The Effects of Mixed Hardwood Biochar, Mycorrhizae, and Fertigation on Container Tomato and Pepper Plant Growth

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Abstract: Biochar (BC) has the potential as a peat moss alternative for container plant growth. Three experiments were conducted to evaluate the effects of mixed hardwood BC, compost types, mycorrhizae, and fertigation on container-grown tomato and pepper growth. In experiment 1 (Exp1), BC at 50%, 70%, and 90% (vol.) were mixed with 5% vermicompost (VC) with the rest being a commercial peat moss-based substrate (CS) and fertigated at 200 or 300 mg L⁻¹ N. In experiment 2 (Exp2), 80% BC was mixed with chicken manure compost (CM; 5% or 10%) and CS and fertigated at 100 or 200 mg L⁻¹ N. In experiment 3 (Exp3), 90% BC was blended with CS and fertigated at 200 or 300 mg L⁻¹ N. Mixes in all the three experiments were added with or without mycorrhizae. Results showed that, compared with CS, in Exp1 tomato and pepper plants grown in BC-VC mixes had similar soil-plant analyses development (SPAD), growth index (GI), and total dry weight (TDW); in Exp2 and Exp3, plants in BC mixes (80% or 90%) had lower GI and TDW. In conclusion, BC (≤70%) amended with VC mixes could be used for container tomato and pepper production without negatively affecting plant growth, while BC (80%, 90%) mixes could have some negative impacts on plant growth.

Keywords: peat moss; substrate; vermicompost; chicken manure compost

1. Introduction

Questions have been raised on peat moss, the most commonly used greenhouse medium with its ideal properties for plant growth, due to environmental impacts and economic concerns [1–3]. Overharvesting peat moss can cause environmental issues such as rare wildlife habitat destruction, wetland ecosystem disturbance, and climate change interference [2,3]. Moreover, the price of peat moss has been rising, which causes economic concerns and could hinder growers’ profits, especially when transportation costs are considered [4].

Therefore, attention has shifted to biochar (BC) as a peat moss alternative due to its numerous advantages [3,5]. Biochar, a carbon-rich material, is a by-product of pyrolysis (a thermo-chemical
reaction in oxygen-depleted or oxygen-limited atmospheres) [6–8]. Biochar can be derived from various sources, such as green waste [9], wood [10], straw [11–15], bark [16], rice hulls [17], and wheat straw [13,18], making it readily available. For the same reason, BC can be generated faster and is not a limited resource like peat moss, presenting great environmental potential as a peat moss alternative. Furthermore, greenhouse gas emissions could be drastically reduced when BC is prepared from agricultural wastes, which otherwise would be incinerated, resulting in greenhouse gas emissions [19]. Additionally, the BC price may be competitive if BC is available locally. The average BC price is $78.57 m$^{-3}$, less than half the price of peat moss ($173.93 m$^{-3}$), presenting a great economic advantage as a peat moss alternative [20,21]. Moreover, different waste biomass and waste heat utilized during BC production process could bring significant savings for the overall economy [22].

Biochar’s potential as an alternative container substrate for peat moss has been documented in many studies. For instance, Guo et al. [23,24] observed that pinewood BC (80%, vol.) with peat moss-based substrate increased the growth of both poinsettia and Easter lily. A study by Huang et al. [25] showed that mixing 70% (vol.) mixed hardwood BC with two composts resulted in similar or better basil and tomato plant growth compared to a peat moss-based commercial substrate. Similarly, Yu et al. [26] showed that up to 70% (vol.) of mixed hardwood BC or sugarcane bagasse BC blended with peat moss can be used to grow container tomato and basil seedlings. Tian et al. [9] stated that 50% (vol.) green waste BC increased the total biomass of Calathea plants by 22% compared to those in 100% peat moss substrate. Additionally, Headlee et al. [27] demonstrated that a red oak BC feedstock mixture with vermiculite increased the total biomass and shoot biomass of hybrid poplar cuttings. Yan et al. [28] showed that 80% (vol.) mixed hardwood BC blended with 20% commercial peat moss-based substrate could be used as mixtures for different types mint plants growth without negative effects. Incorporating compost with BC as a container substrate improves its physical and chemical properties and thus benefits plant growth [29]. Vermicompost (VC; the end product of earthworms breaking down organic waste) [30] and chicken manure compost (CM; the waste resulting from the poultry industry) [31,32] are the composts used in containers. Vermicompost and CM both have fine textures and are rich in nutrients, which could alter substrate properties and provide extra nutrients [25,33]. For instance, Huang et al. [25] demonstrated that adding 5% (vol.) VC or CM to a BC-amended substrate improved tomato and basil growth.

Adding mycorrhizae (MC) to container media, in the presence of BC, could also improve plant growth due to its symbiotic relationship with plants [34,35]. In this symbiosis, MC provide the host plant with mineral nutrients, especially phosphorus (P), and water in exchange for photosynthetic products [36]. Therefore, MC could promote plant growth and plant yield by boosting nutrient uptake [37–39]. Mycorrhizae are commonly known to boost plants’ uptake of P, a nutrient often difficult for plants to absorb due to its insoluble forms [40,41], especially when the substrate pH is higher than 7 [1]. The ideal pH range for P in a soilless substrate is 4 to 6 [1]. However, incorporating BC in the media may limit P availability because most BCs used in greenhouse studies have pH higher than 7 [1,42]. The presence of MC enhancing P availability [41], in addition to a high P content in CM and VC, is expected to compensate for P deficiencies in BC-amended soilless substrates.

