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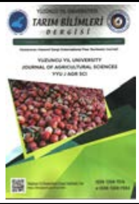

Research Article (Araştırma Makalesi)

The Effects of Arbuscular Mycorrhizal Fungi and Lead (Pb)Applications on Eggplant Seedling Growth and Nutrient Uptake

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Gigaspora margarita (Gm),
Glomus intraradices (Gi),
Heavy metal,
Lead,
Seedling development.

Abstract: There are many factors, which negatively affect plant development. One of these factors is lead (Pb), which causes toxic effects on plants during the seedling period. Mycorrhizas (AMF) contribute positively to plant growth. In eggplant seedlings, it has been researched whether to tolerate the negative effects of lead with mycorrhizas. In the study, *Gigaspora margarita* (Gm) and *Glomus intraradices* (Gi) mycorrhizal races were applied before seeds were sown. After the first true leaves appeared, 5 different doses of Pb (0, 100, 200, 400 and 800 ppm) were given. As a result, the earliest true leaf appearance time, the widest cotyledon and longest cotyledon, were taken from Gm application. In terms of other seedling development parameters, the highest values were obtained from Gm-0 ppm Pb application. In addition, it was observed that the control 0 and 100 Pb ppm applications received high values. As expected, due to the toxic effect of lead, no plants could be obtained in Control-800 ppm Pb application. However, the plants continued to develop by tolerating the toxic effect of lead in Gm-800 ppm and Gi-800 ppm applications especially. The other criteria that we examined were the intake of important nutrients N, P, K and Ca with mycorrhizal even in high-dose lead conditions. It was observed that the lead was taken into the plant by mycorrhizal. Lead content in the root was more than the lead content in the shoot. In general, it was seen that low Pb dose in Gi application can be partially tolerable for seedling growth and nutrient content.

Arbusküler Mikorizal Fungus ve Kurşun (Pb) Uygulamalarının Patlıcan Fide Gelişimi ve Besin Alımı Üzerine Etkileri

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Anahtar kelimeler

Gübreleme,
Gigaspora margarita (Gm),
Glomus intraradices (Gi),
Ağır Metal,

Özet: Bitkilerin gelişimini olumsuz etkileyen birçok faktör vardır. Bu faktörlerden biri de fide döneminde bitkiye toksik etkiye sebep olan kurşun (Pb)'dur. Mikorizalar ise bitki gelişimine olumlu yönde katkı sağlamaktadır. Patlıcan fidesinde, mikorizanın kurşunun olumsuz etkisinin tolere edip etmeyeceği araştırılmıştır. Bu çalışmada, *Gigaspora margarita* ve *Glomus intraradices* mikoriza ırkları tohum ekiminden önce uygulanmıştır. İlk gerçek yaprak göründükten sonra kurşunun 5 farklı dozu (0, 100, 200, 400 ve 800 ppm) verilmiştir. Sonuç olarak gerçek yaprak görünme süresi, en geniş kotiledon ve en uzun kotiledon *Gigaspora margarita* uygulamasından alınmıştır. Diğer fide gelişim parametreleri bakımından, en yüksek değerler *Gigaspora margarita*-0 ppm Pb uygulamasından elde edilmiştir. Ayrıca kontrolde 0 ve 100 ppm Pb

Kurşun,
Fide Gelişimi.

uygulamalarında yüksek değer aldıkları görülmüştür. Beklenildiği gibi kurşunun toksik etkisi sebebiyle Kontrol-800 ppm Pb uygulamasından bitki elde edilememiştir. Fakat her iki mikoriza türünün 800 ppm'lik uygulamalarında kurşunun toksik etkisini tolere ederek gelişimine devam ettikleri görülmüştür. İncelediğimiz diğer kriterler, N, P, K ve Ca gibi önemli besin elementlerin yüksek dozda kurşun şartlarında bile mikorizalar aracılığıyla bitkiye alınmasıdır. Kurşununda, mikorizalar tarafından bitkiye alındığı görülmüştür. Kökteki Pb içeriği, sürgündeki Pb içeriğinden daha fazladır. Genel olarak fide gelişimi ve besin elementleri içerikleri olumlu etki Gi uygulamasında elde edildiği görülmüştür.

