



Improvement of Sandy Soil Fertility by Applying some Organic Amendments and Plant Growth Promoting Bacteria and their Reflection on Crop Productivity

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ABSTRACT

There have been several breakthroughs in recent years aimed at bettering soil conditions by enhancing soil properties that boost agricultural output. So, two field experiments were carried out at the farm of the Ismailia Agriculture Research Station Ismailia Governorate, Egypt, during two successive seasons (2020-2021 and 2021) to study the effect of some soil organic amendments potassium humate (K-H) and calcium humate (Ca-H) at 30 L fed⁻¹, in combined with different rates of biochar (BC) (2, 4 and 6 %) with inoculation with plant growth-promoting rhizobacteria (PGPR) namely (*Bacillus amyloliquefaciens* (B.amy), *Bacillus subtilis* (B.sub) on wheat and peanut crops productivity. Results indicated a slight decrease in soil pH with all treatments in both seasons but showed some increases in electric conductivity (E.C) and organic matter (O.M%) compared to the control. Concerning available N, P and K, they were increased in wheat and peanut when applying 2% biochar plus K- humate (K-H) with mixed bacteria as compared with applied calcium humate (Ca-H) with mixed bacteria. Moreover, soil amendments inoculation with mixed bacteria increased the dehydrogenase activity and total count of bacteria. Application of K-H plus 2% of biochar recorded the highest value of yield components as compared to other treatments. Similar results were recorded with the macronutrients in straw and grain or seeds of wheat and peanut crops, respectively, as well as improved the photosynthetic pigments and carotenoids contents. The results proved that the combination of humic materials (either K-H or Ca-H) with biochar (2%) and mixed bacteria increased soil fertility in sandy soil and consequently on the growth of wheat and peanut plants productivity.

Keywords: Organic Soil amendment; Potassium humate; Calcium humate; Biochar; *Bacillus amyloliquefaciens*; *Bacillus subtilis*.

Introduction

Most of the accessible zones for expanding agricultural activities are characterized as sandy soils; the effect of heightened climatic variability on plants in sandy soils may be attributed to the limited water retention and temperature regulation capabilities of the parent material (Yost and Hartemink, 2019). It is, therefore, necessary to apply different sources of soil amendment to this soil beside the usual fertilizers such as organic acids, biochar and biofertilizer. Soil conditioning refers to any procedure that enhances the capacity of sandy soil to boost crop yields or enhances the overall performance of soil for various purposes. Consequently, any substance used in the process of soil conditioning, regardless of whether it consists of organic elements or not, may be classified as a soil conditioner (Abdel- Fattah and Abdel- Rahman, 2015).

The organic molecules known as humic acids (HA) are crucial to the enhancement of soil quality, plant development, and agronomic characteristics. Coal, lignite, soil, and organic matter are all potential HA sources (Billingham, 2012; Hayes and Swift, 2020). Humic substances (HS) increase plant production, nutrient availability, and absorption by improving membrane permeability, enzyme activities, and hormonal activity in plants, as well as water holding capacity (Kamh and Hedia, 2018). In general, 85-90% negative charge in HS originates from the dissociation of H ion from the functional groups especially carboxyl and phenol, HS is regarded as a supra molecular assembly of small to large

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molecules (Orazio and Senesi, 2009). Also, (HA) has the ability to aggregate both in aqueous environments and in solid form. The stability of these aggregates is attributed to hydrophobic interactions, hydrogen bonding, and the inclusion of metal ions, such as (K), (Ca), (Fe), or (Al) as mentioned by Kotoky and Pandey (2019). In addition, humic compounds have a pronounced attraction for weak acids that possess a phenolic hydroxyl, carboxyl group, or amino sulfhydryl. Several alkaline cations, including Na^+ , K^+ , Ca^{2+} , and Mg^{2+} , are effectively sequestered by a straightforward cation exchange process involving COOH groups. This results in the formation of RCOONa and RCOOK compounds (Zhang *et al.*, 2013). Results obtained by Gümü and Seker (2015) suggest that HA (K-Humate) might be a useful management tool for ensuring the long-term health of the soil environment.

Potassium humate, is an organic substance derived from natural sources. Its use has been shown to enhance the physical and chemical characteristics of soil, as well as increase the dynamics of nutrient availability (Kumar *et al.*, 2014; Abd- All *et al.*, 2017). Also, potassium humate (K-H) is a salt that is obtained from humic acid (HA) and is used for enhancing plant development and productivity via the application of soil and foliar methods (Da Silva *et al.*, 2021). Moreover, Noroozisharaf and Kaviani (2018) has been suggested that the function of HA in promoting plant development and enhancing crop productivity may be linked to its facilitation of element transfer from the soil to the plant, achieved by augmenting the permeability of the cell membrane. In addition, potassium (K) is categorized as a macroelement that is required for the majority of physiological activities occurring inside plants (Mridha *et al.*, 2021). Calcium is an essential nutrient for plants throughout the growth phase, since it serves as a secondary messenger in signal transduction, which is a critical process involved in the environmental conditions and responses associated with plant growth and development. Calcium ions have a crucial role in several cellular processes, including cell wall formation, cell membrane integrity, fruit development, and overall cellular growth. Furthermore, they contribute to enhanced plant growth and improved nutrient uptake efficiency (Pilbeam and Morely, 2007; Shafeek *et al.*, 2013).

In addition, modern agriculture techniques that include soil amendment may boost soils' ecosystem services. Biochar (BC) has been used to enhance the physical and chemical characteristics and fertility of sandy-textured soils that are low in fertility or are polluted (Razzaghi *et al.*, 2020). Biochar is a carbonaceous substance with a fine particle size that is derived from the thermal decomposition of biomass, such as wood, dung, or leaves, under controlled conditions of low or absent oxygen availability. The use of biochar as a soil amendment is a promising strategy for addressing climate change and enhancing agricultural yields (Lehmann *et al.*, 2011). Biochar, when combined with soil, may modify hydrologic characteristics and nutrient availability, both of which have an impact on plant development (Wang *et al.*, 2020). Also, biochar has been studied for its potential benefits because to its high water-holding capacity, high cation exchange capacity, and neutral to high surface area (Zhu *et al.*, 2017). Blanco-Canqui (2017) it has been observed that several farmers have used biochar as a means to amend sandy soils. However, there exists a significant lack of understanding about the advantageous outcomes of using biochar on a large-scale and over an extended period of time, particularly with regards to its impact on soil parameters. Further research is required to assess the enduring impacts of biochar on the physical, chemical, and biological characteristics of sandy soils (Huang and Hartemink, 2020).

Plant growth-promoting rhizobacteria (PGPR) play a significant role in the soil microbiota and are recognized for their ability to enhance biological nitrogen fixation, solubilize phosphate, produce indole acetic acid, augment root surface area, and enhance plant development (Bashan *et al.*, 2014; Viscardi *et al.*, 2016). Plant growth was stimulated after being inoculated with PGPR bacteria such *Bacillus subtilis* and *Bacillus amyloliquefaciens* due to the formation of IAA, siderophores, antifungal activity, HCN, and ACC deaminase (Belnap and Lange 2003; Shaharoon *et al.*, 2008). Plant roots may be colonized by PGPR, making it a useful tool in efforts to increase wheat and peanut growth in sandy soil. During chemotaxis, PGPR was able to identify root exudates such amino acids and carbohydrates. Phosphate solubilization and the production of growth-stimulating hormones are two additional ways in which PGPR promotes expansion (Majeed *et al.*, 2015)

Wheat (*Triticum aestivum* L.) is one of the main cereal crops all over the world and one of the most essential crops in Egypt, which acting as main role in food and nutritive security. Increasing of wheat production is a critical national goal to diminish the gap amongst the Egyptian production and ingesting by increasing the cultivated capacity and wheat productivity per unit area (Badawi *et al.*, 2021).

Peanut (*Arachis hypogaea* L.) is a main legume crop in arid and semi-arid areas of the world (Kheira, 2009). The seed is composed of several components, including oil (48-50%), protein (25-28%), carbs (20-26%), as well as a multitude of minerals, vitamins, dietary fibers, phytosterols, and flavonoids (Bishi *et al.*, 2015).

Therefore, the objective of this work is to study the effect of some organic soil amendments (K-H and Ca-H) plus biochar rates and inoculation by *Bacillus subtilis* and *Bacillus amyloliquefaciens* this study investigates the chemical characteristics of sandy soil and examines the impact of microbial activity on the productivity of wheat and peanut crops.

