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ORIGINAL ARTICLE

Effects of Kaolin (M-99-099) Application on Antioxidant and Phenolic Compounds in Tea Leaves (*Camellia sinensis* L.O. Kuntze)

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Abstract In this study, the effects of kaolin (M-99-099) applications on the total phenolic and antioxidant contents of tea leaves (*Camellia sinensis* (L.) O. Kuntze) harvested in three different periods were investigated. For this purpose, four different strategies including standard fertilizer application (T), 3% kaolin+standard fertilizer application (Ka_1), 6% kaolin+standard fertilizer application (Ka_2), and control (C) (nothing applied) were used to compare the effects of these strategies on total phenolic content, FRAP values, and DPPH radical scavenging capacities of tea leaves. It has been proven that the kaolin applications, Ka_1 and Ka_2, increase the phenolic content and antioxidant contents of tea samples. While the kaolin applications have higher values at 1st harvest than those of T and C, they have the lowest values at 3rd harvest.

Keywords Tea plant · Kaolin · Phenolic content · DPPH · FRAP

Introduction

Tea, *Camellia sinensis* (L.) O. Kuntze, is a drink and food material obtained after the processing of its fresh leaves with different methods. China, India, Kenya, Sri Lanka, Vietnam, and Turkey are the countries where tea is widely cultivated, and tea production is heavily made. With these countries, there are about forty countries

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also producing tea (FAO 2015). Ranking sixth among the tea-producing countries, Turkey has made significant progress in the tea cultivation and tea industry in recent years. Tea has become a strategic product for the country's economy.

Tea is an important source of polyphenols (Gramza and Korczak, 2005). There are studies on the extracts of leaves of green tea (Bu-Abbas et al. 1997; Baptista et al. 1999; Row and Jin 2005; Sharma et al. 2005; Perva-Uzunalic et al. 2006) and fresh tea (Yao et al. 2004). Fresh tea leaves are rich in polyphenols, especially flavan-3-ol (catechin) and flavanol glycosides (Clifford et al. 2000). In recent years, tea catechins have gained attention due to their biological activities (Chen et al. 2001) such as antioxidant (Zandi and Gordon 1999; Mello et al. 2005; Navas et al. 2005), antimutagenic (Halder et al. 2005), anticarcinogenic (Han 1997; Zhu et al. 2005), and antibacterial (An et al. 2004).

Tea is highly consumed after water in Turkey and all over the world due to its beneficial effects such as reducing the risk of cardiovascular disease and cancers, improving antibacterial and anti-inflammatory activity, and regulating food intake. Most of these effects have been attributed to functional components such as polyphenols, polysaccharides, minerals, and amino acids in tea leaves that vary according to genetic strain, climatic conditions, soil profile, growth altitude, horticultural practices, or plucking season (Kosinska and Andlauer 2014; Yang et al. 2014).

Recently, several groups have focused on how total phenol contents, antioxidant capacity, and element contents of tea samples change at different harvest times with some applications. Yang et al. (2014) investigated the effects of fertilizing with N, P, Se, and Zn on functional component and antioxidant activity of tea leaves. In another study, Topuz et al. (2014) investigated the effect of shooting period, shading, and clone on physicochemical properties of Turkish green tea. In 2010, Erturk et al. also reported a paper on how harvest time and clones affect total phenolic, antioxidant activity in Turkish tea shoots. It can be concluded from these studies that total phenolic content, antioxidant capacities, and mineral contents of tea leaves depend on clones, harvest time, climatic conditions, and the materials applied.

