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RESEARCH PAPER

The Effect of the Application of Mycorrhiza on Vegetative Growth, Mineral Element Intake, and Some Biochemical Characteristics of Strawberry Seedlings under Lime Stress

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Abstract

This study aims to determine the effects of vesicular–arbuscular mycorrhiza (VAM) applications on vegetative growth, mineral element intake, and some biochemical characteristics of strawberry seedlings grown under lime stress conditions. The experiment was conducted with frigo seeds of "Albion" strawberry cultivar in pots filled with 1% lime mixture and 1:1 ratio of peat and perlite. In the uprootings performed in three different stages (four leaved, blooming, and fruit stages) to examine the biochemical effects of mycorrhiza applications against the lime stress, vegetative growth criteria (leaf chlorophyll and anthocyanin content, area, crown diameter, fresh and dry plant weights) and mineral contents in the plant parts (leaf, crown, and root) were determined. The proline, total phenolic content, and malondialdehyde (MDA), end product of the lipid peroxidation, analyses were conducted on the leaf samples taken in these uprooting. In all three stages, an increase in crown diameter and leaf area was determined. In uprooting periods, proline and total phenolic amounts increased, and, on the other hand, MDA decreased. Microelement intake, which decreased with the lime application, was detected to be increased with mycorrhiza applications. At the end of the experiment, mycorrhiza application was observed to lessen the effect of lime stress on strawberry seedlings.

1. Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) of the Fragaria kind, the Rosaceae family, is grown in many parts of the world. Due to its taste, health benefits, and suitability for food technology, the production amount has been increasing year by year. The fact that it has no marketing issues encourages growers to strawberry cultivation. While the highest yield is obtained in temperate climatic conditions, the yield decreases as becoming distant from the sea, towards the upcountry (Yılmaz et al., 2006; Balcı et al., 2017). In upcountry areas where the continental climate is dominant, high soil pH is one of the most significant limiting factors for strawberry cultivation. Strawberries are susceptible to high pH level. When cultivation is performed in these areas, severe chlorosis and a decrease in vegetative growth are observed (Yılmaz, 2009).

Mycorrhizal fungi maintain their lives in plant roots by locating in the plant's root surface, root fibers, cells, and intercellular spaces. Mycorrhizal fungi are beneficial to soil microorganisms and are of great importance for healthy plant growth and soil fertility. The role of these fungal species, which play a very important role in 85% of the world's vegetation and establish symbiotic relationships with roots, in agricultural production is rapidly getting stronger day by day (Erzurumlu and Karar, 2014). Many studies demonstrate that mycorrhizal applications have beneficial effects for plants against to biotic or abiotic stress conditions

(Bavaresco and Fogher, 1995; Gianinazzi and Schuepp, 1995; Yano and Takaki, 2005; Sinclair et al., 2014; Latef et al., 2016). Strawberries, which have an important place in world fruit growing, are generally considered among the varieties that respond positively to mycorrhizae. The studies conducted to analyze the effects of the use of mycorrhiza on the growth and yield of strawberries have revealed positive effects in terms of plant development, fruit quality, and early yield in strawberries (Sharma and Adholeya, 2004; Ertan et al., 2007; Bayozen and Yıldız, 2008; Cekic and Yılmaz, 2011). While there are studies stating that mycorrhiza applications positively support vegetative growth, yield, and mineral substance intake in strawberries under stress conditions such as low pH, salinity, high phosphorus content in the soil, and drought (Gupta and Krishnamurthy, 1996; Stewart et al., 2005; Matsubara et al., 2009; Borowicz, 2010; Sinclair et al., 2014; Koç et al., 2016), very few studies examining the effects of mycorrhiza application on biochemical characteristics in the strawberries cultivated under stress conditions (Koç, 2015; Koç et al., 2016; Bahmanbiglo and Eshghi, 2021). There are not enough studies on the effects of mycorriza applications on strawberry under high pH conditions. This study aimed to determine the effects of mycorrhiza applied on strawberries in high pH conditions on vegetative growth, biochemical characteristics, and mineral element intake.

2. Material and Method

This study was carried out in a greenhouse without climate control, located on the field of Yozgat Bozok University (1111 m, 39°35'7" N and 35°09'35" E). In our experiment, the "Albion" cultivar, which is one of the day-neutral strawberries, was used. Albion, which is very productive and high quality, is successfully grown in areas with high altitudes (Balcı et al., 2017). The experiment was carried out by filling the peat perlite mixture at the rate of 1:1 in the 5-liter of pots (265×210 mm). Lime ($CaCO_3$) addition was not done to the pots in the 0% and 1% lime was added to the group to which lime stress was applied in terms of weight. The initial and final pH of the cultivation media in the experiment was determined as 7.74 and 8.41, respectively. The pH values of the environment were determined according to Kacar (2012). The frigo seedlings belonging to the Albion strawberry cultivar were planted in the pots on 28.03.2018. The strawberry seedlings were once fertilized with "Nutritect 18-18-18 TE" commercial fertilizer (15.05.2018).

