

Research Article



Biochar and Biofertilizers Residual Effect on Fertility Status of Soil Two Crop Seasons after their Application

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Abstract | Biochar application is one of the highly discussed mechanisms to enhance soil fertility worldwide. Manipulation of this amendment to enhance its role in soil fertility management is a new area of research. In this study, it was applied to soil in three particle sizes (< 2, 2-5 and 5-10 mm) in combination with two commercial biofertilizers; Bizote-N and Rhizogold to lentils during Winter 2015 in an RCB design and the residual effect was evaluated on soil micro-nutrient status and chemical properties after maize harvest in Summer 2016. Particle sizes in the above order showed 3, 2 and 2% increase in soil pH over the control (7.63) and 15, 10 and 6% increase in soil EC over the control (125 mS cm⁻¹), respectively. Their OM contents (1.73, 1.68 and 1.67%) registered 98, 93 and 91% increase over the OM in the control (0.87%), respectively. Amonium bicarbonate diethylene triamine penta acetic acid (AB-DTPA) extractable soil Fe after < 2, 2-5, 5-10 mm particles size treatments and the control was 2.48, 2.06, 1.99 and 1.81 mg kg⁻¹, Zn was 0.41, 0.36, 0.33 and 0.31 mg kg⁻¹, Mn was 1.23, 1.05, 1.02 and 0.89 mg kg⁻¹, and Cu was 0.51, 0.38, 0.37 and 0.26 mg kg⁻¹, respectively. Bizote-N and Rhizogold treatments OM (1.5 and 1.48%) showed 3 and 0.7% increase over the control (1.47%), respectively. Bizote-N induced increase in Fe, Zn, Mn and Cu (2.16, 0.61, 1.23 and 0.45 mg kg⁻¹) were significantly higher by 7, 238, 45 and 55% while their content in Rhizogold treatment (2.07, 0.24, 1.05 and 0.40 mg kg⁻¹) showed a significant increase (by 33, 24 and 38%) only in Zn, Mn and Cu over the control (2.02, 0.18, 0.85 and 0.29 mg kg⁻¹, respectively). In interaction, Bizote-N was significantly higher in OM content at the < 2 mm biochar particles size, increased soil micronutrients fertility and soil properties over the Rhizogold and the biofertilizer control. It was concluded that Bizote-N in combination with reduced particle size of biochar had significantly higher residual effect on soil fertility improvement that can be used for higher crop production in legume-cereal cropping pattern.

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Introduction

Increasing yield per unit area, decreasing output cost, improving soil health and ensuring sustainable production currently emerged as major concern

for researchers while working for increasing food demand of rising population in the country (Anjum et al., 2011). This may need large application of macro nutrients especially the N being a major limiting factor for agriculture (Li et al., 2008). However, its large

application may decrease its use-efficiency per unit application, may pollute and deteriorate ground water quality (Guo et al., 2008). In the contrary, nutrients application in small amounts and in improper form may reduce soil nutrient reserves and their availability to crop with the passage of time resulting in reduced crop yield. Looking at the capacity of soil, farmer's resources and market demand of the crop, various management techniques can be adopted to avoid soil fertility deterioration and to increase sustainability in crop production. There is a unanimous agreement on some management practices like manures and residues addition for fertility and soil quality enhancement and successful crop production as they are more beneficial for soil C build up (Dong et al., 2006). Use of concentrated nutrient input alone is very costly, especially N-fertilizers, and are subject to losses through various pathways such as leaching, volatilization and denitrification (Hammad et al., 2011) and their use is also associated with hazards to the environment and cause soil degradation (Bhattacharyya et al., 2008).

Biochar, being produced as a result of thermal conversion of organic material under oxygen depleted conditions possesses the properties of the end product that are advantageous for long terms C sequestration and storage in the soil (Maria et al., 2016). This property of biochar improves other soil characteristics like nutrient availability (Atkinson et al., 2010), microbial activity (Steinbeiss et al., 2009) improve soil structure, water holding capacity and nutrient cycling (Harvey et al., 2012). In a nutshell, if scientific and economic technicalities are overcome, biochar has been expected to remediate degraded soil for improved crop production (Spokas et al., 2012).

