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Enrichment of Extract Composition of Merzifon Karası Grape Cultivar with MeJA Elicitor

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Abstract Today, changing lifestyles and conscious consumer behavior force a quality increase and improvements in food. Conscious consumers act sensitively about healthy living and tend to natural products that both protect against diseases and meet their nutritional needs. Some compounds contained in fruits and vegetables are known to have positive effects on human health. Grapes are among these fruits with their high nutritional value and high content of antioxidant compounds, especially phenolic compounds. Therefore, the use of different grape extracts in areas such as food, health, and cosmetics has become popular in recent years. Different methods can be employed to preserve this composition in products with rich content, to minimize extraction losses, as well as to enable the plant to synthesize these components in higher amounts without converting them into extracts. Endogenous hormones are known to be particularly effective in the production of these components by activating different signaling mechanisms. In this study, the potential of using methyl jasmonate as an elicitor in Merzifon Karası grape, a colorful grape variety, was evaluated. Grapes were sprayed once (once application) immediately after veraison, twice (twice applications) immediately after veraison, and 15 days after veraison (doses of 0; 1; 2.5, and 5 mM). At harvest, the clusters were harvested and anthocyanin, phenolic compounds (spectrophotometrically and by HPLC), amino acid and organic acid contents of grape berries, and fatty acid contents of grape seeds were determined. The results showed that methyl jasmonate as an elicitor was highly effective in enriching the berry composition of grapes.

Keywords Grape, Elicitor, Jasmonic acid, Secondary metabolites

1. Introduction

Grapes have a special position because of the many valuable components in their composition. Besides its rich content of vitamins and minerals, grapes are known to have approximately 1600 compounds derived from phenylpropanoids, isoprenoids, and alkaloids that are effective in many diseases. With the effect of these compounds, grapes are known to prevent cancer formation, bad cholesterol, high blood pressure, heart attack risk, Alzheimer's, Parkinson's, dementia, and many other neurodegenerative diseases [1].

The synthesis of these components depends largely on their response to environmental conditions and adaptation to the environment. Endogenous hormones are also effective in the formation of this response. As it is known, hormones are organic compounds that have extremely important roles in plant growth and development, can be transported within the plant, and can show their effects even in very small doses. Jasmonic acid (JA) is one of the plant hormones that play an important role in plant development and many physiological and biochemical processes [2]. It was first isolated in 1962 from the oil extracted from the jasmine (*Jasminum grandiflorum*) plant [3]. JA is known to be effective in activating the natural defense mechanism in plants, stimulating abscission and closure of stomata, and promoting chlorophyll degradation, respiration, ethylene, and protein synthesis [4-6]. In all these processes, it promotes increased secondary metabolite synthesis [7,8].

Methyl jasmonate (MeJA) is an odorous volatile compound that is the methyl ester of JA. It is known that studies on JA or MeJA are mostly conducted to enrich phenolic compounds in the presence or absence of stress. In one of the studies in this field, Larronde et al. [9] treated clusters of Cabernet Sauvignon grape variety with MeJA. As a result of the study, it was found that MeJA application promoted the accumulation of piceid in leaves and trans-resveratrol in berries. In another study conducted on the same variety, MeJA application was applied and it was determined that trans-resveratrol, piceid, ε-viniferin, δ-viniferin and pterostilbenes increased compared to the control [10]. Vezzulli et al. [11] also applied MeJA to the clusters of Barbera grape variety. As a result of their study, they found that MeJA significantly increased the production of trans-resveratrol and ε-viniferin in the berries during the

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ripening period. In another study, Ruiz Garcia et al. [12] applied MeJA at a dose of 10 mM to the Mourvedre grape variety, starting just before the veraison period, 3 times at 3-day intervals. They reported that the amounts of anthocyanins and proanthocyanins in the treated clusters increased significantly compared to the control. In a study conducted in Tempranillo, one of the important wine grape varieties, it was determined that pre-harvest application of MeJA to the vines significantly increased the total anthocyanin content [13]. Wang et al. [14] examined the effect of MeJA on chlorophyll, vitamin C, soluble protein, sugar, nitrate, total phenols, flavonoids, volatile components, enzymatically produced pyruvic acid content and antioxidant activity and stated that MeJA was very effective in increasing quality components.

