Biochar Mediated and Plant Growth Enhancement Through Antioxidant Improvement in Mentha arvensis

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Abstract

The potential of biochar has increased interest in recent decades due to the increasing agricultural yield and nutritional value of products. However, scarce data have been published about the impact of biochar on the nutritional quality of products. Therefore, in this study, we assess the effects of biochar on plant health and productivity with different percentages (%) of the biochar e.g., 0%, 30%, 50%, and 70%. The gas chromatography (GC) analysis of M. arvensis treated with different concentrations of biochar showed different arrays of compounds, especially 33 compounds found in 30% biochar with an abundant source of 2-Propyl-5-acetoxy-5-methyltetrahydropyran, p-Menthan-3-one, 1,2-epoxy, and 2-Hydroxyhexadecyl butanoate. Similarly, the physicochemical characteristics of the plants showed that 30% of biochar application significantly ($p > 0.05$) improved the plant growth attributes e.g., shoot and root length, and fresh and dry biomass. The electrical conductivity (EC), pH, phosphorous, nitrogen, and organic compounds were also significantly ($p > 0.05$) improved with the addition of biochar in the soil and compared to only soil. The nutrient profiling showed that the biochar treatment significantly improved Ca, Mg, Na, and Ni compared to the soil. Antioxidant enzymes (CAT, GST, POX, and SOD) in plant and antioxidant activity of plant extracts such as DPPH, superoxide, Hydroxyl radical scavenging, TPC, and FTC were significantly improved by 30% biochar compared to control as well as 50% and 70% of biochar. The overall conclusion showed that 30% biochar application in the soil improved the valuable compounds in the soil and enhanced nutrients and antioxidant activities in M. arvensis, which improved the growth and development of plants.

Introduction

Biochar is a carbon-rich material derived from biomass pyrolysis under low oxygen conditions. Biochar has been widely used as a soil amendment to enhance soil physical, chemical, and biological properties, such as water-holding capacity, nutrient retention, cation exchange capacity, microbial activity, and diversity. Biochar can also improve plant growth and productivity by increasing water availability, reducing water loss, enhancing nutrient uptake, regulating stomatal conductance, protecting membrane stability, and modulating antioxidant defense and hormonal balance.

Recently, biochar has gained attention due to its significant effects on the biotic and abiotic stress and enhance the phytochemical constituents in plants. Recent research on the impact of biochar on broccoli growth showed that biochar can significantly increase broccoli's nutritional and phytochemical content[1]. Biochar is a versatile material that can be tailored to specific soil and crop needs by varying the feedstock type, pyrolysis temperature, and post-treatment methods. For example, biochar made from woody materials at high temperatures (>500°C) has a higher surface area and porosity, increasing soil water retention and reducing evaporation. Biochar made from manure or sewage sludge has higher nutrient content and availability, which can enhance plant nutrition and growth. Biochar can also be modified by adding minerals, organic acids, or microorganisms to improve its physicochemical properties and biological functions.

The effect of biochar may be that pure biochar does not directly enrich the soil with nutrients. Instead, raising the C/N ratio threatens nitrogen instability, and the soil must be fertilized further to produce beneficial agricultural conditions. Concerning the rising cost of synthetic fertilizers and declining prospects regarding a limited amount of phosphorus and other essential additives, a potential combination of compost and biochar has been proven recently [1]. Surface area, tunable pore, pore size, and dispersion are crucial aspects of biochar because they influence nutrient retention, charcoal adsorption ability, and microbial population activity [2]. A large area is linked to a few other biochar sites, such as cation exchange capacity and water-holding capacity; therefore, in modern agriculture, the number of biochar applications is the first requirement [3]. Volatile compounds are produced as gases during pyrolysis, which can increase the surface area of biochar and create an incredibly porous honeycomb structure.

In recent years, biochar and a wide range of potentially valuable applications have been the subject of extensive research worldwide. In line with the definition stated by the International Biochar Initiative, biochar is a refined carbonization product characterized by high carbon content and low risk of degradation, achieved through the incorporation of biomass pyrolysis and decaying waste. The role of biochar in reducing water stress and the effect of quality (unsaturated fatty acids, proteins, fats, and phytochemicals), yield, and physiological factors (proline, antioxidant enzyme, and chlorophyll content, as well as stomatal extraction) did not occur fully specified in arid and desert areas [4]. Growing plants in soil that is rich in organic matter, with the added qualities of biochar, can result in having larger root mass, better resistance to pests and disease, greater drought tolerance, and more vigorous overall health and growth as well as nutritional value of plant products. Therefore, In this study, we investigated the effects of biochar application on the growth and physiology of *M. arvensis*. Further we analysised the impact of biochar on soil, plant nutrient uptake, antioxidant enzymes and antioxidant activity.

Materials and Methods

4.1 Biochar and Soil preparation

In order to make biochar, a hole was dug, filled with dry plant biomass, and ignited from the top with a fire lighter. The previous layer of biomass was kept ignited at an optimal temperature of 300˚C and new biomass was added to it periodically, making sure that the combustion process did not reach a point where white ash was formed. When the smoke turned gray, it indicated that the biomass had been transformed into biochar. The fire was smothered to stop the burning process. The biochar was cooled overnight, crushed into smaller pieces, and stored in a sealed container away from sunlight and moisture.

At the time of *M. arvensis* harvest, an auger was used to collect soil samples from 0 to 30 cm deep for investigation of the chemical parameters of the soil. Soil samples were air-dried before being put through a 2 mm sieve. EC (dS m^{-1}) was measured in soil paste extract using an EC-meter (Genway, UK), pH was examined using a pH-meter in a suspension of 1:2.5 soil to distilled water (Genway, UK). The

total organic matter was measured by $K_2Cr_2O_7$ volumetric technique with external heating. The quantity of accessible nitrogen was ascertained using the alkaline hydrolysis diffusion method, the amount of accessible potassium was ascertained using NH_4OAC extraction-flame photometry, and the amount of accessible phosphorus was ascertained using the $NaHCO_3$ extraction-Mo-Sb colorimetry method [5]

4.3 Soil minerals analysis

Soil minerals analysis was followed the X-ray diffraction method described by [6].

4.3 Pot Experiment for M. arvensis Growth

The test soil was homogenized and sieved to pass a 20-mesh sieve in preparation for *M. arvensis* pot culture. A 28 cm diameter and 35 cm tall plastic pot was filled with 5.0 kg of soil. Once more, the treatment consisted of control soil and soil filled with 30%, 50%, and 70% of biochar in each pot. The exact weight of the test fertilizers (0.46 g N per pot) was measured out and blended with the soil before being added to each fertilized plant. Plant seeds were inserted into the pot's soil at a depth of 2 cm. One plant per pot was left after seven days of germination, and it was allowed to grow for 90 days, from 2022-03-24 to 2022-06-25 and ach treatment was carried out three times. Throughout the **9**0-day growth period of *M. arvensis*, all 60 pots were moistened to field capacity (the most significant amount of water the soil can store), balanced with tap water every five days. Throughout the whole growing period, *M. arvensis* growth was maintained with 16 hours per day of additional light and a daytime temperature range of 20 to 30°C.

4.4 Antioxdant enzymes actvity

The antioxdant enzymes (CAT, GST, POX, SOD) was carried out according to the method described by [51].

4.5 Extraction of plant extracts

Ten grams from each medicinal plant’s powder was extracted with 200 mL of 80% methanol for 1 h in an ultrasonic bath (Transsonic-700, 37 kHz, Germany) at 50 C. The MPPE was centrifuged at 3,000 rpm for 10 min; the supernatant was obtained, and the residues were discarded. The solvent in the supernatant was evaporated using an R-200-Buchi-rotary evaporator (Switzerland), and the concentrated extracts were obtained, the methanol-free extract was lyophilized and retained in water when used.

4.5 Antioxidant activity of plant extracts

The 2,2-diphenyl-1-picrylhydrazyl (DPPH), superoxide, and hydroxyl radical scavenging activity of *M. arvensis* extracts was utilized to evaluate the antioxidant status using method [7]. The reaction mixture (0.1 mL phenolic extract + 2.9 mL DPPH in methanol) was made in a volume of 3.0 mL and incubated for 20 hours at room temperature (about 27 °C) with stirring. A UV/vis spectrophotometer was used to quantify the decrease in absorbance at 515 nm compared to methanol as a blank. For superoxide, 4.5 ml

of Tri-HCl buffer solution, 0.5 mL blue-tetrazolium chloride, and 0.5 mL NADH were mixed with 0.1 mL of sample extracts and measured at 560 nm. In the hydroxyl scavenging assay, 1 mL FeSO4, 1 mL H2O2, and 0.5 mL methanol-salicylic acid were mixed with 0.1 mL sample extracts and recorded at 520 nm. The proportion of free radical sequestration serves as a quantitative measure of the extracts' capacity to scavenge: DPPH: % DPPH = (absorbance of the blank − absorbance of the sample) /absorbance of blank) × 100. The same formula was used for superoxide and hydroxyl radical inhibition activity. The results are also expressed as mg trolox equivalent per g of freeze-dried sample (mg TE g−1 FW) using a Trolox standard curve (R2 ≥ 0.937).