2. Materials and Methods

The present work was carried out at the Ismailia Agriculture Research Station farm, Ismailia Governorate, Egypt. The farm is located at 30 o 35 ' 41.9 " N latitude and 32 o 16 ' 45.8 E longitude, during two successive seasons of 2021 and 2022. Wheat (*Triticum aestivum* L Giza 168) in the winter and peanut (*Arachis hypogaea* L. Giza 6) in summer were planted in sandy soil under sprinkler irrigation system. This study was conducted to evaluate the effectiveness of some organic soil amendments (i.e., K-H and Ca-H) combined with different rates of biochar (BC) which enriched by *Bacillus amyloliquefaciens* (B.amy, KR149334.1), *Bacillus subtilis* (B.sub, KP196795), and a mixture of them on some soil chemical properties, yield components, and nutritional status of both wheat and peanut crops. The characteristics of the investigated soil before cultivation were done according to the methods described by Page *et al.* (1982) and Cottenie *et al.* (1982). Some physical and chemical soil properties before planting are recorded in Table (1). As well as the characteristics of biochar, potassium humate and calcium humate in Tables (2), (3) and (4).

Table 1: Some physical and chemical properties of the experimental soil.

Particle size distribution					Soil physical properties								
Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Soil texture	Bulk density (gm cm ⁻³)	Total porosity (%)	SP						
70.0	23.65	3.54	2.81	Sandy	1.74	34.7	23						
Soil chemical properties:													
O.M (%)	pH Soil-water suspension ratio (1:2.5)	EC dSm ⁻¹	Soluble cations (meq L ⁻¹)				Soluble anions (meq L ⁻¹)			Available macronutrients (mg kg ⁻¹)			
0.34	7.73	0.44	Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	CO ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻	N	P	K
			1.02	0.99	1.30	1.00	-	1.92	1.2	1.19	39	8.1	50

Table 2: Some chemical properties of biochar.

Parameters	Values	Parameters	Values
pH (1: 2.5 soil: water suspension)	8.73	P (%)	0.37
EC dS m ⁻¹ (1:5)	2.97	K (%)	0.52
CEC (cmol kg ⁻¹)	28.5	H (%)	2.75
OC (%)	58.27	S (%)	-
N (%)	1.21		
C/N ratio	48.16		

Table 3: Some chemical properties of potassium humate.

Parameters	Values	Nutrients (mg L ⁻¹)	Values	Nutrients (mg L ⁻¹)	Values
pH	7.99	P	10.6	Fe	7.58
O.C (%)	0.61	K	3.96	Mn	0.91
O.M (%)	1.03	Na	0.81	Zn	0.30
N (%)	0.52	Ca	5.61	Cu	0.19
C/N ratio	1.21	Mg	321		

Table 4: Some chemical properties of calcium humate.

Parameters	Values	Nutrients (mg L ⁻¹)	Values	Nutrients (mg L ⁻¹)	Values
pH	8.13	P	12.9	Fe	2.11
O.C (%)	0.58	K	0.87	Mn	0.08
O.M (%)	1.01	Na	0.81	Zn	-
N (%)	1.39	Ca	6189	Cu	0.91
C/N ratio	0.41	Mg	228		

2.1. Experimental design and treatments

The field experiment was set up in a split-split plot design with three replicates. The main plots were two organic soil conditioners (potassium humate (K-H) and calcium humate (Ca-H) at 30 L fed⁻¹), which were sprayed on the soil surface at three times which were 15, 30 and 45 days from sowing. The sub main plots represent three rates of biochar (2, 4 and 6 %). The sub-sub plots represent inoculation with bacteria (*Bacillus amyloliquefaciens*, *Bacillus subtilis*) and a mixture of them. Ten L fed⁻¹ from bacteria were added at three doses during the planting period of 15, 30 and 45 days from sowing.

2.2. Bacterial strains

These bacterial strains (*Bacillus subtilis*, KP196795) and *Bacillus amyloliquefaciens*, KR149334.1) were obtained from the Soils, Water and Environment Research Institute, Agricultural Research Center (Ghazal, 2018). The strains were assessed for their quantitative potential in synthesizing plant growth stimulating chemicals, namely indole acetic acid (IAA) and gibberellins (GA). Two strains were cultivated separately in a nutrient broth medium (Difco, 1984). IAA was determined according to Glickmann and Dessoux (1995) and gibberellin (GA) according to Shindy and Smith (1975).

2.3. Fertilization

All treatments were added at the doses recommended by the Egyptian Ministry of Agriculture for wheat and peanut crops. Superphosphate (15% P₂O₅) was added at a rate of 200 Kg fed⁻¹ before cultivation; potassium sulphate (with a K₂O content of 48%) was introduced at a rate of 50 kg per feed unit, given in two equal doses. The first dosage was applied during the sowing process, while the second dose was administered 30 days following sowing. Ammonium nitrate (33.5% N) was used as the nitrogen source, with application occurring in four equal increments at intervals of 15, 30, 45, and 60 days after the seeding of wheat and peanut crops.

2.4. Examined parameters

2.4.1. Soil analysis

At the conclusion of the growing season, soil samples were collected at a depth of 0-15 cm. These samples were then air-dried, crushed, thoroughly mixed, and sieved to ensure passage through a 2mm sieve. The prepared samples were then stored until they could be evaluated for various chemical qualities according to Cottenie *et al.* (1982).

2.4.2. Enzymes Activity

The dehydrogenase activity in soil was determined after 30 and 60 days from sowing according to Thalman (1967).

2.4.3. Bacterial counts

Total bacterial counts was determined after 30 and 60 days from sowing according to Holm and Jensen (1972).

2.4.4. Plant samples

During the harvest period, plant samples were gathered in order to assess the yield components, namely straw weight, seed weight, and/or seed count, for both wheat and peanut crops. The plant specimens were subjected to weighing, followed by oven drying at a temperature of 70°C until a consistent dry weight was achieved. Subsequently, the dried samples were pulverized and subjected to digestion using a combination of H₂SO₄ and H₂O₂ as described by Page *et al.*, (1982). The digested

samples were then analyzed for nutritional content, namely nitrogen (N), phosphorus (P), and potassium (K), using the designated methodology described by Cottenie *et al.* (1982) Moreover, the total nutrient content was determined in kilograms fed⁻¹ in both plant debris and seeds.

2.5. Photosynthetic pigments

The quantification of photosynthetic pigments, including both chlorophyll and carotenoids, was conducted in the foliage of several plant species at 30 and 60 day periods by the method of Niroula *et al.* (2019). The computations were performed using mathematical formulae derived by Hendry and Grime (1993) the concentration of pigments.

2.6. Statistical analysis

The acquired data underwent statistical analysis of variance using a computer software CoStat Software (2004).

3. Results and Discussion

3.1. Indole acetic acid and gibberellins

The ability of *Bacillus amyloliquefaciens* (KR149334.1) and *Bacillus subtilis* (KP196795) to produce indole acetic acid (IAA) and gibberellins (GA) as plant growth promoting substances was examined and the obtained results are shown in Table (5).

Both bacterial strains produced high amounts of IAA and GA in their nutrient liquid cultures. However, *Bacillus amyloliquefaciens* gave slightly higher amounts of both IAA and GA than those produced by *Bacillus subtilis*. Since, IAA values were 272.03 and 169.87 µg/ml, respectively, while in GA they were 119.62 and 102.89 µg/ml in *Bacillus amyloliquefaciens* and *Bacillus subtilis*, respectively. The current results are convenient with those obtained by Bonatelli *et al.*, (2020); Magotra *et al.*, (2021) diverse studies have indicated that many species of *Bacillus* exhibit diverse plant growth-promoting (PGP) activities, including the production of indole-3-acetic acid (IAA) and gibberellic acid (GA). Moreover, several prior research studies have shown the presence of plant growth-promoting (PGP) features in *Bacillus* species, namely via the synthesis of phytohormones like gibberellin (Bottini *et al.*, 2004) and IAA (Idris *et al.*, 2007). Da Silva *et al.*, (2021) has been discovered that plant growth-promoting bacteria (PGPB) are a significant biological alternative that has the ability to enhance crop yields in many agricultural contexts.

Table 5: Indole acetic acid and gibberellins amounts produced by *Bacillus amyloliquefaciens* and *Bacillus subtilis*.

Strains	IAA(µg/ml)	LSD at 0.05	GA(µg/ml)	LSD at 0.05
<i>Bacillus amyloliquefaciens</i>	272.03	1.07	119.62	1.03
<i>Bacillus subtilis</i>	169.87	1.01	102.89	1.04

3.2. Soil chemical properties

The results in Table (6) show the effects of different treatments of humic substances and biochar under inoculation with bacteria on some chemical properties of soil (i.e., pH, EC, O.M % and availability of N, P and K in soil) at the two successive seasons of wheat and peanut yields.

