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Effects of Mycorrhizae and Salicylic Acid on Growth, Cadmium Content and Uptake of Maize (*Zea mays* L.) Seedlings in Cadmium Contaminated Media

Kadmiyum Bulaştırılmış Yetiştirme Ortamda Mısır Fidesinin (*Zea mays* L.) Gelişimine, Kadmiyum İçeriğine ve Alımına Mikoriza ve Salisilik Asit Uygulamalarının Etkisi

Füsun Gülser[1*], Ferit Sönmez[2]

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Abstract. Cadmium (Cd) pollution is an important problem today. In this study, the effects of mycorrhiza (M) and salicylic acid (SA) applications on plant height, fresh weight, dry weight, number of leaves, and Cd content and uptake of maize (*Zea mays* L.) seedlings grown in Cd treated environments were determined. For this purpose, 3 kg pots with and without mycorrhizae were used with two doses of salicylic acid (SA_1: 1.0 mM and SA_2: 2.0 mM). The study was carried out in three replications. As a result of the measurements, mycorrhiza and Salicylic acid applications significantly increased plant height, fresh and dry weight of corn seedlings. On the other hand, mycorrhiza and Salicylic acid applications decreased Cd content and uptake. The lowest plant height was 26.63 cm, the number of leaves was 5.6, the fresh weight was 3.74 g and the dry weight was 1.63 g in the control group, which was not treated with mycorrhiza and SA. The highest plant height was 56.17 cm, the number of leaves was 7.50, and the fresh weight was 21.46 g, with 2.0 m*M* SA application without mycorrhiza treatment. The highest dry weight was determined with 5.70 g in mycorrhiza and 2.0 mM SA application. While the highest Cd content of corn seedlings was 3.37 mg kg^{-1} and its uptake was 5.95 mg kg^{-1} in the control application, the lowest Cd content was 0.307 mg kg^{-1} and uptake were 1.48 mg kg^{-1} with mycorrhiza with 2.0 m*M* SA and 1.0 m*M* SA. detected in applications. When the results of our study were examined, mycorrhiza and SA applications had positive effects on corn seedlings grown in Cd-contaminated environments. The combined application of salicylic acid and mycorrhiza will contribute significantly to yield and nutrient content in agricultural production against heavy metal pollution.

Keywords: Mycorrhiza, salicylic acids, cadmium, maize, nutrient uptake

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Öz. Kadmiyum (Cd) kirliliği önemli bir sorun günümüz için. Bu çalışmada, Cd gulanmış ortamlarda yetişen mısır (*Zea mays* L.) fidelerinin bitki boyu, yaş ağırlık, kuru ağırlık değerleri ile bitkinin Cd içeriği ve alımı üzerine mikoriza (M) ve salisilik asit (SA) uygulamalarının etkileri belirlenmiştir. Bu amaç için salisilik asitin iki dozu (SA_1: 1.0 m*M* ve SA_2: 2.0 m*M*) ile mikoriza uygulanmış ve uygulanmamış olmak üzere 3 kg'lık saksılar kullanılmıştır. Çalışma üç tekerrürlü olarak yürütülmüştür. Yapılan ölçümler sonucunda mikoriza ve Salisilik asit uygulamaları mısır fidelerinin bitki boyunu, yaş ve kuru ağırlıklarını önemli ölçüde artırmıştır. Buna karşılık mikoriza ve Salisilik asit uygulamaları Cd içeriğini ve alımlarını azaltmıştır. Bitki boyunda en düşük değer 26.63 cm, yaprak sayısı 5.6 adet, yaş ağırlık 3.74 gr ve kuru ağırlık 1.63 gr ile mikoriza ve SA uygulanmayan kontrol grubunda belirlenmiştir. En yüksek bitki boyu 56.17 cm, yaprak sayısı 7.50 adet ve taze ağırlık 21.46 g ile mikoriza uygulaması yapılmayan ve 2.0 m*M* SA uygulamasında elde edilmiştir. En yüksek kuru ağırlık 5.70 g ile mikoriza ve 2.0 m*M* SA uygulamasında belirlenmiştir. Mısır fidelerinin en yüksek Cd içeriği 3.37 mg kg^{-1} ve alımı 5.95 mg kg^{-1} ile kontrolde uygulamasında tespit edilmişken, en düşük Cd içeriği 0.307 mg kg^{-1} ve alımı 1.48 mg kg^{-1} olarak mikoriza ile 2.0 m*M* SA ve 1.0 m*M* SA uygulamalarında tespit edilmiştir. Çalışmamızin sonuçları irdelendiğinde mikoriza ve SA uygulamalarının Cd ile kirletilmişmiş ortamlarda yetişen mısır fideleri üzerine olumlu etkileri olmuştur. Salisilik asit ve mikorizanın birlikte uygulanmasının ağır metal krililiğine karşı tarımsal üretimde verim ve besin elementi içeriğine önemli katkı sağlayacaktır.

