

EFFECT OF FISH WASTE-DERIVED BIOCHAR AS A PHOSPHORUS-RICH FERTILIZER ON THE FENNEL (*FOENICULUM VULGARE*) CV. BALADI GROWN ON ALKALINE SOIL

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Abstract

Purpose: Finding natural alternatives to mineral fertilizers, especially in calcareous and alkaline soils, is essential for agricultural sustainability. A suitable alternative for fixing phosphorus (P) in alkaline soils is still unclear. Therefore, acidified fish waste biochar (ABC), which is rich in P and available, cheap, and eco-friendly, was used to improve the availability of P in alkaline soils. **Methods:** This biochar was added at a rate of 1% (BC1), 2% (BC2), and 3% (BC3) compared to super phosphate as chemical fertilizer (CF) in addition to the control (CK). **Results:** The outcomes demonstrated that ABC improves soil chemical characteristics. In comparison to the control, nitrogen (N), phosphorus (P), and potassium (K) were more readily available. The highest available values of N, P, and K were recorded for BC3, while the lowest one was observed with the control treatment. It was observed that N, P and K uptake with CF increased by 31.27, 53.43, and 8.43%, respectively, and the corresponding values were 222.13, 387.90, and 93.93% at the highest biochar rate (BC3). **Conclusions:** The incorporation of ABC improved fennel plant growth characteristics due to suitable management practices. It might be concluded that the application of ABC up to 3% (BC3) improved alkaline soil fertility and fennel crop growth. This study is an attempt to overcome the problem of P unavailability in alkaline soils, and long-term ABC application under field conditions is recommended as a future direction to confirm the findings.

Key words: Acidified biochar; Alkaline soil, Fish waste, Fennel crop, Soil properties.

Introduction

One of the most crucial nutrients is phosphorus (P), followed by nitrogen. It plays a significant and crucial role in the plant, which is evident in plant growth (energy transfer, photosynthesis, the conversion of sugars and starches, the flow of nutrients, and the transfer of genetic traits). Generally, soil P exists in organic or inorganic forms in equilibrium among different phases (Ahmad *et al.*, 2009). In alkaline soils, available P is low due to its fixation as insoluble phosphates, which is mainly affected by high soil reaction (pH) and calcium carbonate content (especially in calcareous soil), although the total P content is high (Cordell *et al.*, 2011).

Globally, calcareous soils are widespread in arid and semi-arid regions and constitute more than one-third of the Earth's surface area. In Egypt, it represents about 25-30% of the total area, according to the Ministry of Agriculture, with a high pH (8.5–9.0) not commensurate with P availability, especially if it contains basic cations (Taalab *et al.*, 2019). This type of soil also contains very low organic matter and low nutrient content, which are the main

reasons for low productivity from its potential yield (Islam *et al.*, 2021). Currently, global food security is under-challenged by an over-increasing human population due to poor soil nutrients and low fertility, which seriously affect crop productivity. Therefore, amendment of calcareous soil with organic materials is crucially important to enhance organic matter content and soil fertility by reducing soil pH in order to increase P availability in the soil, leading to increased crop yield. Additionally, organic materials are also used to reduce global warming (Islam *et al.*, 2022).

Biochar is an important organic material that is a carbon-rich product resulting from the pyrolysis of organic raw material in the absence or presence of little oxygen to convert organic carbon into more stable forms (Song *et al.*, 2019; Pariyar *et al.*, 2020; Li *et al.*, 2024). Besides its organic matter content and nutrients, research has shown that biochar has the potential to improve soil physicochemical and biological properties, prompting suitable conditions for plant growth and nutrient uptake (El-Naggar *et al.*, 2019; Diatta *et al.*, 2020). It also retains soil macronutrients, whether native and/or those added via biochar application, through improved absorption capacity

and increased biochar surface area, which resulted in the slow release of soil nutrients and subsequently enhanced plant uptake (Karimi *et al.*, 2020; Awad *et al.*, 2020).

Biochar may increase P availability via regulating soil pH, changing the adsorption or uptake of certain chelates, and promoting P-solubilizing bacteria (Gao *et al.*, 2016). Therefore, this practice is more suitable to reduce chemical fertilizer utilization and increase nutrient use efficiency (NUE) (Qian *et al.*, 2019). This approach cuts two ways: an indirect way that gradually improves soil properties by increasing stable C, nutrient acquisition, and efficient P use. While the direct way increases organic matter content and nutrient availability (Awad *et al.*, 2022). In addition, using biochar as an alternative organic fertilizer might overcome the excessive use of chemical fertilizers and their high cost (Mukherjee *et al.*, 2014; Kim *et al.*, 2018). Also, adding biochar increased soil anion exchange capacity, enhancing its ability to retain P and its availability and uptake (DeLuca *et al.*, 2015).

Chemically, activated biochar may be a potential process to increase special functional groups by boosting its sorption capacity and its ability to adjust soil acidity to be suit for P (Bhatnagar & Sillanpaa, 2011). The introduction of energizing modifications of biochar, such as acidification, can increase its active sites on a surface, which may increase anion uptake (Bhatnagar *et al.*, 2013). Acidified biochar before the pyrolysis process with HNO_3 and H_3PO_4 acids caused a reduction in soil pH values from 9.38 to 8.57 and from 9.14 to 8.62, respectively. A significant reduction in biochar pH (4.10) as a result of acid additions after pyrolysis may be a beneficial process for use in alkaline calcareous soils, as may increase water-soluble P concentrations (Sahin *et al.*, 2017). Therefore, using acidified fish waste biochar enrich-P is considered an alternative to mineral phosphate fertilizers and may contribute to safe and sustainable agriculture. Fish waste composed mainly of heads, backbones and guts can make up more than half of the total fish by products generated from fish processing. These wastes represent a source of some important minerals, including phosphorus and calcium. However, these wastes are either discarded or sometimes can be incorporated into poultry and fish feeds based on their palatability, safety, and local regulations (Chaklader *et al.*, 2023). On the other hand, the biochar produced from these wastes contains these minerals in high concentrations, making it a source of fertilizer alternative to other chemical fertilizers (Abo-Elyousr *et al.*, 2022). Therefore, it was used as an organic fertilizer alternative to the commercial superphosphate (Ahuja *et al.*, 2020).

