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Conception and Development of Recycled Raw Materials (Coconut Fiber and Bagasse)-Based Substrates Enriched with Soil Microorganisms (Arbuscular Mycorrhizal Fungi, *Trichoderma* spp. and *Pseudomonas* spp.) for the Soilless Cultivation of Tomato (*S. lycopersicum*)

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Abstract: The global production quantity and the utilisation area harvested for the cultivation of tomatoes have significantly increased in the last three decades. Europe still plays an important role in the production of tomatoes, accounting for 12% of global production in 2020. Tomato production can be divided into greenhouse/soilless production and open field production. Greenhouse/soilless tomato production is mostly developed in northern Europe, and open field production in southern Europe. Soilless cultivation serves to improve control of the growing medium and to avoid any likely problems for watering and maintaining proper nutrient concentrations. Beneficial soil microorganisms, particularly arbuscular mycorrhizal fungi (AMF), are increasingly being recognized as key elements of an agro-ecological approach to agricultural production. The use of these beneficial microorganisms on soilless tomato production may improve plant performance and reduce biotic and abiotic stress occurring during production with a consequent decrease of chemicals and increase of sustainability of the production system. In this paper, we tested different substrates composed of coconut fiber and bagasse (S1 to S4) and beneficial microorganisms (AMF, *Pseudomonas fluorescens* and *Trichoderma harzianum*), selecting the most suitable system for the soilless production of tomatoes. Our results showed that substrates S1 (100% coconut fiber) and S2 (66% coconut fiber + 33% bagasse) complementarily used with the consortium of "AMF IP21 + *Trichoderma harzianum* + *Pseudomonas fluorescens* IPB04" seem to be the "best formulation" for this purpose. That confirmed the feasibility of the development of recycled material (coconut fiber and bagasse)-based substrates together with soil microorganisms (AMF and beneficial bacteria) for soilless tomato production.

Keywords: tomato; beneficial soil microorganisms; growing substrates

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1. Introduction

After the Second World War, agriculture faced the problem of providing food and resources to people in order to sustain population growth. In the last decades, agriculture has continued to produce food for such growth (7 billion people). The tomato is one of the most important crops worldwide. Its global production quantity and the utilisation area harvested have significantly increased since 1990 (80 million tons in 1990; 180 million tons in 2020). The production quantity has increased by 125% [1], while harvest area has increased by almost 67% [1]. Europe still plays an important role in the production of tomatoes, accounting for 12% of global production in 2020 [1]. Tomato production can be divided into greenhouse/soilless production and open field production. Greenhouse/soilless tomato

production is mostly developed in northern Europe (France, the Netherlands, Belgium), and open field production in southern Europe (Italy, Spain, Greece, Portugal). Italy is the largest producer with 6.9 million tons (70% in the field, year 2003) [2]. Spain comes second, producing 5.2 million tons. Every year, each family in France consumes 14 kg of tomatoes. Moreover, 642,538 tons of tomatoes were produced in 2020 in France [3], principally in Brittany, Provence-Alpes Côte-d'Azur, and Pays de la Loire [4], with 70% coming from soilless production.

Soilless agriculture in growing media (substrates) is becoming more and more important. In fact, growing media are easier to handle and may provide a better growing environment (in terms of one or more aspects of plant growth) compared to soil culture [5,6]. Substrates can be divided into organic substrates (sawdust, coco peat, peat moss, bark etc.), inorganic substrates of natural origin (perlite, vermiculite, gravel, rockwool, etc.), and synthetically produced substrates (hydrogel, foam mates (polyurethane), etc.) [7–11]. Results of most works show that substrates have a significant effect on the plant growth, composition of leaf, total yield, and fruit quality [12–17]. The use of coconut-based substrates (coconut dust, coconut fiber and its mixtures) together with bagasse is getting more and more importance in soilless agriculture. Both substrates are interesting because they are easy to provide and they are recycled materials. The suitability of coconut-based substrates for the growing of vegetables in greenhouses has been analyzed. Different authors [6,18] compared coconut substrates to other substrates (perlite, rockwool, sawdust) used in greenhouse vegetable growing. Alifar et al. [19] investigated the effect of five different growing media for cucumber growing. Results showed that the largest stem diameter and highest biomass were obtained in cocopeat and perlite-cocopeat media. In the same way, the use of bagasse (fibrous material remaining after removing the sucrose, water, and other impurities (filter mud) from sugarcane) as a growth media component has already shown promise in the soilless production of vegetable, and in particular for tomato production [20–22].

In this context, we investigated the interest of use of coconut fiber and bagasse for soilless tomato production.

Furthermore, agriculture has to face increasing environmental implications, such as the massive use of fertilizers and agro chemicals for plant disease, which pollute the soil, use of large amounts of water and the impoverishing of soils with fewer organic substances, and the production of more and more CO_2, which pollutes the atmosphere.

Against this background, agriculture has had to find a solution to the ever-increasing environmental problems. Extensive agriculture, integrated farming, and organic farming tried to conceive agriculture in a more sustainable way: the rational use of more environmental friendly fertilizers and agro chemicals, no or minimum tillage, the use of tolerant/resistant plant varieties to cope with pests, the use of beneficial microorganisms. In agriculture, microorganisms (bacteria and fungi) are principally used as biocontrol agents, e.g., *Trichoderma* spp., *Bacillus* spp., and *Pseudomonas* spp., and biostimulants/biofertilizers as plant growth promoting bacteria (PGPB) and mycorrhizal fungi (MF) [23–25]. Arbuscular mycorrhizal fungi (AMF) occur in all soils and commonly colonize roots of many plant species. These fungi can increase plant growth and reproduction by enhancing the uptake of nutrients. AMF can also benefit plants by stimulating growth regulating substances, increasing photosynthesis, improving osmotic adjustment under drought and salinity stresses, and increasing resistance to pests [26]. The primary effect of AMF on their host plant is an increase in plant growth and nutrient uptake, mainly phosphorus [27]. Mycorrhizal inoculation reduces the quantity of fertilizer application, making it less than normally required for non-inoculated plant conditions [28]. Furthermore, different studies [29–31] have shown the benefits of using AMF in tomato cultivation against both pathogens and abiotic stresses. Sanchez-Bel et al. [32] showed that tomato plants colonized with AMF *Rhizophagus irregularis* and *Funneliformis mosseae* developed increased resistance against *Botrytis cinerea*. Bitterlich et al. [33] and Chitarra et al. [34] revealed that inoculation with arbuscular mycorrhiza can result in improved water availability and transport within colonised substrates. Physiologically, this indicates that plants may experience or feel

less root surface stress at equal substrate moisture, as substrate moisture decreases. This may be a key element in making soilless tomato production more sustainable by using less water. *Pseudomonas fluorescens* is known as potential P solubilizer [35]. The use of P solubilizers, alone or in combination with other plant growth-promoting microbes as an eco-friendly microbial consortium, could increase the P uptake of crops, increasing their yields for agricultural and environmental sustainability. Furthermore, Bona et al. [36,37] showed that the inoculation of tomato with AMF and *Pseudomonas fluorescens* can help to drastically reduce the use of chemical fertilization, maintaining and, in some cases, even improving the tomato fruit yield and quality.

Woo et al. [38] and Zin et al. [39] listed the different uses of *Trichoderma* spp. in agriculture: the target use is for the control of soilborne fungal pathogens, such as *Rhizoctonia*, *Pythium,* and *Sclerotinia*, and a few foliar pathogens, such as *Botrytis* and *Alternaria*; whereas the minor use indication is for plant growth promotion. An ecological approach to control Fusarium wilt in tomato, using a consortium composed of fluorescent *Pseudomonas*, *Trichoderma harzianum,* and the AMF *Rhizophagus intraradices*, has been able to reduce disease incidence and severity by 74% and 67% in pots and field, respectively [40].