Anahtar kelimeler: Mikoriza, salisilik asit, kadmiyum, mısır, besin elementi alımı

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[1] Prof. Dr. Füsun Gülser. Van Yüzüncü Yıl Üniversitesi, Ziraat Fakültesi, Toprak Bilimi ve Bitki Besleme Bölümü, fgulser@yyu.edu.tr, (Sorumlu Yazar / Corresponding author)

[2] Doç. Dr. Ferit Sönmez, Bolu Abant İzzet Baysal Üniversitesi, Ziraat Fakültesi, Tohum Bilimi ve Teknolojisi Bölümü, sonmezferit@ibu.edu.tr

INTRODUCTION

Heavy metals that come to the fore in environmental pollution are Cd, Cr, Pb, Hg, Cu and Zn. Among them, cadmium is one of the most toxic air, water and soil pollutants that penetrate the environment with phosphate fertilizers used in industrial activities and agriculture. For these reasons, cadmium can reach high levels in agricultural soils and is easily assimilated by plants. As a result, cadmium adversely affects the photosynthesis function of plants, impairs chlorophyll metabolism, inhibits electron transport, and deregulates stomatal activities of plants (Lagriffoul et al., 1998; Liu et al., 2003; Talanova et al., 2001).

Sandalio et al., (2001) reported that cadmium has a toxic effect on the metabolism of plants grown in cadmium-contaminated environments. Gadallah (1995) reported that plants exposed to excessive cadmium regress photosynthesis functions by affecting the chlorophyll mechanism and chloroplast structure. In addition, cadmium pollution affects the opening and closing of stomata (Barcelo and Poschenrieder, 1990). Krantev et al. (2008) and Popova et al. (2009) reported that cadmium toxicity has inhibitory effects on both photosynthetic carbon metabolism and photosystem II. It has been reported by Quariti et al. (1997) that membrane functions are affected by the change in lipid composition of plants exposed to excessive cadmium. Fodor et al. (1995) reported that the activities of enzymes such as H^+-ATPase associated with membranes are adversely affected by excessive cadmium ions.

Arbuscular mycorrhizal fungi (AMF), which is one of the soil microorganisms, can establish a symbiosis with 98% of the plants on the earth (Smith and Read, 1997).

As a result of the research, it was reported that by transforming the forms of Arbuscular Mycorrhiza fungi that cannot be used by plants, such as phosphorus, calcium, magnesium and zinc, the use of these elements by plants improved (Azcon-Aguilar et al., 1979; Al-Karaki and Clark, 1998; Al-Karaki, 2000; Al-Karaki et al., 2001).

It has been reported by Harrier and Sawczak (2000) that plants that establish a symbiosis with mycorrhizae increase their resistance against biotic and abiotic stresses. In addition, it enables plants to cope with abiotic stress by mitigating nutrient deficiencies, improving drought tolerance, overcoming the harmful effects of salinity, and increasing tolerance to pollution (Azcon-Aguliar and Barea, 1997; Juniper and Abbott, 1993).