1. Introduction

Factors such as a rapidly growth in the population, nutritional deficiencies, irregular urbanization, people's desire for excessive consumption, and developing technology cause the environmental pollution problem (Sağlam and Cihangir, 1995). As in many areas, heavy metal pollution is observed in agricultural areas for various reasons. The most important industrial activities that cause the release of heavy metals to the environment are cement production, iron and steel industry, thermal power plants, glass production, garbage and sludge incineration plants. Considering the natural dispersion of heavy metals, it is reported that heavy metal excretion to the biosphere occurs at different process levels of different sectors (Kahvecioğlu et al., 2002). One of the most remarkable elements, especially among heavy metals, is lead (Pb) (Akıncı et al., 2010). According to Aydın (2002), heavy metals have become an environmental problem in terms of human and community health. Lead that enters the plant through plant roots and stomata accumulates in different parts of the plant. In this way, by entering the food chain, it can affect human health indirectly or through respiration (Çavuşoğlu et al., 2009). Lead contamination rates in nature; it is listed as 10% water, 15% air, 20% food and 55% soil (Anonymous, 2010a). Plant species have different capacities in accumulating and transporting heavy metals. There are studies related to subject. It has been reported that, if plants take these heavy metals as essential nutrients, they pose a serious risk to human health. It is not deemed appropriate for the species in the Solanaceae family to be cultivated near industrial areas. The products in the Solanaceae family can take considerable amounts of heavy metals from the soil with their roots, leaves and fruits (Farooq et al., 2008). In a study conducted on plants belonging to the same family, it was reported that the highest Ni and Cu were in tomato, Co and Cd in potato, and Pb, Zn and Mn in eggplant (Shilev and Babrikov, 2005). Plants grown in soils with high lead content become pale and small-leaved (Sesli, 2003). It causes a reduction in the intake of essential nutrients in the roots and immobilization in the roots. Therefore, significant nutrient deficiency appears on the stem. According to Uysal and Taner (2007), heavy metals negatively affect plants during their growth and development periods. In addition, its effects are more important in germination and seedling stages. Because at this stage, the reactions of plants to stress or toxic factors may be more pronounced (Akıncı and Çalışkan, 2010). In the researches, it was determined that the amount of lead in the root is more than the amount of lead in the leaf. It was reported that this difference between them showed a significant restriction in the transportation of metals from roots to shoots and green leaves (Dahmani et al., 2000). The annual eggplant has an important share among the vegetables produced. Studies on lead toxicity are limited. Plants are fertilized from soil with macro and micronutrients. Using the elements found in the soil is a more realistic approach in terms of both environmental health and natural resources. Mycorrhizal fungi contribute significantly to plant development by supplying water (George et al., 1992). Arbuscular mycorrhizal fungus (AMF) contributes to plants' slow uptake of nutrients from the soil, especially phosphorus. It has been found that mycorrhizal intake nutrients such as phosphorus, zinc and copper when they interact effectively with the plant (Ortaş, 1998). Besides, arbuscular mycorrhizal fungus improves uptake of immobilized plant nutrition, especially phosphorus (Goltapeh et al., 2008; Sawers et al., 2008; Eke et al., 2016; Erdinç et al., 2017). In addition, mycorrhizal has undertaken a protective function against the stress factors and pathogens of the plant with the changes in plant physiology. AMF hyphae contribute to soil conservation by entering the areas that the roots cannot penetrate and improving the soil structure in this way (Dodd and Haas, 1983; Ortaş et al., 2000). There are some studies on this subject. In a study where 0, 75, 150 and 300 mg / l lead doses were applied to tomato seedlings, it caused an increase in lead concentration in leaves, shoots and roots. The content of lead in the tissues of seedlings in low-dose lead application has increased. These values were 312 mg/kg at the roots, 130 mg/kg at the shoot and 510 mg/kg at the roots. In addition, they obtained 917–1750 mg/kg in leaves, 750–1022 mg/kg in exile and 1438–2520 mg/kg in root in high and medium dose lead applications. With the increase of lead, nutrient deficiency has emerged by causing a decrease in the presence of elements such as Ca, Mg, K, P, Na, Fe, Zn, Cu and Mn (Akıncı et al., 2010). In another study conducted in Faisalabad of Pakistan, the concentration of lead in the leaves of spinach, lettuce, cauliflower, radish, coriander and cabbage grown around industrial areas is 2.251 mg / kg, 2.411 mg / kg, 1.331 mg / kg, 2.035 mg / kg, 2.652 mg/kg and 1.921 mg/kg respectively (Farooq et al., 2008). Demir (1998), on the other hand, revealed that the compatibility and development parameters of mycorrhizal in tomato, pepper and eggplant plants grown under greenhouse conditions are higher than those without mycorrhizal.

