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Research Article

Determination of fatty oil content, seed yield and some vegetative characteristics of *Laser trilobum* (L.) Borkh

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ABSTRACT

The aim of the study was to determine the fatty oil content, seed yield and selected vegetative characteristics of Laser trilobum belonging to the Apiaceae family grown under Isparta ecological conditions during 2021 and 2022. The initial research material was obtained from the BarlaMountain region of Eğirdir, Isparta at an altitude of 1575 m. The collected seeds were stored at +4°C, sown in autumn, and plant were transferred to the field in spring. The seeds were harvested at full maturity in 2021 and 2022. The fatty oil content of the harvested seeds was extracted using a Soxhlet device, fatty acid components were determined by gas chromatography (GC-FID), and protein content was determined by the Kjeldahl method. The average seed yield per plant was found to be 23.52 g plant-1 in 2021 and 23.34 g plant-1 in 2022. The main fatty acids of the species were petroselinic acid (35.30–37.47%), linoleic acid (16.29–16.68%), and oleic acid (7.65–8.44%). The high levels of petroselinic and unsaturated fatty acids may be a significant finding that could improve the commercial potential of this species. As a result, cultivation of the species instead of collecting it from nature will contribute to making it a sustainable source of oil and protein.

INTRODUCTION

Laser trilobum (L.) Borkh, a species belonging to the Laser genus, is distributed across various regions of Turkey (Davis, 1972). *L. trilobum* is a perennial, white-flowered species of the Apiaceae family, reaching 50-120 cm in height, with herbaceous, glabrous stems, growing in rocky areas and flowering in the natural flora between the 5th-8th months (Karadoğan et al. 2016). The ripe fruits of *L. trilobum*, naturally found in many regions of Turkey, are traditionally used as spice. The species is regionally named Kefe cumin, mountain cumin, and Sıra (Baytop 1997).

Fatty acids in plant seeds play roles in defending against abiotic and biotic stresses, and also serve as an energy source. Fatty acid composition varies with plant diversity, and certain fatty acids are specific to particular plant families (Avato and Tava 2021). For example, hydroxy acids in Euphorbiaceae (Cahoon and Li-Besisson 2020); acetylenic acids in Santalaceae, Olacaceae, Asteraceae, and Caesalpiniaceae (Fatope et al. 2000; Okada et al. 2013; Sun et al. 2017); epoxy acids in Asteraceae and Cruciferae (Spitzer et al. 1996); and cyclic acids in Malvaceae (Bao et al. 2002; Chaves et al. 2012).

The Apiaceae family is generally rich in unsaturated fatty acids, particularly petroselinic acid (Avato et al. 2001; Tosun and Karadoğan 2024). Petroselinic fatty acids have been determined by various researchers to have antidiabetic (Tong et al. 2010), antibacterial (Lee et al. 2022; Soyuçok et al. 2024), and pharmacological applications (Hajib et al. 2023). Although there are various studies on the crude oil content and herbal properties of cultivated species of the Apiaceae family, such as cumin, anise, fennel, quiniche, dill, and parsley, there is very limited literature on naturally distributed *L. trilobum* species. *L. trilobum* is an economically and ecologically significant species with a high petroselinic acid content and diverse applications. Cultivation of the species under controlled conditions will contribute to sustainable resource management, provide raw materials for the industrial and pharmaceutical sectors, and promote biodiversity conservation. This study is among the first to investigate the cultivation of this species under controlled conditions. Adaptation to the Mediterranean climate, characterized by partial drought resistance, confers an advantage in climate change scenarios. The conformation of this species to various cultivation techniques, coupled with its minimal requirement for chemical fertilizers and pesticides, renders it a viable candidate for inclusion among alternative plant resources. This study aimed to determine the fatty oil content and composition, seed yield, and vegetative characteristics of *L. trilobum* cultivated under culture conditions.