Mycorrhizal fungi improve the water uptake capacity of plants by affecting the root hydraulic conductivity of plants, promoting the formation of carbohydrates in the plant, and adjusting the osmotic balance of the cell (Auge et al., 1986; Rosendahl and Rosendahl, 1991). With these changes, the root reduces the negative effects of excess salt (Dixon et al., 1993). Giri et al. (2003) reported that root and shoot weights of AM fungus inoculated plants were significantly higher. Providing much more nutrients, changes in root morphology, and electrical conductivity of soil may be possible mechanisms to protect plants from salt stress in AM colonization.

Many different types of SA conjugates have been found in Plant species have many different types of SA conjugates. They were mostly formed by glycosylation and particularly by esterification (Popova et al., 2012).

Salicylic acid (SA) applications are known as an endogenous growth regulator with many physiological functions in plants. Salicylic acid in the structure of the plant is an important signaling element that plays a role in the formation of local and systematic disease resistance responses in pathogen attack of plants (Alvarez, 2000). In addition, it causes some positive responses to abiotic stress factors such as heavy metals, herbicides, low temperatures, and salinity (Ananieva et al., 2002; Metwally et al., 2003).

Salicylic acid applications protect plants against damage caused by heavy metals membrane damage in rice (Mishra and Chudhuri, 1999), oxidative stress in Medicago sativa (Zhou et al., 2009), Cd damage in barley and maize (Krantev et al., 2008; Metwally et al., 2003; Pal et al., 2002).

Ahmad et al. (2011) and Islam et al. (2016) reported that salicylic acid treatments increased the activities of antioxidant and non-antioxidant enzymes to reduce chromium-induced damage in maize because of co-administration with plant growth promoting bacteria.

In this study, we aimed to determine the effects of arbuscular mycorrhiza and salicylic acid with cadmium applied to the growing medium on the development criteria, cadmium content and uptake of maize (*Zea mays* L.) seedlings.

MATERIAL AND METHOD

In this study, 3:1 ratio of soil:sand mixture was used as a plant growing media. Some properties of the media, determined using the standard analyses methods (Kacar, 1994), can be summarized as; non-saline, slightly alkaline, moderate in lime, low in organic matter content and insufficient in phosphorous and potassium contents (Table 1). After filling the each pot (3000 cm^3) without drainage holes with soil:sand mixtures, 18 pots were autoclaved. The certain dose of cadmium (25 mg kg^{-1}) was added into each pots as $Cd(NO_3)_2.4H_2O$. Inoculum of mycorrhizae, *Glomus intraradices*, consisted of spores was laid into around the seed with 10g. Two different doses salicylic acid (SA_1:1.0 m*M* and SA_2:2.0 m*M*) were applied into pots with and without mycorrhizae (M) as three applications. As a basic fertilizer treatment 40 mg kg^{-1} P_2O_5, 200 mg kg^{-1} K and 200 mg kg^{-1} N also applied into each pot from TSP, K_2SO_4 and $(NH_4)SO_4$ respectively. TTM815 hybrid corn (*Zea mays* L.) variety was used as a plant material. The pots were placed in a growth chamber at 22 ±10C with 12 fluorescent illuminations with 8000 lux light intensity and the seedlings were irrigated with distilled water. The experiment was ended 8 weeks after sowing. The levels of Cd were analyzed in dried and grinded samples (Kacar and Inal, 2008). Variance analysis was carried out in a randomized plots design on six treatments (Control, Cd+SA_1, Cd+SA_2, Cd+M, Cd+SA_1+M, Cd+SA_2+M) with three replications, and pairs of mean values were compared by least significant difference (LSD) using MSTAT (1998)

Table 1. Some properties of the growing media.
Çizelge 1. Yetiştirme ortamına ait bazı özellikler.

