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Tomato Yield, Quality, Mineral Composition and Antioxidants as Affected by Beneficial Microorganisms Under Soil Salinity Induced by Balanced Nutrient Solutions

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Abstract: With the aim of assessing the effects of beneficial microorganisms on greenhouse tomato "plum" grown under salinity conditions, research was carried out in southern Italy from summer to winter, by comparing two arbuscular mycorrhizal fungi (AMF) based formulates (Rizotech Plus, Myco Apply DR) and a non-inoculated control, in factorial combination with four soil electrical conductivities (1.5, 3.0, 4.5, 6.0 mS·cm^{-1} EC). The highest root colonization was 83% at 3.0 mS·cm^{-1} under AMF-based treatments and 34% at 1.5 mS·cm^{-1} in non-treated control; the latter attained lower values than AMF treatments at any soil EC. Harvest occurred 3.5 days earlier in control plants, six days earlier under 6.0 mS·cm^{-1} EC compared to 1.5 mS·cm^{-1}. The inoculated plants always showed higher yield than the control ones and the highest production at 4.5 mS·cm^{-1} EC; control plants attained the highest yield under 3.0–4.5 mS·cm^{-1} EC. The highest values of most fruit quality indicators, mineral elements and antioxidant compounds and activity were recorded under AMF-based formulates inoculation and 6.0 mS·cm^{-1} soil EC. Beneficial microorganisms proved to be an effective environmentally friendly tool for improving tomato yield and quality performances in both normal and soil salinity conditions.

Keywords: *Solanum lycopersicum* L. 'plum' type; root colonization; organic acids; antioxidant content and activity

1. Introduction

Tomato (*Solanum lycopersicum* L.) is the most spread vegetable species worldwide [1], mainly cultivated in Asia (China, India, Turkey, Iran), Africa (Nigeria, Egypt), United States and Europe (Italy, Spain) and is rich in macronutrients, trace-elements and antioxidants [2]. Crop performances of tomato are affected by farming management, which should promote efficient plant development as well as valuable fruit production, quality and antioxidant properties. The latter aspects are key goals of

low environmental impact strategies, within which plant inoculation with beneficial microorganisms is a useful tool for preventing or reducing abiotic and biotic stresses [3]. Arbuscular mycorrhizal fungi (AMF) form symbiotic association with plants, thus changing their nutritional, biochemical and physiological status [4] and showing positive effects on vegetable crops both in open field and greenhouse [5]. In this respect, AMF play an active role in plant nutrition due to their ability to improve mineral element uptake and plant growth, particularly phosphorus (P) which is an essential but low mobile nutrient [6]. Indeed, they interact with mineral and organic fertilizers, increasing phosphorus availability and improving plant physiological conditions, particularly in high salinity conditions causing osmotic stress and toxicity of some ions [7].

AMF inoculation results in the increase of P uptake, tomato plant growth and biomass under low P availability [8,9] and of P content in all tissues [10,11]. However, high fertilizer doses may reduce the mycorrhizal abundance [12] whose effectiveness on yield depends on cultivar or ecotype [13,14], soil P concentration [15], mycorrhizal species [16].

According to Walder and van der Heijden [17], the efficiency of the mycorrhizal symbiosis depends on the association specificity, reciprocity and multi-functionality, and the lifetime fitness should also be taken into account as the behaviour of symbionts may be antagonistic at an earlier life-stage but mutualistic at a later stage; the AMF-plant symbiosis is also influenced by other factors including the environment and the ability to exploit resources. In particular, most AMF have the ability to enhance plant growth and nutrition, and, in this respect, the beneficial effects are strongly dependent on the availability of phosphorus and nitrogen in the soil; in phosphorus-limited soils, AM fungi have been shown to be beneficial to plants, whereas in nitrogen-limited soils, the same AM fungi can suppress growth. Indeed, in nature, plant roots are usually colonized simultaneously by AMF and other beneficial microorganisms belonging to different species, thus forming highly complex partnerships with intense resource exchange activity. In this respect, the co-inoculation of AMF and plant growth promoting bacteria targets to join the different benefits provided by the single microorganisms to plants, leading to yield increase and fruit quality modulation [18].

Though Juniper and Abbott [19] reported the negative effect of soil salinity excess on the growth of AMF hyphae, contrastingly, in research on tomato, cucumber and lettuce [20,21] as well as on olive, apple and Citrus trees [22–24], the plant performances under salt stress conditions were enhanced by AMF inoculation. Indeed, the latter fungi encourage salt tolerance in the colonized plants by increasing water and nutrient uptake, K^+/Na^+ ratio, osmoregulators synthesis, photosynthesis rate, water use efficiency [20]. Under mild salt stress conditions, pot-grown *Arundo donax* plants showed a reduction of photosynthesis and growth, even in symbiosis with AMF such as *Funneliformis mosseae* and *Rhizophagus irregularis*, though the AM fungi elicited some plant metabolic changes consisting of proline and H_2O_2 increase and higher isoprene emission [25]. Sánchez et al. [26] reported that the effects of salinity and water stress on the degree of leaf stomatal closure and photosynthesis reduction in *A. donax* is also dependent on the ecotype. Salt stress is a major cause of water stress and in this respect, Chitarra et al. [27] reported that AMF enhance the tomato leaf stomatal density, which is positively correlated with the plant CO_2 absorption capacity, photosynthetic rate and relative water use efficiency. In addition, the inoculation with *Glomus deserticola*, *Claroideoglomus etunicatum* and *Funneliformis mosseae* both in dicotyledonous and monocotyledonous plants showed a 24% higher effect on stomatal conductance to water vapor than non-mycorrhizal (NM) control and a 100% higher effect during moderate water deficit or over 400% under severe water stress compared to optimally watered conditions [28]. Indeed, the fungi hyphae can capillary explore wider soil volume than the root hair, thus allowing the plants to uptake water even under severe deficit conditions; in addition, the more the root colonization augments the more the stomatal conductance to water vapor increases up to 10 fold compared to lightly colonized plants [28]. Moreover, changes in cell wall composition of both roots and fruits may also be elicited by the inoculation of tomato plants with *Funneliformis mosseae* [29].

