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Research Article

Effects of biochars on wheat yield, soil enzyme activity, and soil productivity parameters

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ABSTRACT- To improve crop and soil productivity, residues of major agricultural and horticultural crops, namely grape waste, wheat straw, and brown walnut shell, were converted into biochars and applied to soil at a rate of 2% (w/w). The experimental treatments included control soil (CS), soil amended with grape waste biochar (GSB), soil amended with wheat straw biochar (WSB), and soil amended with brown walnut shell biochar (NSB). Both biochar-amended and non-amended soils were used for wheat cultivation under pot conditions. Soil enzyme activities, were measured. In addition, soil parameters and plant parameters were evaluated. The results showed that application of GSB significantly increased soil ammonium, nitrate, available phosphorus, and organic carbon concentrations compared with the control treatment. Nitrate concentration increased during wheat growth, whereas ammonium, available phosphorus, and organic carbon concentrations decreased over time. Biochar application increased the activities of all enzymes except phosphatases. Invertase activity in the GSB and WSB treatments increased significantly compared with the control. The addition of GSB increased urease activity by 1.4-fold relative to the control treatment. The geometric mean of enzyme activity (GMEa) was higher in the GSB and WSB treatments than in the other treatments. Based on biochar effects, the percentage of enzyme change (Rch) followed the order GSB > WSB > NSB. The lowest enzyme resistance index was observed in the GSB treatment, whereas the highest was recorded in the NSB treatment. All biochar-amended soils exhibited higher thousand-grain weight and wheat grain yield compared with the control.

INTRODUCTION

In arid and semi-arid regions such as Iran, due to the climatic conditions and lack of water, little vegetation exists, which minimizes the carbon content entering the soil. In this situation, plant production and soil fertility become endangered (Bastida et al., 2008). The use of organic amendments obtained from organic wastes in different soils has been widely studied. One of these organic modifiers is biochar. Biochar has wide-ranging applications (Schmidt and Wilson 2012), but in agriculture, it has been mostly used as a soil additive. In fact, the great significance of biochar is because of its potential to approach agriculture sustainability thorough boosting crop growth, decreasing fertilize runoff, soil remediation, carbon sequestration, and other environmental positive effects (Almaroai and Eissa 2020; Bai et al. 2022; Jatav et al. 2020). Biochar is characterized by a large surface area, high porosity, and numerous surface functional groups. These highly stable residues are strongly resistant to decomposition and may have applications for pollutant removal and soil conditioning as well (Brassard et al. 2019). As an amendment, biochar improves soil aggregation, porosity, and water-holding capacity (Adekiya et al. 2020). The influence of different biochars on soil can vary depending on the source, technique, and temperature of production which all may be linked to the biochar components (Yaashikaa et al. 2020). Biochar is usually produced with raw materials like crop residues, grass, different manures, tree woods, thin branches, plant litter, etc. (Shaaban and Abid 2021).

A significant proportion of climate change and soil carbon degradation is caused by chemical fertilizers and manure. Gao and Cabrera Serrenho (2023) documented that carbon dioxide emissions from these materials have reached approximately 9.6 gigatonnes per year. Furthermore, excess levels of phosphates and nitrates in conventional fertilizers may lead to long-term and cumulative damage to the environment (Craswell 2021). While it has been reported that, manure-based biochar not only draws CO_2 from the atmosphere, reduces CH_4 emotion, and thus increases carbon fixation (due to the unique adsorption properties), but also increases the efficiency of soil nutrients (Bates 2010; Jatav et al. 2020; Rehman et al. 2020). Biochar addition has raised crop yields in different studies. Almaroai and Eissa (2020) reported that biochar improved nutrient uptake by the

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crop, tomato yield, and vegetable quality on metal-polluted soil. It also decreased metal accumulation in the fruit considerably (≥ 20%). Craswell (2021) proved that biochar could improve the phosphorus use efficiency of organic-inorganic fertilizers, maize-wheat productivity, and soil quality on low-fertility soil.

Biochar can also influence microbial activities which in turn affect soil organic matter, nutrient release, and fertility maintenance (Brassard et al. 2019). All these parameters can be assessed by enzyme activity which is a key factor in biogeochemical cycles of soil and then it is considered to be an index of soil fertility (Liu et al. 2021). The correlation between enzyme activities and biochar use has been proved in many research (Jiang et al. 2021; Jing et al. 2020). In the study by Jiang et al. (2021), biochar significantly enhanced microbial biomass carbon (MBC) and nitrogen content of soil and enzymatic activities as well. It seems that biochar increases MBC through the changes in activities of extra- and intracellular enzymes on specific soils (Pokharel, et al., 2020). Zhang et al., (2021a) indicated that the activity of nitrogen-cycling enzymes increased by roughly 20% by biochar addition.

Wheat is a vital crop for global food security, serving as a major dietary component and calorie source for humans (Sadok et al., 2019). Among Middle Eastern countries, wheat yield in Iran (about 2 tons per hectare) is lower than in countries such as Kuwait, Saudi Arabia, Turkey, Cyprus, and Iraq, indicating the need for measures to increase its yield and production (FAO, 2020). In 2020, the leading provinces for irrigated wheat production were Khuzestan (17%), Fars (13%), Golestan (7%), Khorasan Razavi (6.5%), Kermanshah (5.5%), and Hamedan (4.5%) (Ministry of Agricultural Jihad of Iran). Increasing production productivity is crucial to meet growing global food demand given resource constraints (Majiwa et al., 2018). Efficient use of production resources is crucial for sustainable agriculture and reduced resource depletion (Masuda, 2016; Shanmugam and Venkataramani, 2006). In this regard, programs and policies should be oriented towards increasing productivity and reducing environmental impacts.

Wheat production in Iran has exceeded 10 million tons in the last few years (Esfahani 2022). Recycling this wide source of wheat straw can be overpriced and burning it can lead to the greenhouse gas. Accordingly, wheat straw pyrolysis, which produces high-value biochar and helps enhance carbon sequestration, has greatly fascinated researchers. The wheat straw-derived biochar is also highly stable and can be a strong pollutant absorbent (Chen et al. 2022; Wang et al., 2020).

Two other most common horticultural products in Iran (especially in Hamedan province) include walnut and grape. It has been reported that Hamedan is the biggest walnut producer in Iran (Fallah et al. 2022). Hence, walnut wastes like walnut shells are found abundantly. The same condition can be observed for grape residues, since; the production area for grapes in Iran is about 309,000 ha (more than three million tons of grapes) which makes this country the 11th biggest producer of grapes in the universe (Motalebifard 2022). As a result, the grape residue is a great raw material for making biochar in Iran. It is very important to introduce biochar to use these wastes to reduce environmental problems and increase soil fertility.

On the other hand, the low organic matter of calcareous soils in arid and semi-arid regions like Iran is an important fertility problem that may be solved by using biochar. Therefore, this pot study was designed to understand and compare the effects of the grape waste biochar (GSB), wheat straw biochar (WSB), and brown walnut shell biochar (NSB) on wheat yield, soil enzyme activity, and soil productivity parameters in different stages of wheat growth duration.

MATERIALS AND METHODS

Soil sampling

The soil samples were prepared from a wheat field in Kabudarahang, Hamedan, Iran. The coordinates were at a longitude of 48°43' East and latitude of 35°36' North (depth of 0-20 cm). The soil samples were air dried and sieved through 2 mm screens. The chemical properties of soil were measured (Table 1).

Chemical analysis

Chemical experiments carried out on the soil sample were as follows: Electrical conductivity (EC) and pH of the soil sample (1:5 water extraction) were determined using an EC meter, (Jenway 4310), and pH meter, (Metrohm 744), respectively (Rowell 1994). Calcium carbonate ($CaCO_3$)-equivalent was determined with the acid neutralization method (Rowell, 1994). To evaluate soil organic carbon (SOC) of the sample and treatments, Walkley–Black analysis was applied (Nelson and Sommers 1996). The total nitrogen of soil was measured by the Kjeldahl method (Jones and Benton 1991), and total phosphorus was determined based on the yellow color spectroscopic method (Pierzynski 2000). Ammonium and nitrate were determined using the methods proposed by Tan (2005) and Jones and Benton (2001), respectively. Olsen method was applied to estimate available phosphorus in the soil samples (Olsen and Sommers 1982).

Biochar preparation and characteristics

To produce biochar, each plant biomass was washed using distilled water and dried at room temperature. Then, it was pyrolyzed at 400 °C for 2 hours. The oxygen level was very low during this procedure. The prepared biochars were stored at 70 °C for 24 hours in the oven dried completely, and finally pounded and passed through a 0.5 mm sieve. A high-resolution scanning electron microscope (SEM) was employed to provide the pictures of biochar. A dispersive X-ray analyzer (EDX) was also applied to detect the elemental composition of biochars, and Fourier transform infrared spectroscopy (FTIR-Bomem MB) was used to identify their surface functional groups (Fig. 1, Fig. 2, and Fig. 3).

Electrical conductivity (EC) and pH of the biochars were measured in a 1:20 water extraction (Sun et al., 2014). To determine the total phosphorus (TP) content of biochars, the samples were heated at 550 °C for 24 hours in an electric oven and then the TP was extracted from

the prepared ash samples using hydrochloric acid (2 M), and eventually was analyzed using yellow color spectroscopic method (Pierzynski 2000). The total carbon and nitrogen of biochars were evaluated in ash samples the Leco Carbon Analyzer and Kjeldahl method (Jones 1991), respectively. The chemical characteristics of biochars are depicted in Table 2.