3.2.1. Soil reaction

The soil pH was seen to experience a small fall as a result of the application of treatments in comparison to the control. This phenomenon was seen in both seasons. Regarding the effect of different soil amendments, they cause a slight decrease on pH as it ranged from 7.99 to 7.20 in both seasons, respectively. This observation is anticipated since the administration of therapies resulted in heightened biological activity facilitated by the production of organic acid. While the mean values of K-H and biochar BC₁ (0.2%) under inoculation with *Bacillus amyloliquefaciens* and *Bacillus subtilis* were more superior in decreasing pH as compared to other treatments (i.e., Ca-H). The observed phenomenon may be attributed to the introduction of amendments, resulting in a decrease in soil pH. Additionally, potassium humate functions as a buffering agent, aiding in the stabilization of soil pH in response to the application of fertilizers. The findings exhibited concordance with those of Campitelli *et al.*, (2008). It agrees also with those found by Simon *et al.*, (2019) The individual or entity responsible for declaring

that biochar resulted in a modest reduction in pH by 0.22 has not been specified. The alteration in soil pH may occur as a result of fluctuations in climatic circumstances and changes in land-use patterns. Hence, it is advisable to conduct a comprehensive assessment of the impact of biochar on soil pH over an extended period of time in order to get a deeper comprehension of the pH dynamics in soils exhibiting varying levels of acidity. Also, Nasef *et al.*, (2009) and Monowara *et al.*, (2019) the findings suggest that the use of bio-fertilizer and humate in conjunction with nitrogen mineral fertilizer led to a decrease in soil pH. This may be attributed to the presence of diverse acids, including amino acids such as glycine and cysteine, as well as humic acid, together with acid-forming chemicals and active microorganisms. The enzymatic activity of dehydrogenase and the subsequent release of carbon dioxide inside the rhizosphere lead to the generation of carbonic acids, resulting in a reduction in pH levels within the root zone.

3.2.2. Electric conductivity

Regarding the dissolved salt concentration (electrical conductivity), data in Table (6) revealed that the EC value increased with the application of amendment as compared to control. In this study, the highest EC was recorded by using K-H over Ca-H. In addition, the effect of K-H and biochar BC₁ (0.2%) under inoculation with PGPR especially *Bacillus amyloliquefaciens* and *Bacillus subtilis*, had a positive effect, as the values also increased compared to control. On the other hand, the results showed decreases in the EC values in all treatments under Ca-H the EC values in soils tend to decrease but are non-significant. Because of its aromatic character and high carboxylic and phenolic group content, K-H is an excellent soil conditioner. Furthermore, biological activity, chemical interactions, and physical enhancements to soil all contribute to a greater potential for cation exchange. (Dejou, 1987 and Amjad *et al.*, 2010). The presence of many negatively charged functional groups on the surface of biochar, together with an enhanced cation exchange capacity (CEC) of the soil, enables biochar to effectively retain nutritional cations such as NH₄⁺, K⁺, Ca²⁺, and Mg²⁺. (Laird *et al.*, 2010; Wang *et al.*, 2015). The authors provided further clarification that biochar has a higher concentration of soluble salts, which are subsequently released into the soil solution, resulting in an elevation of the soil electrical conductivity (EC). The rise in EC (electrical conductivity) was also attributed by (Shareef *et al.*, 2018) regarding the liberation of loosely bound ions from the biochar into the soil solution.

3.2.3. Organic matter

The effects of applied treatments on soil organic matter content in sandy soil are shown in Table (6). Application of humic substances to soil significantly improved the organic matter for both K-H and Ca-H compared to the control. Regarding soil organic matter content %, data indicated significant increases in all treatments. It also increased by addition of biochar under inoculation with mixed bacteria (*Bacillus amyloliquefaciens* and *Bacillus subtilis*) compared to Ca-H in the two seasons. Similar results were reported by Carpenter *et al.*, (2000) who demonstrated that humic acid (HA) is the predominant component of soil organic matter and plays a crucial role in sustaining soil health by enhancing soil aggregation. The potential cause for the observed rise in organic matter subsequent to the application of biochar may be attributed to the substantial carbon (C) content inherent in biochar. Furthermore, several studies have shown a notable augmentation in the levels of organic matter content, total nitrogen, and carbon-to-nitrogen ratio subsequent to the application of biochar by irrigation. This phenomenon is believed to potentially enhance soil fertility (Mekki *et al.*, 2006; Brunetti *et al.*, 2007 and Mekki *et al.*, 2013). The treatment of K-H shown better efficacy due to the organic nature of potassium humate, which facilitates the proliferation of soil microorganisms (Khaled and Fawy, 2011). HA can be broken down in the environment, but at a slow rate that provides a steady supply of carbon to the soil. In a review made by Sible *et al.*, (2021) when compared to FA, HA are rich in C. This suggests that soil microorganisms may get more of the carbon (C) they need for their biological activity if HA is applied.

Table 6: Some chemical soil properties after harvesting of wheat and peanut plants supplemented by humic substances plus biochar with inoculation of bacteria in sandy soil.

Treatments Organic soil amendments			Wheat			Peanut			
Humic substances	Biochar rates	Inoculation	pH	Ec (dsm ⁻¹)	O.M %	pH	Ec (dsm ⁻¹)	O.M %	
Cont.			7.99	1.13	0.42	7.90	1.17	0.81	
K-H	BC ₁	B.amy	7.29	1.26	0.99	7.84	1.69	1.56	
		B.sub	7.41	1.21	0.97	7.70	1.76	1.29	
		B.amy+B.sub	7.28	1.23	0.97	7.5	1.69	1.31	
		Mean	7.33	1.23	0.98	7.69	1.72	1.39	
	BC ₂	B.amy	7.44	1.22	1.07	7.61	1.70	1.16	
		B.sub	7.32	1.23	0.76	7.69	1.65	1.43	
		B.amy+B.sub	7.20	1.24	0.98	7.33	1.64	1.32	
		Mean	7.32	1.23	0.94	7.54	1.66	1.31	
	BC ₃	B.amy	7.31	1.24	0.99	7.35	1.69	1.34	
		B.sub	7.32	1.23	0.99	7.60	1.68	0.82	
		B.amy+B.sub	7.46	1.22	0.98	7.65	1.66	1.03	
		Mean	7.36	1.23	0.99	7.53	1.67	1.06	
	Ca-H	BC ₁	B.amy	7.36	1.11	0.71	7.67	1.70	1.08
			B.sub	7.31	1.12	0.58	7.80	1.67	1.30
			B.amy+B.sub	7.27	1.12	0.58	7.50	1.73	1.23
			Mean	7.31	1.12	0.62	7.66	1.70	1.20
BC ₂		B.amy	7.32	1.13	0.76	7.70	1.70	1.26	
		B.sub	7.50	1.12	0.79	7.85	1.65	1.34	
		B.amy+B.sub	7.48	1.14	0.77	7.33	1.66	1.08	
		Mean	7.43	1.13	0.77	7.63	1.67	1.22	
BC ₃		B.amy	7.52	1.14	0.73	7.91	1.68	1.33	
		B.sub	7.44	1.14	0.81	7.71	1.68	1.47	
		B.amy+B.sub	7.54	1.12	0.83	7.78	1.70	1.16	
		Mean	7.50	1.13	0.79	7.80	1.69	1.32	
Mean Humic substances		K-H	7.34	1.23	0.97	7.59	1.68	1.25	
		Ca-H	7.42	1.13	0.73	7.69	1.68	1.25	
Mean biochar rates		BC ₁	7.32	1.18	0.80	7.67	1.71	1.30	
		BC ₂	7.38	1.18	0.86	7.58	1.67	1.27	
		BC ₃	7.43	1.18	0.89	7.67	1.68	1.19	
Mean inoculation		B.amy	7.38	1.18	0.88	7.68	1.69	1.29	
		B.sub	7.38	1.18	0.82	7.73	1.68	1.28	
		B.amy+B.sub	7.37	1.18	0.85	7.52	1.68	1.19	
L.S.D. 0.05									
Humic substances (A)			0.16	0.19	0.07	0.14	0.22	0.09	
Biochar rates (B)			0.12	0.16	0.05	0.11	0.19	0.07	
inoculation(C)			0.22	0.29	0.03	0.19	0.28	0.05	
AB			0.13	0.16	0.03	0.15	0.17	0.03	
AC			0.24	0.33	0.05	0.22	0.35	0.04	
BC			0.19	0.24	0.04	0.18	0.48	0.06	
ABC			0.21	0.44	0.07	0.23	0.51	0.12	

3.3. Available nutrients

Data representing the availability of soil N, P and K after wheat and peanut harvesting were shown in Table (7). The available N, P, and K in soil were significantly enhanced by applying the treatments at the two seasons. Addition of different rates of biochar caused higher available nitrogen contents in soil compared with biochar. The highest nutrients content (N, P and K) recorded 160.33, 48.66 and 162.51 mg kg⁻¹, respectively, was found at 2% and 4% of biochar application which was significantly higher, especially K-H under inoculation with mixed bacteria compared to the control.

Table 7: Available macronutrients in soil after harvesting of wheat and peanut plants as affected by humic substances and biochar with inoculation of bacteria in sandy soil.