It is necessary to keep in mind that biochar is not a replacement of fertilizer despite its ability to add plant nutrients. Soils lacking in nutrients respond well to its application since biochar can improve mineral nutrients content in soil and yield (Chan et al., 2008). Its application to cultivated fields has proved helpful to farmers due to its ability to absorb and slowly release the applied inorganic elements in soil. Biochar improves the overall soil environment and nutrients availability and helps in reclaiming degraded soils (Spokas et al., 2012). Biochar's additional advantages include its adoptability in different agricultural setups such as organic, chemical and integrated farming (Cushion et al., 2010). Some published data discuss

the quantity applied and the mechanisms associated for the beneficial biochar related response on growth, production, and soil quality, yet other aspects need to be explored for the prospective improvement of biochar production skills with improved quality and values.

Despite of the aforementioned work on biochar, it was thought that still lot is to be done in exploring its residual role on soil properties and micronutrient status after one year of its application. Furthermore, what the biochar applied in different particle sizes to previously grown legumes has to do with beneficial residual aspects as well as interaction with rhizobia applied to legumes need to be determined. The current research work was, therefore, planned to study of the biochar particle size fraction residual effect on soil chemical properties and micronutrient status of soil after one year of its application to previous legume crop and its interaction with rhizobia applied.

Materials and Methods

The study reported in this paper was undertaken at the Agricultural University Amir Muhammad Khan Campus Mardan research plots in Summer 2016 to evaluate soil chemical properties and micronutrient status under residual biochar applied in different particle sizes and rhizobia inoculated to 2015 Winter (lentil) crop. Soil characteristics before start of the experiment in 2015 are given in Table 1.

Table 1: Analysis of the study site before experiment.

Analysis	Unit	Remarks/value
Texture	-	Silt loam
USDA Classification	Fine loamy, mixed, thermic, Typic Hapludalfs	
Density (Bulk)	g cm ⁻³	1.3
Moisture	%	8.3
Organic Carbon	g kg ⁻¹	3.2
N (Total)	"	0.1
C/N ratio	-	32:1
AB-BTPA extracted		
K	mg kg ⁻¹	72
P	"	4.32
Fe	"	0.9
Cu	"	0.5
Zn	"	0.45
Mn	"	1.52

The experiment was initiated in the existing treatments ($3 \times 4 \text{ m}^2$) layout of previous lentil crop in randomized complete block design (RCBD) with three replications. Wood biochar was obtained from local market while two biofertilizers for lentils with commercial names as Bizote-N prepared by the National Agricultural Research Center, Islamabad (NARC) and Rhizogold prepared by the University of Agriculture, Faisalabad were obtained from their respective organizations. Biozote N contained *Rhizobium leguminosarum* whilst Rhizogold was a mixture of *Rhizobium leguminosarum* and other plant growth promoting rhizobacteria. Biochar (10 Mg ha^{-1}) was applied one year before to winter lentil crop in different particle sizes (< 2 , 2-5 and 5-10 mm) and mixed with 15 cm soil depth whilst rhizobial products were inoculated to lentil seeds designated treatments in 2015. Biofertilizers: biochar particle size combinations include; control, Bizote-N, Rhizogold, < 2 mm, 2-5 mm, 5-10 mm, Bizote-N + < 2 mm, Bizote-N + 2-5 mm, Bizote-N + 5-10 mm, Rhizogold + < 2 mm, Rhizogold + 2-5 mm and Rhizogold + 5-10 mm. Lentil crop was sown on 15th November 2015 and harvested on 15th May, 2016. Maize crop was sown in the same experimental set up on 10th June, 2016 as per recommended practices and harvested on 20th September, 2016 (data not shown in this paper).

Samples from soil 20 cm depth were obtained at each treatment on 3rd October, 2016, cleaned from debris and stones, big root, air dried and threshed with wooden hammer and sieved with 2 mm sieve in the laboratory for further processing and analysis.

Soil texture was estimated by hydrometer technique and bulk density using sampling cores of 100 cm^3 as outline by Tagar and Bhatti (1996). Electrical conductivity (EC) meter was used for measuring the soluble salt in soil. A suspension at the ratio of 1:5 (Soil: H_2O) was prepared by stirring for 30 min then read on EC meter of each soil sample (Rhoades, 1982). A 1:5 (Soil: H_2O) suspension was prepared by stirring for 30 minutes and pH meter (Model German Type B-124) was used to read soil pH (McClean, 1982). A 10g soil was extracted with 20 mL Ammonium bicarbonate diethylene triamine penta acetic acid (AB-DTPA) through 15 minutes shaking on reciprocating shaker and filtered (Soltanpour and Schwab, 1997). Soil micro-nutrients (Fe, Zn, Mn and Cu) concentrations were recorded on atomic

absorption spectrophotometer in the filtrate. For determination of soil organic C, total N extractable P, K and moisture content was determined by following respective procedures outlined in Ryan et al. (2001).

