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Compost and Vermicompost as Substrates Enriched with *Trichoderma asperellum* for the Production of Basic Potato Seed in the Venezuelan Andes

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Abstract: The agricultural sector faces significant pressure to meet the growing global demand for food while managing the planet's limited natural resources. Addressing this challenge requires the strategic use of abundant materials and accessible biotechnologies that farmers can effectively implement. This study evaluated the effects of various substrate mixtures, including combinations of coconut fiber with compost or vermicompost (50:50 *v/v*). It also assessed the impact of heat treatment and inoculation with the antagonist *Trichoderma asperellum* on the production of basic potato seeds grown in 70 L plastic baskets. Statistical analysis revealed that the vermicompost-based mixture outperformed others, demonstrating superior biometric variables for potato plants. The treatment with sterilized vermicompost led to a 41% increase in seed weight, underscoring the beneficial effects of vermicompost. Correlation analysis indicated a positive relationship between the phosphorus content of the mixture and a negative relationship with the E4/E6 ratio—a parameter recognized as a reliable and easily measurable indicator of substrate quality, along with pH and electrical conductivity.

Keywords: vermicompost; plant growth-promoting fungi; *Solanum tuberosum*; substrate; organic matter; bio-waste; composting

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1. Introduction

The increasing global population demands a steady supply of high-quality food. Agronomists, researchers, and agricultural producers have a responsibility to meet this demand while minimizing the environmental impact of their practices. Traditional agriculture, with its heavy reliance on chemical inputs and limited control over environmental variables, often fails to provide sustainable strategies to combat plant diseases and generate sufficient quantities of quality food [1,2]. However, emerging technological developments present a range of possibilities that are beginning to be implemented in Venezuela. These technologies must be validated to generate the necessary knowledge that can positively influence rural development [1]. One of the key challenges facing the agricultural sector is the need for sustainable agronomic practices that can increase crop productivity while addressing the impacts of climate change. Sustainable agronomic practices, such as selecting suitable crop varieties resistant to biotic and abiotic stresses, implementing sustainable

intensification strategies, and adopting resource-conserving agriculture, can play a crucial role in improving food security [1].

The production of high-quality potato seed is a critical aspect of sustainable agriculture, as it ensures the availability of disease-free and genetically superior planting material. Traditional methods of potato seed production can be resource-intensive, often relying on synthetic fertilizers and pesticides. In the technological context described, the production of potato seed developed in Mucuchíes, Venezuela, executed by PROINPA [3], is framed within the Venezuelan National Seed Plan. The objective is to contribute to the supply of quality seed demand at the national level, articulating inter-institutional actions of the public sector and cooperatives that cover the selection of the best germplasm according to national needs, to the commercialization of certified seed among producers [4].

This initiative in Mucuchíes, Venezuela, is part of a broader effort to address the lack of high-quality planting material for farmers' potato varieties, produced locally and at low cost, which is a major constraint limiting the expansion of potato production in the region. The practices implemented in Mucuchíes should be promoted among Andean producers to increase the productivity and sustainability of their potato–grain–pasture systems. Preparing suitable substrates is of utmost importance for the acclimatization of in vitro grown plantlets and the production of pre-basic and basic potato seeds [5]. The production of compost with sufficient quality attributes is extremely valuable to ensure the seed production chain, while also leveraging the beneficial nutrients and biological principles contained within [5,6]. Compost not only nourishes plants but also protects them from pathogens, as these are emergent properties of the agroecosystem that arise from its beneficial interactions [7]. Alongside the use of compost and vermicompost-based substrates, the possibility of enriching the culture medium with plant growth-promoting microorganisms presents additional advantages in terms of nutrition and antagonism against pathogens, such as *Trichoderma* [8–10]. Enriching the beneficial microbiome associated with plants is a promising approach to developing sustainable crop production, as microbes residing in the soil system have immense potential to increase crop growth, nutrient acquisition, stress tolerance, soil fertility, and disease resistance [11]. Rhizospheric soil, the region influenced by plant roots, is typically richer in nutrients compared to bulk soil due to the accumulation of various organic compounds released by roots.

The use of vermicompost in organic farming has been well-documented, with improvements in soil's physical, chemical, and biological properties that enhance crop yield. While vermicompost has been shown to significantly improve plant growth, its application at high concentrations may impede growth due to high levels of soluble salts. Therefore, vermicomposts should be applied at moderate concentrations to obtain maximum plant yield. Additionally, studies have demonstrated the positive effects of soil drenching with vermicompost leachate on the growth of greenhouse-grown tomato seedlings, alleviating deficiencies of phosphorus and potassium.

To address this research gap, the present study evaluated the characteristics of compost and vermicompost and their influence on the growth and yield of potato seedlings in a protected cultivation environment. The integration of these eco-friendly inputs into potato seed production systems could potentially reduce the reliance on synthetic inputs while maintaining or even enhancing crop performance [12–15].

2. Materials and Methods

2.1. Characterization of Compost-Vermicompost Blends with Coconut Fiber for Plant Growth Media

The study was conducted in a greenhouse located at the La Pradera Academic Unit of the Universidad Nacional Experimental del Táchira in the Jáuregui municipality of Táchira state, Venezuela. The site is situated at coordinates 8°11′21.5″ N/71°57′54.4″ W,