According to the World Health Organization, 80% of the world's population relies on medicinal herbs for primary health care (Ekor, 2014). In arid and semi-arid regions, fennel (*Foeniculum vulgare*) is considered one of the oldest spices and the most powerful medicinal herb in Egypt and the world. It is useful in treating digestive disorders because it contains anti-inflammatory, anti-bacterial, carminative, diuretic, and pain-relieving properties. In addition, it is used to treat nervous problems thanks to its anti-ulcer and antioxidant effects (Noreen *et al.*, 2023).

Applications of biochar in alkaline soil almost increase soil pH, which leads to reduced P availability (Awad *et al.*, 2022 Osman *et al.*, 2022). Therefore, our hypothesis is based on producing acidified biochar (ABC) derived from fish waste

that is P-rich, as an alternative source to mineral phosphate as a chemical fertilizer, which may increase P availability and enhance fennel plant growth. Also, biochar can release P slowly and enhance plant growth as well as alkaline soil properties. Therefore, this study aims to characterize the impact of these rates on nutrient availability and vegetative growth of the Fennel-Baladi plants under calcareous soil conditions, as well as assess the changes in soil properties as a result of adding different rates of fish waste-derived biochar compared to the advised superphosphate.

Materials and Methods

Soil material: The experiment and analyses were carried out in the Faculty of Agriculture, Al-Azhar University, Assiut, Egypt, during the winter season of 2020. Surface soil samples (0–30 cm) were collected from the Experimental Farm, Arab Al-Awamer Agric. Res. Station is located 30 km from the eastern province of Assiut, Egypt ($27^\circ 12' 16.67''$ N latitude and $31^\circ 09' 36.86''$ E longitude). According to FAO (1962), the studied soil samples were Aridisols Typic Haplocalsids which were air-dried and sieved through a 2 mm mesh sieve, ready to be used in the pot experiment and for laboratory analysis. Some physical and chemical properties of the tested soil are listed in Table 1.

Table 1. Some physical and chemical characteristics of the studied soil and biochar

Property	Unit	Soil	Biochar
Sand	(%)	87.70	-
Silt	(%)	8.20	-
Clay	(%)	4.10	-
Texture	---	sandy	-
CaCO_3	(g/kg)	110	0.1
pH (1: 2.5)	---	8.42	4.4
EC	(dS/m)	0.39 (1:2.5)	8.34(1:10)
Organic matter	(g/kg)	3.5	540
Available-N	(mg/kg)	10.3	--
Available-P	(mg/kg)	3.16	913
Available-K	(mg/kg)	53	2.32
Total-N	(g/kg)	0.53	--
Total-P	(g/kg)	0.2	51
Total-K	(g/kg)	0.4	17

Each value represents a mean of three replicates

Biochar preparation and characterization: Air-dried fish wastes (viscera, head, and bones) as a powder were obtained from Al-Sharq Company for Feed, Quesna, Menoufia Governorate, Egypt. In order to produce the biochar, the fish waste was pyrolyzed in an electric oven at 400°C for 3 hours. The biochar materials were then crushed using a stainless steel mill, grounded, and sieved using a 0.80 mm sieve. To increase P content, the biochar was mixed well with a fine rock phosphate (33% P_2O_5) at a ratio of 95 biochar: 5 rock phosphate (W/W) to form a homogeneous mixture. To acidify this stuff (the biochar-rock phosphate mixture), 2 molar of sulfuric acid were added to the mixture in a ratio of 1:1, then stirred with an electric stirrer for 12 hours to simulate the manufacture of superphosphate (Sahin *et al.*, 2017). The acidified mixture was then air-dried for 12 hours, oven dried at 105°C for two hours, grounded again, and then sieved with a 0.80 mm sieve and analyzed for chemical properties (Table 1).

Scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX) spectroscopic analysis were performed using Thermo Fischer Scientific, USA. The Fourier transform infrared (FTIR) spectrum was used to evaluate functional groups on biochar surfaces using ATRFTIR Nicolet 50 of Thermo Fischer Scientific, UK (Cantrell *et al.*, 2012). The SEM micrographs were taken at four different magnifications: 1000, 2000, 5000, and 10000.

Pot experiment: Plastic pots (20 x 25 cm) were packed with 4 kg (dry weight) of the collected soil sample that was treated with 1, 2, and 3% (w/w) of the fish waste biochar (FWBC). These rates were chosen based on the previous study (Osman *et al.*, 2023) as well as to meet the added mineral P doses. The experiment was randomly designed with three replicates. Six seeds of fennel cv. Baladi were planted in each pot on October 15, 2020, under greenhouse conditions. The plants were thinned to two plants per pot at 14 days after planting (DAP). The recommended fertilizer doses of nitrogen (N) as ammonium nitrate (33.5%) at a rate of 290.5 kg ha⁻¹ and potassium (K) in the form of potassium sulfate (48%) at a rate 119 kg ha⁻¹, respectively, were added. The N fertilizer was added in two equal doses, the first dose at 30 DAP and the second dose at 45 DAP, while the K fertilizer was added as a single dose at 30 DAP. The control (CK) treatment received the recommended doses of nitrogen (N) and potassium (K) as the other treatments. The recommended dose (714.3 kg ha⁻¹) of superphosphate (12% P₂O₅) was used as a comparison. The experiment was continued in the greenhouse condition (18–25°C and 60–70% relative humidity) until the maturity stage. The plants were irrigated when the soil moisture content reached 70% of field capacity. The treatments were as follows:

1. Control (without any biochar or fertilizers) CK
2. Recommended dose (superphosphate, 12%) CF
3. 1% biochar BC1 (40 gm)
4. 2% biochar BC2 (80 gm)
5. 3% biochar BC3 (120 gm)

$$\text{Nutrient use efficiency (g/g nutrient)} = \frac{\text{Dry shoot weight at applied N, P or K (g pot}^{-1}\text{)}}{\text{Amount of N, P or K applied with biochar (g pot}^{-1}\text{)}}$$

Chemical analysis: From each pot, soil samples were taken at the end of the experiment, which prepared for the following analyses. Soil particle distribution was carried out using the pipette method, according to Page (1982). A soil extract of 1:2.5 (soil weight/volume of water) was used to measure the biochar and soil acidity. The electrical conductivity (EC) of soil was determined in a 1:2.5 (w/v) extract, while it was measured in the biochar in a 1:10 (w/v) extract as described by Burt (2004). Total and available N was measured using the Kjeldhal method, according to Burt (2004). Available P was determined by a spectrophotometer in a 0.5 M sodium bicarbonate solution at pH 8.5, according to Olsen (1954). Flame photometry used to measure available K in ammonium acetate solution, as described by Jackson (1958). A mixture of H₂SO₄ and H₂O₂ was used to analyze the total N, P, and K content in the soil studied and biochar as described by Parkinson and Allen (1975). According to the protocol followed by Walkley-Black, the organic matter was estimated (Jackson, 1958). Calcium carbonate was determined using a calcimeter, according to Burt (2004).

Plant physiological and growth parameters: Five mL of 95% ethyl alcohol in a test tube at 60°C were used to extract the photosynthetic pigments from a definite fresh leaf sample (5g) until colorless. The volume of the extract was supplemented to 10 ml with 95% ethyl alcohol, and a spectrophotometer was used for chlorophylls and carotenoids. The chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoid concentrations were calculated as mg kg⁻¹ fresh weight at 663, 644, and 452 nm using the following equations that were approved by Al-Sayed *et al.*, (2020).

$$\text{Chl. a} = (13.36 * A_{663}) - (5.19 * A_{644})$$

$$\text{Chl. b} = (27.49 * A_{644}) - (8.12 * A_{663})$$

$$\text{Carotenoid} = \{(1000 * A_{452}) - (2.13 * \text{Chl.a}) - (9.76 * \text{Chl.b})\} \div 209$$

The growth parameters (plant height and thickness, branch number/plant, root length and its thickness) were recorded. At the end of the experiment, the grown plants were collected, divided into shoots and roots, and then weighted to obtain the fresh weight. For dry weight determination, the plant material was washed with deionized water, air dried, and placed in an oven at 65°C for 72 h until it reached a constant weight. The plant sample was ground with a pure steel mill and kept for chemical analysis. Plant samples were taken and washed with deionized water, oven-dried at 70°C, mill-ground, and kept for chemical analysis. Dried grounded plant material of 0.2 g was digested using 10 mL of a mixture of a 7: 3 ratio of sulfuric and perchloric acids (Burt, 2004). Total N was measured in the digested sample by distillation with 20 mL of 40% sodium hydroxide using a micro Kjeldahl's distilling unit (Burt, 2004). Phosphorus was measured in the extract by using the chlorostannous, ammonium-molybdate method, while K was measured by a flame photometer (Burt, 2004). For N, P, and K, nutrient use efficiencies were calculated to evaluate their effects with biochar application according to the following equation:

Statistical analysis

The data were statistically analyzed using CoStat software, and the analysis of variance (ANOVA) as well as the Duncan multiple range tests method were used to test the significance of differences at $p < 0.05$.

Results

Biochar characterization: The SEM micrographs were taken at four different magnifications: 1000, 2000, 5000, and 10000. There was a significant difference in the appearance of the biochar before and after acidification on the SEM micrographs. With higher magnification, the details and pores of the used biochar were observed. At 2000 magnification, the appearance was clear sticky filaments of 3 µm for acidification biochar. This image was more visible with different holes in the biochar surface (Fig. 1a, b).

The elemental analyses (SEM-EDX) of acidified fish waste biochar (ABC) showed that it contains many different mineral elements in high quantities, such as oxygen, calcium, sulfur, phosphorus, sodium, and magnesium, with values of 45.30, 15.09, 12.66, 4.51, 4.09, and 1.25%, respectively (Fig. 1b). The corresponding values of these elements before the acidification process were 18.16, 14.74, 0.74, 3.41, 3.08, and 0.55%, respectively (Fig. 1a). A high value of C content (41.46) was quantified before the acidification process, and it decreased to 11.07% after acidification. The K content remained stable before and after acidification. The contents of other elements were slightly decreased after the acidification process, such as chloride, iron, silicon, and aluminum, which decreased from 5.31 to 1.71, from 1.60 to 1.32, from 1.19 to 0.64, and from 0.56 to 0.22%, respectively.

Effects on soil chemical properties: The ABC had a significant ($p<0.05$) effect on soil reaction (pH), electrical conductivity (EC), and soil organic matter (SOM) (Table 2). Soil pH was significantly decreased by adding ABC at any rate compared to the control. Overall, the lowest soil pH value (7.12) was observed at the highest rate of ABC (3%). The EC values were increased gradually by increasing the ABC level. Likewise, ABC significantly ($p<0.05$) increased SOM compared to control and mineral fertilizer treatments (Table 2). The highest SOM content (14.5 gKg^{-1}) was recorded at the highest ABC level, while the lowest one (1.92 gKg^{-1}) was observed at control treatment (CK). It increased by 14.29% using 3%ABC compared to CF treatment. In addition, total calcium carbonate gradually decreased by adding ABC. It relatively decreased by 9.26, 28.70, 32.40, and 34.61% for the CF, BC1, BC2, and BC3 treatment respectively, compared to the control.

