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EFFECTS OF VARIOUS BIOCHARS ON SEED GERMINATION AND CARBON MINERALIZATION IN AN ALKALINE SOIL

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Use of biochar (BC) as soil amendment improves soil physicochemical properties, carbon sequestration and plant growth. However, prior to use as amendment, BC must be evaluated for its potential effects on soil and plants. In this study, various wastes (cotton-sticks, vegetable-market waste, poultry-manure, rice-straw, *Eucalyptus camaldulensis* –leaves, Neem (*Azadirakta indica*) –leaves, citrus-leaves, wheat straw and house-hold waste) were converted into BCs using pyrolysis (350 - 450 °C). Later, the prepared BCs were analyzed for pH, EC, moisture, ash and volatile matter, and nutrients such as carbon, nitrogen, phosphorus, and potassium using recommended methodologies. In first experiment, BCs were applied to an alkaline soil at rate of 1 % and maize seeds were grown. In this study effect of BCs on germination parameters, seedlings growth attributes, soil pH and EC were investigated. In second experiment, the C mineralization of various BCs in alkaline soil was investigated. The results show significant effect of vegetable waste biochar, and eucalyptus-leaves biochar on seedlings dry matter, shoot lengths, and root lengths. Regarding germination, none of the BCs caused detrimental effect. The results of C mineralization study show no significant differences between control treatment and BCs for cumulative CO_2 release. It is concluded that biochars produced from above described feedstock have no negative effect on soil and plant system, rather can improve carbon levels in alkaline soils.
Keywords: organic-wastes, pyrolysis, maize, germination, charcoal

INTRODUCTION

Biochar also termed as charcoal is a carbon (C) rich product of anaerobic and incomplete combustion of organic wastes (Lehmann, 2007). Due to the specific conditions and methodology of biochar production about 50% of the initial C can be sequestered as compared to open-air burning and biological decomposition (Joseph *et al*., 2007). Through extraordinary soil C sequestration-potential and reduced release of greenhouse gases into the atmosphere such as methane, nitrous oxide and carbon oxide, biochar has been proved to be a doorstep in mitigating climate change (Kammann *et al*., 2012). Apart from environmental benefits, biochar significantly influence soil physicochemical properties and fertility status (Verheijen *et al*., 2014). Due to negatively charged surfaces and high surface area biochars can retain nutrients and water in the soils and as compared to conventional organic amendments (Karhu *et al*., 2011; Scott *et al*., 2014).

The feedstock and production conditions affect physicochemical properties of biochar and also determine its beneficial or detrimental effects on soil fertility and plant growth (Manyà, 2012; Ronsse *et al*., 2013). Biochar can be produced using a variety of organic wastes such as agricultural wastes (Vitali *et al*., 2013), food wastes (Li *et al*., 2013), municipal solid wastes (Lu *et al*., 2012), meat and bone meal (Cascarosa *et al*., 2013) etc. Most of the studies cited above refer to generate energy by utilizing these wastes. However, the byproduct (biochar) can be used as an amendment in soils but a careful evaluation of biochar prior to field application is necessary due to some negative effects associated with some biochar (Li *et al*., 2012; Rogovska *et al*., 2012). Various phytotoxic substances, such as polycyclic aromatic hydrocarbon (PAH), phenolic compounds, formic or acetic acid are released from freshly prepared BC (Coûteaux, 2003; Bargmann *et al*., 2013; Quilliam *et al*., 2013) and these substances may pose toxic effects on germination of seeds (Buss and Mašek, 2014). Rogovska *et al*. (2011) conducted germination tests to assess biochar quality and found lesser shoot lengths of corn seedlings in biochar extracts as compared to nutrient solutions; however, the overall growth was better in biochar-extracts than in distilled water.

Pakistan, the sixth largest population of the world (187 million) generates 56,000 tons of municipal solid wastes (MSW) per day (Pak-EPA, 2005) which are comprised of both organic (food wastes, paper, garden trimmings etc.) and inorganic wastes (cardboard, wood, glass, plastic, and special wastes etc.). The organic components of MSW are nutritious, decomposable and can be a used as soil amendment after necessary treatment. Moreover, being an agricultural based country, a tremendous amount of agricultural residues is left after harvest of major crops. Unfortunately there is lack of professionalism and proper waste disposal systems right from collection to disposal. It is proposed that all kinds of organic wastes may be disposed safely in form of biochar. However, due to evidences of both positive and negative effects of biochar on growth of plants, it is recommended to perform basic characterization and germination tests prior to start its utilization on commercial scale. It should be noted that most of the biochars used in previous studies are derived from relatively pure plant based biomass. Most of the well documented studies on biochar research have been done in acidic soils and/or tropical environments. As far as alkaline soils are concerned very less studies have been reported. So far, no study has been reported in Pakistan on biochar production, their characterization and potential use as an organic amendment.

