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Positive Changes in Fruit Quality, Leaf Antioxidant Defense System, and Soil Fertility of Beni-Madonna Tangor Citrus (*Citrus nanko* × *C. amakusa*) after Field AMF Inoculation

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Abstract: Citrus plants rely heavily on arbuscular mycorrhizal fungi (AMF) due to their lack of root hairs. Most experiments have been conducted with AMF inoculation under potted conditions, while field inoculation of AMF on citrus, especially a high economic hybrid tangor variety Beni-Madonna (*Citrus nanko* × *C. amakusa*), has been rarely recorded. This study aimed to analyze the effects of two AMF inoculations (a single *Funneliformis mosseae* and a mixture of *F. mosseae*, *Diversispora versiformis*, and *Rhizophagus intraradices*) on the internal and external fruit quality, leaf antioxidant defense system, and soil fertility and structure of top-worked Beni-Madonna tangor citrus trees. Three and a half years after AMF inoculations, soil hyphal length and root mycorrhizal colonization rate increased by 61.2–101.8% and 15.85–29.6% in inoculated plants, respectively. Inoculated trees had higher external fruit coloration value, fruit horizontal diameter, and fruit weight, and lower fruit rigidity than uninoculated trees. AMF-inoculated trees had higher glucose levels of fruit peels, fructose and sucrose levels of fruit fleshes, and the ratio of fruit soluble solids/titratable acids, as well as lower titratable acids concentrations than non-AMF-inoculated trees. AMF inoculation significantly increased leaf nitrogen balance index, chlorophyll index, peroxidase, catalase, superoxide dismutase, and glutathione reductase activities, as well as reduced glutathione and oxidized glutathione concentrations, resulting in lower hydrogen peroxide and malondialdehyde levels when compared to the uninoculated treatment. In addition, inoculated trees presented higher soil nutrient levels, including organic carbon, available K, and Olsen-P as, well as soil aggregate stability (based on mean weight diameter) than uninoculated trees. This study concluded that field AMF inoculation improved fruit quality, enhanced leaf antioxidant defense system, and improved soil fertility of Beni-Madonna trees, with mixed AMF being prominent in improving fruit quality and *F. mosseae* being prominent in enhancing leaf antioxidant defense system and improving soil fertility.

Keywords: antioxidase; citrus; fruit quality; mycorrhiza; reactive oxygen species; soil nutrient

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1. Introduction

Arbuscular mycorrhizal fungi (AMF) establish common arbuscular mycorrhizae (AM) with roots of most vascular plants [1], in which AMF are involved in many plant functions such as root formation, nutrition acquisition, photosynthesis, and stress tolerance [2]. Earlier studies revealed an increased rate of mycorrhizal colonization in plant roots following exotic AMF inoculation in the field, along with an increase in crop yield [3,4]. This provides a foundation for the use of AMF in the field to increase the economic value of field crops.

Citrus (*Citrus* spp.), one of the world's most important economic fruit crops, is made up of 17 categories, including orange (*Citrus reticulata* Blanco), tangerine (*C. reticulata* Blanco), lemon (*C. lemon* L.), lime (*C. aurantifolia* Linn), and grapefruit (*C. paradise* Macf.) [5]. World citrus production reached 158.5 million tons in 2020, of which China is the largest citrus producer with 44.5 million tons, accounting for 28.1% of global citrus fruit production [6].

Beni-Madonna ('Ehime Kashi No. 28') (*Citrus nanko* × *C. amakusa*) is a hybrid tangor citrus cultivar with high economic value in recent years due to its beautiful appearance, tender taste, great flavor, and early maturity [7]. However, many characteristics of this cultivar, especially the field response to AMF inoculation, are unknown.

Citrus plants have short root hairs, so the AMF colonization rate in field-grown citrus trees is relatively low [8]. Numerous potted studies indicated that AMF inoculation strongly promoted plant growth, nutrient and water uptake, enhanced resistance to drought, low temperature, salinity, root rot, and canker, and improved soil structure and fertility in citrus plants [2,9,10]. Field crops with AMF inoculation have been reported [3,11]. AMF inoculation increased the yield of sweet pepper [3], as well as fruit size, coloration, and hardness in passion fruit [12]. After AMF inoculation in Lane late navel orange, the fruit's titratable acid content reduced, but the content of soluble solids increased significantly [11]. It was also found that *Rhizoglomus irregulare*-treated lettuce plants exhibited higher antioxidant enzyme activities and phenolic levels in leaves than non-inoculated plants [13], suggesting that AMF participate in plant secondary metabolism. Since AMF are beneficial microorganisms for citrus, they are considered in organic citrus production [14]. However, it is unknown whether AMF inoculation can alter the fruit quality of Beni-Madonna tangor citrus, especially antioxidant properties, as well as soil structure and fertility.

Wang et al. [15] investigated three citrus orchards in the Three Gorges Reservoir area of China and found *Funneliformis mosseae* and *Rhizophagus intraradices* to be the dominant AMF strains. *Diversispora versiformis* (formerly *Glomus versiforme*) has been proven to have a good role in promoting plant growth, photosynthesis, and nutrient acquisition of citrus plants [16]. It is uncertain whether these superior AMF strains have a positive effect on field citrus. The aim of this study was to examine the effects of field inoculation of *F. mosseae* and AMF mixture on fruit quality, antioxidant characteristics, soil fertility, structure and stability of Beni-Madonna tangor citrus.

2. Materials and Methods

2.1. Experimental Set-Up

The citrus orchard (30°21′27″ N, 112°3′5″ E) with 3-year-old *C. reticulata* Blanco var. Ponkan mandarin cv. Jinshuigan grafted on trifoliate orange was located in Jingzhou, China, where the plant spacing was 3 m × 4 m. The orchard is situated in the north subtropical monsoon humid climate zone. The total annual solar radiation is 104–110 kcal/cm^2, the annual sunshine hours are 1800–2000 h, the average annual temperature is 15.9–16.6 °C, the annual frost-free period is 242–263 days, and the rainfall in most years is in the range of 1100–1300 mm.

