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DETERMINATION OF SOME PHENOLIC SUBSTANCES IN SIX DIFFERENT POPULATIONS OF TURKISH HAZEL (*CORYLUS COLURNA* L.) LEAVES AND COMPARISON OF PHENOLIC FLUCTUATION WITH WATER DEFICIENCY

UTVRĐIVANJE FENOLNIH SUBSTANCI KOD ŠEST RAZLIČITIH POPULACIJA MEDVJEĐE LIJESKE (*CORYLUS COLURNA* L.) I USPOREDBA FLUKTUACIJE FENOLA U SLUČAJU NEDOSTATKA VODE

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SUMMARY

Turkish hazel (*Corylus colurna* L.) is one of the naturally grown hazelnut species in Turkey. It can be easily separated from other hazel species with its thick single stem and tall appearance. It can be used in afforestation and erosion control studies due to low demand for habitat and strong root system. It contains substances with high medicinal value in its fruits and leaves. Hazel leaves have strong antioxidant activity due to their high phenolic content. Objective of the study was primarily to determine the individual phenolic constituents of six different population of Turkish Hazelnut and then to evaluate the effect of water deficiency stress generated by irrigation regime on phenolic constituents and photosystem II activity on these genotypes. Grafted plants were produced by taking scions from six different populations of Turkish Hazelnut (Oğuzlar, Erenler, Merkeşler, Seben, Güney Felakettin and Pelitcik). The study was started when the grafted seedlings were 7 years old in greenhouse. During the experiment (June and July), two different levels of irrigation were applied (W1: the soil was fully irrigated to reach field capacity in each irrigation; W2: 50% reduction of W1 irrigation water). After the application of two different irrigation regimes, leaves were collected for each month, dried, extracted with methanol and then quantitatively analyzed and compared for individual phenolic constituents (gallic acid monohydrate, caffeic acid, rutin hydrate, luteolin-7-O-β-D glucoside, kaempferol, rosmarinic acid, myricetin, quercetin, coumarin and apigenin) by using high performance liquid chromatography (HPLC) coupled with a diode array detector (DAD). Generally, rutin, kaempferol and luteolin were dominant individual phenols in methanol extracts of Turkish hazelnut leaves. Pelitcik population was noticeable source of rutin and kaempferol in June, and the halved irrigation regime significantly increased the levels of both phenols in July. Similarly, the highest total phenolic content was observed in the Pelitcik population in June and the halved irrigation regime significantly increased the total phenolic content in both months in this population. It was also determined to what extent water deficiency physiologically

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affects the quantum efficiency (Fv/Fm) of photosystem II activity through chlorophyll fluorescence technique in hazelnut leaves. Generally, Fv/Fm value decreased with water deficiency. This study showed that water deficiency stress generally caused an increase in phenolic constituents in Turkish Hazel leaves and they may be proper natural sources of phenolic constituents with abiotic stress applications in pharmaceutical and food industry.

KEY WORDS: *Corylus colurna* L, quantum efficiency, phenol, Turkish Hazelnut, water deficiency

INTRODUCTION
UVOD

Genus *Corylus* belonging to Betulaceae family has three species (*Corylus avellana* L., *Corylus maxima* Mill. and *Corylus colurna* L.) growing naturally in Turkey (Davis, 1982). C. colurna known as Turkish Hazel tree spreads over a wide area from Balkans (Serbia, Bulgaria and Romania), northwest and west Caucasus, north and northwest of Iran, east coast of the Caspian Sea, Afghanistan, Pakistan and the Himalayan Mountains to China. In Anatolia, the most common area is the Northwest Anatolian forests (Polat, 2014). It is also known as Turkish Hazelnut, Turkish Filbert, Tree Nut, Bear Hazelnut, Balkan Hazelnut, Rock Hazelnut Gökbulak Hazelnut and Budağan Hazelnut (Everett, 1988; Polat, 2014). It is the largest hazel species, reaching a height of 35 m and a trunk diameter of up to 1.5 m. It prefers calcareous, well-drained soils (Korkut et al., 2008). It is a species that can be expanded in appropriate places in afforestation and erosion control studies due to its low habitat demand (Arslan, 2005). While it was previously preferred only as a rootstock for cultivated hazelnut varieties for landscape purposes, today it is preferred by hazelnut producers due to its single-stem nature and the low cost of culture in other hazelnut species. As it is a fruity species, it forms the food of wildlife and contributes to biodiversity. Its fruits can be consumed directly as well as used in confectionery (Arslan, 2006). The leaves of *Corylus* species have been used in folk medicine in the treatment of eczema, rash, swelling, phlebitis, varicose veins and haemorrhoidal symptoms (Riethmüller et al., 2016). *C. colurna* leaves have been recorded to possess antibacterial activity against Gram-positive and -negative bacteria (Ceylan et al., 2013) and moderate to high antioxidant activity (Riethmüller et al., 2016). *C. colurna* leaves contain hydroxycinnamic acid derivatives, flavonoid derivatives and diarylheptanoids like quercetin, myricetin, 1-caffeoylquinic acid, 1,3-dicaffeoylquinic acid, catechin and kaempferol (Benov and Georgiev, 1994; Riethmüller et al., 2014; 2016). Benov and Georgiev (1994) isolated mixture of flavonoids from *C. colurna* leaves and reported strong antioxidant activity. Riethmüller et al. (2014) showed that phenolics in the leaves, bark, catkins and involucre of *C. colurna* had strong antioxidant activity. Especially, catkins of *C. colurna* displayed the highest antioxidant capacity, followed by the bark extracts. The catkins had the richest in total polyphenols, tannins, and flavonoids. Riethmüller et al. (2016) indicated that ethyl acetate and methanol extract of *C. colurna* leaves displayed moderate to high antioxidant activity that may be because of antagonistic interaction between the antioxidant components.

