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CHEMICAL COMPOSITION OF TOBACCO GENOTYPES IN RESPONSE TO ZINC APPLICATION UNDER CADMIUM TOXICITY

Ahmet Kınay[1], Halil Erdem[2*], Erdem Karakoç[1]

[1]Tokat Gaziosmanpasa University, Faculty of Agriculture, Department of Field Crops, 60250, Tokat, Turkey
[2]Tokat Gaziosmanpasa University, Faculty of Agriculture, Soil Science and Plant Nutrition Department, 60250, Tokat, Turkey
[*]Corresponding author. E-mail: erdemh@hotmail.com

ABSTRACT

Cadmium (Cd), a non-essential heavy metal that cannot be naturally degraded, causes toxicity for plant, animal and human due to the easy incorporation into the food chain with its high mobility in soil-plant system. Tobacco (*Nicotiana tabacum* L.) contrary to other crops, has ability to absorb and accumulate high concentration of Cd in the leaves without showing phytotoxicity symptom. The purpose of this study was to investigate the effects of zinc (Zn) applications on chemical composition of tobacco leaves under increased doses of Cd under greenhouse conditions. The experimental layout was randomized plots with three replicates, and two different tobacco genotypes (Birlik-124, Xanthi-81) were used as the plant material of the experiment. Plant growing medium was a Zn-deficient calcareous soil, and plants were treated with increasing Cd (0, 2.5, 5.0 and 10 mg kg^{-1} soil) and Zn (0 and 5 mg kg^{-1} soil) doses. The tobacco leaves were harvested in three times and all harvested leaves were dried under sunlight. The leaf samples were analyzed for Cd and Zn concentrations, nicotine, glucose, fructose, chlorogenic and rutin contents. The Cd concentration from leaves of both tobacco genotypes significantly increased ($P<0.05$) with the increase in Cd application rate, but the increase in Cd concentration decreased with Zn application. Application of increased Cd doses significantly ($P<0.05$) increased or decreased the nicotine content of leaves, while Zn application caused a significant increase in nicotine contents of both tobacco genotypes. The nicotine content in Birlik-124 and Xanthi-81 genotypes was 0.41 and 0.48% in control plants (Zn0), and increased up to 0.54% in plants treated with zinc (Zn5 variant). Similar to the nicotine content of both tobacco genotypes, the responses of both genotypes to Cd stress was different form each other, however, Zn application under Cd toxicity caused an increase in sugar synthesis of tobacco varieties. The results revealed that Zn plays an important role in the alleviating the negative effects of Cd stress in tobacco plants.

Keywords: cadmium, zinc fertilization, tobacco, toxicity, nicotine.

INTRODUCTION

Cadmium (Cd), which has a toxic effect on living organisms, is one of the most hazardous heavy metal in the ecosystem. The main sources of Cd in soil are geological material and the anthropogenic activities such as industrial activities and application of phosphorus fertilizer. Application of phosphorus fertilizers is responsible for 54-58% of Cd reaching to soil, while, atmospheric deposition, and sewage sludge and manure applications are responsible for 39-41 and 2-5% of the Cd in the soils, respectively (Wang et al., 2015). The Cd, which causes toxic effects for more than 3 mg kg^{-1} in soils, is rapidly increasing throughout the world especially in the last 20-30 years. The Cd, not an essential element for biological functions, is toxic to humans, animals and plants, and is 2-20 times more toxic effects than other heavy metals (Friberg, 2018). Cadmium is a highly mobile element in soils and is easily included into the nutrient chain of plants. The increased Cd uptake of plants may cause severe changes in biochemical (synthesis of reactive oxygen species) and physiological characteristics (chlorosis and necrosis) of plants (Namdjoyan et al., 2012), impedes growth and alters nutrient uptake (Nedjimi and Daoud, 2009; Gill and Tuteja, 2011). El-Beltagi et al. (2010) indicated Cd accumulation in plants may cause changes in membrane permeability, restriction in

