BIOCHAR AS A WAY TO INCREASE ENVIRONMENTAL FRIENDLINESS FOR POTTED PLANTS PRODUCTION AND DISEASE SUPPRESSION

A Dissertation

by

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ABSTRACT

Greenhouse production uses large amount of peat moss, which causes environmental and economic concerns. Using biochar (BC), a by-product of pyrolysis, to replace peat moss for greenhouse production could potentially address peat moss' environmental and economic concerns. Five experiments were conducted to test the potential of two types of BCs (the sugarcane bagasse biochar (SBB) and mixed hardwood biochar (HB)) as replacement of commercial substrates (CS) at different rates (by vol.) for tomato and basil seedling and plants production; the effects of the BCs, composts (vermicompost (VC), chicken manure (CM)), and mycorrhizae on fertilizer use reduction; and the effects of the BCs and *Trichoderma* spp. on *Phytophthora capsici* (pepper) and *Pythium aphanidermatum* (poinsettia) suppression. Plants grown in the CS were used as the control. Plants growth parameters such as soil-plant analyses development (SPAD) values, growth index (GI), total dry weight (TDW) were measured and disease parameters including disease severity (DS), disease incidence (DI), and area under disease progress curve (AUDPC) were measured.

The results showed that tomato and basil seedlings from all the BC mixes (except SBB30) had similar SPAD and GI to the control. Tomato and basil plants grown in the BC mixes had a similar GI, SPAD, and yield to the control. Tomato and pepper plants grown in the HB-VC mixes had similar SPAD, GI, and TDW to the control. Pepper plants grown in 30%, 50%, and 70% HB and poinsettia plants in 20% HB had significantly lower DS, DI, and AUDPC for *P. capsici* and *P. aphanidermatum*, respectively.

In conclusion, the HB70 mix can be successfully used for tomato and basil seedling production without negative effects on plant biomass; the HB can replace CS at 50% and the SBB at 70% for both tomato and basil plant growth without negative effects; the HB (≤ 70%) amended with VC mixes can reduce fertilizer use in tomato and pepper production without negatively affecting plant growth; HB30, HB50, and HB70 mixes can reduce pepper blight disease caused by *P. capsici* and HB20 can reduce poinsettia root rot disease caused by *P. aphanidermatum*.

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NOMENCLATURE

BC	Biochar
SBB	Sugarcane Bagasse Biochar
HB	Mixed Hardwood Biochar
SPAD	Soil-Plant Analyses Development
GI	Growth Index
NO_3–N	Nitrate Nitrogen
1 WAT	First Week After Transplanting
VC	Vermicompost
CM	Chicken Manure
TDW	Total Dry Weight
SDW	Shoot Dry Weight
GHGs	Greenhouse Gases
CO_2	Carbon Dioxide
N_2O	Nitrous Oxide
CH_4	Methane
C	Carbon
N	Nitrogen
CC	Container Compacity
TP	Total Porosity
BD	Bulk Density

AS	Air Space
EC	Electrical Conductivity
CEC	Cation Exchange Capacity
P	Phosphorus
K	Potassium
UK	United Kingdom
US	United States
PS	Particle Size
MC	Microorganisms
PB	Pinewood Biochar
CS1	Peat Moss-Based Commercial Substrate for Plant Growing
CS2	Peat Moss-Based Commercial Substrate for Plants Propagation
PCS	Pine Bark-based Commercial Substrate
N/A	Not Applicable
NH_4^+	Ammonium
NO_3^-	Nitrate
$H_2PO_4^-$	Dihydrogen Phosphate
HPO_4^{2-}	Hydrogen Phosphate
CAGR	Compound Annual Growth Rate
PAHs	Polycyclic Aromatic Hydrocarbons
P	Perlite (depends on the chapter context)
PM	Peat Moss

CS	Commercial Propagation Substrate (depends on the chapter context)
DI Water	Deionized Water
EP	Emergence Percentage
TFW	Total Fresh Weight
RDW	Root Dry Weight
LDW	Leaf Dry Weight
FDW	Flower or Fruit Dry Weight
CS	Commercial Bark-based Growing Mix (depends on the chapter context)
CS	Commercial Peat Moss-based Substrate (depends on the chapter context)
MC	Mycorrhizae
F	Fertigation
PCA	Principal Component Analysis
HSD	Honest Significance Difference
USDA	United States Department of Agriculture
USGS	United States Geological Survey
DS	Disease Severity
DI	Disease Incidence
PDA	Potato Dextrose Agar
AUDPC	Areas Under Disease Progress Curves

RCBD	Randomized Complete Block Design
LSD	Least Significant Difference
T	*Trichoderma*

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1.1. Introduction

Peatland ecosystem disturbance presents enormous challenges to the environment. Peatland ecosystem disturbance is mainly caused by peat moss harvesting, which causes the emission of three major greenhouse gases (GHGs), carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4), speeding global warming [1, 2]. Among the 17 United Nations Sustainable Development Goals, 8 goals are closely related to ecosystem interference and global warming [3]. Urgent actions need to be taken to deal with the peatland disturbance.

Peatlands only cover around 4% of the earth land area, but they are essential ecosystems to regulate CO_2, N_2O, and CH_4 [4]. Peatlands are the largest natural terrestrial carbon (C) sinks, which can store ~644 Gt of C [5, 6] or 21% of the global total soil organic C stock of ~3000 Gt [7]. Also, peatlands are large organic nitrogen (N) storages [8]. Northern peatlands, cover 3.7 million km^2 of the land area and store 17 Gt N [9]. Additionally, peatlands regulate CH_4 emission. In the peatland system, up to 90% of biologically produced CH_4 is consumed before being released into the atmosphere in this environment [10].

However, peatlands mining and drainage for horticultural and other purposes for centuries have turned peatlands from GHGs storages to GHGs emitters [11]. The damaged peatlands contribute about 10% of GHGs emissions from the land use sector, and CO_2 emissions from the drained peatlands are estimated at 1.3 Gt CO_2 annually, which is equivalent to 5.6% of global anthropogenic CO_2 emissions [4].

Peat moss has long been used in horticulture as a container substrate, but due to the damage caused to peatland for its harvesting, it's urgent to find a suitable peat moss replacement [12, 13]. Biochar is a sustainable carbon-rich material with porous structure produced by the thermo-chemical decomposition of biomass in an oxygen depleted or oxygen-limited atmosphere [14-16]. Previous studies showed that biochar presents promising potential as a peat moss replacement to mitigate environmental issues [12, 17].

Replacing peat moss with biochar as a container substrate brings both environmental and economic benefits [18]. Using biochar as a peat moss placement protects peatland from further drainage for peat moss harvesting, thus protected peatlands ecosystems and reduced GHGs emissions [19-22]. Biochar could increase water and nutrient use efficiency, reduce fertilizer and pesticide runoff, thus reducing negative environmental impacts and economic costs [12]. In addition, using biochar as a container substrate leaded to equivalent plant yield, improving the economic benefits for the industry [17, 23, 24].

Although the number of biochar-related publications increased from 87 (date not shown) to 17,801 in the past two decades (Figure 1.1), studies are still needed specifically on biochar replacing peat moss as a container substrate to benefit the environment. In this

review, we looked at biochar replacing peat moss as a container substrate to tackle with environmental issues. The potential economic values and challenges of replacing peat moss with biochar as a container substrate are also discussed in this study.

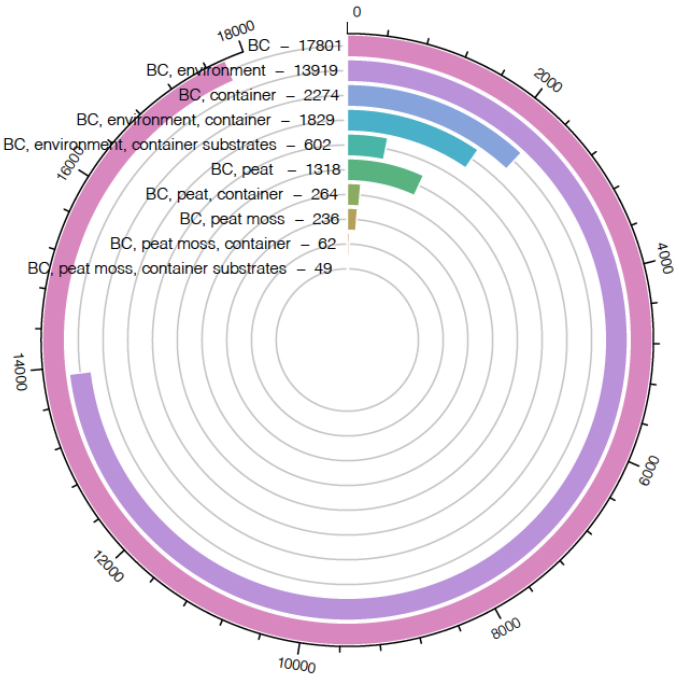

Figure 1.1. Circular bar-plot indicating the number of biochar (BC)-related articles published from 2010~2021 based on key words searching in Science Direct data base.

1.2. Peat Moss and Peatland Ecosystem

1.2.1. Peat Moss Used as a Container Substrate

1.2.1.1. Properties of peat moss as a container substrate

Within a substrate, three types of pore volumes may be present, micropores, macropores, and mesopores, determining nutrient and water flow [25]. Micropores refer to the intra-aggregate spaces less than 2 nm in diameter, responsible for water retention [26]. Macropores consist mostly of interaggregate cavities with a pore size greater than 50 nm in diameter, serving as the major pathways for the infiltration and drainage of water and for aeration [25, 26]. Mesopores refer to pore size between 2-50 nm in diameter, are effective as adsorptive media for liquids while macropores transport adsorbates to the micropores [26].

Container substrate physical properties include air space (%), container compacity (CC, %), total porosity (TP, %), bulk density (BD, $g.cm^{-3}$), and water holding capacity [27, 28]. Air space (AS, %) measures the proportion of macropores after drainage, influencing gas exchange and water holding capacity. Container capacity is the maximum amount of water a substrate can hold after wetting and drainage, determined by micropores and macropores [25]. Total porosity equals container capacity plus air space. Bulk density measures how much one unit of the substrate weighs. Water holding capacity measures a certain type of container substrate's ability to physically hold water against gravity, its maximum value equals container capacity [27, 28].

Pore size distribution correlated to moisture retention curves directly, affecting nutrient and plant performance [25]. For instance, Drzal's study showed that because peat

moss-based substrate contained 11% macropores, more than bark substrate (7%), the nutrient leaching from peat moss-based substrate was larger than bark substrate [25]. Also, since peat moss-substrate had more micropores (9%) than bark (<1%), the wilting and tissue death for plant growing in peat moss-substrate occurred less rapidly under moisture stress than those grown in bark mixes [25]. Similarly, incorporating 10% of perlite into a 60% sphagnum peat and 30% black peat substrate increased the macropores percentage, making it the best substrate for Begonia growth among other substrates tested in Londra, Paraskevopoulou [29] study.

Container substrate chemical properties include pH, electrical conductivity (EC, mS cm^{-1}), and cation exchange capacity (CEC, meq $100g^{-1}$). pH is a measure of the acidity or alkalinity of a container substrate, which determines the availability of nutrients to plants [27, 28]. Electrical conductivity measures all electrical charged ions dissolved in water while CEC measures the total capacity of a substrate to hold exchangeable cations [27, 28].

Peat moss has long been the most widely used container substrate for greenhouse plant production because it has suitable properties such as AS (%), TP (%), and BD ($g \cdot cm^{-3}$), pH, EC (mS cm^{-1}), and CEC (meq $100g^{-1}$) [27, 30]. Suitable properties of peat moss allow it to support plants, hold nutrients, retain water, and change gases [27, 28].

Despite its many suitable properties as a container substrate, peat moss could have rewetting and hydrophobicity issues [31, 32]. The drying process during commercial peat moss production made it hydrophobic and caused rewetting issues [31-33]. As an organic material, peat moss breaks down during greenhouse practices, which changes its

hydrophobicity intensity and causes rewetting issues [34, 35]. Also, after dried out, when the moisture content decreases below 20%, peat moss requires a longer time to rewet as it becomes more hydrophobic [36].

1.2.1.2. Peat moss driven environmental concerns

Harvesting peat moss from peatland has interfered peatland's ecological functions [8]. For instance, peat moss harvesting reduced peatland C capacity, thus hindered its climate change mitigation capacity [37]. Also, harvesting peat moss disturbed N and CH_4 cycles [38]. Additionally, peatland disturbance caused by peat moss harvesting may bring challenges to the native animals, making it harder for them to find new habitats, thus reduce ecosystem biodiversity [37].

Besides interfering with peatland's ecological functions, peat moss, as a container substrate, also creates environmental concerns due to nutrient runoff [32, 36]. In a common nursery production, a 15% leaching fraction was recommended to prevent the buildup of soluble salts in the container substrate [39]. However, extensive irrigation, fertilizers, and pesticides were more often applied to containers to reduce the risk of crop failure [40]. Plants can only use 50% of nitrogenous fertilizers applied even under ideal conditions [41, 42]. Thus, the excessive N, phosphorus (P), and potassium (K) were lost through runoff, causing environmental concerns such as eutrophication, dead zones, and algal blooms [42-44].

Container peat moss substrate-derived pesticide runoff contributes to environmental issues too [45]. Because of the low irrigation efficacy (80% of water runoff) in container production, highly soluble pesticides such as acephate, glyphosate, and

mefenoxam are likely to dissolve and move with runoff water to a containment water body [46]. A 10-year survey of major streams and groundwater found that 97% of stream water and 61% of shallow groundwater near agricultural areas had one or more pesticides present [47].

1.2.2. Challenges of Peat Moss as a Container Substrate

Peat moss encounters production challenges. The total volume of global peatlands has been decreased at a rate of 0.05% annually owing to harvesting and land development [48]. Peat production was estimated to have decreased in 2019 in some peatland-rich countries [48]. Several European countries including Belarus, Ireland, and Sweden, were planning or implementing peatland restoration projects, reducing peat production across Europe in the future [49]. In Canada, among the total of 27, 615 ha peat moss production areas, more than 31% has been or is currently restored or reclaimed, with another 3% converted to other land-use [50].

Peat moss also encounters legislation challenges [30]. For instance, the United Kingdom (UK) and Europe have legislated laws in order to protect the peatland from being over harvested [49, 51]. Also, Ireland announced its plan to stop all peat harvesting by 2028 in 2019 [52]. In the same year, Finland announced its goal to become carbon neutral by 2035 by phasing out peat production [52].

1.2.3. Peatland Ecosystem

Peatlands are natural waterlogged peat-forming ecosystems, where at least 30 cm of peat moss have accumulated, with water weight accounting for 90%~95% of peat moss weight [31]. Besides peat moss accumulation, anaerobic and organic carbon, nitrogen,

sulphur, and phosphate are also accumulated in peatlands [48]. Peatland formation is a two-stage process, a minerotrophic stage (fen) and followed by the ombrotrophic stage (bog) [31]. Bogs are nutrient-poor low species diverse systems and are often dominated by a few sphagnum species because it receives water and nutrients solely from rain and snow [53].

Peatlands provide vital ecological services such as storing C and N, regulating water, providing habitats, supplying food, and preserving information [54]. Peatlands occupied around only around 4% of the terrestrial surface but stored around 644 Gt of C or 21% of the global total soil organic C [8]. With a well grown sphagnum peatland, one single sphagnum farming site takes up N at 35~56 kg ha^{-1} yr^{-1} [48]. By regulating water flows, peatlands help minimize the risk of flooding and drought and prevent seawater intrusion [55]. Peatland provides rare habitats for different wild animals [37]. In many parts of the world, peatlands supply food, fiber and other local products that sustain local economies. Peatlands also preserve important ecological and archaeological information such as pollution records and human artefacts [4].

1.2.4. Peatland Ecosystem Disturbance

Harvesting peat moss from peatlands led to contaminated water and reduced biodiversity [56]. Peatland extraction reduced surface and groundwater quality, and increased land compaction [48]. Also, 15% of global peatland habitats have been lost due to peat extraction [57]. The loss of the Bornean Orangutan's peat swamp habitats is largely responsible for its population decline within a sixty-year period [4, 58].

Moreover, peat moss harvesting degraded peatland and reduced peatland areas. In Germany and Netherlands, peatlands have degraded almost all of the domestic peatland area, with the degradation percentages of 98% and 95%, respectively due to the extensive peat moss harvesting [57]. Peatland drainage has reduced the global peatlands area by 10%~20% since 1800 [59]. Peatland area in Estonia has declined from 22% coverage of the country to only 5.5% for the past decade [56, 60]. In Ireland, around 84% of ombrotrophic peatlands (bogs) have been affected by peat extraction [61].

1.2.5. Peatland Ecosystem Disturbance and Global Warming

Peatland disturbance not only contaminated water, reduced peatland biodiversity and area, but more importantly, turned C-sink into C-emitter, worsening global warming [62]. The drained peatlands cover only 0.4% of the land surface, but they account for 32% of cropland and around 5% of anthropogenic GHGs emissions globally [63, 64]. Large scale peatlands drainage caused CO_2 and N_2O emissions more than 2 Gt CO_2-eq yr^{-1}. Indonesian peat swamp forest fire caused by peatland drainage in 2015, for example, emitted nearly 16 million tons of CO_2 a day, more than the daily emissions from the entire United States (US) economy [4]. If the peatland extraction trend continues, the cumulative of GHGs CO_2 equivalent emission would reach to 249 Gt by 2100 [59].

Unlike being a natural C-sink, peatland is a natural CH_4-emitter. CH_4, which is the second most significant GHGs after CO_2, has a 34-fold stronger effect on global warming than CO_2, and accounts for 20% of the global warming effect [65, 66]. Peatlands, along with other wetlands, contribute around 23% of the total CH_4 budget of 500 to 600 tera gram ($1Tg=10^{\wedge 12}$ gram) per annum [67]. Without peatland disturbance, 10% biologically

produced CH_4 emits from peatland with up to 90% being consumed due to activities of methanogens and methanotrophs [10]. Methanogens responsible for CH_4 production, while methanotrophs for CH_4 consumption [68-70].

Peatland drainage disturbed methanogens and methanotrophs activities, thus increasing CH_4 emissions [69]. The drainage of peatland and other extraction of gas and fossil fuels accounts for roughly a third of CH_4 emissions [71]. In the past decade, the total CH_4 emissions increased from 334 Tg yr^{-1} to 366 Tg yr^{-1} [72]. During peatland mining process, inorganic compounds such as P, K, and Na were removed, leaving a nutrient-deficient environment for the microorganisms, reduced methanotrophs amounts, favoring CH_4 production more than oxidation, making peatland a larger CH_4 emitter [73].

1.3. Biochar Replacing Peat Moss as a Container Substrate

1.3.1. Biochar Properties as a Container Substrate

As a container substrate, the recommended physical properties including TP, CC, AS, and BD are 50~80%, 45~65%, 10~30%, and 0.19~0.7 g cm^{-3}, respectively [28]. For chemical properties, the ideal pH, EC (mS cm^{-1}), and CEC (meq $100g^{-1}$) ranges for plants are 5.4~6.5, <1.5, and 6~15, respectively [27, 28]. The microorganisms in peat moss, biochar, perlite are negligible yet vermicompost and chicken manure could contain many types of microorganisms including bacterial, fungi, and nematodes [74, 75].

Biochar presents similar favorable properties to peat moss as a container substrate. Table 1.1 compared several biochar and peat moss and/or peat moss-based substrates used in containers. Pinewood biochar, mixed hardwood biochar, and sugarcane bagasse biochar used in our previous studies had similar TP (74~85%), AS (3~34%), and BD (0.09~0.17

g cm^{-3}) to peat moss (83%, 19%, and 0.08 g cm^{-3}, respectively) and peat moss-based commercial substrate (71~78%, 3~20%, and 0.11 g cm^{-3}, respectively) [17, 18, 24, 76, 77]. Although biochar properties vary widely depending on feedstocks and production conditions, aforementioned several types of the biochars' physical properties could fall into the recommendation range either by itself or by combining it with other container components such as perlite, peat moss, peat moss-based substrate, bark-based substrate [13].

Table 1.1. The physical properties including total porosity (TP, %), container capacity (CC, %), air space (AS, %), bulk density (BD, g cm^{-3}), and particle size (PS, mm); chemical properties including pH, electrical conductivity (EC, mS cm^{-1}), cation exchange capacity (CEC, meq $100g^{-1}$) and biological properties (microorganisms, MC) of several types of biochar and peat moss-based commercial substrate from our previous studies.

Properties	TP (%)	CC (%)	AS (%)	BD (g cm^{-3})	PS (mm)	pH	EC (mS cm^{-1})	CEC (meq $100g^{-1}$)	MC
Ideal Range	50~85	45~65	10~30	0.19~0.7	N/A	5.4~6.5	<0.75 (seedlings) <1.5(general crops)	6~15	N
PB	83	48.6	34.2	0.17	0.59~2	5.4	N/A	N/A	N
HB	85	60.3	24.4	0.15	67.3% >2	10.8~11.8	0.11	N/A	N
SBB	74	66~85	3~9	0.09~0.11	0.17(mean)	5.9	0.08	N/A	N
Peat moss	83	64	18.9	0.08	N/A	4.3-5	N/A	7~13	N
Perlite	92	59	34	0.05	N/A	7.3	0.01	~0	N
VC	75	72	3	0.38	89.4%<2	4.8	6.7	N/A	Y
CM	64	60	4	0.62	89.4%<2	7.5	32.9	N/A	Y
CS1	74~78	58~71	3~20	0.09~0.1	65.2%<2	N/A	N/A	N/A	N
CS2	71~75	84	15	0.11	N/A	6.8	0.07	N/A	N
PCS	79~97	47~85	12~31	0.15	3~6	6.5~6.75	0.18	N/A	N

Note: Based on the studies from [17, 18, 24, 30, 76, 77]. PB = pinewood biochar, HB = mixed hardwood biochar, SBB = sugarcane bagasse biochar, VC = vermicompost, CM = chicken manure, CS1 = peat moss-based commercial substrate for plants growing, CS2 = peat moss-based commercial substrate for plants propagation, PCS = pine bark-based commercial substrate. N/A = not applicable, N/Y in the microorganism column means mixes do not contain/contain microorganisms.

Unlike peat moss, which may encounter rewetting difficulties, certain types of biochar used in containers are easy to rewet because of larger surface areas and pore size distribution [26]. Most of biochar was produced by pyrolysis, which is a thermochemical process, where biomass subjected to high temperature and /or high pressure, creating a lot of micropores or macropores on the biochar surface, enlarged its surface area [13, 78]. The temperature for biochar (400°C ~1200°C) production is normally higher than that of peat moss (70°C), creating more micropores and larger surface area [13, 31]. As temperature increases, the surface area of biochar also increases as more pores are generated, especially micropores. Micropores contribute largely to biochar surface area, endowing high adsorptive capabilities on the biochar and allowing small dimension molecules, such as gases and solvents to be absorbed [26].

The screening electron microscope images (Figure 1.2) showed the porous structure of peat moss and peat moss-derived biochars produced under different temperatures and times [78].The surface area of biochar increased because high temperatures changed more macropores into mesopores/micropores in biochar [78]. Thus, when the same irrigation practice applied, biochar would encounter less difficulties in rewetting than peat moss or peat moss-based substrate [25].

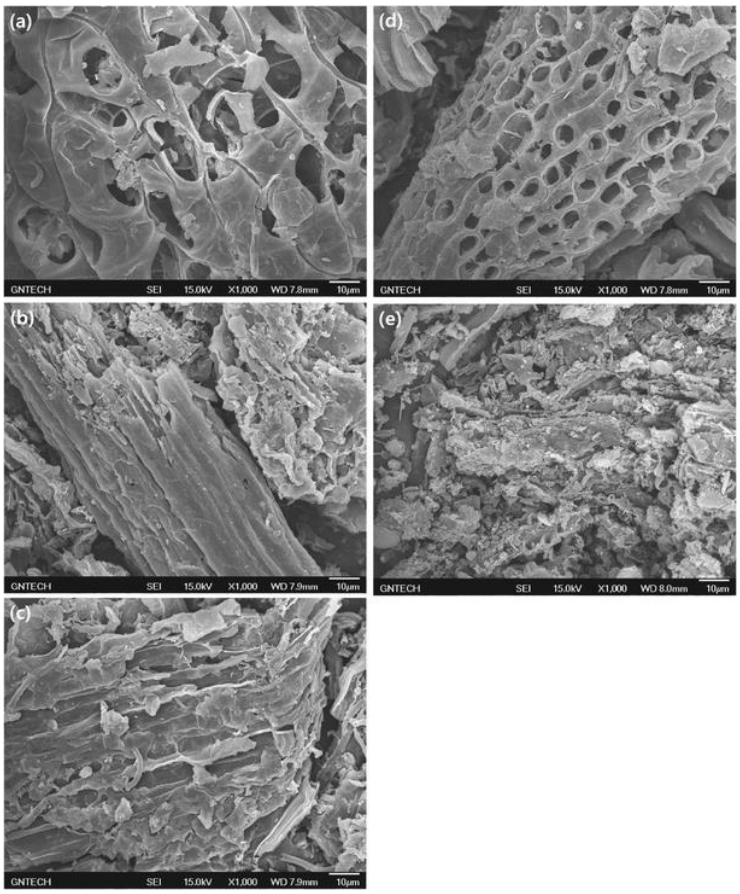

Figure 1.2. Scanning electron microscopy (SEM) images of peat moss (a) and peat moss-derived biochars at different carbonization temperatures (b 400 °C, c 600 °C, d 800 °C, e 1000 °C) based on Lee's study [78] .

1.3.2. Biochar Replacing Peat Moss Effects on Plants Health

Soil-borne diseases affect potted plants marketability and are hard to control [79-81]. There are 10~20% of attainable crop yields loss caused by soil-borne diseases and the economic losses in US are more than $4 billion [80]. Soil-borne diseases control becomes more challenging due to trade globalization [81, 82]. *Phytophthora ramorum* has survived for eight months in root balls and potting substrates of rhododendron plants, affecting the

plants marketability worldwide [83, 84]. *Fusarium oxysporum f. sp papaveris*, a fungi pathogen attacking Papaveraceae plants, largely affected Papaveraceae plants marketability in Italy [85].

As a container substrate to replace peat moss, the effects of biochar on soil borne pathogen derived plant health has been less reported than that of plant growth, which had positive, neutral, and negative effects [13]. To date, there aren't enough studies about the biochar effect on plant health (Figure 1.3), and the dose of biochar is relatively low (ranging in most cases between 0.5~3%, Table 1.2). The highest dose of biochar used in those studies is 50% [86].

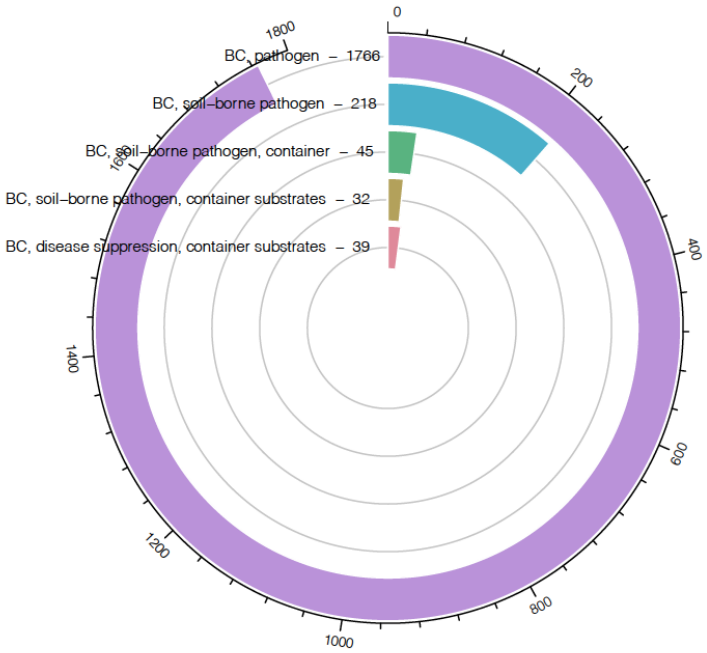

Figure 1.3. Circular bar-plot indicating the number of biochar (BC) pathogen-related articles published from 2010~2021 based on key words searching in Science Direct data base.

