

Effects of Mycorrhiza on Plant Nutrition, Enzyme Activities, and Lipid Peroxidation in Pepper Grown Under Salinity Stress

H. Basak^{1*}, K. Mesut Cimrin², and M. Turan³

ABSTRACT

This study was conducted to determine whether Arbuscular Mycorrhizal Fungi (AMF) [ROOTS-novozymes endo-mycorrhiza fungus (*Glomus* spp.)] increase salt stress tolerance. The effects of mycorrhiza inoculation and salt on root and stem development, mineral nutrition, enzyme activity and lipid peroxidation levels in pepper (*Capsicum annuum* L.) plant was investigated. These effects were explored in pepper plants grown under greenhouse conditions in a randomized block design. Four different doses of salt (0, 50, 100 and 150 mM NaCl) were applied to the soil-filled pots, in addition to two different doses of mycorrhiza (0 and 100 spore mycorrhiza plant⁻¹). It was found that the root and stem dry weights of pepper plants were greatly reduced in the non-mycorrhiza treatments, whereas the presence of mycorrhiza ameliorated these negative effects. N, P, K, Ca, Mg, S, Fe, Mn, Zn and Cu contents of AMF treated pepper were higher than non-mycorrhizal plants. Owing to the presence of AMF colonization, nutrient uptake was increased and, consequently, the nutrient contents of stem and root tissues of mycorrhizal inoculated plants were enhanced as well. On the other hand, the root and stem enzyme activity of plants increased with salinity. AMF inoculation decreased SOD, CAT, POD and APOD enzymes of plant and the MDA and H₂O₂ contents, indicating lower oxidative damage in the inoculated plants. Our results showed that AMF can contribute to protect plants against salinity by alleviating the salt induced oxidative stress and arranging the ion balance in plant via increasing nutrient uptake in saline soils.

Keywords: Arbuscular mycorrhizal fungi, Nutrient uptake, Saline soils, Salt stress tolerance.

INTRODUCTION

Salinity is one of the most destructive stress factors affecting soil fertility and crop yield. Soils that make up about 7% of the global land surface are affected by salinity (Sheng *et al.*, 2011). Approximately 17% of the agricultural areas of the world have been irrigated and 20% of these are reported to have salinity problems (Tuteja, 2007).

Negative effects of salinity in plant growth include osmotic stress, which causes physiological drought and occurs as a result of nutritional imbalance; ion-specific phytotoxic effects; and a combination of

these factors due to irregularities in the ion balance of plants' intake and transport of nutrients (Acosta-Motos *et al.*, 2017). Soil salinity not only affects the uptake of some macronutrients but also prevents the intake of some micronutrients. Therefore, salinity is known to cause metabolic disorders and micronutrient deficiency in the plant (Munns and Tester, 2008).

Different plants can tolerate different doses of salt stress. This tolerance to the accumulation or excretion of toxic ions leads to molecular or biological activity. This activity is governed by physiological and biochemical events that accumulate ions in

¹ Department of Horticulture, Faculty of Agriculture, Kırşehir Ahi Evran University, Kırşehir, Turkey.

² Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Mustafa Kemal University, Hatay, Turkey.

³ Department of Genetics and Bioengineering, Faculty of Engineering, Yeditepe University, Istanbul, Turkey.

* Corresponding author; e-mail: hbasak@ahievran.edu.tr



certain parts of the plant by limiting ion transmission from roots to shoots and increasing osmo-regulators and antioxidant enzymes or activating various genes at the molecular level.

Mycorrhiza, an important soil fungi that forms symbiotic association with the roots of plants, is effective in increasing the uptake of nutrients from the soil and enhancing tolerance to abiotic, biotic, and physiological stress conditions (Salam *et al.*, 2017; Tedersoo *et al.*, 2020). Improvement of plant growth due to mycorrhizal symbiosis might be explained by changes in both the photosynthesis and antioxidant enzyme activities of plants (Li *et al.*, 2019). Mycorrhizal fungi increase the root surface area by forming many hyphae, thus, allowing the roots to absorb more water and nutrients (Barman *et al.*, 2016).

AMF inoculation increases plant resistance to salinity stress by improving nutrient and water uptake in plants under saline conditions, maintaining ionic and osmotic balance, inducing antioxidant system to prevent damage caused by reactive oxygen species, increasing photosynthetic efficiency and modulating phytohormone profile (Evelin *et al.*, 2012; Ruiz-Lozano *et al.*, 2012; Augé *et al.*, 2014; Mirzaei and Moradi, 2016). However, there have been still scientific gaps regarding the effects of AMF on the biochemical status of different parts of pepper plant. Therefore, the present study comprehensively covers biochemical and physiological changes that occur in plants that are inoculated with AMF fungi and exposed to salinity stress. The obtained data will contribute to a better understanding of the mechanisms by which AMF inoculation provides salinity stress alleviation.

Pepper, which belongs to the genus *Capsicum*, has an economic importance in Turkey, as in many countries. According to Turkstat (2020) data, approximately 2.64 million tons (dry or green) pepper is produced in Turkey. Pepper was preferred as a plant material in the study because it is

both widely grown and significantly affected by salinity.

