agronomy

MDPI

Article

Evaluation of Organic Substrates and Microorganisms as Bio-Fertilisation Tool in Container Crop Production

Jose Luis Ruiz [1] and Maria del Carmen Salas [2,*]

[1] Doctoral Program in Protected Agriculture, Almeria University, 04120 Almeria, Spain; jl.ruizz@hotmail.com
[2] Department of Agronomy, Almeria University, Campus de Excelencia Internacional Agroalimentario, ceiA3, La Cañada, 04120 Almeria, Spain
* Correspondence: csalas@ual.es; Tel.: +34-636340621

Received: 11 October 2019; Accepted: 28 October 2019; Published: 1 November 2019

Abstract: Microorganisms are only effective when adequate conditions for their survival and development are provided. Among the factors that influence its effectiveness includes the type of soil or culture substrate, which works as an energy source reserve. Therefore, a tomato and a melon crop were established in different cycles to assess the effect of the physicochemical properties of organic substrates based on coconut fibre and vermicompost in three proportions, 0:100, 40:60 and 60:40 (% *v*:*v*), on the microbial activity in the rhizosphere when the bacteria *Azotobacter vinelandii*, *Bacillus megaterium* and *Frateuria aurantia* were applied. Concentrations of NO_3^-, $H_2PO_4^-$, K^+ and Ca_2^+ in the petiole cellular extract (PCE) were quantified at 60, 90 and 120 days after transplantation (DAT) for tomato and 45 and 65 DAT for melon. We analysed dehydrogenase activity (DHA), acid phosphatase activity (FTA) and β-glucosidase activity (β-GLU). In order to maintain optimal volumetric moisture for the survival of microorganisms, automatic control was used to manage the irrigation frequency between 22%–28%. The results showed that physicochemical substrate properties, by incorporating 40% vermicompost into the coconut fibre mixture, increased enzymatic activity. Plants that were inoculated with *Azotobacter vinelandii* and *Frateuria aurantia* showed an improvement in NO_3^- and K^+ assimilation achieving highest yields.

Keywords: soilless culture; bio-fertiliser; horticulture; plant nutrition; plant growth-promoting bacteria

1. Introduction

Bio-fertilisation practices with plant growth-promoting bacteria (PGPB) have increased considerably in the last decade due to the implementation of changes in the processes of current agriculture. The promoter effect of PGPB occurs because they are an integral part of the biological cycle of nutrients, facilitating their availability for plants in ionic forms. This effect depends on the origin of the organic material, as well as the process to which it has previously been subjected. As described, it is necessary to characterise organic materials to determine their ability to influence microbial activity and processes of mineralisation and/or solubilisation of nutrients, which is a fundamental factor in maintaining sustainability and productivity [1]. One of the most used organic substrates in intensive horticulture is coconut fibre (CF), which is obtained from the extraction of the mesocarp fibres from the fruit, and its physicochemical properties make it a very stable material that can be reused for several consecutive growing cycles. An alternative substrate to CF that can be used in organic horticulture is vermicompost (VC), which has been studied as a substrate for container cultivation and as a source of nutrients, resulting in increases in the production of various plant species and reduced use of mineral fertilisers [2,3]. Research has confirmed that VC modifies the soil structure by improving aeration, drainage, water retention capacity, nutrient supply, organic matter

and microorganisms [4,5]. Therefore, it is crucial to determine the physicochemical and biological properties of substrates that have a direct effect on the survival and activity of microbial populations and that allow their plant growth-promoting abilities to be triggered. In turn, the implementation of microorganisms in agriculture forces the issue of having greater control to ensure the survival and bio-fertilising capacity of PGPB once they are inoculated into the rhizosphere of crops.

Analysis of physicochemical properties of soils/substrates has commonly been used to measure their production potential. However, when working with microorganisms, such as bio-fertilisers, it is necessary to analyse the activity of soil-substrate enzymes since they are direct indicators of the quality of organic material [6]. Enzymatic activity is also considered indicative of changes in soil quality that occur as a result of management practices and the biotic and abiotic factors to which it is subjected and is used to monitor microbial activity [7]. Enzymes are also indispensable in soil function because they play a vital role in organic matter decomposition and nutrient cycle transformation [8,9].

There are different enzymes in the soil, and each catalyses a specific reaction so that the analysis of a single enzyme can be inaccurate [10]. Enzymes involved in the transformation of organic materials are divided into hydrolases and oxidases and are the most studied when assessing soil quality [6]. Among the most common enzymes used to characterise microbial activity are dehydrogenase, amidase, protease, cellulase, glucosidase, urease and phosphatase [6].

The dehydrogenase activity allows, in a global way, knowledge of the microbial processes that occur in the soil as it is only present in living systems, and is used as an indicator of the oxidation rate of organic matter when transferring hydrogen from organic substrates to inorganic acceptors [11]. Phosphatases are a group of enzymes that are produced by certain microorganisms and plant roots, which participate in the biological cycle of phosphorus, mineralising it from organic soil phosphates. The enzyme β-glucosidase is an integral part of the carbon cycle and acts in the last phase of cellulose degradation. It is the most analysed enzyme in the assessment of soil quality [12]. The final product of this reaction is glucose, which is an important source of carbon for microorganisms [13].

The application of VC to soil together with microorganisms has proven to be an efficient practice in crop nutrition and production [14], although the variability in the results is high due to the conditions of each type of soil or substrate. Considering the previous context, the objective of this research was to assess the effect of the physicochemical properties of three substrates according to the VC and CF ratio, on the microbial activity of three PGPB species inoculated into two horticultural crops in containers and assess if there is an improvement in the bio-fertilising capacity of the organic solution as part of the substrate-PGPB set.

2. Materials and Methods

Two independent experiments were conducted to assess the effect of PGPB inoculation on three types of organic substrates according to the ratio of vegetable vermicompost to horticultural waste (VC) incorporated into the CF mixture. The PGPBs' used in this study were: *A. vinelandii* (AV), *B. megaterium* (BM) and *F. aurantia* (FA), at a dose of 10^8 colony forming units (CFU) per plant [15]. Three applications were performed per culture, the first at the time of transplant and the next two at intervals of 30 days. A control treatment (C) was established, without inoculation of bacteria. Twenty-four plants were established per treatment, distributed in random blocks with three replications. The experiments were conducted at the experimental farm of the UAL-Anecoop Foundation, located in south-eastern Spain (36.861905–2.282529), in an area of 900 m^{-2} using a greenhouse with a plastic cover and automated ventilation by zenithal windows. In the first experiment, a tomato crop (*Solanum lycopersicum* cv Ramylee) was established at a density of 1.25 plants m^{-2} in polystyrene containers (27 L), with a transplant date in September 2016 and end of cultivation in March 2017. As substrates, proportional mixtures of VC (Organic matter (OM) 17.20% on dry matter (dm), organic nitrogen (Norg) 1.11%, C:N 7.70] and CF (OM 73.1% dm, Norg 0.89%, C:N 41) were made.

Treatments were established according to the type of PGPB and the proportion of VC and CF (% *v*:*v*) 0:100, 40:60 and 60:40, respectively (Table 1). The substrates were reused for the second crop. For

the second experiment, a melon crop (*Cucumis melo*. var. Brisa) was established in March 2017 on the same substrate and containers of the first experiment with tomatoes, at a density of 1 plants m^{-2}. In both crops, pest control was carried out through the release of natural predators in accordance with the principles and practices of organic farming. As a nutrient solution (NS), we used an aqueous extract of vermicompost tea of vegetable waste aerated between 12 and 16 days (NO_3^-, 6.89; NH_4^+, 0.18; $H_2PO_4^-$, 0.39; K^+, 5.11; Ca^{2+}, 3.81; Mg^{2+}, 0.92).

Table 1. Treatment description according to substrate mixture and microorganisms (PGPB) applied.

Substrate VC:CF (%*v*:*v*)	Treatment	Microorganisms (PGPB)	Concentration (CFU g^{-1})
0:100	AZ	*Azotobacter vinelandii*	1×10^8
	BM	*Bacillus megaterium*	1×10^8
	FA	*Frateuria aurantia*	1×10^8
	C	Control	-
40:60	AZ	*Azotobacter vinelandii*	1×10^8
	BM	*Bacillus megaterium*	1×10^8
	FA	*Frateuria aurantia*	1×10^8
	C	Control	-
60:40	AZ	*Azotobacter vinelandii*	1×10^8
	BM	*Bacillus megaterium*	1×10^8
	FA	*Frateuria aurantia*	1×10^8
	C	Control	-

VC, vermicompost from horticultural waste; CF, coconut fibre; PGPB, Plant growth-promoting bacteria; CFU, colony-forming units.

