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Using Poinsettia and Pepper as Model Plants to Investigate Biochar and *Trichoderma* Suppressing Effects on Plant Diseases

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Summary

Biochar (BC) is a carbon-rich by-product from biomass pyrolysis (thermochemical biomass decomposition under an oxygen-depleted or oxygen-limited environment with specific time and temperature conditions). Biochar is of commercial importance for replacing more costly peat moss-based substrate for greenhouse plant production - and its potential to suppress plant diseases such as *Phytophthora capsici* and *Pythium aphanidermatum.* The application of *Trichoderma* did not significantly reduce disease severity. However, the mixed hardwood biochar (HB) mixed at 20% by volume could replace peat moss-based substrate to reduce poinsettia root rot disease caused by *P. aphanidermatum* without negatively affecting poinsettia plant growth. Incorporating HB by replacing 30% and 50% peat moss in the substrate could also reduce pepper blight disease caused by *P. capsici*.

INTRODUCTION

Potted poinsettia (*Euphorbia pulcherrima*) is one of the most important greenhouse ornamental crops in the United States, with an estimated wholesale value of $170 million in the top 15 states (USDA-NASS, 2018). Pepper is another important crop with a market value around $784 million (USDA-NASS, 2018). *Pythium aphanidermatum* and *Phytophthora capsici* are two common pathogens in greenhouses affecting poinsettia and pepper production significantly. They can both survive and thrive with high humidity and even high temperature. *P. aphanidermatum* is the predominant *Pythium* species causing poinsettia root rot disease, which is a recurrent disease that affects poinsettia production in greenhouses across the US (Lookabaugh et al., 2020; Múnera et al., 2019). *P. capsici* is a destructive hemi-biotrophic pathogen causing disease on a broad range of crops from families including solanaceous, cucurbitaceous, and fabaceous (Kousik et al., 2015). *Phytophthora* blight on pepper caused by *P. capsici* is one of the most serious soil-borne diseases for pepper worldwide (Wang et al., 2019).

Biochar (BC) is a carbon-rich by-product from biomass pyrolysis (thermochemical biomass decomposition under an oxygen-depleted or oxygen-limited environment with specific time and temperature conditions) (Demirbas and Arin, 2002; Lehmann, 2007). Not only can biochar replace peat moss-based substrate for greenhouse plant production (Guo et al., 2018; Huang et al., 2019; Yan et al., 2020; Yu et al., 2020) but it has the potential to suppress plant diseases including the diseases caused by *P. capsici* and *P. aphanidermatum.* For instance, BC-amended soil suppressed disease caused by *Pythium spp.* was reported, although with BC at relatively low rates (≤ 3% w/w) (Jaiswal et al., 2019). Also, incorporating corn stalk biochar (pH 9.73) at 13.7g/kg to soil suppressed pepper blight because it increased the abundance of beneficial microorganisms (Wang et al., 2020).

Trichoderma spp. has been reported as a reliable biological control agent for *P. capsici* and *P. aphanidermatum.* For instance, *Trichoderma harzianum* was proven to suppress pepper root rot caused by *P. capsici* through antimicrobial substances production (Ezziyyani et al., 2007). In a vitro test, *T. harzianum* inhibited *P. capsici* by 65.3% (Das et al., 2019). Also, *Trichoderma spp.* played a role in controlling cucumber damping-off caused by *P. aphanidermatum* (AL-Malikya et al., 2018). Some types of BC have proven to have synergistic effects with other components including *Trichoderma spp.* As such, we conducted two greenhouse trials using poinsettia and pepper plants as model plants to test the BC suppression effects on plant diseases caused by *P. capsici* and *P. aphanidermatum*.