Treatments			Wheat			Peanut		
Organic soil amendments		Inoculation	mg Kg ⁻¹					
Humic substances	Biochar rates		N	P	K	N	P	K
	Cont.		94.86	14.67	44.20	87.02	13.50	46.80
K-H	BC ₁	B.amy	116.03	29.93	68.25	113.29	21.00	78.65
		B.sub	125.83	28.17	76.70	115.95	23.00	72.15
		B.amy+B.sub	160.33	38.33	120.25	121.91	48.67	123.50
		Mean	134.06	32.14	88.40	117.05	30.89	91.43
	BC ₂	B.amy	121.52	31.13	120.90	109.37	30.80	70.20
		B.sub	113.68	30.27	83.20	117.60	28.00	79.30
		B.amy+B.sub	141.51	31.37	162.50	125.17	39.33	93.60
		Mean	125.57	30.92	122.20	117.38	32.71	81.03
	BC ₃	B.amy	139.16	38.07	140.40	126.54	37.00	129.35
		B.sub	119.95	34.87	92.30	120.70	26.20	110.50
		B.amy+B.sub	129.75	28.83	118.95	119.56	23.00	90.35
		Mean	129.62	33.92	117.22	122.26	28.73	110.07
Ca-H	BC ₁	B.amy	117.0	18.13	76.70	123.44	50.67	85.80
		B.sub	119.95	19.97	95.55	119.29	18.67	76.70
		B.amy+B.sub	133.67	24.43	139.75	117.60	24.75	85.80
		Mean	123.74	20.84	104.00	120.11	31.36	82.77
	BC ₂	B.amy	137.98	31.33	109.85	132.85	25.30	117.00
		B.sub	123.48	26.57	93.60	124.85	20.45	66.30
		B.amy+B.sub	150.14	20.53	98.80	118.70	29.27	80.60
		Mean	137.20	26.14	100.75	125.47	25.01	87.97
	BC ₃	B.amy	119.56	29.10	71.50	116.15	17.33	91.00
		B.sub	134.06	31.87	107.90	123.40	26.00	107.90
		B.amy+B.sub	118.38	26.33	87.10	119.91	22.67	80.60
		Mean	124.00	29.10	88.83	119.82	22.00	93.17
Mean Humic substances	K-H	129.75	32.33	109.27	118.90	30.78	94.18	
	Ca-H	128.31	25.36	97.86	121.80	26.12	87.97	
Mean biochar rates	BC ₁	128.90	26.49	96.20	118.58	31.13	87.10	
	BC ₂	131.39	28.53	111.48	121.42	28.86	84.50	
	BC ₃	126.81	31.51	103.03	121.04	25.37	101.62	
Mean inoculation	B.amy	125.31	29.62	97.93	120.27	30.35	95.33	
	B.sub	122.83	28.62	91.54	120.30	23.72	85.48	
	B.amy+B.sub	138.96	28.31	121.23	120.47	31.28	92.41	
L.S.D. 0.05								
Humic substances (A)			0.76	1.36	9.12	0.77	1.47	9.31
Biochar rates (B)			0.49	0.99	5.12	0.48	0.99	6.10
inoculation(C)			0.91	1.09	11.10	0.94	1.12	11.31
AB			0.65	1.71	6.10	0.66	1.61	6.81
AC			0.66	1.80	7.71	0.71	1.83	8.12
BC			0.75	1.69	10.40	0.82	1.66	11.30
ABC			0.61	1.87	12.60	0.71	1.78	13.10

The nutrient retention capacity of soil is contingent upon its cation exchange capacity. The research has shown that the application of humic acid (HA) leads to an augmentation in the cation exchange capacity (CEC) of the soil (Billingham, 2012). Yang *et al.*, (2021) the contribution of humic acids (HA) in enhancing cation exchange capacity (CEC) can be elucidated through the following mechanisms: Firstly, HA facilitates the adsorption of exchangeable cations by offering a substantial surface area for inorganic colloids. Secondly, the dissociation of carboxyl (COOH) and hydroxyl (OH) groups in HA generates polar ends that can form complexes with cations. Lastly, HA promotes the dissolution of soil minerals, resulting in the creation of extensive surface areas for chemical reactions. Additionally, the application of K-humate has been shown to improve the accessibility of vital plant nutrients such as

(N), (P), and (K). Furthermore, whether K-humate is used as a standalone treatment or in conjunction with other soil conditioners, its humic acid concentration has been shown to outperform other treatments (Abdel-Razek *et al.*, 2011). Biochar has potential as a viable potassium (K) source for crop absorption, particularly within the realm of organic agriculture. This is attributable to the fact that over 50% of the overall potassium content within biochar is solubilized in water, rendering it bioaccessible for plant use (Berek *et al.*, 2018). Recently, Karimi *et al.*, (2020) It has been suggested that the effects of biochar application on nutrient availability are contingent upon the rate of addition and the temperature at which pyrolysis occurs. Specifically, it has been shown that higher rates of biochar application result in enhanced availability of phosphorus (P) and potassium (K). Overall, the incorporation of maize residue biochar resulted in a notable enhancement in soil organic carbon levels, microbial biomass, respiration rates, as well as catalase and dehydrogenase activity. These findings suggest potential advantages in utilizing corn residue biochar in dry soils with limited fertility. Plant Growth Promoting Rhizobacteria (PGPR) has the ability to provide more nutrients to plants by solubilizing these nutrients via the synthesis of organic acids, excreting H^+ ions, phytases, and releasing hydrogen cyanide (HCN) (Backer *et al.*, 2018).

3.4. Dehydrogenase activity and bacterial counts

Dehydrogenase activity in soil was taken as a guide for respiration rate and total microbial activity in soil. Data in Figs. (1A and 1B) showed that the soil dehydrogenase activity and total bacterial counts were significantly increased in peanut treatments compared to wheat plants at two periods (30 and 60 day). Based on this, K-H treatments showed significantly higher increases in soil dehydrogenase activity and total bacterial counts than Ca-H treatments. Even though, results in Figs. (1A and 1B) showed the highest values of the soil dehydrogenase activity and total bacterial count in both wheat and peanut plants with soil application of K-H, biochar, and inoculation with *Bacillus amyloliquefaciens* and *Bacillus subtilis* compared with the control treatment. The highest values of soil dehydrogenase activity in wheat plant recorded 147.30 and 106.86 μg Tpf /g soil /24h and peanut plant (190.39 and 146.55 μg TPF/g soil /24h) at day 30 and 60, respectively. Also, the total bacterial counts in wheat plant were 9.20 and 7.54 Log cfu /g soil and peanut plant 9.44 and 9.13 cfu /g soil at day 30 and 60, respectively. These results agree with those obtained by Du *et al.*, (2018) who found that peanut-shell biochar (1%) increased microbial biomass. In that sense, Wang *et al.*, (2020) It has been observed that the application of a large dosage of biochar may have adverse effects on soil microbial communities, whereas a low dosage of biochar may have a beneficial influence.

Regarding soil enzymes, biochar showed positive impacts on soil enzymatic activities (Mierzwa *et al.*, 2016). Addition of biochar at rates of 5 and 10 $t\ ha^{-1}$ in an inception increased the dehydrogenase activity (Ameloot *et al.*, 2013; Mierzwa *et al.*, 2016). Furthermore, it was shown in a greenhouse research that the addition of biochar to the soil resulted in enhanced enzymatic properties. This improvement was found to be significant when biochar was applied at a rate of up to 6% (Yadav *et al.*, 2018). Application of biochar to the soil enhances microbial activity by increasing the soil nutrient contents and the mobility of nutrients. (Meier *et al.*, 2019). Furthermore, humic compounds have the potential to enhance the exudation of organic acids from the root system, hence promoting plant interactions with helpful microbes, such as plant growth-promoting rhizobacteria (PGPR) (Oliveras *et al.*, 2017; Nardi *et al.*, 2021). Furthermore, it has been previously shown that humic compounds have the capacity to enhance microbial proliferation, (Tikhonov *et al.*, 2010).

Finally, all treatments of soil dehydrogenase activity and total bacterial counts at 30 days of the experiment recorded the highest significant values. These results are like those explained by Wang *et al.*, (2008) who noted that the proliferations of plant roots, increase of metabolites and sufficient water have promoted the microorganism's activity. On the other hand, results showed a slight decline in dehydrogenase activity and bacterial count values at 60 days interval in all treatments. A decline in dehydrogenase activity occurring at regular 60-day periods might potentially be linked to reduced microbial populations, which in turn may be attributed to limited substrate supply necessary for sustaining microbial biomass. The microbial community at this particular phase of crop development exhibited reduced levels of metabolic activity, as indicated by Gaind and Nain (2012).