4.6 TPC and TFC content of plant extracts

An altered Folin-Ciocalteu method was used to measure the TPC [7]. In a water bath shielded from light, the phenolic extract was combined with diluted Folin-Ciocalteu reagent (1:10 in water; 100 L) and 7.5 percent sodium carbonate (80 L). The combination was then incubated at 45 °C for 15 minutes. Using a gallic acid standard curve and an absorbance measurement at 750 nm, the results are represented as mg of gallic acid equivalent per g of freeze-dried sample (mg GAEg1 DW). A similar AlCl3 colorimetric method was followed for TFC, as described by [29], using quercetin as a standard.

4.7 Gas Chromatography (GC/MS) Analysis

To separate and distinguish the pyrolyzates, the analytical pyrolyzer was connected to a gas chromatograph and mass spectrometer from Thermo Fisher (Scientific Trace GC Ultra and Thermo ISQ MS). The plant sample mass, approximately 500 g, was loaded into a sample cup and then placed into a pyrolysis tube preheated to a fixed temperature of 750 °C. To separate and distinguish the pyrolyzates, the analytical pyrolyzer was connected to a gas chromatograph and mass spectrometer from Thermo Fisher (Scientific Trace GC Ultra and Thermo ISQ MS). The plant sample, approximately 500 g, was loaded into a sample cup and then placed into a pyrolysis tube preheated to a fixed temperature of 750 °C. By using helium (99.99 mol percent) as the carrier gas and a restrictor capillary tube with an open split interface (split ratio 1:50) at 250 C, the produced pyrolyzates were transported straight to the GC. The GC column was a Restek RTX-1701 with the following specifications: L = 60 m, di = 0.25 mm, and df = 0.25 m. The GC oven's temperature program was as follows: (i) heat at a rate of 5 degrees Celsius per minute to 280 degrees, followed by (ii) maintaining 280 degrees for one minute. After GC separation, the pyrolyzes were led to a single quadrupole MS with electron ionization. The ion source temperature was kept at 230 C while the MS transfer line temperature remained at 280 C. The mass-to-charge ratio (m/z) scan mode was between 29 and 300, the electron ionization energy was 70 eV, and the acquisition rate was 5 spectra per second.

4.8 Statistical analyses

The measured variables were analyzed using ANOVA with the treatment as an independent variable. The post-hoc Tukey test was used to calculate significant differences between treatment means. All statistical analyses were performed using IBP SPSS 23.0. Principal component analysis (PCA) was

performed to visualize the distribution of variables. Further, the partial least square discriminative analysis (PLS-DA) was performed. The variable importance in projection (VIP) score statistically evaluated the contribution of individual bioactive markers.

Results

2.1 Effect of Biochar on Soil Characterization

In this study soil different concentrations biochar mixed with soil was analyzed. The results showed that the pH of soil containing 0% biochar was slightly alkaline, pH=7.6±0.29. However, the pH gradually increased with the successive increase of biochar concentration. The pH of 30% biochar concentration was 8.6±0.08. Moreover, 70% biochar pH was 9.05±0.09, and 50% biochar pH was 9.11±0.09. The significance difference was observed in control group containing 0% biochar was more alkaline pH then the group containing 70% biochar (Table 1).

The addition biochar increased the bulk density and particle density of the soil as shown in Table 1. The bulk density of soil containing 30% biochar was higher than soil containing 0% biochar. Similarly, particle density of soil containing 30% biochar was higher than soil containing 0% biochar. These results showed that the bulk density and particle density of the soil was increased by the addition of biochar.

The porosity and ash content of soil was increased by the addition of biochar. The porosity and ash content of soil was significantly increased ($p < 0.05$) by addition biochar shown in Table 1. The porosity of soil containing 30% biochar was 72.45±5.34 and soil containing 0% biochar was 48.67±2.42. Similarly, Ash content of soil containing 30% biochar was 6.91±0.13 and soil containing 0% biochar was 6.7±0.09. This result show that as the addition of biochar increased the porosity and ash content of soil was increased.

The observed electrical conductivity(EC) of the biochar varied depending on the type and concentration of biochar. The results showed that the EC of soil (% BC) was 1.33±0.05, while EC of soil containing 30% biochar was 0.16 ± 0.02. Moreover, when the biochar was increased to 50%, the EC decreased to 0.26±0.01 and then further reduced to 0.31±0.02 with treated 70% biochar. Similarly, addition of biochar enhances the organic matter, leading to soil fertility. The organic matter of soil was 0.102 ± 0.001, while the organic matter of soil containing 30% biochar was 1.38 ± 0.01. This result showed that addition of biochar increases the organic matter of the soil shown in Table 1.

Table 1: Effects of biochar on physiochemical properties of soil.

Parameter	0% BC	30% BC	50% BC	70% BC
Bulk density (kg m^{-3})	0.28±0.05^{d}	0.31±0.05^{c}	0.51±0.04^{b}	0.72±0.05^{a}
Particle density (kg m^{-3})	1.27±0.03^{d}	1.31±0.04^{c}	1.42±0.02^{b}	1.69±0.03^{a}
Porosity (%)	48.67±2.42^{d}	72.45±5.34^{c}	96.83±7.21^{b}	122.32±10.31^{a}
Maximum water holding capacity (%)	71.35±5.21^{d}	87.91±5.38^{c}	101.21±6.82^{b}	118.31±6.32^{a}
Ash content (%)	6.7±0.09^{d}	6.91±0.13^{cd}	8.62±0.23^{a}	8.12±0.21^{b}
pH	7.60 ± 0.29^{c}	8.60 ± 0.08^{b}	9.05 ± 0.09^{a}	9.11 ± 0.09^{ab}
E.C (dS m^{-1})	1.33± 0.05^{a}	0.16 ± 0.02^{d}	0.26 ± 0.01^{c}	0.31 ± 0.02^{b}
Organic matter (%)	0.102 ± 0.001^{d}	1.38 ± 0.01^{b}	1.27 ± 0.02^{c}	2.76 ± 0.04^{a}
Minerals				
Nitrogen (%)	0.016 ± 0.001^{n}	0.06± 0.001^{l}	0.08±0.004^{m}	0.13 ± 0.001^{a}
Potassium (%)	55.67±3.12^{a}	600.41±19.26^{a}	1100.69±65.57^{a}	1180.18±160.93^{a}
Phosphorus (%)	42.66±7.28^{ab}	48.006±4.64^{d}	52.133±2.10^{d}	54.5±3.34^{a}
Carbon (%)	29.75±1.16^{c}	39.78±1.19^{e}	47.78±1.69^{e}	59.89±3.67^{a}
Silicon (%)	1.49±0.05^{g}	2.13±0.03^{h}	2.59±0.03^{i}	3.87±.0.4^{a}
Calcium (%)	0.99±0.02^{h}	2±0.02^{h}	3.99±0.03^{h}	4.68±0.03^{a}
Cadmium (%)	0.21±0.001^{k}	0.2±0.001^{j}	0.89±0.001^{a}	0.01±0.001
Chromium (%)	0.09±0.001^{l}	0.16±0.001^{k}	0.23±0.001^{l}	0.43±0.001^{a}
Magnesium (%)	0.31±0.01^{ij}	0.12±0.01^{k}	0.51±0.01^{k}	2.52±0.02^{a}
Sodium (%)	0.45±0.02^{i}	1.38±0.02^{i}	1.76±0.05^{j}	2.35±0.05
Sulphur (%)	0.02±0.001^{m}	0.07±0.001^{l}	0.11±0.002^{l}	0.23±0.001
Nickel (%)	0.06±0.001^{l}	0.05±0.001^{l}	0.03±0.001^{n}	0.11±0.001
Manganese (mg kg^{-1})	1.39±0.03^{g}	387.73±7.87^{b}	574.62±8.79^{b}	754.51±11.67^{b}
Copper (mg kg^{-1})	21.56±1.89^{cd}	32.67±2.78^{e}	38.89±2.56^{f}	49.89±2.13

Zinc (mg kg^{-1})	19.89±2.13[d]	23.38±2.78[f]	38.11±3.21[f]	47.67±3.78
Boron (mg kg^{-1})	9.47±0.58[e]	16.78±1.23[g]	4.41±0.78[g]	3.89±0.51
Iron (mg kg^{-1})	46.02±3.67[b]	59.81±6.12[c]	98.01±6.82[c]	134.42±8.49[c]

BC=Biochar, E.C=Electric conductivity,

2.2 Effect of biochar on Minerals Composition of soil

In this study we analyzed the minerals composition of soil after addition of different concentration of biochar. Our results showed that potassium, phosphorus, carbon, iron, copper and zinc concentration was higher than nitrogen, calcium, magnesium, chromium, sodium, sulphur, nickel and boron in soil. However, the addition of biochar significantly increased the minerals content in soil. The total nitrogen contents were also significantly increased ($p < 0.05$) from 0.016 ± 0.001(soil without biochar) to 0.138 ± 0.001 (with the addition of biochar). The potassium contents increased from 55 ± 3.12 in soil (0%), while adding biochar significantly increased the potassium dose to 1180 ± 160.93. Similarly, phosphorus contents were also considerably dose-dependently increased from 42.66 ± 7.28 to 54.5 ± 3.34. The dominant minerals of soil containing 30% biochar were Potassium, phosphorus, iron, and carbon. The mineral composition shows that magnesium, sodium, and zinc concentrations were 0.31±0.01, 0.45±0.02, and 0.02±0.001, while total calcium values 0.99±0.02 was higher than the mean value of magnesium, sodium, and zinc. The proportions of mineral components in soil treated with different concentration of biochar were significantly varied, as shown in Table 1.