MATERIALS & METHODS

Plants Material, Biochar Media, Pathogen & Trichoderma

Poinsettia (*Euphorbia pulcherrima* 'Prestige Sunrise Red') cuttings were stuck in commercial propagation media (BM2 Berger, Saint-Modeste, Quebec, Canada). After the root grew out, uniform cuttings were transplanted into 6-inch azalea pots filled with peat moss-based commercial substrate (CS, Jolly Gardener C/20, Oldcastle Lawn & Garden Inc., Atlanta, GA, USA) incorporated with mix hardwood biochar (HB, Proton Power Inc. Lenoir City, TN, USA)

mixed at 20% and 40% (by vol.). Hot cherry pepper (*Capsicum annuum* 'Capperino') F1 plants were grown in the greenhouse and self-pollinated to get F2 seeds. According to Johnny's seeds, the F1 seeds are susceptible to *P. capsici* (personal conversation). Based on our two previous preliminary studies, there were no patterns of *P. capsici* resistance. Because F1 seeds were not *P. capsici* resistant, F2 plants showed no patterns on *P. capsici* resistance, and the difficulties of passing on the disease resistance to the descendants, we can safely assume that the F2 seeds used in this study are not *P. capsici* resistant. Uniform seedlings were transplanted into 4-inch pots filled with CS blended with either HB at 10%, 30%, 50%, and 70% (by vol.) or sugarcane bagasse biochar (SBB, American Biocarbon LLC White Castle, LA, USA) at 10% (by vol.). The CS used in this study contains 80% Canadian Sphagnum peat moss with the rest being perlite and was used as the control. At transplanting, slow release fertilizer Osmocote Plus (15N-4P-10K, Scotts-Sierra Horticultural Products Company, Marysville, OH, USA) was applied at manufactory rates.

Pythium aphanidermatum and *Phytophthora capsici* were isolated from infected plants and inoculated with actively growing mycelium agar. Root shield Plus-WP (BioWorks, Victor, NY, USA) was used as a biological control agent in this study. The biological control agent contained two active strains of *Trichoderma, T. harzianum* strain T-22, and *T. virens* strain G-41. *Trichoderma*-containing product was applied at the manufactory's recommendation rate four weeks after plant transplanting (WK4, poinsettia, WK1 for pepper). This experiment was designed as random complete block design and was conducted in the greenhouses located on Texas A&M University campus, College Station (poinsettia) and Sommerville (pepper), Texas, USA. The average greenhouse temperature, relative humidity, and dew point were 30.2 ℃, 77.2%, and 25.0 ℃, respectively.

Measurements:

Potting mix physical and chemical properties:

Media physical properties—total porosity (TP), container capacity (CC), air space (AS), and bulk density (BD)—were measured according to North Carolina State University Horticultural Substrates Laboratory Porometer (Fonteno et al., 1995). The leachate electrical conductivity (EC) and pH were measured with a portable EC/pH meter (Hanna Instrument, Woonsocket, RI, USA), according to the pour-through method (LeBude and Bilderback, 2009).

Disease assessment:

Disease symptoms were observed and recorded every 5 days after pathogen inoculation. The disease severity was recorded at a 0-4 scale (no symptom - dead plants) according to Wang's work (Wang et al., 2019). The scale was also visualized in this work as shown in Fig. 1 and 2.

Disease severity index (DS) (Wang et al., 2019) was calculated by the following formula:

$$DS = \sum\left(\frac{\text{number of diseased plants in this index} \times \text{disease index rating from 0 to 4}}{4 \times \text{number of plants investigated}}\right) \times 100\%.$$

Figure 1. 0-4 scales (0 = no symptom, 4 = dead plant) used for the poinsettia root rot caused by *P. aphanidermatum* disease severity rating used in this study, no plant was dead in this study.