Chitarra et al. [30] showed that AMF symbiosis positively affects the tolerance to water deficit in tomato, by improving water use efficiency and, indeed, AMF-colonized plants better withstood

severe water stress conditions (-1.3 MPa), as witnessed by the lower ABA content in roots and leaves compared to non-colonized plants. The inoculation with the *Pseudomonas chlororaphis* also encouraged tomato plant tolerance to mild water stress by enhancing antioxidant activity and proline content and by limiting the accumulation of reactive oxygen species [31]. Moreover, *P. chlororaphis* increased the ABA level in leaves of water-stressed plants, with a consequent improvement of stomatal closure modulation and water use efficiency (WUE) and biomass accumulation. Volpe et al. [32] found that tomato plants inoculated with AMF show different patterns of adaptation to environmental stresses depending on the fungus species: *Funneliformis mosseae* elicited the production of volatile organic compound production and *Rhizophagus intraradices* resulted in a higher water use efficiency under severe water stress.

The present research aimed to assess the ability of beneficial microorganisms to valorise tomato crop nutrition by encouraging the plants to tolerate as high a soil electrical conductivity as possible, thus improving fruit production and quality. In this respect, the interaction between AMF-based formulates and different soil electrical conductivities induced by fertigation with balanced nutrient solutions was assessed on yield, quality, chemical composition and antioxidants content of tomato "plum" fruits grown in Mediterranean climate.

2. Materials and Methods

2.1. Experimental Protocol and Growing Conditions

Research was carried out in 2016-17 and 2017-18 on tomato "plum" (*Solanum lycopersicum* L., cultivar Pixel F_1) grown in greenhouse, at the Experimental Centre of the Department of Agricultural Sciences, University Federico II of Naples, Italy (40°49′ N, 14°20′ E, 63 m above sea level) in the Mediterranean climate. Seedlings were transplanted on 22 August, in both 2016 and 2017, in plastic pots of 24 cm diameter filled with sandy-loam soil and perlite (10% in volume), placed on 10 cm-thick polystyrene sheets, with a density of 4 plants per m^2. The crops were grown under a three-span polytunnel, each span being 5 m wide, 2 and 3.5 m high at wall and roof respectively. The trend of temperature in the greenhouse is shown in Figure 1 as ten-day mean values from transplant to the end of harvests and as an average of 2016-17 and 2017-18 since the year of research did not significantly affect the variables examined.

Comparisons were made between two arbuscular mycorrhizal fungi (AMF) based formulates (Rizotech Plus, Myco Apply DR) and a non-inoculated control, in factorial combination with four soil electrical conductivities (1.5, 3.0, 4.5, 6.0 mS·cm^{-1} EC). A split plot design was used with three replicates and each treatment covered a 4.5 m^2 surface area.