at an elevation of 1750 m above sea level, with an average temperature of 18 °C and a relative humidity of 73%. According to the Holdridge life zone classification, the area is characterized as a premontane wet forest [16]. In a greenhouse with a maximum average temperature of 32.7 °C and a minimum of 15.5 °C with humidity levels ranging from a minimum of 36.07% to a maximum of 90.21%. The laboratory analyses were carried out at the Compostable Waste Valorization Laboratory at UNET. As the first step, 50:50 blends of compost and vermicompost with coconut fiber were prepared. Subsequent to the preparation of compost and vermicompost mixtures, moist heat treatment was applied to half of each of the blends [17–20]. This was completed by transferring 40 L portions into clean nylon bags, which were then placed in a 150 L metal container with a perforated base to create a double-bottom setup. Water was added to the container to a height of 0.25 m, and the level was maintained as needed, similar to a laboratory water bath. The treatment was continued for two hours after the onset of boiling and was repeated twice. The study involved planting five mini-tubers of the Iniafrit potato variety in plastic baskets with a capacity of 70 L, filled with the described substrate mixtures. The initial volume of the mixture was 20 L, with two additional inputs of 10 L each at 30 and 60 days after sowing to simulate hilling. The cultivated potato of the Iniafrit variety was released by the CIP in 2006 and introduced in Venezuela by the Venezuelan Institute of Agricultural Investigations INIA in 2006; by its acronym in Spanish, for industrial processing because it has elongated tubers with characteristics for cane-type processing, tolerant to *P. infestans* [21]. At 60 days after sowing, the number of leaves and the height of the main stem from the base to the tip were measured for each plant [20]. It was measured: thickness of the stems (G-Steam), number of leaves (N° Leaves), dry weight of the roots (radical dry weight), number of tubers per basket (N° tuber box), and weight of the tubers per basket (weight box) were measured. A wettable powder formulation of the rice-based fungus *T. asperellum* [21], containing 1.3×10^9 spores per gram, was used. Suspensions with 10^7 spores mL were prepared from this product and applied as a spray to the point of runoff at the time of sowing in potato seed treatments [22] and plants. Two complementary treatments were performed at 15 and 30 days after planting with 5 mL/plant of the *Trichoderma* suspension. The *T. asperellum* strain was obtained from the Microbiological Control Laboratory of the National Experimental University of Táchira (UNET), Venezuela of soils cultivated with potatoes in the municipality of José María Vargas in the state of Táchira.

The experiment was conducted using a completely randomized factorial design with three treatments and two levels each: (1) two organic materials, Cp (agro-industrial compost) and Cv (vermicompost), both mixed with coconut fiber in a 50:50 *v/v* ratio; (2) two levels of physical treatment: sterilized and non-sterilized; and (3) two levels of biological treatment: with and without *T. asperellum.*

This research was conceived as a continuation of the evaluation of the quality parameters of compost from three compostable mixtures, prepared from agro-industrial waste, biowaste, and agricultural waste [22,23]. The Laboratory for the Recovery of Compostable Waste of the UNET was used to carry out the analyses described unless otherwise indicated. The proportions of waste used to obtain compostable mixtures were calculated according to Richards for their optimization in terms of C/N and moisture content [24]. Therefore, the compost used in the test was wastewater sludge compost from the food industry (Cod. LER 02 01 03) (European Parliament 2014) [25], designated Cp; and vermicompost comprising coffee pulp (Cod. LER 02 01 03), designated Cv. The pulp is a byproduct of the wet processing of the coffee fruit and is composed of the mesocarp of the cherry or berry, which is located just below the pericarp or skin [26]. This product was purchased from the National Institute of Agricultural Research INIA located in the Junín municipality of the state of Táchira, Venezuela.

2.2. Physical, Chemical, and Biological Analysis of Substrate Mixtures

2.2.1. Determination of Organic Carbon in Compost Samples

The determination of organic carbon (OC) in compost was carried out following the procedure described by Sadzawka et al. [27]. This method assumes that the organic matter in the compost contains 56% carbon and uses a conversion factor of 1.8 to calculate the organic carbon content from the organic matter content. Specifically, the organic carbon content was calculated as OC (%): OM/1.8, where OM represents the concentration of organic matter in the sample and was calculated as OM (%) $[(a - b)/a] \times 100$ where **a** is mass (g) of the dry sample at 70 ± 5 °C before calcination and **b** is mass (g) of the calcined sample at 550 °C. To measure the organic matter content, 10 g of the sample, dried at 36 °C and passed through a 16 mm sieve, was placed in a crucible and dried at 70 ± 5 °C for 24 h. The sample was then slowly heated in a muffle furnace up to 550 °C and held at that temperature for 2 h. After this, the temperature was slowly decreased to 200 °C. Finally, the samples were placed in a desiccator until room temperature.

2.2.2. Nitrogen Content (%)

For N determination, the procedure described by [28] was followed. A 0.1 g sample was weighed into a digestion tube. Then, 2 mL of the digestion mixture containing 2.5 g of copper sulfate diluted in 2.5 mL of deionized distilled water was added; subsequently, 0.15 g of selenium was added to the previous preparation. In 100 mL of concentrated sulfuric acid, 2.5 g of potassium sulfate was dissolved, the selenium solution was added, and then 1.0 g of mercury oxide was added. The sample was placed in the digestion apparatus at 350–380 °C for 90 min or until the mixture became transparent. While still warm, distilled water was slowly added to the tubes. To perform the distillation phase, 15 mL of 40% NaOH was slowly added to the flask. The flask was placed in the distillation system for 10 min, and at the opposite end of the condensation system, a flask containing 25 mL of 2% boric acid solution (with methyl red indicator (0.1%) and bromocresol green (0.1%) in 100 mL of 95% ethanol) was placed. The captured ammonium was titrated with 0.1 N H_2SO_4. *N (%)* was calculated as $[(V \times N \times 0.014)/W] \times 100$, where V is sulfuric acid consumption in mL; N is normality of sulphuric acid; milliequivalent of nitrogen; and W: sample weight (g).

