# Interactive effects of biochar and micronutrients on faba bean growth, symbiotic performance, and soil properties

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## Abstract

Leguminous crops are significantly involved in the global symbiotic biological $N_2$ Fixation (BNF), an eco-friendly process in the agriculture system. Biochar is considered as a vital amendment in improving growth and quality of crops and soils. Few investigations have been conducted to determine the combination effect of biochar with microelements on growth of legumes and soil properties. This study was designed to study the effect of soybean straw-derived biochar (SSDB) with or without microelements on soil microbial and chemical properties, growth, yield, and seed chemical composition of faba bean (*Vicia faba* L.). Results revealed that dehydrogenase (DHA) and phosphatase (P-ase) activities were markedly improved with the increase of SSDB rates under addition of microelements and their highest values were recorded after 90 d. Significant increases were noticed in nodulation activities, nodulation numbers (30.1–72.8), concentrations of N (1.62–1.93%), P (0.15-0.21%), and K (0.53-0.67%), and seed chemical constituents due to the addition of SSDB in the presence of microelements. Moreover, the combination of biochar with microelements caused significant changes in microbial counts. Overall, this investigation shows the potential and role of SSDB in enhancing the growth quality of faba bean seeds as well as an improvement of soil characteristics.

**Key words:** dehydrogenase / nitrogenase / $N_2$ fixation / phosphatase / *Vicia faba*

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## 1 Introduction

Biochar is recommended as a soil amendment and can be produced by a thermochemical decomposition process, called pyrolysis, through heating biomass materials at high temperatures (400–800°C) under zero or low oxygen conditions (*Iijima* et al., 2015). Biochar has several potential benefits, including carbon sequestration, adsorbing organic and inorganic pollutants as well as improving soil fertility due to improving nutrient supply and soil physical, chemical, or biological properties (*Parvage* et al., 2013). Addition of biochar can enhance soil fertility through increasing symbiotic $N_2$ fixation, which is highly dependent on different mechanisms such as immobilization of inorganic nitrogen and enhancing the availability of nutrients and the activity and numbers of nodules (*Nelissen* et al., 2012).

Mineral nutrients have important effects on the formation and development of nodules and $N_2$ fixation by legumes. Microelements such as Fe, Zn, B, Mn, Cu, and Mo are considered as essential keys in the growth of living organisms and they are required for the symbiotic fixation of atmospheric $N_2$ in agricultural soils (*O'Hara*, 2001). Furthermore, they contribute to the activities of enzymes such as peroxidase, catalase, and nitrate reductase, and have important functions in the regulation of metabolism, reproduction, and protection of plants against various stresses (*Sharma*, 2006). They also can affect photosynthesis, plant maturity, respiration, cell division, and development of meristematic tissues (*Zeidan* et al., 2006). Foliar application of microelements has a positive effect on growth and production of crops (*George* and *Schmitt*, 2002; *Hoffland* et al., 2006; *Hosseini* et al., 2007), especially in alkaline soils. Therefore, it is considered as a good method to prevent deficiencies when plant roots cannot absorb these nutrients from the soils (*Kinaci* and *Gulmezoglu*, 2007; *Babaeian* et al., 2011; *Zayed* et al., 2011).

Faba bean (*V. faba* L.) is one of the most important legume crops and has a high ability to form a symbiotic association with *Rhizobium leguminosarum* (*Yahia* et al., 2013). Growing faba bean can markedly minimize the application of chemical fertilizers due to the high capability of the symbiosis in the fixation of atmospheric $N_2$. Many studies have been conducted to evaluate the impact of biochar application on growth and quality of legumes (*Lehmann* and *Rondon*, 2006; *Jeffery* et al., 2010; *Güereña* et al., 2015). However, no previous study has investigated the effect of biochar and microele-

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ments on the performance and quality of legume plants grown on clayey soils under arid conditions. The current investigation aims to evaluate the influence of soybean straw-derived biochar with or without foliar application of microelements on soil chemical (nutrients availability, pH, CEC, and EC) and microbial properties (enzymes activity, microbial counts and nodulation), as well as on the growth and chemical composition of faba bean seeds.

## 2 Material and methods

### 2.1 Soil sampling and biochar production

Sub-surface alluvial soil samples with a texture of clayey loam (5.0% coarse sand, 24.3% fine sand, 25.5% silt, and 45.2% clay) were collected at a depth of 0–30 cm from the Agronomy Farm of Faculty of Agriculture, Benha University, Qalyubia Governorate, Egypt (30°24′36′′N, 31°12′36′′E). Chemical and microbial properties of the used soil are presented in Tab. 1. For biochar production, soybean residues (straw) were collected from the Agronomy Farm at the Faculty of Agriculture in Benha University, oven-dried at 60–70°C and finally crushed to small particles 1–2 cm. The dried residues were placed in a muffle furnace for pyrolysis under limited oxygen condition at 450°C for 30 min. Chemical properties of the produced biochar are shown in Tab. 2.

