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Impact of *Arbuscular mycorrhizal* Fungal Strains Isolated from Soil on the Growth, Yield, and Fruit Quality of Tomato Plants under Different Fertilization Regimens

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Abstract: *Arbuscular mycorrhizal* fungi (AMF) have emerged as a promising and environmentally friendly solution for sustainable agriculture, offering a reduction in dependence on chemical inputs. The objective of this greenhouse experiment was to assess the efficacy of a natural endomycorrhizal inoculum obtained from leek root fragments, which acted as a trap plant to capture indigenous fungal spores present in the soil of the Guercif region in Morocco. The investigation aimed to comprehensively evaluate the influence of this inoculum on various parameters related to tomato plant growth, yield, and sensory quality. Additionally, different levels of chemical fertilizers, equivalent to 50%, 75%, and 100% of the recommended dosage, were administered in combination with or without the inoculum. The findings elucidated significant advantages associated with mycorrhizal inoculation. The plants subjected to inoculation exhibited increased plant height, augmented leaf and root dry weights, and improved nutrient uptake compared to the control group. Notably, tomato plants treated with 75% of the recommended chemical fertilizer dosage yielded the highest crop production, with no statistically significant difference observed when compared to those receiving the full dosage (100%). Intriguingly, tomato plants grown in substrates receiving 50% chemical fertilizers demonstrated the highest levels of mycorrhization, exhibiting a frequency (F) of 100% and an intensity (M) of 63%. Importantly, the combination of inoculation with a reduced dose of NPK fertilizer (50% of the recommended amount) resulted in significantly elevated concentrations of calcium (Ca), potassium (K), iron (Fe), zinc (Zn), and phosphorus (P) in the plants, attributable to the heightened mycorrhizal colonization of the roots. In terms of fruit characteristics, no significant variations were detected in pH and electrical conductivity (EC) among the treatment groups. However, the inoculated plants exhibited a notable increase in the Brix index, an indicator of sweetness, compared to the control group across all fertilizer doses. Furthermore, inoculation positively influenced the levels of total carotenoids in the fruits. Remarkably, the values of these compounds in the inoculated plants subjected to 50% of the recommended fertilizer dosage surpassed those recorded in the non-inoculated plants receiving the full dosage.

Keywords: *Arbuscular mycorrhizal* fungi; sustainable agriculture; endomycorrhizal inoculum; tomato growth; yield; fruit quality; fertilization levels

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1. Introduction

The tomato (*Solanum lycopersicum* L.) holds a prominent position among vegetables globally, with its cultivation spanning across 172 countries and territories. As reported by FAOSTAT [1], an extensive 4.76 million hectares are devoted to tomato cultivation annually, resulting in an astonishing production of approximately 3783.8 million tonnes. Leading tomato producers include China, India, the USA, Turkey, Egypt, Iran, Italy, Spain, and Brazil [1].

Remarkably, this crop necessitates substantial amounts of nitrogen, phosphorus, and potassium (NPK) for achieving optimal yields [2]. However, the excessive utilization of chemical fertilizers has engendered significant environmental repercussions, including groundwater contamination and soil degradation. These consequences have disrupted the microbial populations and diversity within the soil, thereby exerting a direct influence on plant growth [3].

In response to these pressing concerns, contemporary agronomic research aims to address the challenges by harnessing the intricate biological processes involving beneficial microorganisms, with a specific emphasis on *Arbuscular mycorrhizal* fungi (AMF). AMF have been extensively documented in the scientific literature to establish mutualistic associations with the roots of approximately 80% of plant species [4–8]. These symbiotic fungi rely on plant-derived carbohydrates and lipids to fuel their growth, while efficiently absorbing essential mineral nutrients from the soil and delivering them to their host plants, particularly under unfavorable conditions such as biotic and abiotic stresses [9–11]. This harmonious alliance not only fulfills the growth requirements of the plants but also significantly enhances their photosynthetic efficiency, overall development, and growth, ultimately optimizing the productivity and biomass of the host plants [12]. As a result, mycorrhizal symbiosis has emerged as a crucial catalyst for productivity in agricultural ecosystems, effectively mitigating the adverse impacts of abiotic stresses, including mineral nutrient deficiencies and water limitations, on the host plants [13–15].

A multitude of previous studies have provided convincing evidence regarding the effectiveness of utilizing AMF inoculum either independently or in combination with NPK fertilizers or varying levels of phosphorus (P) to enhance plant growth, improve mineral nutrient uptake, and increase crop yield [16–19]. Building upon this extensive scientific knowledge, our primary objective was to thoroughly assess the beneficial outcomes of precisely controlled mycorrhization in the context of tomato cultivation, with the overarching goal of promoting a more productive and sustainable agriculture system while reducing reliance on chemical fertilizers. To accomplish this, we conducted a meticulous examination of mycorrhization efficiency using indigenous AMF strains carefully isolated from the fertile soils of the Guercif region in Morocco. Our experimental approach involved cultivating tomato plants in a controlled greenhouse environment, utilizing peat as a growth medium. The naturally sourced inoculum, obtained through the targeted trapping of roots, was skillfully combined with various doses of chemical fertilizers, striking a delicate balance between AMF biofertilization and NPK chemical fertilization. This careful equilibrium sought to optimize fruit production while effectively minimizing the use of chemical inputs within a harmonious and environmentally conscious farming system.

Therefore, the fundamental focus of our study revolved around conducting a rigorous investigation to examine the effects of integrating mycorrhization with different levels of NPK chemical fertilization on the intricate interactions among plant growth, yield, and the sensory characteristics of tomato fruits.

2. Materials and Methods

2.1. Mycorrhizal Inoculum and Experimental Design

The AMF inoculum utilized in this investigation consisted of a composite of strains obtained through trapping spores naturally present in the soil of the Guercif region of eastern Morocco. For this purpose, leek (*Allium ampeloprasum* var. *porrum*) was employed as the trap plant. It is an undemanding species with a high aptitude for root production,

and is also dependent on mycorrhization. However, spore trapping was carried out using the method described by Gerdeman and Nicolson (1963) [20], modified as follows: leek seeds were pre-germinated in a pot containing autoclaved peat for 20 min at 121 °C. Pots were filled halfway with sterilized peat. Spores extracted from Guercif soil were washed to remove as much soil and organic debris as possible. They were then poured onto the surface of the peat filled into the pots, which were then filled with peat and planted with pre-germinated leek seedlings.

After four months, the roots of leek plants, colonized by mycorrhizal fungi, were collected and processed into 2 mm fragments to create the fungal inoculum. At the time of seeding, alveolar boxes were half-filled with sterilized peat, followed by the deposition of a standardized quantity of 10 g of the fungal inoculum. The alveolar boxes were then filled with sterile peat and planted with tomato (*Solanum lycopersicum* L.) seed, var. Feiza F1 (Monsanto Holland B.V).

