

EFFECTS OF DIFFERENT BIOCHAR TYPES ON THE GROWTH AND BIOCHEMICAL CHARACTERISTICS OF BROCCOLI AND PEPPER SEEDLINGS UNDER GREENHOUSE CONDITIONS

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ABSTRACT

This study aimed to verify the effects of biochars produced from chestnut seeds, compost, pine bark, and pine sawdust on the growth and quality of broccoli (*Brassica oleracea* var. *italica* Plenck) and pepper (*Capsicum annuum* L.) seedlings under greenhouse conditions. It was hypothesized that the effects would depend on the biochar feedstock and the plant genotype. Biochars were incorporated into a universal peat substrate at 4.4% (dry weight); an unamended substrate served as the control. Pots experiment was arranged in a completely randomized design, with 10 replicates per treatment. Biochar application generally did not significantly modify the biometric and biochemical parameters of broccoli seedlings. Pine sawdust biochar increased leaf perimeter compared to the control; however, it lowered chlorophyll fluorescence, suggesting a stress response. Compost biochar increased the number of root tips compared to chestnut seed biochar, but roots had the lowest diameter. At the same time, compost biochar-treated broccoli had lower anthocyanin, chlorophyll, and SPAD index contents, contrary to pine bark-treated seedlings. In peppers, pine sawdust biochar provided the best results across plant biometric parameters, compared to pine bark and compost biochar. Plants grown in substrate with chestnut seed biochar had the longest roots and the highest root area, volume, diameter, and number of root tips. Leaf chlorophyll content and the SPAD index were also higher compared to the control. Compost biochar did not positively affect seedling development. Our results suggesting pine sawdust and chestnut seed biochars as promising amendments for peat substrates used in pepper seedling production and highlighting the need to select the appropriate biochar type for each plant species to obtain high-quality planting material.

Keywords: biometric parameters, chestnut seeds, compost, pine bark, pine sawdust, vegetable plants

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INTRODUCTION

Horticulture differs from agriculture in terms of crops (fruit, vegetables, ornamental plants, herbs) and more intensive cultivation systems. Some crops are produced in substrates under protected culture (greenhouses, plastic tunnels), while others are grown in open fields. Currently, the horticultural sector is adopting new strategies to move toward eco-friendly production [Zulfiqar et al. 2022]. One innovative solution gaining particular attention is the use of biochar, which is defined as a renewable fuel that can be produced by heating biomass like farm waste, municipal waste, once considered useless, energy crops, or plants cultivated specifically for this purpose [Patel and Panwar 2023]. Biochar is used primarily for its high content of easily assimilable carbon, in the form of a finely divided, highly porous material. Biochar can increase soil quality and crop yield. It is also an effective, fast way to sequester carbon and reduce greenhouse gas emissions [Patel and Panwar, 2023]. Biochar is most commonly produced by pyrolysis (at temperatures of 350–900 °C) in closed containers with absent or depleted oxygen [Zulfiqar et al. 2022, Khater et al. 2024]. It can be used as a standalone product or as an additive to enhance the properties of growing substrates in horticultural plant cultivation [Amery et al. 2021]. Biochar significantly enhances the physical, chemical, biological, and nutritional qualities of the substrate, prevents fertilizer leaching, and supplies additional nutrients to plants. Biochar's high sorption capacity can also greatly improve water conditions [Zannatul et al. 2023, Simhi Samyukta et al. 2024]. The substrate-biochar mixture is pathogen-free and can transform some heavy metals into less toxic forms for plants. Biochar's disease-control mechanisms include adsorbing virulence factors, inducing systemic resistance in plants, altering soil microbial communities, and/or increasing soil silicon content. However, its final effectiveness varies with factors such as biomass input, pyrolysis conditions, and application rate [Iacomino et al. 2022].

Seedling production is an essential practice in horticulture. High-quality planting material in the initial phase of vegetable plant production is crucial for final yield efficiency and quality. Using seedlings increases shoot and root growth, accelerates maturity, and enhances vegetable yield compared to direct seed sowing. Transplanted seedlings also have higher chlorophyll content than directly seeded plants. Growing seedlings in a controlled environment limits the spread of pests and diseases and reduces the need for plant protection products later on [Gallegos-Cedillo et al. 2024]. Vegetable seedlings should grow uniformly, reach the desired size and growth stage at transplanting, and be free of disease and insect pests. The substrate plays a critical role in producing healthy seedlings [Maynard et al. 2024].

Capsicum annuum L. (pepper) belongs to the Solanaceae family and is the most commonly cultivated plant in the *Capsicum* genus. The species contains both pungent and non-pungent (sweet) fruits. Pepper is one of the most widely used spices worldwide and has valuable medicinal properties due to numerous bioactive compounds [Hernández-Pérez 2020, Liu et al. 2023]. High-quality pepper seedlings should be dark green with sturdy shoots and well-developed root systems. Sweet pepper seedlings have a relatively long growing period (40–60 days), depending on the cultivation conditions and cultivar [Jankauskienė and Laužikė 2023]. Pepper has high nutrient requirements, and the substrate is an important factor affecting seedling quality, plant morphological characteristics, and disease susceptibility [Awuku and Egyir 2018]. Commercial substrates are usually used. Replacing the commercial substrate with alternative substrates can reduce the cost of seedling production [Júnior et al. 2018]. The method of preparing pepper seedlings affects subsequent growth and yield [Jankauskienė and Laužikė 2023]. Container production (also used in this research) provides higher yields and fruit with better characteristics than seedlings produced using the standard method [Markovic et al. 2000].

Broccoli (*Brassica oleracea* L. var. *italica* Plenck), on the other hand, is a representative of the Brassicaceae vegetables and possesses many valuable medicinal properties, high nutritional value, and strong antioxidant activity [Syed et al. 2023]. Its cultivation requires particularly favorable conditions, especially during seedling production, which should ultimately be characterized by a compact habit with no more than five leaves. Strong, healthy seedlings give plants an advantage in competition for water, light, and nutrients, which are essential for proper growth. Well-developed, hardened, and nourished plants have a better chance of adapting to their permanent location and minimizing losses resulting from biotic and abiotic factors [Ptaszek et al. 2021]. According to Moon et al. [2025], 35-day-old broccoli seedlings yield

the best. Generally, earlier-planted seedlings produce higher plants with more leaves and give the lowest share of aberrant curds.

