



Determination of Juglone Content of Some Walnut cultivars and the Light Fastness of Dyestuffs Obtained from These Cultivars in the Dyeing of Cotton

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Abstract: This study was carried out in 2020 to determine the light fastness in dyeing cotton fabrics to test the juglone values in the content of domestic and foreign walnut cultivars as well as the use of these cultivars in natural dyeing. According to the results of the study, the light fastness test results obtained after dyeing the dyestuffs obtained from domestic and foreign cultivars of *Juglans regia* L. walnut species on cotton fabrics were calculated with the lowest 2 values in Serr and Şen 2 walnut cultivars, while the highest 3 were Maraş 18, Şebin, Fernette, and Fernor cultivars. *J. regia* juglone analysis results obtained from domestic and foreign cultivars of walnut species, from lowest to highest, were determined in Şen 2, Maraş 18, Fernette, Şebin, Maraş 12, Pedro, Serr, Sundland, Bilecik, Chandler, Franquette and Fernor cultivars, respectively. In addition, according to the results of the percentage similarity analysis, in which the similarities between the juglone contents of domestic and foreign walnut cultivars were calculated, it was determined that domestic and foreign cultivars were generally grouped among themselves and these groups had higher similarities among themselves. When the tested juglone amounts of domestic and foreign walnut cultivars included in the experiment were compared statistically, it was found that Fernor (0.2595) and Franquette (0.2581) cultivars were not statistically different, but together with Chandler (0.2538), the juglone amounts of these cultivars were statistically significantly different between almost all cultivars.

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1. Introduction

Türkiye is one of the rare countries that has 3 hotspots (Hotspot: priority protection areas) (Aydın, 2005 and 2006; Aydın and Kazak, 2010; Aydın, 2011 and 2018; Aydın and Karaca, 2009 and 2011). For this reason, plant diversity and endemism in Türkiye, which has many endemic species, is quite high compared to European countries (Şekeroğlu and Aydın, 2002; Yaşar et al., 2003; Aydın et al., 2005; Lillig and Aydın, 2006; Dinç et al., 2015; Aydın and Şen, 2020). Walnuts are among the fruit species grown in our country; It shows a wide natural distribution area from the Carpathian Mountains to Türkiye, Iran, Iraq, Afghanistan, Southern Russia, India, Manchuria and Korea. There are 25 walnut

species that are widely accepted in the world, and the most common species grown in Türkiye is *Juglans regia* L. (Akça, 2009; Şen, 2011).

Walnut production in Türkiye is provided from trees grown from seeds and orchards established with grafted saplings in recent years. The presence of walnut trees in Türkiye creates a valuable population in terms of genetic material (Akça, 2009).

Unlike other fruit species, walnuts contain juglone (5-hydroxy-1,4-naphthalenedione). This chemical is found in different amounts in different organs of the plant and shows different effects. There are several studies on the amount of juglone as well as changes in its quantity with time and other factors (Tekintaş et al., 1988; Karadağ, 2007; Turan, 2008; Cosmulescu et al., 2011; Yurtçu, 2014).

Moreover, the fruit, leaves and shells of walnut plant are a type of fruit that has a wide area of use in the paint and cosmetics industry. Considering the richness of species in our country, the number of scientific studies on the possibilities of using especially domestic and foreign walnut cultivars in juglone and natural dyeing is still rare. Present study was aimed to determine the light fastness of cotton fabrics in order to test the juglone values in the content of domestic and foreign walnut cultivars and the use of these cultivars in natural dyeing.