**Table 1**: Chemical and microbiological analyses of the collected soil before cultivation.

| Parameters[a] | Values | Parameters | Values |
|---|---|---|---|
| OM (%) | 1.52 | Available P (mg $kg^{-1}$) | 13.00 |
| $CaCO_3$ (%) | 3.55 | Available K (mg $kg^{-1}$) | 160.0 |
| pH | 7.59 | Total bacterial count ($\times 10^7$) | 21.00 |
| EC (dS $m^{-1}$) | 1.11 | Total free $N_2$ fixers ($\times 10^3$) | 54.00 |
| CEC ($cmol_c$ $kg^{-1}$) | 28.5 | Total yeasts and molds ($\times 10^3$) | 17.70 |
| Total P (%) | 0.12 | Dehydrogenase (mg TPF $kg^{-1}$ soil) | 17.83 |
| Total N (%) | 0.30 | Phosphatase (mg P $kg^{-1}$ soil) | 15.92 |
| Total K (%) | 0.45 | | |
| Available N (mg $kg^{-1}$) | 21.0 | | |

[a]OM = organic matter, EC = electrical conductivity and CEC = cation exchange capacity.

**Table 2**: Chemical analysis of the prepared soybean biochar.

| Parameters[a] | Values | Parameters | Values |
|---|---|---|---|
| pH | 7.89 | Total Fe (mg $kg^{-1}$) | 76.3 |
| EC (dS $m^{-1}$) | 2.49 | Total Mn (mg $kg^{-1}$) | 31.2 |
| OM (%) | 72.0 | Total Zn (mg $kg^{-1}$) | 57.5 |
| Total N (%) | 2.15 | Total Mo (mg $kg^{-1}$) | 0.43 |
| Total P (%) | 0.71 | Available N ($NH_4$+$NO_3$) (mg $kg^{-1}$) | 1150 |
| Total K (%) | 1.12 | Available P (mg $kg^{-1}$) | 610 |
| CEC ($cmol_c$ $kg^{-1}$) | 59.23 | Available K (mg $kg^{-1}$) | 4780 |

[a]OM = organic matter, EC = electrical conductivity and CEC = cation exchange capacity.

### 2.2 Pot experiment

A pot experiment was set up in a complete randomized block design with four replicates and eight treatments (T1–T8) as shown in Tab. 3. The average annual relative humidity and temperature in the experimental greenhouse were 53.7% and 21.1°C and they ranged from 61% and 10°C in December to 46% and 30°C in May, respectively. The pots (30 cm width × 30 cm height) were filled with 10 kg of air-dried soil and then mixed with different biochar rates (0, 20, 40, 80 g $pot^{-1}$) before sowing of faba bean. Seeds of faba bean (*Vicia faba* cv. Giza 40) were obtained from the Agricultural Research Center, Faculty of Agriculture at Benha University, Egypt. The strain of *Rhizobium leguminosarum* bv. viciae was obtained from the bio-fertilization unit, Faculty of Agriculture, Ain Shams University, Egypt. Prior to cultivation, seeds of faba bean were soaked in a solution containing *R. leguminosarum* bv. viciae ($10^9$ CFU $mL^{-1}$) and Arabic gum (20%) at a rate of 1 L $kg^{-1}$ seeds for 60 min. After that, the seeds were left for air drying before sowing. Ten seeds were sown and after their full germination, five strong seedlings were chosen and kept in each pot. Faba bean plants were fertilized with 0.06 g $pot^{-1}$ ammonium sulfate (20.5% N), 2.0 g $pot^{-1}$ calcium superphosphate (6.8% P), and 0.5 g $pot^{-1}$ potassium sulfate (38.4% K) according to recommendations of the Ministry of Agriculture, Egypt. The micronutrient solution was obtained from Sphinx for International Trade Company, Cairo, Egypt. It contained 6% Fe, 0.5% Zn, 0.5% Mn, 0.5% Cu, 0.05% Mo, 4% S, 0.02% B, and 10% citric acid. The microelement solution was applied three times (one month between each time) as a foliar spraying at a rate of (1 mL $L^{-1}$). The experimental pots were irrigated with tap water every 4–5 d and the water content was kept at 60% of the maximum water-holding capacity.

### 2.3 Soil and biochar analyses

Before the start of the experiment and after harvesting of bean plants, soil samples were collected using an auger, mixed thoroughly and finally divided in two parts for further analyses. The first part of the soil samples was left in a fresh status and kept at 4°C in the refrigerator for microbial analyses. The second part was air-dried for a week and then used for physical and chemical determinations. Soil pH was meas-

 

**Table 3**: The experimental treatments.

| Without microelements | | With microelements | |
|---|---|---|---|
| T1 | Control (No biochar addition) | T5 | Control (No biochar addition) |
| T2 | Biochar at 20 (g $pot^{-1}$) | T6 | Biochar at 20 (g $pot^{-1}$) |
| T3 | Biochar at 40 (g $pot^{-1}$) | T7 | Biochar at 40 (g $pot^{-1}$) |
| T4 | Biochar at 80 (g $pot^{-1}$) | T8 | Biochar at 80 (g $pot^{-1}$) |