Drought, high temperature, salinity, heavy metals, UV radiation and nutritional insufficiency are examples of abiotic stressors that can increase the generation and accumulation of reactive oxygen species (ROS) in plants. Plants increase the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) to combat these oxidative stresses. They also produce more low-molecular-mass antioxidants such as phenolic compounds, α-tocopherol, ascorbate, and glutathione as non-enzymatic antioxidant activity (Selmar and Kleinwächter, 2013). The major enzyme in phenol synthesis is phenylalanine ammonia lyase (PAL) that is found in higher plants as a secondary metabolic pathway and it is a defense system in plants that is involved in the production of the phenolic compounds. Plants accumulate phenolic compounds in their tissues as an adaptive response to adverse environmental conditions and play a key role in the regulation of various environmental stresses (Thakur et al., 2019; Ulgen et al., 2021). Today, hazelnut tree leaf, which is a by-product of hazelnut harvest, is seen as a potential natural source of antioxidants (Amaral et al., 2010). During the processing of food and agricultural products, by-products rich in phenolics, which can be natural antioxidant sources, can be recycled. Studies are ongoing to extract and produce adequate amounts of natural antioxidants from most of these sources (Balasundram et al., 2006).

Changes in photosynthetic activity are considered as a stress sensor in advanced plants. Currently, the most modern and reliable technique for measuring photosynthetic activity is chlorophyll a fluorescence to reveal the effect of abiotic stress on photosystem (PS) II activity (Köseoğlu and Doğru, 2021). Genotypes that are drought tolerant under

drought stress have significantly higher values in terms of chlorophyll content, maximum quantum yield of PS II (Fv/Fm values) and variable fluorescence (Fv)/initial fluorescence (F0) parameters than sensitive ones (Rong-hua et al., 2006). *C. avellana* has been reported as a water stress-sensitive species and a decrease in photosynthetic activity, an early cessation of fruit growth and leaf fall, and an increased vulnerability to diseases have all been recorded in this species because of drought stress (Cristofori et al., 2012). We therefore first aimed to reveal the phenolic constituents of *C. colurna* leaves in six different populations and then to investigate the effect of water deficiency stress on the phenolic constituents and quantum efficiency (Fv/Fm) of photosystem II activity in six populations for the first time.

MATERIAL AND METHOD
MATERIJALI I METODE

Plant material – *Biljni materijal*

Scions were taken from naturally grown 6 different Turkish Hazelnut (TH) (*Corylus colurna* L.) populations in Turkey (Fig. 1) and 2 years old *C. colurna* seedlings were used as a rootstock (seeds from Pelitcik population) in 15x25cm polyetylene tube. Information about populations from which scions were taken was presented in Table 1. TH saplings were grafted with whip and tongue grafting method. Scions were provided from three different trees grown in their habitat for each population and six grafted saplings were obtained from each tree. All grafted saplings were nu-

Figure 1. Geographical location of the provenances Oğuzlar (1), Erenler (2), Merkeşler (3), Seben (SE), Güney Felakettin (GF) and Pelitcik (PL) from which the scions were taken.
Slika 1. Geografski položaj provenijencija Oğuzlar (1), Erenler (2), Merkeşler (3), Seben (SE), Güney Felakettin (GF) and Pelitcik (PL) iz kojih su uzete plemke.

Table 1. Information on Turkish hazelnut populations from which scions were taken.
Tablica 1. Informacija o populacijama medvjeđe lijeske iz kojih su uzete plemke.

Populations Populacija	Designations Oznake	Province Regija	Forest Managament Šumsko gospodarstvo	Altitude (m) Visina (m)
Oğuzlar	OG	Çorum	Çorum-Oğuzlar	1263
Erenler	ER	Ankara	Ankara-Nallıhan-Erenler	1521
Merkeşler	ME	Bolu	Bolu-Merkez-Çele	925
Seben	SE	Bolu	Bolu-Seben-Seben	1183
Güney Felakettin	GF	Bolu	Bolu-Merkez	1148
Pelitcik	PL	Bolu	Bolu-Merkez	1065

rtured in a greenhouse (environmentally compatible) found in Western Black Sea Forestry Research Institute, Bolu, Turkey for two years and then were taken to the greenhouse garden. Five years after the grafting process, the grafted young trees were taken back to the greenhouse condition for the experiment. There were 18 grafted young trees for each population. Soil texture in polyetylene tube was sandy clay loam with 58.81 % sand, 22.65 % clay and 18.53 % silt [pH: 7.92, calcium carbonate ($CaCO_3$): 5.95 %, organic matter: 1.93 %, nitrogen (N): 0.08%, potassium (K): 200.25 mg/L, phosphor (P): 0.87 mg/L, field capacity: 21.20, wilting point: 13.72 and electrical conductivity (EC): 0.47].