Despite growing interest in biochar as an ecological additive to horticultural substrates, studies rarely consider how diverse, unconventional biochar sources affect the early stages of vegetable plant development. Knowledge remains limited on selecting appropriate biochar types for specific vegetable species and their effects on seedling quality. Previous studies have often focused on the general effects of biochar, without considering differences resulting from feedstock origin and plant genotype. Chrysargyris et al. [2019] tested commercial biochars in cabbage (*Brassica oleracea* L. var. *capitata*) seedling cultivation. Biochars were mixed with peat at 0, 5, 10, 15, and 20% ratios. Forest wood biochar and bamboo biochar at 20% reduced seed germination and plant length. Forest wood, husks and paper fiber, and fresh wood screening biochars applied at 5% and 10% maintained or improved seedling growth (fresh weight and length). Cucumber (*Cucumis sativus* L.) seedlings cultivated in a substrate with 10% corn stover biochar were characterized by the highest shoot and root growth, while the seedlings treated with 25% biochar were less than 22.23% of the growth found in the 10% biochar treatment, and even less than 1.5% of the growth of the control seedlings [Ma et al. 2024].

This study aimed to investigate the impact of biochars derived from chestnut seeds, compost, pine bark, and pine sawdust on the quality of pepper and broccoli seedlings, with particular emphasis on plant biometric and biochemical parameters. The research hypothesis assumed that supplementing peat substrate with biochar could affect the growth and quality of the tested seedlings. Observed effects will vary depending on the type of biochar and plant genotype. This integrated approach fills a knowledge gap on selecting the right biochar type for a specific vegetable species and supports sustainable production of high-quality vegetable seedlings. The novelty of this study lies in using biochars derived from less explored feedstocks, which have rarely been evaluated as substrate amendments for vegetable seedling production.

MATERIALS AND METHODS

Time and location of the experiment

The experiment was performed in a greenhouse at the Faculty of Agriculture and Biotechnology of the Bydgoszcz University of Science and Technology, Poland (53°07'12.0" N 18°00'29.4" E), from July 1, 2024, to August 5, 2024.

Plant material

Seeds of pepper *Capsicum annuum* L. Marta Polka and broccoli *Brassica oleracea* var. *italica* Plenck – Cezar were purchased from W. Legutko Hobby Ltd. (Rawicz, Poland). Marta Polka is an early cultivar suitable for both indoor and outdoor cultivation. Its stem is stiff and reaches 40 cm in length. It produces large, block-shaped, yellow fruit. The cultivar tolerates unfavorable environmental conditions but prefers fertile soil that warms quickly. Cezar is an early cultivar of broccoli, resistant to ground frosts. It produces large, green heads, prefers fertile, moist soil; during drought, regular watering is recommended.

Experimental design

The biochars were prepared via pyrolysis. The feedstock materials for biochar production were obtained from the following sources: compost from the municipal wastewater treatment plant in Piła, Poland; chestnut seeds from green area maintenance; pine bark; and pine sawdust from a local sawmill. The production of biochars was carried out by the low-temperature pyrolysis (400 °C) of air-dried feedstock biomass (under atmospheric pressure) in the muffle furnace FCF 22 M (Czylok Ltd., Jastrzębie-Zdrój, Poland). Process execution time was 1 h. The obtained biochars were ground and homogenized in a PM100 ball mill (Retsch, Düsseldorf, Germany). Milling parameters: 10 min, 400 rpm, 10 zirconium balls in the milling chamber. The mean size distribution of ground biochar particles was as follows:

- chestnut seed biochar – 2.0–0.05 mm: 30.87%, 0.05–0.002 mm: 66.42%, <0.002 mm: 2.71%,
- compost biochar – 2.0–0.05 mm: 47.22%, 0.05–0.002 mm: 47.05%, <0.002 mm: 5.73%,
- pine bark biochar – 2.0–0.05 mm: 12.86%, 0.05–0.002 mm: 84.23%, <0.002 mm: 2.92%,
- pine sawdust biochar – 2.0–0.05 mm: 34.67%, 0.05–0.002 mm: 62.98%, <0.002 mm: 2.35%.

Biochar particle-size distribution was determined using a Malvern Instruments Mastersizer 2000 analyzer equipped with a dispersing device (Hydro 2000MU; Malvern Panalytical, Malvern, UK).

To provide a comprehensive characterization of the materials used, the physicochemical properties of the biochars and substrate were determined, including the content of total organic carbon (TOC) and total nitrogen (TN), pH, electrical conductivity (EC), ash content, as well as the content of micro- and macroelements. The content of TOC and TN was assayed with the Vario Max CN analyzer (Elementar, Langensfeld, Hesse, Germany). The pH was analysed in accordance with the standard PN-ISO 10390 – soil quality, determination of pH. For this purpose, 0.5 g of biochar/substrate was mixed with 25 mL of deionized water (pH 6.06, EC 0.923 $\mu\text{S}\cdot\text{cm}^{-1}$) and shaken for 5 min; then the flask was set aside for 1 h. The pH was determined using a 540 GLP pH-meter (WTW, Washington, DC, USA) with a glass electrode. Electrical conductivity was measured according to the standard PN-ISO 11265 – soil quality, determination of the specific electrical conductivity. For EC analysis, the extract of water and biochar/substrate described above was used. The EC was determined by the CC-501 conductometer (Elmetron, Zabrze, Poland); see Table 1.

The content of K, Ca, and Na was determined by a flame photometer BWB XP (BWB Flame Technologies, Newbury, UK). The mineralization of biochar/substrate samples was performed in sulphuric acid (96%); 1 g of sample was mixed with 10 mL H_2SO_4 . Mineralization was performed in a DK 20 Digestion Unit (VELP Scientifica Srl, Usmate, Italy) at 265 °C for 6 h. After cooling, the mineralization was continued at 375 °C until the solutions turned dark brown. Then the solutions were decolorized with hydrogen peroxide (30%, 5 mL), and the mixtures were further digested for 30 min (375 °C). After cooling, the solutions were poured into 100-mL volumetric flasks and filled up with deionized water. The contents of Cu, Zn, Mn, Mg, and Fe were measured by atomic absorption spectrometry using an AA240FS spectrometer (Varian, Mulgrave, Australia). Biochar/substrate samples were mineralized in a muffle furnace (750 °C, 150 min.). The obtained ash was further mineralized in a mixture containing 30 mL of 65% nitric acid and 10 mL of 38% hydrochloric acid. The Tecator digester open system (160 °C, 120 min.) was used. After cooling, the solutions were poured into 100-mL volumetric flasks and filled up with deionized water. After filtering, the resulting mineralization solutions were assayed using an AAS. The content of Cu, Zn, Mn, Mg, and Fe was calculated as the concentration of individual elements in ash and finally in pure biochar/substrate (Table 2).