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enzyme activities and reduction in water potential. Gill and Tuteja (2011) reported negative impact of high Cd content on some essential process such as photosynthesis, respiration and protein metabolism (Gill and Tuteja, 2011). The oxidative stress caused by excess Cd in plants restricts the activities of essential functional groups (Schützendübel et al., 2002). Interaction of Cd with the antioxidative defense system through disturbing the electron transport chain was reported as an indirect impact of Cd toxicity (Cuypers et al., 2010). Protection against oxidative stress in plants can be provided by the increasing level of reducing sugars, such as glucose, sucrose and related (Van den Ende and Valluru, 2009). Zinc (Zn) is an essential micronutrient for plants and animals (Alloway, 2013). Contradictory reports have been published on the effects of Zn application on Cd uptake of plants. Some researchers indicated an increase in Cd concentration of plants by the application of Zn. Many researchers claimed that the Cd uptake of plant will decrease with the application of Zn. Studies on the antagonistic and synergistic effects of Zn on Cd uptake of plants are very common in the literature. The initial studies on Zn applications reported significant increase in Cd uptake of plants (Williams and David, 1976). However, Zn application to soil with low Cd content indicated a low Cd uptake of plants, in contrast Cd uptake was increased when soil had high Cd concentration (Page et al., 1981). Higher Cd uptake of plants grown in Zn deficient soils was attributed to the competition of Zn and Cd, which have similar chemical properties, for the absorption points on membranes (Grant et al., 1998; Cakmak et al., 2000; Ozturk et al., 2003), and the increased membrane permeability related to the Zn deficiency (Cakmak and Marschner, 1988). Grain Cd concentration was significantly decreased with the 5 kg ha^{-1} Zn application to Zn deficient soils in Australia (Oliver et al., 1994). Tobacco (*Nicotiana tabacum* L.) has ability to absorb and accumulate high concentration of Cd in the leaves without showing phytotoxicity symptoms (Erdem et al., 2017a; 2017b; Kınay, 2018). Threefold higher Cd accumulation in the leaves of tobacco plants, grown in a soil containing 69 mg kg^{-1} Cd, compared to most of the crops were reported (Davis and Carlton-Smith, 1980). Some of the responses reported upon exposure of plants to high Cd accumulation were retardation of growth, hindrance of photochemical activity of photosystem II, low photosynthetic rate, occurrence of chlorosis and small rust-like circular spots on leaves, leaf chlorophyll a and b contents, chlorophyll a fluorescence and biomass and root damage (Li et al., 1997; Choi et al., 2001; Erdem et al., 2017a; 2017b).

The aim of this study was to investigate the effects of Zn fertilization on reduction of cadmium toxicity and on chemical composition (nicotine, glucose, fructose, chlorogenic and rutin) of tobacco leaves.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse located in the Faculty of Agriculture, Tokat Gaziosmanpasa University, Turkey (40.33°N, 36.47°E, 640 m altitude). Local commonly grown tobacco genotypes of Birlik-124 and Xanthi-81 were used as the plant material of the experiment. Birlik-124 genotype is a medium to late flowered genotype, the number of leaves ranges from 35 to 40 per plant and an average plant height of 120-130 cm. Xanthi-81 genotype is a fine-textured, very fragrant and medium-late flowering tobacco genotype. The number of leaves is 30-32 per plants and the average plant height is between 65 and 105 cm (Ekren and Ilker, 2017; Kinay et al., 2019). The greenhouse was maintained at 33 day/22 night (SD 5) °C temperature. The tobacco seeds were germinated in the flats of sphagnum moss and the seedlings were transferred to the plastic pots filled 2.10 kg air dried Zn deficient soil. The size of plastic pots used in the experiment was 220 by 180 mm (3 L volume). The experimental soil was coarse textured (sandy loam), highly calcareous (19.7%), slightly alkaline (pH=7.83), and low in organic matter content (1.42%).