The objectives of our experiments were to (i) prepare and characterize biochars using various organic feedstock (ii) study potential effect of prepared biochars on seed germination and seedlings growth, and (iii) investigate the carbon stabilization potential of biochars in an alkaline soil.

MATERIALS AND METHODS

Biochar production: Nine various organic-wastes were collected from the Multan district. The material included (i) cotton-sticks from a harvested field, (ii) vegetable-market waste, (iii) poultry manure, (iv) rice straw, (v) eucalyptus (Eucalyptus camaldulensis) leaves, (vi) neem (Azadirachta indica) leaves, (vii) citrus leaves, (viii) wheat straw and (ix) house hold waste. The collected materials were air dried for two days and then pyrolysis was done in an especially designed laboratory scale stainless-steel furnace of 10 kg capacity that was equipped with a gas burner. The ignition was provided through natural gas supply from outside. A top mounded thermo-gauge was used to monitor the temperature. The wastes were processed in such a way that fire was not in direct contact. The time and temperature for each biochar was noted during their preparation.

After completion of pyrolysis, the prepared biochars were allowed to cool for some time in furnace, then taken out from the furnace, ground to pass through 2 mm size and stored in plastic cans.

***Physicochemical characterization of Biochars*:** The volatile matter and ash content were determined by heating the materials at 450 °C and 550 °C respectively, using muffle furnace (Qayyum *et al.*, 2012). The pH and EC of BCs in distilled water (1:20, w/v) were measured using pH and EC meters. The concentrations of total carbon, nitrogen, and hydrogen in BCs were measured using elemental analyzer (Elementar, Germany). To determine total phosphorus, potassium and sodium concentration, the BC samples were digested in di-acid (HNO_3:$HClO_4$). The concentration of P was measured using spectrophotometer while those of K and Na were determined using flame photometer. The data is provided in Table 1. The concentrations of C, H, N, and P in all BCs were almost similar. However, there was slight variation for the concentrations of K and Na among BCs. The other parameters such as pH, EC, ash and volatile matter were highly variable in all BCs. Except poultry manure biochar all biochars were in the range of alkaline pH.

Germination Test: To determine potential positive or detrimental effects of BCs on seed germination, a germination test was conducted using maize seeds. The medium used for germination test was a mixture of loam and sand. The treatments comprised of a control (only potting media, no BC), and nine various BCs [cotton sticks biochar (CSB), vegetable waste biochar (VWB), poultry manure biochar (PMB), rice straw biochar (RSB), eucalyptus leaves biochar (ELB), neem leaves biochar (NLB), citrus leaves biochar (CLB), wheat straw biochar (WSB) and household waste biochar (HWB)]. Prior to sowing, maize seeds were dipped in tap water for 24 hours. Small trays of 10 kg capacity were filled with mixtures of soil and biochar treatments applied at the rate of 1 %. Each treatment was replicated four times using complete randomized design (CRD) for statistical comparison. The moisture content in each tray was maintained at 50 % of the water holding

Table 1. Physicochemical properties of the various biochars

Biochar material	Elemental composition (%, w/w)						Ash (%)	Volatile matter (%)	pH	EC (dS m^{-1})
	C	H_2	N	P	K	Na				
Rice Straw Biochar (RSB)	42.3	1.1	1.5	0.3	2.54	1.1	22.50	24	10.0	2.4
Poultry Manure Biochar (PMB)	43.8	1.1	1.6	0.8	2.0	1.0	19.80	19	6.3	6.6
Neem Leaves Biochar (NLB)	43.0	1.1	1.7	0.5	1.3	0.6	21.30	23	10.6	1.7
Wheat Straw Biochar (WSB)	42.0	1.2	1.7	0.2	2.2	0.7	22.00	23	10.1	2.8
Vegetable Waste Biochar (VWB)	43.5	1.0	1.5	0.8	3.4	1.3	20.35	30	11.3	3.3
Cotton Sticks Biochar (CSB)	46.3	3.6	1.7	0.4	1.6	1.1	15.23	20	9.6	1.7
Citrus Leaves Biochar (CLB)	45.6	3.4	1.8	0.5	1.2	0.7	16.60	23	10.0	0.9
House Hold Biochar (HWB)	44.7	1.1	1.6	0.8	1.3	1.5	18.22	13	9.1	2.1
Eucaplytus Leaves Biochar (ELB)	44.2	2.8	1.6	0.2	1.6	0.7	19.80	28	10.3	1.1