Similar to its effects on plant growth, biochar effects on plant health vary depending on plant species, biochar rates and types [87]. Gravel, Dorais [86] found that adding 50% of balsam fir/ spruce bark biochar caused higher pathogen root colonization rate in all other crops except for coriander. Adding 30% coconut biochar increased plant health [80]. Kadota and Niimi claimed that biochar improved the quality of several plant species, shortened the number of days needed for flowering, and increased plants survival rates [88].

The potential mechanisms on how BC may influence plant disease includes both direct and indirect influence on pathogen: 1) BCs' chemical compounds affect pathogen

growth; 2) BCs' physicochemical properties improve soil nutrients availability and abiotic conditions; 3) BCs' physical properties help absorb toxins and enzymes produced by pathogens, reducing virulence; 4) BCs' presence induces systemic resistance in host plants; 5) BCs' physical properties enhance abundance and/or activities of beneficial microbes [80, 89]

Table 1.2. Biochar effects on plant health.

Host plants	Pathogen	BC feedstock	BC temperature	BC rate	Reference
Bean	*Rhizoctonia solani*	eucalyptus wood, greenhouse wastes	350, 600	0,1%, 3% (w/w)	[90]
Cucumber, tomato, lettuce, sweet pepper etc.	*Rhizoctonia solani*	maple bark biochar		0,1%,3%,5% (w/w)	[91]
Strawberry	*Botrytis cinerea, Colletotrichum acutatum and Podosphaera apahanis*	citrus wood (CW), greenhouse wastes (GWC)	GWC at 450	1% or 3% (w/w)	[92]

Table 1.2. Continued.

Host plants	Pathogen	BC feedstock	BC temperature	BC rate	Reference
Asparagus	*Fusarium oxysporum f. sp. asparagi;*	hardwood dust charcoal	N/A	0,1.5%,3% (w/w)	[93]
Asparagus	*Fusarium oxysporum f. sp. asparagi (Fusarium root rot)*	coconut fiber charcoal	N/A	0,10%,30% (v/v)	[80]
Tomato	*Ralstonia solanacearum*	municipal bio-waste charcoal, wood charcoal	N/A	0, 20% and other not-specified concentrations (v/v)	[94]

Table 1.2. Continued.

Host plants	Pathogen	BC feedstock	BC temperature	BC rate	Reference
Red oak and red maple	*Phytophthora cinnamomi and P. cactorum (stem canker)*	pine	Between 550 and 600	0, 5, 10 and 20% (v/v)	[95]
Sweet pepper, lettuce, basil, geranium and coriander	*Pythium ultimum*	balsam fir bark and spruce bark	475	50% (v/v)	[86]
Tomato	*Fusarium* spp.	eucalyptus wood pepper plant waste	350/600	0,0.5%,1%,3%(w/w)	[96]
Tomato Pepper	*Botrytis cinereal Leveillula taurica*	citrus wood	N/A	1%,3%,5%(w/w)	[97]

Table 1.2. Continued.

Host plants	Pathogen	BC feedstock	BC temperature	BC rate	Reference
Lettuce	*OTC (antibiotic)*	bamboo	600	2%	[98]
Cucumber	*Rhizoctonia solani*	eucalyptus wood and greenhouse wastes	350/600	0%~3%	[99]
Beans	*Rhizoctonia solani*	eucalyptus wood and greenhouse wastes	350/600	0%~3%	[93]
Rice	*Meloidogyne graminicola*	holm oak wood	650	0.6%, 1.2%, 2.5%, 5.0 %	[100]
Tomato	*Botrytis cinerea*	greenhouse wastes	450	0, 1, and 3 %(w/w)	[101]

Table 1.2. Continued.

Host plants	Pathogen	BC feedstock	BC temperature	BC rate	Reference
Lettuce strawberry	*Rhizoctonia solani* *Botrytis cinerea*	holm oak wood	650	0, 1, and 3 % (w/w)	[102]
Carrot	*Pratylenchus penetrans*	pinewood, pine bark, wood pellets, spelt husks	500	0.80%, 0.92%, 1.24%, 0.64%	[103]
Sweet pepper, tomato, lettuce, carrot, radish	*Rhizoctonia solani*	maple wood bark	700	0,1%,3%,5% (w/w)	[90]

1.4. Environmental Benefits of Biochar as a Container Substrate

1.4.1. Biochar as a Container Substrate Protecting Peatland

There is a large peat moss demand in horticulture. Around 0.15 M m^3 of peat moss were used in container plants production, accounting for 86.5% of the total imported peat moss in the US [104]. In the UK, 0.06 M m^3 peat moss were used in horticulture, including container plants, bedding plants, vegetables, soft fruit, and cut flower production. In Europe, around 2.6 M m^3 peat moss were used in horticulture, with the total ratio of peat in media for plant growth being 99% in Estonia, 99% in Lithuania, 92% in Latvia, 88% in Finland, 87% in Ireland, 87% in Denmark, 87 in Sweden, and 81% in Germany [105].

A large peat moss demand leads to large peatland disturbance. Thus, replacing peat moss with biochar protects peatland from further disturbance. The highest rate for biochar replacing peat moss as a container substrate is 80% [76, 106]. If 80% of peat moss can be replaced by biochar, 0.12 M m^3, 0.05 M m^3 and 2.08 M m^3 peat moss can be saved annually in the US, in the UK, and in Europe, respectively. Global average dry biomass Sphagnum production is around 260 g m^{-2} yr^{-1}, depending on species and locations [107]. Considering the commercial peat moss bulk density is 0.1 g cm^{-3}, if 80% of peat moss substrate can be replaced by biochar, 46.2 M m^2, 19.2 M m^2, and 800 M m^2 of peatland can be saved annually from being disturbed for the United States, the United Kingdom, and Europe, respectively.

1.4.2. Biochar as a Container Substrate Reducing Chemical Leaching

1.4.2.1. Biochar as a container substrate reducing nutrient leaching

As aforementioned, fertilizer tends to be over-used in greenhouse production and plants can only use 50% of fertilizers applied [41, 42]. The rest of the other half of fertilizers were either lost in running off /or reactions with organic compounds [42]. Moreover, since the majority of fertilizers haven't been absorbed by plants, they can reach ground water and contaminate ground water [43, 44].

Biochar replacing peat moss as a container substrate reduces nutrient runoff [108]. For instance, adding biochar in a peat-based substrate reduced nutrients such as ammonium (NH_4^+), nitrate (NO_3^-), dihydrogen phosphate ($H_2PO_4^-$), hydrogen phosphate (HPO_4^{2-}), and potassium (K^+) leaching [109]. Jahromi, Walker [110] found that biochar-amended substrates reduced the total nutrients lost from hydrangea containers because biochar addition increased substrate water holding capacity. Altland and Locke [111] demonstrated that adding 10% saw dust biochar to peat moss-based substrate increased nitrate and phosphate retention and subsequently reduced their leaching. Biochar decreased more extractable total N including NO_3-N than peat moss substrates with similar seedlings growth [112]. In a glass columns study, biochar reduced nitrate leaching because biochar captured and retained the nutrient solution then released it over time [113].

1.4.2.2. Biochar as a container substrate reducing pesticide leaching

The over-use of pesticides in greenhouse production also caused environmental concerns [114, 115]. In the US, among the total usage of pesticide, around 90% of pesticide comes from agricultural production [116]. Pesticides contaminate the

environment via surface runoff, spray drift, and subsurface flow, which is the major pathway for pesticides entering water bodies [117]. Leaching can rapidly transport pesticides to surface and subsurface receiving waters [45]. The best management practices are recommended for nurseries to reduce pesticide contamination, yet, the best management practices alone may not completely remove pesticides contamination [118].

Peat moss serves as a good sorbent for efficient removal of chemicals, including heavy metal and pesticides, due to its high sorption capacity [119]. Peat moss high sorption capacity for chemicals is mainly due to 1) a large number of pores, providing large surface area for molecules to adsorb to walls, and 2) a large number of capillary spaces, absorbing and retaining hydrocarbons [120]. Also, the functional groups within peat moss contribute to its the high adsorption capacity for heavy metals and other pollutants [121]. In a pesticide removal efficacy study, peat moss removed nearly 100% of 16 different pesticides due to its high surface area and functional groups [45].

Similar to peat moss, biochar also has been also reported as good sorbent for efficient removal of chemicals, including pesticides. Taha et al. [122] demonstrated that biochar made from corn stover and rice straw adsorbed many types of pesticides including organophosphates (diazinon and malathion) and neonicotinoids (imidacloprid and acetamiprid). Mandal et al. [123] reported that rice straw biochar had the highest adsorption rate for atrazine and imidacloprid.

1.5. Biochar Potential Economic Values

Biochar provides large potential economic values as the market of biochar and biochar supply companies are growing. According to Transparency market research [124,

125], the evaluated worth of global biochar market reached $0.44 M in 2016, and it is expected to experience a Compound Annual Growth Rate (CAGR) of 14.5% from 2017 to 2025 and reach a valuation of $1.48 M by 2025. Also, the number of biochar supply companies increased. There were approximately 150 biochar supply companies in 2013, mostly of them were small garden and specialty retailers, however, the number of biochar companies doubled in 2015 [126, 127].

1.5.1. Biochar Economic Value on Reducing Container Substrate Costs

Replacing peat moss with biochar as a container substrate can bring large economic benefits due to its potential low price and large demand. The average customer price for sphagnum peat increased from $ 22 m^{-3} in 1986 to $172 m^{-3} in 2018 [128, 129]. Customers may have to pay higher prices based on the distributors they chose, for instance, the price of peat moss in Greenhouse Megastore is $ 310.7 m^{-3} [130]. Comparing to peat moss, however, the average biochar price is $100 m^{-3}, half the price of peat moss from BWI, and one third the price of peat moss from Megastore. Aforementioned, 0.15 M m^{-3}, 0.057 M m^{-3}, and 2.6 M m^{-3} of peat moss were used in horticulture in the US, UK, and Europe, respectively [104, 105]. With 80% of biochar being able to replace peat moss as a container substrate [76, 106], $8.64 M, $3.6 M, and $149.76 M can be saved annually in the US, UK, and Europe, respectively if consumers get peat moss from a cheaper distributor. If consumers get peat moss from a more expensive distributor, $25.2 M, $10.5 M, $436.8 M can be saved annually in the US, UK, and Europe, respectively. The actual economic benefits of using biochar to replace peat moss as a container substrate could be

even larger if biochar were produced locally, which may lead to an even lower price than the average.

Also, using BC to replace peat moss as a container substrate brings large economic benefits because of its faster regeneration rates (Table 3). Peat requires thousands of years to be generated, making it a unrenewable resource [131]. With the restoration practices, the average rate of peat moss vertical growth was around 1 mm $year^{-1}$ in the peatland [132]. If no restoration practices are launched, the spontaneous revegetation of abandoned peatlands will take even longer [56]. The best suggested harvesting depth for peat moss is 0.25 m from the top soil, meaning after harvesting, peatland needs 25 years or even longer to be able to harvest again [132]. The 25 years are more than enough to grow pine trees to merchantable size for biochar production [76, 133]. If we grow other biomass such as sugarcane (or other herbs) and shrubs, the generation of biochar can be 25 times faster than peat moss, providing 25 times the economic benefits of peat moss [77].

Table 1.3. The comparison between peat moss and biochar.

	Peat moss	Biochar
Source	Bog plants: moss, sedge…	Any biomass: sugarcane, bark, municipal wastes…
Formation	Plant material not fully decay	Chemical thermal reaction
Condition	Waterlogged, acidic, anaerobic	Oxygen-free, high temperature
Rate of regeneration	0.5~1mm $year^{-1}$ (naturally)	Comparable to generation of biomass
Renewable	Yes	Yes
Regrowth	Yes, 30~40%	Yes, 100%
Main application	Fuel, soil amendments, potting mix	Fuel, soil amendments, potting mix, pollutant filtration
Price	~\$172 m^{-3}	~\$100 m^{-3}
Commercialization	Yes	Limited
Harvesting condition	Depth >2m	N/A
Reclaim rate	~25 yr (harvest wisely)	N/A
Restoration rate	1.5~10 cm $year^{-1}$	N/A

Note: Information based on studies from [56, 77, 128, 129, 132].

1.5.2. Biochar Economic Value on Reducing Peatland Restoration Costs

Peatland restoration requires high economic costs such as techniques costs, rewetting and recurring costs, as well as maintenance costs [54, 56, 134]. The costs associated with restoration range from \$280 ha^{-1} to \$14,016 ha^{-1} [135]. A one-time cost of \$7,000 ha^{-1} for initial rewetting and recurring was estimated, with another cost of \$200 ha^{-1} yr^{-1} maintenance and/or \$140 ha^{-1} yr^{-1} management costs [134].

Replacing peat moss with biochar as a container substrate largely reduces peatland restoration costs because biochar production does not degrade the peatland ecosystem. Biochar is a sustainable product as it can be derived from a wide variety of sources, ranging from plant-based material such as green waste [136], wood, straw [137-141], bark [142], rice hull [143], wheat straw [139, 144, 145] to other sources such as chicken-manure [144], deinking sludge [146]. With around 10.3 M ha peatland area needs to be restored [54], an estimated \$72.1 billion one-time rewetting and recurring costs with another \$2.06 billion and/or \$1.44 billion maintenance and management costs could be saved annually by replacing peat moss with biochar.

1.5.3. Biochar Economic Value on Reducing Chemical Costs

Chemical costs in agriculture are high due to large demands and high prices. Global fertilizer demands were projected to 208 M tons with the US consuming 22 M tons in 2015 at an average price \$719 ton^{-1} [147-149]. Global pesticides use in agriculture was 4.12 M tons with US using 408,000 tons [150]. The total pesticides trade reached approximately 5.9 M tons with a value of \$37.6 billion in 2018 globally. The US was the

top five countries for pesticides imports with trade values ranging $1.4~3.0 billion in 2018 [151].

Replacing peat moss with biochar as a container substrate significantly reduces chemical costs by adding extra nutrients, increasing nutrient use efficiency, and reducing disease incidence. Biochar produced from nutrient-rich raw materials could serve as a source of P and K, reducing the total amount of fertilizer needed for plant growth [17]. If using biochar could increase nutrient use efficiency by 50% [110], $7.91 billion can be saved in the US, and $74.78 billion worldwide (assuming the average price was $719 ton^{-1}) [148]. Also, mixed hardwood biochar used in our previous study could reduce 25% disease incidence, leading to less pesticide consumption (unpublished data). If using biochar could reduce pesticide usage by 25%, $9.4 billion could be saved globally.

1.5.4. Biochar Economic Value on Agricultural Waste Handling

Large amounts of agricultural waste contributed to high waste handling costs. Around 3.9 billion tons of waste were generated annually worldwide with 2.01 billion tons (expected to grow to 3.4 billion tons by 2050) being municipal solid waste (North America contributed 289 M tons) [152]. The operating costs for integrated municipal solid waste management, including collection, transport, treatment, and disposal, generally exceed $100 ton^{-1} yr^{-1} [153].

Using biochar to replace peat moss as a container substrata could significantly reduce agricultural waste handling costs. With pyrolysis for bio-oil purposes, the yield of biochar is from 20%~47% [154] (taking the average as 30%). To produce enough biochar for the horticulture industry in US alone (0.15 M m^3), assuming all the wastes have similar

density as municipal waste, 350 kg m^{-3}[155], nearly 0.18 M tons of agricultural waste can be converted, saving \$18 M yr^{-1}. Similarly, to produce enough biochar for horticulture industry in UK (0.057 M m^3), and Europe (2.6 M m^3), 0.67 M tons, 3.03 M tons of agricultural waste can be converted, respectively, saving millions of dollars on agricultural waste handling.

1.6. Limitations and Possible Solutions

Using biochar as a replacement for peat moss as a container substrate provides many benefits, yet it has several limitations. Biochar limitations are mainly from the varied properties and potential toxic substances it may contain, the non-continuous biochar supply-demand loop, and the lack of awareness and production practice of using it as container substrates [13]. These limitations may be addressed by providing financial and nonfinancial policy support to motivate business practice change, improving biochar commercial availability, to educate consumers, extending biochar demand, and to establish good production and application practice, exploring more biochar application options [156].

1.6.1. Biochar Various Properties and Production

Unlike the well-established sphagnum peat moss, biochar properties vary widely depending on feedstocks, production temperature, and pre- and post-treatment, bringing application difficulties for consumers [13]. Biochar may contain potential toxic compounds such as heavy metals, polycyclic aromatic hydrocarbons (PAHs) and dioxin depending on the raw material and producing conditions [157]. When incorporating

biochar with heavy metals, PAHs and dioxin into container substrates, plant growth could be decreased.

Biochar's various properties could be addressed by implementing standard production practices such as using the same feedstock and temperature every time. Currently, most biochar is produced as a by-product from bio-oil-focused process, leading to various properties and toxic compounds [12, 13]. Also, biochar made from feedstocks containing toxic compounds, either heavy metal, PAHs or chlorine could contain toxic compounds [13]. As such, businesses can avoid producing toxic containing biochar by selecting feedstock material cautiously. Additionally, biochar various properties can be adjusted to an ideal range for container plants growth by incorporating other components such as bark, perlite, and peat [76].

1.6.2. Biochar Non-continuous Supply-demand Loop

Biochar supply and demand have not created a full loop for the industry yet. Consumers are reluctant to switch from peat moss to biochar due to their lack of awareness and poor biochar availability. Because of the unawareness of using biochar as container substrates, consumers tend to use the well-established and well-supplied peat moss as a major container substrate component, lowering biochar demand. In return, the low biochar demand, discourages biochar producing companies due to the low financial benefits. Currently, there are only around 300 biochar companies worldwide, and most of them are small-scale companies, not being able to supply commercial biochar sustainably [127]. Also, due to the lack of financial motivation, companies are not able to invest in biochar facilities, producing large-scale of container substrate-targeted/grade biochar [156].

The non-continuous biochar supply-demand loop can be addressed by establishing related policies to encourage capital investment, providing technology support to reduce the initial production costs [156]. Also, non-financial programs, including extension programs can help educate consumers on biochar economic and environmental benefits and biochar application practices, increasing biochar demand. Additionally, more funding needs to be assigned to biochar research and development programs, exploring more biochar application options to enlarge biochar market margin.

1.7. Conclusions

Using biochar to replace peat moss as a container substrate for plant production provides an environmentally friendly way to address the environmental concerns associated with peatland mining and drainage. Switching peat moss to biochar as a container substrate for plant production protects peatland ecosystem, increases water and fertilizer use efficiency, reduces greenhouse gas emission, and brings economic benefits. However, to reach biochar's full potential, biochar limitations such as the lack of awareness, potential toxic compounds, and the non-continuous supply-demand loop need to be addressed soon by establishing both financial and non-financial supports from governments, companies, and research agencies.

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2. MIXED HARDWOOD AND SUGARCANE BAGASSE BIOCHAR AS POTTING MIX COMPONENTS FOR CONTAINER TOMATO AND BASIL SEEDLING PRODUCTION*

2.1. Introduction

Peat moss (PM) has been widely used as a horticultural substrate due to its ideal physical and chemical properties, such as low bulk density (BD), high water holding capacity, high aeration ratio, and high cation exchange capacity [1–3]. Domestic PM sales in the US were 0.25 M m3 in 2016 and almost 91% PM was sold to the horticultural industry [4]. The marketable PM estimated value in the US was $13.0 million in 2018 [4]. Peat moss mining, however, has been questioned due to the peatland ecosystem disturbance and/or loss, and its environmental consequences. Hence, alternative materials such as pretreated manure composts and processed timber by-products have been introduced as PM replacements [5].

Biochar, a carbon-rich by-product from biomass pyrolysis, has potential for substituting PM as greenhouse growing media [6]. Pyrolysis biochar is generated from biomass thermo-chemical decomposition in oxygen-depleted or oxygen-limited atmosphere [7–9]. Biochar has been considered as a sustainable material because it can be derived from various sources, such as pinewood [3,10,11], green waste [12], wood, sugarcane bagasse [13], straw [14–18], bark [19], rice hull [20], and wheat straw [16,21]. For the same reason, biochar properties can vary widely [22]. Most greenhouse trials have used biochar derived from lignin-based materials, which has

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appropriate properties for plant growth [12]. Graber [23] reported that citrus wood biochar has potential to improve pepper and tomato plant growth in a systematic way, increasing the leaf area, canopy and yield. Guo [6] found that incorporating pinewood biochar with PM-based commercial substrate increased poinsettia growth. Huang's [24] study showed that mixing hardwood biochar with two different composts could lead to similar or better plant growth in basil and tomato plants in comparison to those in PM-based commercial substrates. Tian [12] confirmed that the total biomass could be significantly increased (by 22%) by mixing green waste biochar with a PM-based substrate. When adding biochar in composted green waste, the shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight of *Calathea insignis* were increased by 57.3%, 79.7%, 64.5%, and 82.0%, respectively [25]. Similar works had also been reported on Easter lily [6,26,27]. The biochar from red oak feedstock mixed with vermiculite also increased hybrid poplar total biomass and shoot biomass [28].

Biochar that affects greenhouse seedling production or subsequent seedling growth has seldom been reported. As biochar from different resources has varied properties, some may have adverse effects on plant growth due to possible phytotoxicity [29]. Phytotoxicity assessment is critical for successful soil/soilless amendment with bioenergy by-products such as biochar [30], and the germination test is a reliable procedure for different types of biochar phytotoxicity examinations [30]. We conducted this study to test the phytotoxicity

of two biochars from different raw materials and to explore the use of the two biochars in subsequent container seedling production.

2.2. Materials and Methods

2.2.1. Experiment 1: Media Phytotoxicity and Property Test

Sugarcane bagasse biochar (SBB, American Biocarbon LLC White Castle, Louisiana, USA) was mixed with P (30%, by vol., Kinney Bonded Warehouse, Tyler, Texas, USA) at rates of 10%, 30%, 50%, 70% and 100% (by vol.), with the rest being PM (Voluntary purchasing Group Inc., Bonham, Texas, USA) when SBB and P did not add up to 100%. No P or PM were added to 100% SBB mix. Mixed hardwood biochar (HB, Proton Power Inc. Lenouir City, Tennessee, USA) was mixed with PM at rates of 10%, 30%, 50%, 70% and 100% (by vol.), and no P was incorporated. Another mix was formulated by mixing PM and P at a 7:3 ratio (70%PM:30%P; by vol.). Peat moss, P, and a commercial propagation substrate (CS, BM2, Berger, Saint-Modest, Quebec, Canada) were also included in this study. The commercial propagation mix contained 70–80% of fine sphagnum moss with the rest being fine P and fine vermiculite. The United States Department of Agriculture-Agricultural Research Service, Sugarcane Research Unit (Houma, Louisiana, USA) provided the SBB, which was produced with proprietary methods, and the Proton Power Inc. (Lenouir City, Tennessee, USA) provided HB, which was a by-product from fast pyrolysis of mixed hardwood. Sugarcane bagasse biochar had a pH of 5.9 and HB had a pH of 10.1. The electrical conductivity (EC) of the two biochars were 753 µS cm^{-1} (SBB) and 1,058 µS cm^{-1} (HB), respectively [31]. Because SBB had a

similar pH to PM (SBB 5.9, PM 5.0, Table 1) and the SBB particle size was smaller (mean 0.17mm, resulting in low air space (AS)) [31], when formulating mixes with SBB, 30% P was incorporated to increase the pH of the AS and the mix. As HB had a higher pH (10.1) than PM (5.0), and the HB particle size was larger (67.3% > 2.0 mm, resulting in high AS) [25], no P was incorporated when formulating mixes with HB. The properties of all the components used in this study are shown in Table 1.

All of the mixes were subjected to a phytotoxicity test with Gravel's method [32]. Briefly, water extract was obtained by soaking the mixes with 100 mL deionized (DI) water and shaking for 24 hours. The mixtures were filtered through 11cm-diameter VWR Grade 415 filter paper (quantitative) (VWR International, LLC, Randor, Pennsylvania, USA) and 3 mL extract was used to saturate another filter paper placed in a petri dish. Deionized water was used as the control in this experiment. Twenty-five basil (*Ocimum basilicum*) (Johnny's Selected Seeds, Winslow, Maine, USA) seeds were placed in each petri dish. The emergence percentage (EP) of basil seeds was calculated after incubating the petri dishes at 25 °C in the dark for 7 days by using the following formula: EP = (no. of emerged seedlings/total no. of seeds) × 100%. This experimental design was a complete randomized design with six replicates.

All of the physical properties of the media, including bulk density (BD), total porosity (TP), air space (AS) and container capacity (CC), were determined using the North Carolina State University Horticultural Substrates Laboratory Porometers [33]. The substrate pH and EC were measured by using a handheld pH-EC meter (Hanna Instrument,

Woonsocket, Rhode Island, USA) according to the pour-through extraction method [34]. Three replications of each substrate were measured.

Table 2.1. The pH, electrical conductivity (EC), total porosity (TP), container capacity (CC), air space (AS) and bulk density (BD) of substrate components used in this study.

Substrate Component [z]	pH	EC ($\mu S\ cm^{-1}$)	TP (%)	CC (%)	AS (%)	BD ($g\ cm^{-3}$)
SBB	5.9	753	74 ± 2	71 ± 1	3 ± 1	0.11 ± 0.00
HB	10.1	1,058	87 ± 1	66 ± 1	20 ± 1	0.13 ± 0.00
PM:P (70:30)	5.6	162	79 ± 1	62 ± 1	16 ± 1	0.09 ± 0.00
CS	6.8	745	75 ± 2	66 ± 1	9 ± 1	0.09 ± 0.00
P	7.3	57	92 ± 1	59 ± 1	34 ± 0	0.05 ± 0.00
PM	5.0	179	69 ± 1	58 ± 1	11 ± 0	0.11 ± 0.00

Note: [z] SBB=Sugarcane bagasse biochar; HB=Mixed hardwood biochar; CS=Commercial propagation substrate; P=Perlite; PM=Peat moss. Numbers in parent indicated the ratio of different components, by vol.

2.2.2. Experiment 2: Biochar as Greenhouse Media Amendments for Seedling Production

Tomato (*Solanum lycopersicum* 'Red Robin'™') (Fred C. Gloeckner & Company Inc., Harrison, New York, USA) and basil (*Ocimum basilicum*) (Johnny's Selected Seeds, Winslow, Maine, USA) seeds were soaked in DI water for 24 h before sowing in 72-cell

(cell depth: 5 cm; cell top length and width: 4 cm; volume: 55 ml) plug trays with one seed per cell on 16 February, 2019.

Five SBB:P substrates were formulated by mixing SBB at 10%, 30%, 50%, 70%, and 100% (by vol.) with 30% P (Kinney Bonded Warehouse, Tyler, Texas, USA, except for the 100% SBB) and the rest being Peat moss (PM) (Voluntary purchasing Group Inc., Bonham, Texas, USA) when SBB and P did not add up to 100%. Four HB:PM substrates were formulated by mixing HB at 10%, 30%, 50%, and 70% (by vol.) with PM and a commercial propagation mix (CS, BM2, Berger, Saint-Modest, Quebec, Canada) was used as the control.

All of the mixes had four replications (10 cells per replication), which were arranged in completely randomized blocks in the greenhouse located on Texas A&M University campus, College Station, Texas, USA. The average greenhouse temperature, relative humidity and dew point were 22.8 °C, 79.7% and 18.3 °C, respectively. Before the true leaves (tomato or basil) emerged, the trays were irrigated with DI water. After the true leaves emerged, trays were irrigated with 50 mg N· L^{-1} (20N-4.3P-16.6K) Peters® Professional (Everris NA Inc, Dublin, Ohio, USA) nutrient solution.

At the end of this experiment (27 March, 2019), the height of four randomly-selected seedlings from each mix was measured from the medium surface to the highest point of the plants, and the widest width (width 1) and its perpendicular width (width 2) were measured. The growth index (GI) was calculated as: GI= Height/2 + (width 1+ width 2)/4 [6]. Leave greenness was indicated by Soil-Plant Analyses Development (SPAD)

readings. (SPAD 502 Plus Chlorophyll Meter, Spectrum Technologies, Inc., Plainfield, Illinois USA). For each mix, shoots (stalk and leaf) and roots of four seedlings were harvested separately. The total fresh weight (TFW) was determined at harvest by adding up the fresh weights of the stalk and leaf. Shoot dry weight (SDW) and root dry weight (RDW, after being washed) were determined after drying at 80 °C in an oven until a constant weight was reached. Roots were washed and root length, surface area, root average diameter, and the number of tips were measured by using a root scanner (WinRHIZO, Regent Instruments Inc., Canada). The total dry weight (TDW) was calculated by adding up the SDW and RDW.

