

Indigenous Arbuscular Mycorrhizal Fungi from Benin Improve Growth and Yield of Tomato (*Solanum lycopersicum* L.) and Pepper (*Capsicum annuum*), and Antioxidant Compounds and Capacity in Tomato under Protected Cultivation

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Abstract

Arbuscular Mycorrhizal Fungi (AMF) offer a promising alternative for improving productivity and reducing dependence on external inputs in vegetable farming systems. This study assessed the effect of Glomeraceae strains on the growth, yield and certain biochemical parameters of *Solanum lycopersicum* and *Capsicum annuum* grown in a greenhouse. The experiment was conducted in pots using a Randomized Complete Block Design comprising seven treatments: T0 (control, without inoculation or fertilisation), T1 (AMF), T2 (AMF + 25% NPK-Urea), T3 (AMF + 50% NPK-Urea), T4 (25% NPK-Urea), T5 (50% NPK-Urea) and T6 (100% NPK-Urea) with four replications per treatment for each crop. After four months of cultivation, growth and yield parameters were assessed, including shoot length and stalk diameter, the number of leaves and branches, leaf dimensions, and the number and weight of fruits per plant. In tomatoes, the chlorophyll and carotenoid contents of the leaves were also determined at the end of the vegetative phase, whilst the polyphenol and flavonoid contents, as well as the antioxidant capacity, were assessed in the harvested fruits. The results show that inoculation with AMF, particularly when

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combined with reduced NPK-Urea, significantly improved the performance of both crops. Treatment T3 (AMF + 50% NPK-Urea) stood out in particular for its beneficial effects on the growth and yield of tomatoes and pepper, as well as on the chlorophyll and carotenoid content of tomato leaves. It also promoted the accumulation of polyphenols and improved the antioxidant capacity of the fruit. These results highlight the potential of native AMF derived from the Beninese rhizosphere to improve crop performance whilst reducing the use of mineral fertilisers, thereby offering an interesting prospect for the development of more sustainable fertilisation practices in market gardening systems.

Keywords

AMF, Biostimulants, *Solanum lycopersicum*, *Capsicum annuum*, Antioxydants, Benin

1. Introduction

Tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum annuum*) are among the world's most important vegetable crops due to their economic significance [1], widespread consumption and nutritional value. For example, in the case of the tomato, its fruits are an important source of vitamins, minerals, dietary fibre and numerous bioactive compounds, including carotenoids, phenolic compounds and flavonoids, which contribute to their nutritional and functional properties [2] [3].

In Benin, tomatoes and peppers are the main vegetable crops; they are grown across all the country's agroecological zones and make a significant contribution to food security and the incomes of smallholder farmers. However, their productivity remains limited by several constraints, notably climatic fluctuations, inadequate soil fertility management and pressure from pests and diseases, including bacterial wilt caused by *Ralstonia solanacearum* [4] [5].

In the face of growing demand for food and increasing consumer expectations for food with high nutritional value, improving both the productivity and quality of fruit is a key challenge for sustainable agriculture.

Plant growth and fruit quality are closely linked to the efficiency of photosynthesis and secondary plant metabolism. Chlorophylls and carotenoids are the main photosynthetic pigments involved in capturing light energy and protecting the photosynthetic apparatus from oxidative damage [6] [7]. In addition to their physiological role in plants, carotenoids are of considerable nutritional interest due to their antioxidant properties and their beneficial effects on human health [8] [9]. Furthermore, phenolic compounds and flavonoids represent one of the main families of plant secondary metabolites [10]. Thanks to their strong ability to neutralise reactive oxygen species, these compounds help protect cells against oxidative stress and are associated with numerous biological activities, including antioxidant, anti-inflammatory, antimicrobial and anti-cancer effects [11] [12].

Increasing their accumulation in fruit is therefore an important objective for enhancing the nutritional quality of fruit [13].

In this context, biofertilisers appear to be a sustainable alternative to conventional fertilisation practices [14]. Among these, arbuscular mycorrhizal fungi (AMF) are soil microorganisms capable of establishing a mutualistic symbiosis with the majority of terrestrial plants [15]. This partnership enhances the uptake of mineral nutrients, particularly phosphorus, promotes water absorption and strengthens plants' tolerance to biotic and abiotic stresses [16]. In return, the plant provides the fungi with the carbon compounds necessary for their growth and development [17] [18]. Numerous studies have thus demonstrated that inoculation with AM fungi improves the growth, biomass and yield of various crops [19] [20].

In addition to their contribution to improving mineral nutrition, AMF can also modulate primary and secondary plant metabolism, thereby influencing various physiological and biochemical processes associated with plant growth, development and adaptation to environmental conditions [21] [22]. Improved nutritional status and photosynthetic activity can promote the accumulation of chlorophylls and carotenoids, whilst mycorrhizal symbiosis can stimulate the biosynthetic pathways of phenolic compounds and flavonoids involved in the plant's defence mechanisms [16] [23]. These biochemical changes may result in an increase in the antioxidant capacity of plant tissues and, consequently, an improvement in the nutritional quality of the fruit [21] [24]. However, despite the numerous studies focusing on the effects of AMF on plant growth, their simultaneous impacts on photosynthetic pigments in leaves and on antioxidant compounds in tomato fruit remain insufficiently documented and appear to vary depending on the fungal species, the genotype of the host plant and environmental conditions [25] [26].

The aim of this study is therefore to assess the effect of inoculation with arbuscular mycorrhizal fungi native to Benin on the growth and yield of tomato and pepper plants, chlorophyll and carotenoid levels in the leaves, as well as the levels of phenolic compounds and flavonoids and the antioxidant capacity of whole tomato fruits.

2. Materials and Methods

2.1. Soil Sampling

The soil used for this experiment is a ferrallitic soil from southern Benin, collected from the experimental station of the Department of Biochemistry and Cell Biology at the University of Abomey-Calavi. The trial was carried out in pots, under cover, at the same experimental station, during the main rainy season, from April to August. The soil properties are as described by Houdegbe *et al.* [27] and summarised in **Table 1**. It is a sandy-loam soil with good drainage and permeability. Prior to use, the soil was sterilised in an oven at 121°C for one hour a day for three consecutive days in order to eliminate all present microorganisms.

Table 1. Soil physicochemical characteristics.

Parameter	Sand	Silt	Clay	pH (KCl)	pH (H ₂ O)	C	N	C/N	P	K	Mg	Ca	Ca/Mg
Value	61.98%	25.75%	12.27%	5.48	5.88	1.03%	0.06%	17	23.06 mg/kg	811.2 mg/kg	287.95 mg/kg	126 mg/kg	0.44

2.2. Preparation of AMF-Based Inoculum

The fungal inoculum was prepared from three arbuscular mycorrhizal fungi (AMF) belonging to the family *Glomeraceae*: *Glomus caledonius*, *Rhizophagus intraradices*, and *Funneliformis geosporum*. These strains were originally isolated in southern Benin by Aguégué *et al.* [28] and are maintained in the culture collection of the Laboratory of Biology and Molecular Typing in Microbiology (LBTMM) at the University of Abomey-Calavi, Benin. The strains were reactivated and subsequently multiplied through trap culture using sorghum plants grown under controlled greenhouse conditions. The resulting inoculum consisted of a consortium of the three strains mixed in equal proportions, with a final inoculum density ranging from 100 to 150 spores per gram of inoculum.

