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Use of Arbuscular Mycorrhizal Fungi for Boosting Antioxidant Enzyme Metabolism and Mitigating Saline Stress in Sweet Basil (*Ocimum basilicum* L.)

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Abstract: Salinity is one of the outstanding abiotic stress conditions that a significant part of the world faces. In recent years, beneficial microorganisms started to be utilized in plants to overcome several abiotic factors, including salinity. The effects of arbuscular mycorrhizal fungi (AMF) mixture on growth and enzymatic responses in basil under salt stress were investigated using saline doses of 0 mM (Control), 150 mM, and 300 mM. Results showed that AMF enhanced all growth parameters, but only the leaf number was statistically significant. However, antioxidant enzymes, such as ascorbate peroxidase (APX) by 25%, catalase (CAT) by 25%, and superoxide dismutase (SOD) by 5%, significantly enhanced. At the same time, the accumulation of oxidative enzymes, like hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), was reduced, from 12.05 μmol g^{-1} fw (control) to 11.17 μmol g^{-1} fw (AMF) and from 14.29 μmol g^{-1} fw to 10.74 μmol g^{-1} fw, respectively. AMF also significantly alleviated the chlorophyll loss caused by increasing saline doses. Multivariate analyses revealed the co-occurrence of stress metabolism enzymes as well as the proximate effect of AMF inoculation on basil yield and enzymatic activity. As a result, AMF was considered an appropriate tool for increasing growth and reducing salt stress under both stress-free and saline conditions.

Keywords: oxidative stress; catalase; ascorbate peroxidase; malondialdehyde; structural equational model

Citation: Yilmaz, A.; Yildirim, E.; Yilmaz, H.; Soydemir, H.E.; Güler, E.; Ciftci, V.; Yaman, M. Use of Arbuscular Mycorrhizal Fungi for Boosting Antioxidant Enzyme Metabolism and Mitigating Saline Stress in Sweet Basil (*Ocimum basilicum* L.). *Sustainability* **2023**, *15*, 5982. https://doi.org/10.3390/su15075982

Academic Editor: Xiaowei Li

Received: 3 March 2023
Revised: 25 March 2023
Accepted: 29 March 2023
Published: 30 March 2023

1. Introduction

Modern agriculture is shaped by rigorous changes in the face of many environmental challenges resulting from the rapid growth of the world's population [1]. These changes push people to tackle the challenges of ensuring global food security and maintaining sustainability [2]. In this case, people use fertilizers as a short-term solution [3]. However, unconscious and excessive use of fertilizers (chemical and farm manures) also causes soil salinity, one of the biggest obstacles to agricultural productivity and sustainability, which remains one of today's biggest global agrarian problems [4]. The quickly expanding salinity stress in cultivation areas poses a significant peril to crop yields [2]. Salinity stress in plants is one of the most important abiotic factors limiting agricultural productivity worldwide [5]. It reduces photosynthetic capacity because it causes stoma osmotic pressure and fragment affinity. Plants can suffer from metabolic disorders and general nutrient deficiency due to saline stress [6]. Salinity stress in plants rapidly reduces leaf area, leaf dry matter, and the root and stem biomasses due to indirect effects in osmotic imbalance or direct effects of ion toxicity due to an increase in saline ions between plants and soil [7].

Chemical fertilizers can quickly increase the yield of plants. However, with continuous use, pH and organic matter decrease, and the soil structure becomes hardened; this leads to a reduction in yield in the long term and many biological and environmental problems. In contrast, bioinoculants such as AMF are sustainable and reliable. AMFs are noticeable agents enhancing phytonutrient uptake in a rational economic manner [8]. AMF is a promising technology for researching the plant-soil-atmosphere continuum [9]. In symbiotic systems, AMF has many functions, such as enhancing disease and drought resistance, metabolic and physiological activities of the root system, tolerance to heavy metals, promoting plant growth, and changing the community structure and rhizosphere microbial diversity [10]. AMF improves water regimes and soil water nutrients and increases the tolerance of a plant to temperature extremes and pathogens [11]. AMF increases photosynthesis by colonizing roots and causes changes in the hormonal profiles of plants [12]. AMF species are quite different in terms of their effects on soil biota from other soil management processes that positively impact quality and yield [9]; the greater the number of species in the AMF community, the greater the effect on plant growth [13]. AMF inoculations have generally resulted in increased roots of the host plants and biomasses of aerial parts [1]. Many publications have shown that AMF can enhance the saline tolerance of various plants. Some positive physiological changes occurred in arbuscular mycorrhizal symbiosis under saline stress [14–17].

Basil (*Ocimum basilicum* L.), a significant medicinal plant and fresh vegetable, is widely cultivated in warm regions of Asia, Africa, and the Mediterranean basin [12]. It is used to flavor foods, marinades, cheese, sauces, and meat products and is applied in traditional medicines [18]. This annual plant belongs to the *Lamiaceae* family and constitutes a component of the Mediterranean diet [19–21]. Basil benefits human health through its anti-inflammatory, antiviral, antifungal, antibacterial, and relaxant properties [12].