Total salinity, %	0.02	Organic matter, %	0.75
pH (1:2.5)	8.12	P, mg kg^{-1}	3.93
Lime, %	14.8	K, mg kg^{-1}	0.43

RESULTS AND DISCUSSION

The applications of SA and M and their interactions had significant (P<0.01) effects on plant growth criteria of maize seedlings (Table 2).

Table 2. Variance analysis results of the effects of salicylic acid (SA) and mycorrhiza (M) applications on plant growth criteria and Cd uptake and content of corn seedlings.
Çizelge 1. Salisilik asit (SA) ve mikoriza (M) uygulamalarının mısır fidelerinim bitki gelişim kriterleri ile Cd alım ve içeriğine. etkileri.

Treatments	Shoot Length	Leaf Number	Shoot Fresh Weigth	Shoot Dry Weigth	Cd Content	Cd Uptake
SA	14.1**	9.45**	14.7**	26.8**	122.9**	46.5**
M	28.7**	4.7*	8.1*	5.2*	289.7**	249.5**
MxSA	18.8**	4.2*	13.2**	4.5*	212.7**	57.1**

** * significant at 0.05 level, ** significant at 0.01 level, ns: non significant,

The Cd contents and uptakes decreased while the plant growth criteria increased by the mycorrhizal inoculation. The highest plant length, leaf number and plant fresh weight means were found as 52.7 cm, 7.28, 17.748 g and 4.372 g by mycorrhizae applications, respectively. The lowest Cd contents and uptakes were determined as 0.788 mg kg^{-1} and 2.853 mg kg^{-1} in mycorrhizal inoculated plant. The increases in ratio of 27.6%, 9.5%, 29.3% and 20.5% were obtained for plant length, leaf number, plant fresh weight and plant dry weight in mycorrhizal inoculated plants compared to non-inoculated ones respectively. The decreases obtained in Cd content and uptake by mycorrhizae applications were in ratio of 142.4% and 97.2% respectively (Table 3).

Table 3. The means belong effects of mycorrhizae and saliciylic acid applications on plant growth criteria, cadmium content and cadmium uptake.
Çizelge 3. Mikoriza ve salisilik asit uygulamalarının bitki gelişim kriterleri, kadmiyum içeriği ve kadmiyum alımına ait ortalamalar.

Treatments	Shoot Length	Leaf Number	Shoot Fresh Weigth	Shoot Dry Weigth	Cd Content	Cd Uptake
	cm	No.	g plant^{-1}		mg kg^{-1}	
Salicylic acid, m*M*						
0	40.9±15.9	6.12±0.79	10.733±8.156	2.685±1.272	1.884±1.624	5.421±0.841
1	45.7±6.3	7.02±0.59	16.488±3.181	3.744±0.525	1.513±0.241	3.808±2.533
2	54.5±4.9	7.28±0.46	19.996±2.522	5.568±0.776	0.650±0.385	3.488±1.939
LSD(P<0.05)	5.7	0.61	3.758	0.868	0.176	0.468
Mycorrhizae						
$M_{(-)}$	41.3±13.6	6.65±0.92	13.730±7.896	3.627±1.777	1.910±1.111	5.625±0.551
$M_{(+)}$	52.7±3.6	7.28±0.55	17.748±3.642	4.372±1.131	0.788±0.661	2.853±1.868
LSD(P<0.05)	4.6	0.60	3.068	0.708	0.143	0.382