Table 1. Biochar and peat substrate characteristics: total organic carbon (TOC) and total nitrogen (TN) contents, pH, electrical conductivity (EC), ash share

Tested materials	TOC ($\text{g}\cdot\text{kg}^{-1}$)	TN ($\text{g}\cdot\text{kg}^{-1}$)	pH	EC ($\mu\text{S}\cdot\text{cm}^{-1}$)	Ash (%)
Chestnut seed biochar	714.5	16.74	8.24	777.8	8.47
Compost biochar	298.9	26.38	7.35	632.1	70.52
Pine bark biochar	699.3	2.30	4.72	127.95	2.10
Pine sawdust biochar	642.2	1.30	3.86	108.05	0.47
Peat substrate	538.40	11.10	6.49	356.7	6.92

Table 2. Content of micro- and macroelements in biochars and peat substrate

Tested materials	Macro- and micro-elements content ($\text{mg}\cdot\text{g}^{-1}$)							
	K	Ca	Na	Cu	Zn	Mn	Mg	Fe
Chestnut seed biochar	119.500	5.523	1.724	1.724	1.416	0.527	53.250	2.279
Compost biochar	7.756	4.122	1.000	0.154	0.604	0.458	5.473	4.659
Pine bark biochar	0.139	0.526	4.166	0.217	0.472	2.337	6.527	4.291
Pine sawdust biochar	0.037	0.734	0.638	0.103	0.368	5.267	2.176	2.094
Peat substrate	5.482	59.440	0.769	0.703	1.974	2.003	101.708	36.032

Biochars were added to a universal peat substrate (Hartmann Polska Sp. z o.o., Poznań, Poland) at

a concentration of 4.4% (dry weight). Substrate without biochar was used as a control. Biochar and peat substrate characteristics are provided in Tables 1 and 2. A single biochar application rate of 4.4% was considered sufficient to modify substrate properties while minimizing the risk of adverse effects associated with excessive biochar addition on plant growth (unpublished data). Based on a review of the scientific literature on the effect of biochar on seedling development, the most frequently recommended biochar addition to the substrate ranges from 0.2% to 10% [Chrysargyris et al. 2019, Ma et al. 2024, Kharytonov et al. 2026]. We used the same mass-based biochar doses to simplify preparing the biochar-substrate mixture for commercial gardening.

Seeds were sown individually into 0.25-L plastic pots to a depth of 2 cm, watered evenly, and allowed to germinate in an automated greenhouse under controlled temperature and humidity conditions. No artificial lighting was applied. Plants were cultivated under a natural 16 h photoperiod and light intensity. Air temperature was maintained at 20 °C ± 2 °C, and air humidity at 80%. Plants were watered according to a uniform schedule to maintain optimal substrate moisture (70–80%), and no additional fertilization or fertigation was applied.

The experiment was conducted in a completely randomized design (CRD), in which pots representing individual experimental treatments were randomly arranged in the greenhouse to minimize environmental variability. Each combination included 10 replications (plants).

Assessment of plant biometric and biochemical traits

After five weeks of cultivation, the leaf number, shoot length (cm), shoot fresh weight (g), and shoot dry weight (mg) were determined. Morphological parameters of leaf length (cm), width (cm), perimeter (cm), and area (cm²), as well as total length (cm), area (cm²), root system volume (cm³), root diameter (mm), and number of root tips were assessed using a Perfection V800 scanner (Seiko Epson Corporation, Suwa-shi, Nagano, Japan). The WinFOLIA 2016b program was used to analyze leaf architecture, and WinRHIZO 2016a (Regent Instruments, Canada, USA) was applied to analyze root parameters. The five-week evaluation period was selected as a critical stage of seedling development, allowing the assessment of early effects of biochar amendment on biometric and physiological traits before transplanting. Pepper seedlings of early cultivars have about a 40-day growing period [Jankauskienė and Laužikė 2023]. According to Moon et al. [2025], 35-day-old broccoli seedlings yield the best.

The relative content of flavonols, anthocyanins, chlorophyll, and soil plant analysis development (SPAD) leaf greenness index in the fresh leaves was estimated using a portable MPM-100 multi-pigment meter (Opti-Sciences Inc., Hudson, NH, USA). The relative values of F_0 (minimal fluorescence), F_v (variable fluorescence), F_m (maximal fluorescence), F_v/F_m , and F_v/F_0 ratios in the leaves were measured using the OS-30p+ chlorophyll fluorimeter (Opti-Sciences Inc., Hudson, NH, USA). Measurements were performed on the second pair of fully expanded true leaves to ensure uniform physiological status and comparability among treatments.

Data analysis

The results were statistically processed using Statistica 12.0 (StatSoft Polska, Kraków, Poland). Before the analysis, the Shapiro–Wilk test for normality was performed. The results were analyzed using one-way analysis of variance, and the means were compared using Fisher’s least significant difference (LSD) test at $\alpha = 0.05$. Tables and figures present means ± standard deviations (SD) and are labeled with statistical letters indicating significant differences, separately for each plant species tested.

RESULTS

Biometric traits

Shoot traits. In broccoli, all analyzed shoot biometric traits (leaf number, shoot length, and shoot fresh and dry weights) did not differ statistically with biochar application (Table 3, Figure 1). In pepper, however, adding biochar to the substrate resulted in significant differences in the analyzed traits. Plants grown in

the substrate supplemented with pine bark and compost biochars produced fewer leaves (6.38 and 6.00, respectively) than the control group (7.50), plants cultivated in substrate with pine sawdust biochar (8.17), and chestnut seed biochar (7.43). The application of biochar from compost resulted in a 15.6–25.5% reduction in shoot length compared to control plants and plants cultivated in substrate with biochar from pine sawdust and pine bark. Adding pine sawdust biochar resulted in the highest shoot fresh weight (almost 38% higher than in plants grown with pine bark biochar and 86.0% higher than in plants treated with compost biochar). A similar relationship was observed in the shoot dry weight. The value of this trait was highest with pine sawdust biochar, representing a 37.2% increase compared with the control group, a 42% increase compared to pine bark biochar, and an 85% increase compared to compost biochar (Table 3, Figure 2). Pine sawdust biochar had the most beneficial effect on pepper shoot growth in terms of intensifying and shortening horticultural production. Plants treated with this biochar had a high number of leaves (8.17), length (12.70 cm), and the highest biomass (3.46 g fresh weight and 254.98 mg dry weight).

Table 3. Biometric traits of broccoli Cezar and pepper Marta Polka shoots, depending on the experimental combination

Experimental combination	Number of leaves	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (mg)
broccoli				
Control (substrate without biochar)	4.13 ±0.96 a	11.48 ±2.75 a	1.91 ±1.06 a	153.36 ±88.20 a
Substrate + pine sawdust biochar	3.50 ±0.52 a	11.06 ±1.41 a	1.72 ±0.62 a	140.37 ±49.07 a
Substrate + chestnut seed biochar	3.33 ±1.28 a	10.07 ±3.01 a	1.93 ±1.09 a	152.14 ±91.49 a
Substrate + pine bark biochar	3.38 ±1.02 a	10.06 ±3.03 a	1.72 ±0.93 a	138.34 ±76.06 a
Substrate + compost biochar	3.56 ±0.98 a	9.99 ±3.53 a	1.55 ±0.97 a	124.71 ±81.77 a
pepper				
Control (substrate without biochar)	7.50 ±1.26 a	11.21 ±2.44 a	2.68 ±1.07 ab	185.80 ±83.76 b
Substrate + pine sawdust biochar	8.17 ±1.11 a	12.70 ±1.37 a	3.46 ±0.38 a	254.98 ±33.11 a
Substrate + chestnut seed biochar	7.43 ±0.94 a	11.19 ±1.11 ab	2.89 ±0.58 ab	188.81 ±34.84 ab
Substrate + pine bark biochar	6.38 ±0.50 b	11.28 ±1.11 a	2.51 ±0.49 bc	179.50 ±43.90 b
Substrate + compost biochar	6.00 ±0.84 b	9.46 ±1.78 b	1.86 ±0.67 c	137.56 ±62.30 b

Means ± standard deviation marked with the same letter do not differ significantly at $\alpha = 0.05$ ($n = 10$), separately for each plant species.

