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Comparative investigation of powder and extract of biochar from *Broussonetia papyrifera* on the growth and eco-physiological attributes of *Vigna radiata*

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Abstract

This study compared the impact of biochar (in powder and extract forms) derived from the invasive tree *Broussonetia papyrifera* on the growth and eco-physiological responses of *Vigna radiata* (mung bean) under laboratory and experimental dome environments. The primary objective was to investigate the sustainable utilization potential of biochar derived from invasive plants. Powdered biochar was mixed into garden soil at amendment rates of 0.5%, 1%, 2%, and 4%, while for extract treatments, the seedlings were irrigated with extracts of these concentrations. The responses of plants were found to be dependent on the concentration and type of treatment (i.e., powder or extract) used. The highest levels of growth and eco-physiological responses were observed at a concentration of 1% for biochar extract and 2% for powdered biochar. In addition, the impacts were more pronounced in the roots than in the shoots. The biochar amendment resulted in a 7–73% increase in root length and a 12–148% increase in plant dry biomass when compared to the control. Crop growth, water use efficiency, and leaf area were greater in powdered biochar, but net photosynthesis (Pn), transpiration rate, and stomatal conductance were higher in plants treated with biochar extract. Adding powdered biochar to soil increases its pH, electrical conductivity (EC), moisture content, soil organic C, and amounts of available N, P, and K; however, the effects of applying biochar extracts were less pronounced. Crop growth and eco-physiological responses were found to be positively correlated, regardless of the biochar form used. Following biochar extract treatment, EC was found to be negatively correlated with Pn. The study revealed that powdered biochar had superior growth responses and soil improvement compared to biochar extract at higher concentrations. However, biochar extract also had comparable effects and can be beneficial in short-term cropping systems such as urban farming (e.g., in kitchen gardens and vegetable production) at lower concentrations (up to 2%). The findings of the study provide a baseline for future evaluations regarding the sustainable application of biochar liquor (water extract) as a source of nutrients and the powdered residual biochar as a potential material for adsorbing environmental contaminants or improving soil quality.

Highlights

• Growth of mung bean was highest when treated with 1% biochar extract and 2% powdered biochar.

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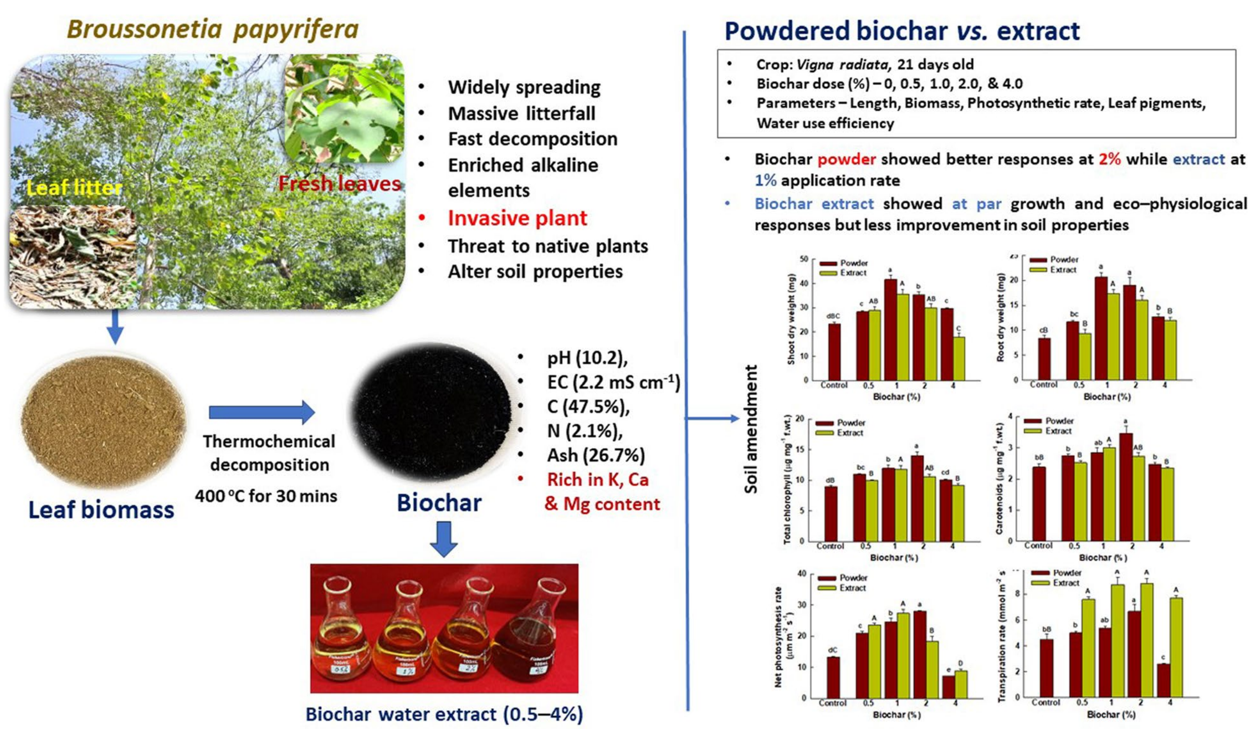
- Use of powdered biochar resulted in better crop growth performance.
- Plants treated with biochar extract had a higher rate of net photosynthesis, transpiration, and stomatal conductance.
- Powdered biochar improved soil physico-chemical properties more effectively than extracts.
- Biochar extract can serve as a prompt nutrient source in urban agriculture systems.

Keywords Carbonaceous materials, Eco-physiological attributes, Growth performance, Invasive plants, Legumes, Soil nutrients

Graphical Abstract



1 Introduction

Biochar is a carbonaceous material produced by pyrolysis of lignocellulosic biomass under oxygen-limited conditions (Jeyasubramanian et al. 2021; Li et al. 2023). It can be produced from a variety of feedstock materials, such as wood, agricultural residues, leftover food, flower residues, cow dung, municipal sewage wastes, etc. (Chen et al. 2018; Singh et al. 2018; Yadav and Singh 2023). The properties of biochar, including pH, surface area, elemental composition, and cation exchange capacity (CEC), are highly dependent on the type of feedstock and pyrolysis conditions (Liu et al. 2015; Ghodake et al. 2021; Gezahegn et al. 2024). For example, biochar produced at moderate pyrolysis temperatures (~400–500°C) is characterized by improved surface area and pore spaces, alkaline pH, higher nutrient content, CEC, etc. (Gupta et al. 2022; Gezahegn et al. 2024). Due to its numerous advantages, the utilization of biochar to improve soil health and crop productivity is widely advocated (Chen et al. 2022; Ibrahim et al. 2023; Zhang et al. 2024). The addition of biochar improves the physico-chemical and biological properties of soils (Ghosh and Maiti 2021; Liu et al. 2023). Biochar possesses a porous structure with functional groups on its surface, which enable it to effectively absorb and retain nutrients (Xiao et al. 2018; Singh et al. 2019a). In a recent study, Kumar et al. (2023a) tested several biochars derived from different waste feedstock materials at the application rates of 0, 2.5%, 5%, and 7.5%, and found an 86% increase in water holding capacity and 31–62% improvement in soil physico-chemical properties. In addition, 71% and 209% in length and 61% and 117% in biomass of Bengal gram and coriander crops, respectively, was reported (Kumar et al. 2023a). Moreover, various other studies have also reported that biochar improves eco-physiological traits of crops such as net photosynthesis, transpiration rate, stomatal conductance, etc. (Zhu et al. 2020; Gao et al. 2021; Wang et al. 2021; Hasnain et al. 2023).