2. Material and Methods

2.1. Material

The study was carried out in 2020. Some domestic cultivars belonging to *Juglans regia* walnut species such as Bilecik, Maraş 12, Maraş 18, Şebin, Şen 2 (Sütyemez, 2016; Sütyemez et al., 2019), as well as some foreign cultivars including Chandler, Fernet, Fernor, Franquette, Pedro, Serr and Sundland were selected for trial. The green dry shells of the domestic and foreign walnut cultivars selected were used as material. These materials were obtained from ten-year-old walnut trees grown under the same conditions in Eğirdir district of Isparta province. The green peels separated from the fruit after harvest were dried in the natural environment with shade and ventilation. The peeled walnut shells were analyzed after they were ground in the mill. Organic knitted cotton fabric was used to determine the light fastness values of domestic and foreign walnut cultivars mentioned above. The light fastness values of the samples obtained after dyeing the natural dyestuffs of domestic and foreign walnut cultivars on cotton fabric were carried out in Türkiye Instrumental Analysis Laboratory (CHT).

2.2. Method

2.2.1. Painting process

The dyeing processes of natural dyestuffs obtained from domestic and foreign walnut cultivars on cotton fabrics were carried out in the Paint Workshop of the Faculty of Fine Arts, Süleyman Demirel University, Türkiye. Because walnut is a direct dye, it has not been subjected to a pre-dyeing process (mordant). The dyestuffs were obtained by grinding the shell part of the walnut fruit into powder. The dyestuffs obtained from walnut cultivars were added separately after the solution, in which 2.5 liters of water was added into a 3-liter beaker, started to boil slightly, and was boiled at 85 °C for one hour with continuous stirring. After the dyeing process was completed, the fabrics were left to cool in the solution overnight. The fabrics were then rinsed with cold water and dried in a shaded and airy environment. These processes were applied to all domestic and foreign walnut cultivars on cotton fabrics and a total of 12 dyeing were carried out (see Table 1 for details).

Table 1. Dyeing recipe of dyestuff obtained from domestic and foreign cultivars of *Juglans regia* L. Walnut on cotton fabrics

Paint Equipment	<i>Juglans regia</i> L. (1753)
Amount Of Paint	20 gr
Mordant Type	-
Mordant Amount	-
Solution Ratio	2.5 lt
pH	7.6
Boiling Temperature	85 °C
Boiling Time	60 min
Painting Type	direct painting
Fabric Used	organic cotton
Painting Process	Add 2.5 liters of water into a 3 liter beaker. 20 gr walnut fruit peel is weighed and put into this solution. After the solution starts to boil slightly, 20 g of cotton fabric is wetted and thrown into the solution and boiled at 85°C for one hour with constant stirring. After the dyeing process is finished, the fabrics are kept in the solution for 1 night. The next day, the fabrics are removed, rinsed and left to dry.

2.2.2. Determination of light fastness

Light fastness measurements were made using the Procedure 3 method on the Atlas Xenotest machine and 8 replications on the D 65 light source and evaluated.

2.2.3. Juglone analyses

2.2.3.1. Sampling and extraction

Juglone was analysed using the method reported by Tekintaş et al. [20]. In order to determine the Juglone content of the seedlings, two-gram samples were weighted from the fruit peels, with 3 replications on the basis of variety. The samples were extracted in 50 ml petroleum ether (40-60%) for a total of 24 hours, including 6 hours in a shaker. For the analysis, the standard curve was prepared as follows.

2.2.3.2. Standard curve preparation

First, crystal juglone stock solution was prepared and this solution was then diluted to different concentrations (0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, 0.55, and 0.60 mg). Briefly, 500 ml of 50 ppm stock solution was prepared by dissolving 0.025 g of crystal juglone in 500 ml of petroleum ether. Samples taken from the stock solution were made up to 25 ml with petroleum ether (Table 2).

The absorbance values of the samples taken from the prepared extracts were measured at 410 nm. A linear relationship emerged between absorbance values and the amount of crystal juglone. The regression equation of the relationship between the determined absorbance values and the amount of crystal juglone in the solutions was created.

$$(y = 0.0455 \cdot X + 0.0429), (R^2 = 0.9945) \quad (1)$$

Crystal juglone values were calculated according to the formula below:

$$\text{Crystal juglone (mg/25ml)} = (0.0455 \cdot \text{absorbance value}) + 0.0429 \quad (2)$$

After preparing the standard curve, 5 ml samples taken from the extracts were completed to 25 ml with petroleum ether and absorbance values were determined at 410 nm wavelength in a spectrophotometer. By adapting these values to the standard curve prepared with crystal juglone, the juglone content of seedling shells was determined with the equation (iii).

$$\text{Juglone (mg/g)} = ((0.0455 \cdot \text{absorbance value}) + 0.0429) \cdot 5 \quad (3)$$

Juglone analysis studies were carried out in Karamanoğlu Mehmetbey University, Central Research Laboratory.