2.2.3. Phosphorous Content (%)

The determination of P followed the procedure described by Sadzawka et al. [27]. Approximately 2 g of fresh sample dried at 70 °C for 24 h was subjected to calcination at 500 °C. The sample was weighed after drying at 70 °C, then placed in a muffle furnace where the temperature was gradually increased to 500 °C and maintained for 2 h. The sample was then allowed to cool slowly and transferred to a desiccator. The ash was analyzed for its P and Ca content [28]. Once the ash from the samples was obtained, 40 mL of HCl 1:3 with a few drops of concentrated HNO_3 was added. The mixture was heated on a hot plate in crucibles until boiling point was reached. After filtration, the extracts were transferred to 250 mL volumetric flasks and topped up with distilled water once cooled. For P determination, 10 mL aliquots were transferred to 50 mL volumetric flasks, followed by the addition of 5 mL of molybdenum vanadate solution [29]. After 10 min, the absorbance of the samples and corresponding standards was read at 400 nm using a 6405 UV-vis brand Jenway (USA).

2.2.4. Calcium Content (%)

Ca (%) was measured by complexometry. In this procedure, the calcium present in the sample is forced to form a calcium oxalate precipitate by adding a saturated solution

of ammonium oxalate to the ash solution of the evaluated material. This precipitate is thoroughly washed with ammonium hydroxide to remove excess ammonium oxalate. Through the action of sulfuric acid, calcium oxalate forms oxalic acid and calcium sulfate. The oxalic acid is determined using a standardized $KMnO_4$ solution [28].

2.2.5. Proportion of Humic and Fulvic Substances: E4/E6 Ratio

A 1 g sample of compost or vermicompost was weighed into 250 mL polyethylene flasks, and 50 mL of 0.5 M NaOH solution was added. The mixture was shaken for 2 h using a horizontal shaker at 60 cycles per min and left to rest for 12 h. The sample was then transferred to 50 mL centrifuge tubes and centrifuged at 3000 rpm for 25 min, followed by filtration. The absorbance of the supernatant was determined at 465 nm (E4) and 665 nm (E6) using a 6405 UV-vis brand Jenway (USA). The E4/E6 ratio was calculated using these values [30].

2.2.6. pH and Electrical Conductivity

To measure the pH, the sample sieved at 16 mm humidity was mixed and stirred with water at a 1:5 ratio. Then, the pH and electrical conductivity of the same extract were measured with a Hanna Instrument brand pH meter. The mass of the sample <16 mm and wet (A) equivalent to 40 g of dry sample at 70 ± 5 °C and the volume of water (B) were calculated to achieve a ratio of 1:5 according to A = (40/ST) × 100 B = 200 − (A − 40), where A = mass, in g, of a sample <16 mm and wet, ST = total solids, in % for a wet sample, and B = volume, in mL. For water, A = mass, in g, of a sample <16 mm and wet [27].

2.2.7. Physical Properties

Total porosity, aeration porosity, moisture retention capacity, bulk density, and particle density were determined using the method of Pire and Pereira [31]. The material was kept in the laboratory at an average temperature of 24 °C during the study to measure the physical properties of the mixtures, including total porosity, TPS (%) = (Va + (WW − DW)/Pa)/Vc × 100; aeration porosity (AP (%) = Va/Vc × 100%); apparent density (Bd ($Mg \cdot m^{-3}$) = DW/Vc); particle density (Pd ($Mg \cdot m^{-3}$) = Bd/(1 − PT/100)); and water retention capacity H_W(%) = (WW − DW)/Vc × 100, where Va is the volume drained (cm^3), WW is the wet weight of the sample (g), DW is the dry weight of the sample (g), Pa is the specific weight of water (1 g cm^{-3}), and Vc is the volume of the tube or cylinder (cm^3). For the determination of physical properties, porometers made of PVC tubes were prepared. Each had a diameter of 7.5 cm, 15 cm, and a foot composed of the same material, which was sealed as a base and perforated on the side to allow water circulation. A ring was added as an extension of the cylinder to prevent sample loss due to expansion when it became wet. The samples were used to fill the porometers to their maximum capacity. Settling was promoted by dropping the porometers twice from a height of 7.5 cm and then again filling them to the upper edge. The cylinders with the samples and the upper rings were placed in a container with a water level just below the upper edge. This process forced moisture into the sample via the bottom holes, allowing the air to escape freely through the upper face. The samples were left in the water until the next day to standardize the saturation process. After 24 h, they were extracted from the container with water and leveled with a spatula. The samples were fitted with a cloth attached to a rubber band on top and completely immersed for 30 min. Rubber plugs were then placed in the bottom holes before removal from the water. Once removed, each porometer with its sample was placed on paths, the trays were removed to measure the volume of water drained in ten minutes, the wet sample was extracted from the porometers, and its dry weight was determined at 105 °C.

2.2.8. Microbial Populations

A suspension was prepared with 1 g of each sample of air-dried substrate mixed in 10 mL of a sterile 0.89% NaCl solution, shaking at 200 rpm for 30 min [32]. The resulting suspension constituted the 1/10 dilution, from which serial dilutions were performed up to 1×10^{-6}. Total fungal counts were carried out using PDA (potato dextrose agar) medium with streptomycin sulfate (100 $\mu g \cdot mL^{-1}$) and chloramphenicol (30 $\mu g \cdot mL^{-1}$). Total bacterial counts were performed using nutrient broth at 1/10 of the standard concentration [32]. For the evaluation of *Trichoderma*, a specific medium was prepared with the following composition: 40 g of PDA, 0.15 g of Bengal Rose, 0.05 g of Captan, and 0.001 g of Benomyl. After sterilization, 0.25 g of chloramphenicol, 0.10 g of streptomycin, and 1 mL of lactic acid per liter were added. In the case of Azotobacter, the culture medium included 20 g mannitol, 0.2 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.2 g NaCl, 0.1 g K_2SO_4, 5 g $CaCO_3$, and 15 g agar. The pH was adjusted to 7.4 $\pm$ 0.2 [33]. The results aim to determine whether agro-industrial waste compost and/or coffee pulp vermicompost, with or without *Trichoderma*, can be used as substrates for producing basic seed potatoes of the Iniafrit variety under the studied conditions.

