RESEARCH ARTICLE

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The effects of foliar zinc application on grain antioxidant traits in some winter durum wheat cultivars at different growth stages

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Abstract

This study was aimed to investigate the grain antioxidant activity (DPPH· and $ABTS^{\cdot+}$ radical scavenging activities and cuprac reducing capacity), contents of total phenolic compounds, flavonoid and total antioxidant capacity of five winter durum wheat cultivars under the foliar application of 0.2% of zinc ($ZnSO_4.7H_2O$) at different growth stages of grain filling (milky or dough ripeness). The study was carried out in randomized blocks according to the split plot design with three replications in the cultivars of Ç.1252, Eminbey, Kızıltan-91, Meram-2002 and Selçuklu-97.

In the study, it was determined that some of the antioxidant traits ($ABTS^{\cdot+}$, total flavonoid and zinc content of grain) were not statistically different between foliar zinc application stages of the milky and dough ripeness, but zinc application in one of these stages showed significantly higher values in terms of these traits compared to the untreated of zinc (control). Also, cultivars and zinc×cultivar interaction for DPPH· radical scavenging activity, total phenolic compounds and total flavonoids showed significant variations. Within the frame of these results, it was found that foliar application of zinc at different stages of grain filling in durum wheat had statistically significant effects on some antioxidant traits; however, in subsequent studies, it was advised that it would be more beneficial to expand the study by increasing the dose and the number of growth stages.

Keywords: Antioxidant traits, Durum wheat, Flavonoids, Phenolics, Zinc

INTRODUCTION

Among the widely grown field crops, wheat plays an important role in daily energy intake, especially in developing countries; it meets about 50% of the daily energy intake in many Central Asian and Middle Eastern countries, and this rate can exceed 70% in rural areas (Çakmak, 2008).

Today, 40% of the world's population, especially in developing countries, is faced with insufficient microelement intake. In these regions, durum wheat is often grown under harsh, drought-prone and even marginal conditions. These vulnerable environments often cause production variability due to variation in annual precipitation. Generally, poverty maps and micronutrient deficiency maps can be used with information on durum wheat production area and per capita wheat intake to identify target regions where biological yield is cost-effective (Çakmak, 2008).

In daily life, irreversible destructions occur in the human body due to various factors such as smoking, alcohol, environmental pollution and stress. Today, phenolic compounds and antioxidant-rich functional foods come to the fore against these damages. Structures such as phenolic substances, carotenoids and vitamin E in wheat are natural powerful antioxidants and are important in preventing some diseases (Menteş-Yılmaz, 2011).

Phenolic substances are aromatic compounds that have hydroxyl groups and can esterify with carbohydrates (Shahidi and Naczk, 1995; Duthie and Crozier, 2000). Phenolic acids, flavonoids, stilbenes, coumarins and tannins are among these compounds (Dinelli et al., 2009). Herbal phenolics are products of normal metabolism, and their amount varies according to plant variety, growing conditions and maturation level (Adom et al., 2005; Kim et al., 2006). Phenolic compounds are important compounds that increase the natural resistance of metabolism against oxidative damage and prevent lipid peroxidation (Gülçin, 2007; 2012). The interest in phenolic acids is increasing due to the protective potential of phenolic acids against oxidative damage such as cancer, stroke and coronary heart disease thanks to a diet rich in fruits and vegetables (Annakkaya, 2012).

Oxygen creates reactive oxygen species (ROS) such as superoxide, singlet oxygen and hydroxyl radical in the respiratory sequence in humans (Prado et al., 2020). If the accumulation of reactive oxygens in the human body is not eliminated with antioxidants, adverse effects such as aging, coronary diseases, wear and tear of cells, cancer, and collapse of the immune system occur under the resulting "oxidative stress". Antioxidants are natural substances that remove these negative effects of free radicals (Liu et al., 2018). Free radicals are reactive structures that contain unstable electrons in their outer orbitals (Fang et al., 2002). In addition, these structures are usually small molecules and can easily pass through cell membranes (Jensen, 2003). Oxidative stress occurs when pro-oxidants, which play a role in accelerating oxidation in tissues, overtake antioxidants. For this reason, the survival of cells under oxygenated conditions is made possible by the introduction of enzymatic and non-enzymatic antioxidants into the system (Sies, 1991).