Among various feedstock materials, converting invasive weeds into biochar has been considered as a potential mechanism for their sustainable management (Liao et al. 2013; Weidlich et al. 2020; Li et al. 2023; Gezahegn et al. 2024). For example, the addition of biochar derived from *Eupatorium adenophorum* Spreng. to weathered Nepalese soil improved soil fertility and *Zea mays* L. growth by increasing available nitrogen (N), phosphorous (P) and potassium (K) contents, soil pH, and soil moisture retention (Pandit et al. 2018). Choudhary et al. (2023) suggested that application of biochar derived from *Parthenium hysterophorus* L. and *Lantana camara* along with inorganic fertilizers improves the quality of fodder, height, and tiller numbers of *Avena sativa* L., along with soil microbial diversity and enzyme activities. Similarly, Ghosh and Maiti (2021) reported improved soil enzymatic activities and productivity of mine spoil soil receiving invasive plant-derived biochar up to 3% dose. In another pot study, the effects of biochar derived from *Bidens pilosa* L., *Wedelia trilobata* (L.) Hitchc., and *Mikania micrantha* Kunth at 1% and 3% doses were proven to be better than lime for improving the soil pH, availability of N, P and K contents, and biological properties under acid-rainfed conditions. In comparison to the high alkalinity induced by liming, biochar amendment was shown to maintain a near neutral pH in acidic soil (Li et al. 2023). However, the impacts of biochar derived from invasive weeds must be thoroughly evaluated at different scales before wider application in the field.

In addition to using biochar as powder (solid), the role of biochar water extract (a water-soluble pool of nutrients) in improving the growth of crops is also currently receiving increased attention (Liu et al. 2021; Gao et al. 2023). Biochar extract has been shown to have a dominating pool of nutrients that improves the growth of crop plants, compared to the washed solid biochar (Liu et al. 2021). In a hydroponic study, Gao et al. (2023) found that biochar-extracted liquor improved the physiological traits of rice seedlings and also upregulated the N metabolism-related genes. Rafique et al. (2022) conducted a Petri dish experiment and found that jujube wood waste biochar improved seed germination and seedling growth of *Triticum aestivum* L. Based on a pot study, Aon et al. (2023) reported that addition of wheat straw biochar in soil enhanced the biomass and grain yield of *Z. mays*, and improved soil properties via increased nutrient retention. Recently, Liao et al. (2023) conducted a global meta-analysis that highlighted the advantages of incorporating biochar into green infrastructure. Urban agriculture systems can potentially utilize biochar in either its solid (powdered) form for soil amendment or as a water-extracted liquor for crop irrigation (Song et al. 2020). This can be a cost-effective strategy, especially for soilless agricultural systems (Gao et al. 2023); however, further investigations are required at different scales.

Broussonetia papyrifera (L.) ĹHér. ex Vent. (Paper mulberry; Moraceae), a subtropical deciduous tree, has been regarded as an invasive plant in India (Maan et al. 2021). The excess litter biomass generated by this tree could alter soil characteristics and also exhibit allelopathic interference with the understorey vegetation (Maan et al. 2021). To address this, innovative applications (such as biochar) of the leaf litter generated by *B. papyrifera* can be extremely beneficial. However, no studies are available on the effect of *B. papyrifera* leaf litter-derived biochar (hereafter Bp-biochar) on the crops. Legumes, the second most cultivated crops globally following cereals (Šerá et al. 2021), have a high nutritive value owing to the presence of abundant proteins, carbohydrates, and dietary fibres, and low levels of cholesterol and fats (Maphosa and Jideani 2017). Despite being resilient, growing aridity and deteriorating soil health in response to climate change scenarios pose a significant challenge to their sustainable cultivation (Kumar et al. 2023b). Therefore, investigating processes (such as biochar amendment) to improve the growth of legumes can be a viable strategy for promoting agricultural sustainability. However, there is no study comparing the benefits of using biochar (powder and/or extract) for legume crop growth and soil properties. In this study, we chose mung bean (*Vigna radiata* (L.) R. Wilczek), because it is the most consumed pulse crop in South Asian countries, particularly India, and is known to provide nutritional security (Mehandi et al. 2019; Kumar et al. 2023b). With the above background, the study hypothesized that the application of Bp-biochar would enhance the growth attributes of mung bean by regulating its eco-physiological responses and improving soil properties. Therefore, the aims of the study were twofold: (i) to investigate the differential impacts of Bp-biochar (in powder form and as an extract) on the initial growth and eco-physiological responses of mung bean, and (ii) to assess the changes in soil physico-chemical properties following the application of Bp-biochar treatments. To the best of our knowledge, the study is the first of its kind to compare the effects of invasive tree-derived biochar powder and extract on the eco-physiological responses of a major legume plant. Such studies examining the benefits of biochar derived from invasive plants can be helpful in their sustainable management.

2 Materials and methods

2.1 Biochar preparation and characterization

The fallen leaves of *B. papyrifera* were collected at P.N. Mehra Botanical Garden, Panjab University, Chandigarh, India, and dried in shade. The biomass was dried in an oven and ground into powder to reduce volume and achieve homogenization, as the properties of biochar are dependent on the size of feedstock material (He et al. 2018). The pulverized biomass was subsequently pyrolyzed in a muffle furnace at 400°C for 30 min (ramp rate ~ 20°C min^{-1}) under oxygen-limited conditions. The pH and electrical conductivity (EC) of the feedstock and biochar were determined using digital pH (EcoScan pH 700; Eutech, Singapore) and EC (Con 700; Eutech) meters. The surface morphology, structure, and nature of biomass and biochar were characterized using scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS). The functional groups and crystalline phases of biochar were identified using a Fourier-transformed infrared (FT-IR) spectrophotometer and an X-ray powder diffractometer (XRD), respectively. The carbon, hydrogen, nitrogen, oxygen, and ash contents of the feedstock material and Bp-biochar were determined via elemental analysis.

2.2 Plant materials

Seeds of mung bean (var. ML-5) were bought from a local store in Chandigarh, India. Prior to sowing, the seeds were surface sterilized with sodium hypochlorite (0.1%) and washed five times with distilled water.

2.3 Laboratory experiment

A Petri plate experiment was performed to assess the preliminary effects of Bp-biochar (powder *vs.* extract) on mung bean growth. A stock of 4% biochar extract was prepared by shaking 20 g of powdered biochar in a conical flask filled with 500 mL of distilled water at room temperature for an hour. The stock solution was diluted by adding distilled water to achieve concentrations of 0.5%, 1%, 2%, and 4%. Petri plates (ø = 15 cm) were disinfected with 100% ethanol and lined with a thin cotton layer on the bottom. A sheet of Whatman filter paper #1 was placed on top of the cotton layer. For biochar powder treatment, filter paper was moistened with 10 mL of distilled water and different amounts of biochar (0, 0.5, 1.0, 2.0, and 4.0 g) were uniformly distributed on the surface of filter paper. The used amounts (0 to 4 g per Petri plate) of biochar powder corresponds to biochar application rates of 0, 5, 10, 20, and 40 t ha^{-1} (for a soil depth of 10 cm) in the field (Solaiman et al. 2012). For biochar extract treatment, filter paper was moistened with 10 ml of varying concentrations (0, 0.5%, 1%, 2%, and 4%) of biochar water extract. Subsequently, 25 mung bean seeds were placed on the surface of filter paper and allowed to germinate for 10 days. Overall, there were nine treatment combinations: four for powder and water extract, and one for control. A total of 27 Petri plates were used, with three plates per treatment. The experiment was conducted at an ambient temperature of 25 ± 2°C, a relative humidity of 80 ± 2%, and a photoperiod of 16/8 h, with a photosynthetic photon flux density of approximately 450 μmol photons m^{-2} s^{-1}. After 10 days, root length, shoot length, and seedling biomass were recorded for each treatment combination.

2.4 Pot experiment

2.4.1 Experimental setup

The growth attributes of mung bean were assessed in an experimental dome using a pot assay to determine the impact of various treatments of powdered and extracted Bp-biochar. In all, there were nine treatments, four each of biochar extracts and biochar powder added to the soil, and a parallel control group with no biochar powder or extract added to the soil. The initial characteristics of the garden soil used for the pot assay were as follows: pH = 7.25 ± 0.02; EC = 203.67 ± 1.76 μS cm^{-1}; soil organic carbon (SOC) = 1.19 ± 0.05%; available nitrogen (N) = 112.90 ± 1.25 kg ha^{-1}; available phosphorus (P) = 40.70 ± 0.47 kg ha^{-1}; and available potassium (K) = 151.42 ± 0.30 kg ha^{-1}.