Both AMF-based formulates predominantly contain *Claroideoglomus etunicatum*, *Funneliformis mosseae*, *Glomus aggregatum*, *Rhizophagus intraradices* (10% in Rizotech Plus and 1% in Myco Apply DR) and, in addition, fungi and bacteria species belonging to genera *Trichoderma*, *Streptomyces*, *Bacillus*, *Pseudomonas* (10^3 UFC·g^{-1} in Rizotech Plus and $2 \cdot 10^6$ UFC·g^{-1} in Myco Apply DR). Notably, Rizotech powder was applied in the soil holes made for seedling transplant, whereas Myco Apply was supplied to the soil as a water solution upon transplanting. The four soil ECs examined (1.5 to 6.0 mS·cm^{-1}) were carried out by supplying nutrient solutions with EC ranging from 1.2 to 4.8 mS·cm^{-1} and pH 6.0, by using drip irrigation method with 2 L·min^{-1} emitters. The ratios between the concentrations (mg·L^{-1}) of N, P, K, Ca, Mg, S in the nutrient solutions were 1.0:0.4:1.4:1.1:0.4:0.4; the microelement concentration (μmol·L^{-1}) was constant in the four nutrient solutions: 35.0 Fe; 1.8 Cu; 24.0 Mn; 11.0 Zn; 82.0 B; 1.0 Mo.
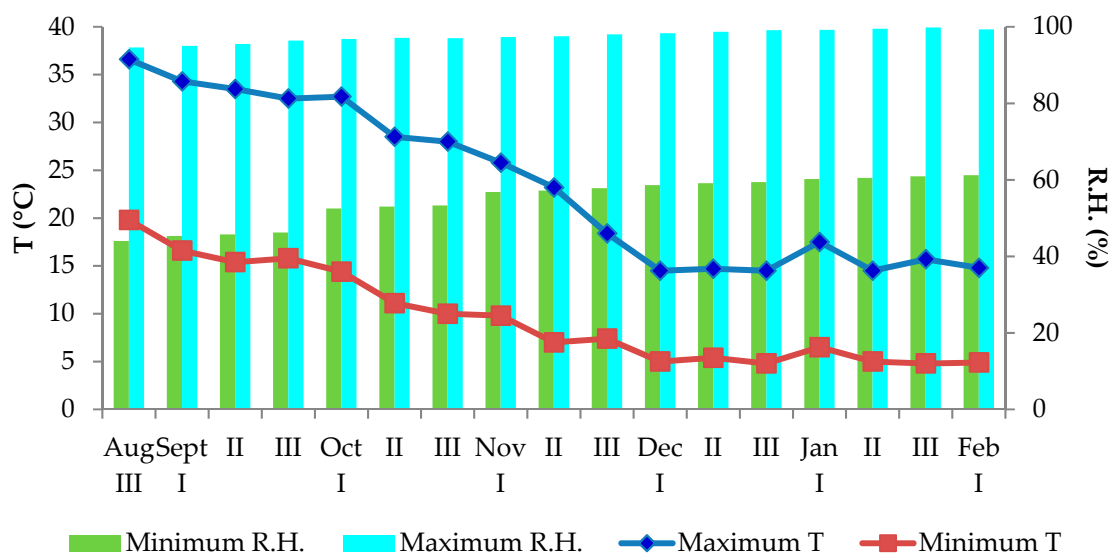
Figure 1. Ten-day means of air temperature (T, °C) and relative humidity (R.H., %) in greenhouse in Portici (Naples, southern Italy) from August to February, as an average of 2016–2017 and 2017–2018.

2.2. Determinations of Root Mycorrhizal Colonization, Plant Growth Indices and Yield Components

The mycorrhizal colonization was assessed twice, sixty days after transplant and at the crop end, according to Trouvelot et al. [33] by calculating the frequency of mycorrhization (%). In this respect, 30 randomly chosen 1 cm-long pieces were cut from the root apparatus of 5 plants per treatment and cleared in 10% KOH for 45 min at 60 °C, stained with 1% methyl blue in lactic acid and mounted on a slide.

At the end of the crop cycles, the following growth determinations were performed on plants taken from all plots: the maximum leaf area, using a bench top electronic leaf area meter (Li-Cor 3000, Li-Cor, Lincoln, NE, USA); the aboveground dry biomass in an oven at 70 °C until constant weight.

Fruit harvest began on 25 or 27 October in 2016 and 2017 respectively and ended on 1 or 5 February in 2017 and 2018 respectively; during this time interval, five ripe fruit trusses were picked up from each plant and, in coincidence with each harvest, total weight, number and mean weight of marketable fruits (regular-shaped and undamaged) from 15 plants per treatment were assessed.

2.3. Determinations of Fruit Quality, Mineral Composition and Antioxidant Compounds and Activity

Fruit samples were collected at the second truss harvest (6 and 9 November in 2017 and 2018 respectively) and transferred to the laboratory, where determinations of dry residue, soluble solids, organic acids (malic, oxalic, citric, isocitric), minerals (K, Ca, Mg, Na, P, S, NO$_3$, Cl), lycopene, total phenols, total ascorbic acid, lipophilic and hydrophilic antioxidant activities were performed by using the following procedures: The dry residue was assessed in oven at 70 °C until constant weight; the soluble solids (°Brix) at 20 °C on the supernatant obtained by centrifuging the raw homogenate, using a digital refractometer by Bellingham and Stanley, model RFM 81. The organic acids were determined as previously described [34]. The mineral elements were assessed according to Rouphael et al. [35]. Lycopene determination was performed referring to De Sio et al. [36]; total phenols and ascorbic acid, as described by Golubkina et al. [37]. The antioxidant activity was assessed according to Brand-Williams et al. [38].

2.4. Statistical Processing

The data statistical processing was performed by the two-way analysis of variance and mean separation through Tukey's test with reference to 0.05 probability level, using SPSS software version 21. Data expressed as a percentage underwent angular transformation before processing. The variables

examined in our research were not significantly affected by the research year and, therefore, only mean data of the two years are reported.