In order to have a constant supply of NS during the crop cycle, five containers of 1000 L each, with automated air injection, were available for the elaboration of aqueous extract to be applied by fertigation. To determine the irrigation demand based on the type of substrate, automatic control by dielectric probes was used to manage the irrigation frequency needed to maintain volumetric moisture of 22%–28%, according to the stage of the crop, and maintain optimal moisture for the survival of microorganisms in the substrate [16]. For irrigation, we used 4 L h^{-1} drippers. The electric conductivity, pH, irrigation and drainage volume were also quantified as measures of fertigation control in soilless cultivation.

2.1. Physicochemical Properties of Substrates

Tests were performed before transplantation of the first crop (tomato) and at the end of the second growing cycle. For each substrate mixture, the OM and Norg contents were determined according to UNE-EN 13039:2012 and UNE-EN13654:2002, respectively. For physical indicators, total porosity (TP), bulk density (BD), air volume percentage (AVP) and readily available water (RAW) were analysed using the methodology established in UNE-EN 13041:2012.

2.2. Production

We randomly selected nine tomato plants and nine melon plants, considering three repetitions per treatment and culture. Production (kg m^{-2}) refers only to fruits considered to be commercial in quality.

Microbial Activity in the Substrate

We analysed dehydrogenase activity (DHA), acid phosphatase activity (FTA) and β-glucosidase activity (β-GLU), which are crucial enzymes in biological and biochemical processes, such as those that occur during the degradation of OM and the mineralisation of nutrients [17,18]. Each sample was isolated from the root zone of the crops, 10 cm deep. Enzyme tests were performed at 60, 90 and 120 days after transplantation (DAT) for tomato and 40, 60 and 80 DAT for melon crops.

DHA allows quantification of the metabolic activity of viable microorganisms in the substrate and their oxidative capacity [19]. This was calculated based on the amount of triphenylformazan (TPF) formed when the microorganisms in the substrate reduced triphenyl tetrazole chloride [20]. The activity of FTA is an indicator of phosphorus mineralisation in soils, while the β-GLU indicates the degradation of OM in the form of cellulose [21], a very important soil-substrate quality index being the most common polysaccharide in nature [22]. The levels of these enzymes were quantified by the concentration of P-nitrophenol (PNP) G^{-1} $soil^{-1}$ H^{-1} [23].

2.3. Nutritional Crop Status

Nutritional diagnosis, by analysing the petiole cellular extract (PCE), is a method that allows knowledge of the ranges of nutrient concentrations assimilated by a crop according to the physiological stage of the plant species, and determination of possible deficiencies or excesses of essential nutrients, which allows modification of nutrition management or correlation of nutrient concentrations to the specific factors involved in a crop's production [24]. Fully developed leaf petiole samples were taken, extracted by mechanical pressing and analysed in the laboratory by potentiometry using a modular probe of specific ions IMA CIMUS (Nt Sensors) and a colourimetry method for phosphates [25]. In this way, concentrations of NO_3^-, NH_4^+, $H_2PO_4^-$, K^+, Ca^{2+}, Mg^{2+}, Cl and Na^+ were quantified at 60, 90 and 120 DAT for tomato and 45 and 65 DAT for melon crops.

2.4. Statistical Analysis

We used a multifactorial analysis to assess the type of PGPB applied, the substrate, as well as the time of sampling during experiments. Variance analysis (ANOVA) was chosen for statistical analysis of the evaluated parameters. A 95% confidence interval was applied in multi-range tests to determine the significance level, using Fisher's comparison test of means with the statistically least significant difference (LSD). To explore the links between the physicochemical properties of substrates with enzymatic activity and crop production, Pearson's correlations were made. Principal component analysis (PCA) was carried out to determine the variability, correlations and synergisms among the different variables. The entire statistical analysis was performed with STATGRAPHICS Centurion XVIII software.

3. Results and Discussion

3.1. Physicochemical Properties of Substrates

The physical and chemical characteristics of the three substrate mixtures show variations when comparing their values before and after the crops. The process of mineralisation of N begins from the Norg content that is transformed by ammonification and nitrification processes to mineral N [5]. Table 2 shows a decrease in the Norg content in the final properties of the three substrate mixtures, possibly due to microorganism mineralisation. The OM percentage was directly related to the CF in the substrates because it contains three to four times more OM than the VC used in this analysis. The OM values were increased, as an effect constant fertigation with vermicompost tea throughout the experiment that represented a frequent addition of OM at a higher rate than degradation. The effect on the substrate 0:100 being more remarkable, which showed an increase of 12% making it a stable substrate [26], which is observed with its C/N ratio much higher than other substrates. Another factor related to the increase in OM at the end was the reuse of the substrates since the second melon culture was transplanted into the same substrate used for tomato cultivation while preserving the previous root system.

Table 2. Initial physicochemical properties (left column) and finals (right column) for each substrate mixture (% *v*:*v*, VC = vermicompost from horticultural crop waste, CF = coconut fibre).

Substrate (VC:CF)	Norg %		OM %		C/N		RAW %		TP %		AVP %		BD g dm/L	
0:100	0.91c	0.89b	73.1a	81.9a	41a	34a	22.4b	33.7a	95.7a	90.4a	36.6a	23.7b	75c	73c
40:60	1.05a	0.95a	19.3b	19.7b	10b	9.4b	29.2a	31.3a	88.1b	80.7b	26.0b	25.0a	454b	448b
60:40	0.99b	0.96a	17.5b	18.9b	9.5b	9.1b	31.7a	28.6b	80.5c	77.2c	19.0c	17.0c	558a	533a

Norg, organic nitrogen; OM, organic matter, C/N, carbon/nitrogen ratio; RAW, readily available water; TP, total porosity; AVP air volume percentage, BD, bulk density. Different letters indicate significant differences between groups ($p < 0.05$).

The C/N ratio is an indicator in OM degradation processes as it is used as an indicator of quality and stability of a material derived from composting processes. The VC is a very stable material, so its C/N ratio is not so modified over time because its material has previously undergone degradation. Studies mention that compost can be of quality when its initial C/N is <16, while over 20 generates N immobilization [27].

In substrates with 40% and 60% VC, the C/N ratio remained below 10, being suitable for mineralising N [28]. Other authors suggest that the optimal C/N ratio for microbial activity in organic materials should be <20 [29], in addition to the high influence of biological N fixation with a decrease in the C/N ratio by up to 16% [30]. RAW is related to soil aeration and its ability to use pore space to be occupied by water and benefit microbial populations in soils [31].

The air volume percentage (AVP) was calculated based on the volume of air retained at 10 cm suction. In this sense, a high correlation between AVP and TP was observed (Table 3). Total porous space is defined as the total volume of the substrate not occupied by organic particles or minerals. The highest values for TP and AVP were obtained in the substrate with coconut fibre, demonstrating the influence of OM on the properties of TP and AVP by increasing the size of the porosities in addition to adding OM, which reduces the compaction effect.

Table 3. Correlations between the initial (I) and final (F) properties of substrates.

	Norg I	OM I	C/N I	RAW I	TP I	AVP I	BD I
Norg F	0.84	−0.99	−0.99	0.99	−0.92	−0.96	1.00
OM F	−0.90	1.00	1.00	−0.97	0.87	0.92	−0.98
C/N F	−0.90	1.00	1.00	−0.97	0.87	0.92	−0.98
RAW F	−0.54	0.86	0.86	−0.96	1.00	0.99	−0.94
TP F	−0.81	0.99	0.99	−1.00	0.94	0.97	−1.00
AVP F	−0.07 *	0.39 *	0.38 *	−0.59	0.78	0.70	−0.55
BD F	0.82	−0.99	−0.99	1.00	−0.94	−0.97	1.00

Norg, Organic nitrogen; OM, organic matter; C/N, carbon/nitrogen ratio; TP, total porosity; AVP, air volume percentage; BD, bulk density. * Non-significant value ($p > 0.05$).

Similarly, the three substrates of the experiment were above 80%, optimal for horticultural crops [32]. Optimal AVP ranges are from 20–30 of volume [33], a range obtained only by substrates 0:100 and 40:60, while the 60:40 mixture was below the recommended lower limit.

BD refers to the relationship between mass and volume under certain conditions. No changes were observed over time on any of the substrates, with very similar values at the beginning and end of the experiment (Table 2). In general, the results demonstrated that the BD for these organic substrates was related to the amount of VC, with a negative correlation with the rest of the properties analysed with the exception of Norg, associated with the VC (Table 3).

3.2. *Effect of Substrate and PGPB on Yield*

Soil microbial populations are directly related to soil biochemical processes and are crucial in soil functioning, as they play a vital role in the decomposition and transformation of OM in the

biological nutrient cycle [9,34]. The type of substrate, according to the mixture and the type of microorganism, had a significant effect on yield. From the results obtained (Table 4), the substrates 40:60 and 60:40 (VC:FC) recorded the highest yield (kg m^{-2}) in the tomato growing cycle with significant differences ($p < 0.05$) compared to 0:100, while in the melon crop, only 40:60 [35] mentioned a significant increase in NO_3^- and NH_4^+ in soil when vermicompost was applied, in addition to increasing the populations of microorganisms in the soil and improving N fixation capacity by microorganisms in the rhizosphere. In the tomato crop, the highest yield was obtained by *Azotobacter vinelandii* inoculation in the 40:60 substrate, achieving increases of 0.84 kg m^{-2} in relation to the control treatment. In this way, when fertigated in a soilless culture with organic solutions of aqueous extracts of VC from plant waste, an improvement in the bio-fertilising effect by AV and organic materials as a substrate was shown.