2.1. Application of mycorrhiza

Preparate in commercial powder form containing mycorrhiza (9 different *Glomus* species) at the rate of 23%, named Endo Roots Soluble, belonging to the Bioglobal firm was used in our study. The fungi and their rates in the content of the preparate were as *Glomus intraradices* (21%), *Glomus aggregatum (*20%), *Glomus mosseage (*20%), *Glomus clarum (*1%), *Glomus monosporus* (1%), *Glomus deserticola (1%*), G*lomus brasilianum (*1%), *Glomus etunicatum (*1%), and *Gigaspora margarita (*1%). Mycorrhiza application to seedlings was performed by soaking plant roots in the solution, which was prepared by mixing the packet of 250 gram-powder with the 10-liter sugared water, for an hour before the planting and inoculating with the fungi. The remaining solution was put in the plant root area as sap. The plants in the control group, on the other hand, were kept in the water for an hour.

2.2. Taking leaf samples

Leaf sampling was performed in three different periods with the aim to determine the VAM effect on lime stress in strawberry seedlings in different development stages. The first leaf sample was taken in the period when the strawberry seedlings had 4 leaves (25.04.2018), the second sample was taken in the blooming period two months after the planting (25.06.2018), and the last sample was taken three months after the planting in the fruiting stage (26.0.2018). Vegetative growth parameters and mineral element contents were determined by uprooting plants in these periods. For mineral element contents analysis, the plants were separated into roots, crowns, and leaves with petioles, and washed. These parts of the plant were oven-dried at 70°C. For biochemical analysis, the leaves that had taken their full size during these periods were cut off, then immediately placed in ice, and kept at -20°C until the analysis. Mineral element analysis was made by the Yozgat Bozok University Application and Research Center of Science and Technology (BILTEM).

2.3. Evaluated criteria

In three different periods, on the leaves just before the removal, the chlorophyll content (SPAD) was determined with the chlorophyll meter (Konica Minolta SPAD-502 Plus Brand Chlorophyll Meter model) and the anthocyanin content (ACI) with the anthocyanin meter (Opti Science ACM-200 Plus Anthocyanin Meter model). After the plants were removed, the leaf area (cm^2 $plant^{-1}$) was determined using the ADC BioScientific Area Meter AM 300 model leaf area meter. Crown diameter (mm) using a digital compass was determined. Leaf fresh and dry weights were determined on a precision balance. For mineral elements, in leaf samples, extraction was prepared according to Falandysz et al. (2001). Mineral elements were determined by using the ICP-MS (ICAP-QC). The amount of proline was calculated according to the method of Bates et al. (1973) and the results were given as

nmol proline g^{-1} (fresh weight). The total phenolic amount was determined according to Singleton and Rossi (1965) by using the Folin Ciocalteu Colorimetric method and the results were given in gallic acid equivalent (mg g^{-1}). The lipid peroxidation was calculated according to Yong et al. (2008) and the calculated results were given as µmol g^{-1} in fresh weight.

2.4. Evaluation of the data

The experiment was set up with three repeats (10 plants in each repeat), two applications (0% and 1% lime application), according to the experimental design in the randomized parcels. For calculating the averages of all data obtained during the research, "Microsoft Office XP EXCEL" was used and the statistical analysis of applications was performed using a t-test, and Cohen's d effect size analysis techniques were used. The d value expressing the effect size was evaluated as 0.20-0.50 small, 0.51-0.80 medium, 0.81-1.00 large (Cohen, 1988). Interactions one ANOVA test SPSS 20.0 package. As a result of the statistical analysis, Duncan Multiple Range Test (Duncan Multiple Comparison Test) was applied by using the same package program to determine the difference between the media. The significance level between the differences in the statistical evaluation of the results was determined as 0.05.

3. Results and Discussion

3.1. Vegetative growth parameters

Vegetative growth parameters were measured in mycorrhizal strawberries exposed to lime stress. While the statistical effect of mycorrhiza and lime applications was insignificant in all three removals, the VAM × lime interactions were significant (Table 1). VAM applications were observed to have positive effects on the leaf area. In every uprooting, the leaf areas decreased with the effect of lime stress (Table 1). VAM application was detected to support the growth of leaf area in plants exposed to lime stress. In a study, of strawberries and pistachio, in which VAM was applied and which were exposed to drought stress, the leaf area has been reported to be positively affected (Borkowska, 2002; Abbaspoura et al., 2012). The thickest crown diameter was measured in mycorrhiza-applied plants (Table 1). Although a decrease in crown diameter was observed in plants exposed to lime stress, VAM applications reduced the severity of these decreases. The negative effect of lime stress on leaf weights can be seen clearly (Table 1). When the fresh weights of the leaves were examined, mycorrhiza applications were observed to increase the fresh weight of the leaves (except for the first uprooting) (Table 1). A similar effect was seen in the dry leaf weights.

The chlorophyll and anthocyanin contents obtained in three extractions are given in Table 2. VAM application on chlorophyll and anthocyanin content in the first removal was found to be insignificant. It was determined that the lime application had a small effect in terms of chlorophyll (d=0.48) and anthocyanin contents (d=0.39). The VAM × lime interaction was significant. (Table 2). In the removal made during the flowering period, while the VAM application had a moderate (d=0.52) effect on chlorophyll content, the lime application was ineffective. In anthocyanin content, while the VAM application was ineffective, the lime application showed a small effect (d=0.30). Considering the VAM × lime interaction, it was found to be significant in the chlorophyll content and insignificant in the anthocyanin content. In the final removal, when the chlorophyll content was examined, it was determined that the VAM application had a small (d=0.29) effect, while the lime application was ineffective. On the anthocyanin content, the VAM application was found to have a small (d=0.27) effect on lime application. The VAM × lime interaction was very important in both examined parameters.