Irrigation regimes – *Režimi navodnjavanja*

Two different irrigation regimes (W1 and W2) were applied to the grafted young trees. There were nine saplings for each irrigation regime and population. After the determination of amount of water as field capacity (W1), 50% reduced amount was applied as W2 to create a water deficiency. Watering (500 ml) was made twice a week for W1 and once a week for W2. The experiment was started in the last week of May. Leaf sampling and chlorophyll fluorescence measurements were made in the second week of June and July.

HPLC-DAD analysis of phenolic constituents – *HPLC-DAD analiza sastava fenola*

Analysis was performed using HPLC system (VWR-Hitachi LaChrom Elite®) with a Hitachi L-2455 diode array detector (DAD), Hitachi L-2130 Pump, Hitachi L-2200 autosampler, Hitachi column oven L-2300 and Venusil XBP C18 column (Bonna-Agela Technologies, particle size 5 µm, 4.6 x 250 mm).Ten phenol standarts (gallic acid monohydrate, caffeic acid, rutin hydrate, luteolin-7-O-β-D glucoside, kaempferol, rosmarinic acid, myricetin, quercetin, coumarin and apigenin) (Sigma®) were performed in the analysis. They were dissolved in acetonitrile to obtain different concentrations (1, 5, 10, 20, 40, 60, 80, 100 and 200 mg/L) for standard curve. Fig. 2 shows the chromatogram of the used standards. HPLC operating conditions were performed using a gradient elution as in previously described method by Turker et al. (2021). Spectra data were recorded from to 200 to 400 nm during the entire run.

Quantum efficiency of photosystem II activity (Fv/Fm) – *Efikasnost kvantnog prinosa fotosustava II (Fv/Fm)*

Chlorophyll fluorescence in the leaves was determined by chlorophyll fluorometer device (HandyPEA+, Hansatech Instruments®). Midday (13:00) was preferred as the measurement time. With the clips attached to the leaves, each leaf was adapted to the dark for 10 minutes, and measurements were made with the saturating light sent after the adaptation. Parameters for the calculation of F_v/F_m values (quantum efficiency of PS II activity) were recorded [F_0: initial/minimal fluorescence; Fm: maximal fluorescence value; Fv: variable fluorescence (F_m-F_0); F_v/F_m: maximum quantum yield of PS II (quantum yield of net photosynthesis)].

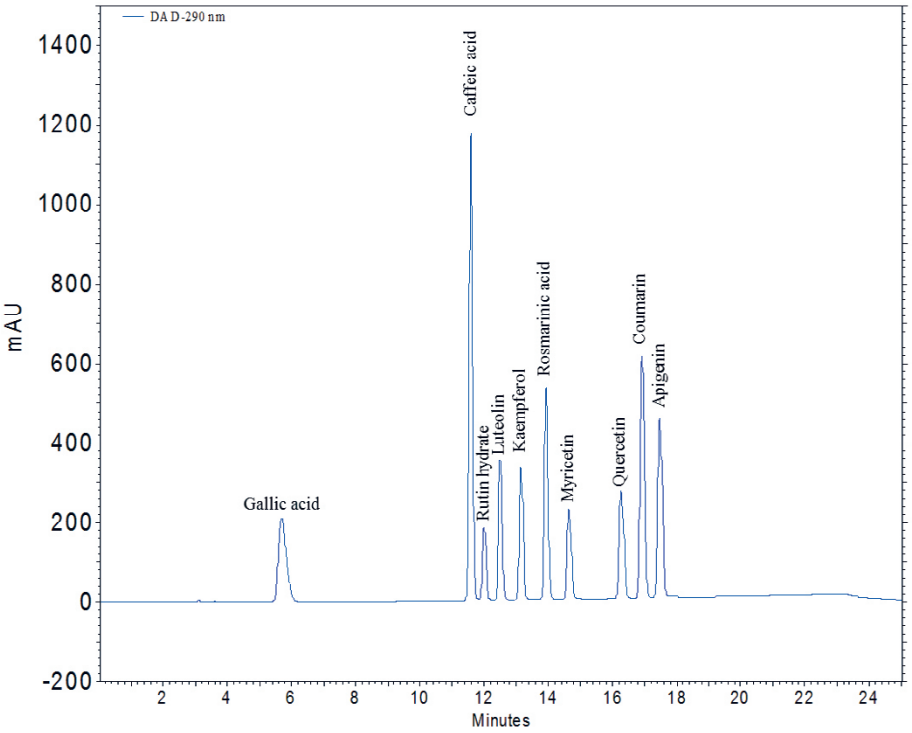

Figure 2. Chromatogram of the standards.
Prikaz 2. Kromatogram standarda.

Data analysis – *Analiza podataka*

All experiments were set up in a completely randomized design. Data analysis was performed using analysis of variance (ANOVA) and Duncan's multiple range tests using SPSS version 26 (SPSS Inc., Chicago, IL, USA). All data in the tables were presented as a mean number ± standard error (SE).