Table 4. Yield (kg m^{-2}) for both crops (tomato and melon) in containers according to the bacteria and substrate mixture (VC:CF % *v*:*v*).

Crop	Treatment	Substrate Mixture										
		0:100			40:60			60:40			[2] PGPB	
Tomato	AV	6.64	a	B	8.19	a	A	7.95	a	A	7.59	A
	BM	6.50	a	B	7.34	b	A	7.34	ab	A	7.06	B
	FA	6.42	a	B	7.61	ab	A	7.53	ab	A	7.18	B
	Control	6.31	a	B	7.35	b	A	7.17	b	A	6.95	B
	[1] substrate	6.46	B		7.62	A		7.50	A			
Melon	AV	4.19	ab	B	6.00	ab	A	5.81	a	A	5.33	B
	BM	3.91	c	B	5.79	b	A	5.45	ab	A	5.05	BC
	FA	4.82	a	B	6.23	a	A	5.90	a	A	5.65	A
	Control	4.03	ab	B	5.68	b	A	5.19	b	A	4.97	C
	[1] substrate	4.24	C		5.93	A		5.59	B			

VC, vermicompost; CF, coconut fibre; AV, *Azotobacter vinelandii*; BM, *Bacillus megaterium*; FA, *Frateuria aurantia*; and control treatment. Lowercase letters express significant differences ($p < 0.05$) between treatments on the same substrate. Horizontal capital letters indicate differences by treatment and substrate. [1] Horizontal italic letters indicate differences between the mean value of substrates and [2] vertically considered mean value for each treatment (PGPB Plant growth promoting bacteria).

In the tomato crop, the 0:100 substrate mixture recorded the lowest yield, besides that there were no statistical differences between treatments, coinciding with the lower levels of enzymatic activity, highlighting the limited ability of this substrate for use in combination with PGPB as analysed under the conditions of this experiment.

In the melon crop, treatments with PGPB application also showed considerably higher production. Higher yields are the result of an improvement in enzymatic activity in the culture medium with successive inoculations over time. The 40:60 substrate, in combination with FA, obtained the largest increases in yield at 6.23 kg m^{-2}. These increases in yield showed the bio-fertiliser effect of applying FA, which can be seen as an improvement of the nutritional status of the crop in the PCE analysis.

3.3. Microbial Activity

An enzymatic activity represents an indicator of the biological transformation of the nutrient cycle and soil quality [7]. Table 5 shows the results of DHA, FTA and β-GLU based on the PGPB and substrate mixture for each plant species. Regarding dehydrogenase activity (DHA) (Table 5), it was observed that a greater amount of VC in the substrate increases the concentrations of triphenylformazan (TPF), due in large part to the high bacterial load and chemical composition of VC that improve crop production [36,37].

Table 5. Mean values of enzymatic activity analysed by treatment and substrate.

Enzyme	Treat	Tomato						Melon					
		0:100		40:60		60:40		0:100		40:60		60:40	
DHA (μ PNP g^{-1} 24 h^{-1})	AV	187 ± 20	a	241 ± 21	ab	264 ± 16	a	206 ± 14	a	455 ± 76	a	310 ± 95	ab
	BM	183 ± 17	a	233 ± 32	b	247 ± 20	ab	166 ± 28	b	421 ± 87	a	323 ± 102	ab
	FA	191 ± 40	a	270 ± 26	a	252 ± 24	ab	207 ± 32	a	470 ± 104	a	358 ± 23	a
	C	168 ± 34	a	211 ± 30	b	233 ± 18	b	155 ± 22	b	380 ± 108	a	293 ± 83	b
	Mean	183 ± 29	B	239 ± 33	A	249 ± 20	A	184 ± 33	C	431 ± 95	A	321 ± 80	B
FTA (μg PNP g^{-1} h^{-1})	AV	390 ± 77	a	479 ± 30	a	474 ± 48	a	471 ± 117	a	660 ± 143	a	769 ± 40	a
	BM	398 ± 52	a	450 ± 56	ab	450 ± 32	a	446 ± 82	a	655 ± 120	a	777 ± 89	a
	FA	359 ± 48	a	480 ± 24	a	471 ± 28	a	454 ± 89	a	681 ± 144	a	761 ± 102	a
	C	361 ± 55	a	424 ± 51	b	400 ± 20	a	413 ± 57	a	518 ± 136	b	734 ± 52	a
	Mean	377 ± 58	B	449 ± 46	A	459 ± 44	A	446 ± 86	C	629 ± 121	B	760 ± 72	A
β-GLU (μg PNP g^{-1} h^{-1})	AV	276 ± 20	a	365 ± 23	a	365 ± 29	a	539 ± 98	a	707 ± 98	a	717 ± 80	a
	BM	277 ± 17	a	328 ± 76	ab	352 ± 19	ab	588 ± 48	a	648 ± 71	a	742 ± 63	a
	FA	262 ± 27	a	303 ± 26	b	361 ± 23	ab	550 ± 76	a	708 ± 82	a	686 ± 48	a
	C	273 ± 32	a	283 ± 36	b	338 ± 38	b	540 ± 83	a	691 ± 89	a	749 ± 61	a
	Mean	272 ± 24	B	321 ± 52	A	335 ± 28	A	554 ± 90	B	689 ± 64	A	724 ± 54	A

AV, *Azotobacter vinelandii*; BM, *Bacillus megaterium*; FA, *Frateuria aurantia*; C, control treatment; DHA, dehydrogenase activity; FTA, acid phosphatase activity; β-GLU, β-glucosidase activity; PNP, P-nitrophenol. Vertical lowercase letters express differences between PGPB for each substrate type. Horizontal capital letters express statistical differences between substrates ($p < 0.05$).

In tomato cultivation, the average values of DHA activity for substrates 60:40 and 40:60 were 152% and 146% higher than 0:100. This showed that the activity of DHA was largely related to the physicochemical and biological properties of substrates or soils. AV and FA treatments recorded the highest levels of this enzyme in 60:40 and 40:60 substrate mixtures, respectively, being statistically significant. These results coincided with the higher production yields and concentration of NO_3^- in the PCE.

In tomato crop, the effect of increase DHA was seen when using AV in 60:40 and FA in 40:60, while in melon were recorded with AV in 0:100 and FA with 0:100 and 60:40 mixture, which shows that applying PGPB promotes an increase of the metabolic activity of the substrate [38]. DHA is proportional to the biomass of microorganisms in the soil, so it is only present in living systems [39]. This experiment found a positive correlation between the proportion of VC in the substrate mixture and enzymatic activity. The correlation rate for DHA with % VC was 0.88 ($p < 0.05$) and 0.68 ($p < 0.05$) for the tomato and melon experiments, respectively.

Similar behaviour was found in melon cultivation among substrates, highlighting 40:60 with an average value of 431 g TPF g^{-1} dm 24 h^{-1}, followed by 60:40 with 321 g TPF g^{-1} dm 24h^{-1}. Among PGPB, the maximum values were obtained with FA and AV at 40:60, although without statistical differences between the rest of the treatments for that substrate. DHA showed a constant increase over time, achieving the highest levels in the second crop cycle (Figure 1).

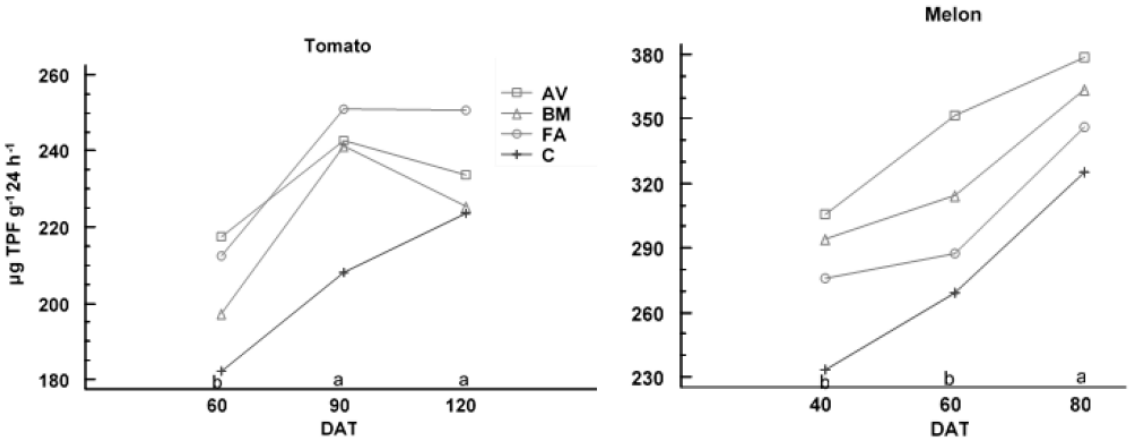

Figure 1. Evolution of dehydrogenase activity (DHA) activity during two growing cycles. AV, *Azotobacter vinelandii*; BM, *Bacillus megaterium*; FA, *Frateuria aurantia*; C, Control; DAT, days after transplant.