In all three uprootings, the highest content of chlorophyll substances was detected in mycorrhiza-applied plants (Table 2). While there was a

Table 1. The effects of VAM on the some vegetative growth criteria of strawberry under calcareous conditions.

Application		Beginning of flowering				Flowering				Harvesting			
		LA (cm^2)	CD (mm)	FW (g)	DW (g)	LA (cm^2)	CD (mm)	FW (g)	DW (g)	LA (cm^2)	CD (mm)	FW (g)	DW (g)
Mycorrhiza	VAM	155	10.5	7.1	1.9	248	10.8	7.8	4.7	294	12.5	10.0	3.2
	N-VAM	121	9.6	6.0	1.4	200	8.5	6.3	3.9	248	11.1	10.5	3.7
$CaCO_3$	0% $CaCO_3$	148	10.8	6.2	1.6	254	10.3	7.5	4.8	293	11.9	11.4	3.7
	1% $CaCO_3$	128	9.3	6.9	1.8	195	9.1	6.6	3.8	250	11.7	9.1	3.2
VAM×$CaCO_3$	VAM×0% $CaCO_3$	162 a	11.6 a	7.6 ns	2.1a	293 a	11.2 a	8.7 a	5.4 ns	307 a	13.1 a	11.8 a	5.4 a
	VAM×1% $CaCO_3$	135 bc	9.4 b	6.5	1.7b	204 b	9.3 ab	6.4 b	3.9	278 b	11.2 b	11.0 ab	3.9 c
	N-VAM×0% $CaCO_3$	149 b	10.0 b	6.1	1.5b	215 ab	10.5 ab	7.0 b	4.1	281 ab	12.8a	11.1 a	4.1 b
	N-VAM×1% $CaCO_3$	107 c	9.2 b	5.9	1.4b	186 b	7.7 c	6.1 b	3.6	219 c	11.0 b	8.2 c	3.6 c
Significance													
VAM		Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
$CaCO_3$		Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
VAM×$CaCO_3$		*	*	Ns	*	*	*	*	Ns	*	*	*	*

LA: Leaf area, CD: Crown diameter, FW: Leaf fresh weight, DW: Leaf dry weight, VAM: Vesicular–arbuscular mycorrhiza, N-VAM: Non vesicular–arbuscular mycorrhiza, $CaCO_3$: Lime

* Significant at the $p < 0.05$ level, Ns: Not significant. Mean followed by different letters within columns differ significantly ($p < 0.05$).

Table 2. The effects of VAM on the chlorophyll and anthocyanin content of strawberries under calcareous conditions.

Application		Beginning of flowering		Flowering		Harvesting	
		Chl (SPAD)	Ant (ACI)	Chl (SPAD)	Ant (ACI)	Chl (SPAD)	Ant (ACI)
Mycorrhiza	VAM	45.2	9.8	48.5	9.6	43.2	8.3
	N-VAM	42.9	8.1	46.1	9.3	39.8	9.5
$CaCO_3$	0% $CaCO_3$	45.9	9.9	47.8	9.7	43.8	9.0
	1% $CaCO_3$	42.1	8.0	46.8	9.1	39.1	8.8
VAM×$CaCO_3$	VAM×0% $CaCO_3$	47.3 a	11.2 a	48.8 a	10.0 ns	46.8 a	10.0 a
	VAM×1% $CaCO_3$	43.0 bc	8.4 b	46.2 ab	9.1	39.7 bc	8.5 b
	N-VAM×0% $CaCO_3$	44.5 ab	8.6 b	46.8 ab	9.5	40.9 b	9.1 a
	N-VAM×1% $CaCO_3$	41.2 c	8.0 b	45.5 b	9.0	38.7 c	8.2 b
Significance							
VAM		Ns	Ns	**	Ns	*	Ns
$CaCO_3$		*	*	Ns	*	Ns	*
VAM×$CaCO_3$		*	*	*	Ns	**	**

Chl: Chlorophyll; Ant: Anthocyanin, VAM: Vesicular–arbuscular mycorrhiza, N-VAM: Non vesicular–arbuscular mycorrhiza, $CaCO_3$: Lime
* Significant at the $p < 0.05$ level, ** Significant at the $p < 0.01$ level, Ns: Not significant. Mean followed by different letters within columns differ significantly ($p < 0.05$).

decrease in chlorophyll content with lime stress, the VAM application to plants exposed to stress was observed to significantly preserve their chlorophyll content as well. In their study, Akay and Kararslan (2012) stated that mycorrhiza applications contributed to the chlorophyll content of the plants, especially in poor soils.

When the anthocyanin content in the leaf was examined, mycorrhiza applications were observed to increase the anthocyanin content (except for the second uprooting). The anthocyanin content (Farrant, 2000; Johnston et al., 2007), known for protecting the chlorophyll structure, increased with mycorrhiza application, in line with the chlorophyll content (Table 2).