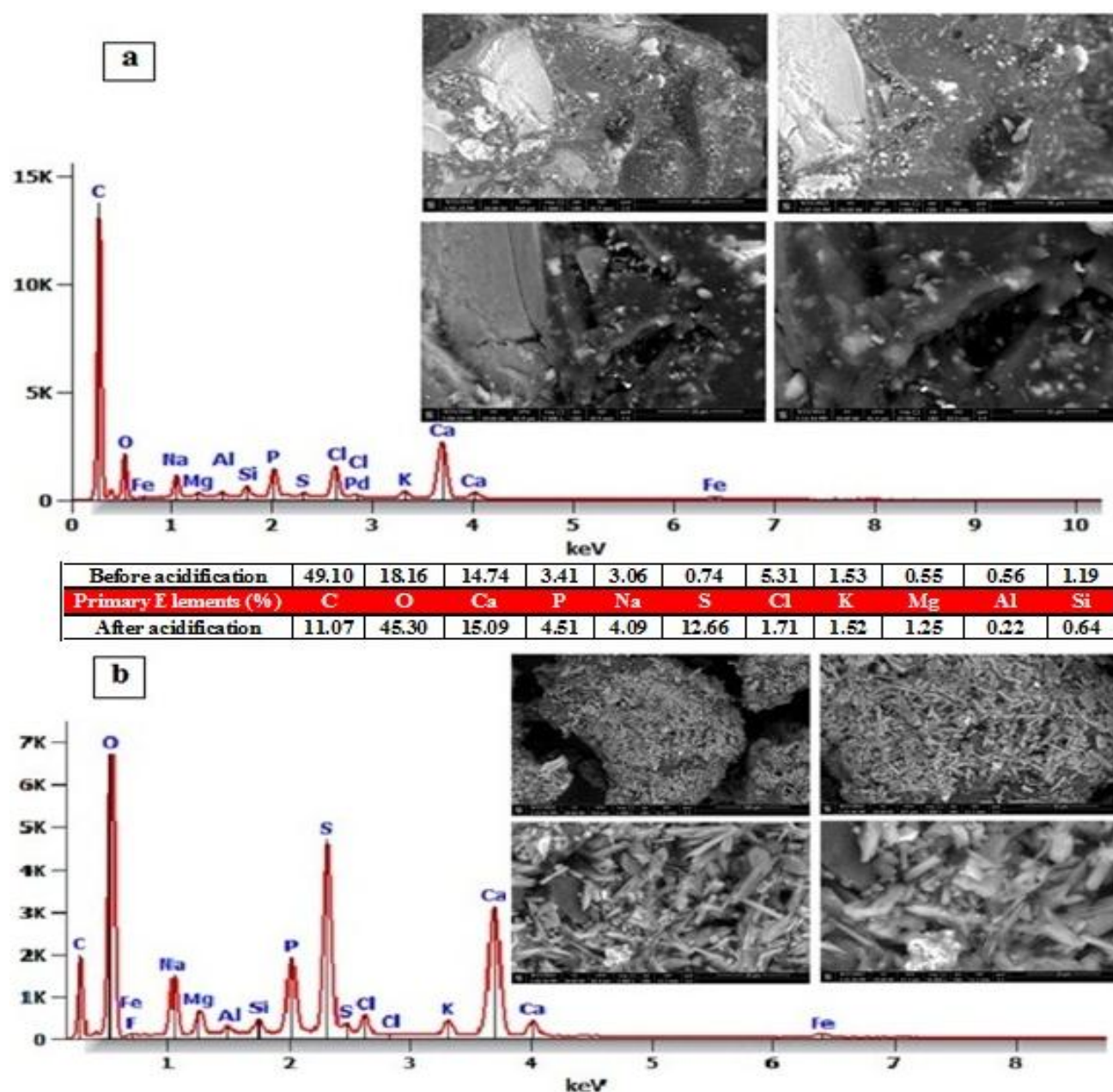


Fig. 1. The energy dispersive X-ray spectra (EDS) of Fish waste-derived biochar shown from the scanning electron microscopy (SEM) before (a) and after (b) activation.

Table 2. Effect of biochar application on soil reaction (pH), electrical conductivity (EC), soil organic matter (SOM) and total calcium carbonate.

Treatments	pH (1:2.5)	EC (1:2.5) (dSm ⁻¹)	SOM (g kg ⁻¹)	Total CaCo3 (%)
CK	8.43 ± 0.04a	0.46 ± 0.01e	3.71 ± 0.04e	10.8 ± 0.40a
CF	8.16 ± 0.02b	0.60 ± 0.02d	4.24 ± 0.04d	9.8 ± 0.16b
BC1	7.58 ± 0.03c	0.91 ± 0.03c	9.78 ± 0.17c	7.7 ± 0.12c
BC2	7.55 ± 0.03c	1.46 ± 0.02b	12.41 ± 0.30b	7.3 ± 0.12cd
BC3	7.12 ± 0.04d	1.95 ± 0.01a	14.29 ± 0.06a	7.06 ± 0.12d

CK, Control (no fertilizer and biochar applied), CF, Chemical fertilizer, BC1 = 1% biochar, BC2 = 2% biochar, BC3 = 3% biochar. Means denoted by the same letter indicate no significant difference according to Duncan's test at $p < 0.05$

Table 3. Vegetative shoot characteristics of Fennel-Baladi (*Foeniculum vulgare*) grown on sandy soil as affected by biochar application.