For the powder treatment, the biochar (at 0.5%, 1%, 2%, and 4%, *w/w* of soil) was thoroughly mixed with garden soil and then filled in polypropylene pots (ø = 10 cm). As described earlier these treatments corresponded to biochar application rates of approximately 5-, 10-, 20-, and 40-ton ha^{-1}, respectively, at a depth of 10 cm in field settings (Solaiman et al. 2012). Throughout the experiment, distilled water was used to irrigate pots treated with biochar powder. For Bp-biochar extract treatments, the pots were irrigated with 40 mL of the respective extract concentrations (0.5%, 1%, 2%, and 4%) on the first day. Then, 10 mL of extract was added on alternate day until a total of 100 mL of extract was added. Afterwards, 10 mL of distilled water was added to the pots on the remaining days. Three pots were maintained for each treatment. A total of 27 pots were used in the pot assay, with 9 treatments and 3 replicates per treatment. On the first day, five mung bean seeds were sown in each pot. The experiment lasted for 21 days under natural conditions at 25 ± 2°C, 70 ± 5% relative humidity, and a 16/8 h day/night photoperiod.

2.4.2 Growth parameters

The effect of Bp-biochar was determined by measuring the growth of 21-day-old mung bean plants. The shoot and root length were measured using a centimeter scale, and their fresh weight was determined using a digital weighing balance (ME104; Mettler Toledo). To determine the dry weight, plant samples were dried in an oven at 60°C for 72 h and then weighed.

2.4.3 Leaf parameters

The area of mung bean leaves was measured using a Leaf area meter (CI-202, CID BioScience, USA). Dimethyl sulfoxide was used to extract chlorophyll and carotenoids from leaves at 60ºC for an hour. The absorbance was measured at 470, 645, and 663 nm, and the amount of total chlorophyll (TC) and carotenoids in leaf samples was calculated using the well-established formulae (Batish et al. 2006). The leaves were dried in an oven for 72 h at 60°C, and the dry weight of the leaves was determined using a digital weighing balance. The foliar water mass was calculated by subtracting the dry weight of the leaves from the fresh weight (Huang et al. 2019).

2.4.4 Eco-physiological parameters

The eco-physiological characteristics such as net photosynthesis rate (Pn, µmol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}), leaf stomatal conductance (C, mmol m^{-2} s^{-1}), and vapor pressure deficit (VPD, kPa) of mung bean were measured to observe the impacts of Bp-biochar using a portable photosynthesis system (CI-340; CID Bio-Science, USA). The data were collected under the following conditions: atmospheric pressure of 96.3 kPa, mass flow rate of 0.9 mol m^{-2} s^{-1}, and photosynthetically active radiation (PAR) of approximately 700 µmol m^{-2} s^{-1}. The air and leaf temperatures during the analysis were around 35 and 36ºC, respectively. The relative humidity at the inlet and outflow was 64 and 75%, respectively. The water use efficiency (WUE) of plants was determined by dividing the value of Pn by E and expressed in µmol $mmol^{-1}$.

2.4.5 Soil analysis

The post-harvest soil was analyzed for physico-chemical characteristics such as pH and EC using digital pH and EC meters (described earlier), soil moisture content using the gravimetric (weight loss) method, and available N, P, K, and SOC contents using standard methods elaborated by Tandon (1993).

2.5 Statistical analysis

The data were examined for normality prior to inferential statistical analysis. A one-way analysis of variance (ANOVA) was used to examine the impact of different doses of Bp-biochar powder and extract (separately) on soil properties and plant responses. The significant differences among the treatments (control with biochar powder and biochar extract, separately) were determined by post hoc Tukey's test at the 95% level of significance ($p \leq 0.05$) using the SPSS package (version 16). Pearson's correlation analysis was performed and represented using the "*ggcorrplot*" package, while principal component analysis (PCA) was conducted using the "*stats*" and "*factoextra*" packages in the R program (version 4.2.2; R Core Team 2022). The graphs were prepared using Sigma Plot (version 11.0) and OriginLab 2022.

3 Results and discussion

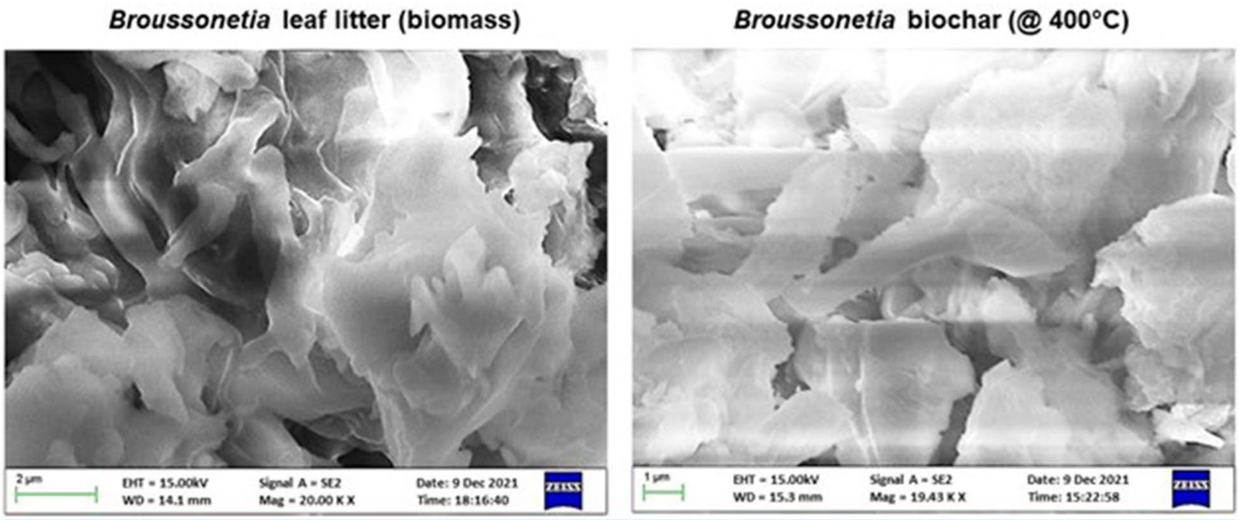
3.1 Biochar characterization

The biochar produced at 400°C exhibited a moderate alkaline nature, with a pH value of 10.17, and an EC of 2.22 mS cm^{-1} (Table 1). The pyrolysis of biomass led to an increase in pH, whereas a reduction in EC was found in relation to the initial feedstock material. In contrast to biomass, biochar exhibited an increase in C, N and ash content levels, while a decline in H and O contents was observed (Table 1). These results are consistent with previous research that have described the characteristics of biochar produced through the pyrolysis of biomass (Mohan et al. 2018; Gezahegn et al. 2024). The observed pH increase in the biochar may be attributed to its higher ash content enriched with various alkaline earth metals like K, Mg, and Ca, as revealed by EDS analysis (Fig. 1). Earlier studies reported that the pyrolysis of biomass is associated with the formation of basic oxides and carbonates of Ca and Mg, which may lead to an increase in pH, whereas the decrease in EC may be attributed to the stabilization of elements on the surface of biochar (Mohan et al. 2018; Singh et al. 2019a; Gezahegn et al. 2024). Crop residues or leaf litter predominantly consist of cellulosic and hemicellulosic constituents, which undergo a more rapid decomposition process in comparison to the woody components that are rich in lignin (Singh et al. 2015). Pyrolysis of biomass at 400°C or higher can cause the hemicellulose and cellulose to degrade more quickly and

Table 1 Properties of *Broussonetia* leaf litter biomass and biochar prepared at 400°C

Parameters	pH	EC (mS cm^{-1})	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Oxygen (%)	Ash content (%)
Biomass	8.31	4.00	37.16	5.17	1.32	36.51	19.84
Biochar	10.17	2.22	47.45	3.97	2.12	19.77	26.69

EC Electrical conductivity



Material	Elements (weight%) based on Energy Dispersive Spectroscopy							
	Aluminium	Carbon	Calcium	Potassium	Magnesium	Oxygen	Phosphorus	Silicon
Biomass	2.52	34.68	4.93	2.14	0.79	48.68	0.74	5.53
Biochar	1.88	--	14.81	3.44	1.81	54.98	--	23.39

Fig. 1 Scanning electron micrograph (SEM) and energy dispersive spectroscopy (EDS) of *Broussonetia* leaf litter biomass and biochar derived at 400°C