2.3. Setting Up and Conducting the Experiment

Tomato seeds (var. BENTO-05) and pepper seeds (var. BENPIM-03), purchased from Living Seed, were used in this study. Sowing was carried out in plastic seed trays containing pre-sterilised soil. One hole was made in each cell, and two seeds were sown per hole. Inoculation was performed immediately after sowing in the cells designated for AMF treatments by applying 0.5 g of inoculum per cell. The cells designated for the non-mycorrhizal treatments (T0: control and T6: 100% NPK-Urea) received the same amount (0.5 g per cell) of a sterilized clay-peat mixture (3:1), which was used as the carrier substrate for AMF inoculum production. Thinning was performed seven days after germination, leaving one healthy plant per cell. The seedlings were monitored and regularly irrigated until transplanting, 30 days after sowing (DAS). On the 30th day after sowing, the seedlings were transplanted into 25 × 25 cm plastic pots, which had been prepared in advance and each contained 8 kg of sterilised soil.

2.4. Treatments

For each crop, seven treatments were applied: T0 = control (No fertilisation or inoculation); T1 = AMF; T2 = AMF + 25% NPK-Urea; T3 = AMF + 50% NPK-Urea; T4 = 25% NPK-Urea; T5 = 50% NPK-Urea; and T6 = 100% NPK-Urea. The experiment was conducted using a randomized complete block design (RCBD), with the seven treatments independently and randomly allocated within each of four blocks, resulting in four replications and a total of 28 experimental units per crop. The recommended dose of NPK-Urea was 200 kg/ha of NPK and 100 kg/ha of urea, equivalent to 0.8 g of NPK and 0.4 g of urea, respectively, per plant. The NPK fertiliser used had a formulation of N₁₄P₂₃K₁₄S₅B₁ and was applied to the bottom of the pot at the time of transplanting. Urea (46% N) was applied as a top

dressing during the flowering stage. Soil moisture prior to the start of the experiment was below 10%. Throughout the experiment, the plants were monitored and irrigated with the same volume of water across all treatments in order to maintain consistent water conditions.

2.5. Assessment of Growth and Yield Parameters

At harvest, growth parameters were determined for each plant. Shoot length, as well as leaf length and width, were measured using a tape measure, whilst the stalk diameter was recorded using a digital calliper (Ingco IP54 Digital Caliper HD28150). The number of leaves and branches per plant was also recorded. The number of fruits per plant was counted at harvest. The morphometric characteristics of the fruits, namely diameter and length, were assessed on a sample of ten fruits taken at random from each plant. The fresh biomass of the shoot and root parts was then measured using a precision electronic balance (Highland™ HCB 302, Max: 300 g × 0.01 g). Before weighing, the roots were thoroughly washed with tap water to remove any residual soil particles. The relevant samples were then dried in an oven at 65°C for 72 hours, until a constant weight was reached, in order to determine the dry biomass of the shoot and root parts.

2.6. Collection of Tomato Leaves and Fruit for Biochemical Analysis

During the experiment, tomato leaves were collected at the end of the vegetative growth stage for the determination of chlorophyll and carotenoid contents. Leaves were collected from plants within each treatment and pooled to obtain one composite sample per treatment. At harvest, fully ripe, uniformly red fruits were collected from plants within each treatment and pooled to obtain a 250 g composite sample per treatment. The fruits were surface disinfected with 70% ethanol, placed in airtight bags, and stored at –80°C until biochemical analysis. Prior to analysis, the fruits were thawed and then blended in 50 g batches using a Moulinex blender. To each 50 g of the resulting purée, 250 mL of 70% ethanol was added; the mixture was left to macerate for 1 hour, then filtered through Whatman filter paper; the resulting filtrate was stored at –80°C until biochemical analysis. For each biochemical analysis, the extract obtained from one 50 g portion was used, and three technical measurements were performed from the same extract.

2.7. Assessment of the Mycorrhization Rate

Root fragments, which had been dried beforehand and were used to determine root biomass, were collected and cut into segments approximately 1 cm long. The roots were clarified using the method described by Phillips and Hayman [29]. The root segments were immersed in a 10% KOH solution and heated to 90°C for 30 minutes in an oven. After clarification, the roots were rinsed five times with tap water to remove any KOH residues. They were then stained in a 0.05% trypan blue solution and incubated at 90°C for 45 minutes to allow visualisation of the struc-

tures of arbuscular mycorrhizal fungi. After staining, the root segments were rinsed with potable water, placed on glass slides and examined under a binocular microscope (Motic XSP-BM-2CEA, 2013). The assessment of mycorrhizal colonisation was carried out using the intersection method described by Giovannetti and Mosse [30], supplemented by the scoring method proposed by Trouvelot *et al.* [31].

Two parameters were used to characterise mycorrhization of root systems: the mycorrhization frequency (F) and the absolute intensity of mycorrhization (m).

- The Mycorrhization Frequency (F), which indicates the degree of root system infection, calculated using Equation (1):

$$F(\%) = (N - n_0) / N \quad (1)$$

where N is the number of observed fragments and n_0 is the number of fragments without signs of mycorrhization.

- The intensity of mycorrhization m (absolute mycorrhization intensity), which expresses the proportion of the cortex colonised relative to the entire root system, calculated using Equation (2):

$$m(\%) = (95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1) / (n - n_0) \quad (2)$$

where n_5 , n_4 , n_3 , n_2 and n_1 are the numbers of fragments respectively classified into the five infection classes, indicating the extent of mycorrhization: 5 = more than 95%, 4 = 50% to 95%, 3 = 30% to 50%, 2 = 1% to 30%, 1 = 1% of the cortex. For each sample, one hundred (100) root fragments were observed under the microscope.

2.8. Chlorophyll a and b and Carotenoid Content in Tomato Leaves

The chlorophyll a, chlorophyll b and carotenoid contents of the leaves were determined using the method described by Lichtenthaler and Buschmann [32]. A 0.25 g sample of ground leaf tissue was homogenised in 2 mL of 95% ethanol. The mixture was then centrifuged at 15,000× g for 15 minutes, after which the absorbance of the supernatant was measured at 664.1, 648.6 and 470 nm using a UV-Vis spectrophotometer (BioMate 3S, Thermo Fisher Scientific, USA), with 95% ethanol as the blank.

The concentrations of chlorophyll a, chlorophyll b and total carotenoids were calculated using Equations (3)-(5) respectively:

$$C_{\text{Chl a}} = (13.36 \times A_{664.1}) - (5.19 \times A_{648.6}) \quad (3)$$

$$C_{\text{Chl b}} = (27.43 \times A_{648.6}) - (8.12 \times A_{664.1}) \quad (4)$$

$$C_{\text{CT}} = ((1000 \times A_{470}) - (2.13 \times C_{\text{Chl a}}) - (97.64 \times C_{\text{Chl b}})) / 209 \quad (5)$$

With $A_{664.1}$, $A_{648.6}$ and A_{470} , the absorbances measured at 664.1 nm, 648.6 nm and 470 nm respectively.

The concentrations obtained were then converted into concentrations expressed in mg/g fresh weight (FW) using Equation (6):

$$\text{Teneur} = (C \times V) / M \quad (6)$$

where C represents the pigment concentration (mg/mL) obtained from one of the preceding equations, V represents the total volume of the extract (mL) and M represents the mass of the fresh sample used for extraction (g).