MATERIALS and METHODS

Plant material

Initial specimens of *L. trilobum* were collected from Isparta, Eğirdiri Barla Mountain an altitude of 1575 m between 2014-2016 as part of the research project titled 'Identification of Plant Species of Umbelliferae Family in Isparta and Burdur Provinces in the Lakes Region and Determination of Essen-

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tial Oil Values'. The plant materials were collected during full anthesis and identified by Prof. Dr. Hasan Özçelik based on 'Flora of Turkey, Volume 4. Type specimens of the species were preserved in Süleyman Demirel University Rose Herbarium under the accession number GUL 63.82.1.1. Seeds collected in 2016 during the full ripened period were stored at +4 °C for one year. The preserved seeds were sown in a 3:1 perlite-peat mixture in 32-cell viol during the autumn of 2017. Field studies were conducted in the experimental areas of Isparta University of Applied Sciences, Faculty of Agriculture, Agricultural Research and Application Center, located in Isparta, within the Lakes Region of the Mediterranean climate. In the spring of 2018, the resultant seedlings were transplanted to field conditions with an intra-row spacing of 30 cm and inter-row spacing of 70 cm. Planting was conducted in two rows, each 6 m in length. Weed control was performed manually, and irrigation was provided to the plants using the drip irrigation method as required. The morphological characteristics of this species were studied in 2019 and 2020. In July 2021-2022, seeds were harvested upon reaching full maturity.

Experimental area's climate and soil characteristics

The long-term average temperature was 12.32 °C; however, it was observed to be approximately 2 °C higher in 2021-2022, during the experimental period. Monthly analysis showed that temperatures in 2021 and 2022 were generally higher than the long-term average, except in March 2021. Examination of the total precipitation over multiple years indicated that the average total precipitation was 568 mm, whereas a decrease of approximately 100 mm was observed in 2021 (468 mm) and 2022 (456 mm). In 2021, the long-term rainfall averages exceeded the mean in January, June, and December, while in 2022, they surpassed the average in February and March. In all other months, rainfall remained below the long-term average (Figure 1).

The experimental soil was classified as clayey loam, with a pH of 8.2, 7.1% lime (Scheibler calcimeter), 1.3% organic matter (Walcley-Black method), and 0.29% total salt. The total nitrogen content was 0.29% (Kjeldahl method), while exchangeable phosphorus and exchangeable potassium contents were 16.7 mg kg-1 (by 0.4 N NaHCO3 extraction) and 179 mg kg-1 (by 1 N NH4OAc), respectively. The available sulfate content was 17.3 mg kg-1.

Measurements were taken from 20 individual plants at full maturity in July. These measurements encompassed the mean quantity of primary branches per plant, average number of umbels (both fruiting and non-fruiting) per plant, and mean seed yield expressed in grams per plant.

Crude fatty content and components

Dried fruit samples were ground and placed in extraction cartridges, then processed using a Soxhlet apparatus with petroleum ether for 4 h. After extraction, the cartridges were dried and reweighed to calculate crude oil content based on weight loss. Five grams of ground dried seeds were mixed with 10 ml of n-hexane and extracted overnight to obtain crude fatty acids. The mixture was subsequently filtered and dried at 45 °C to remove the solvent from the filtrate. After solvent evaporation, the crude oil obtained was converted to methyl esters (FAME) with 0.5% sodium methylate (NaOMe), according to the method recommended by AOAC. From the supernatant, where esterified fatty acids (FAME) were collected, 1 µL was withdrawn and injected into a gas chromatography (GC-FID) device. Chromatograms of fatty acids were obtained, and fatty acid ratios were determined. Gas chromatography was performed using a Shimadzu GC-2010 Plus (Shimadzu Corporation, Kyoto, Japan) equipped with a Restek Rtx-2330 column (50 m × 0.25 mmID, 0.20 µm film thickness), injector temperature 250 °C, detector temperature 200 °C, flow rate (psi) 10, carrier gas H2 (40 ml/min), injector capacity 1.0 µl.