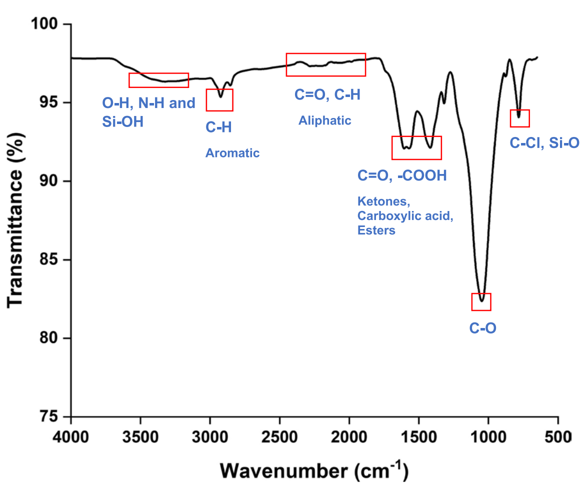


Fig. 2 Fourier transform infrared (FT-IR) spectroscopy of biochar prepared from *Broussonetia* leaf litter (Bp-biochar) at 400°C

releases volatile gases, leading to the rough (amorphous) surface of biochar, as observed in this study (Fig. 1). The amorphous structure of biochar increases its surface area and pore spaces, which may aid in nutrient retention and ion stabilization (Singh et al. 2018). The FT-IR spectrum (Fig. 2) revealed absorption bands primarily at 3526 nm, 2924 nm, 2828 nm, ~ 2300 nm, 1607 nm, 1418 nm, 1048 nm and 783 nm, which indicated the presence of hydroxides, phenolic-OH and amine groups; C-H bonds of aromatic compounds; C-H and C = O groups of aliphatic compounds; ketones, carboxylic acid and esters groups; and C–Cl or Si–O groups, respectively (Singh et al. 2018; Gezahegn et al. 2024). These observations correspond to the breakdown of cellulosic components and the synthesis of aliphatic and aromatic carbonaceous compounds during the pyrolysis process (Singh et al. 2015). The XRD analysis revealed the existence of sharp peaks (2θ) at 26° and 45° which correspond to the specific crystallographic planes of silica and calcite phases, respectively (Mohan et al. 2018; Fig. 3). The EDS results corroborate with the observations reported in XRD spectrum. Overall, the results of FT-IR, XRD, and EDS analyses support that the Bp-biochar used in the present study is alkaline in nature due to the presence of various compounds of alkaline earth elements.

3.2 Growth response of mung bean to Bp-biochar powder and extract: a laboratory assay

Root and shoot length increased significantly ($P \leq 0.001$) with increasing biochar concentrations up to 1% (Table 2 and Table S1). The impacts of biochar powder and extract amendment were more pronounced in the roots than in the shoots. Seedling dry biomass increased by

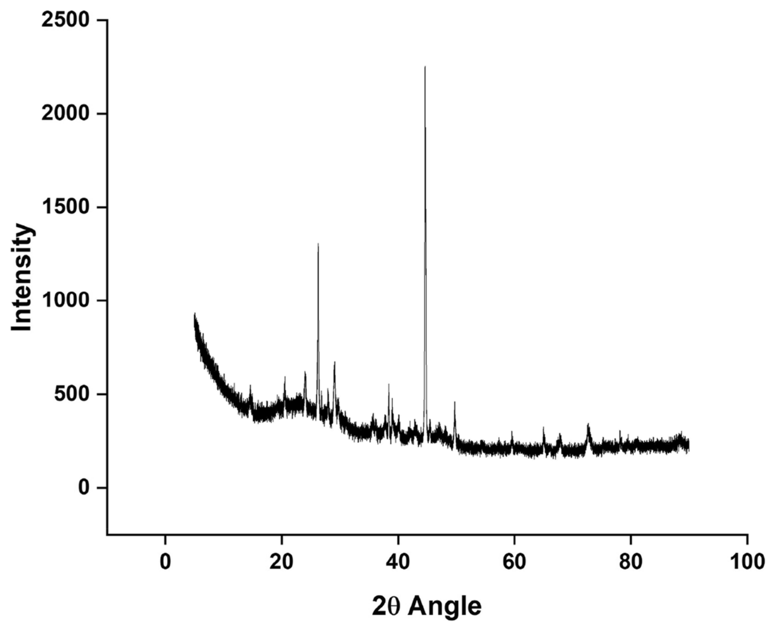


Fig. 3 X-ray diffraction (XRD) spectroscopy of *Broussonetia* leaf litter biochar (Bp-biochar) derived at 400°C

Table 2 Effect of biochar powder and extract on length and biomass of 10-day-old mung bean seedlings grown in Petri plates. Different small alphabets represent the significant (at $p \leq 0.001$) variations among different concentrations of biochar treatment. Values in parenthesis represent the percent increase (+) or decrease (−) at different biochar concentrations with respect to the control

Biochar (%)	Shoot length (cm)		Root length (cm)		Seedling biomass (mg)	
	Powder	Extract	Powder	Extract	Powder	Extract
Control	14.7 ± 0.21c	14.7 ± 0.21d	12.0 ± 0.03b	12.0 ± 0.03b	10.7 ± 0.67d	10.7 ± 0.67d
0.5	17.2 ± 0.29b (+ 17.0%)	16.8 ± 0.52c (+ 14.5%)	13.0 ± 0.56b (+ 8.9%)	12.5 ± 0.27b (+ 4.2%)	17.0 ± 0.58c (+ 59.4%)	14.7 ± 0.67c (+ 37.5%)
1	18.8 ± 0.12a (+ 27.9%)	22.5 ± 0.56a (+ 53.1%)	15.6 ± 0.17a (+ 30.1%)	16.0 ± 0.54a (+ 34.0%)	19.7 ± 0.89bc (+ 84.4%)	22.3 ± 0.67a (+ 109.4%)
2	15.7 ± 0.35c (+ 6.6%)	19.4 ± 0.22b (+ 32.2%)	13.0 ± 0.33b (+ 8.9%)	15.5 ± 0.21a (+ 29.5%)	23.3 ± 0.33a (+ 118.8%)	19.3 ± 0.33b (+ 81.3%)
4	13.3 ± 0.29d (−9.5%)	18.4 ± 0.47bc (+ 25.2%)	9.6 ± 0.22c (−20.1%)	11.6 ± 0.29b (−3.06%)	20.7 ± 0.67ab (+ 93.8%)	14.3 ± 0.33c (+ 34.4%)

109% and 118% ($p < 0.001$) at 1% and 2% concentrations of extract and powdered biochar, respectively. Similar observations were made by Solaiman et al. (2012) and Nazir and Batool (2020), who observed that as biochar concentrations increased, the root and shoot fresh and dry biomass increased. Nevertheless, the growth responses began to decline as biochar concentration increased from 1% or 2%, but they were still greater than the control after 10 days (Table 2). The uniform moist conditions and controlled release of nutrients in the powdered biochar treatments may have aided the growth of seedlings up to 2% concentration (Solaiman et al. 2012). Furthermore, because biochar had a pH of 10.17 (Table 1), it is likely that the high alkalinity of the medium is exerting a significant influence on treatments with biochar concentrations exceeding 2%. Therefore, the application of Bp-biochar at a concentration of up to 2% may help to improve the structural development of crop plants by increasing their resistance to harsh conditions. Based on the laboratory assay results, the study was extended to experimental dome conditions to investigate the impact of Bp-biochar in natural systems.