Table 2. Standard curvature values for Juglone analysis

Standard Curb Sequence No:	Amount of juglone required for standard curd (mg)	Quantity taken from stock solution (ml)	Amount completed with Petroleum Ether (ml)
1	0.05	1	25
2	0.10	2	25
3	0.15	3	25
4	0.20	4	25
5	0.25	5	25
6	0.30	6	25
7	0.35	7	25
8	0.40	8	25
9	0.45	9	25
10	0.50	10	25
11	0.55	11	25
12	0.60	12	25

2.2.4. Evaluation of data

2.2.4.1. Light fastness

As a result of the application of dyestuffs of domestic and foreign cultivars of *Juglans regia* walnut species on cotton fabrics, the "Light Fastness" values were entered into the Microsoft Excel program and X and Y graphic distributions were created.

2.2.4.2. Juglone analysis

The differences between the juglone content of domestic and foreign cultivars of *J. regia* walnut species were evaluated by ANOVA-LSD test. The data obtained were calculated in the JMP Statistics program. In addition, the similarities and differences between the amount of Juglone were subjected to classification analysis. Classification analysis was calculated using the Multi-Variate Statistical Package (MVSP) 3.11c program (Kovach, 1999). The distances between the amounts of juglone were measured with percent similarity.

3. Results and Discussions

3.1. Determination of light fastness

The light fastness test results obtained after dyeing the dyestuffs obtained from domestic and foreign cultivars of *J. regia* walnut species on cotton fabrics were calculated with the lowest 2 values in Serr and Şen 2 walnut cultivars, while the highest 3 values were in Maraş 18, Şebin, Fernette and Fernor cultivars. The light fastness results stained with Bilecik, Maraş 12, Chandler, Franquette, Pedro and Sundland cultivars were found in the range of 2.5 to these two values (Figure 1 and Table 3).

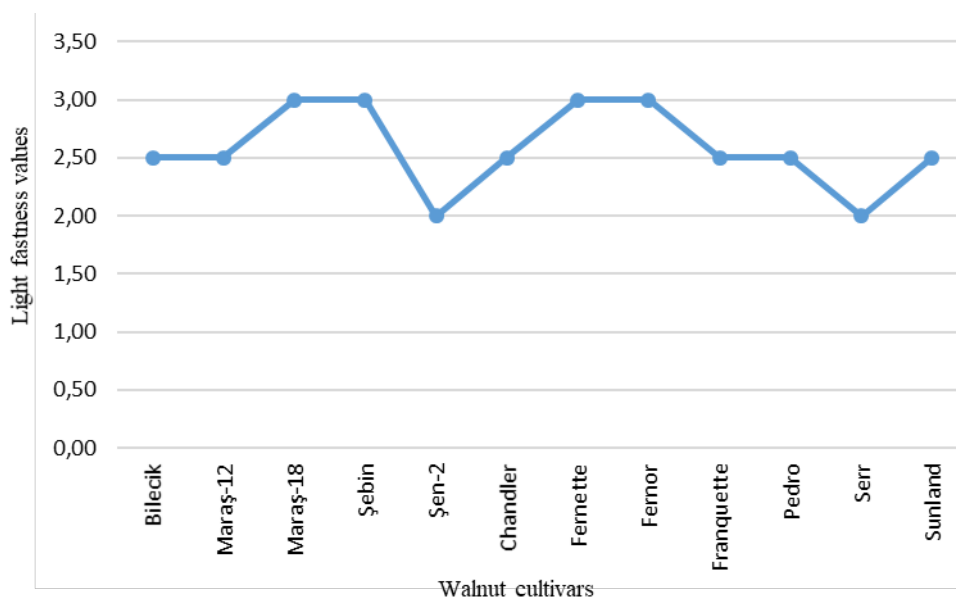


Figure 1. Light fastness values obtained after dyeing materials obtained from domestic and foreign walnut cultivars on cotton fabrics.

