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Supplementation of Manure Compost with *Trichoderma asperellum* Improves the Nutrient Uptake and Yield of Edible Amaranth under Field Conditions

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Abstract: Manure composts can improve soil health and crop production, but their application with *Trichoderma* species has not been well evaluated in amaranth cultivation. This study aimed to determine the effects of manure compost (MC) and MC supplemented with *Trichoderma asperellum* CHF 78 (MC+CHF 78) on the yield and nutrient uptake of amaranth, as well as on soil properties, under field conditions. Four fertilization treatments, including a control without fertilization, chemical fertilization (CF), MC, and MC+CHF 78, were arranged in a randomized complete block design with six replications in the experimental field. MC and MC+CHF 78 significantly increased the yield of amaranth by 96.2–102% in comparison with CF. In addition, MC and MC+CHF 78 significantly increased the soil pH, soil organic matter, soil available P and exchangeable K, and soil microbial activity compared with those in the control and CF treatments. However, only amaranth plants applied with MC+CHF 78 showed a significantly greater P uptake than those with the control and CF treatments, which may be attributed to the phosphate-solubilizing ability of *T. asperellum* CHF 78. In conclusion, manure compost fortified with *T. asperellum* CHF 78 can be used as an alternative to chemical fertilizers for amaranth cultivation.

Keywords: soil fertility; organic farming; chemical fertilizer; soil microbial activity

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1. Introduction

The livestock and poultry industries produce meat, milk, and eggs but also generate large amounts of liquid and solid wastes that may be harmful to human health and the environment [1,2]. Due to the increase in livestock wastes and the impacts on environmental factors caused by improper management, it is vital to find economical alternatives for managing these wastes. In fact, animal manures are valuable sources of nutrients and organic matter for use in the maintenance or improvement of soil fertility and crop production [3]. Composting manures with or without other agricultural wastes provides a low-cost disposal approach, but also represents a way to recycle nutrients [1]. Although inorganic fertilizers provide nutrients that are immediately available to plants to rapidly improve plant growth and crop yield, these fertilizers cannot replace the soil organic matter (SOM) that may be lost during cultivation [4]. Instead, compost application can increase the content of SOM, which improves soil quality and crop production [3]. For organic farming, composted manures have been used as an important alternative to chemical fertilizers because chemical fertilizers are prohibited in organic farming. Given these aforementioned advantages, manure compost has been recognized as an economical alternative for livestock waste management.

Composts inoculated with beneficial microorganisms have been proven to increase crop production and reduce plant diseases [5–7]. Of these beneficial microorganisms,

Trichoderma has been inoculated into compost to effectively reduce the disease severity of several plant diseases [7–9]. The higher degree of disease suppressiveness of composts supplemented with *Trichoderma* may be due to not only its biocontrol ability but also to the changes it induces in both abiotic and biotic soil characteristics [9]. In addition, *Trichoderma* can induce plant resistance to abiotic stresses by enhancing water and nutrient uptakes [10]. It has been suggested that the inoculation of crops with *Trichoderma* species enhances nutrient uptake by plants [11–13]. However, under specific soil nutrient deficiencies, such as P and micronutrients (Fe, Cu, and Zn), *Trichoderma* species may compete for nutrients with plants by suppressing root development [14]. Therefore, the induction of increased or suppressed plant growth by *Trichoderma* species is related to the availability of soil nutrients for these fungi. Accordingly, *Trichoderma*-enriched organic fertilizers have been used to maintain a stable crop yield with reduced use of chemical fertilizers, due to their effect on improving soil microbiota and soil nutrient availability [15,16]. Our previous study suggests that *T. asperellum* can reduce the severity of Fusarium wilt of tomato and promote the nutrient uptake and growth of tomato plants [12]. However, it is not well known whether the application of manure compost fortified with *T. asperellum* can enhance the yield and nutrient uptake of amaranth.

Amaranth (*Amaranthus tricolor* L.), an annual herb in the family Amaranthaceae, is an important vegetable produced in subtropical countries. Its antioxidant capacity has been ranked in the top five for vegetables since amaranth contains abundant bioactive compounds such as *L*-ascorbic acid, beta-carotene, polyphenol, anthocyanins, and lutein [17]. In addition, amaranth is a popular vegetable due to its high nutrient value for human health coupled with its high resistance to various pests and ability to be planted year-round in subtropical countries [17]. However, intensive amaranth cultivation practices need to use large amounts of inorganic fertilizers, partly resulting in over-fertilization, soil degradation, and a decrease in SOM [3]. Therefore, it is necessary to provide alternatives to improve the sustainability of such vegetable cultivation systems. Among these alternatives, composts may provide nutrients and increase crop yield by improving the soil's physical, chemical, and biological properties. Moreover, composts are important sources of nutrients for growing organic amaranth due to the prohibition of manufactured chemical fertilizers. To increase the yield of organic amaranth, however, experimental trials are needed to provide compost recommendations focused on how to supply nutrients in reasonable amounts to achieve high yields without the use of chemical fertilizers [18].

