Soil Environ. 37(2): 143-151, 2018
DOI:10.25252/SE/18/71633
Online ISSN: 2075-1141
Print ISSN: 2074-9546

Effect of biochar particle size and biofertilizers on lentil (*Lense culinarous* M.) yield and available fractions of soil nutrients

Muhammad Mutasim Billah[1], Wiqar Ahmad[1], Murad Ali*[3] and Farmanullah Khan[2]
[1]Department of Soil and Environmental Sciences, The University of Agriculture, AMK Campus, Mardan.
[2]The Agriculture University of Peshawar, Pakistan,
[3]Cereal Crops Research Institute, Pirsabak, Nowshera, Pakistan

Abstract

Suitable management techniques *are required to improve soil nutrient availability and crop yield. This study evaluated biochar particle sizes (<2 mm, 2-5 mm and 5-10 mm) in combination with rhizobia inocula (Biozote-N (Rhizobium leguminosarum (BZ)) and Rhizogold (a mixture of Rhizobium leguminosarum with other plant growth promoting bacteria (RG)) for enhancing lentils yield and nutrients availability in a low fertility alfisol using randomized complete block split plot design during Winter 2015-16. Biochar with lowest particle size (< 2 mm) showed the maximum 13, 17, 6 and 17% increase in bundle weight, straw yield, grain yield and thousand grain weight, respectively, and 27, 43 and 18% increase in soil mineral nitrogen (N), phosphorus (P) and potassium (K) status, respectively, compared to no Biochar plots. The Bizote-N inoculation improved the grain yield by 4%. Combination of Bizote-N and the biochar smallest particle size (< 2 mm) resulted in more mineral N content. The extractable K was highest at < 2 mm particle size and no biofertilizer treatment followed by Bizote-N inoculation. It was concluded that the Biozote-N performance was consistent with the lowest biochar particle size (< 2 mm) for enhanced lentil yield and soil fertility.*

Keywords: Biochar particle sizes, biofertilizer, biozote-N, Lentil, *rhizobium leguminosarum*, rhizogold

Introduction

Due to high population growth, Pakistan's farming community is compelled to follow cereal based cropping systems for ensuring food security (Jalal *et al.*, 2016). Since, cereal crops exhaust soil's nutrient reserves at each harvest, successful crop production on such soils, therefore, requires heavy dose of nutrient fertilizers (Timsina and Connor, 2001; Mahajan and Gupta, 2009). Previously, only NPK fertilization at optimum rates was considered key to higher crop yield (Mojid *et al.*, 2012). Recent studies, however, indicated that not only NPK application to soil was necessary but higher doses of these N, P and K nutrients were required for successful crop production compared to those applied previously for potential yields (Gill *et al*., 2008). This proved gradually deteriorating soil fertility and nutrients balance casting long term impacts on soil quality, sustainable productivity and environmental quality. Legumes are considered essential for poor man's protein, minerals, and vitamins requirements as well as for improvement of soil fertility since they fix atmospheric N biologically through symbiosis, supplement the plant's N requirements and increase soil N reserves. Legumes cultivation not only provides protein rich seed, but its fodder or green manure are rich sources of N for soil and improve soil fertility through nutrient replenishment if included in cereal based cropping systems (Shah and Khan, 2003; Ahmad and Khan, 2014). Supply of N to soil through inclusion of legume crop in cereal based cropping system is a worth considering strategy for reduced dependence on inorganic N fertilizers because cropping systems with legumes are highly sustainable compared to only cereals or fertilizer based systems.

Nutrients transformation in soil and their availability to plants are affected by factors like soil texture, carbon content and other physico-chemical properties. Biochar as soil amendment positively affects soil properties and crop nutrition (Filiberto and Gaunt, 2013). Moreover, C losses from biochar amended soils are very small which does not compromise on its long-term contribution in C sequestration (Jones *et al.*, 2011). Biochar amendment increases the soil's nutrients adsorption capacity and reduces its runoff and leaching to surface and ground water. Besides, it is a source of most plant nutrients itself which are slowly released to growing plants through decomposition. It also lowers soil's bulk density as well as improves its physical properties. In sandy soils, it improves water and nutrients retention. Therefore, addition of biochar as soil amendment has registered significant yield increases (Glaser *et al*., 2002). Glaser *et al*. (2002) termed its C half-life in excess of 1000 years indicating its long lasting contribution to soil quality.

*Email: muradses@gmail.com

The extent of exposed surface area of the applied biochar determines its adsorption capacity, impacts the retention of water, nutrients and organic compounds when added to the soil (Brown *et al*., 2006). This study assumes that decreased particle size of biochar might have greater impact on nutrients adsorption and dynamics in soil plant system. Particle size effect of the biochar amendment has meagrely been studied, either alone or in combination with biofertilizer, for improving lentil productivity and the availability of major nutrients in soil. This research was, undertaken to bring to limelight the biochar particle size and rhizobia inoculation effect for increasing lentils productivity and available fraction of soil N, P and K.

