. Fatty acid profiles in the achenes of *T. tempskyanum* and *T. disciforme* (mean value ± standard deviation, %)
Çizelge 2 T. tempskyanum ve T. disciforme'nin yağ asidi profilleri (ortalama değer ± standart sapma, %)

Fatty acid	*T. tempskyanum*	*T. disciforme*
Capric acid (C10:0)	0.25±0.1	0.93±0.30
Myristic acid (C14:0)	0.41±0.15	0.58±0.06
Palmitic acid (C16:0)	23.13±0.38	19.90±0.47
Stearic acid (C18:0)	3.46±0.36	3.12±0.29
Arachidic acid (C20:0)	1.09±0.08	0.44±0.14
Behenic acid (C22:0)	2.35±0.20	-
Lignoseric acid (C24:0)	2.74±0.21	1.16±0.15
Palmitoleic acid (C16:1)	1.28±0.06	1.50±0.19
Oleic acid (C18:1n9c)	3.49±0.34	8.59±0.47
Linoleic acid (C18:2n6c)	47.62±0.53	43.96±0.69
α-Linolenic acid (C18:3n3c)	14.19±0.29	17.82±0.25
Arachidonic acid (C20:4n6c)	-	2.02±0.02
∑ SFA	33.43±0.46	26.13±0.47
∑ MUFA	4.77±0.29	10.09±0.56
∑ PUFA	61.81±0.65	63.80±0.72
PUFA/SFA	1.85	2.44
n-6/*n*-3	3.36±0.07	2.47±0.05

Eleven FAs were identified and quantified from the achenes of both species in the present study (Table 2).

However, the achenes of *T. disciforme* contain no behenic acid, and the achenes of *T. tempskyanum* contain no arachidonic acid. Similarly, *T. decipiens* has no behenic acid in its achenes (Ayaz et al., 2016). Capric acid is present as a minor FA (0.25%) in *T. tempskyanum*, whereas arachidic acid is found as a minor FA (0.44%) in *T. disciforme*. Similar results have been reported for various other species of *Tripleurospermum*, *Achillea* and *Matricaria* (Ayaz et al., 2016).

The accumulation of PUFAs with linoleic and α-linolenic acids is known to increase the meat quality of animals grazed on pastures, grasslands or meadows. In addition, linoleic and α-linolenic acids are not synthesized by herbivores and other consumers (Wee et al., 2017). The results of the FA analysis show a high accumulation of PUFA in the achenes of both *T. tempskyanum* and *T. disciforme* and that these species may be a rich PUFA source for domestic animals.

Taxonomic Implications

The results of the cluster analysis for *T. tempskyanum*, *T. disciforme* and *T. decipiens* are presented in Figure 2, which shows that these species are connected with each other at several levels depending on their FA profiles. In the dendrogram (Figure 2), *T. tempskyanum* is linked to *T. disciforme* in the same group at a low level, and these species exhibit a high level of similarity in terms of the FA profiles. On the other hand, *T. decipiens* is linked to these species at a high level in another group, and this species is thus similar to *T. tempskyanum* and *T. disciforme* at a low level in terms of the FA profiles. These findings indicate that the FA profiles of the achenes are useful for the delimitation of these species.