The organic matter (OM) contained in composts needs to be mineralized by soil organisms to release nutrients into soils. This mineralization process depends on the characteristics of the composts, soil properties, and environmental conditions such as temperature and soil water content [4,19]. The mineralization rates of some composts may be slow, consequently reducing the risk of nutrient leaching [4]. However, the mineralization rates of the composts should be considered to successfully manage soil fertility to match crop needs [18]. Compost applications that do not consider mineralization rates may fail to supply sufficient nutrients for crop growth, especially under organic farming practices. On the other hand, the amount of compost required to provide the same nutrients that inorganic fertilizers can supply to maintain crop yield may increase the risk of contamination by heavy metal accumulation and nutrient leaching. Therefore, it is vital to determine the appropriate amount of compost to add to soils for a given crop to maintain soil functioning and fertility and to minimize the risk of contamination.

This study aimed to investigate the possibility of using manure composts as an alternative to chemical fertilizers for amaranth cultivation under field conditions and to determine the effects of manure composts with or without *T. asperellum* inoculation on the yield and nutrient uptake of amaranth as well as on soil properties. Our results suggested that manure compost supplemented with *T. asperellum* significantly increased the yield of amaranth by 102% and the uptake of P by 178% in comparison with the chemical fertilization treatment. The results of this study may be of importance in the better utilization of manure composts to recycle nutrients and produce edible amaranth.

2. Materials and Methods

2.1. Manure Compost Production

2.1.1. Preparation of Manure Compost Supplemented with *Trichoderma asperellum* CHF 78

The manure compost used in this experiment was made from cow, chicken, and pig manures mixed with spent mushroom waste (1:3:1:5, *w*/*w*), followed by a composting period of 52 days in a commercial composting facility. These materials were mixed in each pile (approximately 2 tons) to obtain an initial carbon to nitrogen (C/N) ratio of 30.5. In addition, the initial moisture content of the composting piles was adjusted to 60%. Due to the rapid increase in temperature during the first week of composting, the piles were turned every 3 days. After the first week, the piles were turned at weekly intervals. For the manure compost inoculated with *T. asperellum*, a composting pile was mixed with the *T. asperellum* strain CHF 78 [12] inoculum at a ratio of 0.2% (*w*/*w*) at 0 and 45 days of composting. To produce the *T. asperellum* inoculum, rice hull and bran were mixed (1:1, *w*/*w*) and inoculated with the fungus (10^7 spores/mL) at a concentration of 10^5 spores/g, and the water content was adjusted to 45% (*w*/*w*). Three weeks after incubation, the *T. asperellum* inoculum (10^7 spores/g) was used to inoculate the compost pile. Six compost samples of 1.5 L each were collected from each compost pile supplemented with (MC+CHF 78) or without *T. asperellum* (MC) at 52 days of composting and air-dried. Finally, these samples were ground to pass through a 2 mm sieve for the determination of their chemical properties (Table 1) as previously described [3]. Moreover, the fresh compost samples of 1.5 L each were collected from the MC and MC+CHF 78 compost piles at 52 days of composting to quantify the DNA of *T. asperellum* CHF 78 as described below.

Table 1. Chemical properties of manure compost inoculated with *Trichoderma asperellum* CHF 78 and uninoculated manure compost.

	pH [a]	EC [b] (dS/m)	OM [c]	N	P	K	Ca	Mg	Cd	Cr	Cu	Ni	Pb	Zn
Compost						g/kg						mg/kg		
Manure compost	7.81 b [d]	2.08 a	817 a	19.5 a	9.26 a	10.7 a	20.0 a	4.85 a	0.86 a	14.3 a	64.5 a	10.9 a	10.6 a	281 a
Manure compost + *T. asperellum*	7.97 a	1.98 b	837 a	17.6 b	8.82 a	10.5 a	18.6 a	4.55 a	0.75 a	17.6 a	64.2 a	11.0 a	6.99 a	249 b

[a] pH was determined in 1:5 (*w*/*v*) compost: H_2O extracts. [b] Electrical conductivity (EC) was measured in 1:5 (*w*/*v*) compost: H_2O extracts. [c] OM = organic matter. [d] Numbers followed by different letters within a column are significantly different as denoted by the LSMEANS statement of the GLIMMIX procedure in SAS v9.4 at the 5% level of significance using Fisher's least significant difference (Fisher's LSD).