Preparing biochar treatments

Biochar treatments in this study were as follows: soil without biochar as control; soil mixed with wheat straw biochar or WSB (soil and wheat straw were sampled from the same field); soil mixed with walnut shell biochar or NSB (collected from Tuyserkan, Hamedan, Iran); soil mixed with grape residue or GSB (collected from vineyards of Malayer, Hamedan, Iran).

Pot study

To conduct a wheat pot study, 1500 g of the soil sample was mixed with 2% biochar. Triplicate pots were applied for every treatment besides control in a completely randomized block design. The following fertilizers were mixed with all the treatments to simulate field conditions: urea, 0.3 g pot^{-1}; triple superphosphate, 0.15 g pot^{-1}; and potassium sulfate, 0.075 g pot^{-1}. Superphosphate and potassium sulfate were added before plantation, but urea was added in three stages (one-third during seedbed preparation, one-third at the stem elongation stage, and the rest of it at flowering). It was selected based on farmers' application rates (recommended dose for crop cultivation). A winter cultivar of wheat (Pishgam) was chosen for the pot study. Pishgam wheat is recommended for cultivation in cold provinces like Hamedan and areas with limited late-season irrigation, particularly those using sprinkler systems, due to its high cold tolerance and disease resistance. Wheat seeds were soaked in distilled water for 24 hours and then transferred to Petri dishes where they all germinated after two days. After germination, the Petri dishes were stored in the fridge for 21 days at 4 °C (physiological temperature) for vernalization and finally transplanted in the pots (11 seeds per pot). Following plant establishment, the plants in pots were thinned (8 per pot). The research took place in a 700 m greenhouse at 25 °C. Ten irrigation cycles were performed during the wheat growth period. Soil sampling was performed at the following different growth stages: tillering (January 30th), stalk elongation (February 13th), heading (March 11th) flowering (March 18th), and harvest stages (April 22nd). Soil samples were organized in two sets. A set of samples were dried and kept for all experiments (except for enzyme analysis). Another set was stored in sealed containers at a certain soil moisture level at 4 °C for enzyme experiments.

Enzyme activity measurements

Invertase

To assay invertase activity, sucrose was used as a substrate. Soil samples were kept at 50 °C for 3 hours (pH = 5.5). The produced ferric hexacyanoferrate (II) in this process was measured by the colorimetric method (Schinner et al. 2012).

Acid phosphatase and alkaline phosphatase

To assay acid phosphatase and alkaline phosphatase activities, 1 mL 4-nitrophenyl phosphate (as a substrate) was mixed with 1 g soil. Afterwards, a 4 mL buffer solution (pH 6.5 for acid phosphatase and pH 11 for alkaline phosphatase) was added to the mixture. To make a control sample, just 4 mL buffer solution was added to 1 g soil. All the samples were incubated at 37 °C for 1 hour. After adding 1 mL calcium chloride and 4 mL sodium hydroxide to the samples and mixing them, the produced color was assayed using the colorimetric method (400 nm wavelength). Acid phosphatase and alkaline phosphatase were calculated using the following equation (Schinner et al., 2012):

(S - C) * 10 = μg.Np.$g^{-1}.h^{-1}$

(S): Average amount of sample (μg.Np)

(C): Average amount of control (μg.Np) *Urease*

Urease activity was measured by the Margesin method (Schinner et al. 2012). Nine mL Tris buffer and 1 mL urea solution were mixed with 5 g of soil. This mixture was incubated at 37 °C for 2 hours and then 35 mL of potassium chloride was added to it this suspension was shaken for 30 min and eventually distilled water was added to it to a final volume of 50 mL. After passing through a filter paper, 20 mL of the solution was transferred to a distillation flask and mixed with 0.2 g of manganese oxide. After 3.3 min distillation of the sample, ammonium was analyzed in it using sulfuric acid.

Some quality indicators of the soil were calculated using absolute enzyme activity. These indicators were:

A) Geometric mean of enzyme (GME) (Hinojosa et al. 2004)

$$GME = \sqrt[4]{Invertase + ACP + ALP + urease}$$ Eq. (1)

Acid phosphatase = ACP

Alkaline phosphatase = ALP

B) Geometric mean of enzyme activity (GMEa) (Paz-Ferreiro et al. 2012)

$$GMEa = \sqrt[4]{Invertase * ACP * ALP * urease}$$ Eq. (2)

C) The percentage of enzyme changes (Relative change-Rch) about the control soil was calculated according to the formula provided by Chaer et al. (2009).

$$Rch = \left(\frac{T}{C} - 1\right) * 100$$ Eq. (3)

T: Average enzyme activity in the soil after adding biochar

C: Average enzyme activity in the control sample

D) The resistance index (RS) was determined based on the activity of the enzyme based on the method of Orwin and wardels (2004):

$$RS = 1 - \left[\frac{2\,|D_0|}{C_0 + |D_0|}\right]$$ Eq. (4)

$D_0 = C_0 - P_0$

C_0: Enzyme activity in control soil

P_0: Enzyme activity in soil after adding biochar

RS value is from -1 to +1.

Assessment of plant parameters

Wheat Height, seed setting rate, the length of panicles, productive spikelet numbers, thousand seed weight, wheat yield, and straw yield were analyzed in the grown wheat plants. The distance between the soil surface and the tip of the plant, before harvest, was indicated as wheat Height (cm). A ruler was applied to measure the panicle length (cm), as well. The remaining wheat straw after removing the panicles was placed in the oven at 105 °C for 1 hour, and then at 65 °C for 24 hours. During this procedure, the straw reached a steady weight that was measured as straw yield. Productive panicle numbers were also counted. To determine thousand-grain weight (TGW), the grains (after wheat harvest) were dried in the oven at 65 °C for 24 hours to have a steady weight, and then TGW was assayed. The seed setting rate (%) was also calculated from the number of productive panicles/the number of seeds per panicle.

Statistical analysis

The statistical analysis of data was done using SPSS 23.0 for mean value comparison, Duncan test ($P < 0.01$) was performed and Microsoft Office Excel 13.0 was applied to draw the figures.

Table 1. Soil chemical properties

SOM	TN	TP	NH_4^+-N	NO_3^--N	Available P	$CaCO_3$	EC	pH
(%)	(%)	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(%)	(dSm^{-1})	
1.31	0.11	230	32.2	28.7	16.1	16.5	0.21	7.8

EC: Electrical conductivity; TP: Total phosphorus; TN: Total nitrogen; SOM: Soil Organic Matter.

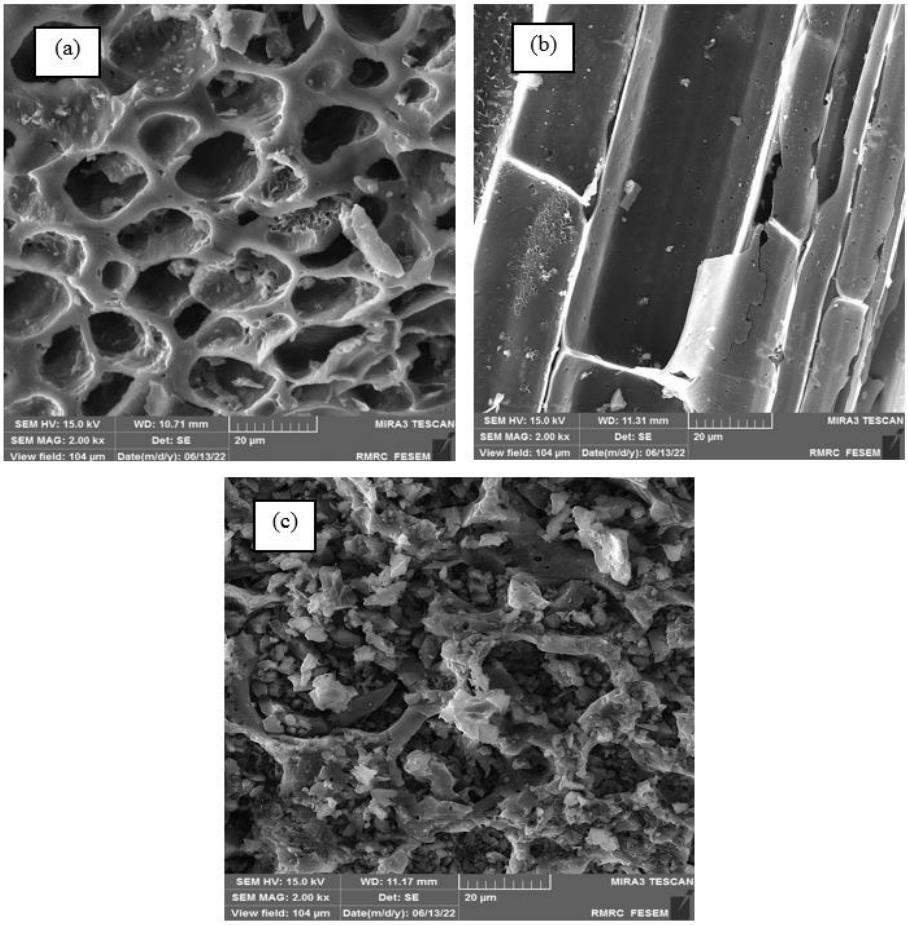

Fig. 1. Scanning Electron Microscopy (SEM) images of biochars, (a) grape residue biochar, (b) wheat straw biochar, and (c) walnut shell biochar.

