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Effect of various application rate of zinc fertilizer with and without fruit waste biochar on the growth and Zn uptake in maize

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Abstract

Deficiency of Zinc (Zn) is a major problem in those agricultural soils that are calcareous and high in pH. Low intake of Zn in plants not only disturb the yield of crops but also consumption of such food developed Zn deficiency symptoms in consumers. To overcome the problem of Zn deficiency in plants scientists recommend to use such amendments that has ability to low soil pHand increase soil nutrients bioavailability to plants. In current study fruit waste biochar (BC) was used to examine its role on the bioavailability of Zn and growth of maize when applied in the presence of half and full recommended dose of Zn fertilizer. Results confirmed that the application of BC significantly enhanced the Zn concentration in maize root and shoot. A significant improvement in maize root fresh weight, photosynthetic pigments and nutrients (NPK) in maize shoot validated the fact that application of fruit waste BC in combination with full recommended doze of Zn has ability to improve the Zn bioavailability in maize. On the basis of results, it is concluded that application of BC with full recommenced doze of zinc is a better approach for improvement in Zn uptake and promotion of growth in maize.

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Introduction

Zinc deficient crops in recent era has become one of major challenge of the agricultural scientist of the world (Rashid, 1996). High pH of soil is a major factor that is contributing towards the less availability of Zn to the plants (Tahir *et al.,* 1991). Mostly Pakistan, China, India, Turkey and Iran are one of leading countries where soils are deficient in bioavailable Zn (Hotz and Brown, 2004; Welch and Graham, 2004). It plays a key role in the regulation of enzymatic activity, stimulation of auxin and many enzymatic reactions (DeDatta, 1981). Optimum intake of Zn not only increase the yield of crops but also improve the health of humans (Hotz and Brown, 2004). It is well-documented fact that the deficiency of Zn in human ranges between 4-73% in different areas of the world.

Due to deficiency of Zn the immune and physical systems become impaired, chances of development of cancer is increased with severe damage to DNA (Hotz and Brown, 2004; Gibson, 2006; Prasad, 2007). It has been noted that low availability of zinc is a major hurdle in agriculture rather than its presence in low concentration (Welch *et al.,* 1982). To overcome the problem of micronutrients deficiency it is mostly recommended to incorporate the organic amendments in combination with inorganic fertilizer by many scientists (Resources and Agricultural, 2008; Bandyopadhyay *et al.,* 2010; Zubair *et al.,* 2012; Iqbal *et al.,* 2015). Biochar is one of such amendment that can ameliorate the soil physical and chemical properties to improve the bioavailability of micronutrients (Amonette and Joseph, 2009; Cornelissen *et al.,* 2013; Bandara *et al.,* 2017).

High surface area and cation exchange capacity of biochar decrease the losses of nutrients and significantly enhanced their uptake in plants (Amonette and Joseph, 2009). It also increases the soil carbon that plays an important role in the enhancement of microbial population in soil (Saxena *et al.,* 2013). Depending upon the feedstock, biochar also releases some of the micronutrients as well that also improves the soil fertility status (Amonette and Joseph, 2009).

As maize is one of most important and 3rd major cereal crop of the world after wheat and rice and widely deficient in zinc, its consumption is also creating deficiency of zinc especiallyin children (Chomba *et al.,* 2015; Imran *et al.,* 2016). Although maize as a staple food provides a significant amount of macronutrients with 15% protein and 20% calories (Nuss and Tanumihardjo, 2011; Chomba *et al.,* 2015) but the demand of time is to enhance its level of Zn to get optimum nutrition from it. The aim of the study was to enhance the uptake of Zn in calcareous and high pH soils by application of low pH nutrients rich fruit waste biochar. Therefore, current experiment was designed with the hypothesis that fruit waste biochar can mitigate the Zn deficiency in maize when applied in combination with inorganic Zn fertilizer.

Material and methods

A pot experiment was conducted in the research area of Soil and Water Testing Laboratory Multan for Research to examin the effect of various application rate of zinc (Zn) in the presence and absence of fruit waste biochar.