In this study, the effects of MeJA applications on the biochemical contents of grapes were examined. Exogenous MeJA applications were made in Merzifon Karası grape variety and the amounts of phenolic compounds, total phenolic matter, total flavonols, total flavanols, and anthocyanins, as well as amino acids and organic acids, and the amounts of fatty acids (in seeds) which are also very important in human health were examined. It was aimed to reveal the potential of MeJA on these components. The composition of the berry was analyzed with its many components, and the potential for conversion into a rich product of extracts will increase in further studies with the increase in the amounts of compounds effective in food, cosmetics, and health in light of the data obtained.

2. Material and Methods

2.1. Material

Merzifon Karası grape variety grafted on 1103 Paulsen (1103 P) American grapevine rootstock was used as material in the study. Merzifon Karası grape variety is one of the wine grape varieties with thin skin, and round and medium-sized berries. The berry skin color is blue and black and it has medium-sized and dense clusters. The treatments were carried out in a 12-year-old vineyard established in the Merzifon District of Amasya Province (Figure 1). MeJA was 99% pure and commercially available (CAS no: 39924-52-2, Sigma Aldrich, Darmstadt, Germany). It was prepared by dissolving in ethyl alcohol and diluted to 1 mM, 2.5 mM, and 5 mM. Deionized water containing equal amounts of alcohol was used as a control.

Figure 1. A view from the vineyard

2.2. Methods

MeJA treatments were applied in two separate application periods as one period and two periods, taking into account the veraison period. The once application (OA) was made once on August 2, 2021, immediately after the veraison period. The twice application (TA) was made twice, the first on the same date and the second two weeks after the first application (August 16). At harvest, clusters were harvested (Figure 2) and analyzed as detailed below.

Figure 2. A view of ripe grape

Extraction of phenolic compounds: An extraction procedure was performed for the determination of phenolic substances [15]. Total phenolic compounds were quantified using the Folin Ciocalteu colorimetric method according to Singleton and Rossi [16]. Results were given in mg/g as gallic acid equivalent (GAE).

Total flavonols were analyzed according to Dai et al. [17] using a Neu solution. The results were expressed as rutin equivalent (RE) in mg/g (Figure 3).

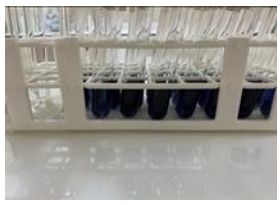

Figure 3. Samples in total flavonol analysis

Total flavanols were estimated according to Arnous et al. [18]. Results were presented as catechin equivalent (CE) in mg/g.

Determination of anthocyanin content: In colored grape varieties, the amount of anthocyanin, which is a color pigment that provides the unique color of the variety, was also determined spectrophotometrically. Anthocyanin analyses in fresh grape samples were performed with Mcllvaine baffle (pH=3) according to the method of Qu et al. [19] (Figure 4). Anthocyanin amounts were calculated as color value (CV) according to the following formula.

$$CV = 0.1 \times \text{absorbance} \times \text{dilution factor}$$

Figure 4. Samples in anthocyanin analysis

Identification of phenolic compounds: The method of Gomes et al. [20] was used for the identification of phenolic compounds and determined in ppm by HPLC (Shimadzu Prominence, Tokyo, Japan).

Determination of amino acids: Amino acid amounts were determined by HPLC. The method of Köse et al. [21] was used in the analysis. The results were given as ppm.

Determination of organic acids: The method of Aktaş et al. [22] was used to determine the amount of organic acids by HPLC and the results were presented as ppm.

Determination of fatty acids: The amounts of fatty acids were determined by GC-MS (AGILENT 7890A GC) and the results were presented as percentages (%). The detector and injector temperature were determined as 24℃ [23].

Statistical analysis: The study was conducted with 3 replications and 5 vines in each replicate. The data obtained at the end of the experiment were evaluated in the SPSS 24 statistical program according to the random blocks experimental design and the differences between the averages were determined according to Duncan's multiple comparison test ($p \leq 0.05$).

3. Results and Discussion

The effects of MeJA treatments on berry composition were examined and the results are detailed below. In terms of total phenolic compounds calculated as gallic acid eq, the highest values were found in the treated grapes (Table 1).

Total flavonol contents were also statistically higher in the treatment groups. It is noteworthy that the highest content of 0.313 mg/g is three times higher than the value (0.098 mg/g) in the control group of TA treatment, which is the lowest content determined numerically. In the statistical analysis of total flavanols calculated as catechin equivalent, the lowest value was found in the control group of OA treatment.