2.1.2. Real-Time PCR Assay for Quantification of *T. asperellum* CHF 78

To detect and quantify *T. asperellum* CHF 78 in the manure compost, a TaqMan real-time PCR assay was developed in this study. The translation elongation factor-1α gene (TEF1α) sequence of *T. asperellum* CHF 78 (GenBank accession number KX377622) was used to design primers and TaqMan probes using Beacon Designer version 8.0 (Premier Biosoft, Palo Alto, CA, USA). The DNA concentration of CHF 78 was determined using the primers TEF_T3_F (CCAAGTACTATGTCACCG) and TEF_T3_R (GGACTTGGAATGTCGATA) and the probe TEF_T3_P (ATTGGTATGTTTTGGACTCTTCTCTCT) dual-labeled with 6-carboxyfluorescein (6-FAM) fluorescent reporter dye and Iowa Black fluorescence quencher. Compost DNA was extracted using a FastDNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA). The real-time PCR assay was performed in a Bio-Rad CFX96 Real-Time PCR system (Bio-Rad, Hercules, CA, USA). A total volume of 20 µL of the reaction mixture included the following components (final concentration): 1 × SsoAdvanced Probes Supermix (Bio-Rad), 0.9 µM forward and reverse primers, 0.2 µM TaqMan probe, 2 µL compost DNA template, and PCR-grade water to make up the final volume. Three simultaneous amplifications were performed for each sample to confirm the reproducibility of the results. A negative control sample consisted of nuclease-free water substituted for the DNA template. The PCR conditions included an initial hot start of 3 min at 95 °C followed by 40 cycles of 95 °C for 15 s and 60 °C for 30 s. Fluorescence emissions were measured at

60 °C during the annealing and extension phase. Threshold cycles were calculated using Bio-Rad CFX Manager 3.0 software. A standard curve was developed by plotting the logarithm of the target DNA concentrations (10-fold dilution series) of *T. asperellum* CHF 78 against the threshold cycle (Cq) values. Cq is defined as the cycle number at which fluorescence increased above the background fluorescence associated with the sample substrate. The Cq value is inversely related to the log of the initial DNA concentration, i.e., the lower the Cq value, the greater the initial DNA quantity.

2.2. Field Experiment

2.2.1. Location and Experimental Design

Two field trials were carried out in two years on a commercial vegetable farm in Taichung, Taiwan (24.08° N, 120.66° E). The annual average temperature ranged from 23.8 to 24.3 °C, and the annual precipitation varied from 1466 to 1526 mm. The soil properties of the experimental site (0–15 cm) are summarized in Table 2. Each plot consisted of a 5 m-long and 1 m-wide bed section with a 1 m buffer zone between adjacent plots. Four treatments, including a control without fertilization, chemical fertilization (CF), MC, and MC+CHF 78, were arranged in a randomized complete block design with six replications. The control was included in the trial because the field soil had high nutrient levels (Table 2). For the CF treatment, each plot was supplied with 14.3 g urea (6 g N), 5.83 g single superphosphate (1.05 g P_2O_5), and 23.8 g potassium chloride (14.3 g K_2O) based on a local chemical fertilization program. For MC and MC+CHF 78 derived from 52 days of composting, the application rates were calculated according to their N and water contents and a mineralization rate of 10% during the growing season to provide the equivalent amount of N that CF can supply to maintain crop yield. Accordingly, the application rates of MC and MC+CHF 78 for each plot were 4.02 and 4.34 kg (on a dry weight basis), respectively. The composts and chemical fertilizers were mixed with the soil by raking to a depth of 15 cm. Ten days after applying these fertilizers, two-week-old amaranth seedlings (Known-You Co., Ltd., Kaohsiung City, Taiwan) were transplanted at 15 cm spacing along the beds in double rows. Each plot contained 60 amaranth plants. The same design and layout of our field trial were performed in the second year.

Table 2. Physicochemical properties of the field soil.

Soil Texture	pH [a]	EC [b] (dS/m)	SOM [c] (g/kg)	Avail. N (mg/kg)	Bray-P (mg/kg)	Exch. K (mg/kg)	Exch. Ca (mg/kg)	Exch. Mg (mg/kg)
Sandy loam	5.75 ± 0.08	0.44 ± 0.03	20.4 ± 1.40	45.1 ± 0.20	96.3 ± 2.39	376 ± 6.11	1122 ± 15.4	185 ± 2.70

[a] pH was determined in 1:1 (*w*/*v*) soil: H_2O extracts. [b] Electrical conductivity (EC) was measured in 1:1 (*w*/*v*) soil: H_2O extracts. [c] SOM = soil organic matter.

The amaranth plants were harvested one month after transplanting. The whole plants in each plot were gently removed and then rinsed with tap water. The yield (on a fresh weight basis) of each plot was recorded. Five plants were randomly collected from each plot and rinsed with deionized water before being oven-dried at 70 °C for 72 h. Accordingly, the dry weight yield was recorded. Dry tissues were used for the following analyses of plant properties.

2.2.2. Soil Property Analysis

Five random soil samples at a depth of 0–15 cm were collected from each plot to make a composite sample, and the composite samples were air-dried and sieved (2 mm). The pH and electrical conductivity (EC) of the rhizosphere soils were determined in 1:1 (*w*/*v*) soil: H_2O extracts [20]. SOM was determined by the Walkley-Black method [21]. The soil available N was extracted using 2.0 M KCl and determined by using the steam distillation method [22]. The available P in soils was extracted by using the Bray P-1 test and determined by using the molybdenum blue method [23]. The exchangeable K in soils was extracted with neutral 1 M NH_4OAc and analyzed with an inductively coupled

plasma-mass spectrometer (ICP-AES; Perkin Elmer, Waltham, MA, USA). The soil microbial activity was determined by measuring the rate of fluorescein diacetate (FDA) hydrolysis as previously described [24]. Since FDA can be hydrolyzed by nonspecific esterases in the cell membrane of soil microbes and their extracellular enzymes, FDA hydrolysis has been suggested as a broad-spectrum indicator of soil biological activity [25].