3.2. Effects on some biochemical characteristics

Some biochemical parameters obtained as a result of the experiment are given in Table 3. At the beginning of flowering, the VAM application had a small effect (d=0.39) on proline content, while the lime application had no effect. When the MDA content was examined, while the VAM application was moderately effective (d=0.66), the lime application was ineffective. In total phenolic substance content, while the VAM application was ineffective, the lime application was moderately effective (d=0.76). The VAM × lime interaction was very important in the investigated parameters. When evaluated on the second removal date, the VAM application had a small (d=0.34 and d=0.35) effect on proline and MDA contents, while it had a moderate (d=0.52) effect on total phenolic substance content. The lime application had little effect on proline and total phenolic content (d=0.39 and d=0.33, respectively) while it had no effect on MDA content. The VAM × lime interaction was very significant in all criteria examined. While no effect was observed on the proline and MDA content of the VAM application during the harvesting period, the effect on the total phenolic content was large (d=0.93). While the effect of lime application on proline and total phenolic substance application was not determined, its effect on MDA content was large (d=0.88). During this uprooting period, the VAM × lime interaction was very important for the evaluated parameters.

Mycorrhiza applications were determined to have significant effects on the amount of the proline throughout the experiment (Table 3).

In the first uprooting period, the highest proline amount (50 nmol proline g^{-1}) was obtained from the mycorrhiza-applied in the control group (Table 4). In the second and third uprooting periods, the highest amount of proline was detected in the mycorrhiza-applied plants in lime stress (30 and 70 nmol proline g^{-1}, respectively). When all experimental groups were evaluated in all uprooting periods, mycorrhiza applications were observed to increase the amount of proline in both groups. Proline, the amount of which is increased in stressful environments in the plant cell (Chen and Murata, 2002; Vardharajula et al., 2011; Cetin and Daler, 2017), is one of the significant osmolites in abiotic stress conditions (Rontein et al., 2002; Aktaş and Akça, 2015). Data we obtained in our research are compatible with many studies reporting that mycorrhiza applications applied in various stress conditions increase the amount of proline in different plants (Krishna et al., 2006; Campanelli et al., 2013; Hazzoumi et al., 2015; Latef et al., 2016).

The mycorrhiza applications were observed to have significant effects on the total phenolic content throughout the experiment (Table 3). When data were examined, the phenolic contents were observed to increase depending on stress in all uprooting periods. The mycorrhiza applications were also detected to positively affect the increase in phenolic content in strawberry seedlings (Table 3).

In plants under stress conditions, phenylpropanoid biosynthesis increases by the means of the PAL enzyme, and many secondary metabolites including phenolic compounds are synthesized (Koç, 2015; Pešaković et al., 2016; Aviova et al., 2017; Cetin and Daler, 2017). There are studies reporting an increase in phenolic compounds when mycorrhiza-applied strawberry

Table 3. The effects of VAM on some the biochemical criteria of strawberry under calcareous conditions.

Application		Beginning of flowering			Flowering			Harvesting		
		P nmol g^{-1}	MDA µmol g^{-1}	TP GAEmg g^{-1}	P nmol g^{-1}	MDA µmol g^{-1}	TP GAEmg g^{-1}	P nmol g^{-1}	MDA µmol g^{-1}	TP GAEmg g^{-1}
Mycorrhiza	VAM	40.0	0.67	3.4	30.0	1.2	5.3	60.0	2.4	4.1
	N-VAM	30.0	1.6	3.4	20.0	2.1	3.4	50.0	3.7	3.6
$CaCO_3$	0% $CaCO_3$	30.0	1.1	3.1	20.0	2.0	3.6	60.0	0.75	3.9
	1% $CaCO_3$	30.0	1.2	3.8	30.0	1.3	5.1	60.0	5.4	3.9
VAM×$CaCO_3$	VAM×0% $CaCO_3$	50.0 a	0.53 b	3.0 b	20.0 b	1.2 b	5.0 b	60.0 ab	0.75 c	4.2 a
	VAM×1% $CaCO_3$	30.0 b	0.98 ab	3.9 a	30.0 a	1.5 b	5.6 a	70.0 a	0.75 c	4.1 a
	N-VAM×0% $CaCO_3$	20.0 b	1.4 a	3.1 b	20.0 b	1.5 b	2.2 d	60.0 ab	4.1 b	3.6 b
	N-VAM×1% $CaCO_3$	30.0 b	1.4 a	3.7 a	20.0 b	2.7 a	4.6 c	40.0 b	6. 7 a	3.7 b
Significance										
VAM		*	**	Ns	*	*	**	Ns	Ns	***
$CaCO_3$		Ns	Ns	**	*	Ns	*	Ns	***	Ns
VAM×$CaCO_3$		**	*	**	**	**	**	**	**	*

P: Proline, MDA: Malondialdehyde, TP: Total phenolic, VAM: Vesicular–arbuscular mycorrhiza, N-VAM: Non vesicular–arbuscular mycorrhiza, $CaCO_3$: Lime
* Significant at the p < 0.05 level, ** Significant at the p < 0.01 level, *** Significant at the p < 0.001 level, Ns: Not significant. Mean followed by different letters within columns differ significantly (p < 0.05).

Table 4. The effects of VAM on some mineral element content of strawberry under calcareous conditions in leaves.