2.9. Phenolic Compound Content of Tomato Fruit

The total phenolic content was determined using a modified version of the method involving the Folin-Ciocalteu reagent, as described by Ainsworth and Gillespie [33]. 125 μL of extract was mixed with 625 μL of Folin-Ciocalteu reagent diluted to 10% (v/v). After vortexing for 5 minutes, 500 μL of a 700 mM sodium carbonate (Na_2CO_3) solution was added to the reaction mixture, which was then incubated at 25°C, in the dark, for 2 hours. The absorbance was measured at 765 nm against a blank. The total phenolic content was determined from a calibration curve prepared using gallic acid and expressed in milligrams of gallic acid equivalents per gram of fresh weight (mg GAE/g FW).

2.10. Flavonoid Content of Tomato Fruit

The total flavonoid content was determined using the colorimetric method described by Ofoe *et al.* [34] with some modifications. To this end, 500 μL of extract was mixed with 500 μL of 10% aluminium chloride (AlCl_3), 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The reaction mixture was incubated at room temperature for 30 minutes, after which the absorbance was measured at 415 nm against a blank prepared under the same conditions but without AlCl_3 . The total flavonoid content was calculated from a calibration curve established using quercetin and expressed in milligrams of quercetin equivalents per gram of fresh weight (mg EQ/g FW).

2.11. Antioxidant Capacity of Tomato Fruit

• 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging

The antioxidant activity of the fruits, assessed by their ability to scavenge the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical, was determined using the method described by Vamanu and Nita [35], 500 μL of extract was mixed with 500 μL of a 0.2 mM DPPH solution. The reaction mixture was homogenised by shaking and then incubated for 30 minutes at room temperature, protected from light. The absorbance was then measured at 517 nm using a spectrophotometer.

The percentage inhibition of the DPPH radical was calculated using the formula (7):

$$I(\%) = ((A_c - A_s) / A_c) \times 100 \quad (7)$$

A_s represents the absorbance of the mixture containing the extract, and A_c represents the absorbance in the absence of the sample.

A calibration curve was plotted using different concentrations of ascorbic acid as standards ($y = 32.2804x + 6.2877$; $R^2 = 0.952$), with concentrations on the x-

axis and inhibition percentages on the y-axis. The DPPH radical scavenging activity (S_a) was expressed in millimoles of ascorbic acid equivalents per gram of fresh weight (mmol AAE·g⁻¹ FW) according to Equation (8):

$$S_a = (X \times V_e) / m_e \quad (8)$$

where X is the antioxidant activity determined from the standard curve (mM AAE), V_e represents the volume of extract used (mL) and m_e the weight of fresh tomato corresponding to the volume of extract analysed (g).

- **Reduction of the phosphomolybdenum complex (APM)**

The antioxidant capacity of the fruit was assessed using the phosphomolybdenum complex reduction method, in accordance with the protocol described by Kedir *et al.* (2023) [36], with some modifications. As with the DPPH assay, 1 mL of a reagent solution containing sulphuric acid (0.6 M), sodium phosphate (28 mM) and ammonium molybdate (4 mM) was added to 500 µL of extract. The tubes were incubated for 90 minutes in a water bath at 95°C. After cooling to room temperature, the absorbance of the solutions was measured at 765 nm using a UV-Vis spectrophotometer. Ascorbic acid was used as the reference standard to establish the calibration curve ($y = 1.4831x - 0.1567$; $R^2 = 0.995$). The reducing power of the extracts was calculated from this curve and expressed in millimoles of ascorbic acid equivalents per gram of fresh weight (mmol AAE/g FM) according to the Equation (9):

$$R_a = (X \times V_e) / m_e \quad (9)$$

where R_a is the reducing power of the extract (mmol AAE·g⁻¹ FW), X corresponds to the reducing activity obtained from the calibration curve (mM AAE), V_e is the volume of extract used (mL) and m_e is the mass in grams (g) of fresh tomato corresponding to that volume of extract.

2.12. Statistical Analysis

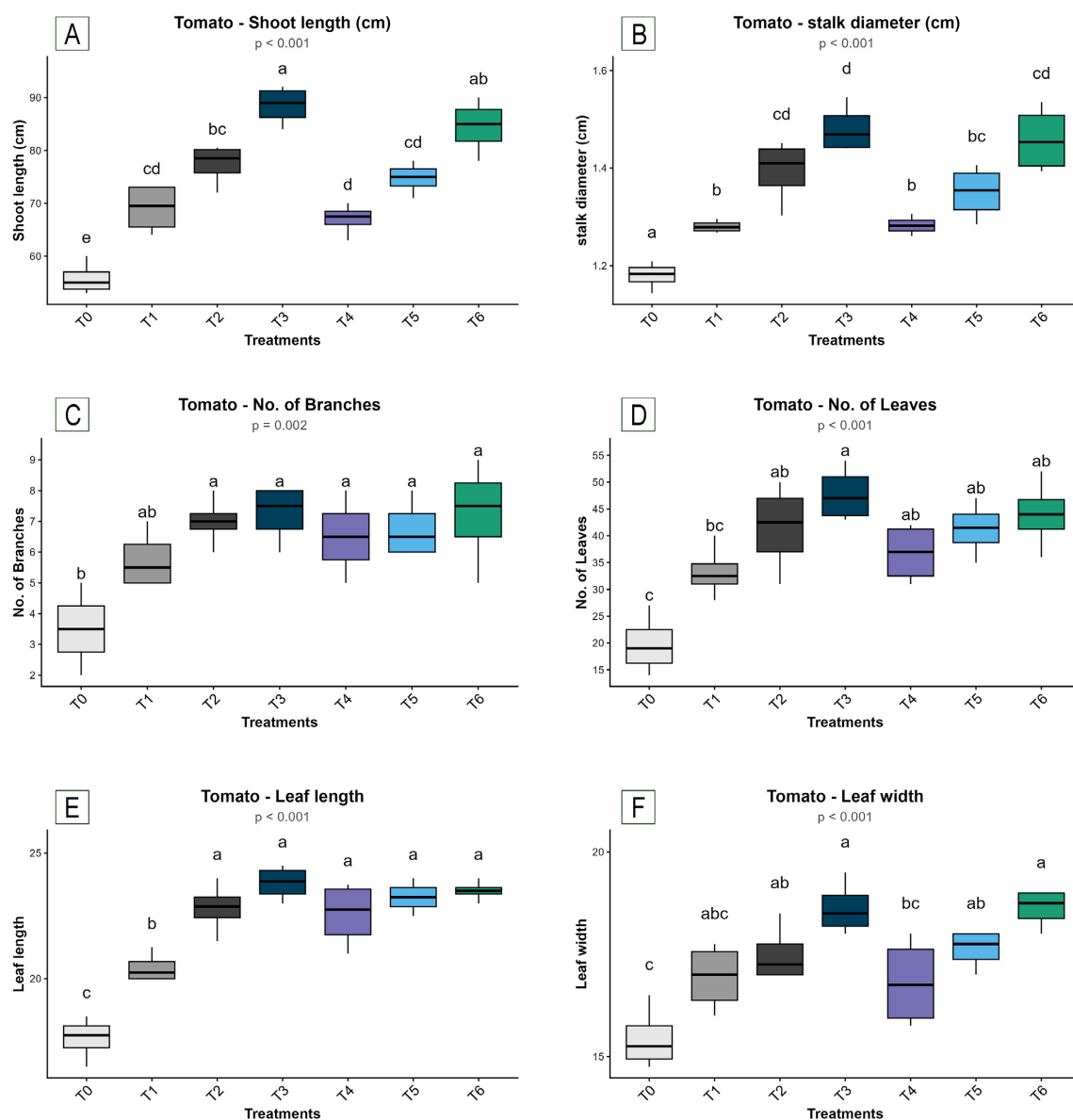
The data were entered and organised using Microsoft Excel 2019 and analysed using RStudio. For each response variable, a one-way analysis of variance (ANOVA) was performed, with treatment as the single factor. Prior to each analysis, the normality of the residuals and the homogeneity of variances were assessed using the Shapiro-Wilk and Levene's tests, respectively. When these assumptions were not met, the data were log-transformed prior to ANOVA. When a significant treatment effect was detected, means were separated using Tukey's honestly significant difference (HSD) test at $\alpha = 0.05$. Results are presented as means $\pm$ standard deviations calculated from the raw data, and differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Plant Growth