3.3 Growth performance of mung bean in Bp-biochar amended soil: pot assay

With different amounts of biochar in both forms, there was a significant ($p \leq 0.01$) variation in the growth characteristics of mung bean (Table S2; Fig. S1). With the exception of shoot length, mung bean growth responses were significantly higher when powdered biochar was used compared to the extract (Fig. 4). The application of extract and powdered biochar at concentrations ranging from 1% to 2% resulted in greater root growth (64–73%) compared to shoot growth (12–17%) (Fig. 4a, b). However, growth was observed to decrease significantly at a concentration of 4%. Similar results were observed in another pot study, where root and shoot length of *Trigonella foenum-graecum* L. and *Cicer arietinum* L. increased significantly when grown in soil amended with biochar powder (Ahmad et al. 2022). The increase in growth resulting from biochar amendment may be attributed to an enhanced nutrient intake from biochar (Gao et al. 2023; Kumar et al. 2023a). This is supported by the higher concentrations of Mg, Ca, K, and N as indicated by the EDS analysis (Fig. 1; Table 1). Similar to length, 1–2% biochar application resulted in a 40–148% increase in the fresh and dry weight of roots compared to 21–52% increase in shoots (Fig. 4c–f). Similar findings were reported by Videgain-Marco et al. (2020) in a greenhouse

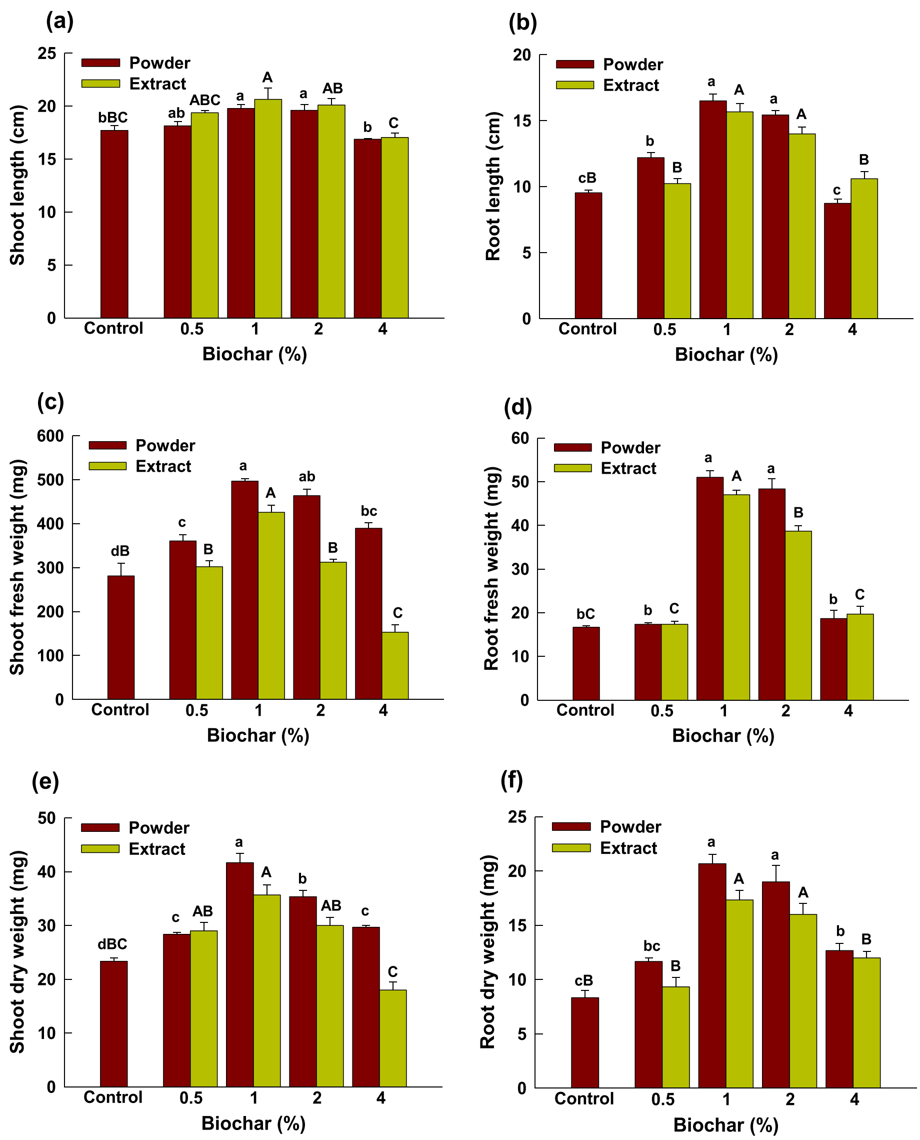


Fig. 4 Variation in primary growth parameters: (**a**) shoot length, (**b**) root length, (**c**) fresh shoot biomass, (**d**) fresh root biomass, (**e**) dry shoot biomass, and (**f**) dry root biomass of mung bean seedlings after 21 days of exposure to different concentrations of Bp-biochar (powder and extract). Different small and capital alphabets represent the significant variations among different concentrations of biochar powder and extract treatments, respectively, according to Tukey's post hoc test at $p \leq 0.05$

study based on exploring the effects of biochar prepared from *Vitis vinifera* L. on the soil–plant system. Biochar produced from *V. vinifera* at 400°C improved the dry biomass of *Sorghum bicolor* L. Moench roots by 52% over control and increased the contents of K, Ca, and Mg in the soil (Videgain-Marco et al. 2020). In another study conducted to explore the impact of biochar amendment on the growth properties of two rice cultivars, Kamara et al. (2015) observed a considerable increase in shoot dry weight of both rice cultivars after biochar amendment compared to the control. However, an increasing dose of biochar has been reported to decrease crop growth by Ibrahim et al. (2023). Therefore, Bp-biochar applied in this study showed an overall positive response to the growth of mung bean up to 2% concentration.

3.4 Eco-physiological responses of mung bean are improved in Bp-biochar amended soils

The growth and adaptability of legumes can be predicted by monitoring their eco-physiological characteristics (Baligar et al. 2020). Many studies have demonstrated that biochar positively affects eco-physiological parameters in plants and contributes to the enhancement of crop growth (Nigam et al. 2019; Gao et al. 2021; Wang et al. 2021; Hasnain et al. 2023). In this study, we observed a significant ($p \leq 0.01$) increase in several eco-physiological parameters (except stomatal conductance) with biochar up to a concentration of 2%, followed by a decline (Figs. 5 and 6; Table S3). Total chlorophyll and carotenoids (Fig. 5a–b), as well as leaf area (Fig. 6f) increased upon application of 1% and 2% of powdered biochar and extract application, respectively. However, leaf dry weight and foliar water content (Fig. 5c–d) were increased up to 2% concentrations of biochar application in both forms. According to Huang et al. (2019), the area of leaf cells expands in response to a greater input of biomass, which in turn increases the active surface area available for photosynthesis. It was confirmed by an increase in leaf dry weight and foliar water mass, which are crucial factors influencing photosynthetic carbon assimilation (Huang et al. 2019). Nevertheless, a significant decline (even lower than the control) was observed at 4% concentration, suggesting the need for careful and thoughtful use of Bp-biochar for promoting crop growth.

In a meta-analysis, Gao et al. (2021) found that the application of biochar at the suggested rates of ~ 2–4%

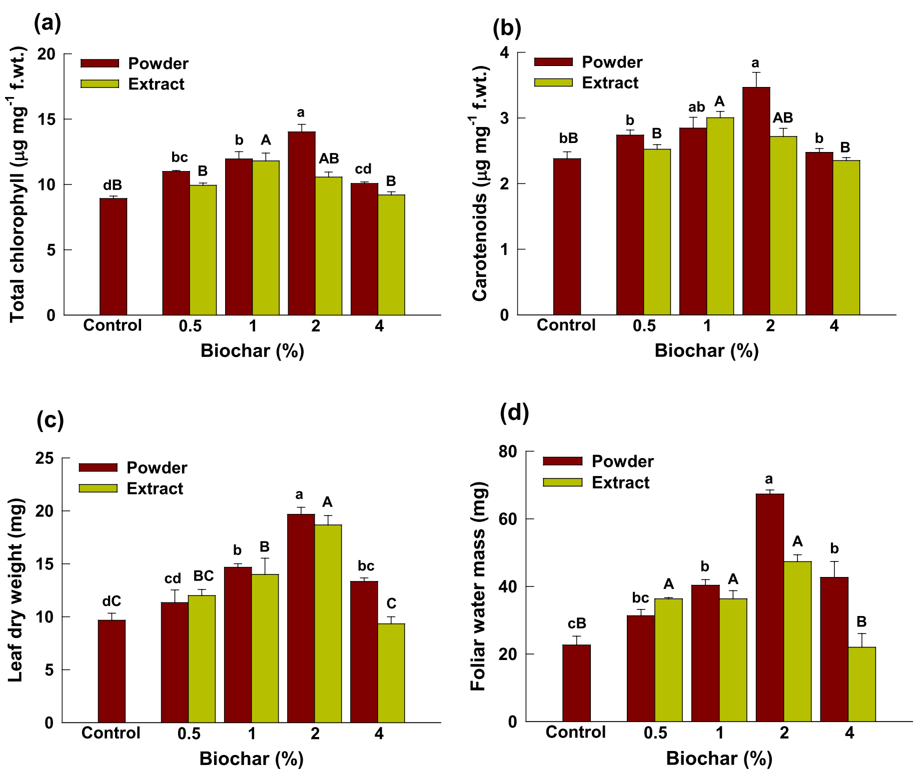


Fig. 5 Variation in leaf parameters: (**a**) total chlorophyll content (**b**) carotenoid content (**c**) leaf dry weight, and (**d**) foliar water mass of mung bean seedlings after 21 days of exposure to different concentrations of Bp-biochar (powder and extract). Different small and capital alphabets represent the significant variations among different concentrations of biochar powder and extract treatments, respectively, according to Tukey's post hoc test at $p \leq 0.05$