Biochar production

For the production of biochar, the methodology of Amonette and Joseph (2009) was followed. Initially, the waste feedstock of fruits was sun-dried. After that, it was pyrolyzed at 377 ^{o}C for 2hours under partially aerobic conditions in a specially designed pyrolyzer. After that biochar was grinded in a manual mortar and passed through 5mm sieve.

Biochar characterization

The pH and EC of biochar were determined by making a dilution of 1:20 w/v following the methodology of Qayyum *et al.* (2014). For determination of nitrogen, phosphorus and potassium in BC di-acid HNO_3 : $HClO_4$ mixture was used according to the method described by Chapman and Pratt (1961). The yellow colour method was used for analysis of phosphorus in BC on spectrophotometer at 420 nm (Jones *et al.,* 1991). The potassium concentration in BC was determined on flamephotometeras described by Nadeem *et al.* (2013). For nitrogen determination, H_2SO_4 digestion (Jones *et al.,* 1991) of BC was done.

However, distillation was done on Kjeldahl's distillation apparatus (Van Schouwenberg and Walinge, 1973). The ash content and volatile matter in BC was assessed according to Qayyum *et al.* (2014) by heating sample in muffle furnace at 550 °C and 450 °C respectively. However, the fixed carbon in BC was calculated by using the equation of Noor *et al.* (2012):

Fixed Carbon (%) = 100 - (%Volatile Matter + %Ash Content)

Zinc, fertilizer and biochar application

For the introduction of Zn 2.5 ppm and 5.0ppm, the analytical grade salt of $ZnSO_4$ was used. For 2.5 and 5.0 ppm, Zn 6.17 and 12.3 mg $ZnSO_4$ was added in one kilogram of soil. The biochar was added at the rate of 1% (w/w) in the soil. The recommended dozes of nitrogen, phosphorus and potassium were applied in the form of urea, DAP and SOP at the time of seeds sowing. Biochar was mixed at the rate of 1% manually by hand. However, Zn was also applied and mixed in the soil along with biochar as per treatment plan.

Pot preparation

In each pot having capacity of 10 Kg soil, the eight-kilogram soil was added. For determination of texture, hydrometer method was followed by USDA textural triangle (Gee and Bauder, 1986) which was sandy loam. Walkley (1935) method was followed for analysis of soil organic matter. However, organic nitrogen in soil was calculated according to the equation:

Organic N (%) = Soil Organic Matter/20

Olsen and Sommers (1982) method was followed for analysis of extractable soil phosphorus. However, extractable soil potassium was analyzed according to the Nadeem *et al.* (2013).

Seeds sowing

The seeds of maize Hybrid-123 were initially screened manually to get healthy seeds. After that seeds were dipped in water for 2h. Finally, 5 seeds per pot were sown. When seeds become germinated after seven days 2 healthy seedlings were maintained in each pot.

Experimental design and treatment plan

There were 6 treatments and 3 replications following the completely randomized design (CRD).

The treatments include: T1 = control (No BC and No Zn), T2 = Zn 2.5 ppm, T3 = Zn 5.0 ppm, T4 = biochar 1%, T5 = biochar 1% + Zn 2.5 ppm and T6 = biochar 1% + 5.0 ppm.

Harvesting and growth attributes

After 55 days of sowing the plants were harvested. The fresh weight of shoot and root were determined by top weight balance soon after harvesting.

Cholorophyll determination

For the analysis of cholorophyll a, cholorophyll b and total cholorophyll the leaves samples were initially grinded in mortar in the presence of 5ml acetone (80%). When the samples of leaves become paste-like then filtration was done and final volume was made by using the acetone. The aliquot absorbance was noted on spectrophotometer at 663 and 645 nm wave length. For final determination of cholorophyll a, cholorophyll b and total cholorophyll following equations of Arnon (1949) were used:

$$\text{Cholorophyll a (mg/g)} = \frac{12.7\,(OD663) - 2.69\,(OD\,645)\,V}{1000\,(W)}$$

$$\text{Cholorophyll b (mg/g)} = \frac{22.9\,(OD645) - 4.68\,(OD\,663)\,V}{1000\,(W)}$$

Total Cholorophyll (mg/g) = Cholorophyll a + Cholorophyll b

Where,

OD = Optical density (wavelength)

V = Final volume made

W = Fresh leaf weight (g)

NPK in maize shoot

For determination of P and K concentration in the shoot of maize samples di-acid HNO_3 : $HClO_4$ mixture was used as a digestor according to the method described by Chapman and Pratt (1961). The yellow colour method was followed for analysis of phosphorus in shoot on spectrophotometer at 420 nm (Jones *et al.,* 1991). The potassium concentration in digested shoot samples was determined on flamephotometeras described by Nadeem *et al.* (2013). For nitrogen determination, H_2SO_4 digestion (Jones *et al.,* 1991) of shootwas done. However, distillation was done on Kjeldahl's distillation apparatus (Van Schouwenberg and Walinge, 1973).