2.2.3. Experiment 3: The Subsequent Growth Evaluation of Seedlings Produced in Biochar-Amended Media

At the end of the second experiment, six seedlings in each mix with similar GI were selected and transplanted into 6-inch azalea pots (depth: 10.8 cm; top diameter: 15.5 cm; bottom diameter: 11.3 cm; volume: 1330 ml) with a commercial growing substrate (CS1, Jolly Gardener, Oldcastle Lawn & Garden Inc. Atlanta, Georgia, USA). The commercial growing mix contained 55% (by vol.) aged pine bark, with the other ingredients being Canadian sphagnum PM, P and vermiculite. The growth index was measured biweekly and the SPAD was measured on 2 April, 2019. After four weeks of growing, plants' leaves and stems were harvested separately, and the shoot DW (SDW), leaf DW (LDW) and flower or fruit DW (FDW) were determined after drying in an oven

at 80 °C until a constant weight was reached. The total dry weight (TDW) was calculated by adding up the SDW, LDW and FDW.

2.2.4. Statistical Analysis

The experiments were set up in a completely randomized block design. Data were analyzed with one-way analysis of variance using JMP Statistical Software (version Pro 12.2.0; SAS Institute, Cary, North Carolina, USA) and means were separated using Dunnett's test when treatments were significantly different from control at $P \leqslant 0.05$.

2.3. Results

2.3.1. Media Phytotoxicity and Properties

The water extract of the commercial propagation mixes and the P had significantly higher EP than DI water (the control). All other biochar-amended mixes, the PM:P mix, and PM had a similar EP compared to the control (Figure 2.1).

The pH of HB-amended mixes had positive linear correlations with the biochar incorporation rate, while SBB-amended mixes showed quadratic correlations. The electrical conductivity (EC) of all biochar-amended mixes increased with an increasing biochar incorporation rate, and had quadratic correlations (Figure 2.2). All of the mixes' TPs were within the recommended range (50% to 85%). The TPs of the HB-amended mixes had positive linear correlations with the biochar incorporation rate; however, the SBB-amended mixes showed quadratic correlations. All of the SBB-amended mixes' CCs were also within the recommended range (45% to 65%), except for 100% SBB mixes (71%). The CC of 10% HB-amended mixes was within the recommended range (62%),

while all the other HB-amended mixes' CCs were slightly beyond the range (68% the highest). The air space of all the biochar-amended mixes was within the recommended range (10% to 30%), except for the 50%, 70%, and 100% SBB-amended mixes. The air space of all SBB-amended mixes decreased as the biochar incorporation rate increased; however, the AS of all HB-amended mixes increased with the biochar rate. The bulk density (BD) of both SBB- and HB-amended mixes were similar (Figure 2.3).

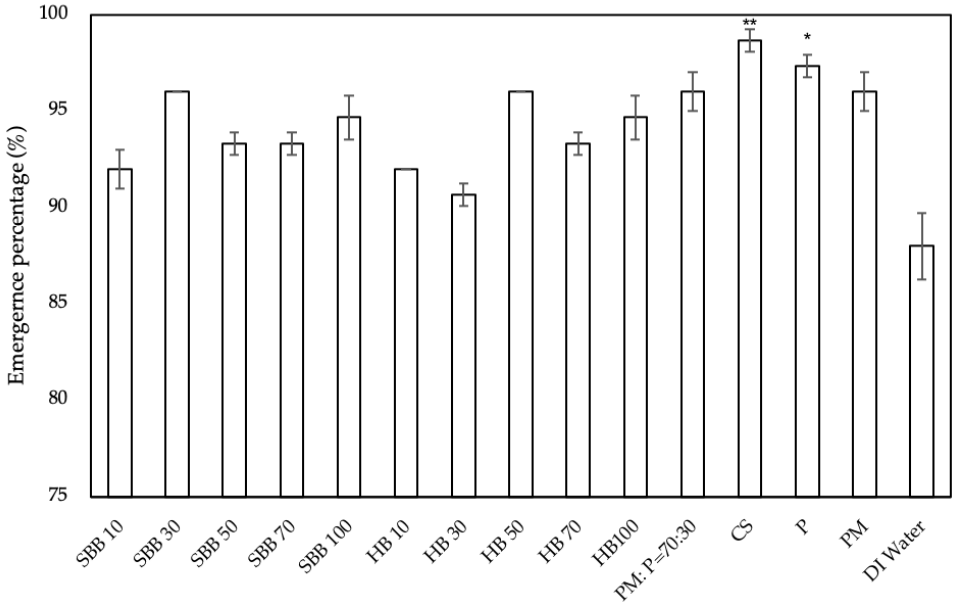

Figure 2.1. The emergence percentage of basil seedlings in the water extract of different mixes. SBB (10%, 30%, 50%, 70% and 100%; by vol.) incorporated with 30% perlite with the rest being peat moss; HB (10%, 30%, 50% and 70%; by vol.) incorporated with peat moss. *, ** indicate a significant difference from the control (DI water) using Dunnett's test at $p \leq 0.05$ and $p \leq 0.01$, respectively.

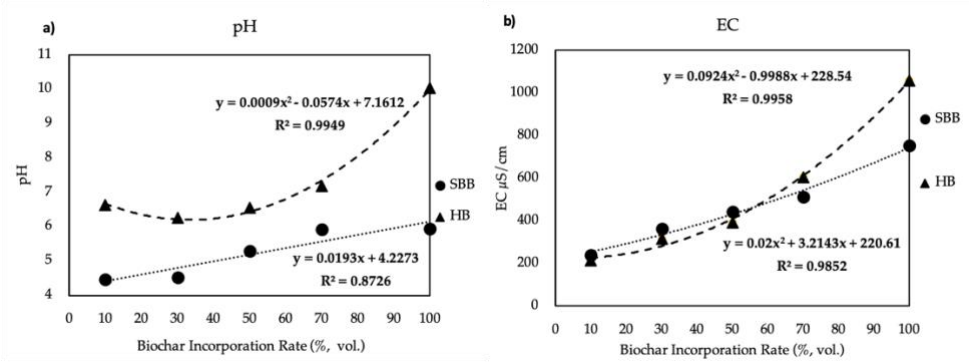

Figure 2.2. The correlation between pH (a) and electrical conductivity (EC) (b) and biochar incorporation rate. SBB (10%, 30%, 50%, 70% and 100%; by vol.) incorporated with 30% perlite with the rest being peat moss; HB (10%, 30%, 50% and 70%; by vol.) incorporated with peat moss.

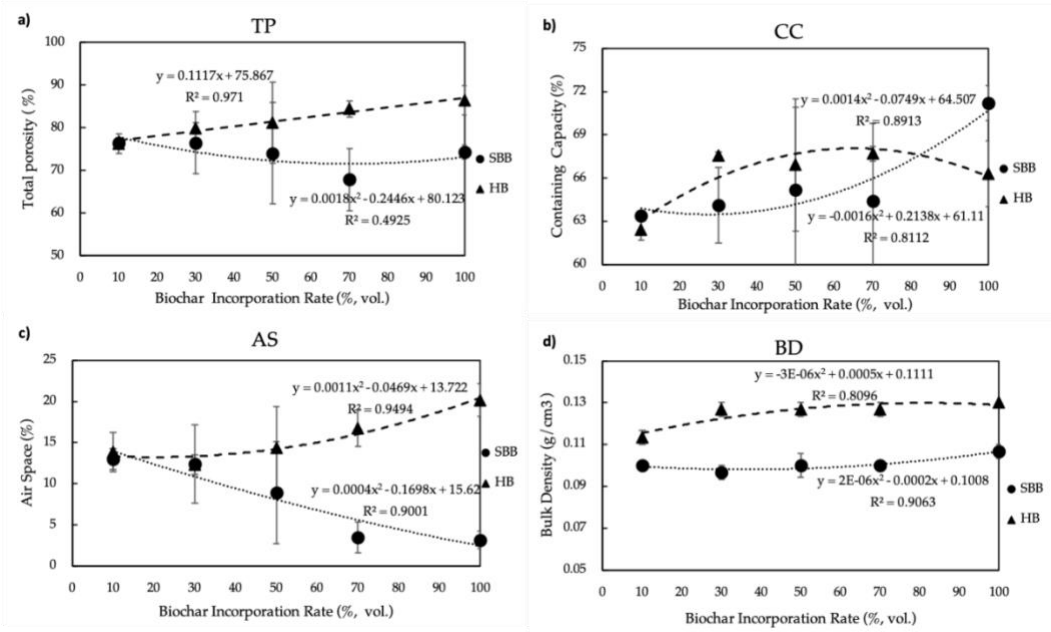

Figure 2.3. The correlation between substrate total porosity (TP, a), containing capacity (CC, b), air space (AS, c) and bulk density (BD, d) and biochar incorporation rate. SBB (10%, 30%, 50%, 70% and 100%; by vol.) incorporated with 30% perlite with the rest being peat moss; HB (10%, 30%, 50% and 70%; by vol.) incorporated with peat moss.

2.3.2. Biochar as Greenhouse Media Amendments for Seedling Production

2.3.2.1. Tomato Seedling Growth

The total fresh weight (TFW), total dry weight (TDW) and GI of SBB-amended mixes had positive linear correlations with the biochar incorporation rate, while HB-amended mixes showed quadratic correlations (Figure 2.4a, b, c). All TFWs, TDWs and GIs in biochar-amended mixes were significantly lower than the control, except for those in 50% HB-amended mixes. Tomato seedlings grown in all SBB-amended mixes had similar SPAD to the control (except 100% SBB, Figure 2.4d); however, seedlings grown in all HB-amended mixes had significantly lower SPAD (except 10% HB).

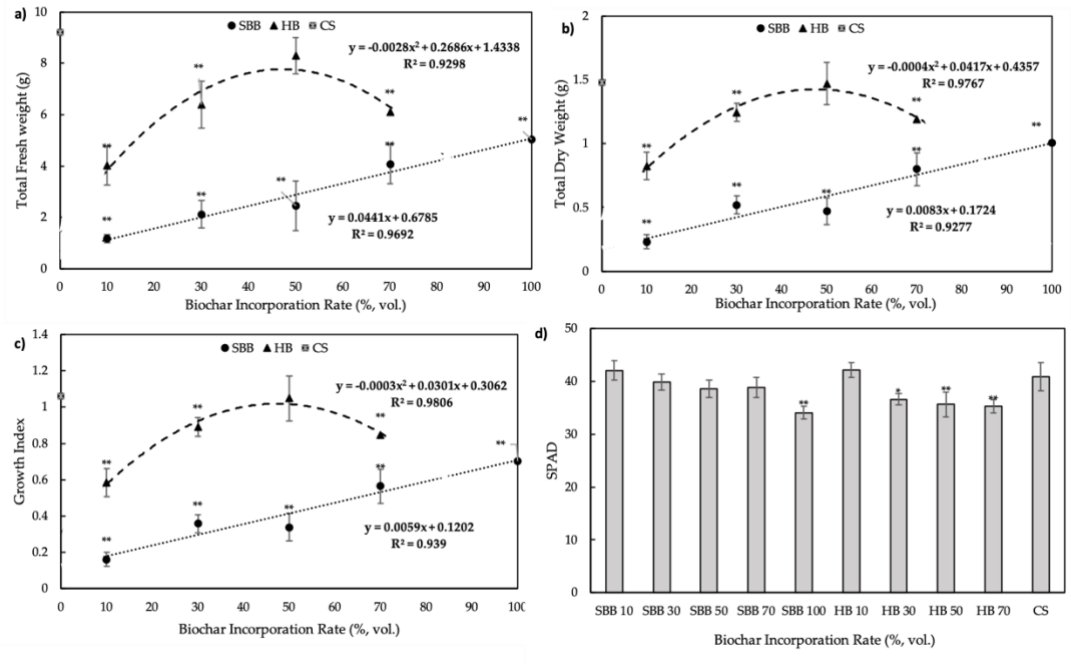

Figure 2.4. The correlations between total fresh weight (TFW, a), total dry weight (TDW, b), growth index (GI, c) and biochar incorporation rate and the soil-Plant Analyses development (SPAD, d) of tomato seedlings grown in biochar-amended mixes. SBB (10%, 30%, 50%, 70% and 100%; by vol.) incorporated with 30% perlite with the rest being peat moss; HB (10%, 30%, 50% and 70%; by vol.) incorporated with peat moss. *, ** indicated significant difference from the control using Dunnett's test at $p \leq 0.05$ and $p \leq 0.01$, respectively.

All tomato seedlings grown in biochar-amended mixes had significantly shorter root lengths than the control (except 30% HB, Table 2). Except for seedlings grown in 50% SBB, 30% HB and 50% HB, all tomato seedlings grown in biochar-amended mixes had significantly smaller root surface areas than the control. Seedlings grown in all biochar-amended mixes had similar or wider root diameters compared to the control; however, they all had fewer root tips (except 30% HB and 50% HB).

Table 2.2. Root growth of tomato seedlings grown in different mixes. (Numbers in parentheses indicate the ratio of different components, by vol. *, ** indicate a significant difference from the control using Dunnett's test at $p \leq 0.05$ and $p \leq 0.01$, respectively)

Mixes [z]	Root Length (cm)	Root Surface Area (cm^2)	Average Diameter (mm)	Number of Tips
SBB:PM:P (10:60:30)	125 ± 10**	27 ± 3**	0.69 ± 0.05**	410 ± 45**
SBB:PM:P (30:40:30)	209 ± 8**	37 ± 1**	0.57 ± 0.01	625 ± 60**
SBB:PM:P (50:20:30)	277 ± 27**	55 ± 4	0.64 ± 0.03*	789 ± 120*
SBB:PM:P (70:0:30)	259 ± 26**	49 ± 4*	0.60 ± 0.02	657 ± 26**
SBB:PM:P (100:0:0)	281 ± 50*	52 ± 7*	0.60 ± 0.03	718 ± 91*
HB:PM (10:90)	243 ± 36**	46 ± 5**	0.62 ± 0.04	648 ± 30*
HB:PM (30:70)	350 ± 26	64 ± 4	0.58 ± 0.04	817 ± 64

Table 2.2. Continued.

Mixes [z]	Root Length (cm)	Root Surface Area (cm^2)	Average Diameter (mm)	Number of Tips
HB:PM (50:50)	278 ± 31**	56 ± 3	0.66 ± 0.05*	1055 ± 148
HB:PM (70:30)	271 ± 21**	50 ± 2*	0.60 ± 0.02	746 ± 47**
Control	432 ± 35	68 ± 4	0.50 ± 0.01	1147 ± 141

2.3.2.2. Basil Seedling Growth

The total fresh weight (TFW), total dry weight (TDW) and GI of seedlings in SBB-amended mixes had positive linear correlations with the biochar incorporation rate, while seedlings in HB-amended mixes showed quadratic correlations (Figure 2.5a, b, c). All TFWs (except 30% and 50% HB), TDWs and GIs (except 50% HB) in biochar-amended mixes were significantly lower than the control. Basil seedlings grown in all biochar-amended mixes had similar or higher SPAD than the control (except 100% SBB, Figure 2.5d).

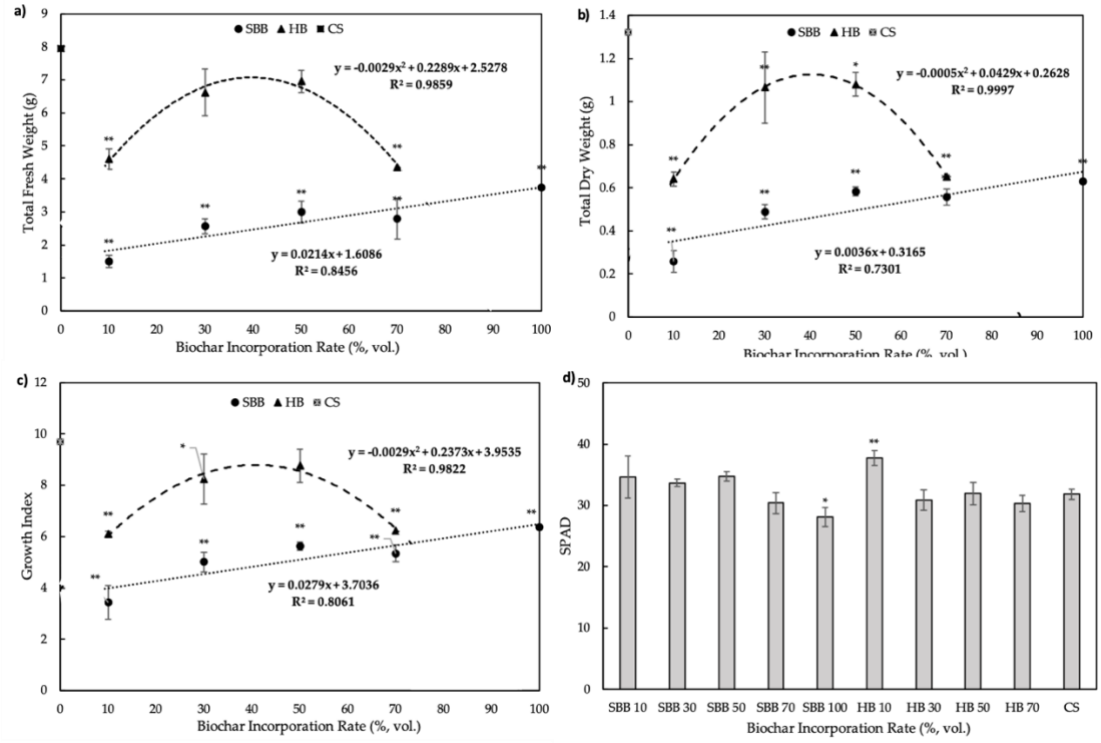

Figure 2.5. The correlations between total fresh weight (TFW, a), total dry weight (TDW, b), growth index (GI, c) and biochar incorporation rate and the SPAD (d) of basil seedlings grown in biochar-amended mixes. SBB (10%, 30%, 50%, 70% and 100%; by vol.) incorporated with 30% perlite with the rest being peat moss; HB (10%, 30%, 50% and 70%; by vol.) incorporated with peat moss. *, ** indicate a significant difference from the control using Dunnett's test at $p \leq 0.05$ and $p \leq 0.01$, respectively.

All basil seedlings grown in biochar-amended mixes had significantly shorter root lengths, smaller root surface areas and fewer root tips than the control (Table 2.3); however, they all had similar root diameters.

Table 2.3. Root growth of basil seedlings grown in different mixes. (Numbers in parentheses indicate the ratio of different components, by vol. *, ** indicate a significant difference from the control using Dunnett's test at $p \leq 0.05$ and $p \leq 0.01$, respectively)

Mixes [z]	Root Length (cm)	Root Surface Area (cm^2)	Average Diameter (mm)	Number of Tips
SBB:PM:P (10:60:30)	121 ± 13**	16 ± 1**	0.43 ± 0.03	480 ± 42**
SBB:PM:P (30:40:30)	295 ± 523**	34 ± 11**	0.37 ± 0.01	819 ± 88**
SBB:PM:P (50:20:30)	433 ± 23**	51 ± 6**	0.37 ± 0.01	1408 ± 235**
SBB:PM:P (70:0:30)	617 ± 92**	72 ± 22**	0.37 ± 0.01	1204 ± 118**
SBB:PM:P (100:0:0)	841 ± 95*	97 ± 15**	0.37 ± 0.02	1584 ± 163**
HB:PM (10:90)	331 ± 29**	40 ± 7**	0.39 ± 0.01	873 ± 45**
HB:PM (30:70)	757 ± 67**	88 ± 19**	0.37 ± 0.01	1758 ± 177**

Table 2.3. Continued.

Mixes [z]	Root Length (cm)	Root Surface Area (cm^2)	Average Diameter (mm)	Number of Tips
HB:PM (50:50)	793 ± 145**	96 ± 35**	0.39 ± 0.01	1761 ± 167**
HB:PM (70:30)	690 ± 44**	85 ± 6**	0.39 ± 0.01	1446 ± 194**
Control	1181 ± 67	145± 21	0.39± 0.02	3001 ± 214

2.3.3. The After-Growth Evaluation of Seedlings Produced in Biochar-Amended Media

2.3.3.1. Tomato Plant Growth

Tomato seedlings from different biochar-amended mixes (except 50% HB) all had significantly lower GI at transplanting compared to those from the commercial propagation mix (Figure 2.6a). However, after growing in CS1 for four weeks, all plants from SBB- and HB-amended mixes had similar GI (except 30% SBB) and SPAD to the control (Figure 2.6a, b). In addition, tomato plants from all biochar-amended mixes had similar SDW (except 10% SBB, 30% SBB and 10% HB) and LDW (except 10% SBB) in

comparison to the control (Table 2.4); however, they had significantly lower FDW and TDW than the control.

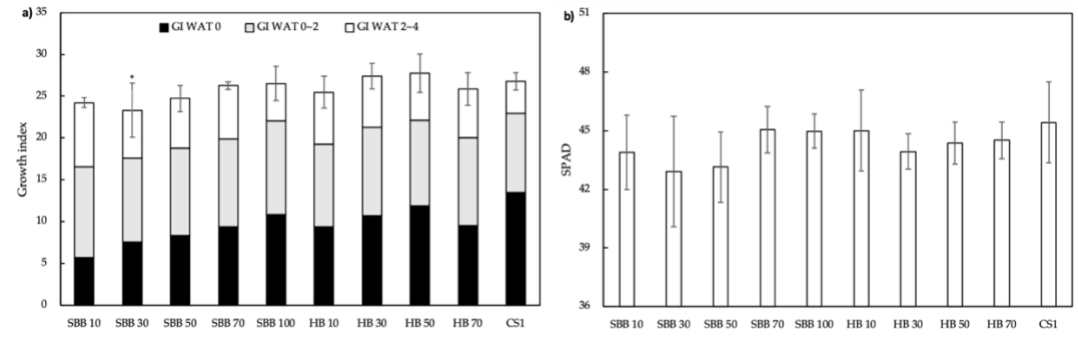

Figure 2.6. The growth index (a) and SPAD (b) of tomato seedlings from biochar-amended mixes after four weeks in commercial substrate. SBB (10%, 30%, 50%, 70% and 100%; by vol.) incorporated with 30% perlite with the rest being peat moss; HB (10%, 30%, 50% and 70%; by vol.) incorporated with peat moss. *, ** indicate a significant difference from the control using Dunnett's test at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Table 2. 1. Stalk, leaf, and fruit dry weight (g) of tomato seedlings from biochar-amended mixes after four weeks in the commercial substrate. (Numbers in parentheses indicate the ratio of different components, by vol. *, ** indicate a significant difference from the control using Dunnett's test at $p \leq 0.05$ and $p \leq 0.01$, respectively)

Mixes [z]	Stalk DW (g)	Leaf DW (g)	Fruit DW (g)	Total DW (g)
SBB:PM:P (10:60:30)	1.7 ± 0.1**	6.5 ± 0.1 **	0.3 ± 0. 0**	8.5 ± 0.2***
SBB:PM:P (30:40:30)	2.0 ± 0.2**	7.6 ± 0.4	1.0 ± 0.3**	10.6 ± 0.9***
SBB:PM:P (50:20:30)	2.7 ± 0.2	8.8 ± 0.5	0.9 ± 0.1**	12.5 ± 0.6***
SBB:PM:P (70:0:30)	3.2 ± 0.3	8.7 ± 0.4	1.3 ± 0.2**	13.2 ± 0.9**
SBB:PM:P (100:0:0)	3.5± 0.2	8.6 ± 0.2	2.4 ± 0.2**	14.5 ± 0.4**
HB:PM (10:90)	2.4 ± 0.3*	8.9 ± 0.5	1.3 ± 0.2**	12.6 ± 0.8***
HB:PM (30:70)	2.9 ± 0.3	7.8 ± 0.4	3.2 ± 0.7**	13.9 ± 1.0**
HB:PM (50:50)	3.2 ± 0.2	8.1 ± 0.2	4.0 ± 0.5*	15.4 ± 0.5*
HB:PM (70:30)	2.6 ± 0.2	8.4 ± 0.2	2.0 ± 0.3**	12.9 ± 0.6***
Control	3.4 ± 0.1	8.1 ± 0.5	7.7 ± 2.3	19.2 ± 2.1

2.3.3.2. Basil Plant Growth

Basil seedlings from different biochar-amended mixes (except 50% HB) all had significantly lower GI at transplanting compared to those from the commercial propagation mix (Figure 2.7a). However, after growing in CS1 for four weeks, all plants

from SBB- and HB-amended mixes (except 10% SBB, 30% SBB and 50% SBB) had similar GI and SPAD (except 30% SBB, 70% SBB, 100% SBB, 30% HB, 50% HB and 70% HB) to the control (Figure 2.7a, b). In addition, basil plants from all biochar-amended mixes had similar LDW (except 10% SBB, 30% SBB and 50% SBB) and FDW (except 10% SBB, 30% SBB, 50% SBB and 10% HB) in comparison to the control (Table 2.5). Plants from SBB-amended mixes all had significantly lower SDW and TDW compared to the control; however, plants from HB-amended mixes all had similar SDW (except 10% HB) and TDW (except 10% HB and 70% HB) to the control.

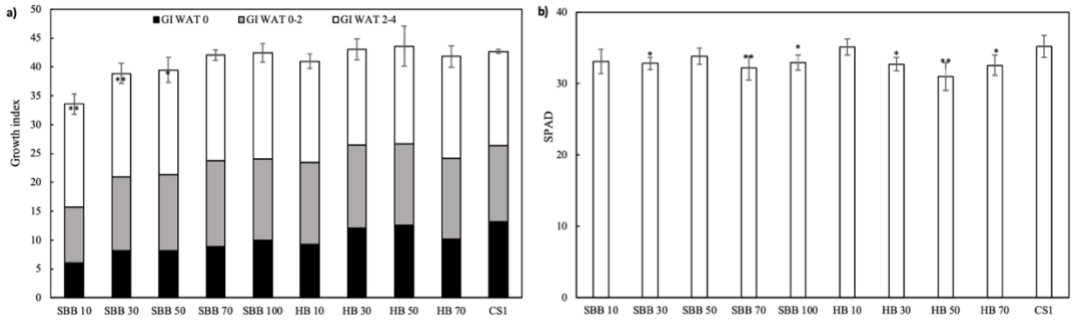

Figure 2.7. The growth index (a) and SPAD (b) of basil plants transplanted from biochar-amended mixes after four weeks. SBB (10%, 30%, 50%, 70% and 100%; by vol.) incorporated with 30% perlite with the rest being peat moss; HB (10%, 30%, 50% and 70%; by vol.) incorporated with peat moss. *, ** indicate a significant difference from the control using Dunnett's test at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Table 2.5. Biomass of basil plants transplanted from biochar-amended mixes after four weeks. (Numbers in parentheses indicate the ratio of different components, by vol. *, ** indicate a significant difference from the control using Dunnett's test at $p \leq 0.05$ and $p \leq 0.01$, respectively)

Mixes [z]	Stalk DW (g)	Leaf DW (g)	Flower DW (g)	Total DW (g)
SBB:PM:P (10:60:30)	1.8 ± 0.2**	4.3 ± 0.4**	0.4 ± 0.1**	6.4 ± 0.7***
SBB:PM:P (30:40:30)	3.3 ± 0.2**	6.2 ± 0.2*	1.1 ± 0.2**	10.5 ± 0.5***
SBB:PM:P (50:20:30)	3.1 ± 0.2**	6.0 ± 0.4**	0.8 ± 0.2**	9.9 ± 0.5***
SBB:PM:P (70:0:30)	3.3 ± 0.1**	5.9 ± 0.2	1.0 ± 0.1**	10.2 ± 0.2***
SBB:PM:P (100:0:0)	3.9 ± 0.08**	6.9 ± 0.3	1.7 ± 0.3	12.5 ± 0.3*
HB:PM (10:90)	3.6 ± 0.1**	6.4 ± 0.1	1.2 ± 0.2*	11.3 ± 0.3***
HB:PM (30:70)	4.3 ± 0.1	7.3 ± 0.3	2.0 ± 0.2	13.5 ± 0.4
HB:PM (50:50)	4.4 ± 0.1	7.3 ± 0.2	1.9 ± 0.4	13.6 ± 0.6
HB:PM (70:30)	4.1 ± 0.3	6.4 ± 0.6	1.5 ± 0.5	11.9 ± 0.7**
Control	4.8 ± 0.2	7.7 ± 0.3	2.5 ± 0.2	14.9 ± 0.6

2.4. Discussion

2.4.1. Media Phytotoxicity and Substrate Properties

Prior to incorporating biochar into any soilless substrate, simple germination tests could be used to test the phytotoxicity of biochar, and a phytotoxicity assessment is essential for successful soilless amendment [30]. The soilless petri dish bioassay (also

known as the germination test) is a rapid and simple preliminary test recommended by Solaiman to test potential biochar toxicity [35]. In this study, the extracts of SBB, HB, their mixes with PM, PM, P, 70%PM:30%P and the commercial propagation mix all showed no phytotoxicity, which is consistent with Taek–Keun's findings [36].