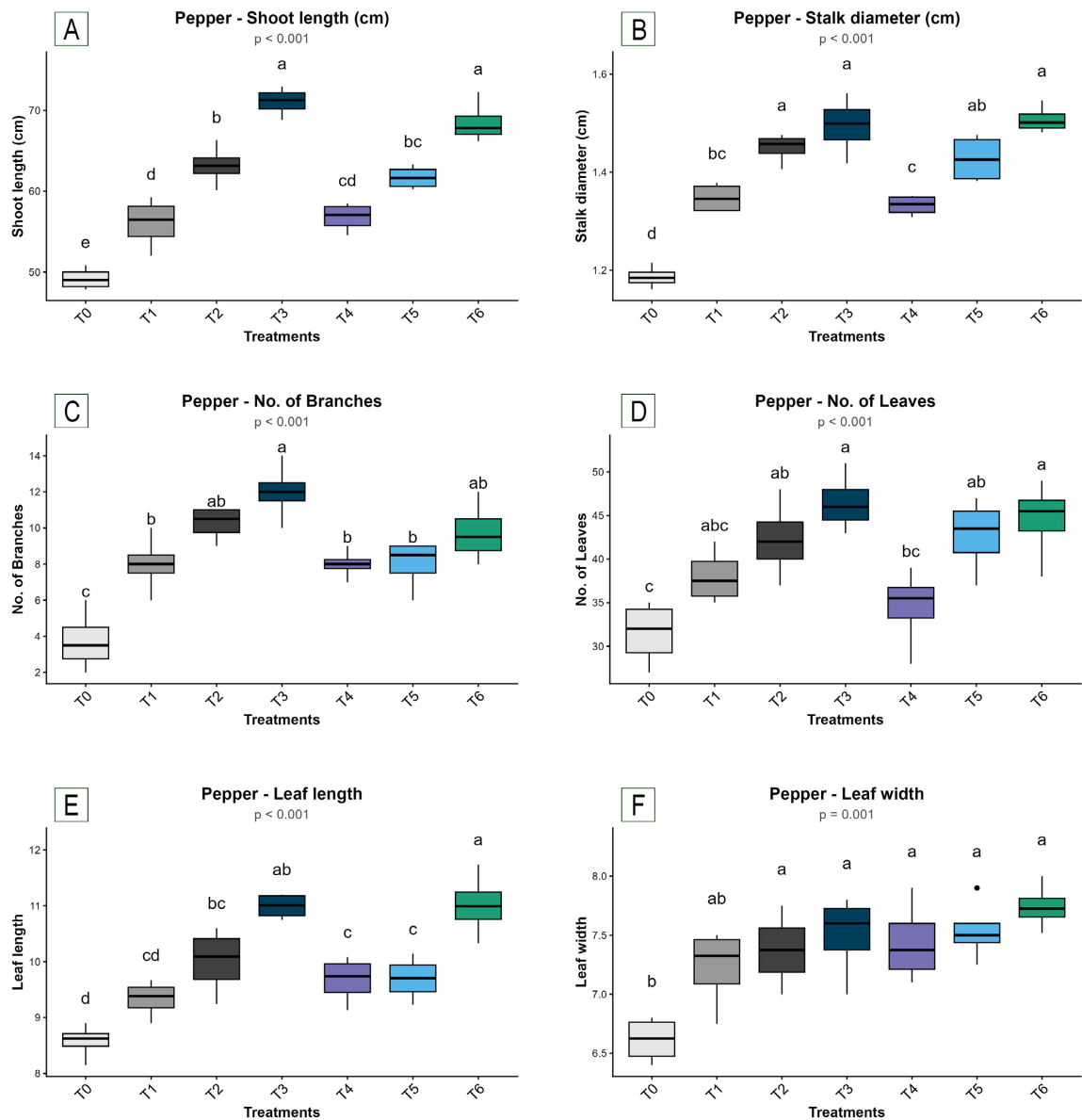
Analysis of variance, combined with Tukey's multiple comparison test, revealed a significant effect ($p < 0.001$) of treatments involving arbuscular mycorrhizal fungi

(AMF) and fertilisers on all growth parameters, in both tomatoes (**Figure 1**) and peppers (**Figure 2**). Inoculation with AMF, even in the absence of fertilisation (T1), was sufficient to significantly improve most growth parameters compared with the untreated control (T0). In tomatoes, T1 thus achieved a shoot length of 69 cm and 33.25 leaves, values comparable to those of T4 (25% NPK-Urea, 67 cm; 36.75 leaves). In peppers, a similar pattern was observed, with T1 (56.06 cm; 9.34 cm leaf length) showing no significant difference from T4 (56.79 cm; 9.67 cm, respectively) for several parameters, reflecting a notable contribution of mycorrhizal symbiosis to growth, independent of any mineral input.



No. of branches = number of branches; No. of leaves = Number of leaves; T0: Control, T1: AMF, T2: AMF + 25% NPK-Urea, T3: AMF + 50% NPK-Urea, T4: 25% NPK-Urea, T5: 50% NPK-Urea and T6: 100% NPK-Urea.

Figure 1. Effects of different treatments on tomato growth parameters. A, Shoot length; B, Stalk diameter; C, No. of branches; D, No. of leaves; E, Leaf length; F, Leaf width.



No. of branches = number of branches; No. of leaves = Number of leaves; T0: Control, T1: AMF, T2: AMF + 25% NPK-Urea, T3: AMF + 50% NPK-Urea, T4: 25% NPK-Urea, T5: 50% NPK-Urea and T6: 100% NPK-Urea.

Figure 2. Effects of different treatments on pepper growth parameters. A, Shoot length; B, Stalk diameter; C, No. of branches; D, No. of leaves; E, Leaf length; F, Leaf width.

