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Combined effect of KSB and biochar on growth indices and phosphorus, potassium and chlorophyll content in sorghum under drought stress

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Abstract

Drought is a big threat to crop production these days, especially in arid and semi-arid regions. Availability and uptake of macro and micronutrients at an adequate level and conservation of soil moisture are relevant strategies in this regard. Potassium (K) plays a vital role to sustain crop growth under drought stress. The plant growth-promoting rhizobacteria with K solubilizing activity have the potential to provide the plants with K in enough quantity to cope with the effects of drought. The role of potassium solubilizing bacteria (KSB) have already been established. On the other hand, biochar is not only a good source of nutrients but also improves porosity and water holding capacity of the soil into which it is added. So, a pot study was designed to assess the combined role of KSB and biochar on growth indices and phosphorus, potassium and chlorophyll content in sorghum crop under water deficit conditions. The data depicted that KSB + biochar improved plant height, root length, shoot fresh biomass, root fresh biomass, potassium, and phosphorus content shoot of plants up to 30, 42, 49, 67, 36 and 44%, respectively over control at 30% of field capacity moisture level. Similarly chlorophyll a and b contents were found to be improved up to 60.8 and 43% over control at the moisture level of 30% of field capacity. It is concluded that the conjoint application of KSB and biochar is quite effective in improving sorghum growth under drought.

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Introduction

World human population is increasing day by day and according to an estimate, by 2050 will reach about 9.7 billion. To feed that huge population, the agriculture sector should improve on a sustainable basis. As far as crop production is concerned, sustainability can't be achieved without replenishing the nutrients depleted from the soil or taken up by the cops. So, the farmers have to use/depend on the chemical fertilizers in a large quantity (Glick, 2012). Although, chemical fertilizers add the essential nutrients to the soil but at the same time pose threats to soil and aquatic environments. Extensive and continuous use of nitrogen, phosphorus and potassium fertilizers deteriorate the soil environment (Adesemoye and Kloepper, 2009). It is essential to adopt more efficient and environmentally friendly techniques to boost up crop yields to feed the ever-increasing population. The focus must be shifted partially from chemical fertilizers to bio-fertilizers.

Moreover, many environmental factors including abiotic stresses like mineral nutrients, light, temperature, relative humidity, salinity and most importantly drought stress affect plant growth and yield. Climate change and drought conditions are the most important challenges for crop production in the 21st century especially in Europe, Central Asia and Africa (Haub *et al.,* 2012). Drought is the main problem for agriculture production and an alarming situation for the environment (Glick, 1998). It may reduce crop yield by up to 50% (Wang *et al.,* 2003). It results in the production of reactive oxygen species in plants that inhibit the production of nucleic acid, proteins and lipids (Kant *et al.,* 2006). Drought affects moisture contents in plants, rate of transpiration, early germination stages, root elongation leaf maintenance and the diffusion process (Hu *et al.,* 2007).

Potassium (K) is the 3rd essential macronutrient required for plant growth and development. It plays a pivotal role in the defense system of plants against diseases, pests and abiotic stresses. Potassium activates 80 different types of enzymes that take part in energy metabolism, nitrate reduction, photosynthesis, sugar degradation, and starch synthesis etc. (Almeida *et al.,* 2015).

Although, K is the seventh most abundant nutrient present in the earth's crust (Total K content in soils ranges between 0.04 and 3%), yet its availability for plant growth is very low. The rest of the K binds with other elements present in the earth's crust and becomes unavailable to plants. About 90-98 % of K present in the soil exists in mineral form and most of that is unavailable for plant growth (Sparks and Huang, 1985). Imbalanced fertilization renders a major part of K applied unavailable to plants. Its deficiency occurs too in certain crops due to depletion from soil solution and system (Meena *et al.,* 2014; Xiao *et al.,* 2017). Extensive use of the fertilizers put dangerous effects on the soil environment, so it is very necessary to find alternate technology to provide the plants with K for sustainable crop production.