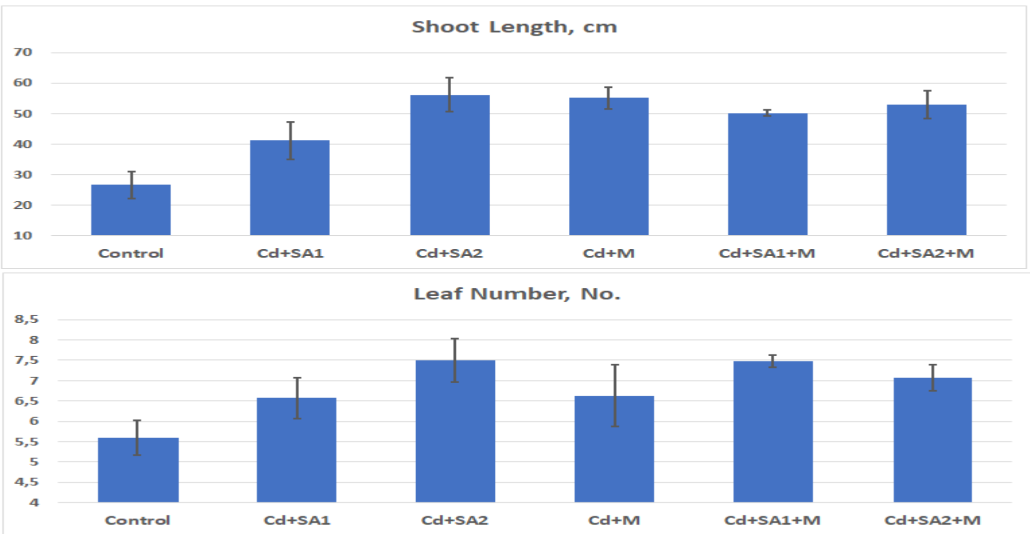
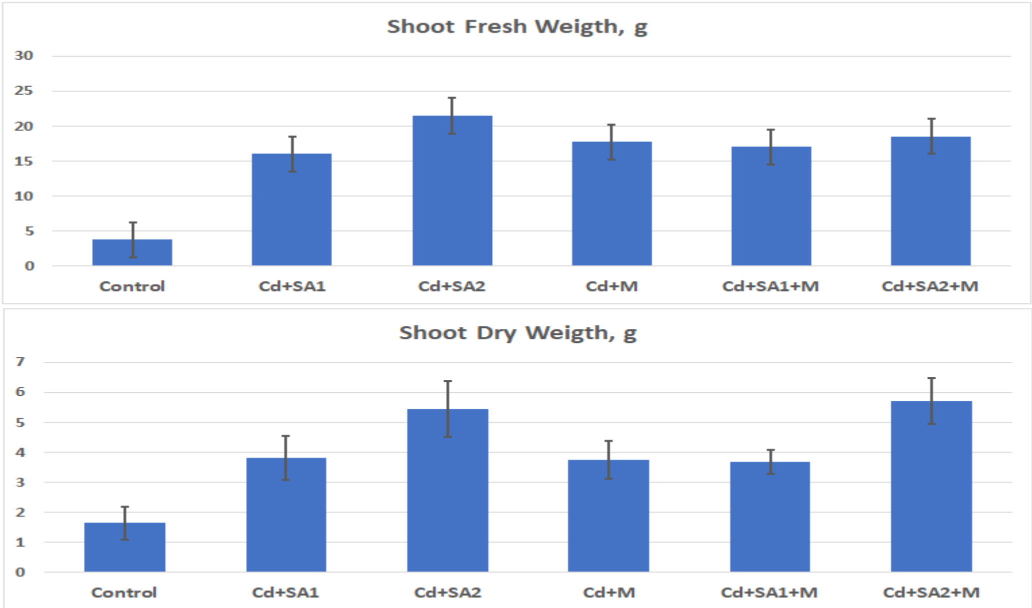


Figure 1. The effects of salicylic acid doses (SA_1: 1.0 m*M*, SA_2: 2.0 m*M*) and mycorrhizae (M) on shoot length and leaf number in maize seedlings.
Şekil 1. Mısır fidelerinin gövde uzunluğu ve yaprak sayısı üzerine salisilik asit dozlarının (SA_1:1.0 m*M*, SA_2: 2.0 m*M*) *ve mikoriza* (M) *uygulamalarının etkisi.*

