Research Article

Subhan Danish*, Fayyaz Ahmad Tahir, Muhammad Khalid Rasheed, Niaz Ahmad, Muhammad Arif Ali, Sidra Kiran, Uzma Younis, Inam Irshad, Beenish Butt

Effect of foliar application of Fe and banana peel waste biochar on growth, chlorophyll content and accessory pigments synthesis in spinach under chromium (IV) toxicity

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Abstract: Chromium (Cr) toxicity is becoming one of a major issue for the cultivation of crops. Toxicity of Cr directly affects synthesis of chlorophyll and restricts Fe intake, which decreases crop growth. It is well documented that the reduction of Cr toxicity through the application of biochar. However, current experiment was carried out to investigate any positive effect of, banana peel waste biochar (BC) and foliar application of Fe (FFe) on growth and chlorophyll content of *Spinacia oleracea* L. under different levels of Cr toxicity. Seeds of *Spinacia oleracea* L. were grown under three levels of Cr i.e. control (Cr0), Cr35 (35 mg Cr kg^{-1} soil) and Cr70 (70 mg Cr kg^{-1} soil). Analyzed data confirmed that *Spinacia oleracea* L. seeds grown in 1% BC amended soils and 1000mM FFe, showed significantly better growth, Fe uptake and chlorophyll content as compared to control at Cr35 and Cr70. A significant improvement in shoot length (16.9 and 26.9%), root length (16.3 and 20.9%), plant fresh (15.5 and 28.3%) and dry weight (70.3 and 77.8%) as compared to control under Cr35 and Cr70, respectively, validated the efficacious functioning of 1% BC and FFe to mitigate Cr toxicity in *Spinacia oleracea* L. It is concluded that both 1% banana peel waste BC and 1000mM FFe have potential but sole application of FFe is more effective to alleviate Cr toxicity in *Spinacia oleracea* L. Fortification of Fe by foliar application is more effective comparative to banana peel waste biochar for improvement in growth, chlorophyll content and accessory pigments synthesis in spinach under chromium (IV) toxicity.

Keywords: Banana; Biochar; Chromium; Fe concentration; *Spinacia oleracea* L.

1 Introduction

Chromium (Cr) is the 2nd largest abundant pollutant that significantly contributes towards environmental pollution (Schiavon et al. 2007; Singh et al. 2013). It has been observed that Cr (VI) is an acute toxic form of Cr that is usually associated with oxygen i.e. chromate or as dichromate (Shanker et al. 2005). Every year a large amount of Cr-rich effluent generated by many industries is disposed on agricultural lands (Adriano 1986; Sharma et al. 2005). Combustion of coal, oil, metallurgical industries and chemical industries wastes are also continuously contributing towards degradation of soil via higher accumulation of Cr (Adriano 1986). Besides all that, overuse of Cr rich sewage water played an imperative role regarding the establishment of Cr toxicity in cultivatable lands and crops (Brar et al. 2000).

Toxic derivatives of Cr usually hampered growth attributes in plants by causing damage to chlorophyll content and poor intake of nutrients (Samantary 2002;

***Corresponding author: Subhan Danish,** Soil, Water and Plant Testing Laboratory, Pakarab Fertilizer Limited, Khanewal Road, Multan, Punjab, Pakistan
Department of Soil Science, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Punjab, Pakistan
E-mail: sd96850@gmail.com
Niaz Ahmad, Muhammad Arif Ali, Department of Soil Science, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Punjab, Pakistan
Fayyaz Ahmad Tahir, Muhammad Khalid Rasheed, Beenish Butt, Soil and Water Testing Laboratory for Research Multan, Punjab, Pakistan
Inam Irshad, Institute of Soil and Environmental Science, University of Agriculture, Faisalabad, Punjab, Pakistan
Uzma Younis, University of Central Punjab, Lahore, Punjab, Pakistan
Sidra Kiran, Mango Research Institute, Multan, Punjab, Pakistan

Shanker et al. 2005). Higher intake of Cr also resulted in poor seed germination (López-Luna et al. 2009), reduction of root growth (Rout et al. 1997) and chlorosis (Pandey and Sharma 2003) that resulted in significant reduction in yield (Sundaramoorthy et al. 2010). Addition of organic amendments for immobilization of toxic heavy metals in soils is mostly suggested by scientists (Park et al. 2011). However, the poor resistance of organic matter against decomposition usually provides a chance for the release of these toxic metals in the soil again (Park et al. 2011).