In March 2018, the citrus trees received two AMF inoculations, including a single *Funneliformis mosseae* (BGC XJ02A) and a mixture of *F. mosseae* (BGC XJ02A), *Diversispora versiformis* (BGC BJ08), and *Rhizophagus intraradices* (BGC HUN02B). The AMF strains were provided by the Institute of Root Biology, Yangtze University. The spore density was 20 spores/g in the *F. mosseae* inoculum and 26 spores/g in the mixed AMF inoculum, respectively. The 600 g (12,000 spores in the *F. mosseae* inoculation and 15,600 spores in the mixed AMF inoculation) of the used mycorrhizal fungal inoculums was applied to two trenches (40 cm × 15 cm × 30 cm) in north–south and east–west directions around 40 cm of the trunk of a selected citrus tree. For the non-AMF inoculation treatment, equal amounts of autoclaved mycorrhizal inoculums were applied. Subsequently, these trees were managed consistently in the field.

The Beni-Madonna variety was top-worked on the main branches of the citrus trees in October 2019. The Jinshuigan variety was removed after the bud viability of the Beni-Madonna variety was checked in March 2020. As a result, in this study, the Beni-Madonna tree top-worked to 4-year-old *C. reticulata* Blanco var. Ponkan mandarin cv. Jinshuigan, after cutting back, with trifoliate orange as the rootstock. The sampling was carried out at the commercial fruit ripening stage in mid-November 2021.

2.2. Experimental Design

This experiment consisted of three treatments, including no exotic AMF inoculation (non-AMF), *F. mosseae* inoculation (Fm), and mixed AMF inoculation (mixed AMF). Each treatment consisted of 5 citrus trees, for a total of 15 trees.

2.3. Determination of Root Mycorrhizal Colonization and Soil Hyphal Length

After removing the top 0–5 cm layer of the soil under the tree canopy, fine roots were collected, and the soil adhering to the root surface, known as rhizosphere soil, was shaken off and placed in sealed bags. The autumn leaves from the current year were collected and stored at −75 °C for antioxidant analysis.

The staining of root arbuscular mycorrhizae was performed using the method described by Phillips and Hayman [17]. Simply, 1 cm long root segments were collected and then cleared in 10% potassium hydroxide solution at 100 °C for 2 h, followed by decolorisation in 10% hydrogen peroxide solution for 5 min and stained using 0.05% trypan blue in lactophenol solution for 2.5 min. The length of hyphae in soil was quantified following the protocol outlined by Bethlenfalvay and Ames [18]. The 2 g soil samples were incubated with 30 mL distilled water for 8 h and passed through 300 μm size sieves. Then, 5 mL of the filtrate was well mixed with 0.1 mL of 0.05% trypan blue solution for 1 min for microscopic observation of the hyphae and determination of hyphae length.

2.4. Determination of Soil Nutrient Levels

Easily extractable glomalin-related soil protein (EE-GRSP) and difficultly extractable glomalin-related soil protein (DE-GRSP) concentrations were determined according to the method of Wu et al. [19]. The distribution of water-stable aggregates (WSAs) was determined via a wet sieving method [20], and then the mean weight diameter (MWD) was calculated on the basis of the formula described by Kemper and Rosenau [21]. Soil organic carbon (SOC) content was determined through the use of potassium dichromate with external heating, as proposed by Walkley and Black [22]. Soil NH_4^+-N, NO_3^--N, Olsen-P, and available K concentrations were analyzed using a Soil Nutrient Detector (HM-TYD, Linyi, China).

2.5. Determination of Fruit Quality

Fruits were collected from all sides of the treated tree canopy, with 12 fruits/tree being immediately transported to the laboratory. The fruits were measured for longitudinal and transverse diameters using a vernier caliper (Guanglu 0-150, Guilin, China), for fruit weight using an electronic balance (ASC-30, Jinhua, China), for coloration value using a colorimeter (CR10, Osaka, Japan), and for rigidity using a hardness tester (GY-B, Hangzhou, China). The soluble solids content of the fruits was measured using a sugar meter (WYT-4, Quanzhou, China). The titratable acid content of the fruits was determined via the titrimetric method, and the glucose, fructose, and sucrose concentrations in the fruit pericarp and sarcacorp were assayed with reference to the spectrophotometric method outlined by Zhang and Zai [23].

2.6. Determination of Leaf Physiological Parameters

Leaf chlorophyll (Chl), flavonoids (Fla), and nitrogen balance index (Nbi) were determined using a portable plant polyphenol–chlorophyll meter (Dualex Scientific+, Orsay, France). Leaf malondialdehyde (MDA) concentrations were determined based on the reaction with thiobarbituric acid [24]. The superoxide anion radical ($O_2^{\bullet-}$) content in the leaves was determined according to the hydroxylamine chloride method [25], and hydrogen peroxide (H_2O_2) concentration was determined via the method of Velikova et al. [26], in the KI reaction. Superoxide dismutase (SOD) activity in leaves was determined via the nitrotetrazolium blue chloride method described by Li et al. [25], catalase (CAT) activity was determined according to the spectrophotometric method (240 nm) [25], peroxidase (POD) activity was determined according to the guaiacol method [25], ascorbate peroxi-

dase (APX) activity was determined according to the method described by Li et al. [25], and glutathione reductase (GR) activity was determined according to the method of Fatemeh et al. [27]. Ascorbic acid (ASC) and dehydroascorbic acid (DHA) concentrations in leaves were extracted with 5 mL of 5% sulfosalicylic acid followed by centrifugation at 12,000× g/min for 10 min, and their concentrations were assayed according to the method described by Gao [28]. Reduced glutathione (GSH) and oxidized glutathione (GSSG) levels were determined according to the method of Li et al. [25].

2.7. Statistical Analysis

One-way analysis of variance, Duncan's multiple range tests ($p < 0.05$), and Pearson's correlation coefficients (r) between variables were performed on the experimental data using SAS software (Version 9.4).