Treatments	Fresh weight (g pot ⁻¹)	Dry weight (g pot ⁻¹)	Plant height (cm)	Stem diameter (cm)	Branches number
CK	8.97 ± 0.35e	1.67 ± 0.16e	29.0 ± 1.00d	0.43 ± 0.07d	5.0 ± 0.00c
CF	14.56 ± 0.17d	3.50 ± 0.28d	40.0 ± 1.00c	0.50 ± 0.05c	5.6 ± 0.57c
BC1	24.38 ± 0.45c	5.14 ± 0.18c	56.3 ± 1.52b	0.61 ± 0.02b	8.0 ± 0.00b
BC2	29.16 ± 0.22b	5.98 ± 0.26b	59.3 ± 1.15a	0.65 ± 0.05ab	8.6 ± 0.57ab
BC3	32.41 ± 0.73a	6.94 ± 0.14a	60.6 ± 1.15a	0.70 ± 0.05a	9.0 ± 0.00a

CK, Control (no fertilizer and biochar applied), CF, Chemical fertilizer, BC1 = 1% biochar, BC2 = 2% biochar, BC3 = 3% biochar. Means denoted by the same letter indicate no significant difference according to Duncan's test at $p < 0.05$

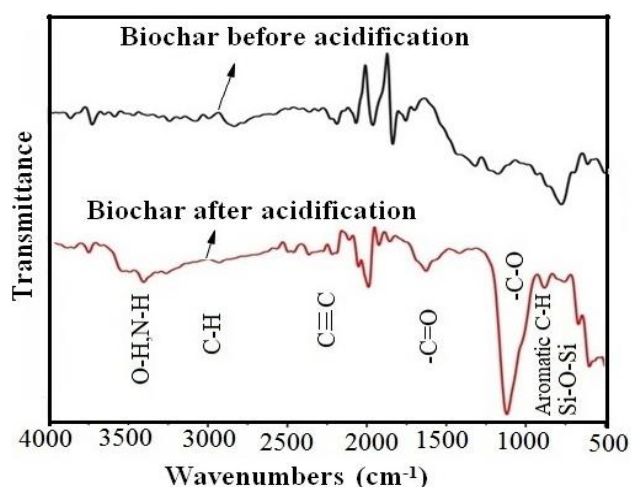


Fig. 2. Fourier-transform infrared (FTIR) spectroscopy of the used biochar.

Effects on nutrient availability and their uptake: The available N, P, and K were significantly ($p < 0.05$) increased due to adding different fertilizers compared to the control (Fig. 3). Although the CF effect on nutrient availability was significantly higher than that of the control, its effect is less than that of the first biochar level (BC1) for all tested nutrients. In general, the data indicated that available NPK increased with increasing biochar addition levels. The highest available values of NPK (38.43, 24.50, and 115.0 mg kg⁻¹, respectively) were recorded for BC3, while the lowest available values of NPK (11.93, 4.69, and 59.30 mg kg⁻¹, respectively) were recorded for control treatment (Fig. 3). The effect of ABC on available NPK could be arranged in descending order: BC3 > BC2 > BC1 > CF > CK.

Also, almost similar results of N, P, and K uptake of Fennel-Baladi plants were detected since NPK uptake was increased at all treatments compared to the control one. The increases were more evident with the increasing BC level (Fig. 3). The amount of N, P, and K uptake at BC3 treatment was the highest compared to other treatments. It is observed that N uptake increased from 0.58 g pot⁻¹ in the control treatment to 1.54 and 8.48 g pot⁻¹ for the CF and BC3

treatments, respectively. However, P uptake increased from 1.12 g pot⁻¹ at the CF treatment to 6.86 g pot⁻¹ at the high biochar level (BC3). Similarly, the amount of K absorbed by the fennel plant increased with all treatments compared to the control, and the high rate of biochar was the most effective one, as it increased from 0.72 g pot⁻¹ to 9.61 g pot⁻¹.

Effects on plant growth: Data presented in Table 3 show the effect of different ABC levels on the fresh and dried weight, plant height, stem diameter, and number of branches for fennel plants. The growth parameters were significantly ($p < 0.05$) affected by using various ABC levels compared to the control treatment. Although the recommended dose of superphosphate (CF) increased fennel plant growth parameters over the control, these increases were less than those noticed at biochar treatments. The fresh, dry weight, plant height, stem diameters, and number of branches were increased by 62.32, 109.58, 37.93, 16.00, and 12.00%, respectively, at CF treatment compared to control. The biochar treatments gradually increased all growth parameters and the increases were pronounced at the highest rate (BC3), which realized higher values of the growth parameters than the other treatments. The corresponding values due to the 3% ABC were 122.60, 98.29, 51.50, 40.00, and 60.71% higher than the CF treatment. The highest fresh, dry weight, plant height, stem diameter, and number of branches were relatively increased by 2.61, 3.15, 1.08, 0.63, and 0.80 times, respectively, at the BC3 treatment over the control treatment.

Effects on fennel plant roots: The root parameters (fresh and dry weight, length, and diameter) increased significantly ($p < 0.05$) in the soil amended with biochar compared to the control (Table 4). The increase was more pronounced at higher levels of biochar application. The recommended P dose significantly ($p < 0.05$) increased the fresh, dry weight, root length, and root diameter over the control treatment by 14.50, 50.0, 17.67, and 92.31%, respectively. Increasing the biochar level up to 3% resulted in gradual increases in the root parameters compared to other treatments. The high biochar level (BC3) caused relative increases in fresh weight, dry weight, root length, and root diameter by about 116, 205, 32, and 164%, respectively, over the CF treatment.

