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Biochar reduces containerized pepper blight caused by *Phytophthora Capsici*

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Phytophthora **blight caused by *Phytophthora capsici* is a serious disease affecting a wide range of plants. Biochar as a soil amendment could partially replace peat moss and has the potential to suppress plant diseases, but its effects on controlling *phytophthora* blight of container-grown peppers have less been explored, especially in combination of biological control using *Trichoderma*. In vitro (petri dish) and in vivo (greenhouse) studies were conducted to test sugarcane bagasse biochar (SBB) and mixed hardwood biochar (HB) controlling effects on pepper *phytophthora* blight disease with and without *Trichoderma*. Sugarcane bagasse biochar and HB were blended with the commercial substrate (CS, peat-based) at 10% (SBB10, by volume), and 10%, 30%, 50%, 70% (HB10, HB30, HB50, and HB70, by volume), respectively, and CS (CS100) was used as the control. Both in vitro and in vivo studies used randomized complete block design with three treatment factors: pathogen (without or with inoculation of *P. capsici*), biochar (different biochar treatments), and *Trichoderma* (without or with inoculation). In vitro results showed that *Trichoderma* inhibited *P. capsici* growth while biochar did not have significant beneficial effects. In vivo results showed that plants grown in HB30 and HB50 had similar or higher plant growth index and shoot dry weight than the control regardless of pathogen presence. In the presence of the pathogen, plants grown in HB30, HB50, and HB70 had significantly lower disease severity, and disease incidence ratings than the control, while *Trichoderma* did not show beneficial effects on controlling the disease. In conclusion, HB replacing 30% and 50% peat moss in substrate could reduce pepper blight disease caused by *P. capsici* without negatively affecting plant growth.**

Keywords Disease incidence, Disease severity, Growth index, In vitro and in vivo, Pathogen inhibition, *Trichoderma*

Phytophthora capsici is a hemi-biotrophic fungal-like oomycete pathogen causing destructive disease across a diverse range of crops in *Cucurbitaceous*, *fFbaceous*, and *Solanaceous* families[1]. *Phytophthora* blight of pepper caused by *P. capsici* is one of the gravest soil-borne diseases affecting pepper production globally[2]. The symptoms of the disease appear on main stem close to the soil line as small brown (early infection) to dark purplish (late infection) water-soaked lesions[3]. Under moist conditions, the disease could affect the whole plant from roots, crown, foliage, to fruit at any growth stages[4,5].

Biochar is a carbon-rich by-product derived from a wide variety of organic materials, involves the thermochemical decomposition of biomass under specific time, temperature, and oxygen-depleted or oxygen-limited conditions[6,7]. Biochar has been used as a soil amendment due to its positive effects on altering the biological and physicochemical properties of soils[8], it also has the potential use as a substrate in containers to improve substrate water and nutrient holding capacity, ameliorate acidity, and provide suitable environments for plant growth[9,10]. Studies had shown that biochar could partially replace peat moss-based substrate for greenhouse ornamental and vegetable plants production[11,12] or used as an additional amendment in substrate to improve crop growth, yield, and quality[13–16].

Biochar has shown the potential to suppress soil-borne diseases, such as *Fusarium* root rot (caused by *Fusarium oxysporum*) of asparagus, bacterial wilt (caused by *Ralstonia solanacearum*) of tomato, damping-off and root rot (caused by *Rhizoctonia solani*) of cucumber and some ornamental crops[17,18]. Studies have also demonstrated that

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amended soil with biochar suppressed the pathogen abundance of *Phytophthora* blight of pepper: amended loam and clay loam soil with 3% (w/w) softwood-derived biochar (pH 6.5) improved root and shoot development of sweet pepper that were infested with *P. capsici*, the population and percentages of root infection by *Phytophthora* spp. were reduced[19]; amended sandy loam soil with 1.33% (w/w) corn straw-derived biochar (pH 9.7) before transplanting reduced the disease index of *Phytophthora* blight of pepper and the population of *P. capsici* while increased the abundance of beneficial bacteria, such as *Bacillus* spp., *Pseudomonas* spp. and *Streptomyces* spp[2] ; their following study using the same setup further illustrated the suppression of pepper blight could also due to the improved abundance of potential rhizosphere-associated biocontrol fungi,enhanced soil organic matter, and available nutrients (P, K)[20]. Interestingly, they found biochar amendment increased the abundance of *Trichoderma* that led to a high control efficacy in *P. capsici*[20].

Trichoderma spp. has been reported as a reliable biological control agent for *P. capsici*: *T. harzianum* was proven to suppress pepper root rot caused by *P. capsici* through antimicrobial substances production[21]; in a vitro test, *T. harzianum* inhibited *P. capsici* by 65.3%[22]; similarly, *T. harzianum*, *T. viride* and *T. reesei* displayed over 85.5% inhibition of mycelial growth of *P. capsici*[23], and *T. longibranchiatum*, *T. asperellum* showed the inhibition on *P. capsici* up to 22.5%[24].

The synergistic use of external *Trichoderma* with biochar could strengthen the overall controlling effects on *P. capsici*. However, studies using biochar to control *P. capsici* were mostly conducted using sandy, loam, or clay soils with low amount of biochar amendment (less than 3% based on weight), while the higher percentage use of biochar to replace soilless substrate (e.g., peat moss) and reduce the severity of pepper blight in container production has been less reported. Our previous study already showed that partially replacing peat moss with biochar could significantly reduce the disease incidence and severity of poinsettia root rot caused by *Pythium aphanidermatum*[25]. Therefore, to evaluate the controlling effects of different types of biochar and *Trichoderma* on pepper blight for container pepper production, we conducted both in vitro petri dish tests and in vivo greenhouse trials. Inhibition of *P. capsici*, disease assessment, and plant growth response were evaluated. This study could provide guidance of using biochar in container soilless substrates production and demonstrate evidence of suppressing soil-borne disease from biochar.

Materials and methods

Biochar-mixed substrate, *Trichoderma*, and pathogen isolation and propagation

Two types of biochar, sugarcane bagasse-derived biochar (SBB; American Biocarbon LLC White Castle, LA, USA) and mixed hardwood-derived biochar (HB; Proton Power Inc. Lenoir City, TN, USA) were used in this study. The SBB was produced using proprietary methods and was provided by USDA-ARS, Sugarcane Research Unit (Houma, LA, USA), it had a pH of 5.9 and an electrical conductivity (EC) of 0.75 dS/m; the HB was a by-product from fast pyrolysis of mixed hardwood and had a pH of 10.1 and an EC of 1.06 dS/m[26,27]. These two types of biochar were separately and thoroughly mixed with the commercial peat moss-based substrates (Jolly Gardener C/20, Oldcastle Lawn & Garden Inc., Atlanta, GA, USA) that contain 80% Canadian Sphagnum peat and 20% perlite. Sugarcane bagasse-derived biochar was mixed with the commercial substrate at a rate of 10% by volume (SBB10), while HB was mixed with the commercial substrate at rates of 10%, 30%, 50%, and 70% by volume (HB10, HB30, HB50, HB70). Commercial substrate alone was also included and used as the control (CS).