Kaolin, its use has increased in agriculture in recent years, generates a cuticle-like protective layer and a reflective white surface on plants and fruit to protect them against biotic and abiotic stress conditions (Glenn et al. 2002; Yazıcı and Kaynak 2009). According to the studies conducted, kaolin applications are used to reduce environmental stresses such as sun damages and temperature stress (Glenn et al. 2001; 2002; Tworkoski et al. 2002; Yazıcı and Kaynak 2009), and insect damages (Glenn et al. 1999; Knight et al. 2000; Puterka et al. 2000a; Unruh et al. 2000) and to prevent the occurrence of diseases (Glenn et al. 1999; Puterka et al. 2000b). Kaolin application increased the fruit size, the amount of dry matter, and fat content in olives (Saour and Makee, 2003), and had a positive impact on the quality of pomegranate fruits (Yazıcı and Kaynak 2009). It was found that kaolin improved photosynthesis in both hot and temperate climates, and it results in increasing yield and quality of plants (Gleen et al. 2001), and also its exogenous application promoting the production of antioxidants, such as phenols, flavonoids, anthocyanins, and vitamin C, which influence fruit quality (Bernardo 2015).

Environmental stresses lead to physiological and metabolic changes which cause loss in yield and quality while they affect the growth of tea negatively as in other plants. Unlike other countries where tea is grown in the world, The Eastern Black Sea Region, where Turkish tea grows, has temperate climatic conditions. Therefore, due to being the northernmost region for tea cultivation in the world, in some years, tea plant can be damaged by cold in early spring, which can cause losses in yield and quality. The foliar application of kaolin has proven effective in mitigating the negative impacts of these abiotic stresses in other fruit crops (Glenn et al. 2001, 2002); however, little is known about its influence on the composition of the tea leaf. Therefore, this study was conducted to determine the effect of Kaolin application on total phenol contents and antioxidant capacity of tea leaves cultivated in the ecological conditions of Rize province of Turkey.

Materials and Methods

Kaolin Application and Harvest

The trial was conducted in experimental orchards of Recep Tayyip Erdogan University, Faculty of Agriculture and Natural Science, Department of Horticulture. The climate of the area is typically temperate and sub-temperate. The annual rainfall ranges between 1800 and 2200 mm. The average air temperature was calculated to be 14.1 °C, and the highest and the lowest air temperatures were measured as 37.9 °C and −7.0 °C, respectively. The orchard soil was sandy in texture with pH 4.50, 390 μhos/cm electrical conductivity (EC), and 3.65% organic matter.

In this study, four situations were compared (standard fertilizer application (T): 70 kg/daa, 25-5-10 composite fertilizer (commercial formulation: 25% NH(4)-N, 5% phosphorus, and 10% potassium oxide); Kaolin Application-1 (Ka_1): %3 Kaolin+70 kg/daa 25-5-10 composite fertilizer; Kaolin Application-2 (Ka_2): %6 Kaolin+70 kg/daa 25-5-10 composite fertilizer); and control (C): nothing applied. The study was conducted according to a completely randomized block design with three replicates. Each plot was approximately 40 m^2. The tea plants were exposed to three kaolin applications with one week of intervals before each of the three harvests. After three weeks from the last kaolin application, the samples were collected at three harvest seasons: May 12, July 12, and September 6, 2016.

Chemicals and Instrumentation

The harvested tea plants were brought to the laboratory for withering and drying. The samples were kept in the drying box until their dry weights became stable. The dried samples were ground to a fine powder and kept at −20 °C until the antioxidant capacities and total phenolic content were carried out.

Gallic acids, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tripyridyl-s-Triazine (TPTZ), and iron (II) sulfate were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Folin–Ciocalteu's phenol reagent, sodium carbonate, hydrochloric acid, methanol, and acetic acid were obtained from Merck (Darmstadt, Germany). Other chemicals and solvents used in the experiments were purchased commercially, and used as received.

UV/vis spectra were recorded on a Shimadzu UV-1800 spectrophotometer (Shimadzu Inc., Kyoto, Japan) at 293 K. The pH measurements were carried out on a Hach Senson PH3 (Hach com., Loveland, USA) pH meter equipped with a Hach micro-combination electrode calibrated with standard buffer solutions.

Extraction

0.2 g of each dried-ground sample was extracted by 80% (v/v) aqueous methanol (20 mL). The extraction was carried out for 2 h using an orbital shaking at 40 °C. The cooled samples were filtered to be used directly for the determination of total phenol contents and antioxidant activities.