In this study, the aim was to evaluate the nutritional elements, enzyme activity, and lipid peroxidation content of pepper (*Capsicum annuum* L. var. *grossum*) plant under salinity conditions and to evaluate the effects of mycorrhizal infection on the plant.

MATERIALS AND METHODS

This study was carried out in a climate-controlled greenhouse at the Kırşehir Ahi Evran University, Faculty of Agriculture, in Turkey. In order to determine the effects of the different doses of salt on the development and nutrition of plants, a randomized block design was planned with 4 replications. The soil samples used in the experiment were taken from the region where the pepper genotype is widely grown, sterilized by autoclaving at 121°C for two hours, and passed through a 2 mm sieve as per the Bouyoucos hydrometer method (Bouyoucos, 1951). The soil reaction (pH) and Electrical Conductivity (EC) were measured with a glass electrode pH meter in the prepared 1: 2.5 soil-water mixture. Lime contents were determined by Scheibler calcimeter and the organic matter by the modified Walkley-Black method (Jackson, 1958). Total N was determined by Kjeldahl method (Bremner, 1965). Available K (extracted with ammonium acetate) was measured by flame photometry (Knudsen *et al.*, 1983) and available P was extracted with sodium bicarbonate (Olsen *et al.*, 1954).

The soil sample characteristics are given in Table 1. Some micro elements (Fe, Zn, Cu, and Mn) were analyzed according to the DTPA method (Lindsay and Norvell, 1978) (Table 1). The soil texture was loamy. It was nitrogen poor, high in lime, non-saline, rich in available phosphorus, and rich in exchangeable potassium. The amounts of useful iron, zinc, manganese, and copper of the trial soil were above the sufficient level. In this study, the Cemele (*Capsicum annuum* cv.) local bell pepper genotype was used.

Table 1. Some physical and chemical properties of the experimental soil.

Clay (%)	22.2	N (%)	0.071
Sand	35.8	Available P (mg kg ⁻¹)	55.0
Loam	42.0	Exchangeable K (mg kg ⁻¹)	600.0
Texture	Loamy	Available Fe (mg kg ⁻¹)	6.46
pH (1:2.5 water)	7.72	Available Zn (mg kg ⁻¹)	4.05
Electrical conductivity (dS.m ⁻¹)	0.209	Available Cu (mg kg ⁻¹)	1.71
Lime (%)	14.6	Available Mn (mg kg ⁻¹)	29.08
Total organic matter (%)	2.15		

The Cemele bell pepper, which is widely grown in Kırşehir Province, is mostly consumed as dried pepper. In this study, 1.5 kg of air dry soil was placed in pots with plastic bags at the bottom to prevent drainage.

In the current study, four different doses of salt (S_0 , S_{50} , S_{100} , and S_{150} mM NaCl) were used and applied in both the control (M_0) and the 100 spore mycorrhizae (M_{100}) treated plants, with the initial electrical conductivities of, respectively, 0.21, 0.54, 3.23 and 5.61 dS.m⁻¹, in both M_0 and the M_{100} plants. The AMF inoculums (*Glomus intraradices*, *mosseae*, *aggregatum*, *clarum*, *monosporus*, *deserticola*, *brasilianum*, *etunicatum* and *Gigaspora margarita*.) consisted of spores named endo roots mycorrhiza inoculant (Novozymes Biologicals, Inc. 5400 Corporate Circle Salem, VA 24153) was applied to the root zone of the plants in the seedling stage during potting.

The experimental conditions in the greenhouse were 25-22°C ambient temperature and 65-70% relative humidity in day and night time, respectively. Throughout the experiment, the plants were irrigated with an average of 200 mL of distilled water every 3 days for 44 days, then, the plant samples were cut from the root collar and washed out of soil. In order to determine the root dry weights, the samples were dried in an oven at 65°C for 48 hours. In addition, some of the harvested and washed plant root and stem samples were stored at -20 °C for analysis of nutrient content, enzyme activity, and lipid peroxidation levels analyses.

Leaf macro (%) and micro (mg kg⁻¹) nutrient contents were determined on a dry weight basis. Nitrogen content was determined via Kjeldahl method (Bremner, 1965). Phosphorus (P) was quantified via the vanadomolybdophosphoric acid colorimetric method (Kacar and İnal, 2008). K, Mg, Ca, Fe, Zn, Cu and Mn contents were determined by using AAS (Atomic Absorption Spectrophotometer) (Kacar and İnal, 2008). Lipid peroxidation was determined by measuring Malondialdehyde (MDA) by the methods of Heath and Packer (1968). The Hydrogen peroxide (H₂O₂) content was determined by the method of Velikova *et al.* (2000). Enzyme activities [Superoxide Dismutase (SOD), Catalase (CAT), Peroxidase (POD) and Ascorbate Peroxidase (AxBOD)] were measured using spectrophotometric method (Sairam and Srivastava, 2002).

Experiment was conducted according to randomized block design. The obtained data were statistically analyzed by GLM procedure of SPSS (Windows version of SPSS, release 20.00). The means were compared by Duncan Multiple Range Test in the same software. Significant differences were accepted at the level $P < 0.05$ and analysis of variance was performed to determine the differences between groups.