ured in a suspension of a 1.0 : 2.5 soil : water (w/v ratio) and electrical conductivity (EC) was determined in the extract from the saturated soil paste. Available N was extracted with KCl (2 M) and total N was obtained after the digestion of soil and biochar samples with a mixture of concentrated $H_2SO_4$ and $HClO_4$ (ratio of 2 : 1, v/v). Both total and available N concentrations were determined using the Kjeldahl method. Values of pH and EC for biochar were determined after mixing 1 g biochar with 5 mL distilled water for 1 h. Total concentrations of Fe, Mn, Zn, and Mo were determined by inductively coupled plasma mass spectrometery (ICP-MS) after the digestion of biochar in the microwave system. The soil particle-size distribution was determined with the pipette method. Soil organic matter and total organic carbon were measured after dichromate oxidation and subsequent titration with acidified ferrous ammonium sulfate. Values of CEC for soil and biochar were determined through using ammonium acetate (1 M), ethanol and 2 N KCl, respectively. Available P and K in soil and biochar were extracted with 0.5 M $NaHCO_3$ (pH 8.5) and 1 M $NH_4OAc$ (pH 7) and then measured with spectrophotometer and flame photometer, respectively. Total P and K concentrations were determined also with spectrophotometer and flame photometer after digestion of soil and biochar samples. Organic matter concentration in the biochar was determined by weight loss during ignition at 450°C for 16 h. Calcium carbonate in the soil was determined by volumetric analysis of the released $CO_2$ after addition of 4 M HCl to the soil.

### 2.4 Microbial counts and soil enzymes

Counts of microorganisms were taken as described in *Hassan* et al. (2013) and *Sadik* et al. (2016). In brief, the total counts of bacteria, yeasts, molds, and free $N_2$-fixing bacteria were determined with the dilution plate count method on nutrient agar medium. In brief, 5 g fresh soil were shaken with 50 mL distilled water for 30 min at 28°C and 180 rpm. After that, 0.1 mL from the above suspension was transferred to a small tube (1 mL) containing 0.9 mL distilled water. Series of dilutions ($10^{-1}$–$10^{-8}$) were prepared and the plates were placed in an incubator at 28°C. Numbers of total bacteria, free $N_2$-fixing bacteria, yeasts, and molds were determined after 4–5 d. Microbiological counts were expressed as the number of colony-forming units (CFUs) $g^{-1}$ of dry soil. Activities of dehydrogenase (EC.1.1.1.1) as μg TPF (g dry soil 24 h)$^{-1}$ and alkaline phosphatase (EC. 3.1.3.1) as μg P $g^{-1}$ dry soil were periodically assayed in the rhizospheric soil after 30, 60, and 90 d after the sowing of bean seeds according to *Casida* et al. (1964) and *Drobrikova* (1961), respectively. Nitrogenase activity ($N_2$-ase, EC.1.18.6.1) as μL $C_2H_4$ $g^{-1}$ dry nodules was measured in nodules using the acetylene reduction technique according to *Silvester* (1983). Nodule numbers on roots of faba bean and their fresh and dry weights were estimated 50 d after thinning.

### 2.5 Plant analysis

Dry weights of straw, seeds, and roots of faba bean plants were determined after harvest. Contents of NPK and microelements (Fe, Mn, Mo, and Zn) of seeds were determined after their digestion using a mixture of $H_2SO_4$ and $HClO_4$ (2 : 1, v/v) as mentioned in *Mohamed* et al. (2015). Total crude protein percentage (%) was calculated by multiplying N concentrations by 6.25, while total carbohydrates were determined colorimetrically using the method of *A.O.A.C.* (2005).

### 2.6 Statistical analysis

Statistical analysis was performed using the CoStat package program, version 6.311 (cohort software, USA). The differences among means of treatments were determined using Tukey's Highly Significant Difference (HSD) at the probability of 5%.

## 3 Results and discussion

### 3.1 Dry weight of faba bean

Dry weights of different faba bean parts were significantly affected by biochar addition at different amounts (Fig. 1). Our data show that application of biochar with or without microelements significantly increased dry weights of faba bean tissues compared with control. The cultivated faba bean in soil under no biochar addition with or without foliar spraying of microele-

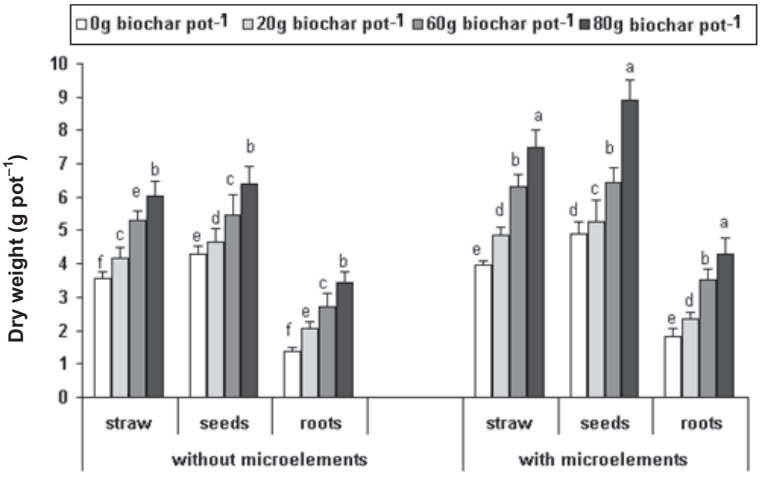


**Figure 1:** Effect of biochar with and without microelements on dry weights of faba bean organs. Different letters in same column indicate a significant difference between treatments at P < 5% according to Tukey's HSD.