RESULTS AND DISCUSSION
REZULTATI I RASPRAVA

Obtained grafted young trees of TH carrying the genetic characteristics of their populations allowed us to apply the experiment under the same environmental conditions in our study. In this way, the phenolic content of each population and its response to water stress were evaluated under the same settings. Among the tested phenols, the amounts of rutin, kaempferol and luteolin were found to be the highest in TH leaves, respectively. Concerning overall phenolic content in stress free groups (W1) of six populations, PL and OG had the highest phenolic content in June and July, respectively (Table 2 and 3). Kaempferol, rutin, apigenin, quercetin and myricetin were found the most in PL population in stress free group (W1-June). The highest amounts of phenol (mg/g dry matter) were determined in the PL population with rutin and kaempferol as 2.77 mg/g and 2.75 mg/g, respectively. The lowest phenol amounts were generally found in GF population. Change in phenol amounts in all populations was monitored as 0.02-0.31 mg/g in apigenin, 0.02-0.36 mg/g in caffeic acid, 0.00-0.07 mg/g in gallic acid, 0.95-2.75 mg/g in kaempferol, 0.00-0.97 mg/g in luteolin, 0.00-0.10 mg/g in myricetin, 0.09-0.27 mg/g in quercetin, 0.00-0.13 mg/g in rosmarinic acid and 0.85-2.77 mg/g in rutin. Coumarin was not detected in all tested TH leaves (Table 2 and 3). Similar to our result, chromatographic analysis of isolated mixture of flavonoids from

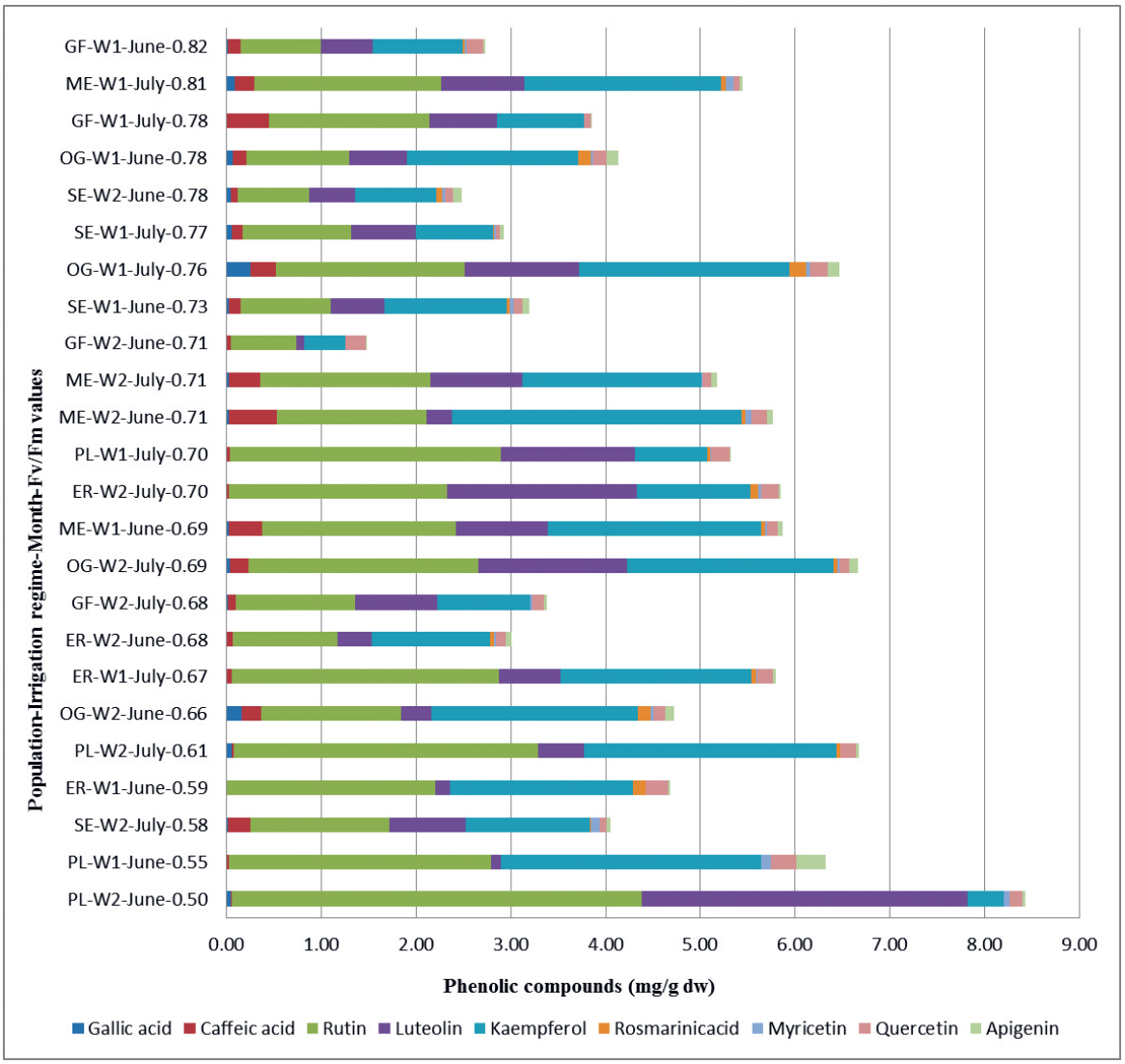

Figure 3. Descending order of Fv/Fm values (from 0.82 to 0.50) with populations, irrigation regimes and months.
Slika 3. Silazni poredak Fv/Fm vrijednosti (od 0.82 do 0.50) s populacijama, režimom navodnjavanja i mjesecima.