When AMF were combined with a fraction of the recommended fertiliser dose: for both crops, T2 (AMF + 25% NPK-Urea) showed no significant differences from T5 (50% NPK-Urea) in terms of shoot length and diameter, and the number of leaves and branches, despite receiving half the amount of fertiliser. Similarly, T3 (AMF + 50% NPK-Urea) recorded the highest values for almost all growth parameters in both species including shoot length (88.5 cm in tomatoes; 71.08 cm for pepper), diameter (1.48 and 1.49 cm), number of branches (7.25 and 12) and leaves (47.75 and 46.5). For these parameters, T3 showed no significant difference from T6 (100% NPK-Urea), despite receiving only half the mineral fertiliser rate. With re-

gard to leaf width, only the tomato showed T3 (18.62 cm) to be the best treatment alongside T6, whilst in the pepper, all fertilised and/or inoculated treatments (T1 to T6) resulted in a statistically consistent improvement compared with the control.

In terms of stem biomass, T3 also performed best in both crops, both for fresh weight (154.25 g in tomatoes; 161.19 g in peppers) and dry weight (24.77 g and 26.52 g), followed by T6. The AMF-based treatments also outperformed the others in terms of root yield: in tomatoes, T1, T2 and T3 recorded the highest fresh root masses (63.88 to 65.28 g). In peppers, T1, T2 and T3 likewise outperformed all other treatments for this parameter (71.85, 73.62 and 74.81 g). A similar finding was observed for root dry weight, where T2 emerged as the most effective treatment for tomatoes (12.45 g), whilst the AMF-fertiliser combinations (T2, T3) dominated for peppers, confirming the predominant role of AMF in root development.

3.2. Yield

Table 2. Effects of different treatments on the yield of tomatoes and peppers.

Parameters	Crops	T0	T1	T2	T3	T4	T5	T6
No. of fruits	Tomato	10.75 ± 1.71 ^c	17.25 ± 1.71 ^b	20.25 ± 1.71 ^{ab}	23.5 ± 1.29 ^a	17.25 ± 2.75 ^b	20.25 ± 2.06 ^{ab}	21.75 ± 1.71 ^a
	Pepper	33 ± 3.16 ^b	36.25 ± 2.22 ^{ab}	36.75 ± 2.22 ^{ab}	40 ± 2.83 ^a	35.75 ± 3.59 ^{ab}	36.75 ± 2.36 ^{ab}	41 ± 1.83 ^a
Fruits weight	Tomato	189.25 ± 17.29 ^d	217.5 ± 20.31 ^{cd}	276.88 ± 32.84 ^b	332.25 ± 26.5 ^a	220.5 ± 23.25 ^{cd}	265.5 ± 25.88 ^{bc}	317.25 ± 14.38 ^{ab}
	Pepper	133.51 ± 12.21 ^c	170.37 ± 15.26 ^{bc}	200.49 ± 9.88 ^{ab}	229.3 ± 14.57 ^a	153.62 ± 18.02 ^c	200.71 ± 18.19 ^{ab}	225.23 ± 21.42 ^a
Fruit length	Tomato	4.68 ± 0.74 ^a	5.38 ± 0.34 ^a	5.43 ± 0.47 ^a	5.48 ± 0.72 ^a	5.24 ± 0.54 ^a	5.39 ± 0.43 ^a	5.55 ± 0.65 ^a
	Pepper	3.08 ± 0.45 ^a	3.37 ± 0.22 ^a	3.39 ± 0.29 ^a	3.56 ± 0.28 ^a	3.34 ± 0.19 ^a	3.4 ± 0.39 ^a	3.6 ± 0.32 ^a
Fruit width	Tomato	2.54 ± 0.29 ^a	2.85 ± 0.4 ^a	2.97 ± 0.45 ^a	3.02 ± 0.6 ^a	3 ± 0.61 ^a	2.83 ± 0.5 ^a	3.03 ± 0.66 ^a
	Pepper	2.68 ± 0.41 ^a	2.85 ± 0.41 ^a	3.11 ± 0.3 ^a	3.07 ± 0.27 ^a	2.8 ± 0.49 ^a	2.96 ± 0.43 ^a	3.16 ± 0.45 ^a
Shoot fresh weight	Tomato	104.75 ± 5.38 ^c	119.75 ± 5.56 ^{bc}	134.25 ± 9.88 ^{ab}	154.25 ± 8.42 ^a	114 ± 13.29 ^{bc}	136 ± 12.3 ^{ab}	154.25 ± 19.38 ^a
	Pepper	78.67 ± 13.37 ^d	100.18 ± 9.3 ^d	134.56 ± 20.67 ^{ab}	161.19 ± 2.69 ^a	104.74 ± 9.37 ^{cd}	127.95 ± 5.79 ^{bc}	145.4 ± 11.71 ^{ab}
Root fresh weight	Tomato	50.74 ± 1.79 ^b	63.88 ± 4.19 ^a	65.28 ± 3.09 ^a	65.06 ± 2.59 ^a	62.1 ± 3.56 ^a	61.3 ± 3.32 ^a	61.54 ± 2.39 ^a
	Pepper	65.51 ± 1.55 ^b	71.85 ± 1.5 ^{ab}	73.62 ± 2.06 ^a	74.81 ± 3.34 ^a	69.19 ± 3.76 ^{ab}	69.85 ± 4.33 ^{ab}	69.69 ± 3.33 ^{ab}
Shoot dry weight	Tomato	11.52 ± 1.6 ^c	15.1 ± 3.52 ^{bc}	19.05 ± 2.38 ^{ab}	24.77 ± 2.11 ^a	14.78 ± 1.8 ^{bc}	19.54 ± 3.76 ^{ab}	23.36 ± 2.87 ^a
	Pepper	15.57 ± 2.56 ^c	18.93 ± 1.73 ^{bc}	23.12 ± 2.77 ^{ab}	26.52 ± 3.85 ^a	24.41 ± 3.31 ^{ab}	26.81 ± 1.64 ^a	26.51 ± 1.11 ^a
Root dry weight	Tomato	7.85 ± 0.53 ^c	11.18 ± 0.36 ^{ab}	12.45 ± 0.46 ^a	11.98 ± 1.15 ^a	9.73 ± 1.32 ^{bc}	10.35 ± 1.11 ^{ab}	11.61 ± 1.08 ^{ab}
	Pepper	6.6 ± 0.73 ^a	8.31 ± 0.73 ^b	8.3 ± 0.51 ^b	8.56 ± 0.89 ^b	7.88 ± 0.54 ^{ab}	8.16 ± 0.88 ^b	8.28 ± 0.77 ^b

No. of fruits = Number of fruits; T0: Control, T1: AMF, T2: AMF + 25% NPK-Urea, T3: AMF + 50% NPK-Urea, T4: 25% NPK-Urea, T5: 50% NPK-Urea and T6: 100% NPK-Urea.

The benefit provided by AMF was also evident in terms of yield. In both tomatoes and peppers, T2 (AMF + 25% NPK-Urea) resulted in a comparable number of fruits and total fruit weight than T5 (50% NPK-Urea) (**Table 2**): for example, fruit weight reached 276.88 g in T2 compared with 265.5 g in T5 for tomatoes, and

200.49 g compared with 200.71 g for peppers; equivalent performance was achieved using half the amount of fertiliser thanks to mycorrhizal inoculation. Treatment T3 confirmed this trend by emerging as the best treatment across all crops, with 23.5 fruits and 332.25 g of fruit weight for tomatoes, and 40 fruits and 229.3 g for peppers, comparable to T6 (100% NPK-Urea), even though the latter was applied at the full dose.