Fertilizer leaching from containers during watering raises environmental concerns, and could be reduced by adding BC to the container substrate [1]. In an open greenhouse production system, excessive fertilizer is commonly used to ensure crop growth and yield, leading to increased nutrient leaching [1]. Nutrient leaching may contaminate groundwater, cause eutrophication, and release nitrous oxide (NO$_2$) [43]. Incorporating BC in a container substrate could reduce nutrient leaching. Yu et al. [44] reported that mixed hardwood BC can retain nutrients due to its porous structure, which may reduce nutrient leaching. Similarly, Guo et al. [23,24] showed that the fertilizer rates could be reduced when pinewood BC was added at 60–80% (vol.) without sacrificing poinsettia’s or Easter lily’s growth.
Peatland has been functioning as carbon sink, playing a significant role in climate change yet its climatic potential has been underappreciated [45]. It was reported that restoring peatland for carbon sequestration was 3.4 times less nitrite costly and less land costly compared to other ways [45]. Due to the urgency of global warming and peatland’s climatic potential, some countries have already taken actions to restrict peatland extraction [3]. For instance, the United Kingdom and Europe have legislated laws to protect the peatland from being overharvested [1,46]. Therefore, peat moss substitutes are needed to reduce the total amount of peat moss used in the horticulture industry.

Based on the environmental and economic concerns of current greenhouse plant production and the potential benefits of using BC, we hypothesized that BC, amended with other components such as composts, MC, could be beneficial for container plants. To test this hypothesis, we conducted three experiments to quantify the effects of BC, compost (VC or CM), MC, and fertigation (F) on container-grown tomato and pepper. The objective of this study was to determine which combination and management practice have the greatest potential for container-grown vegetable production. The study used tomato and pepper due to their widespread usage and economic importance. This study could expand BC usage in the green industry and provide a good peat moss alternative for the future.

2. Materials and Methods

2.1. Substrates and Plant Materials

The BC used in this study was a by-product of the fast pyrolysis of mixed hardwood (Proton Power, Inc., Lenoir City, TN, USA). Two composts, VC (Pachamama earthworm castings, Lady Bug Brand, Conroe, TX, USA) and CM (Back to Nature Inc., Slaton, TX, USA), were chosen as additives to the BC. The commercial substrate Sunshine Mix #1 (CS; Professional Growing Mix #1, SunGro Inc., Agawam, ME, USA) was used when BC and compost volumes did not add up to 100%. The commercial MC product (ENDO/ECTO-MycoApply, Mycorrhizal Applications Inc., Grants Pass, OR, USA) was applied at the recommended rate. The MC product is granular mycorrhizal inoculum consisting of four endomycorrhizae fungi species and seven ectomycorrhizae fungi species. The four endomycorrhizae species were: *Glomus intraradices*, *G. mosseae*, *G. aggregatum*, and *G. etunicatum*. The seven ectomycorrhizae species were: *Rhizopogon villosulus*, *R. luteolus*, *R. amylopogon*, *R. fulvigleba*, *Pisolithus tinctorius*, *Scleroderma cepa*, and *S. citrinum*. The commercial soluble fertilizer (20N-4.3P-16.6K, Peters Professional, Eversis NA Inc., Dublin, OH, USA) was applied through fertigation (F).

The electrical conductivity (EC) and pH of substrate mixtures were measured with a handheld pH-EC meter (HI 98129, Hanna Instruments, Woonsocket, RI, USA) using pour-through extraction method [47]. The chemical and physical properties of BC, VC, CM, and CS, including pH, EC, total porosity (TP, %), container capacity (CC, %), air space (AS, %), and bulk density (BD, g cm$^{-3}$) are presented in Table 1 according to previous studies [24,25]. The carbon and ash content of BC were 88.6% and 5.37%, respectively [25]. The CM had high P and potassium (K) content (Table 2).

| Components       | pH   | EC (ds m$^{-1}$) | TP (%) | CC (%) | AS (%) | BD (g cm$^{-3}$) | Reference |
|------------------|------|------------------|--------|--------|--------|------------------|-----------|
| Biochar          | 11.2 | 2.0              | 85     | 60     | 24     | 0.15             | [25]      |
| Vermicompost     | 4.8  | 6.7              | 75     | 72     | 3      | 0.38             | [25]      |
| Chicken manure   | 7.5  | 32.9             | 64     | 60     | 4      | 0.62             | [25]      |
| Commercial Substrate | -    | -                | 84     | 63     | 22     | 0.11             | [24]      |
Table 2. Nutrient content of the biochar, vermicompost, and chicken manure used in this study according to the work conducted by Huang et al. [25].

| Components | N (%) | P (mg kg$^{-1}$) | K (mg kg$^{-1}$) | Ca (mg kg$^{-1}$) | Mg (mg kg$^{-1}$) | S (mg kg$^{-1}$) | Fe (mg kg$^{-1}$) |
|------------|-------|------------------|------------------|------------------|------------------|----------------|-----------------|
| Biochar    | 0.23  | 456              | 6362             | 27,507           | 1299            | 231            | 2039            |
| Vermicompost| 2.43 | 4901             | 3714             | 25,841           | 3819            | 5996           | 4835            |
| Chicken manure | 2.03 | 17,315           | 28,565           | 71,239           | 11,513          | 7169           | 3703            |

2.2. Experimental Design

Three greenhouse experiments were conducted in the greenhouse with no supplemental light at Texas A&M University, Department of Horticultural Sciences located at College Station, TX, USA. Experiment 1 (Exp1) was conducted from July 2017 to October 2017, while experiment 2 (Exp2) and experiment 3 (Exp3) were conducted from October 2017 to December 2017. The average greenhouse temperature, relative humidity, and dew point were 28.5 °C, 87.33%, and 25.85 °C, respectively, for Exp1, and 22.37 °C, 64.95%, and 14.08 °C, respectively, for Exp2 and Exp3.