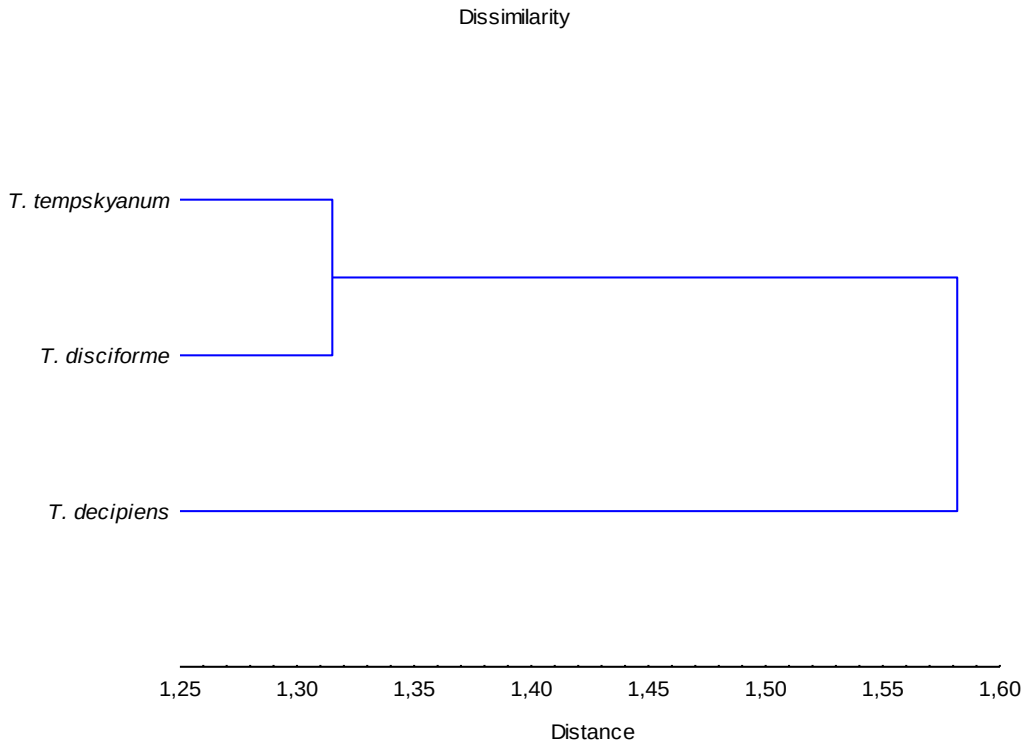

Figure 2. UPGMA clustering of *T. tempskyanum*, *T. disciforme* and *T. decipiens* on the basis of achene fatty acids
Şekil 2. T. tempskyanum, T. disciforme and T. decipiens'in aken yağ asitlerine dayalı UPGMA kümelenmesi

The PCA results showed that two PC factors accounted for 100% of the total variance (Figure 3). PC1 with an 8.14 eigenvalue describes approximately 68% of the variance. Its loadings indicate that it receives high contributions from stearic (-0.99), oleic (0.96), palmitic (-0.93), lignoseric (-0.92) and arachidonic (0.90) acid variables (Figure 4). PC2 with a 3.85 eigenvalue explains 32% of the difference in the data set, showing a high positive loading for capric (0.84) and myristic (0.80) acids (Figure 4). The results indicate that stearic, oleic, palmitic, lignoseric, arachidonic, capric and myristic acids explain most of the total variation among the species.

CONCLUSION

The species *T. tempskyanum*, *T. disciforme* and *T. decipiens* have similar morphological traits. It is therefore difficult to separate these species on the basis of morphological characters. The multivariate analysis results show that the achene FAs may be used as a chemotaxonomic marker to support the morphological separation of these species.