These trends were also reflected in the morphometric characteristics of the fruit (**Table 2**). In the case of tomatoes, T2 produced fruit measuring 5.43 cm in length and 2.97 cm in width, values comparable to those of T5 (5.39 cm × 2.83 cm) and T3 (5.48 cm × 3.02 cm), which was close to T6 (5.55 cm × 3.03 cm), the best treatment at full dose. In peppers, fruit length and width followed the same pattern, with 3.39 cm × 3.11 cm for T2 compared with 3.4 cm × 2.96 cm for T5, whilst T3 (3.56 cm × 3.07 cm) remained close to the values recorded for T6 (3.6 cm × 3.16 cm). However, statistical analysis did not reveal any significant difference between the treatments for these two parameters.

3.3. Mycorrhizal Infection

Analysis of root colonisation confirmed the effectiveness of mycorrhizal inoculation in both crops. A frequency and intensity of mycorrhization of zero (0%) were recorded in the non-inoculated treatments (T0, T4, T5 and T6), regardless of the dose of NPK-Urea applied, as the substrate used had been sterilised beforehand and was therefore free from any pathogens. Conversely, the inoculated treatments (T1, T2 and T3) showed significantly higher mycorrhizal colonisation, with no significant difference between them (**Table 3**). In tomatoes, the frequency of mycorrhization ranged from 53.75% ± 7.14% (T3) to 56.5% ± 9.85% (T1), with corresponding intensities ranging from 41.21% ± 5.38% (T3) to 46.67% ± 9.18% (T1). In peppers, similar trends were observed, with a frequency ranging from 53.75% ± 8.14% (T2) to 57.75% ± 8.46% (T1), and an intensity ranging from 45.08% ± 4.52% (T3) to 51.53% ± 4.27% (T1).

Table 3. Variation in mycorrhizal infection.

Treatments	Tomato		Pepper	
	Frequency	Intensity	Frequency	Intensity
T0	0 ± 0 ^b	0 ± 0 ^b	0 ± 0 ^b	0 ± 0 ^b
T1	56.5 ± 9.85 ^a	46.67 ± 9.18 ^a	57.75 ± 8.46 ^a	51.53 ± 4.27 ^a
T2	55.5 ± 6.61 ^a	46.56 ± 8.5 ^a	53.75 ± 8.14 ^a	46.28 ± 13.39 ^a
T3	53.75 ± 7.14 ^a	41.21 ± 5.38 ^a	54 ± 6.98 ^a	45.08 ± 4.52 ^a
T4	0 ± 0 ^b	0 ± 0 ^b	0 ± 0 ^b	0 ± 0 ^b
T5	0 ± 0 ^b	0 ± 0 ^b	0 ± 0 ^b	0 ± 0 ^b
T6	0 ± 0 ^b	0 ± 0 ^b	0 ± 0 ^b	0 ± 0 ^b

No. of fruits = Number of fruits; T0: Control, T1: AMF, T2: AMF + 25% NPK-Urea, T3: AMF + 50% NPK-Urea, T4: 25% NPK-Urea, T5: 50% NPK-Urea and T6: 100% NPK-Urea.

3.4. Chlorophyll and Carotenoid Contents in Tomato Leaves and the Antioxidant Profile of Tomato Fruits

The various treatments had a significant effect on all the pigment and antioxidant parameters measured ($p < 0.05$) (Table 4). With regard to photosynthetic pigments, chlorophyll a levels were significantly higher in T3 (0.85 mg·g⁻¹ FW), T6 (0.84 mg·g⁻¹ FW) and T5 (0.82 mg·g⁻¹ FW) compared with the control T0 (0.5 mg·g⁻¹ FW), whilst T1, T2 and T4 occupied an intermediate position that was not significantly different from the two groups. A similar pattern was observed for total chlorophyll, with maximum values in T3, T5, T6 and T2 (1.21, 1.24, 1.26 and 1.25 mg·g⁻¹ FW respectively) compared with 0.76 mg·g⁻¹ fresh weight for T0. Chlorophyll b, however, was significantly increased only in T2 (0.46 mg·g⁻¹ FW) compared with the control (0.26 mg·g⁻¹ FW); the other treatments did not differ significantly from T0. For carotenoids, T3 and T6 had the highest levels (1.65 and 1.61 mg·g⁻¹ FW respectively), significantly higher than those of T0 and T1 (1.38 and 1.39 mg·g⁻¹ FW), with T4 and T5 at intermediate levels.

Table 4. Phytochemical composition of tomato leaves and fruits, and the antioxidant capacity of tomato fruits.

Treatment	Pigments in Tomato Leaves				Fruits Antioxydant Compound		Fruits Antioxydant Capacity	
	Chlorophyll a (mg·g ⁻¹ FW)	Chlorophyll b (mg·g ⁻¹ FW)	Total Chlorophyll (mg·g ⁻¹ FW)	Carotenoids (mg·g ⁻¹ FW)	Total Phenolic (mg·GAE·g ⁻¹ FW)	Flavonoids (mg·QE·g ⁻¹ FW)	APM (mmol AAE·g ⁻¹ FW)	DPPH (mmol AAE·g ⁻¹ FW)
T0	0.5 ± 0.1385 ^b	0.26 ± 0.0458 ^b	0.76 ± 0.1819 ^b	1.38 ± 0.0579 ^c	0.54 ± 0.0652 ^c	0.08 ± 0.0089 ^b	1.92 ± 0.1019 ^b	0.65 ± 0.0755 ^c
T1	0.64 ± 0.1261 ^{ab}	0.32 ± 0.0116 ^{ab}	0.95 ± 0.1168 ^{ab}	1.39 ± 0.0550 ^c	0.77 ± 0.0532 ^{ab}	0.09 ± 0.002 ^{ab}	2.48 ± 0.1539 ^a	0.69 ± 0.0868 ^{bc}
T2	0.8 ± 0.0241 ^{ab}	0.46 ± 0.0954 ^a	1.25 ± 0.0785 ^a	1.58 ± 0.0615 ^{ab}	0.86 ± 0.0598 ^a	0.1 ± 0.0151 ^{ab}	2.6 ± 0.0812 ^a	0.91 ± 0.0916 ^a
T3	0.85 ± 0.0139 ^a	0.36 ± 0.0603 ^{ab}	1.21 ± 0.0471 ^a	1.65 ± 0.0033 ^a	0.9 ± 0.0853 ^a	0.1 ± 0.0022 ^a	2.8 ± 0.1227 ^a	0.88 ± 0.0752 ^{ab}
T4	0.56 ± 0.1109 ^{ab}	0.29 ± 0.0539 ^{ab}	0.85 ± 0.1643 ^{ab}	1.4 ± 0.0679 ^{bc}	0.59 ± 0.0967 ^{bc}	0.09 ± 0.0067 ^{ab}	2.55 ± 0.1195 ^a	0.7 ± 0.0593 ^{bc}
T5	0.82 ± 0.0538 ^a	0.42 ± 0.0049 ^{ab}	1.24 ± 0.0528 ^a	1.51 ± 0.1109 ^{abc}	0.63 ± 0.0307 ^{bc}	0.09 ± 0.0053 ^{ab}	2.47 ± 0.2721 ^a	0.67 ± 0.0346 ^c
T6	0.84 ± 0.1759 ^a	0.42 ± 0.0954 ^{ab}	1.26 ± 0.2597 ^a	1.61 ± 0.0579 ^a	0.66 ± 0.0927 ^{bc}	0.09 ± 0.0076 ^{ab}	2.57 ± 0.0714 ^a	0.84 ± 0.0689 ^{abc}
p.value	0.0041	0.0141	0.0022	0.0004	0.0001	0.0494	0.0002	0.0015

T0: Control, T1: AMF, T2: AMF + 25% NPK-Urea, T3: AMF + 50% NPK-Urea, T4: 25% NPK-Urea, T5: 50% NPK-Urea and T6: 100% NPK-Urea.