Material and Methods

A field study was carried out to evaluate the inoculation of rhizobia in combination with biochar of different particle sizes on lentil yield and available fractions of soil nutrients, at Platoo Research Farm of the Amir Muhammad Khan Campus Mardan, The University of Agriculture, Peshawar during Rabi season 2015-16. Pre-sowing soil samples were collected and analysed for physico-chemical properties (Table 1). Randomized complete block design (RCBD) with split plot arrangement was used and replicated thrice with plot size of 3×4 m^2. Recommended N and P_2O_5 were applied as basal at the rate of 30:60 kg ha^{-1}. using urea and single super phosphate (SSP) fertilizers, respectively.

Acacia wood biochar, characterized for physic-chemical characteristics (Table 2), was crushed and sieved in three sizes (<2 mm, 2-5 mm and 5-10 mm) whilst two biofertilizers viz Biozote-N collected from National Agriculture Research Council (NARC), Islamabad and Rhizogold from the University of Agriculture Faisalabad. Biochar was applied to sub-plots at the time of field preparation as per treatment plan (Table 3) at the rate of 10 Mg ha^{-1} (1 kg m^{-2}) for all particle size fractions. Biofertilizers, at the rate of 1 kg ha^{-1}, were mixed with 5% sugar solution to facilitate adherence with seed during mixing and air dried in shade just before sowing in designated main plots (Table 3).

Crop was harvested at maturity and after harvesting of each plot, plants were sun dried and biological yield was recorded and converted into kg ha^{-1} with the help of the following formula. After threshing grains were cleaned and weight and changed into kg ha^{-1}.

$$\text{Biological yield (kg ha}^{-1}) = \frac{\text{(Biological yield plot}^{-1} \times 10000}{\text{Row-row distance (m)} \times \text{row length (m)} \times \text{number of rows}}$$

Straw yield was calculated by subtracting grain yield from biological data. Thousand grains were counted of each

Table 1: Soil texture and nutrient status of the experimental plot

Parameter	Unit	Value
Soil texture	-	Silt loam
Total N	%	0.01
AB-DTPA extractable K	mg kg^{-1}	72
AB-DTPA extractable P	mg kg^{-1}	4.32

Table 2: Characteristics of biochar used in the experiment

Parameter	Unit	Value
Moisture content	%	0.65
Organic C	%	64.3
Total N	g kg^{-1}	0.72
Total K	g kg^{-1}	0.42
Total P	g kg^{-1}	0.25
pH $_{(1:5)}$	-	8.2
EC $_{(1:5)}$	dS m^{-1}	0.65

Table 3: Treatment plan (R1) for the experiment

Rhizobia inoculum (Main Plots)	Biochar particle sizes (Sub-Plots)			
Biozote-N (BF1)	Control (T4)	< 2 mm (T1)	2-5 mm (T2)	5-10 mm (T3)
Rhizogold (BF2)	< 2 mm (T1)	Control (T4)	5-10 mm (T3)	2-5 mm (T2)
Control (BF3)	5-10 mm (T3)	2-5 mm (T2)	Control (T4)	< 2 mm (T1)

plot/treatment and weighed with electric balance.

Soil samples from each plot were collected from 0-30 cm depth after harvesting of crop and analysed in the laboratory of the Department of Soil and Environmental Sciences, University of Agriculture, Peshawar, Pakistan. Soil texture was determined by the standard procedure as mentioned by Gee and Bauder (1986). A 50 g soil sample was suspended in water through a dispersion machine and poured in 1L graduated cylinder. The cylinder was placed on table for 40 seconds to let the sand particles settle down. Hydrometer was inserted in the suspension just after 40 seconds and the reading was noted. The suspension was re-shaken and kept in rest on the table. After 2 hours, the hydrometer was inserted in the suspension and the reading was noted. Temperature of the suspension was noted each time the hydrometer was inserted. A factor of 0.2 was subtracted from the reading for each degree above 25 ºC and added for each degree below 25 ºC (Tagar and Bhatti, 1996). Sand, silt and clay content in soil were calculated as follows and the textural triangle was used to determine the soil texture.

$$\text{Sand (\%)} = \frac{\text{Soil Sample weight (g)} - \text{hydrometer reading at 40 Sec after correction}}{\text{Soil Sample weight}} \times 100$$

$$\text{Clay (\%)} = \frac{\text{hydrometer reading at 2 hr after correction for temperature}}{\text{Soil Sample weight}} \times 100$$

$$\text{Silt (\%)} = 100 - (\text{Sand (\%)} + \text{Clay (\%)})$$

Available soil nitrogen was determined by the method mentioned by Mulvaney (1996) while Bremner (1996) procedure was adopted for determination of total N content in biochar samples. and both were calculated by the following formula,

$$\text{Clay (\%)} = \frac{\text{hydrometer reading at 2 hr after correction for temperature}}{\text{Soil Sample weight}} \times 100$$

Soil phosphorus (P) and potassium (K) were determined by the procedure as suggested by Soltanpour and Schwab (1977).