2.2.3. Plant Nutrient Analysis

The dry plant tissues were ground using a sample mill and stored in 20-mL plastic scintillation vials. The digestion of the plant tissues was performed using sulfuric acid in the presence of hydrogen peroxide [26]. Briefly, 0.2 g of dry tissues were digested with 12 N H_2SO_4 and 30% H_2O_2 at a temperature of 275–300 °C in digestion blocks. The digest was finally diluted with distilled water. The total N concentration was analyzed by using the steam distillation method [22]. In addition, P and K were analyzed by using ICP-AES.

2.3. Statistical Analyses

The amplification efficiency (E) of the real-time PCR assay was calculated according to the equation $E = [10^{(-1/slope)}] - 1$ [27]. The data were analyzed using generalized linear models employed in PROC GLIMMIX of SAS (version 9.4; SAS Institute, Cary, NC, USA) to determine the effects of the treatments. The effect of repeated field trials was considered random [12]. The least squared means (LSMEANS) statement in the GLIMMIX procedure in SAS was used to compare treatment means at the 5% level of significance according to Fisher's least significant difference (Fisher's LSD). Pearson's correlation analysis was carried out to determine the relationships between amaranth yield and soil properties at harvest using SAS.

3. Results

3.1. Chemical Properties of Manure Composts

No significant difference was detected between the MC and MC+CHF 78 treatments in most chemical properties except pH, EC, N, and Zn (Table 1). The pH value of MC+CHF 78 was significantly higher than that of MC. In contrast, EC, N, and Zn in MC were significantly higher than those in MC+CHF 78. Inoculation of the manure compost with *T. asperellum* significantly reduced EC, N, and Zn by 5.05, 10.8, and 12.9%, respectively. There was no significant difference in the concentrations of the five heavy metals, including Cd, Cr, Cu, Ni, and Pb, between the MC and MC+CHF 78 treatments. In addition, the concentrations of the six heavy metals (Cd, Cr, Cu, Ni, Pb, and Zn) of the MC and MC+CHF 78 treatments were under the limits established by the Taiwan Fertilizer Regulations, USEPA, and WHO [28].

3.2. Quantification of T. asperellum CHF 78 in Compost

The standard curve using the primer set and TaqMan probe developed in this study showed a linear correlation between the Cq values and DNA quantities of *T. asperellum* CHF 78, with a correlation coefficient (R^2) of 0.999 at $p < 0.0001$ and an amplification efficiency of 96.5%, described by the equation $y = 35.6 - 3.41 \log(x)$ (Figure 1). Therefore, the detection limit of the real-time PCR assay was calculated at a *T. asperellum* CHF 78 DNA quantity of 1.50 pg in 20 µL of the real-time PCR or at a Cq value of 35 cycles. Although MC did not contain the DNA of *T. asperellum* CHF 78 analyzed by the real-time PCR assay, MC+CHF had the fungal DNA at a concentration of 14.4 ng/g compost at 52 days of composting.

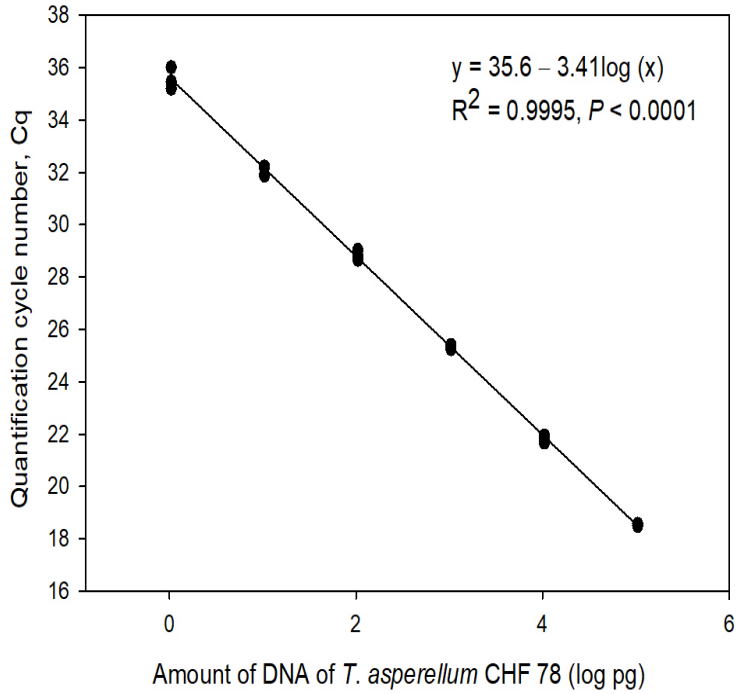

Figure 1. Real-time polymerase chain reaction (PCR) assay standard curve of *Trichoderma asperellum* CHF 78 DNA extracted from 7-day-old mycelia grown on PDA. Various amounts of DNA, ranging from 1.05 pg to 105 ng, were used to develop the standard curve. Each point represents three replicates.