Application		Beginning of flowering					Flowering					Harvesting				
		Ca (%)	P (%)	Mg (%)	Fe (ppm)	Zn (ppm)	Ca (%)	P (%)	Mg (%)	Fe (ppm)	Zn (ppm)	Ca (%)	P (%)	Mg (%)	Fe (ppm)	Zn (ppm)
Mycorrhiza	VAM	2.8	0.11	0.66	300	12.6	2.4	0.16	0.46	224	21.1	2.5	0.12	0.41	316	25.7
	Non VAM	2.7	0.03	0.55	245	9.3	2.3	0.12	0.47	224	20.3	2.5	0.09	0.43	262	14.0
$CaCO_3$	0% $CaCO_3$	2.2	0.08	0.61	320	12.9	2.2	0.20	0.49	244	27.2	2.2	0.16	0.41	314	24.0
	1% $CaCO_3$	3.3	0.06	0.60	225	9.0	2.4	0.08	0.44	205	14.2	2.8	0.05	0.43	265	15.7
VAM×$CaCO_3$	VAM×0% $CaCO_3$	2.5 c	0.16 a	0.74 a	340 a	13.8 a	2.5 a	0.23 a	0.49 a	264 a	26.6 a	2.0 d	0.18 a	0.46 a	358 a	29.6 a
	VAM×1% $CaCO_3$	3.0 b	0.04 b	0.58 c	259 c	11.3 b	2.3 b	0.09 c	0.43 b	222 b	15.6 b	2.9 a	0.07 c	0.35 c	269 b	21.9 b
	N-VAM×0% $CaCO_3$	1.8 d	0.08 b	0.63 b	299 b	11.9 b	2.0 c	0.17 b	0.50 a	226 b	27.8 a	2.4 c	0.15 b	0.46 a	275 b	18.5 c
	N-VAM×1% $CaCO_3$	3.5 a	0.02 b	0.48 d	191 d	6.7c	2.5 a	0.07 d	0.44 b	187 c	12.8 c	2.7 b	0.03 d	0.40 b	255 b	9.5 d
Significance																
VAM		Ns	**	Ns	Ns	*	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	*	**
$CaCO_3$		**	Ns	Ns	**	**	Ns	***	***	**	***	**	***	Ns	*	*
VAM×$CaCO_3$		**	**	**	**	**	**	**	**	**	**	**	**	**	**	**

Ca:Calcium, P:Phosphate, Mg:Magnesium, Fe:Iron, Zn:Zinc, VAM:Vesicular–arbuscular mycorrhiza, N-VAM:Non vesicular–arbuscular mycorrhiza, $CaCO_3$:Lime
* Significant at the p < 0.05 level, ** Significant at the p < 0.01 level, *** Significant at the p < 0.001 level, Ns: Not significant. Mean followed by different letters within columns differ significantly (p < 0.05).

seedlings are exposed to different stresses (Koç, 2015).

The mycorrhiza application in lime stress was detected to have a significant effect on the total MDA content throughout the experiment (Table 3). When data were examined, while the amount of MDA increased in stress conditions, mycorrhiza applications were observed to decrease significantly the amount of the MDA content. When plants are exposed to stress, first of all, the effects of malondialdehyde (MDA), one of the end products of lipid peroxidation, on membranes are clearly observed (Hodges et al., 1999), and it is known that MDA content increases when exposed to stress in almost all plants (Krupa et al., 1986; Quarti et al., 1997; Nouairi et al., 2006; Zhang et al., 2008; Büyük et al., 2012; Yekbun and Kabay, 2017). As in our research, there are many studies in which mycorrhiza applications reduce the MDA content and preserve the membrane permeability (Baozhong et al., 2010; Koç, 2015; Moradtalab et al., 2019).

3.3. Some nutrient element contents

Many factors affect the obtainability of nutrients in the soil and their usefulness to the plant. Soil reaction is one of the most important of these factors (Yakupoğlu et al., 2010). Leaf mineral element contents are given in Table 4. In the first removal, the VAM application had a moderate (d=0.59) effect on P content and a small (d=0.36) effect on Zn content. The lime application had a moderate effect on Ca, Fe, and Zn contents (d=0.77, d= 0.72, and d=0.52, respectively). VAM application had no effect on the mineral element content in the uprooting during the flowering period. The lime application had a large effect on P, Mg, and Zn contents (d=0.89, d=0.92, and d=0.96, respectively) and moderately effect on Fe content (d=0.53). In the final removal, the VAM application had a small effect on Fe content (d=0.44) and moderately effect on Zn content (d=0.33). The lime application had a medium effect on Ca content (d=0.74), large effect on P content (d=0.90) and a small effect on Fe and Zn content (d=0.36 and 0.33). VAM × lime interaction was very important in leaf element contents in all removals.

In the experiment, the Ca content in the leaves was found to be in the range of 1.83-3.46% at beginning of flowering, 2.47-2.03% in flowering, and 2.94-1.98% in harvesting (Table 4). The Ca content of the leaves increased with the increase in the air

Table 5. The effects of VAM on some mineral element content of strawberry under calcareous conditions in the crown.