One gram biochar was dried in oven for 24 hours at 105 °C and re-weighed. Percentage reduction in weight after drying was recorded as percent moisture. For determination of carbon (C) content, the dried biochar sample was ignited in a muffle furnace with controlled heating of up to 750 °C and re-weighed and the loss in weight expressed in percentage was the percent C of the biochar. Electrical conductivity ($EC_{1:5}$) was measured by preparation of 1:5 (biochar: H_2O) suspension subjected to 30 min of stirring and centrifugation for 5 minutes at 3000 rpm. The suspension was filtered and read on EC meter in dS m^{-1} (Rhoades, 1982). In the filterate prepared for EC, pH was read on pH meter (Model German Type B-124 using glass and calomel electrodes) (Mclean, 1982). The biochar total P and K contents were measured through extraction by the perchloric acid-nitric acid digestion method as described by Kue (1996) and read on spectrophotometer at 880 nm wavelength and flame photometer, respectively.

The recorded data obtained were statistically analyzed using Analysis of Variance (ANOVA) procedure for RCB design (Jan *et al*., 2009) using Statistic 8.1 computer software. Least significant difference (LSD) test were used to compare treatment means (Steel *et al*., 1997).

Results

Results showed that biochar particle size and biofertilizer (Biozote-N and Rhizogold) significantly (p< 0.05) increased the biological yield, straw yield, grain yield and 1000 grain weight over the control plots (Table 4). The straw yield, however, did not show significant variation amongst biochar particle sizes (Table 4). Data showed the highest (13%) increase in biological yield (2126 kg ha^{-1}) with the biochar particle size < 2 mm, followed by 2-5 mm size exhibiting 9% increase (2053 kg ha^{-1}) and 5-10 mm (2023 kg ha^{-1}) with 7% increase over the biochar control (1886 kg ha^{-1}). Amongst the biochar particle sizes, the smallest particles size (< 2 mm) treatment was significantly (p< 0.05) higher over the medium particle size (2-5 mm) and maximum particle size (5-10 mm) with 4 and 6% increase, respectively. Statistically similar biological yield was recorded from both the medium (2-5 mm) and high (5-10 mm) biochar particle size treatments. Biozote-N exhibited a significant (p< 0.05) 12% increase in biological yield whilst increase in biological yield in the Rhizogold treated plots was 8% over the biofertilizer control plot.

All biochar particle sizes showed significant (p< 0.05) increase in straw yield over the biochar control, however, the biochar particle sizes were non-significant among themselves. None of the biofertilizers showed significant increase in straw yield over the biofertilizer control plot (Table 4). Application of Biochar having < 2 mm particle size recorded highest straw yield (17% higher over the control) followed by straw yield noted in treatments with biochar particle size of 2-5 and 5-10 mm, where each particle size exhibited 11% increase in straw yield over treatment having no Biochar. Biofertilizers (Biozote-N and Rhizogold) inoculation enhanced the straw yield by 6.7 and 6.3%, respectively, over un-inoculated control.

Table 4: Biochar particle size and bio-fertilizer treatment effect on lentil yield parameters

Biochar Particle Sizes	Biological yield	Straw yield	Grain yield	1000 grain weight (g)
	…………………..(kg ha^{-1})………….…….			
<2mm	2126 a	1345 a	781 ab	34.77 a
2-5mm	2053 b	1269 a	784 a	34.55 a
5-10mm	2023 b	1270 a	753 bc	31.66 b
Control	1886 c	1144 b	742 c	29.77 c
$LSD_{(p<0.05)}$	71.2	77.2	29.1	1.27
Biofertilizer				
Bizote-N	2108 a	1285	822 a	33.16 a
Rhizogold	2038 a	1281	756 b	33.00 a
No Biofertilizer	1920 b	1204	716 c	31.91 b
$LSD_{(p<0.05)}$	94.0	ns	24.0	0.85
Biochar x Biofert	Ns	Ns	Ns	Ns

Table 5: Biochar particle size and biofertilizers effect on nodules $plant^{-1}$ and nodules weight $plant^{-1}$

Biochar Particle Sizes	No of nodules $plant^{-1}$	Nodule weight (g) $plant^{-1}$
<2mm	14.44	0.14
2-5mm	14.77	0.14
5-10mm	15.11	0.15
Control	14.33	0.13
$LSD_{(p<0.05)}$	Ns	Ns
Biofertilizers		
Bizote-N	22.41 a	0.22 a
Rhizogold	21.58 a	0.21 a
No Biofertilizer	0.00 b	0.00 b
$LSD_{(p<0.05)}$	1.29	0.02
Biochar x Biofert	Ns	Ns

Means followed by different letters in each column are differ from each other significantly, NS= non-significant.

Lentil grain yield obtained from biochar treated plots with < 2 and 2-5 mm particle size were significantly ($p<0.05$) higher (by 5.2 and 5.6%, respectively) while the high biochar particle size (5-10 mm) was statistically non-significant in grain yield to the biochar control plots (Table 4). Grain yield in biochar particle 2-5 mm in size was significantly ($p< 0.05$) enhanced over the plots treated with maximum particle size (5-10 mm). A Significant ($p< 0.05$) variation was observed in grain yield due to biofertilizer application. The plant inoculated with Biozote-N recorded significantly ($p< 0.5$) higher grain yield over Rhizogold and un-inoculated control (by 9 and 15%, respectively) and the Rhizogold significantly ($p< 0.05$) enhanced lentil yield (by 6%) over un-inoculated control.