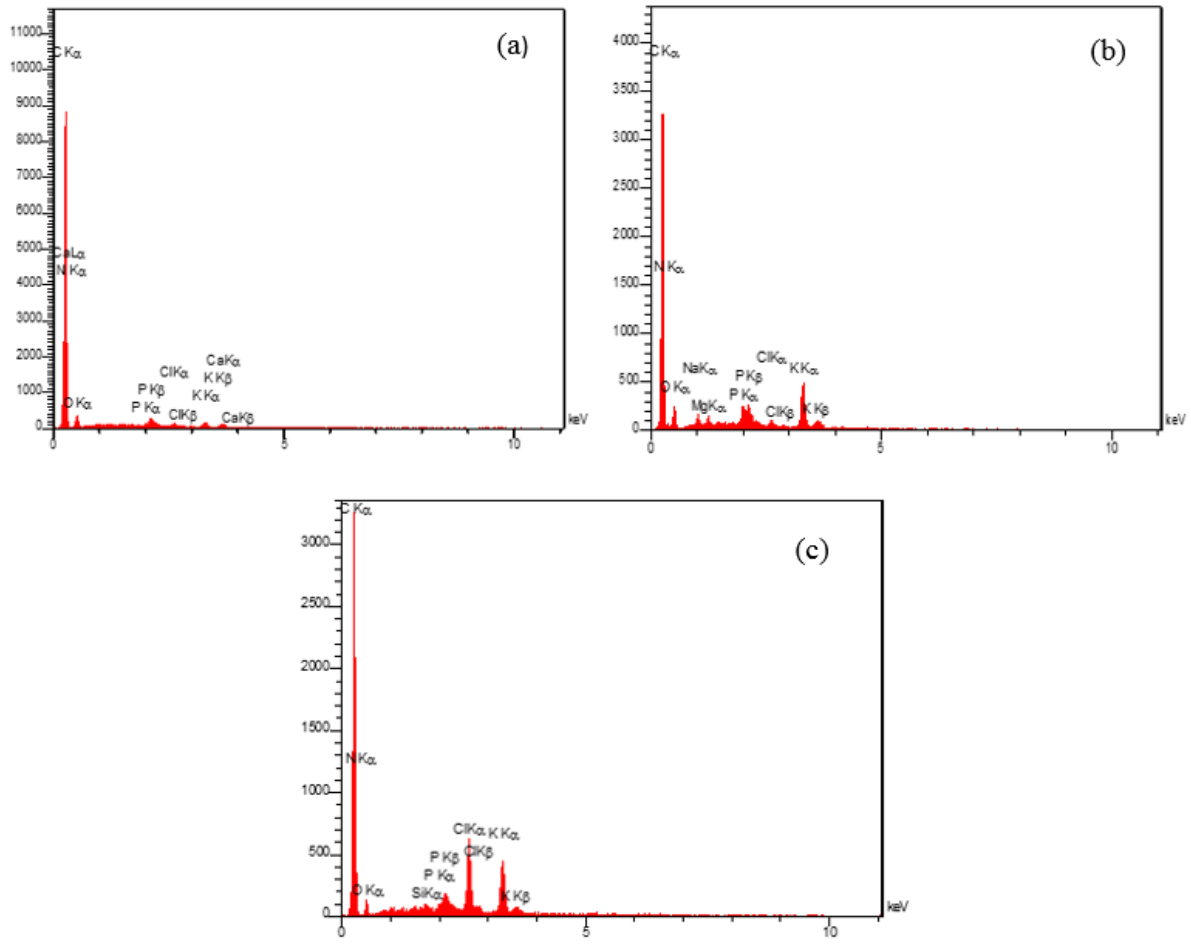

Fig. 2. Energy Dispersive X-Ray Spectroscopy (EDX) graphs of biochars, (a) grape residue biochar, (b) wheat straw biochar, and (c) walnut shell biochar.

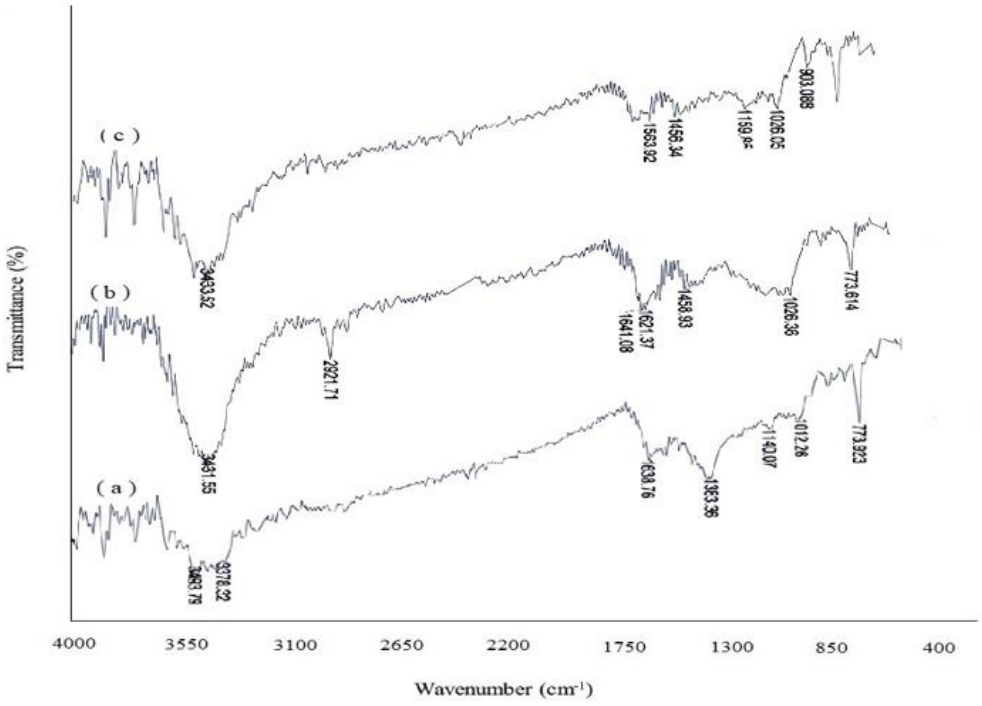

Fig. 3. Fourier Transform Infrared Spectrometer (FTIR) of biochars, (a) grape residue biochar, (b) wheat straw biochar, and (c) walnut shell biochar.

Table 2. Chemical properties of used biochars

Biochar type	pH	EC	TN	TP	TC
		(dS m^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})
Grape waste biochar	9.7	2.3	9.8	63.7	937.0
Wheat straw biochar	9.9	1.8	6.3	6.9	467.0
Walnut waste biochar	7.5	0.52	7.7	4.7	422.0

EC: Electrical conductivity; TN: Total nitrogen; TP: Total phosphorus; TC: Total carbon.

RESULTS AND DISCUSSION

SEM, EDX, and FTIR analyses of biochars

The porous structures of biochars were recognizable in SEM images (Fig. 1), which consisted of a wide range of pores in variable sizes. The abundant pores of grape residue biochar were shaped like a bee hive (Fig. 1a), but the number of pores in the NSB seemed to be fewer and they were bigger than the GSB ones (Fig. 1c). Overall, the largest pores were observed in WSB (Fig. 1b).

The walnut biochar exhibited a honeycomb-like structure, possibly due to the tubular remnants of plant cells (Nartey and Zhao, 2014). Hemati Matin et al. (2020) observed spongy, filamentary polygon- and skew polygon-like porous structures in almond and walnut brown shell biochar particles. These structures resulted from volatile gases released during pyrolysis, leading to a brittle skeletal structure with parallel slots in certain biochar parts, possibly due to the component decomposition.

The EDX analysis results indicated that biochars were composed of carbon, nitrogen, potassium, and phosphorus (Fig. 2). Although the quantitative analysis suggested that the amount of composed elements in GSB was three times higher than that detected in other biochars (Fig. 2a).

Piash et al. (2021) used EDX analysis to determine the elemental composition of fertilizer and wood-derived biochar, reporting calcium, carbon, and oxygen contents of 9.79%, 39.66%, and 18.88%, respectively.

Surface functional groups of used biochars are displayed in Fig. 3. Overall, five functional groups were identified in biochars. An aromatic C-H stretch was noticed at 400 to 800 cm^{-1}. The aliphatic C-O single bond that occurred at 1026 cm^{-1} was also one of the main chemical structures in biochars which was related to aliphatic esters and cellulose and hemicellulose polarity. At 1400 cm^{-1} the aromatic C-O bond, corresponded to lignin, produced a peak (Rasoulpoor et al., 2020). In addition, a carbonyl C=O stretch appeared at 1600 cm^{-1}, (Tipson and Cohen, 1968) and the O-H bond showed absorption at 3400 cm^{-1} which led to a broad peak (Fleming and Williams, 1966).

Chen and Chen (2009) observed bands in orange peel biochar spectra indicative of -OH (3000-3690 cm^{-1}), CH_2 (2927 and 1446 cm^{-1}), aromatic C=C, and C=O (1613 cm^{-1}). Biochar spectra peaks have been attributed to methyl C-H stretching compounds (2916 cm^{-1}), carbonyl/carboxyl C=O (1699 cm^{-1}), aromatic C=C and C=O (1595 cm^{-1}), and aliphatic C-O-C and alcohol –OH (1030 cm^{-1}) (Sun et al., 2011; Trazzi et al., 2016).