2.3. Data Analysis

The experimental design was factorial with three factors, two levels each, and three replicates: (1) Two levels of organic materials: Cp (agro-industrial compost) and Cv (vermicompost), both mixed with coconut fiber in a 50:50 *v/v* ratio; (2) Two levels of physical treatment: heat treated and non-heat treated; and (3) Two levels of biological treatment: with and without *T. asperellum.* The objective was to measure the effect of compost type in each mixing ratio and its interaction with the antagonist *T. asperellum*. Descriptive analyses were performed, followed by tests to verify the assumptions of normality, homogeneity of variances using the Kolmogorov–Smirnov and Levene tests, and error independence through graphical analysis. To identify the variables with the greatest influence on the measured biological response, principal component analysis (PCA) was applied, followed by analysis of variance, correlation analysis, and regression analysis using the statistical software InfoStat v. 2022 [34].

3. Results

3.1. Evaluation of Biometric Variables Measured on Plants and Substrate Characteristics Affected by Treatments and Their Interactions

All the results obtained were subjected to a principal component analysis (PCA) (Figure 1) with the intention of determining the best associations between the variables, as well as with the treatments that have shown the best performance, measured as the number of tubers per basket. First, it was verified how much variability was represented in the first components after the analysis. It was verified that the cumulative variability of the first two components, Cp1 (56.0%) and Cp2 (20.5%), exceeded 70%, reaching 76.5%. It was also confirmed that the correlation of the variables included in the analysis with the original variables was greater than 70%. According to the PCA biplot (Figure 1), treatments with vermicompost, both sterilized and non-sterilized, are concentrated in quadrants 1 and 3, close to the biometric variables and specifically to the number of mini tubers. This variable was selected for its importance in seed potato production, being close to the variables P and OM, revealing the importance of good P supply and organic matter content in the biometric variables measured. On the opposite side, in quadrant 4, the E4/E6 proportion is located, showing the importance of the evolution of the humification of organic matter in compost for its performance as a substrate and its benefit to yield.

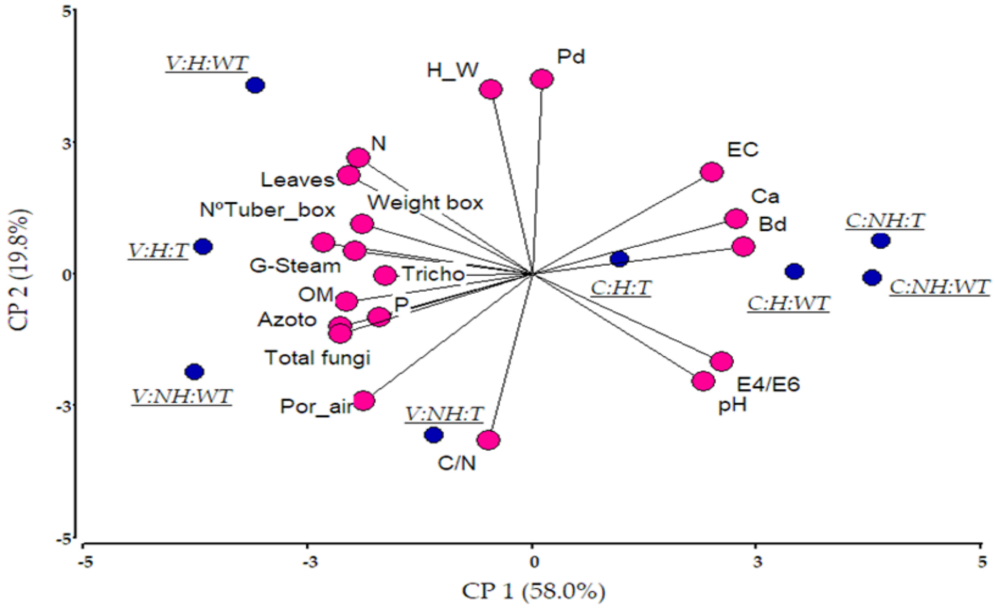

Figure 1. Biplot of the PCA applied to the set of variables measured on the plants, substrates, and mini tubers produced in the trial. Where C: compost; V: vermicompost; NH: non-heat; H: heat treatment; T: with *T. asperelllum*; WT: without *T. asperelllum*.

The best treatments were the substrates composed of sterilized vermicompost, both with and without *T. asperellum*, followed by treatments with vermicompost, but not sterilized. It was also observed that the other biometric variables measured were notably associated with the number of mini tubers produced per basket. Likewise, the variables N (%) and P (%) content, total fungal count, *Trichoderma*, and *Azotobacter* revealed a positive association with yield, but a negative one with E4/E6, pH, Ca, and EC. The latter was associated with the worst biological expression. Given the need to better identify the most favorable treatments, a Multivariate Analysis of Variance (MANOVA) (Table 1) was conducted for the variables number and weight of mini tubers, and the number of stems per basket, due to their great agronomic importance. The results show the significance of the sources of variation as well as the compost variable in combination with the antagonist.

Table 1. Result of the Multivariate Analysis of Variance (Wilks, df num = 2, df den = 15) for the variables number and weight of mini tubers per basket (with five plants and 40 L of substrate) in the trial for the evaluation of substrate type (compost and vermicompost), heat treatment, and inoculation with *T. asperellum* for the production of potato seed tubers of the INIAFrit variety.