3.3. Effects of Compost on Amaranth Production

The MC and MC+CHF 78 treatments significantly increased the yield of amaranth in comparison with both the control and CF, whereas there was no significant difference in yield between MC and MC+CHF 78 (Figure 2A). Compared to CF, MC and MC+CHF 78 remarkably enhanced the amaranth yield (fresh weight) by 96.2 and 102%, respectively. In comparison with CF, MC and MC+CHF 78 significantly increased the dry weight of amaranth by 75.7 and 101%, respectively (Figure 2B). Although no significant difference was detected in dry weight between the control and MC, MC+CHF 78 showed a significant increase in dry weight by 61.4%, compared to the control.

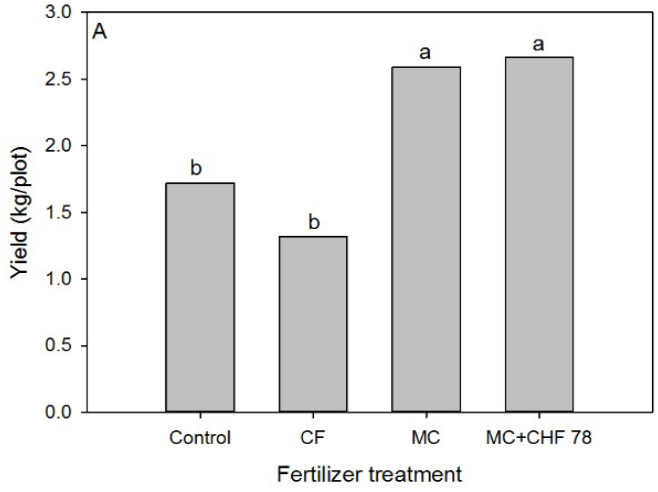

Figure 2. *Cont.*

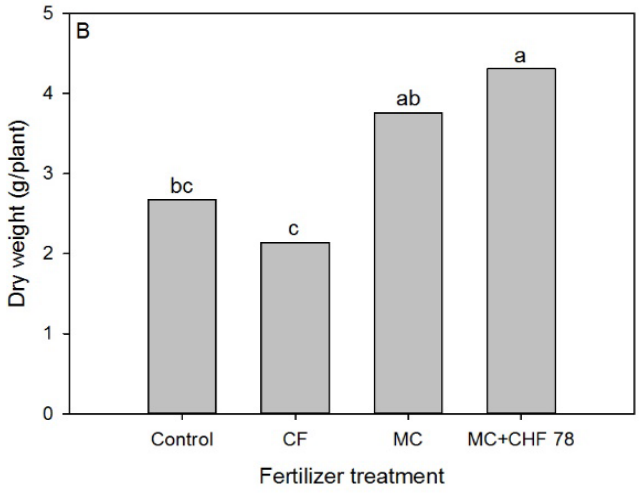

Figure 2. Effects of manure composts supplemented with *Trichoderma asperellum* CHF 78 on the yield (fresh weight) (**A**) and dry weight (**B**) of amaranth. Control = no fertilization; CF = chemical fertilizer; MC = manure compost; MC+CHF 78 = manure compost supplemented with *Trichoderma asperellum* CHF 78. Bars with different letters are significantly different at a 5% level of significance according to Fisher's least significant difference (Fisher's LSD).

3.4. Effects of Compost on Soil Properties

Significant differences were found in the pH, EC, SOM, available N, Bray-P, and exchangeable K among the treatments (Table 3). In comparison with the other treatments, CF significantly reduced the soil pH. At harvest, the MC and MC+CHF 78 treatments slightly increased the soil pH levels to 5.78–5.93, but the soil pH in the CF treatment decreased to 5.58. No significant difference was detected in EC among the CF, MC, and MC+CHF 78 treatments. However, the EC value of the control was significantly lower than that of the soil supplied only with MC+CHF 78. The control and CF treatments showed a significantly lower content of SOM than the MC and MC+CHF 78 treatments.

Table 3. Effects of manure composts supplemented with *Trichoderma asperellum* CHF 78 on soil properties at harvest.