RESULTS AND DISCUSSION

Salt stress significantly reduced root and stem dry weights compared to the control (Table 2). As can be seen from this data, there were statistically significant ($P < 0.01$)



Table 2. The effects of mycorrhiza and salt applications on root and stem dry weights of pepper plants (g plant⁻¹).^a

Applications	N	Root weights	dry	Stem weights	dry	Stem:Root ratio
M ₀	S0	4	2.53a	27.75b		10.96
	S50	4	0.87bc	19.93c		22.91
	S100	4	0.42d	9.25de		22.02
	S150	4	0.26d	2.74f		10.54
M ₁₀₀	S0	4	2.73a	33.05a		12.11
	S50	4	1.11b	21.40c		19.27
	S100	4	0.56cd	10.71d		19.13
	S150	4	0.35d	5.85ef		16.71
SEM			0.237	2.512		-
P			0.000**	0.000**		-
M ₀	16		1.03B	14.92B		16.61
M ₁₀₀	16		1.19A	17.75A		16.81
P			0.011**	0.000**		-

^a (a-b) and (a-f) The differences between mean values indicated by different letters are significant ($P < 0.05$). The different letters in the same column show the statistical difference between applications according to Duncan Multiple Range Test. Std. Error Mean (SEM), M0: 0 spore Mycorrhiza plant⁻¹, M100: 100 spore Mycorrhiza plant⁻¹. * and **: Significant at $P < 0.05$ and $P < 0.01$ levels.

linear decreases in the root and stem dry weights of the pepper plant due to the increased salt content in both mycorrhiza and non-mycorrhiza trials (Table 2). Control plants were affected more by salt stress compared to mycorrhiza treated ones. Both the mycorrhizal plants and the non-mycorrhizal plants experienced a reduction in dry weight in stems and roots, but the reduction in weight was lower in the mycorrhizal plants. This result indicates that AMF colonization increased dry matter content in plants under salt stress. Supporting our findings, Souza *et al.* (2020) reported that *Hyptis suaveolens* plant inoculated with AMF increased dry matter compared to plants without mycorrhiza inoculation, and plants colonized with AMF on 35 mM NaCl treatment increased biochemical and physiological responses. The effect of AMF on dry matter content was more pronounced in the stem and leaf biomass than root biomass.

In addition, the stem to root ratio of plants increased by the S₅₀ dose and decreased by the S₁₀₀ and S₁₅₀ doses, medium and high salinity, respectively (Table 2). In other words, although the plant roots were directly

exposed to salinity, the growth of stem was more negatively affected than root due to the salt stress. Daei *et al.* (2009) reported that the reduced water uptake in plants due to the effect of salt stress increased with mycorrhizal inoculation, and thus the negative effect of salt stress could be partially or fully alleviated.

As shown in Tables 3, 4, and 5, N, P, K, Ca, Mg, S, Fe, Mn, Cu and Zn concentrations of stem were significantly decreased by increased salinity. The concentration of Na increased with increasing salinity levels in non-mycorrhizal plants. However, N, P, K, Ca, Mg, S, Fe, Mn, Cu and Zn concentrations of AMF treated plant were higher than untreated plants. On the other hand, the N, P, Ca, Mg, S, Fe and Zn contents of the roots increased significantly by increased salinity in mycorrhiza treated and untreated plants. A similar situation was reported by Taban and Katkat (2000), who applied increasing doses of NaCl (0, 15, 30, 45, and 60 mM) to the corn plant. It was observed that AMF inoculation increased nutrient uptake in control plants untreated salt stress.

Table 3. The effects of mycorrhiza and salt applications on root N, P, K and Na contents of pepper plants (mg kg^{-1}).^a

			N		P		K		Na	
Applications	N	Root	Stem	Root	Stem	Root	Stem	Root	Stem	
M ₀	S ₀	4	50.99cd	313.7ab	7.73b	35.28b	137.8a	279.8ab	2.18a	4.11g
	S ₅₀	4	59.01bcd	273.7bc	8.67b	26.63d	147.9a	202.9c	1.87ab	7.84d
	S ₁₀₀	4	61.91bc	197.9d	14.03a	20.82e	91.2bc	123.5e	1.58bc	15.55b
	S ₁₅₀	4	81.31a	131.5e	12.64a	17.92f	100.9b	77.5f	1.15cd	28.04a
M ₁₀₀	S ₀	4	45.11d	342.8a	8.22b	38.51a	147.7a	302.7a	2.14a	4.31fg
	S ₅₀	4	49.01cd	301.8ab	13.24a	30.31c	78.8c	254.5b	1.51bcd	4.92ef
	S ₁₀₀	4	68.89ab	251.9bcd	12.37a	27.89cd	78.3bc	171.5d	1.16cd	5.41e
	S ₁₅₀	4	79.16a	235.4cd	11.96a	21.91e	71.5c	127.9e	0.99d	8.85c
SEM			2.474	12.218	0.468	1.213	5.768	13.796	0.082	1.386
P			0.000**	0.000**	0.002**	0.000**	0.000**	0.000**	0.000**	0.000**
M ₀	Mean	16	63.30	229.2B	11.76	25.16	119.5A	170.9	1.70	13.89A
M ₁₀₀	Mean	16	60.54	282.9A	11.44	29.66	94.1B	214.2	1.45	5.87B
P			0.586	0.025*	0.470	0.063	0.025*	0.119	0.207	0.002**

^a (a-b) and (a-f) The differences between mean values indicated by different letters are significant ($P < 0.05$). Std. Error Mean (SEM).