Although the effects of AMF and salinity stress on many plants have been investigated in detail, there are no studies on the interaction of these two on basil. Therefore, this study determined the growth, biomass production, and antioxidant enzyme activities of basil treated with AMF under different salinity levels for the first time. The study hypothesized that with increasing salinity levels, AMF would create a solution for suppressed growth and enzymatic activities.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

One of the Turkish registered varieties, Dino, was used as plant material purchased from the AG TOHUM® seed company (Antalya, Turkey). This research was carried out at the Department of Field Crops, Faculty of Agriculture, Bolu Abant Izzet Baysal University. Seeds were sowed in pots and grown under controlled climate room conditions. Each pot included a single plant. Plants were harvested after the 45-day growing period. The photoperiod was 16:8 light and dark. The temperature was kept at 25 °C during the night and day, with a relative humidity of 75% and a light intensity of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

2.2. Saline and Mycorrhiza Applications

Sodium chloride (NaCl) was utilized for salinity. The salt used for the stress conditions was obtained from Sigma-Aldrich (Darmstadt, Germany), with a purity of 99.5%. Saline stress of 0, 150, and 300 mM was applied to the plants two times for ten days intervals with irrigation water after the one-month growing period [22]. The Endo Roots Soluble (ERS) mixture package used in the experiment containing nine different arbuscular mycorrhizal fungi was obtained from Bioglobal® company (Antalya, Turkey) in powder form. The guaranteed total living organism presence of the fungi in the package is 23.5%. The package includes *Rhizophagus irregularis* (21%), *Glomus aggregatum* (20%), *Funneliformis mosseae* (20%), *Gigaspora margarita* (1%), *Glomus monosporum* (1%), *Glomus etunicatum* (1%), *Glomus deserticola* (1%), *Glomus clarum* (1%), and *Paraglomus brasilianum* (1%) fungi. AMF application was made with a solution prepared by mixing 25 g of ERS with 1 L of distilled

water. The solution consisted of about 250,000 spores. AMF was applied to the seedling beds of the 10-day-old seedlings in viols just before planting in pots. 4 mL of the AMF solution was inoculated with approximately 1000 spores in each pot, and the planting process was carried out quickly without wasting time [12].

2.3. Physical Analyses and Chlorophyll Contents

As a physical analysis, the height (cm) of each plant in the experiment was measured, and fresh herb (g plant^{-1}), dry herb (g plant^{-1}), fresh leaf (g plant^{-1}), and dry leaves (g plant^{-1}) were weighed in the precision balance. Dry weights were determined by drying weighted plant parts under shade for two weeks and weighing them.

Chlorophyll content measurements were made from the middle leaves of the plant with the 'spad' unit using the 'Apogee MC 100 Chlorophyll Concentration Meter' device [6].

2.4. Enzyme Analyses

For the enzyme analysis, three grams of leaves obtained from the middle of the plant were used for each replication.

2.4.1. Malondialdehyde (MDA) Analysis

The range of MDA was used to determine lipid peroxidation. 0.5 g of the leaf was placed in 10 mL of trichloroacetic acid (TCA, 0.1%) solution to determine the MDA content. For 5 min, the suspension was centrifuged at 15,000× *g*. The supernatant was then supplemented with 4 mL of 0.5% thiobarbituric acid (TBA) dissolved in 20% TCA to make a total volume of 5 mL. After 30 min of incubation at 95 °C, the mixture was rapidly cooled in an ice bath. The clear portion of the solution after centrifuging at 10,000× *g* for 10 min was measured between 532 and 600 nm wavelengths. The MDA content was calculated by the coefficient reported by Sairam and Saxena [23].

2.4.2. Hydrogen Peroxide (H_2O_2) Analysis

Leaf samples weighing 0.25 g were homogenized in 2.5 mL of 1% (*w*/*v*) TCA. The homogenate was then centrifuged for 15 min at 12,000 g. Following centrifugation, 1 mL of 10 mM KH_2PO_4 (pH = 7) phosphate buffer and 1 mL of 1 M potassium iodide were added to 0.5 ml supernatant. The absorbance of the resulting mixture was measured at 390 nm. The amount of H_2O_2 was then determined using the standard curve obtained from 50, 100, 200, 300, 400, 500, and 700 µL of 163 µM H_2O_2 stock solutions absorbance. Each tube received 1 mL of 1 M potassium iodide. After that, the final volumes were increased to 2 mL with 10 mM phosphate buffer. The absorbance of the prepared tubes was measured and recorded at 390 nm [24].

2.4.3. Preparation of Plant Extracts for the Analysis of Antioxidant Enzymes

Leaf samples were kept in the −80 °C refrigerator until analysis. A frozen 1 g leaf sample was homogenized with 5 mL cold 0.1 M Na-phosphate, 0.5 mM Na-EDTA, and 1 mM ascorbic acid (pH: 7.5). The homogenate was centrifuged at 4 °C for 30 min at 18,000× *g*. Catalase (CAT) and ascorbate peroxidase (APX) were determined immediately. The same procedure was used to determine SOD activity, but after the last centrifugation, some of the homogenate extracts were kept at −20 °C [25].

2.4.4. Catalase (CAT) Analysis

CAT activity was determined by measuring the disappearance of H_2O_2 at 240 nm. The reaction solution consisted of 0.05 M phosphate buffer (KH_2PO_4) and 1.5 mM H_2O_2 (pH: 7.0). 2.5 mL of the reaction solution and 0.2 mL of plant extract were combined. In the spectrophotometer, readings at 240 nm wavelength were taken at the 0th and 60th seconds. The reaction started by adding 0.1 mL of enzyme extract, and the absorbance change was measured after 1 min [25].

2.4.5. Ascorbate Peroxidase (APX) Analysis

The reduction of ascorbic acid-bound H_2O_2 was used to measure APX activity at 290 nm. The reaction solution consisted of 50 mM phosphate buffer (KH_2PO_4), 0.5 mM ascorbic acid, 0.1 mM Na-EDTA, and 1.5 mM H_2O_2 (pH: 7.0). 3 mL of the reaction solution was mixed with 0.1 mL of plant extract. In the spectrophotometer, the 0th and 60th-second readings were recorded at 290 nm wavelength. The reaction was started with 0.1 mL of enzyme extract, and the absorbance change within 1 min was used to evaluate the reaction [26].