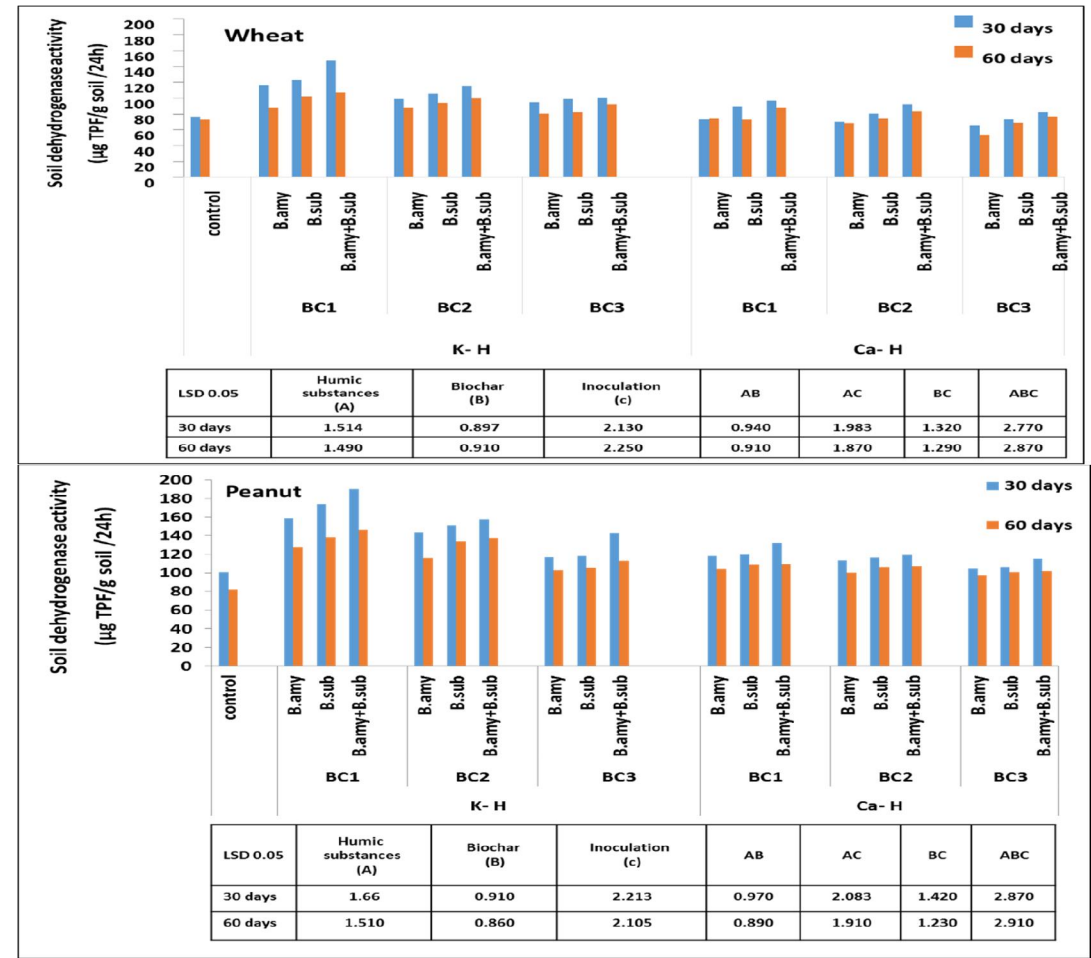
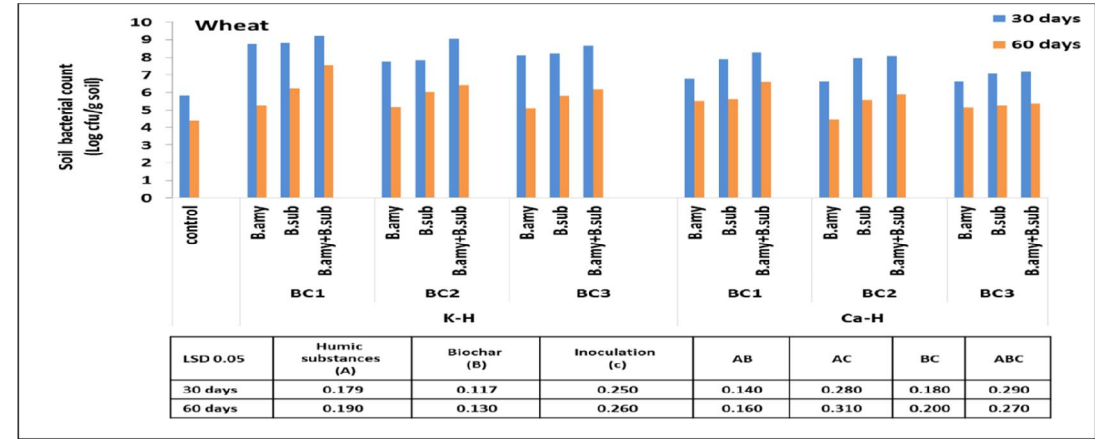


Fig. 1A: Dehydrogenase activity in soil of wheat and peanut plants as affected by humic substances and different rates of biochar with inoculation of bacteria in sandy soil after 30 and 60 days.



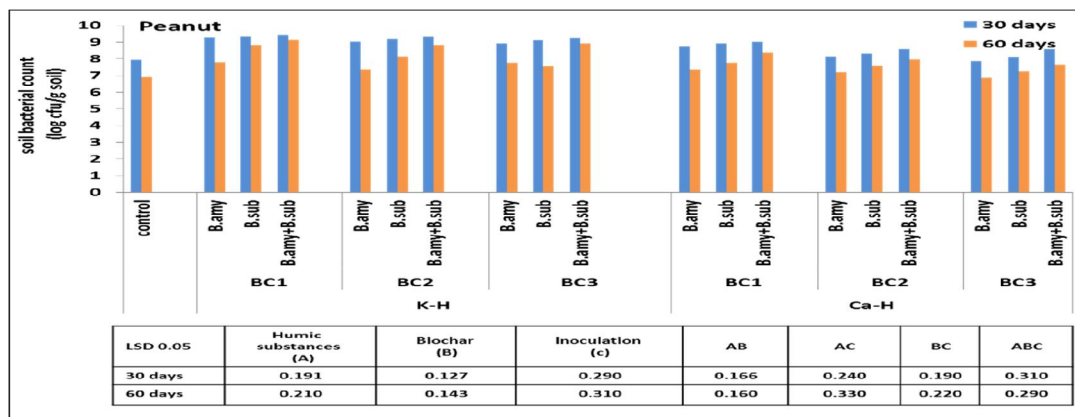


Fig. (1B): Bacterial count in soil of wheat and peanut plants as affected by humic substances and biochar with inoculation of bacteria in sandy soil after 30 and 60 days.

3.5. Yield components

Fig. (2) showed the effect of humic substances (K-H and Ca-H) and rates of biochar (BC) with inoculation by bacteria on wheat and peanut yield components (biological yield, grain, and straw). Generally, applied humic substances plus biochar with bacteria led to significantly increase in yield component as compared to control treatment. Yield components in the first and second seasons were increased by application of K-H compared to Ca-H and biochar BC₁ combined with bacteria, respectively. The observed enhancements in wheat and peanut output might potentially be attributed to the indirect impacts of (HS), biochar, and the application of (PGPR). These factors have been shown to stimulate soil enzyme activity and facilitate the proliferation of microorganisms in the rhizosphere, hence fostering improved crop development. With respect to the effect of different soil amendment, data indicated that application of humate potassium (K-H) and biochar generally, the study observed a significant increase in the favorable yield components of wheat and peanuts. These results are in harmony with those of Canellas *et al.*, (2008) the individual who reported the potential correlation between the rise in yield components and the stimulation of root development by HA, as well as its impact on root morphology via organic acid exudation, resulting in enhanced nutrient absorption and subsequent improvements in growth and yield. Also, Lehmann (2011) has been shown that the use of biochar has a beneficial impact on both the growth and yield components. It is important to carefully evaluate the optimal biochar rate and materials for application in order to prevent a reduction in crop yields or the degradation of soil qualities. Furthermore, Gaskin *et al.*, (2010) and Barrow (2012) A favorable correlation between the application of biochar and plant growth and biomass output was seen, but only when biochar was used in conjunction with a supplemental fertilizer source.

Furthermore, the yield components for both seasons exhibited a considerable increase when subjected to inoculation with a combination of bacteria. Moreover, the use of plant growth-promoting rhizobacteria (PGPR) has been shown to enhance plant development in both optimal and challenging environmental conditions. (Canellas and Olivares, 2014 and 2017). Prior studies have shown that alterations in root shape generated by humic substances might enhance the colonization of (PGPR), hence promoting their establishment and persistence on the surface of plant roots. (Canellas *et al.*, 2013; Canellas and Olivares, 2017). The application of (PGPR) has been shown to enhance the colonization zone in the soil rhizosphere and promote their proliferation in plants. This effect is mediated by (HSS) and is believed to contribute to the overall improvement in plant establishment (Canellas *et al.*, 2020).