Application		Beginning of flowering					Flowering					Harvesting				
		Ca (%)	P (%)	Mg (%)	Fe (ppm)	Zn (ppm)	Ca (%)	P (%)	Mg (%)	Fe (ppm)	Zn (ppm)	Ca (%)	P (%)	Mg (%)	Fe (ppm)	Zn (ppm)
Mycorrhiza	VAM	2.2	0.20	0.59	718	23.6	1.6	0.44	0.27	726	43.6	2.5	0.11	0.44	639	47.6
	Non VAM	2.0	0.15	0.49	355	23.0	1.6	0.16	0.22	634	36.7	2.1	0.11	0.36	530	20.9
$CaCO_3$	0% $CaCO_3$	1.5	0.17	0.50	760	23.8	1.2	0.42	0.31	1057	44.7	2.0	0.13	0.40	846	48.3
	1% $CaCO_3$	2.7	0.19	0.58	313	22.9	2.0	0.19	0.19	302	35.7	2.6	0.08	0.40	323	20.2
VAM×$CaCO_3$	VAM×0% $CaCO_3$	1.6 c	0.20 a	0.55 b	1043 a	24.2 a	1.3 c	0.63 a	0.29 d	1064 a	63.5 a	2.0 d	0.14 a	0.40 b	896 a	67.3 a
	VAM×1% $CaCO_3$	2.8 a	0.20 a	0.63 a	393 c	23.1 a	2.0 a	0.25 b	0.33 a	389 b	42.6 b	2.2 a	0.08 b	0.48 a	381 c	28.0 b
	N-VAM×0% $CaCO_3$	1.4 d	0.18 b	0.45 d	477 b	23.4 a	1.2 d	0.21 c	0.32 b	1051 a	44.7 b	2.0 d	0.13 a	0.40 b	795 b	29.3 b
	N-VAM×1% $CaCO_3$	2.6 b	0.13 c	0.53 c	232 d	22.6 b	1.9 b	0.12 d	0.30 c	216c	28.8 c	2.2 a	0.08 b	0.33 c	264 d	12.5 c
Significance																
VAM		Ns	*	**	*	Ns	Ns	**	Ns	Ns	*	Ns	Ns	**	Ns	*
$CaCO_3$		***	Ns	*	**	Ns	***	*	Ns	***	**	**	***	Ns	***	*
VAM×$CaCO_3$		**	**	**	**	**	**	**	**	**	**	**	**	**	**	**

Ca: Calcium, P: Phosphate, Mg: Magnesium, Fe: Iron, Zn: Zinc, VAM: Vesicular–arbuscular mycorrhiza, N-VAM: Non vesicular–arbuscular mycorrhiza, $CaCO_3$: Lime

* Significant at the $p < 0.05$ level, ** Significant at the $p < 0.01$ level, *** Significant at the $p < 0.001$ level, Ns: Not significant. Mean followed by different letters within columns differ significantly ($p < 0.05$).

temperature. Daugaard (2001) reported that the amount of Ca accumulated in the leaves increased with the increase in the air temperature. The Ca content in the strawberry leaves in the range of 0.20-1.50% was considered sufficient (May and Pritss, 1990).

In the present study, the P content in the leaves was found to be in the range of 0.02-1.14% at beginning of flowering, 0.07-0.23% in flowering, and 0.03-0.18% in harvesting (Table 4). To get sufficient efficiency in strawberry cultivation, the amount of P in the leaf to be 0.25-0.40% has been reported to be enough (May and Pritts, 1990). The VAM-applied plants were seen to be in or close to this range. It was observed that the addition of lime to the growing medium decreased the P uptake, whereas the application of mycorrhiza alleviated the effect of this decrease.

Looking at the Mg content of the leaves, it was found to be in the range of 0.74%-0.48% at beginning of flowering, 0.50-0.43% in flowering, and 0.46-0.35% in harvesting. May and Pritts (1990) stated that Mg content between the range of 0.20-0.50% was sufficient (Table 4). It is seen that Mg decreased with the addition of lime to the growth medium and that Mg intake increased with mycorrhiza application (Table 4).

We determined in our study that Fe in the leaves changed in the range of 340.15-190.50 ppm at beginning of flowering, 260.35-187.1 ppm in flowering, and 358.21-254.76 ppm in harvesting. The Fe content in strawberry leaves has been reported to change in the range of 70-1383.20 ppm (May and Pritts, 1990; Stanisavljevic et al., 1997). It was detected that the addition of lime to the growing medium decreased the Fe uptake, whereas the application of mycorrhiza alleviated the effect of this decrease.

Throughout our experiment, the Zn content of the leaves changed in the range of 13.83-6.71 ppm at beginning of flowering, 26.64-12.81 ppm in flowering, and 29.55-9.53 ppm in harvesting (Table 4). Ersoy and Demirsoy (2006) stated that the Zn content of the leaves was in the range of 22.9–54.9 ppm. May and Pritts (1990) determined that the Zn content of the leaves in the range of 20-50 ppm was sufficient for normal development and growth in strawberries. It decreased lime stress, mycorrhiza applications increased Zn intake.

The mineral element contents of the crown obtained during the experiment are given in Table 5. It was determined that the VAM application had a small (d=0.21) effect on the P content of the crown, medium (d=0.60) on the Mg content, and a small (d=0.36) effect on the Fe content in the crown during the uprooting before flowering. It was determined that the lime application had a large (d=0.98) effect on the Ca content of the crown, a small (d=0.39) effect on the Mg content, and a moderate (d=0.54) effect on the Fe content. In the second extraction, the VAM application had a moderate (d=0.51) effect on P content and a small (d=0.43) effect on Zn content. The lime application, on the other hand, had a large (d=0.99 and 0.97) effect on Ca and Fe content, moderate (d=0.55) on Zn content, and small (d=0.35) on P content. In the harvesting period, the VAM application had a medium (d=0.54) effect on the Mg content and a small (d=0.48) effect on the Zn content. Lime application, on the other hand, affected the Ca content at a moderate (d=0.53), P and Fe content at a large level (d=0.99 and d=0.96, respectively), and the Zn content at a small level (d=0.48). The VAM × lime interaction is essential in terms of crown mineral content in all dismantling.