Following an incubation period of eight weeks, the root systems of the tomato seedlings were meticulously examined to evaluate the extent of mycorrhizal colonization. Subsequently, the seedlings were transplanted into plastic pots, each containing a standardized volume of seven liters of sterilized peat medium (the substrate was autoclaved for 20 min at 121 °C). With peat had the following characteristics: structure super fine; pH 6.1; salinity 1.2 g L^{-1}; N 210 mg L^{-1}; P,O 120 mg L^{-1}; K,O 260 mg L^{-1}; NPK balance 1-0.6-1.2.

The tomato plants were cultivated within a semi-controlled greenhouse environment, ensuring exposure to natural sunlight. In order to investigate the influence of different nutrient regimes on mycorrhizal and non-mycorrhizal tomato plants, three distinct NPK fertilization treatments were implemented: 50%, 75%, and 100% of the recommended dosage. One week after transplanting, the plants were fertilized weekly as described in Table 1 and watered if necessary.

Table 1. Fertilization inputs in the different treatments.

		I−, 100%			**I−, 75%**			**I−, 50%**			**I+, 100%**			**I+, 75%**			**I+, 50%**		
		VP	**F/L**	**H**	**VP**	**F/L**	**H**	**VP**	**F/L**	**H**	**VP**	**F/L**	**H**	**VP**	**F/L**	**H**	**VP**	**F/L**	**H**
Fertigation	N (g/plant)	10	5	5	7.5	3.75	3.75	5	2.5	2.5	10	5	5	7.5	3.75	3.75	5	2.5	2.5
	P_2O_5 (g/plant)	4	2	1.2	3	1.5	0.9	2	1	0.6	4	2	1.2	3	1.5	0.9	2	1	0.6
	k_2O (g/plant)	9	14	17	6.75	10.5	15.75	4.5	7	8.5	9	14	17	6.75	10.5	15.75	4.5	7	8.5
	CaO (g/plant)	6	7	11	4.5	5.25	8.25	1.8	3.5	5.5	6	7	11	4.5	5.25	8.25	1.8	3.5	5.5
	MgO (g/plant)	2	2	1.6	1.5	1.5	1.2	1	1	0.8	2	2	1.6	1.5	1.5	1.2	1	1	0.8

Plant treatments: I−, 100%: non-inoculated plants received 100% of dose recommended; I−, 75%: non-inoculated plants received 75% of dose recommended; I−, 50%: non-inoculated plants received 50% of dose recommended; I+, 100%: non-inoculated plants received 100% of dose recommended; I+, 75%: non-inoculated plants received 75% of dose recommended; I+, 50%: non-inoculated plants received 50% of dose recommended. Phenological phases of tomato plants: VP, vegetative phase; F, flowering to ripening; H, harvest.

During the growth period, diseases and insects were controlled according to standard, conventional practices.

These treatments facilitated a comparative assessment of the growth, yield, and sensory attributes of the tomato fruits.

2.2. Evaluation of Growth and Mycorrhization

2.2.1. Growth Parameters

After a four-month cultivation period, the influence of *Arbuscular mycorrhizal* fungi (AMF) inoculation and different levels of NPK fertilization on tomato plants was evaluated by quantifying parameters such as plant height, total yield per plant, and percentage dry weight of leaves and roots. Root and leaf dry weights were determined by oven drying (70 °C, 48 h) and the percentage of leaf and root dry weights was calculated.

2.2.2. Mycorrhization Parameters

To analyze the root samples of tomato plants, a meticulous procedure was followed. Thirty randomly chosen 1 cm-long pieces were cut from each root system and thoroughly washed to eliminate any extraneous material. Subsequently, they underwent a clearing process using a 10% KOH solution at a temperature of 90 °C for one hour. The medium was then acidified by adding a few drops of lactic acid and left for five minutes. Following this, the roots were stained with Trypan blue, following the methodology outlined by Phillips and Hayman [21]. Microscopic examination of the fungal colonization was performed according to the protocol established by Trouvelot et al. [22]. The frequency of mycorrhizal colonization (F%) and the intensity of mycorrhizal infection (M%) within the root system were quantified using the method described by Derkowska et al. [23]. Specifically, ten randomly selected root fragments, each measuring one centimeter in length, were mounted between a slide and coverslip and examined under a light microscope.

Frequency of mycorrhization

$$F\ \% = ((N - No)/N) \times 100$$

N = number of fragments observed and No = number of fragments with no trace of mycorrhization.

Intensity of mycorrhization

$$M\ \% = [(95 \times n5) + (70 \times n4) + (30 \times n3) + (5 \times n2) + n1]/N$$

where N: number of observed fragments; n represents fragments with an index of 0, 1, 2, 3, 4 or 5 with the following infection rates: 100 > n5 > 90; 90 > n4 > 50; 50 > n3 > 10; 10 > n2 > 1; and 1 > n1 > 0.

2.3. Richness of Culture Substrates in AMF, at the End of the Experiment

At the end of the experiment, the diversity of *Arbuscular mycorrhizal* fungi (AMF) present in the culture substrates was evaluated. The extraction of AMF spores from the culture substrate was conducted using the method described by Gerdemann and Nicolson [20]. Subsequently, the extracted spores were carefully examined under a light microscope at 40x magnification, using a slide and coverslip. The classification of spores was based on their color, shape, wall structure, and hyphal attachments, enabling the identification of their respective genera. The classification process drew upon the research of Ferrer and Herrora [24], Schenck and Smith [25], Walker and Mize [26], Schenck and Perez [27], Morton and Benny [28], as well as information available in various databases such as INVAM [29].

The isolated spores were quantified by direct counting using a binocular loupe, enabling the estimation of spore densities in 10 g of substrate. This quantification process was conducted in triplicate to ensure the reliability and robustness of the results.

2.4. Physicochemical Composition of Plant Leaves

2.4.1. Total Chlorophylls and Soluble Sugars in Leaves

The total chlorophyll concentration was measured using spectrophotometry, following the method described by Tran et al. [30]. The extraction of chlorophyll was performed by treating the plant samples with 80% ice-cold acetone. Optical density (O.D.) readings were obtained at specific wavelengths (λ) of 645 nm, 652 nm, and 663 nm to determine the chlorophyll content.

2.4.2. Soluble Sugars

To determine the sugar content, the following procedure was followed. Fresh material weighing 10 mg was mixed with 80% ethanol and allowed to extract for 24 h in a dark environment. The obtained extract was then diluted 10 times with 80% ethanol. Afterwards, 4 mL of a 0.2% anthrone reagent was added to the diluted extract. The anthrone reagent

was prepared by combining 0.2 g of anthrone with 100 mL of 98% sulfuric acid. The mixture was thoroughly vortexed and kept on ice. Subsequently, the tubes containing the mixture were placed in a water bath set at 92 °C for 8 min. After the incubation period, the tubes were allowed to cool for 30 min in the dark. The optical density (O.D.) of the samples was measured at a wavelength (λ) of 530 nm using a spectrophotometer. The methodology described by Dubois et al. [31] was employed for the sugar content analysis.