3. Results and Discussion

3.1. Root Mycorrhizal Colonization

The frequency of root fragments colonized by mycorrhizal hyphae in tomato plants did not significantly change between the two determinations performed sixty and eighty-four days after transplant and therefore only their mean values are shown in Figure 1. This index was significantly affected by the interaction between AMF-based formulate and soil EC (Figure 2): the increase of nutrient availability in the soil from 1.5 to 3.0 mS·cm^{-1} EC enhanced the mycorrhizal root colonization from 60% to 83% in plants inoculated with AMF; interestingly, the further soil enrichment with balanced nutrient solution up to 6.0 mS·cm^{-1} EC did not inhibit the root colonization by mycorrhizal hyphae. In non-treated plants, soil salinity over 1.5 mS·cm^{-1} EC impaired the symbiotic relationship between AMF and tomato roots, which was lowest at 6.0 mS·cm^{-1} EC (26%). The percentages of colonization recorded in the present research in the roots of tomato plants inoculated with AMF-based consortia fell in the middle of the range including reports from other authors relevant to the same species. Indeed, a 55.7% and 63% root colonization were recorded upon the inoculation with *Funneliformis mosseae* and *Claroideoglomus etunicatum* respectively, in a clay-silty soil in the Mediterranean region of Adana, Turkey [39]. The 84.1% and 100% of roots were colonized by mycorrhizal hyphae upon AMF inoculation in a silty-loam soil along Basilicata coast (southern Italy), compared to 51% and 92.6% of non-inoculated control, at mid and end growing season respectively [40]. Contrary to the conditions of soil medium-high phosphorus content set up in our research, Kowalska et al. [9] recorded the 51% of root colonization induced by AMF application under low P availability in Poland.
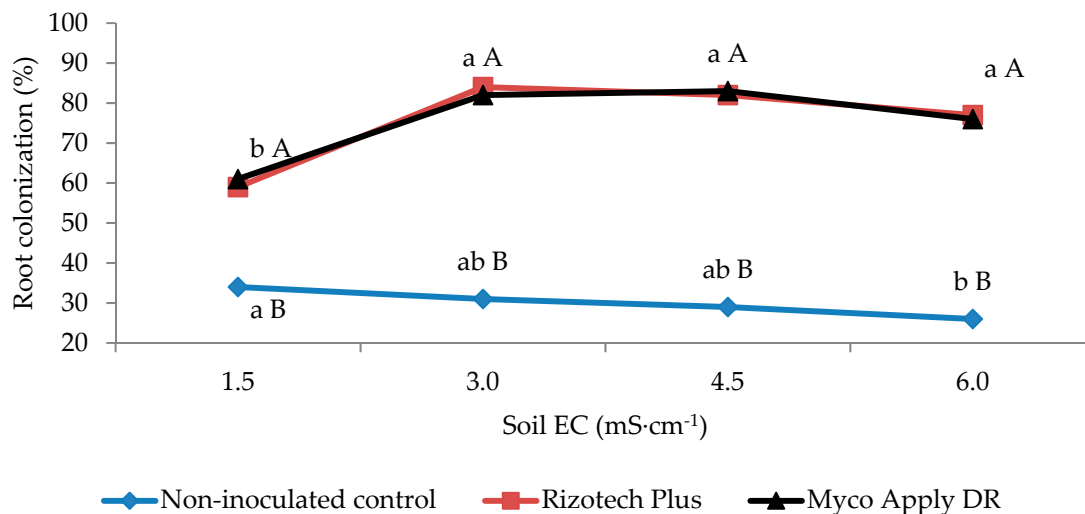

Figure 2. Interaction between arbuscular mycorrhizal fungi (AMF)-based formulate and soil electrical conductivity (EC) on tomato root colonization (%). Different letters mean significant difference in the comparison between soil ECs (lowercase letters) or between AMF-based formulates (capital letters), according to Tukey's test at $p \leq 0.05$.

3.2. Plant Growth and Yield

The harvest of the first fruit truss began 64 days after transplant in the non-inoculated plants and 3.5 days later in those treated with mycorrhizal-based formulates (Table 1).

Regarding soil EC (Table 1), the earliest fruit ripeness was elicited by the highest EC (6 mS·cm^{-1}), whereas the most diluted soil solution (1.5 mS·cm^{-1}) caused a six-day delay.

The described physiological behaviour is explainable by the fact that the inoculated plants showed a more enhanced vigour compared to control ones, with higher leaf surface area and total dry matter (0.38 m^2 and 89.7 g per plant vs 0.34 and 58.7) and this resulted in a longer vegetative phase with consequent delay in fruit ripeness. The plants grown in the most diluted soil solution had the smallest leaf expansion and dry matter accumulation (0.33 m^2 and 47.7 g respectively), whereas those subjected to 4.5 mS·cm^{-1} the highest (0.40 m^2 and 107.2 g respectively).

Balestrini et al. [41] reported that, after 3 months of greenhouse rearing, no difference in grapevine plant growth was recorded between the inoculation with sole *Funneliformis mosseae* and with an AMF-PGPB consortium containing *Trichoderma* spp., *Pochonia chlamidosporia*, *Streptomyces* spp., *Bacillus subtilis*, *Pseudomonas* spp., *Funneliformis mosseae*, *Glomus* spp. After 3 months, the roots of the plants inoculated with the mixed inoculums showed a low frequency of AMF colonization (2.6%) compared to the high colonization frequency in the roots inoculated solely with *Funneliformis mosseae* (80.7%). Moreover, several AM marker genes were upregulated upon the *F. mosseae* treatment, with the mixed inoculum however eliciting an important transcriptional regulation. The expression of the genes associated to nutrient transport, transcription factors and cell wall was significantly but differently affected by both the two treatments.