2.4.6. Superoxide Dismutase (SOD) Analysis

The inhibition of nitroblue tetrazolium (NBT) at 560 nm was used to determine SOD activity. The reaction solution consisted of 50 mM Na-phosphate buffer ($Na_2HPO_4 \times 2H_2O$), 0.1 mM Na-EDTA, 33 mM NBT, 5 mM riboflavin, and 13 mM methionine (pH: 7.0). 2.5 mL of the reaction solution was mixed with 0.1 or 0.2 mL of plant extract. For 10 min, the reaction was carried out at 25 °C under 75 mol m^{-2} s^{-1} (40 W) light. The control solution, which did not contain an enzyme, was kept in the dark for the same duration. The control and reaction solutions were measured at 560 nm. SOD activity was defined as the activity that reduces 50% of NBT as a unit of measurement [27].

2.5. Statistical Analysis

The study was carried out in randomized parcels of experimental design consisting of three biological and three technical replicates for each treatment. Effects of salinity stress and AMF inoculation were determined by performing a two-way analysis of variance (ANOVA). Differences between the means of control and the AMF treatment were evaluated according to the Student's *t*-test for each saline level. Correlation analysis was utilized by eliminating endogenous correlations of yield parameters and enzymes, and correlations were only performed between yield traits and enzymes/chlorophyll for each salinity level. Pearson's coefficient was used in the correlation analyses, and data were visualized by the R package 'corrplot' [28]. The relationship between salt and the AMF applications with the studied traits was determined using principal component analysis (PCA) and structural equational model (SEM) statistical approaches by R packages 'ggplot2' and 'lavaan' [29,30]. The SEM model was constructed using two formative indicators (salinity and AMF inoculation) and two reflective indicators, one of them including yield parameters (latent: Yield) and the other consisting of oxidative enzymes and chlorophyll (latent: Enzymes). Each latent variable consisted of six indicator variables (traits).

3. Results

3.1. Plant Height, Fresh Herbal, Dry Herbal, Fresh Leaf, and Dry Leaf Yields

Yield characteristics of basil were significantly affected by saline doses and AMF application, whereas saline dose × AMF interaction was insignificant. Increasing saline doses caused a notable reduction in plant height. The AMF application significantly mitigated reductive effect of salinity, particularly in the 150 mM dose. However, two-way ANOVA results indicated the benefit of the AMF regardless of the stress existence. Fresh and dry herb weights, the crucial characteristics for leaf crops, were also significantly reduced by increasing saline in the growing media. The AMF application enhanced fresh and dry herb weights in both stress- and stress-free conditions. Leaf numbers per plant was mainly affected by the AMF application, with higher numbers in inoculated plants. On the contrary, leaf numbers, both fresh and dry, and leaf weights were primarily affected by salinity intensity (Table 1).

Table 1. Plant height, fresh herbal, dry herbal, fresh leaf, and dry leaf weights (mean ± standard deviation) according to saline doses and the AMF application (n = 3).

Saline Dose		DHW (g plant^{-1})	DLW (g plant^{-1})	FHW (g plant^{-1})	FLW (g plant^{-1})	NoL (plant^{-1}) (adet plant^{-1})	PH (cm)
0 mM		1.63 ± 0.25 a	0.89 ± 0.12 a	10.78 ± 0.99 a	6.34 ± 0.94 a	13.33 ± 1.63 a	37.85 ± 2.67 a
150 mM		1.28 ± 0.14 b	0.71 ± 0.10 b	8.67 ± 0.42 b	5.38 ± 0.45 b	11.67 ± 1.51 ab	31.70 ± 3.37 b
300 mM		1.08 ± 0.14 b	0.63 ± 0.05 b	7.57 ± 0.87 c	4.92 ± 0.36 b	11.00 ± 1.10 b	27.65 ± 3.06 c
Treatment							
AMF		1.43 ± 0.32 a	0.76 ± 0.17 a	9.52 ± 1.54 a	5.69 ± 0.97 a	12.89 ± 1.45 a	34.52 ± 4.75 a
Control		1.23 ± 0.23 a	0.72 ± 0.12 a	8.49 ± 1.49 a	5.40 ± 0.76 a	11.11 ± 1.45 b	30.28 ± 4.93 a
Saline Dose × Treatment							
AMF	0 mM	1.77 ± 0.30 a	0.95 ± 0.10 a	11.41 ± 0.93 a	6.72 ± 0.96 a	14.00 ± 2.00 a	39.50 ± 2.91 a
	150 mM	1.37 ± 0.16 bc	0.71 ± 0.16 bc	9.00 ± 0.09 c	5.27 ± 0.55 bc	12.67 ± 1.15 ab	34.40 ± 2.35 b
	300 mM	1.16 ± 0.04 cd	0.64 ± 0.01 c	8.16 ± 0.31 c	5.07 ± 0.31 bc	12.00 ± 0.00 abc	29.67 ± 1.92 c
Control	0 mM	1.49 ± 0.07 ab	0.83 ± 0.13 ab	10.14 ± 0.63 b	5.96 ± 0.93 ab	12.67 ± 1.15 ab	36.20 ± 1.11 ab
	150 mM	1.20 ± 0.05 cd	0.70 ± 0.04 bc	8.34 ± 0.34 c	5.49 ± 0.41 bc	10.67 ± 1.15 bc	29.00 ± 1.00 cd
	300 mM	1.00 ± 0.16 d	0.62 ± 0.08 c	6.97 ± 0.85 d	4.76 ± 0.41 c	10.00 ± 0.00 c	25.63 ± 2.74 d
ANOVA							
F_{AMF}		7.48 *	1.01 ns	13.15 **	0.84 ns	10.67 **	17.74 **
$F_{Salinity}$		0.25 ns	0.58 ns	0.44 ns	0.87 ns	0.17 ns	0.37 ns
$F_{AMF \times Salinity}$		18.54 ***	11.41 **	43.25 ***	7.49 **	6.5 *	34.62 ***

Different letters in the same column indicate significant differences according to Student's t-test ($p \leq 0.05$). ns: not significant, *, **, and *** indicates significance at $p \leq 0.05$, 0.01, and 0.001, respectively. PH: plant height, FHW: fresh herb weight, DHW: dry herb weight, FLW: fresh leaf weight, NoL: number of leaves.