Biochar with small and medium particle sizes (< 2 and 2-5 mm, respectively) significantly ($p< 0.05$) enhanced the 1000 grain weight (by 17 and 16%, respectively) over the biochar control whilst both were statistically similar to each other. Moreover, biochar particle size of 5-10 mm improved 1000 grain weight significantly, but the increase was only 6% over biochar control (Table 4). Both biofertilizers (Biozote-N and Rhizogold) were statistically similar in 1000 grain weight, yet significantly ($p< 0.05$) higher with 4 and 3.4% increase in 1000 grain weight over un-inoculated control (Table 4). Results (Table 4) further revealed that interaction between the biochar and biofertilizer was non-significant for yield and yield components, confirming their individual ability to affect the yield of lentil crop.

Results showed that, neither biochar nor its particles size variation affected the number of nodules and nodules dry weight $plant^{-1}$ significantly. However, the biofertilizer inoculation effect on number of nodules and nodules dry weight $plant^{-1}$ was highly significant ($p< 0.01$) (Table 5). The difference in number of nodules and nodules dry weight $plant^{-1}$ recorded from the Bizote-N and Rhizogold biofertilizers treatments was non-significant to each other (Table 5). The number of nodules $plant^{-1}$ were 22.41 and 21.58 and nodule dry weight $plant^{-1}$ were 0.22 g and 0.21 g in the Bizote-N and Rhizogold treatments, respectively, whilst the number of nodules $plant^{-1}$ with no-biofertilizer inoculation was zero and hence with zero nodule weight.

There was no interaction between the biochar different particle sizes and biofertilizer strains to affect the number of nodules and nodules dry weight plant^{-1} (Table 5). Application of biochar as a soil amendment significantly (p< 0.05) enhanced available nitrogen (N), phosphorous (P) and potassium (K) over the biochar control whilst all these nutrients were non-significantly affected with biofertilizer inoculation over the biofertilizer control plot (Table 6). All biochar particle sizes were statistically similar in response to their effect on N availability, however, maximum mineral N (55.27 mg kg^{-1}) was recorded with the particle size 5 to 10 mm followed by 2-5 mm size (53.9 mg kg^{-1}) and < 2 mm size (52.79 mg kg^{-1}) showing 33, 29 and 27% increase in mineral N, respectively, over the minimum available N (41.68 mg kg^{-1}) noted in biochar control (Table 6). Despite non-significant individual effect on mineral N content by the biofertilizers, the Biozote-N enhanced the mineral N by 1.7% over the Rhizogold (Table 6). Furthermore, the interaction between biochar particle size and biofertilizers on mineral N content was significant (p < 0.05) (Table 7) showing maximum mineral N in 5-10 mm biochar particle size with no biofertilizer (59 mg kg^{-1}) followed by < 2 mm size along with Bizote-N treatment (57.2 mg kg^{-1}).

Increase in available P with < 2, 2-5 and 5-10 mm biochar particle sizes was 43, 33 and 22%, respectively, over the biochar control (Table 6). Application of small particle size (< 2 mm) enhanced the available P (by 21%) over the largest particle size (5-10 mm), and by 10% over the medium particle size (2-5 mm). Biofertilizer response was observed non-significant to AB-DTPA extractable P, however, inoculation with Bizote-N improved the available P by 16% as compared to biofertilizer control plot (Table 6). The interaction between the biochar and biofertilizer on available P was observed non-significant.

Biochar particle size significantly, while biofertilizer non-significantly affected the extractable K. The maximum K (95.06 mg kg^{-1}) was noted in treatment with small biochar particle size (< 2 mm) that showed 18% increase whilst the medium (2-5 mm) and large (5-10 mm) particle size induced increase in available K up to 9 and 13%, respectively, over the biochar control (Table 6). In case of biofertilizers, the Biozote-N was found superior over Rhizogold. The Biozote-N enhanced available K by 4% while the Rhizogold by 1.2% over un-inoculated control. There was significant (p< 0.05) interaction between biochar particle size and biofertilizer on the availability of K fraction in soil (Table 7) and maxixum available K (105.7 mg kg^{-1}) was found in < 2 mm size of biochar without biofertilizer followed by Biozote-N with the same particle size of biochar (96.4 mg kg^{-1}).