 

ments had the lowest values of dry matter accumulations. Increasing biochar doses up to 80 g pot$^{-1}$ caused the highest improvements in dry weights of faba bean organs. The increases of dry matter might be related to high enhancements of nodulation, $N_2$ fixation, and nutrient availability after biochar application. In this respect, *Rondon* et al. (2007) and *Mia* et al. (2014) reported that biochar might increase legume growth and yield due to improving biological $N_2$ fixation. *Egamberdieva* et al. (2016) hypothesized that increasing nutrient availability after biochar application could cause high improvements in plant biomass yield and nutrients concentrations. Overall, these results demonstrated the potential role of biochar application in improving plant growth performance. Many studies provided different hypotheses to explain the effect of biochar application on plant performance. In this context, *Suppadit* et al. (2012) found high increments in plant height, dry weight, yield, and nutrient amounts in some legumes as a result of biochar application. Also, *Lanza* et al. (2016) reported that biochar addition caused marked shifts in microbial populations, especially for plant growth-promoting microorganisms (PGPM).

## 3.2 Nutrient status in bean organs

Table 4 indicates that the lowest concentrations of NPK in straw and seeds were observed in plants of the control treatment, whereas highest records appeared when the soil was treated with 80 g biochar pot$^{-1}$ in the presence of microelement application. The results clearly show that biochar addition at different amounts with spraying microelements led to significant effects on total N values in straw and seeds. The combination effects of biochar and micronutrients on total P and K in straw or seeds of faba bean were significant. This could be explained by the ability of biochar to enhance the availability of soil nutrients and their contents in faba bean plants. In this respect, *Steiner* et al. (2008) and *Hunt* et al. (2010) showed that biochar application had a marked contribution in increasing nutrients in plants.

The effect of biochar on the absorption of nutrients by plants highly depended on its production conditions (*Hass* et al., 2012) and its important role in decreasing nutrient losses by leaching from soils (*Major* et al., 2010). Our data also illustrated that total amounts of NPK were significantly affected by the different treatments and were gradually increased with increasing biochar doses to reach their maximum at 80 g pot$^{-1}$. In this respect, *Laufer* and *Tomlinson* (2012) reported that the amounts of nutritional elements such as phosphorus and potassium were positively influenced by biochar application as compared with the control. Two mechanisms could explain the marked effect of biochar on plant growth: (1) biochar was a nutrient source and (2) biochar improved nutrient availability. The high efficiency of biochar in adsorption of $NH_4^+$ and $NO_3^-$ might inhibit their leaching from soils and this improved its absorption by plants (*Güereña* et al., 2013).

## 3.3 Seed chemical constituents and 100 seed weight

The chemical compounds of faba bean seeds, including microelements (Fe, Mo, Mn, and Zn), carbohydrates, protein, and fat were higher under biochar alone or biochar + microelements than those of the control (Tab. 5). Increasing biochar amounts with or without microelements led to significant increases in carbohydrates and microelements. This trend was same in protein concentration but when biochar was added at doses of 20 or 40 g pot$^{-1}$ with the foliar spraying of microelements, there were no significant differences. Fats concentrations of faba bean seeds were significantly affected by increasing biochar doses under foliar application of microelements, whereas no significant effect was observed when biochar was applied at a rate of 20 or 40 g pot$^{-1}$ without microelements. Application of microelements such as Zn in foliar forms resulted in high enhancements in the absorption of Zn by different parts of bean plants. In turn, it might protect the plasma membrane against the harmful effects of higher temperature/oxidative stresses (*Bajgiran*, 2013). Also, Tab. 5

**Table 4**: Total concentrations of NPK (%) in faba bean parts under different treatments.[a]

| Treatments | In straw | | | In seeds | | |
|---|---|---|---|---|---|---|
| | N | P | K | N | P | K |
| T1 | 1.61d ± 0.01 | 0.14 f ± 0.001 | 0.53 c ± 0.004 | 2.10 e ± 0.02 | 0.15 e ± 0.002 | 2.46 ± d0.03 |
| T2 | 1.66 d ± 0.02 | 0.16 d ± 0.001 | 0.55 b ± 0.006 | 2.30 c ± 0.04 | 0.17 d ± 0.002 | 2.51 ± cd0.01 |
| T3 | 1.71 c ± 0.01 | 0.18 c ± 0.002 | 0.55 b ± 0.006 | 2.45 b ± 0.03 | 0.20 c ± 0.001 | 2.68 ± b0.02 |
| T4 | 1.84 a ± 0.01 | 0.19 b ± 0.002 | 0.62 a ± 0.004 | 2.51 a ± 0.03 | 0.25 a ± 0.003 | 2.74 ± b0.05 |
| T5 | 1.62 d ± 0.02 | 0.15 e ± 0.001 | 0.53 c ± 0.006 | 2.14 d ± 0.02 | 0.17 d ± 0.002 | 2.52 ± cd0.03 |
| T6 | 1.83 b ± 0.02 | 0.16 d ± 0.003 | 0.55 b ± 0.005 | 2.42 b ± 0.03 | 0.19 c ± 0.002 | 2.57 ± c0.04 |
| T7 | 1.84 b ± 0.03 | 0.19 b ± 0.003 | 0.56 b ± 0.007 | 2.58 a ± 0.05 | 0.23 b ± 0.001 | 2.70 ± b0.05 |
| T8 | 1.93 a ± 0.03 | 0.21 a ± 0.003 | 0.67 a ± 0.007 | 2.60 a ± 0.05 | 0.26 a ± 0.001 | 2.81 ± a0.06 |