Determination of Total Phenolic Content

The total phenolic content was analyzed using the Folin–Ciocalteau method as described previously by Waterhouse (2002). Briefly, 20 µL of the extract was mixed with 1.58 mL of distilled water and subsequently with 100 µL of Folin–Ciocalteau reagent. After 3–5 min, 300 µL of sodium carbonate solution (75 g L^{-1}) was added into the mixture. Then the mixture was incubated at 50 °C for 15 min, and the absorbance was measured at 765 nm using a Shimadzu UV-1800 spectrometer (Shimadzu Inc., Kyoto, Japan). Gallic acid was used as the standard, and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dried weight (DW) of the sample.

Determination of Antioxidant Activity by FRAP

The FRAP assay was carried out according to a modified previous method (Benzie and Strain 1996). The FRAP solution was prepared by mixing 25 mL of 300 mM acetate buffer (3.1 g of $CH_3COONa{\cdot}3H_2O$ and 16 mL of CH_3COOH pH 3.6), 2.5 mL TPTZ solution (10 mM TPTZ in 40 mM HCl), and 2.5 mL of 20 mM $FeCl_3{\cdot}6H_2O$ solution. 50 µL of the extract (or 50 µL of distilled water as blank) was mixed with 1.95 mL of the FRAP solution, and then the mixture was allowed to warm to 37 °C for 3 min. Absorbance was determined at 593 nm using a Shimadzu UV-1800 spectrometer. $FeSO_4$ was used as the standard to establish a standard curve. The results were expressed as mmol of iron (II) sulfate ($FeSO_4$) equivalents per gram of dried weight (DW) of the sample.

Determination of Antioxidant Activity by DPPH

The antioxidant activity of the sample was performed using DPPH assay according to slightly modified method of Topuz et al. (2014). Diluted sample extract (100 µL) was added to 2 mL of freshly prepared DPPH (2,2-diphenyl-1-picryhydrazyl radical) solution (0.12 mM in 80% of MeOH). The mixtures were shaken and kept in the dark at room temperature for 30 min. Absorbance values of the final solutions were recorded at 517 nm using a Shimadzu UV-1800 spectrometer. The antioxidant activity of the samples was expressed as percent inhibition of the DPPH radical and calculated by equation below:

$$I(\%) = \frac{Ac - Au}{Ac} \times 100,$$

where I is the inhibition percentage, and Ac and Au are the absorbance values of the control and test samples, respectively.

Statistical Analysis

The study was conducted according to a completely randomized block design with three replicates. Analysis of variance (ANOVA) was used to evaluate the effects of harvesting time and kaolin application on the quality parameters and chemical components of the tea samples and the Duncan's multiple range tests were used to compare the averages (Gomez and Gomez 1984).

Results

In this study, the effects of the kaolin applications on the amount of the total phenolic content and DPPH radical scavenging capacity (%) were found to be statistically significant. However, the FRAP values were not statistically significant (Table 1).

Total Phenolic Content

The total phenolic contents (TP) of the green tea leaves are shown in Fig. 1. The highest total phenolic content in terms of the application averages was determined for C (125.32 mg GAE/g DW) and Ka_2 (124.48 mg GAE/g DW), while the total phenolic content of Ka_1 was calculated 123.91.7 mg GAE/g DW. The lowest value was calculated for T (117.04 mg GAE/g DW). In terms of the harvest time averages, the highest phenolic content (125.78 mg GAE/g DW) was determined at 1st harvest time, while the lowest (119.71 mg GAE/g DW) was found at 3rd harvest time (Table 1).

The highest total phenol values were determined in the first harvest, while the lowest values were determined in the second harvest for T, Ka_1, and Ka_2. TP for T, Ka_1, and Ka_2 were 120.60 ± 2.40, 130.13 ± 1.46, 131.64 ± 4.52 mg GAE/g DW at 1st harvest and decreased to 117.95 ± 4.46, 124.41 ± 2.24, 122.83 ± 2.22 mg GAE/g DW at 2nd harvest and then decreased further to 112.88 ± 4.19, 117.20 ± 2.39, 118.98 ± 3.25 mg GAE/g DW at 3rd harvest, respectively. However, for C, the highest value (129.75 ± 1.69) was calculated in the third harvest and the lowest value (120.73 ± 3.13) was determined in the first harvest. On the other word, the orders of TP for T, C, Ka_1, Ka_2 were $Ka_2 > Ka_1 > C \approx T$ at 1st harvest, $Ka_1 > Ka_2 > C > T$ at 2nd harvest, and $C > Ka_2 > Ka_1 > T$ at 3rd harvest (Fig. 1).