Biochar may or may not have phytotoxic effects on plants depending on the original feedstock and process conditions [30]. For instance, the biochar from hardwood, corn and switchgrass under different process conditions had no effect on germination rate [29], while biochar from olive mill waste was phytotoxic [37]. In this study, the germination rates of all basil seeds in the aqueous extracts of biochar-amended mixes were higher than those in DI water, which indicated no phytotoxicity for the biochar used in this study. This is similar to what had been found in Rogovska's work [29]. However, biochar-amended mixes having no effects on seedling germination rates does not necessarily mean they had no inhibition on shoot growth because plant's shoot growth can be inhibited by polycyclic aromatic hydrocarbons present in aqueous extracts [29], or by the poor physical–chemical properties of the mixes [2]. The seedlings grown in SBB-amended mixes had significantly lower DW than the control, which may be due to their low AS [38].

Even though the effects of biochar on substrate properties also varies depending on original feedstock and process conditions [22,30], some biochar types have been proven to improve the physical properties of the growing media [39]. For instance, pinewood biochar from fast pyrolysis of pinewood at 450 °C can make the substrate better

for poinsettia and Easter lily to grow [6,27]. Mixed hardwood biochar from fast pyrolysis can also improve the substrate properties for tomato and basil plant growth [24]. Sugarcane bagasse biochar and pinewood biochar improved the growing mix properties for bean and cucurbit seedlings production [31]. The pruning residue biochar produced from pyrolysis at 500°C can improve growing media properties for soilless vegetable production [13,39]. The biochar could also replace perlite and has a liming effect when incorporated into a soilless substrate [40,41]. These improvements were also observed in this study, especially for HB.

2.4.2. Biochar Effects on Plant Growth

The effects of biochar on plant growth could be positive, null, and negative [6,42,43], depending on the types of biochar and the incorporation rates. Incorporating biochar made from woodchips of *Pinus densiflora* and *Quercus acutissima* and rice husk at 20% (by vol.) with growing media, *Zelkova serrata* seedlings showed better performance than the control in a containerized production system; however, biochar made from crab shell had negative effects on seedling growth [44]. In this study, seedling biomass increased with the SBB (10–100%) and HB (10–50%) incorporation rate, which is slightly different from Webber's results [31]. Tomato plants from all biochar-amended mixes had significantly lower FDW (yield), basil plants from biochar-amended mixes (except for 10% SBB, 30% SBB and 50% SBB) had similar LDW (yield) to the control.

Even though the effects of biochar on plant biomass can be variable [6,42,43], the effects of biochar on plant GI is more often positively reported [6,27,45]. The GI of plants

can be an important parameter for landscape plants such as *Magnolia*, *Ilex*, *Lagerstroemia* and other species [46]. Biochar has also been reported to have positive effects on some ornamental plant GIs, such as poinsettia, Easter lily and "Firework" *Gomphrena* [6,27,45]. In this study, even though seedlings grown in biochar-amended mixes (SBB-, 10%, 30% and 70% HB-amended) had significantly lower TDW than the control, after growing in CS1 for four weeks, plants from biochar-amended mixes (except 30% SBB for tomato, 10%, 30% SBB for basil) all had a similar GI to the control. As landscape plants need more time to grow from seedling to a marketable size, the biochar-amended mixes used in this study might be used more successfully for landscape plants seedling production. More biochar studies on landscape plants should be conducted in the future.

Detailed studies on biochar–root interactions are few [47], but plant roots are the first contacting points to biochar particles. Plants with longer root length, larger surface area and more root tips may be able to obtain more nutrients and grow better [47,48]. In this study, root length, surface area and the number of tips of seedlings grown in biochar-amended mixes (except for 50%HB-amended) were all shorter, smaller or less than those grown in the control, which can explain why seedlings grown in most biochar-amended mixes did not perform as well as the control.

2.5. Conclusions

The biochar-amended mixes used in this experiment had acceptable BD, CC, AS, and TP (except 50%, 70% and 100% SBB). The results from this experiment indicated PM mixed with up to 50% HB could be used for tomato and basil seedling production in

a greenhouse. Both tomato and basil seedlings grown in 50% HB-amended mixes exhibited greater or similar growth compared to those in a commercial propagation mix, as reflected by similar seedling FW, DW, GI, SPAD and root development. Seedlings grown in 70% HB-amended mixes had significantly lower DW than the control, however, after growing in commercial growing media for four weeks, their DWs were similar to the control. Up to 70% of HB could be amended with PM for tomato and basil seedling production without negative effects on plant biomass.

2.6. References

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3. EFFECTS OF MIXED HARDWOOD AND SUGARCANE BIOCHAR AS BARK-BASED SUBSTRATE SUBSTITUTES ON CONTAINER PLANTS PRODUCTION AND NUTRIENT LEACHING*

3.1. Introduction

Both tomato and basil are important crops and 95% of tomato and basil are produced in soilless cultivation systems using different horticultural growing substrates [1]. Tomato is one of the most important horticulture crops, with a total production estimated to be at 164 MT worldwide [2]. Tomato can be grown in coconut fiber, and perlite alone or in mixture with peat, and produce good yields [3]. Additionally, 50% coco–peat mixed with 50% perlite was recommended for tomato seedling production [4]. Basil is an annual herb that is commercially important for its medical and culinary purposes [5,6]. Basil plants can be grown in 75% sphagnum peat moss mixed with 25% coarse perlite [7]. Additionally, the mix of 60% sphagnum peat and 10% biochar with compost, has proven to be suitable for basil production [8].

Container plant production has become a major source of N leaching and runoff that can be a potential contamination source [9,10]. Container plant production requires a large amount of fertilizer, with nitrogen as the key component, making container plant production a major source of N leaching or runoff [9]. The leachate of N can be a potential

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contamination source for surface and underground water, resulting in environmental and health concerns [11]. NO_3–N, the main form for plants absorption, contributes in large to the N leaching and runoff in soilless production systems.

Bark has become one of the most commonly used container organic components in horticulture [12]. The reason for bark being commonly used in horticulture is because it has suitable properties for container plants to grow well and it is easy to get access to [13,14]. Compared to peat moss, another most commonly used container component, bark, is a byproduct of the forestry industry, is less expensive because it is available locally and does not require extra shipping costs [15,16]. In the US, Douglas fir bark is mainly used in the pacific northwest, while pine bark is mainly used in the southwest [17,18].

Although bark has been a good container component, besides peat moss, its inconstant and unpredictable supply in recent years has limited its usage in horticulture industry [16,19,20]. Bark supply competes with many other markets, including alternatives of industrial fuel, timber production, housing, and paper market, all of which prevent bark from being a constant source for the horticulture industry [20–22]. Since the supply of bark is fluctuating and unpredictable, it would be beneficial for the horticulture industry to explore less expensive and more constant alternatives with similar properties [16,22].

Biochar (BC), a by-product from thermochemical biomass decomposition under an oxygen-depleted or oxygen-limited environment [23–25] with specific time and temperature conditions and from certain carbon-rich raw materials, can be a potential

alternative to common substrates for plant growth, as has been documented in many trials [16,26–29]. Research has shown that BC can increase water and nutrient holding capacity, ameliorate substrate acidity, and provide suitable environments for plants [30–32]. It, thus, improves greenhouse crop growth, yield, and quality, under appropriate conditions [32–36].

Biochar has been considered to be a sustainable component of a growing substrate because it can be derived from various agriculture by-products, such as green waste [33], wood, straw [31,37–40], bark [41], rice hull [42], and wheat straw [31,43]. Additionally, due to the significant variation in pyrolysis conditions, the BC properties could vary significantly, and there is no universal standard for BC addition to plant production and BC's effects on container substrates vary, as a result [28]. Research on BC as a substrate amendment is still in its infant stage [29]. In this present study, a trial was conducted to determine whether two types of BCs had the potential to be a replacement of bark-based substrate amendments for container plant production.

3.2. Materials and Methods

3.2.1. Plant Materials

Plant seeds (tomato, *Solanum lycopersicum* 'Red Robin™', Fred C. Gloeckner and Company Inc., Harrison, New York, USA; basil, *Ocimum basilicum*, Johnny's Selected Seeds, Winslow, Maine, USA) were sown in 72-cell plug trays (one seed per cell, cell dimension: 5 cm*4 cm*4 cm, depth/length/width; volume: 55 mL) with a commercial germination substrate (BM2 Berger, Saint-Modeste, Quebec, Canada), on 26 February

2019. After the first pair of true leaves expanded, uniform seedlings were transplanted into 6-inch azalea pots (dimension: 10.8 cm* 15.5 cm*11.3 cm, depth/top/bottom diameter; volume: 1330 mL) with a commercial growing substrate (Jolly Gardener, Oldcastle Lawn & Garden Inc., Atlanta, Georgia, USA) that was incorporated with either sugarcane bagasse biochar (SBB, American Biocarbon LLC White Castle, Louisiana, USA) at two different rates (50% and 70%; by vol.), or with mixed hardwood biochar (HB, Proton Power Inc. Lenoir City, Tennessee, USA) at 50% (by vol.), on 27 March 2019.

The composition used in this study was chosen because a previous study had showed that 70% of HB can be successfully incorporated with peat moss based commercial substrates and with composts for tomato and basil production [29], and 50% of SBB can be used for petunia growth (not published). We wanted to do further tests of HB with different compositions, on tomato and basil, using tests of SBB with different plant species. The main components for the commercial growing substrate was aged pine bark (55%; by vol.), the other ingredients in the substrate were Canadian sphagnum peat moss, perlite, and vermiculite. The commercial substrate was used as the control. The pH of SBB and of HB were 5.9 and 10.1, respectively (Table 3.1). The SBB and HB had electrical conductivity (EC) of 753 µS cm^{-1} and 1,058 µS cm^{-1}, respectively [44]. During transplanting, slow-release fertilizer Osmocote Plus (15N-4P-10K, Scotts-Sierra Horticultural Products Company, Marysville, Ohio, USA) was surface-dressed at the rate of 4.8 g pot^{-1} for basil and 7.7 g pot^{-1} for tomato. All mixes were placed in a greenhouse at Texas A&M University, College Station, Texas, USA. The average greenhouse

temperature, relative humidity, and dew point were 23.7 °C, 82%, and 19.6 °C, respectively.

Table 3.1. The pH, electrical conductivity (EC), total porosity (TP), container capacity (CC), air space (AS), and bulk density (BD) of biochars and the substrate mixes used in this study.

Composition	pH	EC ($\mu S\ cm^{-1}$)	TP (%)	CC (%)	AS (%)	BD ($g\ cm^{-3}$)
SBB	5.9	753	74	71	3	0.11
HB	10.1	1058	87	66	20	0.13
50%SBB + 50%CS	6.3	2073	81	75	7	0.13
50%HB + 50%CS	7.5	1370	78	62	17	0.13
70%SBB + 30%CS	6.4	1830	89	76	13	0.14
CS	6.5	1819	97	85	12	0.15
Suitable range [z]	-	-	50–80	45–65	10–30	0.19–0.7

Note: SBB = Sugarcane Bagasse Biochar; HB = Mixed hardwood Biochar; and CS = Commercial bark-based growing mix; [z] Recommended physical properties of container substrate by Yeager et al. [45].

3.2.2. Measurements

3.2.2.1. Potting Mix Physical and Chemical Properties

Mix 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 [46]. The leachate EC and pH were measured every other week, starting at one week after transplanting (1 WAT), with a portable EC/pH meter (Hanna Instrument, Woonsocket, Rhode Island, USA), according to the pour-through method [47].

Nutrient leachate was collected whenever EC and pH were measured and was stored in the refrigerator (4 °C) until analysis. A HQ440d Benchtop Meter and ISENO3181 nitrate electrode (Hach Company, Loveland, Colorado, USA) were used for leachate NO_3–N measurements.

3.2.2.2. Plant Growth

Plant height and two widest canopy widths (width 1: horizontal, width 2: perpendicular) were measured at 1, 3, 5, and 7 WAT. The plant growth index (GI) was calculated according to the formula: GI = plant height/2 + (width 1 + width 2)/4 [26]. Plants' leaf greenness was measured at 1 WAT with a portable soil-plant analyses development (SPAD) meter (SPAD 502 Plus Chlorophyll Meter, Spectrum Technologies, Inc., Plainfield, Illinois, USA). Each plant's leaf greenness was determined by taking averages of readings from three random mature leaves. Plant stem, leaf, and fruit were harvested separately. After being dried at 80 °C in an oven until a consistent weight was

reached, their dry weights (shoot dry weight (SDW), leaf dry weight (LDW), fruit dry weight (FDW)) were measured. Plant roots were washed under running water, after harvest. Root length, root surface area, average root diameter, and the number of root tips were measured by using a root scanner (WinRHIZO, Regent Instruments Canada Inc., Quebec, Canada). Root dry weights (RDW) were determined after being dried at 80 °C in an oven, until a constant weight was reached. Total dry weights (TDW) were calculated by adding up the SDW, LDW, FDW, and RDW.

3.2.3. Statistical Analysis

This experiment was designed as a completely randomized block design with six replications for each mix. A one-way analysis of variance using JMP Statistical Software (version Pro 14.2.0; SAS Institute, Cary, North Carolina, USA) was used for data analysis. All the means were separated by using Dunnett's test when treatments were significantly different from control at $p \leqslant 0.05$. A principle component analysis (PCA) was conducted to evaluate the relationship between the selected variables and were treated using R programing software (version 3.5.1).

3.3. Results

3.3.1. Potting Mix Physical and Chemical Properties

Most of the mixes' physical properties were within the recommended range [45], except for the SBB-incorporated mixes, which had a slightly higher TP and CC than the recommended value (Table 1). The 50% SBB mix had a slightly lower AS, as compared

to the recommended value. All the mixes had slightly lower BD in comparison to the recommended value and the commercial mix had the lowest BD among all the mixes.

Tomato and basil plants grown in all BC-incorporated pots had similar EC as compared to the control, throughout the experiment, except for the tomato plants in 50% HB at 1 WAT (Figure 3.1). The 50% HB mixes with tomato plants had a significantly higher pH than the control at 1, 3, and 7 WAT (Figure 3.2A). The SBB-incorporated mix with tomato plants (50% at 1 WAT, 70% SBB at 7 WAT) had a significantly lower pH, compared to the control. Plants in all the other BC-incorporated mixes had a similar pH, throughout the experiment. Basil plants grown in 50% HB mixes had a significantly higher pH compared to the control, throughout the experiment (Figure 3.2B). However, basil plants grown in SBB-incorporated mixes (50% and 70%, at 5 and 7 WAT) had a significantly lower pH, compared to the control.

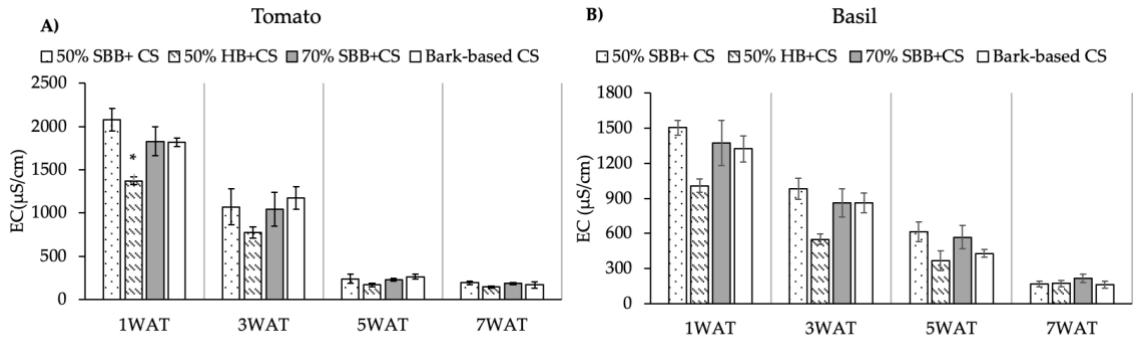

Figure 3.1. The EC (mean ± standard error) of potting mixes with 50% sugarcane bagasse biochar (SBB), 50% mixed hardwood biochar (HB), and 70% SBB (by vol.) mixed with bark-based commercial substrate (CS) with tomato (A) and basil (B) plants at 1, 3, 5, and 7 week(s) after transplanting (WAT). *indicated significant differences from CS using Dunnett's test at $p \leq 0.05$.

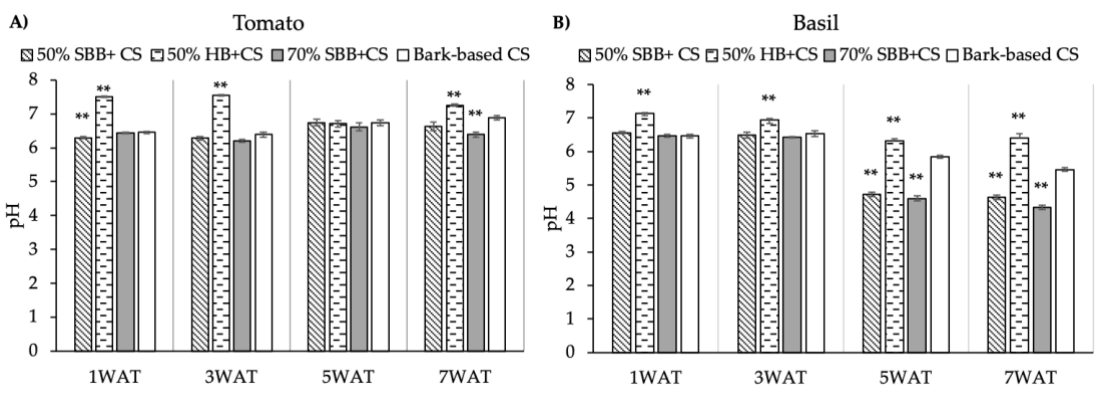

Figure 3.2. The pH (mean ± standard error) of container mixes, with 50% sugarcane bagasse biochar (SBB), 50% mixed hardwood (HB), and 70% SBB (by vol.) mixed with bark-based commercial substrate (CS) grown with tomato (A) and basil (B) plants at 1, 3, 5, and 7 week(s) after transplanting (WAT). **indicated significant differences from CS using Dunnett's test at $p \leq 0.01$.

3.3.2. Leachate NO_3–N

The leachate of all BC-incorporated mixes (both with tomato and basil plants) had a similar or higher NO_3–N concentration compared to the control. The leachate NO_3–N concentration generally decreased from 1 WAT to 7 WAT, for each mix (Figure 3.3).

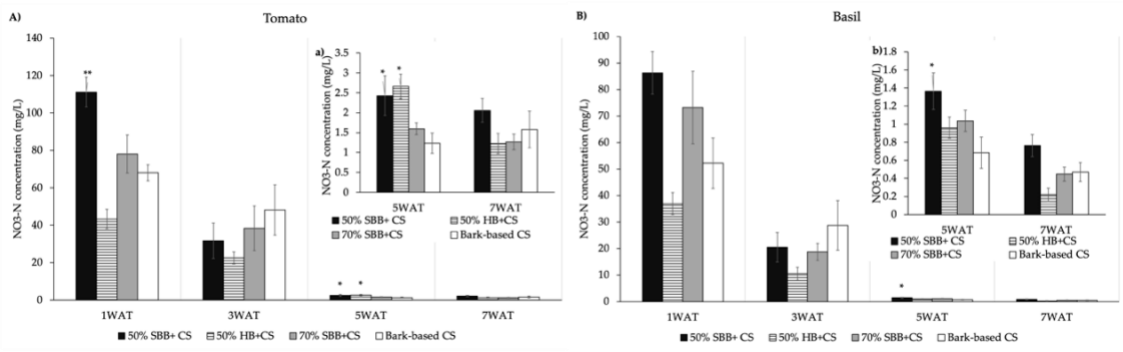

Figure 3.3. Leachate NO_3–N (mean ± standard error) of tomato (A) and basil (B) plants grown in container mixes with 50% (by vol.) sugarcane bagasse biochar (SBB), 50% mixed hardwood biochar (HB), and 70% SBB mixed with bark-based commercial substrate (CS). (A, B) Amplified figure for tomato (a) and basil (b) from 5 WAT to 7 WAT. *, **indicated significant differences from CS using Dunnett's test at $p \leq 0.05$ and $p \leq 0.01$, respectively.

3.3.3. Plant Growth

In the BC-incorporated mixes, both tomato and basil plants had a similar or higher GI, in comparison to the control, throughout the experiment (Figure 3.4). Tomato plants in all BC-incorporated mixes had similar SDW and FDW (yield), compared to the control, however, tomato plants in SBB-incorporated mixes had significantly lower TDW, RDW,

and LDW compared to the control (Figure 5A). Basil plants grown in all BC-incorporated mixes had similar RDW, SDW (except 50% HB), LDW, FDW, and TDW to the control (Figure 5B). The SPAD of tomato and basil plants grown in all BC-incorporated mixes was no different from the control (Figure 6).

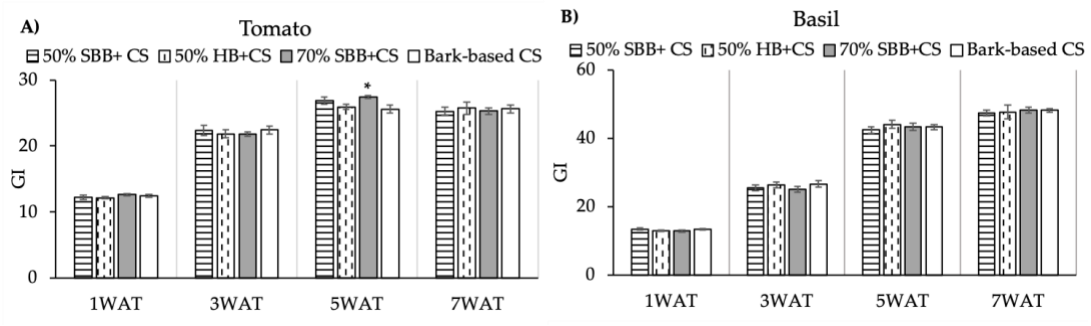

Figure 3.4. Growth index (mean ± standard error) of plants tomato (A) and basil (B) grown in container mixes with 50% sugarcane bagasse biochar (SBB), 50% mixed hardwood biochar (HB), and 70% SBB (by vol.) mixed with bark-based commercial substrate (CS) at 1, 3, 5, and 7 week(s) after transplanting (WAT). *indicated significant differences from CS, using Dunnett's test at $p \leq 0.05$.

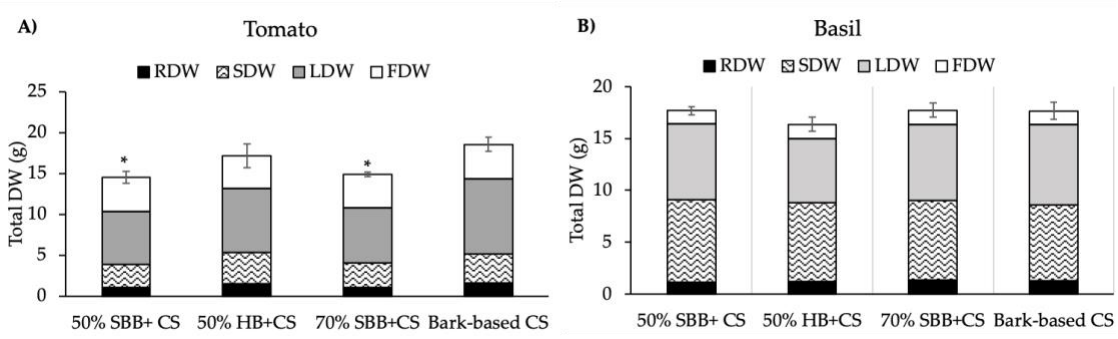

Figure 3.5. Total dry weight (Total DW = root dry weight (RDW) + shoot dry weight (SDW) + leave dry weight (LDW) + fruit dry weight (FDW); mean ± standard error) of tomato (A) and basil (B) grown in container mixes with 50% sugarcane bagasse biochar (SBB), 50% mixed hardwood biochar (HB), and 70% SBB (by vol.) mixed with bark-based commercial substrate (CS). *indicated significant differences on the total DW from CS using Dunnett's test at $p \leq 0.05$.

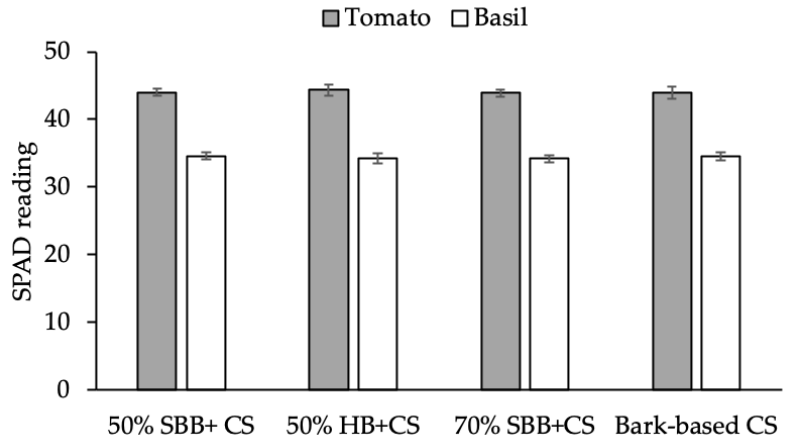

Figure 3.6. The soil-plant analyses development (SPAD) (mean ± standard error) of tomato and basil grown in container mixes with 50% sugarcane bagasse biochar (SBB), 50% mixed hardwood biochar (HB), and 70% SBB (by vol.), mixed with bark-based commercial substrate (CS).

Similar root length, average root diameter, and number of root tips were observed between tomato plants grown in all BC-incorporated mixes and the control (except 50% SBB), however, significantly smaller root surface area of tomato plants grown in all SBB-incorporated mixes were noticed (Table 3.2). Basil plants grown in all BC-incorporated mixes had significantly shorter root length but bigger diameter than the control. Basil plants in all BC-incorporated mixes had similar root surface area to the control, yet those in 50% BC-incorporated mixes had significantly fewer root tips than the control (Table 2).

Table 3.2. The root development (mean ± standard error) of plants grown in potting mixes with 50% sugarcane bagasse biochar (SBB), 50% mixed hardwood biochar (HB), and 70% SBB (by vol.) mixed with bark-based commercial substrate (CS). *, **, and ***indicated significant differences from CS using Dunnett's test at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

Mixes	Root Length (cm)	Root Surface Area (cm^2)	Average Root Diameter (mm)	Number of Root Tips
		Tomato		
50%SBB + 50%CS	1214 ± 60	442 ±37 *	1.2 ± 0.1	2650 ± 94 *
50%HB + 50%CS	1454 ± 67	557 ± 24	1.2 ± 0.1	3349 ± 171
70%SBB + 30%CS	1234 ± 74	421 ± 25 *	1.1 ± 0.1	2970 ± 196
CS	1324 ± 40	543 ± 19	1.3 ± 0.1	3227 ± 157
		Basil		
50%SBB + 50%CS	1415 ± 48 ***	819 ± 18	1.9 ± 0.1 ***	3092 ± 166 **
50%HB + 50%CS	1887 ± 117 *	866 ± 23	1.5 ± 0.1 *	3006 ± 149 **

Table 3.2. Continued.