Variable	Statistic	F	*p*-Value
Substrate	0.03	237.16	<0.0001
Heat treatment	0.08	89.83	<0.0001
T. asperellum	0.010	68.79	<0.0001
Substrate*Heat treatment	0.65	4.04	0.0395
Substrate**T. asperellum*	0.37	12.52	0.0006
Heat treatment**T. asperellum*	0.06	117.42	<0.0001
Heat treatment*Substrate**T. asperellum*	0.08	82.82	<0.0001

In the global analysis, the effect of the type of compost used to prepare the substrate stands out, with respect to sterilization and inoculation with *T. asperellum*, measured as the number and weight of mini tubers per basket, all at $p < 0.001$. Similarly, the analysis detected an interaction between the type of compost and inoculation, as well as between sterilization and inoculation. Sterilization had a significant impact on the yield in both composts. In

the expression of the antagonist, double significance was found with the sterilization of the mixture and triple significance among the evaluated variables. To determine which treatment configuration resulted in the best yield, a Bonferroni-type mean comparison test was applied, with the results summarized in the corresponding table (Table 2). The tests for the individual factors provided significant information according to the Ho telling test with Bonferroni correction ($\alpha = 0.05$) regarding the effect of the type of compost on the expression of the variable number of mini tubers. A 151.70% increase ($p < 0.05$) in the number of mini tubers was produced in vermicompost compared to compost. As for the heat treatment factor (humid heat treatment), a 67% effect ($p < 0.05$) was quantified in the overall measurement of the number of mini tubers. Treatments such as VHT and VHWT show the highest values for the number and weight of mini tubers, positioning themselves as the best resulting options.

Table 2. Hotelling test with Bonferroni-corrected $\alpha = 0.05$ and 16 df for the variables weight number of mini tubers and weight produced per basket with 5 plants from the treatments in the evaluation of compost and vermicompost as substrates for potato seed production in a protected environment.

Treatment	N°Tuber Box	Weight Box (g)
VHWT	51.67 ± 0.58 *a*	345.2 ± 4.44 *c*
VHT	45 ± 3 *ab*	434.3 ± 33.2 *b*
CHT	43.67 ± 4.51 *abc*	176.89 ± 3.92 *d*
VNHT	37 ± 4 *bcd*	82.27 ± 24.84 *ef*
VNHWT	34.33 ± 3.79 *cd*	540.52 ± 36.89 *a*
CHWT	26.67 ± 3.51 *de*	193.6 ± 17.06 *d*
CNHWT	23 ± 4 *e*	159.1 ± 42.53 *de*
CNHT	5.67 ± 2.52 *f*	27.28 ± 8.78 *f*

Data presented are means ± SE, n = 3. Means within the same column in each studied factor followed by the different letters indicate significant differences at $p \leq 0.05$ according to Bonferroni test. C: compost; V: vermicompost; NH: Non-heat; H: heat treatment; T: with *T. asperelllum;* WT: without *T. asperelllum.*

The variables associated with the best yields are displayed in the Spearman correlation analysis in Table 3. There, the positive relationship of the measured biometric variables can be seen, including the number of leaves, number of stems, number of tubers per basket, and total tuber weight (per basket with five plants).

Table 3. Spearman correlations for the variables measured in the trial.

	Leaves	Steams	N°-Tuber	Weight Box	Total Fungi	Azoto-Bacter	Bd	pH	EC	N	P	E4/E6	OM	Ca^{++}
Leaves	1													
Steams	0.61	1												
No tuber	0.7	0.64	1											
Weight box	0.64	0.63	0.55	1										
Total fungi	0.59	0.72	0.61	0.71	1									
Azotobac	0.53	0.35	0.54	0.7	0.69	1								
Bd	−0.51	−0.74	−0.52	−0.66	−0.71	−0.51	1							
pH	−0.62	−0.66	−0.66	−0.59	−0.35	−0.21	0.69	1						
EC	−0.33	−0.52	−0.42	−0.5	−0.66	−0.64	0.84	0.39	1					
N	0.63	0.55	0.4	0.56	0.34	0.16	−0.59	−0.72	−0.24	1				
P	0.39	0.39	0.57	0.47	0.49	0.63	−0.63	−0.42	−0.68	0.21	1			
E4/E6	−0.62	−0.7	−0.62	−0.68	−0.45	−0.32	0.76	0.9	0.46	−0.85	−0.47	1		
OM	0.36	0.41	0.41	0.56	0.47	0.39	−0.8	−0.68	−0.66	0.43	0.67	−0.66	1	
Ca^{++}	−0.46	−0.77	−0.54	−0.54	−0.64	−0.47	0.78	0.51	0.68	−0.34	−0.53	0.5	−0.4	1

The results showed a positive correlation of the four measured biometric variables with the total fungal count, *Azotobacter*, the nitrogen content of the mixture, phosphorus,

and organic matter. In contrast, yield was negatively affected, according to the analyzed correlations, by the bulk density of the mixture, electrical conductivity, E4/E6 ratio, pH, and Ca content of the substrate. Some of these relationships were assessed through regression analysis (Table 3) to better understand the effect of the variables on yield. Likewise, it is observed that as the pH value increases, the yield of mini tubers decreases, just as it decreases with the increase in electrical conductivity, the E4/E6 proportion, and the concentration of Ca in the mixture used in the treatments. On the other hand, the higher the P and N content of the mixtures, the greater the number of mini tubers. The correlations with the highest values, both positive and negative, were selected for the application of linear regression analysis: pH, EC, E4/E6, total fungi, *Azotobacter*, N, and P.

3.2. Evaluation of Growth Indices in Potato Plants

The information derived from the calculation of physiological growth indices helps to more clearly explain the mechanisms activated in the plant through the mediation of the physics, chemistry, and biology of the substrates. Figure 2A shows an expression of the efficiency of plants that grew in vermicompost-based substrates, treated or not with heat, and in the absence of *T. asperellum*.