[a]Data in the table are mean values ± standard errors. Different letters in same column indicate a significant difference between treatments at P < 5% according to Tukey's HSD.

 

**Table 5**: Chemical composition of faba bean seeds and 100-seed weight (g) in different treatments.

| Treatments | (%) | | | mg kg$^{-1}$ | | | | 100 seed weight |
|---|---|---|---|---|---|---|---|---|
| | Protein | Carb.[a] | Fats | Fe | Mn | Zn | Mo | |
| T1 | 10.06 e ± 0.32 | 42 b ± 0.43 | 1.8 b ± 0.02 | 24.4 e ± 0.45 | 5.34 f ± 0.04 | 20.3f ± .34 | 0.65e ± 0.01 | 28.67 f ± 0.32 |
| T2 | 10.38 d ± 0.35 | 43 b ± 0.56 | 1.9 b ± 0.03 | 27.3 d ± 0.24 | 6.25 e ± 0.05 | 21.7 e ± 0.27 | 0.77d ± 0.02 | 40.33 e ± 0.42 |
| T3 | 10.69 c ± 0.23 | 44 ab ± 0.53 | 1.9 b ± 0.02 | 30.1 c ± 0.46 | 8.17 d ± 0.06 | 22.2 d ± 0.25 | 0.93b ± 0.02 | 54.16 d ± 0.24 |
| T4 | 11.50 a ± 0.19 | 45 a ± 0.45 | 2.1 b ± 0.03 | 30.2 c ± 0.35 | 9.98 c ± 0.06 | 23.5 c ± 0.35 | 0.99b ± 0.02 | 56.53 c ± 0.67 |
| T5 | 10.13 e ± 0.14 | 44 ab ± 0.34 | 2.0 b ± 0.04 | 26.6 d ± 0.47 | 6.12 e ± 0.04 | 21.1 e ± 0.45 | 0.71d ± 0.03 | 32.21f ± 0.51 |
| T6 | 11.44 b ± 0.24 | 45 a ± 0.54 | 2.3 ab ± 0.02 | 30.8 c ± 0.27 | 8.91d ± 0.07 | 23.3 c ± 0.29 | 0.87 c ± 0.03 | 67.84 b ± 0.89 |
| T7 | 11.50 b ± 0.21 | 46 a ± 0.56 | 2.8 a ± 0.05 | 34.4 b ± 0.32 | 11.7 b ± 0.09 | 26.5 b ± 0.41 | 0.98b ± 0.04 | 85.62 a ± 0.92 |
| T8 | 12.06 a ± 0.34 | 46 a ± 0.37 | 2.9 a ± 0.03 | 39.1 a ± 0.41 | 13.3 a ± 0.11 | 28.6 a ± 0.29 | 1.47a ± 0.06 | 91.73 a ± 0.95 |

[a]Carbohydrates. Data in the table are mean values ± standard errors. Different letters in same column indicate a significant difference between treatments at P < 5% according to Tukey's HSD.

shows that the combination of biochar with microelements had higher influences on the weight of 100 seeds as compared to no-microelements addition and the maximum values were recorded at 80 g biochar pot$^{-1}$. The high improvements of growth parameters such as 100-seed weight after biochar application could be attributed to its marked effect on nutrient supply (*Silber* et al., 2010).

### 3.4 Nodulation and nitrogenase activity

Table 6 shows the interaction effects of different rates of soybean biochar combined with microelements as foliar spraying on nodulation parameters (number, fresh weight, dry weight plant$^{-1}$). All faba bean plants under different treatments showed nodulation due to the inoculation of their seeds with rhizobia, which were responsible for nodulation. The lowest values of nodulation parameters were observed in faba bean under no-biochar application. On the other hand, higher values of nodule number and nodule fresh weight were observed in plants cultivated in soil amended with biochar at different amounts and they were gradually enhanced with increasing biochar amounts. This might result from promoting the efficiency of inoculants or native microorganisms (*Mete* et al., 2015). Also, *Güereña* et al. (2015) reported that the biochar additions resulted in 35.75% increase in nodule biomass and 21.26% increase in N derived from the atmosphere over the control.

Nitrogenase activity in root nodules of faba bean was gradually enhanced with the increase of biochar doses and the highest readings were observed in soil treated with 80 g pot$^{-1}$ followed by 40 g pot$^{-1}$. These results reflect the beneficial effect of biochar on the native microorganisms and rhizobia to colonize the rhizosphere of legumes. In addition, the efficient impact of biochar treatments on nodulation could be attributed to the $N_2$-fixing capacity of rhizobia and plant growth-promoting substances produced by native and introduced microorganisms (*Badri* et al., 2009; *Quilliam* et al., 2013).