* and **: Significant at $P < 0.05$ and $P < 0.01$ levels.

Table 4. The effects of mycorrhiza and salt applications on root and stem Ca, Mg and S contents of pepper plants (mg kg^{-1}).

Applications		N	Ca		Mg		S	
			Root	Stem	Root	Stem	Root	Stem
M ₀	S ₀	4	48.3c	169.2bc	5.29c	14.04ab	2.11bcd	11.52b
	S ₅₀	4	56.5bc	153.2cd	5.38c	12.73b	2.03cd	9.59cd
	S ₁₀₀	4	89.4ab	87.5ef	7.14b	8.72c	2.65a	8.23ef
	S ₁₅₀	4	96.5a	61.2f	8.48a	8.54c	2.57ab	7.75f
M ₁₀₀	S ₀	4	55.5bc	239.3a	5.53c	15.08a	1.78d	13.86a
	S ₅₀	4	72.9abc	197.9b	6.12bc	13.97ab	2.63a	11.47b
	S ₁₀₀	4	76.3abc	123.7de	6.41bc	12.79b	2.45abc	10.46bc
	S ₁₅₀	4	69.7abc	86.8ef	5.89bc	10.35c	2.38abc	9.13de
SEM			3.756	10.587	0.201	0.446	0.062	0.348
P			0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
M ₀	Mean	16	72.7	117.8B	6.57	11.01B	2.35	9.27B
M ₁₀₀	Mean	16	68.6	161.9A	5.99	13.05A	2.31	11.23A
P			0.597	0.035*	0.147	0.020*	0.790	0.003*

^a (a-b) and (a-f) The differences between mean values indicated by different letters are significant ($P < 0.05$). Std. Error Mean (SEM).

* and **: Significant at $P < 0.05$ and $P < 0.01$ levels.

Sallaku *et al.* (2019) reported that inoculation with

AMF enhanced the nutrient uptake of cucumber seedlings, through extending their root system and enhancing the photosynthetic rate.

These results show that the negative effects of salt stress in the plants were significantly reduced in all mycorrhizal treatments, but this effect decreased at high

salinity levels. While plants can tolerate salinity up to 100 mM with AMF application under salt stress conditions, damage occurs above this salinity level. However, when these losses are compared to the control, it is seen that AMF applications prevent 30-70% product decrease under salt stress conditions. Since mycorrhiza has vesicles resembling fungal vacuoles and accumulates large amounts of heavy metals in them, it

**Table 5.** The effects of mycorrhiza and salt applications on root and stem Fe, Cu, Mn and Zn contents of pepper plants (mg kg^{-1}).

Applications	N	Fe		Cu		Mn		Zn		
		Root	Stem	Root	Stem	Root	Stem	Root	Stem	
M ₀	S ₀	4	10.66c	124.7bc	2.53a	8.75a	3.34bc	28.00ab	22.45c	14.00ab
	S ₅₀	4	29.17a	113.8c	1.36b	6.87ab	2.85cd	22.93abc	48.74a	12.09b
	S ₁₀₀	4	25.08a	75.7d	1.31b	3.53c	2.40d	14.57de	44.27a	6.66c
	S ₁₅₀	4	24.18ab	62.5d	1.20b	3.13c	2.59cd	8.33e	39.99ab	5.41c
M ₁₀₀	S ₀	4	11.94c	166.9a	2.56a	8.99a	4.04ab	29.47a	24.54bc	16.59a
	S ₅₀	4	14.76bc	143.1b	2.95a	7.09a	4.22a	21.83bc	20.03c	13.45ab
	S ₁₀₀	4	32.88a	119.8c	1.41b	4.62bc	2.77cd	17.23cd	49.84a	8.59c
	S ₁₅₀	4	26.76a	78.0d	1.46b	3.63c	3.02cd	13.54de	46.09a	7.88c
			1.538	6.240	0.134	0.432	0.123	1.325	2.370	0.709
	P		0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
M ₀	Mean	16	22.27	94.2B	1.61	5.57	2.80B	18.46	38.87	9.54
M ₁₀₀	Mean	16	21.59	126.9A	2.10	6.09	3.52A	20.52	35.13	11.63
	P		0.829	0.006**	0.066	0.561	0.002**	0.446	0.440	0.144

^a (a-b) and (a-f) The differences between mean values indicated by different letters are significant ($P < 0.05$). Std. Error Mean (SEM).

** : Significant at $P < 0.01$ level.

provides toxic tolerance to the host plant (Dhalaria *et al.*, 2020). The decrease of leaf Na content from 13.89 mg kg^{-1} in mycorrhiza untreated plants to 5.87 mg kg^{-1} in mycorrhizal plants was significant in terms of mycorrhiza's reducing effect on Na toxicity (Table 3).