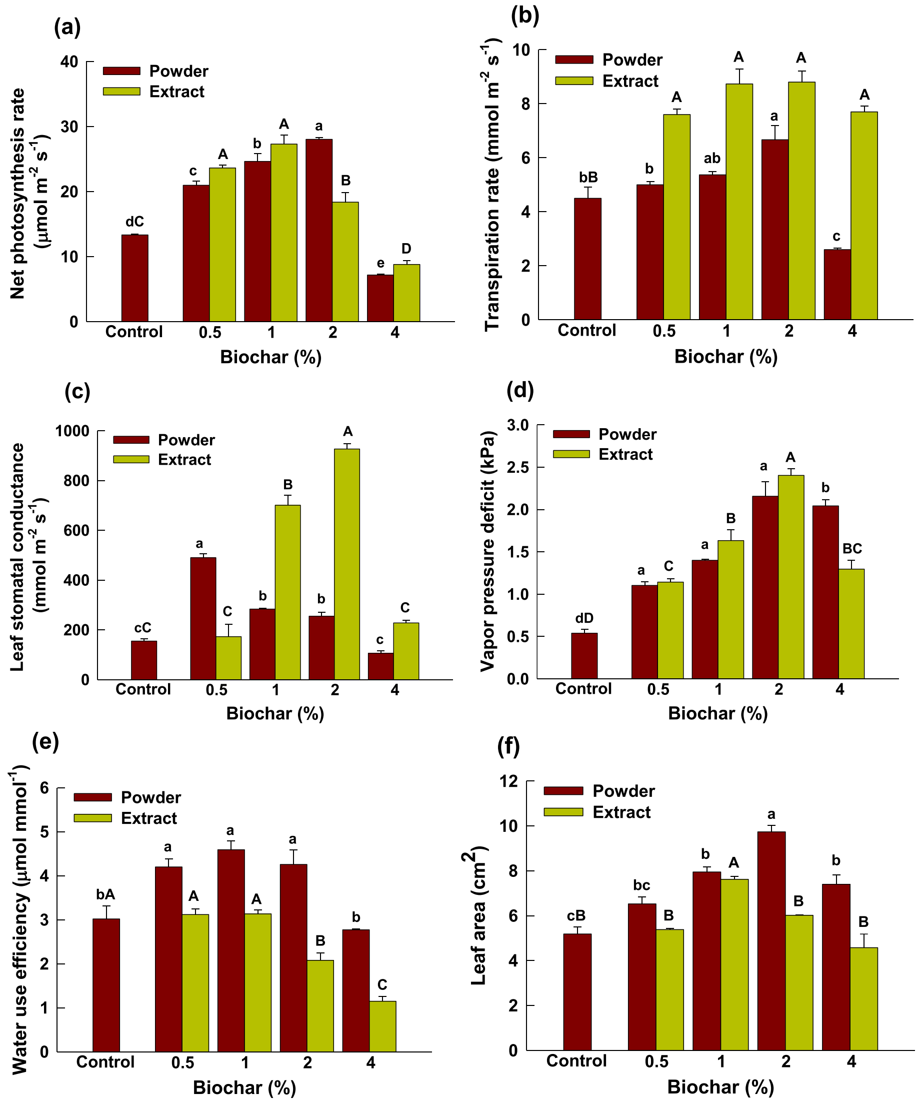


Fig. 6 Variation in eco–physiological attributes: (a) net photosynthetic rate, (b) transpiration rate, (c) stomatal conductance, (d) vapor pressure deficit, (e) water use efficiency, and (f) leaf area of mung bean seedlings after 21 days of exposure to different concentrations of *Broussonetia* biochar. Different small and capital alphabets represent the significant variations among different concentrations of biochar powder and extract, respectively, according to Tukey's post hoc test at $p \leq 0.05$

results in an average increase of 23% in the photosynthetic rate. In the present study, we observed an increase in the net photosynthetic rate of mung bean by over 38% when biochar powder and extract concentrations were applied at a rate of ≤ 2% (Fig. 6a). The transpiration rate was observed to be approximately two times higher in extract-applied treatments compared to powdered biochar application (Fig. 6b). The stomatal conductance exhibited remarkably high increase of 352% and 498% at 1% and 2% extract concentrations, respectively (Fig. 6c). In contrast, an initial increase in stomatal conductance was observed upon application of 0.5% powdered biochar but it decreased sharply as the biochar concentration increased (Fig. 6c). Likewise, the extract-applied treatment exhibited a greater vapour pressure deficit than the powdered biochar (Fig. 6d). High vapor pressure deficit implies that the air surrounding the leaf is dry, increasing the atmospheric water demand, and consequently, the transpiration rate of the leaf increases (Massmann et al. 2019). This was corroborated by our results, which indicated that the extract-applied treatments exhibited a greater transpiration rate in the presence of a high vapour pressure deficit. However, contrasting results were observed with the application of biochar powder. Plants may have adopted this strategy in an attempt to decrease the evapotranspiration resulting from a high vapour pressure deficit by closing their stomata and lowering their conductance (Massmann et al. 2019; Singh et al. 2020). Furthermore, the relatively greater foliar water mass observed in seedlings growing in biochar powder may contribute to the enhancement of WUE and the reduction of water demand, i.e., transpiration loss, under such treatments. In order to observe the efficacy of photosynthesis in terms of water loss, instantaneous WUE was also calculated. Both forms of biochar application showed a concentration-dependent increase in WUE ($p \leq 0.01$); however, the powdered biochar treatment was more effective (Fig. 6e).

In general, the eco-physiological responses of mung bean seedlings treated with extract were comparable to those observed with powdered biochar (Figs. 5 and 6). With the exception of photosynthetic rate (Pn), which declined at > 1% extract concentration, transpiration rate, stomatal conductance, and vapor pressure deficit were significantly higher under extract applied treatments up to 2% (Fig. 6). This might be because roots absorb nutrients in the form of biochar extract more quickly than nutrients released by powdered biochar in the soil matrix. Consequently, using extracts might increase stomatal conductance and transpiration rate, facilitating the rapid transportation of nutrients to the leaves (Singh et al. 2020). This suggests that biochar extract has high potential for utilization in soil-less hydroponic agriculture or irrigating urban crops.

3.5 Biochar powder and water extract improve the soil properties

The rhizospheric soil serves as the principal medium for plant nutrition and mineral absorption, and crop growth is directly impacted by the availability of nutrients to the plants (He et al. 2022). Biochar significantly influences the physico-chemical properties and nutrient composition of soil through the improvement of microclimatic conditions and mineralisation activities (Singh et al. 2019a; Amoah-Antwi et al. 2020). The majority of research on biochar has utilized powdered biochar for studying its effects on plant growth. Further, in comparison with the washed biochar residue, the water-soluble pool of biochar, i.e., its extract, has been shown to be a dominant source that improves the growth of crop plants (Liu et al. 2021). In this 21-day short-term study, we found that applying biochar in both powder and extract form significantly ($p \leq 0.01$) increased the levels of physico-chemical properties like pH, EC, moisture content, and available nutrient concentrations (Table 3 and Table S4). An increase in soil pH and EC was observed with increasing amounts of both forms of biochar. Likewise, soil moisture content increased by up to 200% over the control with the powdered biochar. At 4% powdered biochar concentration, there was a significant ($p \leq 0.01$) increase in soil available N (46.7%), P (281.5%), K (157.3%), and SOC (55.8%) contents compared to the control (Table 3). However, the increase in nutrient concentrations was marginal in extract treatments. These observations agree with the previous studies reporting the impacts of biochar on soil properties (Singh et al. 2019a, b; He et al. 2022). In general,