Treatment	pH [a]	EC [b] (dS/m)	SOM [c] (g/kg)	Avail. N (mg/kg)	Bray-P (mg/kg)	Exch. K (mg/kg)	Microbial Activity [d]
Control	5.79 a [e]	0.35 b	18.3 b	21.8 a	104 b	190 b	42.7 b
Chemical fertilizers	5.58 b	0.45 ab	16.9 b	22.3 a	97.4 b	194 b	38.3 b
Manure compost	5.78 a	0.42 ab	23.0 a	23.5 a	134 a	245 a	56.5 a
Manure compost + *Trichoderma asperellum*	5.93 a	0.52 a	25.1 a	20.3 a	154 a	267 a	60.8 a

[a] pH was determined in 1:1 (w/v) soil: H_2O extracts. [b] Electrical conductivity (EC) was measured in 1:1 (w/v) soil: H_2O extracts. [c] SOM = soil organic matter. [d] Microbial activity represents μg fluorescein diacetate hydrolyzed at 37 °C h^{-1} g^{-1} dry weight of soils. [e] Numbers followed by different letters within a column are significantly different as denoted by the LSMEANS statement of the GLIMMIX procedure in SAS v9.4 at the 5% level of significance using Fisher's least significant difference (Fisher's LSD).

Although no significant difference was observed in soil available N among the treatments, soil available P and exchangeable K were significantly enhanced by the manure composts. Compared to the CF treatment, the MC and MC+CHF 78 treatments significantly increased the soil available P and exchangeable K by 37.6–58.1% and 26.3–37.6%, respectively. The microbial activity was significantly variable among the treatments at harvest. The MC and MC+CHF 78 treatments significantly increased soil microbial activity compared to the other treatments, whereas no significant difference was found between the MC and MC+CHF 78 treatments. The MC and MC+CHF 78 treatments significantly enhanced soil microbial activity, by 47.5–58.7% in comparison with CF. The control also showed a

significantly lower level of soil microbial activity compared to the MC and MC+CHF 78 treatments. Interestingly, there were significant positive correlations between amaranth yield and soil pH, SOM, exchangeable K, and microbial activity (Table 4).

Table 4. Correlation between amaranth yield and soil properties at harvest as influenced by fertilizer treatments.

	pH [a]	EC [b] (dS/m)	SOM [c] (%)	Avail. N (mg/kg)	Bray-P (mg/kg)	Exch. K (mg/kg)	Microbial Activity [d]
r	0.75	−0.14	0.59	−0.38	0.27	0.60	0.55
p value	0.0002	0.5556	0.021	0.1001	0.2511	0.0052	0.0139

[a] pH was determined in 1:1 (*w*/*v*) soil: H_2O extracts. [b] Electrical conductivity (EC) was measured in 1:1 (*w*/*v*) soil: H_2O extracts. [c] SOM = soil organic matter. [d] Microbial activity represents μg fluorescein diacetate hydrolyzed at 37 °C h^{-1} g^{-1} dry weight of soils.

3.5. Amaranth Nutrient Uptake

Significant differences were found in N, P, and K uptake by amaranth among the treatments (Figure 3). In general, the MC and MC+CHF 78 treatments significantly increased N, P, and K uptake by the amaranth plants. Although no significant difference was detected in N uptake by amaranth plants between the MC and MC+CHF 78 treatments, the two treatments significantly increased N uptake by 116–121% in comparison with CF (Figure 3A). The same trend was observed for P and K uptake. The MC and MC+CHF 78 treatments significantly increased P and K uptake by 98.4–178% (Figure 3B) and 131–155% (Figure 3C), respectively, compared to CF. However, there was no significant difference in P uptake between the control and MC (Figure 3B). Instead, MC+CHF 78 showed a significantly greater P uptake than the control.