In a study presented by [1], they analysed the effect of time on enzymatic activities for 13 crops over five years, finding constant increases during the first nine crops. In another trial by [3], the highest levels of TPF derived from DHA were obtained in the second year of cultivation after four crops were established on the same substrate. In this research, the maximum values were reached during the final stage of melon cultivation with a steady increase in all treatments possibly due to the increase in ambient temperature for the second growing cycle, which was performed during the onset of spring season with an increase from the previous crop, as DHA is influenced by environmental temperature [40].

Regarding the activity of the phosphatase enzyme (FTA), significant differences were found in the treatments with PGPB with respect to the control only in the 40:60 substrate in both crops.

In 0:100 and 60:40, no effect was observed when applying PGPB (Table 5). The FTA is usually influenced by a large number of factors, so its interpretation depends on the analysis of other variables, such as the properties of the substrate, in addition to the FTA also produced by the plant roots [41].

The values of our experiment coincide with the ranges presented by [42] (55.6 to 4017 µg PNP g^{-1} h^{-1}) and [12] (324 to 2191 µg PNP g^{-1} h^{-1}), although well below those presented by [43] (10.4–307 µmol g^{-1} h^{-1}). No relationship was observed between the activity of this enzyme and

treatment with BM bacteria to which phosphate solubilisation effects are attributed. Ref [44] mentioned that interactions between microorganisms and plant species affect FTA, which is usually higher in the rhizosphere through the activity of root exudates that stimulate bacterial activity, and that it depends on the type of exudate. The factor that most influenced FTA activity was the type of substrate when incorporating VC, where we obtained a correlation rate of 0.86 ($p < 0.05$) for tomato and 0.95 ($p < 0.05$) for melon crops.

Glucosidase is a very common enzyme found in soils, which is used as a biochemical indicator to measure processes of degradation and stabilisation of OM [45] as it is involved in the carbon cycle [39]. In this context, it is necessary to know the activity of this enzyme to determine the stability of the substrate since it is proven that β-GLU hydrolyses cellulose into glucose [46]. Table 5 presents the mean values of β-GLU activity where statistically significant differences between the type of PGPB and substrate mixture are shown. Greater activity was observed on substrates when incorporating VC, being higher in the substrate 60:40 in all cases.

As in the rest of the enzyme activities analysed, a correlation was found between the VC ratio and β-GLU, with 0.87 for tomato and 0.88 for melon. In any case, the higher values of β-GLU were associated with VC substrates, and in the two crops, these increments, in turn, had a negative correlation with the AVP and TP. These properties decreased with an increase of microbial activity, behaviour that should be considered as they are important physical properties for materials that are used as substrates in soilless cultures.

3.4. Pearson Correlations between Microbial Activity and Yield

The Pearson correlation rate between the yield of each crop and the three enzymatic activity parameters showed a positive correlation with statistically significant differences ($p < 0.05$) in all cases. The increase in production was highly correlated with all enzymatic activities, coinciding with the results presented by [1], where they mentioned a positive correlation between soil enzyme activity and yield in a tomato crop in a greenhouse. As described above, the chemical composition of VC improves microbial activity, which promotes enzyme synthesis and provides nutrients in assimilable forms, while CF is typically used for its suitability and the stability of its properties for the production of vegetables in containers and not as a source of nutrients.

DHA reveals the overall metabolic activity of all three substrate mixtures. The detected concentrations increased over time, which according to [47] might be due to a higher rate of mineralisation of OM coinciding with a decrease in the C/N ratio and the Norg content in the substrates at the end of the experiment. The total correlations between enzymatic activities and production were statistically significant ($p < 0.05$). In the tomato crop, correlations between yield and enzymatic activity were 0.76, 0.83 and 0.76 for DHA, FTA and β-GLU, respectively; while in the melon crop, the correlation rates for the same enzymes were 0.94, 0.77 and 0.75, respectively (Table 6).

Table 6. Pearson correlation between yield and enzymatic activity.

Melon	**kg/m^{-2}**	**DHA**	**FTA**	**β-GLU**	**kg/m^{-2}**	**DHA**	**FTA**	**β-GLU**
Yield		0.76	0.83	0.76		0.94	0.77	0.75
p-Value		0.0040	0.0008	0.0044		0.0000	0.0033	0.0045
DHA	0.76		0.94	0.81	0.94		0.64	0.72
p-Value	0.0040		0.0000	0.0016	0.0000		0.0250	0.0081
FTA	0.83	0.94		0.88	0.77	0.64		0.79
p-Value	0.0008	0.0000		0.0002	0.0033	0.0250		0.0024
B-GLU	0.76	0.81	0.88		0.75	0.72	0.79	
p-Value	0.0044	0.0016	0.0002		0.0045	0.0081	0.0024	

DHA, dehydrogenase; FTA, phosphatase, β-GLU, β-glucosidase.

The yield increase in DHA and β-GLU is largely due to the increased amount of nutrients in mineral forms derived from biological processes of fixation, solubilisation and mineralisation of the organic forms present in the substrates, coinciding with [3], who mentioned that substrates with higher vermicompost ratios contain more anions and cations forms compared than coconut fibre as substrate. On the other hand, [8] mentioned that the higher enzymatic activity could be explained in terms of a larger microbial population, which in addition to fixing atmospheric N and mineralisation and solubilisation of essential nutrients, metabolises amino acids, sugars, acids phytohormones and other compounds from the roots of plants and soil OM.

3.5. Pearson Correlations between Microbial Activity and Substrate Properties

Enzyme activity correlates strongly with soil physicochemical parameters. Studies mentioned that soil properties influenced the level of response of enzymatic activities being, in many cases, strongly correlated [48,49] with the pH, OM and total N [46]. In the correlation analysis (Table 7), we found an inverse relationship between OM content and enzymatic activity, largely due to the high amount of OM contained in the CF, and a positive correlation with the initial and final Norg content.

Table 7. Correlations between initial physicochemical (I) and final (F) properties of the substrates and enzymatic activity.

	Norg		**OM**		**C/N**		**RAW**		**TP**		**AVP**		**BD**	
Crop 1	**I**	**F**	**I**	**F**	**I**	**F**	**I**	**F**	**I**	**F**	**I**	**F**	**I**	**F**
DHA	0.744	0.892	−0.887	−0.858	−0.885	−0.885	0.886	−0.817	−0.701	−0.892	−0.840	−0.888	0.891	0.892
FTA	0.718	0.874	−0.866	−0.864	−0.864	−0.864	0.870	−0.808	−0.675	−0.874	−0.816	−0.868	0.873	0.874
β-GLU	0.561 *	0.833	−0.802	−0.796	−0.797	−0.795	0.859	−0.863	−0.504 *	−0.844	−0.703	−0.807	0.849	0.842
Crop 2	**I**	**F**	**I**	**F**	**I**	**F**	**I**	**F**	**I**	**F**	**I**	**F**	**I**	**F**
DHA	0.964	0.799	−0.851	−0.858	−0.857	−0.859	0.722	−0.505 *	−0.962	−0.772	−0.931	−0.844	0.757	0.775
FTA	0.614	0.912	−0.878	−0.871	−0.872	−0.871	0.941	−0.946	−0.551 *	−0.924	−0.770	−0.803	0.930	0.923
β-GLU	0.811	0.918	−0.920	−0.920	−0.921	−0.920	0.900	−0.805	−0.772	−0.913	−0.891	−0.921	0.910	0.914

Crop 1 – Tomato, Crop 2 – Melon. Norg, organic nitrogen; OM, organic matter; C/N, carbon/nitrogen ratio; TP, total porosity; AVP, air volume percentage; BD, bulk density. * Does not represent significant value (p <0.05).

It is found that very low relationships between the effect of nutrients available in the soil on the enzyme rate [47]. They analysed the effect of applying mineral nitrogen and phosphorus to the soil and found no clear effect on enzymatic activity when applying fertilisers and so concluded that the greatest influence is given by the physical-chemical properties of the soil. In other experiments, it was shown that applying mineral sources of fertilisation decreased the activity of FTA and β-GLU while incorporating organic materials, such as compost and VC, significantly increased FTA activity [44], β-GLU [50] and DHA [38], coinciding with the results of this experiment.

Regarding the C/N ratio, an inverse correlation with enzymatic activity was found, as the substrate 100% CF had the highest C/N ratio and was the one that recorded the lowest enzyme values in this experiment. This relationship indicates the degree of stability of the OM and decreases with its degradation. The results for all substrates showed a decrease in C/N, indicating mineralisation processes, being an important factor to consider when working with organic materials to avoid N immobilisation problems in immature materials [33].