Soil properties

The amounts of soil NH_4^+-N and NO_3^--N were significantly different among treatments at five stages of wheat growing (Fig. 4). The highest levels of NH_4^+-N in all samples were related to the samples taken in tillering stage (9-16.5 mg kg^{-1}, $P < 0.05$), and after this stage ammonium decreased drastically in all treatments (Fig. 4a). As it was predicted, a reverse trend was observed for the levels of NO_3^--N in samples (Fig. 4b). The lowest nitrate concentration was reported in tillering stage for all treatments (12.03-14.20 mg kg^{-1}, $P < 0.05$), and the highest NO_3^--N content was related to the soil samples in harvest stage. During the whole period of plant growth, the GSB treatment was indicated to contain the maximum level of ammonium and nitrate (16.5 and 27.8 mg kg^{-1}) followed by the WSB treatment (20.1 and 13.7 mg kg^{-1}). The lowest levels of NH_4^+-N and NO_3^--N were reported for the control in all steps of growth.

This study investigates the use of grape residue and walnut shell biochars, rarely studied in Iran, to improve wheat yield. Results indicated that biochar treatments increased soil nitrogen levels, in both tested forms, compared to the control. This demonstrates biochar amendments can significantly impact the soil nitrogen cycle. For example, nitrite (NO_2^-) release and thus nitrogen loss decreased in soils amended with biochar. It has been related to the fact that ammonium and nitrate are absorbed by biochar functional groups and this elevates the retention capacity of nitrogen in the soil (Zhang et al. 2021b). This additive can also boost microbial activity, which in turn, provides more available nitrogen for plants. Nitrogen-fixing, nitrification, denitrification, and ammonification (the processes involved in the nitrogen cycle) are mostly done by soil microorganisms and thus biochar can positively influence them by enhancing the number and diversity of soil microbial communities (Liao et al. 2020). GSB treatment had the maximum level of nitrate and ammonium among all samples. It may be associated with the biochar characteristics or its higher nitrogen content compared to other treatments. The positive effect of wheat straw biochar on soil nitrogen has been reported in many studies (Jing et al. 2020; Qu et al., 2012).

The respond of soil available phosphorus (AP) to biochar addition is shown in Fig. 5. GSB treatment contained the highest AP amount among all treatments during all stages of wheat growth (14.6-60.5 mg kg^{-1}, $P < 0.05$). The WSB and NSB treatments showed a significant ($P < 0.05$) increased AP content from tillering to stalk stages compared with the control, but there were no significant differences in soil AP among these treatments and control from heading, flowering, and harvest stages ($P \geq 0.05$). Additionally, a downward trend in soil AP was observed in all samples from the tillering to the harvest stage which was more pronounced for GSB treatment.

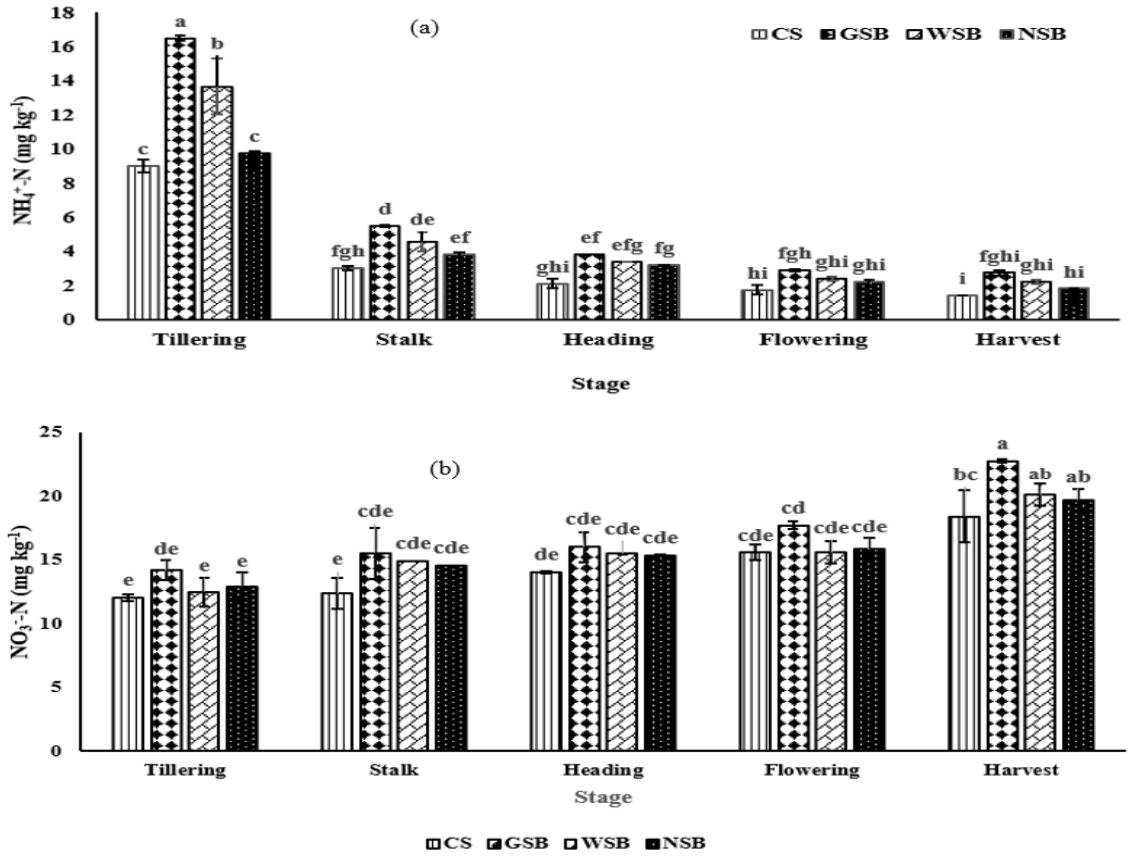

Fig. 4. (a) NH_4^+-N and (b) NO_3^--N contents on biochar-amended and non-amended soils in different wheat growth stages (values accompanied by different letters are significantly different within columns at the level of $P < 0.05$) (CS, GSB, WSB, and NSB, respectively control soil, soil treated with grape residue biochar, soil treated with wheat straw biochar, and soil treated with brown walnut shell biochar).

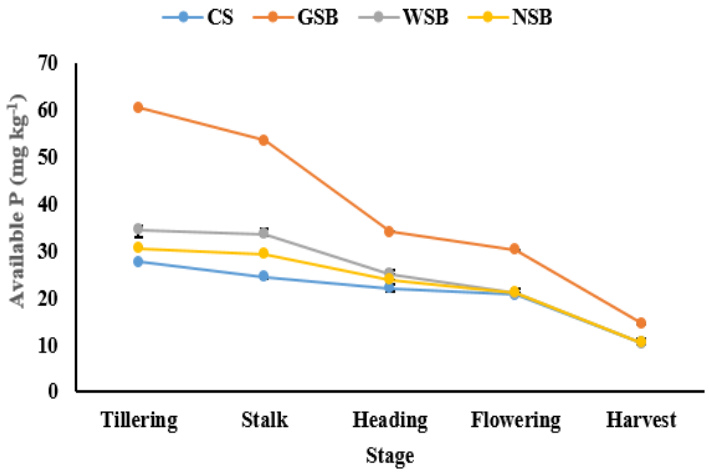

Fig. 5. Available phosphorus content on biochar-amended and non-amended soils in different wheat growth stages (CS, GSB, WSB, and NSB, respectively control soil, soil treated with grape residue biochar, soil treated with wheat straw biochar, and soil treated with brown walnut shell biochar).

Biochar use can have contradictory effects on the soil available phosphorus (AP) (Nobaharan et al., 2021). In this study, biochar increased AP in all samples, but GSB indicated the greatest effect on soil AP among all biochars. This can be due to the highest phosphorus content of this biochar among all samples. Yao et al. (2021) and Kong et al. (2021) demonstrated that biochar amendment significantly enhances soil phosphorus availability. Accordingly, apart from indirect impacts on AP, biochars can also act as a source of bioavailable P in soil. It has been reported that biochar increases labile or available fractions of phosphorus which are mostly absorbed by plants and thus reduced over time (Wang et al., 2014). This may also explain the decline in available phosphorus over the growth duration of wheat. Biochar addition can impressively alter all biochemical reactions of the soil phosphorus cycle in

different ways. It influences phosphorus sorption, desorption, speciation, and the activity of soil microbial communities taking part in the phosphorus cycle. Overall, the fixation of phosphorus in soil makes less than 20% of the phosphorus fertilizers accessible to plants (Ghodszad et al., 2021). It has been proven that the functional groups on biochar surfaces may stabilize phosphorus as well, but some studies demonstrated that this stable phosphorus may turn into a long-term source of phosphorus which releases it gradually and makes it soluble for plants (Torres-Dorante et al., 2005), and meanwhile has the potential to diminish phosphorus loss on soil runoff (Feng et al., 2017). However, the findings on the role of biochar in phosphorus leaching from soils are inconsistent and a considerable number of investigations reported increased soil phosphorus loss after biochar amendment (Madiba et al., 2016; Yang et al., 2021; Yuan et al., 2016).

All biochars increased SOC at the beginning of wheat growth compared with the control (Fig. 6), although the effects of GSB and WSB were more significant among all treatments (1% vs. 0.9% in control, $P < 0.05$). The SOC level declined over time for all biochar-treated soils besides control, and thus, the lowest contents of soil organic carbon were detected in the samples acquired at the end of a growth cycle. The reduction rate of SOC during the growth cycle was lower for GSB than for other treatments (0.9-1%).

The SOC was significantly higher in soils treated with biochars than control. Among all biochars, GSB had the highest efficacy in terms of soil carbon enrichment probably because of higher carbon content than other treatments. A principal role of biochar in soil is increased carbon sequestration. Extensive research has documented the positive influence of biochar on soil organic carbon (Mukherjee and Zimmerman, 2013). A ten-year experiment by Gross et al. (2021) indicated that SOC increased by 29% on average after the application of biochar at different levels. This amount was about 75% in pot experiments. They also reported that plant-based biochar is more pronounced in rising SOC than biochar made from animal feces given higher C/N. On the other hand, the organic carbon of the treatments dropped as plants grew further which can be the result of biochar decomposition on the soil. Similar findings were reported by Jing et al. (2020) using straw biochar on paddy soil.