3.2. Enzymatic Responses to AMF Inoculation

Antioxidant enzymes and chlorophyll content were significantly affected by inoculation, salinity severity, and AMF × saline doses interaction (Supplementary Table S1). Among antioxidant enzymes, APX and CAT were considerably enhanced by AMF inoculation in all doses. These enzymes were almost three- and five-fold higher in 150 mM and 300 mM saline doses than in stress-free plants. On the other hand, the SOD activity was lower in AMF-inoculated saline-free plants, while there was enhanced activity in AMF-inoculated plants under saline conditions. However, SOD activity involvement was limited compared to other antioxidant enzymes.

Increasing saline stress significantly enhanced two oxidative enzymes, H_2O_2 and MDA. However, MDA involvement was considerably higher, reaching almost a 5-fold increment in 300 mM saline conditions, while H_2O_2 increased up to forty percent. AMF inoculation significantly reduced H_2O_2 activity under 300 mM saline stress. Lipid peroxidation exhibited almost a linear enhancement by increasing saline dose. AMF inoculation reduced lipid peroxidation to 30% under 150 mM and 25% under 300 mM salinity, while the reduction was 14% in stress-free plants. Moreover, lipid peroxidation reached a 4.30-fold value under 300 mM salinity in uninoculated basil plants, while the involvement was 3.70-fold in AMF-inoculated plants. The results suggested a decrease compared to the control and relative mitigation in the involvement of reactive oxygen species.

The chlorophyll content of basil leaves was statistically higher in non-inoculated plants under zero saline conditions. Under 150 mM salinity, non-inoculated plants also had higher chlorophyll, although the difference was insignificant. However, AMF-inoculated plants yielded higher chlorophyll contents under 300 mM salt stress (Figure 1).