Shoot and root Zn concentration

For determination of Zn concentration in shoot and root of maize samples di-acid HNO_3 : $HClO_4$ mixture was used as a digestor according to the method described by Chapman and Pratt (1961). After digestion of samples, the Zn concentration was assessed on pre-calibrated atomic absorption spectrophotometer.

Statistical Analysis

Statistical analysis of collected data was done by following standard statistical procedures described by Steel *et al.* (1997). All the treatments were compared using Tukey's test at $p \leq 0.05$.

Table 1. Soil and Biochar characteristics.

Material	Property	Unit	Value
Soil	pH_s	-	8.14
	EC_e	dSm^{-1}	2.76
	Organic Matter	%	0.45
	Organic nitrogen	%	0.023
	Extractable phosphorus	$\mu g\ g^{-1}$	8.14
	Extractable potassium	$\mu g\ g^{-1}$	196
Biochar	pH	-	7.29
	EC	dSm^{-1}	3.26
	Volatile compounds	%	10.0
	Ash content	%	20.9
	Fixed carbon	%	63.1
	Zinc	$\mu g\ g^{-1}$	53.9

Results

Main effect of various levels of zinc (Zn) application rate and interactive effect of Zinc and biochar (BC1) remained non-significant for soil pH and EC (Table 2). However, the main effect of BC1 differs significantly ($p \leq 0.05$) for pH of soil. Application of Zn5 and Zn15 remained statistically alike to each other as compared to control for soil pH and EC. As compared to control (No BC1), the application of BC1 remained significantly best for soil pH and EC. All the treatments of Zn including control were statistically alike to each other for soil pH and EC. On an average, maximum reduction of 1.6% in soil pH was noted as compared to control where BC1 was applied as an amendment. However, for soil EC application of BC1 gave maximum increase of 25.1% as compared to control (No BC).

Main effect of BC and Zn remained significant ($p \leq 0.05$) but their interaction remained non-significant for root fresh weight. However, for shoot fresh weight only main effect of BC was significant (Table 2). Both main effect of Zn and interactive effect of Zn × BC differ non-significantly.

As compared to control (No BC), application of BC1 remained significantly best for shoot fresh weight. All the treatments of Zn including control were statistically alike to each other for shoot fresh weight. Maximum increase of 33.4% in shoot fresh weight was noted where BC1 was applied as an amendment. Both Zn5 and Zn15 remained statistically alike to each other but only Zn15 differ significantly as compared to control for root fresh weight. On an average, application of BC1 also differs significantly as compared to control (No BC) for root fresh weight. Maximum increase of 17.7% in root fresh weight was noted where Zn15 was applied as an amendment. However, on an average application of BC1 gave maximum increase of 79.4% in root fresh weight as compared to control.

Main effect of Zn and BC were significant ($p \leq 0.05$) but their interaction remained non-significant for Zn concentration in shoot and root (Table 2). As compared to control, Zn5 and Zn15 differ significantly for Zn concentration in shoot and root. Application of Zn15 performed significantly better as compared to Zn5 for Zn concentration in shoot and root. On an average, as compared to control (No BC), the application of BC1 remained significantly better for Zn concentration in shoot and root. Maximum increase of 47.0 and 79.6% Zn concentration in maize shoot and root respectively as compared to control where Zn15 was applied as an amendment. On an average, application of BC1 gave maximum increase of 23.1 and 29.3% in shoot and root Zn concentration respectively as compared to control (BC1).