3. Results

3.1. Root AMF Colonization and Soil Hyphal Length

Both inoculations increased root AMF colonization and soil hyphal length in top-worked Beni-Madonna tangor citrus (Figure 1). Compared with the uninoculated treatment, soil hyphal length was increased by 61.2% in the Fm-inoculated trees and 101.8% in the mixed AMF-inoculated trees, respectively (Figure 1). The root AMF colonization rate increased by 15.8% in the Fm-inoculated trees and 29.6% in the mixed AMF-inoculated trees, respectively, compared with the non-AMF-inoculated trees (Figure 1).

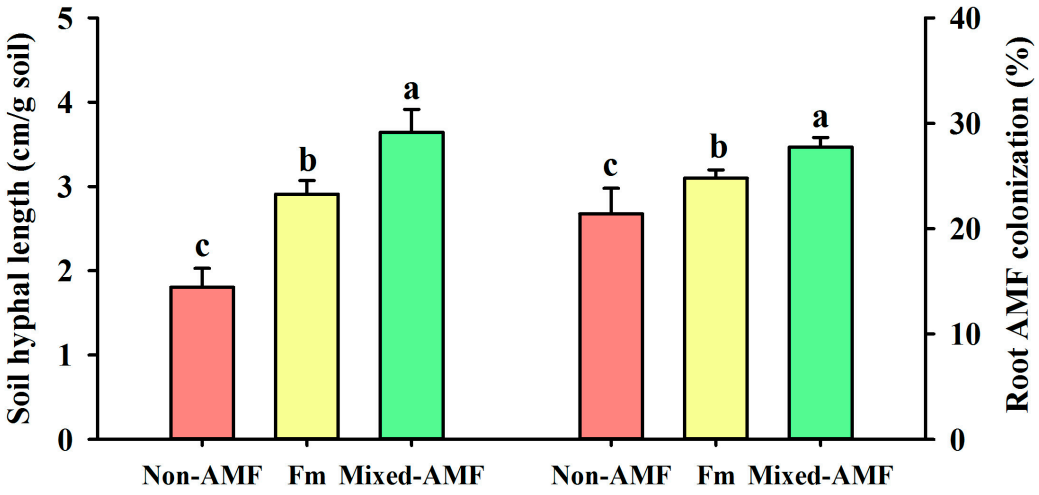

Figure 1. Effects of field inoculation of arbuscular mycorrhizal fungi on soil hyphal length and root mycorrhizal colonization rate in Beni-Madonna tangor citrus. Means ± SD (n = 5) followed by different letters at the bar indicate significant ($p < 0.05$) differences between treatments. Abbreviations: Non-AMF, the citrus trees inoculated without exotic arbuscular mycorrhizal fungi; Fm, the citrus trees inoculated with exotic *Funneliformis mosseae*; mixed AMF, the citrus trees inoculated with exotic *Diversispora versiformis*, *F. mosseae*, and *Rhizophagus intraradices*.

3.2. External Quality of Fruits

AMF treatments significantly improved the external quality of Beni-Madonna fruits (Figure 2; Table 1). Compared with the uninoculated treatment, coloration value, pericarp thickness, horizontal diameter, sarcocarp weight, pericarp weight, and single fruit weight were significantly increased by 6.2%, 8.3%, 4.2%, 40.3%, 25.8%, and 24.6%, respectively, under single *F. mosseae* inoculation and by 6.9%, 10.6%, 5.4%, 46.8%, 19.4%, and 41.2% under mixed AMF inoculation (Table 1). Compared with the uninoculated treatment, fruit rigidity significantly decreased by 31.5% under single *F. mosseae* inoculation conditions and 45.2% under mixed AMF inoculation conditions. In addition, the decrease in fruit rigidity

and the increase in single fruit weight under mycorrhization were higher under mixed AMF inoculation conditions than under single *F. mosseae* inoculation conditions.

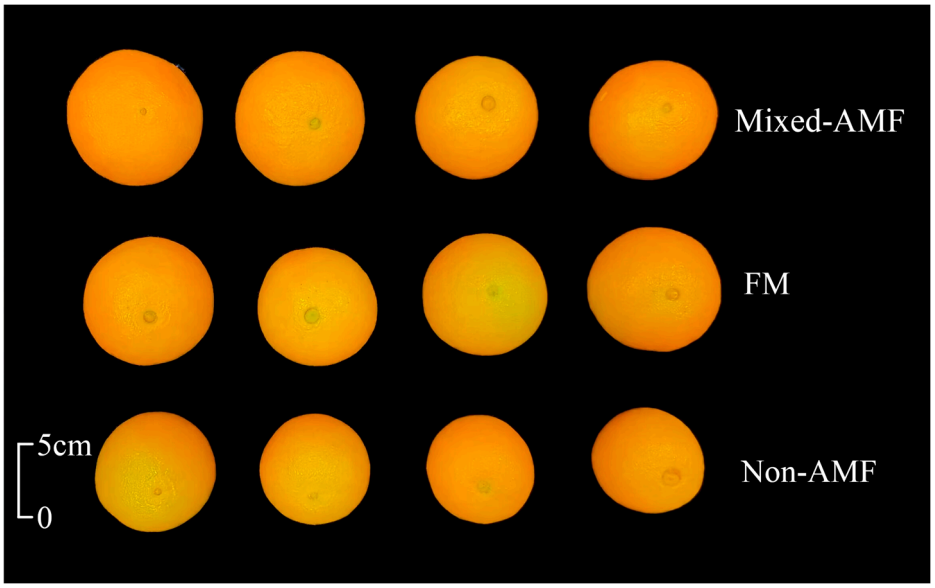

Figure 2. Changes in fruit appearance of Beni-Madonna tangor citrus inoculated with arbuscular mycorrhizal fungi in the field.

Table 1. Effects of field inoculation of arbuscular mycorrhizal fungi on fruit external quality of Beni-Madonna tangor citrus.