Due to the phytochemicals (vitamin E, phenolic compounds and carotenoids), wheat is considered as a natural antioxidant source (Menteş-Yılmaz, 2011). Researchers focusing on this subject focused on wheat bran and stated that there is not only a load fiber ratio in the bran; they also reported that it increases the total antioxidant activity with the phenolic acids it contains (Kim et al., 2006). The phenolic acids do not result in a uniform distribution in the layers of the grain; it is stated that it is mostly found in aleurone, fruit peel and germ, and a small amount is located in the starchy endosperm layer (Menteş-Yılmaz, 2011).

In this study, it was aimed to examine the effects of foliar application of zinc on the antioxidant properties at two different growth periods, such as milky or dough ripeness, in winter durum wheat cultivars, and to determine the effects of application period×cultivar interactions in terms of these properties to be examined.

MATERIALS AND METHODS

Materials

Grains of five durum wheat cultivars (Meram-2002, Selçuklu-97, Kızıltan-91, Eminbey and Ç-1252) grown under field conditions of Şiran/Gümüşhane/TÜRKİYE, at the 2013-2014 growth season and stored under storage conditions at a temperature of around 18 °C and a relative humidity of 50-60% were used as material. Foliar application of zinc (0.2% of $ZnSO_4.7H_2O$) at two different growth stages, such as milky or dough ripeness, in these winter durum wheat cultivars were done.

Methods

Determination of DPPH· free radicals removal activity

DPPH· free radical scavenging activity was determined using the method of Blois (1958). 1 mM solution of DPPH· was used as a free radical. Stock solutions prepared previously at a concentration of 1 mg mL^{-1} were used as samples. Stock solutions were transferred to test tubes to form solutions at concentrations of 20 µg $µL^{-1}$, respectively, and the total volume was made up with ethanol to reach 2000 µL. Then, 500 µL of the stock DPPH· solution was added to each sample tube, followed by incubation for 30 minutes at room temperature and in the dark, and the absorbance at 517 nm was measured against an ethanol blank. As controls, 2000 µL of ethanol and 500 µL of DPPH· solution was used. The decreased absorbance compared to the control gave the remaining amount of DPPH· solution, that is, the free radical scavenging activity.

$ABTS^{\cdot+}$ radical removal activity

$ABTS^{\cdot+}$ radical scavenging activity was determined according to the method of Re et al. (1999). First, 7 mM $ABTS^{\cdot+}$ solution was prepared. $ABTS^{\cdot+}$ radicals were produced by adding 2.45 nM persulfate solution to this solution. Before using the $ABTS^{\cdot+}$ radical solution, the control solution was adjusted to 0.700±0.025 nm with a phosphate buffer with an absorbance of 0.1 M at 734 nm and a pH of 7.4. The 20 µg mL^{-1} concentrations of the extracts whose $ABTS^{\cdot+}$ radical scavenging activity will be examined were completed to 1500 µL with ethanol. Then, 500 µL of ABTS.+ radical solution was added and incubated for 30 minutes at room temperature. Absorbances were recorded at 734 nm against the blank consisting of ethanol.

Cu^{2+}-Cu^{+} reduction capacity

In the prepared extracts, the Cu^{2+} reduction activities were performed with a slight modification (Ak and Gülçin, 2008) of the copper ion reduction method (Apak et

al., 2006). 125 µL of $CuCl_2$ solution (0.01 M), 125 µL of ethanolic neocuprine solution ($7.5x10^{-3}$ M) and 125 µL of CH_3COONH_4 buffer solution (1 M) were added to the tubes containing the extracts prepared at a single concentration (20 µg $µL^{-1}$), respectively. Final volumes were made up to 1 mL with distilled water; after 30 minutes, absorbance values were measured against the blank at 450 nm. Pure water was used as the blank.