The plant growth criteria increased while the Cd contents and uptakes decreased by increasing salicylic acid doses. The plant length, leaf number, plant fresh weight, plant dry weight, Cd content and uptake means were found as 4.09 cm, 6.12, 10.733 g, 2.685 g, 1.884 mg kg^{-1} and 5.421 mg kg^{-1} in control groups. These parameters were obtained as 54.5 cm, 7.28, 19.996 g, 5.568 g, 0.650 mg kg^{-1} and 3.488 mg kg^{-1} in 2.0 m*M* SA application respectively. These changes obtained by SA applications were determined as increases in ratio of 33.3%, 18.9%, 86.3% and 107.4% for the plant length, leaf number, plant fresh weight and plant dry weight and were found as decreases in ratio of 189.8% and 55.4% for Cd content and uptake respectively (Table 3).

The lowest shoot length (26.63 cm), leaf number (5.60 No.), shoot fresh (3.74 g) and dry weights (1.63 g) were obtained in the control treatment without M and SA application. While the highest mean of shoot length (56.17 cm), leaf number (7.50 No.), and shoot fresh weight (21.46 g) means were obtained in 2.0 m*M* SA application without M application, the highest shoot dry weight means (5.70 g) was determined in 2.0 m*M* SA treatment with M application (Figure 1, 2).



Figures 2. The effects of salicylic acid doses (SA_1: 1.0 m*M*, SA_2: 2.0 m*M*) and mycorrhizae (M) on shoot fresh and dry weights in maize seedlings.

Şekil 2. Mısır fidelerinin gövde yaş ve kuru ağırlıkları üzerine salisilik asit dozlarının (SA_1:1.0 m*M*, SA_2: 2.0 m*M*) *ve mikoriza* (M) *uygulamalarının etkisi.*

The effects of the applications on Cd contents and uptake in maize seedlings are given in Figure 3. The Cd content and uptake were significantly ($P<0.01$) influenced by M and SA applications. While the highest Cd content (3.67 mg kg^{-1}) and uptake (5.95 mg kg^{-1}) of the seedlings were determined in the control, the lowest Cd content (0.307 mg kg^{-1}) and uptake (1.48 mg kg^{-1}) were in 2.0 m*M* SA and 1.0 m*M* SA treatments with M applications, respectively. According to the results, it can be concluded that mycorrhizae and salicylic acids applications had positive effects on plant growth criteria. Several researchers (Mosse, 1977; Rabie, 2005) reported that the Mycorrhizae application increased plant growth in different cultivars. Major reason for this increase in the growth can be attributed to the ability of plants in association with M to uptake some nutrients efficiently. It was also reported (Ramanujam et al., 1998; Türkyılmaz et al., 2005) that the salicylic acid applications had positive effects on plant growth.

The M and SA applications decreased Cd content and uptake in maize seedlings. The decreases in heavy metal contents and uptake with M applications were reported by several researchers. Li and Christe (2001) and Malcova et al. (2003) reported that in slightly metal contaminated soil, M increased shoot uptake of metals (Weissenhorn et al., 1993; Weissenhorn et al., 1994). Mycorrhiza can reduce the shoot metal concentration of plants grown in heavily polluted soils with heavy metals and protect the plants against the harmful effects of these metals (Li and Christe, 2001). These findings also support our results. Many studies have reported that Mycorrhiza protects the shoots of plants against toxic elements such as high levels of Al (Rufyikiri et al., 2000), Cd (Yu et al., 2004) and As (Fitz and Wenzel, 2002).

Jamalabad and Khara (2008) declared that sugar and proteins content increased in wheat plant under cadmium stress inoculated with Glomus intraradices compared to not inoculated plants.

Similarly, it was reported that salicylic acid alleviates the cadmium toxicity in barley seedlings (Metwally et al., 2003), in wheat (Moussa and El-Gamal, 2010) in maize (Güneş et al., 2007; Krantev et al., 2008; Pal et al., 2002); in rice (Mishra and Choudri, 1999).