Soil enzyme activity

The activity of the tested enzymes reacted differently to biochars. Urease, alkaline phosphatase, and acid phosphatase were enhanced significantly in most treatments getting close to the harvest stage (Fig. 7, Fig. 8, and Fig. 9). However, there was no significant difference between the alkaline phosphatase contents of control samples collected in stalk, heading and flowering stages. Additionally, alkaline phosphatase activities in all biochar-treated soils did not alter significantly during the heading and flowering stages (March) ($P < 0.05$). The activity of acid phosphatase was also constant throughout the last two stages of growth for GSB treatment. And no difference in this enzyme was reported during heading and flowering between GSB and WSB. Altogether, the samples of the harvest stage had the highest concentrations of these three enzymes, and the lowest amounts of them were observed at the start of the growth season. Contrary changes were detected in invertase activity (Fig. 10). As a result, for all treatments, the lowest and highest activity of this enzyme were observed in the last and first days of the growth period, respectively.

Although in some cases the differences among the activity of the phosphatase enzymes were not statistically significant for different treatments, alkaline phosphatase and acid phosphatase activities showed the highest range in control followed by NSB treatment. Grape waste biochar was also found to lead to lower activities of these enzymes in soil compared with other biochars. Urease and invertase activities were positively influenced most by GSB treatment ($P < 0.05$). On the other hand, an increase in this enzyme activity was found to be induced by walnut-based biochar less than other residues.

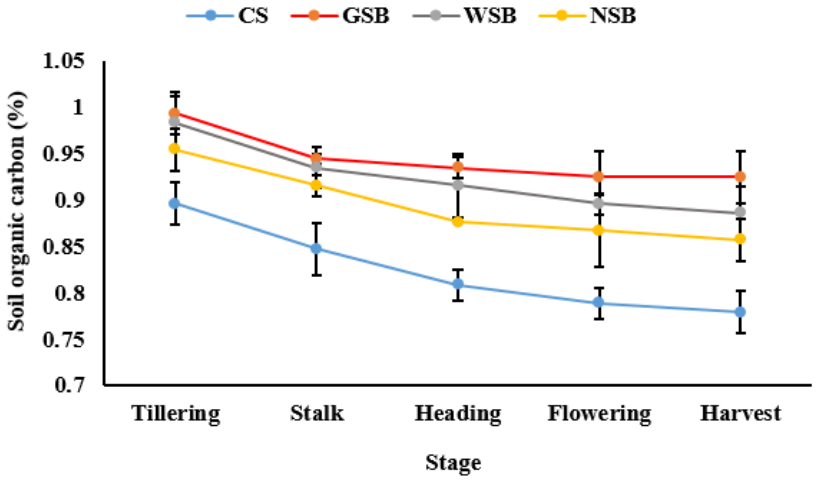

Fig. 6. Soil organic carbon of biochar-amended and non-amended samples in different wheat growth stages (CS, GSB, WSB, and NSB, respectively control soil, soil treated with grape residue biochar, soil treated with wheat straw biochar, and soil treated with brown walnut shell biochar).

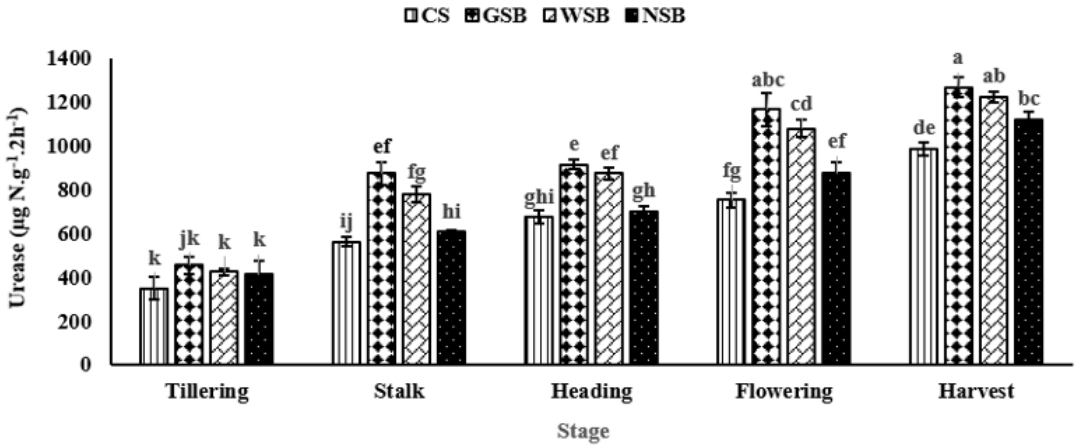

Fig. 7. Urease activity on biochar-amended and non-amended soils in different wheat growth stages (Values accompanied by different letters are significantly different within columns at the level of $P < 0.05$) (CS, GSB, WSB, and NSB, respectively control soil, soil treated with grape residue biochar, soil treated with wheat straw biochar, and soil treated with brown walnut shell biochar)

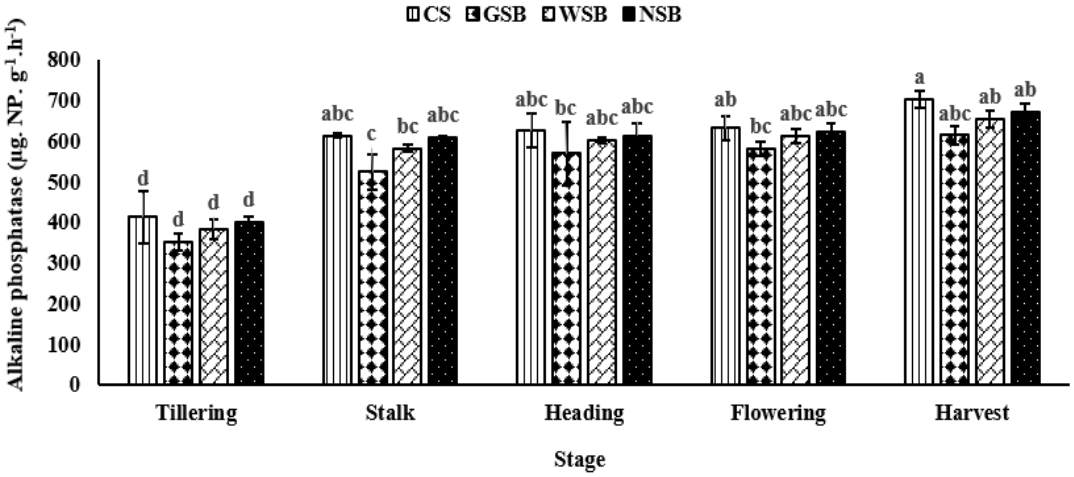

Fig. 8. Alkaline phosphatase activity on biochar-amended and non-amended soils in different wheat growth stages (Values accompanied by different letters are significantly different within columns at the level of $P < 0.05$) (CS, GSB, WSB, and NSB, respectively control soil, soil treated with grape residue biochar, soil treated with wheat straw biochar, and soil treated with brown walnut shell biochar).

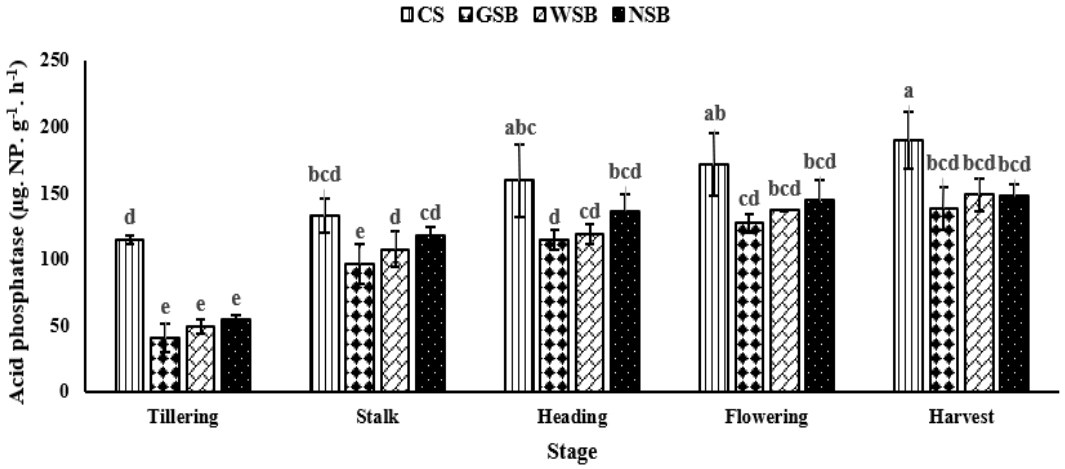

Fig. 9. Acid phosphatase activity on biochar-amended and non-amended soils in different wheat growth stages (Values accompanied by different letters are significantly different within columns at the level of $P < 0.05$) (CS, GSB, WSB, and NSB, respectively control soil, soil treated with grape residue biochar, soil treated with wheat straw biochar, and soil treated with brown walnut shell biochar)

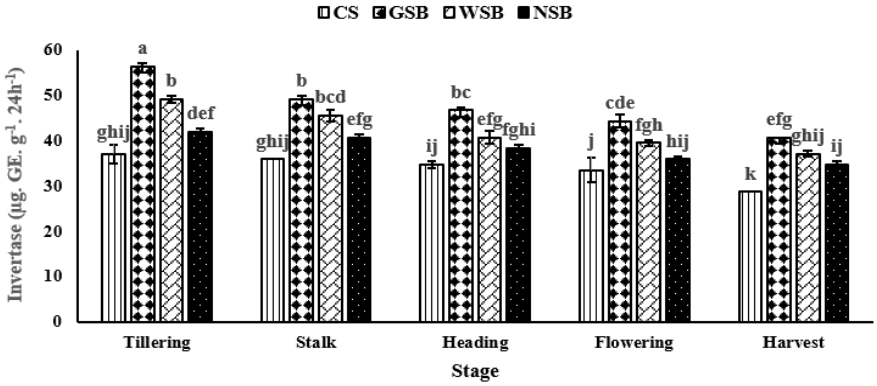

Fig. 10. Invertase activity on biochar-amended and non-amended soils in different wheat growth stages (Values accompanied by different letters are significantly different within columns at the level of $P < 0.05$) (CS, GSB, WSB, and NSB, respectively control soil, soil treated with grape residue biochar, soil treated with wheat straw biochar, and soil treated with brown walnut shell biochar).