2.2.1. Experiment 1: Biochar, Vermicompost, Mycorrhizae, and Fertigation Effects on Plant Growth

The substrates were formulated by mixing BC (50%, 70%, 90%, or 0%; vol.) with 5% VC. The remaining volume in each BC-VC mix was a peat moss-based commercial substrate. The 8 mixture combinations were treated with a commercially available MC product applied at the recommended rate. Another set was not treated with MC. Finally, a fertilizer was applied to the mixture/MC combinations through fertigation at 200 mg L$^{-1}$ or 300 mg L$^{-1}$ nitrogen (N) (Table 3) for a total of 16 treatments.

Table 3. List of treatments used in experiment 1 including biochar, vermicompost, and commercial peat moss-based substrate, mycorrhizae (Y/N = with/without), and fertigation rate (mg L$^{-1}$ N).

| Treatment | Biochar (%) | Vermicompost (%) | Commercial Substrate (%) | Mycorrhizae | Fertigation (mg L$^{-1}$ N) |
|-----------|-------------|------------------|--------------------------|-------------|--------------------------|
| 50 X:Y:200 | 50          | 5                | 45                       | Y           | 200                      |
| 50:Y:300   | 50          | 5                | 45                       | N           | 200                      |
| 50:N:200   | 50          | 5                | 25                       | Y           | 300                      |
| 50:N:300   | 50          | 5                | 25                       | N           | 300                      |
| 70:Y:200   | 70          | 5                | 25                       | Y           | 200                      |
| 70:Y:300   | 70          | 5                | 25                       | N           | 300                      |
| 70:N:200   | 70          | 5                | 25                       | N           | 200                      |
| 70:N:300   | 70          | 5                | 25                       | N           | 300                      |
| 90:Y:200   | 90          | 5                | 5                        | Y           | 200                      |
| 90:Y:300   | 90          | 5                | 5                        | Y           | 300                      |
| 90:N:200   | 90          | 5                | 5                        | N           | 200                      |
| 90:N:300   | 90          | 5                | 5                        | N           | 300                      |
| 0:Y:200    | 0           | 0                | 95                       | Y           | 200                      |
| 0:Y:300    | 0           | 0                | 95                       | Y           | 300                      |
| 0:N:200    | 0           | 0                | 100                      | N           | 200                      |
| 0:N:300    | 0           | 0                | 100                      | N           | 300                      |

X indicates biochar rate at 0%, 50%, 70%, or 90% (vol.); Y indicates whether mycorrhizae was added (Y) or not added (N).

Tomato (*Solanum lycopersicum* ‘Tumbling Tom Red’, Morgan County Seeds, Barnett, MO, USA) and pepper (*Capsicum annuum* ‘Nippon Taka 108F1’, self-collected) seeds were sown in 102-cell plug trays (volume = 20.5 cm$^3$, upper diameter = 1.70 cm, and height = 4.20 cm) filled with Sunshine Mix #1 on 26 July 2017. Tomato was transplanted on August 8, 2017 and pepper on 11 August 2017 (the week after transplanting (WAT) 0) into 6” azalea pots (top diameter 15.5 cm, bottom diameter 11.3 cm, and depth 10.8 cm).
The experiment was arranged as a three-way full factorial randomized complete block design (RCBD) with six replicates. The three factors were mix type (BC-VC mixes), MC (with or without), and F (200 mg L\(^{-1}\) or 300 mg L\(^{-1}\) N), respectively.

2.2.2. Experiment 2: Biochar, Chicken Manure Compost, Mycorrhizae, and Fertigation Effects on Plant Growth

Substrate mixtures were prepared by mixing BC (80% or 0%) with 5% or 10% CM. The remaining volume in each BC-CM mix was amended with CS to reach 100%. The plant materials used were the same as in Exp1 except they were planted on different dates (sown on October 10, 2017 and transplanted on 14 October 2017). Fertilizer was applied through F at 100 mg L\(^{-1}\) or 200 mg L\(^{-1}\) N (Table 4).

**Table 4.** List of treatments used in experiment 2 including biochar, chicken manure compost, commercial peat moss-based substrate, mycorrhizae (Y/N = with/without), and fertigation rate (mg L\(^{-1}\) N).

| Treatment | Biochar (% vol.) | Chicken Manure (% vol.) | Commercial Substrate (% vol.) | Mycorrhizae | Fertigation (mg L\(^{-1}\) N) |
|-----------|-----------------|-------------------------|------------------------------|-------------|-------------------------------|
| 80-S:Y:100| 80              | 5                       | 15                           | Y           | 100                           |
| 80-Y:200  | 80              | 5                       | 15                           | Y           | 100                           |
| 80-5:N:100| 80              | 5                       | 15                           | N           | 100                           |
| 80-5:N:200| 80              | 5                       | 15                           | N           | 200                           |
| 80-10:S:100| 80             | 10                      | 10                           | Y           | 100                           |
| 80-10:S:200| 80             | 10                      | 10                           | Y           | 200                           |
| 80-10:N:100| 80             | 10                      | 10                           | N           | 100                           |
| 80-10:N:200| 80             | 10                      | 10                           | N           | 200                           |
| 0-Y:100   | 0               | 10                      | 100                          | Y           | 100                           |
| 0-Y:200   | 0               | 10                      | 100                          | Y           | 200                           |
| 0-N:100   | 0               | 10                      | 100                          | N           | 100                           |
| 0-N:200   | 0               | 10                      | 100                          | N           | 200                           |

\(X\) indicates 80% (vol.) biochar mixed with 5% or 10% (vol.) chicken manure; \(Y\) indicates whether mycorrhizae was added (Y) or not added (N).

The experiment was arranged as a three-way full factorial RCBD with six replicates. The three factors were mix type (BC-CM mixes), MC (with or without), and F (100 mg L\(^{-1}\) or 200 mg L\(^{-1}\) N).

2.2.3. Experiment 3: Biochar, Mycorrhizae, and Fertigation Effects on Plant Growth

Substrate mixtures were formulated by mixing BC (90% or 0%) with CS. Plant material and sowing/transplanting dates were the same as Exp2. Fertilizer was applied through F at 200 mg L\(^{-1}\) or 300 mg L\(^{-1}\) N (Table 5).