It is seen that the highest value of Merzifon Karası, a colorful grape variety, in terms of anthocyanins was obtained from a 1 mM MeJA dose of TA application (Table 1). Therefore, MeJA has a positive effect on the production of total phenolics, total flavonols, total flavonols, and anthocyanins.

In studies investigating the effect of MeJA applications on the phenolic compound composition of plants or plant parts, positive results are generally obtained similar to our study. It is known that MeJA can stimulate phenylalanine, an enzyme involved in the synthesis of phenolics and flavonoids through the phenylpropanoid pathway [24]. The data showing that the total phenolic contents of fresh grapes, apples, plums, and strawberries increased with the increase in PAL activity associated with MeJA are in line with the results of this study [25,26].

Studies have been conducted on loquat [27], plum [28], apple [29], medlar [30], and pomegranate [31] fruits in which total phenolic content was determined to increase with MeJA treatments.

In a study conducted in this field, Ghasemnezhad and Javaherdashti [32] stated that MeJA may increase phenolic compounds in raspberry fruits and thus trigger the defense mechanism. Wang et al. [33] also reported that PAL activity was increased by MeJA treatment in Chinese blueberry plants. Flores and Castillo [34] reported that MeJA application promoted the accumulation of phenolic compounds such as ellagic acid, quercetin, and myricetin in raspberry plants in the pre-harvest period and stated that this was due to the effect of MeJA on PAL enzyme activity.

Andrys et al. [35] stated that JA added to the in vitro culture medium increased the amounts of phenolic compounds. In another study, MeJA was applied to cell cultures of marsh willowherb (*Persicaria minor*) and its effect on sesquiterpene production was examined. Among the 15 identified sesquiterpene compounds, α-muurolene was found to reach the highest level in all MeJA-treated groups. They also stated that sesquiterpene production varied depending on the dose of MeJA in suspension culture and incubation time [36]. Wang et al. [14], who conducted a study on the yield and quality components and antioxidant properties of MeJA in Chinese Chives, found that 500 μM MeJA treatment significantly increased the levels of total chlorophyll, phenolic compounds, flavonoids, vitamin C, and volatile compounds.

Table 1. Changes in the amounts of total phenolic compounds, total flavonols, total flavanols and anthocyanin

Applications	*Dose (mM)*	*Total phenolic compounds (mg/g GAE)*	*Total flavonols (mg/g RAE)*	*Total flavanols (mg/g CAE)*	*Anthocyanin (mg/g CV)*
OA	***Control***	0,577 b*	0,133 cd	0,072 b	3,418 b
	1,0	0,566 b	0,201 bc	0,081 ab	3,778 b
	2,5	0,866 a	0,313 a	0,085 a	3,623 b
	5,0	0,806 a	0,208 ab	0,083 a	3,926 b
TA	***Control***	0,537 b	0,098 d	0,076 ab	3,737 b
	1,0	0,765 a	0,165 cd	0,085 a	4,663 a
	2,5	0,806 a	0,244 ab	0,081 ab	3,825 b
	5,0	0,627 b	0,147 cd	0,078 ab	3,806 b

*There is a statistical difference between the means with different letters in the same column ($p<0.05$).

Table 2. Changes in the amounts of some phenolic compounds (ppm)

Applications	*Dose (mM)*	*Gallic acid*	*3,4 dihydroxy benzoic acid*	*Chlorogenic acid*	*4-hydroxy benzoic acid*	*2,5-dihydroxy benzoic asit*	*Vanillic acid*	*Caffeic acid*	*Epicatechin*	*p-coumaric acid*	*Ferulic acid*	*Rutin*	*Ellagic acid*	*Cinnamic acid*	*Quercetin*
OA	***Control***	4,92* de	20,06 c	74,63 a	42,94 d	1089,68 d	195,40 c	5544,24 bc	395,25 c	14,60 b	716,52 e	135,28 c	211,43 e	3,57 e	3,34 d
	1,0	7,82 ab	20,22 c	74,94 a	42,80 d	1055,79 d	249,99 b	5616,13 bc	391,63 c	33,47 a	1152,80 b	180,59 b	473,01 b	4,38 d	8,86 a
	2,5	8,52 a	18,32 cd	45,99 d	28,06 e	2037,46 c	489,13 a	2890,70 d	1081,29b	25,99 ab	907,39 d	139,62 c	209,97 e	3,81 e	5,85 c
	5,0	6,63 b-d	20,90 bc	49,12 cd	41,97 d	1077,00 d	98,76 f	4903,80 c	255,06 d	12,87 b	5294,90 a	116,79 c	214,28 e	13,95 a	1,32 g
TA	***Control***	5,19 de	16,54 de	54,94 bc	39,89 d	1596,28 cd	95,74 f	5065,20 bc	343,49 c	15,56 b	582,43 f	73,56 d	273,79 d	3,73 e	3,54 d
	1,0	4,58 e	14,12 e	58,50 b	58,67 c	1184,93 d	136,90 e	6456,81 b	181,68 e	21,32 ab	1056,37c	208,47 a	885,85 a	8,70 b	6,20 b
	2,5	7,08 a-c	36,6 a	37,07 e	72,50 b	3346,04 b	144,22 e	2600,11 d	1133,43 b	19,83ab	925,78 d	62,23 d	418,63 c	6,88 c	2,69 e
	5,0	5,54 c-e	23,28 b	44,52 d	76,87 a	4378,57 a	175,63 d	8567,16 a	1365,63a	17,34 b	793,37 e	57,46 d	286,43 d	3,26 e	1,79 f