2.5. Mineral Content of Leaves

The fourth leaf from the growing point of three plants per replicate per treatment was selected. These leaves were oven-dried at 70 °C for 48 h and ground.

Mineral element analysis was performed using X-ray fluorescence spectrometry, specifically the EDX 7000 Shimadzu instrument. This analytical technique allowed for the determination of the mass concentration of chemical elements present in the samples. The X-ray fluorescence spectrometer provided quantitative data on the elemental composition of the samples.

2.6. Organoleptic Characterization of Tomato Fruits

Representative samples of ten uniform fruits of similar sizes and colors were chosen for each treatment to determine organoleptic parameters of the tomato. Results were then treated as mean values. Therefore, each analysis consisted of three replicates.

2.6.1. pH

The pH of tomatoes is measured using a pH meter. This involves immersing the pH probe directly into the tomato purée to obtain a direct reading of the pH value.

2.6.2. Brix Level

The determination of soluble dry residue or Brix level in the tomato samples was carried out using a refractometer. In our study, a small quantity of fresh tomato purée was delicately placed between the two surfaces of the refractometer prism to measure the Brix value. The Brix level represents the percentage by mass of soluble solids in the tomato purée and provides an indication of its sweetness or overall quality.

2.6.3. Titratable Acidity

The titratable acidity of the samples was determined following the procedure outlined by [32]. Titratable acidity was assessed through a titration process using a 0.1 N NaOH solution, with phenolphthalein employed as a color indicator. The calculation of titratable acidity was carried out using the following formula:

$$A^\circ = \% \, (100 \times V1 \times 100)/(V0 \times M \times 10) \times 0.07 = 175 \times V1/V0 \times M$$

where:

M represents the mass in grams of the sampled material,

V0 denotes the volume in milliliters of the test sample,

V1 signifies the volume in milliliters of the 0.1 N NaOH solution, and

0.07 represents the conversion factor for titratable acidity, expressed in citric acid equivalent ("$C_6H_{12}O_6$") per 100 g of tomato.

2.6.4. Total Carotenoids

The determination of total carotenoid content in the tomato samples was conducted according to the methodology outlined by Lin and Chen [33]. A 32 mL volume of an ethanol/hexane solution (4/3, *v*/*v*) was added to 8 g of tomato purée. The mixture was stirred for 30 min, and the upper phase was collected. Subsequently, a second extraction was performed by adding 15 mL of hexane. The combined extracts were mixed with 100 mL of 10% NaCl solution and 150 mL of distilled water, and the resulting mixture was separated using a separating funnel.

After the extraction process, an incubation period of 17 h in the dark was conducted. Following this, 5 mL of hexane was added, and after 1 min, 5 mL of 1% (*w*/*v*) sodium sulfate solution was introduced. The mixture was allowed to settle in the dark for 1 h, and the absorbance was measured at a wavelength of 450 nm. This allowed for the quantification of the total carotenoid content in the tomato samples.

2.7. Statistical Analysis

The significance of differences between treatments was evaluated using an analysis of variance (ANOVA). In instances where significant differences ($p \leq 0.05$) were identified, a multiple comparison test of means, Tukey's test, was employed. Differences between treatments were analyzed using the Student–Newman–Keuls test.

All analyses were performed considering a significance level of 5%.

3. Results

3.1. Evaluation of Mycorrhization Parameters

During the nursery stage, a comprehensive assessment was undertaken to evaluate the extent of colonization in tomato roots, revealing that all tomato plants exhibited mycorrhization. The frequency of mycorrhization was established at 100%, signifying that each plant had successfully formed a mycorrhizal association. Furthermore, the intensity of mycorrhizal infection was determined to be 70%, indicating a notable level of colonization within the root system.

Figure 1 showcases a compilation of images that clearly illustrate the diverse structures of endomycorrhizal fungi observed in the roots of tomato plants that underwent mycorrhization during the nursery stage. These visual depictions offer an informative overview of the various morphological characteristics exhibited by the endomycorrhizal fungi present in the roots of mycorrhized tomato plants.

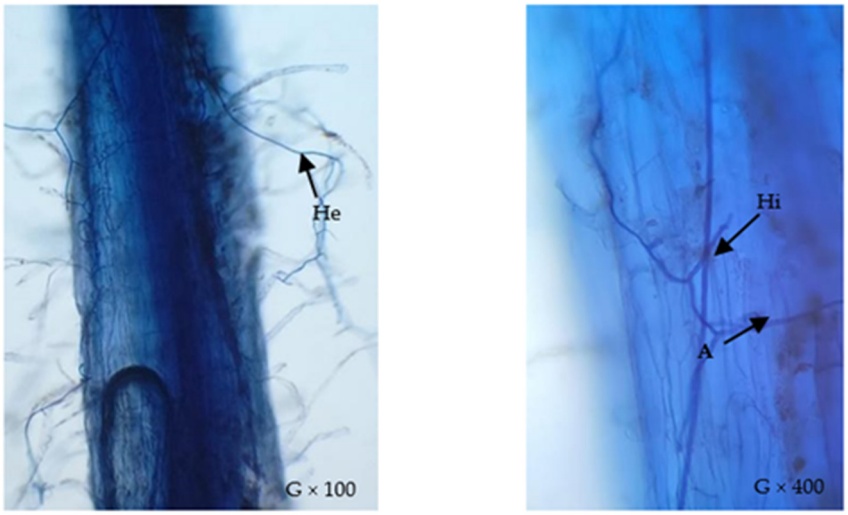

Figure 1. Mycorrhized tomato plant roots with intracellular hyphae (Hi); extracellular hyphae (He), and arbuscules (A).

The application of NPK fertilizers had a significant impact on the levels of root colonization. The highest frequency and intensity of mycorrhizal colonization (100% and 63%, respectively) were observed in the 50% NPK treatment (Figure 2). This indicates that a reduced dosage of NPK fertilizers promoted a more favorable environment for mycorrhizal fungi to colonize the roots of the tomato plants (Figure 3).