The response of enzyme activity to biochar highly depends on the enzymes and biochar type. Biochars that are richer in carbon and nitrogen (GSB in the present study) may induce more remarkable changes in extracellular enzyme activities and subsequently provide plants with higher rates of nutrients (Oladele, 2019). The dosage of biochar and the incubation time are also factors affecting enzyme activity (Oladele, 2019). In this study, alkaline and acid phosphatase decreased with biochar addition. It may be related to the functional groups on the biochars which could inhibit enzyme reaction with absorbed substrates (Czimczik and Masiello, 2007). Although, the dynamics of soil phosphatases can usually reflect the rate of phosphorus mineralization of the soil, in the present study the content of available phosphorus and the activity of phosphatases correlated negatively in most stages of wheat growth (Table 3). Acid phosphatase correlated with pH negatively last three growth stages (Table 3), but alkaline phosphatase significant correlated with it only in the harvest stage. The dependence of phosphatase enzymes on soil pH has been proved in other studies (Mazorra, Rubio, and Blasco 2002). Overall, increased available phosphorus in soils immediately after biochar use could be considered a direct result of soil amendment with phosphorus-rich additives. On the other hand, as the AP declined with time, enhanced alkaline and acidic phosphatase activities were observed and they peaked in the last days of growth. It is assumed that plant phosphorus demand triggers phosphatase production by them (Nannipieri et al., 2012).

The function of invertase is to break down sucrose into simple sugars which in turn increase the microbial population of soil (Hu et al., 2011). In the present study, a positive correlation was found between invertase and organic carbon, available phosphorus, and ammonium throughout growth seasons (Table 3). These findings have been supported by other researchers as well (Liu et al., 2021). Hence, decreased invertase activity with time could be predicted based on the change patterns of OC, AP, and NH_4^+ in samples. Decomposed biochars seem to limit invertase substrates over time and cause a decrease in this enzyme activity (Martens, et al., 1992). The variable effects of different biochars on this enzyme activity were also affected by the concentrations of carbon, nitrogen, and phosphorus of biochars and thus the highest invertase activity among all samples occurred in grape waste biochar-amended soils. Urease, as an enzyme that degrades soil organic matter, significantly correlated with SOC, AP, and NH_4^+ contents of samples in all stages, except for tillering samples (Table 3). GSB treatment having the highest organic carbon and phosphorus among treatments accelerated urease activity more than WSB and NSB. Increased urease activities by rice residue biochar have been reported in another study (Oladele 2019). Biochars with high porosity and surface area provide habitats for soil microorganisms. They directly increase microbial populations by serving as a food source and indirectly by creating a large internal surface area that enhances organic matter absorption, resulting in more diverse microbial habitats (Ghodszad et al., 2021). In arid and semi-arid soils with low biological activity, biochar amendment can enhance microbial diversity, population, activity, and enzymatic activity (Zhang et al., 2020).

In the majority of cases, GME and GMEa enhanced significantly ($P < 0.05$) during wheat growth days, as presented in Fig. 11 and Fig. 12. These indices reached the highest rate in the last phase of cultivation (6.6-6.7 and 247.3-257.7, respectively). However, there were no significant differences in GME among treatments in the tillering and the harvest stage. All treatments also had the same GMEa in the tillering stage, but it started to elevate significantly over time. In cases where the differences among biochar effects on the GME and GMEa were significant, grape waste and wheat straw biochars showed the highest rates of these indexes during the growth of wheat. There was a positive correlation between urease and GME in all sampling times (Table 3). NH_4^+-N and SOC also positively correlated with this index in some growth stages ($r = 0.37$-0.91, $P < 0.05$). However, the results of the correlation between soil parameters and GMEa were so inconsistent in different stages of growth and the positive correlation between urease and this index was observed only in stalk elongation and flowering stages ($P < 0.05$ and $P < 0.01$, respectively) (Table 3).

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

Authors should discuss the results and how they can be interpreted from the perspective of previous studies

and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

Two geometric indices (GME and GMEa) were used for a better understanding of the relation between investigated enzyme activities and biochar use. GME and GMEa are considered to be sensitive multiparametric indicators of soil quality which reflect both enzyme and microbial activities (Yu et al. 2019). Based on the results, both (especially GMEa) were positively affected by all kinds of biochars and time. Increased GMEa after straw biochar addition has also been displayed (Jing et al., 2020). These two indices are greatly sensitive to soil variation, and thus, are usually applied for the evaluation of soil quality after using amendments like biochars, land use change, and other management practices (García-Ruiz et al., 2008). GMEa can also be a practical index to combine information on different enzymes having various ranges and units (Wang et al., 2012). In the present survey, GSB and WSB proved to increase GMEa which confirmed the advantages of these biochars for SOC and soil fertility.

Rch showed that urease and invertase were the enzymes that were stimulated after the application of all three types of biochar (Fig. 13). This index for urease varied from 11.7% in NSB treatment to 40.8% in GSB treatment and for invertase from 12.7% in NSB treatment to 39.4% in GSB treatment. The negative value of Rch for acid and alkaline phosphatase enzyme activity was recorded after the application of all three biochars (-21.7 to -32.6 for acid phosphatase and -2.2 to -11.6 for alkaline phosphatase). The value of the Rch index for the tested enzymes was as follows:

Acid phosphatase < alkaline phosphatase < invertase < urease

Considering the effect of biochar, the value of the Rch index was as follows:

GSB > WSB > NSB

Rch shows the direct effect of experimental factors (inhibition or activation) on soil enzyme activity (Lemanowicz et al., 2021). According to the obtained results, two enzymes, urease, and invertase, were stimulated after the application of all three biochars. The value of Rch for acid and alkaline phosphatase was negative throughout the growth period. Wojewodzki et al. (2023) investigated the effect of using biochar obtained from different plant biomass wastes (one-month compost biochar, pine bark biochar, began needle biochar, pine cone biochar, and maple leaf biochar) to improve soil fertility and activity enzyme and increased carbon deposition concluded that protease was the only enzyme stimulated by biochars. The value of Rch for protease ranged from 0.5% (for soil treatment + pine bark biochar) to 57.84% (for soil treatment + one-month compost biochar). Negative Rch values for acid phosphatase enzyme were recorded after the application of all biochars (-4.49 to -37.49%). Lemanowicz et al. (2021) by investigating the effect of saline soils (layers S1-S8) on some chemical properties and on the activity of selected enzymes, concluded that the value of Rch for catalase enzyme in layers S1, S2, S3, S4, and S5 increased compared to the control and decreased in S6, S7, and S8 compared to the control, which ranged from 2.88 to 63.94 percent. Rch values for acid phosphatase were negative in all layers.

The values of R_S presented in Fig. 14 show that the sensitivity of enzymes to adding biochar varies during the growth period. The highest value of R_S was observed in the NSB treatment (average urease R_S = 0.79, average invertase R_S = 0.78, average acid phosphatase R_S = 0.65, and average alkaline phosphatase R_S = 0.85). The lowest value of R_S was observed in the GSB treatment. It means that the enzymatic activity of the soil has increased more with the addition of this biochar compared to other biochars (medium alkaline phosphatase R_S = 0.76, medium acid phosphatase R_S = 0.57, medium invertase R_S = 0.44, and urease R_S = 0.44). Regardless of the type of biochar used, the lowest resistance to alkaline phosphatase, acid phosphatase, and invertase was seen in the tillering stage and urease in the flowering stage.

The stability (resistance and flexibility against a disturbance) of the soil system is the key factor that affects the characteristics and processes of the ecosystem. To compare the stability of different systems, it is necessary to have indicators that provide a relative quantitative measure of both resistance and flexibility of a response variable in all possible states. This index accurately shows the response of soil properties (for example, microbial biomass) to a disturbance and can determine the difference in stability between different soils (Orwin and Wardle 2004). In this study, the highest enzyme resistance (Rs) was observed in NSB treatment and the lowest in GSB treatment. Higher values of the RS index show that biochar has a minor effect (maximum resistance) on the activity of soil enzymes and the activity of these enzymes has not changed much with the addition of biochar, and lower values of the index indicate that biochar has a great effect (at least resistance) on the activity of soil enzymes and has increased their activity (Orwin and Wardle 2004).