Table 3 Effect of different concentrations of biochar powder and extract on soil physico-chemical properties and nutrient concentrations. The data is presented in the form of the mean ± SE of three replicates tested. Different small and capital alphabets in one column represent significant differences among different powdered biochar and extract treatments, respectively, according to Tukey's post hoc test at $p \leq 0.05$

Treatment	pH	Electrical conductivity (μS cm^{-1})	Moisture (%)	Available K (Kg ha^{-1})	Available N (Kg ha^{-1})	Available P (Kg ha^{-1})	Soil organic carbon (%)
Control	7.48 ± 0.01dC	206.00 ± 2.65eE	28.25 ± 1.67 dB	157.70 ± 0.34dD	115.40 ± 0.72dC	41.69 ± 0.77dC	1.27 ± 0.03 dB
0.5% Powder	7.75 ± 0.02c	479.00 ± 1.73d	37.66 ± 1.68c	172.78 ± 3.01 cd	122.93 ± 2.51d	53.31 ± 1.15c	1.64 ± 0.02c
1% Powder	7.96 ± 0.03b	498.33 ± 3.67c	42.88 ± 1.18c	193.58 ± 8.17c	137.98 ± 0.72c	56.35 ± 0.78c	1.77 ± 0.05bc
2% Powder	8.03 ± 0.02b	530.0 ± 2.08b	53.12 ± 2.05b	221.31 ± 2.20b	153.04 ± 2.17b	104.01 ± 1.98b	1.80 ± 0.04ab
4% Powder	8.14 ± 0.01a	665.67 ± 1.67a	64.88 ± 1.79a	405.18 ± 6.60a	169.34 ± 2.51a	159.03 ± 1.33a	1.97 ± 0.07a
0.5% Extract	7.87 ± 0.01B	242.33 ± 2.96D	34.53 ± 0.60AB	165.91 ± 1.37D	119.17 ± 2.61C	44.51 ± 0.31B	1.31 ± 0.03AB
1% Extract	7.89 ± 0.02B	219.33 ± 1.67C	35.75 ± 0.62A	178 ± 1.29C	123.77 ± 1.11BC	45.47 ± 0.36AB	1.35 ± 0.01AB
2% Extract	7.90 ± 0.01B	334.67 ± 2.03B	37.00 ± 1.08A	194.88 ± 3.09B	131.29 ± 3.42AB	45.71 ± 0.36AB	1.40 ± 0.04AB
4% Extract	7.96 ± 0.01A	402.67 ± 2.73A	37.71 ± 2.57A	207.50 ± 2.22A	136.73 ± 1.45A	47.47 ± 0.87A	1.45 ± 0.06A

the addition of biochar powder significantly improved the physico-chemical properties of the soil in comparison to the application of biochar extract (Table 3). The surface characteristics of biochar, including its larger surface area, pore spaces, and presence of exchangeable cations, have a significant impact on enhancing the physicochemical attributes of soil when powdered biochar is applied (Mohan et al. 2018; Singh et al. 2019a). The powdered biochar is rich in nutrients and effectively retains these elements within its matrix for gradual release. Biochar has the ability to attract and retain nutrients from the soil matrix, making them available to crops for an extended period of time (He et al. 2022). This could result in an initial shortage of nutrient concentration for the plants when high doses of biochar are applied alone (Singh et al. 2020). On the other hand, in the case of soil irrigation with biochar extract, plants can only utilize the extracted amount of nutrients at a time, compared to the slow-release of nutrients from powdered biochar for a longer duration. Therefore, in this study, after a 21-day treatment period, the soil amended with powdered biochar exhibited significantly greater amounts of available nutrients compared to the soil treated with extract.

3.6 Interrelationships between mung bean growth parameters and soil properties: mechanism of action under biochar powder and extract application

Due to its stable biochemical structure, resistance to breakdown, and ability to increase the organic carbon pool in soil, biochar has the potential to improve soil fertility and nutrient composition, and thus, enhance crop yields (Rehman et al. 2021). It was observed that plants grown in biochar powder performed better at concentrations up to 2% than plants that were irrigated with biochar extract, where the effect peaked at 1%. This may be explained by the fact that a significant portion of nutrients are extracted during the preparation of extract; therefore, irrigating with such solutions provides plants with a first-hand, direct source of nutrients that supports the early growth of seedlings (Liu et al. 2021). In contrast, biochar powder allows a gradual release of nutrients in accordance with the needs of the plant or mineralisation rate, resulting in optimal plant growth at a concentration of 2%. This assumption is supported by a strong positive correlation between photosynthetic rate and growth parameters (Fig. 7a, b). Nevertheless, a strong negative correlation was found between the photosynthetic rate and EC under extract-applied treatment (Fig. 7b). This

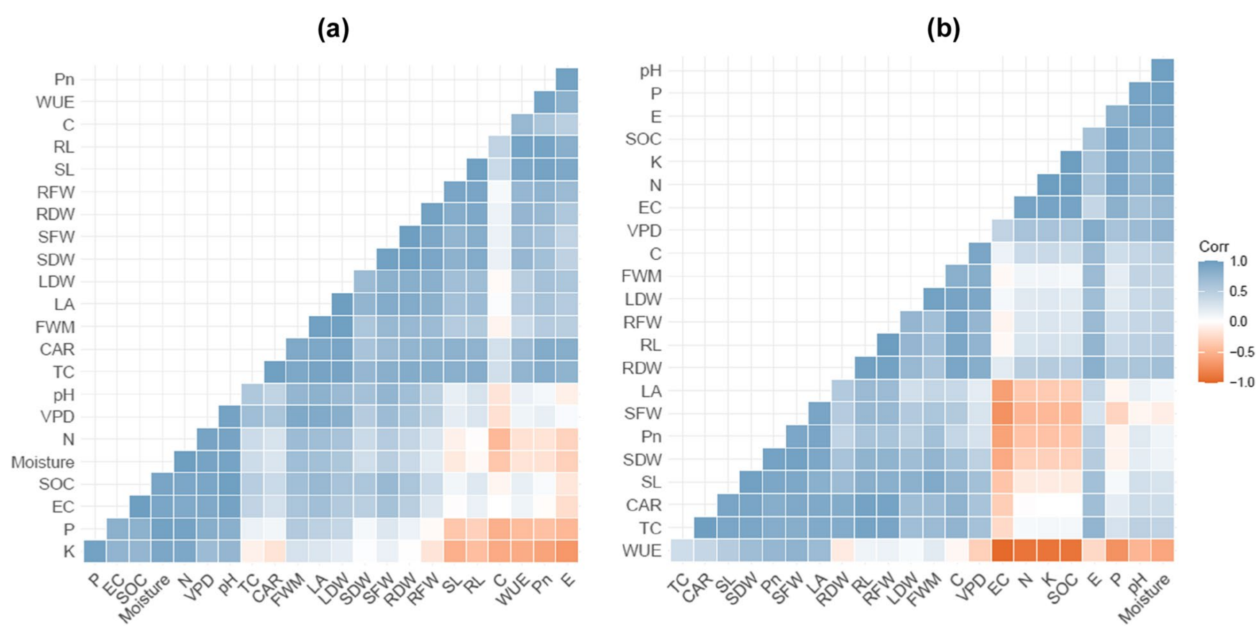


Fig. 7 Correlation heatmap of (**a**) powdered biochar and (**b**) extract treatment of various plant (growth and eco-physiological) attributes and post-harvest soil parameters of mung bean. *Abbreviations: C – Stomatal conductance; CAR – Carotenoid content; E – Transpiration rate; EC – Soil electrical conductivity; FWM – Foliar water mass; K – Soil available potassium;, LA – Leaf area; LDW – Leaf dry weight; N – Soil available nitrogen; P – Soil available phosphorous; pH – Soil pH; Pn – Net photosynthesis rate; RDW – Root dry weight; RFW – Root fresh weight; RL – Root length; SDW – Shoot dry weight; SFW – Shoot fresh weight; SL– Shoot length; SOC – Soil organic carbon; TC – Total chlorophyll content; VPD – Vapour pressure deficit; WUE – Water use efficiency*

may be the result of increased alkaline stress conditions on the plants at greater concentrations of biochar extract, which lowers the photosynthetic rate and, consequently, the growth responses. Further, a negative relationship between soil nutrients and growth parameters revealed that crops responded positively upto some concentration, and after that, either nutrient saturation or adsorption of nutrients on the biochar surface may hinder crop growth (Singh et al. 2019a). Previously, Gale and Thomas (2019) demonstrated that the dose-dependent effects of biochar on the growth and eco-physiological characteristics of two plants, *Abutilon theophrasti* Medik. and *Trifolium repens* L., are primarily caused by changes in nutrient availability. In this context, we propose that altering the aforementioned soil characteristics can enhance plant growth, photosynthesis, and other eco-physiological parameters.