**Table 5.** List of treatments used in experiment 3 including biochar, commercial peat moss-based substrate, mycorrhizae (Y/N = with/without), and fertigation rate (mg L\(^{-1}\) N).

| Treatment | Biochar (% vol.) | Commercial Substrate (% vol.) | Mycorrhizae | Fertigation (mg L\(^{-1}\) N) |
|-----------|-----------------|------------------------------|-------------|-------------------------------|
| 90-X:Y:200| 90              | 10                           | Y           | 200                           |
| 90:Y:300  | 90              | 10                           | Y           | 300                           |
| 90:N:200  | 90              | 10                           | N           | 200                           |
| 90:N:300  | 90              | 10                           | N           | 300                           |
| 0-Y:200   | 0               | 100                          | Y           | 200                           |
| 0-Y:200   | 0               | 100                          | Y           | 300                           |
| 0-N:300   | 0               | 100                          | N           | 200                           |
| 0-N:300   | 0               | 100                          | N           | 300                           |

\(X\) indicates 90% (vol.) biochar; \(Y\) indicates whether mycorrhizae was added (Y) or not added (N).
The experiment was arranged as a three-way full factorial RCBD with six replicates. The three factors were mix type (BC mixes), MC (with or without), and F (200 mg L$^{-1}$ or 300 mg L$^{-1}$ N).

2.3. Measurements

For each experiment, the growth index (GI) was determined by measuring plant height and two perpendicular widths on the 8th week after transplanting (WAT 8) using the following formula: $GI = \text{Height}/2 + (\text{Width}_1 + \text{Width}_2)/4$ [24].

Leaf greenness was quantified as soil-plant analyses development (SPAD) readings (SPAD-502 Minolta Camera Co., Osaka, Japan) at WAT 7. Plant fruits and shoots were harvested at WAT 8, fruit dry weight (FDW) and shoot dry weight (SDW) were determined after plant tissues were oven-dried till constant weight was reached. Total dry weight (TDW) was calculated as the sum of FDW and SDW.

2.4. Statistical Analysis

Data were analyzed with ANOVA using JMP Statistical Software (version Pro 14.2.0; SAS Institute, Cary, NC, USA) to test the effects of substrate mixtures, MC, and F rate on container plant development. Mean separation was conducted using Tukey honest significance difference (HSD) multiple comparison test at $p \leq 0.05$ level of significance. A cluster dendrogram and a principal component analysis (PCA) were conducted to evaluate the relationship among all the treatments and between the selected variables, using R programming software (version 3.5.1).

3. Results

3.1. Experiment 1: Biochar, Vermicompost, Mycorrhizae, and Fertigation

There were three-factor (mix, MC, and F) interactions in GI 8, FDW, and TDW for tomato and only GI 8 for pepper (Table 6). MC $\times$ F interaction was only significant for TDW in tomato and SPAD in pepper. Mix $\times$ F interaction was not significant for all variables in tomato and only for FDW in pepper. Significant interactions for Mix $\times$ MC were observed in GI 8, FDW, and TDW in tomato, and SPAD and TDW in pepper. All main factors had significant effects on tomato SPAD only, with Mix having significant effects on all variables in tomato and pepper. For pepper, F only had significant effects on SPAD and TDW, while MC only had significant effect on GI 8.

Table 6. A summary of the statistical significance of treatment factors on growth index at the eighth week after transplanting (GI 8), soil-plant analyses development (SPAD), fruit dry weight (FDW), and total dry weight (TDW) for tomato and pepper plants.

| Tomato | GI 8 | SPAD | FDW | TDW | Pepper | GI 8 | SPAD | FDW | TDW |
|-------|-----|------|-----|-----|--------|-----|------|-----|-----|
| Significance $^X$ | | | | |  | | | | |
| Mix | *** | *** | *** | *** | Mix | *** | *** | *** | *** |
| MC | NS | ** | NS | NS | MC | ** | NS | NS | NS |
| F | NS | ** | NS | NS | F | NS | NS | NS |
| Mix $\times$ MC | * | NS | * | ** | Mix $\times$ MC | NS | *** | NS | * |
| Mix $\times$ F | NS | NS | NS | NS | Mix $\times$ F | * | ** | NS | * |
| MC $\times$ F | NS | NS | NS | ** | MC $\times$ F | NS | * | NS | NS |
| Mix $\times$ MC $\times$ F | * | NS | ** | *** | Mix $\times$ MC $\times$ F | * | NS | NS | NS |

$^X$Significance of mixes type, mycorrhizae, and fertigation on plant growth parameters and SPAD. NS means not significant. *, **, *** indicate significance at $p \leq 0.05, 0.01$, and 0.001, respectively.

At BC rates of 0%, 50%, or 70%, MC addition or F rates did not significantly affect GI 8, FDW, and TDW of tomato, or GI 8 of pepper (Table 7). The lowest GI 8 was observed when no BC or MC were added at 300 mg L$^{-1}$ N (0:N:300) and the highest in 90:N:200. At 90% BC rate, the addition of MC or F rates did not significantly affect GI 8 and FDW for tomato yet with MC at higher F rates, tomato TDW was significantly improved. In general, plants in 50% BC and 70% BC mixtures had better growth than those in 90% BC.
Table 7. Growth index of tomato and pepper plant grown in Sunshine Mix #1 amended with biochar (0%, 50%, 70%; and 90%, vol.) at the eighth week after transplanting (GI 8), tomato fruit dry weight (FDW) and total dry weight (TDW) at two fertigation levels (200 mg L\(^{-1}\) and 300 mg L\(^{-1}\) N).