Table 2. Fluctuation of individual phenolic constituents concerning irrigation regimes in 6 populations in June. Pop: Populations; ND: not detected. Means with the same letter within columns are not significantly different at $P>0.05$.

Tablica 2. Fluktuacija pojedinačnih sastavnih dijelova fenola vezano uz režim navodnjavanja u 6 populacija u lipnju. Pop: populacija; nd: nije otkriveno. Srednje vrijednosti s istim slovom u stupcu nisu značajno različite u odnosu na $P>0.05$.

Phenolic compounds (mg/g dry weight) Spojevi fenola (mg/g suhe tvari)											
June Lipanj	Pop Pop	Apigenin Apigenin	Caffeic acid Kofeinska kiselina	Gallic acid Galna kiselina	Kaempferol Kaempferol	Luteolin Luteolin	Myricetin Miricetin	Quercetin Kvercetin	Rosmarinic acid Rozmarinska kiselina	Rutin Rutin	OVERALL HENOLIC CONTENT UKUPNI SADRŽAJ FENOLA
	OG	0.117 ± 0.015^{b}	0.140 ± 0.040^{cd}	0.070 ± 0.029^{ab}	1.810 ± 0.064^{bcde}	0.607 ± 0.147^{bc}	0.027 ± 0.009^{bc}	0.137 ± 0.009^{abcd}	0.127 ± 0.038^{a}	1.080 ± 0.040^{de}	**0.46**
	ER	0.017 ± 0.003^{ef}	ND	ND	1.930 ± 0.497^{bcd}	0.157 ± 0.032^{cd}	ND	0.240 ± 0.092^{ab}	0.130 ± 0.040^{a}	2.200 ± 0.664^{bc}	**0.52**
W1	ME	0.050 ± 0.000^{cdef}	0.357 ± 0.055^{b}	0.020 ± 0.006^{ab}	2.250 ± 0.566^{abc}	0.970 ± 0.433^{b}	0.020 ± 0.006^{bc}	0.120 ± 0.040^{bcd}	0.037 ± 0.020^{b}	2.037 ± 0.574^{bcd}	**0.65**
	SE	0.070 ± 0.012^{cd}	0.127 ± 0.066^{cd}	0.017 ± 0.009^{ab}	1.290 ± 0.006^{cdef}	0.570 ± 0.156^{bc}	0.040 ± 0.006^{bc}	0.090 ± 0.017^{cd}	0.027 ± 0.009^{b}	0.947 ± 0.090^{de}	**0.35**
	GF	0.023 ± 0.012^{ef}	0.140 ± 0.006^{cd}	0.010 ± 0.010^{b}	0.947 ± 0.119^{def}	0.543 ± 0.206^{bc}	0.023 ± 0.023^{bc}	0.180 ± 0.086^{abcd}	0.023 ± 0.023^{b}	0.850 ± 0.072^{e}	**0.30**
	PL	0.310 ± 0.000^{a}	0.020 ± 0.000^{d}	ND	2.750 ± 0.000^{ab}	0.100 ± 0.015^{cd}	0.100 ± 0.000^{a}	0.270 ± 0.000^{a}	ND	2.770 ± 0.000^{b}	**0.70**
OVERALL MEAN SREDNJA VRIJEDNOST		**0.10**	**0.13**	**0.02**	**1.83**	**0.49**	**0.04**	**0.17**	**0.06**	**1.65**	
	OG	0.087 ± 0.038^{bc}	0.203 ± 0.092^{c}	0.163 ± 0.149^{a}	2.177 ± 0.524^{abc}	0.317 ± 0.083^{cd}	0.040 ± 0.021^{bc}	0.120 ± 0.021^{bcd}	0.130 ± 0.023^{a}	1.477 ± 0.481^{cde}	**0.52**
	ER	0.057 ± 0.012^{cde}	0.070 ± 0.029^{cd}	0.003 ± 0.003^{b}	1.247 ± 0.163^{cdef}	0.357 ± 0.162^{cd}	0.020 ± 0.006^{bc}	0.107 ± 0.032^{bcd}	0.037 ± 0.017^{b}	1.103 ± 0.364^{de}	**0.33**
W2	ME	0.060 ± 0.006^{cde}	0.510 ± 0.104^{a}	0.017 ± 0.009^{ab}	3.050 ± 0.635^{a}	0.270 ± 0.058^{cd}	0.060 ± 0.029^{ab}	0.167 ± 0.032^{abcd}	0.050 ± 0.029^{b}	1.580 ± 0.393^{cde}	**0.63**
	SE	0.090 ± 0.006^{bc}	0.083 ± 0.003^{cd}	0.040 ± 0.023^{ab}	0.847 ± 0.066^{ef}	0.490 ± 0.040^{bcd}	0.027 ± 0.015^{bc}	0.077 ± 0.015^{d}	0.070 ± 0.006^{ab}	0.747 ± 0.251^{e}	**0.28**
	GF	0.010 ± 0.000^{f}	0.050 ± 0.000^{cd}	ND	0.430 ± 0.000^{f}	0.080 ± 0.000^{cd}	ND	0.220 ± 0.000^{abc}	ND	0.690 ± 0.000^{e}	**0.16**
	PL	0.033 ± 0.000^{def}	0.017 ± 0.000^{d}	0.041 ± 0.000^{ab}	0.382 ± 0.000^{f}	3.440 ± 0.000^{a}	0.062 ± 0.00^{ab}	0.142 ± 0.000^{abcd}	ND	4.316 ± 0.000^{a}	**0.94**
OVERALL MEAN SREDNJA VRIJEDNOST		**0.06**	**0.16**	**0.04**	**1.36**	**0.83**	**0.04**	**0.13**	**0.05**	**1.65**	