The AMF, PGPB, and biocontrol agents as *Trichoderma* spp. are absent in soilless substrates. The use of these beneficial microorganisms on soilless tomato production may improve plant performance and reduce biotic and abiotic stress occurring during production, with a consequent decrease of chemicals and increase in the sustainability of the production system [41,42].

In this paper, we tested different substrates composed with coconut fiber and bagasse and beneficial microorganisms for selecting the most suitable system for the soilless production of tomatoes.

2. Materials and Methods

2.1. Inoculum Production

Different beneficial soil microorganisms have been tested in this work: (i) arbuscular mycorrhizal fungi (AMF) (one strain of *Rhizophagus intraradices* and a mix of 6 *Glomus* strains), (ii) a bacteria, *Pseudomonas fluorescens,* and (iii) a strain of the fungi *Trichoderma harzianum*. We used a strain of *Trichoderma harzianum* in mix with AMF and *Pseudomonas fluorescens* for testing a potential synergetic effect on growth and abiotic stresses.

The AMF strain IP21 (*Rhizophagus intraradices*) and *Pseudomonas fluorescens* IPB04 were produced by INOCULUMplus from its own microorganisms collection. *Rhizophagus intraradices* IP21 inoculum was produced in vivo on lucerne using a growing substrate composed of peat, vermiculite, and oyster shells. *Pseudomonas fluorescens* IPB04 has been produced in the laboratory, in vitro in liquid medium (LB broth). Its final concentration was 10^9 UFC/mL. The interest of this strain is its capacity to solubilize phosphorus present in the soil/substrate. The strain of *Trichoderma harzianum* was provided by the firm Microgaia (SP), as a powder with a fungal concentration of 10^8 UFC/g. A commercial AMF product has been also tested. It contained 6 AMF strains: *Claroideoglomus etunicatum* (*G. etunicatum*), *Glomus microaggregatum, Rhizophagus intraradices (G. intraradices), Claroideoglomus claroideum (G. claroideum), Funneliformis mosseae (G. mosseae), Funneliformis geosporum (G. geosporum).* This was a granulated product (1–2 mm) with 200 propagules AMF/g.

2.2. Experimental Design

NUCEA SUBSTRATE delivered to INOCULUMplus 200 clods made of different mixtures of coconut fiber and bagasse. These clods were placed on benches in a greenhouse and a drip system was set up for the irrigation of plants. Before planting, clods were washed abundantly with reverse osmosis water until obtaining a conductivity between 0.15 and 0.2 S/m.

This experiment was carried out in INOCULUMplus greenhouse for 8 weeks: 168 tomato plants ("Plaisance" cultivar), delivered by NUCEA SUBSTRATE, were planted in different growing substrates for comparing the impact of 6 types of microorganisms modalities

on their development: modality 1 = AMF (strain IP21); modality 2 = AMF (strain IP21) + *Trichoderma harzianum*; modality 3 = AMF (strain IP21) + *Pseudomonas fluorescens* (strain IPB04); modality 4 = AMF (strain IP21) + *Trichoderma harzianum* + *Pseudomonas fluorescens* (strain IPB04); modality 5 = commercial AMF product containing 6 AMF strains; modality 6 = commercial AMF product containing 6 AMF strains + *Trichoderma harzianum;* modality 7 = control (no addition of microorganisms).

We applied 20 g of inoculum AMF strain IP21 (*Rhizophagus intraradices*) caracterized by high mycorrhization rates (F%, which corresponds to the percentage of roots with AMF structures, 100% in minimum; and M%, which corresponds to the percentage of colonization inside the roots, 80% in minimum) in planting holes for plants of modalities 1, 2, 3, and 4. *Pseudomonas fluorescens* IPB04 has been applied in substrates for modalities 3 and 4 by adding 10 µL of inoculum (final concentration: 10^7 UFC/plant). The strain of *Trichoderma harzianum* was applied at 1 g/plant for modalities 2, 4 and 6.

We applied 20 g of commercial AMF product in substrates for modalities 5 and 6.

Four growing substrates containing different proportions of coconut fiber and bagasse were tested (Table 1): S1 (100% coconut fiber), S2 (66% coconut fiber + 33% bagasse), S3 (50% coconut fiber + 50% bagasse) and S4 (33% coconut fiber + 66% bagasse). The experimental scheme is described in Table 1. There were 7 blocks of plants, corresponding to 6 treatments + 1 control, which were planted in the 4 substrates. For each block, 6 plants were planted in each substrate, for a total of 24 plants per block. The different modalities were brought near roots of each tomato seedling at the time of planting. The greenhouse cultivation conditions were set as follows: temperature 21 °C day, 17 °C night, and minimum illumination of 100 watt/m^2/16 h per day.

Table 1. Table showing the experimental conditions: 4 different substrates tested (S1, S2, S3, S4), 7 treatments tested (6 modalities + Control), and 6 replicates per treatment. For example, for substrate S1, 42 plants (7 treatments × 6 replicates) were planted. So that gives a total of 168 plants (42 plants per substrate × 4 substrates).

Substrate	Coconut Fiber	Bagasse	Number of Modalities	Number of Replicates	Total Number of Plants
S1	100%	0%	6 modalities + 1 control	6	42
S2	66%	33%	6 modalities + 1 control	6	42
S3	50%	50%	6 modalities + 1 control	6	42
S4	33%	66%	6 modalities + 1 control	6	42

2.3. Physical and Chemical Analyses

The pH and conductivity were measured every day in the drainage water before planting up to 10 weeks after planting. Substrates were analyzed for their physicochemical properties by an independent laboratory (INRAE, Arras). Attention has been focused on the concentration of phosphorus, which plays a key role in mycorrhizal development 43–45.

2.4. Nutrient Diet

Phosphorus content in the soil, substrates or in the nutrient solution is essential for the development of the AMF. The available phosphorus would be used by the plant and the AMF would not develop [43–45]. Since the P content in the coconut fiber is high (335 ppm, see Section 3.1), in order to allow the microorganisms (especially AMF) to better establish themselves in the roots of the plants, plants were watered with reverse osmosis water for the first 9 days (200 cc per day). From the tenth day, the plants were watered 5 days out of 7 (200 cc per day) with a nutrient solution without adding phosphorus (225 g of SCN My (1.5% N, 37% K_2O, 5.5% MgO, 0.03% B, 0.006% Cu, 0.15% Fe, 0.074% Mn, 0.005% Mo, 0.03% Zn) were diluted in 1.5 L of reverse osmosis water; 262 g of Calcium Nitrate were diluted in 1.5 L of reverse osmosis water by adding 3 mL of the iron-rich "Fertiligène" solution. The two solutions were then mixed in the drip tray by adding reverse osmosis water until having a conductivity of 0.29 S/m. The pH of this solution was

5.5), and the two remaining days of each week with reverse osmosis water. From the 6th week after planting, phosphorus was added to the nutrient solution as monopotassium phosphate (240 mg/plant), as the plants showed some symptoms of phosphorus deficiency (appearance of violet leaves [46]).

2.5. Assessment of Plant Development

Plant development has been defined by morphological measurement, including plant height and number of branches. Manual calculation of plant height from each treatment was performed using a measuring tape (±1 mm error). The height of each selected plant was taken from the base of the stem to the growing tip of the last leaf.

At 4, 6, and 8 weeks after planting (T4, T6 and T8 respectively), the height of stem, branch development, and phosphorus deficiency (percentage of violet leaves) were measured for 8 plants/modality and 14 plants/substrate. Fruiting potential (percentage of flowers, wilted flowers, fruits on the total of buds) and roots were also analyzed: root development and AMF colonization evaluation (following the procedure described in Phillips and Hayman [47] for coloration of roots and Trouvelot et al. [48] for the microscopic root colonization evaluation). These analyses being destructive, only one plant was taken for each modality in each substrate at T4, T6 and T8. So, we had 4 root systems for the modalities 1, 2, 3, 4, 5, 6 and the control (from S1, S2, S3 and S4); we had 7 root systems for the substrates S1, S2, S3, and S4 (from the 7 modalities tested).