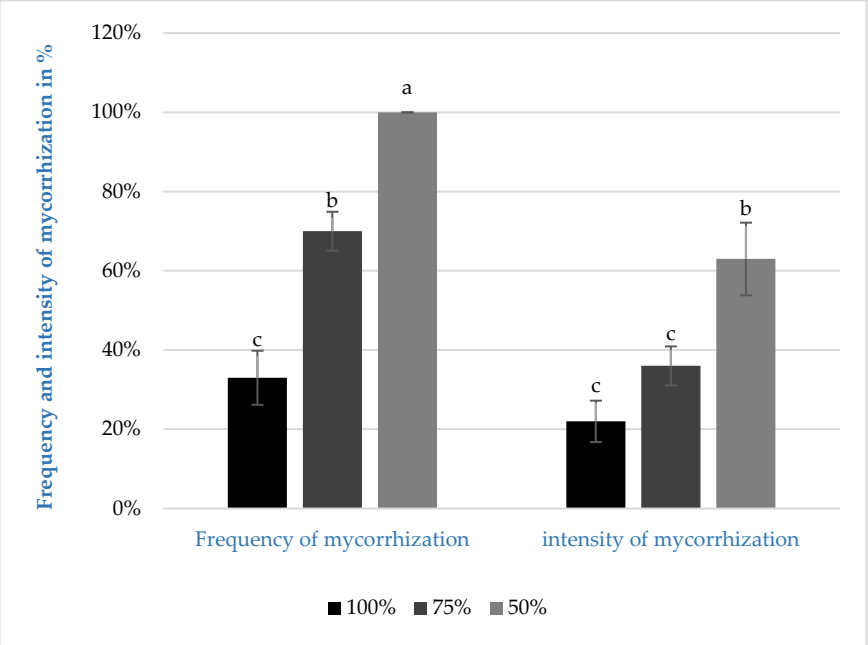

Figure 2. Frequency and intensity of mycorrhization of tomato roots at the end of the crop cycle 100%: 100% of recommended dose of NPK; 75%: 75% of NPK; 50%: 50% of NPK. Different letters indicate statistically significant differences ($p < 0.05$). Mycorrhization parameters (frequency and intensity) were statistically treated independently.

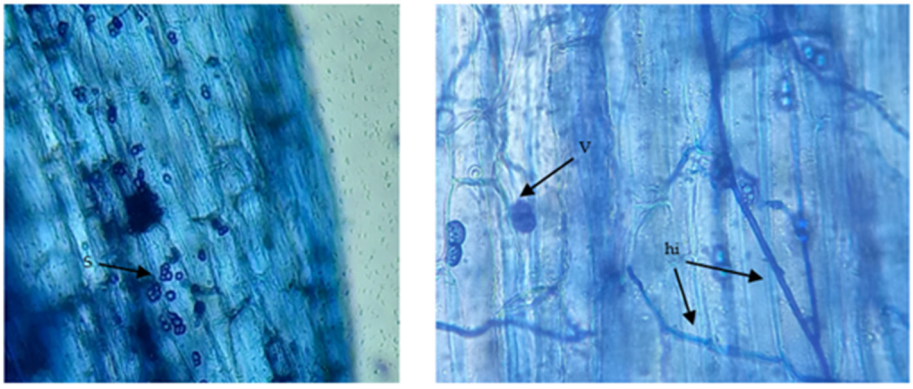

Figure 3. Structures of endomycorrhizal fungi observed in the roots of mycorrhized tomato plants: spores (s); intracellular hyphae (hi); vesicle (V) (G × 400).

3.2. Richness of Culture Substrates in AMF, at the End of the Experiment

The quantification of *Arbuscular mycorrhizal* fungi (AMF) spore density in the growing medium of tomato plants was conducted following inoculation with a natural inoculum. The recorded spore densities were 74, 170, and 279 spores per 10 g of growing medium for the treatments corresponding to 100%, 75%, and 50% of the recommended dose of NPK fertilization, respectively. These results are visually presented in Figure 4, providing a clear depiction of the observed variations in spore density among the different fertilization treatments.

A thorough morphological analysis of the spores was undertaken, leading to the identification of five distinct morphotypes, which corresponded to the following species: *Glomus fasciculatum*, *Glomus versiforme*, *Glomus constructum*, *Glomus microcarpum*, and *Glomus macrocarpum*. These findings are succinctly summarized in Table 2 and visually represented in Figure 5. It is noteworthy that all of these species belong to the Glomaceae family and are classified within the order Glomerales.

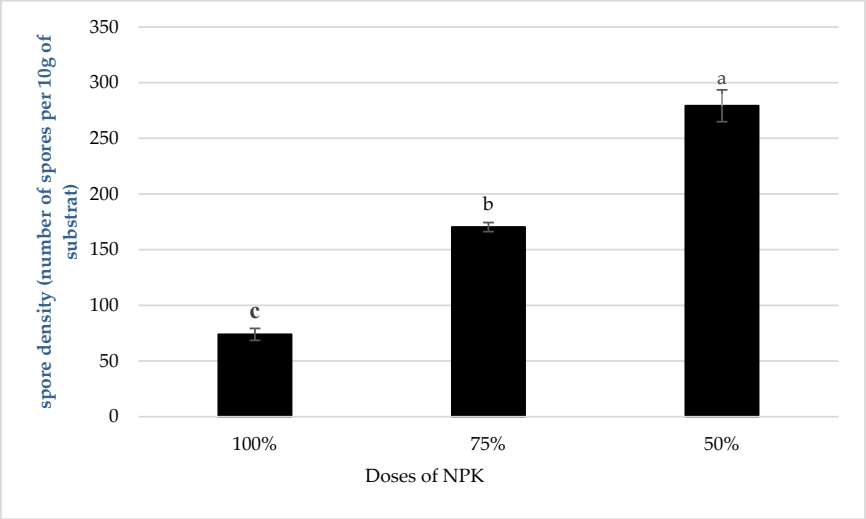

Figure 4. Density of AMF species in the culture substrate of tomato plants inoculated with natural inoculum, at the end of the trial. Different letters indicate statistically significant differences ($p < 0.05$).

Table 2. Characteristics of all AMF isolated from the growing medium inoculated with natural inoculum at the end of the tomato growing cycle.

Number	Species	Form	Color	Spore Size (µm)	Wall Size (µm)	Spore Surface
1	*Glomus fasciculatum*	Globular	Brown	90	1.2	Smooth
2	*Glomus versiforme*	Globular	Brown	130	1.3	Smooth
3	*Clomus constructum*	Globular	Almost black	100	1	Smooth
4	*Glomus microcarpum*	Globular	Yellow	86	1.1	Granular
5	*Glomus macrocarpum*	Globular	Orange	147	3.3	Smooth
6	*Glomus versiforme*	Globular	Yellow	60	0.3	Smooth
7	*Glomus macrocarpum*	Globular	Brown	139	1.5	Smooth