| Treatment | Tomato GI 8 | Tomato FDW | Tomato TDW | Pepper GI WAT 8 |
|-----------|-------------|------------|------------|-----------------|
| 0\(^{X}\):Y:200 | 63 ± 2 abc\(^{Z}\)  | 7.6 ± 1.0 abc | 33.2 ± 1.4 ab | 46 ± 2 ab |
| 0:Y:300 | 59 ± 2 bc | 7.2 ± 1.0 abc | 32.3 ± 1.4 ab | 46 ± 2 ab |
| 0:N:200 | 61 ± 2 bc | 4.4 ± 1.0 bc | 32.3 ± 1.4 ab | 47 ± 2 ab |
| 0:N:300 | 57 ± 2 c | 7.8 ± 1.0 abc | 34.1 ± 1.4 a | 45 ± 2 ab |
| 50:Y:200 | 62 ± 2 bc | 8.1 ± 1.0 ab | 35.9 ± 1.4 a | 53 ± 2 ab |
| 50:Y:300 | 63 ± 2 abc | 8.1 ± 1.0 ab | 37.9 ± 1.4 a | 53 ± 2 a |
| 50:N:200 | 67 ± 2 ab | 7.8 ± 1.0 abc | 36.8 ± 1.4 a | 50 ± 2 ab |
| 50:N:300 | 64 ± 2 abc | 9.8 ± 1.0 a | 38.1 ± 1.4 a | 52 ± 2 ab |
| 70:Y:200 | 67 ± 2 ab | 7.7 ± 1.0 abc | 35.6 ± 1.4 a | 52 ± 2 ab |
| 70:Y:300 | 62 ± 2 bc | 9.6 ± 1.0 a | 37.5 ± 1.4 a | 51 ± 2 ab |
| 70:N:200 | 64 ± 2 abc | 7.5 ± 1.0 abc | 34.7 ± 1.4 a | 50 ± 2 ab |
| 70:N:300 | 64 ± 2 abc | 9.9 ± 1.0 a | 39.1 ± 1.4 a | 50 ± 2 ab |
| 90:Y:200 | 63 ± 2 abc | 3.0 ± 1.0 c | 18.5 ± 1.4 d | 28 ± 2 c |
| 90:Y:300 | 68 ± 2 ab | 5.9 ± 1.0 abc | 32.4 ± 1.4 ab | 43 ± 2 b |
| 90:N:200 | 72 ± 2 a | 5.4 ± 1.0 abc | 26.6 ± 1.4 bc | 28 ± 2 c |
| 90:N:300 | 63 ± 2 abc | 4.0 ± 1.1 bc | 23.8 ± 1.6 cd | 27 ± 2 c |

\(^{X}\) indicates biochar rate at 0%, 50%, 70%, or 90% (vol.); \(^{Y}\) indicates whether mycorrhizae was added (Y) or not added (N); \(^{Z}\) numbers within a column followed by the same letter are not significantly different according to Tukey honest significance difference (HSD) multiple comparison at \(p \leq 0.05\).

Biochar rate, MC, and F had significant effects on tomato SPAD (Table 6; Figure 1A–C). All tomato plants grown in BC-amended mixes had significantly lower SPAD compared to those grown in CS. Tomato plants grown with MC or 300 mg L\(^{-1}\) N had significantly higher SPAD compared to those without MC or 200 mg L\(^{-1}\) N.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** The effects of % biochar rates (BC; A), mycorrhizae (B), and fertigation (C) on tomato soil-plant analyses development (SPAD). The same letter indicates not significantly different according to Tukey HSD multiple comparison at \(p \leq 0.05\).

At 200 mg L\(^{-1}\) N, pepper plants grown in BC-amended mixes had significantly lower SPAD compared to those in the CS, while at 300 mg L\(^{-1}\) N, those in 50% BC had similar SPAD to the CS (Figure 2A). Pepper plants grown in 50% and 70% BC-amended mixes had similar FDW (Figure 2B) and TDW (Figure 2C) compared to those grown in the CS.

3.2. Experiment 2: Biochar-Chicken Manure, Mycorrhizae, and Fertigation

There were three-factor (mix, MC, and F) interactions in FDW and TDW for tomato and no interactions in GI 8, SPAD, FDW or TDW for pepper (Table 8). MC × F interaction was not significant for all variables in tomato and only for FDW in pepper. Mix × F interaction was not significant for all variables in tomato and pepper. Significant interactions for Mix × MC were observed in SPAD and TDW in tomato, and GI 8, SPAD, and TDW in pepper. Main factor F had no significant effects on all variables in tomato and pepper while MC only had significant effects on SPAD and TDW for tomato.
and GI 8, SPAD for pepper. Mix had significant effects on SPAD and FDW for tomato and all variables in pepper.

**Figure 2.** The effects of biochar on pepper soil-plant analyses development (SPAD) (A), fruit dry weight (FDW; B) and total dry weight (TDW; C). The same letter indicates not significantly different according to Tukey HSD multiple comparison at $p \leq 0.05$.

**Table 8.** A summary of the statistical significance of treatment factors on growth index at the eighth week after transplanting (GI 8), soil-plant analyses development (SPAD), fruit dry weight (FDW), and total dry weight (TDW) for tomato and pepper plants.

| Tomato | GI 8 | SPAD | FDW | TDW | Pepper | GI 8 | SPAD | FDW | TDW |
|--------|------|------|-----|-----|--------|------|------|-----|-----|
| Significance$^X$ | Mix | NS | *** | NS | Mix | *** | *** | *** | *** |
| MC | NS | *** | NS | ** | MC | * | * | NS | NS |
| F | NS | NS | NS | NS | F | NS | NS | NS | NS |
| Mix $\times$ MC | NS | * | NS | *** | Mix $\times$ MC | * | * | NS | NS |
| Mix $\times$ F | NS | NS | NS | NS | Mix $\times$ F | NS | NS | NS | NS |
| MC $\times$ F | NS | NS | NS | NS | MC $\times$ F | NS | NS | * | NS |
| Mix $\times$ MC $\times$ F | NS | NS | * | NS | Mix $\times$ MC $\times$ F | NS | NS | NS | NS |

$^X$Significance of mix type, mycorrhizae, and fertigation on plant growth parameters and SPAD. NS means not significant. *, **, *** indicate significance at $p \leq 0.05, 0.01$, and $0.001$, respectively.