The concentrations of metals extracted by DTPA were 0.05, 0.28, 3.74, 8.44 and 0.84 mg kg^{-1} for Cd, Zn, Fe, Mn and Cu, respectively.

Experimental Setup

The greenhouse experiment was designed in a randomized plots with three replicates. Four-six leaf stage tobacco seedlings were planted into the plastic pots. Fertilizer solution containing 250 mg N kg^{-1} soil as $Ca(NO_3)_2.4H_2O$, 100 mg P kg^{-1} soil as KH_2PO_4, 2.0 mg Fe kg^{-1} soil as Fe-EDTA was applied and thoroughly mixed with the soil prior to filling the free-draining pots. Four doses of Cd (0, 2.5, 5.0 and 10 mg kg^{-1}) as $3(CdSO_4).8H_2O$ and two doses of Zn (0 and 5 mg kg^{-1}) as $ZnSO_4.7H_2O$ were used as the treatments of the experiment. Tobacco plants were irrigated as needed using deionized water. The duration of the experiment was 92 days and all matured tobacco leaves were harvested in three times. Harvested tobacco leaves were air dried under sunlight and powdered with an agate mill for chemical analysis.

Cadmium and zinc analysis of plant leaves

The weights of tobacco leaves air dried was expressed as g dry matter (DW). The powdered tobacco leaves were digested in a microwave oven using 2 ml of 35% H_2O_2 and 5 ml of 65% HNO_3. The Cd and Zn concentrations in solutions were determined by an inductively coupled plasma atomic emission spectroscopy (Varian Vista) (Bataglia et al., 1978). The concentrations of Cd and Zn in tobacco leaves were double-checked by using the reference leaf samples purchased from the National Institute of Standards and Technology (Gaithersburg, MD, USA).

Nicotine content of tobacco leaves

Nicotine content of tobacco leaves was determined using HPLC analysis by an Agilent technology 1260 series HPLC system (Agilent Technologies, Boeblingen, Germany) with a diode array detector. The separation of nicotine was carried out on a reversed-phase ACE C18 column (Agilent Technologies) by 250 x 4.6 mm i.d. dimensions and 5 μm particle size. The mobile phase of the system contained 1% acetic acid in water (solvent A) and acetonitrile (solvent B). Alkaloids of the leaf samples were recorded at 324 nm UV and the nicotine content was determined using a certified standard (Kinay, 2018).

Phenolics (chlorogenic and rutin) analysis of tobacco leaves

Chlorogenic and rutin contents of tobacco leaves were determined using a HPLC system by a poroshell 120 EC C18 column (2.7 μm, 150 mm × 3.0 mm i.d.) with guard precolumn. The mobile phase of the system contained 1% acetic acid in water (solvent A) and acetonitrile (solvent B) (Kınay, 2018).

Reducing sugars (glucose and fructose) analysis of tobacco leaves

Reducing sugars contents of tobacco leaves were determined using the application note for Agilent Hi-Plex Columns for Carbohydrate, Alcohols and Acids by a Zorbax Carbohydrate column (4.6 x 250 mm and 5 μm particle size) (Kinay, 2018).

Statistical Analysis

The effects of Zn and Cd application doses on Zn concentration and nicotine, glucose, fructose, chlorogenic and rutin contents of two tobacco genotypes were assessed using the variance analysis (ANOVA). Duncan's homogeneity test ($P<0.05$) was used to differentiate the means in case ANOVA denoted significant differences. The results obtained in Zn application were compared with the t-test. The data were analyzed using MSTAT-C statistical software.