Main effect of Zn and BC differ significantly ($p \leq 0.05$) but interactive effects of Zn × BC remained non-significant for cholorophyll a, cholorophyll b and total cholorophyll (Table 2). Application of Zn5 and Zn15 remained statistically similar to each other but differ significantly as compared to control for cholorophyll a and total cholorophyll. For cholorophyll b, Zn15 differ significantly as compared to control. However, Zn5 remained statistically alike with control. Maximum increase of 1.91, 33.3 and 23.4% in cholorophyll a, cholorophyll b and total cholorophyll respectively as compared to control.

Statistical analysis showed that main effect of Zn and BC were significant for the N, P and K concentration in the maize shoot but their interaction remained non-significant (Fig 1-3). Application of BC1 significantly enhanced the N, P and K in the shoot regardless of level of Zn in the soil. Plants which were amended with BC1 performed significantly better for the intake of N, P and K in the maize as compared to those which were not amended with BC1. Maximum increase of 25.2, 70.0 and 46.4% in the N, P and K were noted as compared to control where BC1+ Zn15 was applied.

Table 2. Effect of various application rate of Zn in the presence and absence of biochar on soil and maize growth attributes.

Various levels of Zinc	Soil pH			Soil EC (dSm⁻¹)			Shoot Fresh Weight (g)		
	Various levels of Biochar								
	Zn × BC		ME (Zn)	Zn × BC		ME (Zn)	Zn × BC		ME (Zn)
	No BC	BC1		No BC	BC1		No BC	BC1	
Control	8.15	8.03	8.09	2.77	3.68	3.23	80.3	104.3	92.3
Zinc5	8.14	8.00	8.07	3.0	3.79	3.40	83.4	109.0	96.2
Zinc15	8.17	8.03	8.10	3.19	3.75	3.47	80.3	112.6	96.5
ME (BC)	8.15^{A}	8.02^{B}		2.99^{B}	3.74^{A}		81.4^{B}	108.6^{A}	
	Root Fresh Weight (g)			Shoot Zinc (μg g⁻¹)			Root Zinc (μg g⁻¹)		
Control	9.24	15.5	12.4^{B}	31.06	31.28	31.17^{C}	11.22	11.82	11.52^{C}
Zinc5	9.77	17.6	13.7^{AB}	31.29	31.64	31.47^{B}	11.98	12.66	12.32^{B}
Zinc15	10.1	19.0	14.6^{A}	31.56	31.88	31.72^{A}	12.53	12.92	12.73^{A}
ME (BC)	9.70^{B}	17.4^{A}		31.30^{B}	31.60^{A}		11.91^{B}	12.47^{A}	
	Cholorophyll a (mg g⁻¹)			Cholorophyll b (mg g⁻¹)			Total cholorophyll (mg g⁻¹)		
Control	0.75	1.03	0.89^{B}	0.34	0.62	0.48^{B}	1.08	1.66	1.37^{B}
Zinc5	0.89	1.21	1.05^{A}	0.42	0.62	0.52^{B}	1.31	1.83	1.57^{A}
Zinc15	0.96	1.15	1.06^{A}	0.58	0.70	0.64^{A}	1.54	1.85	1.69^{A}
ME (BC)	0.87^{B}	1.13^{A}		0.44^{B}	0.65^{A}		1.31^{B}	1.78^{A}	

Means sharing different letter are statistically different at ($p \leq 0.05$). Non-significant attributes have no letter. ME (BC) = Main effect of biochar; ME (Zn) = Main effect of zinc.

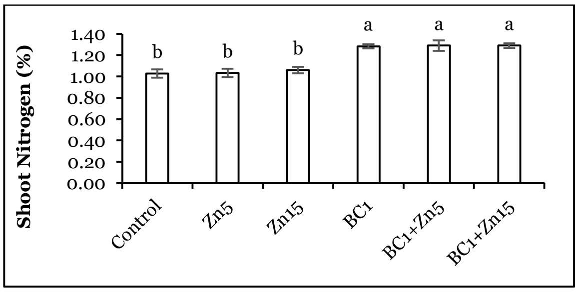

Fig. 1. Effect of various levels of zinc (5 and 15 ppm) in the presence and absence of biochar (BC=1%) on shoot nitrogen concentration of maize. Means sharing different letter are compared by Tukey's test and differ significantly at $p \leq 0.05$.