Statistical procedure

Analysis of variance for the data collected was performed was determined using STATIX 8.1 software. In case of significant difference amongst means, Least Significant Difference (LSD) test was applied (Steel and Torrie, 1980). Significant interactions amongst the treatments was demonstrated in graphs with help of M.S. Excel software.

Results and Discussion

Data on soil $\text{pH}_{1:5}$ indicated significant ($p < 0.05$) difference in residual effect of the applied biochar different particle sizes and biochar control, whilst non-significant difference in soil pH was observed among the biochar particle sizes (Table 2). The 2-5 mm and 5-10 mm size particle pH value of 7.85 and the < 2 mm particle pH of 7.86, were 2 and 3% higher, respectively, over biochar control pH (7.63). Lori and Harpole (2012) and Knox et al. (2015) revealed that biochar raised soil pH while Arif et al. (2016) reported that biochar had no negative impact on soil pH. Results (Table 2) further indicated that neither biofertilizer strains nor the interaction between biochar and biofertilizer strains significantly affected the soil pH. Results (Table 2) regarding soil EC indicated significant ($p < 0.05$) difference in residual biochar particle sizes effect over the biochar control whilst a significant variation was also observed among particle sizes. The < 2 mm size particles showed maximum soil EC (144 mS cm^{-1}) followed by 2-5 mm sized particle where soil EC was 138 mS cm^{-1} whilst the maximum particle size had minimum soil EC (133 mS cm^{-1}) amongst the biochar particles sizes and each particle size showed 15, 10 and 6% increase in soil EC, respectively, over the biochar control (125 mS cm^{-1}). Such increase in soil EC could be due to releasing cations by biochar as well as retention of salt in soil to prevent its leaching. Results (Table 2) further indicated that neither biofertilizer strains nor the interaction between biochar and biofertilizer strains significantly affected the soil EC. Arif et al. (2016) suggested that biochar may solve soil fertility problem on permanent basis since its addition increased levels of fertility and showed no negative impact on EC of alkaline soil.

Table 2: Residual biochar different particle sizes and biofertilizer effect on soil fertility and chemical properties.

Soil Properties	Particle Sizes (mm)					Biofertilizers				Interxn
	< 2	2-5	5-10	Cont.	LSD (p<0.05)	Bizote-N	Rhizo gold	Con	LSD (p<0.05)	
pH	7.86	7.85	7.85	7.63	0.16	7.85	7.81	7.74	ns	ns
EC mS cm ⁻¹	144	138	133	125	8.22	136	134	134	ns	ns
Organic matter (%)	1.73	1.68	1.67	0.87	0.026	1.52	1.48	1.47	0.225	0.014

Cont: Control; Interxn: Interaction; Means lying in rows with given LSD value are statistically different at the $p < 0.05$.

Table 3: Residual biochar in different particle sizes and biofertilizers effect on soil microneutrients content.

Nutri- ents	Particle Sizes (mm)					Biofertilizers				Interxn
	< 2	2-5	5-10	Cont.	LSD (p<0.05)	Bizote-N	Rhizogold	Cont.	LSD (p<0.05)	
Fe	2.48	2.06	1.99	1.81	0.07	2.16	2.07	2.02	0.06	ns
Zn	0.41	0.36	0.33	0.31	0.06	0.61	0.24	0.18	0.05	0.1
Mn	1.23	1.05	1.02	0.89	0.06	1.23	1.05	0.85	0.05	0.108
Cu	0.51	0.38	0.37	0.26	0.01	0.45	0.40	0.29	0.01	0.026

Cont: Control; Interxn: Interaction; Means lying in rows with given LSD value are statistically different at the $p < 0.05$.

Residual effect of biochar in different particle sizes on soil organic matter was highly significant ($p < 0.01$) (Table 2). The < 2 mm particle size treatment OM content (1.73%) registered 98% increase, 2-5 mm particle size (1.68%) recorded an increase of 93% whilst the lowest increase by 91% was recorded in soil OM in the 5-10 mm particle size fraction (1.67%) over the biochar control plots where OM content was 0.87%. Highest OM content in < 2 mm particle plots can be ascribed to more soil volume dilution through uniform biochar C distribution compared to 2-5 and 5-10 mm biochar particles. McElligott (2011), Qayyum et al. (2014) and Tian et al. (2016) showed increase OM content after addition of biochar in soils.