AMF colonization evaluation gave us different parameters. The most reliable are: (i) F% corresponds to the percentage of roots with AMF structures; (ii) M% corresponds to the percentage of colonization inside roots; (iii) a% corresponds to the content in arbuscules of mycorrhized parts. The higher M% is, the more effective the establishment of AMF inside roots. Furthermore, a high value of a% means that a lot of arbuscules (exchange place between the plant and the fungi) have been observed.

2.6. Statistical Analyses

For ground-up parameters, two-way ANOVA (p-value < 0.05) was performed in order to compare groups of results according to two crossed categories of sampling procedure: the substrate in one hand and the modality in the other. Thus, it was possible to reveal any individual effect from those two cultivation condition categories, as well as any cross-effect between them. We did not integrate temporality (T4, T6 and T8) as a statistical factor because of the destructive sampling (root analyses) realized during this work. Thus, we performed the two-way ANOVA for each time step, and we compared them afterwards. Because of the sparse data for fruiting potential and mycorrhization rates, one-way ANOVA (p-value < 0.05) was performed for evaluating whether significant differences were observed between the substrates on the one hand and the modalities on the other. When a p-value < 0.05 was obtained, a Tukey–Kramer post-hoc test was performed in order to indicate which substrate or/and which modality demonstrated significant differences from the others.

3. Results and Discussion

3.1. Physical Parameters Evolution

The pH was analyzed in the drainage water before planting up to 10 weeks after planting. Values reached a low of 5.3 and a high of 7.6. Generally, they stabilized around 6.0, except for the small decrease measured after 5 weeks (Figure 1).

The electrical conductivity reached values between 0.48 to 0.68 S/m before planting. After watering with reverse osmosis water, they stabilized between 0.15 and 0.2 S/m. During the experiment, it changed between 0.08 S/m and 0.28 S/m depending on the substrate (Figure 2).

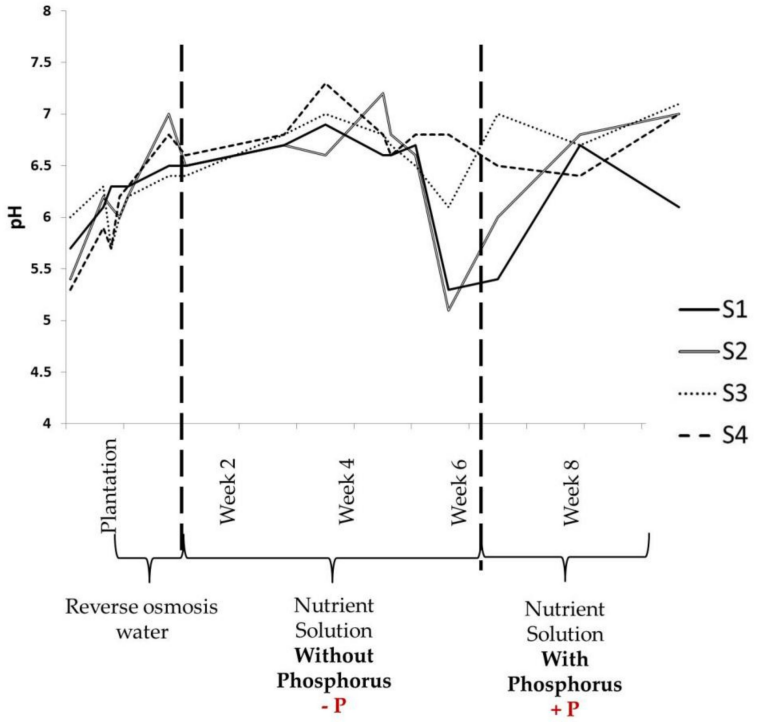

Figure 1. Evolution of pH value of the drainage water collected at the level of the different substrates (S1: 100% coconut fiber; S2: 66% coconut fiber + 33% bagasse; S3: 50% coconut fiber + 50% bagasse; S4: 33% coconut fiber + 66% bagasse) before planting and up to 10 weeks after planting.

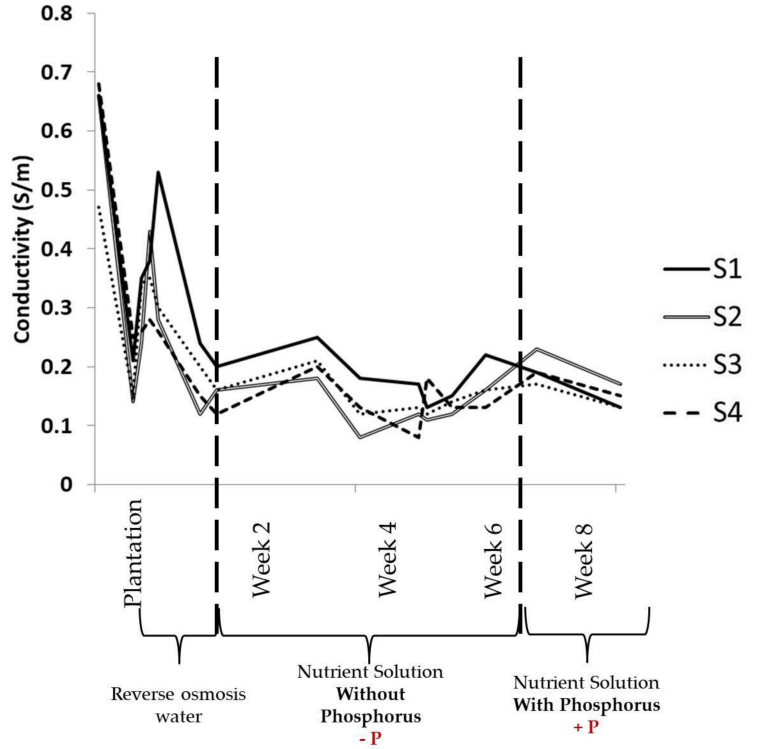

Figure 2. Evolution of conductivity value (S/m) values of the drainage water collected at the level of the different substrates (S1: 100% coconut fiber; S2: 66% coconut fiber + 33% bagasse; S3: 50% coconut fiber + 50% bagasse; S4: 33% coconut fiber + 66% bagasse) before planting and up to 10 weeks after planting.

The coconut fibers used in this study were analyzed for their physicochemical properties. Attention has been focused on the concentration of phosphorus, which plays a key role in mycorrhizal development [43–45]. The results of the analysis (Table 2) showed that the concentration of phosphorus available for plants is 355 ppm. For different reasons, the analysis of bagasse was not possible during this work. Nevertheless, recent studies [49,50] showed that the average phosphorus content of bagasse can be estimated to 260 ppm. So, we hypothesize that the phosphorus content in each substrate was 355 ppm for S1, 291 ppm for S2, 307 ppm for S3, and 262 ppm for S4. The fertilization was designed in relation to these data.

Table 2. Physicochemical properties of washed coconut fiber with a focus on phosphorus content.

	Whashed Coco Fiber
pH	7.02
Organic material	75%
Total content of Phosphorus	563 ppm
Available Phosphorus	329 ppm
Phosphorus content in solution	355 ppm
Phosphorus content in solution	100%

3.2. Plant Growth Effects

3.2.1. Root Development

The development of the root system of each plant was observed and estimated using an arbitrarily defined qualitative scale as follows (Figure 3): (i) "+" corresponds to weak development (sporadic development over the entire root ball), (ii) "++" corresponds to medium development (significant development on the side surfaces of the root ball, but less on the upper surface), (iii) "+++" corresponds to significant development (homogeneous development on the upper surface of the root ball and also on the side surfaces).

Root development inside the clods was not taken into account, because it appeared in the same way in all cases and did not constitute a criterion of distinction.

No difference in root system development was observed between the different substrates tested.