Plant parameters

Productivity parameters of wheat for different treatments are shown in Table 4. Overall, biochar use increased thousand-grain weight and grain yield significantly. Plant height was enhanced in all biochar treatments compared with control, but the difference between grape residue biochar and control was significant. Among all biochars, GSB was demonstrated to be the most effective biochar in increasing the mentioned parameters ($P < 0.05$). The highest panicle length was also observed in soils amended with grape residue biochars (7.3 cm). Conversely, amendments reduced the number of productive spikelets in GSB and NSB treatments (7 vs. 7.4 in other soils). GSB and NSB also had the lowest amounts of straw yield among treatments (1.74 and 1.86 g pot^{-1}, respectively). All biochars caused a significant reduction in the setting rate and thus the highest rate of this factor was observed in the non-amended soil (14.2%).

Table 3. Pearson correlation coefficient between soil properties in different biochar-amended samples in different stages of wheat growth

	SOC	Invertase	Ap	Alkaline	Acid	NO_3	NH_4	Urease	GME	GMEa	pH
Tillering											
SOC	1	**0.75****	0.50	-0.20	-0.69*	0.28	0.75**	0.44	0.04	-0.33	-0.30
Invertase		1	0.87**	-0.52	-0.73**	0.53	0.93**	0.43	-0.11	-0.41	0.65*
AP			1	**-0.61***	**-0.68***	0.57	0.84**	0.40	0.005	-0.17	-0.71**
Alkaline				1	0.25	-0.32	-0.37	-0.21	0.55	0.39	0.25
Acid					1	-0.60*	-0.54	-0.43	0.03	0.72**	0.11
NO_3						1	0.23	0.42	0.009	-0.52	-0.33
NH_4							1	0.36	-0.02	-0.16	-0.70*
Urease								1	0.66*	0.15	-0.22
GME									1	0.61*	-0.06
GMEa										1	0.08
pH											1
Stalk											
SOC	1	**0.84****	0.62*	-0.41	0.10	0.59*	0.59*	0.64*	0.63*	0.35	-0.71**
Invertase		1	0.87**	-0.68*	0.34	0.45	0.81**	0.86**	0.80**	0.45	-0.81**
AP			1	**-0.68***	**-0.60***	0.49	0.65*	0.80**	0.71**	0.32	0.73**
Alkaline				1	-0.48	0.15	0.56	-0.84**	-0.66*	-0.38	0.41
Acid					1	0.15	0.55	0.52	0.39	-0.11	-0.23
NO_3						1	0.55	0.32	0.48	0.29	-0.40
NH_4							1	**0.90****	0.91**	0.62*	-0.43
Urease								1	0.95**	0.61*	-0.52
GME									1	0.78**	0.41
GMEa										1	-0.007
pH											1
Heading											
SOC	1	**0.61***	0.64*	-0.24	-0.52	0.49	0.75**	0.69*	0.48	0.17	-0.77**
Invertase		1	0.75**	-0.51	-0.52	0.15	0.80**	0.83**	0.46	0.34	-0.71**
AP			1	-0.18	**-0.63***	0.49	0.68*	0.71*	0.56	0.34	0.70*
Alkaline				1	0.33	-0.30	-0.35	-0.38	0.31	0.31	0.13
Acid					1	-0.18	-0.57	-0.51	-0.08	0.44	**-0.64***
NO_3						1	0.44	0.36	0.17	-0.05	-0.45
NH_4							1	**0.75****	0.51	0.26	-0.93**
Urease								1	0.73**	0.42	-0.82**
GME									1	0.80**	-0.66*
GMEa										1	-0.28
pH											1
Flowering											
SOC	1	**0.76****	0.53	-0.18	-0.45	0.53	0.83**	0.69*	0.67*	-0.58*	-0.84**
Invertase		1	0.74**	-0.21	-0.63*	0.26	0.91**	0.75**	0.70*	0.58*	0.69*
AP			1	**-0.62***	-0.44	0.61*	0.71**	0.63*	0.52	0.38	0.47
Alkaline				1	0.35	-0.49	-0.27	-0.43	-0.19	0.06	0.35
Acid					1	0.50	-0.57	-0.39	0.20	-0.15	**-0.60***
NO_3						1	0.52	0.46	0.40	0.39	-0.31
NH_4							1	**0.75****	0.70*	0.58*	-0.74**
Urease								1	0.96**	0.77**	-0.72**
GME									1	0.91**	-0.64*
GMEa										1	-0.44
pH											1
Harvest											
SOC	1	**0.86****	0.50	-0.85**	-0.52	0.60*	0.88**	0.66*	0.37	0.23	-0.82**
Invertase		1	0.62*	-0.71**	-0.55	0.55	0.93**	0.81**	0.64*	0.46	-0.88**
AP			1	**-0.60***	-0.40	0.41	0.74**	0.59*	0.43	0.12	-0.49
Alkaline				1	0.56	-0.54	-0.81**	-0.65*	-0.26	0.05	**0.67***
Acid					1	-0.34	-0.53	-0.69*	-0.37	0.39	**-0.70***
NO_3						1	0.44	0.39	0.19	0.09	-0.31
NH_4							1	**0.80****	0.58*	0.35	-0.86**
Urease								1	0.88**	0.28	-0.82**
GME									1	0.57	-0.62*
GMEa										1	-0.23
pH											1

SOC (soil organic carbon), AP (available phosphorus), Alkaline (alkaline phosphatase), Acid (acid phosphatase), GME (Geometric mean of enzyme), and GMEa (geometric mean of enzyme activity). *$P < 0.05$. **$P < 0.01$.

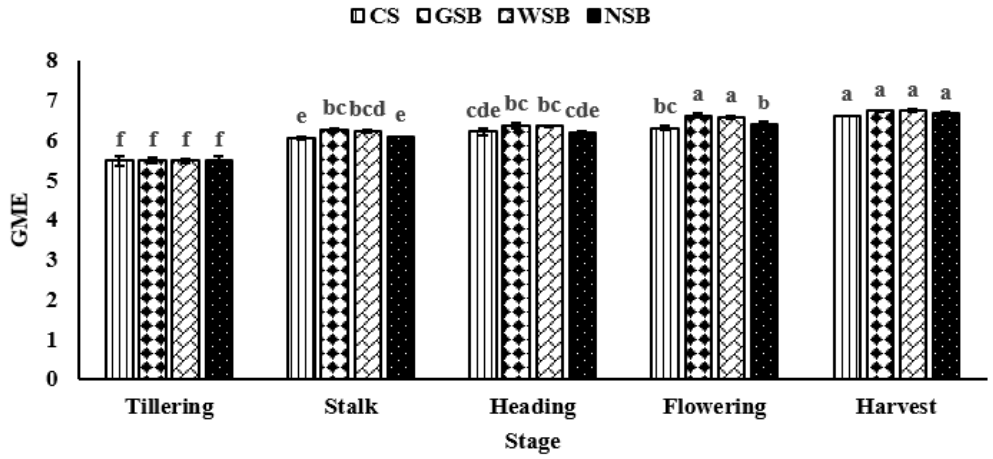

Fig. 11. Geometric mean of enzyme (GME) on biochar-amended and non-amended soils in different wheat growth stages (Values accompanied by different letters are significantly different within columns at the level of $P < 0.05$) (CS, GSB, WSB, and NSB, respectively control soil, soil treated with grape residue biochar, soil treated with wheat straw biochar, and soil treated with brown walnut shell biochar)

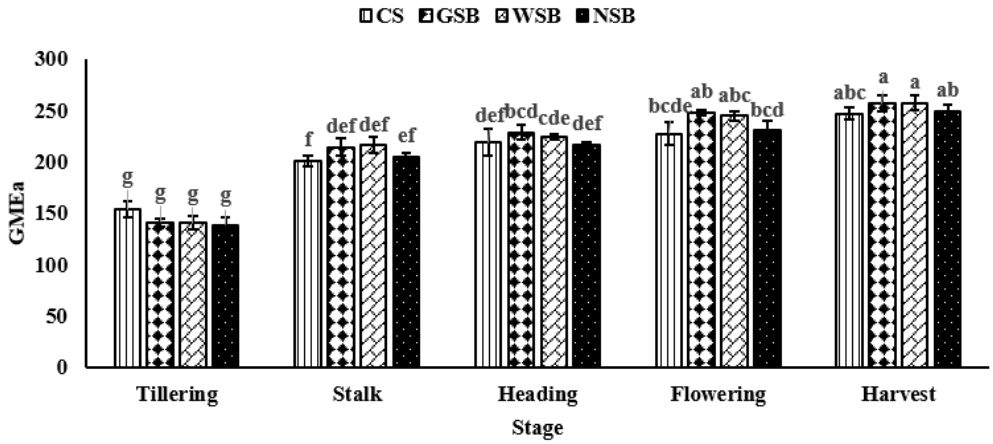

Fig. 12. Geometric mean of enzyme activity (GMEa) on biochar-amended and non-amended soils in different wheat growth stages (Values accompanied by different letters are significantly different within columns at the level of $P < 0.05$) (CS, GSB, WSB, and NSB, respectively control soil, soil treated with grape residue biochar, soil treated with wheat straw biochar, and soil treated with brown walnut shell biochar).