Mixes	Root Length (cm)	Root Surface Area (cm^2)	Average Root Diameter (mm)	Number of Root Tips
70%SBB + 30%CS	1850 ± 115 *	870 ± 19	1.5 ± 0.1 *	3528 ± 222
CS	2240 ± 74	832 ± 26	1.2 ± 0.0	4003 ± 80

3.4. Discussion

3.4.1. Potting Mix Physical and Chemical Properties

Despite the fact that BC can have various effects on substrate properties contingent on the types of feedstocks and the pyrolysis conditions of BC [28,48], many types of BC have been proven to be suitable replacements for commercial growing substrates, without negatively affecting the plant [28,35]. Biochar from fast pyrolysis (pinewood, 450 °C), for instance, could replace commercial substrate at up to 80%, providing suitable properties for the poinsettia and Easter lily growth [26,27]. Biochar from fast pyrolysis (mixed hardwood) could be suitable for tomato and basil plant growth, due to the proper properties it created [29]. Sugarcane bagasse BC and pinewood BC mixes had similar physical properties to commercial growing mix, allowing them to be acceptable for bean and cucurbit seedlings production, even though some of the TP and CC in the SBB-

incorporated mixes were slightly higher than the recommended values [44]. Adding pruning residue BC (fast pyrolysis, 500 °C) to soilless mixes can render appropriate physical properties for vegetable production [35,49]. In this study, even though 50% SBB and 70% SBB mixes had slightly higher TP (81%, 89%, respectively) and CC (75%, 76%, respectively) than the recommended value (TP 50%–80% and CC 45%–65%) [45], the growth of tomato and basil plants was not affected, as observed in Webber's study [44].

Different initial BC pH (HB: 10.05, SBB: 5.94) resulted in differences in pH levels in the different BC mixes. Mixes with HB (50%, by vol.) and commercial bark-based substrates (initial pH: 6.81) had a pH lower than the initial HB but higher than the initial commercial bark-based substrate. The same was true for all SBB mixes. Since SBB had an acidic initial pH, adding 30% to 50% of the commercial substrate (pH: 6.81) resulted in mixes with a pH that was lower than the commercial substrate but was higher than the SBB.

3.4.2. Biochar Effects on Leachate NO_3–N

Plant species, plant stage, and substrate properties can influence NO_3–N leaching [9,50,51]. Tomato, as a heavy feeder fertilizer crop, require more nutrients throughout the growing season than other lighter feeder fertilizer crops, such as snapdragon and bedding plants [52,53]. As a result of administering the same amount of fertilizer to different plant species due to their divergent nutrient requirements, the final NO_3–N leaching varies. Additionally, the nutrients demand for plant at different stages also vary. During the growing period, plants' requirement for nutrients presents a skewed "s" curve—vegetative

periods need less nutrient yet when entering the flowering/fruit-set period, the demand for nutrients increases dramatically [54]. Nitrate leaching can be also affected by soil or substrate texture and normally, coarse textured mixtures lead to more nitrate leaching [55]. Substrate properties affecting nitrate leaching can explain why leachate from 50% HB (in both case of tomato and basil) had the lowest NO_3–N concentration (except tomato at 5 WAT), among all mixes.

3.4.3. Biochar Effects on Plants Growth

Biochar can have positive, null, and negative effects on plant growth [26,56,57], contingent on plant species, BC types, incorporation rates, and the interactions of both. For instance, pinewood BC had positive effects on bell pepper growth [58], similar results were reported on Easter lily, poinsettia, and "Firework" *Gomphrena*. Mixed hardwood BC can positively affect tomato and basil plants growth [16,26,27,29]. The null and negative effects of BC (from tomato crop waste or wood pellet) on tomato plant growth have also been reported [56,57]. This study obtained similar results to some previous studies that found that BC does not negatively affect plant growth at high incorporation rates [16,26,27,29].

There are few studies with detailed information on BC–root systems [59]. Since roots are essential parts for water and nutrients uptake, plants with better roots were desired [59,60], and the effects of BC on root development is an eventuality. In this study, tomato plants grown in all the BC-incorporated mixes had similar root length, root surface area (except 50% and 70% SBB), average root diameter, and number of tips, in

comparison to the control. Basil plants had similar root surface area to the control, which can explain why plants grown in BC-incorporated mixes performed as well as those in the control.

3.4.4. Treatment Factors Determined Plants and Mix Properties

As the effect of biochar on plants and mix properties can be complex and difficult to explain, given the fact that two types of biochars and multiple variables were included in this study, a principal component analysis (PCA) was used to depict variables shaped by different biochars with tomato (Figure 3.7A) and basil (Figure 3.7B) plants. For tomato plants, 88.9% of the variability was explained by the first two components (Figure 3.7A). PC1 accounted for 65.8% variance, with SBB differing from HB and CS. Sugarcane bagasse biochar was associated more with yield (FDW) and NO_3–N leaching, while CS and HB was related more to plant growth (RDW, LDW, and GI). PC2 accounted for 23.1% variance, distinguishing the CS and BC mixes. Commercial substrate tended to be affiliated with plant biomass, however, BC mixes appeared to be related to nutrient leaching. For basil plants, the first two components explained 77.1% of the variability (Figure 3.7B). PC1 accounted for 42.9% variance, SBB 50% differing from HB and CS mixes. A 50% sugarcane bagasse biochar mix showed a greater association with NO_3–N leaching and SDW, while CS, 70% SBB, and HB showed a greater relation to plant growth, including root parameters (RDW, root length (RL), root tip (RT), and root surface area (RSA)) and chemical properties of the mixes (EC, pH). PC2 accounted for 34.2% variance, distinguishing between the CS and BC mixes. Commercial substrates tended to

be affiliated with plant biomass, however, BC mixes appeared to be related to the chemical properties of the mixes (EC, pH, NO_3–N).

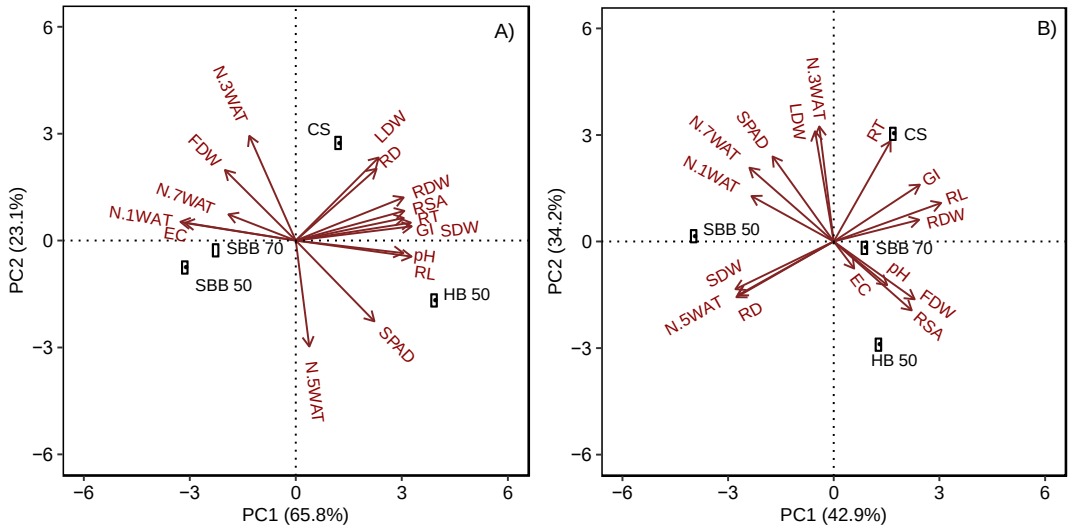

Figure 3.7. Principal component analysis (PCA) depicting the relationships between selected variables and treatment factors with tomato (A) and basil (B). Selected variables are displayed by arrows and include plant growth parameters—SPAD, growth index (GI), fruit dry weight (FDW), leave dry weight (LDW), shoot dry weight (SDW), root length (RL), root dry weight (SDW), root diameter (RD), root surface area (RSA), and number of root tips (RT); substrate chemical parameters were pH, EC, and NO_3–N leachate at different weeks. Treatment factors are displayed by filled grey circles: 50% sugarcane bagasse biochar (SBB 50), 50% mixed hardwood biochar (HB 50), 70% SBB (SBB 70) mixed with bark-based commercial substrate, and bark-based commercial substrate (CS).

3.5. Conclusions

The mixed hardwood biochar and sugarcane bagasse biochar used in this experiment could be used as bark-based substrate amendments for container plant production. The mixed hardwood biochar could replace the bark-based substrate at 50% and the sugarcane bagasse biochar at 70%, as growing mixes for tomato and basil production. More than 5.4 M ft^3 container substrates were used in horticulture industry in 2017 and the current container substrate major components—peat moss and bark are causing serious environmental concerns [61]. As can be seen from the results of this study, if mixed hardwood biochar or sugarcane bagasse biochar was chosen for greenhouse production, around 1.35 M ft^3 fewer peat moss or 1.94 M ft^3 fewer bark could be used annually (assuming container substrate contains 50% peat moss or bark).

3.6. References

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4. THE EFFECTS OF MIXED HARDWOOD BIOCHAR, MYCORRHIZAE, AND FERTIGATION ON CONTAINER TOMATO AND PEPPER PLANT GROWTH*

4.1. 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,

*P. Yu, Q. Li, L. Huang, K. Qin, G. Niu, M. Gu. 2020 The effects of mixed hardwood biochar, mycorrhizae, and fertigation on container tomato and pepper plant growth, Sustainability, 2020, 12, 7072; https://doi.org/10.3390/su12177072.

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 sequestrate 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.

4.2. Materials and Methods

4.2.1. 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, Tennessee, USA). Two composts, VC (Pachamama earthworm castings, Lady Bug Brand, Conroe, Texas, USA) and CM (Back to Nature Inc., Slaton, Texas, USA), were chosen as additives to the BC. The commercial substrate Sunshine Mix #1 (CS; Professional Growing Mix #1, SunGro Inc., Agawam, Maine, 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, Oregon, USA) was applied at the recommended rate. The MC product is granular mycorrhizal inoculum consisting of four endomycorrhizal fungi species and seven ectomycorrhizal 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, Everris NA Inc., Dublin, Ohio, 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, Rhode Island, 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 4.1 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 4.2).

Table 4.1. Physical (total porosity (TP, %), container capacity (CC, %), air space (AS, %), and bulk density (BD, g cm^{-3})) and chemical (pH, EC) properties of biochar, vermicompost, chicken manure, and commercial peat moss substrate used in this study according to previous studies [24,25].

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 4.2. Nutrient content of the biochar, vermicompost, and chicken manure used in this study according to the work conducted by Huang et al. [25].

	N	P	K	Ca	Mg	S	Fe
Components	**(%)**	**(mg kg^{-1})**					
Biochar	0.23	456	6,362	27,507	1,299	231	2,039
Vermicompost	2.43	4,901	3,714	25,841	3,819	5,996	4,835
Chicken manure	2.03	17,315	28,565	71,239	11,513	7,169	3,703

4.2.2. 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, Texas, USA. Experiment 1 (exp.1) was conducted from July 2017 to October 2017, while experiment 2 (exp.2) and experiment 3 (exp.3) 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 exp.1, and 22.37 °C, 64.95%, and 14.08 °C, respectively, for exp.2 and exp.3.

4.2.2.1. 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 4.3) for a total of 16 treatments.

Tomato (*Solanum lycopersicum* 'Tumbling Tom Red', Morgan County Seeds, Barnett, Missouri, 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 July 26, 2017. Tomato was transplanted on August 8, 2017 and pepper on August 11, 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.

Table 4.3. List of treatments used in experiment 1 including biochar (BC), vermicompost (VC), and commercial peat moss-based substrate (CS), mycorrhizae (MC, Y/N = with/without), and fertigation (F) rate (mg L^{-1} N).

Treatment	BC (%, vol.)	VC (%, vol.)	CS (%, vol.)	MC	F (mg L^{-1} N)
50 [X]:Y[Y]:200	50	5	45	Y	200
50:Y:300	50	5	45	Y	300
50:N:200	50	5	45	N	200
50:N:300	50	5	45	N	300
70:Y:200	70	5	25	Y	200
70:Y:300	70	5	25	Y	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	5	95	Y	200
0:Y:300	0	5	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).

4.2.2.2. 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 October 14, 2017). Fertilizer was applied through F at 100 mg L^{-1} or 200 mg L^{-1} N (Table 4.4).

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).

Table 4.4. List of treatments used in experiment 2 including biochar (BC), chicken manure compost (CM), commercial peat moss-based substrate (CS), mycorrhizae (MC, Y/N = with/without), and fertigation (F) rate (mg L^{-1} N).

Treatment	BC (%, vol.)	CM (%, vol.)	CS (%, vol.)	MC	F (mg L^{-1} N)
80-5[X]:Y[Y]:100	80	5	15	Y	100
80-5:Y:200	80	5	15	Y	200
80-5:N:100	80	5	15	N	100
80-5:N:200	80	5	15	N	200
80-10:Y:100	80	10	10	Y	100
80-10:Y:200	80	10	10	Y	200
80-10:N:100	80	10	10	N	100
80-10:N:200	80	10	10	N	200
0-0:Y:100	0	0	100	Y	100
0-0:Y:200	0	0	100	Y	200
0-0:N:100	0	0	100	N	100
0-0:N:200	0	0	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).

4.2.2.3. 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 4.5).

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).

Table 4.5. List of treatments used in experiment 3 including biochar (BC), commercial peat moss-based substrate (CS), mycorrhizae (MC, Y/N = with/without), and fertigation (F) rate (mg L^{-1} N).

Treatment	BC (%, vol.)	CS (%, vol.)	MC	F (mg L^{-1} N)
90[X]:Y[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).

4.2.3. 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 = Height/2 + (Width1 + Width2)/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.

4.2.4. Statistical Analysis

Data were analyzed with ANOVA using JMP Statistical Software (version Pro 14.2.0; SAS Institute, Cary, North Carolina, 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).

4.3. Results

4.3.1. 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 4.6). MC × F interaction was only significant for TDW in tomato and SPAD in pepper. Mix × F interaction was not significant for all

variables in tomato and only for FDW in pepper. Significant interactions for Mix × 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 4.6. 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]					**Significance**				
Mix	***	***	***	***	**Mix**	***	***	***	***
MC	NS	**	NS	NS	**MC**	**	NS	NS	NS
F	NS	**	NS	NS	**F**	NS	**	NS	**
Mix × MC	*	NS	*	**	**Mix × MC**	NS	***	NS	*
Mix × F	NS	NS	NS	NS	**Mix × F**	*	**	NS	*
MC × F	NS	NS	NS	**	**MC × F**	NS	*	NS	NS
Mix × MC × F	*	NS	**	***	**Mix × MC × 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 4.7). 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 4.7. 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	Tomato	Tomato	Pepper
	GI 8	FDW	TDW	GI 8
0[X]:Y[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 4.6; Figure 4.1). 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.

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 4.2A). Pepper plants grown in 50% and 70% BC-amended mixes had similar FDW (Figure 4.2B) and TDW (Figure 4.2C) compared to those grown in the CS.

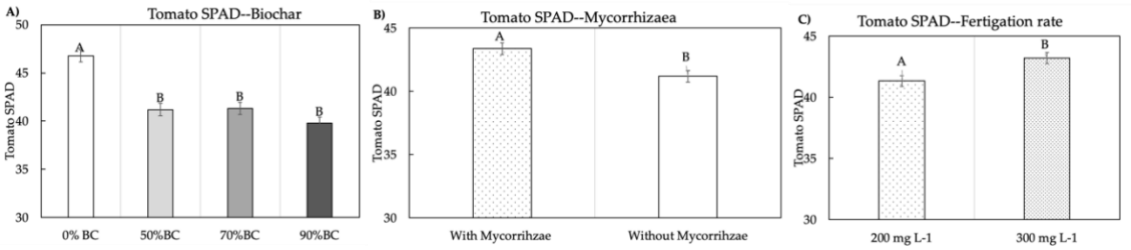

Figure 4.1. The effects of % biochar rates (BC; A), mycorrhizae (B), and fertigation (C) on tomato soil-plant analyses development (SPAD). The same letter indicates not significantly different according to Tukey HSD multiple comparison at $p \leq 0.05$.

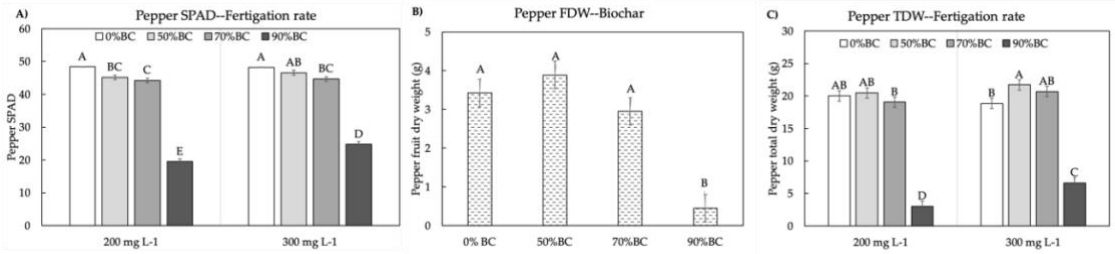

Figure 4.2. The effects of biochar on pepper soil-plant analyses development (SPAD) (A), fruit dry weight (FDW; B) and total dry weight (TDW; C). The same letter indicates not significantly different according to Tukey HSD multiple comparison at $p \leq 0.05$.

4.3.2. 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 4.8). 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.

Table 4.8. 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]					**Significance**				
Mix	NS	**	***	NS	**Mix**	***	***	***	***
MC	NS	***	NS	**	**MC**	*	*	NS	NS
F	NS	NS	NS	NS	**F**	NS	NS	NS	NS
Mix × MC	NS	*	NS	***	**Mix × MC**	*	**	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	NS	**	**	**Mix × MC × F**	NS	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.

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 4.9). 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 4.9. 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[X]:Y[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 $p \leq 0.05$.

Adding MC did not significantly impact SPAD of tomato plants grown in BC-amended mixes with 5% CM (Figure 4.3A). Mix type did not significantly influence tomato GI 8 (Figure 4.3B). With MC, mix type had no significant effects on pepper GI, SPAD, or TDW (Figure 4.4A−C). Similarly, at 200 mg L^{-1} N, mixes with 5% CM did not significantly impact pepper FDW either (Figure 4.4D).

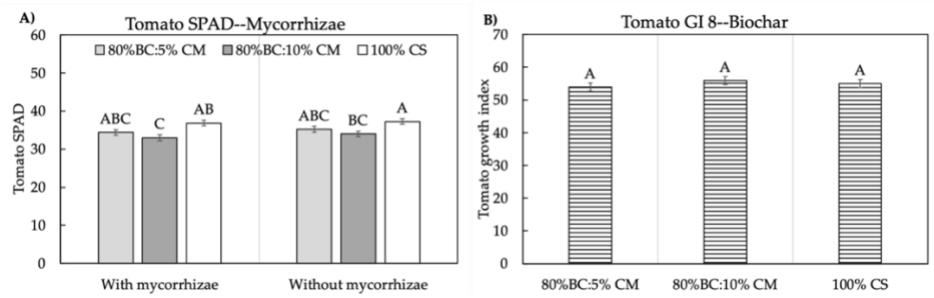

Figure 4.3. The effects of mycorrhizae on tomato SPAD (A) and mixes on tomato growth index at the eighth week after transplanting (GI 8; B). The same letter indicates not significantly different according to Tukey HSD multiple comparison at $P \leq 0.05$.

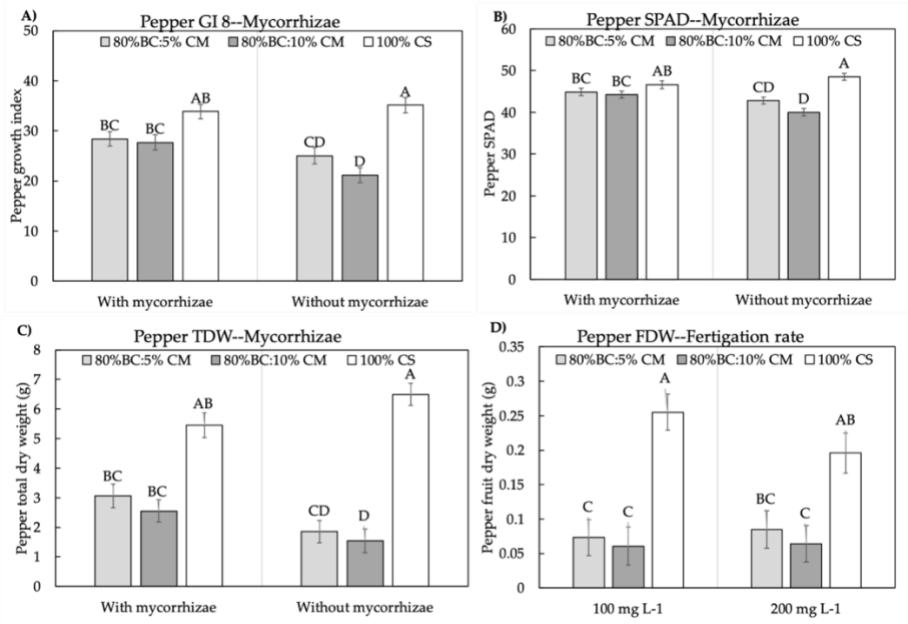

Figure 4.4. The effects of mycorrhizae on pepper growth index at the eighth week after transplanting (GI 8; A), soil-plant analyses development (SPAD) (B), total dry weight (C), and the effects of mixes on pepper plant fruit dry weight (D). The same letter indicates not significantly different according to Tukey HSD multiple comparisons at $p \leq 0.05$.

4.3.3. 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 4.10). 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 GI8 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 4.10. 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 [x]					**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	*
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 $P \leq 0.05$, 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 4.11). 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 4.11. 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[X]:Y[Y]:200	52.0 ± 3 ab[Z]	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 $p \leq 0.05$.

Biochar-amended mix significantly reduced tomato FDW (Figure 4.5). Without MC, the mix type significantly impacted pepper TDW and SPAD: BC-amended mixes had no significant impact on pepper GI (Figure 4.6A) but led to lower TDW and FDW

(Figure 4.6C, 4.7A). Fertigation rate did not significantly impact pepper SPAD (Figure 4.6B). Adding MC significantly decreased pepper FDW (Figure 4.7B).

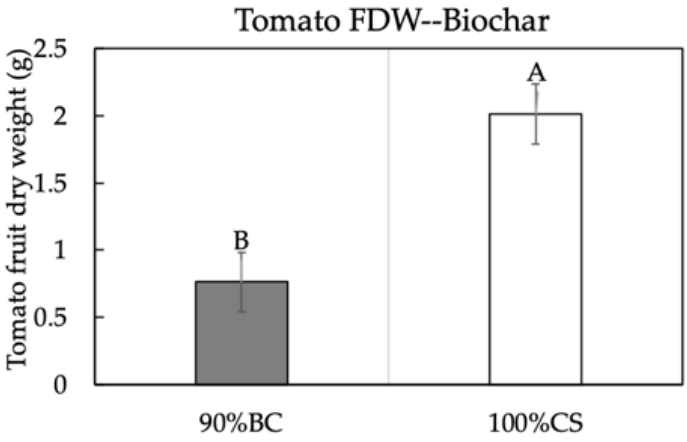

Figure 4.5. The effects of biochar on tomato plants fruit dry weight (FDW). The same letter indicates not significantly different according to Tukey HSD multiple comparisons at $p \leq 0.05$.

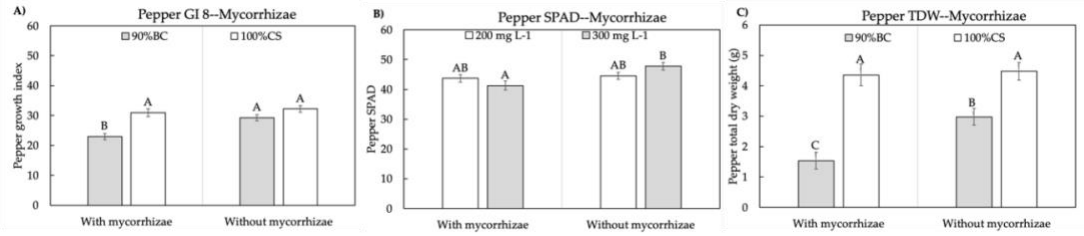

Figure 4.6. The effects of mycorrhizae on pepper plants growth index at the eighth week after transplanting (GI 8; A), SPAD (B), and total dry weight (TDW; C). The same letter indicates not significantly different according to Tukey HSD multiple comparisons at $p \leq 0.05$.

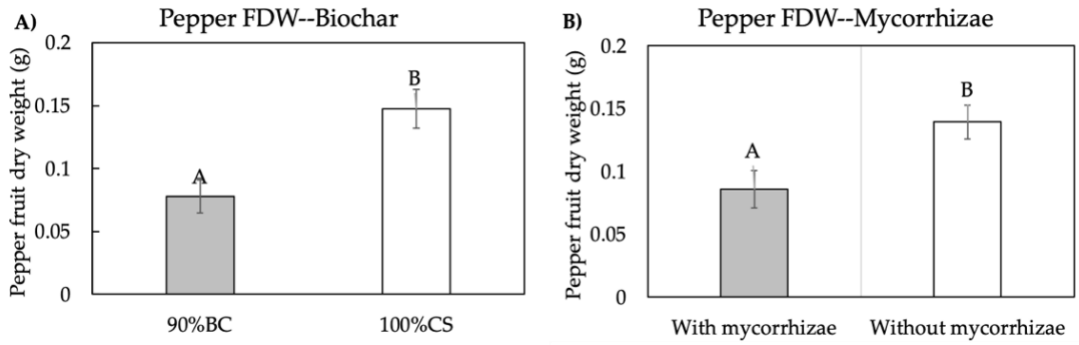

Figure 4.7. The effects of biochar (A) and mycorrhizae (B) on pepper plant fruit dry weight (FDW). The same letter indicates not significantly different according to Tukey HSD multiple comparisons at $p \leq 0.05$.

4.3.4. 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 4.6,4.8,4.10), 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 4.8) were created separately for tomato and pepper based on the similarities (Jaccard's similarity coefficient) among treatments.

We drew a line at height 25 (Figure 4.8), and treatments fell into three groups for both tomato (Figure 8A) and pepper plants (Figure 4.8B). 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 4.9A) and pepper (Figure 4.9B) plants. For tomato plants, 91.7% of the variability was explained by the first two components (Figure 4.9A). 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.

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.

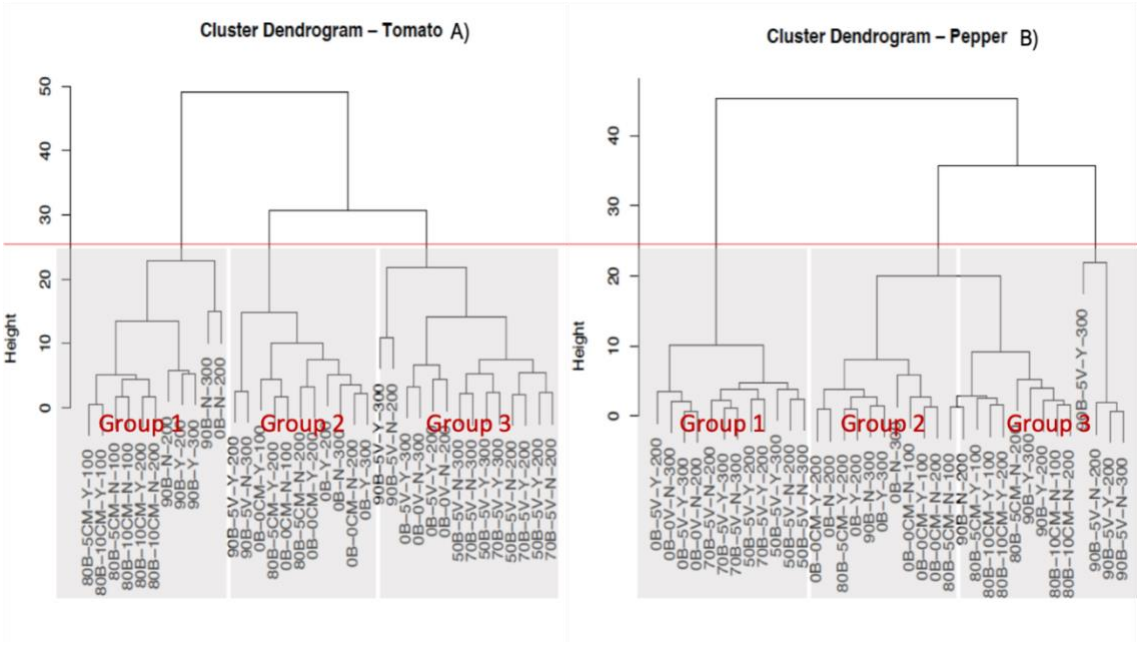

Figure 4.8. The cluster dendrogram for tomato (A) and pepper (B) plants. Group 1, 2, and 3 in tomato (A) represent 11 treatments with high biochar rates (BC, 80% or 90%), 11 treatments with low composts rate (0% or 5%), and 14 treatments with BC-5% vermicompost (VC) mixes, respectively. Group 1, 2, and 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.