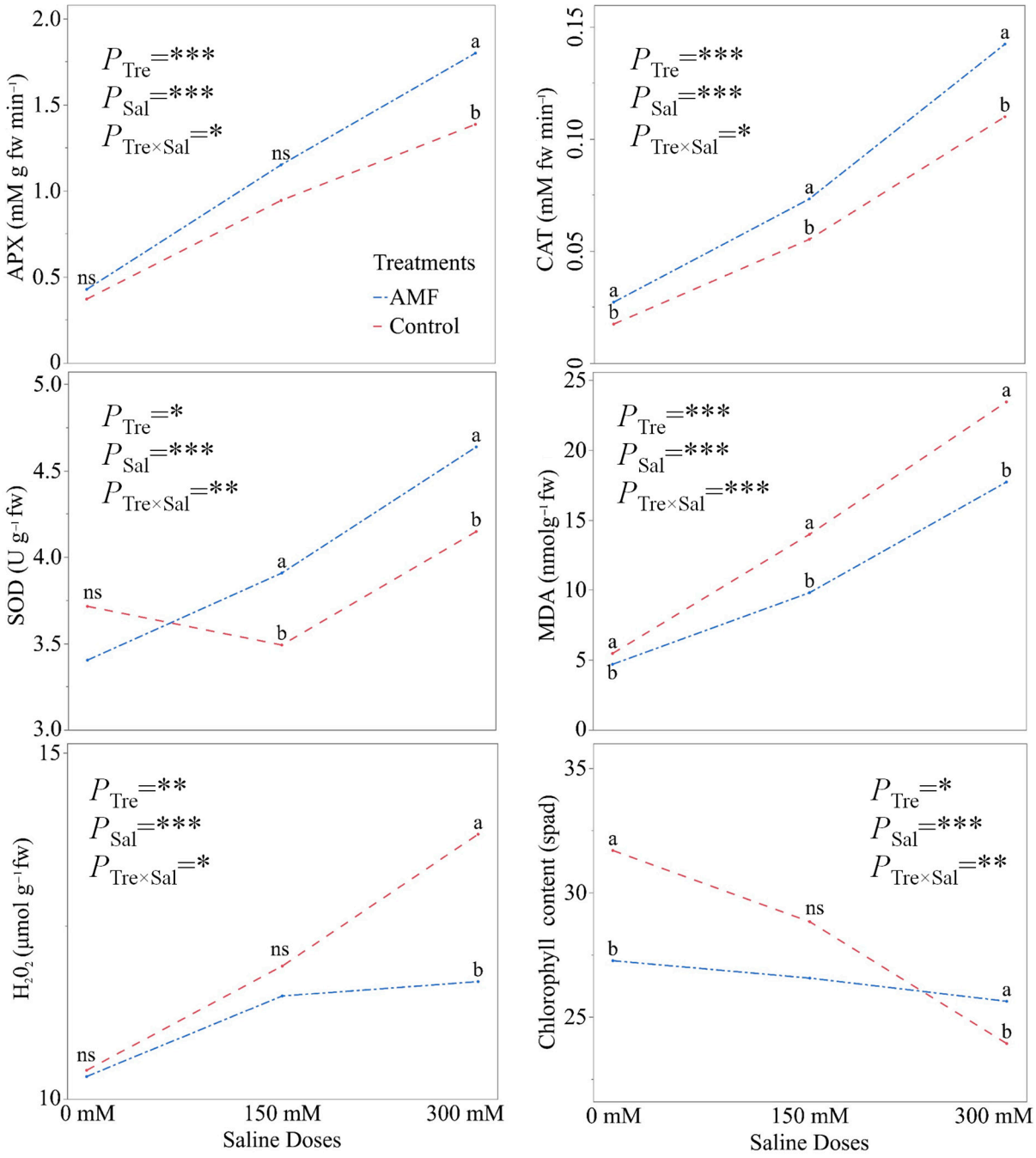

Figure 1. Oxidative stress enzymes and chlorophyll responses of basil plants under different salinity (n = 3). Different letters in the same doses indicate significant differences according to Student's t-test ($p \leq 0.05$). ns: not significant, *, **, and *** indicates significance at $p \leq 0.05$, 0.01, and 0.001, respectively. CAT: catalase, SOD: superoxide dismutase, APX: ascorbate peroxidase, MDA: malondialdehyde, H_2O_2: hydrogen peroxide, Tre: treatment, Sal: saline level, Tre × Sal: treatment-saline level interaction.

3.3. Relationships between Yield Parameters and Enzymes Affected by Saline Stress

Plant height, leaf number, fresh herb weight, and dry herb weight were positively correlated to APX and CAT at all salinity levels. Similarly, dry leaf weight was positively related to APX and CAT, with almost negligible correlations in 150 mM and 300 mM saline doses. Contrarily, fresh leaf weight exhibited moderate negative correlations with CAT under 150 mM saline stress, while the relationship was positive in other doses of salinity. H_2O_2 had weak associations with fresh and dry leaf weights at almost all saline levels. On

the other hand, plant height, leaf number, and dry herb weight possessed an exact opposite relationship with H_2O_2 under saline stress and stress-free conditions. The associations under zero salt conditions were positive, while strong negative relations were determined under 150 mM and 300 mM salt stress. MDA content was almost negatively related to yield parameters at all salinity levels except for negligible positive correlations with fresh and dry leaf weights under 150 mM saline. SOD displayed strong negative associations with all yield parameters under stress-free conditions. However, there were strong positive relationships with yield parameters under stress conditions, particularly for plant height and leaf number, except for fresh leaf weight in the 150 mM saline dose. Interestingly, chlorophyll content was negatively associated with yield parameters under stress-free and 150 mM saline conditions, while it exhibited positive correlations under 300 mM saline stress (Figure 2).

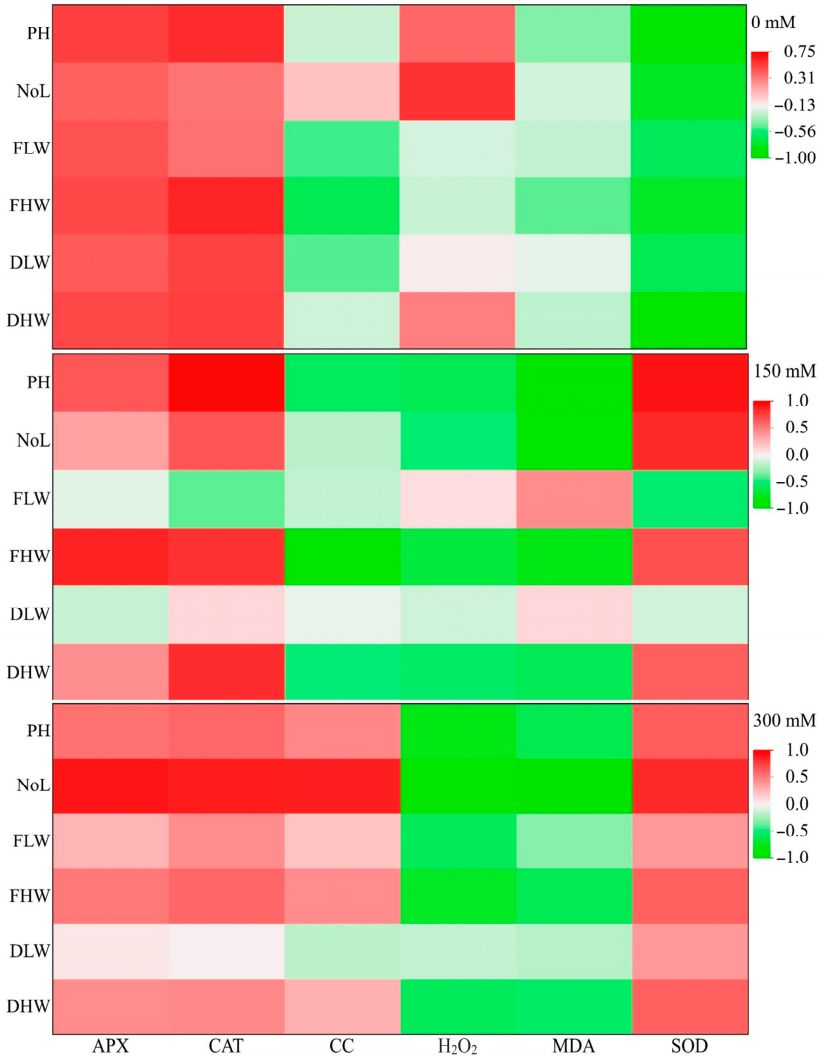

Figure 2. Heatmap illustration of correlations between yield parameters and enzymes/chlorophyl content (n = 3). CAT: catalase, SOD: superoxide dismutase, APX: ascorbate peroxidase, MDA: malondialdehyde, H_2O_2: hydrogen peroxide, PH: plant height, FHW: fresh herb weight, DHW: dry herb weight, FLW: fresh leaf weight, DLW: dry leaf weight, NoL: number of leaves, CC: chlorophyll content.