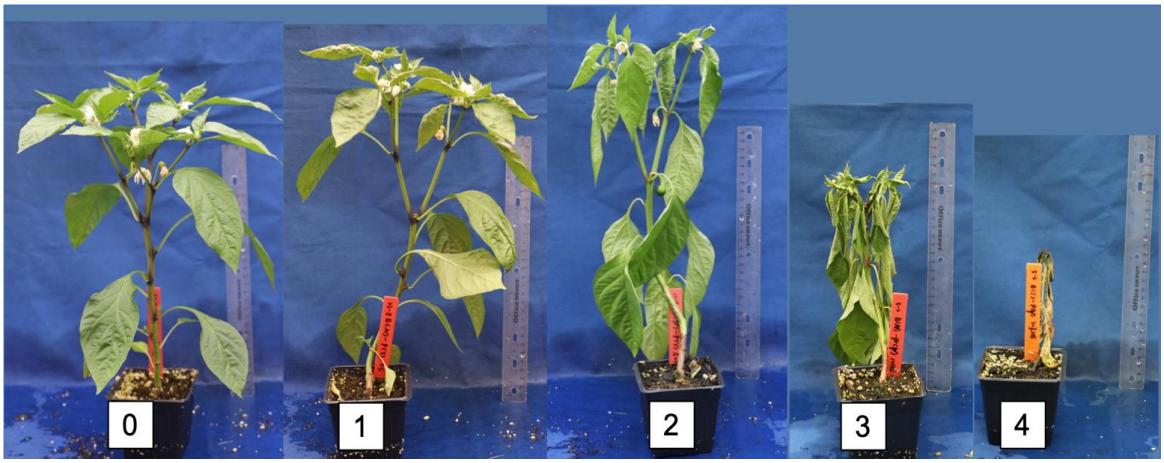

Figure 2. Visual scales (0-4; 0 = no symptom; 4 = dead) used for the pepper blight caused by *Phytophthora capcisi* disease severity rating used in this study.

Disease incidence (DI) was evaluated by counting the number of diseased plants in each pot twice during the trials, according to the formula:

$$\text{DI} = \frac{number\ of\ diseased\ plants}{number\ of\ total\ plants} \times 100$$

(Bellini et al., 2020).The disease severity obtained at different times after inoculation was used to calculate areas under disease progress curves (AUDPC) following the formula:

$$\text{AUDPC} = \sum_{i=0}^{n-1} \frac{(y_i + y_{i+1})}{2}(t_{i+1} - t_i).$$

Where y_i is the scale rating at the ith observation, $t_i$is the day of the ith observation, and n is the total number of observations (Madden et al., 2007).

RESULTS

Potting mix physio-chemical properties:

Most of the mixes' physical properties were within the recommended range (Yeager et al., 2007), except for the BDs in all the treatments, which were lower than the recommended value (Table1).

The HB20 and HB40 mixes had a significantly lower TP, and pH, as compared with the control (CS100). The HB50 and HB70 mixes had significantly lower TP, CC and BD, while they had significantly higher pH as compared with the control (CS100).

Table 1. Container compacity (CC), air space (AS), total porosity (TP), and bulk density (BD) of the commercial substrate (CS), mixed hardwood biochar (HB), and sugarcane bagasse biochar mixes (SBB).

Trt.	TP (%)	CC (%)	AS (%)	BD (g cm^{-3})	pH	EC (µS cm^{-1})
Poinsettia						
CS100	74±0.3	56±0.2	18±0.5	0.09±0.00	6.8±0.05	2,058±29
HB20	72±0.3*	54±1.2	17±1.5	0.09±0.00	7.6±0.05***	2,022±26
HB40	70±0.5*	52±1.0	18±0.6	0.11±0.00**	8.2±0.01***	1,457±11***
Pepper						
SBB10	73±0.1	61±1.7	13±1.6	0.10±0.00	6.6±0.03	1,065±72***
HB10	72±0.3	54±1.2	17±1.5	0.09±0.00	7.5±0.04***	1,960±18
HB30	70±0.5	52±1.0	18±0.6	0.11±0.00**	7.9±0.03***	1,830±32
HB50	68±3.0*	50±1.2*	18±4.0	0.12±0.00***	8.0±0.08***	1,575±178**
HB70	68±0.8*	47±1.5***	21±2.0	0.13±0.00***	8.4±0.10***	1,395±67***
Suitable range[z]	50-80	45-65	10-30	0.19-0.7	5.4-6.5	<1,500

Note: Numbers after CS, SBB, and HB indicated the ratio of different components, by vol. *, **, and *** indicates significant difference from the commercial substrate (CS100) according to Dunnett's test at $p \leq$ 0.1,0.05, and 0.01, respectively. [z]Recommended physical properties of container substrate by (Nelson, 2012); Yeager et al. (2007).

Disease parameters

Disease symptoms of *Pythium* poinsettia root rot appeared in transplants in all the treatments at 5 days after inoculation (Fig. 3. A). Compared with CS100 treatments, HB20 treatments maintained a low disease severity throughout the experiment and reduced the disease severity at 5, 10, 15, 20, and 25 days after inoculation by 10.9%, 10.9%, 18.8%, 21.9%, respectively.

The application of *Trichoderma* did not significantly reduce disease severity throughout the experiment (Fig. 3. B). Also, HB20 treatments reduced disease incidence by 31.3% starting at 5 days after inoculation (data not shown).