In our study, the Ca content of the crown was seen to be on the level of 2.79-1.35% at beginning of flowering, 1.98-1.15% in flowering, and 2.20-1.96% in harvesting (Table 5). The Ca content of the crown was observed to be relatively low in the uprootings made during the blooming period, compared to other uprootings. The reason for this has been thought to be Ca transferring to other organs (particularly flowers) in this period (Ersoy and Demirsoy, 2006). Throughout our experiment, the P content of the crown was seen to be on the level of 0.20-0.13% at beginning of flowering, 0.63-0.12% in flowering, and 0.14-0.08% in harvesting

(Table 5). It has been stated in the studies conducted on strawberries that P content on the level of 0.21-0.35% in the crown is sufficient (Ersoy and Demirsoy, 2006; Demirsoy et al., 2010; Demirsoy et al., 2012). When data were examined, the P content of the crown was detected to be mildly low in the harvesting period. It is known that towards the end of development, plants take relatively less P from the soil and transfer the P that they absorb at the beginning of development to the fruit (Kacar, 2012).

Looking at the Mg content of the crown, it was determined as 0.63-0.45% at beginning of flowering, 0.33-0.29% in flowering, and 0.48-0.33% in harvesting (Table 5). In studies conducted on strawberries, the amount of Mg in the crown has changed in the range of 0.09% and 0.19% (Stanisavljevic et al., 1997; Demirsoy et al., 2010).

In our study, the Fe contents in the strawberry crown changed in the range of 1043.14-232.42 ppm at beginning of flowering, 1063.63-216.36 ppm in flowering, and 896.28-264.12 ppm in harvesting (Table 5). In the study of Ersoy and Demirsoy (2006), the Fe content of the strawberry crown was reported to change in the range of 408.3-2362.3 ppm. May et al. (1994) in their study conducted in New York determined the Fe content of the crown in the Earlyglow strawberry kind as approximately 300-1300 ppm.

Throughout our research, the Zn content of the strawberry crown was in the range of 24.15-22.57 ppm at beginning of flowering, 63.50-28.81 ppm in flowering, and 67.26-12.48 ppm in harvesting (Table 5). In a previous study, the Zn content was determined as 225.6-48 ppm in the crown throughout the experiment period (Ersoy and Demirsoy, 2006). May et al. (1994) have determined the Zn content of the crown in the range of 160-250 ppm.

The root mineral substance contents obtained in our study are given in Table 6. It was determined that VAM application had a moderate effect on P content (d=0.67) in the root, large and small effects on Mg and Fe content (d=0.98 and d=0.96, respectively) (d=0.37) in the root during flowering, and a moderate effect on Ca content (d=0.58). The lime application, on the other hand, had a moderate (d=0.53 and d=0.78) effect on Mg and Zn contents, and a small (d=0.48) effect on Fe content. In the final removal, the VAM application had a moderate (d=0.68) effect on the Zn content. The lime application had a large (d=0.97 and d=0.91, respectively) effect on Ca and Fe content. During the experiment, the VAM × lime interaction significantly affected the root mineral element contents.

While the content of Ca in roots in our experiment was seen between the range of 2.69-1.44% at beginning of flowering, 2.04-1.73% in flowering, and 3.06-1.53% in harvesting (Table 6). The amount of Ca decreased in the blooming period, similarly to the crown.

In the experiment, the P content changed in the range of 0.07-0.02% at beginning of flowering, 0.10-0.03% in flowering, and 0.08-0.05% in harvesting (Table 6). While Demirsoy et al. (2010) have determined the P content of the root as 0.33-0.22%, Stanisavljevic et al. (1997) have determined it as 0.09%. As the pH of the growth medium increases, P intake decreases (Hazelton and Murphy, 2007), and also mycorrhiza supports the intake of P (Giri et al., 2003; Tüfenkci et al., 2006; Sönmez et al., 2013).

The Mg content obtained in the uprootings performed at different times throughout the experiment is seen in Table 6. The Mg content in the roots of strawberry seedlings was determined in the range of 0.93-0.37% at beginning of flowering, 0.72-0.51% in flowering, and 0.72-0.64% in harvesting (Table 6). When studies conducted on strawberries were analyzed, the Mg content in the root has been in the range of 0.15-0.23% (Stanisavljevic et al., 1997; Ersoy and Demirsoy, 2006; Demirsoy et al., 2012).

Table 6. The effects of VAM on some mineral element content of strawberry under calcareous conditions in the root.