Treatments	Coloration Value	Rigidity ($\times 10^5$ kg/cm^3)	Pericarp Thickness (mm)	Size (mm)		Weight (g)		
				Vertical Diameter	Horizontal Diameter	Sarcocarp	Pericarp	Single Fruit
Non-AMF	69.2 ± 8.2 b	31.4 ± 3.9 a	250.8 ± 24.5 b	65.5 ± 4.4 a	71.7 ± 3.4 b	154 ± 8 b	31 ± 4 b	187 ± 9 c
Fm	73.5 ± 7.5 a	21.5 ± 2.4 b	271.7 ± 28.5 a	66.2 ± 3.8 a	74.7 ± 3.7 a	216 ± 18 a	39 ± 2 a	233 ± 16 b
Mixed AMF	74.0 ± 7.4 a	17.2 ± 2.8 c	277.3 ± 20.7 a	66.6 ± 5.9 a	75.6 ± 4.7 a	226 ± 15 a	37 ± 4 a	264 ± 12 a

Means ± SD (n = 5) followed by different letters in the column indicate significant ($p < 0.05$) differences between treatments.

3.3. Internal Quality of Fruits

The two AMF inoculation treatments differentially affected the internal fruit quality (Figures 3a–d and 4). Compared with the uninoculated treatment, the titratable acids content of the fruit decreased by 17.2%, while fruit vitamin C content increased by 37.8% under single *F. mosseae* inoculation, but the soluble solids content of the fruit did not change significantly (Figure 3). Mixed AMF inoculation significantly reduced the fruit titratable acid content by 8.6% but had no significant effect on fruit vitamin C and soluble solids content compared with the uninoculated treatment.

In addition, compared with the uninoculated treatment, fructose and sucrose concentrations of sarcocarps were significantly increased by 59.1% and 5.4%, respectively, and glucose concentrations of pericarps were increased by 49.6% under single *F. mosseae* inoculation conditions; glucose and fructose concentrations of sarcocarps were significantly increased by 43.4% and 61.2%, respectively, and glucose and fructose concentrations of pericarps were significantly increased by 34.9% and 26.8%, respectively, under mixed AMF inoculation conditions (Figure 4).

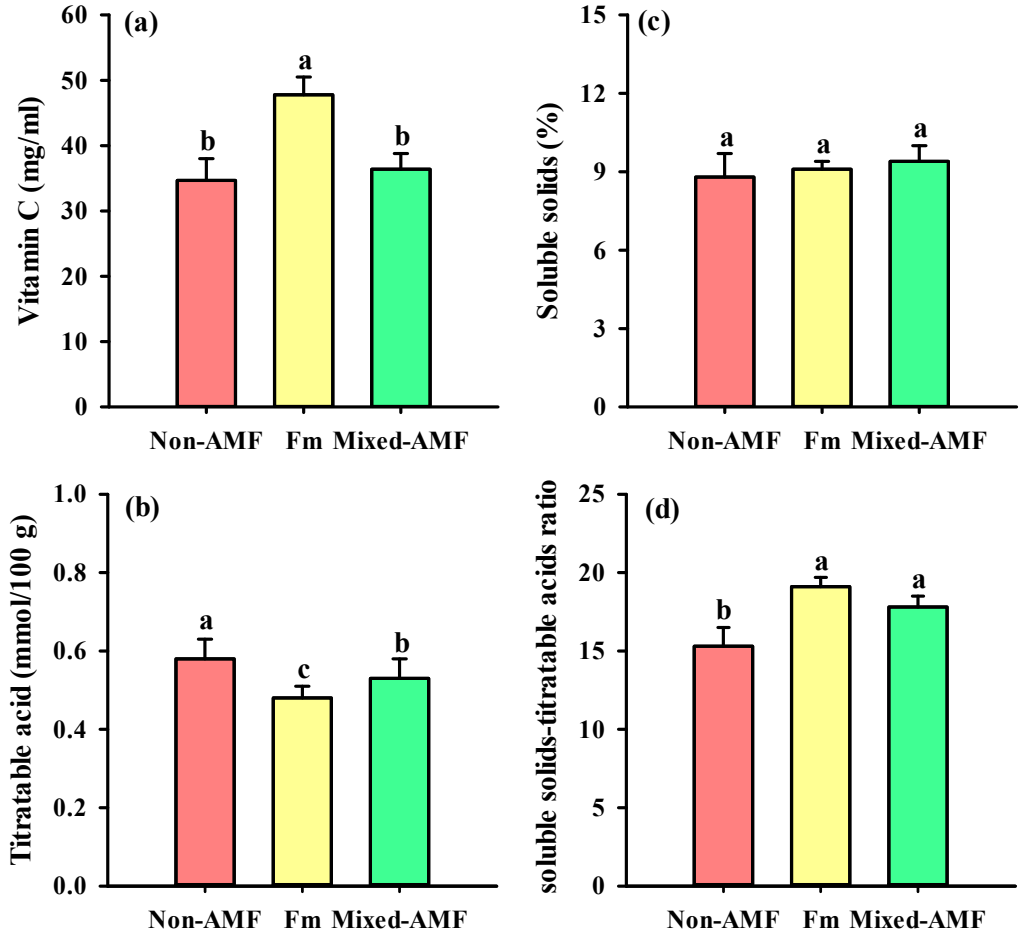

Figure 3. Effects of field inoculation of arbuscular mycorrhizal fungi on fruit vitamin C (**a**), titratable acids (**b**), soluble solids contents (**c**) and soluble solids-titratable acids ratio (**d**) in Beni-Madonna tangor citrus. Means ± SD (n = 5) followed by different letters at the bar indicate significant ($p < 0.05$) differences between treatments.