Determination of total antioxidant amount

500 µL of flour was taken from the extract sample and 2500 µL of distilled water was added; 1000 µL of molybdate reagent was added to the resulting mixture; after vortexing the mixture, it was incubated for 90 minutes in a 95 °C water bath with the mouths closed. It was taken from the water bath and waited for 20-30 minutes to come to room temperature and 500 µL of distilled water was used instead of the sample as a blank. The absorbance of the obtained reaction mixtures was read in the spectrophotometer at 695 nm (Kasangana et al., 2015). The total antioxidant amount was calculated from the unit of ascorbic acid equivalent (AAE) by using the regression equation in the standard ascorbic acid graph (Figure 1).

Determination of total phenolic compound

The amount of phenolic compounds in the prepared extracts was determined by Singleton et al. (1999) and gallic acid was used as the standard phenolic compound. For this, firstly, a standard graph of gallic acid was created (Figure 2). In order to determine the amount of phenolic compounds in durum flour extracts, the prepared stock solution was used. 750 µg of the extract was taken from the stock solution and placed in a metric cup and the volume was made up to 23 mL with distilled water. 500 µL of Folin-Ciocalteu reagent and 1500 µL of 2% Na_2CO_3 were added to the mixture after 3 minutes. The samples were mixed for 2 hours at room temperature. Then, the absorbance of the samples at 760 nm was read against a blank consisting of pure water. The amount of gallic acid equivalent (GAE) corresponding to the absorbance values of the samples was determined with the help of the equation obtained from the standard graph (Figure 2). The results are given as gallic acid equivalents (Köksal and Gülçin, 2008).

Determination of total flavonoids

In the prepared extracts; total amount of flavonoids were made according to the method of Park et al. (1997). 750 µg of extract was added to a vezin cup. The extract, which was then transferred to the test tube, was diluted with 4300 µL of ethanol solution containing 100 µL (1 M) of CH_3COOK and 100 µL (10%) $Al(NO_3)_3$ solutions and mixed in a vortex. After incubation at room temperature for 40 minutes, absorbance at 415 nm was recorded. Quercetin was used as a standard for the determination of total flavonoid concentration and the total flavonoid concentration was determined as microgram quercetin equivalent (QE) from the equation obtained from the standard quercetin graph (Figure 3).

Determination of grain zinc content

It has been determined according to the EPA 6020 method; 0.5 grams of each flour sample was weighed, 4 mL of 65% HNO_3 and 6 mL of H_2O_2 were added to it, burned in the microwave (Milestone Start D) and diluted with 50 mL of ultrapure water. The elemental content of the samples was analyzed by inductively coupled plasma-mass spectrometry (inductively coupled plasma mass spectrometry: ICP-MS; Agilent brand ICP-MS 7700e series) technique. Agilent mix 2a standard was used in the analyses.

Evaluation of Data

The data obtained from the laboratory studies, according to the randomized blocks split plot design, with three replications; control and zinc application periods were arranged by placing in the main plots and the cultivars in the sub plots. Statistical analyzes of the data (F test and EGF test) were performed with the JMP statistical package program.

RESULTS AND DISCUSSION

The mean squares obtained from the variance analyzes of the antioxidant traits of the grain that zinc applied from the leaves during the stage of milky or dough ripeness of some winter durum wheat varieties were given in Table 1; the mean values for these traits were given in Table 2-8.

DPPH· Free Radical Scavenging Activity

DPPH· free radical scavenging activity was determined as the inhibition rate (%) by comparing the absorbance data of each sample in 20 µg mL^{-1}. This value was determined according to the following formula:

DPPH· free radical scavenging activity (%) = $[(\lambda_{517(C)} - \lambda_{517(S)})/ \lambda_{517(C)}] \times 100$

In the above formula, $\lambda_{517(s)}$ is the absorbance value determined after the sample is added to the DPPH· free radical solution; $\lambda_{517(C)}$ shows the absorbance value of the control containing only DPPH· free radical solution. BHA, BHT, α-tocopherol and trolox were used as positive controls in the studies.