Gözütok (2017) determined the light fastness value obtained from walnut shell in the range of 5.5 in the application without mordant. In the study, it was also mentioned that the walnut species included in the experiment was *J. regia*, but walnut cultivars were not mentioned. It can be said that the difference between the light fastness values is also caused by the dyestuff applied on the woolen fabric samples (Gözütok, 2017). In the study where light fastness values were measured, it was determined that the light fastness results painted on the skin by applying a single dose were between 2 and 4, and the light fastness results painted on the skin by applying multiple doses were between 2 and 4 (Arabacıoğlu, 2017). Ghaheh et al. (2012) emphasized that the light fastness value varies between 3 and 4 as a result of the dyeing process without pre-dyeing (without mordant). Ghaheh et al. (2012) and our results were found to be similar, however, walnut cultivars were not specified in this study, and only *J. regia*, which is a walnut type, was specified. Eser et al. (2015) stated in their study that the light fastness values of the dyestuffs obtained from walnut leaves by using different mordant substances changed between 1 and 6, and the light fastness values of the dyestuffs applied without using mordant changed between 4 and 5. The difference between the results obtained in our study and the results of Eser et al. (2015) obtained the natural dyestuff from walnut leaves.

Mirjalili et al. (2010) calculated the light fastness values of fabrics treated with walnut natural dyestuff on silk fabrics boiled in ethanol and water as 6 and 5, respectively. The high light fastness values obtained in the mentioned study may be due to the silk fabric. Sharma and Grover (2011) stated in their study that the light fastness values of walnut natural dyestuff treated with different mordant substances on cotton fabrics vary between 6 and 8. The light fastness values obtained in our study were obtained as a result of application without mordant. Sadeghi-Kiakhani et al. (2019) measured the light fastness values in the range of 4 to 8 on fabrics dyed with walnut natural dyestuff using different mordant substances. Khan et al. (2016) found all the light fastness values measured as a result of the application of walnut natural dyestuff on wool yarn with and without mordant method as 5. Zakaria et al. (2017) reported in their study that the light fastness values measured as a result of the application of walnut natural dyestuff on knitted cotton fabrics before, during and after mordanting generally vary in the range of 2-3. Doğan-Sağlamtimur et al. (2017) determined that the light fastness values obtained as a result of the application of the walnut natural dye obtained from the dry and wet shells of Kaman variety on the leather were 2 in the control group and in the wet shell test, and 3.5 in the dry shell test. As it can be understood from previous studies, it can be said that light fastness is affected by many factors such as the difference in the material to which the natural dyestuff is applied, the properties of the plant part used in dyeing, applications with and without mordant, among the reasons for the different results of light fastness values.

3.2. Juglone analysis

Juglans regia juglone analysis results obtained from domestic and foreign cultivars of walnut species, from lowest to highest, were determined in Şen 2, Maraş 18, Fernette, Şebin, Maraş 12, Pedro, Serr, Sundland, Bilecik, Chandler, Franquette and Fernor cultivars, respectively (Figure 2). Analysis results showed that the juglone content values of foreign origin cultivars were higher than domestic cultivars. However, the domestic Bilecik walnut variety was among the juglone values obtained from foreign cultivars with a value of 0.2536. The measured juglone values of all other domestic cultivars were generally lower than those obtained from foreign cultivars. It is thought that the difference between the amount of juglone between domestic and foreign cultivars may be due to the genetic structure between cultivars and the different foliation periods and harvest times of many foreign cultivars. Chandler, Franquette and Fernor foreign walnut cultivars are the last leafing cultivars compared to other walnut cultivars tested. The highest amount of juglone was calculated in these cultivars. This may be due to late leafing. Among the foreign cultivars, since Pedro is a mid-leafing variety, the amount of juglone is between late leafing foreign cultivars and early leafing domestic cultivars. In addition, among the reasons for measuring the amount of juglone between cultivars with different values, it is a fact that although values such as location, tree age, soil characteristics were taken as homogeneous in the experiment, the juglone values may be affected by some ecological factors.