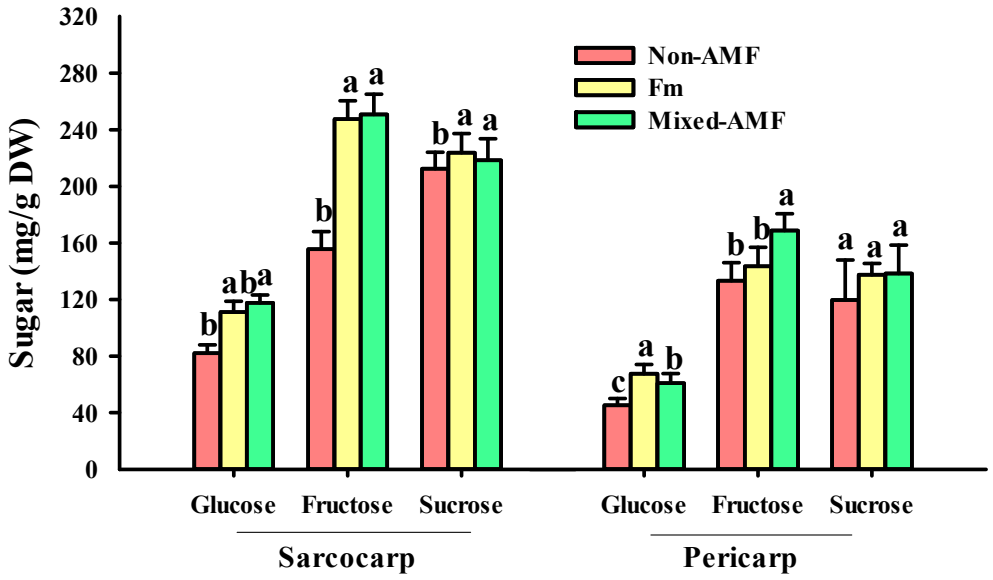

Figure 4. Effects of field inoculation of arbuscular mycorrhizal fungi on fructose, glucose, and sucrose concentrations in pericarps and sarcocarps of Beni-Madonna tangor citrus. Means ± SD (n = 5) followed by different letters at the bar indicate significant ($p < 0.05$) differences between treatments.

3.4. Leaf Chlorophyll Index, Flavonoid Index, and Nitrogen Balance Index

The two AMF treatments significantly increased the leaf Chi by 12.4% and 22.7% under single *F. mosseae* inoculation and mixed AMF inoculation conditions, respectively (Figure 5). In addition, the inoculated plants also presented 24.7% and 15.1% significantly higher Nbi under single *F. mosseae* inoculation and mixed AMF inoculation conditions compared with the uninoculated treatment. However, the treatments inoculated with mixed AMF did not significantly affect leaf Fla, but single AMF inoculation inhibited leaf Fla by 10.2%, compared with the uninoculated control.

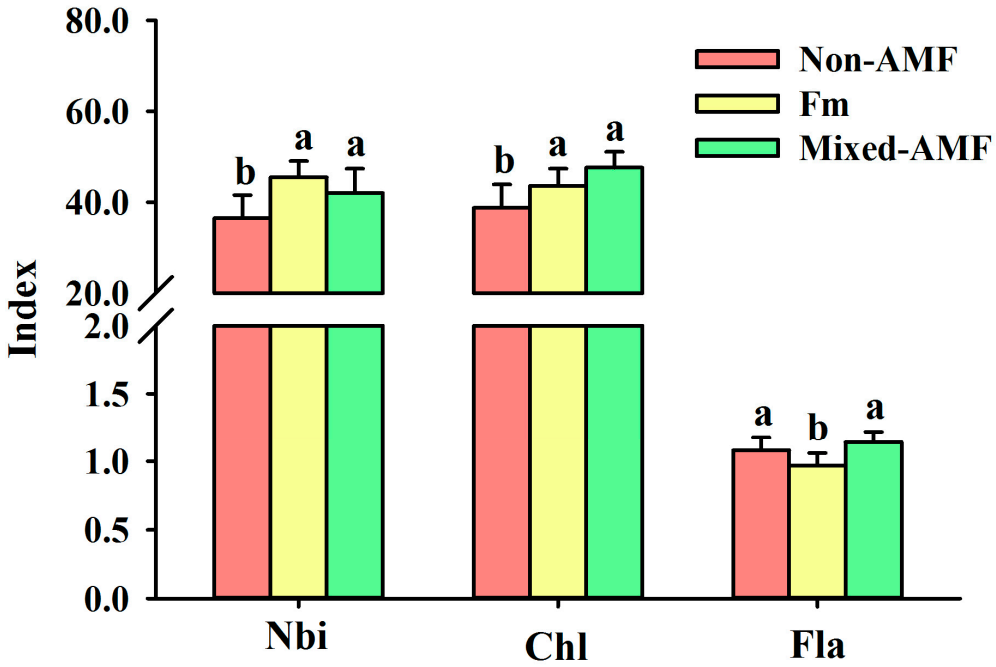

Figure 5. Effects of field inoculation of arbuscular mycorrhizal fungi on leaf Nbi, Chl, and Fla in Beni-Madonna tangor citrus. Means ± SD (n = 5) followed by different letters at the bar indicate significant ($p < 0.05$) differences between treatments.

3.5. Leaf Antioxidant Enzyme Activities and Antioxidant Concentrations

Leaf CAT, POD, SOD, APX, and GR activities were significantly increased by 46.2%, 34%, 24.7%, 37%, and 24.5%, respectively, under *F. mosseae* inoculation treatment compared with non-AMF treatment (Figure 6a,b). Leaf CAT, POD, SOD, and GR activities were significantly increased by 11.7%, 18%, 23.7%, and 13.6%, respectively, under the mixed AMF inoculation conditions compared with non-AMF treatment, while leaf APX activity was not significantly affected by mixed AMF inoculation. In addition, *F. mosseae*-inoculated trees recorded significantly higher leaf POD, SOD, APX, CAT, and GR activities than mixed AMF-inoculated trees.

AMF also had an effect on leaf antioxidant concentrations, as evidenced by leaf GSH, GSSG, and DHA concentrations being significantly increased by 56.5%, 53.1%, and 42.2% under *F. mosseae* versus non-AMF conditions, and leaf GSH and GSSG being significantly increased by 41.3% and 21.9% under mixed AMF versus non-AMF conditions (Figure 7a,b). Both fungal inoculations did not significantly affect leaf ASC concentrations (Figure 7b).