Discussion

In the current study a significant enhancement in root and shoot Zinc concentration in the presence of BC1 (1% BC) validated the imperative role of BC for better intake of Zn in maize plants. Our findings were in agreement with the findings of Turan *et al.* (2017) as they observed a significant improvement in the zinc bioavailability with significant immobilization of Ni toxicity by application of biochar (BC) and chitosan (CH) in the presence of Zinc. The increase in the bioavailability of Zn in current experiment was might be due to an average reduction in the pH of soil where BC1 was applied as an amendment.

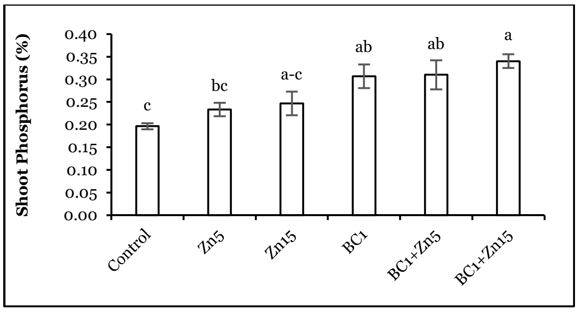

Fig. 2. Effect of various levels of zinc (5 and 15 ppm) in the presence and absence of biochar (BC=1%) on shoot phosphorus concentration of maize. Means sharing different letter are compared by Tukey's test and differ significantly at $p \leq 0.05$.

According to He *et al.* (2017) it is pH and soil nitrogen contents that significantly affect the bioavailability of metallic ions in the soil and their bioavailability to plants.

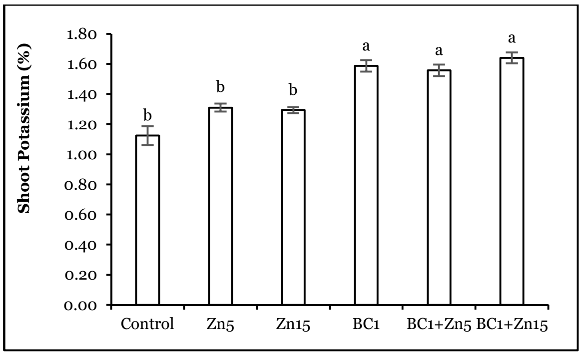

Fig. 3. Effect of various levels of zinc (5 and 15 ppm) in the presence and absence of biochar (BC=1%) on shoot potassium concentration of maize. Means sharing different letter are compared by Tukey's test and differ significantly at $p \leq 0.05$.

Cakmak *et al.* (1996) suggested that the low level of organic matter and high pH significantly decrease the availability of Zn to the plants. Cakmak (2008) observed and documented that the low moisture level, especially in the cereal cultivated crops, also decreased the Zn uptake in the plants. In current study, the application of biochar might also enhance the soil water holding capacity as well as organic carbon due to which Zn bioavailability of maize was enhanced. Curaqueo *et al.* (2014) suggested that biochar has micropores that play a signified role in the retention of water.

As biochar is produced at high temperature and less availability of oxygen, the level of organic carbon the biochar is tremendously high that also improve the soil carbon level (Amonette and Joseph, 2009; Danish *et al.*, 2014). A significant improvement in the N, P and K in maize shoot validated that fact of better synthesis of photosynthetic pigments in the maize leaves. The findings of Younis *et al.*, (2014) also justified our argument regarding the better uptake of nutrients after application of cotton sticks biochar. According to Danish *et al.*, (2015) better availability of nutrients in the plants play an imperative role in the synthesis of cholorophyll a, cholorophyll b and total cholorophyll in maize plants when applied with phosphorus solubilizing bacteria. The better intake of N, P and K in the maize plants and improvement in water holding capacity of soil might enhance the root and shoot fresh weight in biochar amended soils. Younis *et al.*, (2014b) also reported similar kind of improvement in the weight of plants where they use biochar as an amendment.

Conclusion

On the basis of results, it may be concluded that biochar with low pH might be an efficacious amendment for the improvement in the availability of Zn in the plants as well as to improve their growth attributes. The photosynthetic pigments might also be improved by the better intake of macronutrients in the shoot where biochar is applied as an amendment.

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