The crop system plays a major role in determining the effect of AMF on the growth of plants which, in particular, may benefit from mycorrhizal contribution to valorise the nutrient supply [22]. Indeed, the increase of soil EC by enhancing the salt concentration of the balanced nutrient solutions applied through fertigation may improve yield and produce quality up to a salinity threshold depending on both the genotype and the growing management [23]. Among the latter, soil P availability is a key factor [42] and, in this respect, in our research the effect of AMF application increased with the increase of nutrient uptake including P up to 4.5 mS·cm^{-1}, whereas other authors [43] reported that the effect of AMF is independent on soil P content.

The fruit yield was significantly affected by the interaction between AMF-based formulate and soil EC (Figure 3). Indeed, both the 3.0 and 4.5 mS·cm^{-1} soil ECs resulted in the highest production of non-inoculated plants, whereas the 4.5 mS·cm^{-1} EC led to the best performances of those treated with beneficial microorganisms, independently on mycorrhizal formulate. However, both in mycorrhized plants and in non-inoculated ones a yield reduction was recorded when the 6.0 mS·cm^{-1} EC treatment was applied. Moreover, AMF inoculation was more effective than non-treated control on yield at any soil EC but there were no significant differences between the two formulates (Figure 3).

Table 1. Tomato precocity, growth indices and yield components as affected by mycorrhizal-based formulate and soil electrical conductivity.

Experimental Treatment	Precocity Days from Transplant to First Harvest	Leaf Area m^2 per Plant	Dry Matter g per Plant	Marketable Fruits Yield (g per Plant)	Number	Mean Weight (g)
Mycorrhizal-based formulate						
Rizotech	69.5 a	0.37 a	58.7 b	520.8 a	21.6 a	24.0 a
Myco Apply	69.5 a	0.38 a	88.8 a	536.2 a	22.0 a	24.3 a
Non-inoculated control	66.0 b	0.34 b	90.5 a	395.8 b	18.2 b	21.7 b
Soil electrical conductivity						
1.5 mS·cm^{-1}	71.3 a	0.33 c	47.7 c	373.6 c	17.5 c	21.3 c
3.0 mS·cm^{-1}	69.7 ab	0.37 ab	79.1 b	509.4 b	21.2 b	24.0 ab
4.5 mS·cm^{-1}	67.0 bc	0.40 a	107.2 a	568.0 a	22.8 a	24.7 a
6.0 mS·cm^{-1}	65.3 c	0.36 bc	83.3 b	485.9 b	20.8 b	23.2 b

Within each column, means followed by different letters are significantly different according to Tukey's test at $p \leq 0.05$.

The fruit yield was significantly enhanced by AMF-based formulate application that indeed promoted a higher establishment of fruits as well as higher mean weight, compared to non-inoculated control; the two production components were also significantly affected by the interaction between AMF-based formulate and soil EC, showing similar trends as yield (Figure 3).
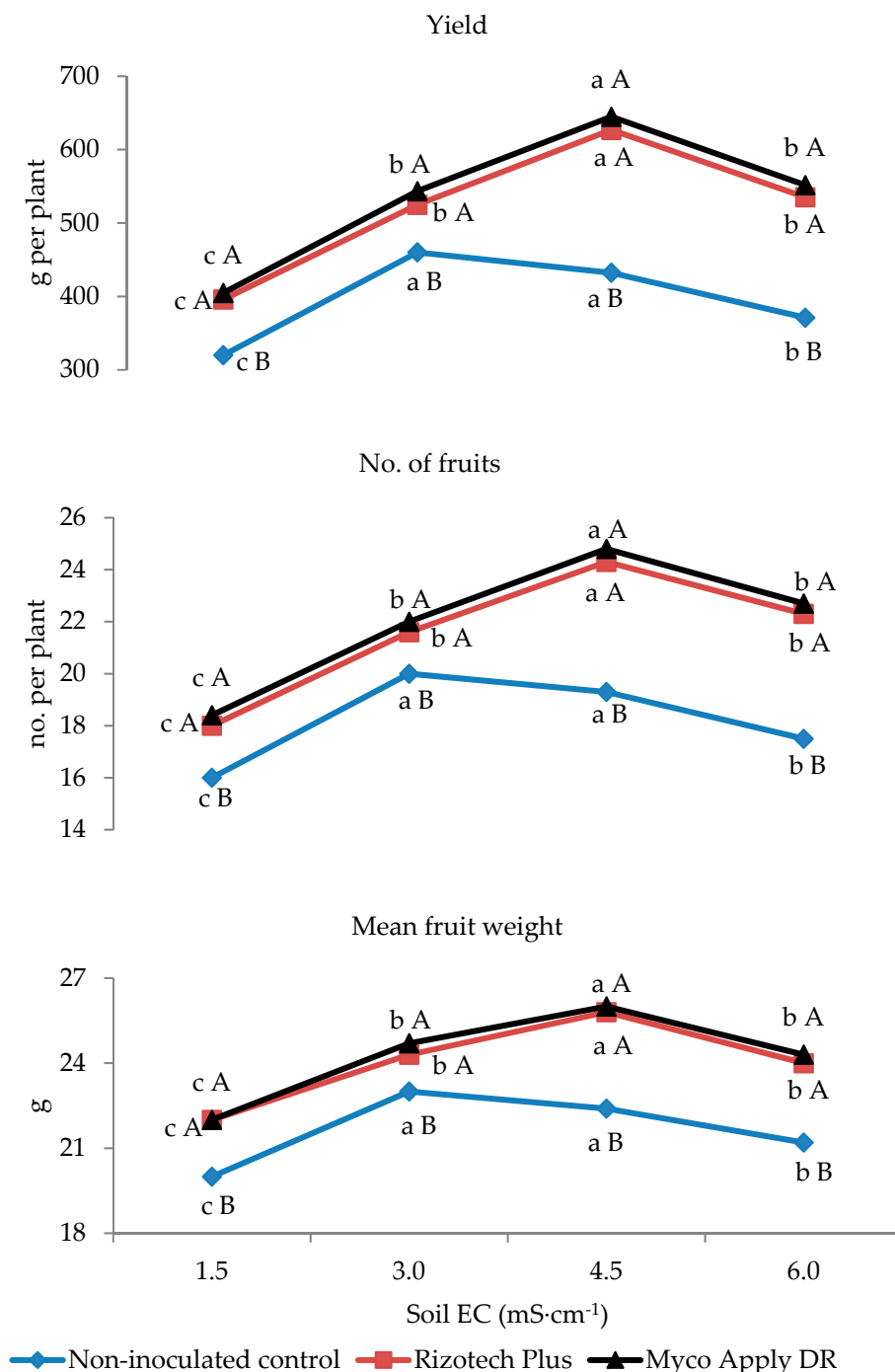