3.6. Leaf H_2O_2, $O_2^{\bullet -}$, and MDA levels

AMF treatments reduced leaf H_2O_2, $O_2^{\bullet -}$, and MDA levels to different degrees, as shown by leaf H_2O_2 and MDA levels being reduced by 29.0% and 19.7% under *F. mosseae* versus non-AMF conditions and leaf H_2O_2 and $O_2^{\bullet -}$ being reduced by 13.4% and 36.4% under mixed AMF versus non-AMF conditions, respectively (Figure 8a,b).

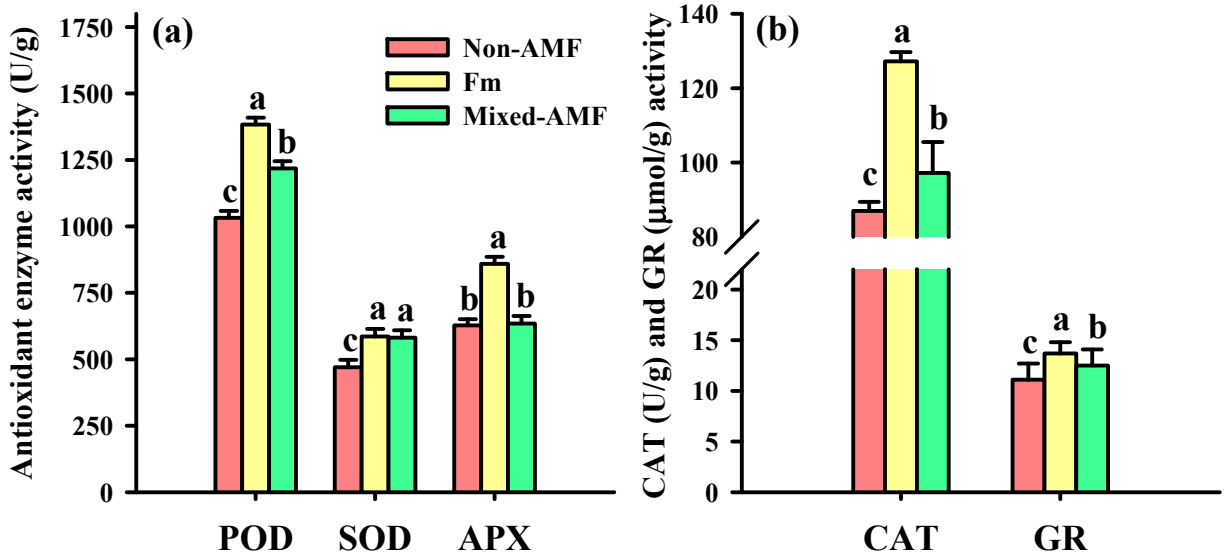

Figure 6. Effects of field inoculation of arbuscular mycorrhizal fungi on leaf SOD (**a**), POD (**a**), APX (**a**), CAT (**b**), and GR (**b**) activities in Beni-Madonna tangor citrus. Means ± SD (n = 5) followed by different letters at the bar indicate significant ($p < 0.05$) differences between treatments.

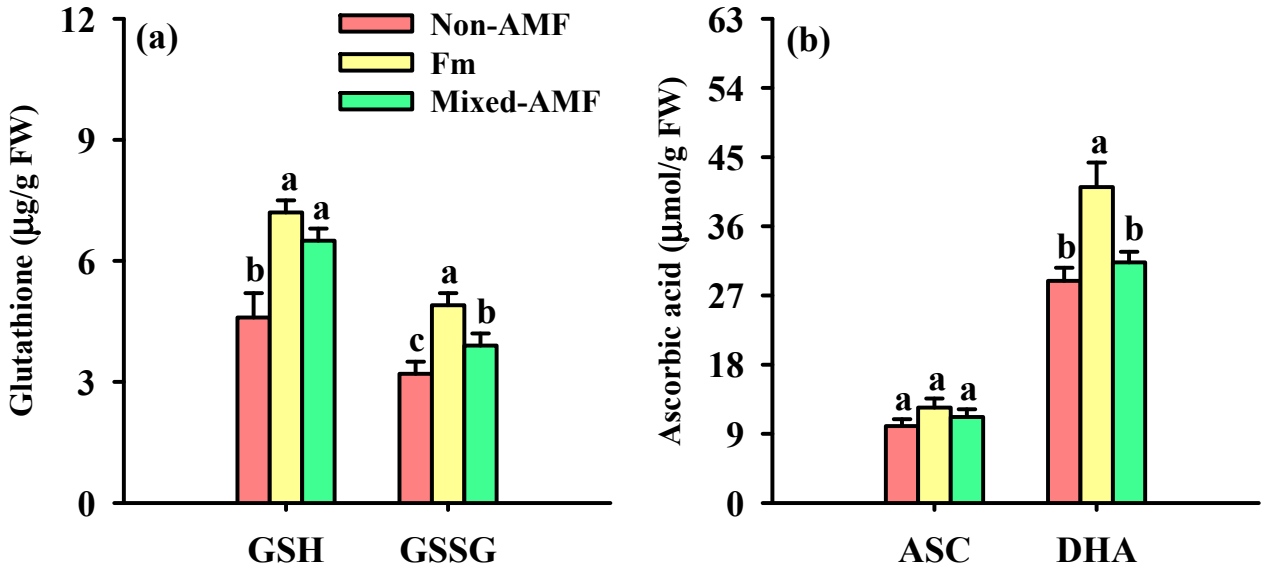

Figure 7. Effects of field inoculation of arbuscular mycorrhizal fungi on leaf GSH (**a**), GSSG (**a**), ASC (**b**), and DHA (**b**) concentrations in Beni-Madonna tangor citrus. Means ± SD (n = 5) followed by different letters at the bar indicate significant ($p < 0.05$) differences between treatments.

3.7. Soil Nutrient Levels and Aggregate Stability

AMF-treated citrus trees showed significant changes in soil nutrient levels and aggregate stability, as evidenced by MWD, SOC, EE-GRSP, DE-GRSP, NO_3^--N, Olsen-P, and available K levels being elevated by 23%, 27%, 14.8%, 15.3%, 25.8%, 39.1%, and 26.7% in *F. mosseae* versus non-AMF conditions and MWD, SOC, Olsen-P, and available K levels being elevated by 8.2%, 17.0%, 29.4%, and 13.0% under mixed AMF versus non-AMF conditions, respectively (Table 2).