Table 1 Statistical analysis on total phenolic content, FRAP values, and DPPH values of the green tea samples

Varieties	Strategies	1st harvest	2nd harvest	3rd harvest	Mean of strategies
Phenolic content (mg GAE/g DW)	C	120.73 ± 3.13b	121.47 ± 3.19b	129.75 ± 1.69a	125.32 A
	T	120.60 ± 2.40ab	117.95 ± 4.46b	112.88 ± 4.19b	117.04 B
	Ka1	130.13 ± 1.46a	124.41 ± 2.24b	117.20 ± 2.39c	123.91 AB
	Ka2	131.64 ± 4.52b	122.83 ± 2.22b	118.98 ± 3.25bc	124.48 A
	Mean of harvest	125.78 A	121.68 B	119.71 B	
S: *, H: *, S × H: **					
DPPH radical scavenging capacity (%)	C	89.25 ± 1.19	86.61 ± 1.09	85.76 ± 1.53	87.21 B
	T	88.51 ± 1.20	85.88 ± 1.07	84.61 ± 1.53	86.33 B
	Ka1	92.60 ± 1.20	88.45 ± 0.68	87.98 ± 1.53	89.68 A
	Ka2	93.60 ± 1.19	89.60 ± 1.08	87.47 ± 1.07	90.22 A
	Mean of harvest	90.99 A	87.63 B	86.46 C	
S: **, H: **, S × H: NS					
FRAP values (mmol FeSO4\g DW)	C	5.51 ± 0.23	5.45 ± 0.09	5.93 ± 0.45	5.63
	T	5.44 ± 0.31	5.44 ± 0.01	5.16 ± 0.43	5.35
	Ka1	5.53 ± 0.16	5.37 ± 0.18	5.36 ± 0.56	5.42
	Ka2	5.47 ± 0.16	5.42 ± 0.20	5.00 ± 0.90	5.3
	Mean of harvest	5.49	5.42	5.36	

S: NS, H: NS, S × H: NS

S Strategies, *H* harvest, *NS* not significant

** Significant level 1%, * significant level 5%

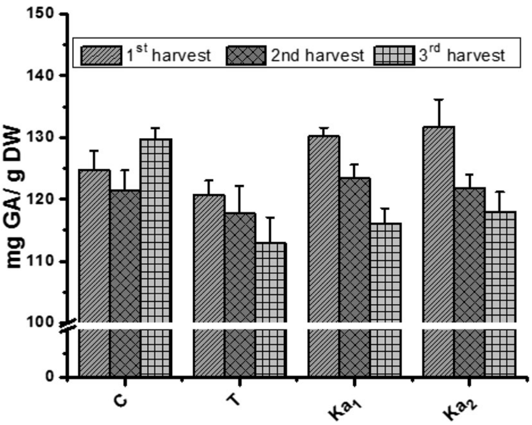

Fig. 1 Total phenolic content of the green tea powders. *T* traditional, Ka_1 3% kaolin, Ka_2 6% kaolin, and C was the control group (statistical information given in Table 1)

DPPH Radical-Scavenging Capacity

In terms of the application averages, the highest DPPH radical scavenging capacities were determined for the tea leaves applied with Ka_2 (90.22%) which was statistically in the same group as Ka_1 (89.68%). The lowest DPPH radical scavenging capacities were calculated for C and T (87.21% for C; 86.33% for T). In terms of the harvest time averages, the highest DPPH radical scavenging capacity (90.99%) was found at 1st harvest time, while the lowest DPPH radical scavenging capacities were determined at 3rd (86.46) harvest times (Table 1).