RESULTS AND DISCUSSION

Cadmium can be easily transferred to the green parts fallowing the uptaken by plant roots. The mobility of Cd in the soils is higher than other heavy metals (Mn, Zn, Mo and Se) metals and can be easily taken by many plant species (Moral et al., 2002). Therefore, a positive correlation has often

been reported between the increasing doses of Cd application to the soil and Cd concentration of the green components. Leaf Cd concentration of two tobacco genotypes in Zn0 and Zn5 treatments significantly increased ($P<0.05$) with the increase in Cd application rates (Table 1). The leaf Cd concentration, which was 1.92 mg kg^{-1} in the Cd0 dose, of Birlik-124 genotype increased to 88.0 mg kg^{-1} at the Cd10 dose of Zn0 treatment, and the leaf Cd concentration, which was 1.80 mg kg^{-1} in the Cd0 dose, increased to 74.9.mg kg^{-1} in Zn5 treatment. Similar trend was recorded for Xanthi-81 genotype. The leaf Cd concentration in Zn0 treatment was 1.29 mg kg^{-1} at Cd0 dose and increased to 63.2 mg kg^{-1} at Cd10 dose. The leaf Cd concentration in Zn5 treatment was 0.83 mg kg^{-1} at Cd0 dose and increased to 88.4 mg kg^{-1} at Cd10 dose (Table 1). The results are in accordance with the previous reports which indicated that tobacco plants remove relatively higher Cd from Cd contaminated soils compared to many other crops (Lugon-Moulin et al., 2004; Erdem et al., 2012a; Kinay, 2018). Erdem et al. (2017b) conducted a greenhouse study to investigate the effects of Cd applications at 0, 2.5, 5.0 and 10 mg kg^{-1} doses in two separate forms ($CdS0_4$)$3.8H_20$, Cd(NO3)$2.4H_2O$ and $CdCl_2.H_2O$ on Cd uptake of Katerini tobacco cultivar. The Cd concentration in green components of tobacco plants significantly increased with the applications of different forms and increased doses of Cd. The highest Cd concentration in the 10 mg kg^{-1} Cd application compared to the control was obtained in the applications of $CdCl_2.H_2O$ (176.3 mg kg^{-1}) and ($CdS0_4$)$3.8H_20$ (170.47 mg kg^{-1}) forms, while the lowest Cd concentration was reported in the application of Cd(NO_3)$2.4H_2O$ (129.42 mg kg^{-1}) form. Contradictory opinions have been reported about the effects of Zn application on the Cd uptake of plants. Some researchers suggested that the Cd concentration of plants will increase with the application of Zn, while majority of researchers indicated that Cd uptake of plants will decrease with the Zn application. Leaf Cd concentrations of Birlik-124 and Xanthi-81 tobacco genotypes decreased significantly with the application of 5 mg kg^{-1} Zn compared to the Zn0 mg kg^{-1}. The Cd concentration of the Birlik-124 genotype, which was 63.4 mg kg^{-1} under Zn0xCd5 interaction, decreased to 53.5 mg kg^{-1} in the Zn5xCd5 interaction, and in the Xanthi-81 variant decrease from 50.7 mg kg^{-1} to 42.4 mg kg^{-1} (Table 1). The results are in agreement with the statement of Muramoto et al. (1990) who reported an antagonistic effect of Zn application on Cd uptake. Inorganic or organic Zn application to Zn-deficient soil significantly reduced Cd concentration in corn plants (*Zea mays* L.) grown under greenhouse conditions (Abdel-Sabour et al., 1988). Similar increase and decrease in Zn concentration were reported for Cd applications in rice genotypes by Wu et al. (2006) who indicated that Zn concentration of shoots increased with Cd application dose up to 0.1 mM, in contrast higher Cd application doses caused to a significant decrease in Zn concentrations.

Zinc application caused significant increases in leaf Zn concentrations of both tobacco varieties. The mean leaf Zn concentration of the Birlik-124 cultivar in the Zn0 and Zn5 treatments was 52.4 and 79.6 mg kg^{-1}, and for Xanthi-81 cultivar, 68.2 and 92.2 mg kg^{-1}, respectively (Table 1).