Multivariate statistics analysis showed significant difference between different groups. The principal component analysis PC1 was 99.2% and PC2 was 0.7% variance (Fig. 1A). Fig. 1B shows the PLS-DA score plot on physiochemical and minerals of soil containing different concentration of biochar with an entire variation 100%. These variance score plot indicate that all groups of biochar are clearly separated from each other. The relevance of physiochemical parameters and minerals ingredients for the separation of biochar different concentration was ranked using the variable importance in the projection (VIP) score, which was obtained using the integrated discriminating model. As result, Bulk density, particle density, porosity, water holding capacity, EC, pH, organic matter, ash, and minerals content were screened out using VIP > 1 (Fig. 1C). based on the ANOVA ($p < 0.05$) and PLS-DA (VIP > 1) 5 marker i.e., potassium and manganese were reported as a potential biomarker for the differentiation of soil containing different concentration of biochar.

2.3 Plant growth parameters

Different concentrations of the biochar, i.e., 30%, 50%, and 70%, were used to evaluate the growth-promoting effect of biochar on *M. arvensis*. The soil containing 0% of biochar was used as control group. The results showed that the addition of the biochar significantly ($p < 0.05$) improved the *M. arvensis* growth in a dependent manner (Table 2). No significant difference showed in shoot length between

control and 30% biochar group. The results exhibited that each successive increase of biochar doses, e.g., from 50% to 70%, reverse the improvement in shoot length compared to control and 30% biochar (Table 2). Similarly, compared to control group (without biochar), the plant root length was significantly increased after treated with 30% biochar, while the root length of control group was higher then 50% and 70% biochar. Total plant shoot and root biomass was also increased after adding the successively 30% doses of biochar. Results also showed that biochar doses, e.g., from 50% to 70% impoverish the shoot and root biomass as compared to control as well as compared to 30% doses of biochar. In addition, total fresh and total dry biomass of the plants were significantly increased with the successive addition of 30% biochar, while 50% and 70% of biochar in the soil reduced the total mass.

Table 2: Effects of different concentrations of biochar on plant growth attributes.

M. arvensis 15 Plants per plat with temperatures 65F to 70F for total 90 days				
Parameters	Control	30%	50%	70%
Plant height (cm)	12.34 ± 1.23^{c}	12.03 ± 1.04^{c}	8.78 ± 0.68^{c}	6.78 ± 0.57^{c}
Total fresh weight (g)	360 ± 8.56^{a}	474 ± 11.32^{a}	386 ± 8.67^{a}	109 ± 5.35^{a}
Total dry weight (g)	120 ± 4.23^{b}	154 ± 3.67^{b}	125 ± 3.21^{b}	36 ± 2.89^{b}
Shoot dry weight (g)	1.83 ± 0.04^{e}	2.21 ± 0.05^{e}	1.61 ± 0.04^{e}	1.16 ± 0.03^{e}
Root dry weight (g)	0.42 ± 0.02^{f}	0.48 ± 0.02^{f}	0.32 ± 0.02^{f}	0.21 ± 0.02^{f}
Root length (cm)	4.37 ± 0.12^{d}	5.21 ± 0.19^{d}	3.95 ± 0.16^{d}	2.97 ± 0.09^{d}

2.5 antioxidant enzymes

The antioxidant enzymes were significantly different in plants treated with different concentration of biochar. The CAT enzymes activity was significantly higher in plants treated 30% biochar than control group, while the plants grown in 50% and 70% biochar had significantly lower CAT enzymes activity as compared to control. Similarly, GST, POX, and SOD enzymes activity was significantly higher in 30% biochar treated plants as compared to control, while 50% and 70% biochar treated plants had lower GST, POX, and SOD enzymes activity than control and 30% biochar. This result showed that 30% significantly increase the antioxidant enzymes activity in *M. arvensis* plants (Fig. 2).

2.6 Antioxidant activity

2.6.1 DPPH Analysis

Scavenging the stable DPPH radical molecule has been a popular way to assess antioxidant activity with its characteristic absorbance at 517 nm. When the antioxidant interacts with DPPH, 2,2-diphenyl-1-picrylhydrazine is produced. The antioxidant activity of biochar was evaluated using a DPPH free radical

hydrogen acceptor. The results showed (Fig. 3) that the total antioxidant activity was significantly higher in the soil containing 30% biochar than in the control (only soil). However, the activities were subsequently decreased as biochar concentration increased from 30% to 50% and then 70% compared to control. These findings suggested that 30% biochar concentration was optimum for best antioxidant activity.

2.6.2 Superoxide radical scavenging activity

Results (Fig. 3) showed that the activity of the antioxidant enzyme superoxide radical scavenging activity was significantly different ($p < 0.05$) with varying concentrations of biochar. There was a significant difference between stressful and non-stressful circumstances. With 12.4%, 22%, and 34% increases in antioxidant enzyme concentration at 30% biochar concentration, respectively, without biochar application. Biochar amendment increased the superoxide scavenging activity of plant samples growing under the 30% biochar, respectively. Under the conditions of 50% and 70%, biochar levels reduced superoxide radical scavenging activity up to 6.8% and 11% concerning their respective control.

2.6.3 Hydroxyl radical scavenging activity

The results showed that hydroxyl radical activity was significantly ($p < 0.05$) increased with the addition of 30% biochar as compared to control (soil without biochar). However, the hydroxyl radical activity was significantly ($p < 0.05$) decreased when the concentration of the biochar was increase up to 50% as compared to control (Fig. 3). Similarly, the addition of 70% biochar decreased the hydroxyl radical activity as compared to control and also compared to 30% of biochar. The results revealed that 30% biochar increased the hydroxyl radical activity significantly but further increase of biochar from 50% to 70% decreased the hydroxyl radical activity compared to control.

2.6.4 TPC Analysis

The results demonstrated a significant ($p < 0.05$) increase in TPC when 30% biochar was added to the soil in comparison to the control (soil without biochar). When the biochar concentration increased from 30% to 50%, the TPC activity significantly decreased ($P < 0.05$) compared to the control. Additionally, compared to control, the activity of TPC was reduced by 70% biochar, 30% biochar, and 50% biochar. The results revealed that adding 30% biochar significantly increased TPC activity, whereas adding 50% to 70% decreased TPC activity compared to control (Fig. 4).

2.6.5 TFC Analysis

The results showed that adding 30% biochar to the soil increased TFC significantly ($p < 0.05$) more than the control (soil without biochar). The TFC activity considerably decreased ($p < 0.05$) when the biochar concentration rose from 30% to 50% compared to the control. In addition, 70% biochar and 50% biochar decreased TFC activity compared to control. The findings showed that a biochar concentration of 30% considerably boosted TFC activity but a 50% to 70% lower TFC activity relative to control (Fig. 4).

2.5 Volatile compounds Analysis

In this study we identify sixty-five compounds in M. arvensis (extracts) grown with different concentrations of biochar including control (Table 3). Following GC/MS results, four groups of M. arvensis in descending order have the most detected volatile compounds i.e., 30% > control group > 50% > 70%. In the GC/MS profile of extracts from M. arvensis treated with 30% biochar, 35 compounds were found. The main compounds of 30% biochar plant extract were p-Menthan-3-one, 1,2-epoxy (13.57%), also found in the control group (12.96%), 50% biochar group (5.3%) and 70% biochar group (2.58%). Similarly, 2-Propyl-5-acetoxy-5-methyltetrahydropyran was found 11.80 % in 30% biochar plant extract while this compound is only found in control group 5.79%, and didn't find other group of plant extracts treated with 50% and 70% biochar. Overall, 30% group were found thirty-five compounds, control group were found twenty-six compounds, 50% group found twenty-two compounds and 70% group found only nineteen compounds.

Multivariate statistics were applied to the data on physiochemical and minerals of soil containing different concentration of biochar to classify the different groups biochar. The principal component analysis PC1 was 29.2% and PC2 was 42.9% variance (Fig. 5A). Fig. 1B shows the PLS-DA score plot on volatile compounds of *M. arvensis* (grown in soil containing different concentration of biochar) entire variation was 72%. These variance score plot indicate that all groups of biochar are clearly separated from each other. The distinct colored lines in the heatmap plot demonstrate these overall variances. The proportion of volatile compounds contained in the *M. arvensis* (extracts) grown in soil containing different concentration of biochar is shown in Fig. 5C. The relevance of chemical ingredients for the separation of *M. arvensis* grown in soil containing different concentration of biochar was ranked using the variable importance in the projection (VIP) score, which was obtained using the integrated discriminating model. As result, volatile compounds of *M. arvensis* were screened out using VIP > 1 (Fig. 5D). based on the ANOVA ($p < 0.05$) and PLS-DA (VIP > 1) 5 marker i.e., Cyclohexanone, 2-isopropylidene, p-Menthan-3-one, Hexadecanoic acid, and Methyl petroselinate were reported as a potential biomarker for the differentiation *M. arvensis* grown in soil containing different concentration of biochar.