As far as, environmental friendly approaches are concerned, using soil microbial community is one of the effective tools to improve soil fertility and plant growth through certain mechanisms (Parmar and Sindhu, 2013: Danish *et al.,* 2020). It is documented that some soil microorganisms including bacteria, actinomycetes and fungi have the ability to render K available to the plants through different mechanisms which may include the production of inorganic acids, enhancing exchange reactions in soil, production of polysaccharides and chelates or through acidolysis or complexolysis reactions. Potassium solubilizing bacteria (KSB) can help in K uptake by the plants and promote the yield of crops. They solubilize organic and inorganic reserves of K, present in the soil (Saha *et al.,* 2016). It is published that KSB has a very good role in the enhancement of the growth of various crops (Bakhshandeh *et al.,* 2017).

On the other hand, biochar is an organic amendment that is environment-friendly and a new unmatchable soil enrichment material (Budai *et al.,* 2016). Biochar is synthesized through pyrolysis which is a carbon sequestration method. Agricultural and industrial wastes are used to produce biochar (Chen *et al.,* 2010). So, biochar not only enhances crop productivity through the addition of organic matter and nutrients to the soil but also cleans the environment by using the wastes with minimum greenhouse gas emissions (Park *et al.,* 2011). Biochar is an active organic material with a high soil pore volume and cation exchange capacity and is being reported to decrease drought effects on plants (Wacal *et al.,* 2019).

The combined use of rhizobacteria and biochar provides a good interaction in the improvement of soil health and a good source of micro and macro-nutrient availability in the soil as well (Glick, 2012; Tahir *et al.,* 2013). Soil microbes and biochar simultaneously take part in the improvement of soil physical and chemical properties and increase the amount of organic matter in soil and ultimately promote the crop yield under drought conditions (Ngo *et al.,* 2014; Zafar-ul-Hye *et al.,* 2019).

Sorghum is a very important crop and considered as 5th most important crop in the world, it is used as a staple food in most parts of Asia and Africa and it is a major fodder crop in certain regions of USA, South Africa, Australia, and Mexico. Excellent drought-resistant properties of sorghum include it in the most important food and fodder crops in the whole world (Mann *et al.,* 1983).

A very little literature is available on the combined role of KSB i.e., *Bacillus* sp. and biochar under drought condition. So, the present trial was conducted with the hypothesis that KSB like *Bacillus sp.* in combination with biochar would grant resistance against drought to sorghum plants through K availability and improve growth and yield.

Materials and methods

Potassium solubilizing bacteria

A pre-isolated potassium solubilizing bacterial strain i.e. *Bacillus sp.* (ZM20) was collected from the Soil Microbiology Laboratory of University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan.

Experimental conditions

A pot experiment was designed to evaluate the role of KSB strain in combination with biochar on growth attributes and nutrients uptake by the sorghum in normal and drought conditions. The experiment was conducted in the experimental area of the Department of Soil Science, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University Multan, Pakistan. An 8 kg soil was taken in each pot. The soil was analyzed before the sowing of the crop for the physicochemical properties.

The properties of soil are given as follows: Textural class was clay loam with sand 30%, silt 38.6% and clay 34.3%. The pH was 8.23, EC was 0.45 dSm^{-1} and the saturation percentage was 33%. Total N, P and extractable K were 0.06%, 5.66% and 580 $mgkg^{-1}$, respectively.

Rhizobacteria inoculation

The selected seeds were inoculated using peat, clay and sugar solution (10%) as a carrier material. The treatments applied consisted of control (uninoculated), *Bacillus sp.* KSB, biochar 0.1%, biochar 0.3%, KSB + biochar 0.1% and KSB+ biochar 0.3% along with two moisture levels i.e., 70 and 30 % of field capacity (FC).

Sowing of seeds

Four seeds of sorghum variety JOHAR were sown in each pot in the September 2018 and pots were arranged in CRD design with three replications.