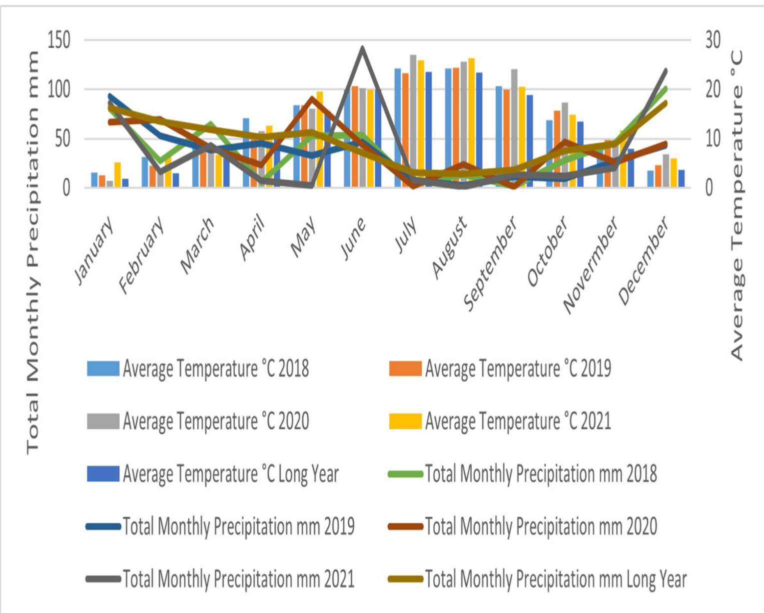

Figure 1. 2018-2021 diagram of climate

Table 1. t-test results and means of the vegetative characteristics of L. trilobum species

	Main branches (per/plant)		Fruiting umbrellas (per/plant)		Non-fruiting umbrellas (per/plant)		Fruit yield (g/ plant)	
Harvest years	2021	2022	2021	2022	2021	2022	2021	2022
Simple number	20	20	20	20	20	20	20	20
Avarage	1.50	1.70	3.85	3.55	3.10	3.25	23.52	23.34
Standard deviations	0.37	0.80	1.27	1.90	1.48	1.68	2.47	4.13
p value	0.105		0.562		0.767		0.868	

Table 2. Fatty acid component, crude protein and crude fat content of L. trilobum.

Retention Time		**Component**	**2021**	**2022**
7.553		Unidentified	23.97	21.80
18.450	C13:0	Tridecanoic Acid	0.16	0.17
20.076	C14:0	Myristic Acid	11.39	9.86
21.957	C15:0	Pentadecanoic Acid	0.02	0.01
22.334		Unidentified	0.34	0.28
24.401	C16:0	Palmitic Acid	3.27	3.60
26.101	C16:1	Palmitoleic Acid	0.05	0.06
27.332	C17:0	Heptadecanoic Acid	0.02	0.01
29.590	C17:1	cis-10-Heptadecanoic Acid	0.01	0.01
31.350	C18:0	Stearic Acid	1.03	1.07
33.440	C18:1n6c	Petroselinic Acid	35.30	37.47
33.823	C18:1n9c	Oleic Acid	7.65	8.44
38.321	C18:2n6c	Linoleig Acid	16.29	16.68
41.537	C20:0	Arachidic Acid	0.03	0.03
43.353	C20:1	cis-11-Eicosenoic Acid	0.18	0.15
47.534	C20:2	cis-11,14 Aicosadienoic Acid	0.01	0.02
49.453	C22:0	Behenic Acid	0.05	0.06
53.290	C20:4n6	Arachidonic Acid	0.01	
54.268	C23:0	Tricosanoic Acid	0.04	
54.325	C22:2	cis-13,16 Docosadienoic Acid		0.14
62.140	C22:6n3	cis-4,7,10,11,16,19-Docosahexaenoic Acid	0.17	0.15
Defined component			75.69	77.92
Fatty acid number			20	19
Total saturated fatty acid (∑DY)			16.01	14.81
Total monounsaturated fatty acid (∑DY)			43.19	46.13
Total polyunsaturated fatty acid (∑ÇDY)			16.48	16.99
Crude fatty content			8.29±0.06	7.77±021
Crude protein content			16.34±0.25	15.62±0.28

After maintaining 140 °C for 10 min, the oven temperature was increased to 240 °C at a rate of 3 °C per minute and maintained at this temperature for 10 min. The peaks in the chromatograms obtained were labeled according to the commercial standard fatty acid methyl ester mixture (Sigma, Supelco® 37 Component FAME Mix) (Tosun ve Karadoğan 2024).

Crude protein content

The crude protein content of the dried, ground samples was determined using the Kjeldahl method using a KJELDATHERM KT 20s unit and VAPODEST 50s distillation and titration unit, (Gerhardt GmbH& Co. KG, Königswinter, Germany).

Statistical analysis

A t-test for independent variables (Minitab statistical software) was used to compare the number of main branches, fruit yields, and fruiting and non-fruiting umbels across 2021–2022. Standard deviations were calculated for crude oil and protein contents.