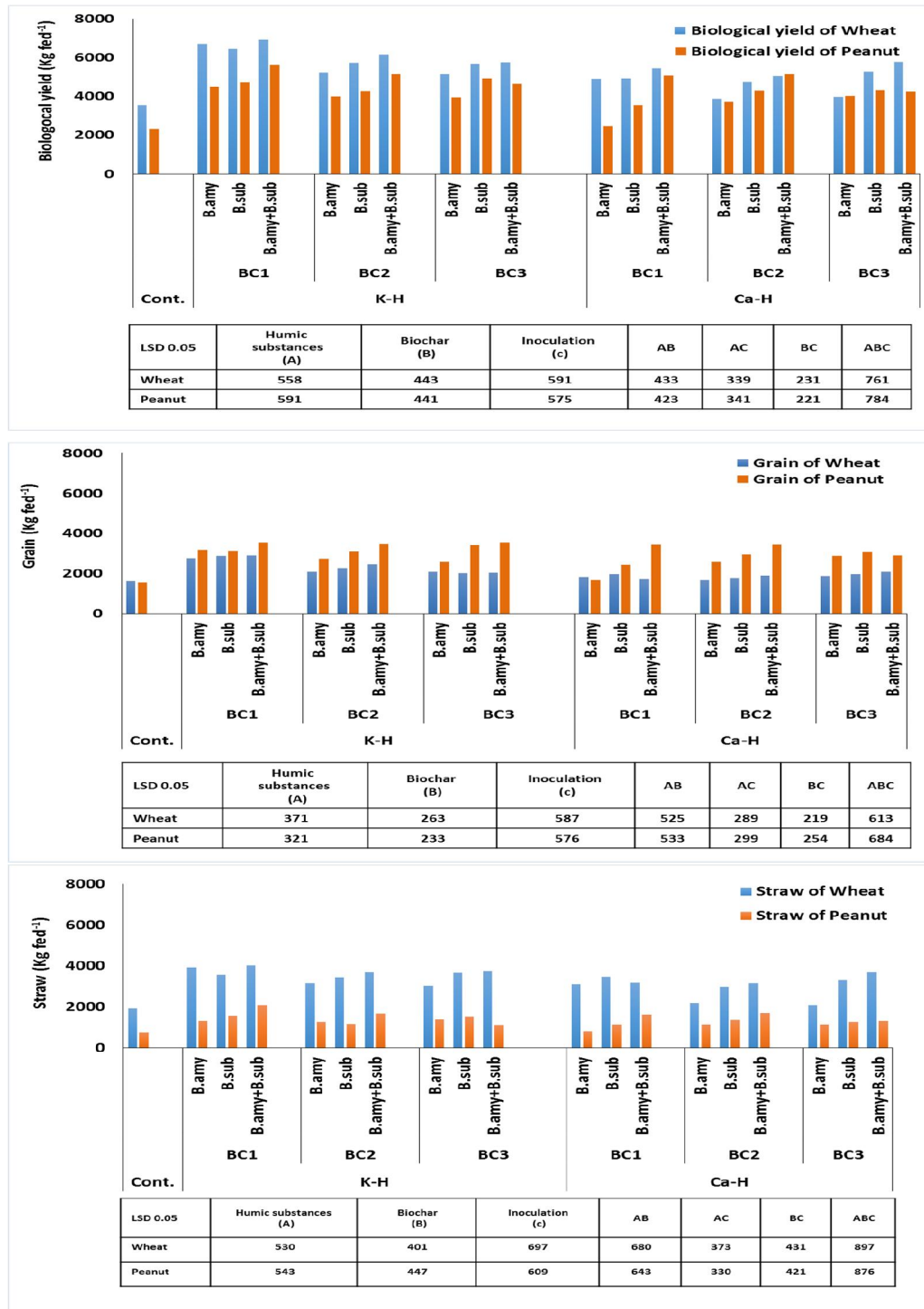


Fig. 2: Yield component of wheat and peanut as affected by humic substances and different rates of biochar with inoculation of bacteria in sandy soil.

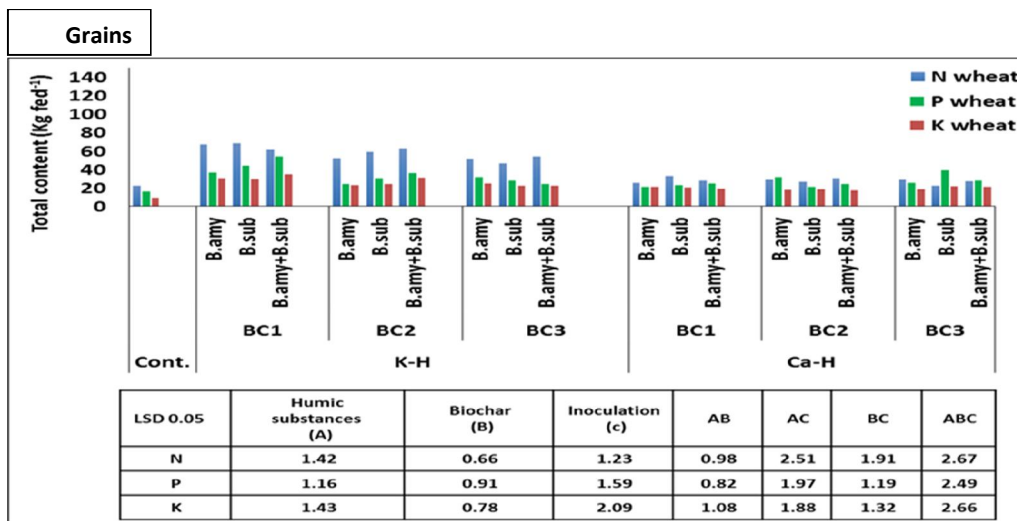
3.6. Macronutrients total content

Data presented in Fig. (3) indicated the effect of humic substances (K-H and Ca-H) and different rates of biochar under inoculation with bacteria on the total content of nitrogen, phosphorus and potassium in wheat and peanut crop (straw and grains). The findings indicated a noteworthy augmentation in the overall nitrogen, phosphorus, and potassium levels for both the grain and straw of both crops across all treatments administered, as compared to the control group. These findings are

consistent with those acquired by Muhammad *et al.*, (2021) They discovered that by applying humic substances (HS) and biochar, soil physiochemical characteristics are improved, which in turn increases grain production and the quantity of soil nitrogen available, which in turn increases plant development and yield (Farooq *et al.*, 2019) Additionally, the observed increase in grain production ranging from 43% to 68% may be attributed to the application of nitrogen (Farooq *et al.*, 2020a and 2020b).

The maximum value of macronutrients was observed with K-H combined with biochar BC₁ in the presence of mixed bacteria compared to Ca-H at the first and second seasons. Also, the behavior of the total macronutrients' contents showed a similar trend to those recorded by yield components. In general, NPK concentrations in plants are reflective of their accessible amounts in soil and the length of time it takes for these nutrients to be leached away from the soil when using various amendments. This outcome may be related to the effect of soil amendments in enhancing soil microbial activity in releasing nutrients essential for plant development. Microorganisms, which have increased in abundance at the cost of organic matter from foreign sources or produced from plant root activity, are the agents of nutrient release and mobilization among their many other roles in the soil (Abdallah *et al.*, 2016).

Concerning the treatment with K-H, it showed a clear effect on the total content of nitrogen and potassium. This may be due to an improvement in plant physiology because of adding the treatments. The observed rise in overall content as a result of the K-H phenomenon is likely attributable to the capacity of humic chemicals to enhance microbial activity (Mayhew, 2004), allowing more water and nutrients to enter cells and improving their capacity to divide (Sibanda and Young, 1986; Valdrighi *et al.*, 1996). It may also be related to K-H in increasing root development in hydroponic systems and produce an increase in root volume, which may be attributable to quicker absorption and more efficient nutrients. An increase in root development is one factor that has been linked to increased plant nutrient intake. Functional groups found in HA, such as carboxyl, phenol, and hydroxyl, may contribute to K⁺ binding by HA, which may explain why HS treatment reduced K⁺ leaching (Wang and Huang, 2001). One possible explanation for this finding is that calcium serves as a second messenger in the signaling of nutrient availability, therefore facilitating nutrient uptake by the plant's roots and facilitating their transportation throughout the plant (Jorg *et al.*, 2018).



