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Full Length Article

Broccoli Seedling Production in Response to Recognised Organic Inputs

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Abstract

This study evaluated the production of seedlings broccoli (*Brassica oleracea* var. *Italica*) with organic inputs. The inputs were as follows; a) growth medium, consisting of *Sphagnum* peat (Pro Moss TBK®) mixed with poultry manure compost (Vertia® brand) in a) 90:10 and 80:20 ratios; b) biofungicide *Trichoderma harzianum* Rifai (Natucontrol® brand) at doses of 1.5 and 3 g/L water per 338-cavity polystyrene tray; and c) complementary nutrition applied in irrigation with poultry manure tea at doses of 0.5 and 1 dS/m per tray every two days. Control set was a 'typical management' control based on peat (100%) as a growing medium with the application of conventional fertiliser (1 g/L of Tricel® 20 every two days) and conventional fungicide Mancozeb as a damping-off preventative (1 g/L per tray). The seedling growth, relative chlorophyll content, photosystem II quantum yield, and morphological indicators showed that the eight treatments with recognised organic inputs performed significantly better than the control ($p<0.05$). The use of peat substrate mixed with poultry manure (80:20 ratio) with inoculation of *T. harzianum* at a dose of 1.5–3 g/L and with application of poultry manure tea at a dose of 1 dS/m yielded the best results. We determined that it is possible to obtain quality broccoli seedlings with the inputs recognised for certified organic agriculture.

Keywords: Growing medium; Horticulture; Organic agriculture; Peat; Poultry manure; *Trichoderma harzianum*

Introduction

Organic agriculture (OA) is concerned with the health of soils, ecosystems, and people. The principles on which it is based are ecology, biodiversity and the cycles that occur in production sites rather than the use of harmful inputs. OA combines tradition, innovation, and science for the benefit of the environment and a high quality of life for all involved (IFOAM 2019). In Mexico, organic crops are focused on the production of cereals (38%), green fodder (26%), coffee (20%), olives (16%), nuts (13%), oilseeds (12%) and others (19%) (Lernoud and Willer 2018). Certified organic production in 2017 took place across 1,127,000 hectares of land, with a total production of 329,656 tonnes, yielding more than 70 different products.

Broccoli is an economically important vegetable in Mexico, representing 3.6% of national production, with an average annual production of 403,000 tonnes from 2012 to 2017 (SIAP 2018). Globally, Mexico is among the main broccoli-exporting countries, where 70% of broccoli production is exported to the United States of America (Rocha and Cisneros-Reyes 2019). Certified organic products are those that are produced, stored, handled, and marketed in accordance with precise technical standards, and whose recognition as "organic" products is carried out by a specialised agency. Once such entity verifies compliance with the standards governing the field of organic products before recognition is granted to the product. This label will vary according to the certification body issuing it but can be taken as a guarantee of compliance with the fundamental requirements of an "organic" product from farm to market. It is important to note that the organic quality label applies to the production process and guarantees that the product has been created and processed in a way that does not harm the environment (FAO 2020).

The first step in the broccoli production process is seedling production, which is carried out in a special care area with specialised inputs such as substrate (growth medium), fertilisers (nutrient sources), and preventive pesticides (fungicides). Conventional seedling management uses organic substrates, such as peat moss or coconut fibre, conventional compound fertilisers (e.g., 20-20-20, 20-30-10) and synthetic preventive fungicides that prevent

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damping-off (e.g., metalaxyl, propamocarb hydrochloride). The organic input market recognised by the Organic Materials Review Institute (OMRI) offers a wide range of products that can be used for seedling production. The use of these materials facilitates organic production processes and ensures certification of the product. However, there is a need to understand the performance of some products to assure growers of their efficacy. Thus, a study was conducted to evaluate the production of broccoli seedlings (*Brassica oleracea* var. *italica*) with three technological components recognised by the OMRI: a) growing medium, *Sphagnum* peat (Moss TBK®) with poultry manure (Vertia® brand); b) biofungicide *Trichoderma harzianum* Rifai (Natucontrol® brand); and c) application of poultry manure tea as a supplement to nutrition.