The study utilized the Root shield Plus-WP (BioWorks, Victor, NY, USA) that consisted of two active strains of *Trichoderma*, *T. harzianum* strain T-22 and *T. virens* strain G-41, as the biological control agent. Pathogen *Phytophthora capsici* was isolated and identified from an infected pepper plant, followed by pathogen propagation, where *P. capsici* was isolated and maintained in the darkness on a V8 juice agar that is selective for oomycete organisms[30].

In vitro test

Water extracts of substrate

Water extracts of commercial substrate and biochar-mixed substrates were obtained following the method outlined by Gravel, et al.[31]. In summary, commercial substrate and five types of biochar-mixed substrate were separately mixed with deionized water in a 1:1 ratio by volume in 500 mL flasks and agitated for 24 h using a shaker (Orbital; Laboratory Supply Network, Atkinson, NH, USA). The resulting mixtures were filtered through filter papers, and 25 mL of the extracts from each type were collected and sterilized (autoclaved for 1 h) for the in vitro test. An equal amount of sterilized deionized water was used as a control. The potato dextrose agar (PDA) media was therefore produced in petri dishes (100 × 15 mm) by either incorporating the sterilized deionized water (DI water control) or commercial substrate and biochar-mixed water extracts (CS, SBB10, HB10, HB30, HB50, HB70) into a 25% PDA sterilized solution before the media solidified.

Trichoderma treatment and pathogen growth

Half of the PDA petri dishes produced using different water extracts were inoculated with 5 mm square plugs of actively growing *P. capsici* in the center of each petri dish, while the other half petri dishes were introduced with dual confrontation technique for the inoculation of *Trichoderma* and *P. capsici*[32]. Specifically, we placed a drop of *Trichoderma*-containing solution mixed at the recommended rate by the manufacturer (0.30 g/L) opposite to a 5 mm square plug of actively growing *P. capsici* within the petri dish, such that both elements positioned at equal distances from each other and to the border of the petri dish. All the petri dishes were then placed in a dark environment at room temperature. After four days, the radial growth of mycelium was measured, and the inhibition percentage of pathogen growth was then computed using this formula from Nawaz, et al.[23]: Inhibition = ((A1 - A2) × 100%) / A2, where A1 represents the area of pathogen growth in commercial substrate or biochar-mixed water extracts, and A2 represents the area of pathogen growth in the DI water control.

In vivo greenhouse trial

Plant materials, treatment setup, and growth environment

Hot cherry pepper (*Capsicum annuum* cv. 'Capperino') seeds (F1 self-selected seeds from Johnny's Selected Seeds, Fairfield, ME, USA) were pretreated with 10% bleach for 3 min and then rinsed with DI water. Treated-seeds were sown in the commercial propagation media (BM2 Berger; Saint-Modeste, Quebec, Canada) and grown for 3 weeks until the true leaves came out. Uniform seedlings were then transplanted into growing pots with dimensions of 7.5 cm at the top, 6 cm at the bottom, and 8.2 cm in depth, with a total volume of 375 mL. These pots were previously filled with either commercial or biochar-mixed substrates. After transplanting, slow-released fertilizer (15 N-4P-10 K Osmocote Plus; Scotts-Sierra Horticultural Products Company, Marysville, OH, USA) was incorporated with the surface substrate at the rate of 2 g/container.

One week after transplanting, *Trichoderma* treatment was introduced by drenching the solution to the substrate surface at the rate of 0.30 g/L. Pathogen *P. capsici* was inoculated with actively growing mycelium at the rate of 5 plugs with plastic inoculation loops (VWR, Radnor, PA, USA). When conducted pathogen inoculation, a 5-mm diameter agar plug was taken from the margin of an actively growing colony of the pathogen and placed on the surface of the substrate contacting plant stem. Standard propagation trays were placed underneath the pots to create a moisturized environment. All the pots were placed in a *P. capcisi*-permitted greenhouse at Texas A&M University, Sommerville, TX, USA. During the experiment, plants were regularly watered. Inside the greenhouse, the average temperature was 30.2 °C, with a relative humidity of 77.2% and a dew point of 25.0 °C.

Substrate physical and chemical property measurement

These substrates were measured for their physical properties based on Fonteno, et al.[28] used the North Carolina State University Porometer (Raleigh, NC, USA), including bulk density, air space, container capacity, and total porosity. Their chemical properties of leachate electrical conductivity (EC) and pH were measured following the pour-through method[29] with a portable EC/pH meter (Hanna Instrument, Woonsocket, RI, USA).

Plant growth measurements and disease assessment

From 3 weeks after transplanting, weekly measurements were conducted for plant height (PH) and two-sided widths (PW1 and PW2). Plant growth index (GI) was then calculated by: GI = PH/2 + (PW1 + PW2)/4[25]. Pepper plants shoots were harvested and dried at 80 °C for 3 days in the oven at the end of the experiment, shoot dry weight (SDW) was recorded thereafter.

Symptoms of the disease were monitored and documented every 5 days, starting 3 days after inoculating the pathogen. Disease severity was assessed on a scale of 0–4, following the methodology outlined by Wang, et al.[2]. The scales were illustrated in Fig. 1: 0 indicated healthy plants, 1 represented plants with small brown lesions on the stem or slightly wilted leaves, 2 denoted plants with moderate brown lesions on the stem and moderate wilted leaves, 3 indicated plants with large brown lesions on the stem and significantly wilted leaves, and 4 represented dead plants. Disease Severity Index (DS) was calculated using the following formula: $\mathrm{DS} = \sum \left(\frac{\text{number of diseased plants in this index} \times \text{disease index rating from 0 to 4}}{4 \times \text{number of plants investigated}} \right) \times 100\%$[2].

The disease severity obtained at different times after inoculation was used to calculate areas under disease progress curves (AUDPC) following the formula: $\mathrm{AUDPC} = \sum_{i=0}^{n-1} \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i)$. 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[33]. During the trials, Disease Incidence (DI) was assessed by tallying the number of diseased plants in each pot, following the specified formula: $\mathrm{DI} = \frac{number\ of\ diseased\ plants}{number\ of\ total\ plants} \times 100$[34].