Figure 1. Broccoli Cezar seedlings after five weeks of cultivation: A – control (substrate without biochar), B – substrate + pine sawdust biochar, C – substrate + chestnut seed biochar, D – substrate + pine bark biochar, E – substrate + compost biochar (bar = 15 cm)

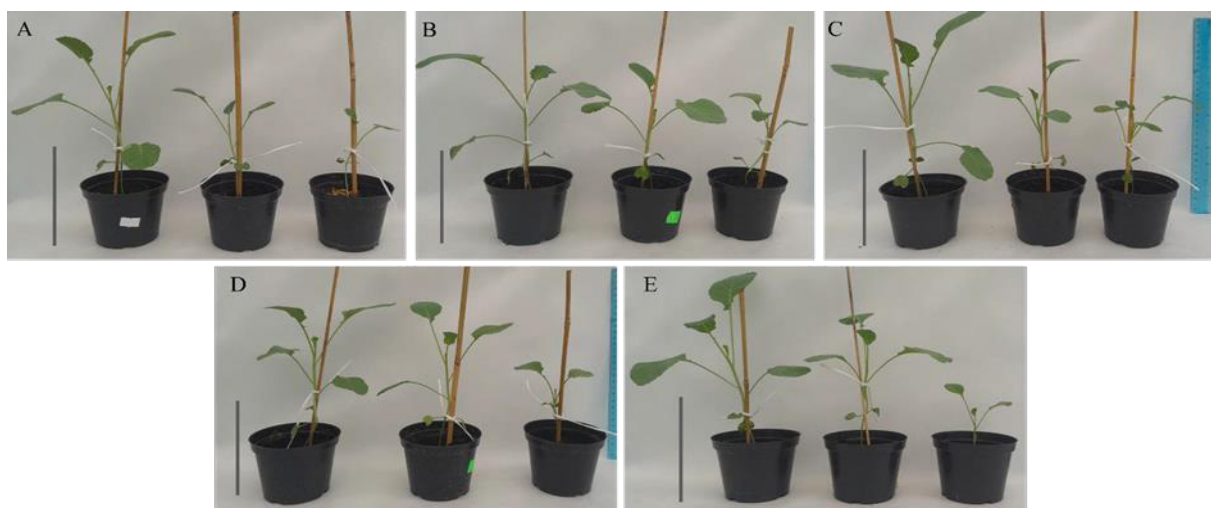


Figure 2. Pepper Marta Polka seedlings after five weeks of cultivation: A – control (substrate without biochar), B – substrate + pine sawdust biochar, C – substrate + chestnut seed biochar, D – substrate + pine bark biochar, E – substrate + compost biochar (bar = 15 cm)

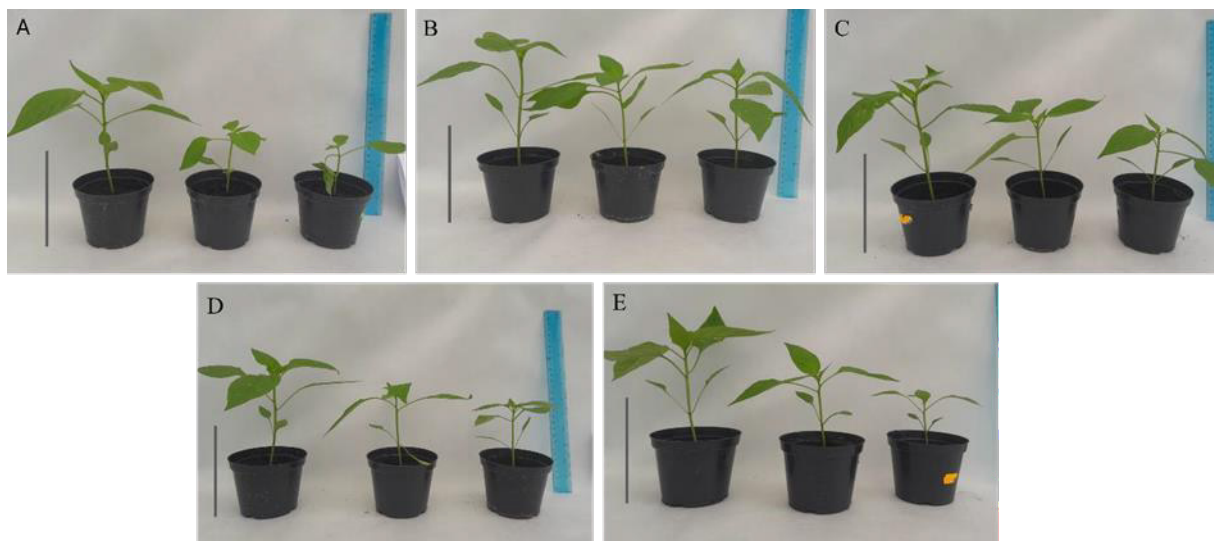


Figure 3. Morphological traits of broccoli Cezar and pepper Marta Polka root systems, depending on the experimental combination; means $\pm$ standard deviation marked with the same letter do not differ significantly at $\alpha = 0.05$ ($n = 10$), separately for each plant species