*There is a statistical difference between the means with different letters in the same column ($p<0.05$).

Table 3. Changes in the amounts of amino acids (ppm)

Applications	*Dose (mM)*	*Arginine*	*Serine*	*Glycine*	*Alanine*	*Proline*	*Valine*	*Threonine*	*Methionine*	*Isoleucine*	*Leucine*	*Phenylalanine*	*Tyrosine*	*Aspartic acid*	*Glutamic acid*	*Lysine*	*Glutamine*	*Cysteine*	*Tryptophan*
OA	***Control***	18,80 e	32,59 e	72,70 c	52,17 f	1,84 g	6,01 e	15,89 e	61,27 h	72,44 f	20,08 c	2,24 f	5,31 d	7,30 b	6,28 e	0,019 f	5,19 g	0,33 c	0,08 h
	1,0	17,94 f	30,80 f	78,23 b	57,03 e	7,70 f	10,76 b	80,91 a	76,30 e	108,69 c	18,69 d	5,45 c	6,59 c	7,20 b	5,22 f	0,038 c	11,64 d	0,39 a	0,23 f
	2,5	21,31 d	40,77 b	82,80 a	79,64 b	7,69 f	5,25 g	18,64 d	89,32 b	132,34 a	33,33 a	3,01 e	14,75 b	14,84 a	7,38 d	0,032 e	7,73 e	0,37 b	0,41 e
	5,0	32,35 b	36,28 c	84,67 a	94,25 a	11,63 e	5,63 f	11,11 f	122,88 a	125,61 b	33,59 a	3,54 d	6,84 c	7,34 b	4,70 g	0,061 a	19,19 a	0,33 c	0,53 d
TA	***Control***	6,77 h	34,32 d	77,76 b	72,11 c	12,46 d	3,73 h	18,92 d	67,49 f	85,32 e	17,11 f	1,48 h	5,30 d	5,12 c	5,91 e	0,037 d	5,61 f	0,26 e	0,11 g
	1,0	8,22 g	22,72 g	85,51 a	73,74 c	43,66 a	7,30 d	50,99 b	82,91 d	101,09 d	17,89 e	2,02 g	3,01 e	3,64 d	8,88 c	0,041 b	3,30 h	0,33 c	0,58 c
	2,5	28,96 c	74,22 a	53,61 d	47,04 g	22,18 c	9,19 c	41,68 c	65,09 g	100,44 d	5,72 g	6,55 b	28,28 a	7,12 b	11,51 a	0,008 h	15,83 b	0,25 f	0,72 a
	5,0	77,14 a	29,91 f	41,29 e	65,37 d	29,29 b	14,88 a	41,71 c	86,83 c	129,51 a	23,53 b	9,91 a	6,41 c	7,12 b	10,64 b	0,012 g	13,99 c	0,30 d	0,59 b

*There is a statistical difference between the means with different letters in the same column ($p<0.05$).