Figure 3. Interaction between AMF-based formulate and soil EC on yield performances of tomato 'plum' Pixel F_1. Different letters mean significant difference in the comparison between soil ECs (lowercase letters) or between AMF-based formulates (capital letters), according to Tukey's test at $p \leq 0.05$.

In the present research, the soil EC values best enhancing fruit number and mean weight resulted accordingly in the highest yield. In this respect, the AMF-based formulate application allowed the plants to better tolerate the salinity increase, thus valorising the fertigation up to 4.5 mS·cm^{-1} soil EC,

which corresponded to the highest production; whereas the non-inoculated plants suffered from mild salt stress over 3.0 mS·cm^{-1}, showing a tendency to yield decrease. However, the production of both the mycorrhized and control plants was depressed at 6 mS·cm^{-1} EC, as a consequence of plant adaptation to water stress through vegetative growth reduction [44]. As a confirmation of the importance of genotype and crop system on plant tolerance to salinity, different findings compared to our results arose from previous research: a production drop with higher than 2.5 mS·cm^{-1} nutrient solution EC, made of either balanced element composition or sodium chloride addition [45]; no negative effects of nutrient solution EC increase from 4 to 7 dS·m^{-1} [46].

The benefits from the application of mixed microorganisms inocula can be targeted to improve crop performances by enhancing the fertigation as carried out in the present study or to valorise the AMF ability to enhance the plant nutrient use efficiency by reducing the fertilization rate. The latter goal was achieved by Bona et al. [18], who recorded higher tomato mean fruit weight upon the co-inoculation of AMF and *Pseudomonas fluorescens* C7 or *Pseudomonas* sp. 19 Fv1T along with 30% reduction of the traditional fertilization, compared to the controls with full or 30% reduced fertilization; the latter effect stemmed from the significant interaction between the arbuscular mycorrhizal fungi and the bacteria applied, as the co-inoculation was more effective than the inoculation with the sole AMF or with the sole *Pseudomonas*. However, in another investigation on tomato [40] a mixed AMF-based inoculum did not result in better yield than the sole AMF inoculation, which witnessed the prevailing effect of arbuscular mycorrhizal fungi on plant response; a 10.8% production increase upon the beneficial microorganism application was recorded in comparison with the non-inoculated control, as a consequence of the higher fruit number. Consistently with our results, in research carried out on lettuce and zucchini [8], the co-inoculation with *Rhizophagus intraradices* and *Trichoderma atroviride* led to a yield increase compared to non-inoculated control; indeed, the beneficial microorganisms showed a synergic effect in enhancing the uptake of both macronutrients (N, P, K, Mg) and micronutrients (Fe, Mn, Zn and B) and better promoted plant growth compared to the inoculation with the sole *R. intraradices* and *T. atroviride*.

The intensification of activity and biomass of soil microbial community [47] resulted in improving the nutrient absorption efficiency, thus increasing the fruit number, weight and yield [48]. In particular, the yield increase promoted by AMF inoculation is connected to the biostimulant action of these fungi on plant uptake and growth [16,49], which is the consequence of eliciting the root auxin production in mycorrhized plants [50]. In this respect, plant growth and yield are dependent on the nutrient availability during the phenological development and, indeed, in previous research [51,52] the effect of AMF inoculation was emphasized by soil P deficiency. Similarly, in Mediterranean field conditions, Rafique and Ortas [39] recorded the increase of tomato yield by as much as 37.8% and 76.1% upon the inoculation of *Funneliformis mosseae* and *Claroideoglomus etunicatum* respectively with no P supply, whereas with the soil application of 100 kg·ha^{-1} P the production was only 29.1% and 6.8% higher compared to the non-inoculated control; moreover, a 31.8% increase of plant P uptake was recorded only with no P supply. However, consistently with the production increase which in the present investigation has been connected to the enhanced nutrient supply up to a certain threshold, in previous study [53] the number of flowers and fruits in tomato was encouraged by phosphorus availability, thus leading to yield increase; in this respect, Mahanta et al. [54] found a positive correlation between P and production.