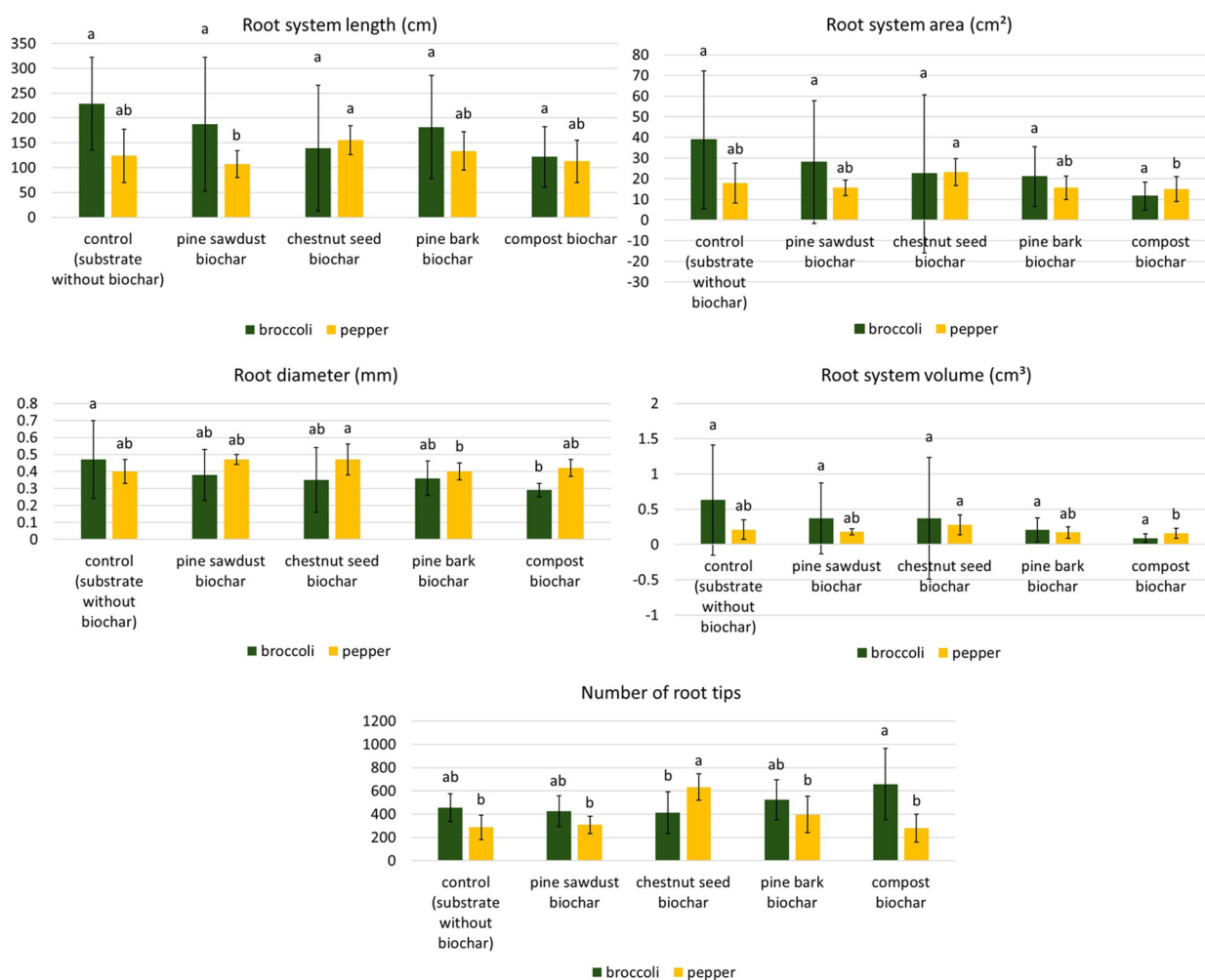
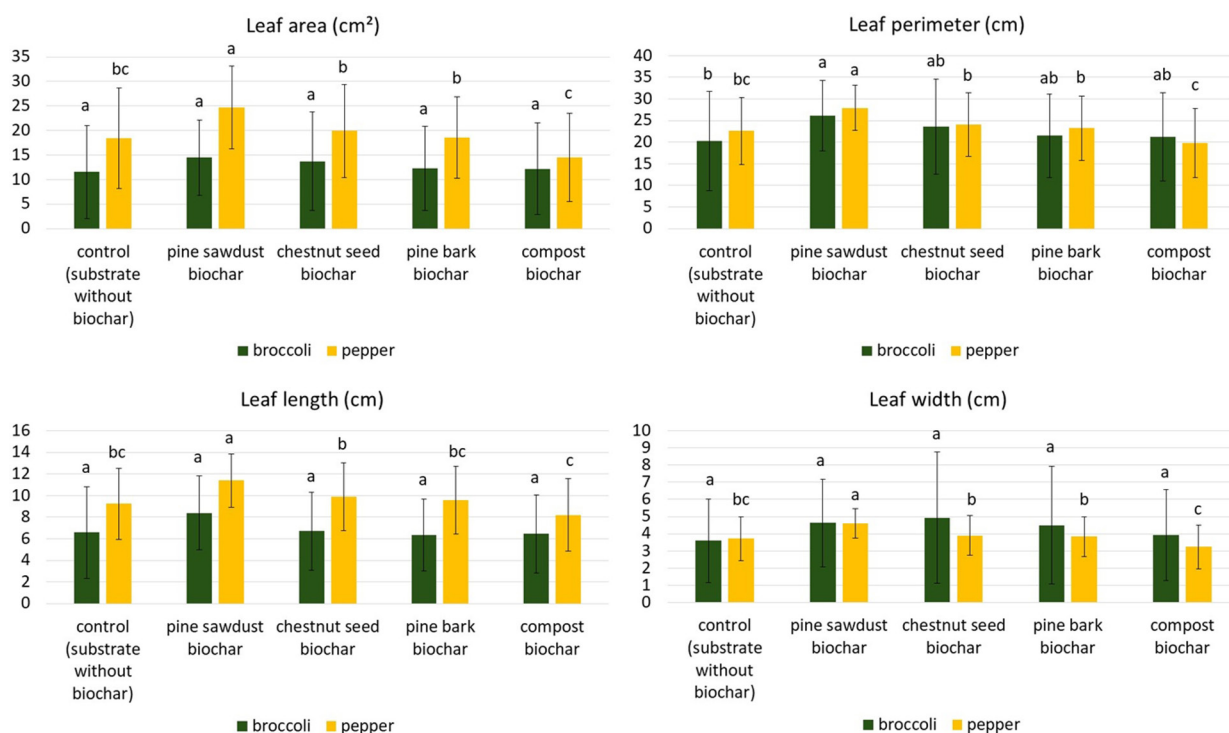


Figure 4. Morphological traits of broccoli Cezar and pepper Marta Polka leaves, depending on the experimental combination; means $\pm$ standard deviation marked with the same letters do not differ significantly at $\alpha = 0.05$ ($n = 10$), separately for each plant species



Root traits. In broccoli, experimental combinations showed no significant differences in root system length, area, or volume. However, control plants had the highest root diameter (0.47 mm), while plants treated with compost biochar had a root diameter reduced by more than 38%. Statistically significant differences were also noted in the number of root tips. The use of compost biochar resulted in the development of the highest number of root tips. This number was reduced by almost 40% in the root systems of plants growing in the substrate with chestnut seed biochar (Figure 3).

Pepper plants cultivated in the substrate supplemented with chestnut seed biochar had the longest root systems (155.40 cm), i.e., a 45.4% increase compared with plants from the substrate with pine sawdust biochar. The root system area and volume in plants treated with chestnut seed biochar were significantly higher than in plants treated with compost biochar, indicating a positive effect of this biochar on root development. The addition of chestnut seed biochar also resulted in a higher root diameter (0.47 mm) than pine bark biochar (0.40 mm), showing a 17.5% increase, and a higher number of root tips compared to other experimental combinations (Figure 3).