Application		Beginning of flowering					Flowering					Harvesting				
		Ca (%)	P (%)	Mg (%)	Fe (ppm)	Zn (ppm)	Ca (%)	P (%)	Mg (%)	Fe (ppm)	Zn (ppm)	Ca (%)	P (%)	Mg (%)	Fe (ppm)	Zn (ppm)
Mycorrhiza	VAM	2.4	0.05	0.89	3034	63.2	1.8	0.08	0.62	5218	48.9	2.3	0.07	0.68	5094	66.2
	Non VAM	2.0	0.02	0.39	1911	53.1	2.0	0.06	0.54	2814	40.2	2.2	0.07	0.66	4411	52.2
$CaCO_3$	0% $CaCO_3$	1.7	0.05	0.67	3118	64.0	1.9	0.06	0.64	5558	52.5	1.6	0.06	0.69	6388	63.1
	1% $CaCO_3$	2.6	0.03	0.60	1827	52.4	2.0	0.08	0.52	2474	36.5	2.9	0.07	0.65	3117	55.4
VAM×$CaCO_3$	VAM×0% $CaCO_3$	2.0 c	0.07 a	0.93 a	4192 a	66.2 a	1.7 d	0.10 a	0.72 a	7809 a	54.3 a	1.5 d	0.05	0.72 a	6467 a	72.5 a
	VAM×1% $CaCO_3$	2.7 a	0.04 b	0.84 b	1876 c	60.3 b	1.9 c	0.07 a	0.52 c	2627 c	43.4 c	3.1 a	0.08	0.64 c	3721 b	60.0 b
	N-VAM×0% $CaCO_3$	1.4 d	0.02 c	0.41 c	2045 b	61.8 b	2.0 b	0.03 b	0.56 b	3307 b	50.6 b	1.6 c	0.08	0.66 b	6310 a	53.7 c
	N-VAM×1% $CaCO_3$	2.6 b	0.02 c	0.37 d	1778 d	44.4 c	2.0 a	0.09 a	0.51 c	2321 d	29.7 d	2.7 b	0.06	0.67 b	2513 c	50.7 c
Significance																
VAM		Ns	**	***	***	*	**	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	**
$CaCO_3$		***	Ns	Ns	*	*	Ns	Ns	**	*	**	***	Ns	Ns	***	Ns
VAM×$CaCO_3$		**	**	**	**	**	**	**	**	**	**	**	Ns	**	**	**

Ca: Calcium, P: Phosphate, Mg: Magnesium, Fe: Iron, Zn: Zinc, VAM: Vesicular–arbuscular mycorrhiza, N-VAM: Non vesicular–arbuscular mycorrhiza, $CaCO_3$: Lime

* Significant at the $p < 0.05$ level, ** Significant at the $p < 0.01$ level, *** Significant at the $p < 0.001$ level, Ns: Not significant. Mean followed by different letters within columns differ significantly ($p < 0.05$).

The Fe content of the strawberry roots was determined in the range of 4191.71-1777.45 ppm at beginning of flowering, 7808.60-2321.20 ppm in flowering, and 6467.10-2512.95 ppm in harvesting (Table 6). In the study of Ersoy and Demirsoy (2006), the Fe content of the root changed in the range of 987.10–2643.30 ppm. In their study, May et al. (1994) determined the Fe content of the root in strawberries in the range of 900-2700 ppm. It is known that, as the pH of the environment increases, the plants' Fe intake decreases (Hazelton and Murphy, 2007). While Fe intake decreases with lime stress, mycorrhiza application was observed to increase the Fe intake.

The Zn content of the strawberry roots was determined in the range of 66.21-44.36 ppm at beginning of flowering, 54.39-29.71 ppm in flowering, and 72.45-50.70 ppm in harvesting (Table 6). In another study, the Zn content of the root changed in the range of 45.9–160.2 ppm (Ersoy and Demirsoy, 2006). May et al. (1994) determined in their study that the Zn content of the roots of the strawberry is in the range of approximately 110-140 ppm.

In all uprootings, the Ca content in the plant parts was observed to increase in direct proportion with the addition of Ca in the growth medium in the pots. Mg uptake is the highest at pH 7-8.5 (Kacar, 2012), therefore, since the pH range of our growth media was within these levels in our experiment, it is thought that the Mg content was higher than that of other studies.

The mycorrhiza applications were determined to significantly affect iron intake in lime stress. Significant differences were found between the applications in terms of the Fe content of the leaf throughout the experiment period. While lime stress decreases the Fe intake in the leaves, crowns, and roots, mycorrhiza applications were detected to increase the Fe intake (Table 4, 5, 6). The effect of mycorrhiza applications in lime stress on the Zn content of the leaves, crowns, and roots was significant (Table 4, 5, 6). While the intake of Zn, which is one of the microelements whose intake by plants is affected by the pH of the growth medium (Hazelton and Murphy, 2007). Many studies have revealed that mycorrhizal applications support the growth and development of the plant by supporting the intake of mineral elements in plants growing in stress conditions (Medeiros et al., 1994; Almaca, 2014; Latef et al., 2016).

4. Conclusion

Strawberries are cultivated in many parts of the world due to their adaptation ability. The most important factor in the increasing importance of strawberry in the world and in Türkiye, especially in recent years, is the breeding of varieties suitable for different climatic and soil conditions. However, despite the newly developed varieties, soil pH causes significant problems in strawberry cultivation. Mycorrhizal fungi in the soil play an active role in plant growth and use of nutrients in the environment. When the data obtained from our experiment were examined, it was determined that the amount of chlorophyll and leaf area decreased with the application of lime and increased to the same values as the plants in normal growing conditions with the application of VAM to the growing medium with lime. This situation was also detected in Fe intake. In addition, while mycorrhiza application to strawberry seedlings exposed to lime stress increased proline and total phenolic contents, it decreased MDA content. The use of mycorrhiza can be recommended to reduce the negative effects of strawberry cultivation in calcareous soils far from the sea.

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