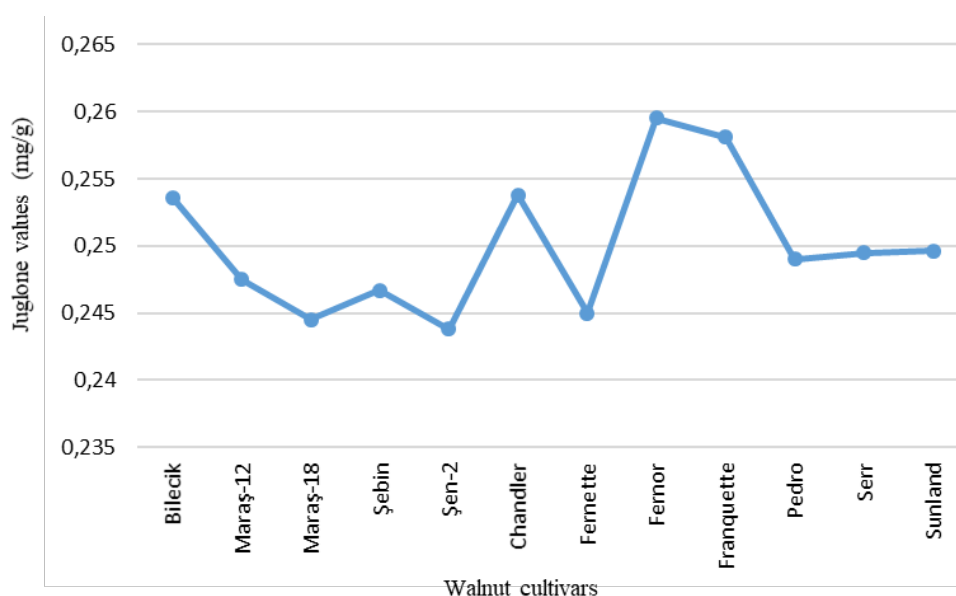


Figure 2. Juglone values calculated from domestic and foreign walnut cultivars.

Uğurlu et al. (2019) examined the juglone amounts of walnuts harvested in different periods and grown in different locations in their study and determined it in the range of 0.132 to 0.278 mg g⁻¹, which are different than the results of our study. These results are reasonable due to the fact that regional and variety differences. Cosmulescu et al. (2011) evaluated the juglone content between cultivars in their study. In the study, the average of the juglone values measured from the leaf was found to be 0.4879±0.028 mg g⁻¹, and the average of the juglone values measured from the green bark was 0.4877±0.012 mg g⁻¹. The measured juglone values of the Franquette variety, which is the common variety with our study, were found to be 0.1521±0.043 and 0.4034±0.123 mg g⁻¹ in the leaf and green fruit peel, respectively. Although the juglone values were measured from the dry bark in our study, it is among the values measured from the leaf and green bark in the study of Cosmulescu et al. (2011). In the study of Sesli (2016), the juglone values calculated from the fruits of different walnut cultivars vary between 0.22 and 0.29 mg g⁻¹. When the walnut cultivars used in Sesli (2016) were compared with our study results, Bilecik (0.292 mg g⁻¹), Chandler (0.258 mg g⁻¹), Fernor (0.268 mg g⁻¹), Pedro (0.277 mg g⁻¹) and Serr (0.244 mg g⁻¹) were found to be very close to the juglone values obtained from cultivars. There are not many studies with juglone values measured from dried fruit shells of different walnut cultivars.