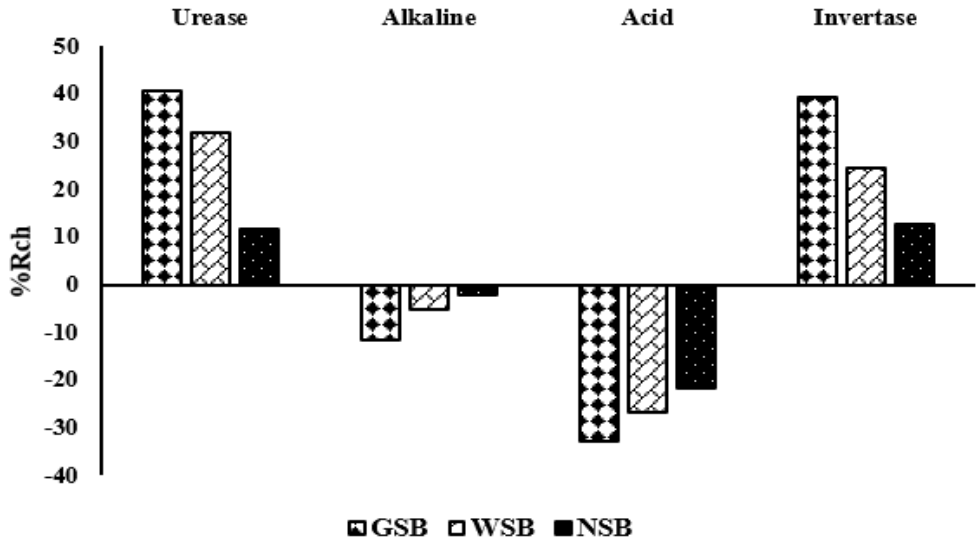

Fig. 13. Ratio of enzyme changes (Rch) during the growth period (GSB, WSB, and NSB are soil treated with grape residue biochar, soil treated with wheat straw biochar, and soil treated with brown walnut shell biochar, respectively). Alkaline (alkaline phosphatase) and Acid (acid phosphatase).

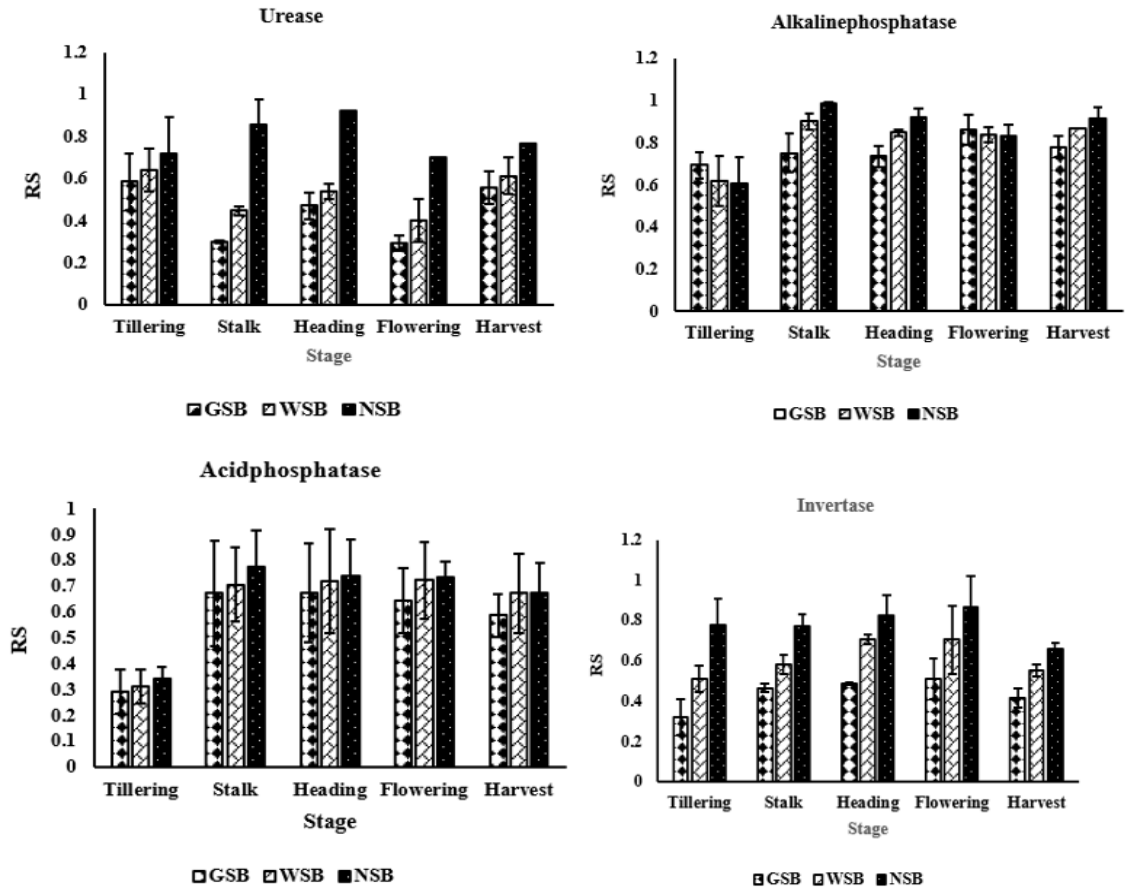

Fig. 14. Enzyme resistance (RS) during the growth period in different treatments (GSB, WSB, and NSB, respectively soil treated with grape residue biochar, soil treated with wheat straw biochar, and soil treated with brown walnut shell biochar).

Table 4. Biochar effects on wheat productivity parameters

Treatment	Number of productive spikelets	Setting rate (%)	Thousand-grain weight (g)	Grain yield (g pot^{-1})	Panicle length (cm)	Plant height (cm)	Straw yield (g pot^{-1})
CS	7.4^{a}	14.2^{a}	39^{c}	2.24^{d}	5.2 ± 0.40^{b}	32.1 ± 1.18^{b}	4.37^{a}
GSB	7^{a}	4.5^{c}	51^{a}	6.87^{a}	7.3 ± 0.37^{a}	37.0 ± 0.58^{a}	1.74^{b}
WSB	7.4^{a}	6.3^{b}	48^{ab}	5.49^{b}	5.3 ± 0.29^{b}	34.9 ± 1.10^{ab}	4.20^{a}
NSB	7^{a}	7.5^{b}	44^{bc}	3.60^{c}	5.6 ± 0.30^{b}	33.1 ± 0.72^{b}	1.86^{b}

CS, GSB, WSB, and NSB, respectively control soil, soil treated with grape residue biochar, soil treated with wheat straw biochar, and soil treated with brown walnut shell biochar (values accompanied by different letters are significantly different within columns at the level of $P < 0.01$).

Although biochars produced negative effects on setting rate and straw yield for unknown reasons, these additives had a positive effect on most wheat productivity parameters. This effect was significant on thousand grain weight, grain yield, panicle length, and plant height especially on GSB-treated soil which could match the higher nitrate, ammonium, available phosphorus, and organic carbon of grape residue biochar than those in other biochars. Generally, biochar can also accelerate nitrogen (Fallah et al., 2022) and phosphorus (Mian et al. 2021) uptake by plants which in turn promotes the growth of plants. In research by Pourmansour et al. (2019) biochar use (1.25%, w/w) could enhance wheat height by about 12%. They also noticed that 1.2% was the appropriate rate of biochar addition and higher rates showed a negative effect on plant parameters because of salinity caused by biochar. Zaheer et al. (2021) used biochar to mitigate the detrimental effects of drought stress on wheat and observed an increase in wheat yield on biochar-amended soil. In an investigation conducted by Sun et al. (2019) grain yield of wheat improved by about 3-20% after using 5-20 t ha^{-1} straw biochar, but higher rates of addition were not recommended. Similar results for wheat were also observed by Curaqueo et al. (2021) using oat hull and pine bark biochars.

CONCLUSION

Identifying solutions to mitigate the adverse effects arising from the excessive use of fertilizers in Iran is crucial. Soil amendment with biochar represents an affordable and environmentally beneficial approach. This study focused on biochars produced from the most common crop residues in Hamedan, Iran, and evaluated their effects on soil properties

and wheat performance. The highest concentrations of NH_4^+–N, available phosphorus, and soil organic carbon across all treatments were observed at the tillering stage, whereas the highest NO_3^-–N concentrations occurred at the harvest stage. Throughout the entire growth period, the grape waste biochar treatment consistently exhibited the highest levels of ammonium, nitrate, available phosphorus, and organic carbon. Although contradictory responses were observed for some measured parameters, the overall positive effects of the applied biochars on most soil and plant characteristics justify further investigation, particularly through field studies. Such studies could be designed to examine different biochar application rates and a wider range of crops. Given that the effects of brown walnut shell biochar and wheat straw biochar were not significant in some cases, future research should consider applying higher rates of these biochars produced under different pyrolysis temperatures. In addition, assessing the long-term impacts of brown walnut shell biochar and grape waste biochar, as well as their potential applications in the remediation of contaminated soils, is essential.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization: Zahra Varasteh Khanlari; Methodology: Zahra Varasteh Khanlari and Ali-Reza Kazemi; Software: Zahra Varasteh Khanlari and Ali-Reza Kazemi; Validation: Zahra Varasteh Khanlari, Ali-Reza Kazemi, and Mahboubeh Zarabi; Investigation: Zahra Varasteh Khanlari, Ali-Reza Kazemi, and Mahboubeh Zarabi; Resources: Zahra Varasteh Khanlari; Data curation: Zahra Varasteh Khanlari and Ali-Reza Kazemi; Writing—original draft preparation: Zahra Varasteh Khanlari; Writing—review and editing: Zahra Varasteh Khanlari, Ali-Reza Kazemi, and Mahboubeh Zarabi; Visualization: Zahra Varasteh Khanlari; Project administration: Zahra Varasteh Khanlari.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ETHICAL STATEMENT

All authors have approved the manuscript and agree with the submission to "Iran Agricultural Research" Journal. Manuscript was prepared in compliance with Ethics in Publishing Policy and we used Guide for Authors in preparing submitted manuscript.

DATA AVAILABILITY

Not applicable

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