With regard to the antioxidant compounds in the fruit, the total polyphenol content was significantly higher in T3 (0.9 mg GAE·g⁻¹ FW), T2 (0.86 mg GAE·g⁻¹ FW) and T1 (0.77 mg GAE·g⁻¹ FW) than in T0 (0.54 mg GAE·g⁻¹ FW), whilst T4, T5 and T6 (0.59 to 0.66 mg GAE·g⁻¹ FW) remained comparable to the control (Table 4). Flavonoids followed a similar but less pronounced trend, with a maximum value in T3 (0.1 mg QE·g⁻¹ FW) significantly higher than in T0 (0.08 mg QE·g⁻¹ FW), whilst the other treatments did not differ clearly from this. With regard to antioxidant activity, the phosphomolybdenum reduction capacity (APM) was significantly enhanced by all treatments compared with the control (1.92 mmol AAE·g⁻¹ FW), with values ranging from 2.47 (T5) to 2.8 mmol AAE·g⁻¹ FW

(T3). Finally, DPPH radical scavenging activity was significantly higher in T2 (0.91 mmol AAE·g⁻¹ FW) and T3 (0.88 mmol AAE·g⁻¹ FW) than in their respective non-inoculated counterparts (T4 and T5). However, no significant differences were observed between T2 or T3 and T6 (0.84 mmol AAE·g⁻¹ FW), despite T6 receiving the full mineral fertilisation rate.

4. Discussions

Vegetable Solanaceae, notably the tomato (*Solanum lycopersicum*) and the pepper (*Capsicum annuum*), play a central role in agricultural production systems in Benin. They contribute both to household food security and to the income of family farms. However, their cultivation relies on the intensive use of mineral fertilisers, the harmful effects of which on soil fertility, water quality and, ultimately, human and environmental health are now well documented [37] [38]. From an agroecological and One Health perspective, which recognises the interdependence between the health of soils, plants, animals and human populations [39]), the utilisation of AMF native to the local rhizosphere appears to be a promising biological alternative. It would help to reduce dependence on chemical inputs whilst improving, or failing that, maintaining, productivity [16] [40].

It is within this framework that the present study evaluated, under controlled conditions, the effect of AMF strains belonging to the Glomeraceae family, isolated from soils in Benin, applied either alone or in combination with reduced doses of NPK-Urea fertiliser, on the growth and yield of tomatoes and peppers. The experimental design comprised a mixture of three strains (*Glomus caledonius*, *Rhizophagus intraradices* and *Funneliformis geosporum*) tested alone (T1) or combined with 25% (T2) or 50% (T3) of the recommended dose of NPK-Urea. These treatments were compared with equivalent doses of mineral fertiliser alone (T0: absolute control; T4: 25% NPK-urea; T5: 50% NPK-urea) as well as with the full dose (T6: 100% NPK-urea). Growth and yield parameters were measured and then subjected to an analysis of variance followed by Tukey's test to identify significant differences.

The results reveal a significant effect of the treatments ($p < 0.001$) on all the parameters assessed, showing that both mycorrhizal inoculation and mineral fertilisation influence the development of both crops. More specifically, inoculation with AMF alone (T1), in the absence of any mineral fertiliser, equalled the effect of applying 25% NPK-urea (T4) for all growth parameters, with a marked effect on peppers. This result illustrates the ability of native strains to improve the uptake of poorly mobile nutrients, particularly phosphorus, through the expansion of the extra-root hyphal network. This mechanism, established since the work of Smith and Read [41], has been confirmed in Solanaceae by several studies reporting comparable growth gains in the absence of phosphate fertilisation [42] [43]. The more pronounced response observed in peppers could be explained by this species' inherently higher dependence on mycorrhizae, linked to a less branched root system that is therefore less effective at exploring the soil, as has been de-

scribed in other species with a high dependence on mycorrhizae [44] [45]. Recent studies confirm that *Capsicum annuum* often exhibits a higher degree of mycorrhizal dependence than the tomato, particularly in soils low in available phosphorus [46] [47].

The absence of any significant difference between treatments in terms of fruit length and width, despite contrasting levels of NPK-Urea and AMF, suggests that these traits are relatively stable under the trial conditions, probably due to their strong genetic component [48] [49]. By contrast, overall yield, particularly fruit number and weight, appears to be more sensitive to nutritional conditions and mycorrhization [50]. However, the trend observed in T2 and T3, whose dimensions remain comparable to those of T5 and T6, supports the hypothesis that the reduction in fertiliser is partially offset by improved phosphorus uptake via the hyphae of the AMF [51]. This finding is consistent with the work of Candido *et al.* [52], who demonstrated that, in tomatoes, the effects of mycorrhization are more pronounced on yield and fruit number than on fruit characteristics. The agronomic benefit of mycorrhizal symbiosis therefore lies more in maintaining productivity whilst reducing inputs than in improving fruit size [53] [54].

The increasing synergistic effect observed between AMF and mineral fertilisation is one of the most striking findings of this study. Treatment T2 (AMF + 25% NPK-Urea) achieved performance levels statistically comparable to those of T5 (50% NPK), whilst T3 (AMF + 50% NPK-Urea) proved equivalent to T6 (100% NPK-Urea) for virtually all growth and yield parameters in both crops. This phenomenon of partial compensation of mineral fertilisation by AMF is consistent with the improved nutrient uptake and utilisation induced by mycorrhizal symbiosis [16] [52]. This research highlights that AMF can reduce the need for phosphate fertilisers by 30% to 50% without any loss of yield in various crops. Recent studies on tomatoes and peppers confirm this substitution potential: inoculation with AMF combined with 50% of the NPK and Urea dose resulted in yields not statistically different from those achieved with the full dose [42] [55]. The magnitude of the gain observed here, with treatments receiving half or even the full mineral fertilisation rate showing no statistically significant differences from their corresponding inoculated treatments, remains nevertheless noteworthy and should be interpreted with caution. The absence of a statistically significant difference does not demonstrate equivalence or superiority. The practical significance of these findings would therefore benefit from further evaluation using an economic indicator, such as a cost-benefit ratio incorporating the cost of the inoculum and the fertiliser savings achieved [56] [57].