3.4. Interrelations of Yield Traits, AMF Treatment, and Salinity

PCA analysis accounted for 82% of the total variation by the first two components, 71.3% for PC1 and 10.7% for PC2. Saline doses were separated entirely into different groups

in the PCA. The plants grown under 0 mM salinity were identified by high values of plant height, dry and fresh herb weight, dry and fresh leaf weight, and chlorophyll content. The high salinity level, 300 mM, was characterized by the enhanced enzymatic response of basil plants. The moderate saline dose, 150 mM, was between them and was not represented by any trait (Figure 3a). PCA demonstrated AMF inoculation's superiority on yield traits and antioxidant enzymes, while high oxidative enzymes and chlorophyll content defined non-inoculated plants (Figure 3b).

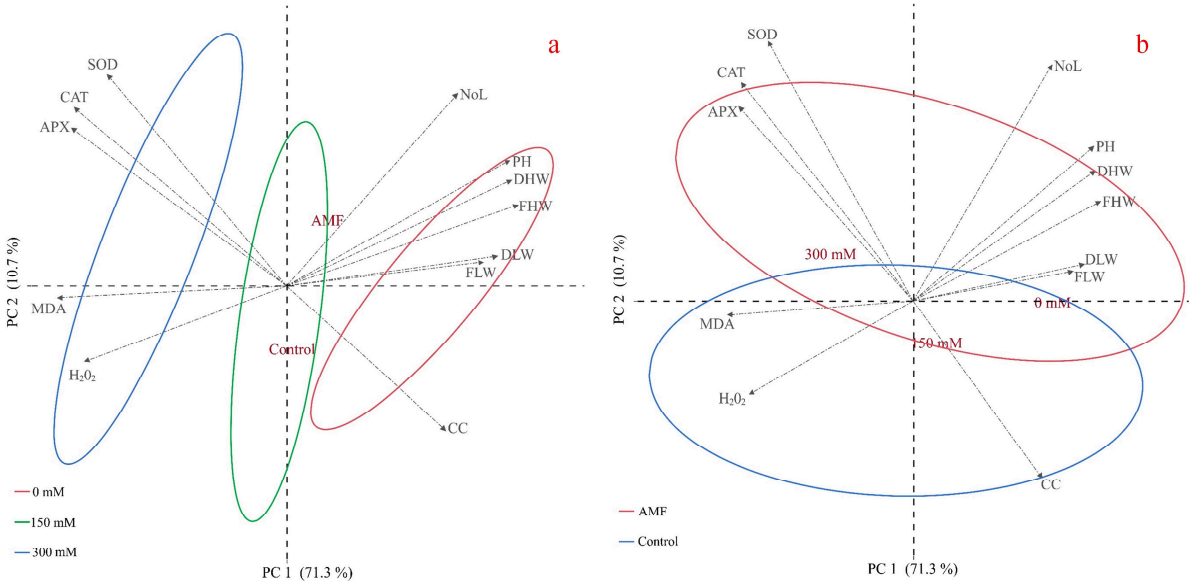

Figure 3. Distribution of yield traits according to the AMF treatment (**a**) and saline doses (**b**) on the biplot. Circles were created according to 95% confidence intervals. CAT: catalase, SOD: superoxide dismutase, APX: ascorbate peroxidase, MDA: malondialdehyde, H_2O_2: hydrogen peroxide, PH: plant height, FHW: fresh herb weight, DHW: dry herb weight, FLW: fresh leaf weight, NoL: number of leaves, CC: chlorophyll content.

Antioxidant and oxidative enzymes were grouped as enzymes in SEM (Figure 4). Variable loadings for enzymes varied from 0.84 (SOD) to 0.99 (CAT), and all enzymes had a significant effect on the latent ($p < 0.001$). The other latent, the yield, comprised five dry and fresh herb characteristics and chlorophyll. Variable loadings of the yield traits changed between 0.50 (chlorophyll) and 0.98 (plant height). Yield variables were highly influential on the latent ($p < 0.001$) besides the chlorophyll ($p < 0.05$). The enzymes and yield exhibited a robust negative relationship ($r = -0.75$). The AMF treatment and salinity positively correlated with enzymes by $cov = 1.14$ and $cov = 0.75$, respectively. The AMF treatment also had a positive association ($cov = 0.75$) with the enzymes, whereas it possessed a negative relationship with the yield ($cov = -0.29$).