Experimental design and data analysis

The in vitro test followed a randomized complete block design (RCBD) with five blocks. Within each block, treatments were randomly assigned to petri dishes based on a 7 × 2 factorial design involving substrate treatments

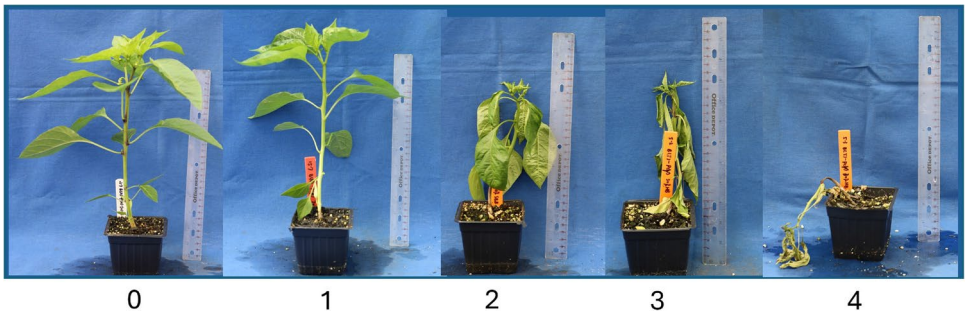

Figure 1. Visual scales used for the pepper blight caused by *Phytophthora capcisi* disease severity rating used in this study. 0 indicated healthy plants, 1 represented plants with small brown lesions on the stem or slightly wilted leaves, 2 denoted plants with moderate brown lesions on the stem and moderate wilted leaves, 3 indicated plants with large brown lesions on the stem and significantly wilted leaves, and 4 represented dead plants.

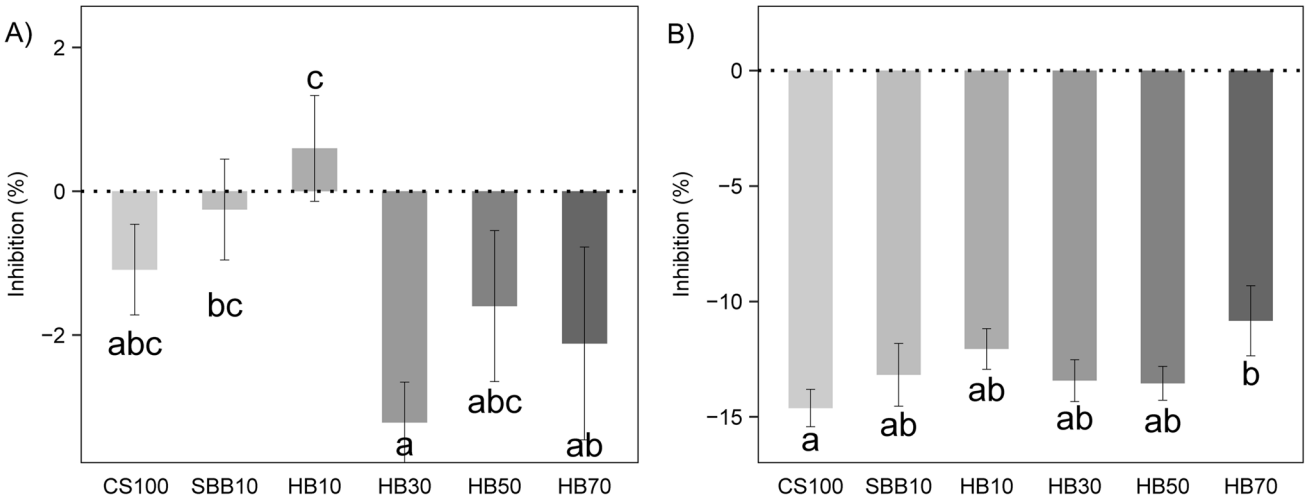

Figure 2. Inhibition percentage of *Phytophthora capsici* from in vitro test ($n = 5$) without (**A**) and with the presence of *Trichoderma* (**B**), values with the same letters are not significantly different based on LSD's multiple comparison test at $p \leq 0.05$. CS100 represents water extract from peat moss-based commercial substrate; SBB10, HB10, HB30, HB50, and HB70 represent water extracts from 10% sugarcane bagasse biochar, 10%, 30%, 50%, and 70% mixed hardwood biochar-mixed substrates (by volume), respectively.

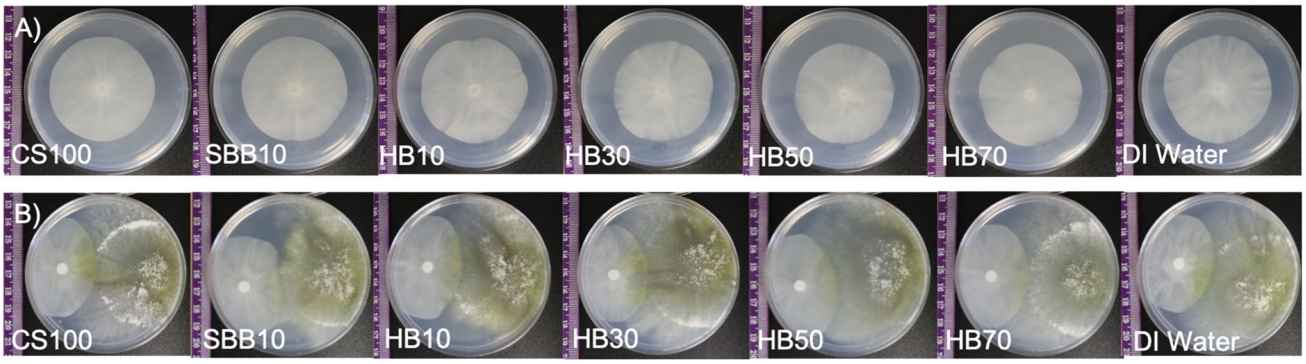

Figure 3. *Phytophthora capsici* grown on potato dextrose agar (PDA) media without (**A**) and with the presence of *Trichoderma* (**B**) after four days in the dark environment and room temperature. CS100 represents water extract from peat moss-based commercial substrate; SBB10, HB10, HB30, HB50, and HB70 represent water extracts from 10% sugarcane bagasse biochar, 10%, 30%, 50%, and 70% mixed hardwood biochar-mixed substrates (by volume), respectively. DI water indicates deionized water as the control.