Biofertilizer treatments residual effect also recorded significant ($p < 0.05$) increase in soil OM. Bizote-N treated soil OM (1.5%) showed 3% increase whilst the Rhizogold treated soil OM (1.48%) recorded 0.7% increase over the biofertilizer control (1.47%). Biofertilizer treatments might have increased the crop growth as well as the microbial population which might have resulted in higher organic matter content in soil. Highly significant interaction effect between the bio-fertilizer and biochar particles sizes treatments was recorded on soil OM content (Table 2). Bizote-N was more pronounced in OM content at all biochar particles sizes than the Rhizogold and the biofertilizer control. However, all biofertilizer treatments were uniformly superior at the < 2 mm biochar particles sizes with respect to soil OM whilst uniformly lowest at the biochar control plots (Figure 1).

Residual biochar particle sizes resulted in significant ($p < 0.05$) increase in AB-DTPA extractable micronutrients (Fe, Zn, Mn and Cu) (Table 3). Improvement in extractable Fe by each particles were 37, 13 and 10% in extractable Zn were 32, 16 and 6%, in extractable Mn were 38, 17 and 14 and in extractable Cu were 96, 46 and 42%, respectively, over biochar control. Jones et al. (2011) reported the changes brought about by the biochar in physical properties of soil like bulk density, increased OM breakdown and the biochar liberated organic C suggesting solution for C sequestration when applied to soil, its carbon releasing the nutrients effectively and reduce nutrient leach down (Lori and Harpole, 2012). Laird et al. (2010b) reported higher extractable nutrients content after biochar amendment application to soil. Ali et al. (2015) incorporated the biochar to agriculture field and reported it to have got an importance for soil fertility.

Residual effect of the biofertilizers (Bizote-N and Rhizogold) also recorded significant variations in extractable Fe, Zn, Mn and Cu contents with their significantly higher and maximum values in the Bizote-N (2.16, 0.61, 1.23 and 0.45 mg kg⁻¹, respectively) compared to the Rhizogold (2.07, 0.24, 1.05 and 0.45 mg kg⁻¹) and the biofertilizer control (2.02, 0.18, 0.25 and 0.29 mg kg⁻¹, respectively). Bizote-N induced increase in extractable Fe, Zn, Mn and Cu contents were 7, 238, 45 and 55% over the control, respectively. Rhizogold showed significant increase in Zn, Mn and Cu (by 33, 24 and 38,

respectively) over the biofertilizer control whilst with regard to Fe, the Rhizogold effect was statistically similar to the biofertilizer control. Legume (lentil-maize) in cropping pattern increase the nutrient budget of soil (Shafi et al., 2010). Significant residual effect of preceding legumes on maize growth parameters was reported by Ali et al. (2015) while the treatment with no previous crop showed significantly reduced per ear grains, 1000 grain weight and yield when compared with legume crop. Such Improvement in growth parameters can be due to overall improvement in soil quality including soil OM and micronutrient content as a result of the residual biofertilizer effect inoculated to legumes might be attributed to higher crop growth and subsequently higher organic matter addition to the soil.

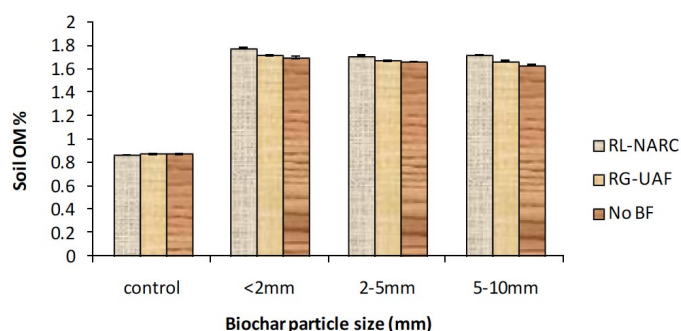


Figure 1: Interactive effect of the residual biochar particle sizes and rhizobia on soil OM. RL-NARC: Bizote-N, RG-UAF: Rhizogold, No BF: Biofertilizer control.