**Table 6**: Nodule Parameters and nitrogenase activity (μL $C_2H_4$ g$^{-1}$ dry nodule) under different treatments.[a]

| Treatments | Nodules No. | Nodules FW (g plant$^{-1}$) | Nodules DW (g plant$^{-1}$) | $N_2$-ase activity |
|---|---|---|---|---|
| T1 | 24.2 ± 0.45 f | 2.38 ± 0.01 d | 0.01 ± 0.0001 d | 20.2 ± 0.13 e |
| T2 | 30.1 ± 0.24 e | 3.30 ± 0.02 c | 0.02 ± 0.0001 c | 38.8 ± 0.45 d |
| T3 | 30.8 ± 0.32 e | 3.99 ± 0.03 bc | 0.02 ± 0.0002 c | 48.9 ± 0.54 c |
| T4 | 48.8 ± 0.51 d | 4.60 ± 0.04 b | 0.04 ± 0.0002 b | 62.9 ± 0.78 b |
| T5 | 30.3 ± 0.21 e | 2.02 ± 0.01 d | 0.01 ± 0.0001 d | 39.1 ± 0.65 d |
| T6 | 54.9 ± 0.54 c | 3.48 ± 0.02 c | 0.02 ± 0.0001 c | 47.2 ± 0.39 c |
| T7 | 60.8 ± 0.61 b | 4.52 ± 0.04 b | 0.03 ± 0.0002 b | 62.3 ± 0.71 b |
| T8 | 72.8 ± 0.56 a | 5.76 ± 0.06 a | 0.05 ± 0.0003 a | 89.3 ± 0.98 a |

[a]Data in the table are mean values ± standard errors. Different letters in same column indicate a significant difference between treatments at P < 5% according to Tukey's HSD. No = Number, FW = Fresh weight, DW = Dry weight and $N_2$-ase = nitrogenase.

 

Treating plants with microelements caused higher $N_2$-ase activity than untreated plants. Microelements such as Fe could play a vital role in activities of many enzymes and also in the structure of the nitrogenase, so it caused marked increases in its activity with high quality of root nodules. In this respect, *Bilen* et al. (2011) reported that leguminous plants require boron (B) for $N_2$ fixation because it played a role in nitrogen assimilation. Also, *Sarkar* et al. (2007) indicated that application of microelements such as Zn, Fe, and Mn in a foliar form played an important role in increasing $N_2$ fixation. In addition, rhizobia required some microelements, including B, Co, Cu, Fe, Mn, Mo, Ni, Se, and Zn for their survival as free-living soil saprophytes, and for their symbiotic relationships with legumes (*O'Hara*, 2001). Also, Fe might be involved in many metabolic processes at several key stages in the symbiotic $N_2$ fixation due to its role in enhancing photosynthesis and carbohydrate synthesis.

Foliar application of Zn fertilizers seems to be an effective way of increasing Zn concentration in grains of maize by 49.56% as compared to the control treatment (*Tariq* et al., 2014), and could be implemented for higher yield and quality of soybean by influencing the number of seeds per plant and seed weight (*Kobraee* et al., 2011). Spraying micronutrients such as Fe, Mo, and Co showed positive effects on fresh weight and numbers of nodules, growth and yield parameters of soybean and mungbean (*Heidarian* et al., 2011). These influences could result from the importance of Fe and Mo in photosynthesis, $N_2$ fixation, nodulation, nodule activity, and in the utilization of specific plant enzymes that play roles in oxidative and reduction reactions (*Grzebisz* et al., 2008). Also, Fe and Mo are key components of nitrate reductase and nitrogenase enzymes that enhance the growth of bacteria and their symbiotic efficiencies. Foliar applications of Mo to legumes increased $N_2$ fixation and formation of root nodules with high masses and this led to high increases in the yield of soybean and nutrient concentrations of seeds (*Yanni*, 1992; *Vieira* et al., 1998; *Wiedenhoeft*, 2006). It was reported by *Zeidan* et al. (2006) that yield components of lentil were markedly improved by foliar application of Mn and Cu. Spraying of Zn enhanced soybean yield, and number and weight of seeds per plant (*Kobraee* et al., 2011). Manganese played a significant role in the synthesis of polyamines and then increased growth and development of plants (*Evans* and *Malmberg*, 1989), and also in the infection and binding of rhizobia with young root hairs (*Kijne* et al., 1988). Copper could affect the efficacy of bacteroid and *nif*-genes. Strong alterations were found in $N_2$ fixation by soybean plants due to the foliar application with B. Moreover, B had significant effects on nodule development and cell-surface interaction of *Rhizobium* on pea roots (*Bolanos* et al., 1996).