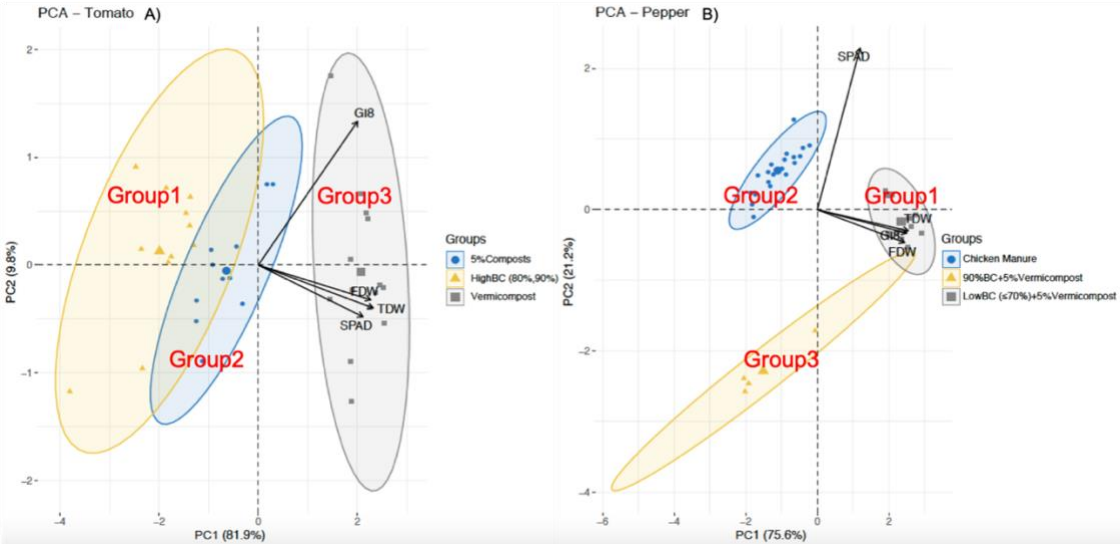

Figure 4.9. Principal component analysis (PCA) depicting the relationships between selected variables and treatment factors with tomato (A) and pepper (B). Selected variables are displayed by arrows and include plant growth parameters—soil-plant analyses development (SPAD), growth index (GI), fruit dry 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).

4.4. Discussion

4.4.1. 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.

4.4.2. 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].

4.4.3. Biochar Potential Climatic Value

Using biochar as a peat moss alterative 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].

4.5. Conclusions

Among all the treatments, adding mycorrhizae did not have a significant impact on plant growth (except 90% biochar-5% vermicopost 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.

4.6. References

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5. BIOCHAR, *TRICHODERMA* REDUCE CONTAINERIZED PEPPER BLIGHT CAUSED *BY PHYTOPHTHORA CAPSICI*

5.1. Introduction

Phytophthora capsici is a destructive hemi-biotrophic pathogen causing disease on a broad range of crops from families including solanaceous, cucurbitaceous, and fabaceous [1]. *Phytophthora* blight on pepper caused by *P. capsici* is one of the most serious soil-borne diseases for pepper worldwide [2]. The symptoms of the disease appear on the 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].

Trichoderma spp. has been reported as a reliable biological control agent for *P. capsici. Trichoderma harzianum* was proven to suppress pepper root rot caused by *P. capsici* through antimicrobial substances production [6]. Also, in an in vitro test, *T. harzianum* inhibited *P. capsici* by 65.3% [7]. Similarly, *T. harzianum* and *T. virens* inhibited *P. capsici* growth in other studies [8, 9].

Biochar (BC), 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) [10, 11], could replace peat moss-based substrate for greenhouse plant production [12-15]. Biochar can increase water and nutrient holding capacity, ameliorate substrate acidity, and provide suitable environments for

plants [16, 17]. Biochar could improve greenhouse crop growth, yield, and quality, under appropriate conditions [18-21].

Replacing peat moss-based substrate with BC could provide both environmental and economic benefits. Replacing peat moss-based substrate with BC could reduce the environmental concerns associated with peat moss such as rare wildlife habitat destruction, wetland ecosystem disturbance, and climate change interference [22, 23]. Also, incorporating BC in the substrate could reduce the initial investment for growers as the price of peat moss has been rising, which hindered growers profits, especially when transportation costs are considered [24].

Not only can BC replace peat moss-based substrate to produce plants but also has the potential to suppress pepper blight caused by *P. capsici.* For instance, incubating sandy soil for 20 days and then adding 1.33% (w/w) corn straw BC (pH 9.73) in the container before transplanting suppressed pepper blight disease because it improved soil chemical properties and increased beneficial microorganisms [2]. Also, adding 3% (w/w) softwood BC (pH 6.5) pre-charged with beneficial microorganisms mixed with soil in containers brought down the abundance of *P. capsici* in the soil, thus reducing pepper blight infection [25]. Similarly, incorporating corn stalk BC (pH 9.73) at 13.7g/kg into soil suppressed pepper blight because it increased the abundance of beneficial microorganisms [26].

Biochar rates used in plant disease suppression studies were low, but more studies need to be conducted before accepting the BC dosage dependent theory. As aforementioned, BC used in most pepper blight disease studies were ≤ 3% (w/w). Also, BC and *Pythium* spp. disease studies have also been reported, but with BC also at relatively

low rates (≤ 3% w/w) [27]. The highest rate of BC used in a phytopathogenic system was 50% (by vol.) [28]. Among the small amount of BC and plant disease control studies, many of them demonstrated BC dosage dependent effects, indicating high BC dose (>3%, w/w) may have a negative effect on plant disease control [29]. However, due to the paucity of related data, it is still too early to be certain about the BC dosage-dependent response of plant disease, and more related studies should be conducted.

The potential mechanisms on how BC may influence the plant disease includes both direct and indirect influence on pathogens: 1) BCs' chemical compounds affect pathogen development; 2) BCs' physicochemical properties improve nutrients availability and abiotic conditions ; 3) BCs' physical properties help absorb toxins and enzymes produced by the pathogens, reducing virulence; 4) BCs' presence induces systemic resistance in the host plants; 5) BCs' physical properties enhance the abundance and/or activities of beneficial microbes [29, 30].

Due to the complexity of the BC-plant-media-microorganisms system, it's hard to decipher which mechanism is responsible for BC impacts on disease development in a given phytopathogenic system, especially knowing some of the mechanisms work synergistically [29]. Except for the chemical compound mechanism, which can be identified and measured separately by removing BCs' physical and chemical properties and their influences on pathogen and host plants, other mechanisms are hard to identify or measure separately. Herein, we conducted an in vitro test and a greenhouse trial to identify whether BC's chemical compounds affect *P. capsici* or other mechanisms were involved.

5.2. Material and Methods

5.2.1. Biochar Amended Media and Biochar Water Extracts

Two types of biochar: mix hardwood biochar (HB, Proton Power Inc. Lenoir City, Tennessee, USA) and sugarcane bagasse biochar (SBB, American Biocarbon LLC White Castle, Louisiana, USA) were mixed with peat moss-based commercial substrate (CS, Jolly Gardener C/20, Oldcastle Lawn & Garden Inc., Atlanta, Georgia, USA). The CS used in this study contains 80% Canadian Sphagnum peat moss with the rest being perlite. Mixed hardwood biochar was mixed with CS at 10%, 30%, 50%, and 70% (by vol.) and SBB at 10% (by vol.). The HB was a by-product of fast pyrolysis of mixed hardwood and the SBB was produced using proprietary methods. The SBB was provided by USDA, ARS, Sugarcane Research Unit (Houma, Louisiana, USA). Sugarcane bagasse biochar had a pH of 5.9 and HB 10.1[31]. The electrical conductivities (EC) of the two BCs were 753 $\mu S\ cm^{-1}$ (SBB) and 1,058 $\mu S\ cm^{-1}$ (HB), respectively [32].

The water extracts were obtained according to Gravel, Dorais [28]. Briefly, the mixtures were mixed with deionized water (DI water) at ratio of 1:1 (by vol.) and shook for 24 h. Mixture was filtered through filter paper and a 25 mL extract was collected and sterilized for the in vitro test. The same amount of DI water was used as the control.

The BC-amended potato dextrose agar (PDA) media was prepared by adding the sterilized mixture water extracts in the 25% PDA sterilized solution prior to media hardening. The control medium contained the same amount of sterilized DI water only.

5.2.2. Plant Material, *Trichoderma*, and *P. capsici*

Several 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 *Phytophthora capsici* (personal conversation). Generally, it is very difficult to find genotypes that can resist to *P. capsici* in all circumstances [33]. 'Criollo de Morelos 334' (CM334), displayed a high level of resistance towards *P. capsici* and has been used commonly for disease resistant breeding [34]. Many literatures involving CM334 have found that several major chromosomal regions affect many of the resistance components to *P. capsici* [33-35]. 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.

Root shield Plus-WP (BioWorks, Victor, New York, USA) was used as a biological agent in this study. The biological agent contained two active strains of *Trichoderma*, *T. harzianum* strain T-22 and *T. virens* strain G-41. *Phytophthora capsici* was isolated from an infected pepper plant. Dr. Thomas Isakeit from the Department of Plant Pathology and Microbiology, Texas A&M University helped with the *P. capsici* isolation and identification. Cultures of pathogens were isolated and maintained in the dark on a V8 juice agar selective for the culture of oomycete organisms [36].

5.2.3. In Vitro Test

5.2.3.1. Biochar-amended Extracts and Pathogen Growth

One plug of actively growing *P. capsici* was placed in the center of each petri dish (100 mm*15 mm). Petri dishes were then placed in a dark environment at room temperature (~25°C). Radial mycelium growth was measured four days later. The percentage inhibition of pathogen growth was calculated using the following formula [9]: $Inhibition = \frac{(A1-A2)\times 100\%}{A2}$, where A1 = area of pathogen growth in BC-amended media, A2 = area of pathogen growth in the control.

5.2.3.2. Biochar-amended Extracts, *Trichoderma*, and Pathogen Growth

The dual confrontation technique [37] with slight modifications was used in this trial. Briefly, a drop of *Trichoderma* containing solution (mixed at the manufacture rate) was paired against a 5 mm plug of actively growing *P. capsici* at equal distances opposite to each other in a 100 mm diameter petri dish containing BC-amended 25% PDA. Petri dishes were then placed in a dark environment at room temperature(~25°C). Radial mycelium growth was measured four days later. The percentage inhibition of pathogen growth was calculated using the following formula [9]: $Inhibition = \frac{(A1-A2)\times 100\%}{A2}$, where A1 = area of pathogen growth in BC-amended, *Trichoderma* added media, A2 = area of pathogen growth in the control.

5.2.4. Greenhouse Trial

After the bleach residues were rinsed away with DI water, seeds were sown in commercial propagation media (BM2 Berger, Saint-Modeste, Quebec, Canada) on June 31st, 2020. After the true leaves came out, uniform seedlings were transplanted into 4”

pots (dimension: top 7.5 cm, bottom 6 cm, depth 8.2, volume 375 mL) filled with BC-amended mixes on July 22nd, 2020. At transplanting, slow-released fertilizer Osmocote Plus (15N-4P-10K, Scotts-Sierra Horticultural Products Company, Marysville, Ohio, USA) was applied at manufacturer's rate.

Trichoderma-containing product was applied at manufacturer's recommendation rate one week after plant transplanting on July 29th, 2020. Plants were inoculated with *Phytophthora capsici* with plastic inoculation loops (VWR, Radnor, Pennsylvania, USA) on August 4th, 2020. The inoculum was a 5-mm diameter agar plug of *P. capsici* taken from the margin of an actively growing colony of the pathogen. 5 plugs were placed on the surface of the substrate contacting the plant stem.

5.2.4.1. Substrate 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 [38]. The leachate electrical conductivity (EC) and pH were measured with a portable EC/pH meter (Hanna Instrument, Woonsocket, Rhode Island, USA), according to the pour-through method [39].

5.2.4.2. Disease Assessment

Disease symptoms were observed and recorded every 5 days starting at 3 days after pathogen inoculation. The disease severity was recorded on a 0-4 scale according to Wang's work [2]. The scale was also visualized in this work as shown in Figure 5.1.: 0 = healthy plants, 1 = plants with small brown lesions in the stem or slightly wilted leaves, 2

= plants with moderate brown lesions in the stem and moderate wilted leaves, 3 = plants with big brown lesions in the stem and significantly wilted leaves, and 4 = dead plants. Disease severity index (DS) was calculated by the following formula: DS = $\sum\left(\frac{\text{number of diseased plants in this index} \times \text{disease index rating from 0 to 4}}{4 \times \text{number of plants investigated}}\right) \times 100\%$ [26].

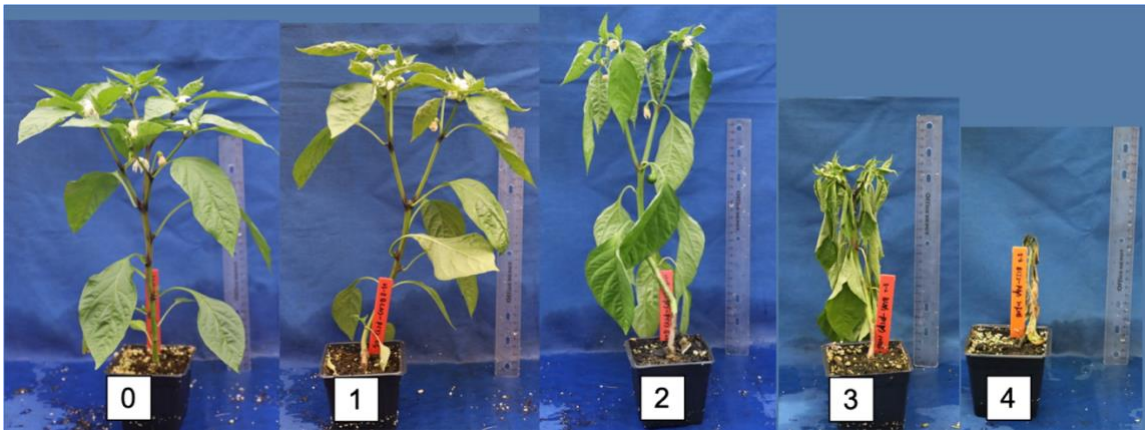

Figure 5.1. Visual scales (0-4; 0 = no symptom; 4 = dead) used for the pepper blight caused by *Phytophthora capsici* disease severity rating used in this study.

The disease severity obtained at different times after inoculation was used to calculate areas under disease progress curves (AUDPC) following the formula: 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 [40]. Disease incidence (DI) was evaluated by counting the number of diseased plants in each pot twice during the trials, according to the formula: DI = $\frac{number\ of\ diseased\ plants}{number\ of\ total\ plants} \times 100$ [41].

5.2.5. Experimental Design and Maintenance

The in vitro test was arranged as a randomized complete block design (RCBD) with five blocks. Within each block, we randomly applied the treatments to the petri dish from the 7 x 2 factorial design with BC treatments (water extracts of CS100, SBB10, HB10, HB30, HB50, HB70, and DI water) and *Trichoderma* application (with/without). The greenhouse experiment was a randomized complete block design (RCBD) with eight blocks. Inside the greenhouse, we assigned a dimension of 1.5 m x 1 m area as a block on the raised bench. Within each block, we randomly applied our treatments to the pots from the 6 x 2 factorial design with BC treatments (CS100, SBB10, HB10, HB30, HB50, and HB70), *Trichoderma* application (with/without).

Standard propagation trays were placed underneath the pots to create a moisturized environment. All the pots were placed in a *P. capsici*-permitted greenhouse at Texas A&M University HORTTREC, Somerville, Texas, USA. Plants were watered as needed throughout the experiment. The average greenhouse temperature, relative humidity, and dew point were 30.2 °C, 77.2%, and 25.0 °C, respectively.

5.2.6. Data Collection and Analysis

Image J (version 1.53a) was used to estimate the area of pathogen growth in the in vitro test. All the data was analyzed with the one-way analysis of variance using R program software (version 3.5.1). All the means were separated by using Dunnett's test when treatments were significantly different from the control at $p \leq 0.05$ or the least significant difference (LSD) when treatments were significantly different from each other at $p \leq 0.05$.

5.3. Results

5.3.1. Biochar-amended Extracts, *Trichoderma*, and Pathogen Growth

In the absence of *Trichoderma*, BC-amended mixtures' extracts had no significant effect on *P. capsici* growth compared with the CS extracts (Figure 5.2, 5.3 A). Except for HB10 extracts, which stimulated (indicated by the positive inhibition value) *P. capsici* growth, all the other extracts suppressed (indicated by the negative inhibition value) pathogen growth. Among all the pathogen-suppressed BC extracts, HB30 had a significantly higher inhibition percentage compared with SBB10.

In the presence of *Trichoderma*, BC-amended mixtures' extracts had no significant effect on *P. capsici* growth compared to the CS extracts except for HB70, which had a significantly lower inhibition percentage (Figure 5.2, 5.3 B). All the extracts suppressed *P. capsici* growth.

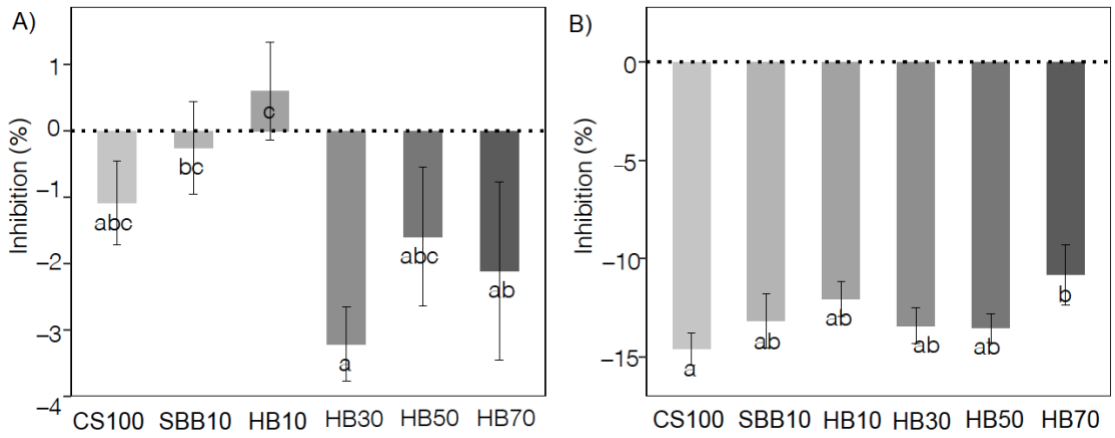

Figure 5.2. Inhibition percentage of *Phytophthora capsici* growth on 25% PDA amended with 25 mL of liquid extraction from peat moss-based commercial substrate (CS100), 10% (by vol.) sugarcane bagasse biochar (SBB10), 10%, 30%, 50%, and 70% mixed hardwood biochar-amended mixes (HB10, HB30, HB50, and HB70, respectively) without (A) and with the addition of *Trichoderma* (B). Data are mean of five replications. Values followed by the same letters are not significantly different according to LSD's multiple comparison test at $p \leq 0.05$.

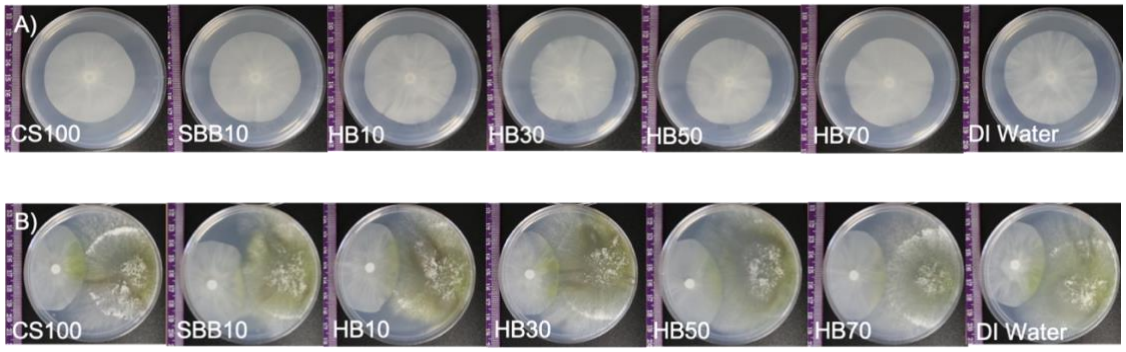

Figure 5.3. *Phytophthora capsici* grown on 25% PDA amended with 25 mL of liquid extraction from peat moss-based commercial substrate (CS100), 10% sugarcane bagasse biochar (SBB10), 10%, 30%, 50%, and 70% (by vol.) mixed hardwood biochar-amended mixes and deionized water (HB10, HB30, HB50, HB70, and DI water respectively in the absence (A) and presence of *Trichoderma* (B, light green) after four days in a dark environment.

5.3.2. Substrate Physical and Chemical Properties

Most of the mixes' physical properties were within the recommended range [42], except for the BDs in all the treatment , which were lower than the recommended value (Table 5.1). The HB50 and HB70 mixes had significantly lower TP, CC and BD, as compared with the control (CS100). All the HB mixes had significantly higher pH compared with the control (except SBB 10). Except for HB10 and HB30, all the BC-amended mixes had significantly lower EC than the control.

Table 5.1: Substrate physical properties including total porosity (TP), container compacity (CC), air space (AS), bulk density (BD) and chemical properties including pH and electrical conductivity (EC).

Trt.	TP (%)	CC (%)	AS (%)	BD (g cm^{-3})	pH	EC (µS cm^{-1})
CS100	74±0.3	56±0.2	18±0.5	0.10±0.00	6.8±0.05	2,058±29
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: 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. *, **, 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 Yeager and Nelson [42, 43].

5.3.3. Disease Parameters

Plants grown in all the treatments showed disease symptoms 3 days after transplanting except for HB70, which showed symptoms 7 days after transplanting (Figure 5.4 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 application of *Trichoderma* did not significantly reduced disease severity over the entire experiment (Figure 5.4 B).

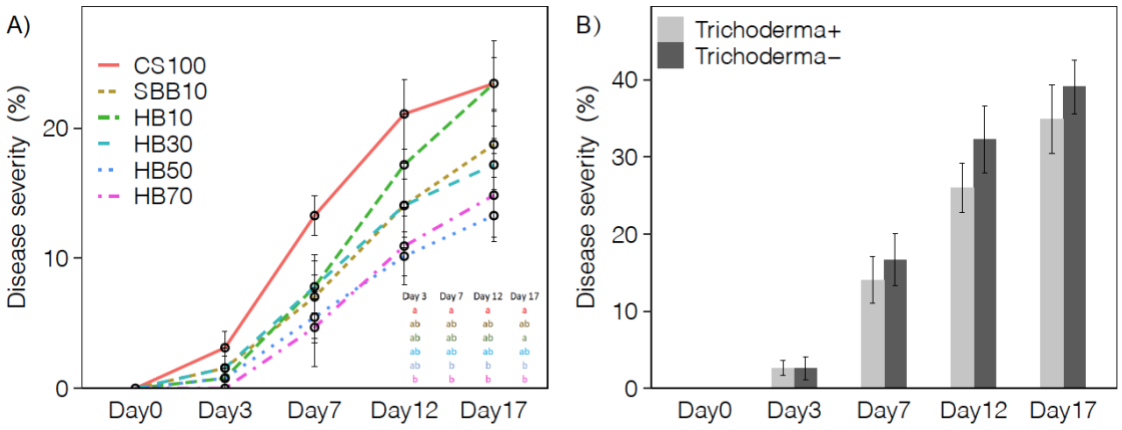

Figure 5.4. The effect of biochar rates (A) and *Trichoderma* (B) on disease severity for pathogen-inoculated treatments. SBB = Sugarcane bagasse biochar, HB = Mixed hardwood biochar, CS = Peat moss based commercial substrate. Numbers after CS, SBB, and HB indicate the ratio of different components, by vol. The same letter indicates not significantly different from each other on the same day according to LSD multiple comparison test at $p \leq 0.05$.

Biochar mixes had significant impacts on disease incidence, especially HB-amended (30%-70%) mixes (Figure 5.5 A). 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. The application of *Trichoderma* did not significantly reduced disease incidence over the entire experiment (Figure 5.5 B).

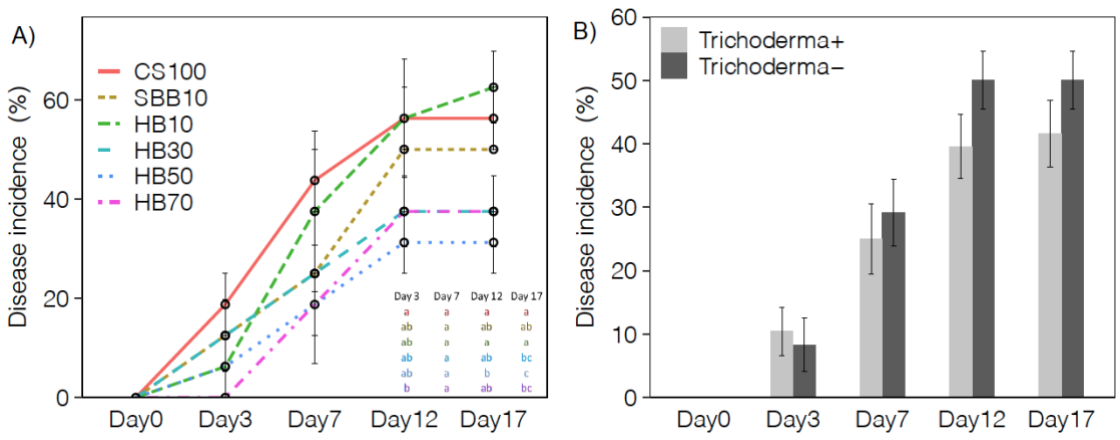

Figure 5.5. The effect of biochar rates (A) and *Trichoderma* (B) on disease incidence for pathogen-inoculated treatments. SBB = Sugarcane bagasse biochar, HB = Mixed hardwood biochar, CS = Peat moss based commercial substrate. Numbers after CS, SBB, and HB indicated the ratio of different components, by vol. The same letter indicates not significantly different from each other on the same day according to LSD multiple comparison test at $p \leq 0.05$.

All the BC-amended mixes 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 (Figure 5.6 A). The application of *Trichoderma* did not significantly reduced AUDPC the entire experiment (Figure 5.6 B).

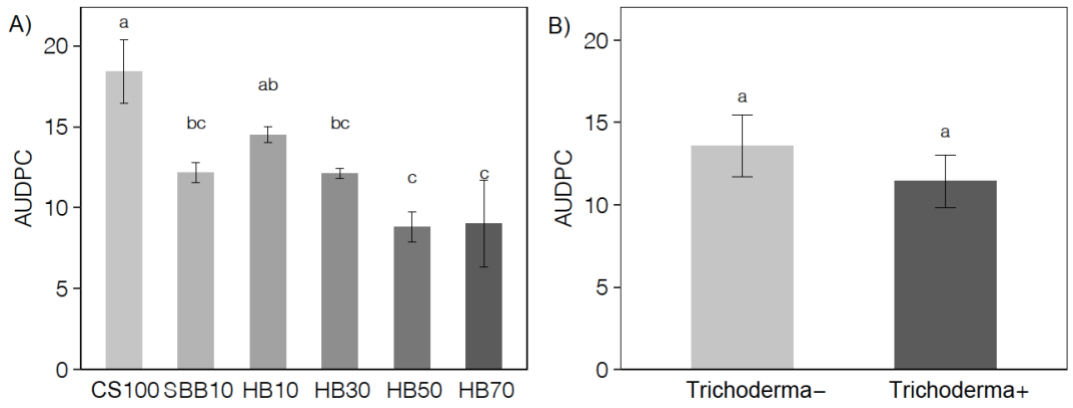

Figure 5.6. The effect of biochar types and rates (A) and *Trichoderma* (B) on the area under disease progress curve. SBB = Sugarcane bagasse biochar, HB = Mixed hardwood biochar, CS = Peat moss based commercial substrate. Numbers after CS, SBB, and HB indicate the ratio of different components, by vol. The same letter indicates not significantly different from each other according to LSD multiple comparison test at $p \leq 0.05$.