There are no statistically significant differences in foliar zinc application stages in durum wheat cultivars (Table 1); however, the lowest DPPH· inhibition rate was obtained from the milky ripeness stage (3.28%) while the highest value was obtained from the zinc application stage of dough ripeness (3.54%) (Table 2). Cultivars showed significant changes at $p<0.05$ level (Table 1); the highest DPPH· inhibition rate was obtained from Ç.1252 cultivar (3.76%), and the lowest value was obtained from Selçuklu-97 cultivar (3.06%) (Table 2). Zilic et al. (2013) reported that the antioxidant capacity measured as DPPH· radical scavenging activity was similar in bread and durum whe

ats, but there were significant differences between genotypes within the species; it supports our study showing that statistically significant changes in DPPH· radical scavenging activity were observed among the cultivars.

In addition, Zinc×Cultivar interaction for DPPH· radical scavenging activity showed significant differences (p<0.01, Table 1); this interaction is due to the fact that the cultivars other than Kızıltan-91 are in statistically different groups in terms of high DPPH· radical scavenging activity in the control, milky and dough ripeness (Table 2).

Table 1. Mean squares and coefficients of variation related to the effects of zinc application at the stages of milky or dough ripeness on the antioxidant traits in durum wheat cultivars.

Variation Source	Df	Mean Squares						
		DPPH	ABTS	Kuprac	Total Phenolic	Total Flavonoid	Total Antioxidant	Zinc
Replicate (R)	2	0.704	17.215	0.00207	6.910	0.062	95.92	18.432
Zinc (Zn)	2	0.265	35.271*	0.00001	31.027	2.184**	47.49	655.62**
R*Zn&Random (Error 1)	4	0.207	3.144	0.00559	5.520	0.047	56.31	33.999
Cultivar (C)	4	0.595*	11.875	0.00094	16.652*	0.336**	92.87	150.564**
Zn×C	8	0.783**	13.565	0.00201	14.744*	0.305**	49.93	22.822
Error 2	24	0.184	5.854	0.00114	5.405	0.069	46.10	23.412
CV (%)		12.52	8.13	12.43	4.22	20.24	7.85	4.69

Df, degree of freedom; CV, coefficient of variation; *, ** show the probability levels of p<0.05, p<0.01, respectively.

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In addition, Zinc×Cultivar interaction for DPPH· radical scavenging activity showed significant differences (p<0.01, Table 1); this interaction is due to the fact that the cultivars other than Kızıltan-91 are in statistically different groups in terms of high DPPH· radical scavenging activity in the control, milky and dough ripeness (Table 2).

Table 2. Mean values of DPPH. inhibition rates of grain in zinc applications at the stage of milky or dough ripeness in durum wheat cultivars (%)

Cultivars (C)	Zinc Application Stages (Zn)			
	Control	Milky ripeness	Dough ripeness	Mean
Ç.1252	3.66 a-d	3.31 c-e	4.30 a	3.76 a
Eminbey	2.87 ef	4.07 ab	3.45 b-e	3.46 a-c
Kızıltan-91	3.49 b-e	3.49 b-e	3.02 d-f	3.33 bc
Meram-2002	3.96 a-c	2.97 d-f	3.65 a-d	3.53 ab
Selçuklu-97	3.35 b-e	2.55 f	3.27 c-f	3.06 c
Mean	3.47	3.28	3.54	3.43
LSD_{Zn}		ns		
LSD_{C}		0.42		
$LSD_{Zn×C}$		0.72		

*There is no difference at the 0.05 probability level between the mean values with the same letter groups. LSD shows the least significant difference between the mean values. ns means not important.