capacity. The trays were placed in laboratory conditions (25 °C temperature). During the whole experiment, the germinated seedlings were counted every day. Three germination parameters namely germination percentage (GP), mean emergence time (MET) and the coefficient of uniformity of emergence (CUE) were determined following Hatamzadeh *et al*. (2012). After 10 days of germination, the seedlings were harvested to determine dry mass per pot, average shoot and radicle lengths.

Carbon mineralization study: In this study, all prepared BCs were applied to an alkaline soil at an application rate of 2 % w/w. The experimental setup comprised of same treatments as used in germination study. The mixtures of soil (50 g soil) and BCs (1 g) were added in china dishes which were placed inside the incubation jars on the iron stands. The moisture content of each dish was maintained at 60% WHC. The CO_2 released from all treatments at various intervals of time (1, 5, 15 and 30 days of incubation period) was captured in KOH that was placed inside each jar. After each sampling, the sampled KOH was back titrated against 0.1M HCl using phenolphthalein as an indicator in the presence of $BaCl_2$.

Statistical analyses: The data of germination experiment and carbon mineralization were analyzed statistically employed for statistical comparisons using SPSS (PASW version 18.0). The treatments were analyzed for analysis of variance (ANOVA) and means were compared following Tukey HSD test.

RESULTS

Effect of biochars on soil pH and EC: Soil samples taken after completion of germination test were analyzed for pH. The statistical analysis show significant effect of BC on soil pH (Table 2). Among all tested-BCs, VWB, CSB, and ELB increased the soil pH from 7.35 to 7.83, 7.75, and 7.85 respectively. All other BCs influenced soil pH but the differences between themselves and control were statistically non-significant.

The BCs also significantly affected soil electrical conductivity (EC). Application of NLB and VWB significantly decreased the values of EC by 31 % and 13 % respectively. Two BCs (CSB and CLB) did not affect soil EC when compared with the control treatment, while the remaining BCs significantly increased soil EC values (Table 2).

Effect of biochars on germination of maize seeds

Germination parameters: The data regarding germination parameters are provided in Table 3. The germination percentages range from 69 to 85 % in different treatments but the statistical analysis revealed no significant differences. Similarly, other parameters such as mean emergence time and coefficient of uniformity of emergence were also not affected by any of the BC when compared with the control treatment. None of the BCs increased or decreased germination parameters.

Table 2. Effect of control, rice straw biochar (RSB), poultry manure biochar (PMB), neem leaves biochar (NLB), wheat straw biochar (WSB), vegetable waste biochar (VWB), cotton sticks biochar (CSB), citrus leaves biochar (CLB), household waste biochar (HWB), and eucalyptus leaves biochar (ELB) on seed germination (%), mean emergence time (days), and Coefficient of uniformity of emergence.

	control	RSB	PMB	NLB	WSB	VWB	CSB	CLB	HWB	ELB
Germination (%)	75.00 ±6.74	69.17 ±6.14	71.67 ±2.15	70.83 ±10.92	68.33 ±13.71	85.83 ±5.34	76.67 ±1.92	69.17 ±11.89	70.838. 75	75.003. 97
Mean emergence time (days)	2.91 ±0.25	3.31 ±0.37	3.26 ±0.15	3.19 ±0.17	2.98 ±0.32	2.73 ±0.19	2.94 ±0.21	3.23 ±0.37	3.37 ±0.21	2.95 ±0.10
Coefficient of uniformity of emergence	0.65 ±0.07	0.61 ±0.08	0.66 ±0.04	0.73 ±0.06	0.89 ±0.03	0.72 ±0.07	0.70 ±0.07	0.60 ±0.06	0.65 ±0.06	0.75 ±0.07

The values are mean ± standard error of four replicates.

Table 3. Effect of control, rice straw biochar (RSB), poultry manure biochar (PMB), neem leaves biochar (NLB), wheat straw biochar (WSB), vegetable waste biochar (VWB), cotton sticks biochar (CSB), citrus leaves biochar (CLB), household waste biochar (HWB), and eucalyptus leaves biochar (ELB) on soil pH and soil EC.