In the case of RAW, a direct correlation between enzymatic activity and initial RAW, and inverse with final RAW, i.e., enzymatic activity was constantly increasing during the two cultures while RAW decreased during the second crop, was found.

TP and AVP had an inverse relationship to enzymatic activity in general, derived from the fact that these properties are attributed to a greater 0:100, which had no significant changes in these properties during the two crop cycles, confirming the good stability of CF in terms of degradation over time.

To analyse the contribution of each factor to the total variability of treatments, the analysis of the main components (PCA) was performed. A small number of linear combinations of the total variables was obtained that explained the greatest variability in the data. The criterion for determining the number of major components was to consider those groups with an eigenvalue >1. Figure 2 shows the influence of the factors on the total variability of the analysed parameters, which were grouped by weights of each component in the tomato crop. In this case, two components were extracted that together explain 96.71% of the variability in the original data.

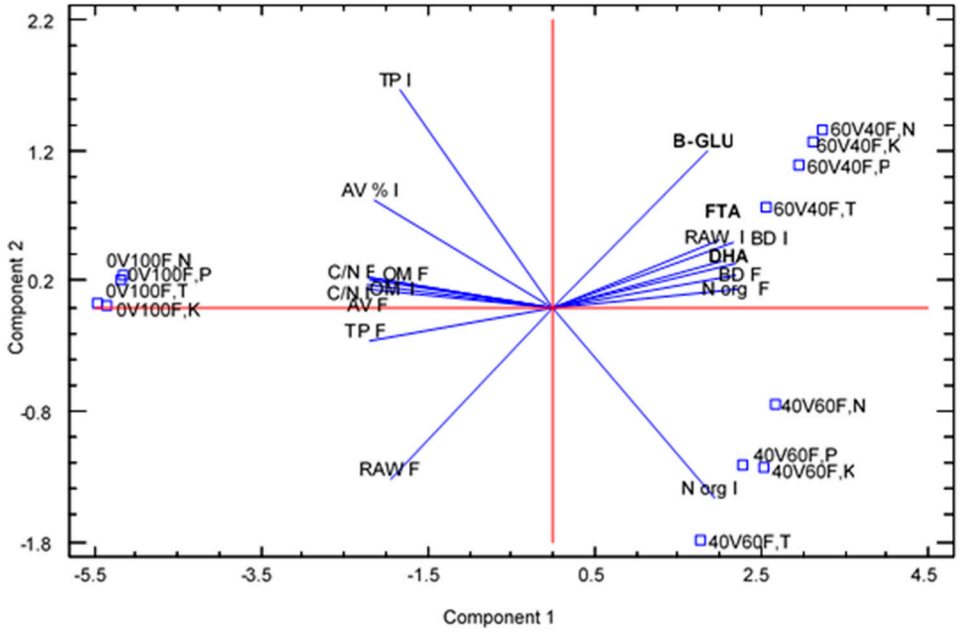

Figure 2. Principal component analysis in the tomato experiment. Two components were extracted with an eigenvalue >1, which together explain 96.71% of the variability in the original data. DHA, dehydrogenase; FTA, phosphatase; β-GLU, β-glucosidase; N, *Azotobacter vinelandii*; P, *Bacillus megaterium*; K, *Frateuria aurantia*; C, control treatment; Norg, organic nitrogen; OM, organic matter; C/N, carbon/nitrogen ratio; TP, total porosity; AVP, air volume percentage; BD, bulk density.

Within component 1, DHA, FTA, β-GLU, RAW I, BD I, BD F and Norg F were grouped together, which shows a correlation or synergism between them and explains more than 90% of the variance, while in component 2, the rest of the substrate properties were integrated and represent 6.43% of the variance. These data showed that the activity of β-GLU and DHA were related to soil moisture [38,51]. In addition, it was found that β-GLU increased considerably by providing OM with a low C/N ratio [6]. The results matched those of [46], confirming that physicochemical properties influence the response of enzymatic activity by finding a high correlation between soil N content and enzymatic activities. In the PCA results for the melon crop, two main components were identified with an eigenvalue >1, which together explain more than 98% of the variability in the original data, where component 1 explains almost 91% of the total variance, while the second component influences 7.51% of the total (Figure 3). Enzymatic activities with the highest percentage weight were grouped into the first component.

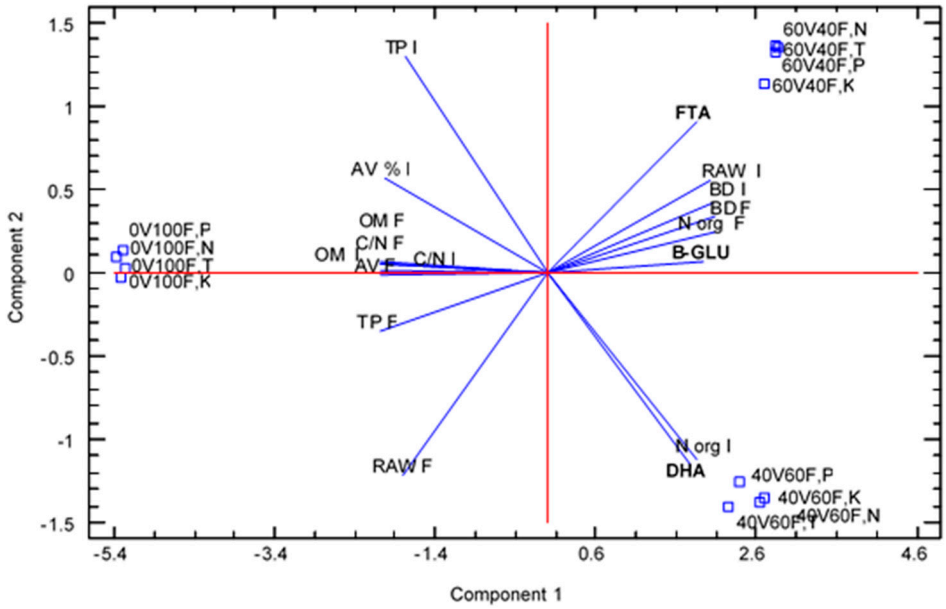

Figure 3. Principal component analysis in the melon crop. Two components were extracted with an eigenvalue >1, which together explain 98.23% of the variability in the original data. DHA, dehydrogenase; FTA, phosphatase; β-GLU, β-glucosidase; N, *Azotobacter vinelandii*; P, *Bacillus megaterium*; K, *Frateuria aurantia*; C, control treatment; Norg, organic nitrogen; OM, organic matter; C/N, carbon/nitrogen ratio; TP, total porosity; AVP, air volume percentage; BD, bulk density.

A strong correlation between Norg and DHA was observed ($r = 0.96$, $p < 0.5$). The results concur with those of [52] who found a high correlation between moisture content with β-GLU and FTA, which was observed in the component graphic (Figure 2). It is clear that there is a relationship between the type of OM and DHA, but it is necessary to highlight that not only is the amount of OM in the soil important but especially its quality, since the OM affects the energy supply for microbial growth and enzyme production [53]. AVP and TP were not determining factors for DHA as the aeration of substrates was not limited; on the contrary, it has been found that DHA activity is increased under conditions of limited O_2 and even in flood situation anaerobiosis [40].

3.6. Crop Nutritional Status

The elemental macronutrient content in the PCE was analysed according to the PGPB bacteria, substrate mixture and growth phase, finding significant statistical differences ($p < 0.05$) between treatments. The average concentration for NO_3^- (mg L^{-1}) in decreasing order was 830, 772 and 653 for substrate mixtures 60:40, 40:60 and 0:100, respectively, with maximum values at 90 DAT. PGPB showed an improvement in the assimilation of NO_3^- in plants that were inoculated with N-fixative bacteria, *Azotobacter vinelandii* (AV) and with the K^+ solubiliser, *Frateuria Aurantia* (FA). The effect of these bacteria was most clearly observed in VC substrates, where NO_3^- concentrations were found to be around 20% more than in control. These values were within the reference levels proposed by [54]; however, they were lower than those reported by [24] who mentioned a range from 700 to 1200 ppm NO_3^- for a tomato crop with mineral nutrition in a greenhouse. In melon, a similar behaviour to tomato was observed with substrates 60:40 and 40:60, which had a higher Norg content to be mineralised by PGPB for plant disposal. The bacteria FA (1006 mg L^{-1} NO_3^-) and AV (929 mg L^{-1} NO_3^-) were highlighted in ranges suitable for melon [24]. A high correlation was observed between the concentration of NO_3^- in PCE and the yield of the tomato crop ($r = 0.77$, $p = 0.0031$) and melon ($r = 0.59$, $p = 0.0416$), as this is an essential element in horticultural nutrition.

Regarding $H_2PO_4^-$, higher concentrations in PCE were detected in the plants established in the 60:40 (VC:CF) substrate in all samplings. Among bacteria, BM stood out by increasing the phosphate concentration in the PCE by 19% compared to the control in the global average.