Hydrogen peroxide and MDA contents were used to determine the effects of salt stress on plants, while enzyme analyses were done to determine the efficacy of mycorrhiza application on the elimination of salinity stress.

In this study, it was determined that the application of mycorrhiza induced the activity of antioxidant defense enzymes to a great extent (Table 6). Despite the symbiotic nature of AMF associations, the induction of antioxidant enzymes observed during appressoria formation was attributed to the defense responses of plants during the early stage of symbiosis development (Blilou *et al.*, 2000; Hajiboland *et al.*, 2010).

While the highest enzyme activity was determined at 50 mM Salt dose (S_{50}) in plants treated with mycorrhiza, the difference between the enzyme activities detected at the 100 mM and 150 mM salt

doses was not statistically significant. This situation was determined to be due to the mycorrhiza inoculation in the pepper plant activating the enzymatic antioxidant defense system at the 50 mM (moderate) salt dose ($M_{100}S_{50}$). In other words, the supportive effect of mycorrhiza decreased with excessive salt doses ($M_{100}S_{100}$; $M_{100}S_{150}$). Similarly, arbuscular mycorrhizal fungal symbiosis at 0 mM and 50 mM salt doses was reported to increase the antioxidant enzyme activities of plants and alleviate the negative impact of salt stress (Alguacil *et al.*, 2003) (Table 6).

In this study, SOD, CAT, POD and AxPOD enzyme activities determined in the mycorrhizal plants at 100 mM and 150 mM salt concentrations ($M_{100}S_{100}$; $M_{100}S_{150}$) were at a lower level than the plants without mycorrhiza inoculation. AMF inoculation is known to alleviate salt stress by enhancing the rhizospheric soil characteristics (Ahanger *et al.*, 2014), but it is known that the formation of mycorrhizal symbiosis is negatively affected by high salt doses (Wang *et al.*, 2021).

While CAT, SOD, and POD enzyme activities in stem tissues were

Table 6. The effects of mycorrhiza and salt applications on root and stem enzyme activities and lipid peroxidation of pepper plants.

Applications		N	SOD (EU g leaf ¹)		CAT (EU g leaf ¹)		POD (EU g leaf ¹)	
			Root	Stem	Root	Stem	Root	Stem
M ₀	S ₀	4	56.19c	189.4c	148.48c	479.8c	21.07c	72.3c
	S ₅₀	4	62.44bc	208.9bc	159.91c	563.9bc	26.48bc	89.2bc
	S ₁₀₀	4	103.92a	265.7a	279.23a	714.0ab	57.90a	147.8a
	S ₁₅₀	4	77.31b	257.9a	255.06ab	848.3a	41.13b	136.4a
M ₁₀₀	S ₀	4	64.91bc	193.5c	197.44bc	553.0bc	29.72bc	87.2bc
	S ₅₀	4	79.23b	263.7a	184.12c	612.4bc	39.85b	132.2a
	S ₁₀₀	4	63.97bc	240.3ab	177.71c	641.5bc	32.39bc	118.7ab
	S ₁₅₀	4	67.40bc	230.8abc	172.29c	589.5bc	34.14bc	109.9abc
			2.877	5.890	9.077	21.999	2.164	5.300
P			0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
M ₀	Mean	1€	74.97	230.5	210.67	651.5	36.65	111.4
M ₁₀₀	Mean	1€	68.88	232.1	182.89	599.1	34.03	112.1
P			0.298	0.894	0.128	0.240	0.554	0.954
Applications		N	AxPOD (EU g leaf ¹)		MDA (EU g leaf ¹)		H ₂ O ₂ (EU g leaf ¹)	
			Root	Stem	Root	Stem	Root	Stem
M ₀	S ₀	4	22.01e	10.6b	49.91b	26.5b	19.13e	12.9c
	S ₅₀	4	28.98e	18.9b	107.48a	53.7a	36.02d	24.2b
	S ₁₀₀	4	86.89bc	49.8a	98.54a	44.3a	60.77b	30.1a
	S ₁₅₀	4	120.60a	53.9a	99.98a	54.9a	75.29a	28.4ab
M ₁₀₀	S ₀	4	25.39e	13.4b	46.59b	24.5b	13.44e	11.9c
	S ₅₀	4	73.90cd	47.7a	40.06b	20.1b	18.09e	15.5c
	S ₁₀₀	4	69.40d	42.6a	99.69a	47.3a	29.66d	25.4ab
	S ₁₅₀	4	101.11b	42.7a	97.19a	51.1a	44.61c	26.8ab
			6.352	3.201	5.378	2.553	3.725	1.280
P			0.000	0.000**	0.000	0.000**	0.000	0.000**
M ₀	Mean	1€	64.62	33.3	88.98	44.9	47.81A	23.9
M ₁₀₀	Mean	1€	67.46	36.6	70.89	35.8	26.45B	19.9
P			0.828	0.615	0.093	0.074	0.003**	0.120

^a (a-b) and (a-f) The differences between mean values indicated by different letters are significant (P<0.05). Std. Error Mean (SEM).