	Control	RSB	PMB	NLB	WSB	VWB	CSB	CLB	HWB	ELB
Soil pH	7.35 ±0.03 (a)	7.55 ±0.06 (ab)	7.625 ±0.11 (ab)	7.55 ±0.03 (ab)	7.5 ±0.04 (ab)	7.83 ±0.10 (b)	7.75 ±0.09 (b)	7.58 ±0.06 (ab)	7.68 ±0.09 (ab)	7.85 ±0.06 (b)
Soil $EC_{(1:10)}$, μSm^{-1}	284.06 ±39.25 (C)	293.14 ±14.55 (D)	308.98 ±5.80 (E)	176.56 ±4.31 (A)	361.1 ±22.03 (F)	289.55 ±68.5 (B)	273.785 ±16.24 (C)	262.445 ±10.01 (C)	276.365 ±10.78 (D)	262.41 ±17.40 (D)

The values are mean of four replicates ± standard error followed by various letters in case of significance.

Yield parameters: The dry matter of maize seedlings is provided in Fig. 1. The results show significant effect of BCs on dry matter yield but in most of the cases the effects were not significantly different when compared with the control treatment. Only three BCs (NLB, VWB and ELB) significantly increased the dry matter of seedlings. The shoot and root lengths were also influenced significantly by the application of various BCs (Fig. 2). Except PMB, all BCs significantly increased the shoot length of maize seedlings.

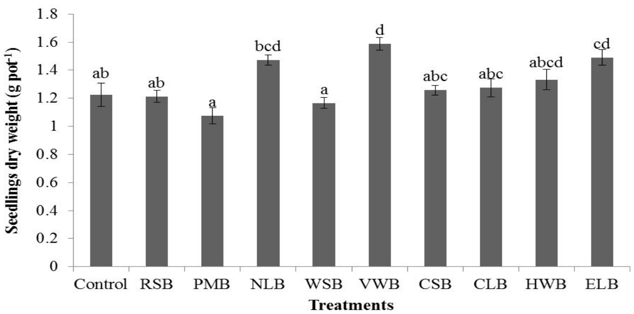

Figure 1. Effect of control, rice straw biochar (RSB), poultry manure biochar (PMB), neem leaves biochar (NLB), wheat straw biochar (WSB), vegetable waste biochar (VWB), cotton sticks biochar (CSB), citrus leaves biochar (CLB), household waste biochar (HWB), and eucalyptus leaves biochar (ELB) on seedlings dry weight (g pot^{-1}). The various letters provided above the bars show significant differences at P ≤ 0.05.

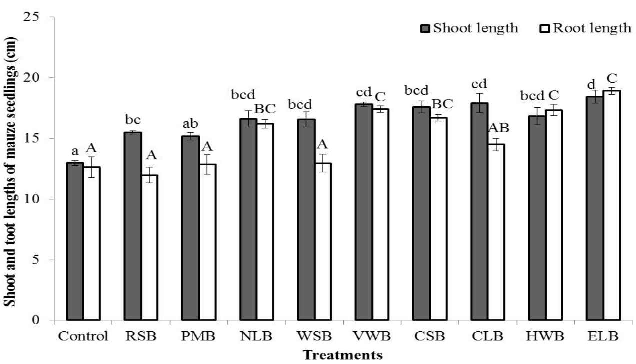

Figure 2. Effect of control, rice straw biochar (RSB), poultry manure biochar (PMB), neem leaves biochar (NLB), wheat straw biochar (WSB), vegetable waste biochar (VWB), cotton sticks biochar (CSB), citrus leaves biochar (CLB), household waste biochar (HWB), and eucalyptus leaves biochar (ELB) on shoot and toot lengths of maize seedlings (cm). The various letters provided above the bars show significant differences at P ≤ 0.05.

Carbon mineralization in biochar amended soil: The results show no significant differences between control treatment and biochars for cumulative CO_2 release between any of the treatments (Fig. 3). In some biochar treatments, the C mineralization rates were higher in the start of incubation but with the passage of time, the CO_2 release rates were decreased and become similar in all treatments. This show, the presence of some easily degradable organic materials in the biochars which were later disappeared. At the end of incubation, all of the biochars were statistically similar to the control treatment for C mineralization (cumulative CO_2).

