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GROWTH OF SPINACH AS INFLUENCED BY BIOCHAR AND *BACILLUS ENDOPHYTICUS* IGPEB 33 IN DROUGHT CONDITION

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Abstract

Drought is a major problem in the world, cause of various crops yield loss among abiotic factor. The impact of biochar and *Bacillus endophyticus* IGPEB 33 on spinach growth and root parameters and soil enzyme activities in drought conditions was studied. A net-house experiment was carried out at IGPEB, Kibray, Uzbekistan. Four treatments, such as control, biochar alone, *B. endophyticus* IGPEB 33 alone, and a combination of biochar and *B. endophyticus* IGPEB 33, were used in the experiment. After sixty days, plant growth and root traits were measured. The results showed that the combined application of biochar and *B. endophyticus* IGPEB 33 significantly enhanced plant height by 45%, leaf number by 61%, and leaf length by 58% compared to those of the control plants under drought conditions. Moreover, *Bacillus endophyticus* IGPEB 33 with biochar significantly enhanced root length, projected area, root surface area, root volume, root diameter, and soil enzyme activities (catalase, urease, invertase) compared to the controls. The results concluded that the joint application of biochar 1% and *Bacillus endophyticus* IGPEB 33 positively influences soil enzyme activities, root parameters, and growth in spinach under drought stress.

Key words: *Spinacia oleracea*, Plant growth, Promoting bacteria, Water scarcity, Growth parameters, Key enzymes.

Introduction

The most prominent abiotic stress in semi-arid and arid regions of the world is agricultural drought stress. Furthermore, droughts are becoming more severe due to climate change (Rakesh *et al.*, 2019). By 2025, a 60% rise in the demand for water for agriculture is anticipated worldwide (Boretti & Rosa, 2019). The sustainability of global food production is severely affected by drought mediated by water constraints (Lobell *et al*., 2014; Parvin *et al*., 2019; Khatun *et al*., 2021). Due to inadequate nutrient levels, poor photosynthesis, and depleted water supplies, drought stress typically results in decreased crop growth and yields (Fang *et al.*, 2020; Jabborova *et al.*, 2021a; Zafar-ul-Hye *et al*., 2021; Jabborova *et al.,* 2022a). Drought is thus likely to affect the world's ability to meet the agricultural needs of a growing global population (Wang *et al*., 2003). Vegetables are generally sensitive to drought stress, which could also lead to substantial production reductions, particularly in dry and semi-arid environments. Recent studies have revealed that drought stress has a detrimental impact on tomato growth and physiological traits (Ors *et al*., 2021).

Climate change mitigation through sustainable conservation practices greatly improves soil properties and plant growth (Rakesh *et al.*, 2021; Khan *et al.*, 2021a; Jabborova *et al.*, 2021a; Rakesh *et al*., 2022). A carbon-rich substance called biochar, produced by the pyrolysis of diverse biomasses, is known as biochar. In addition to lowering atmospheric CO_2 concentrations and mitigating global warming, biochar also helps to improve agricultural soils (Lehman *et al.*, 2006). Biochar improves soil ability to hold water, reducing the effects of drought stress (Akhtar *et al.*, 2015). According to earlier studies, adding biochar to soil has massively improved overall plant growth and root parameters (Major *et al.*, 2010; Jabborova *et al.*, 2021b; Jabborova *et al.*, 2021c; Jabborova *et al.*, 2021d). Recently, a promising new strategy to increase long-term yield and water use potency utilizing biochar has been proposed (Singh *et al*., 2019). The application of biochar has been revealed to promote the soil qualities and enhance the activity of soil microbes (El Nahhas *et al.*, 2021; Elsaeed *et al.*, 2021). Numerous studies have shown that using biochar alone boosted the activity of several enzymes (Trupiano *et al.*, 2017; Jabborova *et al.*, 2020a; Jabborova *et al.*, 2021e). The availability and adsorption of soil nutrients are other key functions of biochar. The soil nutrients N, K, Ca, and total carbon are increased by biochar (Saxena *et al.*, 2013; Wang *et al.*, 2014).