### 3.5 Soil microbial counts

Table 7 shows that all estimated total microbial counts (bacteria, yeasts, molds, and $N_2$ fixers) were affected by biochar addition compared to control. The highest significant records were observed in T8 (biochar 80 g $pot^{-1}$ with microelements). This could be attributed to the positive effect of biochar as an organic amendment on microbial counts through providing beneficial substrates for soil microbes (*Elad* et al., 2011). Biochar could cause high enhancements in microbial populations in the rhizosphere and marked promotions in plant growth (*Rondon* et al., 2007). In addition, biochar amendment increased legume growth and yield through improving biological $N_2$ fixation (*Quilliam* et al., 2013; *Mia* et al., 2014; *Lane* et al., 2015). Moreover, *Lehmann* et al. (2011) reported that incorporation of biochar in soils had strong impacts on microbial populations, which could have beneficial functions in improving soil fertility (*Graber* et al., 2010; *Ding* et al., 2016). Moreover, they found that microbial growth rates significantly increased with application of biochar.

### 3.6 Soil enzyme activities

Soil enzymatic activities can be used as indicators for soil fertility and quality because they reflect the beneficial effects on soil microorganisms in the presence of organic compounds. Data in Figs. 2 and 3 indicate that the rhizosphere soil of faba bean without the application of biochar had the lowest values of dehydrogenase (DHA) and phosphatase (P-ase), while the

**Table 7**: Microbial counts of soil in different treatments.[a]

| Treatments | Total bacterial count ($\times 10^7$) | Total yeasts and molds ($\times 10^3$) | Total free $N_2$ fixers ($\times 10^3$) |
|---|---|---|---|
| T1 | 30.3 ± 2.61 c | 22.7 ± 0.21d | 55 ± 2.12 g |
| T2 | 70.7 ± 8.13 b | 34.3 ± 1.35 c | 79 ± 2.10 f |
| T3 | 82.0 ± 8.24 a | 42.7 ± 0.32 b | 110 ± 4.23 d |
| T4 | 83.0 ± 9.11a | 47.0 ± 2.81 a | 220 ± 5.23 b |
| T5 | 33.2 ± 1.56 c | 29.3 ± 0.95 d | 64 ± 1.10 g |
| T6 | 76.4 ± 8.32 b | 42.0 ± 1.67 b | 92 ± 1.23 e |
| T7 | 84.0 ± 11.5 a | 44.0 ± 1.14 b | 160 ± 2.45 c |
| T8 | 85.6 ± 9.65 a | 50.5 ± 1.96 a | 240 ± 3.06 a |

[a]Data in the table are mean values ± standard errors. Different letters in same column indicate a significant difference between treatments at $P < 5\%$ according to Tukey's HSD.

 

addition of biochar was responsible for higher activities in the estimated enzymes compared to the control treatment. This could be attributed to the positive effect of biochar on the microbial community through providing beneficial substrates for soil microbes (*Deenik* et al., 2010). The results also show that DHA values in various treatments were gradually increased from the initial time to reach their maximum records at flowering stage and then decreased. In this respect, *Badri* et al. (2009) showed that root exudates caused significant changes in the natural microbial community and increased their activities. Also, the amplification of P-ase activity might contribute to the stimulatory effect of biochar on the release and availability of phosphate.

Foliar spraying with microelements led to higher and significant values of DHA than the control (without foliar spraying). *Grotz* and *Guerinot* (2006) found that microelements played an important role as major components or as functional, structural or regulator co-factors of large numbers of enzymes. For example, Fe and Mn had vital roles in enzymes function involved in respiration and biosynthesis of cells as well as activators for oxidation and reduction, while Zn acts as a co-factor of many enzymes.

### 3.7 Soil chemical properties

Table 8 indicates that the highest values of available nutrients (N, P, and K) were observed in soil amended with biochar at a rate of 80 g $pot^{-1}$, whereas the lowest values were found when the soil did not receive biochar. These results are in agreement with *Güereña* et al. (2015) who reported that biochar application resulted in increasing N retention in soils. Also, *Sohi* et al. (2009) mentioned that biochar addition had positive effects on nutrients availability in the soil and could be used effectively as a soil conditioner. Application of biochar to soils could increase their pH values and enhance amounts of available K, Ca, and Mg (*Gaskin* et al., 2010). Our results reveal that the electric conductivity (EC) and cation exchange capacity (CEC) were highly affected by biochar addition to soils. Biochar at different doses caused increases for all estimated chemical parameters relative to the control. In this respect, *Amonette* and *Joseph* (2009) reported that biochar application to soils markedly influenced their chemical properties such as pH, EC, CEC, and nutrient concentrations. Moreover, *Mete* et al. (2015) found an increase in CEC and this can improve nutrient retention in soils. The beneficial effect of biochar in increasing retention of nutrients could result from its high porous structure and associated surface area. In addition, the high surface area of biochar could increase cation exchange capacity and enhance the ability of soils to retain and supply nutrients (*Barbosa de Sousa* et al., 2014).