Principal component analysis (PCA) showed the arrangement of different plant growth parameters and soil variables according to their concentration (Fig. 8). The majority of crop growth and eco-physiological parameters were found to be aligned towards PC axis-1, while soil properties were aligned with PC axis-2. As regards the powdered biochar, the majority of the parameters investigated were aligned towards a concentration of 2% (Fig. 8a), whereas for extract treatment, the majority of the parameters were aligned with concentrations ranging from 1% to 2% (Fig. 8b). In both cases, PC axes 1 and 2 together accounted for over 75% of the variability in the observed parameters (Fig. 8a, b). Nevertheless, it is noteworthy that, despite the reduced availability of soil nutrients, a significant increase in plant growth was observed in response to biochar extract treatment. This suggests that the extract ensures immediate nutrient supply to the crop plants, making biochar extract suitable for short-term urban cropping systems. In general, extract offers nutritional roles that may help plants grow; whereas, powdered biochar gives adaptation benefits to plants, in addition to retaining its surface characteristics. For example, a strong correlation was found between the rate of photosynthesis and the moisture content of the soil when powdered biochar was applied. The surface characteristics of biochar, such as its large surface area, porosity, and cation exchange capacity, are advantageous for adsorption (Gope et al. 2022), wastewater treatment (Qambrani et al. 2017), and crop growth in abiotically stressed soils (Mansoor et al. 2021). Overall, the findings of this study will contribute to the control of the invasive tree and expand our understanding about the benefits of biochar extract on crop health. The assessment of eco-physiological characteristics will serve as a definitive indicator of the impacts of biochar in future studies, and a decision can be made regarding whether to use powdered or extracted biochar based on the specific needs of the study. Nevertheless, it is necessary to conduct long-term studies including several plant species under both treatment regimes to establish a definitive conclusion.

4 Conclusions

Based on the findings of the study, we can conclude that applying biochar as an extract enhances crop growth and eco-physiological responses comparable to those observed with powdered biochar. Nevertheless,

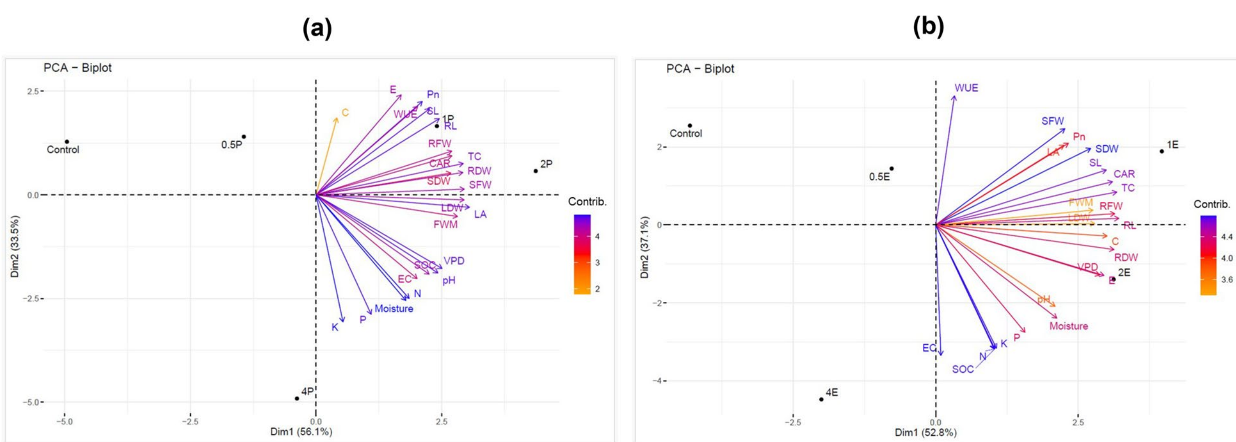


Fig. 8 Principal component analysis (PCA) biplot of (a) powdered biochar and (b) extract treatment of various plant (growth and eco-physiological) attributes and post-harvest soil parameters of mung bean. *Abbreviations: C – Stomatal conductance; CAR – Carotenoid content; E – Transpiration rate; EC – Soil electrical conductivity; FWM – Foliar water mass; K – Soil available potassium;, LA – Leaf area; LDW – Leaf dry weight; N – Soil available nitrogen; P – Soil available phosphorous; pH – Soil pH; Pn – Net photosynthesis rate; RDW – Root dry weight; RFW – Root fresh weight; RL – Root length; SDW – Shoot dry weight; SFW – Shoot fresh weight; SL – Shoot length; SOC – Soil organic carbon; TC – Total chlorophyll content; VPD – Vapour pressure deficit; WUE – Water use efficiency*

the enhancement of soil properties is relatively more pronounced when biochar is applied in powdered form. While powdered biochar is advantageous for long-term growth enhancement and soil improvement, the application of biochar extract may be beneficial for short-term growth improvement due to immediate supply of available nutrients. The results indicate that application of biochar extract derived from invasive plants, at concentrations as low as 1% to 2%, may be effectively used for crop cultivation, while the residual biochar powder can be used for adsorption purposes. However, further comprehensive research is required, taking into account various crop types, application rates and soil conditions under different agroclimatic regimes. Further, the utility of *B. papyrifera* for biochar preparation may also serve as an economically-viable method for its management from the urban and peri-urban areas where it is spreading rapidly.

Abbreviations

ANOVA	Analysis of variance
C	Leaf stomatal conductance
CAR	Carotenoid content
E	Transpiration rate
EC	Electrical conductivity
EDS	Energy dispersive spectroscopy
FWM	Foliar water mass
K	Available potassium
LA	Leaf area
LDW	Leaf dry weight
N	Available nitrogen
P	Available phosphorus
PCA	Principal component analysis
Pn	Net photosynthesis rate
RDW	Root dry weight
RFW	Root fresh weight
RL	Root length
SDW	Shoot dry weight
SEM	Scanning electron microscopy
SFW	Shoot fresh weight
SL	Shoot length
SOC	Soil organic carbon
TC	Total chlorophyll content
VPD	Vapour pressure deficit
WUE	Water use efficiency

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s44246-024-00112-5.

Additional file 1: Table S1. One-way analysis of variance (ANOVA) depicting differences in different plant growth parameters between biochar concentrations at 10 days after commencement of the laboratory experiment. **Table S2.** One-way ANOVA depicting differences in different plant growth parameters between biochar concentrations at 21 days after commencement of the dome experiment. **Table S3.** One-way ANOVA depicting differences in different plant eco-physiological parameters between biochar concentrations at 21 days after commencement of the dome experiment. **Table S4**. One-way ANOVA depicting differences in different post-harvest soil parameters between biochar concentrations at 21 days after commencement of the dome experiment. **Fig. S1**. Photograph showing the effect of biochar (a) powder and (b) extract on the growth of mung bean seedlings at different concentrations.

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Ipsa Gupta conducted the experiment, data collection and analysis, and prepared the initial draft and the finalized manuscript; Rishikesh Singh participated in preparing the draft, conducting analysis, and reviewing and finalizing the manuscript; Anupama Kaushik conducted analysis and contributed to the review and finalization of the article; Harminder Pal Singh designed the study and participated in reviewing and finalizing the article; Daizy R. Batish contributed to the conception and design of the study, and reviewing and finalizing the manuscript.

Statements and declarations

The authors declare that the data presented in this manuscript is their own work. The data would be made available on request to the corresponding author.

Authors' contributions

IG: Performed the experiment, Data Collection and analysis, Initial drafting and finalization; RS: Drafting, Analysis, Review and finalization; AK: Analysis, Review and finalization; HPS: Designing, Review and finalization; DRB: Conception, Designing, Review and finalization.

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Availability of data and materials

Relevant data has been presented in the manuscript. The raw data can be made available on request to the corresponding author.

Declarations

Competing interests

The authors declare that there is no conflict of interest. All the authors have seen the final version of the manuscript and agreed to its submission to the journal.

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