(water extracts of CS100, SBB10, HB10, HB30, HB50, HB70, and DI water as control) and *Trichoderma* application (with/without).

In the greenhouse experiment, an RCBD with eight blocks was utilized. Each block consisted of a 1.5 m x 1 m area on a raised bench. Treatments were randomly allocated to pots within each block, following a $6 \times 2 \times 2$ factorial design involving substrate treatments (CS100 as control, SBB10, HB10, HB30, HB50, and HB70 as biochar-mixed treatments), *Trichoderma* application (with/without), and pathogen presence (non-pathogen, pathogen-inoculated).

The pathogen growth area in the in vitro test was estimated using Image J, version 1.53a[35]. Data analysis was conducted using one-way analysis of variance (ANOVA) in R program software, version 3.5.1[36]. In the greenhouse trial, treatments with non-pathogens and those inoculated with pathogens were analyzed separately. Means were separated using Dunnett's test when treatments significantly differed from the control at $p \leq 0.05$ or the least significant difference (LSD) when treatments significantly differed from each other at $p \leq 0.05$.

Results

Pathogen growth from in vitro test

Results from the in vitro test showed that without *Trichoderma* (Figs. 2A and 3A), the extracts from biochar-mixed substrate showed no significant difference in inhibiting *P. capsici* growth compared to CS extracts. However, their inhibition rate varies from (0.59% to -3.22% HB10 and HB30, respectively). The positive inhibition rate exhibited from HB10 extracts indicated a stimulation of *P. capsici* growth, while all the negative inhibition values

Substrates	TP (%)	CC (%)	AS (%)	BD (g/cm^3)[b]	pH	EC (dS/m)
CS100	74.3 ± 0.3	55.9 ± 0.2	18.4 ± 0.5	0.09	6.8 ± 0.0	2.06 ± 0.03
SBB10	73.4 ± 0.1	60.8 ± 1.7	12.6 ± 1.6	0.10	6.6 ± 0.0	1.07 ± 0.07***
HB10	71.6 ± 0.3	54.3 ± 0.11.2	17.2 ± 1.5	0.09	7.5 ± 0.0***	0.63 ± 0.03***
HB30	70.1 ± 0.5	52.4 ± 1.0	17.6± 0.6	0.11**	7.9 ± 0.0***	0.66 ± 0.05***
HB50	68.0 ± 3.0*	49.8 ± 1.2*	18.2 ± 4.0	0.12***	8.0 ± 0.1***	0.81 ± 0.05***
HB70	67.8 ± 0.8*	46.5 ± 1.5***	21.3 ± 2.0	0.13***	8.4 ± 0.1***	0.91 ± 0.07 ***
Suitable range[a]	50–80	45–65	10–30	0.19–0.7	5.4–6.5	<1.5

Table 1. Physical and chemical properties of commercial and biochar-mixed substrates: total porosity (TP), container compacity (CC), air space (AS), bulk density (BD), pH and electrical conductivity (EC). 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 volume *, **, 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. Numbers after ± are standard errors. [a]Recommended container substrate properties[37], [b]the standard error of bulk density were all less than <0.00, so we did not include them.

Factors	G-IWK3	GI-WK4	GI-WK5	SDW
Non-Pathogen				
Substrate	*	*	**	**
Trichoderma	***	NS	NS	***
Substrate × *Trichoderma*	NS	NS	NS	NS
Pathogen-inoculated				
Substrate	NS	NS	NS	NS
Trichoderma	NS	NS	NS	NS
Substrate × *Trichoderma*	NS	NS	NS	NS

Table 2. Statistical analysis of pepper plant growth index (GI) from 3, 4, and 5 weeks after transplanting (WK 3, 4, 5) and final shoot dry weight (SDW) as affected by substrate and *Trichoderma* treatment factors under non-pathogen and pathogen-inoculated conditions. NS means not significant. *, **, *** indicate significance at $p \leq 0.05$, 0.01, and 0.001, respectively.

from other mixes, indicated a suppression of the pathogen growth. Among all the pathogen-suppressed biochar extracts, HB30 had a significantly higher inhibition percentage compared with SBB10. When *Trichoderma* was present, all the extracts effectively suppressed *P. capsici* growth, while the biochar-mixed substrate extracts showed no significant difference in inhibiting *P. capsici* growth compared to the CS extracts, except for HB70, which exhibited a relatively lower inhibition percentage (Figs. 2 and 3B).

Substrate physical and chemical properties

Among all the physical and chemical properties, except for the bulk density and pH, all the mixes fall within the recommended range for container substrate properties, as detailed in Yeager, et al.[37](Table 1). The HB50 and HB70 mixes exhibited significantly lower while other mixes had similar total porosity and container compacity compared to the control (CS100). There was no significantly difference in the air space for all the substrates. Except for the SBB10 and HB10 mixes, all the HB mixes had significantly higher bulk density than the control. All the biochar mixes showed significantly higher pH levels than the control except for SBB10 mixes, and all biochar-mixed substrates displayed significantly lower EC levels than the control.

Plant growth from in vivo greenhouse trial

There were no significant interaction effects between substrate and *Trichoderma* treatments on any of the growth parameters regardless the presence or absence of the pathogen (Table 2). For non-pathogen treatments, pepper plant growth index from 3 weeks after transplanting (11.3 and 12.2 for substrate and *Trichoderma*, respectively) and final shoot dry weight (6.05 g and 6.05 g, respectively) were both significantly influenced by substrate and *Trichoderma* treatments. However, plant growth index from 4 to 5 weeks after transplanting was only significantly influenced by substrate treatment (25.5 and 29.9, respectively). Under pathogen-inoculated conditions, none of the factors significantly influenced any of the growth parameters.