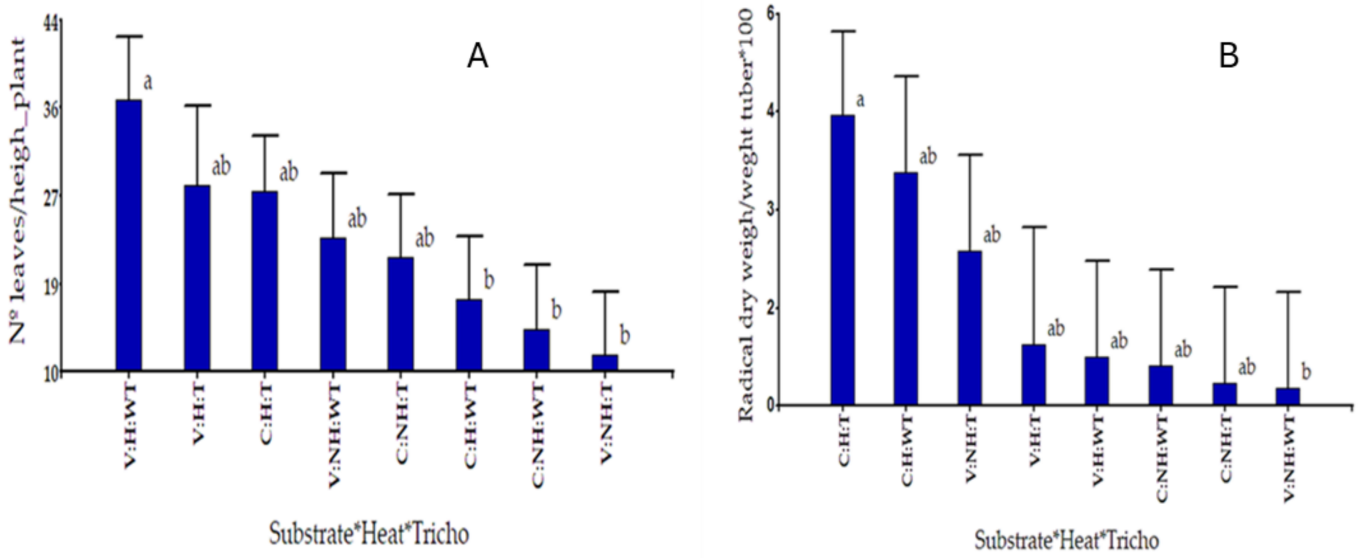

Figure 2. Effect of the factors: substrate type, heat treatment, and inoculation with *T. asperellum* on the physiological growth indices of potato plants. Number of leaves/plant height (**A**) and root dry weight/total number of mini tubers (**B**) and measured two months after planting. C: compost; V: vermicompost; NH: non-heat; H: heat treatment; T: with *T. asperelllum*; WT: without *T. asperelllum*. Different letters indicate significant differences at $p \leq 0.05$ according to LSD test.

The results reveal that the plants required less root mass per unit weight of mini tubers. Both heat-treated substrates, with or without inoculation of *T. asperellum*, exhibited greater leaf development per unit of plant height, indicating increased foliage (Figure 2B and 3).

3.3. Effect of Substrate Characteristics on the Yield of Seed Potatoes of the INIAfrit Variety

Two important variables that have been shown to influence mini tuber yield are the ratio of E4/E6 (Figure 4A), which is an indicator of substrate humification, and the phosphorus (P) (Figure 4B) content of the growing medium.

Figure 3. Differential aspects of potato plants developed under the treatments applied in the trial. C: compost; V: vermicompost; NH: non-heat; H: heat treatment; T: with *T. asperelllum*; WT: without *T. asperelllum*.

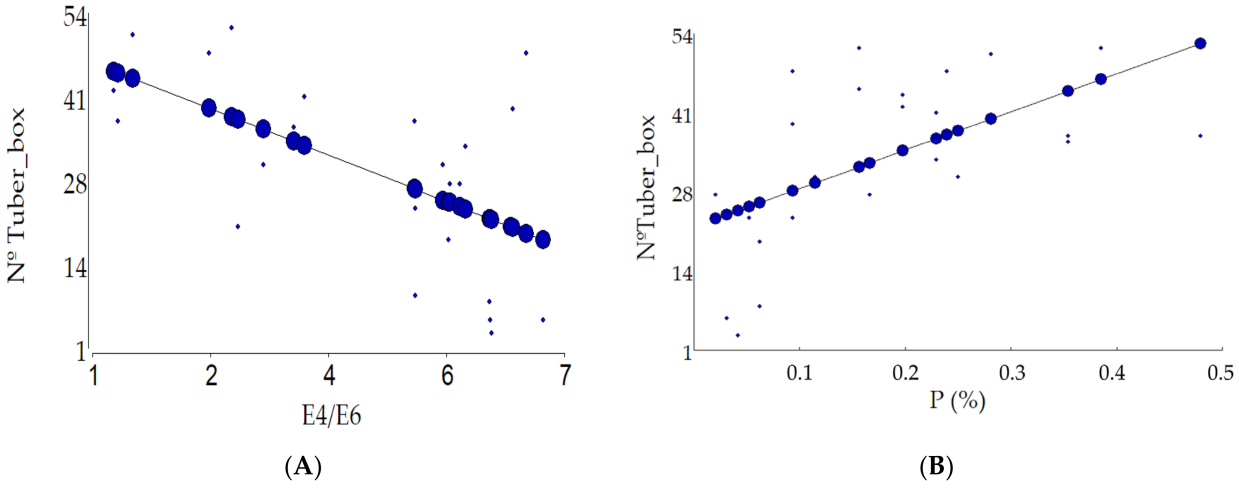

Figure 4. Adjustment of the linear regression analysis for the variable number of mini tubers per box against the variables (**A**) E4/E6 proportion and (**B**) P concentration in the substrate as regressor variables.