Looking at Figure 3, we can see that the root system of the plants treated with microorganisms is more developed than in control: (i) significant development (+++) in modalities 5 and 6; (ii) medium development (++) in modalities 1, 2, 3, 4; (iii) weak development (+) in the control. Modalities 1, 2, 3, and 4 allowed the roots to develop well on the outside and on the surface in a homogeneous way. Modalities 5 and 6 allowed the roots to develop well on the outside and especially on the upper surface. These observations occurred as well at week 4, then week 6 and week 8.

Figure 3. Pictures showing the different types of root development (+, ++, +++) observed in clods, (**a**) 4 weeks after planting, (**b**) 6 weeks after planting and (**c**) 8 weeks after planting.

3.2.2. Mycorrhization

AMF root colonization was assessed at six and eight weeks after planting. Variance analysis (one-way ANOVA with *p*-value threshold 0.05) was performed (Table 3). No significant difference on mycorrhization rates between used substrates was highlighted at T6 and T8 (*p*-values more than 0.05). Considering modalities, a significant difference was revealed for F% and a% (*p*-value less than 0.05).

Table 3. Probabilities obtained with one-way ANOVA (*p*-value threshold 0.05) performed on mycorrhization rates (F%, M% and a%) at T6 and T8 considering substrates used in one hand and modalities in the other.

	F%	M%	a%
T6			
Substrate	0.17	0.18	0.24
Modality	0.34	0.80	0.72
T8			
Substrate	0.97	0.53	0.34
Modality	0.04	0.09	0.02

Based on the Tukey–Kramer post-hoc test, we found that the difference in means between each modality was finally not statistically significant (all absolute mean differences > Q critical value). These results are due to high values of variance for some samples, due to a heterogeneous development of plants inside each modality and the low number of samples. Nevertheless, a descriptive analysis allows for highlighting some trends (Figures 4 and 5).

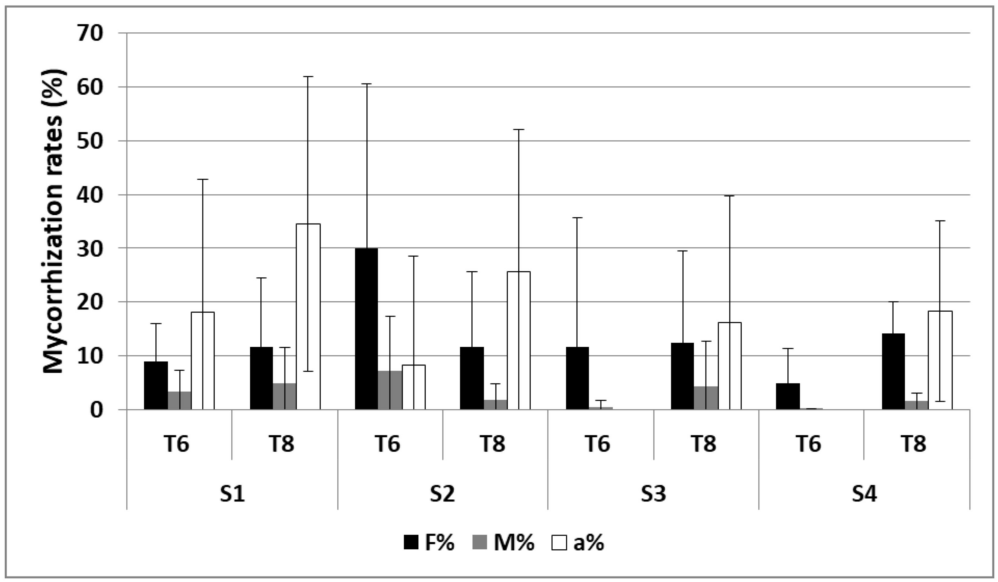

Figure 4. Mycorrhization rates (F%, M%, and a%) for plants at week 6 (T6) and week 8 (T8) after planting taking into account the different substrates used (S1: 100% coconut fiber; S2: 66% coconut fiber + 33% bagasse; S3: 50% coconut fiber + 50% bagasse; S4: 33% coconut fiber + 66% bagasse). F% (in black) corresponds to the percentage of roots with AMF structures; M% (in grey) corresponds to the percentage of colonization inside roots; a% (in white) corresponds to the content in arbuscules of mycorrhized parts.

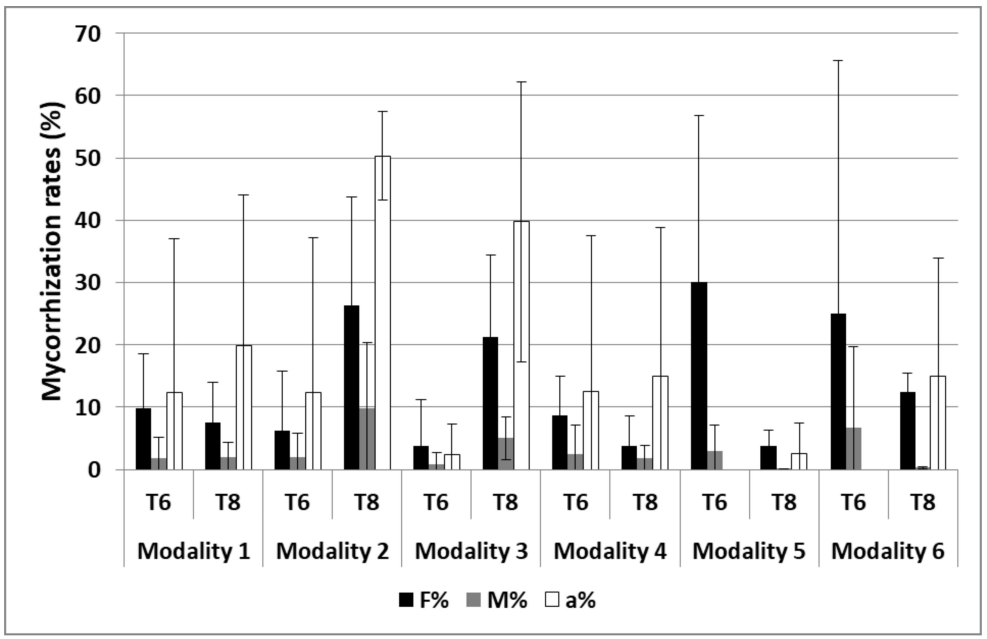

Figure 5. Mycorrhization rates (F%, M%, and a%) for plants of modalities 1 to 6 at week 6 (T6) and week 8 (T8) after planting independently of the substrates. F% (in black) corresponds to the percentage of roots with AMF structures; M% (in grey) corresponds to the percentage of colonization inside roots; a% (in white) corresponds to the content in arbuscules of mycorrhized parts. Modality 1 = AMF (strain IP21); modality 2 = AMF (strain IP21) + *Trichoderma harzianum*; modality 3 = AMF (strain IP21) + *Pseudomonas fluorescens* (strain IPB04); modality 4 = AMF (strain IP21) + *Trichoderma harzianum* + *Pseudomonas fluorescens* (strain IPB04); modality 5 = commercial AMF product containing 6 AMF strains; modality 6 = commercial AMF product containing 6 AMF strains + *Trichoderma harzianum.*

If we consider only the substrates, globally, mycorrhization rates (F% and M%) increased between T6 (six weeks after planting) and T8 (eight weeks after planting) (Figure 4). Thus means that despite the supply of P, AMF colonization kept going to progress in roots. Substrates S1 (100% coconut fiber) and S2 (66% coconut fiber + 33% bagasse) showed higher values of a% (34.5% and 25.7% at T8, respectively) in comparison with substrates S3 (50% coconut fiber + 50% bagasse) and substrate S4 (33% coconut fiber + 66% bagasse) which showed a% values of 16.2% and 18.4% at T8, respectively. With the parameter a% giving the percentage of arbuscules in mycorrhized parts of roots, we can say that substrates S1 and S2 seem to be the best substrates for optimizing exchanges between AMF and tomato plants. In these substrates (S1 and S2), the uptake of nutrients by plants could be better than in S3 and S4 [51].