Table 5, of the enzyme results, shows that substrate 0:100 (VC:FC) had the lowest FTA, linked to phosphate mineralisation processes, as well as the lower concentration in PCE, and that by its very nature, this substrate did not have a positive effect on PGPBs as there were no statistical differences from the control (Table 8). The maximum average value obtained by PGPB was 292 mg L^{-1} for BM, followed by FA (278 mg L^{-1}) and AV (267 mg L^{-1}), which are also associated with inorganic phosphate solubilisation capabilities [55], while the control recorded the lowest phosphate value of 245 mg L^{-1} in PCE. The solubilising capacity of phosphates by BM in substrates with VC was observed, by improving the nutritional status of the plants in the analyses at 90 and 120 DAT.

Table 8. Petiole cellular extract analysis for the tomato crop (mg L^{-1}). DAT, days after transplant.

Ion	Treatment	60 DAT			90 DAT			120 DAT		
		0:100	40:60	60:40	0:100	40:60	60:40	0:100	40:60	60:40
NO_3^-	AV	644a	863a	830a	739a	817a	961a	691a	839a	853a
	BM	681a	758ab	825a	667ab	703c	932a	611a	744b	816a
	FA	682a	839ab	800a	759a	773b	999a	591a	829a	908a
	C	587a	736b	795b	610b	672c	702b	575a	702b	849a
	Mean	649B	799A	813A	694C	741B	899A	617C	778B	856A
$H_2PO_4^-$	AV	254a	237a	284ab	256a	260a	274B	234a	252b	348a
	BM	266a	253a	320ab	287a	271a	356a	234a	271a	367a
	FA	277a	241a	357a	300a	215ab	270b	269a	257ab	319a
	C	221a	245a	231b	240a	192b	241b	247a	247b	337a
	Mean	255B	244B	298A	271A	234B	286A	246B	257B	343A
K^+	AV	2111a	2517a	2301a	2027a	3209a	2375a	3806ab	4200ab	4354ab
	BM	2106a	2558a	2309a	2014a	3150a	2338a	3813ab	3780bc	4338ab
	FA	2118a	2504a	2491a	2027a	3173a	2381a	3853a	4308a	4377a
	C	2097a	2473a	2506a	2024a	3101a	2350a	3602b	3600c	4308b
	Mean	2108B	2513A	2402A	2023C	3158A	2361B	3768C	3972B	4344A
Ca_2^+	AV	259a	288a	297a	516b	562a	570a	484a	565a	706a
	BM	274a	288ab	317a	645a	605a	649a	399a	557a	545a
	FA	270a	316ab	321a	632a	586a	523a	346ab	513a	561a
	C	253a	286b	289a	419c	556a	566a	243b	478a	536a
	Mean	264A	294A	306A	553A	577A	577A	368B	528A	587A

AV, *Azotobacter vinelandii*; FA, *Frateuria aurantia*; BM, *Bacillus megaterium*; average value. Different lowercase letters represent statistically significant differences between PGPB, while capital letters refer to differences between substrate mixture ($p < 0.05$).

A significant increase in H_2PO_4 concentrations was also observed in the melon culture in PCE when applying BM and FA in substrate 40:60 (VC:FC) at 50 and 80 DAT and AV in 40:60 and 60:40 at 80 DAT (Table 9). This stands out as, in the tomato crop, the BM treatment was superior to the rest of treatments with a statistical significance ($p = 0.05$). In Figure 4, the correlation rate between the phosphate content in PCE and yield is presented, with a positive correlation observed only in the second crop.

Table 9. Petiole cellular extract analysis for the melon crop (mg L^{-1}.). DAT, days after transplant.

Ion	Treatment	50 DAT						80 DAT					
		0:100		40:60		60:40		0:100		40:60		60:40	
NO_3^-	AV	878	a	946	a	942	b	884	a	967	ab	956	a
	BM	876	a	855	b	854	b	757	a	817	c	861	a
	FA	902	a	1014	a	1212	a	989	a	1047	a	873	a
	Control	807	a	863	b	705	b	805	a	910	bc	808.5	a
	[1] substrate	866	B	920	A	928	A	859	B	935	A	875	B
$H_2PO_4^-$	AV	264	a	337	b	389	a	407	a	425	a	450	a
	BM	318	a	438	a	378	a	403	a	436	a	432	ab
	FA	359	a	424	a	292	a	334	a	394	a	392	b
	Control	347	a	344	b	342	a	324	a	338	b	356	ab
	[1] substrate	322	A	386	A	350	A	367	A	398	A	407	A
K^+	AV	2653	a	2521	a	2818	a	3879	a	5180	a	5514	a
	BM	2419	b	2422	a	2484	a	3906	a	5184	a	4487	b
	FA	2513	ab	2281	a	2771	a	3803	a	5232	a	5536	a
	Control	2759	a	2498	a	2593	a	3518	b	4915	a	5092	ab
	[1] substrate	2586	A	2430	B	2666	A	3776	B	5128	A	5157	A
Ca^{2+}	AV	246	a	356	b	507	a	421	a	523	ab	554	a
	BM	280	a	520	a	424	b	457	a	623	a	646	a
	FA	238	a	552	a	413	b	433	a	592	a	620	a
	Control	241	a	361	b	390	b	384	a	386	b	566	a
	[1] substrate	251	B	447	A	433	A	423	C	531	B	596	A

AV, *Azotobacter vinelandii*; FA, *Frateuria aurantia*; BM, *Bacillus megaterium*. Lowercase letters express significant differences ($p < 0.05$) between treatments on the same substrate. [1] Horizontal capital letters indicate differences by treatment and substrate.

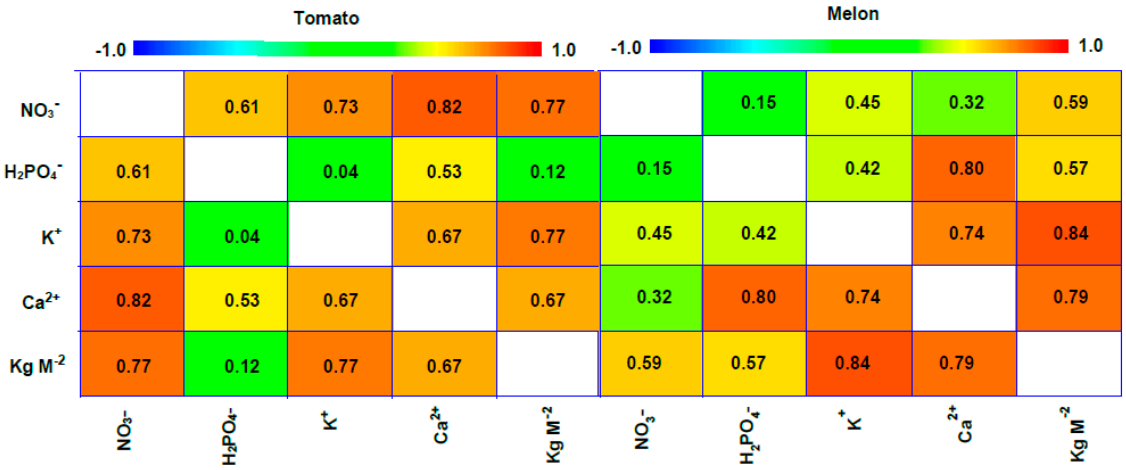

Figure 4. Pearson correlation analysis between yield and petiole cellular extract (PCE) concentrations.

Regarding the concentration of K^+ in PCE (Table 9), only in 0:100, we saw statistical differences in the effect of applying PGPB to substrates. The greatest variation was positively correlated with the percentage of VC in the substrate, as it is a substrate with a high K^+ content [16]. In the tomato crop, the average concentrations for the substrates were 2633, 3214 and 3055 mg L^{-1} for (VC:FC) 0:100, 40:60 and 60:40, respectively. Substrates with %VC of 40 and 60 achieved acceptable K^+ levels in PCE. In the melon crop, values of 3180, 3779 and 3912 mg L^{-1} (0, 40, 60% VC) were recorded; values very close to the lower limit of K^+ in PCE [24]. In both cases, there was no improvement in the concentration of K^+ with FA, associated with K^+ solubilisation processes in soils [56].

In the case of Ca^{2+} differences in PCE, the content was mostly associated with the substrate mixture than the bacteria. No clear effect was observed when applying PGPB, as it did not show significant differences from the control. However, the recorded levels were suitable for the recommendations for tomatoes. In addition, there was no Ca^{2+} deficiency due to the fertigation with VC tea, so it was not

a limitation. On the contrary, the values of Ca^{2+} and K^{+} showed a high correlation with production (Figure 4).

4. Conclusions

According to the physical and chemical properties of the substrate mixtures used in the experiment, the initial Norg content presents the highest positive correlation with microbial activity, which is also related to the nutritional status of the crop yield.