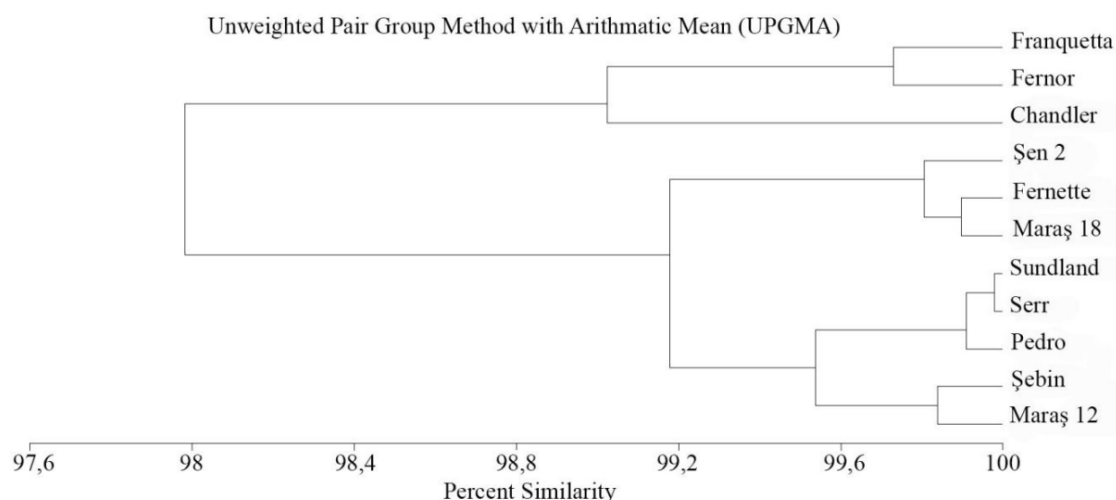


Figure 3. Percent similarity analysis of juglone values calculated from domestic and foreign walnut cultivars.

According to the results of the percentage similarity analysis, in which the juglone content of domestic and foreign walnut cultivars is calculated, it is seen that domestic and foreign cultivars are generally grouped among themselves. It was determined that the most similar cultivars were between Serr and Sundland foreign cultivars with 99.98%. 99.91% similarity of Pedro cultivar to the group consisting of Serr and Sundland cultivars was calculated. Figure 3 shows that there is 99.90% similarity between Maraş 18 and Fernette, another similar group, and that Şen 2 is 99.81% similar to this group. It has been calculated that two local cultivars, Maraş 12 and Şebin, are 99.84% similar to each other in terms of juglone content. Fernor and Franquette cultivars were 99.73% similar to each other, and Chandler's was 99.03% similar to this group they formed.

Table 3. Light fastness values on cotton fabrics and juglone values measured from fruit peels of natural dyestuff obtained from domestic and foreign walnut cultivars

Cultivars	Light Fastness Values	Amount of Juglone (mg/g) $\pm$ standart deviation
Fernor	3	0.2595 a*
Franquette	2.5	0.2581 ab
Chandler	2.5	0.2538bc
Bilecik	2.5	0.2536 cd
Serr	2	0.2495 de
Sunland	2.5	0.2496 e
Pedro	2.5	0.2491ef
Maraş-12	2.5	0.2475efg
Şebin	3	0.2467efg
Fernette	3	0.2450fg
Maraş-18	3	0.2445 g
Şen-2	2	0.2438 g
P		0.0001
F		11.7621

* Means within a column followed by the same letters are not significantly different by the LSD test at $P < 0.01$.

While the group consisting of Sundland, Serr and Pedro cultivars and the groups formed by Şebin and Maraş 12 cultivars were found to be 99.54% similar to each other, the similarity of all these cultivars was calculated to be 99.18% to the group consisting of Şen 2, Fernette and Maraş 18. Finally, the group consisting of Franquette, Fernor and Chandler cultivars was determined to be 97.98% similar

to the group consisting of all other cultivars. The reason for this dissimilarity is thought to be due to the different genetic structure as well as the different foliation and harvest periods.