Discussion

Inadequate nutrients availability is one of the major abiotic constraints reducing productivity of legume crops grown on nutrients deficient soils. Pulses have shown better response to 30-40 kg ha^{-1} N applied (Ali *et al.*, 2000). Biochar induced alteration in soil nutrient content may not affect plant growth in the short-run, the significant improvement may, therefore, be ascribed to more exposed surface area with reduced particle size of the biochar that might have enhanced nutrients adsorption and release for crop growth (Hardy *et. al.*, 2015). These results, therefore, not only confirm the biochar's role in the crop growth and development but also the enhancement of this effect with decreasing the biochar particle size (Table 4). However, particle size variation effects may not be visible on plant at the early growth stages. Reducing particle size is an easily adoptable strategy to reduce loss of nutrients due to more adsorption with correspondingly increased surface area and the exposition of increased number of functional groups compared to the biochar control or soil treated with larger biochar particles sizes. The associated improvement in soil physical properties (bulk density and water storage) with biochar addition (Liang *et. al.*, 2014; Abdurrab *et. al*., 2016) might have improved the straw yield. Biochar amendment should be chopped to smaller size before application in order to get support for growth and yield in the short run. Small particle size amendment may hold more water and nutrients compared to the larger particles due to their increased surface area. Release of such adsorbed nutrient in due course of plant growth become available to plants compared to large particles and ensures water and nutrient availability till the crop physiological maturity. Results of the current study with improved growth and yield attributes of the crop confirm the previous findings reported by Ali *et al*. (2015) and Abdurrab *et al.* (2016) for biochar addition. Biochar contribution towards positive implications for bioenergy production is well established through previous research as well (Utomo 2012 and Sarah, 2013; Hardy *et al.*, 2015; Abdrrab *et al.*, 2016). Furthermore, improvement in soil's physico-chemical properties by biochar amendment (Paz-Ferreiro *et al.*, 2014) and its potential to impart plant friendly environment to soil (Park *et al.* 2011) might have further been reinforced with increased surface area through reduced biochar particle size. Despite significantly (p< 0.05) higher nutrients (N, P, K) availability in biochar treatments over the no-biochar plots (Table 4), the decreased N availability with reducing biochar particle size might be because of increasing charged surfaces and functional groups associated with biochar amendment resulting in enhanced adsorption/retention and slow release of these nutrients for the crop throughout growth and development compared to higher-biochar particle size fractions. Biochar has been described a universal sequester (Baidoo et al., 2016) that retain with itself the ammonium and nitrate ions (Yao et al., 2012) and lengthen their retention time in soil for plant uptake (Major *et al*., 2012; Jien and Wang, 2013; Morales *et al*., 2013).

Table 6: Biochar particle size and biofertilizer treatment effect on available fraction of soil NPK

Biochar Particle Sizes	Mineral N	AB-DTPA ext. P	AB-DTPA ext. K
	……………….. (mg kg^{-1})……………….		
<2mm	52.79 a	4.02 a	95.06 a
2-5mm	53.9 a	3.74 ab	87.84 b
5-10mm	55.27 a	3.42 b	90.94 ab
Control	41.68 b	2.81 c	80.26 c
LSD(p<0.05)	3.35	0.36	4.15
Biofertilizer			
Bizote-N	51.4	3.85	90.42
Rhizogold	50.43	3.33	88.14
No Biofertilizer	50.9	3.32	87.02
LSD(p<0.05)	Ns	Ns	Ns
Biochar x Biofert	** (Table 7)	Ns	* (Table 7)

Means followed by different letters in each column are differ from each other significantly, NS= non-significant

Table 7: Interaction data of mineral N and AB-DTPA extractable K content in soil as affected by biochar particle sizes and biofertilizer inoculum

Biofertilizers	Control	<2mm	2-5mm	5-10mm
	Mineral N (mg kg^{-1})			
Bizote-N	39.59	57.23	55.13	53.64
Rhizogold	46.85	50.31	51.36	53.20
No Biofertilizer	38.58	50.84	55.21	58.98
	AB-DTPA ext. K (mg kg^{-1})			
Bizote-N	83.55	96.41	91.10	90.62
Rhizogold	90.00	83.06	90.58	88.92
No Biofertilizer	67.24	105.72	81.85	93.29

Grains being the final sink for metabolites and although non-significant, yet 0.6% higher 1000 grain weight in Biozote-N treatments indicated improved availability of the metabolites during the grain formation and ensuring grain yield higher by 9% than the Rhizogold (Table 4). Statistically similar straw yield amongst the Bizote-N and Rhizogold (Table 4) is indicative of similar vegetative growth. Biofertilizer inoculation increased the number of nodules and nodules dry weight plant^{-1} significantly (p< 0.01) (Table 5). Statistically similar number of nodules and nodules dry weight plant^{-1} by Bizote-N and Rhizogold biofertilizers treatments (Table 5) indicated that both the biofertilizers were equally effective for nodulation in lentils. Since both biofertilizers were statistically similar in number of nodules and nodules dry weight plant^{-1} (Table 5) and the straw yield recorded by both biofertilizers is statistically similar, this showed that both biofertilizers performed equally in N fixation during vegetative growth period. In a nutshell, biofertilizer improved the vegetative and reproductive growth as well as nutrient uptake significantly over the plants where no biofertilizer was used. These results confirm the findings of Aslam *et al*. (2010) and Chaichi (2015) who reported increased crop growth and yield after biofertilizer application.