Biochar types and application rates did not significantly affect the shoot dry weights of healthy pepper plants (non-pathogen), whether with or without *Trichoderma*, except for HB70 mix, which showed significantly lower biomass compared to the control in both non-*Trichoderma* and *Trichoderma*-treated conditions (Fig. 4). Furthermore, Biochar did not significantly influence the growth index from 3 to 4 weeks after transplanting. However, plants grown under HB30 and HB50 substrates showed significantly higher growth index from 5 weeks after transplanting compared to the control (Fig. 5A). The use of *Trichoderma* did not have a significant

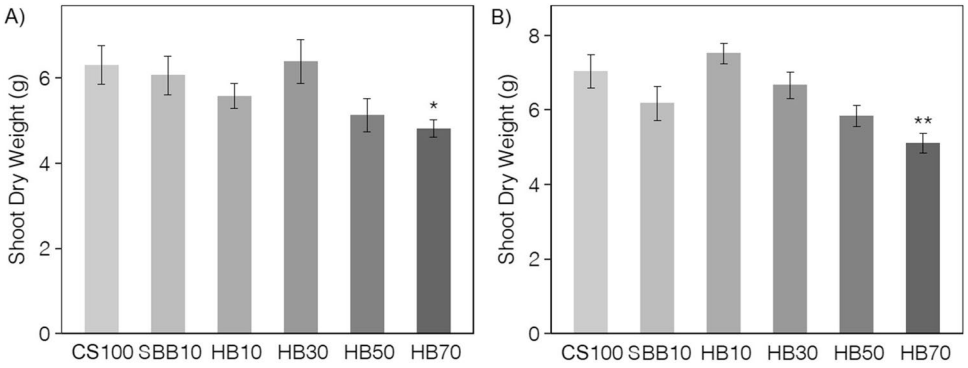

Figure 4. The effect of substrate on pepper plant shoot dry weight under non-pathogen conditions without (**A**) or with (**B**) *Trichoderma* application. CS100 represents water extract from peat moss-based commercial substrate; SBB10, HB10, HB30, HB50, and HB70 represent water extracts from 10% sugarcane bagasse biochar, 10%, 30%, 50%, and 70% mixed hardwood biochar-mixed substrates (by volume), respectively. *, ** indicate significantly different from the control (CS100) according to the Dunnett test at $p \leq 0.05$, 0.01.

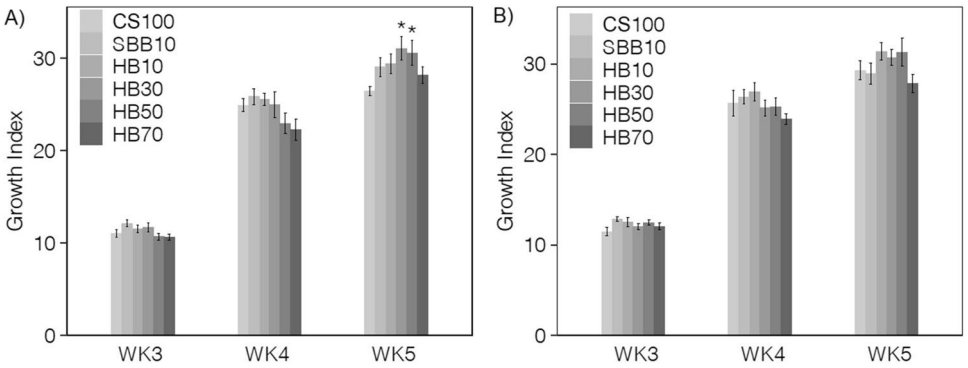

Figure5. The effect of substrate on pepper plant growth index at week 3, 4 and 5 after transplanting (WK3, WK4, and WK5) under non-pathogen conditions without (**A**) or with (**B**) *Trichoderma* application. CS100 represents water extract from peat moss-based commercial substrate; SBB10, HB10, HB30, HB50, and HB70 represent water extracts from 10% sugarcane bagasse biochar, 10%, 30%, 50%, and 70% mixed hardwood biochar-mixed substrates (by volume), respectively. * indicates significantly different from the control (CS100) according to the Dunnett test at $p \leq 0.05$.

impact on any of the growth indexes (Fig. 5B). While under pathogen-inoculated conditions, neither substrate nor *Trichoderma* treatments had significant impacts on pepper plant growth index and biomass accumulation (date not shown).

Disease development from in vivo greenhouse trial

Under pathogen-inoculated conditions, plants grown in all the treatments showed disease symptoms 3 days after transplanting except for HB70, which showed symptoms 7 days after transplanting (Fig. 6A). Compared with CS100 treatment, HB50 and HB70 treatments reduced disease severity at 12 days after transplanting by 10.94% and 10.16%, respectively, and at 17 days after transplanting by 9.59% and 9.59%, respectively. The application of *Trichoderma* did not significantly reduce disease severity during the entire experiment (Fig. 6B).

Biochar-mixed substrates had significant impacts on disease incidence, especially HB-amended (30-70%) mixes (Fig. 7A). Compared with CS100 treatment, HB50, HB70, and SBB10 treatments reduced disease incidence at 7 days after transplanting by 25.0%, 25.0%, and 18.8%, respectively, and at 12 days after transplanting by 25.0%, 18.8%, and 6.3%, respectively. The application of *Trichoderma* did not significantly reduce disease incidence during the entire experiment (Fig. 7B).

All the biochar-mixed substrates had significantly lower AUDPC values (except for HB10) than the CS100. The HB50 and HB70 mixes reduced the AUDPC value by 9.6 and 9.4 respectively (Fig. 8A). The application of *Trichoderma* did not significantly reduce AUDPC during the entire experiment (Fig. 8B).

Discussions

Effects of biochar on controlling pathogen growth and disease development involve both direct and indirect mechanisms. Directly, chemical compounds naturally contained in biochar, such as benzoic acid, ethylene