$ABTS^{\cdot+}$ Radical Scavenging Activity

Similar to $DPPH^{\cdot}$ free radical scavenging activity; $ABTS^{\cdot+}$ radical scavenging activity was also determined as the inhibition rate (%) by comparing the absorbance data of each sample at 20 µg mL^{-1}. This value was calculated according to the following formula:

$ABTS^{\cdot+}$ radical scavenging activity (%) = $[(\lambda_{734(C)} - \lambda_{734(S)})/\lambda_{734(C)}] \times 100$

In the above formula, $\lambda_{734(S)}$ is the absorbance value determined after adding the sample to the $ABTS^{\cdot+}$ free radical solution; $\lambda_{734(C)}$ shows the absorbance value of the control containing only $ABTS^{\cdot+}$ free radical solution. BHA, BHT, α-tocopherol and trolox were used as positive controls in the studies.

Statistically significant differences ($p<0.05$) were found in durum wheat cultivars in terms of foliar zinc application stages (Table 1); while the lowest $ABTS^{\cdot+}$ inhibition rate was obtained from the control application without zinc application (28.10%), the highest value was obtained from the zinc application stage of dough ripeness (31.17%) (Table 3). Although there are no statistically significant differences in $ABTS^{\cdot+}$ inhibition rate in durum wheat cultivars (Table 1); it varied between 28.82% (Kızıltan-91) and 31.56% (Eminbey) (Table 3). While data are not for durum wheat, Ragaee et al. (2006) are in agreement with the findings. The Zinc×Cultivar interaction did not show statistically significant differences in $ABTS^{\cdot+}$ radical scavenging activity (Table 1).

Table 3. Mean values of ABTS.+ inhibition rates of grain in zinc applications at the stage of milky or dough ripeness in durum wheat cultivars (%)

Cultivars (C)	Zinc Application Stages (Zn)			
	Control	Milky ripeness	Dough ripeness	Mean
Ç.1252	23.67	31.43	31.70	28.93
Eminbey	30.22	32.18	32.28	31.56
Kızıltan-91	29.47	28.81	28.20	28.82
Meram-2002	28.88	29.68	32.01	30.19
Selçuklu-97	28.29	27.62	31.67	29.19
Mean	28.10 b*	29.94 a	31.17 a	29.74
LSD_{Zn}		1.80		
LSD_{C}		ns		
$LSD_{Zn×C}$		ns		

*: There is no difference at the 0.05 probability level between the mean values with the same letter groups. LSD shows the least significant difference between the mean values. ns means not important.

Cu^{2+}-Cu^{+} (Cuprac) Reducing Capacity

In durum wheat, the cupric ion (Cu^{2+}) reducing capacity of the flour sample extracts taken from the grains that were ground with their bran was determined as the absorbance of the solutions at 20 µg mL^{-1} concentration at 450 nm, and these absorbance values are given in Table 4. In durum wheat cultivars, cuprac reducing capacity did not show a statistically significant difference in terms of foliar zinc application stages (milky and dough ripeness) (Table 1 and Table 4). Although there is no statistically significant difference in cuprac reducing capacity of durum wheat cultivars (Table 1); mean absorbance value varied between 0.262 (Eminbey) and 0.287 (Meram-2002) (Table 4). Zinc×Cultivar interaction did not show significant differences in terms of cuprac reduction capacity (Table 1).

Total Antioxidant Content

Ascorbic acid was used as a standard in the determination of total antioxidant amounts in flour samples taken from grains ground with bran in durum wheat cultivars. Using the regression equation in the standard graph given in Figure 1, the total antioxidant amount was calculated from the unit of ascorbic acid equivalent (AAE) according to the formula below, and the mean values are given in Table 5.