Involement of plant growth-promoting bacteria (PGPB) in growth promotion is one of their properties (Zafar-ul-Hye *et al*., 2020; Gupta *et al.*, 2022). PGPB are symbiotic, root-dwelling bacteria that can either directly or indirectly stimulate plant development (Vimal *et al.*, 2017; Sarkar & Rakshit, 2021). Natural processes by which PGPB execute the processes including mainly N fixation, K solubilization, and phytohormone functioning (Sarkar *et al.,* 2021; Jabborova *et al.,* 2022b; Mamarasulov *et al.,* 2022; Jabborova *et al.,* 2022c). Numerous PGPB have been identified and have been demonstrated to reduce of the adverse effects of various environmental stresses on plants (Jabborova *et al.,* 2020b; Kim *et al.,* 2022; Admassie *et al.,* 2022). Extensive research has looked into the efficacy of PGPB for a sustainable agro-ecosystem in drought conditions (Tiepo *et al.,* 2018; Naderi *et al.,* 2022).

The nutritional content of spinach (*Spinacia oleracea* L.), which includes bioactive substances, vitamins, and minerals, is high (Nemadodzi *et al.,* 2017; Bukhari *et al.,* 2021). It has a lot of bioactive substances that support its anticancer, anti-obesity, hypoglycemic, and hypolipidemic activities (Roberts & Moreau, 2016). In this study, we hypothesized that joint application of biochar and *B. endophyticus* IGPEB 33 would improve spinach growth, promote physiological processes and increase soil enzyme activities under drought conditions.

Materials and Methods

Soil and biochar were analyzed by Jabborova *et al.,* (2021f). The municipal solid waste biochar was obtained from the Soil Sciences department of Biology faculty, National University of Uzbekistan (41.35040N, 69.20585 E). Pyrolysis of municipal solid waste biochar was carried out at 500◦C for 40 min. The municipal solid waste biochar properties are shown in Tables 1 and 2.

Experimental design: The impact of biochar and *Bacilus endophyticus* IGPEB 33 on spinach growth and root morphological traits was conducted under drought stress in the net house at IGPEB. Total 4 treatments: control, biochar alone, *B. endophyticus* IGPEB 33 alone and a combination of biochar and *B. endophyticus* IGPEB 33 were used in an experiment. Spinach seeds were cultivated into plastic pots (diameter 26 cm, depth 22 cm) containing 8.0kg of soil. After sowing 60 days, plants were harvested, and plant growth parameters were measured.

Measurement of root traits: The whole root parameters were determined using by scanning system (STD4800, Epson, CA). Digital images of the root parameters were determined using Win RHIZO software.

Photosynthetic pigment measurement: "Photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents) of younger leaves in spinach were measured by the method of Hiscox & Israelstam (1979).

Soil enzyme activities measurement: Urease activity was analyzed using Pancu & Gautheyrou (2006). Soil samples (2.5 g) were added with toluene (0.5) mL for 15 min. Invertase and catalase activity in soil have also been analyzed by Xaziev (2005) method. The urease, invertase, and catalase of soil were determined in a spectrophotometer. Data were analyzed with the StatView Software using ANOVA. The significance of the effect of treatment was determined by the magnitude of the p-value ($p<0.05<0.01$).

Results and Discussion

Plant growth in drought conditions: In our results, biochar and *B. endophyticus* IGPEB 33treatments promoted plants' growth compared to the control in drought stress (Table 3). The plant height, leaf length, leaf number, and leaf width increased sharply, which significantly enhanced by 36%, 27%, 48%, and 22% at biochar treatment, respectively, in control. The *B. endophyticus* IGPEB 33 alone significantly promoted plant height by 22%, leaf length by 19%, leaf number by 13%, and leaf width by 10% than the control. Combination of biochar and *B. endophyticus* IGPEB 33 significantly enhanced plant height by 45%, leaf length by 58%, leaf number by 61%, and leaf width by 30 % in drought stress.

Root morphological traits: Root morphological traits were promoted by biochar and *B. endophyticus* IGPEB 33 treatments under drought stress (Table 4). Biochar significantly increased the total root length by 52%, projected area by 26%, root surface area by 38%, root volume by 13%, and root diameter by 48% than the control. Inoculated *B. endophyticus* IGPEB 33 alone significantly promoted the total root length, projected area, root surface area, and root diameter by 25%, 18%, 18%, and 18%. Combination with biochar and *B. endophyticus* IGPEB 33 significantly stimulated the total root length by 61%, projected area by 36%, root surface areaby59%, root volume by19%, and root diameter by 69% over the control.