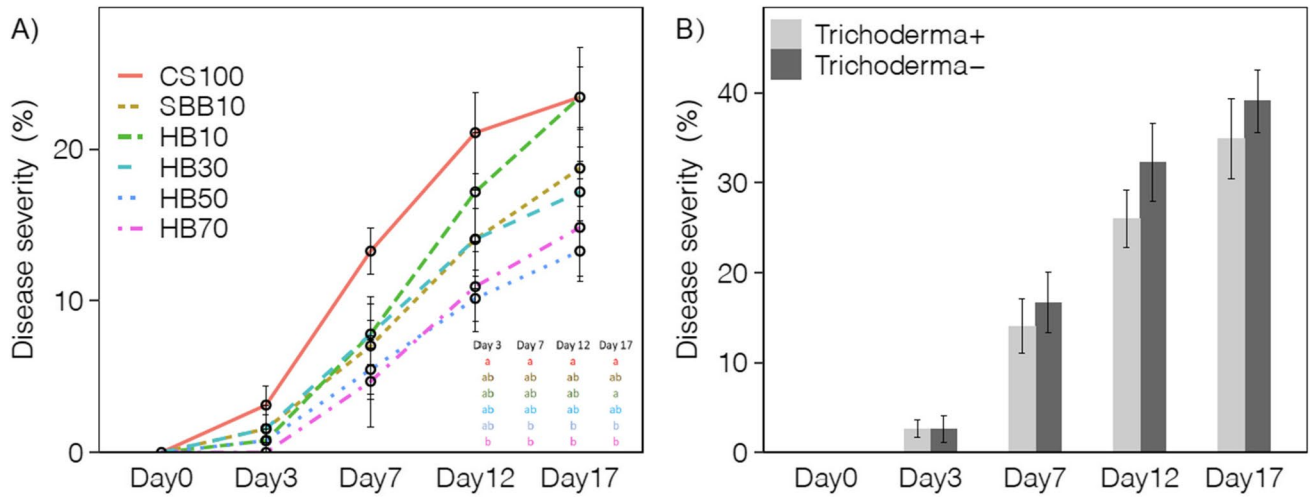

Figure 6. The effect of substrate (**A**) and *Trichoderma* (**B**) treatments on disease severity under pathogen-inoculate conditions. CS100 represents water extract from peat moss-based commercial substrate; SBB10, HB10, HB30, HB50, and HB70 represent water extracts from 10% sugarcane bagasse biochar, 10%, 30%, 50%, and 70% mixed hardwood biochar-mixed substrates (by volume), respectively. The same letter indicates not significantly different from each other on the same day according to LSD multiple comparison test at $p \leq 0.05$.

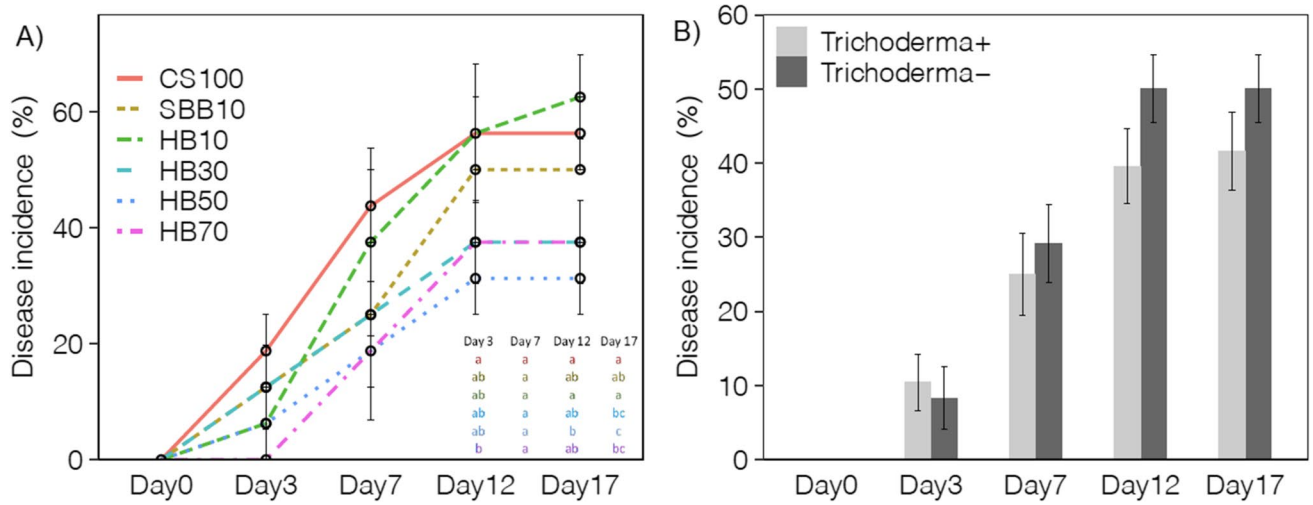

Figure 7. The effect of substrate (**A**) and *Trichoderma* (**B**) treatments on disease incidence under pathogen-inoculate conditions. CS100 represents water extract from peat moss-based commercial substrate; SBB10, HB10, HB30, HB50, and HB70 represent water extracts from 10% sugarcane bagasse biochar, 10%, 30%, 50%, and 70% mixed hardwood biochar-mixed substrates (by volume), respectively. The same letter indicates not significantly different from each other on the same day according to LSD multiple comparison test at $p \leq 0.05$.

glycol, propylene glycol, hydroxypropionic acid, quinones, or some specific phenols and carboxylic acids that generated during the pyrolysis process, these compounds have shown the capacity to inhibit microbial growth; indirectly, synergistic effects from biochar's physical and chemical properties could lead to the beneficial outcomes: biochar absorbs and deactivates toxic metabolites or enzymes (e.g., cell wall degrading enzymes) produced by pathogens, reducing their virulence, boosting plant growth, inducing systemic plant resistance, and enhancing the abundance and activity of beneficial bacteria and fungi[2,18,20,38–40].

In vitro test isolated the physical properties of biochar by only testing the effects of chemical compounds on *P. capsici* development using water extracts from biochar-mixed substrates. Results showed that the extracts from high percentage biochar mixes suppressed *P. capsici* growth when using hardwood as the raw material. Our previous study found that used the water extracts from hardwood-derived biochar led to neutral or negative effects on suppressing pathogen *Pythium aphanidermatum* growth in poinsettia[25], indicated the chemical compounds from the same type of biochar could impose different effects on different soil-borne pathogens. Different types of raw material-derived biochar could also lead to different pathogen controlling effects, as eucalyptus wood and organic waste-derived biochar extracts showed no effects on suppressing *Fusarium* colonization[41]. In the

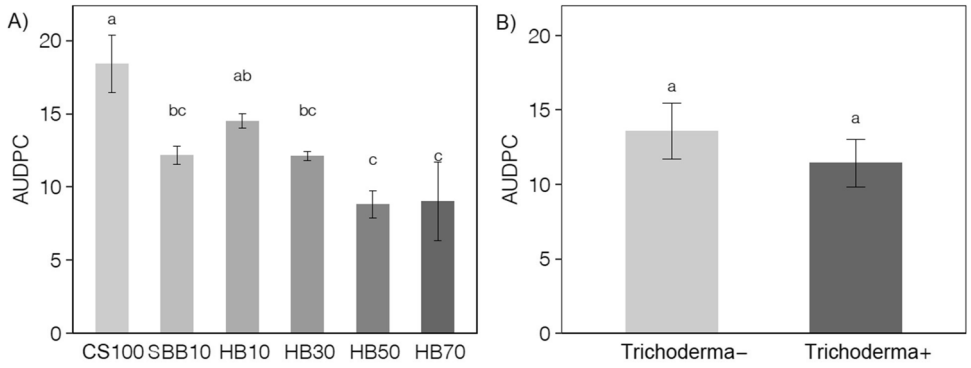

Figure 8. The effect of substrate (**A**) and *Trichoderma* (**B**) treatments on the area under disease progress curve (AUDPC) under pathogen-inoculate conditions. CS100 represents water extract from peat moss-based commercial substrate; SBB10, HB10, HB30, HB50, and HB70 represent water extracts from 10% sugarcane bagasse biochar, 10%, 30%, 50%, and 70% mixed hardwood biochar-mixed substrates (by volume), respectively. The same letter indicates not significantly different from each other on the same day according to LSD multiple comparison test at $p \leq 0.05$.

presence of *Trichoderma*, the inhibiting effects of biochar-mixed extracts on *P. capsici* growth were enhanced due to *Trichoderma*'s biocontrol impact on suppressing the growth of *P. capsici*. This in vitro test showed that synergistically using the chemical compounds extracted from biochar and biocontrol components could provide positive effects on controlling pathogen *P. capsici*.