In the recent past, most of the scientists noted that biochar is an alternative resistant organic amendment that has better potential to sorp toxic heavy metals (Van Zwieten et al. 2010). Biochar is a carbonaceous material that is produced through pyrolysis under a limited supply of oxygen at high temperature (Abid et al. 2017; Chen et al. 2011; Lehmann et al. 2006; Woolf et al. 2010). It acts as a carbon pool that is quite stable in nature and effectively sequesters CO_2 that makes it an environment-friendly amendment (Lehmann et al. 2006). Besides all that, application of biochar also improves physio-chemical properties of soil, fertility status and microbial population (Danish and Zafar-ul-Hye 2019) that play an important role in the improvement of crops yield (Danish et al. 2015, 2014; Lehmann 2007; Liang et al. 2006; Younis et al. 2015, 2014). However, production and application of biochar in large quantity is a big hurdle regarding its use as an amendment.

Instead of root crops (turnip, carrot, potato and radish etc.), higher accumulation of Cr beyond toxic limits has been reported mostly in leafy vegetables i.e. spinach (Hundal and Arora 1993). Spinach (*Spinacia oleracea* L.) is one of the richest source of calcium, beta-carotene, vitamin C, P, K and Fe (Dicoteau 2000). It is also consumed as a supplement for p-coumaric acid derivatives (that showed antioxidant activity) and flavonoids derivatives named glucuronic acid (Bergman et al. 2001; Edenharder et al. 2001; Pandjaitan et al. 2005). As higher concentration of Cr can significantly decrease the uptake of Fe in crops (Turner and Rust 1971), therefore, current study was conducted with aim to examine the effectiveness of Fe foliar application and biochar on growth and chlorophyll content in *Spinacia oleracea* L. under Cr toxicity.

2 Materials and methods

2.1 Production of banana peel waste biochar

From the local vegetable and fruit market (30°11'20.9"N 71°31'02.0"E) in Multan, Punjab, Pakistan the banana peel was collected for the manufacturing of biochar (BC). First, waste material was sun dried for 6 days and after that pyrolyzed at 463°C for 68 min in pyrolyzer, as described by Qayyum et al. (2014). Then, BC was ground and passed through a 2 mm sieve to obtain a fine powder (≤ 2 mm). Prepared BC was then stored in air-tight plastic jars for future experimentation.

2.2 Characterization of biochar

For the determination of pH and EC, biochar to water ratio of 1:20 *w*/*v* was made according to Tahir et al. (2018). Biochar was digested with a di-acid mixture of HNO_3 : $HClO_4$ in 2:1 ratio (Chapman and Pratt 1961). Yellow color method was used for determination of phosphorus (P) using a spectrophotometer (Jones et al. 1991) while potassium (K) in digests sample was analyzed using a flame-photometer (Nadeem et al. 2013). For nitrogen, H_2SO_4 digestion (Jones et al. 1991) was done while distillation was carried out on Kjeldahl's distillation apparatus (Van Schouwenberg and Walinge 1973). For ash content and volatile matter in BC methodology of Qayyum et al. (2012) was adopted. Biochar sample was heated in a muffle furnace at 450°C and 550°C. However, fixed carbon (FC) in BC was calculated by Noor et al. (2012) equation:

$$FC\ (\%) = 100 - (\%\ \text{Volatile Matter} + \%\ \text{Ash Content}) \quad (1)$$

The characteristics of biochar are provided in Table 1.

2.3 Soil characteristic

The bulk soil was collected from plough layer near the nursery site of Mango Research Institute, Multan, Punjab, Pakistan (30°153593′ N and 71°445645′ E). The soil of the selected area was previously characterized as river alluvium, brown, moderately calcareous, weakly structured, hyperthermic Fluventic Haplocambic, Ochric epipedon and cambic subsurface horizon (Miani Soil Series). Hydrometer method was used for textural analysis of soil (Gee and Bauder 1986), Olsen and Sommers (1982) for available soil P, Nadeem et al. (2013) for extractable K and

Table 1. Pre-experimental characteristics of soil and banana peel waste biochar (BC)

Soil	Unit	Value	Biochar	Unit	Value
Sand	%	55	pH	-	8.26
Silt	%	35	EC_e	dS m^{-1}	3.41
Clay	%	10	Volatile Matter	%	28.96
Texture	Loam		Ash Content	%	8.9
pH_s	-	8.03	Fixed Carbon	%	62.14
EC_e	dS m^{-1}	1.81	Total N	%	0.08
Organic Matter	%	0.70	Total P	%	0.94
Extractable P	μg g^{-1}	7.26	Total K	%	3.61
Extractable K	μg g^{-1}	195	Total Cr	μg g^{-1}	3.29

Walkley (1935) for soil organic matter. The pre-experimental soil characteristics are provided in Table 1.