The negative effects of heavy metals that are more effective during germination and seedling period on plants have been seen in researches. For this reason, the research was carried out during the seedling period. In this study, *Gigaspora margarita* and *Glomus intraradices* AMF inocula, which are known to have good interaction with eggplant, were studied. The effects of these mycorrhizas on eggplant seedling growth and plant nutrient content in high doses of lead applications were investigated.

2. Materials and Methods

This study was carried out in the greenhouses of Selcuk University, Faculty of Agriculture, Department of Horticulture. Plant nutrient analysis was done in the Soil Science and Plant Nutrition Department laboratories. Kemer eggplant variety was used as plant material. *Glomus intraradices* race were observed 165 spores in 10 g soil. *Gigaspora margarita* race were determined 100 spores in 10 g of soil. The growth medium was used for germinating seeds and growing seedlings consists of 1: 1 peat and perlite. This growth medium was filled into 250 ml pet cups. The Gi and Gm types were applied in the specified amounts. At this stage, real leaf appearing time, hypocotyl length, cotyledon length and cotyledon widths were measured. Then, when the seedlings started to see the first true leaves, lead (Pb) doses were determined and applied (0, 100, 200, 400 and 800 ppm). Lead acetate ((CH_3COO) $2Pb.3H_2O$) (Pb = 207.34 g) was used as the lead source. 50 ml of lead acetate was given to plants with injector. Applications were given by irrigation times in three stages, 20 to 26 days after sowing. In this process, cultural procedures were carried out to continue the development of seedlings (Vural et al., 2000). Plants fertilized twice with a Hoagland nutrient solution (about 200-250 cc $plant^{-}$). Study was set up with 3 repetitions according to the random parcels trial pattern. Twenty pots (20 plants) were kept in each repeat. Seedling transplanting stage, shoot diameter, shoot fresh weight, shoot dry weight, leaf number, root fresh weight, root dry weight, contents of N, P, K, Ca, and Pb elements in shoot and root were examined. At this stage after the plant samples were dried in the oven at 65 °C for 48 hours, was ground with a robot. Grinded shoots and roots are weighed in sensitive scales, weighed 0.2 g, and placed in linear tubes. 5 ml nitric acid and 2 ml hydrogen peroxide were added to them and the tubes were tightly closed. The tubes were then left in the microwave (MARSXpress device) for 15 minutes. The tubes coming out of the microwave oven were opened under the fume hood and placed in falcon tubes. Then 20 ml of distilled water was added. The samples were poured on filter papers and filtered. The filtered samples were read N, P, K, Ca and Pb on the ICP-AES device (Lindsay and Norwell, 1978). Determination of nitrogen was determined by Kjeldahl (6.25 x N) method (Bayraklı, 1987).

In order to determine the effect of the values obtained because of the study according to the application topics, the JMP statistical analysis package program was subjected to variance analysis (Howell, 1987). Trial subjects that were statistically significant in F control were grouped with 5% LSD test.

3. Results and Discussion

The data that we obtained from all parameters were subjected to variance analysis. The results, which were found significant, were compared with the literature below. Before lead application, the tree leaf appearance time was obtained from Gm application with the earliest 16.73 days. Also at this stage, the longest hypocotyls were taken from Gm and control applications with 20.27 mm and 19.29 mm. The maximum cotyledon width (11.03 mm) and cotyledon length (30.50 mm) were measured from Gm applications (Table 1). Fytianos et al. (2001) reported that high heavy metal concentrations in the soil can disrupt important physiological functions in plants and may cause food imbalance.