Photosynthetic pigments: Biochar and *B. endophyticus* IGPEB 33 significantly promoted the photosynthetic pigments in spinach under drought stress (Fig. 1). The biochar alone significantly increased the chlorophyll a, chlorophyll b content, total chlorophyll content, and carotenoid content of the leaf by 83%, 34%, 62%, and 107%. *B. endophyticus* IGPEB 33 alone significantly stimulated the chlorophyll a, total chlorophyll, and carotenoid contents of the leaf by 31%, 22% and 37%, respectively. Combined *B. endophyticus* IGPEB 33 and biochar showed more positive effects on the photosynthetic pigments of spinach under drought stress.

Soil enzyme activities: Data in Table 5 showed that combined with *B. endophyticus* IGPEB 33 and biochar treatment stimulated soil enzyme activities in drought conditions. Combined with *B. endophyticus* IGPEB 33and biochar significantly promoted the catalase, invertase, and urease activities in soil by 13%, 13%, and 43%, respectively. Biochar treatment significantly boosted the urease activity in soil by 38%. *B. endophyticus* IGPEB 33alone promoted reuse activity in the soil. It resulted in a 23% increase in urease enzyme in soil under drought stress.

Table 1. The municipal solid waste biochar characteristics.

Biochar	BOC (g/kg)	BOM (g/kg)	TN (g/kg)	TP (g/kg)	TK (g/kg)	AN (mg/kg)	AP (mg/kg)	AK (mg/kg)	pH
Mean contents	330.6	570.0	0.26	4.48	42.6	237.8	0.77	688.9	8.01

Note BOM: Biochar organic matter; BOC: Biochar organic carbon; TN: Total nitrogen; TP: Total phosphorus; TK: Total potassium; AN: Available nitrogen; AP: Available phosphorous; AK: Available potassium

Table 2. Biochar properties (ash contents, volatile matter, fixed carbon and EC).

Biochar	Ash contents (%)	Volatile matter (%)	Fixed carbon (%)	EC (µS/cm)
Mean contents	10.8	40.2	49.0	609

Table 3. Plant growth of spinach as influenced by drought stress.

Treatments	Shoot length (cm)	Leaf length (cm)	Leaf number	Leaf width (cm)
Control	1.92 ± 0.01	15.03 ± 0.03	31.00 ± 0.13	4.21 ± 0.01
Biochar alone	2.62 ± 0.01*	19.12 ± 0.05*	46.00 ± 0.56*	5.13 ± 0.01*
B. endophyticus 33	2.35 ± 0.01	17.83 ± 0.07*	35.00 ± 0.34*	4.65 ± 0.01
Biochar + *B. endophyticus* 33	2.79 ± 0.01*	23.71 ± 0.10**	50.00 ± 0.48**	5.48 ± 0.02*

Asterisk differed significantly at p<0.05*, p<0.01**

Table 4. Root parameters in spinach as influenced by drought stress.

Treatments	TRL (cm)	PA (cm^2)	RSA (cm^2)	RV (cm^3)	RD (mm)
Control	97.51 ± 3.14	8.13 ± 0.11	18.11 ± 0.16	0.63 ± 0.01	0.86 ± 0.01
Biochar alone	148.14 ± 5.97*	10.28 ± 0.13*	25.04 ± 0.22*	0.71 ± 0.01*	1.27 ± 0.01*
*B.endophyticus*33	122.18 ± 6.13*	9.56 ± 0.12*	21.43 ± 0.18*	0.68 ± 0.01	1.08 ± 0.01*
Biochar + *B. endophyticus* 33	157.30 ± 7.28**	11.05 ± 0.09*	28.87 ± 0.20	0.75 ± 0.01	1.45 ± 0.01*