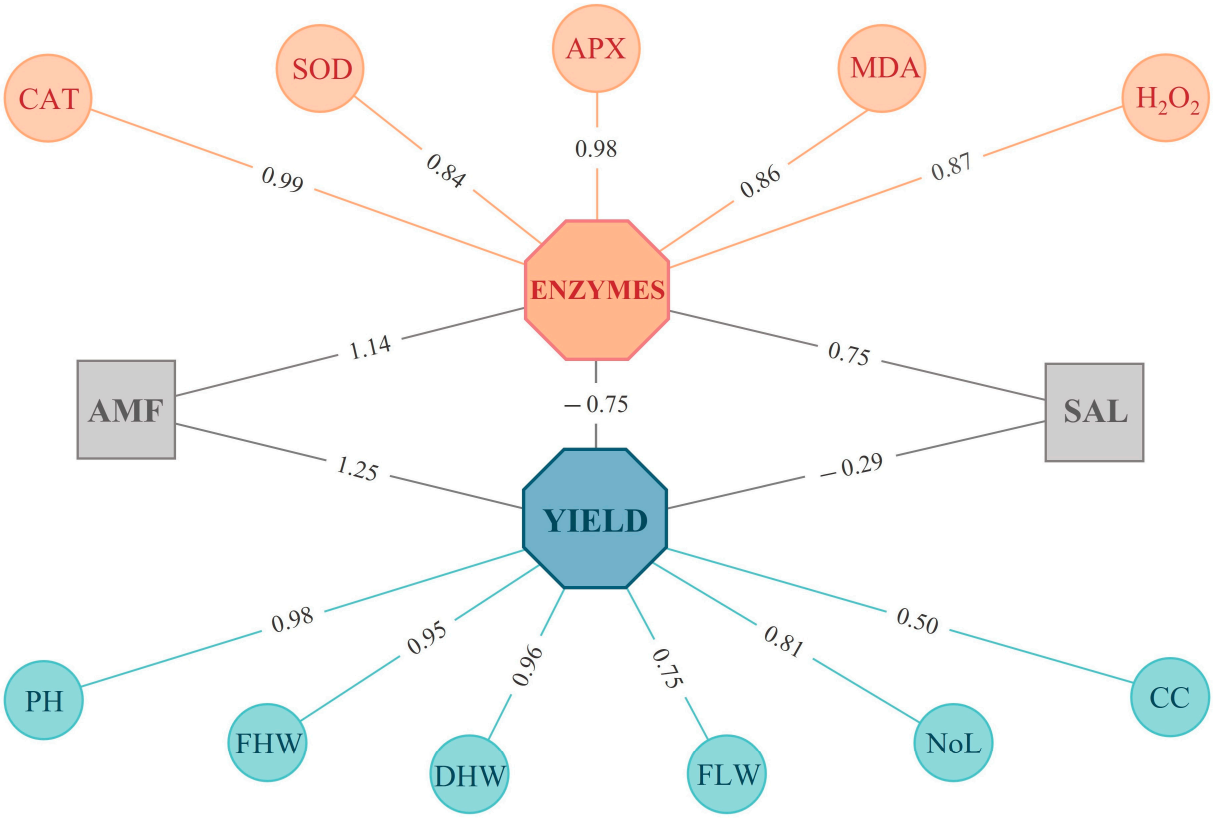

Figure 4. Path diagram illustrating the effects of the salinity and AMF inoculation on enzymes and yield parameters. Values between circles (traits) and hexagons (latents) indicate factor loadings. Values between squares (treatments) and hexagons indicate covariates. SAL: Salinity, CAT: catalase, SOD: superoxide dismutase, APX: ascorbate peroxidase, MDA: malondialdehyde, H_2O_2: hydrogen peroxide, PH: plant height, FHW: fresh herb weight, DHW: dry herb weight, FLW: fresh leaf weight, NoL: number of leaves, CC: chlorophyll content.

4. Discussion

Salt stress reduces the growth and development of plants by involving osmotic potential reduction, excessive uptake of toxic ions, and nutrient imbalance [2]. Plants are naturally colonized by some beneficial internal and external bioinoculants in their habitat, improving plants' stress tolerance; hence, the development and yield. Recently, many studies have shown the reductive effects of salinity and the AMF's salt stress ameliorative effects on plant growth in various plants such as *Valeriana officinalis* L. [31], *Phoenix dactylifera* L. [32], *Euonymus maackii* Rupr. [33], and *Zelkova serrata* (Thunb.) and *Gleditsia sinensis* Lam [34].

In this study, basil plants subjected to moderate and severe saline levels (150 mM and 300 mM) yielded lesser growth parameters than stress-free plants. The growth reduction severity increased by saline dose, in line with the mentioned researchers. AMF inoculation's effects on salinity stress alleviation also supported the previous reports. However, Li et al. [33] reported a reduced/no impact of AMF on plant growth of *Euonymus maackii* Rupr under 200 mM NaCl concentration, while Ait-El-Mokhtar et al. [32] and Amanifar and Toghranegar [31] in different plants, and Saia et al. [35] and the current study in basil showed the efficacy of AMF inoculation under all saline doses. The dissimilarities in different species should be attributed to either genetic responses differences to salinity or microorganism colonization ability on divergent roots.

Chlorophyll concentrations are significantly reduced due to the suppression of specific enzymes responsible for photosynthetic pigment synthesis under salt stress conditions [36]. The reduced intake of minerals (such as Mg) required for chlorophyll biosynthesis reduces

chlorophyll concentration in the leaf [37]. Various researchers indicated higher chlorophyll contents in the leaves of plants inoculated with mycorrhiza in saline conditions [38–40]. In our study, salt stress significantly reduced the chlorophyll content of basil leaves in the control group. The AMF inoculation preserved chlorophyll content, while there was a slight decrease in both saline levels. Furthermore, contrary to this study, some studies reported increased chlorophyll content under AMF-inoculated saline stress conditions [40,41], while some other studies reported similar chlorophyll reduction in line with salinity increment in basil [22,35]. Abeer et al. [42] demonstrated that the salt stress-tolerant basil genotype's chlorophyll content increased when inoculated with the AMF, whereas the susceptible genotype showed a dramatic decline even with the AMF. The differences between the previous notes and this study could be attributed to the genetic material studied.