Table 1. Effect of Zn application on leaf yield, leaf Cd and Zn concentration in tobacco cultivars grown under Cd toxicity

Genotype	Cd Dose mg kg^{-1}	Cd Concentration (mg kg^{-1})**			Zn Concentration (mg kg^{-1})**			Leaf Yield (g plant^{-1})*		
		Zn0	Zn5	T test	Zn0	Zn5	T test	Zn0	Zn5	T test
Birlik-124	0	1.92^{f}	1.80^{f}	*	58.5	85.8	**	7.64	6.96	*
	2.5	36.8^{de}	25.6^{e}	**	44.3	80.0	**	6.95	6.92	ns
	5	63.4^{bc}	53.5^{cd}	**	47.6	78.8	**	7.21	7.19	ns
	10	88.0^{a}	74.9^{ab}	**	59.4	74.0	**	5.39	7.52	**
Mean		***47.5A***	***39.0B***		***52.4B***	***79.6A***		***6.80B***	***7.15A***	
Xanthi-81	0	1.29^{f}	0.83^{f}	*	83.0	97.5	**	7.65	6.97	*
	2.5	25.5^{e}	26.8^{e}	ns	58.7	87.1	**	8.08	7.53	*
	5	50.7^{cd}	42.4^{cde}	**	62.5	91.7	**	6.75	8.62	**
	10	63.2^{bc}	88.4^{a}	**	68.5	92.7	**	7.37	7.82	*
Mean		***35.2B***	***39.6A***		***68.2B***	***92.2A***		***7.46B***	***7.74A***	

*Significant at P<0.05 level; **Significant at P<0.01 level; ns: non significant.

The increasing Cd application doses significantly decreased the Zn concentrations of tobacco leaves in Zn0 and Zn5 treatments. The Zn concentration of Birlik-124 genotype was 58.5 mg kg^{-1} in the Zn0xCd0 interaction and decreased to 47.6 mg kg^{-1} in Zn0xCd5 interaction. Similarly, the leaf Zn concentration of Birlik-124 genotype was 85.8 mg kg^{-1} in Zn5xCd0 treatment and decreased to 78.8 mg kg^{-1} in Zn5xCd5 treatment. A similar situation was recorded for the Xanthi-81 variety (Table 1). The decrease of Zn concentrations in leaves with increasing Cd application rates can be attributed to the antagonistic relationship between Cd and Zn. Several studies reported reduced Zn uptake of plants due to the Cd applications (Grant and Bailey, 1997; Grant et al., 2002; Erdem et al., 2012b; Eker et al., 2013). Higher Cd uptake of plants in Zn deficiency has been associated to the competition between Zn and Cd, which have similar chemical properties, for absorption points on membranes (Grant et al., 1998; Welch et al., 1999; Cakmak et al., 2000; Harris and Taylor, 2001; Ozturk et al., 2003) and the increased membrane permeability (Cakmak and Marschner, 1988).

Leaf yields of tobacco varieties increased and decreased with increasing Cd and Zn application rates, however these increases and decreases were statistically insignificant. Leaf yield of Birlik-124 cultivar in Cd0xZn0 interaction was 7.64 g plant^{-1} and decreased to 5.39 g plant^{-1} in Zn0xCd10 interaction. Similarly, leaf yield of Xanthi-81 variety in Cd0xZn0 interaction was 7.65 g plant^{-1} and decreased to 7.37 g plant^{-1} in Zn0xCd10 interaction. Khurana and Jhanji (2014) reported a significant dry matter yield of corn with the application of Cd to soil. The decrease in dry matter yield was 11.9 and 23.5% with 10 and 20 mg kg^{-1} Cd application doses, respectively. The decrease in dry matter yield with Cd application was attributed to the phytotoxic effect of Cd on plant growth (Yang et al., 1996; Pereira et al., 2011). The mean leaf yield of cultivars increased with the increasing Zn application rates. The mean average leaf yield of the Birlik-124 cultivar in Zn0 was 6.80 g plant^{-1}, which increased to 7.15 g plant^{-1} in Zn5 treatments. Similarly, mean leaf yield of Xanthi-81 variety in Zn0 rate was 7.46 g plant^{-1} and increased to 7.74 g plant^{-1} variant in Zn5 application rate (Table 1). Zhu et al. (2003) applied different doses of Cd (0, 15, 30 and 50 mg kg^{-1}) and Zn (0, 2, 10, 100 and 1000 mg kg^{-1}) to a soil, where a winter wheat (Kenong 9209) was grown. The researchers indicated a dry matter yield decrease with the increasing doses of Cd, however, the decrease

in dry matter yield reduced with the Zn application (up to 10 mg Zn kg^{-1}).