** : Significant at P< 0.01 level.

approximately 3 times higher than the values in the root tissues, the AxPOD, MDA, and H₂O₂ levels were determined approximately 2 times higher than the values determined in stem tissues. Root H₂O₂ level of plants inoculated with mycorrhiza decreased by 44.7% compared to those without mycorrhiza, while there was only 16.7% decrease in the stem tissues. This decrease may be due to the increased AxPOD activity in root by mycorrhiza inoculation (Table 6). In addition to CAT, peroxidases (POD and AxPOD) also serve a function in the detoxification of H₂O₂, which increases

under stress conditions. Because AxPOD has a higher substrate affinity than catalase due to the presence of ascorbic acid, it is known to be more effective in the degradation of H₂O₂ (Willekens *et al.*, 1994).

Supporting the findings obtained in this study, Rai *et al.* (2004) reported that AxPOD enzyme protected plants from oxidative damage by eliminating toxic H₂O₂ from plant cells. Ghorbanli *et al.* (2004) stated that mycorrhizal plants removed higher amounts of H₂O₂ when compared to non-mycorrhizal, due to their higher AxPOD activities. On the other



hand, the adverse effect of free oxygen radicals on cell membrane occurs via lipid peroxidation. Due to the increased salt concentrations, the increased electrolyte leakage in stem and root tissues enhanced H_2O_2 and MDA, signs of lipid peroxidation. However, these parameters showed higher values in root tissues. The H_2O_2 content of mycorrhizal plants was at lower level than those of non-mycorrhizal for all salt doses, including the control.

MDA content is an important parameter in determining the extent of stress damage in salt stress studies (Gossett *et al.*, 1994). In this study, the MDA content increased significantly as the salt concentration increased in both the mycorrhizal and the non-mycorrhizal plants. While the stem and root SOD, CAT and POD enzyme activities of mycorrhizal plants increased up to 50 mM NaCl dose, they decreased at higher salt doses. However, root and stem H_2O_2 and MDA levels of mycorrhizal inoculated plants did not show any significant change at 50 mM NaCl compared to the control plants, but increased statistically significantly with the effect of increasing salt doses. This situation implies that the enzymatic antioxidant defense system's inhibitory effect on lipid peroxidation is insufficient for a dose of salt exceeding 50 mM.

CONCLUSIONS

In this study, activity of antioxidant enzymes in AMF colonization of pepper plants compared with non-mycorrhizal plants was associated with lower activity of antioxidant enzyme such as SOD, CAT, POD and AxPOD. This indicated lower oxidative damage in the colonized plants and stimulate plant nutrient uptake. According to our results, AMF can contribute to protect plants against salinity by alleviating the salt induced oxidative stress and arranging the ion balance in plant via stimulating the nutrient in saline soil.

REFERENCES

1. Acosta-Motos, J., Ortuño, M., Bernal-Vicente, A., Diaz-Vivancos, P., Sanchez-Blanco, M. and Hernandez, J. 2017. Plant Responses to Salt Stress: Adaptive Mechanisms. *Agronomy*, **7**: 1-38.
2. Ahanger, M. A., Hashem, A., Abd_Allah, E. F. and Ahmad, P. 2014. Arbuscular mycorrhiza in Crop Improvement under Environmental Stress. In: "Emerging Technologies and Management of Crop Stress Tolerance", (Eds.): Ahmad, P. and Rasool, S. Academic Press, Elsevier, San Diego, CA, **2**: 69-95.
3. Alguacil, M., Hernandez, J. A., Caravaca, F., Portillo, B. and Roldan A. 2003. Antioxidant Enzyme Activities in Shoots from Three Mycorrhizal Shrub Species Afforested in a Degraded Semi-Arid Soil. *Physiol. Plant.* **118**: 562-570.
4. Augé R. M., Toler, H. D. and Saxton, A. M. 2014. Arbuscular Mycorrhizal Symbiosis and Osmotic Adjustment in Response to NaCl Stress: A Meta-Analysis. *Front. Plant. Sci.*, **5**: 1-14.
5. Barman, J., Samanta, A., Saha, B. and Datta, S. 2016. Mycorrhiza: The Oldest Association between Plant and Fungi. *Resonance*, **21(12)**: 1093-1104.
6. Blilou, I., Bueno, P., Ocampo, J. A. and García-Garrido, J. M. 2000. Induction of Catalase and Ascorbate Peroxidase Activities in Tobacco Roots Inoculated with the Arbuscular Mycorrhizal Fungus *Glomus mosseae*. *Mycol. Res.* **104**: 722-725.
7. Bouyoucos, G. J. 1951. A. Recalibration of the Hydrometer Method for Making Mechanical Analysis of the Soil. *Agron. J.*, **43**: 434-438.
8. Bremner, J. M. 1965. Total Nitrogen. Part 2. *Agronomy*. In: "Methods of Soil Analysis", (Eds.): Black, C. A. *et al.* American Society of Agronomy, Inc. Madison, Wisconsin, USA, PP. 1149-1178.
9. Daei, G. 2009. Alleviation of Salinity Stress on Wheat Yield, Yield Components, and