The oxidative enzymes MDA and H_2O_2 increase in response to stress [43]. Plant cells have several defense mechanisms and repair systems to reduce the formation of oxidative damage caused by reactive oxygen species (ROS) [44]. The most common mechanism plants use to detoxify the ROS synthesized during the stress response is an increase in ROS-scavenging enzymes such as SOD, CAT, and APX. While mycorrhizal inoculation increased all antioxidant enzymes, the rise of oxidative enzymes was suppressed in this study. Jakovljević et al. [45] reported that basil plants exposed to salt stress during the early development period exhibited enhanced CAT activity according to increasing salt doses, while SOD did not represent similar activity. Abeer et al. [42] reported that the AMF application under stress conditions significantly reduced the amount of MDA in both tolerant and sensitive basil genotypes to salt stress. CAT accumulation was observed only when plants were subjected to moderate salinity stress, whereas SOD was significantly increased in AMF plants regardless of salinity stress [46]. The researchers stated that AMF's effects were noticeable when the host plants were subjected to saline stress, and the impact ratio depends on the plant and fungi species. The AMF inoculation significantly increased antioxidant enzymes while decreasing oxidative enzymes in this study. The findings corroborate the phenomena described in previous notes. Furthermore, the fact that the AMF mixture used in this study suppresses salt stress at the enzymatic level has given rise to the notion that appropriate AMF application can be determined by creating new microorganism mixtures based on plant species and sensitivity level.

In recent years, multivariate analyses have been utilized comprehensively to discriminate the direct and indirect relationships among the investigated characteristics [8,47]. Multivariate analysis is typically used to detect hidden structures in data sets by combining data from multiple sources and applying mathematical tools to problems involving multiple variables [48]. The correlation, PCA, and SEM analyses were used to evaluate the relationships in this study. The APX detoxifies H_2O_2 that was proportionally altered via catalyzing by SOD [49], supported by the alterations in the correlation analysis (see Figure 2). The negative correlations between leaf weights and both APX and H_2O_2 refer to the overproduction of H_2O_2 that was unable to be detoxified by the increment in the APX. CAT is a primary enzyme that neutralizes H_2O_2 and contributes to cellular signaling metabolism by ensuring ROS homeostasis [50]. The findings suggest that CAT upregulates plant growth even under stress-free conditions, and is proportionally affected by the severity of stress. MDA content was the most consistent oxidative enzyme in all conditions. However, the negative relationships between MDA and growth parameters were negligible under non-stress conditions, while there were significant correlations with plant height and leaf number under salt stress. MDA is derived from the peroxidation of polyunsaturated fatty acids in plant membranes as a response to oxidative stress [51]. The remarkable rise in the MDA content, 3-fold in AMF-inoculated and 5-fold in non-inoculated, suggests that the cell membrane damage caused by oxidative stress leads to growth inhibition.

The biplot PCA and SEM analyses were utilized to discriminate the relationships among the factors and variables. The results demonstrated that either oxidative or antioxidant enzymes are involved with rising stress. Jaleel et al. [52] reported the simultaneous enhancement of the enzymes, which was also proven in SEM analysis, as the latent "EN-

ZYMES" had more than factor loadings of 0.84 by all enzymes. SEM also indicated that the enhancement of yield and enzymes by AMF inoculation is relatively harmonious, which was supported by Chen et al. [53]'s study that the improvements in growth parameters and some key enzymes in cucumber plants by various mycorrhizae species were similar. Moreover, SEM results suggest that the involvement in enzyme metabolism is more prominent than the decline in growth. Chen and Hoehenwarter [54] reported an alteration in the levels of some photosynthetic metabolites like fructose, sucrose, amino acids, and glycolysis intermediates according to oxidative stress response to salinity. They also noted a rearrangement in primary metabolism by early signaling responses induced by salt. Plants are known to have adaptation potential against salt stress by regulating ionic homeostasis [55]. Plant biology's pivotal functions, such as establishing cell turgor are determined by ion homeostasis [56]. Altogether, the results and previous studies propose that an enhanced oxidative stress response to enzymatic activity can alleviate the reduction of vegetative growth.

5. Conclusions

This study clarified the effects of AMF under salinity stress in basil plants in terms of growth and enzymatic responses. AMF improved yield parameters in all conditions compared to the control. Mycorrhizae inoculation also enhanced antioxidant enzymes such as APX, CAT, and SOD, while also decreasing the accumulation of the oxidative enzymes, H_2O_2 and MDA. AMF inoculation also significantly alleviated the decline in chlorophyll, which indicates mitigated damage in chloroplasts. Multivariate analyses revealed that oxidative stress enzymes co-occur. Moreover, SEM analysis proved the importance of AMF inoculation for improving either growth or antioxidant enzyme status. Consequently, this study suggests AMF inoculation is an excellent tool for basil plant (cv. Dino) in both saline and non-stress conditions. The effect metabolism may be evaluated in a wide range of basil varieties to create a general overview of AMF symbiosis in the *Ocimum* species in future studies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su15075982/s1, Table S1: ANOVA table according to oxidative stress enzymes and chlorophyll responses of basil plants under different salinity conditions.

Author Contributions: Conceptualization, A.Y. and H.Y.; methodology, H.Y.; software, A.Y., H.Y. and E.G.; validation, E.G., M.Y. and A.Y.; formal analysis, A.Y., H.Y., H.E.S. and E.G.; investigation, A.Y., E.G. and M.Y.; resources, A.Y.; data curation, E.G.; writing—original draft preparation, A.Y., E.G. and H.Y.; writing—review and editing, A.Y., E.G., V.C., M.Y. and E.Y.; visualization, A.Y., H.E.S. and E.G.; supervision, V.C. and E.Y.; project administration, A.Y.; funding acquisition, A.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This study was financially supported by The Scientific Research Projects Coordination Unit of Bolu Abant Izzet Baysal University with the project number 2021.10.07.1527. The authors are grateful for the support.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Conflicts of Interest: Authors have no conflict of interest that may influence this study.

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