Table 3. volatile compounds *M. arvensis* (extracts) treated with different concentration of biochar.

No	Compounds	(Relative peak area %)				
		R. Time	Control	30%	50%	70%
1	Isobomeol	7.567	3.60	n.d	n.d	n.d
2	p-Menthan-3-one, 1,2-epoxy-	9.560	12.96	13.57	5.30	2.58
3	Cinnamaldehyde, (E)-	9.906	n.d	3.00	n.d	n.d
4	7-Pentadecanone Phenol, 2-ethyl-4,5-dimethyl	10.264	n.d	2.09	n.d	n.d
5	Phenol, 2-ethyl-4,5-dimethyl-	10.367	4.41	3.61	n.d	n.d
6	2,5-Diethylphenol	10.369	n.d	n.d	2.51	n.d
7	alpha-Limonene diepoxide	11.571	n.d	0.65	n.d	n.d
8	1,3,3-Trimethyl-2-oxabicyclo [2.2.2] octan-6-one	10.786	10.42	n.d	11.22	n.d
9	2-Oxabicyclo[2.2.2]octan-6-one, 1,3,3-trimethyl-	10.815	n.d	n.d	n.d	10.34
10	4,4-Biscyclohexanone, 2,2,6,6-tetramethyl-	11.724	n.d	1.07	n.d	n.d
11	2-Isopropylidenecyclohexanone	12.273	n.d	n.d	19.14	n.d
12	Cyclohexanone, 2-(1-methylethylidene)	12.282	31.19	n.d	n.d	n.d
13	3-Decyne	12.284	n.d	n.d	n.d	11.95
14	3-Methyl-6-propyltetrahydro-2H-pyran-3-yl acetate	12.724	n.d	n.d	n.d	8.93
15	Cyclohexanone, 2-isopropylidene	11.742	n.d	17.57	n.d	n.d
16	2-Propyl-5-acetoxy-5-methyltetrahydropyran	12.719	5.79	11.80	n.d	n.d
17	2-Hydroxyhexadecyl butanoate	12.716	n.d	0.61	n.d	n.d
18	2H-Pyran-3-ol, tetrahydro-3-methyl-6-propyl-, acetate	12.719	n.d	n.d	6.50	n.d
19	4-Hydroxymethylene-2,6-dimethyl-oct-7-en-3-one	12.719	2.36	n.d	n.d	n.d
20	Cyclooctanol, acetate	13.037	n.d	3.32	2.91	n.d
21	1,7-Nonadien-4-ol, 4,8-dimethyl-	13.518	1.51	n.d	n.d	n.d
22	4,8-Dimethyl-1,7-nonadien-4-ol	13.343	n.d	1.41	n.d	n.d
23	2,3,6-Trimethyl-7-octen-3-ol	13.959	1.72	n.d	n.d	n.d
24	11-Tetradecen-1-ol, acetate, (Z)-	14.349	n.d	0.77	n.d	n.d

25	(Z)-4-Chloro-2,3-dimethyl-1,3-hexadiene	14.884	1.30	1.82	1.00	n.d
26	3-Pentanol, 2,3,4-trimethyl-	15.119	2.14	1.35	n.d	n.d
27	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxy-7-octalin	15.178	n.d	0.61	n.d	n.d
28	1,3-Hexadiene, 3-ethyl-2-methyl-, (z)	15.712	o.53	n.d	n.d	n.d
29	Calamenene	16.124	n.d	0.48	n.d	n.d
30	Tridecanoic acid, methyl ester	16.017	2.29	n.d	n.d	n.d
31	Caryophyllene oxide	17.581	n.d	0.53	n.d	n.d
32	Cyclopropane, 1-chloro-2,2-dimethyl-3-(3,3-dimethyl-1-butynyl)-	17.258	0.53	0.29	0.45	n.d
33	Methyl tetradecanoate	20.573	0.45	n.d	n.d	n.d
34	11,11-Dimethyl-spiro[2,9]dodeca-3,7-dien	20.689	0.16	n.d	n.d	n.d
35	Tetradecanoic acid	21.307	0.57	n.d	0.56	n.d
36	5-Isopropyl-6-methyl-hepta-3,5-dien-2-ol	21.478	0.82	n.d	n.d	n.d
37	4a,7,7-Trimethyloctahydro-2(1H)-naphthalenone	22.466	n.d	0.25	n.d	n.d
38	Tridecanoic acid	21.310	n.d	1.25	n.d	n.d
39	Tridecane	22.193	n.d	0.26	n.d	n.d
40	Pentadecanoic acid	21.310	n.d	0.23	n.d	n.d
41	Docosanoic acid, 1,2,3-propanetriyl ester	22.804	n.d	0.08	n.d	n.d
42	1-Octadecyne	23.019	2.41	1.69	2.17	1.85
43	6,10,14-Trimethyl-2-pentadecanone	23.119	n.d	n.d	0.87	n.d
44	2-Undecanone, 6,10-dimethyl-	23.125	n.d	1.23	n.d	1.19
45	Hexahydropseudoionone	23.123	0.28	n.d	n.d	n.d
46	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	23.527	0.40	n.d	0.42	0.45
47	7-Hexadecenoic acid, methyl ester, (Z)-	24.320	n.d	n.d	0.52	n.d
48	9-Hexadecenoic acid, methyl ester, (Z)-	24.329	n.d	0.25	n.d	n.d
49	Z-10-Tetradecen-1-ol acetate	24.667	n.d	0.46	n.d	n.d
50	Pentadecanoic acid, 14-methyl-, methyl ester	24.745	n.d	n.d	10.29	n.d
51	Methyl isoheptadecanoate	24.746	2.34	n.d	n.d	n.d

52	Hexadecanoic acid, 15-methyl-, methyl ester	24.748	n.d	7.31	n.d	n.d
52	Hexadecenoic acid, methyl ester	24.751	n.d	n.d	n.d	15.40
53	9-Hexadecenoic acid	24.995	0.17	0.46	n.d	n.d
54	n-Hexadecanoic acid	25.408	n.d	n.d	n.d	3.89
55	Hexadecenoic acid, dioctyl ester	25.819	n.d	n.d	n.d	9.15
56	Linolelaidic acid, methyl ester	27.974	0.66	n.d	4.61	6.75
57	Methyl cis-6-octadecenoate	28.091	n.d	n.d	9.43	n.d
58	Methyl petroselinate	28.096	n.d	n.d	n.d	13.65
59	10-Octadecenoic acid, methyl ester	28.193	n.d	n.d	0.69	1.32
60	13-Octadecenoic acid, methyl ester	28.2	n.d	n.d	n.d	1.32
61	Phytol	28.341	10.32	n.d	10.30	5.61
62	Octadecanoic acid, methyl ester	28.581	n.d	n.d	1.36	2.81
63	Stearic acid, methyl ester	28.588	0.64	n.d	n.d	n.d
64	Linolenic acid	28.780	n.d	n.d	n.d	1.56
65	Octadecanoic acid	29.259	n.d	n.d	1.60	0.56

Discussion

Biochar is a carbon-rich material derived from the pyrolysis of organic matter. It has been proposed as a soil amendment to improve soil quality and crop productivity. However, the effects of biochar on plant growth may vary depending on the type and dose of biochar, the plant species, and the soil conditions. In this study, we investigated the effects of biochar on the growth of *M. arvensis*, a medicinal and aromatic plant. Plant height, shoot dry weight, and root dry weight all decrease at high biochar doses. Different crops saw reduced germination rates and plant growth due to high concentrations of biochar[8, 9]. Similarly, Elhakem[10] reported that massive reductions in plant growth and the dry weight of *M. arvensis* were caused by high dose of biochar and salinity of soil. Water stress condensed the plant growth conditions such as plant height, number of leaves, and branches [11, 12]. Similarly, the plant growth of *Abelmoschus esculentus* was reduced by the water stress reported by. The present study assessed the effect of biochar on plant growth and phytochemical content in *M. arvensis* [13].

The results showed that biochar significantly increased the plant growth and phytochemical content of *M. arvensis* compared to the control. Biochar improved soil properties such as pH, cation exchange capacity, nutrient availability, and organic matter content. Biochar enhanced soil microbial activity and diversity, influencing plant growth and phytochemical production through various direct and indirect mechanisms. Biochar induced systemic resistance in plants against biotic and abiotic stresses by

modulating the expression of genes and pathways associated with plant defense and growth such as jasmonic acid, brassinosteroids, cytokinins, auxin and synthesis of flavonoid, phenylpropanoids and cell wall. The findings of this study suggested that biochar was used as a sustainable soil amendment to improve the growth and quality of *M. arvensis*. Biochar also enhanced the phytochemical content of *M. arvensis*, such as total phenolics, flavonoids, and antioxidant activity. The positive effects of biochar on *M. arvensis* growth and phytochemistry were attributed to the improved soil properties, such as pH, electrical conductivity, organic carbon, available phosphorus, and cation exchange capacity.