Fertilizer application

Fertilizers were applied uniformly rate according to the production technology of sorghum at a rate of 50 mg N, 50 mg of P and 40 mg of K per kg of soil in each pot. The sources of fertilizers were urea for nitrogen (N), murat of potash (MOP) for potassium (K) and single superphosphate (SSP) as phosphorus (P). The fertilizers were applied at the time of sowing. After 20 days of seed emergence, urea was applied again as 20mg per kg of soil.

Biochar

Two levels of biochar were used i.e., 0.1 and 0.3% in 8 kg soil. The soil was mixed well to make it homogeneous with respect to NPK and biochar application.

Drought stress

Drought stress was given to the selected treatments by calculating the saturation percentage of soil and amount of water for 30% and 70% moisture level of FC as per treatment plan. After 90 days of sowing, plant samples were taken for analyses.

Plant growth attributes

The plant physical parameters i.e. plant height, root length, shoot fresh biomass and root fresh biomass were recorded at the time of harvesting.

Phosphorus (P) and potassium (K) content

Shoot and root samples of sorghum were cleaned and oven-dried. After grinding the samples digestion was done according to the procedure as described by the Wolf (1982) for the full recovery of P and K contents. A 0.1gram of completely ground plant sample was taken in a digestion flask, 2ml of H2SO4 were added into it and left for overnight. These incubated plant samples were heated on the digestion block until fumes appeared from it. Then 1ml of H_2O_2 was added after every 20min interval until the plant material became a colorless solution. The volume of the extract was made to 10ml using distilled water. The phosphorus concentration was recorded in plant and soil samples by following the procedure, malachite green method (Ohno and Zibilske, 1991) at the spectrophotometer. The potassium contents in soil and plant samples were found out using a flame photometer (*Jenway PFP-7*) for comparison of the readings of samples with standard solutions of KCl. A curve was drawn and sample readings were compared with.

Chlorophyll content

For the determination of chlorophyll contents two mature green leaves were collected from each plant and chlorophyll contents were measured (Arnon, 1949; Ravelo-perez *et al.*, 2008). A 0.1g plant fresh leaf sample was weighed and dipped into 10ml of 80% acetone solution and left overnight after covering the tubes. Readings were noted down at 645 and 663nm wavelengths using a spectrophotometer. Actual chlorophyll contents were recorded using the formula.

Statistical analysis

The data were statistically analyzed. Least significant difference (LSD) test was applied at 5% probability to compare the means (Steel *et al.*, 1997). Computer-based software “Statistix 8.1” was used.

Results

Growth parameters

Plant growth parameters recorded (Table 1) included plant height, root length, shoot dry biomass, root dry biomass. All these growth parameters were noted to be significantly higher with the combination of 0.3% biochar and KSB as compared to other treatments at both moisture levels i.e. 70% and 30% FC. Plant height increased up to 29% due to the application of 0.3% biochar + KSB at 70% FC moisture level and up to 30% increase was recorded with the same treatment at 30% FC moisture. Root length was increased up to 42% with the 0.3% biochar + KSB at 70% FC and up to 49% at 30% FC. Shoot dry biomass was also increased to 66 % due to the same combination at 70% FC and to 67% at 30% FC. Root dry biomass was increased up to 54% at 70% FC and up to 61% at 30% FC, when 0.3% biochar was applied in combination with KSB as compared to the control.

Potassium concentration in root and shoot

Maximum K concentration i.e., 30% and 46% in root was also observed with the combination of 0.3% biochar and KSB at 70% and 30% FC, respectively as compared to the control. Similarly, maximum and significantly improved K content were noted in a shoot with the application of 0.3% biochar in combination with KSB at both the moisture levels. It was observed that at 70% FC there was 35.1% increase and at 30% FC there was 36% increase in shoot K, over the control (Table 2).

Table 1. Effect of KSB solubilizing *Bacillus* strain inoculation and biochar on growth attributes of sorghum.