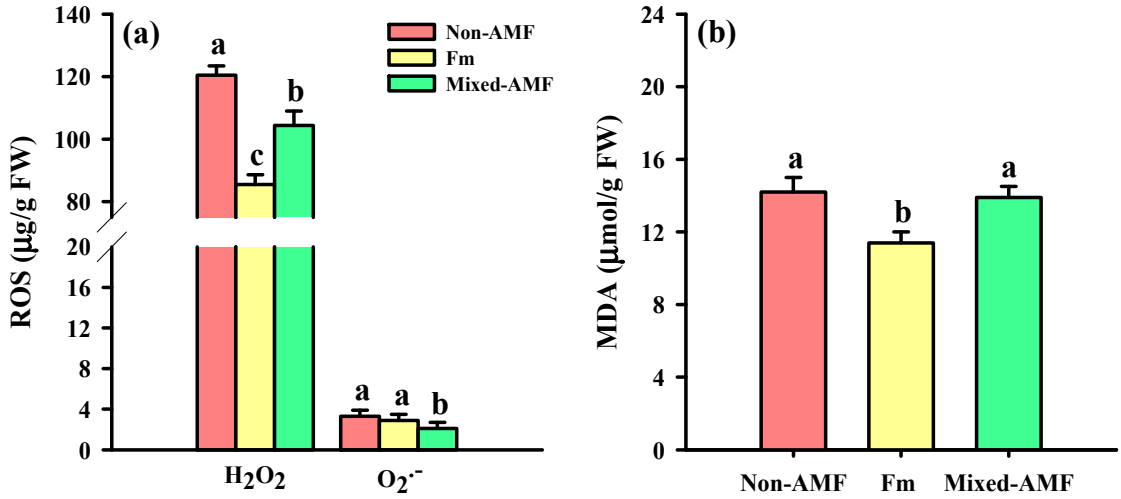

Figure 8. Effects of field inoculation of arbuscular mycorrhizal fungi on leaf H_2O_2 (**a**), $O_2^{\bullet -}$ (**a**), and MDA (**b**) levels in Beni-Madonna tangor citrus. Means ± SD (n = 5) followed by different letters at the bar indicate significant ($p < 0.05$) differences between treatments.

Table 2. Effects of field inoculation of arbuscular mycorrhizal fungi on soil traits of Beni-Madonna tangor citrus.

Treatments	MWD (mm)	SOC (mg/g)	EE-GRSP (mg/g)	DE-GRSP (mg/g)	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	Olsen-P (mg/kg)	Available K (mg/kg)
Non-AMF	1.83 ± 0.05 c	14.1 ± 0.9 c	0.54 ± 0.03 b	0.72 ± 0.03 b	38.8 ± 7.6 a	65.9 ± 7.2 b	50.6 ± 2.6 c	283.7 ± 10.1 c
Fm	2.25 ± 0.10 a	17.9 ± 0.2 a	0.62 ± 0.06 a	0.83 ± 0.06 a	40.2 ± 4.3 a	82.9 ± 5.1 a	70.4 ± 3.3 a	359.4 ± 25.6 a
Mixed AMF	1.98 ± 0.08 b	16.5 ± 0.7 b	0.56 ± 0.02 ab	0.75 ± 0.02 b	39.9 ± 2.7 a	73.9 ± 1.1 b	65.5 ± 2.9 b	320.6 ± 21.9 b

Means ± SD (n = 5) followed by different letters in the column indicate significant differences ($p < 0.05$) between treatments.

3.8. Correlation Analysis

AMF colonization rate was significantly and positively correlated with SOC, MWD, NO_3^--N, Olsen P, available K, EE-GRSP, and DE-GRSP (Table 3). In addition, MWD was significantly and positively correlated with DE-GRSP and SOC. The root AMF colonization rate was significantly and positively correlated with flesh sucrose, glucose, and fructose concentrations but not with fruit soluble solids content (Table 4). The root AMF colonization rate was significantly and positively correlated with leaf SOD, POD, CAT, APX, GR, GSH, GSSG, ASC, and DHA concentrations, while it was significantly and negatively correlated with leaf MDA and H_2O_2 (Table 5).

Table 3. Correlation analysis between AMF colonization and soil parameters.

	AMF Colonization	SOC	DE-GRSP	MWD	NO_3^--N	Olsen P	Available K	EE-GRSP
AMF colonization	1	0.87 **	0.69 *	0.82 **	0.77 **	0.83 **	0.86 **	0.64 *
MWD	0.82 **	0.81 **	0.85 **					

*, $p < 0.05$; **, $p < 0.01$.

Table 4. Correlation analysis between AMF colonization and fruit internal quality.

	Sucrose	Glucose	Fructose	Soluble Solids
AMF colonization	0.73 **	0.59 *	0.74 **	0.43

*, $p < 0.05$; **, $p < 0.01$.

Table 5. Correlation analysis between AMF colonization and leaf antioxidant defense system.

	CAT	POD	SOD	APX	GR	MDA	ASC	DHA	GSH	GSSG	H_2O_2
AMF colonization	0.83 **	0.86 **	0.79 **	0.74 **	0.89 **	−0.78 **	0.68 *	0.88 **	0.86 **	0.81 **	−0.90 **

*, $p < 0.05$; **, $p < 0.01$.

4. Discussion

4.1. Effects of AMF on Soil Nutrient Levels and Aggregte Stability

The present study showed that both AMF inoculations significantly increased root AMF colonization rate and soil hyphal length in top-worked Beni-Madonna tangor citrus, with the mixed AMF inoculation showing a higher increase scale than the single *F. mosseae* inoculation. The same results were reported by Li et al. [11]. This also implies that there was a synergistic effect between the three mixed AMF species, which promotes root colonization. Exotic AMF inoculation may alter the structure of native AMF communities and thus promote colonization by AMF in roots and soil hyphal formation [3,4].