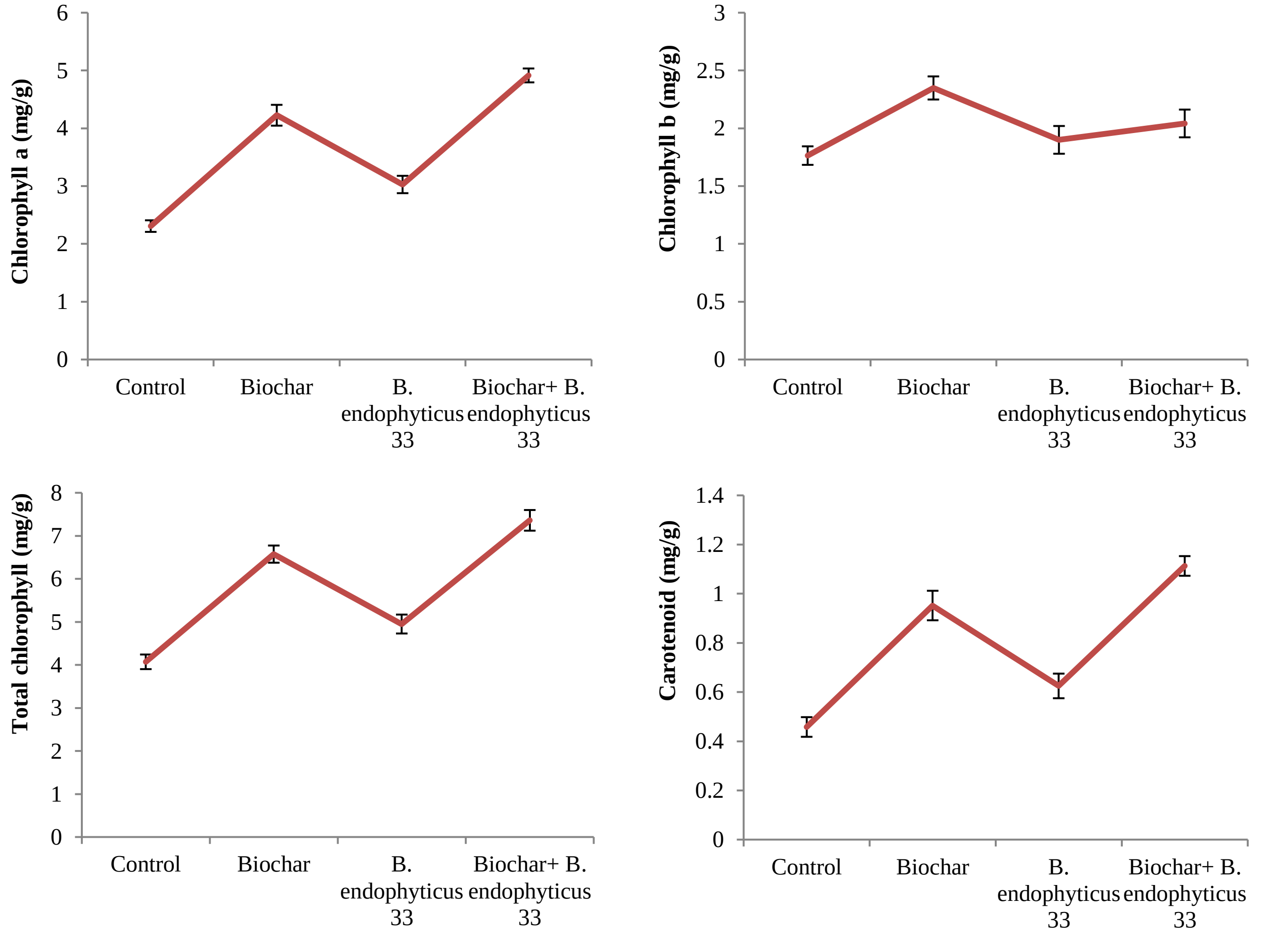

Fig. 1. Photosynthetic pigments of spinach as influenced by drought stress.

Table 5. Enzyme activities of soil as influenced by drought stress.

Treatments	Activity of catalase ($mL\ KMnO_4\ g^{-1}$ soil h^{-1})	Activity of invertase (µg glucose·g^{-1} soil·h^{-1})	Activity of urease (1 µg pNP/h/g of soil)
Control	7.85 ± 0.02	8.11 ± 0.01	18.36 ± 0.04
Biochar alone	8.22 ± 0.01	8.98 ± 0.02*	25.42 ± 0.03*
*B.endophyticus*33	8.16 ± 0.02	8.28 ± 0.03	22.63 ± 0.05*
Biochar + *B. endophyticus* 33	8.84 ± 0.03*	9.13 ± 0.02*	26.19 ± 0.05*

Asterisk differed significantly at $p<0.05$, $p<0.01$**

Discussion

In the current study, drought decreased plant growth, root parameters, and physiological traits in spinach. Drought decreases plant growth (Vishnupradeep *et al.*, 2022; Kiran *et al.*, 2022), root parameters (Hosseini *et al.*, 2022), and yield in many crops (Ors & Suarez, 2017; Bheemanahalli *et al.*, 2022). Nawaz *et al.*, (2020) observed decreased photosynthesis and transpiration rate of maize in drought stress. Similarly, Tng *et al.*, (2022) reported that plants' photosynthesis, fresh leaf mass, and leaf tough decreased by drought. Seymen (2021) informed that drought reduced carotenoid, chlorophylls a and b content in *Spinacia oleracea* L. Increased root length and chlorophyll of red cabbage due to application of *Pseudomonas fluorescens* and *B. subtilis* under reduced fertilizer dose was reported (Sarkar *et al.*, 2022). Numerous studies found that drought stress reduced soil enzyme activities (Siebielec *et al.*, 2020; Abdul Rahman, 2021).