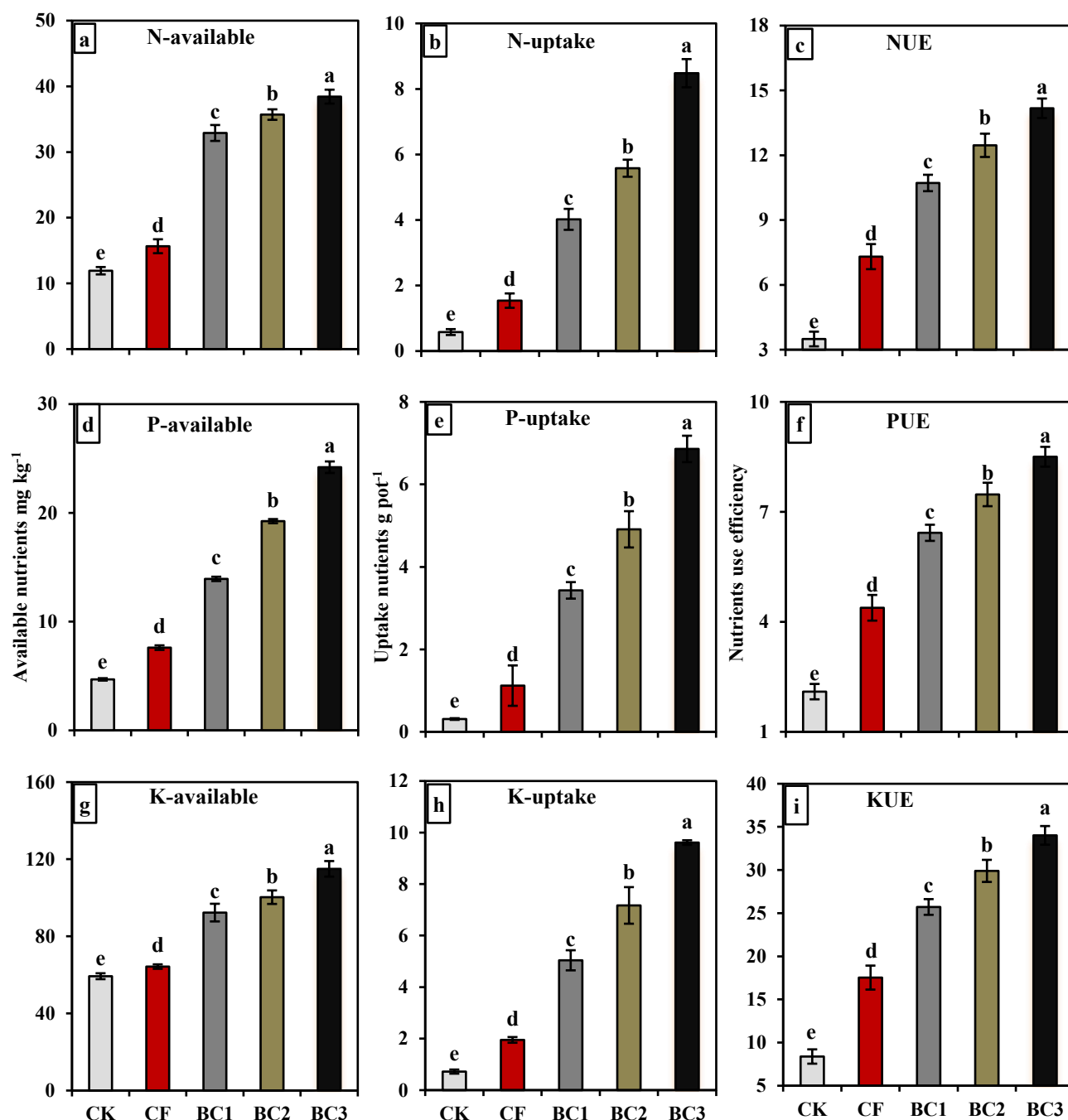


Fig. 3. Effect of biochar application on available nitrogen (N, a), phosphorous (P, c) and potassium (K, e), their uptake (b, d and f) in the shoots of Fennel plants and nutrients use efficiencies, nitrogen use efficiency (NUE c), phosphorus use efficiency (PUE f), potassium use efficiency (KUE i). CK, control (no biochar applied); CF, recommended fertilizers dose; BC1, BC2 and BC3 fish waste biochar at rate of 1, 2, and 3%. Error bars are standard deviations of the means (n= 3). Different letters above the columns indicate significant (p<0.05) difference between.

Table 4. Vegetative root characteristics of Fennel-Baladi (*Foeniculum vulgare*) grown on sandy soil as affected by biochar application.

Treatments	Fresh weight (g pot ⁻¹)	Dry weight (g pot ⁻¹)	Root length (cm)	Root diameter (cm)
CK	4.62 ± 0.21e	1.36 ± 0.03e	26.6 ± 1.52d	0.13 ± 0.03e
CF	5.29 ± 0.22d	2.04 ± 0.08d	31.3 ± 1.15c	0.25 ± 0.00d
BC1	9.46 ± 0.19c	4.88 ± 0.16c	36.6 ± 1.52b	0.51 ± 0.03c
BC2	10.70 ± 0.15b	5.99 ± 0.15b	39.6 ± 0.57a	0.60 ± 0.05b
BC3	11.45 ± 0.10a	6.23 ± 0.10a	41.6 ± 0.57a	0.66 ± 0.03a

CK, Control (no fertilizer and biochar applied), CF, Chemical fertilizer, BC1 = 1% biochar, BC2 = 2% biochar, BC3 = 3% biochar. Means denoted by the same letter indicate no significant difference according to Duncan's test at p < 0.05

Table 5. Photosynthetic pigments (Chlorophylls a, b; Total chlorophylls; Carotenoids; a/b ratio; and Chl/Carot. ratio) of Fennel-Baladi (*Foeniculum vulgare*) leaves grown on sandy soil as affected by biochar application.