The number of root tips showed a different trend across the plant species studied, depending on whether chestnut seed biochar or compost biochar was used. In broccoli, the highest number of root tips was formed in the substrate with compost biochar, and the lowest in the substrate with chestnut seed biochar. In peppers, the relationship was reversed.

Leaf morphology. In broccoli, leaf area, length, and width did not differ across the experimental combinations; however, adding pine sawdust biochar to the substrate significantly increased leaf perimeter (26.12 cm) compared to the control (20.23 cm), representing a 29.1% increase (Figure 4).

In pepper plants, those grown in a substrate with pine sawdust biochar had the highest leaf area (24.68 cm²), perimeter (27.94 cm), length (11.38 cm), and width (4.59 cm), in contrast to plants treated with compost biochar, which presented the lowest values of these traits. Compared to the control plants, leaves of plants grown in the substrate supplemented with pine sawdust biochar had almost 38% higher surface area, almost 24% higher perimeter, and 23% higher length and width (Figure 4).

Physiological parameters

Chlorophyll fluorescence. In broccoli, the highest F_0 value was recorded in plants grown in the substrate supplemented with pine sawdust biochar (139.40), which was 7.5% higher than in plants treated with compost biochar (129.67). Control plants had the highest F_v value (611.33). The F_m in the control plants was significantly higher (744.93) than in the plants supplemented with chestnut seed, pine bark, and compost biochars. The lowest F_v/F_m and F_v/F_0 values were recorded in the plants supplemented with pine sawdust biochar (0.808 and 4.243, respectively) (Table 4).

In pepper, depending on the experimental combination, the F_0 did not differ statistically, ranging from 196.80 to 202.00. At the same time, F_v and F_m in plants grown in a substrate with pine bark biochar were higher than in the control plants, representing increases of 4.3% and 3.8%, respectively. Both the F_v/F_m and F_v/F_0 ratios in plants grown on the chestnut seed biochar substrate were significantly lower than those in plants treated with the other biochars, indicating slightly poorer photosynthetic apparatus condition (Table 4).

Table 4. Chlorophyll fluorescence parameters of broccoli Cezar and pepper Marta Polka plants, depending on the experimental combination

Experimental combination	F_0	F_v	F_m	F_v/F_m	F_v/F_0
broccoli					
Control (substrate without biochar)	133.60 ± 4.98 b	611.33 ± 24.51 a	744.93 ± 27.97 a	0.820 ± 0.005 a	4.577 ± 0.154 a
Substrate + pine sawdust biochar	139.40 ± 4.94 a	591.20 ± 15.49 b	730.60 ± 19.49 ab	0.808 ± 0.004 c	4.243 ± 0.100 c
Substrate + chestnut seed biochar	131.53 ± 4.47 bc	575.93 ± 14.53 c	707.47 ± 18.02 c	0.814 ± 0.004 b	4.380 ± 0.103 b
Substrate + pine bark biochar	130.80 ± 4.09 bc	576.73 ± 19.73 c	707.53 ± 22.15 c	0.815 ± 0.005 b	4.410 ± 0.143 b
Substrate + compost biochar	129.67 ± 5.11 c	592.53 ± 12.01 b	722.20 ± 14.71 bc	0.820 ± 0.006 a	4.577 ± 0.175 a
pepper					
Control (substrate without biochar)	196.80 ± 14.12 a	785.53 ± 56.34 b	982.33 ± 66.54 b	0.799 ± 0.010 ab	3.998 ± 0.229 ab
Substrate + pine sawdust biochar	200.93 ± 7.15 a	812.80 ± 44.83 ab	1013.73 ± 51.43 ab	0.801 ± 0.004 a	4.043 ± 0.109 a
Substrate + chestnut seed biochar	201.67 ± 7.77 a	785.73 ± 31.94 b	987.40 ± 38.06 ab	0.795 ± 0.005 b	3.897 ± 0.115 b
Substrate + pine bark biochar	200.00 ± 9.16 a	819.73 ± 37.25 a	1019.73 ± 45.68 a	0.803 ± 0.003 a	4.099 ± 0.088 a
Substrate + compost biochar	202.00 ± 5.83 a	812.73 ± 27.27 ab	1014.73 ± 31.20 ab	0.800 ± 0.004 a	4.024 ± 0.115 a

Means ± standard deviation marked with the same letter do not differ significantly at $\alpha = 0.05$ ($n = 10$), separately for each plant species. F_0 – minimal chlorophyll fluorescence, F_v – variable chlorophyll fluorescence, F_m – maximal chlorophyll fluorescence

Table 5. Relative pigment content and SPAD index in broccoli Cezar and pepper Marta Polka plants, depending on the experimental combination

Experimental combination	Flavonols	Anthocyanins	Chlorophyll	SPAD index
broccoli				
Control (substrate without biochar)	0.105 ± 0.056 a	0.025 ± 0.012 ab	14.83 ± 2.20 ab	39.17 ± 2.39 ab
Substrate + pine sawdust biochar	0.102 ± 0.043 a	0.027 ± 0.009 ab	14.95 ± 2.78 ab	39.23 ± 2.80 ab
Substrate + chestnut seed biochar	0.089 ± 0.018 a	0.026 ± 0.012 ab	14.16 ± 1.50 ab	38.52 ± 1.70 ab
Substrate + pine bark biochar	0.109 ± 0.053 a	0.028 ± 0.009 a	15.72 ± 2.84 a	40.06 ± 3.27 a
Substrate + compost biochar	0.111 ± 0.051 a	0.020 ± 0.010 b	13.55 ± 2.59 b	37.59 ± 2.61 b
pepper				
Control (substrate without biochar)	0.083 ± 0.053 a	0.017 ± 0.008 ab	7.32 ± 0.89 b	27.95 ± 1.94 b
Substrate + pine sawdust biochar	0.078 ± 0.051 a	0.014 ± 0.012 b	7.78 ± 0.91 b	28.91 ± 1.93 b
Substrate + chestnut seed biochar	0.089 ± 0.069 a	0.021 ± 0.009 a	8.63 ± 0.80 a	30.63 ± 1.54 a
Substrate + pine bark biochar	0.101 ± 0.054 a	0.013 ± 0.007 b	7.61 ± 1.18 b	28.49 ± 2.57 b
Substrate + compost biochar	0.075 ± 0.046 a	0.019 ± 0.013 ab	7.74 ± 0.94 b	28.88 ± 1.82 b

Means ± standard deviation marked with the same letter do not differ significantly at $\alpha = 0.05$ ($n = 10$), separately for each plant species.