Table 3. Fluctuation of individual phenolic constituents concerning irrigation regimes in 6 populations in July. Pop: Populations; ND: not detected. Means with the same letter within columns are not significantly different at $P>0.05$.

Tablica 3. Fluktuacija pojedinačnih sastavnih dijelova fenola vezano uz režim navodnjavanja u 6 populacija u lipnju. Pop: populacija; nd: nije otkriveno. Srednje vrijednosti s istim slovom u stupcu nisu značajno različite u odnosu na P>0.05.

Phenolic compounds (mg/g dry weight)											
July Srpanj	Pop Pop	Apigenin Apigenin	Caffeic acid Kofeinska kiselina	Gallic acid Galna kiselina	Kaempferol Kaempferol	Luteolin Luteolin	Myricetin Miricetin	Quercetin Kvercetin	Rosmarinic acid Rozmarinska kiselina	Rutin Rutin	OVERALL PHENOLIC CONTENT UKUPNI SADRŽAJ FENOLA
	OG	0.127±0.012^{a}	0.2667±0.042^{ab}	0.247±0.055^{a}	2.217±0.498^{ab}	1.210±0.060^{ab}	0.040±0.00^{abc}	0.183±0.054^{ab}	0.183±0.095^{a}	1.993±0.212^{ab}	**0.72**
	ER	0.030±0.021^{cd}	0.0567±0.028^{ab}	0.003±0.003^{c}	2.010±0.705^{abc}	0.657±0.244^{ab}	0.013±0.009^{bc}	0.170±0.061^{ab}	0.053±0.022^{b}	2.807±1.077^{ab}	**0.64**
W1	ME	0.040±0.012^{cd}	0.200±0.110^{ab}	0.090±0.0236^{b}	2.077±0.130^{abc}	0.870±0.040^{ab}	0.077±0.026^{ab}	0.057±0.003^{ab}	0.050±0.029^{b}	1.977±0.061^{ab}	**0.61**
	SE	0.047±0.017^{cd}	0.1067±0.047^{ab}	0.063±0.058^{bc}	0.813±0.030^{bc}	0.687±0.236^{ab}	0.020±0.010^{bc}	0.043±0.009^{b}	0.010±0.010^{b}	1.137±0.410^{b}	**0.33**
	GF	0.007±0.007^{d}	0.4467±0.358^{a}	0.003±0.003^{c}	0.923±0.235^{bc}	0.710±0.246^{ab}	ND	0.083±0.042^{ab}	ND	1.690±0.495^{ab}	**0.43**
	PL	0.007±0.003^{d}	0.0333±0.003^{b}	ND	0.760±0.208^{c}	1.420±0.664^{ab}	0.007±0.003^{bc}	0.197±0.026^{a}	0.030±0.017^{b}	2.860±0.531^{ab}	**0.59**
OVERALL MEAN SREDNJA VRIJEDNOST		**0.05**	**0.19**	**0.07**	**1.47**	**0.93**	**0.03**	**0.12**	**0.05**	**2.08**	
	OG	0.087±0.009^{b}	0.2033±0.102^{ab}	0.033±0.024^{bc}	2.177±0.424^{abc}	1.573±0.585^{ab}	0.023±0.014^{bc}	0.100±0.021^{ab}	0.043±0.018^{b}	2.430±0.741^{ab}	**0.74**
	ER	0.020±0.012^{cd}	0.0233±0.009^{b}	ND	1.197±0.350^{bc}	2.003±0.909^{a}	0.027±0.022^{bc}	0.193±0.096^{ab}	0.083±0.044^{b}	2.310±0.777^{ab}	**0.65**
W2	ME	0.057±0.009^{bc}	0.3267±0.061^{ab}	0.020±0.011^{bc}	1.890±0.341^{abc}	0.967±0.413^{ab}	0.010±0.006^{bc}	0.100±0.023^{ab}	ND	1.797±0.078^{ab}	**0.58**
	SE	0.040±0.010^{cd}	0.240±0.032^{ab}	0.007±0.007^{bc}	1.307±0.286^{abc}	0.803±0.169^{ab}	0.103±0.061^{a}	0.070±0.036^{ab}	0.013±0.003^{b}	1.467±0.385^{ab}	**0.45**
	GF	0.027±0.007^{cd}	0.090±0.046^{ab}	0.007±0.003^{bc}	0.983±0.295^{bc}	0.863±0.438^{ab}	0.027±0.018^{bc}	0.120±0.015^{ab}	ND	1.257±0.423^{b}	**0.38**
	PL	0.027±0.017^{cd}	0.0167±0.009^{b}	0.063±0.009^{bc}	2.670±0.849^{a}	0.477±0.211^{b}	ND	0.173±0.055^{ab}	0.037±0.020^{b}	3.210±0.618^{a}	**0.74**
OVERALL MEAN SREDNJA VRIJEDNOST		**0.05**	**0.15**	**0.02**	**1.71**	**1.11**	**0.03**	**0.13**	**0.03**	**2.08**	