Biochar has been shown to enhance plant growth in various ways, such as improving soil properties, retaining soil nutrients, and remediating soil contaminants. One of the mechanisms by which biochar enhances plant growth is by storing nutrients in crystals inside the micropores of its insoluble carbon matrix[14]. The notion that nutrients in biochar will be released to soil solution during crop growth is the basis for the correlation between nutrient elements in comparable biochar and fertilizer rates[15]. This theory and the mechanisms governing the release of nutrients from biochar have been studied in the current research. In contrast to most chemical fertilizers, only a tiny to moderate part of the K, Ca, P, and other nutrient elements in biochar is easily soluble in straightforward water extraction. This is due in part to the fact that nutrients are stored in crystals inside the micropores in the insoluble carbon matrix of biochar, which prevents dissolution within the time frame of traditional water extraction[16]. This mechanism has been observed for nutrients such as N, P, Ca, K and Mg. This hypothesis was supported by several studies that have used advanced analytical techniques to characterize the nutrient forms and distribution in biochar and biochar-amended soils. In damp soils, biochar particles have pores filled with soil solution ingested by capillary from the soil [17]. Biochar is similar to KCl and K_2SO_4 fertilizers in that most K diffused from biochar particles into the soil within a month[18]. Biochar is unlikely to be a valuable fertilizer for these substances.

The pH values of biochar are positively correlated with the formation of carbonates and the presence of inorganic alkalis[19]. The main culprits of alkaline pH are these groups [7]. There have been reports of total base cation and carbonate concentrations rising with warmth, which affects pH (ranging from 6.5 to 10.8) [7]. Ash content and oxygen functional group increases during pyrolysis have been linked to higher pH with rising temperature[20]. This study showed that the pH of 30% biochar concentration was more alkaline and reached to pH=8.6 ± 0.08. Moreover, 50% biochar pH was reached to 9.05 ± 0.09 and 70% biochar pH was even more alkaline and reached up to 9.11 ± 0.09.

The kind of feedstock affects the EC of biochar. This distinction was noticed because biochar with a higher EC can be produced from biomass with a high ash concentration [21]. This capacity may be explained by the fact that alkali and alkali metals in biomass encourage synthesizing surface functional groups that contain oxygen . Consequently, the EC of biochar made from manure is larger than that of woody biochar[22]. When compared to biochar made from wood, non-wood-derived biochar had higher EC and surface acidity [23]. Our investigation showed that when the biochar was increased up to 50% the EC was decrease up to 0.31 ± 0.02 and then further was decreased to 0.26 ± 0.01 when in 70% biochar. Similar results were reported by Gaskin, Steine[24], who found a significantly higher EC for biochar from

poultry litter than for biochar produced from peanut hulls. The number of functional groups containing oxygen on the biomass surface may have decreased, which may account for some of this decline[25]. According to Suliman, Harsh [26] douglas fir wood biochar had a higher EC than Douglas fir bark-biochar and hybrid poplar-biochar. This distinction may be explained by the interaction between particular surface area and carboxylic functional groups, which account for the majority of the EC among acidic functional groups [25]. Similarly, our finding showed that adding biochar significantly increased the content of potash, nitrogen, and phosphorus, as well as organic matter shown in Table 1.

As for the antioxidant activity, our studies have shown that 30% and control group have a positive effect on *M. arvensis* increasing the activity of antioxidant enzymes. Recent study showed that application of biochar enhanced the antioxidant enzymes such as CAT, POD, and SOD [49]. Similarly, our study demonstrated that 30% biochar potentially increased the antioxidant enzymes activity.

Phenolic compounds are the diverse metabolites generated by the secondary metabolism. These compounds are biological active and are used to alleviate different philological disease such as cancer, metabolic disorders, and cardiovascular disease[27]. Besides this biological activity, phenolic compounds also play a pivotal role in regulating plant growth, development, environmental adaptation, and stress conditions [28]. In recent work, we found that *Mentha piperita* plants that had undergone direct inoculation as well as treated with biochar had higher TPC and phenylalanine ammonia-lyase (PAL) activity, the latter of which is necessary for the synthesis of phenolic compounds[29]. In agreement, the TPC was observed to increase in different plant species by supplementing biochar[1]. For example, application of biochar increases the nutritional value of plant by increasing the phytochemical content such as in broccoli [1], spinach [41], and S. rechingeri Jamzad [30]. Our result reported that the application of biochar with 30% has significantly increased the phytochemicals value of *M. arvensis*. However, Jayapala et al. [31] discovered the development of infection resistance in chili plants treated with *Bacillus sp*. by enhancing the activities of defense-related enzymes and a greater buildup of TPC. Furthermore, Tahir et al. [32] found that *Bacillus sp. mVOCs* activate ISR in tobacco plants, which has been shown to have a detrimental effect on the development of the pathogen R. solanacearum. According to Tahir et al. [32], a rise in SM levels and defense-related enzymes, such as PAL, leads to resistance.

Antioxidants called phenolic compounds may be necessary to scavenge ROS and guard the lipid membrane from oxidative stress [33]. The buildup of phenolic compounds, for instance, increased in a concentration-dependent manner in Fagopyrum esculentum plants cultivated in conditions with increasing salt concentrations, resulting in a larger DPPH free radical scavenging capability[34]. This result was supported by the current study's observations of plants had treated with different concentration biochar. These plants displayed increased antioxidant capacity as seen by their high levels of DPPH, superoxide, and hydroxyl radical scavenging activity. The *M. arvensis* plant exposed to 30% biochar exhibited the most increased antioxidant activity.

Additionally, it was discovered that peppermint grown under standard conditions was more efficient at scavenging DPPH free radicals and had a higher reducing power than when it was subjected to high dose of some specific biochar. This finding indicates that peppermint tissues under heat and/or water stress have lower levels of antioxidants and reducing chemicals. The degree of discoloration reveals the antioxidant extract's capacity to scavenge free radicals, which is brought on by its capacity to donate hydrogen. The addition of biochar to a nutrient-poor, soilless substrate was sufficient to support fruit growth, but the biochar feedstock source impacted the average weight of tomato fruits[35].

Biochar promoted the formation of cell wall and secondary metabolites in addition to hormone defense mechanisms. The secondary cell wall, which is mainly made of cellulose, hemicelluloses (largely xylans), and lignin, is essential for the ability of plants to fend off diseases[36]. Several genes involved in the synthesis of cellulose and xylan were increased by biochar. Similarly, some genes essential for producing phenylpropanoid, phenylalanine, flavonoids, and lignin were also markedly elevated. These activated genes may boost the synthesis of ferulic acid, flavonoids (naringenin, kaempferol, and quercetin), and lignin in plants[36]. These compounds help plants defend themselves by acting as antibacterial agents and strengthening and stabilizing cell walls.

Some properties of biochar, such as the increased retention of nutrients or the reduced loss of nutrients through leaching and the maintenance of a higher level of moisture in the root zone, may have had a substantial impact on the fruit weights, compensating for the nutrient shortage[33, 35]. The present study showed that the potential antioxidant activity, such DPPH, superoxide, and hydroxyl radical scavenging activity was recorded in 30% biochar. Montoya reported a similar result, and Fernández [1] that enrich-biochar application has increased broccoli's phenolic compounds and glucosinolates. In GC/MS analysis of *M. arvensis* after application with different concentrations of biochar showed that the *m. arevensis* with 30% biochar have enrich source of bioactive compounds then that of 50% and 70%. The total 35 main compounds were founded in plants with 30% biochar are cyclohexanone, 2-isoprophylidene 17.57%, and p-Menthan-3-one, 1,2-epoxy 13.57%, and with 50% biochar have total 22 phytochemicals, in which 2-isoprophylidenecyclohaxanone 19.14% and phytol 10.3%. While the plants with 70% biochar was found very low content of phytochemicals. These results show that plant treated with specific amount biochar can significantly increase the nutritional value of plant. Further study is needed to confirm phytochemical enhancement with biochar treatment.

Conclusion

Based on the results of the present study, it has been concluded that adding different concentrations of biochar (0%, 30%, 50%, and 70%) was extremely important in plant growth promotion and augmentation of valuable nutrients and compounds. All the physical, chemical and biochemical analyses showed 30% biochar provided a variety of beneficial chemicals to soil and applying 30% biochar considerably ($p > 0.05$) enhanced the growth characteristics of the plant, such as shoot length, root length, fresh and dry biomass. Overall, the findings indicated that adding 30% biochar to the soil enhanced the necessary chemicals e.g., Ca, Mg, Na, and Ni, minerals e.g., P, N, and organic matter and antioxidant including

DPPH, superoxide, TPC, FTC, and hydroxyl radical scavengers. Based on the conclusion, it has been recommended that the application of the biochar improves soil fertility, so adding the biochar is recommended. Biochar has many valuable chemical compounds that benefit the soil for plant growth. Applying the biochar improves plant growth by providing desired nutrients to the plants. Only specific percentage of the biochar is important if the percentage is higher and/or lower, it has either no effect or an adverse effect on plant growth.