2.4 Polythene bags

Black color polythene bags having dimensions of 60 cm deep × 45 cm diameter were used as pots. Each bag has the capacity to carry 7 kg soil.

2.5 Polythene bags preparation

In each polythene bag, 6 kg of soil was filled. Nitrogen, P (as P_2O_5) and K (as K_2O) were applied at the rate of 22:15.6:13.8 kg ha^{-1} (ZTBL 2018). Urea was applied in 3 splits during the growth period. However, diammonium phospahate (DAP) and sulphate of potash (SOP) fertilizer were applied at the time of sowing in a single dose.

2.6 Experiment site and treatment plan

The pot experiment was conducted in the research area (30°153738′ N, 71°445168′ E) of Mango Research Institute Multan, Pakistan under Cr toxicity on *Spinacia oleracea* L. There were 3 treatments (application of biochar, foliar Fe and no application, n=3) applied under 3 levels of Cr following factorial completely randomized design. For the development of artificial Cr toxicity analytical grade salt of $K_2Cr_2O_7$ was used as described by Sharma et al. (2005). There were three levels of Cr: control (Cr0), 35 mg kg^{-1} (Cr35) and 70 mg kg^{-1} Cr (Cr70). Both 35 and 70 mg kg^{-1} Cr were applied and homogenized manually in the soil at the time of pot preparation keeping in mind the Cr concentration of BC. Similarly, 1% BC was also mixed in soil manually at the time of pot preparation. However, foliar Fe was applied in three splits at 13, 26 and 39 days after sowing.

2.7 Seed collection and sowing

The seeds of spinach (*Spinacia oleracea* L.) were purchased from certified seed dealer of the Government of Punjab, Pakistan. Weak and damaged seeds were initially screened out manually. For the experiment, five seeds were sown in each pot manually by hand at 15 August, 2018.

2.8 Harvesting

For shoot and root length, plant fresh weight and dry weight, electrolyte leakage, chlorophyll content, carotenoids, anthocyanin and lycopene determination harvesting was done after 50 days of sowing. The shoot and root length were measured by using the measuring tape. However, plant fresh weight was taken soon after harvesting. For determination of plant dry weight samples were oven dried at 65ºC for 48 h in an air circulating oven.

2.9 Electrolyte leakage

The methodology of Lutts et al. (1996) was used for analysis of electrolyte leakage (EL). Initially, leaves of *Spinacia oleracea* L. were washed with distilled water (DW) and then 1 cm diameter discs were cut with a steel cylinder. Uniform size discs of 1g were immersed in a 20 ml DW containing test tube and incubated at 25°C for 24h. After that

first electrical conductivity (EC1) was determined while second EC (EC2) was observed after heating test tubes at 120°C for 20 min in a water bath. Finally, EL was calculated using the equation as follows:

$$EL\ (\%) = EC1 / EC2 \times 100 \quad (2)$$

2.10 Chlorophyll content

The chlorophyll a, chlorophyll b and total chlorophyll contents were examined in the fresh leaves of *Spinacia oleracea* L. by following the methodologies of Arnon (1949) and Ravelo-Pérez et al. (2008). The extract was taken in acetone (80%) solution. For the estimation of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, lycopene and anthocyanin absorbance was noted at optical densities of 663, 645, 530, 503 and 480 nm wavelength on spectrophotometer. Final calculations were made using the following relations:

$$\text{Chlorophyll a (mg/g)} = 12.7\ (OD\ 663) - 2.69\ (OD\ 645)\ V / (1000 \times W) \quad (3)$$

$$\text{Chlorophyll b (mg/g)} = 22.9\ (OD\ 645) - 4.68\ (OD\ 663)\ V / (1000 \times W) \quad (4)$$