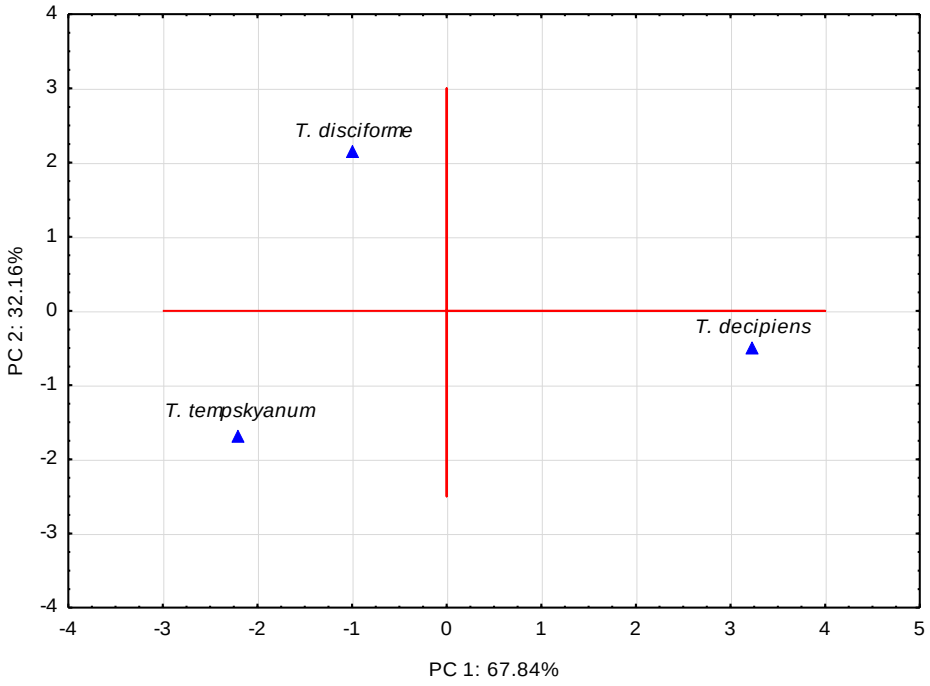

Figure 3. Results from principal components analysis of *T. tempskyanum*, *T. disciforme* and *T. decipiens* based on achene fatty acids

Şekil 3. T. tempskyanum, T. disciforme and T. decipiens'in aken yağ asitlerine dayalı temel bileşenler analiz sonuçları

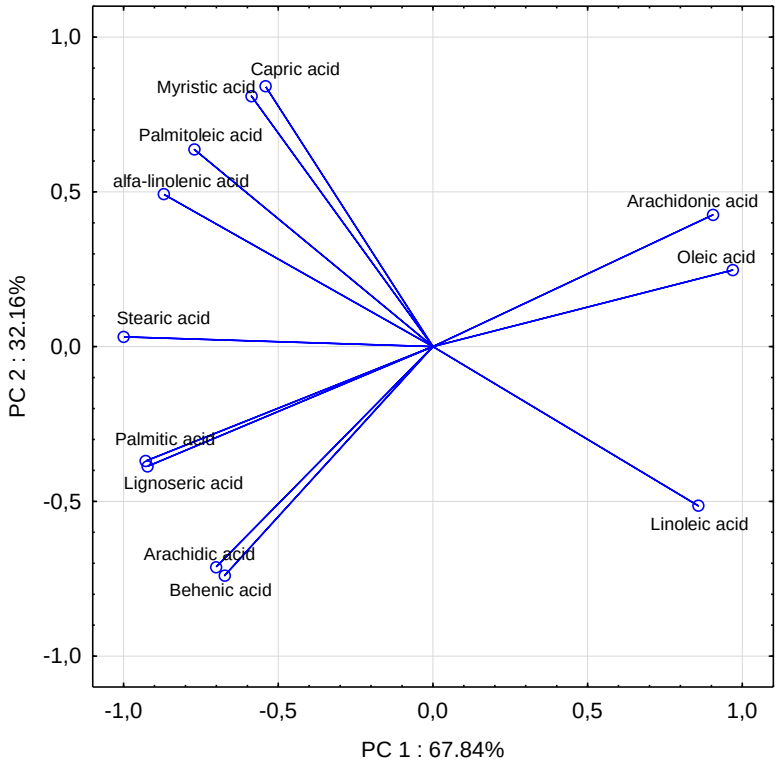

Figure 4. Results from principal components analysis of achene fatty acid composition in *T. tempskyanum*, *T. disciforme* and *T. decipiens*

Şekil 4. T. tempskyanum, T. disciforme and T. decipiens'de yağ asidi içeriklerinin temel bileşenler analiz sonuçları

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Researchers Contribution Rate Declaration Summary

The authors declare that they have contributed equally to the article.