Significantly higher mineral N in biochar amended plots than the biochar control indicated that either the N was released from the biochar inherent N content (Table 2) or biochar prevented the losses of externally applied inorganic N from the applied fertilizers. Previous research indicated that biochar retained the ammonium and nitrate with it (Yao *et al*., 2012) and increased their retention time in soil for plant uptake (Major *et al*., 2012; Jien and Wang, 2013; Morales *et al*., 2013). However, 5% less available N in the <

2 mm particle treated plots (Table 6) pointed out increased N retention because of more exposed surface area and vice versa. Mineral N with medium size (2-5 mm), being higher than the smallest size (< 2 mm) confirms this fact. Results with higher mineral N in biochar plots has previously been communicated by researchers (Chan *et al.*, 2008, Zheng and Brewer, 2015), whilst this study suggest the consideration of variation in biochar particle size for improved mineral N content as well as its retention in soil. In case of interaction (Table 7), Less vegetative growth as indicated by lower straw yield in the maximum biochar particle size (5-10 mm) than medium (2-5 mm) and smallest (< 2 mm) particles sizes combined with biofertilizer control (Table 4) might be responsible by less N uptake by the crop and higher soil mineral N content (Table 7). Higher mineral content in the < 2 mm particle size coupled with Bizote-N might be because of higher N retention by the smallest particles size biochar and greater N fixation by the Biozte-N than the Rhizogold.

The AB-DTPA ext. P in the < 2 mm particle size of biochar plot was 10% higher over the 5-10 mm and 2-5 mm particle size plots (Table 6). These could have been due to more exposed surface area of the < 2 mm size applied biochar and suspected higher immobilization of the basic cations reducing their reaction with PO_4 anions. Higher P availability might also be due to the retention of the P anions applied through fertilizer on exposed surfaces of the biochar and reducing its fixation in high pH soils. Results from Sika (2012) and George *et al.* (2014) report higher available P content with biochar amendment.

Higher surface area per unit weight of the applied < 2 mm biochar particles might have adsorbed and retained K ions to prevent its fixation in soil. Although, 2-5 and 5-10 mm biochar particle size did not significantly vary in AB-DTPA ext. K content (Table 6). Yet, higher extractable K content (by 4%) in the 5-10 mm particles size plots than the 2-5 mm particle size plots might be ascribed to low K adsorption on larger size (5-10 mm) biochar particles due to comparatively smaller exposed surface area than the medium size (2-5 mm) biochar particles. Sadhana (2014) revealed higher total N, P and K contents in biofertilizers inoculated chickpea plants than the control, at all stages of growth. Thus, high NPK content can be expected in such crop post-harvest soil compared to the control. Lentils shed leaves and produce a thin layer of litter on soil surface at maturity which increase the soil organic matter content (Ahmad and Khan, 2014) and soil nutrient content upon its decomposition (Courtney an dMullent, 2008). Soils after inoculation might have higher microbial activity and increased cycling of the available nutrients through micro-organisms bodies, thus preventing their fixation or losses (Ayaga, *et al*., 2006). Favourable effect on the AB-DTPA extractable P and K in the post-harvest legume crop soil might also be attributed to higher organic matter returns to soil by the legume crop (Sharma and Sharma, 2004) which upon gradual decomposition release the nutrients contained in it (Guo, *et al*., 2008) resulting in increased nutrient content in soil solution. Legumes crops are effective way for improving soil N and other soil properties (Yang, 2006). The production of more organic acids by the micro-organisms during decomposition of this organic matter alters the rhizosphere pH (Wilson, *et al.*, 1982) and creates a favourable soil environment for their release into the soil solution. The extractable K at the < 2 mm particle size was highest in biofertilizer control closely followed by Biozote-N within the same particle size (Table 7). Perhaps, less K was up taken by the crop in biofertilizer control due to less vegetative growth thus resulting in more K residual effect of the applied K in the soil. More K in the < 2 mm biochar particle size plot indicates its greater retention due to adsorption on the higher exposed surface area of < 2 mm biochar particles. At higher particle sizes (2-5 mm and 5-10 mm), less K adsorption due to comparatively less exposed biochar surface area might have less retention and higher K losses through leaching and runoff compared to < 2 mm particle size. The bio-fertilizer net effect was previously reported either superior to or at par with biofertilizer control (George *et al.*, 2014).

Conclusion

In the biochar induced 1.5 to 17% increase in yield parameters, the maximum limit pertained to small particle size (< 2 mm). Biochar also significantly increased the available fraction of N, P and K in soil where the < 2 mm particle size again showed significantly higher soil available P and K contents than higher particle size. Both biofetilizer were significantly higher in yield parameters than the biofertilizer control, Bizote-N, however, showed significantly higher grain yield than the Rhizogold. Interaction of Biozote-N with < 2 mm biochar particle showed significantly improved soil mineral N content. These results, therefore, suggested that Biozote-N inoculation and the lowest particle size of biochar (< 2 mm) obtain greater benefits of the biochar amendment and biofertilizer for the lentil yield and soil fertility.

Acknowledgement

National Agriculture Research Center, Islamabad, the University of Agriculture, Faisalabad are acknowledged for providing biofertilizers for this study.

References

Abdurrab, M.R. Khan, S.U. Haq, S. Zahid, M. Asim, M.Z. Afridi, M. Arif and F. Munsif. 2016. Impact of biochar on mungbean yield and yield components. *Pure and Applied Biology* 5(3): 632-640.