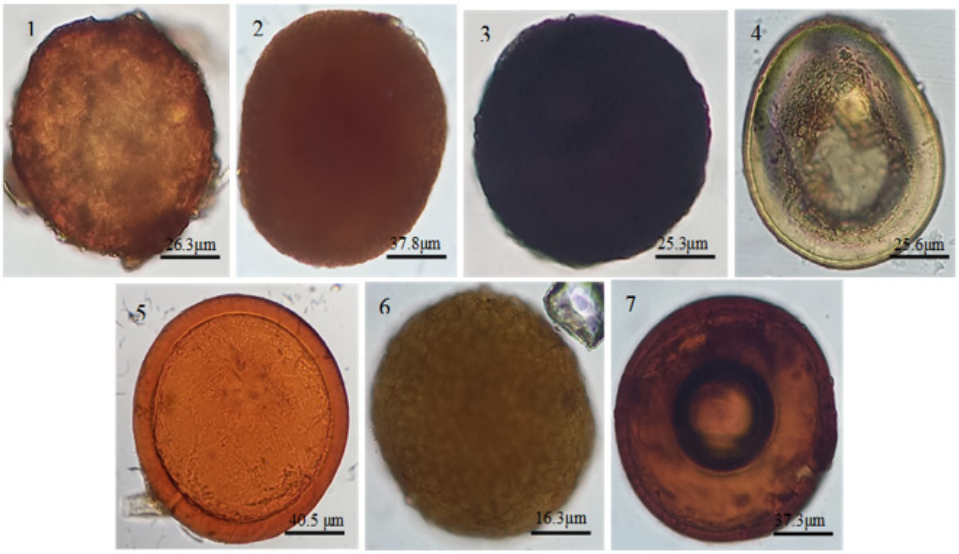

Figure 5. Photographs of spores of endomycorrhizal fungal species isolated from tomato growing medium at the end of the trial. 1: Glomus fasciculatum, 2: Glomus versiforme, 3: Clomus constructum, 4: Glomus microcarpum, 5: Glomus macrocarpum, 6: Glomus versiforme, 7: Glomus macrocarpum.

3.3. Effect of Mycorrhization and Fertilization Rates on Growth Parameters and Physicochemical Composition of Plant Leaves

This study aimed to investigate the influence of mycorrhization and fertilization rates on various growth parameters.

The analysis of variance showed that both fertilizer dose and inoculation had a significant effect on plant height (Table 3). The height of non-inoculated tomato plants did not show any significant variations regardless of the applied level of chemical fertilization, as indicated in Table 4.

Table 3. Analysis of variance of growth parameters and physicochemical composition of plant leaves.

Source of Variation	df	Mean Squares	*F*-Value	*p*-Value
PH				
Fertilizer dose (F)	2	81.056	17.228	0.000
Inoculation (I)	1	57.781	12.281	0.004
F × I interaction	2	15.875	3.374	0.069
error	12	4.705		
Percentage of LDW				
Fertilizer dose (F)	2	1.633	0.317	0.734
Inoculation (I)	1	65.628	12.748	0.004
F × I interaction	2	9.887	1.921	0.189
error	12	5.148		
Percentage of RDW				
Fertilizer dose (F)	2	35.229	1.437	0.276
Inoculation (I)	1	384.014	15.669	0.002
F × I interaction	2	45.883	1.872	0.196
error	12	24.508		
Chlorophyll				
Fertilizer dose (F)	2	0.000	8.231	0.006
Inoculation (I)	1	0.001	61.202	0.000
F × I interaction	2	1.380E-5	0.907	0.430
error	12	1.522E-5		
Soluble sugar				
Fertilizer dose (F)	2	1926.878	6.000	0.016
Inoculation (I)	1	79850.736	248.643	0.000
F × I interaction	2	2569.170	8.000	0.006
error	12	321.146		

Abbreviations: PH: plant height; percentage of LDW: percentage of leaf dry weight; percentage of RDW: percentage of root dry weight; F: fertilizer dose; I: inoculation.

However, for mycorrhized plants, a noteworthy difference was observed between the 75% NPK and 50% NPK treatments. Inoculated plants receiving 75% of the recommended dose of NPK displayed the greatest height, surpassing even the height of control plants that were not mycorrhized but received 100% of the recommended NPK dose, as shown in Table 4.

Furthermore, analysis of variance showed that only inoculation with isolated strains had an effect on leaf and root dry weight percentages. Leaf and root dry weight percentages were higher in inoculated plants compared to non-inoculated plants, regardless of the NPK dose applied, as indicated in Table 4. Notably, mycorrhized plants, even with 50% fertilization, had higher leaf and root dry weights than non-mycorrhized plants receiving 100% fertilization, but not with a significant difference. Among the treatments, the highest value for root dry weight percentage (38.37%) was recorded for inoculated plants receiving 75% of the recommended NPK dose. Similarly, the highest value for leaf dry weight percentage (22.76%) was obtained for mycorrhizal plants fertilized at 100% of the recommended NPK dose.

Table 4. Height, percentage of dry weight of leaves and roots of tomato plants, chlorophyll, and soluble sugar levels.

Treatments	Plant Status	PH (cm)	Percentage of LDW (%)	Percentage of RDW (%)	Total Chlorophyll (mg g^{-1} f.w)	Soluble Sugar (µg g^{-1} f.w)
Fertilizer Dose						
100%	I−	81.17 ± 2.268 ab	18.60 ± 0.650 ab	30.18 ± 1.892 ab	0.0416± 0.003 bcd	365.76± 20.320 c
	I+	81.58 ± 1.443 ab	22.76 ± 1.602 a	34.50 ± 8.785 ab	0.0586 ± 0.008 a	535.09 ± 11.732 a
75%	I−	80.08 ± 3.761 bc	19.61 ± 0.514 ab	23.15 ± 1.617 b	0.0387 ± 0.001 cd	352.21± 31.039 c
	I+	87.00 ± 1.323 a	20.71 ± 4.259 ab	38.37 ± 4.333 a	0.0497 ± 0.002 ab	494.45 ± 11.732 ab
50%	I−	74.67 ± 1.756 c	16.54 ± 1.649 b	23.50 ± 2.192 b	0.0336 ± 0.002 d	372.53 ± 11.732 c
	I+	78.08 ± 1.422 bc	22.73 ± 2.603 ab	31.67 ± 6.332 ab	0.0486 ± 0.003 abc	460.59 ± 11.732 b

Note: Different letters in the same column indicate significant differences ($p < 0.05$) between treatments based on Tukey's test. Abbreviations: PH, plant height; percentage of LDW, percentage of leaf dry weight; percentage of RDW, percentage of root dry weight; I−, non-inoculated tomato plants; I+, inoculated tomato plants.

The total chlorophyll content of both mycorrhizal and non-mycorrhizal tomato plants (I+ and I−, respectively) demonstrated an upward trend with increasing levels of NPK fertilization, as depicted in Table 4. Notably, the chlorophyll content of inoculated plants receiving 50% and 75% of the recommended NPK dose surpassed that of non-inoculated plants fertilized with NPK at 100% of the recommended dose. These findings underscore the positive impact of higher NPK fertilization levels on the total chlorophyll content in tomato plants, regardless of mycorrhization. Furthermore, the observation of enhanced chlorophyll levels in mycorrhizal plants even at lower NPK fertilization rates suggests the potential of mycorrhization in promoting efficient chlorophyll synthesis and accumulation. However, analysis of variance showed that both fertilizer dose and inoculation had a significant effect on leaf total chlorophyll content, as shown in Table 3.