- Nutrient Uptake Using Arbuscular Mycorrhizal Fungi under Field Conditions. *J. Plant. Physiol.*, **166**: 617-625.
10. Dhalaria, R., Kumar, D., Kumar, H., Nepovimova, E., Kuřca, K., Torequel Islam, M. and Verma, R. 2020. Arbuscular Mycorrhizal Fungi as Potential Agents in Ameliorating Heavy Metal Stress in Plants. *Agronomy*, **10**: 815.
 11. Evelin, H., Giri, B. and Kapoor, R. 2012. Contribution of *Glomus intraradices* Inoculation to Nutrient Acquisition and Mitigation of Ionic Imbalance in NaCl-Stressed. *Trigonella foenum-graecum*. *Mycorrhiza*, **22**: 203–217.
 12. Ghorbanli, M., Ebrahimzadeh, H. and Sharifi, M. 2004. Effects of NaCl and Mycorrhizal Fungi on Antioxidative Enzymes in Soybean. *Bio. Plant.*, **48**: 575–581.
 13. Gossett, D. R., Milhollon, E. P. and Lucas, M. C. 1994. Antioxidant Response to NaCl Stress in Salt-Tolerant and Salt-Sensitive Cultivar of Cotton. *Crop Sci.*, **34**: 706–714.
 14. Hajiboland, R., Aliasgharzadeh, N., Laiegh, S. F. and Poschenrieder, C. 2010. Colonization with Arbuscular Mycorrhizal Fungi Improves Salinity Tolerance of Tomato (*Solanum lycopersicum* L.) Plants. *Plant Soil*, **331**: 313–327.
 15. Heath, R. L. and Packer, L. 1968. Photoperoxidation in Isolated Chloroplasts. I. Kinetics and Stoichiometry of Fatty Acid Peroxidation. *Arch. Biochem Biophys.*, **125**: 189-198.
 16. Jackson, M. 1958. *Soil Chemical Analysis*. Prentice-Hall Inc., Englewood Cliffs, New Jersey, USA, 498 PP.
 17. Kacar, B. and İnal, A. 2008. *Plant Analysis*. Nobel Publications, Ankara, 891 PP.
 18. Knudsen, D., Peterson, G. A. and Pratt, P. F. 1983. Lithium, Sodium and Potassium. Methods of soil analysis, Part 2. *Chem. Microbiol. Prop.*, **9**: 225–246.
 19. Li, J., Meng, B., Chai, H., Yang, X., Song, W., Li, S., Lu, A., Zhang, T. Sun, W. 2019. Arbuscular Mycorrhizal Fungi Alleviate Drought Stress in *C₃* (*Leymus chinensis*) and *C₄* (*Hemarthria altissima*) Grasses via Altering Antioxidant Enzyme Activities and Photosynthesis. *Front. Plant Sci.*, **10**: 1-12.
 20. Lindsay, W. L. and Norvell, W. A. 1978. Development of a DTPA Soil Test for Zinc, Iron, Manganese and Copper. *Soil Sci. Soc. Am. J.*, **42**: 421-428.
 21. Mirzaei J, Moradi M. 2016. Single and Dual Arbuscular Mycorrhiza Fungi Inoculum Effects on Growth, Nutrient Absorption and Antioxidant Enzyme Activity in *Ziziphus spina-christi* Seedlings under Salinity Stress. *J. Agric. Sci. Technol.*, **18** (7): 1845-1857.
 22. Munns, R. and Tester, M. 2008. Mechanism of Salinity Tolerance. *Annu. Rev. Plant Biol.*, **59**: 651-681.
 23. Olsen S. R., Cole, C. V., Watanabe, F. S. D. and Dean, L. A. 1954. *Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate*. United States Department of Agriculture, Washington, DC
 24. Rai, V., Vaypayee P., Singh S. N. and Mehrotra, S. 2004. Effect of Chromium Accumulation on Photosynthetic Pigments, Oxidative Stress Defence System, Nitrate Reduction, Proline Level and Eugenol Content of *Ocimum tenuiflorum* L. *Plant Sci.*, **167** (5): 1159-1169.
 25. Ruiz-Lozano, J. M., Porcel, R., Azcón, C., and Aroca, R. 2012. Regulation by Arbuscular Mycorrhizae of the Integrated Physiological Response to Salinity in Plants: New Challenges in Physiological and Molecular Studies. *J. Exp. Bot.*, **63**: 4033–4044.
 26. Salam, E. A., Alatar, A. and El-Sheikh, M. A. 2017. Inoculation with Arbuscular Mycorrhizal Fungi Alleviates Harmful Effects of Drought Stress on Damask Rose. *Saudi J. Biol. Sci.*, **25** (8): 1772–1780.
 27. Sallaku, G., Sandén, H., Babaj, I., Kaciu, S., Balliu, A. and Rewald, B. 2019. Specific Nutrient Absorption Rates of Transplanted Cucumber Seedlings Are Highly Related to