Ahmad, W. and F. Khan. 2014. Remediation of soil past erosion effects through amendments and agronomic practices. *Journal of The National Science Foundation of Sri Lanka*. 42 (1): 45-62. DOI: http://dx.doi.org/10.4038/jnsfsr.v42i1.6680.

Ali, K., A. Muhammad, A. Waheed, A. Wiqar, M. Rabnawaz, L.S. Ayeni, M. Amin and M. Jehangir. 2015. Influence of organic and inorganic amendments on weeds density and chemical composition. *Pakistan Journal of Weed Science Research* 21(1): 47-57.

Ali, M., J.P. Mishra and M. Ali. 2000. Nutrient management in pulses and pulse-based cropping system. *Fertiliser News* 45: 57-69.

Aslam, M., H.K. Ahmad, Himayatullah, M. Ayaz, E. Ahmad, A.G. Sagoo, Inayatullah, A. Hussain and M. Manzoor. 2010. Nodulation, grain yield and grain protein contents as affected by rhizobium inoculation and fertilizer placement in chickpea cultivar Bittle-98. *Sarhad Journal of Agriculture* 26(4). 467-474.

Ayaga, G., A. Todd and P.C. Brookes. 2006. Enhanced biological cycling of phosphorus increases its availability to crops in low-input sub-Saharan farming systems. *Soil Biology and Biochemistry* 38(1): 81 – 90.

Baidoo, I., D.B. Sarpong, S. Bolwig, and D. Ninson. 2016. Biochar amended soils and crop productivity: A critical and meta-analysis of literature. *International Journal of Development and Sustainability* 5(9): 414-432.

Bremner, J.M. 1996. Nitrogen-Total. p.1085-1092. In: Methods of Soil Analysis. Part 3. Chemical Methods. D.L. Spark ed. Soil Science Society of America and American Society of Agronomy, Madison, Wisconsin, USA.

Brown, R.A., A.K. Kercher, T.H. Nguyen, D.C. Nagle. and W.P. Ball. 2006. Production and characterization of synthetic wood chars for use as surrogates for natural sorbents. *Organic Geochemistry* 37:321-333.

Chan, K.Y., B.L. Van Zwieten, I. Meszaros, D. Downie and S. Joseph. 2008. Using poultry litter biochars as soil amendments. *Australian Journal of Soil Research* 46: 437- 444.

Chaichi, M.R. 2015. Effect of bio fertilizers on the growth, productivity and nutrient absorption of fenugreek (*Trigonella foenum graecum* L.). *International Journal of Agriculture Innovations and Research* 3(5): 2319-1473.

Courtney, R.G. and G.J. Mullen. 2008. Soil quality and barley growth as influenced by the land application of two compost types. *Bioresource Technology* 99(8): 2913 −2918.

Filiberto, D.M., and J.L. Gaunt. 2013. Practicality of biochar additions to enhance soil and crop productivity. *Agriculture* 3:715-725. *DOI:10.3390/agriculture3040715* ISSN 2077-0472.

Gee, G.W. and J.W. Bauder. 1986. Particle-size analysis. p. 383–411. *In:* Methods of Soil Analysis Part 1—Physical and Mineralogical Methods; A. Klute ed.; ASA and SSSA: Madison, WI, USA.

Gill, M.V., L.F. Calvo, D. Blanco and M.E. Sanchez. 2008. Assessing the agronomic and environmental effects of the application of each cattle manure compost on soil by multivariate methods. *Bio Resource Technology* 13:5763-5772.

Glaser, B., J. Lehmann, and W. Zech. 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal—A review. *Biology and Fertility of Soils* 35: 219– 230.

GUO, S.L., T.H. Dang and M.D. Hao. 2008. Phosphorus changes and sorption characteristics in a calcareous soil under long-term fertilization. *Pedosphere* 18(2): 248--256.

Hardy, S., G. Dunst and B. Glaser. 2015. Positive effects of composted biochar on plant growth and soil fertility. p.817-827. *In:* Agronomy for Sustainable Development, Springer Verlag/EDP Sciences/INRA.

Jan, M.T., P. Shah, P.A. Hollington, M.J. Khan and Q. Sohail. 2009. Agriculture Research: Design and Analysis, a monograph. NWFP Agricultural University Peshawar, Pakistan.

Jalal, F., M. Arif, K. Ali, F. Munsif, M. J. Khan and B. Khan. 2016. Integration of biochar and legumes in summer gap for enhancing productivity of wheat under cereal based cropping system. *Pakistan Journal of Botany* 48(6): 2281-2288.

Jien, S.H. and C.S. Wang. 2013. Effects of biochar on soil properties and erosion potential in a highly weathered soil. *Catena* 110:225–233.

Kue, S. 1996. Phosphorus. p.869-919. *In:* Methods of Soil Analysis Part-3. Chemical Methods, D.L. Spark ed. SSSA, Inc., ASA, Inc., Madison, Wisconsin, USA.

Liang, F., L.G. tong, L. Qi-mei, Z.X. Rong. 2014. Crop yield and soil properties in the first 3 years after biochar application to a calcareous soil. *Journal of Integrative Agriculture* 13(3): 525-532.