In non-inoculated tomato plants, the concentrations of leaf soluble sugars remained relatively consistent across the different NPK fertilizer doses applied, as depicted in Table 4. However, mycorrhizal tomato plants demonstrated a significant positive response by exhibiting an increased accumulation of foliar sugars compared to non-mycorrhizal plants, irrespective of the level of NPK fertilization (Table 4). Notably, the leaf soluble sugar content in mycorrhized plants receiving 50% of the recommended NPK dose surpassed that of non-inoculated plants fertilized with 100% of the recommended NPK dose. The results of the analyses also show that inoculation, the dose of fertilizer applied, and their interactions have a significant effect on leaf sugar content (Table 3).

These findings indicate that mycorrhization exerts a positive influence on the accumulation of soluble sugars in tomato leaves, regardless of the level of NPK fertilization applied. Additionally, mycorrhized plants at a lower fertilization rate exhibited higher leaf soluble sugar content compared to non-inoculated plants at a higher fertilization rate, thus suggesting the potential of mycorrhization in enhancing sugar metabolism in tomato plants.

3.4. Mineral Content of Leaves

The symbiotic association of mycorrhizal fungi with indigenous strains derived from the soil of Guercif, Morocco, exerted a beneficial influence on the uptake of vital mineral elements, specifically potassium (K), phosphorus (P), calcium (Ca), iron (Fe), zinc (Zn), and copper (Cu). Consequently, mycorrhized plants that received 50% of the recommended NPK dose exhibited notably elevated leaf concentrations of Ca, K, Fe, Zn, and P, approaching the levels observed in both mycorrhized and non-mycorrhized plants that received 100% of the recommended NPK dose (Table 5). The results in Table 6 showed that inoculation has a significant effect on all mineral elements, and that the dose of fertilizer applied has a significant effect on P, K, Ca, and Mn. The interaction of the two factors also had a significant effect on P, K, Ca, Zn, and Mn.

Table 5. Mineral element content of tomato leaves (% per d.w).

Treatments		P	K	Ca	Fe	Zn	Cu	Mn
Fertilizer Dose	Plant Status							
100%	I−	0.10 ± 0.060 ab	13.59 ± 0.153 b	66.65 ± 1.433 ab	1.89 ± 0.365 b	1.10 ± 0.164 abc	0.12 ± 0.006 a	2.21 ± 0.104 ab
	I+	0.29 ± 0.069 ab	15.15 ± 0.404 ab	69.63 ± 0.411 a	4.00 ± 0.466 a	1.11 ± 0.014 abc	0.14 ± 0.021 a	1.83 ± 0.258 b
75%	I−	0.05 ± 0.026 ab	8.72 ± 0.189 c	60.32 ± 4.321 bc	1.86 ± 0.076 b	0.94 ± 0.008 c	0.12 ± 0.029 a	2.31 ± 0.357 ab
	I+	0.08 ± 0.033 b	16.41 ± 0.160 a	67.76 ± 4.068 a	4.02 ± 0.016 a	1.39 ± 0.086 a	0.16 ± 0.003 a	2.48 ± 0.098 a
50%	I−	0.04 ± 0.035 ab	8.15 ± 1.556 c	57.92 ± 0.085 c	1.89 ± 0.222 b	1.01 ± 0.031 bc	0.15 ± 0.010 a	2.67 ± 0.034 a
	I+	0.58 ± 0.249 a	15.31 ± 0.235 ab	69.46 ± 0.127 a	4.02 ± 0.088 a	1.28 ± 0.195 ab	0.16 ± 0.004 a	1.87 ± 0.003 b

Note: Different letters in the same column indicate significant differences ($p < 0.05$) between treatments based on Tukey's test. Abbreviations: I−, non-inoculated tomato plants; I+, inoculated tomato plants.

Table 6. Analysis of variance of mineral element content of tomato leaves (% per d.w).

Source of Variation	df	Mean Squares	*F*-Value	*p*-Value
P				
Fertilizer dose (F)	2	0.092	7.493	0.008
Inoculation (I)	1	0.289	23.670	0.000
F × I interaction	2	0.101	8.259	0.006
error	12	0.012		
K				
Fertilizer dose (F)	2	10.961	24.137	0.000
Inoculation (I)	1	134.655	296.525	0.000
F × I interaction	2	17.296	38.089	0.000
error	12	0.454		
Ca				
Fertilizer dose (F)	2	36.681	5.874	0.017
Inoculation (I)	1	241.260	38.634	0.000
F × I interaction	2	27.458	4.397	0.037
error	12	6.245		
Fe				
Fertilizer dose (F)	2	0.000	0.003	0.997
Inoculation (I)	1	20.499	297.885	0.000
F × I interaction	2	0.001	0.012	0.988
error	12	0.069		
Fe				
Fertilizer dose (F)	2	0.006	0.450	0.648
Inoculation (I)	1	0.262	21.425	0.001
F × I interaction	2	0.075	6.159	0.014
error	12	0.012		
Cu				
Fertilizer dose (F)	2	0.001	3.428	0.066
Inoculation (I)	1	0.002	8.138	0.015
F × I interaction	2	0.000	1.148	0.350
error	12	0.000		
Mn				
Fertilizer dose (F)	2	0.001	6.097	0.015
Inoculation (I)	1	0.002	14.616	0.002
F × I interaction	2	0.000	10.054	0.003
error	12	0.000		

Abbreviations: F, fertilizer dose; I, inoculation.

These findings emphasize the capability of mycorrhizal symbiosis to enhance the acquisition and assimilation of essential mineral elements, thereby bolstering the nutritional status of tomato plants even when subjected to reduced NPK fertilization levels.

3.5. Yield and Organoleptic Quality of Tomato Fruits

The yield of tomato plants was profoundly influenced by mycorrhizal inoculation, demonstrating a statistically significant increase compared to non-inoculated plants, regardless of the NPK fertilization doses applied (Table 7). Moreover, there was a notable positive correlation between increasing NPK fertilization rates and enhanced yield.

Table 7. Yield, pH, EC (electrical conductivity), Titratable acidity, °Brix, and total carotenoid of tomato fruit puree.