The highest yield was obtained in tomato and melon plants when VC was used in the substrate mixture (40:60 and 60:40% VC:FC). The increases in production were positively correlated with the enzymatic activity of the substrate and were reflected in an improvement in the nutritional status of the crops, with higher concentrations of NO_3^- and K^+ in the cellular extract of the petioles.

There was a positive correlation between DHA, FTA and β-GLU activity with the RAW, BD and Norg of the substrate, which was given by the ratio of VC.

The results of this research showed that the potential of the bio-fertilisation effect by PGPB to improve crop nutrition and productivity is in function with the physical-chemical properties of the substrate used as a growing medium.

The highest production, with significant statistical differences, was obtained in tomato and melon plants grown on substrates 40:60 and 60:40 (VC:FC), inoculated with AV and FA bacteria. BM bacteria did not affect production, however, presents the highest concentrations of $H_2PO_4^-$ in PCE.

Higher levels of NO_3^- in PCE improves the nutritional status of plants when inoculated with the bacterium *Frateuria aurantia* in melon and tomato crops, so it is necessary to investigate this bacterium in more detail in processes related to the nitrogen cycle since the historical research has only identified it as a potassium solubiliser.

The results of this experiment showed that it is possible to provide the nutrient needs of long-cycle horticultural crops in a soilless culture with good yields, by using vermicomposted horticultural waste as a substrate and as an organic nutrient solution in fertigation combined with the application of PGPB.

The enzyme activity of the substrate should be considered as an additional control parameter (Electric conductivity, pH, ionic concentration) for the nutritional management of the crop when organic solutions and PGPB are used in culture without soil in the container.

When working with organic nutritious solutions and PGPB in soilless cultivation with coconut fibre as a substrate in 100% proportion, the production of tomato and melon crops decreases due to lower microbial activity, and lower nutrient assimilation according to the petiole cellular extract (PCE) analysis regarding mixing with vermicompost.

Based on this research, the importance of monitoring enzymatic activity, being highly correlated with crop production, should be a factor to consider.

Author Contributions: Conceptualization, J.L.R. and M.d.C.S.; methodology, J.L.R. and M.d.C.S.; software, J.L.R. and M.d.C.S.; validation, J.L.R. and M.d.C.S.; formal analysis, J.L.R. and M.d.C.S.; investigation, J.L.R. and M.d.C.S.; resources, J.L.R. and M.d.C.S.; data curation, J.L.R.; writing—original draft preparation, J.L.R.; writing-review and editing, J.L.R. and M.d.C.S.; visualization,, J.L.R. and M.d.C.S.; supervision, M.d.C.S.; project administration, M.d.C.S.; funding acquisition, M.d.C.S.

Funding: This research was funded by Consejo Nacional de Ciencia y Tecnología (Mexico), grant number 010050.

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

References

1. Fu, H.; Zhang, G.; Zhang, F.; Sun, Z.; Geng, G.; Li, T. Effects of continuous tomato monoculture on soil microbial properties and enzyme activities in a solar greenhouse. *Sustainability* **2017**, *9*, 317. [CrossRef]
2. Wang, X.; Zhao, F.; Zhang, G.; Zhang, Y.; Yang, L. Vermicompost Improves Tomato Yield and Quality and the Biochemical Properties of Soils with Different Tomato Planting History in a Greenhouse Study. *Front. Plant Sci.* **2017**, *8*, 1978. [CrossRef]

3. Mejía, P.; Salas, M.; López, M. Evaluation of physicochemical properties and enzymatic activity of organic substrates during four crop cycles in soilless containers. *Food Sci. Nutr.* **2018**, 1–13.
4. Jindo, K.; Chocano, C.; De Aguilar, J.M.; Gonzalez, D.; Hernandez, T.; Garcia, C. Impact of compost application during 5 years on crop production, soil microbial activity, carbon fraction, and humification process. *Commun. Soil Sci. Plant Anal.* **2016**, *47*, 1907–1919. [CrossRef]
5. Haouvang, L.C.; Ngakou, A.; Yemefack, M.; Mbailao, M. Effect of organic fertilizers rate on plant survival and mineral properties of Moringa oleifera under greenhouse conditions. *Int. J. Recycl. Org. Waste Agric.* **2019**. [CrossRef]
6. Adetunji, A.; Lewu, F.; Mulidzi, R.; Ncube, B. The biological activities of β-glucosidase, phosphatase and urease as soil quality indicators: A review. *J. Soil Sci. Plant Nutr.* **2017**, *17*, 794–807. [CrossRef]
7. Zhou, X.G.; Wu, F.Z. Changes in soil chemical characters and enzyme activities during continuous monocropping of cucumber (Cucumissativus). *Pak. J. Bot.* **2015**, *47*, 691–697.
8. Sekaran, U.; McCoy, C.; Kumar, S.; Subramanian, S. Soil microbial community structure and enzymatic activity responses to nitrogen management and landscape positions in switchgrass (*Panicum virgatum* L.). *GCB Bioenergy* **2019**, *11*, 836–851. [CrossRef]
9. Sherene, T. Role of soil enzymes in nutrient transformation: A review. *Bio Bull.* **2017**, *3*, 109–131.
10. Nannipieri, P.; Giagnoni, L.; Renella, G.; Puglisi, E.; Ceccanti, B.; Masciandaro, G.; Fornasier, F.; Moscatelli, M.C.; Marinari, S. Soil enzymology: Classical and molecular approaches. *Biol. Fertil. Soils* **2012**, *48*, 743–762. [CrossRef]
11. Zhang, N.; He, X.; Gao, Y.; Li, Y.; Wang, H.; Ma, D.; Zhang, R.; Yang, S. Pedogenic Carbonate and Soil Dehydrogenase Activity in Response to Soil Organic Matter in Artemisia Ordosica Community. *Pedosphere* **2010**, *20*, 229–235. [CrossRef]
12. Gil-Sotres, F.; Trasar-Cepeda, C.; Leirós, M.C.; Seoane, S. Different approaches to evaluating soil quality using biochemical properties. *Soil Biol. Biochem.* **2005**, *37*, 877–887. [CrossRef]
13. Merino, C.; Godoy, R.; Matus, F. Soil enzymes and biological activity at different levels of organic matter stability. *Soil Sci. Plant Nutr.* **2016**, *16*, 14–30.
14. Das, D.; Bhattacharyya, P.; Ghosh, B.C.; Banik, P. Bioconversion and biodynamics of Eisenia foetida in different organic wastes through microbially enriched vermiconversion technologies. *Ecol. Eng.* **2016**, *86*, 154–161. [CrossRef]
15. Ahmed, Y.E.; Zaied, K.A.; Kash, K.S.; Farid, M. Evaluation of fruit quality of tomato inoculated with genetically modified strains of pgpr (Azospirillum SP.). *Egypt. J. Agric. Res.* **2008**, *86*, 1187–1194.
16. Mondal, T.; Data, J.K.; Mondal, N.K. Chemical fertilizer in conjunction with biofertilizer and vermicompost induced changes in morpho-physiological and bio-chemical traits of mustard crop. *J. Saudi Soc. Agric. Sci.* **2017**, *16*, 135–144. [CrossRef]
17. Dorodnikov, M.; Blagodatskaya, E.; Blagodatsky, S.; Marhan, S.; Fangmeier, A.; Kuzyakov, Y. Stimulation of microbial extracellular enzyme activities by elevated CO_2 depends on aggregate size. *Glob. Chang. Biol.* **2009**, *15*, 1603–1614. [CrossRef]
18. Li, Y.-T.; Rouland, C.; Benedetti, M.; Li, F.; Pando, A.; Lavelle, P.; Dai, J. Microbial biomass, enzyme and mineralization activity in relation to soil organic C, N and P turnover influenced by acid metal stress. *Soil Biol. Biochem.* **2009**, *41*, 969–977. [CrossRef]
19. Watts, D.; Torbert, H.; Feng, Y.; Prior, S. Soil Microbial Community Dynamics as Influenced by Composted Dairy Manure, Soil Properties, and Landscape Position. *Soil Sci.* **2010**, *175*, 474–486. [CrossRef]
20. Casida, L.E. Microbial Metabolic Activity in Soils as Measured by Dehydrogenase Determinations. *Appl. Environ. Microbiol.* **1977**, *34*, 630–636.
21. Ahmed, A.; Nasim, F.; Batool, K.; Bibi, A. Microbial β-Glucosidase: Sources, Production and Applications. *J. Appl. Environ. Microbiol.* **2017**, *5*, 31–46. [CrossRef]
22. Acosta-Martínez, V.; Pérez-Guzmán, L.; Johnson, J.M.F. Simultaneous determination of β-glucosidase, β-glucosaminidase, acid phosphomonoesterase, and arylsulfatase activities in a soil sample for a biogeochemical cycling index. *Appl. Soil Ecol.* **2019**, *142*, 72–80. [CrossRef]
23. Tabatabai, M.A.; Bremner, J.M. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* **1969**, *4*, 301–307. [CrossRef]