5.4. Discussions

5.4.1. Biochar Chemical Compound and Pathogen Inhibition

The chemical compound in BC could have direct influence on disease development. Biochar could contain chemical compounds such as ethylene glycol and propylene glycol, hydroxypropionic and hydroxybutyric acids, benzoic acid and o-cresol, quinones (resorcinol and hydroquinone), and 2-phenoxyethanol, which could adversely affect microbial growth and survival. Also, compounds such as methoxyphenols, phenols, carboxylic acids, furans, and ketones, which could form during pyrolysis process, have suppressive impacts on microbial activity [44, 45]. In the in vitro test where the pathogen growth was not affected by BC physical properties, all the BC mixtures' extracts suppressed *P. capsici* growth except for HB10, which was different from the study by

Jaiswal, Elad [46], where the eucalyptus wood BC extracts stimulated *Fusarium oxysporum* growth. The difference between these two studies can be explained by the different chemical compounds caused by different BC types. Also, different pathogens may contribute to the different results too. In the presence of *Trichoderma*, the inhibiting effects of BC-amended mixes' extracts on *P. capsici* growth was enhanced due to *Trichoderma'*s suppressive impact on *P. capsici*. This study has proven that BC and other components provide synergistic effects on pathogens, which was also proven by Debode, Ebrahimi [47].

5.4.2. Biochar Properties and Disease Development

Adding BC to the substrate may profoundly influence the complex rhizosphere–root–media–pathogen system by the BC's physical and chemical properties such as nutrient content, water holding capacity, redox activity, adsorption ability, pH and content of toxic and hormone-like compounds, which can affect the disease triangle factors directly and indirectly [29]. The BCs with high pH (>9) could contain phenolic groups such as phenolic acid and alkali, which alter the microorganism's environment [48]. The adsorption ability of many BCs could also reduce the toxic acids near plant roots, boosting host plant growth [29].

5.4.3. Treatment Factors Determine Plant Disease Development

For pathogen-inoculated plants, 94% of the variability was explained by the first two components (Figure 5.7). PC1 accounted for 77% variance, differing CS100, HB10, HB70-TN, and SBB10-TY mixes from the rest BC-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.

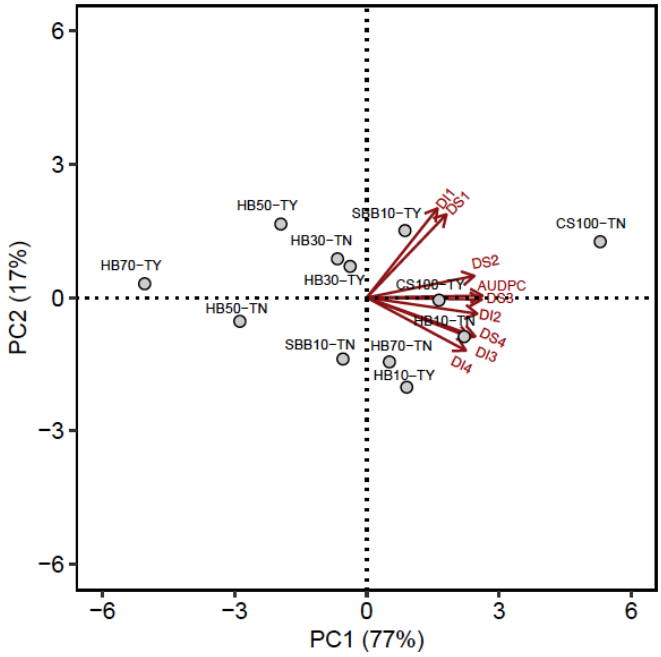

Figure 5.7. Principal component analysis (PCA) depicting the relationships between selected variables and treatment factors with pathogen-inoculated plants. Selected variables are displayed by arrows and include disease parameters—disease severity after 3, 7, 12, and 17 days of transplanting (DS1, DS2, 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: Peat moss-based substrate (CS100), biochar-amended mixes at different rates (by vol., SBB10, HB10, HB30, HB50, HB70) with (TY) or without *Trichoderma* (TN).

5.5. Conclusions

The mixed hardwood biochar blended with commercial peat moss-based substrate at 30%, 50 %, and 70% (by vol.) could significantly reduce *Phytophthora* pepper blight disease severity and/or disease incidence with or without *Trichoderma* addition. Compounds contained in biochar contributed to its disease inhibition capability to a certain extent.

5.6. References

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6. BIOCHAR, *TRICHODERMA* REDUCE CONTAINERIZED POINSETTIA ROOT ROT CAUSED *BY PYTHIUM APHANIDERMATUM*

6.1. 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 [1]. Being one of the most popular holiday flowers worldwide, limiting the losses of poinsettia plants from disease is critical in production [2]. *Pythium 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 [2, 3]. Under favorable environmental conditions, *P. aphanidermatum* causes stunting, root rot, wilting, defoliation, chlorosis, and in severe cases, plant death [4].

Soilless substrate can be conducive to *Pythium* root rot as it is limited in microbial activity [5], however, greenhouses may purchase plantlets or cuttings that are infected but asymptomatic from propagation greenhouses [6]. Once the *Pythium* successfully intrudes into greenhouses, it can infect the whole greenhouse and become a source of primary inoculum [7]. Also, due to greenhouses' monocultural and humid *Pythium* favorable environment, mycelium is easy to survive and reproduce, making *Pythium* an intractable problem [7].

Replacing peat moss, a commonly used soilless substrate for poinsettia production, with biochar (BC) provides several benefits. Biochar is a carbon-rich by-product from

biomass pyrolysis (thermochemical biomass decomposition under an oxygen-depleted or oxygen-limited environment with a specific period of time and temperature conditions) [8, 9]. Several studies have shown that BC can replace peat moss-based substrate for greenhouse plant production [10-13]. Also, replacing peat moss-based substrate with BC was proven to reduce the environmental concerns associated with peat moss such as rare wildlife habitat destruction, wetland ecosystem disturbance, and climate change interference [14, 15]. Additionally, incorporating BC in the substrate could reduce the initial investment for growers as the price of peat moss has been rising, which hindered growers' profits, especially when transportation costs are considered [16].

Not only can BC replace peat moss for poinsettia plant production [17] but also has the potential to suppress plant diseases in different plant-pathogen systems. For instance, incubating sandy soil for 20 days and then adding 1.33% (w/w) corn straw BC (pH 9.73) in the container before transplanting suppressed pepper blight disease due to the improvement of soil chemical properties and increase of beneficial microorganisms [18]. Other studies with BC-amended soil control disease caused by *Pythium* spp. were also reported with BC at relatively low rates ($\leqslant$ 3% w/w) [19].

In most cases, BC provides synergistic effects with other components and *Trichoderma* spp. has been reported as a reliable biological control agent for a wide range of pathogens including *P. aphanidermatum* [20]. For instance, *T. asperellum* was proven to suppress tomato damping-off caused by *P. aphanidermatum* [21]. Also, AL-Mailkya's work showed that the efficacy of spent mushroom substrate against cucumber damping-

off caused by *P. aphanidermatum* was related to the presence of *Trichoderma* spp. in the substrate [22].

To date, there are not enough studies focusing on BC suppressing plant disease development and BC incorporation rate is relatively low (ranging in most cases between 0.5~3%). The highest rate of BC used in the phytopathogenic system was 50% (by vol.) testing its effects on *Pythium ultimum* with different crops [23]. The majority of the BC-pathogen studies have shown sensible responses to BC dosages, indicating that the high dose of BC may contribute a negative effect on plant disease [24]. However, due to the paucity of soilless related data, it is still too early to be certain about the BC dosage depending response of plant disease, more research on the subject is needed.

The potential mechanisms on how BC may influence plant disease includes both direct and indirect influence on pathogen: 1) BCs' chemical compounds affect pathogen growth; 2) BCs' physicochemical properties improve soil nutrients availability and abiotic conditions; 3) BCs' physical properties help absorb toxins and enzymes produced by pathogens, reducing virulence; 4) BCs' presence induces systemic resistance into host plants; 5) BCs' physical properties enhance abundance and/or activities of beneficial microbes [24, 25].

Due to the complexity of the BC-plant-media-microorganisms system, it is hard to decipher which mechanism is responsible for BC impact disease development in a given phytopathogenic system [24]. Except for the chemical compound mechanism, which can be identified and measured separately by removing BCs' physical and chemical properties and their influences on pathogen and host plants, other mechanisms are hard to identified

and measured separately. Herein, we conducted an in vitro test and a greenhouse trial to identify which mechanism was involved in BC-poinsettia-*P. aphanidermatum* system and test BC effects on poinsettia root rot disease development.

6.2. Material and Methods

6.2.1. Biochar Amended Media and Biochar Water Extracts

Mixed hardwood biochar (HB, Proton Power Inc., Lenoir City, Tennessee, USA) was mixed with peat moss-based commercial substrate (CS, Jolly Gardener C/20, Oldcastle Lawn & Garden Inc., Atlanta, Georgia, USA) at 20% and 40% (by vol.). The CS used in this study contains 80% Canadian Sphagnum peat moss with the rest being perlite. The HB was a by-product of fast pyrolysis of mixed hardwood and had a pH of 10.1 and an electrical conductivity (EC) of 1,058 μS cm^{-1} [26, 27].

The water extracts was obtained according to Gravel, Dorais [23]. Briefly, the mixtures were mixed with deionized water (DI water) at a ratio of 1:1 (by vol.) and shook for 24 h. The mixture was filtered through filter paper and 25 mL extract was collected and sterilized for the in vitro test. The same amount of DI water was used as the control.

The BC-amended potato dextrose agar (PDA) media was prepared by adding the sterilized mixture water extracts in the 25% PDA sterilized solution before media hardening. The control medium contained the same amount of sterilized DI water only.

6.2.2. Plant Material, *Trichoderma*, and *P. aphanidermatum*

Root shield Plus-WP (BioWorks, Victor, New York, 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. *Pythium*

aphanidermatum was isolated from an infected poinsettia plant. Cultures of pathogens were isolated and maintained in the dark on a cornmeal agar selective for the culture of oomycete organisms [28].

6.2.3. In Vitro Test

6.2.3.1. Biochar-amended Extracts and Pathogen Growth

One plug of actively growing *P. aphanidermatum* was placed at the center of each petri dish (100 mm×15 mm). Petri dishes were then placed in a dark environment at room temperature (~25°C). Radial mycelium growth was measured two days later. The percentage of pathogen growth inhibition was calculated using the following formula [29]: $Inhibition = \frac{(A1-A2)\times 100\%}{A2}$, where A1 = area of pathogen growth in BC-amended media. A2 = area of pathogen growth in the control.

6.2.3.2. Biochar-amended Extracts, *Trichoderma* and Pathogen Growth

The dual confrontation technique [30] with slight modifications was used in this trial. A drop of *Trichoderma* containing solution (mixed at the manufacture rate) was paired against a 5 mm plug of actively growing *P. aphanidermatum* at equal distances opposite to each other in 100 mm diameter petri dish containing BC-amended 25% PDA. Petri dishes were then placed in a dark environment at room temperature(~25°C). Radial mycelium growth was measured four days later. The percentage inhibition of pathogen growth was calculated using the following formula [29]: $Inhibition = \frac{(A1-A2)\times 100\%}{A2}$, where A1 = area of pathogen growth in BC-amended, *Trichoderma* added media. A2 = area of pathogen growth in the control.

6.2.4. Greenhouse Trial

Poinsettia (*Euphorbia pulcherrima* 'Prestige Sunrise Red') cuttings were stuck in commercial propagation media (BM2 Berger, Saint-Modeste, Quebec, Canada) on June 5th, 2020. After the root grew out, uniform cuttings were transplanted into 6-inch azalea pots (depth: 10.8 cm; top diameter: 15.5 cm; bottom diameter: 11.3 cm; volume: 1,330 mL) filled with BC-amended mixes on June 21st, 2020. At transplanting, slow release fertilizer Osmocote Plus (15N-4P-10K, Scotts-Sierra Horticultural Products Company, Marysville, Ohio, USA) was applied at manufacturer's rates.

Trichoderma-containing product was applied at the manufacturer's recommendation rate four weeks after plant transplanting (WK4) on July 23rd, 2020. Plants were inoculated with *Pythium aphanidermatum* with plastic inoculation loops (VWR, Radnor, Pennsylvania, USA) on July 31st, 2020. The inoculum was a 5-mm diameter agar plug of actively growing mycelium. 5 plugs were placed on the substrate's surface contacting plant stem.

6.2.4.1. Substrate Physical and Chemical Properties

Media physical properties of the BC mixes—total porosity (TP), container capacity (CC), air space (AS), and bulk density (BD)—were measured according to the North Carolina State University Horticultural Substrates Laboratory Porometer [31]. The leachate electrical conductivity (EC) and pH were measured with a portable EC/pH meter (Hanna Instrument, Woonsocket, Rhode Island, USA), according to the pour-through method [32].

6.2.4.2. Plant Growth

Plant height and two canopy widths (width 1: the widest widths, width 2: the width perpendicular to width 1) were measured biweekly starting at WK4, when *Trichoderma* was applied. The plant growth index (GI) was calculated according to the formula: GI = plant height/2 + (width 1 + width 2)/4 [17]. On August 25, 2020 at the end of this experiment, poinsettia plant shoots were harvested. After being dried at 80 °C in an oven until a consistent weight was reached, the shoot dry weight (SDW) was measured.

6.2.4.3. Disease Assessment

Disease symptoms were observed and recorded every 5 days starting at 5 days after pathogen inoculation. The disease severity was recorded on a 0-4 scale according to Lookabaugh's work with modification [4]. The scale was also visualized in this work as shown in Figure 6.1.: 0 = healthy plants, 1 = slightly stunted or wilted, 2 = chlorosis, moderate stunting and/or defoliation, 3 = wilting and/or severe stunting, 4 = dead plants. The disease severity index (DS) was calculated by the following formula: DS= $\sum\left(\frac{\text{number of diseased plants in this index} \times \text{disease index rating from 0 to 4}}{4 \times \text{number of plants investigated}}\right) \times 100\%$ [18].

Figure 6.1. 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.

The disease severity obtained at different times after inoculation was used to calculate areas under disease progress curves (AUDPC) following the formula: 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]. Disease incidence (DI) was evaluated by counting the number of diseased plants in each pot during the trial, according to the formula: DI = $\frac{number\ of\ diseased\ plants}{number\ of\ total\ plants} \times 100$ [34].

6.2.5. Experimental Design and Maintenance

The in vitro test was arranged as a randomized complete block design (RCBD) with five blocks. Within each block, we randomly applied the treatments to the petri dish from the 4 x 2 factorial design with BC treatments (water extracts of CS100, HB20, HB40, and DI water) and *Trichoderma* application (with/without). The greenhouse experiment was a randomized complete block design (RCBD) with eight blocks. Inside the greenhouse, we assigned a dimension of 1.6 m x 1.2 m area as a block on the benches. Within each block, we randomly applied our treatments to the pots from the 3 x 2 x 2 factorial design with BC treatments (CS100, HB20, and HB40), *Trichoderma* application (with/without), and pathogen (non-pathogen, pathogen-inoculated).

All the pots were placed in a *P. aphanidermatum*-permitted greenhouse at Texas A&M University, College Station, Texas, USA. Plants were watered as needed throughout the experiment. The average greenhouse temperature, relative humidity, and dew point were 30.2 °C, 77.2%, and 25.0 °C, respectively.

6.2.6. Data Collection and Analysis

Image J (version 1.53a) was used to estimate the area of pathogen growth in the in vitro test. All the data was analyzed with the one-way analysis of variance using R program software (version 3.5.1). For the greenhouse trial, non-pathogen and pathogen-inoculated treatments were analyzed separately. All the means were separated by using Dunnett's test when treatments were significantly different from the control at $p \leqslant 0.05$ or the least significant difference (LSD) when treatments were significantly different from each other at $p \leqslant 0.05$.

6.3. Results

6.3.1. Biochar-amended Extracts and Trichoderma on Pathogen Growth

In the absence of *Trichoderma,* BC-amended mixtures' extracts had significantly lower inhibition effects on *P. aphanidermatum* growth compared with the CS extracts (Figure 2, 3 A). All the mixes' extracts stimulated (indicated by the positive inhibition rate) *P. aphanidermatum* growth.

In the presence of *Trichoderma,* BC-amended mixtures' extracts had similar effects on *P. aphanidermatum* growth compared to the CS extracts (Figure 6.2, 6.3 B). All the mixture extracts suppressed *P. aphanidermatum* growth (indicated by the negative inhibition rate).

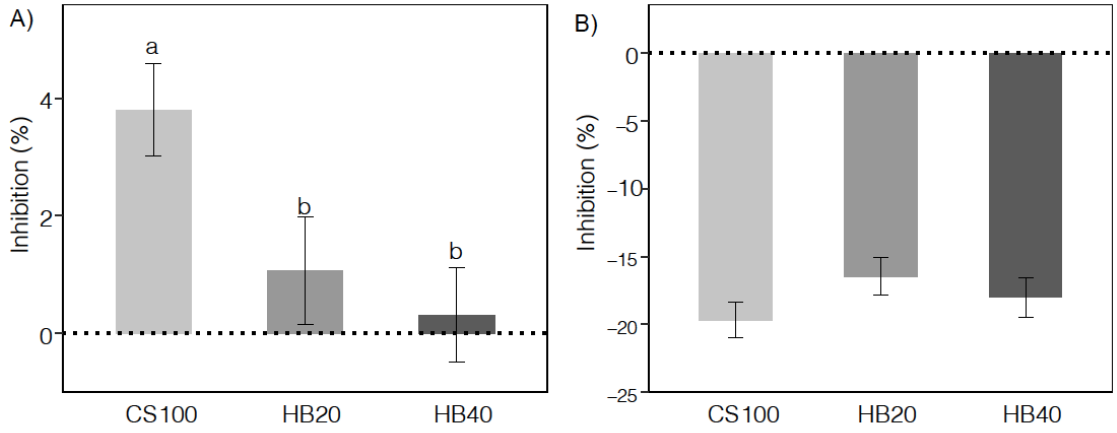

Figure 6.2. Inhibition percentage of *Pythium aphanidermatum* growth on 25% PDA amended with 25 mL of liquid extracts from peat moss-based commercial substrate (CS100), 20% and 40% (by vol.) mixed hardwood biochar-amended mixes (HB20, and HB40, respectively) without (A) and with the addition of *Trichoderma* (B). Data are mean of n=5. Values followed by the same letters are not significantly different from each other according to LSD' multiple test at $p \leqslant 0.05$.

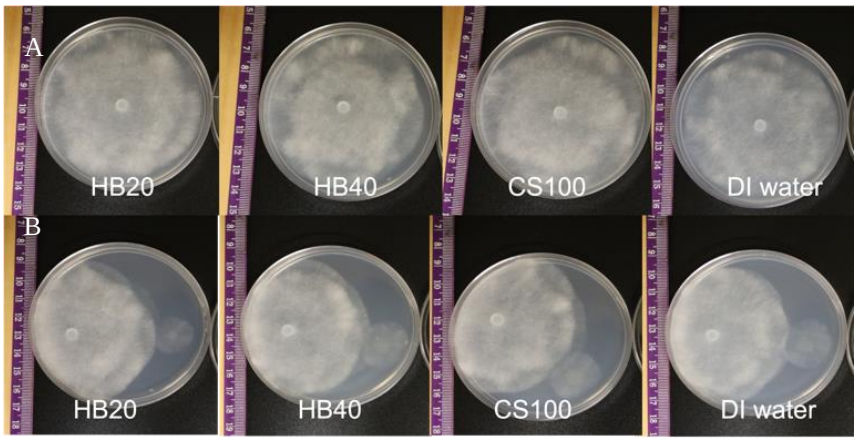

Figure 6.3. *Pythium aphanidermatum* grown on 25% PDA amended with 25 mL of liquid extraction from peat moss-based commercial substrate (CS100), 20%, and 40% (by vol.) mixed hardwood biochar-amended mixes and deionized water (HB20, HB40, and DI water respectively) in the absence (A) and presence of *Trichoderma* (B, on the right, small circle) after two days setting in the dark environment.

6.3.2. Substrate Physical and Chemical Properties

Most of the mixes' physical properties were within the recommended range [35], except for the BDs in all the treatments, which were lower than the recommended value (Table 6.1). The HB20 and HB40 mixes had a significantly lower TP, and pH, as compared with the control (CS100). The HB40 mixes had significantly higher BD and lower EC compared with the control.

Table 6.1: Substrate physical properties including total porosity (TP), container compacity (CC), air space (AS), bulk density (BD), and chemical properties including pH and electrical conductivity (EC).

Trt.	TP (%)	CC (%)	AS (%)	BD (g cm^{-3})	pH	EC (µS cm^{-1})
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**
Suitable range[z]	50-80	45-65	10-30	0.19-0.7	5.4-6.5	<1,500

Note: HB=Mixed hardwood biochar; CS=Peat moss based commercial substrate. Numbers after CS and HB indicated the ratio of different components, by vol. *, **, and *** indicates a significant difference from the commercial substrate (CS100) according to Dunnett's test at $p \leqslant 0.1, 0.05$, and 0.01, respectively. [z]Recommended physical properties of container substrate by Yeager and Nelson [35, 36].

6.3.3. Plant Growth

There was no main factor (BC and T) interactions in any of the growth parameters for any of the treatments with or without pathogen (Table 6.2). For the non-pathogen treatments, SPAD WK8 and SPAD WK10 were both significantly influenced by BC. For the pathogen-inoculated treatments, BC significantly influenced GI WK10, SPAD WK8, SPAD WK10, and SDW.

Table 6.2: A summary of the statistical significance of treatment factors on growth index at four, six, eight, and ten weeks after transplanting (GI WK4, GI WK6, GI WK8, GI WK10), SPAD at eight, and ten weeks after transplanting (SPAD WK8, SPAD WK10), and shoot dry weight (SDW).

		Significance[X]					
Factors	**GI WK4**	**GI WK6**	**GI WK8**	**GI WK10**	**SPAD WK8**	**SPAD WK10**	**SDW**
Non-Pathogen							
BC	NS	NS	NS	NS	**	*	NS
T	NS	NS	NS	NS	NS	NS	NS
BC×T	NS	NS	NS	NS	NS	NS	NS
Pathogen-inoculated							
BC	NS	NS	NS	*	***	***	***
T	NS	NS	NS	NS	NS	NS	NS
BC×T	NS	NS	NS	NS	NS	NS	NS

[X] Significance of biochar (BC) and *Trichoderma* (T) on plant growth parameters for non-pathogen and pathogen-inoculated treatments. NS means not significant. *, **, *** indicate significantly different from each other at $p \leqslant 0.05$, 0.01, and 0.001, respectively.

Biochar rates had no significant impacts on the non-pathogen poinsettia plants' SDW either with or without *Trichoderma* (Figure 6.4). Biochar had no significant influences on any of the GIs and *Trichoderma* had no significant impact on any the GIs either (Figure 6.5). Poinsettia plants grown in the BC mixes (both HB20 and HB40) had

significantly lower SPAD at WK8, but had no significant impact on SPAD WK10. *Trichoderma* did not significantly influence SPAD WK8 or SPAD WK10 (Figure 6.6).

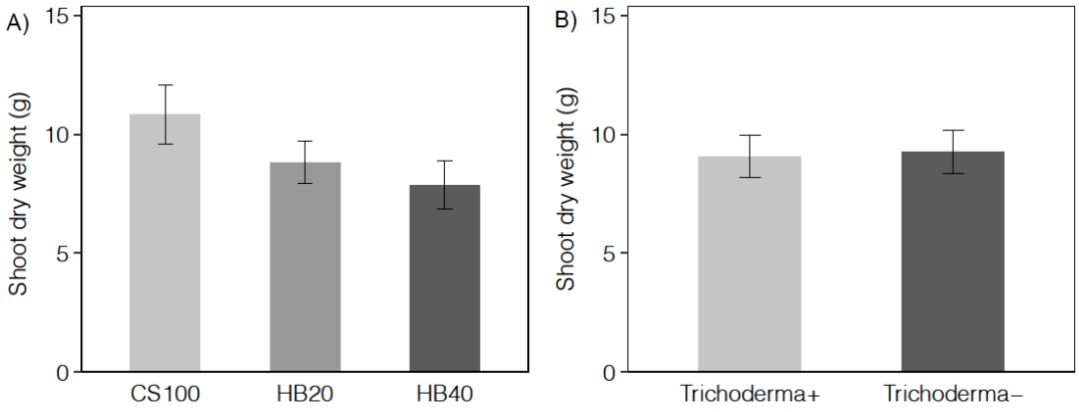

Figure 6.4. The effect of biochar rate (A) and *Trichoderma* application (B) on shoot dry weight for non-pathogen treatments. CS100 = peat moss-based commercial substrate, HB20 and HB40 = 20% and 40% (by vol.) mixed hardwood biochar-amended mixes, respectively.

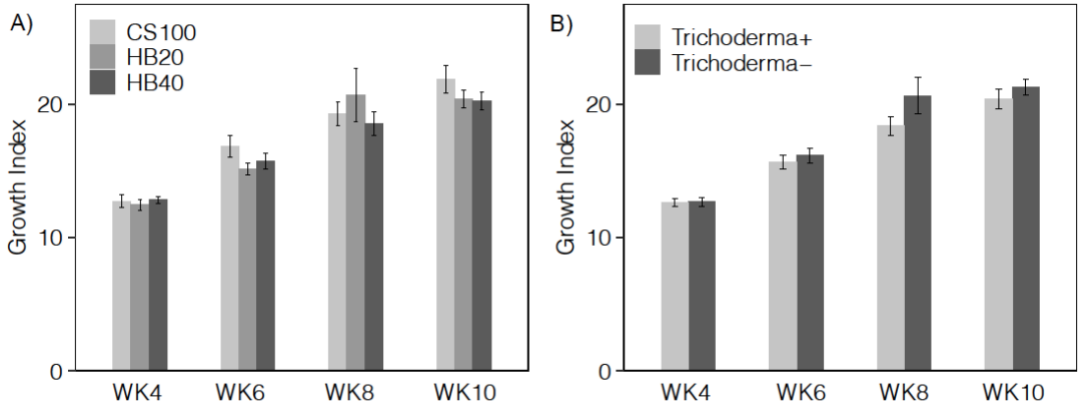

Figure 6.5. The effect of biochar rate (A) and *Trichoderma* application (B) on growth index at week 4, 6, 8, and 10 after transplanting (WK4, WK6, WK8, and WK10) for non-pathogen treatments. CS100 = peat moss-based commercial substrate, HB20 and HB40 = 20% and 40% (by vol.) mixed hardwood biochar-amended mixes, respectively.

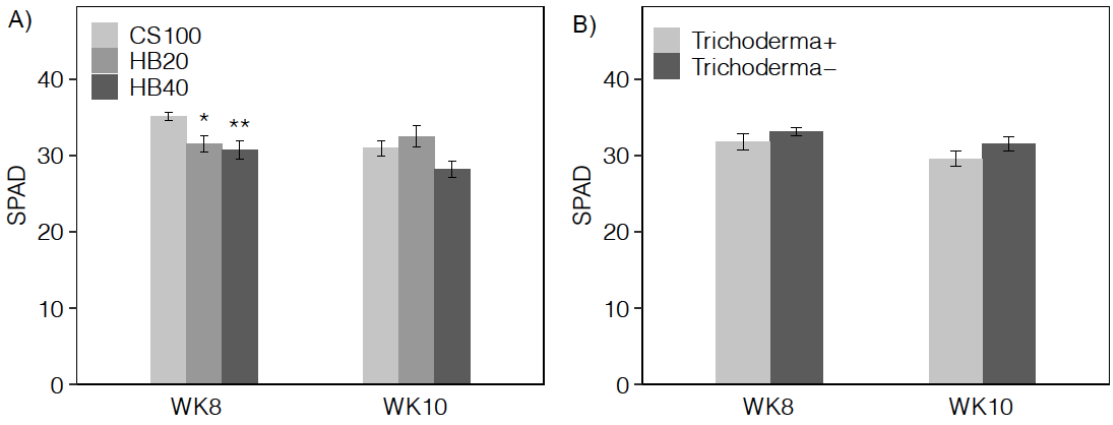

Figure 6.6. The effect of biochar rate (A) and *Trichoderma* application (B) on SPAD at week 8 and 10 after transplanting (WK8 and WK10) for non-pathogen treatments. CS100 = peat moss-based commercial substrate, HB20 and HB40 = 20% and 40% (by vol.) mixed hardwood biochar-amended mixes, respectively. *, indicates significantly different from the control (CS100) according to the Dunnett test at $p \leqslant 0.05$ and $p \leqslant 0.01$, respectively.**

The pathogen-inoculated poinsettia plants grown in the HB20 mixes had significantly higher SDW compared with those in the CS100 (Figure 6.7 A). *Trichoderma* had no significant influence on the SDW (Figure 6.6 B). Neither BC nor *Trichoderma* had significant impacts on any of the GIs (Figure 6.8). Poinsettia plants grown in the HB40 mixes had significantly lower SPAD values at WK8 and WK10 compared with the CS100, but *Trichoderma* had no significant influence on the SPADs (Figure 6.9).