Declarations

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Author Contributions

M.I: Conceptualization, methodology, software, writing—original draft; A.M: methodology, software, writing—review and editing; H.M: software, writing—review and editing; G.L: supervision, project administration, funding acquisition, writing—review and editing; Q.W: supervision, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Data availability

All data generated or analyzed during this study are included in this article. The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declaration

Ethics approval and consent to participate

All experimental studies on plants complied with relevant institutional, national, and international guidelines and legislation. This project uses cultivated plant materials and does not utilize wild species and transgenic technology. All the varieties are cultivated varieties given by the department of Forestry, Bamboo Research Institute, Nanjing Forestry University (Nanjing, China).

Consent for publication

Not Applicable

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Montoya, D., et al., *Enriched-biochar application increases broccoli nutritional and phytochemical content without detrimental effect on yield.* 2022. **102**(15): p. 7353-7362.
2. Shakya, A., T.J.B.a.i.a. Agarwal, and e. management, *Potential of biochar for the remediation of heavy metal contaminated soil.* 2020: p. 77-98.
3. Seleiman, M.F., et al., *Effects of ZnO nanoparticles and biochar of rice straw and cow manure on characteristics of contaminated soil and sunflower productivity, oil quality, and heavy metals uptake.* 2020. **10**(6): p. 790.
4. Seleiman, M.F., et al., *Integrative effects of rice-straw biochar and silicon on oil and seed quality, yield and physiological traits of Helianthus annuus L. grown under water deficit stress.* 2019. **9**(10): p. 637.
5. Pansu, M., J.J.H.o.s.a.m. Gautheyrou, organic, and i. methods, *Mineralogical separation by selective dissolution.* 2006: p. 167-219.
6. Singh, V. and H. Agrawal, *Qualitative soil mineral analysis by EDXRF, XRD and AAS probes.* Radiation Physics and Chemistry, 2012. **81**(12): p. 1796-1803.
7. Mueed, A., et al., *Extraction, characterization of polyphenols from certain medicinal plants and evaluation of their antioxidant, antitumor, antidiabetic, antimicrobial properties, and potential use in human nutrition.* 2023. **10**: p. 1125106.
8. Zaid, A., et al., *Plant growth regulators improve growth, photosynthesis, mineral nutrient and antioxidant system under cadmium stress in menthol mint (Mentha arvensis L.).* 2020. **26**: p. 25-39.
9. Nabi, A., et al., *Triacontanol protects Mentha arvensis L. from nickel-instigated repercussions by escalating antioxidant machinery, photosynthetic efficiency and maintaining leaf ultrastructure and root morphology.* 2021. **40**: p. 1594-1612.
10. Elhakem, A.H.J.J.o.B.S., *Impact of salicylic acid application on growth, photosynthetic pigments and organic osmolytes response in Mentha arvensis under drought stress.* 2019. **19**(6): p. 372-380.
11. Mohd Nor, N., F. Pa'ee, and N. Manan, *The effect of water stress on growth rate in Mentha arvensis.* 2022.
12. Nor, N.M., F. Pa'ee, and N. Manan. *The effect of water stress on growth rate in Mentha arvensis*. in *IOP Conference Series: Earth and Environmental Science*. 2021. IOP Publishing.
13. Jabborova, D., et al., *Biochar and Arbuscular mycorrhizal fungi mediated enhanced drought tolerance in Okra (Abelmoschus esculentus) plant growth, root morphological traits and physiological properties.* 2021. **28**(10): p. 5490-5499.
14. Bolan, S., et al., *The potential of biochar as a microbial carrier for agricultural and environmental applications.* 2023: p. 163968.
15. Novak, J.M., M.G. Johnson, and K.A.J.F.i.S.F.S. Spokas, *Concentration and release of phosphorus and potassium from lignocellulosic-and manure-based biochars for fertilizer reuse.* 2018. **2**: p. 54.

16. Gupta, A., *Extraction and characterization of phosphorus from organic algal biochar*. 2014, University of Kansas.
17. Prendergast-Miller, M., M. Duvall, and S.J.E.j.o.s.s. Sohi, *Biochar–root interactions are mediated by biochar nutrient content and impacts on soil nutrient availability.* 2014. **65**(1): p. 173-185.
18. Khalil, R.A., et al., *Sulfur abatement in pyrolysis of straw pellets.* 2008. **22**(4): p. 2789-2795.
19. Ding, W., et al., *Pyrolytic temperatures impact lead sorption mechanisms by bagasse biochars.* 2014. **105**: p. 68-74.
20. Tomczyk, A., et al., *Biochar physicochemical properties: pyrolysis temperature and feedstock kind effects.* 2020. **19**(1): p. 191-215.
21. Yang, F., X.-q. Lee, and B.J.C.J.o.G. Wang, *Characterization of biochars produced from seven biomasses grown in three different climate zones.* 2015. **34**: p. 592-600.
22. Tag, A.T., et al., *Effects of feedstock type and pyrolysis temperature on potential applications of biochar.* 2016. **120**: p. 200-206.
23. Mukome, F.N., et al., *Use of chemical and physical characteristics to investigate trends in biochar feedstocks.* 2013. **61**(9): p. 2196-2204.
24. Gaskin, J.□., et al., *Effect of low-temperature pyrolysis conditions on biochar for agricultural use.* 2008. **51**(6): p. 2061-2069.
25. Singh, B., B.P. Singh, and A.L.J.S.R. Cowie, *Characterisation and evaluation of biochars for their application as a soil amendment.* 2010. **48**(7): p. 516-525.
26. Suliman, W., et al., *Influence of feedstock source and pyrolysis temperature on biochar bulk and surface properties.* 2016. **84**: p. 37-48.
27. Mueed, A., et al., *A comprehensive review of flaxseed (Linum usitatissimum L.): health-affecting compounds, mechanism of toxicity, detoxification, anticancer and potential risk.* 2023. **63**(32): p. 11081-11104.
28. Petruccelli, R., et al., *Influence of biochar application on nutritional quality of tomato (Lycopersicon esculentum).* 2015. **66**(7): p. 747-755.
29. Cappellari, L.d.R., et al., *Volatile organic compounds from rhizobacteria increase the biosynthesis of secondary metabolites and improve the antioxidant status in Mentha piperita L. grown under salt stress.* 2020. **10**(8): p. 1094.
30. Machado, R.M., et al., *Effects of coir-based growing medium with municipal solid waste compost or biochar on plant growth, mineral nutrition, and accumulation of phytochemicals in spinach.* 2022. **11**(14): p. 1893.
31. Jayapala, N., et al., *Rhizobacteria Bacillus spp. induce resistance against anthracnose disease in chili (Capsicum annuum L.) through activating host defense response.* 2019. **29**(1): p. 1-9.
32. Tahir, H.A., et al., *Plant growth promotion by volatile organic compounds produced by Bacillus subtilis SYST2.* 2017. **8**: p. 236091.

33. Khan, N., et al., *Crosstalk amongst phytohormones from planta and PGPR under biotic and abiotic stresses.* 2020. **90**: p. 189-203.
34. Lim, J.-H., et al., *Effect of salinity stress on phenolic compounds and carotenoids in buckwheat (Fagopyrum esculentum M.) sprout.* 2012. **135**(3): p. 1065-1070.
35. Alhaithloul, H.A., et al., *Changes in ecophysiology, osmolytes, and secondary metabolites of the medicinal plants of Mentha piperita and Catharanthus roseus subjected to drought and heat stress.* 2019. **10**(1): p. 43.
36. Jaiswal, A.K., et al., *Molecular insights into biochar-mediated plant growth promotion and systemic resistance in tomato against Fusarium crown and root rot disease.* 2020. **10**(1): p. 13934.