Conflicts of Interest Statement

The article authors declare that they do not have any conflict of interest.

REFERENCES

Ayaz, F.A., Hayırlıoglu-Ayaz, S. & Beyazoglu, O. (1999). Fatty acid composition of leaf lipids of some *Alchemilla* L. (Rosaceae) species from Northeast Anatolia (Turkey). *Grasas y Aceites 50*, 341-344.

Ayaz, F.A., Inceer, H., Hayırlıoglu-Ayaz, S. & Aksu-Kalmuk, N. (2016). Achene fatty acid composition in the tribe Anthemideae (Asteraceae). *Romanian Biotechnological Letters 21*, 11576-11584.

Ayaz, F.A. & Olgun, A. (2000). Fatty acid composition of leaf lipids of some *Carex* L. (Cyperaceae) species from Northeast Anatolia (Turkey). *Grasas y Aceites 51,* 307-310.

Ayaz, F.A., Ozcan, M., Kurt, A., Karayigit, B., Ozogul, Y., Glew, R. & Ozogul, F. (2017). Fatty acid composition and antioxidant capacity of cypselas in *Centaurea* s.l. taxa (Asteraceae, Cardueae) from NE Anatolia. *South African Journal of Botany 112,* 474-482.

Enayet Hossain, A.B.M. (1975). *Tripleurospermum* Schultz Bip. In P.H. Davis (Ed.), *Flora of Turkey and the East Aegean Islands* 5. Edinburgh University Press, Edinburgh.

Folch, J., Lees, M. & Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biolological Chemistry 226*, 497-509.

Inceer, H. (2021a). Lectotypification of two Boissier's names in *Chamaemelum* (Anthemideae, Asteraceae). *Candollea 76*, 259-260.

Inceer, H. (2021b). Lectotypification of the name *Chamaemelum heterolepis*, the basionym of *Tripleurospermum heterolepis* and taxonomic notes on *T. tempskyanum* (Asteraceae). *Botanica Serbica 45,* 347-352.

Inceer, H. & Ozcan, M. (2021). Taxonomic evaluations on the anatomical characters of leaf and achene in Turkish *Tripleurospermum* with its relative *Matricaria* (Asteraceae). *Flora 275,* 151759.

IUPAC, (1989). *Standard methods for the analysis of oils, fats and derivatives*. Blackwell Scientific Publications, Oxford.

Janaćković, P., Gavrilović, M., Vujisić, L., Matevski, V. & Marin, P.D. (2017). Fatty acid composition of the cypselae of two endemic *Centaurea* species (Asteraceae). *Botanica Serbica 41,* 3-9.

Marin, P.D., Sajdl, V., Kapor, S., Tatic, B. & Petkovic, B. (1991). Fatty acids of the Saturejoideae, Ajugoideae and Scutellarioideae (Lamiaceae). *Phytochemistry 30,* 2979-2982.

Oberprieler, C., Vogt, R. & Watson, L.E. (2007). *Tribe Anthemideae* Cass. In J. W. Kadereit, & C. Jeffrey (Eds.), Kubitzki's *The families and genera of vascular plants* 8. Springer-Verlag, Berlin Heidenberg.

Shorland, F.B. (1963). *The distribution of fatty acids in plant lipids*. In T. Swain (Ed.), *Chemical plant taxonomy*. Academic Press, New York.

Teksen, M., Kahraman, Erkul, S., Duman, H., Alev Ates, M.& Sagıroglu, M. (2022). *Tripleurospermum eskilensis* (Asteraceae): a new halophytic species from Central Anatolia, Turkey. *Turkish Journal of Botany 46,* 160-175.

Wee, C.D., Hashiguchi, M., Anai, T., Suzuki, A. & Ryo Akashi, R. (2017). Fatty acid composition and distribution in wild soybean (*Glycine soja*) seeds collected in Japan. *Asian Journal of Plant Sciences* 16, 52-64.