Nicotine, the primary commercial source of tobacco, is an addictive alkaloid in humans (Moghbel et al., 2017). Nicotine concentrations, which is a very important alkaloid component for tobacco, had statistically significant increases and decreases ($P<0.05$) with the increasing application doses of Cd to soil. The increasing doses of Cd in Zn deficiency caused a decrease in nicotine content of Birlik-124 variety, in Xanthi-81 variety a decrease in Cd2.5 dose, while an increase in Cd5 and Cd10 doses. A similar trend was recorded for increasing Cd applications in Zn5 treatment. The results indicated that the responses of tobacco varieties to Cd stress are different from each other. However, Zn applications caused a significant increase in nicotine concentrations in both tobacco varieties. The mean nicotine concentration in the Zn0 dose of the Birlik-124 variety was 0.41% and increased to 0.54% in Zn5 dose. Similar increase in nicotine concentration was observed for Xanthi-81 variety (Table 2). The increase in alkaloid accumulation with metal application have not been clearly explained. Several mechanisms have been reported to explain the mechanism. Srivastava and Srivastava (2010) pointed to the significance of metals, especially the essential micronutrients, as cofactors of enzymes involved in biosynthetic pathway for the accumulation of alkaloids. The researchers also indicated that the primary metabolic pathways act the initial metabolic building blocks for the continuation of alkaloid biosynthetic pathway.

Table 2. Effect of Zn application on nicotine, glucose and fructose concentration on tobacco cultivars grown under Cd toxicity

Genotype	Cd Dose mg kg^{-1}	Nicotine Concentration (%)**			Glucose Concentration (%)**			Fructose (%)*		
		Zn0	Zn5	T test	Zn0	Zn5	T test	Zn0	Zn5	T test
Birlik-124	0	0.45^{b-e}	0.52^{a-d}	*	0.32^{h}	3.94^{b-e}	**	6.07^{de}	4.84^{efg}	*
	2.5	0.44^{b-e}	0.49^{a-e}	*	0.34^{h}	5.14^{abc}	**	4.30^{g}	4.31^{g}	ns
	5	0.37^{de}	0.56^{abc}	**	1.67^{gh}	5.50^{ab}	**	5.38^{d-g}	4.59^{fg}	ns
	10	0.37^{de}	0.58^{ab}	**	1.58^{gh}	6.64^{a}	**	3.95^{g}	5.33^{d-g}	**
Mean		***0.41B***	***0.54A***		***0.98B***	***5.30A***		***4.93***	***4.77***	
Xanthi-81	0	0.48^{a-e}	0.36^{e}	*	3.31^{def}	4.37^{bcd}	**	5.75^{def}	9.38^{c}	**
	2.5	0.43^{cde}	0.62^{a}	**	4.39^{bcd}	2.56^{efg}	**	6.34^{d}	10.69^{bc}	**
	5	0.49^{a-e}	0.43^{b-e}	ns	3.56^{cde}	2.41^{efg}	**	5.86^{def}	11.95^{b}	**
	10	0.53^{abc}	0.58^{abc}	ns	5.40^{ab}	3.85^{b-e}	**	6.04^{de}	13.65^{a}	**
Mean		***0.48***	***0.50***		***4.16A***	***3.30B***		***6.00B***	***11.42A***	

*Significant at $P<0.05$ level; **Significant at $P<0.01$ level; ns: non significant.