- RGR and Influenced by Grafting Method, AMF Inoculation and Salinity. *Sci. Hortic.*, **243**: 177–188.
28. Sairam, R. and Srivastava, G. 2002. Changes in Antioxidant Activity in Sub-Cellular Fractions of Tolerant and Susceptible Wheat Genotypes in Response to Long Term Salt Stress. *Plant Sci.*, **162**: 897-904.
29. Sheng, M., Tang, M., Zhang, F. and Huang, Y. 2011. Influence of Arbuscular Mycorrhiza on Organic Solutes in Maize Leaves under Salt Stress. *Mycorrhiza*, **21**: 423–430.
30. Souza, M. C. G., Morais, M. B., Andrade, M. S., Vasconcelos, M. A., Sampaio, S. S. and Albuquerque, C. C. 2020. Mycorrhization and Saline Stress Response in *Hyptis suaveolens*. *Ciê. Rur.*, **50**: e20190533.
31. Taban, S. and Katkat, A. V. 2000. Effect of Salt Stress on Growth and Mineral Elements Concentrations in Shoots and Roots of Maize Plants. *J. Agric. Sci.*, **6**: 119-122.
32. Tedersoo, L., Bahram, M. and Zobel, M. 2020. How Mycorrhizal Associations Drive Plant Population and Community Biology. *Science*, **367**: 1-10.
33. Turkstat 2021. *Value of Pepper Production*. 2020. Available form: <https://data.tuik.gov.tr/Bulten/Index?p=Bitkisel-Uretim-Istatistikleri-2020-33737> (Accessed 2021 July 30)
34. Tuteja, N. 2007. Mechanisms of High Salinity Tolerance in Plants. *Meth. Enzymol.*, **428**: 419-438.
35. Velikova, V., Yordanov, I. and Edreva, A. 2000. Oxidative Stress and Some Antioxidant System in Acid Rain Treated Bean Plants: Protective Role of Exogenous Polyamines. *Plant Sci.*, **151**: 59–66.
36. Wang, Y. H., Zhang, N. L., Wang, M. Q., He, X. B., Lv, Z. Q., Wei, J., Su, X., Wu, A. P., Li, Y. 2021. Sex-Specific Differences in the Physiological and Biochemical Performance of Arbuscular Mycorrhizal Fungi-Inoculated Mulberry Clones Under Salinity Stress. *Front. Plant Sci.*, **18(12)**: 1-13.
37. Willekens, H., Van Camp, W., Van Montagu, M., Inzé, D., Langebartels, C. and Sandermann, H. 1994. Ozone, Sulfur Dioxide, and Ultraviolet B Have Similar Effects on mRNA Accumulation of Antioxidant Genes in *Nicotiana plumbaginifolia* L. *Plant Physiol.* **106**: 1007–1014.

اثرهای قارچ میکوریزا بر تغذیه گیاه، فعالیت آنزیمی و پراکسیداسیون لیپیدی در فلفل کشت شده در تنش شوری

ه. باشاک، ک. مسعود چیمیرین، و م. توران

چکیده

این پژوهش برای تعیین اینکه آیا قارچ‌های میکوریزا آربوسکولار (AMF) (ROOTS-novozymes endo-) mycorrhiza fungus (*Glomus spp.*) تحمل به تنش شوری را افزایش می‌دهند انجام شد. بنا بر این، اثرهای تلقیح میکوریزا و نمک بر رشد ریشه و ساقه، تغذیه معدنی، فعالیت آنزیمی و سطوح پراکسیداسیون لیپیدی در گیاه فلفل (*Capsicum annuum* L.) بررسی شد. این اثرها در گیاهان فلفل رشد کرده در شرایط

گلخانه در یک طرح بلوک‌های تصادفی بررسی شد. به این منظور، به گلدان‌های پر از خاک، چهار تیمار مختلف نمک (۰، ۵۰، ۱۰۰ و ۱۵۰ میلی مولار NaCl) افزون بر دو تیمار مختلف میکوریزا (۰ و ۱۰۰ اسپور میکوریزا در هر گیاه) اعمال شد. مشخص شد که وزن خشک ریشه و ساقه گیاهان فلفل در تیمارهای غیر میکوریزا به میزان زیادی کاهش یافت، در حالی که حضور قارچ این اثرات منفی را بهبود بخشید. محتوای N، P، K، Ca، Mg، S، Fe و نیز Zn و Cu فلفل تیمار شده با AMF بیشتر از گیاهان غیرمیکوریزی بود. به خاطر وجود کلونیزاسیون AMF، جذب عناصر غذایی افزایش یافته و در نتیجه محتوای مواد مغذی بافت‌های ساقه و ریشه گیاهان تلقیح شده با میکوریز نیز افزایش یافت. از سوی دیگر، فعالیت آنزیم ریشه و ساقه گیاهان با شوری افزایش یافت. تلقیح AMF باعث کاهش آنزیم‌های SOD، CAT، POD و AxPOD گیاه و محتوای MDA و H₂O₂ شد که نشان دهنده آسیب کمتر اکسیداتیو در گیاهان تلقیح شده است. نتایج ما نشان داد که AMF می‌تواند با کاهش تنش اکسیداتیو ناشی از نمک و تنظیم تراز یونی در گیاه از طریق افزایش جذب مواد مغذی در خاک‌های شور، به محافظت از گیاهان در برابر شوری کمک کند.