Treatments	Chlorophyll a (mgkg ⁻¹)	Chlorophyll b (mgkg ⁻¹)	Total Chl. (mgkg ⁻¹)	Chl.a/Chl.b	Carotenoids (mgkg ⁻¹)	Chl/Carot
CK	3.56 ± 0.06e	54.88 ± 0.09e	58.45 ± 0.07e	0.07 ± 0.006e	93.47 ± 0.004a	0.63 ± 0.006e
CF	5.01 ± 0.06d	55.42 ± 0.18d	60.44 ± 0.25d	0.09 ± 0.000d	93.43 ± 0.009b	0.65 ± 0.003d
BC1	8.98 ± 0.08c	61.68 ± 0.02c	70.66 ± 0.10c	0.15 ± 0.006c	93.10 ± 0.006c	0.76 ± 0.001c
BC2	9.82 ± 0.06b	62.46 ± 0.13b	72.29 ± 0.09b	0.16 ± 0.000b	93.06 ± 0.000d	0.78 ± 0.001b
BC3	11.00 ± 0.02a	64.02 ± 0.10a	75.02 ± 0.11a	0.17 ± 0.001a	92.97 ± 0.001e	0.81 ± 0.001a

CK, Control (no fertilizer and biochar applied), CF, Chemical fertilizer, BC1 = 1% biochar, BC2 =2% biochar., BC3 =3% biochar. Means denoted by the same letter indicate no significant difference according to Duncan's test at p<0.05

Effects on photosynthetic pigments of fennel plant:

Photosynthetic pigments of fennel plants in the treated soil were significantly ($p<0.05$) increased compared to untreated soil (Table 5). Chl a and Chl b at CF treatment were increased by 40.73 and 0.98%, respectively, over the control. At the high biochar rate, the corresponding increases were 208.99 and 16.65, respectively. It increased by 119.56 and 16.65% over the CF treatment. In contrast, the carotenoids showed the highest value at the control treatment, while the lowest value was observed at the high biochar rate.

Nutrient use efficiency: The use efficiencies of nitrogen (NUE), phosphorus (PUE), and potassium (KUE) by fennel plants were increased by biochar application (Fig. 3c,f,i). The CF increased the NUE from 3.49 to 7.31, the PUE from 2.10 to 4.38, and the KUE from 8.38 to 17.53 g g⁻¹. Moreover, at the high biochar rate, it increased up to 14.17, 8.5, and 34.02 g g⁻¹ for NUE, PUE, and KUE, respectively.

Discussion

According to the SEM microscopic images, there was a significant difference in the appearance of biochar before and after acidification, and it was more evident with 2000 magnification where visible sticky filaments of 3 µm appeared. These variations were the result of sulfuric acidification and pores in the body of the geophyte (Fig. 1a, b). In addition, as a result of acidification with sulfuric acid, the elemental composition (SEM-EDX) had a direct role in increasing some elements, such as oxygen, which increased by about 27%, calcium by 35%, sulfur by 12%, phosphorus and sodium by 1%, and magnesium by 0.75%, compared to before acidification. Fourier-transform infrared (FTIR) spectroscopy is a recognized tool for detecting functional groups to further study adsorption behavior (Yenisoy-Karaka *et al.*, 2004). In our study, the FTIR spectra were different for biochar before and after acidification, especially for the peak that appeared in acidified biochar related to the stretching vibration of C=O (Fig. 2). Also, the broad peak associated with the stretching vibration of C-O in acidified biochar is higher than before acidification. The appearance of these oxygen-containing surface functional groups may be attributed to H₂SO₄, which can cause the oxidation of carbonized surfaces, thereby enhancing the biochar's ability to adsorb and nutrient retention.

Through the direct addition of organic matter, biochar directly delivers nutrients. Indirectly, it improves soil chemical properties. The ABC significantly ($p<0.05$) increased SOM, which leads to a significant improvement in soil properties. Also, a significant decrease in soil pH was observed with the addition of high biochar levels (Table 2).

The decrease in pH value may be attributed to the huge protons (H⁺) released from the exchangeable sites of ABC. According to Figure 1, the ABC has high sulfur content (a hundredfold increase) compared to before acidification, which has acidic tendencies when it reacts with water, which leads to a positive effect on reducing the pH values. Also, ABC may produce acidic functional groups in the oxygen abundance state plus hydrogen release from the wetted biochar surface (Fig. 2). Oxygen has a vital role in plant life as it is involved in the processes of photosynthesis, and respiration, and the associated oxidation and reduction. So, it also combines with many other elements to form organic materials and oxides. This process leads to the formation of some acids that neutralize soil alkalinity. Similar results were obtained by Blackwell *et al.*, (2010) and Khalafalla *et al.*, (2024) whom confirmed that ABC was effective in reducing the pH of calcareous soils, which is consistent with our research hypothesis. Soil salinity depends on the composition and ash content of added biochar and the ionic composition of the soil solution. The gradual increase in EC values is related to the added biochar level, as biochar itself already has a high salt content (Table 1). Although the highest recorded electrical conductivity (1.95 dS/m) in BC3 is still below the critical limit for most crops, continuous or long-term use of these treatments in fields over multiple growing seasons may lead to a gradual accumulation of dissolved salts. Therefore, while this acidified biochar technology offers an effective solution for phosphorus fixation, close monitoring of soil salinity dynamics is recommended for sustainable management. Also, adding ABC to the soil caused an increase in the EC value due to the release of weakly bound nutrients (cations and anions) from biochars into the soil solution (Gundale & DeLuca, 2007; Chan *et al.*, 2008; Awad *et al.*, 2021 a, b). We hypothesized that adding ABC to alkaline soils can reduce soil alkalinity and enhance carbon sequestration, which may reduce CO₂ elevation and increase organic matter contents in calcareous soils. It has been confirmed by many researchers (Cross & Sohi, 2011; Ahmed *et al.*, 2021; Saranya *et al.*, 2011) that biochar addition directly led to an increase in SOM content due to its high carbon content, or indirectly via enhancing the microbial communities and its activity.