In our study, as the heavy metal density increases, the development of plants and the distribution of the amount of elements changed negatively (Tables 1, 2 and 3). If we examine the interactions of lead doses and mycorrhizal applications in Table 2, the highest values were obtained from Gm-0 ppm application. Shoot length, shoot diameter, number of leaves, shoot and root fresh weight, shoot and root dry weight, were measured as 14.27 mm, 2.80 mm, 5.32 pcs / plant, 76.10 g, 13.29 g, 7.64 g, and 1.10 g, respectively in Gm-0 ppm application. Control-0 and control-100 ppm applications follow these values. Şen (2008) observed that Gi applications have positive effects on seedling shoot length, shoot diameter, number of leaves, shoot age weight, shoot dry weight, root fresh weight and root dry weight. In another study conducted by Keskin (2009), it was stated for some of the criteria examined higher values were obtained from Gm application, while for some other criteria Gi application was better. It is similar in our study. The positive interactions of mycorrhizal with those in the Solanaceae family are mentioned in Demir (1998). In our study, it was seen that there was coherence between AMF and eggplant. If we examine Table 3, the application of Gi was better than Gm application for K element uptake. Similarly, the increase in shoot P content (3322.4 ppm) is the highest in Gi-0 ppm application. The root phosphorus content (3682.9 ppm) and nitrogen content (6.69%) has reached the highest values in Gm -800 ppm application. However, in general, when the parameters were examined, it was seen that the data obtained with increasing lead doses in Gi application had higher values than Gm and control. According to these results, the data we obtained shows the efficacy of mycorrhizal in different lead doses. Mycorrhizal symbiosis plays an effective role in the uptake of nitrogen (N), phosphorus (P) and potassium (K), which are essential elements in plant nutrition (Ames et al., 1983; Ortaş, 1998). In the study conducted by Şen (2008), it is reported that the possible negative effects of NaCl on eggplant seedling development and seedling nutrient content can be significantly reduced by Gi applications in salty soil conditions. Similarly, in our study, *Glomus intrarasices* (Gi) and *Gigaspora margarita* (Gm) applications significantly reduced the negative effects of Pb. In another study conducted by Dahmani et al., (2000) the major differences in leaf and root concentrations of lead showed a significant restriction in transporting metals from roots to shoots and green leaves. Akıncı et al. (2010), the intake of elements by roots and leaves of tomato seedlings, was negatively affected by the increase of lead concentration, especially in 300 mg / l Pb. Low lead levels in the plant were found to be 510 mg/kg in the roots, 130 mg/kg in the shoots and 312 mg/kg in the leaves. In addition, medium and high lead application of the plant was found to be 917–1750 mg/kg in leaves, 750–222 mg/kg in shoots and 1438–2520 mg/kg Pb in shoots. Shilev and Babrikov (2005) reported that Solanaceae family plants can take considerable amounts of heavy metals from soil by their roots, leaves and fruits, and the amounts of Pb, Zn and Mn were found to be the highest. Our study shows parallel results with this study. The highest values of Ca (33538.8 ppm in shoot and 24165.6 ppm in root) and Pb (1692.14 ppm in shoot and 29601.6 ppm in root) were obtained from Gm application. As shown in Table 3, Pb content has reached high values in eggplant, and AMF have been found to increase the value of Pb content in shoots and root.

The results of Al-Chaarini et al., (2009) showed that the rate of unwashed samples of heavy metal changed from high to undetectable to 3.0904 mg / g. Moreover, in this study leafy vegetables, subsoil vegetables or aboveground vegetables were compared, and it was seen that all heavy metals polluted the leafy vegetables at significantly higher levels. In Ergün and Öncel (2009), it was determined that in bread wheat the inhibition of root and seedling growth was in parallel with the increase of heavy metal concentration and application time. Zengin and Munzuroğlu (2004) reported that copper and lead applied in the form of chlorine salt has a significantly negative effect on the growth of root, stem and leaf of bean seedlings. Dodd and Haas (1983) mentioned indirect effects of mycorrhizal resistance for heavy metal toxicity. Our findings have been parallel to this study.

Table 1. Effects of mycorrhizal on some growth parameters of seedling period.

Applications	True leaf appearance time (day)	Hypocotyl length (mm)	Cotyledon width (mm)	Cotyledon length (mm)
Gigaspora margarita	16.73c	19,29a	11.03a	30.50a
Glomus intraradices	20.47a	15.84b	8.61b	24.74b
Control	19b	20.27a	10.36a	29.13a
LSD	0.70	0.94	0.66	3.23

Table 2. The effects of lead and mycorrhizal applications on some growth parameters of seedling and root.