Similarly, DPPH radical scavenging capacity of the tea samples at different harvest times were 1st harvest > 2nd harvest > 3rd harvest (Fig. 2). The strongest DPPH antioxidant activity was found for Ka_1 and Ka_2 at 1st harvest time (92.60% and 93.60%, respectively). The lowest DPPH values were obtained from 3rd harvest for all the strategies. The values were found as follows: for C: 89.25 ± 1.19, 86.61 ± 1.09, 85.76 ± 1.53; for T: 88.51 ± 1.20, 85.88 ± 1.07, 84.61 ± 1.53; for Ka_1: 92.60 ± 1.20, 88.45 ± 0.68, 87.98 ± 1.53; and for Ka_2: 93.60 ± 1.19, 89.60 ± 1.08, 87.47 ± 1.07at 1st, 2nd, and 3rd harvest times, respectively (Fig. 2).

FRAP Assay

In terms of the application averages, the highest FRAP values (mmol $FeSO_4$/g DW) were determined from the tea leaves applied with C (5.63), T (5.35), Ka_1 (5.42), and Ka_2 (5.30) which are not statistically significant. In terms of the harvest time averages, the highest FRAP value (5.49) was found at 1st harvest time, while the lowest FRAP value (5.36) was determined at 3rd harvest time (Table 1).

The orders of FRAP levels for C, T, Ka_1, and Ka_2 were similar to that seen in total phenolic content and DPPH radical scavenging capacities. The highest FRAP values (mmol $FeSO_4$/g DW) were obtained from the tea samples collected at 1st harvest time. The lowest FRAP values of the tea samples were found at 3rd harvest time, except for C. These values for the strategies T, Ka_1, and Ka_2 were determined to be 5.44 ± 0.31, 5.53 ± 0.16, 5.47 ± 0.16 mmol $FeSO_4$/g DW, respectively, at

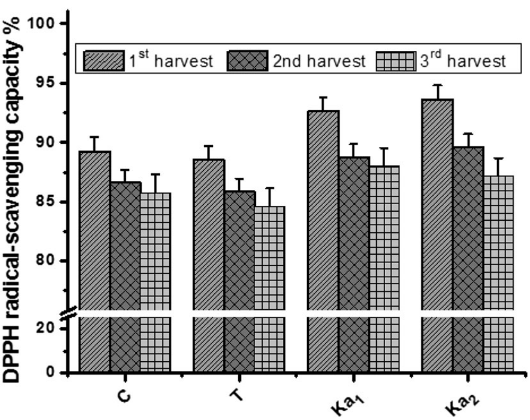

Fig. 2 Free radical scavenging activity (by DPPH) of green tea powder. *T* traditional, *Ka_1* 3% kaolin, *Ka_2* 6% kaolin, and C was the control group (statistical information given in Table 1)

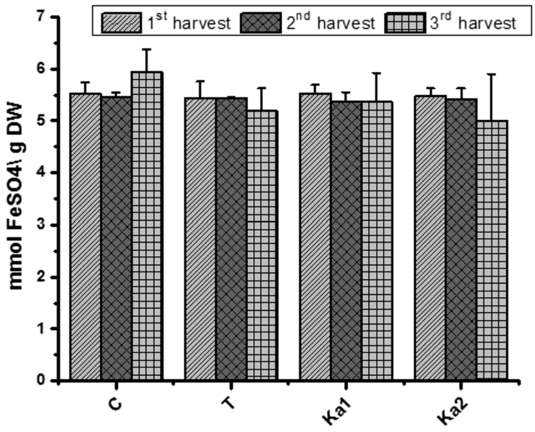

Fig. 3 FRAP values of the green tea powders. *T* traditional, *Ka*$_1$ 3% kaolin, *Ka*$_2$ 6% kaolin, and C was the control group (statistical information given in Table 1)

1st harvest; 5.44 ± 0.01, 5.37 ± 0.18, 5.42 ± 0.20 at 2nd harvest; and 5.16 ± 0.43, 5.36 ± 0.56, 5.00 ± 0.90 at 3rd harvest (Fig. 3; Table 1). FRAP values in C were 5.51 ± 0.23, 5.45 ± 0.09, 5.93 ± 0.45 mmol $FeSO_4$/g DW at 1st, 2nd, and 3rd harvest times, respectively (Table 1; Fig. 3).