In general, increasing biochar application rate improved the disease suppression percentage, and using hull, wood, straw and organic waste as the raw material has better controlling effects for disease compared to using bark as the raw material[42]. Results from the in vivo greenhouse study showed that compared with commercial peat-based substrate and sugarcane-derived biochar, using high-percentage hardwood-derived biochar significantly reduced disease incidence, severity, and disease intensity over time (AUDPC) of *P. capsici* induced Phytophthora blight. Although the highest percentage biochar-mix (HB70) significantly decreased the final plant biomass, using a moderate rate of biochar (HB50) maintained and even promoted plant growth index. Biochar application has been reported to improve crop biomass and yield by enhancing soil structure with increased nutrient availability and water holding capacity, immobilize inorganic (heavy metals) and organic contaminants, and reduce abiotic and biotic stresses[43]. Our results have further encouraged the use of biochar as a substitute for peat-based substrates[12], offering additional benefits in suppressing soil-borne diseases due to its chemical and physical properties.

Unlike the in vitro test, *Trichoderma* treatment only numerically decreased the disease development in the greenhouse study. And the interaction effects between biochar and *Trichoderma* were marginally significant, indicating that under the in vivo conditions, other factors could reduce the beneficial effects from *Trichoderma*. In order to better understand the complexed effects of *Trichoderma* and two types of biochar on plant growth and disease development, we employed a principal component analysis (PCA) to illustrate the distinct variables influenced by different treatment factors. For non-pathogen plants, 86% of the variability was explained by the first two components (Fig. 9A). PC1 accounted for 65% variance, with HB50-TN (HB50 without *Trichoderma*), HB70-TN, HB70-TY (HB70 with *Trichoderma*), and CS100-TN differing from the rest of treatments. Biochar-mixed substrate at relatively lower rates (HB10-TN, HB10-TY, HB30-TN, HB30-TY, and HB50-TY) along with CS100 were associated more with the yield (SDW) and GIs (GI WK3, GI WK4, and GI WK5). PC2 accounted for 21% variance, distinguishing the CS100 and SBB10 from HB mixes. Commercial substrate and SBB-amended mixes tended to be affiliated with plant biomass and GI WK3 and GI WK4, however, HB30, HB50 mixes appeared to be related to GI WK5. For pathogen-inoculated plants, 87% of the variability was explained by the first two components (Fig. 9B). PC1 accounted for 63% variance, differing biochar-mixed substrate with *Trichoderma* (except for HB30-TN) and CS100-TY from those without *Trichoderma* treatments. The treatment HB30-TN and CS100 were associated more with the yield (SDW) and plant late growth (GI WK4 and GI WK5). PC2 accounted for 24% variance, distinguishing mixes without *Trichoderma* from those with *Trichoderma*. Commercial substrate and biochar-amended mixes with *Trichoderma* tended to be affiliated with GI WK3 while HB30-TN appeared to be related to GI WK4.

For pathogen-inoculated plants, 94% of the variability was explained by the first two components (Fig. 10). PC1 accounted for 77% variance, differing CS100, HB10, HB70-TN, and SBB10-TY mixes from the rest biochar-amended treatments. The treatments CS100, HB10, HB70-TN, and SBB10-TY were positively associated with all the disease parameters while the rest of the treatments were negatively associated with them. PC2 accounted for 17% variance, distinguishing CS100, SBB10-TY, HB30, HB50-TY and HB70-TY mixes from the rest of the treatments. CS100-TY, SBB10-TY, HB30-TY, and HB30-TN mixes tended to be affiliated with DI1, DS1, DS2 and AUDPC while HB10-TN, HB10-TY, and HB70-TN, appeared to be related to DI2, DI3, DI4, DS3, and DS4. This indicated that *Trichoderma*-treated plant (TY) tended to have an early disease development, while during the plant late growth period, *Trichoderma* positively suppressed disease development and intensity when biochar was present. Although studies testing the effects of combined use of biochar with other materials on pathogen