Figures

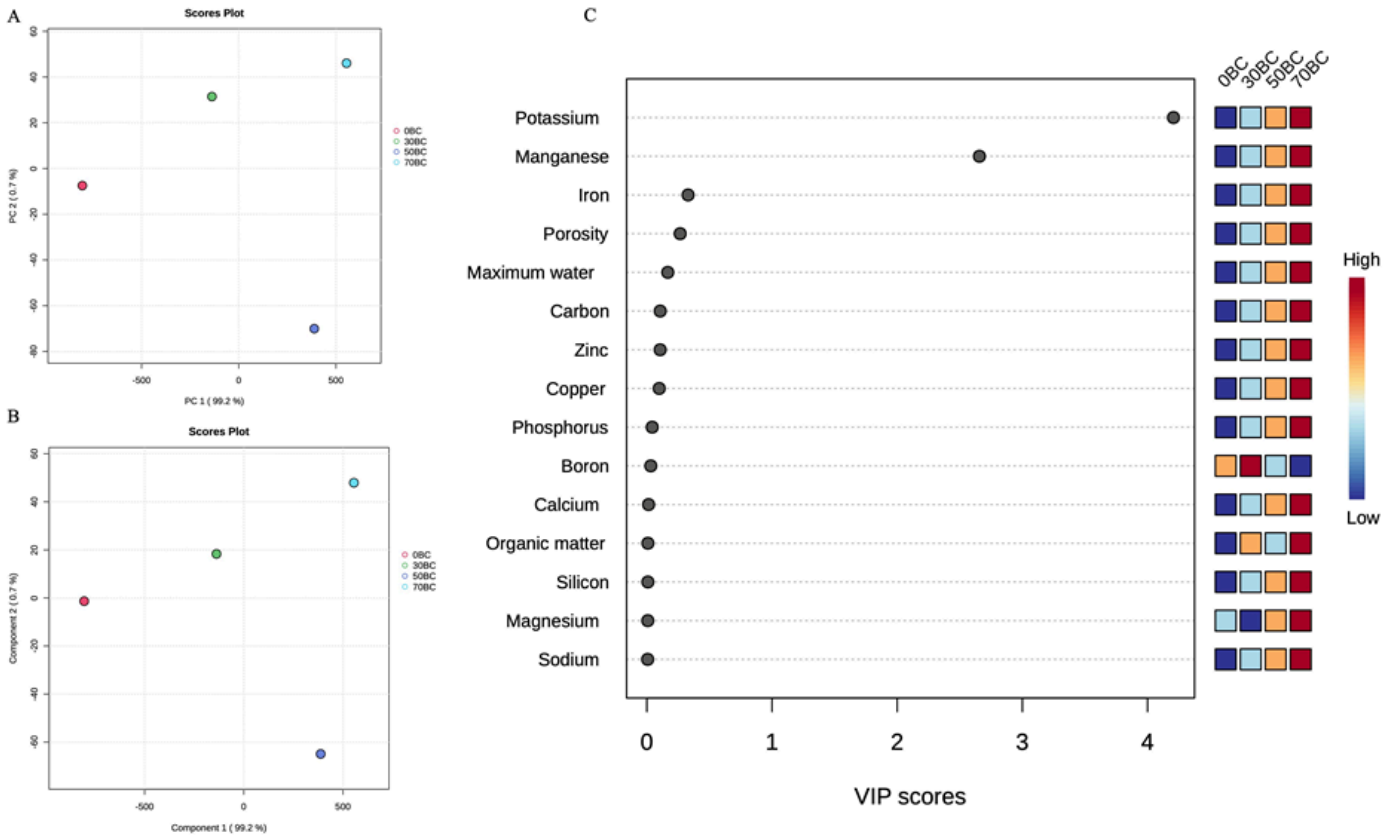

Figure 1

(A) Principal components analysis (PC1 and PC2) based on physiochemical parameters and minerals content in soil containing different percentage of biochar. (B) PLS-DA score plot model established based physiochemical parameters and minerals content in soil containing different percentage of biochar. (C) VIP score plot model established to separate the soil based on their potential biomarkers.

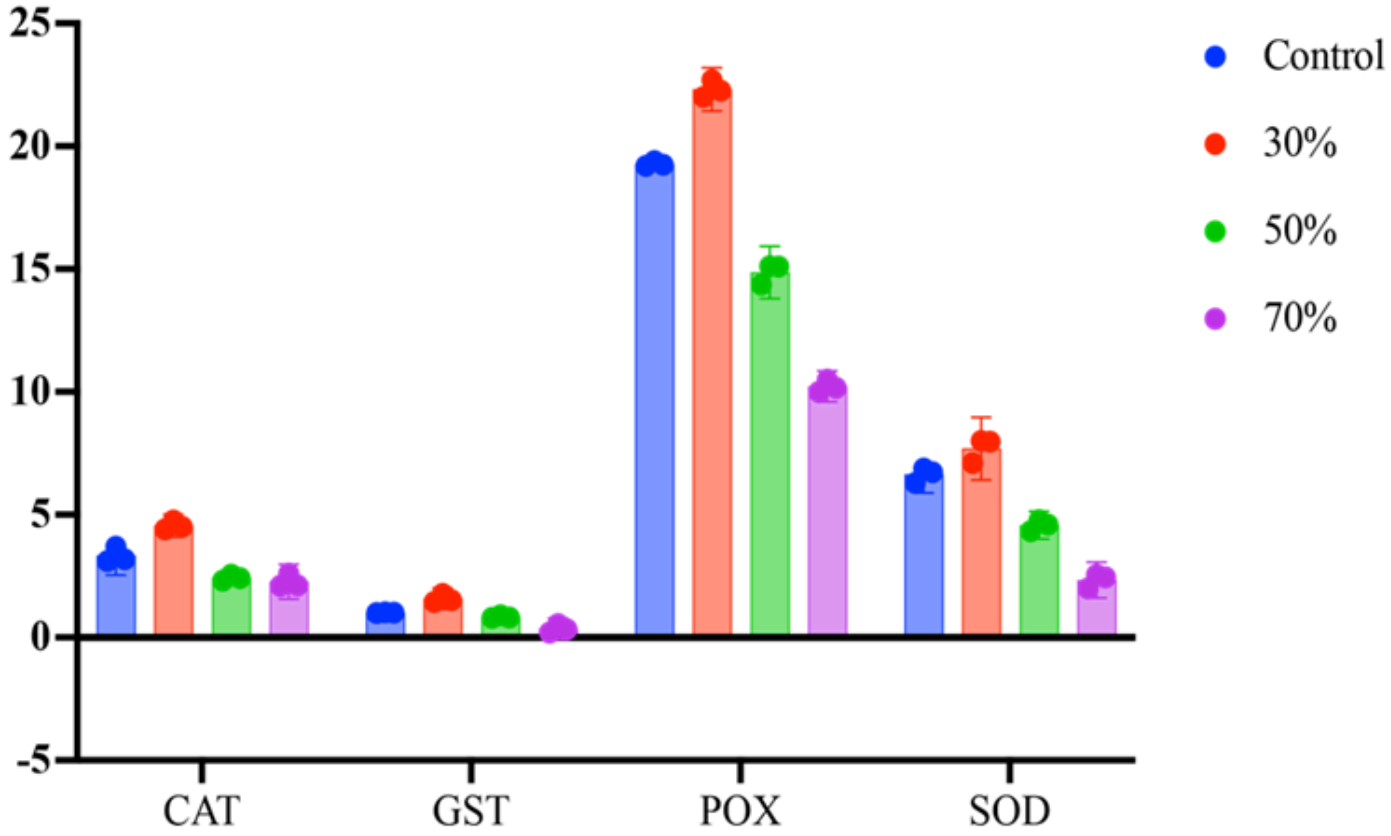

Figure 2

The antioxidants enzymes in plants.

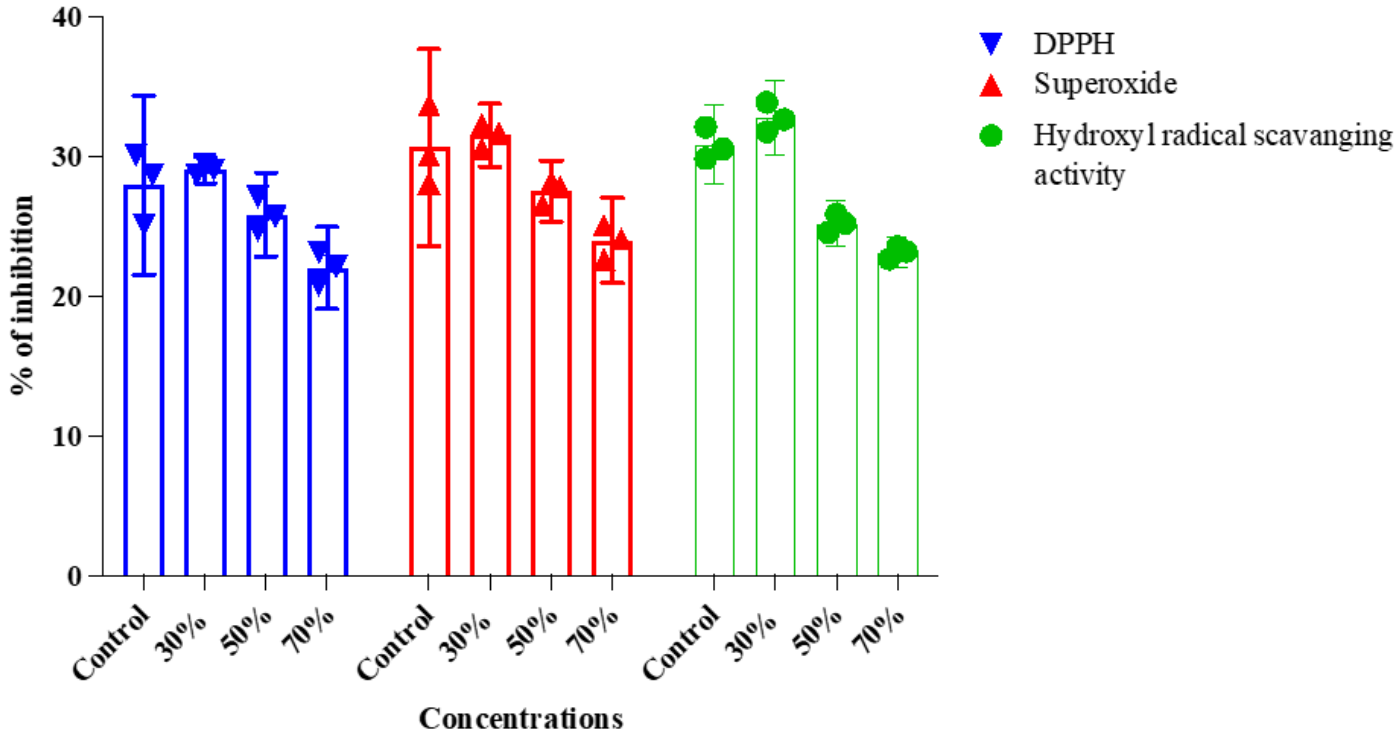

Figure 3

Antioxidant activity (DPPH, Hydroxyl radical activity, and Superoxide activity) of *M. arvensis* plant extracts grow under different concentrations of biochar.