Treatments	Plant Height (inch)		Root Length (inch)		Shoot Dry Biomass (g)		Root Dry Biomass (g)	
	Moisture (%)							
	70	30	70	30	70	30	70	30
Control	54.3 a	49.6 a	11.6 f	14.0 ef	6.5 f	6.0 f	3.8 gh	2.9 h
KSB	68.0 a	63.6 a	15.0 e	14.3 e	10.1 de	8.4 ef	5.1 de	4.6 d-g
0.1% Biochar	57.7 a	54.6 a	18.8 cd	13.0 ef	12.1 cd	10.1 de	4.8 d-g	4.0 fg
0.3% Biochar	58.6 a	56.0 a	20.2 b-d	18.2 d	13.8 bc	11.3 c-e	4.8 d-f	4.18 e-g
0.1% Biochar + KSB	66.3 a	64.0 a	21.3 ab	19.8 b-d	14.5 bc	12.0 cd	5.5 cd	6.0 c
0.3% Biochar + KSB	77.0 a	71.3 a	23.0 a-c	20.8	19.4 a	18.5 ab	8.4 a	7.4 b

Different letters showed statistical difference at $p \leq 0.05$.

Phosphorus content in root and shoot

Combined use of 0.3% biochar and KSB increased P content up to 75% in roots at 70% FC and up to 75.5% at 30% FC. Similarly in the shoot, 45% increase in P content at 70% FC and 44% increase at 30% FC as compared to the control were noted (Table 2).

Chlorophyll a content

Results showed that KSB significantly affected the chlorophyll a content of plants. The 0.3% Biochar + KSB gave chlorophyll a content in both moisture levels i.e. 70% and 30% of FC. There was an increase of 54.8% in chlorophyll a content with a combination of 0.3% biochar and KSB as compared to the control. At 30% FC. 60.8% increase in chlorophyll a content was noted through a combination of 0.3% biochar and KSB as compared to the control (Table 3).

Chlorophyll b content

The results showed that the KSB significantly also affected the chlorophyll b content of plants. The 0.3% Biochar + KSB bacteria produced maximum chlorophyll b content at 70% and 30% FC. There was an increase of 30.6 percent in chlorophyll b content at 70% FC through a combination of 0.3% biochar and KSB as compared to the control. At 30% FC 43% increase in chlorophyll b content was observed when 0.3% of biochar and KSB were applied conjointly (Table 3).

Discussion

In the current study, the results revealed that the KSB in combination with biochar along with recommended fertilizers at two different moisture levels (i.e., 70% and 30% FC) improved growth indices and nutrients content of sorghum plants. Biochar always proved to be very effective when applied especially in combination with mineral fertilizers (Lehmann *et al.,* 2003). The combined use of biochar and mineral fertilizers gave better results as compared to the sole application of either of them (Uzoma *et al.,* 2011; Naeem *et al.,* 2018).

Table 2. Effect of Potassium solubilizing Bacillus strain and biochar on potassium root and shoot of sorghum.

Treatments	Potassium contents (ppm)			
	Shoot		Root	
	Moisture (%)			
	70	30	70	30
Control	29.5 ef	27.7 f	93.9 i	90.4 i
KSB	39.1 bc	38.8 bc	157.8 cd	153.7 de
0.1% Biochar	35.9 cd	33.5 de	144.1 fg	132.2 h
0.3% Biochar	37.6 cd	37.0 cd	149.6 ef	140 g
0.1% Biochar + KSB	40.3 bc	38.2 cd	162.2 bc	156.6 c-e
0.3% Biochar + KSB	45.5 a	43.5 ab	173.9 a	167.4 ab

Different letters showed statistical difference at $p \leq 0.05$.

As compared to the other treatments 0.3 % biochar + KSB showed a significant increase in the phosphorus and potassium concentration in the shoot and roots of sorghum as compared to other treatments. Bacterial strains help in the conversion of nutrients from organic to inorganic form; that might be one of the reasons for the improvement of phosphorus and potassium content in sorghum, especially potassium content (Lehmann *et al.*, 2003).