Relative pigment content and SPAD index. In both plant species, adding biochar did not affect flavonol content. In broccoli, pine bark biochar application increased anthocyanin, chlorophyll, and SPAD index content to the greatest extent. Plants grown in the substrate with compost biochar had the lowest content of these pigments. Pine sawdust and chestnut seed biochars did not change these parameters compared to the control (Table 5).

In pepper, adding chestnut seed biochar resulted in higher anthocyanin content than pine sawdust and pine bark biochars, as well as the highest chlorophyll content (8.63) and SPAD index (30.63) among the other experimental combinations. Chestnut seed biochar-treated plants accumulated almost 18% more chlorophyll and had an SPAD index higher by almost 10% compared to the control seedlings, confirming better physiological condition and nutrition in chestnut seed biochar-amended plants (Table 5).

DISCUSSION

Plant biometric traits after biochar treatment

A review of scientific publications confirms that biochar modifies plant growth, but the magnitude and direction of the effect depend on the dosage, plant type, feedstock, and soil conditions [Xiang et al. 2017, He et al. 2020, Joseph et al. 2021]. Widowati et al. [2025] recommend using rice husk biochar at 4% for optimal plant growth in wild chili pepper (*Capsicum frutescens* L.) cultivation. Adding biochar adjusted soil pH and significantly increased organic carbon content, water retention capacity, and total N, P, and K content. The use of biochar increased the plant growth rate and leaf number, and improved photosynthesis. In our study with *C. annuum*, four different biochars were added to the substrate at 4.4% concentration, resulting in significant differences in the values of the seedling traits. Biochar from pine sawdust and chestnut seed did not affect the number of leaves compared with the control plants, whereas biochar from pine bark and compost reduced it (on average by 1.31). Furthermore, adding compost biochar shortened shoots compared to control plants (by an average of 1.75 cm), which in turn reduced shoot fresh weight by over 30%. No such relationship was observed in broccoli; none of the biochars used affected shoot growth (Table 3). Leaf area, length, and width were similar, but adding pine sawdust biochar increased their perimeter compared to the control (Figure 4). The results regarding the use of biochars in broccoli are inconclusive. Nayem et al. [2024] demonstrated that corn stover biochar improved broccoli plant growth parameters, including leaf length and width. However, this did not apply specifically to plant length, which is consistent with our study. In the study performed by Graber et al. [2010], pepper growth (leaf area, leaf weight, number of leaves, and yield) was stimulated by wood-derived biochar. In our research, pepper plants cultivated in a substrate amended with pine sawdust biochar also had the highest leaf area, perimeter, length, and width. Effects of biochar on germination and seedling growth range from inhibition to stimulation. Hormesis is commonly observed (high rates of biochar can inhibit, while low rates can stimulate plant growth). The most important factors determining whether biochar impacts seed germination and seedling growth are: (i) release of salts from biochar to the soil solution; (ii) release of phytotoxins; (iii) release of germination-inducing hormones; (iv) change in water capacity and porosity of the soil. Biochar feedstock, production method, and application amount have a range of impacts on germination speed and rate. The specific sensitivity of seeds from different plant species to salinity, toxins, hormone-like compounds, and water availability also leads to highly variable results [Joseph et al. 2021, Bednik et al. 2022].

The use of different biochars resulted in varying responses in pepper root system growth, with the best results after applying chestnut seed biochar (Figure 3). In broccoli, root system length, area, and volume showed no significant differences, and compost biochar reduced root diameter compared with the control. Xiang et al. [2017] conducted a meta-analysis with 2108 paired observations from 136 articles to evaluate root traits under biochar application. Overall, biochar application increased root biomass (+32%), root volume (+29%), and surface area (39%). The biochar-induced increases in root length (+52%) and number of root tips (+17%) were much larger than the increase in root diameter (+9.9%); this result suggests that biochar application benefits root morphological development to alleviate plant nutrient and water deficiency rather than to maximize biomass accumulation.

The physicochemical properties of biochar, such as pH, electrical conductivity, porous structure, and density, are strongly modified by biomass type and thermal parameters of the production process [Ahmad et al. 2021, Tomczyk et al. 2020]. The elemental and mineral composition of biochar depends directly on biomass type, and thermochemical conversion affects nutrient concentrations [Campos et al. 2020, Sayğan et al. 2025]. Biochars derived from biomass rich in organic components exhibit higher potassium and phosphorus contents [Uroić Štefanko and Leszczyńska 2020]. Typical biochar contains 45% to 60% carbon, 2–5% hydrogen, and 10–20% oxygen, although these proportions vary significantly depending on the feedstock [Fakhar et al. 2025]. Biochars derived from agricultural waste exhibit high concentrations of phosphorus (up to 134.6 mg·kg⁻¹) and calcium (up to 649.0 mg·kg⁻¹), as well as magnesium, sodium, and sulfur [Khater et al. 2024]. Much of the N within the biochar C matrix is unavailable to plants, whereas most K in biochar is present in soluble forms, released in the short term after application to soil, and is readily available to plants [Joseph et al. 2021]. In the study by Küçükyumuk et al. [2017], biochar produced from wood debris, combined with chemical fertilizers, increased soil pH, organic matter, N, P, K, Fe, and Mn content, and plant dry weight, while reducing the soil EC value, which suggests that biochar can be used with chemical fertilizers to increase yields and nutrient concentrations in pepper. Laurentino et al. [2025] found that adding biochar to pepper cultivation resulted in higher vitamin C and soluble solids content than mineral fertilization. In our study, pepper shoot dry weight increased significantly (by 37.23%) only after adding pine sawdust biochar, compared with plants grown without biochar (Table 3).