The use of biochar significantly stimulated the growth and physiological characteristics of spinach. Similarly, Bu *et al.*, (2020) found that biochar significantly stimulated *Robinia pseudoacacia* L. Numerous studies have shown that applying biochar stimulated the development and photosynthesis of crops in drought stress (Abideen *et al.*, 2020; Beiranvandi *et al.*, 2022). Integrating biochar into soil has been found to promote soil fertility and stimulate plant growth and the physiological processes under drought stress (Khan *et al.*, 2021b). In addition, biochar improves photosynthesis and promotes soil quality in drought stress (Hafez *et al.*, 2020). The use of biochar enhanced the catalase, invertase, and urease activities. Similar results demonstrating that biochar boosted soil dehydrogenase activity and catalase activity, as well as soil microbial biomass carbon, were reported by Kumar *et al.*, (2013).

The present experiment investigated that *Bacillus endophyticus* IGPEB 33 improved spinach growth and enzyme activity's growth, and physiological traits in soil under drought stress. Presently, PGPB such as *Bacillus subtilis*, *Bacillus endophyticus*, *Pseudomonas putida*, and *Azospirillum lipoferum* have been reported to benefit the crops in drought stress (Bano *et al.*, 2013; Kumar *et al.*, 2017; Kour & Yadav, 2022). Similarly, *B. subtilis* Rhizo SF 48 inoculated promoted plant height and chlorophyll of tomatoes in drought conditions (Gowtham *et al.*, 2020). Similarly, Tiwari *et al.*, (2016) confirmed that Pseudomonas putida MTCC5279 improvedplant growth, increasing chickpea's biochemical traits in drought stress. Lastochkina *et al.*, (2020) reported similar findings *B. subtilis* 10-4 increased wheat chlorophyll a, b, and carotenoids in drought stress. Similarly, plant growth promoting *P. putida* increased plant growth, the leaves chlorophyll of *Medicago sativa* and soil phosphatase, β-galactosidase, and arylamidase activities under stress condition was informed by Tirry *et al.*, (2021). Mutumba *et al.*, (2018) informed that inoculated *Bacillus* sp. enhances shoot and root weight, chlorophyll index of wheat, and urease enzyme activity in soil under water stress.

In particular, the combined treatment of biochar and *Bacillus endophyticus* IGPEB 33 resulted in significantly higher morphological traits in spinach than in control. Combined with B. endophyticus IGPEB 33 and biochar, it had greater favorable impacts on its photosynthetic pigments in spinach and soil enzyme activities. Combined with biochar and PGPB improve plant growth, increase physiological properties, and enhance plant tolerance to drought stress in plants (Nadeem *et al.*, 2017; Ullah *et al.*, 2020; Waqar *et al.*, 2022). Similarly, Danish & Zafar-ul-Hye, (2019) investigated combined with *B. amyloliquefaciens* and biochar stimulated shoot length, chlorophyll a, chlorophyll b, and photosynthetic rate of wheat under drought conditions. He was similarly reported by Ren *et al.*, (2020), combined with *Bacillus megaterium* and wheat-derived biochar, improved soil properties. Hafez *et al*., (2019) informed that combining biochar and PGPR increases rice's chlorophyll content and stomatal conductance and promotes soil properties under drought stress.

Conclusion

Bacillus endophyticus IGPEB 33 promoted the growth of spinach effectively as it was seen to increase the number of leaves, length of leaves, width of leaves, and length of shoot under drought stress. The impact of biochar was also seen on the photosynthetic pigments in spinach while the soil enzyme activities were promoted. However, joint inoculation of biochar and *B. endophyticus* IGPEB 33 significantly improves plant growth, spinach physiological traits, and soil enzyme activities in drought conditions. The combined inoculation of biochar and plant growth promotes beneficial bacteria as bio-fertilizer, as it can potentially increase tolerance for drought stress in spinach cultivation and enhances soil enzyme activities.

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