Materials and Methods

The study was conducted at the Protected Agriculture Training Center of the Universidad Autónoma de San Luis Potosí, Mexico (22°13'54' N, 100°51'28' W), which is located at an altitude of 1872 m above sea level, with a climate classified as 'cold steppe dry'. Nine treatments were evaluated for the production of broccoli seedling Broccoli F1 Hybrid Avenger from the Sakata® company. The first eight treatments were obtained by combining three input-based technology components recognised by the OMRI. The first was the growth medium, composed of *Sphagnum* peat (Pro Moss TBK®) mixed with poultry manure compost (Vertia® brand) in proportions of 90:10 and 80:20 peat and poultry manure respectively. The second component was the biofungicide *T. harzianum* (Natucontrol® brand) at doses of 1.5 and 3 g/L water per 338-cavity polystyrene tray (34 × 66 cm, alveolus volume 13 mL). Each gram of biofungicide contains 1×10^9 CFU, an application was made to obtain 100% emergence of broccoli seedlings (2 cotyledonal leaves). The third component was the application of poultry manure tea through irrigation as a form of complementary nutrition, in doses of 0.5 and 1 dS/m per tray every two days. Poultry manure tea was obtained by mixing poultry manure and water in a 1:1 ratio. After 24 h at rest, it was diluted with water at 0.5 and 1 dS/m for application. The poultry manure tea solution at 1 dS/m was analysed and yielded values of 26.13 ppm of NO^3-N, 6.13 ppm of P, and 71.33 ppm of K. In the control treatment, conventional materials were applied: 100% *Sphagnum* peat (Pro Moss TBK®) without OMRI registration was used as the growth medium; Mancozeb fungicide was applied at 1 g/L; as well as Tricel 20 (20-20-20) applied as fertilizer in irrigation at a dose of 1 g/L. The justification of the technological components that comprised the treatments was as follows: the growing medium mixture provided the broccoli seedlings with good oxygenation and a stock of available nutrients; the biofungicide prevented damage associated with damping off (root-choking), whilst the tea was used as nutritional supplement. Descriptions of the nine treatments are presented in Table 1. The properties of the recognised organic inputs, peat and poultry manure are presented in Table 2.

The experiment was established using a randomized block experimental design with three replicates. The experimental units consisted of polystyrene trays with 338 cavities. Each experimental unit occupied one-third of the polystyrene tray; that is, 112 cavities represented one replicate. The trays were filled with the substrate mixture according to the treatments described. Once the seedlings had the first true leaves (not cotyledonal), *T. harzianum* was applied. Fertigation (tea and Tricel 20) was initiated when the seedlings presented the development of their first two true leaves. Irrigation was applied every two to three days, visually considering the substrate humidity and water status of the plants (a total of 20 irrigations were made). The volumes of nutrient solution were an average of 1 to 1.5 L per application per tray.

Growth variables

The treatments were evaluated 55 days after planting (dap). Seven seedlings from each experimental unit were taken randomly when they had three true leaves and roots with a complete root ball and were ready to be transplanted to the field. The growth variables measured were: 1) plant height, which was determined from the base of the stem to the end of the leaves; 2) leaf area, considering all true leaves, and the area was estimated using the ImageJ program (Newton *et al*. 2013) and 3) stem diameter, determined using a digital vernier, placing it between the base of the plant and the cotyledons; and 4) dry weight-the substrate was carefully removed from the root ball of each seedling to obtain only the roots. They were then washed, dried and placed in a forced air oven at 65°C until a constant weight was obtained. Afterwards, the total weight of each seedling was measured using an analytical balance, with the roots then separated from the aerial portion of the plant. The root weight was recorded and the difference between the total weight and root weight was considered as the dry weight of the aerial portion of each seedling.

Physiological variables

Two physiological variables were measured: 1) relative chlorophyll content (Soil Plant Analysis Development (SPAD) units) was determined using a chlorophyll meter (Konica Minolta model 502); and 2) photosynthesis ("fluorescence" photosynthesis) was determined using the MINI-PAM II (photosynthesis yield analyser from WALZ). Physiological variables were measured at 55 days, using the most developed leaf, placing the sensor between the veins and the leaf edge, in the central part of the leaf.