Tomato FDW (except for 0% CM at 200 mg L$^{-1}$ N) was not significantly affected by MC addition, F rates, or CM rates (Table 9). At 5% CM rate, MC addition did not significantly affect tomato TDW. Fertigation rates, however, significantly affected TDW: higher F led to higher TDW. At 10% CM rate, F rates and MC did not significantly impact tomato TDW. At 0% CM rate with MC, F rates did not significantly impact tomato FDW or TDW. At 0% CM rate without MC, however, F rates significantly influenced tomato TDW; higher F led to higher TDW.

**Table 9.** Fruit dry weight (FDW) and total plant dry weight (TDW) of tomato grown in Sunshine Mix #1 amended with biochar (80%, vol.) and chicken manure (5% and 10%, vol.) at two fertigation levels (100 mg L$^{-1}$ and 200 mg L$^{-1}$ N).

| Treatment | Tomato FDW | Tomato TDW |
|-----------|------------|------------|
| 80-5;Y:100 | 1.0 ± 0.2 b$^Z$ | 11.9 ± 0.8 d |
| 80-5;Y:200 | 1.2 ± 0.2 ab | 16.3 ± 0.8 bc |
| 80-5:N:100 | 1.1 ± 0.2 b | 11.1 ± 0.8 d |
| 80-5:N:200 | 1.3 ± 0.2 ab | 17.4 ± 0.8 abc |
| 80-10;Y:100 | 0.8 ± 0.2 b | 11.9 ± 0.8 d |
| 80-10;Y:200 | 0.8 ± 0.2 b | 14.2 ± 0.8 cd |
| 80-10:N:100 | 1.0 ± 0.2 b | 12.0 ± 0.8 d |
| 80-10:N:200 | 0.9 ± 0.2 b | 14.7 ± 0.8 acd |
| 0-0;Y:100 | 1.5 ± 0.2 ab | 17.0 ± 0.8 bc |
| 0-0;Y:200 | 1.3 ± 0.2 ab | 19.3 ± 0.8 ab |
| 0-0:N:100 | 1.2 ± 0.2 ab | 15.9 ± 0.8 bc |
| 0-0:N:200 | 2.0 ± 0.2 a | 20.9 ± 0.8 a |

$^X$indicates 80% (vol.) biochar mixed with 5% or 10% (vol.) chicken manure; $^Y$indicates whether mycorrhizae was added (Y) or not added (N); $^Z$numbers within a column followed by the same letter are not significantly different according to Tukey HSD multiple comparison at $p \leq 0.05$. 

Sustainability 2020, 12, x FOR PEER REVIEW 2 of 17
Adding MC did not significantly impact SPAD of tomato plants grown in BC-amended mixes with 5% CM (Figure 3A). Mix type did not significantly influence tomato GI 8 (Figure 3B). With MC, mix type had no significant effects on pepper GI, SPAD, or TDW (Figure 4A–C). Similarly, at 200 mg L⁻¹ N, mixes with 5% CM did not significantly impact pepper FDW either (Figure 4D).

Figure 3. The effects of mycorrhizae on tomato SPAD (A) and mixes on tomato growth index at the eighth week after transplanting (GI 8; B). The same letter indicates not significantly different according to Tukey HSD multiple comparisons at p ≤ 0.05.

Figure 4. The effects of mycorrhizae on pepper growth index at the eighth week after transplanting (GI 8; A), soil-plant analyses development (SPAD) (B), total dry weight (C), and the effects of mixes on pepper plant fruit dry weight (D). The same letter indicates not significantly different according to Tukey HSD multiple comparisons at p ≤ 0.05.

3.3. Experiment 3: Biochar, Mycorrhizae, and Fertigation

There were three-factor (mix, MC, and F) interactions in GI 8, SPAD, and TDW for tomato but no interactions for all variables in pepper (Table 10). MC × F interaction was not significant for all variables in tomato and only for SPAD in pepper. Mix × F interaction was not significant for all variables in tomato and pepper. Significant interactions for Mix × MC were only observed in GI 8 and TDW in pepper. All main factors had significant effects on tomato SPAD only, with Mix having significant effects on all variables in tomato and pepper and MC not having significant effects on GI 8 and TDW in tomato only.
Table 10. A summary of the statistical significance of treatment factors on growth index at the eighth week after transplanting (GI 8), soil-plant analyses development (SPAD), fruit dry weight (FDW), and total dry weight (TDW) for tomato and pepper plant.

| Tomato | GI 8 | SPAD | FDW | TDW | Pepper | GI 8 | SPAD | FDW | TDW |
|--------|------|------|-----|-----|--------|------|------|-----|-----|
| Significance | Mix | ** | *** | *** | Mix | *** | *** | *** | *** |
| MC | NS | ** | NS | ** | MC | ** | ** | * | * |
| F | NS | ** | NS | NS | F | NS | NS | NS | NS |
| Mix × MC | NS | NS | NS | NS | Mix × MC | * | NS | NS | NS |
| Mix × F | NS | NS | NS | NS | Mix × F | NS | NS | NS | NS |
| MC × F | NS | NS | NS | NS | MC × F | NS | * | NS | NS |
| Mix × MC × F | ** | ** | NS | * | MC × F × Mix | NS | NS | NS | NS |

Note: X Significance of mixes type, mycorrhizae, and fertigation on plant growth parameters and SPAD. NS means not significant. *, **, *** indicates significance at p ≤ 0.05, 0.01, and 0.001, respectively.