According to Lévesque et al. [2020], adding 5% maple bark and pine chip biochars improved plant water-use efficiency, increased the dry weight of tomato (*Solanum lycopersicum* L.) and pepper fruits, and increased N and P uptake efficiency. Majeed et al. [2022] showed that single-plant biochar derived from corn cobs was insufficient to provide adequate nutrients for optimal pepper growth. While biochar alone increases soil carbon sequestration, most biochars do not contain sufficient available plant nutrients, so the authors suggest the use of biochar in conjunction with mineral or organic fertilizers rich in available plant nutrients. Biochar application alone had no significant effect on most growth parameters, but combining 0.5% biochar with 2% poultry litter resulted in the highest plant height and stem diameter. According to Montoya et al. [2022], using enriched biochar in broccoli cultivation is advisable because it does not cause yield losses and increases nutrient content in inflorescences. In contrast, in our study, biochar use in broccoli cultivation did not cause nearly any changes in plant biometric or biochemical traits (Tables 3–5). In contrast, in pepper, the effect varied, confirming plants' genotypic sensitivity to specific biochar types, as reported by Liao et al. [2024]. This may be attributed to differences in species' tolerances to changing soil/substrate conditions. The results should also be related to the carbon and nitrogen content determined in the tested biochars (Table 1). Compost biochar generally did not increase the analyzed traits of broccoli and pepper plants. This biochar had the lowest total organic carbon content. The other biochars contained more than twice as much carbon. However, compost biochar had the highest total nitrogen content. The high nitrogen content of the biochars does not appear to be a key factor determining the growth of pepper and broccoli in this experiment. Pine sawdust and chestnut seed biochar had similar total carbon content, but chestnut seed biochar had thirteen times more nitrogen (Table 1). While pine sawdust, characterized by low pH (3.86) and EC (108.05 μS·cm⁻¹), stimulated more intensive shoot development in pepper seedlings, chestnut seed biochar with high pH (8.24) and EC (777.8 μS·cm⁻¹) stimulated more intensive development of the root system (Table 3, Figure 3). A large portion of the nutrients, including N, P, K, and Ca, in the feedstocks can be retained in the biochar's porous structure during pyrolysis. These nutrients promote soil fertility and plant growth. The elemental composition of biochar plays an important role in modulating plant growth [Guo et al. 2023]. The chemical analysis of the tested biochars showed that, compared to peat substrate, chestnut seed biochar was rich in K, Na, Cu and Zn. The high content of these elements in chestnut seed biochar may be associated with its alkaline pH and high EC value. Moreover, compared to the other types of biochar tested, chestnut seed biochar contained the highest levels of K, Ca, Cu, Zn, and Mg – elements that stimulate root system development. On the other hand, pine sawdust biochar had very low content of K, Ca, Na, Cu, Zn, Mg, and Fe, but it was characterized by a high Mn content. Manganese is an essential micronutrient for pepper plants, playing a major role in growth, enzyme functions, and stress responses [Silber et al. 2009, Yasuor et al. 2015].

Plant physiological parameters after biochar treatment

He et al. [2020] synthesized 74 publications to evaluate the overall tendency of plant photosynthesis and growth following biochar amendment. They found that biochar significantly increased photosynthetic rate by 27.1% and improved stomatal conductance, transpiration rate, water use efficiency, and chlorophyll concentration by 19.6%, 26.9%, 26.8%, and 16.1%, respectively. Corn stover biochar applied at 2–8 ton per ha improved chlorophyll content in broccoli [Nayem et al. 2024]. In our study, chlorophyll content in broccoli did not differ in treatments with biochar compared to the control. In contrast, pepper showed a higher chlorophyll content only in plants treated with chestnut biochar (Table 5). Chestnut seed biochar contained 53.25 mg·g⁻¹ Mg, 5–8 times higher than the other biochars tested, and half the content found in the peat substrate. Magnesium is a central building block of chlorophyll. Without it, plants cannot produce chlorophyll, capture sunlight, and carry out photosynthesis. Magnesium (Mg²⁺) is pivotal for the vitality, yield, and quality of horticultural crops. Central to plant physiology, Mg²⁺ stimulates growth and biomass accumulation [Ishfaq et al. 2022, Ahmed et al. 2023]. Under Mg deficiency, pepper plants show decreased relative growth rate, total dry weight, stem and root mass fractions, and leaf area, whereas the shoot-to-root ratio increased [Riga and Anza 2003].

The broccoli plants treated with pine sawdust, chestnut seeds, and pine bark biochars had slightly lower F_v/F_m and F_v/F_0 ratios. As for pepper, the lowest F_v/F_m and F_v/F_0 values were found for chestnut seed biochar (Table 4). The ratio of variable to maximum fluorescence (F_v/F_m) represents the maximum potential quantum efficiency of open photosystem II centers. Its average optimal value for most plant species is 0.79 to 0.83, with lowered values indicating plant stress [Maxwell and Johnson 2000]. The values obtained in our study fall within the reference range, confirming the absence of stress resulting from the application of the tested biochars. In contrast, F_v/F_0 is a more sensitive plant stress detector, normalized over the minimum fluorescence measurement. It defines the efficiency of the water-splitting reaction (photolysis) and the performance of the electron donor for photosystem II following dark adaptation of the plant. It highlights even minor disruptions in electron transport and damage to the oxygen-evolving complex, for example, by drought, salinity, or toxins. For healthy plants, this parameter typically falls within the range of 4–6 [Baker and Oxborough 2004, Banks 2018]. In our study, all F_v/F_0 values in all broccoli plants were above 4, whereas in pepper, only plants treated with chestnut seed biochar and the control group had F_v/F_0 values below 4. Therefore, biochar treatment did not induce stress in the studied plants.

CONCLUSION

In broccoli, using various biochars during seedling production had an ambiguous effect on plant cultivation. None of the tested biochar treatments significantly stimulated plant growth for horticultural production, allowing for shortening the seedling cultivation period or increasing the commercial quality of the plants. The use of pine sawdust, chestnut seeds, pine bark, and compost biochars at a concentration at 4.4% in peat substrate does not seem justified in the production of broccoli seedlings under greenhouse conditions. Perhaps higher concentrations of the tested biochars would have positive effects, which is worth testing in future studies.

Pepper plants grown in peat substrate supplemented with pine sawdust biochar showed improved shoot growth parameters. Chestnut seed biochar positively affected root system development. This biochar also stimulated the highest accumulation of anthocyanins and chlorophyll in leaves. Chlorophyll fluorescence parameters of biochar-treated pepper seedlings were statistically similar to those in the control. The use of biochars from pine sawdust and chestnut seeds may therefore be a beneficial modification in pepper seedling production. The observed stimulation of plant growth can shorten the seedling production period, thereby ensuring high-quality seedlings. The obtained results are worth implementing in horticultural production.

Overall, various biochar types affect shoot and root development differently. Plant genotype is an important factor in the mechanism of plant response to biochar treatment. Future research should focus on broader testing of different vegetable species and cultivars, as well as examining different combinations of

biochars at higher concentrations. It is important to bear in mind the relationship between the chemical composition of biochars and their impact on plants. An important research direction is also to determine the microbiological and biochemical activity of substrates supplemented with biochars.

DECLARATION OF THE USE OF GENERATIVE AI TOOLS IN THE PROCESS OF PREPARING THE PAPER

The authors did not use genAI tools during the research and manuscript preparation.

AUTHOR CONTRIBUTIONS

A.T. – developing a concept, methodology, conducting research, data curation, visualization, writing, review, supervision; D.K. – methodology, conducting research, data curation, review; P.W. – methodology, conducting research, data curation, review; A.K. – conducting research, visualization; A.M. – conducting research; M.A. – software, analysing the results, visualization, writing, review.

PATENTS

The elaborated method of pine sawdust biochar and chestnut seed biochar use in pepper seedlings cultivation was sent to the Patent Office of the Republic of Poland as a patent application P.455823 [WIPO ST 10/C PL455823]: “Method for producing pepper seedlings using biochar from pine sawdust or chestnut seeds” (Polish title: “Sposób produkcji rozsady papryki rocznej z wykorzystaniem biowęgla z trocin sosnowych lub kasztanów”); in Polish.

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