Morphological index variables

Morphological indices consider the combination of two or more morphological parameters, which were designed to

Table 1: Treatments evaluated in broccoli seedling production with recognized organic inputs

Treatment	Growth medium (v/v)		*T. harzianum*	Poultry manure tea
	Peat	Poultry manure	(g/L)	(dS/m)
1	90%	10 %	1.5	0.5
2	90%	10%	1.5	1.0
3	90%	10%	3.0	0.5
4	90%	10%	3.0	1.0
5	80%	20%	1.5	0.5
6	80%	20%	1.5	1.0
7	80%	20%	3.0	0.5
8	80%	20%	3.0	1.0
9 (Control)	100%	0%	Mancozeb (1 g/L)	Triple 20 (1 g/L)

Table 2: Physico-chemical characteristics of amendments

Amendment type	Physico-chemical characteristics	Value/content	Unit
Peat Pro Moss TBK®.	pH	3.96	
	Electrical conductivity	0.0	dS/m
	Organic matter	63.57	%
	Bulk density	0.25	g/cm^3
	Particle density	1.78	g/cm^3
	Total porosity	83.48	%
	Osmotic potential	-0.10	kPa
	Dry weight	35.47	%
Poultry manure Meyfer®	pH	7.7	
	Electrical conductivity	4.9	dS/m
	Organic matter	75.5	%
	Nitrogen	2.16	%
	Phosphorus	5.36	%
	Potassium	2.87	%
	Calcium	2.87	%
	Magnesium	1.08	%
	Sulfur	0.65	%
	Iron	1802	ppm
	Copper	40	ppm
	Manganese	514	ppm
	Zinc	299	ppm

point out an abstract attribute of a plant, for example, balance and vigour. The combination of morphological parameters, given below, is relevant as it expresses the field performance of some individual parameters more relationally:

Stem/root index (SRI), which is the ratio of the aerial part and the root part, with an optimum range of 1.5 to 2.5, in places without environmental limitations, varying according to the species. This ratio allowed for the measurement of the balance between a seedling's water absorption and transpiration area, which can guarantee greater survival because transpiration is prevented from exceeding the absorption capacity (Iverson 1984):

$$SRI = \frac{\text{stem dry weigth } (g)}{\text{root dry weigth } (g)} \quad (1)$$

Slenderness index (SI), which is the ratio between the height of the plant (in cm) and its diameter (in mm), which is an indicator of crop density. It is an important parameter in containerised plants, where plants can be tapered. High values of this index are indicative of a more robust plant that can tolerate physical damage (Schmidt-Vogt 1990):

$$SI = \frac{\text{stem diameter } (mm)}{\left(\frac{\text{stem height } (cm)}{10}\right)+2} \quad (2)$$

Leaf area ratio (LAR), the ratio of leaf area (cm²) to total dry matter (g). Low values of this index imply greater resistance to transplant shock (Masson *et al*. 1991):

$$LAC = \frac{\text{leaf area } (cm^2)}{\text{aereal dry weigth } (g)} \quad (3)$$

Specific leaf area (SLA), the ratio between leaf area (cm²) and leaf dry matter (g). Low values give rise to plants that are more resistant to transplant shock (Urrestarazu *et al.* 2016):

$$SLA = \frac{\text{leaf area } (cm^2)}{\text{leaf dry weigth } (g)} \quad (4)$$

Pre-transplant horticultural quality index (PHQI), which attempts to compile all the information related to the desired or sought-after parameters in pre-transplant seedlings dedicated to intensive horticultural production was measured. The method of evaluating whether a plant is going to resist stress better or worse is related to the dry matter content, so it is considered that high values of this index indicate seedlings with lower transplanting stress (Carrillo 2011):

$$PHQI = 10^4 * \frac{\text{shoot dry weigth } (g)}{\text{leaf area } (cm^2)} * \frac{\text{root dry weigth } (g)}{\text{total dry weigth } (g)} * \frac{\text{stem diameter } (cm)}{\text{stem height } (cm)} \quad (5)$$

Statistical analysis

The data obtained was subjected to an analysis of variance (ANOVA). In the variables where a statistical difference was shown, a comparison of means was performed using Tukey's test (p<0.05), using the SPSS statistical package, IBM Corp Released (2013), version 22.

Results

Growth variables

Table 3 shows that the organic treatments to produce broccoli seedlings favoured greater stem diameter (20–32%), aerial part dry weight (20–114%) and total dry weight (20–94%) (p<0.05). The conventional treatment was statistically equal to some organic treatments in the variables of plant height, root dry weight and leaf area (p<0.05). Overall, the organic treatments resulted in better broccoli seedling sizes. Treatments 6 and 8 demonstrated the best overall results.