Mahajan, A. and R.D. Gupta. 2009. Role of integrated nutrient management (INM) in a sustainable rice-wheat cropping system. Springer Science + Business Media B.V. Pp. 139-140.

Major, J., M. Rondon, D. Molina, S.J. Riha, and J. Lehmann. 2012. Nutrient leaching in a Colombian Savanna Oxisol amended with biochar. *Journal of Environmental Quality* 41(4): 1076-1086.

McLean, E.O. 1982. Soil lime requirement. p.209-223. *In*: Methods of Soils Analysis. Part 2. 2nd Ed. A.L. Page, R.H. Miler and D.R. Keeney (eds.). Agronomy 9.

Mojid, M.A., G.C.L. Wyseure and S.K. Biswas. 2012. Requirement of nitrogen, phosphorus and potassium fertilizers for wheat cultivation under irrigation by municipal wastewater. *Journal of Soil Science and Plant Nutrition* 12 (4): 655-665

Morales, M.M., N. Comerford, I.A. Guerrini, N.P.S. Falcão, and J.B. Reeves. 2013. Sorption and desorption of phosphate on biochar and biochar-soil mixtures. *Soil Use and Management* 29(3): 306–314.

Mulvaney, R.I. 1996. Nitrogen-inorganic forms. p.1123-1184. *In:* Methods of Soil Analysis, D.L. Sparks (ed). Part 3. American Society of Agronomy. USA.

Park, J. H., G.H. Choppala, N.S. Bolan, J.W. Chung and T. Chuasavathi. 2011. Biochar reduces the bioavailability and phytotoxicity of heavy metals. *Plant and Soil* 348, 439–451.

Paz-Ferreiro, J., H. Lu, S. Fu1, A. Méndez, and G. Gascó. 2014. Use of phytoremediation and biochar to remediate heavy metal polluted soils: a review. *Solid Earth* 5: 65–75., doi:10.5194/se-5-65-2014.

Rhoades, J.D. 1982. Soluble salts. p.167–179. *In:* Methods of Soil Analysis Part 2—Chemical and Microbiological Properties; A.L. Page, ed. ASA and SSSA: Madison, WI, USA.

Sarah, C. 2013. The impact of biochar application on soil properties and plant growth of pot grown lettuce (*Lactuca sativa*) and cabbage (*Brassica chinensis*) simon shackley. University of Edinburgh, UK Biochar Research Centre, School of Geo Sciences, Edinburgh, UK. Agronomy 3: 404-418; doi:10.3390/agronomy3020404.

Shah, Z. and A.A. Khan. 2003. Evaluation of crop residues for mineralizable N in soil. *Sarhad Journal of Agriculture* 19: 81-92.

Sharma, S.N. and S.K. Sharma. 2004. Role of crop diversification and integrated nutrient management in resilience of soil fertility under rice-wheat cropping system. *Archives of Agronomy and Soil Science* 50(3): 345—352.

Sika, M.P. 2012. Effect of biochar on chemistry, nutrient uptake and fertilizer mobility in sandy soil. M.Sc. (Agriculture) Thesis, University of Stellenbosch. http://scholar.sun.ac.za.

Soltanpour, P.N. and A.P. Schawab. 1977. A new soil test for simultaneous extraction of macro and micronutrients in alkaline soil. *Communication in Soil Science and Plant Analysis* 8: 1995-207.

Steel, R.G.D., J.H. Torrie and D.A. Dicky. 1997. Principles and Procedures of Statistics- A Biometrical Approach, (3rd Ed.) McGraw Hill Book International Company, Singapore.

Tagar S. and A.U. Bhatti. 1996. Soil physical properties. p.250. *In:* Soil Science, B. Elena and R. Bantel eds. National Book Foundation, Islamabad, Pakistan.

Timsina, J. and D.J. Conner. 2001. Productivity and management of rice-wheat cropping system: issues and challenges. *Field Crops Research* 69:93-132.

Utomo, W.H. 2012. The Effect of Biochar on the growth and N fertilizer requirement of maize (*Zea mays* L.) International Research Centre for Management of Degraded and Mining Land. University of Brawijaya, Malang, Indonesia. doi: 10.5539/jas.v4n5p255.

Wilson, G.F., R. Lal. and B.N. Okigbo. 1982. Effects of cover crops on soil structure and on yield of subsequent arable crops grown under strip tillage on an eroded Alfisol. *Soil and Tillage Research* 2: 233 − 250.

Yang, Z., B.R. Singh and S. Hansen. 2006. Aggregate associated carbon nitrogen and sulphur and their ratios in long-term fertilized soils. *Soil and Tillage Research* 95(1-2): 161-171.

Yao, Z., X. Zheng, H. Dong, R. Wang, B. Mei, J. Zhu. 2012. A 3-year record of N_2O and CH_4 emissions from a sandy loam paddy during rice seasons as affected by different nitrogen application rates. *Agricultural Ecosystem and Environment* 152: 1–9.

Zheng, C. and C. Brewer. 2015. A Review of Biochar’s Applications in the Soil Nitrogen Cycle. Department of Chemical & Material Engineering. New Mexico State University.