RESULTS

Analysis of variance revealed no statistically significant differences between 2021 and 2022 in the number of main branches, fruiting and non-fruiting umbels, or seed yields (Table 1). *L. trilobum* exhibited a mean of 1.50 main branches per plant in 2021, which increased to 1.70 in 2022. The number of fruiting umbels decreased slightly from 3.85 in 2021 to 3.55 in 2022, while non-fruiting umbels increased from 3.10 to 3.25. Seed production continued relatively constant, with yields of 23.52 g per plant in 2021 and 23.34 g per plant in 2022 (Table 1).

Crude protein content of *L. trilobum* fruits was 16.34% in 2021 and 15.62% in 2022. The crude oil content was 8.29% in 2021 and 7.77% in 2022. A total of 20 fatty acids were identified in 2021 and 19 in 2022 in the extracted crude oil, with 75.69-77.92% of the total fatty acids successfully characterized in both years. The predominant fatty acids were petroselinic acid (35.30-37.47%), linoleic acid (16.29-16.68%), myristic acid (11.39-9.86%), and oleic acid (7.65-8.44%), with 21.80–23.97% of the fatty acids remaining unidentified. The fatty acid composition comprised 14.81-16.01% saturated, 43.19-46.13% monounsaturated, and 16.48-16.99% polyunsaturated fatty acids (Table 2).

DISCUSSION

Tosun and Karadoğan (2024) reported a fatty oil content of 12.36% in *L. trilobum*, with 14.40% saturated, 48.08% monounsaturated, and 22.17% polyunsaturated fatty acids. Major components included petroselinic (34.96%), linoleic (21.09%), oleic (10.40%), palmitic (7.09%), and myristic acid (4.10%). Additionally, 11.80% of the fatty acids remained unidentified, with a retention time of 14.491 minutes. Parlatan et al. (2009) determined the fatty acid composition of the species as capric (59.01-69.27%), myristic (1.77-3.16%), lignoceric (1.75-8.83%) and myristoleic acid (3.01-31.09%). While our results are consistent with those of Tosun and Karadoğan (2024), they differ from the findings of Parlatan et al. (2009), likely due to differences in solvent extraction methods. Hajib et al. (2023) reported that the solvents and extraction methods used influenced the fatty oil content and ratio.

Petroselinic and oleic acids, two principal fatty acids in *L. trilobum*, differ in the position of their double bonds. While carbon bonds are double bonds in petroselinic acid (18:1 cis Δ6) or cis-6-octadecenoic acid ($C_{18}H_{34}O_2$) at C6-C7, oleic acid (18:1 cis Δ9) is an isomer at the C9-C10 positions and both are commonly found in plant oils (Avato et al., 2001; Sayed-Ahmad et al., 2017; Avato and Tava, 2022). Petroselinic acid content has been shown to vary with environmental and developmental factors, including climate, soil conditions, seed maturation stage, and genetic variability. It tends to increase as seeds mature (Msaada et al., 2009; Nguyen et al., 2015; Nguyen et al., 2020), but decreases under drought stress (Laribi et al., 2009; Rebey et al., 2012). Variability in petroselinic acid levels has also been linked to genotype differences (Yaldiz and Camlica, 2019).

CONCLUSION

This study presents an initial investigation of the seed yield, crude oil content, and fatty acid composition of *L. trilobum* cultivated under controlled conditions, contributing novel data to the existing literature. The high proportion of petroselinic and unsaturated fatty acids highlights the species' potential for commercial exploitation. It is anticipated that this species will be incorporated among alternative oil source plants by enhancing its fatty oil component and content in future breeding studies. Consequently, it is hypothesized that the cultivation of *L. trilobum* under controlled conditions, as opposed to harvesting from natural habitats, will facilitate the conservation of the species while simultaneously allowing its utilization as a protein and oil source.

DECLARATIONS

Ethics Approval

This study does not require an ethics approval

Conflict of Interest

Author declare no conflict of interest.

Consent for Publication

Not applicable

Author contribution

Idea, concept and design: BT

Data collection and analysis: BT

Drafting of the manuscript: BT

Critical review: BT

Data Availability

The data sets generated and/or analyzed during the current study are available from the corresponding author on a reasonable request.

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