Treatments		Yield (g/Plant)	pH	EC at 25 °C (mS/cm)	Titratable Acidity (g L^{-1})	°Brix	Total Carotenoid (mg 100 g^{-1} f.w)
Fertilizer Dose	Plant Status						
100%	I−	1635.42 ± 41.329 bc	4.40 ± 0.100 a	12.28 ± 0.155 ab	1.45 ± 0.05 b	8.73± 0.651 b	12.02± 0.427 d
	I+	2026.83 ± 52.522 a	4.38 ± 0.053 a	12.02 ± 0.060 b	1.33 ± 0.058 c	8.80 ± 0.100 ab	20.06 ± 0.295 b
75%	I−	1424.42 ± 28.729 d	4.48 ± 0.025 a	12.67 ± 0.535 ab	1.75 ± 0.00 a	8.63 ± 0.153 b	10.63 ± 0.843 d
	I+	1789.50 ± 106.337 b	4.48 ± 0.026 a	12.65 ± 0.044 ab	1.75 ± 0.030 a	9.37 ± 0.153 ab	26.02 ± 0.830 a
50%	I−	1191.17 ± 77.203 e	4.49 ± 0.020 a	13.51 ± 1.070 a	1.75 ± 0.02 a	8.83 ± 0.058 ab	7.98 ± 0.225 e
	I+	1496.00 ± 74.833 cd	4.46 ± 0.020 a	12.91 ± 0.480 ab	1.74 ± 0.032 a	9.57 ± 0.058 a	18.40 ± 0.065 c

Note: Different letters in the same column indicate significant differences ($p < 0.05$) between treatments based on Tukey's test. Abbreviations: I−, non-inoculated tomato plants; I+, inoculated tomato plants; EC, electric conductivity.

Specifically, mycorrhizal plants that received 50%, 75%, and 100% of the recommended NPK dose exhibited substantial total weight gains of 20%, 20%, and 19%, respectively, compared to their non-inoculated counterparts. The results of the analysis of variance show that both the dose of fertilizer applied and inoculation have a significant effect on yield. These findings emphasize the synergistic effects of mycorrhizal inoculation and NPK fertilization in promoting tomato plant productivity, with mycorrhizal plants consistently exhibiting greater yield gains across all levels of NPK fertilization.

Analysis of fruit characteristics showed no noteworthy variations in pH and electrical conductivity (EC) across the different treatment groups (Table 7). However, the introduction of mycorrhizal strains had a significant impact on the titratable acidity of tomato plants (Table 8). Tomato plants inoculated with mycorrhizal strains and receiving 100% of the recommended NPK dose exhibited a decrease in titratable acidity. In contrast, when the NPK dose was reduced to 75% or 50%, the titratable acidity values were comparable between mycorrhized and non-mycorrhized plants, although higher than those observed in plants fertilized with 100% NPK (refer to Table 7).

Moreover, the inoculation with mycorrhizal strains had a positive influence on the Brix index, which is an indicator of tomato fruit quality, across all NPK doses (refer to Table 7). The highest Brix index value was recorded in inoculated plants that were fertilized with 50% of the recommended NPK dose, surpassing the value obtained in non-inoculated plants receiving 100% of the recommended NPK dose (Table 7).

Table 8. Analysis of variance of yield, pH, EC, titratable acidity, °Brix, and total carotenoid of tomato fruit puree.

Source of Variation	df	Mean Squares	*F*-Value	*p*-Value
Yield				
Fertilizer dose (F)	2	357,313.962	76.133	0.000
Inoculation (I)	1	563,214.222	120.004	0.000
F × I interaction	2	2955.045	0.630	0.550
error	12	4693.295		
pH				
Fertilizer dose (F)	2	0.015	6.049	0.015
Inoculation (I)	1	0.002	0.644	0.438
F × I interaction	2	0.000	0.116	0.892
error	12	0.002		

Table 8. *Cont.*

Source of Variation	df	Mean Squares	*F*-Value	*p*-Value
EC				
Fertilizer dose (F)	2	1.702	6.039	0.015
Inoculation (I)	1	0.381	1.353	0.267
F × I interaction	2	0.127	0.451	0.647
error	12	0.282		
Titratable acidity				
Fertilizer dose (F)	2	0.252	185.229	0.000
Inoculation (I)	1	1.176	14.493	0.002
F × I interaction	2	0.006	4.494	0.035
error	12	0.001		
°Brix				
Fertilizer dose (F)	2	0.282	3.479	0.064
Inoculation (I)	1	1.176	14.493	0.002
F × I interaction	2	0.222	2.740	0.105
error	12	0.081		
total carotenoid (mg 100 g^{-1} f.w)				
Fertilizer dose (F)	2	39.789	138.575	0.000
Inoculation (I)	1	572.742	1994.719	0.000
F × I interaction	2	21.121	73.560	0.000
error	12	0.287		

Abbreviations: F, fertilizer dose; I, inoculation; EC, electric conductivity.

A two-factor analysis of variance demonstrated a significant effect of inoculation on titratable acidity, a significant effect of NPK fertilizer dose, and a significant interaction effect between inoculation and NPK fertilizer (Table 8). These findings collectively highlight the beneficial influence of mycorrhizal inoculation on titratable acidity and the Brix index, indicating its potential to improve the sensory and nutritional attributes of tomato fruits.

The examined fruit quality indicators, namely total carotenoid, was significantly enhanced through mycorrhizal inoculation, as depicted in Table 7.

The highest concentration of total carotenoid was observed in plants that were both inoculated and fertilized with 75% of the recommended NPK dose, as illustrated in Table 7. Remarkably, even when plants were inoculated and fertilized with only 50% of the recommended NPK dose, the recorded total carotenoid value exceeded that of non-inoculated plants receiving 100% of the recommended NPK dose (Table 7).

Analysis of variance shows that fertilizer and inoculation, as well as the interaction of these two factors, have a significant effect on total carotenoid content in fruit (Table 8).

4. Discussion

The current investigation involved the utilization of a biostimulant sourced from natural inoculum originating from the Guercif, Oriental Morocco region. This biostimulant was administered in an actual tomato production crop, in conjunction with varying doses of fertilizer. The objective of this approach was to acquire agronomic data concerning the application of the biostimulant, with the goal of minimizing the need for chemical inputs, while also evaluating its impact on tomato production and nutritional quality.

As anticipated, the roots of the tomato plants were completely colonized by *Arbuscular mycorrhizal* fungi (AMF) during the nursery stage. This colonization exhibited a notable frequency and intensity. However, upon evaluating the root colonization of the inoculated plants at the conclusion of the trial, using the parameters of mycorrhization frequency and intensity, a significant decrease was observed as the doses of NPK chemical fertilizer increased.

A significant correlation was observed between the frequency of mycorrhizal colonization (expressed as F%) and the different doses of NPK fertilization administered to the

plants. This finding is consistent with prior research studies that have indicated a negative impact of increased phosphorus (P) fertilization on the colonization of plant roots by mycorrhizal fungi [18,34,35]. Additionally, the plants' fruit production plays a substantial role as a carbon sink, depleting the available carbohydrates for the fungal partner and subsequently leading to a decrease in mycorrhizal colonization [36].

Examination of the rhizosphere of tomato plants inoculated with mycorrhizal fungi unveiled the presence of five distinct morphotypes: *Glomus fasciculatum*, *Glomus versiforme*, *Glomus constructum*, *Glomus microcarpum*, and *Glomus macrocarpum*. These morphotypes are members of the *Glomus* genus, which typically dominates the *Arbuscular mycorrhizal* fungal (AMF) communities observed in Guercif soil [37]. Similar investigations conducted in Morocco have also reported the prevalence of the Glomus genus within the rhizosphere of diverse plant species [38–42].