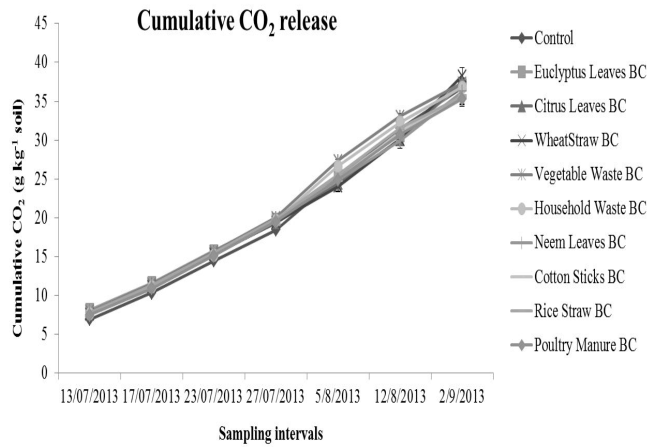

Figure 3. Effect of control, rice straw biochar (RSB), poultry manure biochar (PMB), neem leaves biochar (NLB), wheat straw biochar (WSB), vegetable waste biochar (VWB), cotton sticks biochar (CSB), citrus leaves biochar (CLB), household waste biochar (HWB), and eucalyptus leaves biochar (ELB) on the cumulative CO2 release from soil

DISCUSSION

The characterization data show alkaline pH of all BCs (Table 1), and it was obvious that these would cause alkalinity in soil. The alkaline effect is due to accumulation of mineral elements in the BCs (Jeffery *et al*., 2011; Chintala *et al.*, 2014). The soil used in our experiment was a mixture of sand and loam with high pH (7.35) and the BCs were applied at the rate of 1 percent (20 tons per ha), but it is also astonishing that most of the BCs did not increase pH significantly (Table 2) which indicates that these biochars can be used as an amendment in alkaline soils if applied at the rate of one percent or less. During pyrolysis, basic cations are not lost rather accumulated in the BC (Antal and Grønli, 2003). This is the reason why wheat straw biochar (WSB), poultry manure biochar (PMB), rice straw biochar (RSB), household waste biochar (HWB), and eucalyptus leaves biochar (ELB) significantly increased EC of soil. Artiola *et al*. (2012) also stated significant increase in soil EC values with BC derived from pine forest waste (applied at 2 % and 4 %). In our case decrease in soil EC with NLB and VWB may be attributed due to lower amounts of minerals in feedstock materials. Such deviating effects of BC on soil chemical properties (pH and EC) need more attention.

The increased values of biochar pH, and EC could be attributed due to high ash content and basic oxides which are accumulated as a result of pyrolysis. Among the nutrient elements, C and N are volatilized during pyrolysis, while P and K are accumulated. The enhancement in growth of maize seedlings in some treatments may be attributed to their respective high nutrient status. Previously, to evaluate potential toxicity/effects of biochars, germination tests have been performed by various scientists (Busch *et al*., 2012; Rogovska *et al*., 2012; Bargmann *et al*., 2013). Taek-Keun *et al*. (2012) compared three biochars derived from orange-peel (OP), residual-wood (RW), and water treatment-sludge (WS) on germination and growth of lettuce and reported inhibitory effects on germination in the treatment where biochar derived from orange-peel was applied. Bargmann *et al*. (2013) investigated potential effects of biochar, hydrochar and process water on seed germination. They found no detrimental effect of biochar, however, hydrochar caused decline in germination parameters. Our results show that prepared biochars did not harm growth of seedlings and did not influence dry matter. In most of the studies where negative effects of biochars have been reported, the application rates of biochars were in the range of 2 % to 10 % (Bargmann *et al*., 2013). However such higher application rates are not applicable in field conditions. Therefore we applied all biochars at a lower rate (1 %).

The cumulative release of CO_2 from BCs-amended soil was not significantly different to that of control (only soil) that shows higher carbon sequestration potential of studied BCs (Fig. 3). Biochars have higher stability due to formation of aromatic carbon compounds during pyrolysis. Previously, many studies have reported variable C mineralization potential of biochars (Qayyum *et al*., 2012; Gajić *et al*., 2012). Harris *et al*. (2013) found variable carbon mineralization in several BCs and reasoned the feedstock as an important factor. The differences between BCs are due to their production methodologies (temperature). Hydrochars are reported to have lower stability as compared to charcoals. In our case, the temperature of BC production was 400-500 °C that yielded a product having higher stabilization potential compared with that of hydrochars.

From the above discussion, it is concluded that the prepared biochars have potential to be used in soil as suitable amendments for carbon sequestration in alkaline soil. However, to avoid toxic effects on soil pH and EC, higher application rates should be avoided.

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