4. Discussion

The study of plant biometric variables, substrate characteristics, and their interactions is crucial for understanding the complex relationships between plants and their growing environment. This research paper aims to investigate the effects of various treatments on plant biometric variables and substrate characteristics, as well as the interactions between these factors. The composition of compost can vary significantly depending on the materials from which it is derived, leading to diverse effects on plant growth and disease suppression [35,36]. Compost can directly provide plants with essential nutrients, while also indirectly influencing the soil microbiome in ways that promote plant health and protect against pathogens [37–39]. Several studies have highlighted the benefits of compost application to soil. Compost can help reduce the financial costs associated with chemical fertilizers and mitigate the environmental impacts of their production and use [38].

Furthermore, compost-amended soils have shown significant improvements in overall soil health compared to untreated soils [38]. The physical, chemical, and biological properties of the growth medium are crucial factors that shape the complex web of interactions decisive for the development and agronomic performance of crops [40–43]. Linear Discriminant Analysis (LDA) was employed to identify the minimal set of variables capable of effectively discriminating between groups and establishing quality ranges, as illustrated in Figure 5. The analysis suggests that biological and chemical variables were the most influential in the observed response, as they could not be disregarded, unlike other factors such as apparent density or real density.

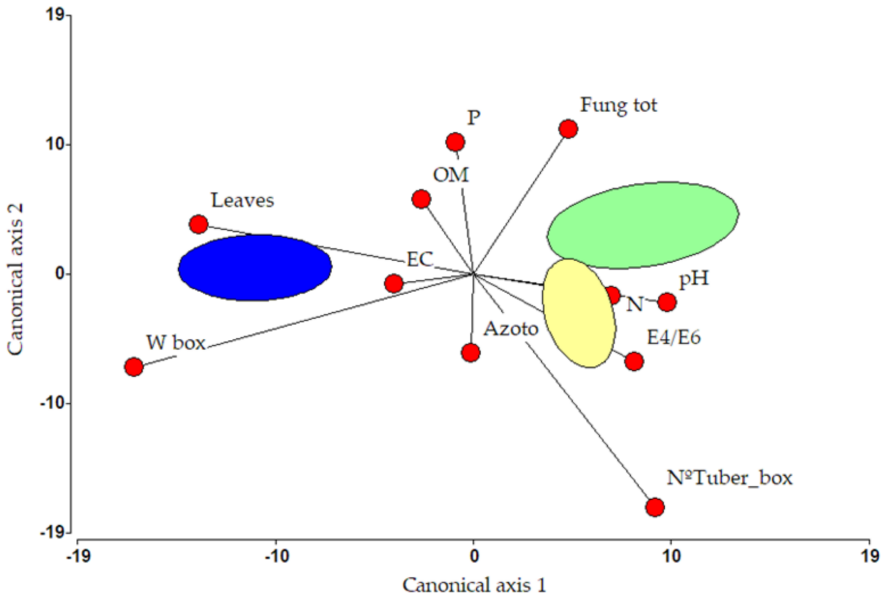

Figure 5. Groups for three qualities of substrates obtained according to an LDA applied to the variables measured on Iniafrit potato plants. Blue = a; green = b; yellow = c.

The use of bioorganic fertilizers has emerged as a promising approach to promote sustainable agriculture and address the growing concerns associated with the excessive use of chemical fertilizers [44]. These bioorganic fertilizers, often combined with compost, have been found to enhance plant growth and yield by improving the composition and functionality of the rhizosphere microbiome. The rhizosphere, the zone immediately surrounding plant roots, harbors a diverse community of microorganisms that play a crucial role in nutrient cycling, plant development, and disease resistance [45]. By modulating the rhizosphere microbiome, bioorganic fertilizers can improve the availability and uptake of essential nutrients, leading to increased plant growth and productivity [46].

Several studies have demonstrated the benefits of bioorganic fertilizers in enhancing plant performance. It has been observed that the application of bioorganic fertilizers alters the soil's chemical properties, thereby selectively favoring specific microbial taxa and functions within the rhizosphere microbiome. This targeted manipulation of the rhizosphere microbiome composition and activity can lead to improved nutrient availability, increased biological nitrogen fixation, and enhanced phosphorus solubilization, all of which contribute to the stimulation of plant growth and development [45]. Furthermore, the presence of plant growth-promoting microorganisms, such as *Trichoderma*, in combination with compost, has been found to be particularly promising in reducing the reliance on chemical fertilizers while maintaining high yields and protecting soil environments from agricultural contamination [45,46].

As suggested by various authors, *Trichoderma* strains can be defined as symbiotic opportunistic organisms of plants, capable of colonizing plant roots through mechanisms similar to those of mycorrhizal fungi to stimulate plant growth [47,48]. Previous research has demonstrated that *Trichoderma* species can rapidly colonize niches left vacant by other organisms, and their TasHyd1 gene plays a crucial role in the colonization of plant roots [49,50]. Additionally, *T. harzianum* can efficiently colonize tomato roots and soil under field conditions, independent of the inoculation formulation. The presented experiment aimed to assess the impact of applying a spore suspension to an organic substrate on the growth-promoting potential of the microorganism within the root system of potato plants. However, the observed response did not align with the expected outcomes in terms of agronomic performance under the prevailing conditions of the trial.

Trichoderma spp. can also act as biocontrol agents and induce disease resistance in symbiotic plants. The introduction of *Trichoderma* has been found to correlate positively with the abundance of microorganisms and the level of available nutrients, similar to the findings in this study where an organic substrate and potato were used instead of tomato [50]. The results of our study suggest that *Trichoderma* can stimulate plant growth primarily through the production of hormone-like substances and/or the supply of phosphorus, which promotes root system development and overall plant growth.

The characteristics of compost and/or substrate play a crucial role in mediating the development and performance of the plant grown in it [51]. For instance, in the case of our study, the vermicomposted coffee pulp residue was a better substrate than the composted wastewater sludge from the food processing industry. Similarly, the treatment applied to control or limit pathogens in the mixtures can either promote or hinder the desired growth-promoting effect, as observed in the ranges presented in Table 4 [52]. The characteristics of compost and substrate have a significant influence on the development and yield of plants [53]. The composition and quality of compost can vary depending on the raw materials used and the composting process, which can impact its effects on plant growth [54]. For instance, in the case of our study, the vermicomposted coffee pulp residue was a better substrate than the composted wastewater sludge from the food processing industry. Similarly, the treatment applied to control or limit pathogens in the mixtures can either promote or hinder the desired growth-promoting effect, as observed in the ranges presented in Table 4.