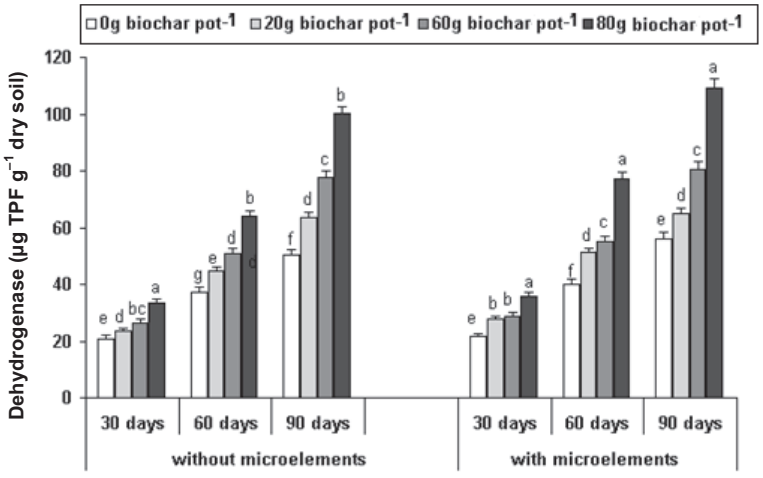


**Figure 2:** Changes in soil dehydrogenase activity in faba bean rhizosphere in different biochar treatments with and without microelements at three experimental times. Different letters indicate a significant difference between treatments at P < 5% according to Tukey's HSD.

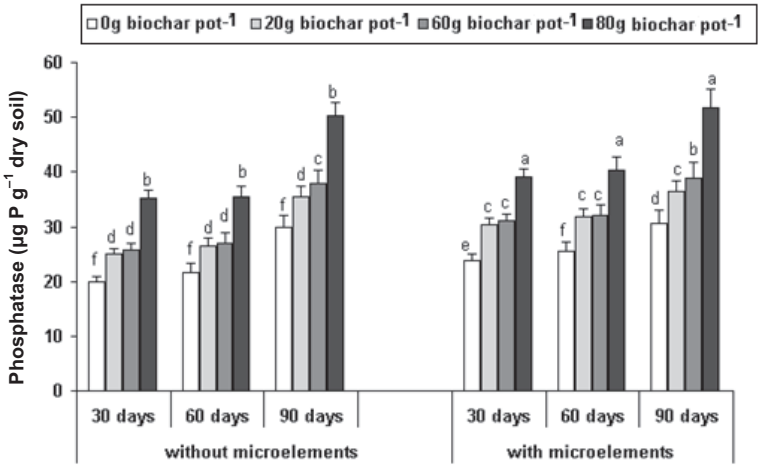


**Figure 3:** Changes in soil phosphatase activity in faba bean rhizosphere in different biochar treatments with and without microelements at three experimental times. Different letters indicate a significant difference between treatments at P < 5% according to Tukey's HSD.

## 4 Conclusion

Application of biochar with microelements caused positive effects on growth and seed quality of faba bean. Values of DHA and P-ase, nodulation, and $N_2$-ase activity in the rhizosphere of faba bean were markedly affected by the combination of biochar with microelements. Biochar addition in the chosen clayey loam soil increased dry weights of faba bean organs and concentrations of carbohydrates, protein, fat, and microelements (Fe, Mo, Mn, Zn) in the seeds. Our results confirm the potential of biochar and microelement application in enhancing the performance of bean plants and soil fertility.

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**Table 8**: Soil chemical properties under different treatments.[a]

| Treatments | Available nutrients (mg $kg^{-1}$) | | | pH | EC ($ds\ m^{-1}$) | CEC ($cmol_c\ kg^{-1}$) |
|---|---|---|---|---|---|---|
| | N | P | K | | | |
| T1 | 21.8 ± 0.32 d | 13.74 ± 0.12 d | 165 ± 3.14 d | 7.56 ± 0.01b | 1.09 ± 0.04 c | 28.4 ± 0.43 d |
| T2 | 24.7 ± 0.24 c | 14.83 ± 0.14 d | 170 ± 2.13 d | 7.61 ± 0.01 b | 1.14 ± 0.05 c | 30.1 ± 0.42 c |
| T3 | 26.3 ± 0.25 b | 17.28 ± 0.23 c | 188 ± 1.98 b | 7.69 ± 0.02 a | 1.32 ± 0.03 b | 31.9 ± 0.51 b |
| T4 | 30.7 ± 0.61 a | 19.59 ± 0.21 b | 211 ± 3.67 a | 7.74 ± 0.01a | 1.40 ± 0.03 a | 34.2 ± 0.13 a |
| T5 | 22.1 ± 0.43 d | 14.13 ± 0.11 d | 170 ± 4.11 d | 7.48 ± 0.02 c | 1.12 ± 0.05 c | 29.3 ± 0.24 d |
| T6 | 25.4 ± 0.54 c | 17.52 ± 0.25 c | 180 ± 3.29 c | 7.57 ± 0.02 b | 1.18 ± 0.03 c | 31.0 ± 0.31 c |
| T7 | 28.7 ± 0.32 b | 19.03 ± 0.34 b | 191 ± 2.87 b | 7.60 ± 0.03 b | 1.39 ± 0.06 b | 32.8 ± 0.56 b |
| T8 | 31.9 ± 0.63 a | 21.02 ± 0.16 a | 214 ± 1.89 a | 7.69 ± 0.01 a | 1.45 ± 0.03 a | 35.6 ± 0.45 a |

[a]Data in the table are mean values ± standard errors. Different letters in same column indicate a significant difference between treatments at probability level of P < 5% according to Tukey's HSD.

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