The rhizosphere of tomato plants exhibited a general abundance of AMF spores at the end of the trial. However, the number of spores decreased with increasing NPK fertilizer inputs. These results align with previous studies suggesting that fluctuations in AMF spore numbers can be attributed to soil chemical properties [35,43,44] and the sampling season [45,46].

Inoculation of tomato plants with AMF from the nursery exhibited a positive impact on all the assessed growth parameters, regardless of the fertilizer dosage administered. Notably, plants that were both inoculated with AMF and fertilized with 75% of the recommended NPK dose demonstrated enhanced growth, exemplified by increased stem height, as well as greater leaf and root dry weights, in comparison to plants receiving 100% of the recommended NPK fertilization. Surprisingly, even mycorrhizal plants fertilized with only 50% of the recommended NPK dose exhibited growth parameters on par with those of non-inoculated plants receiving the full recommended NPK dose. These outcomes align with prior studies demonstrating significantly higher levels of dry biomass in tomato plants that received AMF inoculation compared to their non-inoculated counterparts [36]. Similar findings have been reported in nutrient-poor soil scenarios, where the addition of both AMF and NPK resulted in substantial increases in plant biomass [47]. Berta et al. [48] also concluded that AMF inoculation played an essential role in root development.

Inoculation of tomato plants with the natural inoculum sourced from the Guercif region in eastern Morocco resulted in a notable elevation in leaf sugar content, with variations observed based on the dosage of NPK fertilizer administered. This finding suggests an enhancement in photosynthetic activity within mycorrhizal plants compared to non-mycorrhizal counterparts. Particularly noteworthy is the discovery that mycorrhization, in conjunction with the application of only 50% of the recommended NPK dose, proved sufficient to significantly augment chlorophyll content, matching or even surpassing the levels observed in plants receiving the complete recommended NPK dose (100%). Previous research attributes this increase to the pivotal role of fungal symbionts in facilitating the uptake of magnesium and nitrogen, thereby promoting heightened chlorophyll synthesis [49], or alternatively, to the enhanced activity of enzymes involved in chlorophyll synthesis [50].

Inoculation of tomato plants with AMF also resulted in elevated leaf concentrations of potassium (K), phosphorus (P), calcium (Ca), iron (Fe), zinc (Zn), and copper (Cu) when compared to non-inoculated plants. Notably, the highest levels of Ca, K, Fe, Zn, and P were observed when mycorrhization was combined with the application of NPK fertilizer at only 50% of the recommended dose, a condition that promoted robust mycorrhizal root colonization. Similar findings have been reported in previous studies, which have demonstrated enhanced leaf mineral contents through AMF inoculation in soil-grown tomato plants [51,52]. Balliu et al. [52] also showed that the use of an inoculum made up of a mixture of species (*Glomus intraradices*, *Glomus etunicatum*, *Glomus mosseae*, *Glomus geosporum*, and *Glomus clarum*) led to an improvement in mineral content (N, P, Mg, Ca, Mn, and Fe).

The optimal tomato plant yields were attained when the plants were both inoculated and fertilized with 100% of the recommended dose of NPK. This observation aligns with

previous studies that have demonstrated enhanced tomato plant yields through the combined application of inoculation with *Arbuscular mycorrhizal* fungi (AMF) and the full dose of phosphorus (P) fertilizer [16]. These findings emphasize the advantage of a strategic approach that combines inoculation with *Arbuscular mycorrhizal* fungi and application of NPK chemical fertilizer improved crop production. Additionally, the study reveals that in the presence of a natural inoculum, applying 75% of the recommended dose of chemical fertilizer to tomato plants can achieve fruit yields comparable to those of non-inoculated plants receiving the full recommended NPK dose. This suggests a potential reduction of nearly 25% in chemical fertilizer usage without compromising yield. Previous research has also demonstrated the positive impact of mycorrhizal inoculation on increasing tomato fruit yield [3,53–57]. The yield increase promoted by AMF inoculation is linked to the biostimulant action of these fungi on plant uptake and growth [58]. Another study showed that *Funneliformis mosseae* increased total tomato yield [59], while Felföldi et al. [60] recorded an increase in the yield of various tomato varieties inoculated with a mixture of AMF species *Rhizophagus irregularis*, *Claroideoglomus etunicatum*, *Claroideoglomus claroideum*, *Funneliformis mosseae*, and *Funneliformis geosporum*.

The pH levels of the tomato purée remained relatively stable across all treatments, with no notable significant differences observed, consistently measuring around 4.4. While mycorrhization resulted in a slight decrease in electrical conductivity, mycorrhization has a significant impact on titrable acidity, similar results have been reported by Regvar et al. [58]. However, the inoculation of tomato plants with mycorrhizal fungi did have a noteworthy effect on the °Brix value, which serves as an indicator of dissolved solids, sucrose, and fructose, and reflects fruit sweetness. Inoculation significantly influenced the °Brix value across all NPK doses compared to non-inoculated plants. These findings align with previous research that has also demonstrated increased °Brix values associated with mycorrhization [59].

In terms of the antioxidant properties of tomato fruit, mycorrhizal inoculation had a positive effect on the total carotenoids concentrations, contributing to improved fruit quality and external appearance. This finding is consistent with a previous research study [36]. Other studies have also reported an increase in carotenoid content as a result of mycorrhization, although the molecular mechanisms underlying this augmentation in carotenoid levels are yet to be fully understood [59–61].

5. Conclusions

In conclusion, our study highlights the potential benefits of utilizing *Arbuscular mycorrhizal* fungi (AMF) from the Guercif Eastern region of Morocco for the inoculation of tomato plants. This approach not only increased the yield of greenhouse-grown tomato fruits but also improved their nutritional quality. Furthermore, the incorporation of AMF inoculation allowed for a significant reduction in the use of chemical fertilizers without compromising plant growth and yield. Specifically, tomato plants that received AMF inoculation and were fertilized at 75% of the recommended NPK dose displayed superior growth and yield compared to non-inoculated plants receiving the full recommended dose of fertilizer. Additionally, our findings demonstrate that this combination of AMF inoculation and reduced fertilizer doses enhances the production of secondary metabolites, such as total carotenoids and lycopene, thus improving the nutritional value of the tomato fruits. This study also shows that fertilizer levels did not influence the diversity of AMF communities, while high fertilizer levels led to lower AMF colonization due to lower C investment by the plant.

The results of our study indicate that the utilization of the arbuscular AMF isolates, in combination with reduced chemical fertilizer doses, shows great potential as an effective strategy for large-scale tomato production. This approach not only leads to the cultivation of tomatoes with enhanced nutritional quality but also reduces the dependence on chemical fertilizers. These findings highlight the viability of implementing this approach in

commercial tomato production, offering the opportunity to obtain high-quality fruits while minimizing the environmental impact associated with excessive fertilizer usage.

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