If we consider only the microorganisms, in modalities 2 and 3, mycorrhization rates (F% and M%) increased between T6 and T8, while they decreased in modalities 1, 4, 5, and 6 (Figure 5). In the same way, highest a% values were observed in modalities 2 and 3 (50% and 39% at T8, respectively). The consortia "AMF IP21 (*Rhizophagus intraradices*) + *Trichoderma harzianum*" and "AMF IP21 (*Rhizophagus intraradices*) + *Pseudomonas fluorescens* IPB04" seem to be the most appropriate for optimizing the root colonization of tomato plants.

3.2.3. Ground-Up Development

Two-way ANOVA realized on ground-up parameters of plants are shown in Table 4.

Table 4. Probabilities obtained with two-way ANOVA (*p*-value threshold 0.05) performed on stem length, branch number and P deficiency at T4, T6 and T8.

	Stem Length	**Branch Number**	**P Deficiency**
T4			
Substrate	0.31	0.06	variance 0
Modality	0.95	0.70	variance 0
Interaction	1.00	0.66	variance 0
T6			
Substrate	9.629460×10^{-07}	0.61	variance 0
Modality	0.11	0.39	variance 0
Interaction	0.87	0.99	variance 0
T8			
Substrate	2.805976×10^{-27}	0.000171038	variance 0
Modality	4.091049×10^{-12}	0.26	variance 0
Interaction	1.717594×10^{-19}	0.47	variance 0

Globally, four weeks after planting (T4), no significant difference was highlighted. Six weeks after planting (T6), only substrates could have an effect on the stem length of plants (p-value < 0.05). When we take a look at the graph of means in Figure 6, we can see that, despite high values of confidence intervals, the substrates S1, S3, and S4 allowed a better growth of plants for three modalities (1, 2, and 3). Eight weeks after planting (T8), substrates, modalities, and both together could impact on the plant growth (Table 4; p-values < 0.05). The substrates S1, S2, and S4 allowed a better growth of plants (Figure 7). Modality 1 seems to have the best effect on growth when it was used in substrates S1 and S2. In the same way, modality 2 and modality 3 gave good plant development (more than global mean) when they were used in substrate S4 and substrates S2, S3, and S4, respectively. Regardless of substrate or treatment, plant growth was highest between weeks 6 and 8 (>100% development) compared to the period between week 4 and week 6 (<20% development). This difference could be explained by the addition of phosphorus in the nutrient solution six weeks after planting.

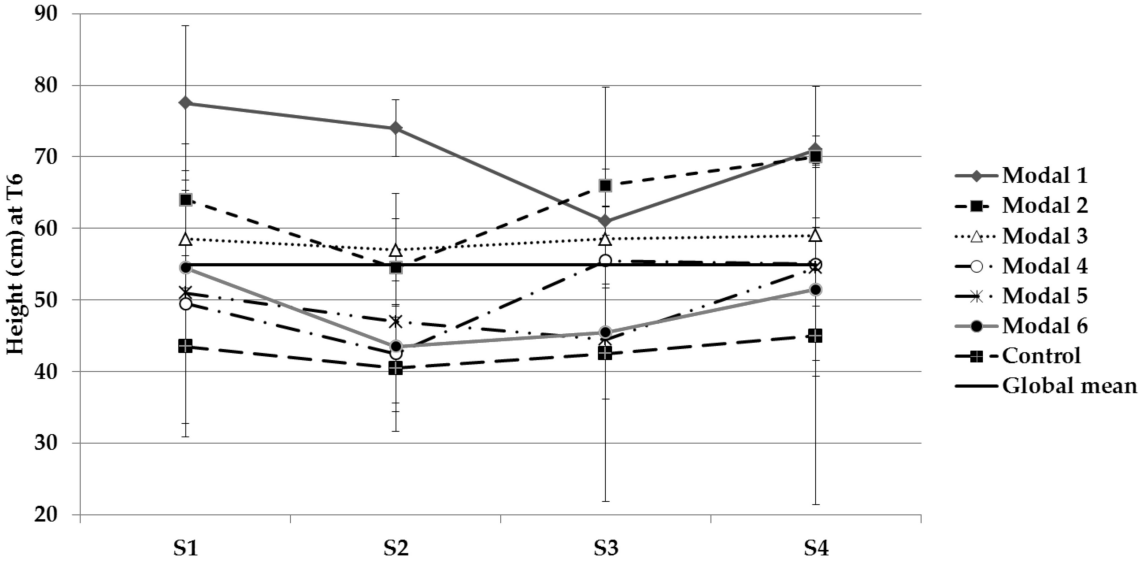

Figure 6. Graph of means of stem length (on the vertical axis) for each modality tested according to the different substrates (on the horizontal axis), 6 weeks after planting. S1 = 100% coconut fiber; S2 = 66% coconut fiber + 33% bagasse; S3 = 50% coconut fiber + 50% bagasse; S4 = 33% coconut fiber + 66% bagasse). Modality 1 = AMF (strain IP21); modality 2 = AMF (strain IP21) + *Trichoderma harzianum*; modality 3 = AMF (strain IP21) + *Pseudomonas fluorescens* (strain IPB04); modality 4 = AMF (strain IP21) + *Trichoderma harzianum* + *Pseudomonas fluorescens* (strain IPB04); modality 5 = commercial AMF product containing 6 AMF strains; modality 6 = commercial AMF product containing 6 AMF strains + *Trichoderma harzianum.* Confidence level was 95%.

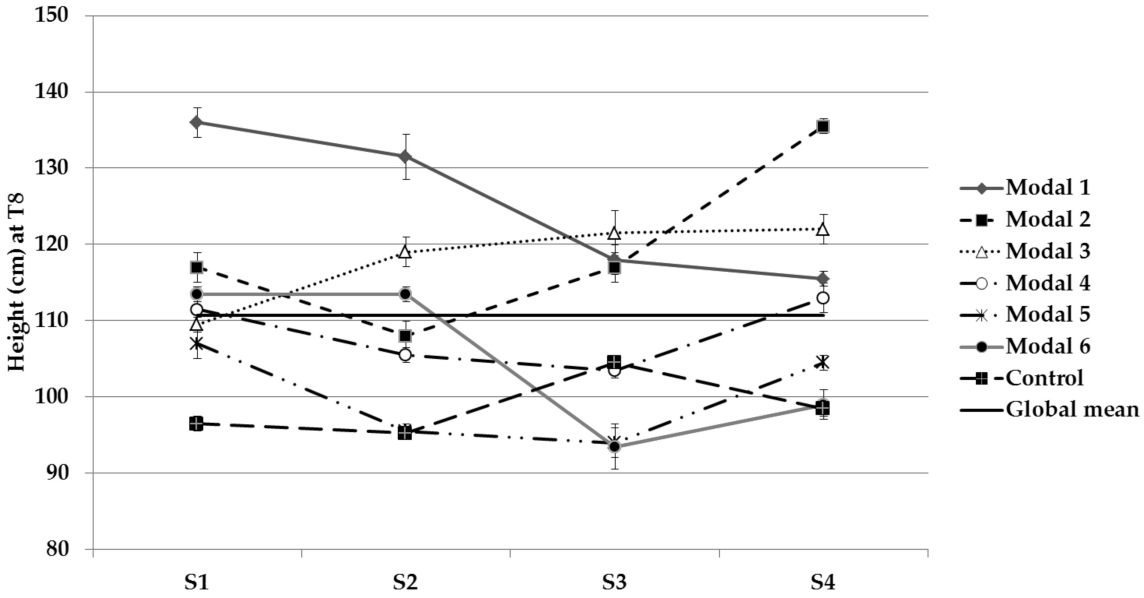

Figure 7. Graph of means of stem length (on the vertical axis) for each modality tested according to the different substrates (on the horizontal axis), 8 weeks after planting. S1 = 100% coconut fiber; S2 = 66% coconut fiber + 33% bagasse; S3 = 50% coconut fiber + 50% bagasse; S4 = 33% coconut fiber + 66% bagasse). Modality 1 = AMF (strain IP21); modality 2 = AMF (strain IP21) + *Trichoderma harzianum*; modality 3 = AMF (strain IP21) + *Pseudomonas fluorescens* (strain IPB04); modality 4 = AMF (strain IP21) + *Trichoderma harzianum* + *Pseudomonas fluorescens* (strain IPB04); modality 5 = commercial AMF product containing 6 AMF strains; modality 6 = commercial AMF product containing 6 AMF strains + *Trichoderma harzianum.* Confidence level was 95%.