Physiological variables

Fig. 1 shows significant differences between treatments in SPAD units and photosystem II quantum yield (p<0.05). Most of the organic treatments had higher SPAD units than the conventional treatments (Fig. 1A). In the case of

Table 3: Effects of treatments on growth variables in broccoli seedlings

Treatment	Stem diameter (mm)	Stem height (cm)	Shoot dry weight (mg)	Root dry weight (mg)	Total dry weight (mg)	Leaf area (cm$^{2)}$
1	1.6±0.35ab	10.0±0.65bc	134.7±5.03b	28.0±6.01	159.4±7.50bcd	5.5±0.67ab
2	1.5±0.09ab	10.6±1.20abc	209.0±57.00a	23.1±10.30	229.0±47.00a	4.7±0.65b
3	1.5±0.07ab	9.3±0.31c	121.0±7.00b	26.1±4.30	143.0±11.00cd	6.3±1.05ab
4	1.6±0.12ab	10.7±1.60abc	137.3±4.16b	33.8±2.72	167.3±6.43bcd	7.9±1.60a
5	1.5±0.04ab	10.1±0.40bc	117.5±12.55b	27.4±1.42	141.3±12.70cd	4.8±2.68 b
6	1.7±0.16a	11.3±0.84abc	150.0±28.00b	33.5±3.70	178.0±31.75abc	5.8±1.03ab
7	1.6±0.02ab	9.4±0.30bc	126.0±10.00b	27.0±2.00	143.0±7.00cd	5.7±1.50ab
8	1.7±0.02a	11.7±0.65ab	133.6±14.40b	31.3±1.70	203.0±21.0ab	7.2±0.85 ab
9	1.3±0.08b	12.7±0.55a	97.8±6.00b	25.8±1.60	118.0±8.00d	7.5±0.60ab

Means ± standard deviation. Values with different letters per column are significantly different (Tukey, p<0.05).

Table 4: Effects of treatments on morphological indices in broccoli seedlings

Treatment	SRI	SI	LAR	SLA	PHQI
1	1.7±0.39a	0.54±0.13a	34.2±3.15bc	40.5±4.65bc	0.69±0.14ab
2	3.8±2.77c	0.50±0.01ab	24.5±17.85c	28.1±22.44c	0.62±0.22ab
3	1.6±0.01a	0.51±0.01ab	43.4±4.85bc	51.3±6.32bc	0.54±0.06abc
4	1.4±0.18ab	0.53±0.02a	47.2±10.99ab	57.5±13.21ab	0.53±0.04abc
5	1.4±0.12ab	0.53±0.06a	33.6±1.82bc	40.5±1.57bc	0.81±0.16a
6	1.5±0.13ab	0.53±0.06a	32.6±5.51bc	38.7±7.02 bc	0.78±0.23a
7	1.6±0.17a	0.55±0.01a	39.7±8.49bc	45.0±8.28bc	0.74±0.14a
8	1.4±0.08a	0.53±0.02a	35.4±4.52bc	53.9±7.01ab	0.42±0.06bc
9	1.3±0.00ab	0.38±0.02b	63.5±9.45a	76.6±10.90a	0.29± 0.03c

SRI: stem/root index, SI: slenderness index, LAR: leaf area ratio; SLA: specific leaf area; PHQI: pre-trasplant horticultural quality index.
Means ± standard deviation. Values with different letters per column are significantly different (Tukey, p<0.05).