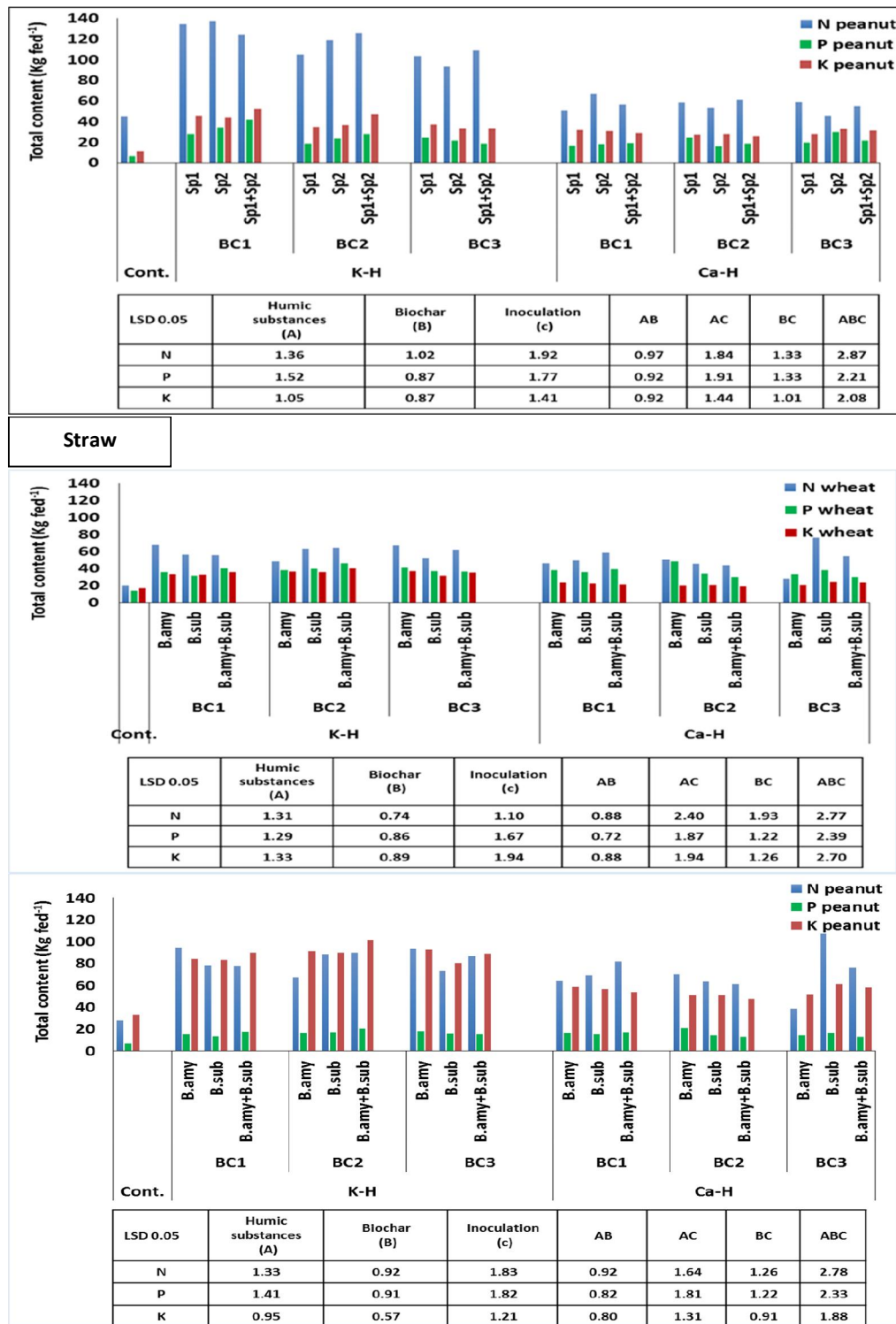


Fig. 3: Macronutrients total content of wheat and peanut as affected by humic substances and rates of biochar with inoculation of bacteria in sandy soil.

3.7. Correlations between the studied parameters

To make the picture clear the obtained results were expressed as linear correlation between grains yield and total contents of N, P and K as shown in Fig. (4). Significant positive linear correlations (R^2) were found between total contents of N, P and K and grains yield of both wheat and peanut. They were

0.914, 0.916 for N; 0.951, 0.948 for P and 0.938, 0.928 for K, respectively. This indicated the positive effect between the studied parameters and grains yield productivity for both wheat and peanut yields, respectively.

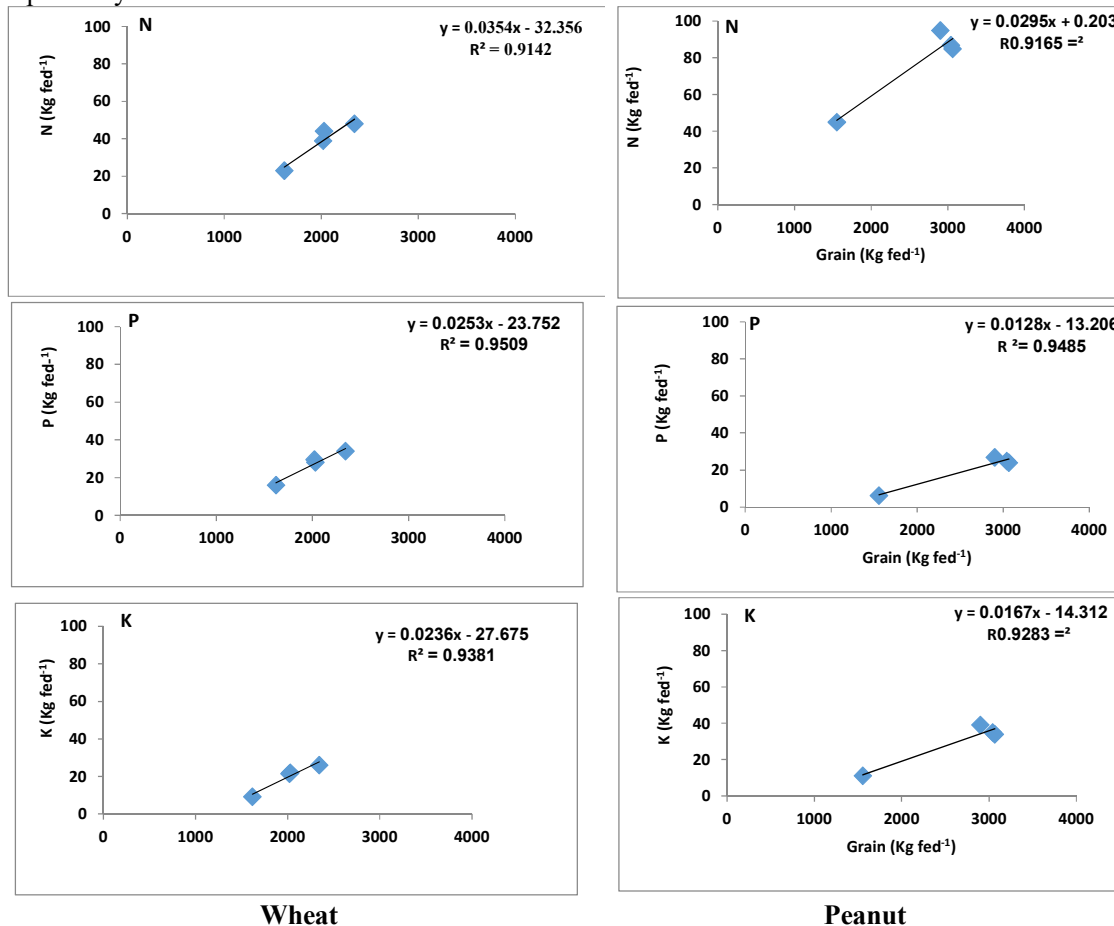
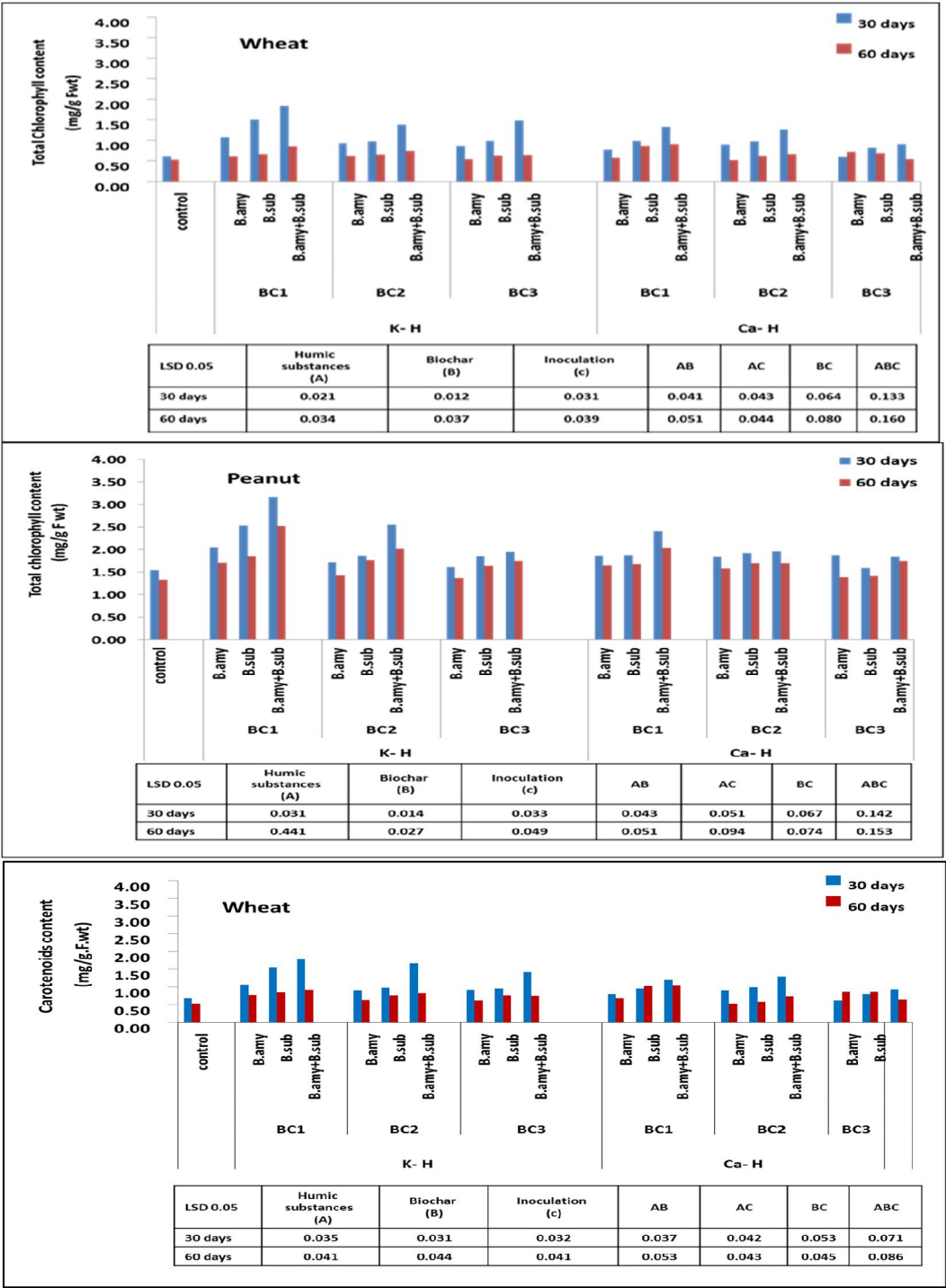