The amount of available P depends on the type of biochar, and the increased availability of P may be attributed to one or more of these reactions and mechanisms like i) Biochar is represent a direct source of available phosphorus in the soil (Zhang *et al.*, 2016) (Table 1 and Fig. 1), ii) The decrease in soil pH when adding biochar increases the uptake of phosphorus in the soil due to stronger anionic repulsion (Chen *et al.*, 2018), iii) Due to the activities of Ca⁺², Fe⁺², Fe⁺³ and Al⁺³ ions that are able to precipitate P which pH depended, hence, the

available P values are changed with changing pH (Gao *et al.*, 2019), and iv) Through forming complexes of these ions with dissolved organic matter from biochar and reducing the active amount of them (Liu *et al.*, 2018). In the same regard, the availability of N, P, and K increased with increasing biochar levels and fertilizer applications. These increases may be due to adding biochar, which increases the active surface area of tested soils, and other nutrients released from biochar (Major *et al.*, 2010).

As a result, the yield and absorption were greatly boosted by the nutrients in biochar. The current study suggests that the highest amount of N, P, and K taken by the fennel plant was recorded at BC3 application. Added ABC may positively affect nutrient amounts, and neutralize soil alkalinity, so ensure conditions more suitable for N, P, and K availability and their uptake. In addition to its nutrient contents, there is encouragement between these nutrients; an increase in the element magnesium may increase the absorption of P and N. Similarly, Wang *et al.*, (2012) stated that the tissue content of N, P, and K in maize, okra, and cassava was significantly increased as a result of adding biochar (Gao & DeLuca, 2016; Syuhada *et al.*, 2016; Rekaby *et al.*, 2020, 2021).

Growth parameters (fresh weight, dry weight, and photosynthesis pigments) are very important indicators that show the response of the growing plant to agricultural processes and fertilization, which ultimately affect the yield. The application of ABC gradually enhanced the plant height and biomass production compared to control treatment (Table 3). Adding biochar improves soil properties, increasing nutrient availability and their use efficiency, and consequently improving Fennel-Baladi growth characteristics (shoot and root). These results are consistent with those reported by Ahmed *et al.*, (2021), who confirmed that P levels positively affected plant height and dry weight of maize. Bashir *et al.*, (2021) noticed that activated biochar additives can speed up corn growth and yield. Also, biochar-rich P application improves soil properties that ensure an appropriate and optimal environment for plant growth quantity and quality. Similar findings were obtained by Lehmann *et al.*, (2011), who reported that the biochar stimulated root growth. Correspondingly, Imran (2015) suggested that adding biochar in conjunction with the mineral P doses showed a significant increase in the root system that increased the plant's ability to absorb more phosphorous and other elements, which reflected on plant growth. Jabborova *et al.*, (2021) reported that biochar positively affected all growth parameters under water deficiency.

The characteristics of pigments are the most significant indicators of agricultural management. There were significant ($p < 0.05$) increases in both Chl a and Chl b with biochar addition (Table 5). This result may be attributed to the fact that biochar supplies the soil and plants with sufficient amounts of essential nutrients that generate optimal growth conditions, thus increasing leaf pigment content and enhancing photosynthesis and chlorophyll accumulation, which reflect on vegetative growth. These results are consistent with those reported by Feng *et al.*, (2021), who reported that biochar application enhanced the chlorophyll content of tomato leaves. Jabborova *et al.*, (2021) found that adding biochar significantly increased the chlorophyll content in okra compared to the control under drought condition, and had a positive and significant effect on photosynthesis and production of peanut (Wang *et al.*, 2021). Concurrently, the significant decrease in carotenoid

levels observed alongside the chlorophyll increase (Table 5) further elucidates this physiological shift. Carotenoids typically function as photo protective agents and antioxidants synthesized under environmental or nutrient stress conditions. By optimizing nutrient availability and mitigating soil-induced stress, the acidified biochar matrix allowed the plants to reallocate their metabolic energy and resources away from stress-defense pigments and toward primary photosynthetic machinery (chlorophyll synthesis). This resource reallocation underscores a physiological optimization that directly benefits vegetative growth and overall biomass production.

Conclusion

The low phosphorus availability in the alkaline soil represents an obstacle to the agricultural sustainability of this soil type, and reaching a suitable option is a constructive contribution. According to the study's findings, the chemical fertilizers and acidified biochar improve the chemical characteristics of alkaline soils by lowering their pH levels while increasing their organic matter content and electrical conductivity compared to the control. Among all treatments, the acidified biochar was the most effective one to improve soil properties and plant growth parameters of Fennel (cv. Baladi). This improvement in the soil properties due to ABC resulted in an improvement in soil fertility as NPK availability and their uptake increased by Fennel (cv. Baladi) at the vegetative stage of growth. In the treated soil, the nutrient use efficiencies increased with an increasing biochar rate compared to the chemical fertilizer treatment and the untreated one. It can be concluded that adding acidified biochar at a high level (BC3) is considered the best treatment that ensures favorite cultivation conditions for fennel plant traits and yield via improving alkaline soil properties and soil fertility. Therefore, the incorporation of acidified biochar is a promising tool and an appropriate management practice that reduces the use of chemical fertilizers and thus promotes clean food and the environment. This study represents an important and innovative model to address the low availability of phosphorous in alkaline soil, and it is more important for arid and semi-arid regions as well as those interested in organic agriculture and safe food.

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Declarations

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