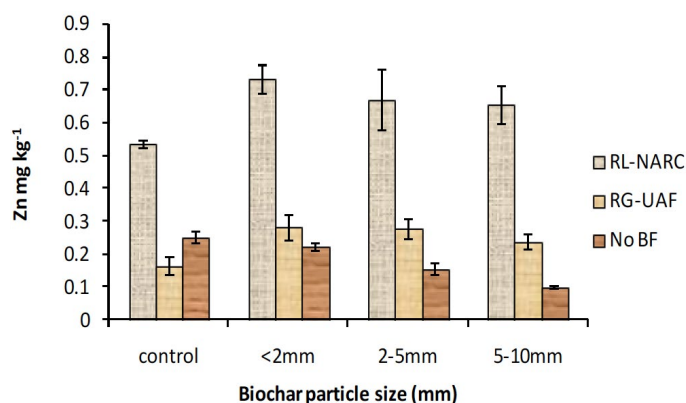


Figure 2: Interactive effect of the residual biochar different size particles and rhizobia on extractable Zn content. RL-NARC: Bizote-N, RG-UAF: Rhizogold, No BF: Biofertilizer control.

Interaction effect of the biochar and Biofertilizer strains on extractable Fe content was non-significant but it was significant upon Zn, Mn and Cu content of soil. Results (Figures 2, 3 and 4) showed the Bizote-N performance was significantly higher over the Rhizogold and the biofertilizer control throughout the biochar particle size treatments. However, amongst the particle sizes, it revealed the

highest concentrations for extractable Zn, Mn and Cu in the < 2 mm size particles and the same was true for Rhizogold as well. Biofertilizer showed the lowest performance with respect to these nutrients throughout the biochar particle sizes (Figures 2, 3 and 4).

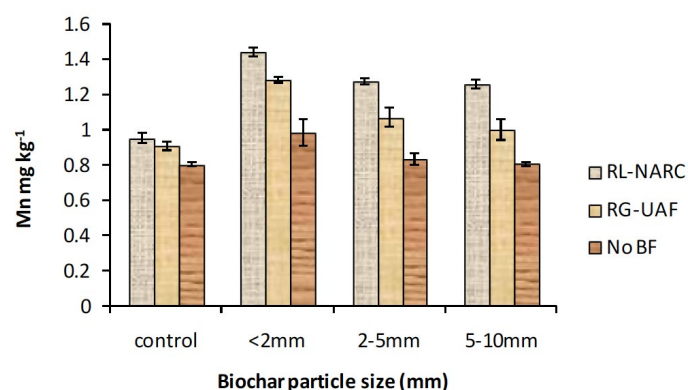


Figure 3: Interactive effect of the residual biochar different size particles and rhizobia on extractable Mn content. RL-NARC: Bizote-N, RG-UAF: Rhizogold, No BF: Biofertilizer control.

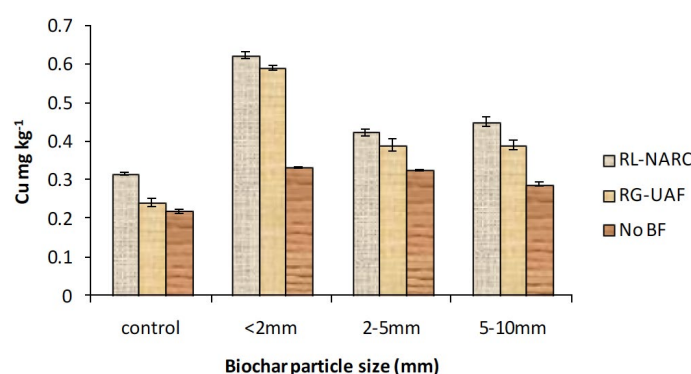


Figure 4: Interactive effect of the residual biochar different size particles and rhizobia on extractable Cu content. RL-NARC: Bizote-N, RG-UAF: Rhizogold, No BF: Biofertilizer control.

Conclusions and Recommendations

Bizote-N was more pronounced in improving micronutrient content and soil OM at all biochar particles sizes. However, amongst the particle sizes, it showed the maximum value of extractable micronutrients at the < 2 mm biochar particle treatment and the same are recommended for field application and inoculation of the legume crop in order to maintain soil fertility on sustained basis.

Novelty Statement

Biochar beneficial aspects reported so far by the prior research are based on the quantity of biochar applied and mechanism of its application. This research in-

investigated the biochar impact with a new dimension of biochars particle size (<2, 2-5 and 5-10) mm variation and its combination with biofertilizer. The current manuscript, therefore, reports novel information with regard to biochar different particles sizes and rhizobia strains applied to previous lentil crop and their residual impact on subsequent maize crop and soil properties.

Author's Contribution

Amjid Ali conducted the field work, Wiqar Ahmad supervised the experiment and data collection and helped in manuscript write up, Muhammad Zeeshan helped in statistical analysis, Farman Ullah Khan helped in laboratory analysis and Motasim Billah helped in experiment installation and data collection and manuscript write up.

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