Fig. 4: Correlations between N,P, and K and grains yields of wheat and peanut crops. Photosynthetic pigments contents

Photosynthetic pigments contents.

Data in Fig. (5) Showed that the total chlorophyll and carotenoids contents were significantly higher in peanut treatments compared to wheat plant at 30 and 60 days. Also, K-humate treatments showed significant increases in total chlorophyll and carotenoids than Ca-humate treatments. However, all treatments inoculated with *Bacillus subtilis* showed non-significant increase in total chlorophyll and carotenoids compared to the treatments inoculated with *Bacillus amyloliquefaciens* and the highest total chlorophyll and carotenoid contents in wheat and peanut plant were recorded with soil application with K-humate, biochar and inoculation with mixed bacteria. Data fully agreed with the previous studies made by Radhakrishnan and Lee (2016) They studied the impact of PGPB (*Bacillus methylotrophicus* KE2) on the development and nutritional metabolites of lettuce and concluded that PGPB treatment boosts leaf pigments (total chlorophyll and carotenoids) content. The results are in agreement with those found by Han and Lee (2005) who observed that well-watered treatment with PGPMs and K-humate in salty soil resulted in the greatest levels of chlorophyll and carotenoids. Plants' chlorophyll and carotenoid content is inversely proportional to their mineral content (Pinto *et al.*, 2014).



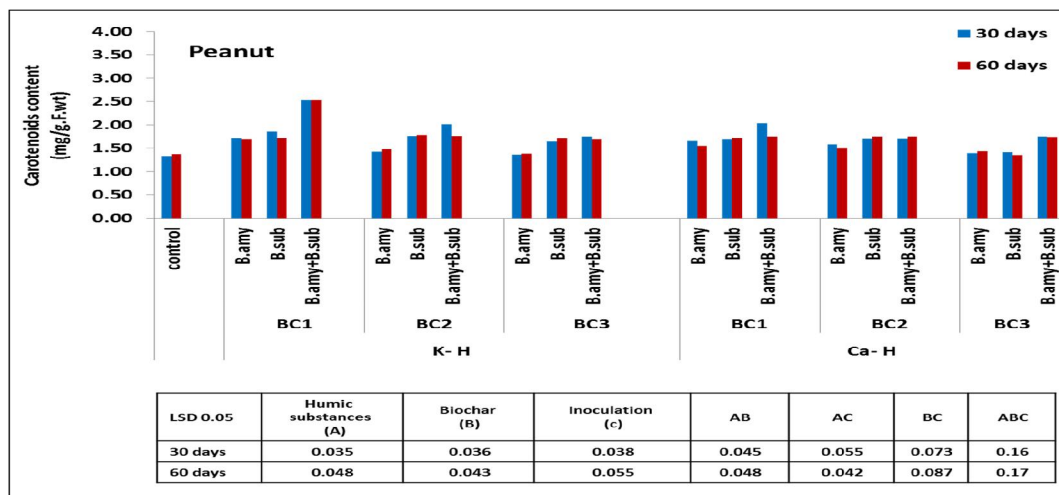


Fig. 5: Photosynthetic pigments contents of wheat and peanut as affected by humic substances and rates of biochar with inoculation of bacteria in sandy soil.

3.8. Correlation coefficient analysis between the investigated biological yield and enzymatic parameters for the different treatments under study in wheat and peanut plant

Data in Table (8) showed that all K-H treatments in both crops (wheat and peanut plant) yields are in strong positive significant correlation with total chlorophyll, carotenoids, dehydrogenase, and bacterial counts. The values for wheat were 0.09, 0.64, 0.63 and 0.72 and were 0.56, 0.85, 0.85 and 0.58 for peanut plants, respectively. Total chlorophyll and carotenoids gave strong positive correlations with yields. Moreover, data declared that peanut plants in both K-H and Ca-H showed strong positive correlation between yields and Total chlorophyll and carotenoids. These results are like those explained by Morais *et al.*, (2015) who first noticed that a substantial link exists between chlorophyll concentration and maize grain yield. Also, Pérez *et al.*, (2012) the author claimed that the strong link seen between carotenoids and grain production may perhaps be rationalized by their participation in the photosynthetic process. The strong positive correlation observed between the overall chlorophyll levels and grain yields has led to the utilization of chlorophyll as a reliable indicator for crop evaluation and estimation of grain yields. This is due to the fact that photosynthesis, a crucial process for plant energy production and subsequent grain formation, is reliant on the presence of chlorophylls and carotenoids. The results indicated, in general, that total chlorophyll as evidence of plant health and dehydrogenase are an indicator of microbiological redox systems are more important in k- humate treatment in both wheat and peanut plant.

On the contrary Ca-H treatments in the case of wheat plant, the correlation between yield and total chlorophyll. and carotenoids are negative (-0.25 and -0.19) and the correlation between yields and dehydrogenase and bacterial count are positive non-significant (0.04 and 0.05). While in the case of peanut plant the correlation between yields and dehydrogenase and total chlorophyll, and bacterial counts are positive non-significant correlation (0.41, 0.05 and 0.19 respectively).

Finally, in all studied treatments were with both wheat and peanut plants there were strong positive significant correlation between dehydrogenase and total chlorophyll, carotenoids and bacterial count, the correlation between chlorophyll and carotenoids and bacterial counts. And the correlation between carotenoids and bacterial counts are strong positive correlation. Generally, the results indicated that K-H treatment is more effective than Ca-H.

Conclusions Biological strategies, such as a combination of PGPR and HSs, provide hope for enhancing plant performance and metabolic processes while decreasing the economic and environmental costs associated with food production Olivares *et al.*, (2017).

Table 8: Correlation coefficient analysis between the investigated biological yields and enzymatic parameters for studied treatments with wheat and peanut plants.

	Soil Application	Variables	Soil Dehydrogenase (De-ase)	Total Chlorophyll Content (Total Chlorol.)	Carotenoids Content (Carot.)	Soil Bacterial Count
Wheat plant	K-H	Yield	0.90	0.64	0.63	0.72
		De-ase		0.81	0.77	0.77
		Total Chlorol.			0.96	0.85
		Carot.				0.89
	Ca-H	Yield	0.04	-0.25	-0.19	0.05
		De-ase		0.91	0.88	0.93
		Total Chlorol.			0.98	0.86
		Carot.				0.83
Peanut plant	K-H	Yield	0.56	0.85	0.85	0.58
		De-ase		0.87	0.82	0.90
		Total Chlorol.			0.97	0.90
		Carot.				0.86
	Ca-H	Yield	0.41	0.50	0.75	0.19
		De-ase		0.85	0.88	0.89
		Total Chlorol.			0.84	0.60
		Carot.				0.68

Pearson's correlation coefficient (r) is shown in the figures. Correlation is shown from most positive (yellow) to least positive (green) to most negative (blue). The color density and numbers show the scale of association *Significant level ($P < 0.05$).

4. Conclusions

Based on the above discussion it can be concluded that soil amendments with potassium humate and biochar combined with two bacterial strains (*Bacillus amyloliquefaciens* and *Bacillus subtilis*) soil characteristics including pH, organic matter, cation exchange capacity, and nutrient availability saw significant improvements. So, they improve the soil quality of sandy soil. As well as, increase plant growth, nutritional elements contents such as, dehydrogenases activity, total counts of bacteria and photosynthetic pigments. Soil amendments plus PGPR inoculation succeeded in increasing the growth as consequently yield of wheat and peanut plants grown in sandy soil.

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