Regulation of internal osmolarity and protection of biomolecules and membranes are also induced by sugar accumulation (Sinniah et al., 1998; Verma and Dubey, 2001). In addition, carbohydrate storage reserves, which support basal metabolism under stressed environment, also are increased by an increase in soluble sugars content of plants (Hurry et al., 1995; Dubey and Singh, 1999). The reducing sugar (glucose and fructose) concentrations of tobacco leaves significantly ($P<0.05$) changed with increasing doses of Cd to the soil (Table 2). The increasing doses of Cd in Zn deficiency caused and increase in glucose concentrations of tobacco leaves and an increase and decrease in fructose concentrations. The glucose concentration of Birlik-124 genotype in Zn0xCd0 interaction was 0.32 and increased to 3.31% in the Zn0xCd10 interaction. Similarly, glucose concentration of the Xanthi-81 genotype

increased from 3.31 to 5.41%. The fructose concentration of the Birlik-124 genotype, which was 6.07% in the Zn0xCd0 interaction, decreased to 3.95% in the Zn0xCd10 interaction, while it increased from 5.75 to 6.04% for the Xanthi-81 genotype. Similar to the nicotine concentrations, the response both tobacco genotypes to Cd stress in terms of sugar concentrations was different from each other. However, the reducing sugar content (fructose + glucose) in both varieties increased with the increase in Zn application doses. Verma and Dubey (2001) applied 100 and 500 µM Cd to two different rice cultivars and reported that total and reducing sugar concentrations of both rice varieties significantly increased. Kinay (2018) reported that increasing application doses of Cd to the soil (0, 0.25, 2.5 and 10 mg kg^{-1} Cd) caused an increase in glucose concentration and a decrease in fructose concentration (especially in Cd 10 application rate) of Xanthi-2A tobacco genotype. The change in glucose and fructose concentrations of tobacco genotypes with the Zn application was statistically important. Zn application led to an increase in the glucose concentrations of the Birlik-124 genotype, while a decrease in the Xanthi-81 genotype. Zinc application resulted in a significant increase in the fructose concentration of Xanthi-81 genotype, whereas significant increase in Birlik-124 genotype was obtained only in the Cd10 treatment (Table 2). The results showed that Zn application under Cd toxicity, caused a significant increase in sugar synthesis of tobacco genotypes, which revealed the importance of Zn in alleviating the Cd stress. Zinc plays an important role in carbohydrate metabolism, synthesis of proteins, expression and regulation of genes, structural integrity of ribosomes and phosphate metabolisms, therefore Zn is essential metal for a large number of enzymes such as Cu-Zn superoxide dismutase, alcohol dehydrogenase, RNA polymerase and DNA-binding proteins (Broadley et al., 2007). In addition, Zn increases the defense system and provides an excellent protection against the oxidation of several vital cells component (Cakmak, 2000). Phenolics compounds have strong antioxidant activity due to their redox potential, and exert their antioxidant activity by scavenging some reactive species (Halliwell, 2007). Kapoor (2016) indicated that modulated activities of antioxidants, enzyme activities, and radicals scavenging activities reported under stressed conditions demonstrate the active participation of phenolic compounds in oxidative stress management.

The increasing application rate of Cd in Zn deficiency caused a significant decrease in chlorogenic concentrations of tobacco genotypes, and an increase and decrease in rutin concentrations. The chlorogenic concentration of Birlik-124 genotype was 548 mg kg^{-1} in Zn0xCd0 interaction, while decreased to 300 mg kg^{-1} in Zn0xCd10 interaction. The rutin concentration of the Xanthi-81 genotype was 56.3 mg kg^{-1} in Zn0xCd0 interaction, decreased to 53.9 mg kg^{-1} at Cd2.5, while increased to 62.0 mg kg^{-1} at Cd10 dose (Table 3). Kinay (2018) reported that the chlorogenic concentration in the leaves of Xanthi-2A genotype decreased from 37.78 mg kg^{-1} at Cd0 dose to 25.8 mg kg^{-1} at Cd5 dose and to 27.91 mg kg^{-1} at Cd10 dose. The results reported by Kováčik and Klejdus (2008) for *Matricaria chamomilla* treated with Cu and by Irtelli and Navari-Izzo (2006) for *Brassica juncea* exposed to Cd showed that chlorogenic concentration decreased with the increase in Cd application rate. In contrast to the results obtained, higher chlorogenic acid concentrations were reported with the Cd application which indicates that Cd increases the production of chlorogenic acid. The results indicated that chlorogenic acid is a protective response to the oxidative damage caused by the metal.