Concerning branches, only substrates S1 and S2 seem to have an effect on at T8 (Table 4 and Figure 8). Regardless of the substrates or treatments, branch development of the plant was higher between six and eight weeks (development between 80% and <100%, respectively) than development between four and six weeks (development < 5%). This difference could be explained by the addition of phosphorus in the nutrient solution six weeks after planting.

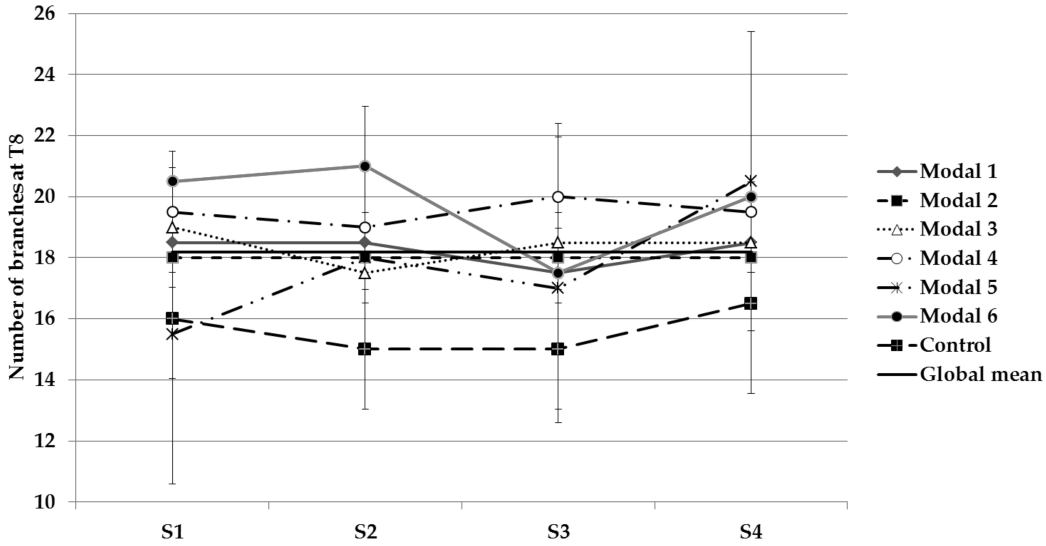

Figure 8. Graph of means of number of branches (on the vertical axis) for each modality tested according to the different substrates (on the horizontal axis), 8 weeks after planting. S1 = 100% coconut fiber; S2 = 66% coconut fiber + 33% bagasse; S3 = 50% coconut fiber + 50% bagasse; S4 = 33% coconut fiber + 66% bagasse). Modality 1 = AMF (strain IP21); modality 2 = AMF (strain IP21) + *Trichoderma harzianum*; modality 3 = AMF (strain IP21) + *Pseudomonas fluorescens* (strain IPB04); modality 4 = AMF (strain IP21) + *Trichoderma harzianum* + *Pseudomonas fluorescens* (strain IPB04); modality 5 = commercial AMF product containing 6 AMF strains; modality 6 = commercial AMF product containing 6 AMF strains + *Trichoderma harzianum.* Confidence level was 95%.

As the period of the experiment was only 8 weeks, it was not possible to obtain yield data. For this reason, we chose to evaluate the fruiting potential (percentage of flowers, wilted flowers, knotted fruits on the total of buds) at T6 and T8. Due to few data available, one-way ANOVA has been performed for highlighting significant effect of substrates or/and modalities on this parameter (Table 5).

Table 5. Probabilities obtained with one-way ANOVA (*p*-value threshold 0.05) performed on fruiting potential at T6 and T8 considering substrates used in one hand and modalities in the other.

	Fruiting Potential
T6	
Substrate	0.23
Modality	0.10
T8	
Substrate	0.0156
Modality	0.0098

At T6, no significant effect of substrates or treatments was shown for fruiting potential. On the contrary, at T8, significant differences were revealed (*p*-value less than 0.05).

Based on the Tukey–Kramer post-hoc test, we found that the difference in means between each modality and each substrate was finally not statistically significant (all absolute mean differences > Q critical value). These results are due to high values of

variance for some samples, due to a heterogeneous development of plants inside each modality and substrate. Nevertheless, a descriptive analysis allows for highlighting some trends (Figures 9 and 10).

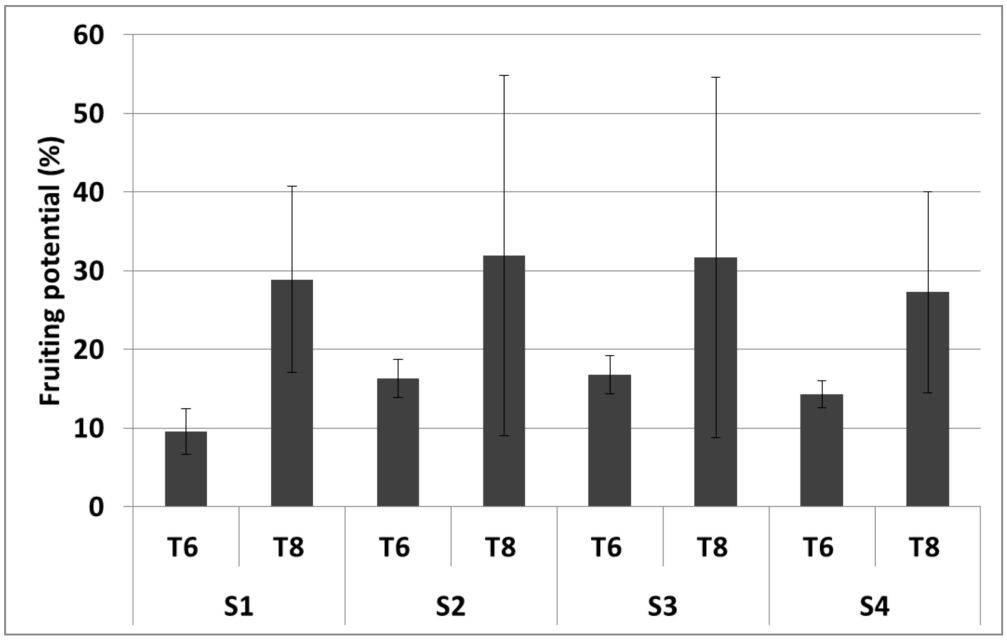

Figure 9. Evolution at T6 and T8 of the fruiting potential in % (percentage of flowers, wilted flowers, fruits on the total of buds) considering only growing substrates (S1: 100% coconut fiber; S2: 66% coconut fiber + 33% bagasse; S3: 50% coconut fiber + 50% bagasse; S4: 33% coconut fiber + 66% bagasse).