Discussion

The results obtained from this study show that the kaolin applications on tea leaves enhanced the amount of total phenol content, DPPH radical scavenging capacity, and FRAP values of tea leaves at all three different harvest times.

The composition of bioactive compounds, and therefore antioxidant capacity of tea, might be influenced by several parameters associated with growth conditions: genetic strain, climatic conditions, soil profile, growth altitude, horticultural practices, or plucking season (Kosinska and Andlauer 2014). The temperature in the region, where tea is cultivated in Turkey, is high at 3rd harvest and low at 1st harvest. For this reason, the kaolin was applied before the 1st harvest where the temperature was low, which is effective in stress conditions, has a positive effect on the amount of the total phenolic content and antioxidant capacities of tea leaves in 1st harvest. Erturk et al. (2010) investigated the total phenolics, antioxidant activity, and mineral contents of seven tea clones cultivated in Turkey at different harvest times. The total phenolics of all clones were lower in cool months of May for two years. Thereafter, the levels of total phenolics increased throughout the warmer months from July to September. Ercisli et al. (2008) studied seasonal variation of total phenolics, antioxidant activity, and element assay in fresh tea leaves grown in Turkey. The highest values of total phenolics and antioxidant activity were obtained at 2nd harvest time. Jamal and his colleagues worked on seasonal variation of phenolic components in two clones of tea grown in Iran. The results of this study showed that phenolic components of both tea clones increased from 1st harvest to 3rd harvest (Mohammadian et al. 2014).

The yields and quality in tea cultivation are significantly affected by climate factors. It has been shown that the biosynthesis of phenolic compounds can be effectively induced by sunlight (Harbowy and Balentine 1997). Especially, precipitation, temperature, sunlight, day length, and moisture directly affect photosynthesis in tea plants; hence, it results in differences in quality and yield of tea in different harvest times. The effect of temperature on growth and yield of tea for different areas cannot be generalized as the optimum range would possibly vary depending on the overall environmental condition of an area (Panda 2011). Wijeratnc and Fordham (1996) reported that the decline in shoot extension rates appeared to begin with increasing temperatures above approximately 26 °C. In a multiple linear regression relationship with all three environmental factors, only the temperature effect was significant. In Turkey, quality and yield in tea plant are adversely affected by temperature under 13 °C and over 32 °C. That is why the total phenolic content and antioxidant capacities of tea samples in this study varied at 1st, 2nd, and 3rd harvest times. The great difference of tea leaves for antioxidant activity at different harvest times is supposed to be due to the effect of change of ecological parameters. It was previously reported that the composition of tea leaves varies with climate, variety, and age of the leaf (Leung and Foster 1996).

There are several studies reporting positive impact of kaolin application on plants. Dinis et al. (2016) investigated the effect of kaolin on the antioxidant system of grapevine (cv. Touriga Nacional) leaves and fruits extracts to summer stress, using chemical (ABTS, FRAP, DPPH) and biochemical methods. The results show that kaolin exogenous application enhanced the activity of enzymatic and non-enzymatic antioxidants systems, reducing reactive oxygen species (ROS) levels and lipid peroxidation levels (TBARS) and promoting the production of antioxidants, such as phenols, flavonoids, anthocyanins, and vitamin C, which influence grapes quality. Also, in Merlot grape (*Vitis vinifera* L.), kaolin application enhanced the total amount of berry anthocyanins (Song et al. 2012).

Conclusion

Temperature, depending on its severity and duration, affects metabolic activities, growth, and development of plants, which limits the distribution of plants in countries and regions. Most plants generally develop at temperatures between 15 and 45 °C. The growth and metabolism of plants as well as the quality and quantity of the products are seriously affected under and above these temperatures. In Turkey, quality and yield in tea plant are adversely affected by temperature under 13 °C. These low temperatures, which are usually measured before the first harvest season, cause low tea quality in the harvest season. In this study, it was determined that the kaolin applications especially made during this period increased the phenolic content and antioxidant contents of tea samples. Kaolin has promising results for further studies.

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