24. Hochmuth, G.; Maynard, D.; Vavrina, C.; Hanlon, E.; Simonne, E. Plant Tissue Analysis and Interpretation for Vegetable Crops in Florida. In *Nutrient Management of Vegetable and Row Crops Handbook*; University of Florida: Gainesville, FL, USA, 2004; pp. 45–92.
25. Murphy, J.; Ripely, J.P. Determination single solution method for the in natural waters. *Anal. Chim. Act.* **1962**, *27*, 31–36. [CrossRef]
26. Abad, M.; Fornes, F.; Carrión, C.; Noguera, V.; Noguera, P.; Maquieira, A.; Puchades, R. Physical Properties of Various Coconut Coir Dusts Compared to Peat. *HortScience* **2005**, *40*, 2138–2144. [CrossRef]
27. Truong, T.H.; Marschner, P. Respiration, available N and microbial biomass N in soil amended with mixes of organic materials differing in C/N ratio and decomposition stage. *Geoderma* **2018**, *319*, 167–174. [CrossRef]
28. Shinohara, M.; Aoyama, C.; Fujiwara, K.; Watanabe, A.; Ohmori, H.; Uehara, Y.; Takano, M. Microbial mineralization of organic nitrogen into nitrate to allow the use of organic fertilizer in hydroponics. *Soil Sci. Plant Nutr.* **2011**, *57*, 190–203. [CrossRef]
29. Wang, L.; Li, Y.; Prasher, S.O.; Yan, B.; Ou, Y.; Cui, H.; Cui, Y. Organic matter, a critical factor to immobilize phosphorus, copper, and zinc during composting under various initial C/N ratios. *Bioresour. Technol.* **2019**, *289*, 121745. [CrossRef]
30. Abul-Soud, M.; Emam, M.; El-Rahman, N. The Potential Use of Vermicompost in Soilless Culture for Producing Strawberry. *Int. J. Plant Soil Sci.* **2015**, *8*, 1–15. [CrossRef]
31. Cabrera, M.L.; Kissel, D.E.; Vigil, M.F. Nitrogen Mineralization from Organic Residues: Research Opportunities. *J. Environ. Qual.* **2005**, *34*, 75–79. [CrossRef]
32. De Boodt, M.; Verdonk, O.; Cappaert, I. Method for measuring the water release curve of organic substrates. *Acta Hort.* **1974**, *37*, 2054–2062. [CrossRef]
33. Abad, M.; Fornes, F.; Carrión, C. Los sustratos en los cultivos sin suelo. In *Tratado De Cultivo Sin Suelo*; Mundi-Prensa: Madrid, Spain, 2004; pp. 113–158.
34. Zeng, J.; Liu, X.; Song, L.; Lin, X.; Zhang, H.; Shen, C.; Chu, H. Nitrogen fertilization directly affects soil bacteria nitrogen-use-efficiency of maize. *Appl. Soil Ecol.* **2016**, *107*, 324–333.
35. Singh, R.; Varshney, G. Effects of carbofuran on availability of macronutrients and growth of tomato plants in natural soils and soils amended with inorganic fertilizers and vermicompost. *Commun. Soil Sci. Plant Anal.* **2013**, *44*, 2571–2586. [CrossRef]
36. Manh, V.H.; Wang, C.H. Vermicompost as an important component in substrate: Effects on seedling quality and growth of muskmelon (Cucumis melo L.). *APCBEE Proc.* **2014**, *8*, 32–40. [CrossRef]
37. Karuna, S.H.; Pramod, S.; Eric, M.A.; Kerry, B.W.; Keith, H.; Andrew, S.B. Changes in microbial and nutrient composition associated with rumen content compost incubation. *Bioresour. Technol.* **2011**, *102*, 3848–3854.
38. Wolińska, A.; Stępniewska, Z. Dehydrogenase Activity in the Soil Environment. In *Dehydrogenases*; Canuto, R.A., Ed.; In Tech: Rijeka, Croatia, 2012; pp. 183–209. [CrossRef]
39. Henríquez, H.; Uribe, L.; Valenciano, A.; Nogales, R. Actividad enzimática del suelo, Deshidrogenasa, -ß Glucosidasa, Fosfatasa y Ureasa, bajo diferentes cultivos. *Agron. Cost.* **2014**, *38*, 43–54.
40. Wolińska, A.; Stępniewska, Z. Microorganisms Abundance and Dehydrogenase Activity As a Consequence of Soil Reoxidation Process. In *Soil Tillage & Microbial Activities*; Miransari, M., Ed.; Research Singpost: Kerala, India, 2011; pp. 111–143.
41. Hayes, J.E.; Richardson, A.E.; Simpson, R.J. Phytase and acid phosphatase activities in extracts from roots of temperate pasture grass and legume species. *J. Plant Physiol.* **1999**, *26*, 801–809.
42. Paz-Ferreiro, J.; Trasar-Cepeda, C.; Leirós, M.C.; Seoane, S.; Gil-Sotres, F. Biochemical properties of acid soils under native grassland in a temperate humid zone. *N. Z. J. Agric. Res.* **2007**, *50*, 537–548. [CrossRef]
43. Turner, B.L.; Baxter, R.; Whitton, B.A. Seasonal phosphatse activity in three characteristic soils of the English uplands polluted by long-term atmospheric nitrogen deposition. *Environ. Pollut.* **2002**, *120*, 313–317. [CrossRef]
44. Nannipieri, P.; Giagnoni, L.; Landi, L.; Renella, G. Phosphorus in Action. *Soil Biol.* **2011**, *26*, 215–243.
45. Ali, J.; Chandra, S.D.; Bano, A.; Gupta, A.; Sharma, S.; Bajpai, P.; Pathak, N. Exploiting Microbial Enzymes for Augmenting Crop Production. *Enzym. Food Biotechnol.* **2019**, *29*, 503–519.
46. Sinsabaugh, R.L.; Lauber, C.L.; Weintraub, M.N.; Ahmed, B.; Allison, S.D.; Crenshaw, C.; Contosta, A.R.; Cusack, D.; Frey, S.; Gallo, M.E.; et al. Stoichiometry of soil enzyme activity at global scale. *Ecol. Lett.* **2008**, *11*, 1252–1264. [CrossRef] [PubMed]

47. Li, Z.; Cui, J.; Mi, Z.; Tian, D.; Wang, J.; Ma, Z.; Wang, B.; Chen, H.Y.H.; Niu, S. Responses of soil enzymatic activities to transgenic Bacillus thuringiensis (Bt) crops—A global meta-analysis. *Sci. Total Environ.* **2019**, *651*, 1830–1838. [CrossRef] [PubMed]
48. Haynes, R.J. Size and activity of the soil microbial biomass under grass and arable management. *Biol. Fertil. Soils* **1999**, *30*, 210–216. [CrossRef]
49. García-Ruiz, R.; Ochoa, V.; Viñegla, B.; Hinojosa, M.B.; Peña-Santiago, R.; Liébanas, G.; Linares, J.C.; Carreira, J.A. Soil enzymes, nematode community and selected physico-chemical properties as soil quality indicators in organic and conventional olive oil farming: Influence of seasonality and site features. *Appl. Soil Ecol.* **2009**, *41*, 305–314. [CrossRef]
50. Meyer, A.H.; Wooldridge, J.; Dames, J.F. Variation in urease and β-glucosidase activities with soil depth and root density in a 'cripp's pink'/m7 apple orchard under conventional and organic management. *S. Afr. J. Plant Soil.* **2015**, *32*, 227–234. [CrossRef]
51. Zhang, Y.; Chen, L.; Wu, Z.; Sun, C. Kinetic parameters of soil β-glucosidase response to environmental temperature and moisture regimes. *Rev. Bras. Ciênc do Solo.* **2011**, *35*, 1285–1291. [CrossRef]
52. Fontaine, S.; Marotti, A.; Abbadie, L. The Priming Effect of Organic Matter: A Question of Microbial Competition. *Soil Biol. Biochem.* **2003**, *35*, 837–843. [CrossRef]
53. Teimouri, M.; Mohamadi, P.; Jalili, A.; Dick, W. Assessing soil quality through soil chemical properties and enzyme activities in semiarid area, Iran. *Appl. Ecol. Environ. Res.* **2018**, *16*, 2113–2127. [CrossRef]
54. Cadahía, C. *La savia como índice de fertilización*; Mundi-Prensa: Madrid, Spain, 2008; p. 256.
55. Ferreira, C.M.; Soares, H.M.; Soares, E.V. Promising bacterial genera for agricultural practices: An insight on plant growth-promoting properties and microbial safety aspects. *Sci. Total Environ.* **2019**, *682*, 779–799. [CrossRef]
56. Subhashini, D.V.; Anuradha, M.; Damodar Reddy, D.; Vasanthi, J. Development of bioconsortia for optimizing nutrient supplementation through microbes for sustainable tobacco production. *Int. J. Plant Prod.* **2016**, *10*, 479–490.