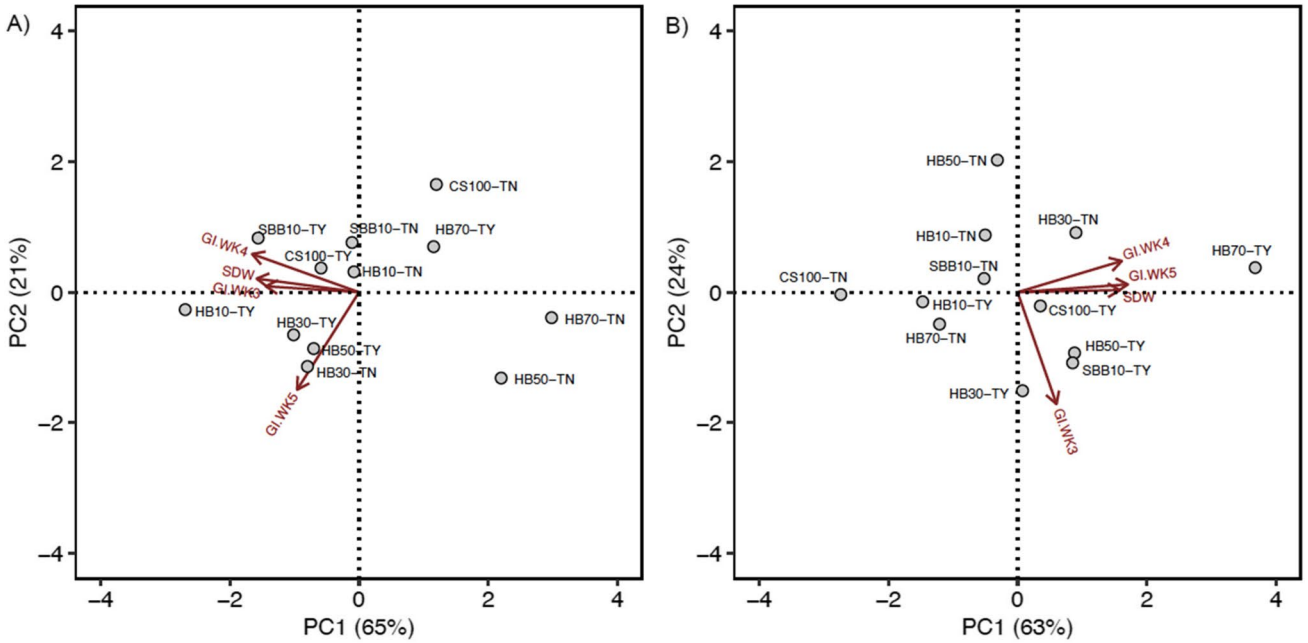

Figure 9. Principal component analysis (PCA) depicting the relationships between selected variables and treatment factors in non-pathogen (**A**) and pathogen-inoculated (**B**) plants. Selected variables are displayed by arrows and include plant growth parameters—growth index after 3, 4, and 5 weeks of transplanting (GI WK3, GI WK4, and GI WK5) and shoot dry weight (SDW). Treatment factors are displayed by filled grey circles: CS100 represents peat-based commercial substrate, SBB10, HB10, HB30, HB50, and HB70 represent biochar-mixed substrates at different rates (by volume) with (TY) or without *Trichoderma* (TN).

suppression have been less reported, promising research had shown that the combined use of compost and biochar could suppress *Rhizoctonia solani* induced disease[44], and the combined use of biochar and arbuscular mycorrhizal fungi could control *Fusarium oxysporum* by altering root exudates[43]. Future studies could focus on exploring more reliable bioactive materials that could be combined used with biochar to promote the synergistic effects.

Conclusions

The mixed hardwood biochar blended with commercial peat moss-based substrate at 50% and 70% (by volume) could significantly reduce *Phytophthora* pepper blight disease severity, incidence, and intensity over time. The chemical compounds contained in biochar could contribute to its disease inhibition capability for *P. capsici* to a certain extent. In the absence and presence of the pathogen, mixed hardwood biochar could replace commercial peat moss-based substrate up to 50% (by volume) for container pepper plant production without negative impact on its growth. Combined use of moderate rate of biochar amendment (up to 50% by volume) and *Trichoderma* could improve plant growth while reducing pathogen *P. capsici* growth.

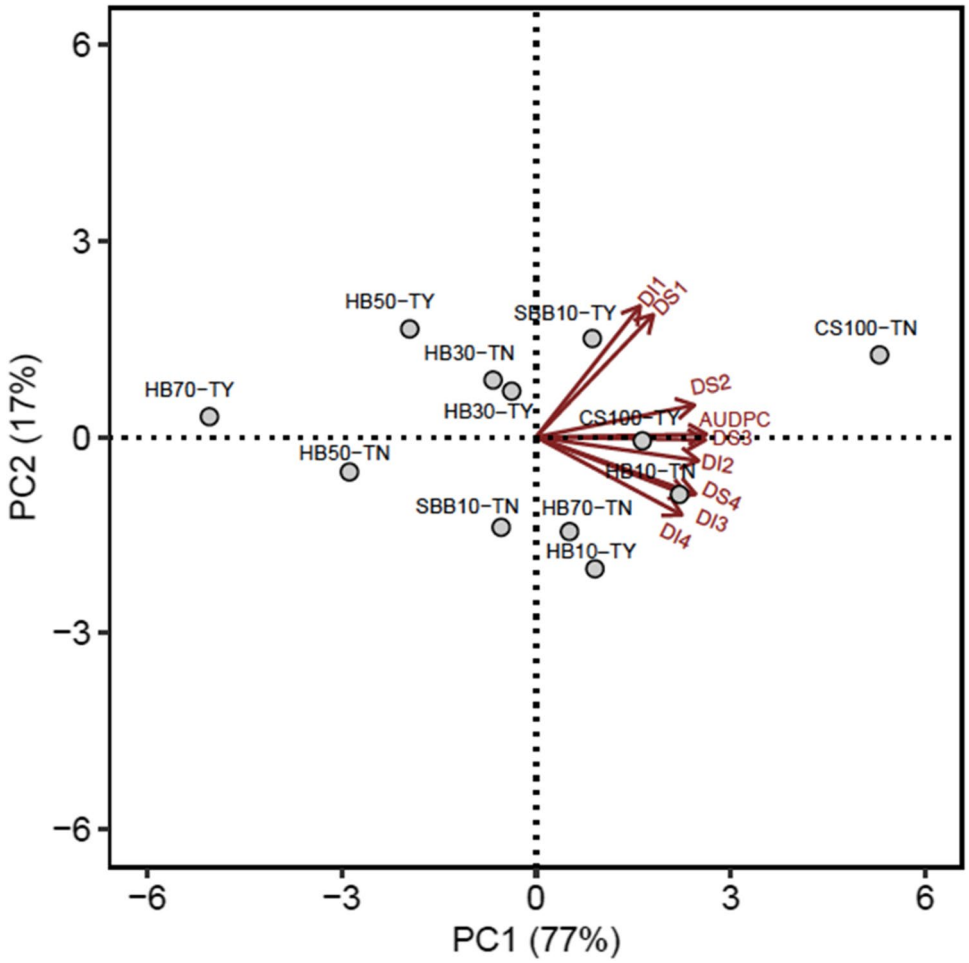

Figure 10. Principal component analysis (PCA) depicting the relationships between selected variables and treatment factors in pathogen-inoculated plants. Selected variables are displayed by arrows and include disease parameters—disease severity after 3, 7, 12, and 17 days of transplanting (DS1, DS2, DS3, and DS4), disease incidence after 3, 7, 12, and 17 days of transplanting (DI1, DI2, DI3, and DI4), and area under disease progress curve (AUDPC). Treatment factors are displayed by filled grey circles: CS100 represents peat-based commercial substrate, SBB10, HB10, HB30, HB50, and HB70 represent biochar-mixed substrates at different rates (by volume) with (TY) or without *Trichoderma* (TN).

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

This work was a product of the combined effort of all the authors. All authors conceptualized and designed the study. PY performed the experiments, collected and analysed the data, and wrote the manuscript with assistance from all other authors, mainly MG. KQ, KC and KO provided technical advice and assistance when the study was conducted and revised and improved the manuscript. TG revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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