At BC rates of 0% and 90%, MC and F had no significant impacts on tomato GI (except for 90% BC with MC at 300 mg L⁻¹ N; Table 11). Mycorrhizae addition and F had no significant effects on tomato SPAD or TDW. Mix type, however, significantly influenced tomato SPAD as well as TDW: BC-amended mixes caused significantly lower SPAD and TDW.

Table 11. Growth index at the eighth week after transplanting (GI 8), soil-plant analyses development (SPAD), and total dry weight (TDW) of tomato plants grown in Sunshine Mix #1 amended with biochar (90% and 0%, vol.) and at two fertigation levels (200 mg L⁻¹ and 300 mg L⁻¹ N).

| Treatment | Tomato GI 8 | Tomato SPAD | Tomato TDW |
|-----------|-------------|--------------|------------|
| 90:Y:200 | 52.0 ± 3 a | 26 ± 2 cd | 12 ± 2 bc |
| 90:Y:300 | 57 ± 3 a | 28 ± 2 cd | 13 ± 2 bc |
| 90:N:200 | 56 ± 3 a | 24 ± 2 d | 10 ± 2 c |
| 90:N:300 | 41 ± 3 b | 23 ± 2 d | 7 ± 2 c |
| 0:Y:200 | 56 ± 3 a | 39 ± 2 a | 24 ± 2 a |
| 0:Y:200 | 56 ± 3 a | 35 ± 2 ab | 20 ± 2 a |
| 0:N:300 | 55 ± 3 a | 31 ± 2 bc | 19 ± 2 ab |
| 0:N:300 | 58 ± 3 a | 37 ± 2 ab | 21 ± 2 a |

X indicates 90% (vol.) biochar; Y indicates whether mycorrhizae was added (Y) or not added (N); Z numbers within a column followed by the same letter are not significantly different according to Tukey HSD multiple comparison at p ≤ 0.05.

Biochar-amended mix significantly reduced tomato FDW (Figure 5 A–C). Without MC, the mix type significantly impacted pepper TDW and SPAD: BC-amended mixes had no significant impact on pepper GI (Figure 6A) but led to lower TDW and FDW (Figure 6C, Figure 7A). Fertigation rate did not significantly impact pepper SPAD (Figure 6B). Adding MC significantly decreased pepper FDW (Figure 7B).

Figure 5. The effects of biochar on tomato plants fruit dry weight (FDW). The same letter indicates not significantly different according to Tukey HSD multiple comparisons at p ≤ 0.05.
3.4. Treatment Grouping and Their Correlation to Plant Growth

Treatments with different components such as BC mix types, F rates, and MC applications had varied responses. From the ANOVA table (Table 6, Table 8, Table 10), we observed some of the treatments could be grouped together for their closely related characteristics, which were reflected in their similar effects on plant growth. Therefore, based on the obtained data from our study, we can use hierarchical analysis to cluster these 36 treatments in all the three experiments. Using a complete linkage method, dendrograms (Figure 8) were created separately for tomato and pepper based on the similarities (Jaccard’s similarity coefficient) among treatments.

Figure 8. The cluster dendrogram for tomato (A) and pepper (B) plants. Group 1, 2, and 3 in tomato (A) represent 11 treatments with high biochar rates (BC, 80% or 90%), 11 treatments with low compost rate (0% or 5%), and 14 treatments with BC–5% vermicompost (VC) mixes, respectively. Group 1, 2, and 3 in pepper (B) represent 12 treatments with BC-VC mixes, 20 treatments with composts (chicken manure compost (CM) and VC), and 4 treatments with 90% BC–5% VC mixes, respectively. Red line indicates the height at 25 in the cluster dendrogram.
We drew a line at height 25 (Figure 8A,B), and treatments fell into three groups for both tomato (Figure 8A) and pepper plants (Figure 8B). Tomato plant treatments were grouped into high BC rate group (80%, 90%) with 11 treatments (group 1), low compost rates (0%, 5%) with 11 treatments (group 2), and BC–5%VC group with 14 treatments (group 3). Pepper plant treatments were grouped into BC–VC group (BC ≤ 70%) with 12 treatments (group 1), compost group (CM and VC) with 20 treatments (group 2), and 90%BC–5%VC group with 4 treatments (group 3).

These identified treatments within a group shared similar effects on plant growth, but how could we qualify these effects on the measured responses (SPAD, GI, FDW, TDW)? Based on the group information, we used principal component analysis (PCA) to qualify these effects and depict variables shaped by different mix types (treatments) for tomato (Figure 9A) and pepper (Figure 9B) plants. For tomato plants, 91.7% of the variability was explained by the first two components (Figure 9A). PC1 accounted for 81.9% variance. Vermicompost treatments (group 3) were associated with yield (FDW, TDW) and growth (SPAD, GI), while compost group (group 2) was correlated more to plant growth (GI). PC2 accounted for 9.8% variance, however it did not distinguish differences from the groups.

![Figure 9](https://example.com/figure9.png)

**Figure 9.** Principal component analysis (PCA) depicting the relationships between selected variables and treatment factors with tomato (A) and pepper (B). Selected variables are displayed by arrows and include plant growth parameters—soil-plant analyses development (SPAD), growth index (GI), fruit dry weight (FDW), and total dry weight (TDW). Treatment factors are displayed by filled blue circles: 5% composts (group 2 for tomato; A), or chicken manure compost (CM, group 2 for pepper; B); orange triangle: high biochar 80%, 90% (BC, group 1 for tomato; A) or 90% BC + 5% vermicompost (VC, group 3 for pepper; B); and grey square: VC (group 3 for tomato; A) or low BC (≤70%) + 5% VC (group 1 for pepper; B).