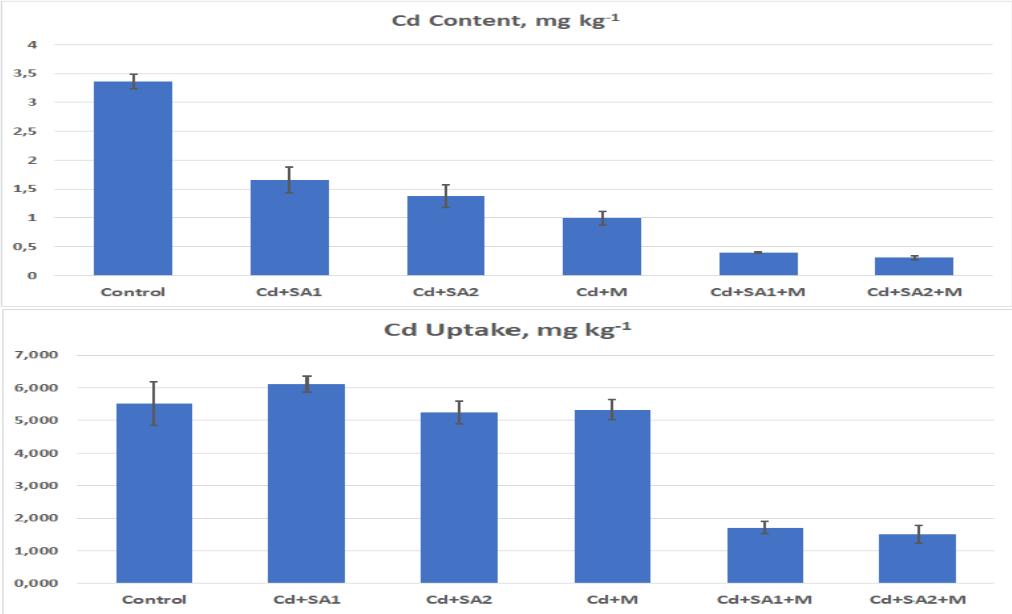


Figure 3. The effects of salicylic acid doses (SA_1:1.0 m*M*, SA_2: 2.0 m*M*) and mycorrhizae (M) on Cd content and Cd uptake in maize seedlings.
Şekil 3. Mısır fidelerinin Cd içeriği ve alınımı üzerine salisilik asit dozlarının (SA_1:1.0 m*M*, SA_2: 2.0 m*M*) *ve mikoriza* (M) *uygulamalarının etkisi.*

CONCLUSION

It has been revealed that salicylic acid applications have positive and important effects against heavy metal and other stress factors of plants. Studies have reported that salicylic acid applications affect photosynthesis by affecting the chlorophyll content of plants, stomatal conductivity and enzyme activities related to photosynthesis (Rivas-San Vicente et al., 2011).

It has been reported that the cadmium content of the plant decreased and the contents of glutathione, nonprotein thiol and phytochelatins increased with salicylic acid applications to plants exposed to cadmium stress (Gu et al., 2018).

Salicylic acid enhances photosynthetic efficiency and improves photosynthetic processes under heavy metals stress (Shi et al., 2009). At the end of the study in which salicylic acid and mycorrhiza applications were applied together, it was determined that there was an increase in the sugar and proline content of the plant against aluminum stress (Enteshari and Mirzaiyan, 2012). In this study obtained data were in line with referred research results.

As a result, the mycorrhizae and salicylic acid applications increased plant growth and decreased Cd content and uptake in maize seedlings although the harmful effects of Cd on plant growth.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this article.

DECLARATION OF AUTHOR CONTRIBUTION

FG and FS designed and performed the experiments, analyzed data, and wrote the manuscript; FG and FS provided feedback and run statistical analysis. All authors read and approved the final manuscript.

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