Mycorrhizal hyphae and spores can secrete and deposit a glycoprotein in the soil called GRSP [29]. GRSP is divided into two types, EE-GRSP and DE-GRSP, and also is the main source of SOC [19]. In this study, two GRSP concentrations were significantly increased after *F. mosseae* inoculation but not mixed AMF, suggesting that the increase in GRSP was dependent on the type of AMF inoculation. SOC had a significantly positive correlation with root AMF colonization rate and DE-GRSP since DE-GRSP is mainly a component of stored nutrients [19]. SOC was also higher under mycorrhization than under non-mycorrhization conditions, demonstrating that AMF inoculation promoted the synthesis and secretion of GRSP by the host plant and improved the microenvironment by increasing SOC. In addition, AMF increase plant photosynthesis and facilitate the release of root exudates and stabilize SOC sequestration, thus contributing to the increase in SOC [30].

AMF affects soil available nutrient levels by altering the abundance of soil bacterial and fungal communities [31]. Moreover, soil available nutrients, such as NO_3^-N, have an impact on GRSP and SOC content, which in turn change the stability of the aggregates [32]. In this experiment, soil Olsen-P, available K, and MWD of citrus trees were significantly higher under AMF inoculation conditions than under non-AMF inoculation conditions, and citrus trees inoculated with *F. mosseae* also showed higher levels of NO_3^--N than uninoculated trees. Zhu et al. [33] inoculated AMF on *Paris polyphylla* seedlings and observed that the soil Olsen-P, available K, SOC, and NO_3^--N levels of inoculated plants were higher than uninoculated plants. A significantly positive correlation was also found between MWD and DE-GRSP, SOC and root mycorrhizal colonization rate, suggesting that mycorrhizal improvement of soil aggregate stability is the combined result of mycorrhizal mycelium, DE-GRSP, and SOC. Therefore, AMF inoculation in the field increased the soil nutrient levels of Beni-Madonna trees and provided a favorable environment for tree growth. Soil nutrient levels of citrus trees inoculated with *F. mosseae* were significantly better than those inoculated with mixed AMF, indicating the preference of *F. mosseae* for Beni-Madonna trees in the field.

4.2. Effects of AMF on Fruit Quality

The economic value of fruits depends mainly on external qualities, such as hardness and size, and internal qualities, such as soluble solids and titratable acids content [34]. The present study showed that two AMF inoculations improved the external and internal quality of Beni-Madonna fruits to different degrees but varied according to AMF inoculations. In terms of external fruit quality, mixed AMF inoculation presented a relatively better effect than *F. mosseae* inoculation. Horvath et al. [35] inoculated a mixture of AMF on field tomatoes and found that fruit setting, fruit size and color were significantly improved after AMF inoculation. In addition, inoculated trees also showed lower titratable acids contents and higher ratios of soluble solids, titratable acids, glucose concentrations of pericarps, and fructose and sucrose concentrations of sarcocarps than uninoculated trees, along with higher Vc content in *F. mosseae* versus non-AMF treatment and higher glucose

of sarcocarps and fructose of pericarps, suggesting that inoculated citrus trees represented greater internal qualities in terms of fruit than uninoculated trees. Li et al. [11] found a significant increase in fruit glucose concentrations and a reduction in titratable acids contents of fruits after AMF inoculation on field-grown Lane late navel oranges. Root AMF colonization rate was positively correlated with sarcocarp sucrose, glucose, and fructose levels. These results suggest that mycorrhizal fungi form a carbon pool in citrus trees, which, in turn, alters the partitioning of sugars to fruits and roots, thus facilitating sugar accumulation in the fruit [36]. Li et al. [11] found that AMF induced the up-regulation of invertase and *SWEET* (sugar exported transporter) gene in citrus fruits, which, in turn, promotes sugar accumulation. AMF are also involved in the synthesis, transport and cleavage of fruit sugars [11], indicating that mycorrhizae play an important role in regulating the change of fruit sugars, which is worthy of further investigation.

4.3. Effects of AMF on Leaf Antioxidant Defense Systems

Plants usually develop antioxidant enzymes and antioxidant defense systems to mitigate the damage of reactive oxygen species caused by adverse environments [37]. Our study indicated that two AMF inoculations distinctly increased antioxidant enzyme activities and GSH and GSSG concentrations in leaves, which, in turn, allowed the inoculated plants, especially *F. mosseae*-inoculated trees, to maintain lower leaf H_2O_2 and MDA levels. Moreover, root mycorrhizal colonization rate was significantly positively correlated with leaf antioxidant enzyme activities and antioxidant concentrations while significantly negatively correlated with MDA and H_2O_2 levels. This explains why mycorrhizal plants have a strong antioxidant capacity and thus have higher resistance than uninoculated plants [2]. The reasons for the enhanced antioxidant defense system in mycorrhizal plants are the combined activation of AMF's own defense system and the enhancement of antioxidant defense system in host plants by activating metabolites, such as polyamines, flavonoids, and fatty acids [2]. This showed that AMF-inoculated plants have a higher ability to resist stress in the field. However, the underlying mechanisms, especially at the molecular level, need to be further investigated.

5. Conclusions

The field inoculation of AMF distinctly increased citrus fruit quality, leaf antioxidant defense system, soil fertility, and structure, among which *F. mosseae* was prominent in enhancing the leaf antioxidant defense system and soil fertility, and mixed AMF was prominent in terms of fruit quality improvement. Overall, *F. mosseae* presented a relatively higher effect on the observed parameter. Such a result provides an important reference for the field application of mycorrhizal fungi. However, this experiment is the result of the third year after AMF inoculation, and a longer-term validation of experimental results is needed. In addition, this study was only performed on five trees per inoculation treatment, and the sample of inoculated trees should be increased in the future to confirm the role of AMF in citrus plants. Multi-year periodic data to analyze the benefits of AMF on field citrus trees are more likely to support the future application of AMF for organic citrus production.

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