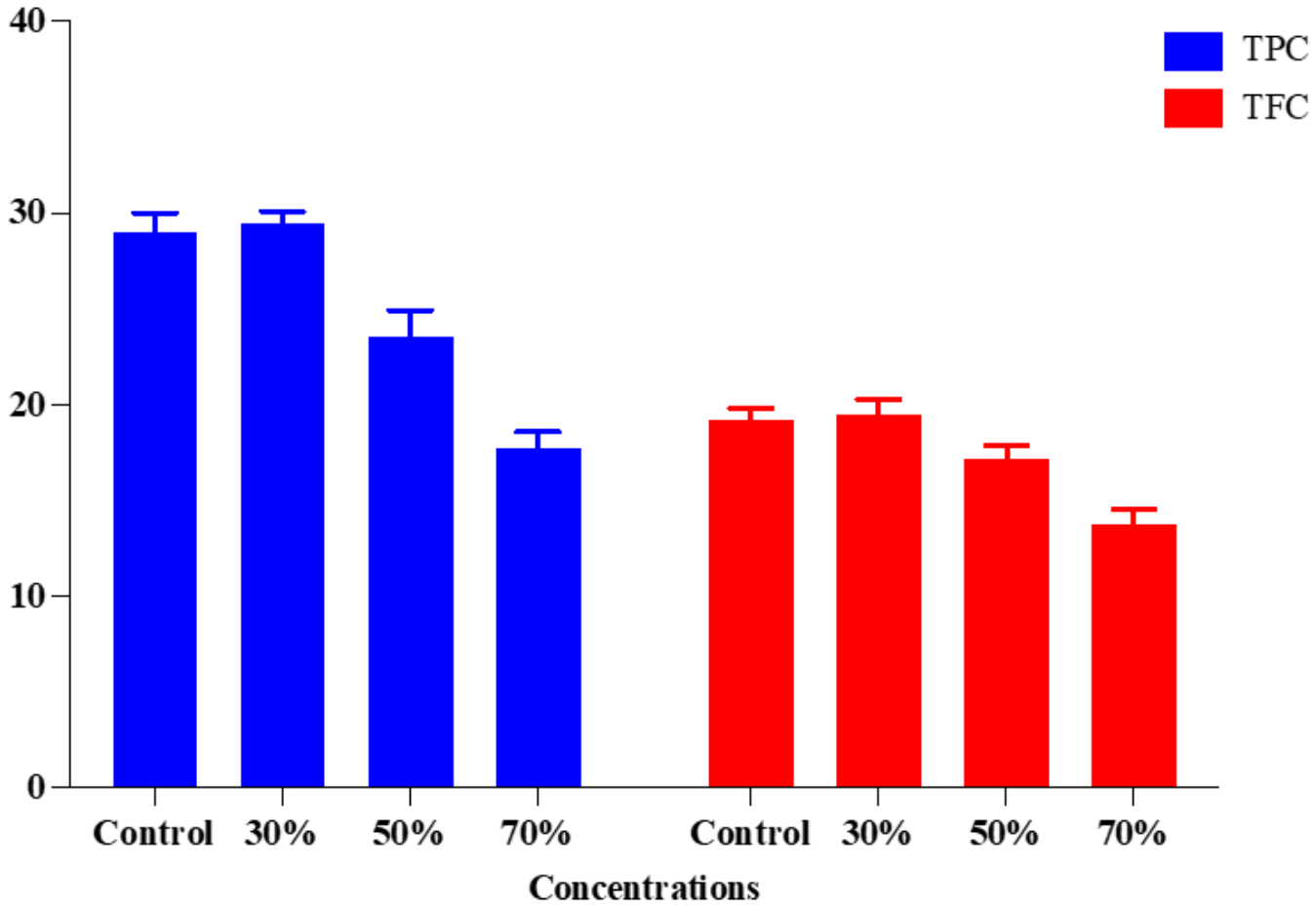

Figure 4

TPC and TFC activity of *M. arvensis (extracts)* grown in different concentrations of biochar.

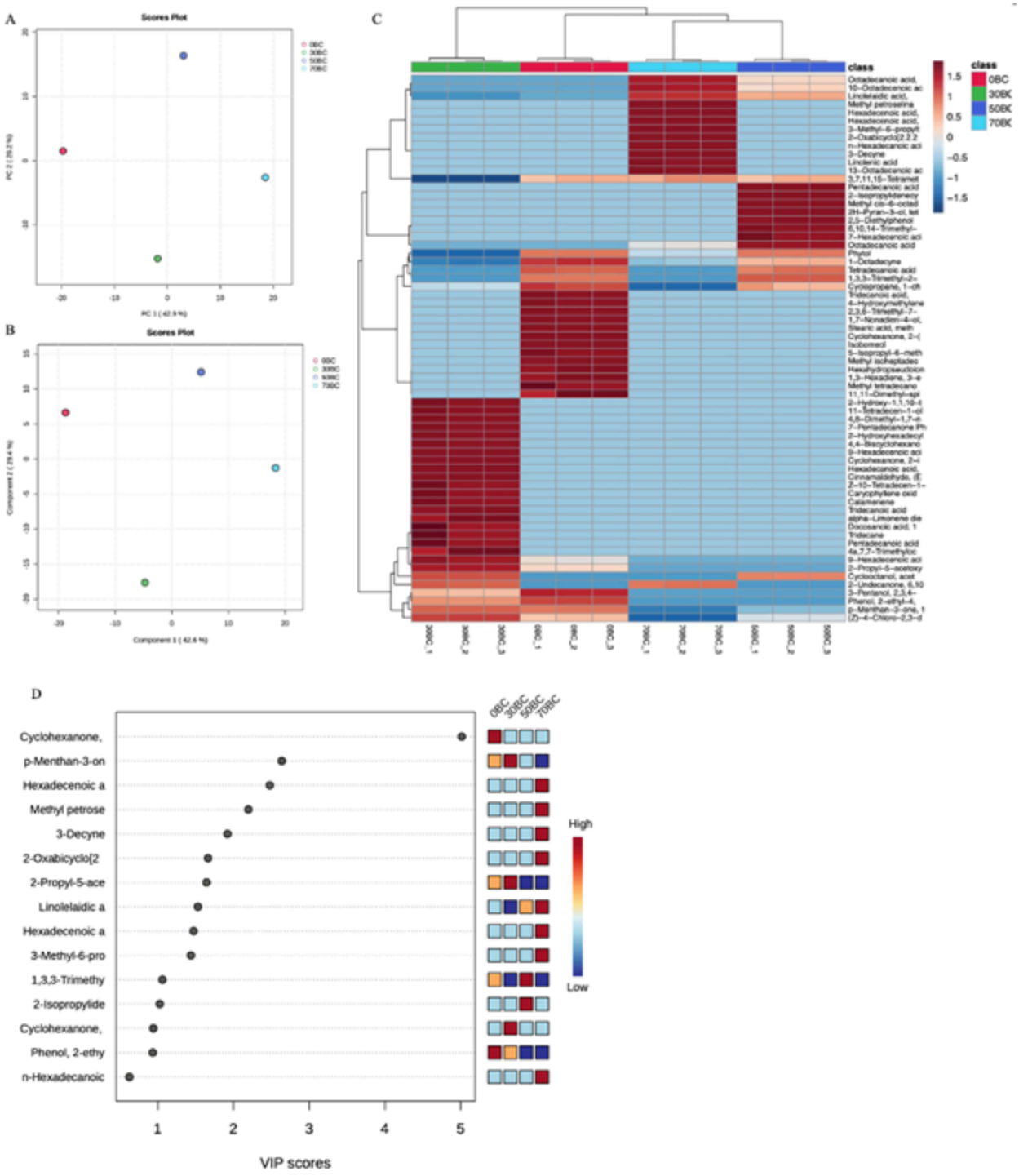

Figure 5

(A) Principal components analysis (PC1 and PC2) based on volatile compounds in *M. arvensis*. (B) PLS-DA score plot model established based on volatile compounds in *M. arvensis*. (C) VIP score plot model established to separate *M. arvensis* plant grown in soil containing different concentration of biochar based on their potential biomarkers. (D) Volatile composition of *M. arvensis* plant grown in soil containing different concentration of biochar. Each column represents the individual group of plant, whilst each row represents the individual volatile compound detected in different group of *M. arvensis*.