AMF Application	Pb Dose	Shoot length (cm)	Shoot diameter (mm)	Number of leaf (number/plant)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Gigaspora margarita	0	14.27a	2.80a	5.32a	76.10a	13.29a	7.64a	1.10a
	100	11.87b	2.33bc	4.62bc	50.88c	10.30bc	4.76b	0.73cde
	200	11.27b	2.07def	4.13ef	40.28d	9.22cd	3.37c	0.69cde
	400	8.85cde	1.94fgh	4.06fg	31.04ef	7.60de	2.85cd	0.57ef
	800	4.24h	1.41j	2.72ı	4.87ı	0,70g	0.42g	0.07h
Glomus intraradices	0	9.34cd	2.14de	4.42cd	38.05de	6.56ef	3.21cd	0.44fg
	100	8.14ef	1.99efg	4.43cd	30.21f	5.68ef	2.44de	0.39g
	200	8.30def	1.90gh	4.35de	29.19fg	6,16ef	2.64cde	0.42fg
	400	7.65fg	1.72ı	3.83g	22.38gh	4.45f	1.74ef	0.31g
	800	6.78g	1.78hı	3.38h	16.08h	4,42f	1.38f	0.34g
Control	0	13.31a	2.45b	4.72b	66.08b	12.32ab	5.50b	0.78cd
	100	13.59a	2.20cd	4.75b	54.57c	13.16a	5.31b	1.01ab
	200	9.69c	1.96fg	4.18def	32.52ef	11.17abc	3.50c	0.84bc
	400	9.17cde	1.91fgh	3.96fg	29.95f	9.15cd	2.81cd	0.63de
	800	-	-	-	-	-	-	-
LSD		1.20	0.17	0.25	7.10	2.25	0.95	0.17

Table 3. The effects of lead and mycorrhizal applications on macro and micronutrient element contents of seedling and root development.

AMF Application	Pb Dose	Shoot N (%)	Shoot P (ppm)	Shoot Pb (ppm)	Shoot K (ppm)	Shoot Ca (ppm)	Root N (%)	Root P (ppm)	Root Pb (ppm)	Root K (ppm)	Root Ca (ppm)
Gigaspora	0	5.89a	3268.46a	14.31ı	28858.5g	31210.3abc	2.74c	2939.5b	15.1g	13793.8f	10460.7c
	100	3.56cd	2537.46b	225.88gh	34468.4efg	33538.8a	2.57c	2572.9bcd	2846.4def	19599.2e	10381.2c
	200	4.25c	2292.05bcd	306.6fg	40971.9de	27990.6cd	2.79c	2103.9ef	4593.4cde	20298.4e	11047.7c
	400	3.63cd	1797.01e	594.21c	36718.4efg	30940.9abc	2.69c	2010.1f	6901.6c	26849.2d	11388.8c
	800	5.49ab	1898.33de	1692.14a	39604.2def	31846.4ab	6.69a	3682.9a	29601.6a	32912.4c	24165.6a
Glomus intraradic	0	3.79cd	3322.40a	29.90ı	59004.1ab	21149.4fg	3.82bc	2994.8b	264.8fg	50320.5a	10917.3c
	100	4.31bc	2960.42a	122.34hı	59973.3a	20252.3g	4.41bc	2946.9b	1075.4fg	52769.9a	11073.4c
	200	3.53cd	2365.25bc	248.59fg	56673.6ab	25370.3de	4.57bc	2457.6cdef	2077.6efg	51436.0a	11183.3c
	400	3.75cd	2410.15bc	506.26cd	57975.0ab	21409.3fg	6.28a	2543.7bcde	5889.1 c	53050.1a	12174.2c
	800	3.41cd	2043.24cde	1083.07b	51483.3bc	25554.7de	4.45b	2199.9def	12559.8b	43490.1b	18792.5b
Control	0	3.81cd	3204.27a	26.08ı	52468.9abc	24116.7ef	2.78c	2919.4bc	25.1g	40856.2b	11830.3c
	100	2.92d	2324.02bc	205.96gh	32677.6fg	28589.1bcd	2.49c	2715.6bcd	4892.6cd	24486.3de	11770.2c
	200	2.74d	2200.11bcde	350.47ef	34297.2efg	26376.6de	2.42c	2439.8cdef	4321.0cde	24058.6de	10591.9c
	400	3.24cd	1856.63e	451.98de	45971.7cd	25260.8de	2.88c	2183.1def	6660.4c	39880.5b	11414.4c
	800	-	-	-	-	-	-	-	-	-	-
LSD		1.24	422.70	115.56	8054.9	3480.0	1.46	451.1	2709.7	5132.7	2070.0

4. Conclusion

In our study, the negative effects of lead, which is one of the important heavy metals, on seedling growth and seedling parameters in eggplant were determined. In addition, until control-400 ppm the intake of lead was observed in the eggplant seedling period in both shoot and root. In the control-800 ppm application, the plants did not develop and dried due to the high toxicity of lead. According to the data obtained in our study, AMF was found to be effective in soils containing lead. *Glomus intraradices* were found to be more effective among mycorrhizal species especially in eggplant

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