There is some information that MeJA applications increase anthocyanin content [37,38]. Researchers state that anthocyanin accumulation increases with increased ethylene synthesis. Researchers who examined the effect of MeJA application on the anthocyanin, apigenin, luteolin, quercetin, and proanthocyanin contents of buckwheat (*Fagopyrum esculentum* Moench) plants stated that the synthesis of these compounds increased with MeJA application [39]. Similarly, Shafiq et al. [40] reported that MeJA application promoted the accumulation of anthocyanins such as cyanidin 3-galactoside in apples. Ozturk et al. [29] stated that the content of anthocyanins (cyanidin-3-galactoside), ethylene, total phenolic (gallic acid) and antioxidant capacity increased linearly with increasing MeJA doses. In contrast to the results of these studies, studies conducted in cherry [41] and orange [42] showed that MeJA-treated fruits had lower total phenolic, flavonoids and antioxidant activity compared to the control. Boonyaritthongchai and Supapvanich [43] also found the effect of MeJA on pineapple to be insignificant. Therefore, it is possible to conclude from these results that the effect of MeJA varies according to the dose applied, the plant, and even the growing conditions of the plant.

In this study, phenolic compound amounts were also analyzed on a component basis. While selecting these compounds determined by HPLC, care was taken to ensure that they were the most common compounds found in grapes.

The amounts of gallic acid, 3,4-dihydroxy benzoic acid, chlorogenic acid, 4-dihydroxy benzoic acid, 2,5- dihydroxy benzoic acid, vanillic acid, caffeic acid, epicatechin, p-coumaric acid, ferulic acid, rutin, ellagic acid, cinnamic acid and quercetin were determined in grape samples (Table 2). In Table 2, it is noteworthy that the highest values of all phenolic compound contents except chlorogenic acid, which was found at the highest level in both control and OA groups and at 1 mM dose, were obtained only from the treated clusters. In addition, the difference between the lowest and highest values of ferulic acid 9.09 fold, epicatechin 7.51 fold, quercetin 6.71 fold, and vanillic acid was 5.10 fold on the basis of the components, which shows that the applied MeJA elicitor is very effective in this respect.

In another study, JA was applied to root cultures of calendula (*Calendula officinalis*) and its effects on the biosynthesis and accumulation of triterpenoids were investigated. JA treatment increased the production of triterpenoid saponins by 86-fold [44].

In this study, the effects of MeJA treatments on the amino acid profile of grape berries were also examined and the data obtained are given in Table 3. Amino acids are the building blocks of proteins. Since some of them are not produced by the human body, they must be taken with food. These amino acids are defined as essential amino acids. Essential amino acids are valine, leucine, isoleucine, threonine, methionine, phenylalanine, tryptophan, and lysine. Their deficiency is known to cause various problems in metabolism, neurological disorders, and suppression of normal mental development in humans. Some amino acids produced by the body but not in sufficient quantity are semi-essential amino acids, including arginine and histidine. These are amino acids that need to be supplemented to meet the need. Some amino acids are produced in the body and do not require additional supplementation. These are called non-essential amino acids. These are alanine, arginine, aspartic acid, asparagine, cystine, cysteine, glutamic acid, glutamine, glycine, proline, and serine [45].

In the study, it is clearly seen that MeJA application had a positive effect on all amino acids, and none of the control groups without MeJA had higher amino acid content than the others. While some amino acids showed a slight increase in response to MeJA, the levels of others increased significantly. It is noteworthy that the difference between the highest and lowest values is 23.74 times for proline, 11.4 times for arginine, and 8.27 times for tryptophan amino acid. Only a limited number of studies were found on the effect of these components on amino acids, which are the building blocks of protein. Van Dam and Oomen [46], who examined the effect of JA applications on primary metabolites such as sugars and amino acids in cabbage (*Brassica oleracea*), reported that JA application affected glucosinolates, amino acid, and sugar content in the plant and the results also varied according to the organ to which JA was applied. Wang et al. [14] examined the effect of pre-harvest MeJA applications on growth, quality, essential oil components, and antioxidant defense mechanism in Chinese Chives and stated that 500 µM MeJA significantly increased protein content compared to control.

In the study, the effect of MeJA treatments on the organic acid content of grape berries was also examined (Table 4). Organic acids are an important indicator of the taste and nutritional quality of fruits [47]. They provide chemical stability of water-soluble vitamins B and C by reducing their sensitivity to heat and light. In terms of human health, they are effective in increasing appetite, facilitating digestion, increasing potassium absorption, facilitating the uptake of copper, zinc, and calcium by the body, and providing resistance against diseases [48,49].

Oxalic acid, malic acid, fumaric acid, tartaric acid, and citric acid contents from organic acids were analyzed (Table 4). It was observed that there was no statistical difference between the treatments in terms of fumaric acid content. Except for malic acid, all other three organic acids were synthesized at the highest level in the TA treatment group and with MeJA application at a dose of 2.5 mM. Malic acid was synthesized at the highest level in the OA group and at a dose of 1.0 mM. It is seen that the MeJA application is an effective application in terms of these properties. However, no research was found on the effect of MeJA applications on the organic acid content of the fruits.