Table 4. Ranges generated in Linear Discriminant Analysis (LDA) classifications (Group a, b, and c) applied to biometric variables.

Variable	Group a		Group b		Group c	
	V:H:T * V:H:WT V:NH:WT		**C:H:T C:H:WT C:NH:WT**		**C:H:T V:NH:T**	
	Min	Max	Min	Max	Min	Max
NºTuber_box	34.33	51.67	29.67	33.67	5.67	37
Nº Leaves/plant	37.33	47.33	17	28.33	14.67	20
Weight mini tubers box	345.2	540.52	149.55	196.39	27.28	82.27
E4/E6	1.79	4.07	5.78	6.89	5.06	6.25
EC	1.41	2.59	3.32	3.72	1.62	3.9
OM	74.14	78.6	28.46	39.35	43.07	74.55
N	2.53	3.76	1.16	2.36	1.79	2.07
P	0.17	0.29	0.05	0.09	0.02	0.28
pH	3.90	6.70	6.70	6.90	5.80	6.90

* C: compost; V: vermicompost; NH: Non-heat; H: heat treatment; T: with *T. asperelllum*; WT: without *T. asperelllum*.

The positive effect of sterilizing composted and vermicomposted materials is explained by the elimination of nutrient competition, including pathogenic competition, by the native microbiota [55]. Other authors have reported that heat treatment, even microfiltration, impairs the biological effect of compost [53]. In our case, we evaluated the growth-promoting

effect, which persisted in sterilized treatments and even surpassed that of non-sterilized ones. The E4/E6 proportion, which indicates the degree of humification of the composted material, showed an inverse linear relationship with yield. This is understood as the ratio between lower molecular weight chain compounds detected at 465 nm absorbance and more humified, condensed compounds with higher molecular weight measured at 665 nm [56]. An E4/E6 proportion of less than 5 is typically associated with humic acids, while a ratio between 6.6 and 8.5 indicates the presence of fulvic acids [57–59]. The study found that the lower values of the E4/E6 proportion were obtained in the treatments classified as quality "a", while the higher values were observed in the quality "c" group, which had the lowest agronomic yield. How does this affect growth? The relationship between the E4/E6 index and plant growth can be interpreted as follows: Low E4/E6: substrate rich in humified organic matter, providing better growth conditions due to higher nutrient availability and improved soil structure. High E4/E6: soils with organic matter in a less advanced stage of humification. A low E4/E6 value indicates that compost is highly mature and stabilized. In this study, we observed that the promotion of plant growth was attributed to the use of compost, whether treated with *T. asperellum* or not, and always with heat treatment. The growth-promoting effect can be attributed to the combined benefits of the addition of nitrogen (N) and phosphorus (P), primarily provided in a stable matrix with a higher content of humic acids. The data presented in the analysis indicates that the total nitrogen levels ranged from 1.16 to 3.76, which corresponds to a ratio of 10:6:8 (C:H:N) to 37.6 kg per ton (V:H:N). Similarly, the total phosphorus levels were observed to be in the range of 0.46 to 6.64 kg/ton P_2O_5. These findings suggest that the treatments with the highest levels of these essential nutrients (a and b) exhibited the best response in terms of crop quality and yield. The importance of phosphorus in crop production is further supported by the literature. Phosphorus is the second most critical plant nutrient after nitrogen, as it directly affects root proliferation, nitrogen fixation, and overall plant growth and development [59]. Moreover, the efficient utilization of atmospheric nitrogen and the symbiotic process are enhanced by adequate phosphorus levels, reducing the dependence on nitrogen-based fertilizers and promoting sustainable farming practices [59]. The present study investigated the nutrient content of substrates prepared with vermicompost as a soil amendment. Vermicompost has been shown to increase the uptake of essential nutrients in greenhouse crops [60] and improve soil fertility through its physical, chemical, and biological properties [6]. In particular, the incorporation of vermicompost has been reported to increase the availability of nitrogen, phosphorus, and other micronutrients in the growing medium [61,62]. The results of this study indicated that both nitrogen and phosphorus levels in substrates amended with vermicompost are above the regulatory requirements established in Spain and Germany [6,20,63–65]. The nutrient concentrations were not only sufficient to meet the needs of the target crops but were present in markedly higher quantities in the vermicompost treatments. The nutrient concentrations were not only sufficient to meet the needs of the target crops but were present in markedly higher quantities in the vermicompost treatments.

5. Conclusions

Ultimately, compost and vermicompost can be used as substrates for potato seed production, with vermicompost being the most effective. Vermicompost, especially when heat-treated and enriched with beneficial microorganisms such as *T. asperellum*, was found to be the most effective in increasing both the quantity and weight of mini tubers per plant. This approach ensures higher seed yield and quality, thereby improving crop productivity and profitability. The nutrient content of the compost played a key role, particularly adequate levels of nitrogen (N) and phosphorus (P), along with good organic matter

content. An important advantage is the application of heat treatment to the substrate: heat treatment demonstrated a significant benefit to productivity as optimal humification level (E4/E6 ratio) improves the plant's efficiency in tuber production. Based on these findings, it is recommended that producers choose high-quality substrates, preferably with treated vermicompost and adequate levels of key nutrients. Additionally, monitoring factors such as pH, density, humification, and nutrient content—particularly N and P—of the substrate can significantly improve harvest outcomes. Implementing these practices can help increase both the quantity and quality of potato seeds, making the crop more competitive in terms of yield and sustainability.

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