When the classification analysis is examined in general terms, it is seen that mostly domestic and foreign cultivars have higher similarities among themselves (Figure 3).

When the tested juglone amounts of domestic and foreign walnut cultivars included in the experiment were compared, there was no statistical difference between Fernor (0.2595 mg g^{-1}) and Franquette (0.2581 mg g^{-1}) cultivars, but the juglone amounts of these cultivars were statistically significantly different from Chandler (0.2538) and almost all cultivars. Tested juglone values ranged from 0.2595 to 0.2438 mg g^{-1} , standard errors are reflected in Figure 4.

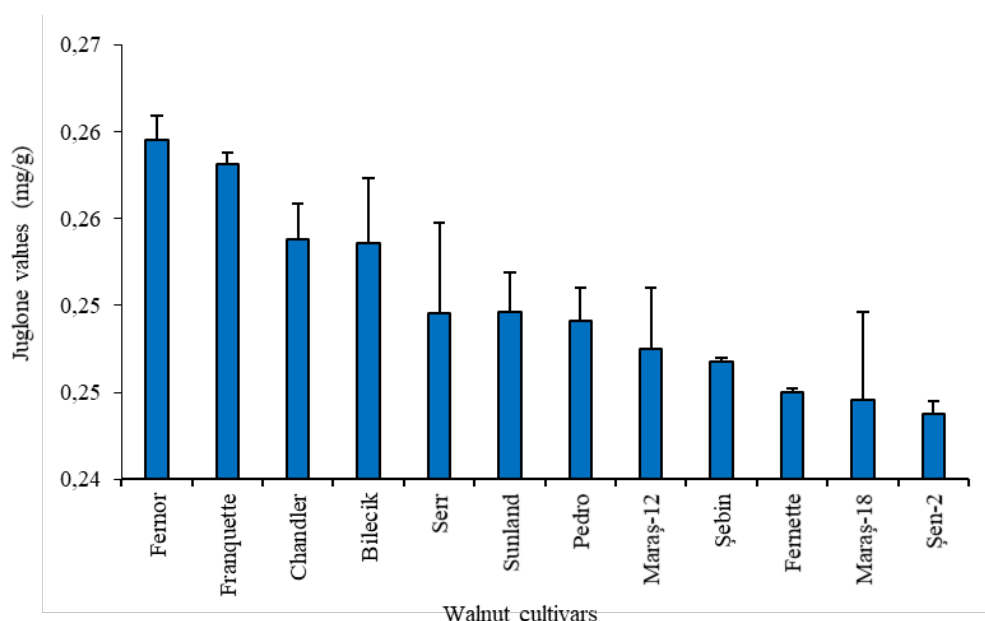


Figure 4. Juglone values and standard deviations measured from walnut cultivars.

Conclusion

In this study, the juglone amounts of different walnut cultivars including domestic and foreign cultivars were calculated, and differences between the juglone amounts and different walnut cultivars selected were determined. The origin of the cultivars, the cultivar characteristics, the material used and the ecology have an effect on the formation of the differences. Among the cultivars included in the study, the juglone content of the native cultivars is generally at a lower level. On the other hand, according to the early or late leafing characteristics of the cultivars in terms of vegetative period; it is seen that the juglone content amounts of late leafing cultivars are higher, it is thought that new studies can be done on this subject. In addition, it was concluded that it would be beneficial to work with the same cultivars in different ecologies in order to determine the effect of ecology.

Considering the relationship between juglone amounts and light fastness, although there is no significant difference between the juglone amounts of the cultivars, the light fastness values are different. In this case, the use of different dyeing materials with the same cultivars or the use of different dyeing materials with a single variety in new studies to be conducted may be beneficial for studies

Ethical Statement

The authors state that ethical approval is not required for the present study, as the study consists of walnut materials that do not require ethical approval.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Author Contributions

Each author made an equal contribution to the article (33.3%).

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