In the study, the fatty acid composition in the seeds of grape berries was also analyzed. The changes in fatty acids were determined by GC-MS (Table 5). The amounts of tetradecanoic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, 11-hexadecanoic acid, stearic acid, oleic acid, linoleic acid, linolenic acid and eicosanoic acid were determined. It is noteworthy that all fatty acids examined were at the highest values in the treated groups. Especially pentadecanoic acid, 11-hexadecanoic acid, linolenic acid, and eicosanoic acid were synthesized at the highest level at 5.0 mM MeJA dose in the OA group.

The effect of the treatments was especially evident with significant increases in the levels of eicosanoic acid (6.9-fold) and tetradecanoic acid (4-fold) (Table 5).

4. Conclusions

It is known that plant extracts in particular have a significant consumption as food and nutritional supplements. Studies aiming to increase the components in the main product from which they are obtained in order to further enrich the content of extracts, which are rich in nutrients and components that have a significant effect on human health, are also extremely important. It is also known that many studies have been carried out on different plants in this field. Especially in plant materials that are already known to be rich in these components, these studies will make the final product composition more valuable by tolerating the losses in the extract process. This study showed that MeJA applications had positive effects on all parameters examined. It is possible to transfer this application to practice by taking the period and doses used to increase the amount of the desired target compound in studies in this field as a reference. However, it is also thought that the diversity of applications is limited in the studies on compound enhancement. Comprehensive studies using different pre-applications are needed.

Table 4. Changes in the amounts of organic acid (ppm)

Applications	*Dose (mM)*	*Oxalic acid*	*Malic acid*	*Fumaric acid*	*Tartaric acid*	*Citric acid*
OA	***Control***	2122,024* g	543,883 e	4,303	6,247 h	177,519 f
	1,0	2915,153 e	1328,038 a	5,385	9,531 c	106,973 h
	2,5	2923,882 e	483,161 f	6,750	7,172 g	209,403 e
	5,0	2974,094 d	609,155 d	5,050	8,844 d	340,761 b
TA	***Control***	2342,566 f	632,511 c	4,595	9,862 b	324,789 c
	1,0	3164,648 b	614,726 d	4,599	7,590 f	217,982 d
	2,5	3521,571 a	687,848 b	4,872	21,030 a	570,092 a
	5,0	3089,553 c	461,012 g	5,738	8,715 e	119,549 g

*There is a statistical difference between the means with different letters in the same column ($p<0.05$).

Table 5. Changes in the amounts of fatty acids (%)

Applications	*Dose (mM)*	*Tetradecanoic acid*	*Pentadecanoic acid*	*Palmitic acid*	*Palmitoleic acid*	*11-hexadecanoic acid*	*Stearic acid*	*Oleic acid*	*Linoleic acid*	*Linolenic acid*	*Eicosanoic acid*
OA	***Control***	0,771 g	0,114 f	15,945 f	0,107 b	0,077 c	3,689 f	20,324 c	43,129 d	0,637 f	0,406 e
	1,0	1,177 d	0,109 g	14,437 g	0,108 b	0,081 b	8,623 b	19,615 d	58,232 a	1,218 b	0,208 f
	2,5	1,090 e	0,155 c	19,324 d	0,115 a	0,055 d	8,469 b	20,953 b	48,648 c	1,152 c	0,574 c
	5,0	1,538 c	0,248 a	19,854 c	0,085 e	0,096 a	6,584 d	17,726 f	52,266 b	1,295 a	1,440 a
TA	***Control***	0,918 f	0,157 c	21,364 b	0,080 f	0,040 f	4,394 e	18,443 e	47,863 c	0,616 f	0,473 de
	1,0	1,173 d	0,163 b	18,203 e	0,084 e	0,045 e	7,043 c	19,753 d	48,771 c	0,772 d	0,516 cd
	2,5	3,707 a	0,140 d	19,154 d	0,092 d	0,030 h	6,520 d	29,613 a	42,644 d	0,484 g	0,654 b
	5,0	2,044 b	0,122 e	24,762 a	0,095 c	0,035 g	8,822 a	15,147 g	48,983 c	0,741 e	0,556 c

*There is a statistical difference between the means with different letters in the same column ($p<0.05$).

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Conflict of interest: E.S. Çetin and A.T. Ersenel declare that they have no competing interests.

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