C. colurna leaves contained mainly quercetin and myricetin (Benov and Georgiev, 1994). Riethmüller et al. (2014) investigated the phenolic compounds and antioxidant activities in different parts of the *C. colurna*. In parallel to our results, they detected quercetin, kaempferol and myricetin in methanolic leaf extract. Some phenolic compounds such as 3-, 4- and 5-caffeoylquinic acids, caffeoyltartaric acid, p-coumaroyltartaric acid, myricetin 3-rhamnoside, quercetin 3-hexoside, quercetin 3-rhamnoside, kaempferol 3-rhamnoside and derivatives of p-coumaric acid, myricetin and quercetin were identified in the cultivars of *C. avellana* leaves by HPLC-DAD (Amaral et al., 2005; Oliveira et al., 2007; Amaral et al., 2010).

Water deficiency stress (W2) was applied in June and July having average temperatures as 16.2 ºC and 19.2, respectively. Generally, it was observed that water stress generated by halved irrigation regime (W2) elevated the phenolic constituents in both months, and the highest increase was observed in July due to the higher average temperature. Among the populations, PL was the most affected by water stress, and overall phenolic content was enhanced from 0.70 mg/g to 0.94 mg/g in June. Noticeable increase in individual phenol was obtained with luteolin level (34.4 times) in June in PL population (Table 2 and 3; Fig. 3). A steady increase was monitored with rutin level in OG population and with luteolin level in ER population from June to July with water stress. In addition, halved irrigation regime affected and elevated rutin level significantly in June and July in PL population (Table 2 and 3; Fig. 3).

Enhancements were observed significantly with water stress with most of the tested phenols. For example, rutin increased from 2.77 mg/g to 4.32 mg/g in PL population (56% rise) and from 1.08 mg/g to 1.48 mg/g in OG population (37% rise) in June. Similarly, 2.33-fold increase with rosmarinic acid in SE population in June, and 3-fold increase with apigenin in GF and PL populations in July were observed. Gallic acid showed 2.29 times and 2.00 times increase in OG and SE populations in June, respectively. Caffeic acid was elevated 1.42 times in OG and ME populations in June. Myricetin showed 3-fold rise in ME population in June and 5-fold rise in SE population in July. Kaempferol increased 1.36 times in ME and 1.20 times in OG in June, and 1.62 times in SE and 3.51 times in PL populations in July. Luteolin was ascended 3.03 times in ER and 1.30 times in OG in July (Table 2 and 3; Fig. 3).

Photosystem quantum efficiency (photosynthetic efficiency) in chlorophyll fluorescence measurements (Fv/Fm ratio) were also performed to determine the effect of water deficiency stress on photosystem II in TH leaves. The Fv/Fm ratio has long been regarded as a sensitive indicator of plant photosynthetic performance and as a numerical value in many developed plants is around 0.83. Decreases in this index indicate a decrease in PS II efficiency, i.e. photoinhibition. The main effect of abiotic stress is that PS II becomes inclined to photoinhibition (Rong-hua et al., 2006; Köseoğlu and Doğru, 2021). Overall, drought stress-induced photoinhibition (W2) was observed as a significant reduction in the maximum quantum efficiency (Fv/Fm) of PS II in TH leaves comparing with control (W1) (Fig.3). In June, the Fv/Fm values varied between 0.55-0.82 according to the populations in W1 irrigation, and this value decreased to 0.50-0.78 with W2 irrigation. In July, values between 0.67-0.82 in W1 irrigation decreased to 0.58-0.71 values in W2 treatment (Fig. 3). These results showed that water deficiency created a stress in the trees and it can be interpreted that this stress caused an increase in some phenol levels and populations. Similarly, Galle et al. (2007) determined photosynthetic performance in young pubescent oak (*Quercus pubescens*) trees during drought stress and reported the decrease in Fv/Fm values in stress-treated trees comparing with control. Wang et al. (2018) created drought stress in young apple tree with less irrigation and showed the decrease in Fv/Fm value to 0.375. However, Fv/Fm ratios in other words photosynthetic performance differed in each population in our study. In connection with this result, Rong-hua et al. (2006) reported that under drought stress in Barley, Fv/Fm values in drought tolerance genotypes were considerably greater than those in drought sensitive genotypes. The lowest Fv/Fm ratio (0.50) was obtained with PL population in June in our study (Fig. 3). The fact that the Fv/Fm value that is an indicator of photosynthetic activity was low in PL population that has the highest phenolic level, showed that this population was the most affected by drought stress. On the other hand, Fv/Fm values of GF population (W1) were higher than other populations. In addition, this population had the lowest overall phenolic content and interestingly, applied water stress in both months (W2) in this population did not cause an increase in phenolic content. This population should be more tolerant to water stress than other populations and able to cope with abiotic stress more easily without giving stress-induced increase in phenolic substances. Similarly, SE population with the second lowest phenolic content had higher Fv/Fm ratios. In SE population, water stress application in June could not increase the phenolic content and even the phenolic content decreased. It can be deduced that SE population is second stress-resistant population after GF population.