For pepper plants, the first two components explained 96.8% of the variability (Figure 9B). PC1 accounted for 75.6% variance, BC–5% VC (BC ≤ 70%) mixes (group 1) showed a greater association with TDW, GI, and FDW, while CM mixes (group 2) showed a greater relation to SPAD. PC2 accounted for 21.2% variance, distinguishing between the 90% BC–5% VC (group 3) and CM mixes (group 3). Group 3 (90% BC–5% VC) tended to be affiliated with FDW, GI, as well as TDW, however, CM mixes appeared to be related to the SPAD.
4. Discussion

4.1. Treatment Effects on Plant Growth

Biochar mixes, MC, and F rates and their synergistic impacts can beneficially influence plant growth. Biochar can aid plant growth both directly by supplying nutrients [48] and indirectly by influencing nutrient availability via changing substrate total porosity and pH [23,49]. For instance, for poinsettia and Easter lily, adding 20–60% (vol.) pinewood BC to peat moss-based substrate increased the total stem length and the number of leaves due to the suitable total porosity and pH, which improved nutrient uptake at given F rates [9]. Peng et al. [3] demonstrated the mix of BC (20–60%) and peat moss-based substrate (80–40%) had no negative effects on basil, tomato, or chrysanthemum because suitable physical properties helped nutrient absorption. Furthermore, pinewood BC can replace a commercial peat moss-based substrate from 5–30% (vol.) without any negative impacts on gomphrena plant growth [4], resulting from mix properties and F integrated effects. Moreover, mixed hardwood BC can replace 70% (vol.) of a commercial peat moss-based substrate without negatively impacting on tomato or basil plant growth [25] due to the enhanced nutrient uptake.

Biochar can impact substrate pH, making nutrients, especially P, less available to the plant, which could be compensated by adding MC [1,50]. For example, Conversa et al. [51] showed that 30% of BC, even with a pH at 8.6 which made P less available, increased geranium plant growth because MC compensated P uptake. However, high percentage of BC (70%; vol.) induced high pH and led to N and P deficiency, which could not be compensated by MC, reducing geranium plant growth. Part of the Conversa et al. [51] results were similar to ours: tomato and pepper plants grown in BC-amended mixes (lower than 70%) had similar growth compared to those in the commercial substrate. However, in our study, the high BC rate (70% for pepper, 80% for tomato) did not result in any negative impacts on plant growth as Conversa et al. [51] reported. The difference may be due to the presence of composts (VC or CM) in our study. Additionally, our study had similar results to the study by Huang et al. [25], which showed that 70% (vol.) of mixed hardwood BC with 5% VC or CM can be used for tomato and basil plant growth with no negative impacts on plant growth. However, in our study, the results in 90% BC–5% VC mix with MC and 300 mg L$^{-1}$ N differed from those of Huang et al. [25]. The differences could be explained by the MC, which improved nutrient uptake.

In this study, we only tested one type of biochar. Since biochars from different feedstocks have varied properties [44], we can test different types of biochar in the future. Moreover, we only tested tomato and pepper plants in this study, more horticultural plants should be tested in the future.

4.2. Biochar Potential Economic Value

According to the United States Department of Agriculture (USDA) and the United States Geological Survey (USGS) [52], around 0.15 M m$^{3}$ of container substrates were used for the horticulture industry with 91% (by vol.) being peat moss-based or just peat moss [52,53]. The Sunshine Mix #1 used in this study contains 80% peat moss. The estimated prices of the 70% biochar–5% vermicompost mix and Sunshine Mix #1 are $119.7 m^{-3}$ and $176.9 m^{-3}$, respectively [25]. With the results in this study, if the mix 70% biochar–5% vermicompost were chosen for container plant production, 0.1 M m$^{3}$ of peat moss with an estimated value of $5.98 M could be saved annually, in addition to the reduced fertilizer costs. This study showed one aspect of the economic value of biochar by replacing peat moss-based substrate; other studies also proposed the economic value of biochar by introducing it into wastewater, farming, and municipal industries [54–56].

4.3. Biochar Potential Climatic Value

Using biochar as a peat moss alternative could have significant potential to slow down global warming. Peatland, accounting only for around 3% of the terrestrial surface, may store 21% of the global total soil organic carbon stock of around 3000 Gt [57–59] and provide natural habitats for wild animals. However, the potential climatic value of peatland has been underappreciated [45]. Using alternative
substrate materials such as biochar could slow down peat moss harvest, and thus slow down depleting peat bogs, which could conserve their carbon sink capability and contribute to slower global warming. According to the literature, 20–80% of peat moss can be replaced by biochar [9,28,44]. With those numbers (assuming the commercial substrate contains 75% of peat moss), an estimated 0.02 M–0.08 M m$^{-3}$ of peat moss can be saved annually. Furthermore, with pyrolysis for bio-oil purposes, the yield of biochar ranges from 20–47% [60]. Assuming biochar yield at 30%, to produce the same amount of biochar used sufficiently for the horticulture industry (assuming replacing 50% of peat moss), nearly 0.28 M m$^{-3}$ of agriculture waste can be converted annually, which otherwise would be incinerated and aggravate global warming [61].

5. Conclusions

Among all the treatments, adding mycorrhizae did not have a significant impact on plant growth (except 90% biochar–5% vermicompost with fertigation at 300 mg L$^{-1}$ N for tomato). The biochar can replace commercial peat moss-based substrate when used at 50% to 70% (vol.) for both tomato and pepper plants. At these mixture rates, biochar had a similar growth index, SPAD, fruit dry weight, and total plant dry weight as the unmixed control when used with either 200 or 300 mg L$^{-1}$ N. Among the mixes, the best plant performance was observed when biochar was ≤70% with additional 5% vermicompost, and had similar results when plants were fertilized with 200 mg L$^{-1}$ or 300 mg L$^{-1}$ N. The hypothesis that BC, amended with composts, MC, could be beneficial for container plants was confirmed.

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