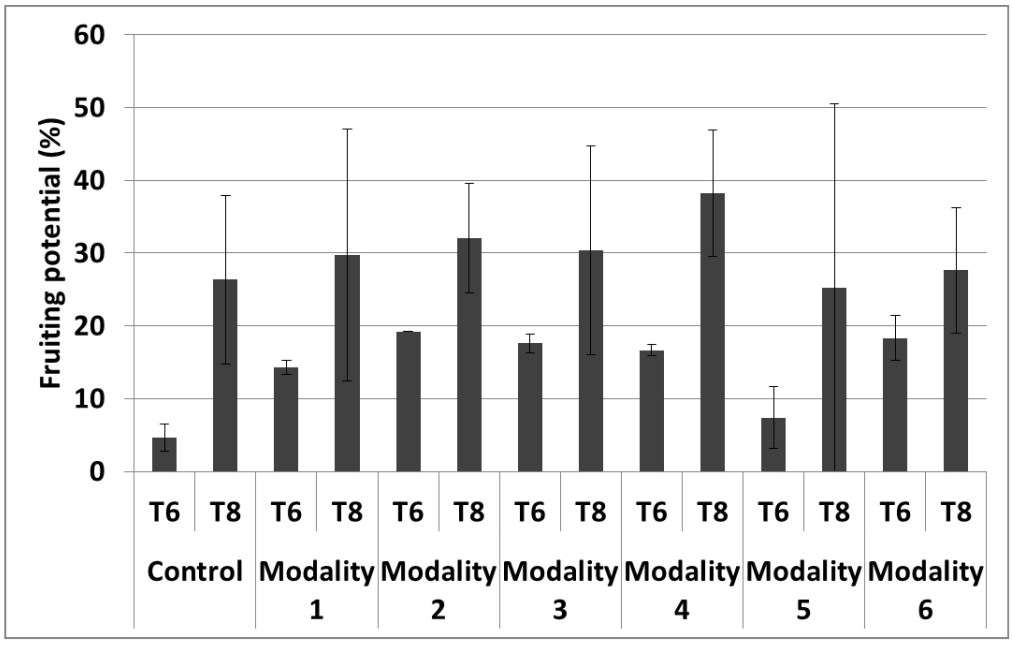

Figure 10. Evolution at T6 and T8 of the fruiting potential in % (percentage of flowers, wilted Figure 1. = AMF (strain IP21); modality 2 = AMF (strain IP21) + *Trichoderma harzianum*; modality 3 = AMF (strain IP21) + *Pseudomonas fluorescens* (strain IPB04); modality 4 = AMF (strain IP21) + *Trichoderma harzianum* + *Pseudomonas fluorescens* (strain IPB04); modality 5 = commercial AMF product containing 6 AMF strains; modality 6 = commercial AMF product containing 6 AMF strains + *Trichoderma harzianum.*

Regardless of the substrates or treatments, control plants showed the lowest fruiting potential at T6. This difference disappeared at T8, having globally the same level of fruiting potential.

When we focus only on substrates (Figure 9), only plants on S1 showed lower fruiting potential at T6. Within two weeks, fruiting potential doubled in substrates S2, S3, and S4 (from 14–15% to 27–32%) and tripled in substrate S1 (from 9% to 28%), reaching comparable values.

When we focus on treatments (Figure 10), plants in control and modality 5 gave the lowest fruiting potential at T6 (4.5% and 7%, respectively), while the other modalities gave similar values (between 14% and 18%). At T8, plants of modality 4 showed the highest fruiting potential with 38% while the other modalities showed values between 25% and 32%.

Concerning the observation of phosphorus deficiency, because of the homogeneous distribution of purple colour on leaves, no variance has been obtained. So, a descriptive analysis was realizaed (Figures 11 and 12). After increasing between four and six weeks (between 5% and <40%), phosphorus deficiency (indicated by the presence of purple leaves on the plants) strongly decreased between six and eight weeks after planting (between 60% and <40%), by adding phosphorus to the nutrient solution.

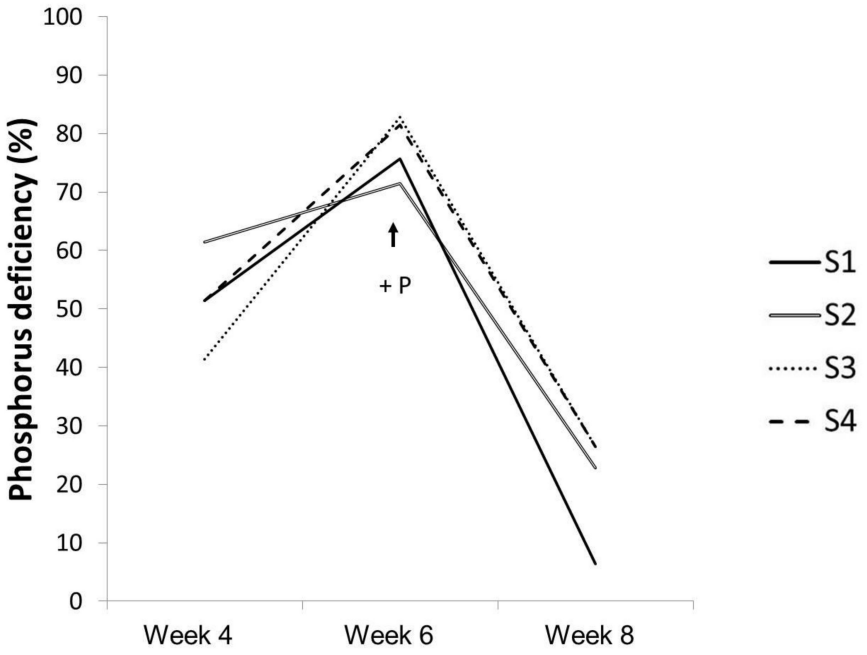

Figure 11. Temporal evolution of the percentage of phosphorus deficiency (purple leaves) in plants from 4 weeks after planting up to 6 and 8 weeks considering only growing substrates (S1: 100% coconut fiber; S2: 66% coconut fiber + 33% bagasse; S3: 50% coconut fiber + 50% bagasse; S4: 33% coconut fiber + 66% bagasse).

If we consider only the growing substrates, P deficiency symptoms appeared more prominently in substrates S3 and S4 (more than 80% of purple leaves) at week 6. Substrates S1 and S2 showed lower P deficiency with 60% and 70% of purple leaves, respectively. As these substrates contain more coconut fiber (and so, more native P), they allowed the plants to feel less P deficiency. Furthermore, substrate S1 seemed to be more effective in recovering from deficiency symptoms compared to the other substrates (Figure 11): P deficiency was less than 10% in S1 at week 8 while it was still 25% and in S2, S3, and S4.

Considering only the treatments, the plants of modalities 1, 4, and 6 showed lower deficiencies at each step of the experimentation (between 55% and 65% at week 6; between 5% and 15% at week 8). On the other hand, control plants showed the highest symptoms at each step of the experimentation (Figure 12).