C = [((Absorbance + 0.1917) / 0.0052) ×10]

C: Concentration (mg AAE 100 g^{-1} DM)

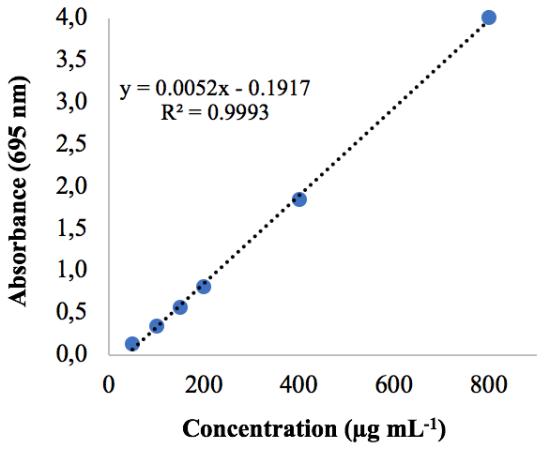

Figure 1. Standard graph of total antioxidant content

Table 4. Mean values of cuprac reduction capacity of grain in zinc applications at the stage of milky or dough ripeness in durum wheat cultivars (absorbance value)

Cultivars (C)	Zinc Application Stages (Zn)			
	Control	Milky ripeness	Dough ripeness	Mean
Ç.1252	0.257	0.267	0.269	0.265
Eminbey	0.234	0.306	0.245	0.262
Kızıltan-91	0.296	0.248	0.279	0.274
Meram-2002	0.287	0.292	0.283	0.287
Selçuklu-97	0.287	0.242	0.274	0.268
Mean	0.272	0.271	0.270	0.271
LSD_{Zn}			ns	
LSD_{C}			ns	
$LSD_{Zn\times C}$			ns	

LSD shows the least significant difference between the mean values. ns means not important.

There was no statistically significant difference between foliar zinc application stages (milky and dough ripeness) in terms of total antioxidant content in durum wheat cultivars (Tables 1 and 5). However, the highest value was obtained from zinc application stage of dough ripeness (89.01 mg AAE 100 g^{-1} DM).

Although there are no statistically significant differences in total antioxidant content of durum wheat cultivars (Table 1); it varied between 81.76 mg AAE 100 g^{-1} DM (Meram-2002) and 90.01 mg AAE 100 g^{-1} DM (Selçuklu-97) (Table 5). Menteş-Yılmaz (2011) also reports that the total amount of antioxidants significantly varies according to the cultivars. Zinc×Cultivars interaction did not differ in terms of total antioxidant content (Table 1).

Total Phenolic Compound Content

Gallic acid was used as a standard in the determination of the total phenolic content in the evaporated ethanol extracts of the flour samples taken from the grains ground with the bran in durum wheat, and the total phenolic compound content was calculated as gallic acid equivalent (GAE) unit from the regression equation in the standard graph given in Figure 2.

Absorbance = 0.0019×[GAE]

The total phenolic compound content of durum wheat cultivars were obtained by using the above formula is given in Table 6.

Although the total phenolic content of durum wheat cultivars did not show statistically significant differences in terms of foliar zinc application stages (Table 1); while the lowest total phenolic content was obtained from the milky ripeness stage (34.5 µg GAE mg^{-1} extract), the highest value was obtained from dough ripeness (37.1 µg GAE mg^{-1} extract) (Table 6). Cultivars showed significant changes at the $p<0.05$ level (Table 1); the highest total phenolic compound content was obtained from the cultivars of Ç.1252 (37.6 µg GAE mg^{-1} extract) and Eminbey (37.2 µg GAE mg^{-1} extract), while the lowest value was obtained from Kızıltan-91 (34.2 µg GAE mg^{-1} extract) (Table 6). Mpofu et al. (2006) also reported that the total phenolic compound content showed significant changes according to the genotypes. Our thesis findings showed values close to the lower limit (37.1 µg GAE mg^{-1}) stated by Sedej et al. (2010) for total phenolic content in whole wheat flour. This may be due to the fact that the plant material in our study was grown under minimum input conditions.

In addition, Zinc×Cultivar interaction for total phenolic content shows significant differences ($p<0.05$) (Table 1); this interaction is due to the fact that the Ç.1252 cultivar showed high total phenolic compound values in the control and dough ripeness stage, while low values obtained in the milky ripeness stage (Table 6).