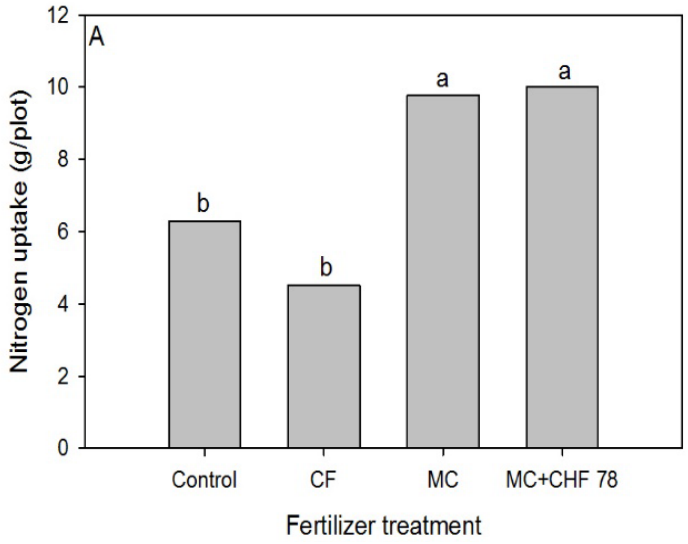

Figure 3. *Cont.*

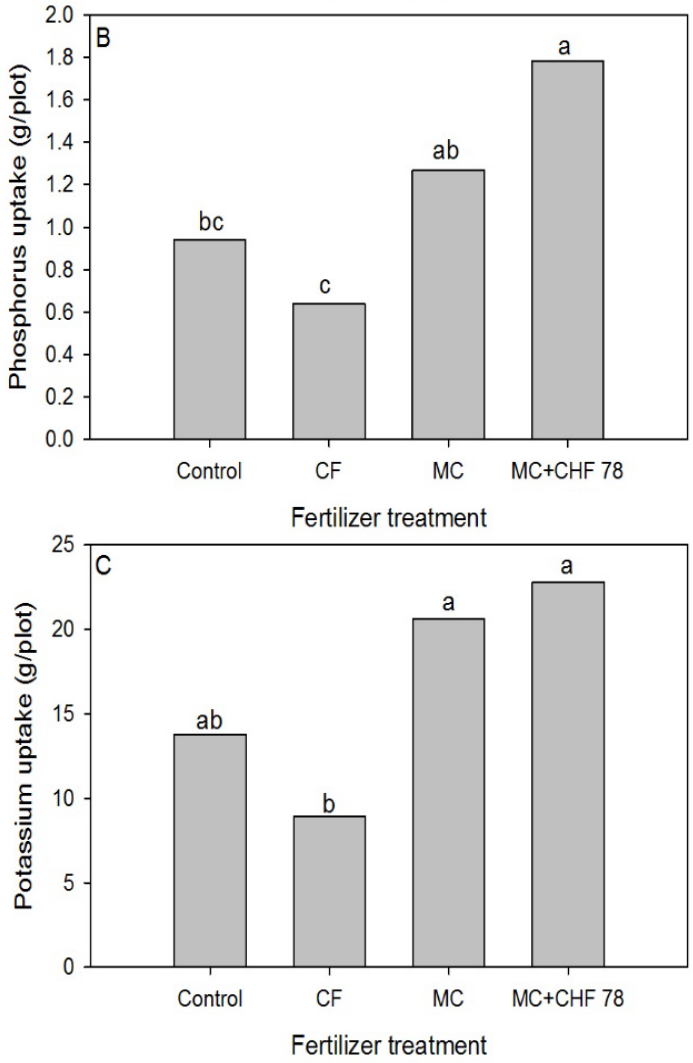

Figure 3. Effects of manure composts supplemented with *Trichoderma asperellum* CHF 78 on the uptake of N (**A**), P (**B**), and K (**C**) by amaranth. Control = no fertilization; CF = chemical fertilizer; MC = manure compost; MC+CHF 78 = manure compost supplemented with *Trichoderma asperellum* CHF 78. Bars with different letters are significantly different at a 5% level of significance according to Fisher's least significant difference (Fisher's LSD).

4. Discussion

This study demonstrates that the MC and MC+CHF 78 treatments can be used as an alternative to chemical fertilizers for amaranth cultivation under field conditions because they significantly improve soil quality as a result of increasing soil pH, SOM, Bray-P, exchangeable K, and microbial activity compared to CF. Consequently, these changes in soil properties enhance the yield and nutrient uptake of amaranth. Although the MC and MC+CHF 78 treatments significantly enhanced N and K uptake by amaranth plants compared with those under CF, only amaranth plants treated with MC+CHF 78 resulted in a significantly greater P uptake than those with the control and CF. Therefore, the application of manure compost supplemented with *T. asperellum* can enhance the yield and nutrient uptake of amaranth. The results of this study may be useful when applying manure composts as a complete or partial substitute for mineral fertilizers in amaranth cultivation.

To be applied as a replacement for inorganic fertilizers, composts should supply a rate of available N similar to that provided by inorganic fertilization, but the most important condition is to meet the N requirement of a given crop. Although it is difficult to calculate

the amount of compost to be used as a total substitute for inorganic fertilizers, the most rational way to establish comparisons between these two kinds of fertilizers may be based on their similar N availability [4]. However, the mineralization rate of organic N depends on the properties of the composts applied and other environmental characteristics, such as soil water content, temperature, and soil type [29]. Reliable mineralization rate estimates for various manure composts are lacking; the total N mineralization of cattle manure compost is approximately 15% over 33 weeks [30]. In our study, the application rate of the MC and MC+CHF 78 treatments was calculated assuming a mineralization rate of 10% due to the warmer weather during the growth period of amaranth in the summer. This assumption was proven to be appropriate because the soils applied with MC and MC+CHF 78 showed significantly higher yields than the soil treated with CF in this study. This finding suggests that manure composts can be used in organic amaranth cultivation as a complete substitute for mineral fertilizers if they are used at an adequate rate.