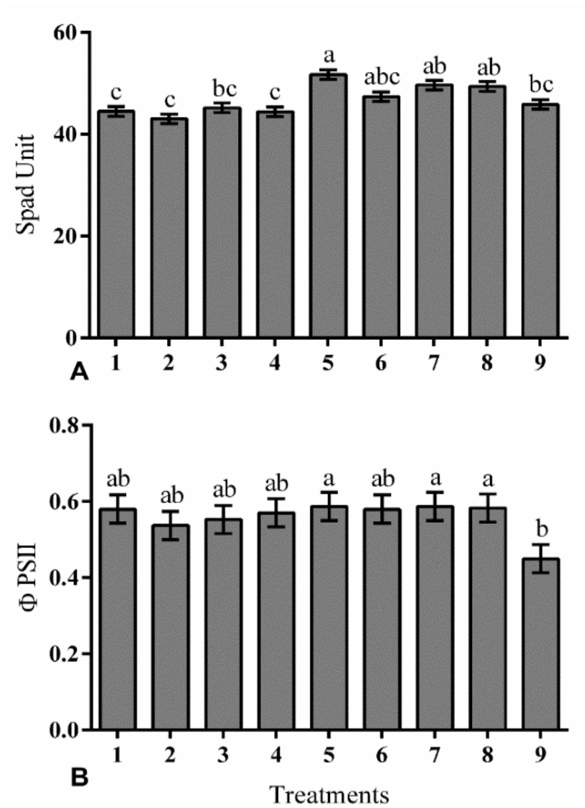

Fig. 1: A) Relative chlorophyll content (SPAD units); B) Effective quantum yield of photosystem II (PSII). The lines above the bars represent the standard error. Bars with different literals denote significant difference (Tukey, p<0.05)

photosystem II quantum yield, all organic treatments had significantly (p<0.05) higher values (19–30%) than the conventional treatment (Fig. 1B).

Morphological index variables

Table 4 shows that, with the exception of ITR, the conventional treatment was outperformed by the organic treatments. The differences are significant (p<0.05), the ranges for each variable are 32–42% in IE; 74–159% in CAF; 35–159% in IAFE; 82–179%. Among the organic treatments, the results in morphological index values are similar.

Discussion

The results obtained in growth, physiology and morphological indices showed that the treatments applied with organic production management techniques had better broccoli seedling quality than those managed with conventional techniques. Considering the performance of each study variable, the treatments with the best results were treatment 6: peat-based growing medium mixed with poultry manure (80:20) with inoculation of *T. harzianum* at a dose of 1.5 g/L and with application of poultry manure tea at a dose of 1 dS/m; and treatment 8: peat-based growing medium mixed with poultry manure (80:20) with inoculation of *T. harzianum* at a dose of 3 g/L and with applications of poultry manure tea at a dose of 1 dS/m. Both treatments comprised the same culture medium and dosed with the same amounts of poultry manure tea. The results obtained by these treatments were due to the individual and joint effects of the three technological components. The poultry manure provided nutrients to the seedlings in an available and constant manner, *T. harzianum* prevented the presence of microorganisms that cause damping off, and the tea helped supplement nutrition. Adekiya *et al.* (2020) reported that poultry manure provides essential nutrients that are used by the plant, including nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, manganese, copper, zinc, chlorine, boron, iron and molybdenum; although the percentage of nutrients can vary depending upon factors, such as age and diet of the birds, as well as humidity and age of the manure (Dróżdż *et al*. 2020). Poultry manure has high phosphorus content, which is important for seedling growth. This is due to the role that phosphorus plays in adenosine triphosphate (ATP) production. Phosphorus is also responsible for the storage and transport of energy for organic compound synthesis processes and active nutrient absorption (Muktamar *et al.* 2020). Arancon *et al.* (2012) found that poultry manure provides nitrogen (in the form of nitrates and ammonium), which has been related, in addition to chlorophyll, to nitrogen levels in the leaves of various horticultural crops (Zhu *et al*. 2012). Lizardo and Gómez (2015) reported that the highest growth results for paprika (*Capsicum annuum* L.) seedling production were obtained in the treatments of a mixture of litter (75%), poultry manure (25%) and *T. harzianum,* and the soil treatment (50%), litter (25%), poultry manure (25%) and *T. harzianum*.

It is evident that the nutrients provided by the poultry manure improved nitrogen and chlorophyll levels in broccoli seedlings than those that did not receive it, as verified from the results of the SPAD units. The seedlings under organic treatment also presented less stress than those that received the conventional treatment, possibly due to availability of nutrients and water retention in the growth medium containing the organic fertiliser. This was evidenced by the photosystem II quantum yield variable. This variable measures the ability of the plant at the photosystem level to transport electrons beyond photosystem II. This signifies the photosystem's level of performance in converting light energy into chemical energy. A decrease in this value is attributed to higher environmental stress, as the plant begins to fail to convert energy (Sasi *et al*. 2018).