All application doses of Cd in Zn application to soil caused a statistically significant increase in the leaf chlorogenic and rutin concentrations of tobacco genotypes. The mean chlorogenic concentration of the Birlik-124 variety in Zn0 treatment was 481 mg kg^{-1} and this value increased to 666 mg kg^{-1} in the Zn5 application rate. Similarly, the mean rutin concentration increased from 35.7 mg to 50.0 mg kg^{-1} (Table 3).

Table 3. Effect of Zn application on chlorogenic and rutin concentration on tobacco cultivars grown under Cd toxicity

Genotype	Cd Dose mg kg^{-1}	Chlorogenic Concentration (mg kg^{-1})**			Rutin Concentration (mg kg^{-1})**		
		Zn0	Zn5	T test	Zn0	Zn5	T test
Birlik-124	0	548^{b-e}	469^{c-g}	*	34.8^{d}	52.8^{cd}	**
	2.5	515^{b-f}	702^{ab}	**	41.2^{d}	41.7^{d}	ns
	5	563^{b-e}	625^{bc}	*	34.7^{d}	42.5^{d}	*
	10	300^{g}	869^{a}	**	32.3^{d}	61.2^{bcd}	**
Mean		***481A***	***666B***		***35.7B***	***50.0A***	
Xanthi-81	0	464^{c-g}	574^{b-e}	*	56.3^{bcd}	79.8^{abc}	**
	2.5	392^{efg}	589^{b-e}	**	53.9^{cd}	80.5^{abc}	**
	5	308^{fg}	608^{bcd}	**	60.5^{bcd}	85.6^{ab}	**
	10	404^{d-g}	563^{b-e}	**	62.0^{bcd}	96.5^{a}	**
Mean		***392B***	***584A***		***58.2B***	***85.6A***	

*Significant at P<0.05 level; **Significant at P<0.01 level; ns: non significant.

The decrease in phenolic compounds of tobacco varieties under Cd stress and Zn deficiency conditions increased with Zn fertilization, which reveals the importance of Zn fertilization in alleviating the negative impacts of Cd stress. The phenolic compounds cause the chelation of metals under heavy metal stress or can alleviate the oxidative damage by displacing the free radicals. An increase in the concentration of phenolic compounds in both leaves and roots of different plants treated with metals have been reported (Tolra et al., 2005; Zouari et al., 2016). The phenolic metabolites accumulation has been attributed to the activation of phenyl alanine ammonia-Liaza enzyme, which is the main biosynthetic pathway of phenolic compounds.

CONCLUSIONS

This study provides new information about the effects of Zn application on nicotine, glucose, fructose, chlorogenic and rutin content of tobacco genotypes under conditions of different cadmium doses application. The results revealed that Cd applications caused a statistically significant (P<0.01) increase of Cd content in tobacco leaves for studied genotypes, but the increase in Cd content decreased with Zn application. The application of Cd increased the Cd content of tobacco leaves, and this increase caused a statistically significant (P<0.05) increase and decrease in nicotine concentrations of tobacco leaves. However, nicotine content of both tobacco genotypes significantly increased with the Zn application. Similar to the nicotine concentrations, the sugar concentrations of both tobacco genotypes under Cd stress was different from each other. Reducing sugar (fructose + glucose) concentrations in the leaves of both tobacco genotypes significantly increased with the Zn application. The results revealed that the mean chlorogenic and rutin concentrations of tobacco varieties in zinc deficiency significantly increased with the Zn application. The results indicated the importance of Zn application in reducing the negative effect of Cd toxicity in tobacco plants.

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