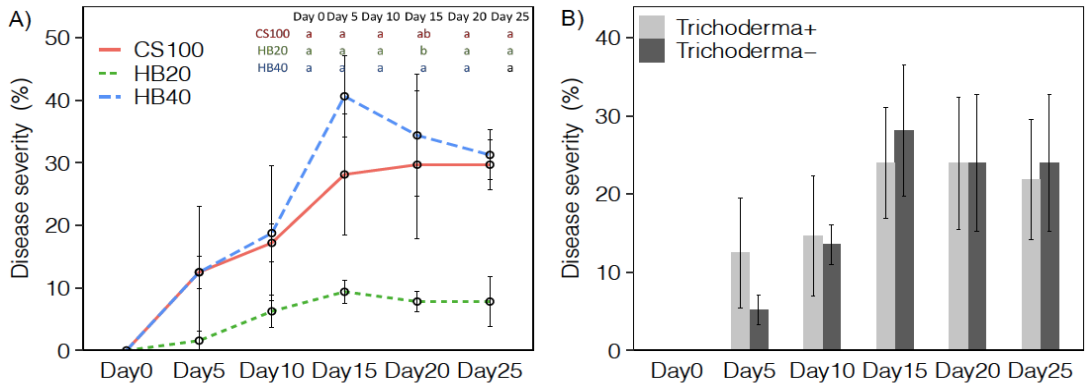

Figure 3. The effect of biochar rates (A) and *Trichoderma* (B) on disease severity for pathogen inoculate poinsettia plants. CS100 = peat moss-based commercial substrate, HB20 and HB40 = 20% and 40% (by vol.) mixed hardwood biochar-amended mixes, respectively. The same letter indicates not significantly different from each other according to LSD multiple comparison test at $p \leq 0.05$ on the same day.

Biochar mixes had significant impacts on pepper plants disease severity, especially HB-amended (30%-70%) mixes (Fig. 4. A). Compared with CS100 treatment, HB50 and HB70 treatments reduced disease severity at 12 days after transplanting by 10.94% and 10.16%, respectively. The HB 50 and HB 70 also significantly reduced disease incidence by 25.0%, respectively (data not shown).

The application of *Trichoderma* did not significantly reduced disease incidence the entire experiment (Fig. 4. B). All the BC-amended mixes had significantly lower AUDPC values (except for HB10) than the CS100 (data not shown).

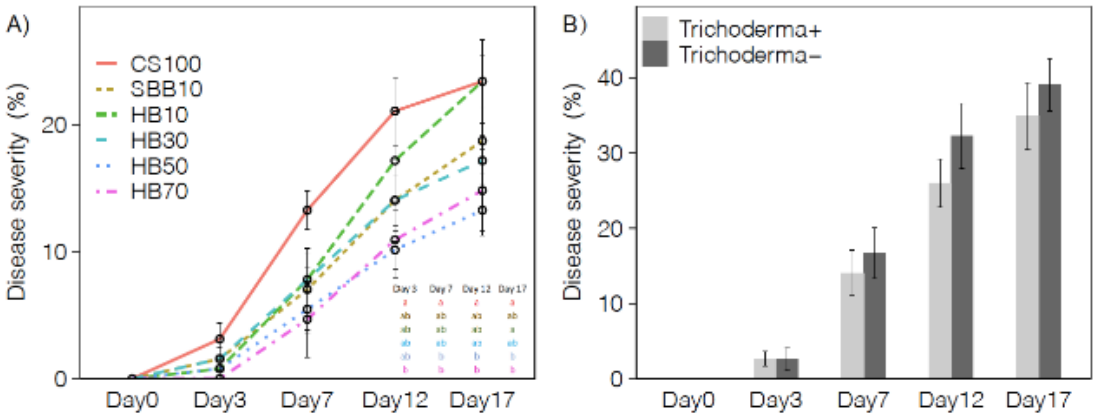

Figure 4. The effect of biochar rates (A) and *Trichoderma* (B) on disease severity for pathogen-inoculate treatments. SBB = Sugarcane bagasse biochar, HB = Mixed hardwood biochar, CS = Peat moss based commercial substrate. Numbers after CS, SBB, and HB indicated the ratio of different components, by vol. The same letter indicates not significantly different from each other on the same day according to LSD multiple comparison test at $p \leq 0.05$.

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