Barbaro *et al*. (2013) evaluated ten treatments of substrates formulated with different proportions (20, 50 and 80%) of three types of poultry manure mixed with pine bark compost and a commercial substrate for growing *Impatiens walleriana* and *Salvia splendens*. These authors reported that substrates with 20 and 50% of the three types of poultry manure demonstrated the highest water retention capacity; whilst 80% mixture demonstrated the highest air porosity. Araméndiz-Tatis *et al*. (2013) obtained the best results in the production of eggplant seedlings with substrates consisting of a mixture of 20% vermicompost or poultry manure, 40% sand and 40% alluvium. These results were based on a greater nutritional contribution and good aeration, which favoured root length since seedlings with longer roots take better advantage of the physical, chemical, and nutritional conditions provided by the volume of the substrate. They also observed a clear relationship between root length, seedling height, stem diameter, fresh and dry weight of the aerial portion of the plant, as well as the fresh and dry weight of roots. Carballo *et al*. (2017) reported that in the production of zucchini and cucumber seedlings (produced with substrate mixtures in combination with peat moss-poultry manure 84–16% and 88–12%, respectively, seedlings showed an increase in leaf area and biomass production. Similarly, poultry manure can contribute to diverse beneficial microorganisms that help plant nutrition and health. Sarmiento and Velandía (2013) found biocontrol microorganisms corresponding to the genera *Trichoderma*, *Penicillium* and *Bacillus*.

On the other hand, the results of the organic treatments showed that poultry manure tea applied at a dose of 1 g/L was better than 0.5 g/L dose. This practice is an alternative to the supply of minerals in an easily absorbable form. Jandaghi *et al*. (2020) noted that the variables shoot length, stem diameter, true leaf length and width, shoot fresh and dry weights, and chlorophyll content, days until flowering and total fruit weight of cucumber were increased by the application of poultry manure tea. Arancon *et al*. (2012) reported that seed germination and seedling growth of tomato and lettuce with 20% vermicompost and 20% poultry manure teas showed better growth. Outstanding results in favour of the organic fertilization treatments can be attributed to the presence of plant hormones in addition to the nutrients provided. Among the hormones reported in poultry manure are indole acetic acid (IAA), cytokinins and three types of gibberellins.

T. harzianum has beneficial effects on plants. Patkowska *et al*. (2020) reported an improved the growth, development and health of this vegetable plant. Silletti *et al*. (2021) mentioned that inoculation with *Trichoderma* improves the solubility and absorption of nutrients in the soil, favours the plants growth and development. Guzmán-Guzmán *et al*. (2019) mentioned that plants grown in soil amended and inoculated with *Trichoderma* sp. had a marked increase in the number of leaves, leaf area, chlorophyll, as well as in the concentrations of Ca^{2+}, Mg^{2+} and K^+ ions.

Pascale *et al*. (2020) noted that *Trichoderma* sp. influence the nutritional status and the promotion of plant growth and development, increase soil exploration beyond the nutrient and water depletion zone, solubilize organic compounds and produce secondary metabolites, which act analogously to phytohormones. Conversely, the establishment, adaptation, and possible synergistic interaction between the antagonists in the rhizospheric zone protect the plant against edaphic pathogens attributed to the antagonists, as a direct mode of action, in the competition for space and nutrients. Indirectly, the production of antimicrobial compounds is stimulated, which regulate or truncate the advance of the pathogens towards the root system. In addition, it also stimulates the defence system of plants, conferring a certain type of tolerance to the attack of pests and aerial pathogens. Labrador *et al*. (2014) reported that biopreparation based on *T. harzianum* was effective in the regulation of *Pieris brassicae* in broccoli and resulted in an increase of the number healthy plants and the estimated yields of the crop. Manganiello *et al*. (2018) examined the effects of *T. harzianum* on tomato plants infected with *Rhizoctonia solani* where an increase in the expression of genes related to plant protection was observed. Another possible benefit of using *T. harzianum* in seedling inoculation is stress support, as demonstrated in this study by measuring the quantum yield of photosystem II. The positive effects of the combined *T. harzianum* and compost are alternates to increased plant nutrition and growth.

Conclusion

Broccoli seedling production using organic inputs performed better than the conventional treatment, as evidenced by growth, physiology and morphological indices. The most beneficial treatment for broccoli seedling production with organic inputs was with a *Sphagnum* peat-based growing medium mixed with poultry manure (80:20) with inoculation of *T. harzianum* at a dose of 1.5–3 g/L and with application of poultry manure tea at a dose of 1 dS/m. Thus, it is possible to obtain high-quality broccoli seedlings with the inputs recognised for certified organic agriculture.

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Author Contributions

JCR and FJC designed experiment and prepared the manuscript. MCH, PP, HR and CJL collected and analyzing the data. All authors reviewed and approved the final draft of manuscript.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable in this paper.

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