Total Flavonoid Content

Quercetin was used as a standard for the determination of the total flavonoid content in the evaporated ethanol extracts of the flour samples taken from the grains ground with the bran in durum wheat cultivars. The total flavonoid content was calculated from the quercetin equivalent (QE) unit according to the formula below from the regression equation in the standard graph given in Figure 3, and the mean values were given in Table 7.

Absorbance = 0.0103×[QE]

Total flavonoid content in durum wheat cultivars showed statistically significant differences ($p<0.01$) in terms of foliar zinc application stages (Table 1); the lowest total flavonoid content was obtained from the control without zinc application (0.857 µg QE g^{-1} extract), while the

highest values were from the stage of milky ripeness (1.504 µg QE g^{-1} extract) and dough ripeness (1.532 µg QE g^{-1} extract) (Table 7). The cultivars showed a significant change at the $p<0.01$ level in terms of total flavonoid content (Table 1); the highest flavonoid content was obtained from Ç.1252 cultivar (1,590 µg QE g^{-1} extract), while the lowest value was obtained from Kızıltan-91 cultivar (1,061 µg QE g^{-1} extract) (Table 7). These findings are under the findings of Murathan and Özdinç (2018), who reported that the total flavonid content was in the range of 99.67-302.1 mg $100g^{-1}$.

In addition, Zinc×Cultivar interaction showed significant differences ($p<0.01$) for flavonoid content (Table 1); because of this interaction, cultivars other than Ç.1252 showed low values in terms of total flavonoid content in the control group that did not receive zinc; in other words, it is understood that it is due to being in the lower group statistically. Indeed, it is observed that other cultivars, except Ç.1252, show higher values for total flavonoid content at the stages of milky and dough ripeness compared to the control (Table 7).

Zinc Content

Statistically significant differences ($p<0.01$) were found in zinc content in durum wheat cultivars in terms of foliar application stages (Table 1); as expected, the lowest zinc content was obtained from the control application without zinc (39.39 mg kg^{-1}), while the highest values were from zinc application at the milky ripeness (51.85 mg kg^{-1}) and dough ripenes (49.46 mg kg^{-1}) stages (Table 8). Cultivars showed significant changes at the $p<0.01$ level (Table 1); this change was determined between 39.68 mg kg^{-1} (Ç.1252) and 49.61 mg kg^{-1} (Selçuklu-97) (Table 8). Zinc×Cultivar interaction did not differ in terms of grain zinc content.

Table 5. Mean values of total antioxidant content of grain in zinc applications at the stage of milky or dough ripeness in durum wheat cultivars (mg AAE 100 g-1 DM)

Cultivars (C)	Zinc Application Stages (Zn)			
	Control	Milky ripeness	Dough ripeness	Mean
Ç.1252	84.42	89.49	85.76	86.56
Eminbey	85.82	84.46	92.80	87.69
Kızıltan-91	93.39	88.07	85.60	89.02
Meram-2002	78.05	82.38	84.84	81.76
Selçuklu-97	86.42	87.54	96.06	90.01
Mean	85.62	86.39	89.01	87.01
LSD_{Zn}			ns	
LSD_{C}			ns	
$LSD_{Zn×C}$			ns	

LSD shows the least significant difference between the mean values. ns means not important.

Table 6. Mean values of total phenolic compound content of grain in zinc applications at the stage of milky or dough ripeness in durum wheat cultivars (µg GAE mg-1 extract)

Cultivars (C)	Zinc Application Stages (Zn)			
	Control	Milky ripeness	Dough ripeness	Mean
Ç.1252	38.1 a-c*	33.1 e	41.6 a	37.6 a
Eminbey	35.7 c-e	36.1 b-e	39.7 ab	37.2 a
Kızıltan-91	34.6 c-e	34.6 c-e	33.5 e	34.2 b
Meram-2002	37.4 b-d	33.9 de	34.7 c-e	35.4 ab
Selçuklu-97	38.1 a-c	34.5 c-e	36.0 b-e	36.2 ab
Mean	36.8	34.5	37.1	36.1
LSD_{Zn}			ns	
LSD_{C}			2.3	
$LSD_{Zn×C}$			3.9	

*There is no difference at the 0.05 probability level between the mean values with the same letter groups. LSD shows the least significant difference between the mean values. ns means not important.