MC and MC+CHF 78 used in this study significantly improved soil properties, suggesting that they can be included in the integrated soil fertility management for amaranth cultivation. In this study, the original field soil was acidic, pH 5.75, which is close to the lower limit of pH 5.50 for most vegetable growth, including amaranth [31]. At harvest, the MC and MC+CHF 78 treatments slightly increased the soil pH levels to 5.78–5.93, probably due to their alkalinity and high levels of basic cations. In contrast, the soil pH in the CF treatment decreased to 5.58. The increased salt concentration in soils that accompanies the application of manure composts is a major environmental concern [3]. However, our study suggested that manure compost application had no significant effect on soil EC. This finding may have resulted from the considerable uptake of nutrients by amaranth plants in plots treated with either MC or MC+CHF 78, as well as the possibility of leaching during rainfall and irrigation. Although no significant difference was observed in soil available N among treatments, soil available P and exchangeable K were significantly enhanced by supplying either MC or MC+CHF 78. This could be attributed to the higher levels of P and K that accompanied the application of manure composts, as the application rate of these composts was calculated based on the N requirement estimated by the N content and assumed mineralization rate during the amaranth growing period. In other words, the amounts of P and K added through the compost were higher than those from the conventional inorganic fertilizers. Although manure compost can improve soil fertility and amaranth yield, the upper limit for compost application should be further determined for each soil to avoid the excess accumulation of P and K.

The manure composts with high levels of organic matter used in this study could increase SOM, improving soil quality [31]. The control and CF showed a significantly lower content of SOM than MC and MC+CHF 78. SOM has long been considered the main factor influencing the physical, chemical, and biological properties of soils [32]. Previous studies have suggested that increasing SOM enhances soil microbial activity, as determined by fluorescein diacetate hydrolysis [32–34]. Our results showed that the application of MC and MC+CHF 78 significantly increased SOM and soil microbial activity, in agreement with these previous studies. In contrast, the chemical fertilization treatment did not increase SOM, consequently reducing FDA hydrolysis activity. Therefore, the considerable increase in SOM and soil microbial activity probably resulted in promoting amaranth yield. This is not surprising, as it has been suggested that crop yield is closely related to the increases in SOM and other biological properties that accompany the application of compost [33,35].

Although SOM management through the use of manure composts can influence crop yield, the residual effects of manure or compost application can sustain crop production for several years through the continual release of nutrients from OM after manure or compost application ends [36]. The residual effects occur because only a fraction of the N and other nutrients mineralized from OM in the first year after manure or compost application become available to plants [37,38]. Since the residual effects of manure or compost application can influence soil properties for several years after application ceases, soil fertility management should be adjusted according to soil analysis results. In addition,

fertilization adjustment based on residual effects after manure or compost application will help to prevent nutrient pollution.

Most *Trichoderma* species are unable to grow at temperatures of 37 °C or greater [39]. Therefore, the thermophilic period, during which temperatures were greater than 55 °C up to 28 days for our composting study at the commercial facility, likely destroyed the *T*. *asperellum* introduced at the beginning of composting. However, our results showed that the second inoculation with *T*. *asperellum* for MC+CHF 78 at 45 days of composting resulted in an evident increase in the fungal DNA (14.4 ng/g compost) at 52 days of composting at the time of the application of the composts to the field plots. Conversely, MC did not show any signal from the fungal DNA analyzed by the real-time PCR assay, suggesting its specificity and reliability. Therefore, the real-time PCR assay developed in this study may be used to quantify the population changes in *T*. *asperellum* CHF 78 in soils when new management approaches are used, contributing to the better use of this beneficial microorganism.

Amaranth plants treated with MC+CHF 78 showed a significantly greater P uptake than those with the control and CF, which may be attributed to the phosphate-solubilizing ability of *T*. *asperellum* based on our previous study [12]. It has been suggested that *Trichoderma* species can promote nutrient uptake by plants in fields in addition to providing biocontrol efficacy [11,14,40]. This increase in plant nutrient uptake induced by *Trichoderma* strains usually depends on root colonization, and these fungi have a superior capacity to mobilize and take up soil nutrients [41]. Since our study did not quantify the population density of *T*. *asperellum* CHF 78 in soils after applying MC+CHF 78, in the future, it is necessary to determine the relationship between the population dynamics of this beneficial microorganism and nutrient availability in the rhizosphere. Moreover, the mechanisms for enhancing nutrient uptake in plants treated with MC+CHF 78, if any, should be further investigated.

5. Conclusions

This study demonstrates that MC and MC+CHF 78 can be used as a complete substitute for mineral fertilizers in amaranth cultivation under field conditions, because they significantly improve soil quality as a result of increasing soil pH, SOM, Bray-P, exchangeable K, and microbial activity compared to CF. These changes in soil properties, in turn, enhance amaranth nutrient uptake and yield. Only amaranth plants treated with MC+CHF 78 showed a significantly greater P uptake than those treated with the control and CF, which may be resulted from the phosphate-solubilizing ability of *T*. *asperellum* CHF 78. The results of this study may be beneficial when applying manure composts as a complete or partial substitute for mineral fertilizers in amaranth growth under field conditions. Moreover, the real-time PCR assay developed in this study may be useful in quantifying *T*. *asperellum* in composts and soils, resulting in better use of this fungus in promoting crop growth and reducing plant diseases. Studies on reducing disease severity with MC+CHF 78 should be further conducted to evaluate the effectiveness of *T*. *asperellum* CHF 78 in disease suppression, which is important for increasing the attractiveness of using manure composts.

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