3.3. Fruit Quality, Mineral Composition and Antioxidant Compounds and Activity

The fruit quality indicators examined, that is, dry residue, soluble solids and organic acids were significantly affected by AMF application to rhizosphere. In fact, these parameters attained higher values in the fruits obtained from inoculated plants, as compared to control ones but the two AMF-based formulates did not differ from each other (Table 2).

Consistently with our findings, in previous research [18] a higher dry residue was recorded in tomato fruits harvested from plants co-inoculated with AMF and *Pseudomonas* sp. The increase of

soluble solids but not of dry residue was recorded in shallot bulbs upon the application of a mixed beneficial microorganism formulate [55]. Contrastingly to the results of the present investigation, Candido et al. [40] did not record significant differences between AMF-inoculated and control plants in terms of tomato fruit dry weight and soluble solids.

The increase of soil EC from 1.5 to 6.0 mS·cm^{-1} enhanced the fruit quality attributes (Table 2). Presumably, the highest availability of nutrients corresponding to the highest salt concentrations led to more enhanced accumulation in tomato fruits [52] and, accordingly, to higher dry residue and soluble solids, the latter being notoriously correlated to sugar content. Similar effects of salinity on fruit quality properties also arose in previous research [52] and, in particular, Adams and Ho [56] recorded enhancement in sugars as an effect of applied salinity increase. However, in other investigations fruit quality worsening was detected with over 5 mS·cm^{-1} EC [57] or sugar content lowering caused by the fruit respiration enhancement under salt stress [58].

Table 2. Quality indicators of tomato fruits as affected by mycorrhizal-based formulate and soil electrical conductivity.

Experimental Treatment	Dry Residue (%)	Soluble Solids (°Brix)	Organic Acids (g·kg^{-1} d.w.)			
			Malic	Oxalic	Citric	Isocitric
Mycorrhizal-based Formulate						
Rizotech	8.9 a	7.6 a	5.7 a	2.2 a	40.1 a	0.71 a
Myco Apply	8.9 a	7.6 a	6.0 a	2.4 a	41.6 a	0.70 a
Non-inoculated control	8.5 b	7.3 b	4.6 b	1.5 b	31.5 b	0.49 b
Soil electrical Conductivity						
1.5 mS·cm^{-1}	8.2 c	7.0 d	4.2 d	1.2 d	31.7 d	0.49 c
3.0 mS·cm^{-1}	8.5 c	7.3 c	4.8 c	1.7 c	35.3 c	0.55 c
4.5 mS·cm^{-1}	9.0 b	7.7 b	5.7 b	2.2 b	39.1 b	0.66 b
6.0 mS·cm^{-1}	9.4 a	8.0 a	7.0 a	2.9 a	44.8 a	0.83 a

d.w.: dry weight. Within each column, means followed by different letters are significantly different according to Tukey's test at $p \leq 0.05$.

As for fruit mineral composition (Table 3), the application of AMF-based formulates resulted in higher content of K, Ca, Mg, P, S and NO$_3$ compared to the control, whereas Cl was not significantly affected by the beneficial microorganisms. Interestingly, the fruits produced by the mycorrhized plants showed a lower Na concentration than those obtained from the non-inoculated ones, presumably due to the concurrent higher accumulation of K, Ca and Mg.

Zouari et al. [59] found that the nutrient content of tomato fruits produced by mycorrhized plants under low fertilization is similar to that of fruits from non-inoculated plants grown in optimal nutrient conditions, which means that the use of AMF can reduce the negative environmental impact of mineral fertilizers. Moreover, 712 genes were found to be differentially expressed in fruits from mycorrhized or control plants. In particular, the fruits of mycorrhized plants showed genes characteristic of a climacteric fleshy fruit and genes related to mycorrhizal status, such as phosphate and sulphate transporters. In other research [60], plants inoculated with *Glomus intraradices* produced fruits with higher content of potassium, calcium, phosphorus and zinc compared to control plants. Consistently with our findings, Ndung'u Magiroi et al. [61] reported that the beneficial microorganism inoculation led to increased fruit content of calcium, potassium and magnesium, the latter soil exchangeable form being positively correlated with bacteria community solubilizing phosphorus. Indeed, the presence of AMF results in the siderophore release in the rhizosphere, which promotes P solubilization and availability for plants, thus leading to higher P concentration in plant tissues [62]. The high level of trehalose in mycorrhized plants could be the reason for high intracellular P concentration, which mobilizes polyphosphates [63]. Thompson et al. [64] also recorded an improved P status in tomato upon the inoculation with *Funneliformis mosseae* under field conditions and low soil phosphorus.

Other authors [65] reported that the composition of soil microbial community can contribute to nutrient accumulation in plants more than the microbial biomass.

Table 3. Mineral composition of tomato fruits as affected by mycorrhizal-based formulate and soil electrical conductivity.