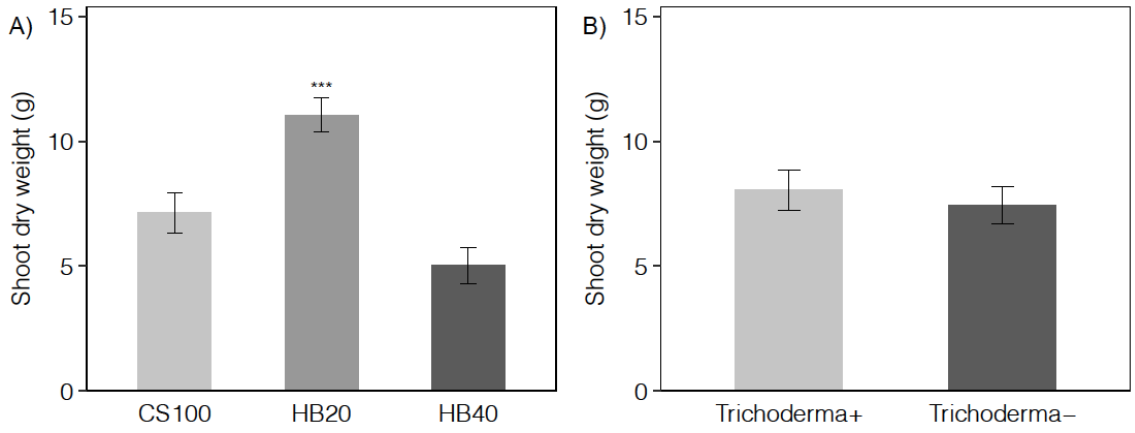

Figure 6.7. The effect of biochar rate (A) and *Trichoderma* application (B) on shoot dry weight for pathogen-inoculated treatments. CS100 = peat moss-based commercial substrate, HB20 and HB40 = 20% and 40% (by vol.) mixed hardwood biochar-amended mixes, respectively. * indicates significantly different from the control (CS100) according to the Dunnett test at $p \leqslant 0.001$.**

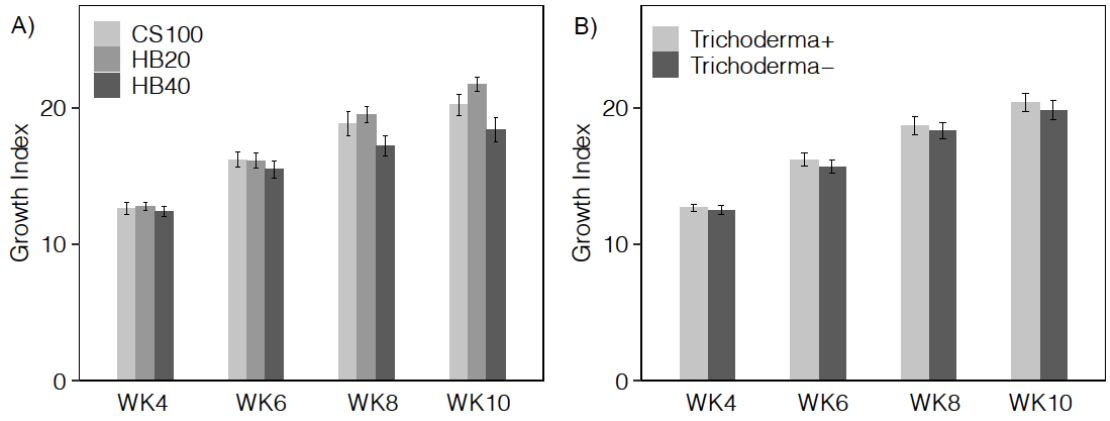

Figure 6.8. The effect of biochar rate (A) and *Trichoderma* application (B) on growth index at 4, 6, 8, and 10 weeks after transplanting (WK4, WK6, WK8, and WK10) for pathogen-inoculated treatments. CS100 = peat moss-based commercial substrate, HB20 and HB40 = 20% and 40% (by vol.) mixed hardwood biochar-amended mixes, respectively.

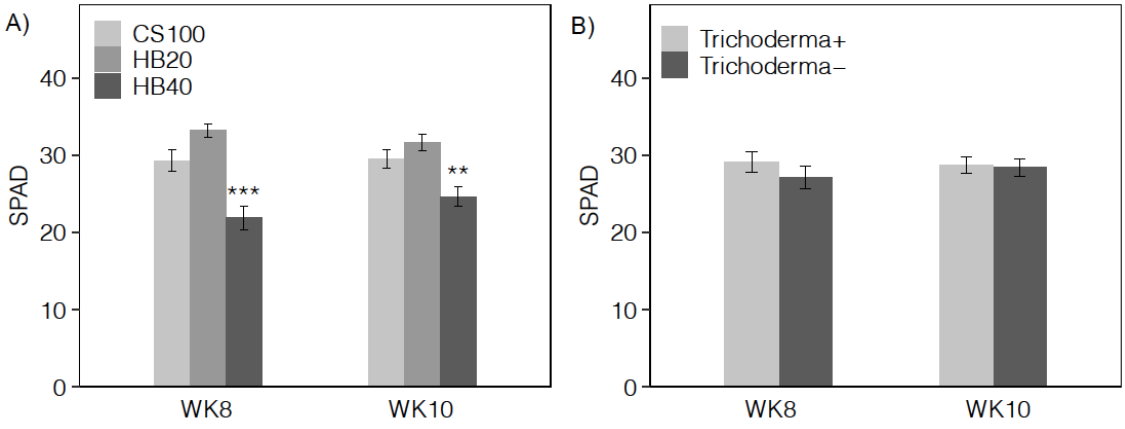

Figure 6.9. The effect of biochar rate (A) and *Trichoderma* application (B) on SPAD at 8 and 10 weeks after transplanting (WK8 and WK10) for pathogen-inoculated treatments. CS100 = peat moss-based commercial substrate, HB20 and HB40 = 20% and 40% (by vol.) mixed hardwood biochar-amended mixes, respectively. **,*indicates significantly different from the control (CS100) according to the Dunnett test at $p \leqslant 0.01$ and at $p \leqslant 0.001$, respectively.**

6.3.4. Disease Parameters

Disease symptoms of *Pythium* poinsettia root rot appeared in transplants in all the treatments at 5 days after inoculation (Figure 6.10 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 HB40 treatments, however, increased the disease severity at 15, 20, and 25 days after inoculation by 12.5%, 4.7%, and 1.6%, respectively. The application of *Trichoderma* did not significantly reduce disease severity throughout the experiment (Figure 6.10 B).

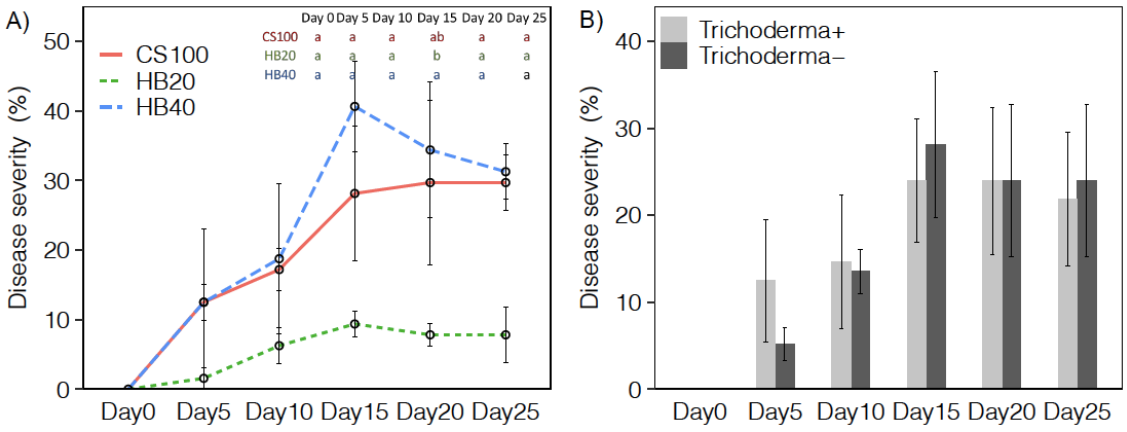

Figure 6.10. The effect of biochar rates (A) and *Trichoderma* (B) on disease severity for pathogen inoculate treatments. CS100 = peat moss-based commercial substrate, HB20 and HB40 = 20% and 40% (by vol.) mixed hardwood biochar-amended mixes, respectively. The same letter indicates not significantly different from each other according to LSD multiple comparison test at $p \leqslant 0.05$ on the same day.

Biochar mixes had a significant impact on disease incidence, especially the HB-amended (20% and 40%) mixes (Figure 6.11 A). Compared with the CS100 treatments, HB20 treatments reduced disease incidence by 31.3% starting at 5 days after inoculation. The HB40 mixes, however, increased disease incidence at 15, and 20 days after inoculation by 12.5% and 6.3%, respectively. The application of *Trichoderma* did not significantly reduce disease incidence for the entire duration of the experiment (Figure 6.11B).

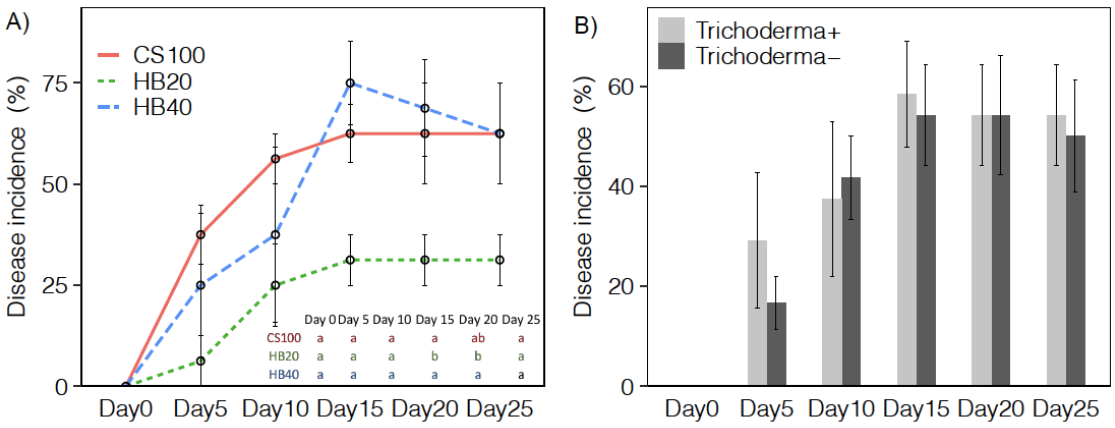

Figure 6.11. The effect of biochar rates (A) and *Trichoderma* (B) on disease incidence for pathogen-inoculate treatments. CS100 = peat moss-based commercial substrate, HB20 and HB40 = 20% and 40% (by vol.) mixed hardwood biochar-amended mixes, respectively. The same letter indicates not significantly different from each other according to LSD multiple comparison test at $p \leqslant 0.05$ on the same day.

The HB20 mixes had significantly lower AUDPC than the CS100 while HB40 had similar AUDPC to the CS100 (Figure 6.12 A). The HB20 reduced the AUDPC value by

13.6 yet the HB40 slightly increased the AUDPC value by 3.9 (Figure 6.12 A). *Trichoderma* did not significantly reduce AUDPC the entire experiment (Figure 6.12 B).

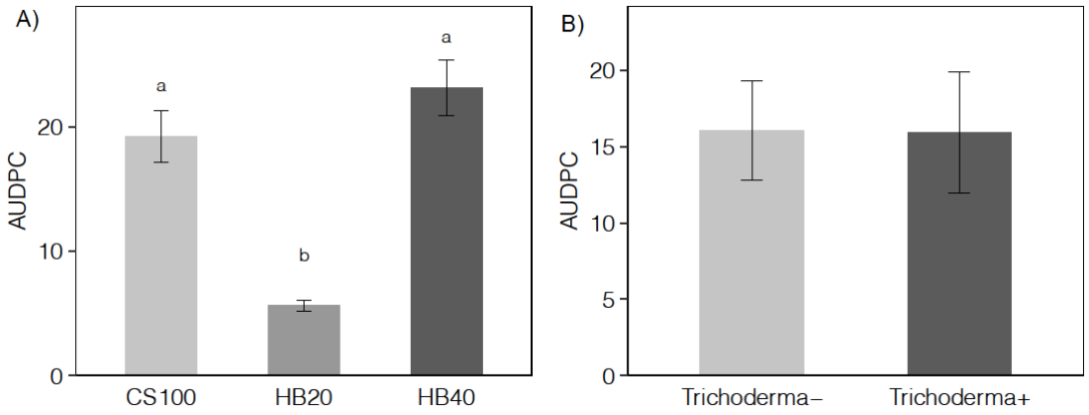

Figure 6.12. The effect of biochar types and rates (A) and *Trichoderma* (B) on the area under disease progress curve. CS100 = peat moss-based commercial substrate, HB20 and HB40 = 20% and 40% (by vol.) mixed hardwood biochar-amended mixes, respectively. The same letter indicates not significantly different from each other according to LSD multiple comparison test at $p \leqslant 0.05$.

6.4. Discussions

6.4.1. Biochar Chemical Compound and Pathogen Inhibition

The chemical compounds in BC could have a direct influence on disease development. Some types of biochar contain chemicals compounds such as ethylene glycol and propylene glycol, hydroxypropionic and hydroxybutyric acids, benzoic acid and o-cresol, quinones (resorcinol and hydroquinone), and 2-phenoxyethanol, which could adversely affect microbial growth and survival. Also, compounds such as

methoxyphenols, phenols, carboxylic acids, furans, and ketones, which could form during pyrolysis process, have a suppressive impact on microbial activity [37, 38]. In the in vitro test where the pathogen growth was not affected by BC physically properties, all the BC mixtures' extracts stimulated *P. aphanidermatum* growth, which was similar to Jaiswal, Elad [39] study, where eucalyptus wood BC or greenhouse pepper plant waste BC extracts did not inhibited *Fusarium oxysporum* growth. However, different from this study, Gravel, Dorais [23]'s study showed that softwood BC mixed with organic potting mix extracts suppressed *P. ultimum* growth. In the presence of *Trichoderma*, the stimulating effects of BC-amended mixes' extracts on *P. aphanidermatum* was reversed due to *Trichoderma'*s suppressive impacts on *P. aphanidermatum*. This study has proven that BC and other components provide synergistic effects on pathogen, which was also proven by Debode, Ebrahimi [40].

6.4.2. Biochar Properties and Disease Development

When a BC is added to the substrate, it may profoundly influence the complex rhizosphere–root–media–pathogen system by its physical and chemical properties such as nutrient content, water holding capacity, redox activity, adsorption ability, pH, and content of toxic and hormone-like compounds, which can affect the disease triangle factors both directly and indirectly (via its influence on the rhizosphere microbiome). In turn, the direct and indirect impacts of BC on the environment, host plant, pathogen, and rhizosphere microbiome can have domino effects on plant and disease development [24]. The BCs with high pH (>9) could contain some phenolic groups, such as phenolic acid and alkali,

which mainly exist as organic anions [41]. The high pH and buffer capacity of many BCs could also reduce the toxic acids near plant roots [24].

6.4.3. Biochar and Plant Growth

As the effects of BC and *Trichoderma* application on plants can be complex and difficult to explain, and two rates of BC and multiple variables were included in this study, a principal component analysis (PCA) was used to depict variables shaped by different BC rates with non-pathogen and pathogen-inoculated (Figure 6.13) plants. For the non-pathogen plants, 80% of the variability was explained by the first two components (Figure 6.13 A). PC1 accounted for 51% variance, with BC mixes (HB40-TN, HB40-TY, HB20-TY) differing from the CS mixes (CS100-TY, CS100-TN). Biochar-amended mixes were associated more with early GI (GI WK4). PC2 accounted for 29% variance, distinguishing the CS100 and HB40 from HB20 mixes. Commercial substrate and HB40 mixes tended to be affiliated with plant yield (SDW), early and later GIs (GI WK4, GI WK6, and GI WK10), and SPAD WK8.

For the pathogen-inoculated plants, 95% of the variability was explained by the first two components (Figure 6.13 B). PC1 accounted for 80% variance, differing HB40 and CS100-TN from HB20 and CS100-TY. Treatment HB20 and CS100-TY were associated more with yield (SDW), early and later GIs, and SPAD values. PC2 accounted for 15% variance, distinguishing HB20-TN, HB40-TY, CS100-TN from HB20-TY, HB40-TN, and CS100-TY. Treatment HB20-TN, HB40-TY, and CS100-TN tended to be affiliated with early GI (GI WK4), plant yield (SDW), and SPADs.

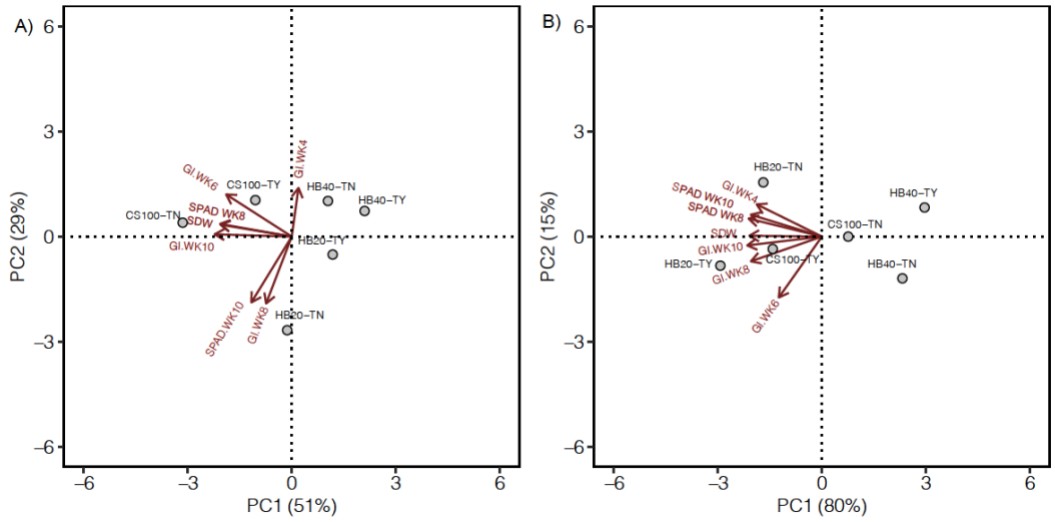

Figure 6.13. Principal component analysis (PCA) depicting the relationships between selected variables and treatment factors with non-pathogen (A) and pathogen-inoculated (B) plants. Selected variables are displayed by arrows and include plant growth parameters—growth index after 4, 6, 8, and 10 weeks of transplanting (GI WK4, GI WK6, GI WK8, and GI WK10), SPADs after 8, 10 weeks of transplanting (SPAD WK8, SPAD WK10) and shoot dry weight (SDW). Treatment factors are displayed by filled grey circles: peat moss-based substrate (CS100), biochar-amended mixes at different rates (by vol., HB20, and HB40) with (TY) or without *Trichoderma* (TN).

6.4.4. Treatment Factors Determine Plant Disease Development

For the pathogen-inoculated plants, 89% of the variability was explained by the first two components (Figure 6.14). PC1 accounted for 73% variance, differing HB20 from HB40 and CS treatments. The treatments CS100 and HB40 were positively associated with all the disease parameters while the HB20 treatments were negatively associated with them. PC2 accounted for 16% variance, distinguishing HB20-TY, HB40-TN, and CS100-TN treatments from the rest of the treatments. Treatment HB20-TY, HB40-TN, and CS100-TN tended to be affiliated with later DIs (DI 3-5) and DSs (DS 4-

5) and AUDPC while HB20-TN, HB40-TY, and CS100-TY appeared to be related to early DIs (DI 1-2) and DSs (DS 1-2).

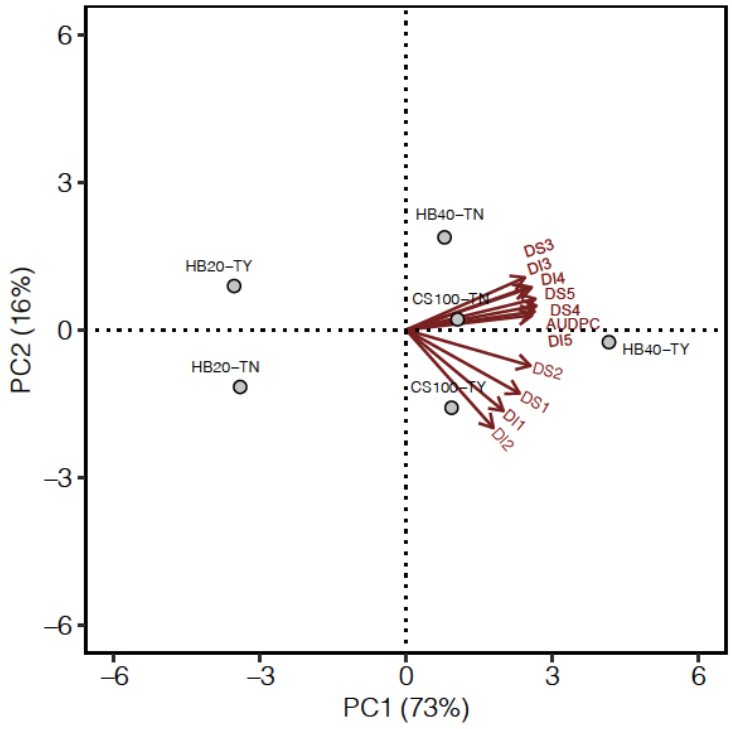

Figure 6.14. Principal component analysis (PCA) depicting the relationships between selected variables and treatment factors with pathogen-inoculated plants. Selected variables are displayed by arrows and include disease parameters—disease severity after 5, 10, 15, 20, and 25 days of transplanting (DS1, DS2, DS3, DS4, and DS5), disease incidence after 5, 10, 15, 20, and 25 days of transplanting (DI1, DI2, DI3, DI4, and DI5), and area under disease progress curve (AUDPC). Treatment factors are displayed by filled grey circles: peat moss-based substrate (CS100), biochar-amended mixes at different rates (by vol., HB20 and HB40) with (TY) or without *Trichoderma* (TN).

6.5. Conclusions

The mixed hardwood biochar blended with commercial peat moss-based substrate at 20% (by vol.) could significantly reduce *Pythium aphanidermatum* poinsettia root rot disease severity and disease incidence with or without *Trichoderma* addition. Compounds contained in biochar did not contribute to its disease inhibition capability given the fact it stimulated pathogen growth in the in vitro test. In the absence of the pathogen, mixed hardwood biochar could replace commercial peat moss-based substrate at 20% and to 40% (by vol.) for container poinsettia plant production without any negative impacts on its growth. In the presence of the pathogen, 20% (by vol.) of mixed hardwood biochar could replace commercial peat moss-based substrate for container poinsettia plant with reduction of disease severity and incidence.

6.6. References

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7. CONCLUSIONS

Biochar, a byproduct of pyrolysis, has the potential to replace peat moss, a commonly used container substrate in greenhouse production. Using BC to replace peat moss as a container substrate for plant production provides an environmentally friendly way to address the environmental concerns associated with peatland mining and drainage. Switching peat moss to BC as a container substrate for plant production protects peatland ecosystem, increases water and fertilizer use efficiency, reduces greenhouse gases emission, and brings economic benefits. Although the number of BC-related publications increased in the past two decades, studies are still needed specifically on BC replacing peat moss as a container substrate to benefit the environment. The purpose of this study was to test the effects of different BC mixes on plant growth and plant disease suppression.

In the first study, we found that none of the mixes caused phytotoxicity and the HB mixed at 70% (by vol.) with the rest being peat moss can be successfully used as the potting mix for tomato and basil seedling production without negative effects on plant biomass. Tomato seedlings from all the BC mixes had significantly lower total fresh weights (TFWs), TDWs and GIs than the control except for HB50 mixes. Tomato seedlings from all the BC mixes (except SBB30) had similar SPAD values and GI to the control. Basil seedlings from all the BC mixes had significantly lower TFWs (except HB30 and HB50), TDWs and GIs (except HB50) than the control yet similar or higher SPAD (except SBB100).

In the second study, we found that the HB could replace bark-based substrates at 50% and the SBB at 70% for both tomato and basil plant growth without negative effects. Most of the mixes' physical properties were within the recommended range. The leachate of all the BC mixes (both with tomato and basil plants) had a similar or higher NO_3–N concentration compared to the control. Also, both tomato and basil plants from the BC mixes, all had a similar GI to the control. Tomato plants in all BC mixes had similar SDW and fruit dry weight (FDW, yield) to the control, yet those in SBB mixes had significantly lower TDW, root dry weight [159], and leaf dry weight [158]. Basil plants grown in all BC mixes had similar RDW, SDW (except HB50), LDW, FDW, and TDW to the control. The SPAD of tomato and basil plants grown in all BC mixes was similar to the control.

In the third study, we found that adding mycorrhizae did not have a significant impact on plant growth (except HB90-VC5 with fertigation at 300 mg L^{-1} N for tomato). The HB can replace commercial peat moss-based substrate when used at 50% to 70% (vol.) for both tomato and pepper plants. At the BC rates of 0%, 50%, or 70%, mycorrhizae addition or fertigation rates did not significantly affect GI at week 8 (WK8), FDW, and TDW of tomato, or GI WK8 of pepper. At 90% HB rate, the addition of mycorrhizae or fertigation rates did not significantly affect GI WK 8 and FDW for tomato yet with mycorrhizae at higher fertigation rates, tomato TDW was significantly improved. Pepper plants grown in the HB 50 and HB70 mixes had similar FDW and TDW to the control.

In the fourth study, we found that the HB replacing 30% and 50% peat moss in substrate could reduce pepper blight disease caused by *P. capsici* without negatively affecting plant growth. Most of the mixes' physical properties were within the

recommended range, except for the BDs. In the in-vitro trial, all the BC extracts suppressed *P. capsici* growth with and without *Trichoderma* (except HB10). The HB50 and HB70 treatments reduced disease severity at 12 days after transplanting by 10.94% and 10.16%, respectively. Also, the HB50, HB70, and SBB10 treatments reduced disease incidence at 7 days after transplanting by 25.0%, 25.0%, and 18.8%, respectively. The HB50 and HB70 mixes reduced the area under disease progress curve (AUDPC) value by 9.6 and 9.4 respectively. The application of *Trichoderma* did not significantly reduced disease severity, disease incidence, or AUDPC of the entire experiment.

In the fifth study, we found that the HB20 could replace peat moss-based substrate to reduce poinsettia root rot disease caused by *P. aphanidermatum.* All the BC water extracts suppressed pathogen growth with *Trichoderma* yet stimulated pathogen growth without *Trichoderma.* The HB20 treatments reduced the disease severity at 5, 10, 15, 20, and 25 days after inoculation by 10.9%, 10.9%, 18.8%, 21.9%, respectively. Also, the HB20 treatments reduced disease incidence by 31.3% starting at 5 days after inoculation and the AUDPC value by 13.6. The HB40 treatments, however, increased the disease severity, disease incidence, and AUDPC. The application of *Trichoderma* did not significantly reduce disease severity, disease incidence, or AUDPC throughout the experiment.

In conclusion, most of the mixes' physical properties were within the recommended range and adding mycorrhizae did not affect plant growth. The HB70 can be used for tomato and basil seedling and tomato and pepper plant production by mixing with peat moss or peat moss-based substrates. Also, the HB50 and the SBB70 mixed with

bark-based substrates could be used for tomato and basil plant production. The HB30, HB50, and HB70 could reduce pepper blight disease caused by *P. capsici* and the HB20 could reduce poinsettia root rot disease caused by *P. aphanidermatum.*