Table 7. Mean values of total flavonoid content of grain in zinc applications at the stage of milky or dough ripeness in durum wheat cultivars (µg QE g^{-1} extract)

Cultivars (C)	Zinc Application Stages (Zn)			
	Control	Milky ripeness	Dough ripeness	Mean
Ç.1252	1.694 a*	1.597 a	1.481 a	1.590 a
Eminbey	0.522 cd	1.748 a	1.375 ab	1.215 bc
Kızıltan-91	0.388 d	1.359 ab	1.437 a	1.061 c
Meram-2002	0.728 cd	1.392 ab	1.764 a	1.294 bc
Selçuklu-97	0.955 bc	1.424 a	1.602 a	1.327 b
Mean	0.857 b	1.504 a	1.532 a	1.298
LSD_{Zn}	0.221			
LSD_{C}	0.256			
$LSD_{Zn×C}$	0.443			

*There is no difference at the 0.05 probability level between the mean values with the same letter groups. LSD shows the least significant difference between the mean values. ns means not important.

Table 8. Mean values of zinc content of grain in zinc applications at the stage of milky or dough ripeness in durum wheat cultivars (mg kg-1)

Cultivars (C)	Zinc Application Stages (Zn)			
	Control	Milky ripeness	Dough ripeness	Mean
Ç.1252	35.12	43.72	40.20	39.68 b*
Eminbey	41.08	55.10	49.67	48.61 a
Kızıltan-91	38.23	54.03	51.03	47.76 a
Meram-2002	42.78	49.68	54.05	48.84 a
Selçuklu-97	39.76	56.71	52.35	49.61 a
Mean	39.39 b	51.85 a	49.46 a	46.90
LSD_{Zn}		5.91		
LSD_{C}		4.71		
$LSD_{Zn×C}$		ns		

*There is no difference at the 0.05 probability level between the mean values with the same letter groups. LSD shows the least significant difference between the mean values. ns means not important.

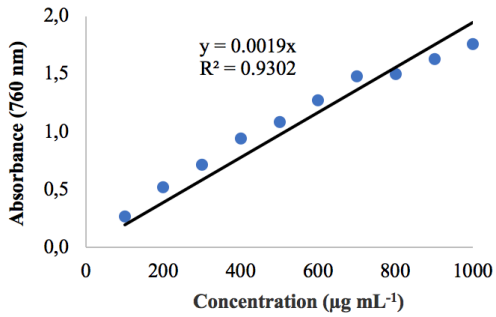

Figure 2. Standard graph for total phenolic compound content

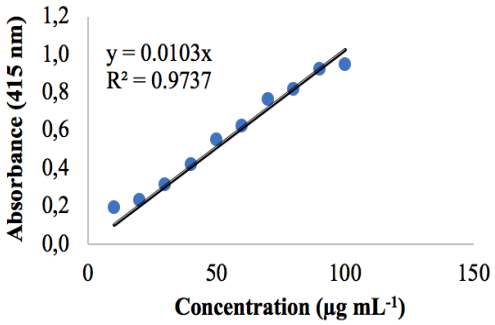

Figure 3. Standard graph of total flavonoid content

Conclusion and Recommendations

With this study, it is concluded that some antioxidant properties of grain such as flavonoid content, $ABTS^{+}$ radical scavenging activity and zinc content can be further increased by foliar zinc application at the stage of dough ripeness of grain. In addition, it is understood that it would be more beneficial to carry out this study on larger plots, with more genotypes, and even by increasing the application periods. Moreover, including the pasta properties in the scope of the study in addition to the physical and chemical quality criteria, it will provide new contributions to the literature and pasta industry.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. However, Aut. G. COŞKUN done his MSc thesis; Aut. F. TOPAL made much supports for the laboratory works; and Aut. B. BAHAR made statistical analysis and the paper writing together with the coordination.
All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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