Experimental Treatment	K	Ca	Mg	Na	P	S	NO$_3$	Cl
	g·kg^{-1} d.w.							
Mycorrhizal-Based Formulate								
Rizotech	32.4 a	0.66 a	1.31 a	0.60 b	1.06 a	0.60 a	0.17 a	5.46
Myco Apply	32.5 a	0.70 a	1.32 a	0.63 b	1.00 a	0.64 a	0.18 a	5.43
Non-inoculated control	28.8 b	0.49 b	1.06 b	0.69 a	0.64 b	0.36 b	0.09 b	5.42
								n.s.
Soil Electrical Conductivity								
1.5 mS·cm^{-1}	29.0 c	0.52 d	1.10 b	0.75 a	0.77 c	0.42 c	0.10 c	5.40
3.0 mS·cm^{-1}	30.4 bc	0.59 c	1.15 b	0.69 b	0.86 b	0.53 bc	0.11 c	5.47
4.5 mS·cm^{-1}	31.8 ab	0.65 b	1.32 a	0.60 c	0.93 b	0.57 b	0.17 b	5.32
6.0 mS·cm^{-1}	33.7 a	0.72 a	1.35 a	0.52 d	1.04 a	0.62 a	0.21 a	5.57
								n.s.

d.w., dry weight. Within each column, n.s. no statistically significant difference; means followed by different letters are significantly different according to Tukey's test at $p \leq 0.05$.

Similar to the quality indicators and mineral elements described above, both the antioxidant compounds and activities examined in tomato fruits were better affected by the beneficial microorganism inoculation compared to non-treated control, but no significant difference was recorded between the two AMF-based formulates (Table 4).

Consistently with our findings, in previous research the content of lycopene in tomato fruits was enhanced by the inoculation of *Glomus intraradices* [60], *Funneliformis mosseae* or *Rhizophagus irregularis* [10].

A positive effect of beneficial microorganisms was recorded on ascorbic acid and polyphenols with increasing nitrogen [66], whereas conversely Le Bot et al. [67] reported the polyphenols synthesis limitation caused by nitrogen increase in soil solution. Other authors [68] reported the effect of *Glomus intraradices* inoculation in enhancing the phenolic profile of rosemary leaves. Amanifar et al. [69] recorded the increased antioxidant synthesis promoted by *Funneliforms mosseae* in liquorice under salt conditions, compared to non-inoculated control. Unlike the results of the present research, Nzanza et al. [70] did not detect overall benefits for antioxidant compounds or the activity of tomato fruits upon inoculation with *Trichoderma harzianum* and *Glomus mosseae*.

Soil salinity also had a significant effect on the fruit antioxidant status, as the values of all the variables (lycopene, phenols and ascorbic acid as well as hydrophilic and lipophilic antioxidant activities) increased from 1.5 to 6.0 mS·cm^{-1} EC. Consistently with our results, in previous investigation [71] similar trends were recorded for lycopene as a response to salinity. Navarro et al. [72] reported that the use of a moderately saline water was beneficial to pepper red fruits by increasing both the hydrophilic and lipophilic antioxidant activities but it did not affect the concentration of lycopene, ascorbic acid and total phenolics; the two latter antioxidant compounds along with carotenoids and α-tocopherol were positively affected up to 4.1-4.4 mS·cm^{-1} EC in green pepper type [73].

Table 4. Antioxidant content and activity in tomato fruits as affected by mycorrhizal-based formulate and soil electrical conductivity.

	Lycopene mg·100 g^{-1} f.w.	Total Phenols mg Gallic Acid·100 g^{-1} d.w.	Ascorbic Acid mg·100 g^{-1} f.w.	Lipophilic Antioxidant Activity mmol Trolox eq·100 g^{-1} d.w.	Hydrophilic Antioxidant Activity mmol Ascorbic Acid eq·100 g^{-1} d.w.
		Mycorrhizal-based formulate			
Rizotech	338.2 a	1.93 a	18.5 a	10.2 a	8.6 a
Myco Apply	350.0 a	2.04 a	20.4 a	11.3 a	9.0 a
Non-inoculated control	285.6 b	1.63 b	14.4 b	7.5 b	7.8 b
		Soil electrical conductivity			
1.5 mS·cm^{-1}	207.7 d	1.75 b	11.0 d	7.5 d	7.4 c
3.0 mS·cm^{-1}	297.3 c	1.80 b	15.3 c	9.1 c	8.2 b
4.5 mS·cm^{-1}	360.6 b	1.95 a	20.6 b	10.0 b	8.8 ab
6.0 mS·cm^{-1}	432.9 a	1.97 a	24.2 a	11.9 a	9.4 a

f.w., fresh weight; d.w., dry weight. Within each column, means followed by different letters are significantly different according to Tukey's test at $p \leq 0.05$.

4. Conclusions

From research carried out in southern Italy, enhancement of tomato fruit yield, quality, mineral composition and antioxidant status arose upon the application of arbuscular mycorrhizal fungi (AMF) based formulates to plants grown in saline soils (1.5 mS·cm^{-1} to 6.0 mS·cm^{-1}). Taking into account both the higher consumer expectations for healthy products and the current policies oriented to environmentally friendly crop systems, the use of beneficial microorganisms represents an effective and eco-compatible farming technique aiming to reduce chemical inputs, even more under salt stress conditions.

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