An increase in plant P content might be due to P provision through biochar. The KSB might have the potential too to decompose organic matter in soil and make soil P available to the plants (Doan *et al.*, 2015). Biochar itself has a considerable amount of phosphorus which becomes available to the plants (Nigussie *et al.,* 2012; Agegnehu *et al.,* 2016).

The KSB were used in the current study to increase the potassium availability and mitigate the drought stress in the plant that were in stress condition due to low moisture level. Potassium helps to minimize the drought effects in plants. Drought stress decreased the vegetative growth of plants, but K availability controlled that stress and sustained the plant growth (Gitelson, 2012).The treatment, 0.3% biochar + KSB remained better regarding K content in plants.

Biochar may have plant nutrients in a good amount (Yaun *et al.,* 2011). That's why potassium amount was increased through biochar addition and became available when added with KSB strain.

Table 3. Effect of Potassium solubilizing Bacillus strain and biochar on phosphorus root and shoot of sorghum.

Treatments	Phosphorus in Root (%)		Phosphorus in Shoot (%)		Chlorophyll a ($mg\ g^{-1}$)		Chlorophyll b ($mg\ g^{-1}$)	
	Moisture (%)							
	70	30	70	30	70	30	70	30
Control	11.6 fg	10.4 g	14.7 ef	12.9 f	0.028 d-f	0.024 g	0.052 f	0.042 h
KSB	12.4 e-g	13.2 d-g	46.5 a-c	42.9 a-d	0.031 cd	0.030 c-e	0.066 b	0.061 d
0.1% Biochar	15.2 b-f	13.9 c-g	34.8 cd	29.8 de	0.027 f	0.026 ef	0.057 e	0.048 g
0.3% Biochar	17.1 a-d	15.9 b-f	50.4 a-c	45.4 a-d	0.028 ef	0.026 f	0.066 bc	0.072 a
0.1% Biochar + KSB	17.9 a-c	16.7 a-d	42.9 a-d	39.3 b-d	0.039 a	0.033 c	0.062 cd	0.063 b-d
0.3% Biochar + KSB	20.9 a	18.7 ab	56.4 a	52.7 ab	0.041 a	0.037 b	0.075 a	0.073 a

Different letters showed statistical difference at $p \leq 0.05$.

Photosynthetic pigment, chlorophyll is the main part of plant food production. The combined use of biochar and KSB increased the K solubility and availability and showed a significant increase in chlorophyll content. Plant height, root length, number of leaves per plant and chlorophyll content improvement may be due to increase in photosynthesis rate of plants which is directly related to the improvement in stomata conductivity and high activity of Ribulose-1,5-bisphosphate carboxylase which results in the increase rate of CO_2 fixation (Cakmak and Engels, 1999).

Nutrients availability to the plant during growth stages is the main point which has an impact on the improvement of plant growth (Arif *et al.,* 2013). Plant dry matter content mainly depends on the nutrient availability to the plants (Okon, 1985). Physical parameters which include plant height, number of leaves per plant, root length, shoot length, dry biomass of plant remained relatively better in the treatments having a combination of biochar (0.3%) and KSB. Drought condition affected the root and shoot growth but this effect was minimized by the addition of KSB strain and KSB strain performance was increased up to a great extent when added in a combination of biochar (Schimidhalter *et al.,* 1991). Addition of organic matter into soil in the form of biochar also played a positive role in the growth of plants by providing nutrients as well as improved the physical condition of soil which directly impacted on the availability of nutrients to plants and improved growth of plants and in drought stress proved best by increasing the water holding capacity of the soil (Liu *et al.,* 2005).

Conclusion

From the current experiment, it is well proved that with the addition of biochar and KSB, significant improvement all the growth indices of sorghum may be obtained under water deficit condition.

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