CONCLUSION
ZAKLJUČAK

Among the tested populations, PL had the highest phenolic content, and their levels enhanced at most with water deficiency stress. Rutin, kaempferol and luteolin were determined as the most abundant phenols in all populations of Turkish hazel leaves and remarkable increases were observed with drought stress in luteolin and rutin levels in PL

population in June. The lowest phenolic content was observed in GF and SE populations, and drought stress applications did not increase their phenolic content overall. It was determined that the phenol levels of TH populations were different and the populations reacted differently with water deficiency concerning phenolic constituents and photosynthetic efficiency (Fv/Fm ratio). GF and SE populations may be more tolerant, and PL population may be more sensitive to drought stress when comparing Fv/Fm values among populations. Future studies should be focused on antioxidant enzyme activities like SOD, CAT and PAL to clarify the endurance of populations against abiotic stresses. It was observed that TH leaves, byproduct of hazelnut harvesting, have potential as a source of natural antioxidants in nutraceutical industry and can be improved with drought stress depending on genetic differences.

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SAŽETAK

Medvjeđa lijeska (*Corylus colurna* L.) jedna je od prirodno rasprostanjenih vrsta lijeske u Turskoj. Lako se može razlikovati od drugih vrsta zahvaljujući svojoj visini i promjeru debla. Vrlo često se koristi za pošumljavanje terena sklonih eroziji zbog snažnog korijenja i skromnih ekloških zahtjeva. Njezino lišće i plodovi sadrže mnogo tvari koje se koriste u medicinske svrhe. Lišće medvjeđe ljeske ima snažno antioksidativno djelovanje zbog visokog sadržaja fenola. Cilj ovoga istraživanja bio je utvrditi sadržaj fenola u lišću šest populacija medvjeđe lijeske te procijeniti učinak sušnoga stresa na sadržaj fenola i kvantnu učinkovitost fotosustava II. Istraživane biljke proizvedene su cijepljenjem nakon sakupljanja plemki iz šest populacija medvjeđe lijeske (Oğuzlar, Erenler, Merkeşler, Seben, Güney Felakettin i Pelitcik). Istraživanje je provedeno u stakleničkim uvjetima kad su kalemljene biljke bile stare 7 godina. Tijekom pokusa (lipanj i srpanj), primijenjena su dva različita načina navodnjavanja (W1: tlo je navodnjavano do poljskog vodnog kapaciteta; W2: tlo je navodnjavano s 50 % manje vode u odnosu na W1 način navodnjavanja). Nakon primjene dva različita režima navodnjavanja, lišće je uzorkovano u lipnju i srpnju. Nakon toga je osušeno, ekstrahirano s metanolom te kvantitativno analizirano s ciljem utvrđivanja njegova fenolnog sastava (galna kiselina monohidrat, kofeinska kiselina, rutin, hidrat, luteolin-7-O-β-D glukozid, kaempferol, rozmarinska kiselina, miricetin, kvercetin, kumarin i apigenin) uz korištenje tekuće kromatografije visoke djelotvornosti (HPLC) i detektora s nizom dioda (DAD). Rutin, kaempferol i luteolin bili su dominantni fenoli u ekstraktu metanola iz lišća medvjeđe lijeske. Populacija Pelitcik bila je značajan izvor rutina i kaempferola u lipnju, a W2 režim navodnjavanja značajno je povećao razine oba fenola u srpnju. Isto tako, najviši sadržaj fenola zabilježen je u populaciji Pelitcik u lipnju, a W2 režim navodnjavanja značajno je povećao ukupan sadržaj fenola u lipnju i srpnju. Također je utvrđeno u kojoj mjeri nedostatak vode utječe na kvantnu učinkovitost fotosustava II (Fv/Fm) . Općenito, Fv/Fm vrijednosti opadaju s nedostatkom vode. Ova studija pokazala je da nedostatak vode kod medvjeđe ljeske potiče produkciju fenola te da se na taj način može osigurati prirodni izvor fenola koji se može koristiti u farmaceutskoj i prehrambenoj industriji.

KLJUČNE RIJEČI: *Corylus colurna* L, kvantna učinkovitost, fenol, medvjeđa lijeska, nedostatak vode