The performance of T3, which outperforms T6 in terms of root biomass and fruit yield whilst using half the amount of fertiliser, highlights a ceiling on efficiency once NPK-Urea levels exceed 50% in the presence of AMF. Beyond this threshold, the additional mineral input no longer yields a proportional agronomic benefit, as the symbiosis alone ensures the nutritional coverage required for optimal production. This plateau reflects the genuine agronomic and economic added

value of inoculation: AMF do not merely compensate for a reduction in fertilisation; they enable the performance level achieved with a full dose of fertiliser to be matched or even exceeded, whilst limiting the quantity of chemical inputs used. This type of plateau response, where yield ceases to increase beyond a certain level of mineral input combined with inoculation, is consistent with the principle of increased nutrient use efficiency associated with mycorrhizal symbiosis, which has been widely reported in vegetable crops [43] [58]. Trials under controlled conditions and in the field have shown that moderate levels of fertilisation (around 50% of the recommended rate) maximise both root colonisation and agronomic benefits, whilst high rates of phosphorus inhibit the symbiosis [42] [59].

With regard to mycorrhizal infection, the complete absence of root colonisation in the non-inoculated treatments (T0, T4, T5 and T6) demonstrates the effectiveness of prior sterilisation of the substrate. This observation confirms that the mycorrhization observed in the inoculated treatments is primarily linked to the addition of inoculum [30] [60]. The rates of mycorrhizal frequency and intensity recorded in T1, T2 and T3 showed no significant differences between them. This result can be explained by the application of an identical dose of inoculum, providing a comparable quantity of infectious propagules per plant [19] [60]. The good viability of these propagules promotes effective root colonisation [61]. This uniformity in mycorrhization thus suggests that, under the conditions of the present study, the quality and viability of the inoculum contributed significantly to the establishment of the symbiosis [60].

The significant increase in chlorophyll a and total chlorophyll content observed in plants that received treatments combining AMF with a moderate nitrogen input (T2 and T3) reflects an improvement in the photosynthetic apparatus, as nitrogen is an essential component of chlorophyll and a key determinant of photosynthetic capacity [16]. The fact that T3 (AMF + 50% NPK-Urea) achieved chlorophyll a and carotenoid levels that were statistically comparable to those of T6 (100% NPK-Urea), whilst using half the amount of mineral fertiliser, suggests that AMF has a beneficial effect on nutrient uptake and utilisation. This mechanism is consistent with the role of the extraradicular mycelial network in the uptake and transfer of nitrogen and phosphorus, as well as in maintaining the photosynthetic performance of mycorrhizal plants [62]. These results suggest improved fertiliser use efficiency, as AMF-associated treatments achieved responses that were not statistically different from those obtained with higher mineral fertiliser inputs, an aspect of major agronomic interest with a view to reducing mineral inputs [62] [63]. The relative stability of chlorophyll b across treatments, in contrast to the more marked variations in chlorophyll a, could be explained by differential regulation of the chlorophyll a/b ratio in response to nitrogen availability [64] [65]. These pigment dynamics may influence photosynthetic efficiency and, by extension, biomass production and yield, as chlorophyll content serves as an indicator of the photosynthetic capacity of leaves [66].

With regard to the antioxidant compounds in the fruit, the trend observed is

somewhat different from that for leaf pigments. Indeed, total polyphenol and flavonoid contents were higher in the treatments combining AMF and reduced fertilisation (T1, T2, T3), whilst mineral fertilisation alone (T4, T5, T6) did not result in levels exceeding those of the control for these compounds. This observation is consistent with the hypothesis that moderate nitrogen availability may promote the accumulation of certain phenolic compounds in tomatoes [67]. Conversely, mycorrhization may stimulate the accumulation of phenolic compounds in the fruit, likely in connection with the activation of secondary metabolism and the plant's defence responses [68] [69].

The higher phenolic compound content observed in the AMF-associated treatments was accompanied by greater in the antioxidant capacity of the fruit, particularly in terms of DPPH radical scavenging activity, which was higher in T2 and T3 than in the treatments using mineral fertiliser alone (T4, T5 and T6). This result suggests that AMF plays an important role in enhancing the antioxidant potential of the fruit, with phenolic compounds and flavonoids contributing significantly to this activity through their ability to neutralise free radicals [68] [70]. Phosphomolybdenum reducing power (APM), on the other hand, was improved more uniformly across all treatments, suggesting that various antioxidants, notably phenolic compounds, carotenoids and vitamin C, contribute to this reducing capacity [71].

Ultimately, these results highlight the role of AMFs as an agroecological tool capable of reconciling agronomic performance with crop quality. Their application improved the plants' photosynthetic status, with effects comparable to those achieved with full mineral fertilisation, whilst promoting the accumulation of bioactive compounds and the antioxidant capacity of the fruit, which was in some cases higher than that observed with mineral fertilisation alone. The combined effects observed on nutritional efficiency and fruit quality reinforce the value of AMF in strategies aimed at reducing mineral inputs, particularly through the potential to halve the application of mineral fertilisers without compromising crop performance. It is fully in line with an agroecological and One Health approach, potentially helping to limit nitrogen and phosphorus losses to the environment, reduce the environmental footprint associated with the use of synthetic fertilisers, and preserve the quality of agroecosystems. The use of native AMF offers additional benefits in this regard, owing to their adaptation to local soil and climate conditions and the promotion of indigenous fungal diversity. Research carried out in Benin and West Africa, particularly on consortia of indigenous Glomeraceae, further corroborates the potential of these microorganisms to improve crop productivity whilst reducing levels of mineral fertilisation [72] [73]. These findings therefore support the integration of native AMF into vegetable farming practices, particularly in low-input systems, where they could simultaneously contribute to crop productivity, nutritional quality and the sustainability of agricultural practices. However, the transfer of these results, obtained under controlled conditions, now requires validation in the field, over several growing seasons and in

contrasting soil and climate conditions. These trials will also need to take into account the interactions between the introduced inocula and indigenous microbial communities, which may influence their effectiveness, as well as economic and environmental indicators such as the cost-benefit ratio, nutrient use efficiency and the risks of mineral elements leaching into water bodies. Such an approach would enable a better assessment of the feasibility, profitability and sustainability of using native AMF and confirm their potential as a component of more productive, input-efficient and environmentally friendly market gardening systems in Benin.

5. Conclusion

This study demonstrated the efficacy of native arbuscular mycorrhizal fungal (AMF) strains, isolated from the Beninese rhizosphere, on two vegetable crops of economic and nutritional importance, *Solanum lycopersicum* and *Capsicum annuum*, grown in greenhouses. The results show that inoculation with these AMF not only improves crop growth and yield, but also promotes the accumulation of bioactive compounds and enhances the antioxidant capacity of tomato fruits. Combining them with reduced doses of mineral fertilisers, in particular, 50% of the recommended fertiliser rate, thus enables good agronomic performance to be maintained whilst reducing the use of mineral inputs. These results highlight the potential of native AMF as an agroecological tool for more efficient and sustainable vegetable production in Benin. However, field trials are needed to confirm the reproducibility and agronomic relevance of these results under real production conditions, perhaps across different soil types, seasons and cropping cycles.

Author Contributions

A. Agonkoun, R.M. Aguégué, O. Amogou, S.A. Assogba, and Y.M. Adoko carried out the experimental work, data collection and analysis. A. Agonkoun, S.M.I. Hoteyi, A. Adjanohoun, N. Desoignies and L. Baba-Moussa contributed to the designing, supervision, and interpretation of the results. A. Adjanohoun, N. Desoignies and L. Baba-Moussa reviewed the final project. N. Desoignies and L. Baba-Moussa wrote the global project. All authors contributed to the study conception, the article writing and approved the submitted version.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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