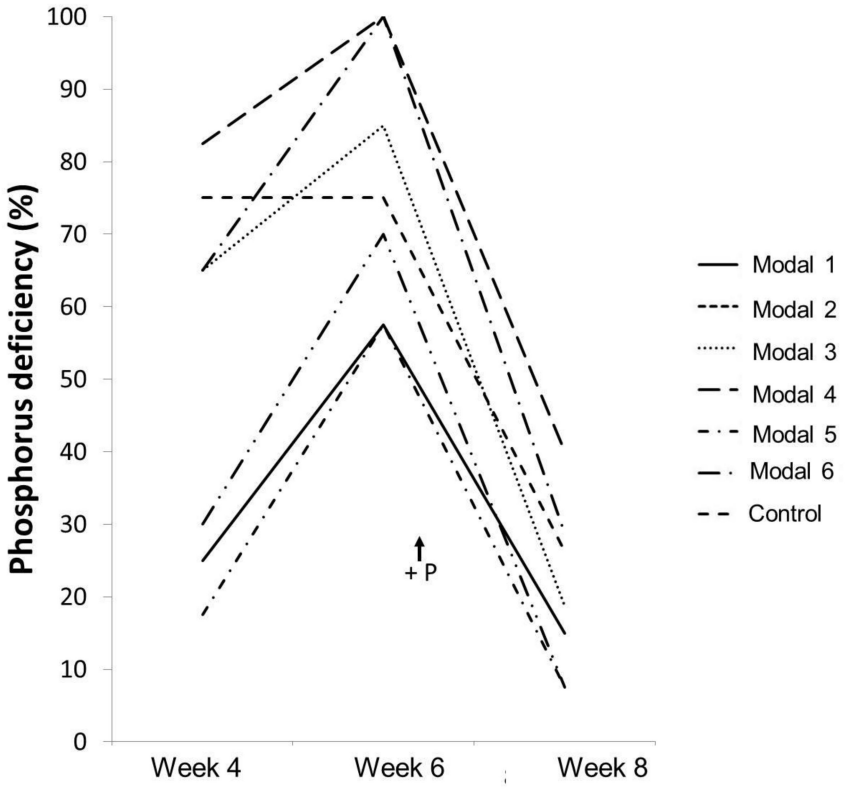

Figure 12. Temporal evolution of the percentage of phosphorus deficiency (purple leaves) in plants from 4 weeks after planting up to 6 and 8 weeks only considering applied treatments. Modality 1 = AMF (strain IP21); modality 2 = AMF (strain IP21) + *Trichoderma harzianum*; modality 3 = AMF (strain IP21) + *Pseudomonas fluorescens* (strain IPB04); modality 4 = AMF (strain IP21) + *Trichoderma harzianum* + *Pseudomonas fluorescens* (strain IPB04); modality 5 = commercial AMF product containing 6 AMF strains; modality 6 = commercial AMF product containing 6 AMF strains + *Trichoderma harzianum.*

Because of heterogeneous plant development inside modalities, we obtained high confidence intervals for most parameters. Nevertheless, it has been possible to highlight some trends: if we compile information on the effect of substrates and treatments on plant development (Table 6), we see that substrates S1 (100% coconut fiber) and S2 (66% coconut fiber + 33% bagasse) are the most suitable for a good mycorrhization and a better development of plants. These substrates seem also to be suitable for decreasing the effect of P deficiency. For the other measured parameters (root development and fruiting potential), no significant difference has been observed between substrates. Furthermore, the addition of bagasse to the substrate did not seem to have a significant beneficial effect on the development of beneficial microorganisms and plants.

Table 6. Summary of substrates and treatments with a positive effect or no effect (-) on plants: root development, mycorrhization rates; stem length, branch number, fruiting potential and P deficiency.

	Substrate	**Modality**
Root development	-	5 and 6
Mycorrhization	S1 and S2	2 and 3
Stem length	S1, S2 and S3	1, 2 and 3
Branch number	S1 and S2	-
Fruiting potential	-	4
P deficiency	S1 and S2	1, 4 and 6

Modalities 2 (AMF IP21 + *Trichoderma harzianum*) and 3 (AMF IP21 + *Pseudomonas fluorescens* IPB04) seemed to be the most suitable for a good establishment of AMF inside

roots and a better development of plants. On the contrary, modalities 1 (AMF IP21), 4 (AMF IP21 + *Trichoderma harzianum* + *Pseudomonas fluorescens* IPB04), and 6 (AMF six strains + *Trichoderma harzianum*) seemed to be the best to decrease the effect of P deficiency. The use of modality 4 (AMF IP21 + *Trichoderma harzianum* + *Pseudomonas fluorescens* IPB04) resulted in a higher fruiting potential than in other modalities. The modalities 5 (commercial AMF product containing 6 AMF strains) and 6 (commercial AMF product containing 6 AMF strains + *Trichoderma harzianum*) seemed to have a positive effect on root development.

Following the results obtained, substrates S1 (100% coconut fiber) and S2 (66% coconut fiber + 33% bagasse) complementarily used with the consortium of "AMF IP21 + *Trichoderma harzianum* + *Pseudomonas fluorescens* IPB04" seem to be the "best formulation" for the optimal growth and productivity of tomato plants in a soilless production system. In fact, S1 and S2 seem the most appropriate substrates for almost all the parameters evaluated (Mycorrhization, stem length, branch number, P deficiency). Apparently, coconut fiber is the most suitable raw material for the composition of sustainable substrates in agriculture, because it is a recycled product with a large amount of phosphorus which can be exploited by the plant. The addition of bagasse doesn't affect plant development and allow the plant to grow properly when its concentration in the substrate does not exceed the percentage of 33%. In our experimentation, the interest of bagasse as a part of substrate (Substrate S2) has been demonstrated. So, the physio-chemical analysis of bagasse is essential in order to understand the real potential utilisation of such material, which is also a recycled material like the coconut fiber.

Concerning the microorganisms used in this study, the consortium "AMF IP21 + *Trichoderma harzianum* + *Pseudomonas fluorescens* IPB04" allowed the plant to have potentially more fruit and to recover better from P deficiency. This means that the use of such microorganisms in the substrates is useful for plant development.

In agriculture, phosphorus is one of the key elements (macronutrient together with nitrogen and potassium) essential for plant development. Unfortunately, phosphorus is a limited and expensive resource. So, sustainable alternatives need to be found and developed. The utilisation of AMF and/or phosphorus solubilizing bacteria can be a real alternative for the optimization of phosphorus resources. In the present study, the percentage of phosphorus deficiency could be an indicator of the beneficial contribution of mycorrhizal fungi to the plant. As the coconut fiber already contained a high concentration of phosphorus (355 ppm), it can be exploited by the plant due to the presence of AMF inside their roots [35,52]. For this reason, the treated plants grew in the first six weeks and showed lower P deficiency symptoms. Then, at six weeks, phosphorus had to be added to the nutrient solution, because we hypothesize that the plants had exploited all the phosphorus reserves present in the substrate. The synergistic effect "AMF + *Trichoderma harzianum* + *Pseudomonas fluorescens*" allowed the plants to use the subsequent phosphorus supply better than the control. This allowed the plants to grow better and recover faster from deficiency symptoms.

4. Conclusions

In this paper, we tested different substrates composed with coconut fiber and bagasse (S1 to S4) and beneficial microorganisms (AMF, *Pseudomonas fluorescens* and *Trichoderma harzianum*), selecting the best suitable system for soilless production of tomatoes. Our results showed that substrates S1 (100% coconut fiber) and S2 (66% coconut fiber + 33% bagasse) complementarily used with the consortium of "AMF IP21 + *Trichoderma harzianum* + *Pseudomonas fluorescens* IPB04" seem to be the "best formulation" for this purpose. That confirmed the feasibility of the development of recycled material (coconut fiber and bagasse)-based substrates together with soil microorganisms for soilless tomato production.

Following these promising results, the use of resilient, bio-based nutrient sources, such as wastewater-derived algae in the frame of minerals nanofertilizer application [53], could be a complementary technology for a more sustainable soilless production. In fact, Handan [13] demonstrated that algae addition in tomato is beneficial for tomato plant

growth and mineral enrichment (Calcium uptake). Gulia et al. [54] demonstrated the potential use of cyanobacteria in the soilless cultivation of tomato concerning fruit and flower count.

Currently, INOCULUMplus is involved in the European project Excalibur (2019–2024; grant number 817946). This will allow us to improve knowledge concerning the effects of the use of micro-organisms (AMF, bacteria, fungi) on different crops (yield, health of plants, and resistance to biotic and abiotic stresses): apple, strawberry, tomato. At the end of this project, conclusions will be made regarding the management of bio-fertilizers and bio-control for cultivation in soil. The results obtained for the tomato crop could be a start for new experimental designs to test for soilless tomato production.

5. Patents

Currently, a patent is being written by the company NUCEA SUBSTRATE in order to integrate beneficial microorganisms in their substrates.

Author Contributions: S.M. and T.S. contributed equally to this paper. S.M., J.B. and T.S. contributed to the experimental design. S.M. and T.S. acquired and interpreted the data. S.M. drafted the manuscript. T.S., J.C.B. and J.-M.D. critically revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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