$$\text{Total Chlorophyll (mg/g)} = \text{Chlorophyll a} + \text{Chlorophyll b} \quad (5)$$

$$\text{Carotenoids (mg/g)} = OD\ (480) + (0.114 \times OD\ 663) - (0.638 \times OD\ 645)\ V / (1000 \times W) \quad (6)$$

$$\text{Anthocyanin (µmol/ml)} = (0.817 \times OD\ 537) - (0.00697 \times OD645) - (0.002228 \times OD663) \quad (7)$$

$$\text{Lycopene (µg/g)} = OD503 \times 31.2 / \text{g tissue} \quad (8)$$

Where,
OD = Optical density (nm)
V= Final volume made (ml)
W= Fresh weight of sample (g)

2.11 Fe and Cr determination

Collected leaves and roots samples were digested by using a di-acid mixture of (HNO_3: $HClO_4$) (Chapman and Pratt 1961) for the determination of Fe and Cr concentration on atomic absorption spectrophotometer (Jones et al. 1991).

2.12 Statistical Analysis

Statistical analysis was carried out using standard statistical procedures as given by Steel et al. (1997). Two factorial ANOVA was applied by using Statistix 8.1 software for calculation of significance of treatments. All the treatments were compared using Tukey's test at $P \leq 0.05$.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

Statistical analysis confirmed that the effects of various levels of Cr and treatments (T) were significant for shoot length, root length, plant fresh and dry weight of *Spinacia oleracea* L. (Table 2). It was observed that at Cr35 and Cr70 shoot length, plant fresh and dry weight remained statistically alike to each other but differ significantly as compared to control (Cr0). However, for root length, only Cr70 differ significantly as compared to control (Table 1). A significant maximum reduction in shoot length (16.0%), root length (18.3%), plant fresh (32.4%) and dry weight (27.8%) was noted where *Spinacia oleracea* L. was grown at Cr70 as compared to control (Cr0). Application of biochar and foliar Fe remained statistically alike to each other but performed significantly better (Table 1) for shoot length (16.9 and 26.9%), root length (16.3 and 20.9%), plant fresh (15.5 and 28.3%) and dry weight (70.3 and 77.8%), respectively as compared to control.

Both main and interactive effects of T and Cr remained significant for Cr concentration in root and shoot of *Spinacia oleracea* L. For root Cr concentration, BC and FFe remained statistically alike to each other but performed significantly the best as compared to control at Cr70 (Table 3). It was noted that at the Cr35, application of BC and FFe remained statistically similar to each other but only FFe performed significantly best as compared to control for root Cr concentration. However, at Cr0 both BC and FFe did not differ significantly as compared to control for root Cr concentration. A significant maximum reduction of 43.4 and 40.2% in root Cr concentration was noted as compared to control where FFe was applied at Cr35 and Cr70, respectively. In the case of shoot Cr concentration, FFe performed significantly best as compared to control at Cr70. Similarly, application of BC also differed significantly for shoot Cr concentration as compared to control at Cr70 (Table 3). It was noted that at the Cr35, application of BC and FFe remained statistically similar to each other

Table 2: Main effects of biochar and foliar application of Fe on shoot length, root length, plant fresh weight and plant dry weight of *Spinacia oleracea* L. under various levels of Cr induced toxicity

Chromium levels (mg kg^{-1})	Shoot length (cm)	Root length (cm)	Plant FW (g plant^{-1})	Plant DW (g plant^{-1})
0	24.63 a	19.01 a	27.48 a	2.41 a
35	21.29 b	17.19 ab	21.05 b	1.91 b
70	20.67 b	15.53 b	20.75 b	1.74 b
Treatments				
Control	19.37 b	15.34 b	20.15 b	1.35 b
Biochar	22.64 a	17.84 a	23.28 a	2.30 a
Foliar Fe	24.58 a	18.54 a	25.85 a	2.40 a

Means of main effect not sharing the same letter, within a column, differ significantly from each other at $p \leq 0.05$ (n=3)
FW = Fresh weight; DW = Dry Weight

Table 3: Main and interactive effects of biochar and foliar application of Fe on shoot and root Cr and Fe concentration in *Spinacia oleracea* L. under various levels of Cr induced toxicity

Treatments	Root Cr concentration (µg g^{-1}) Various levels of Chromium (mg kg^{-1}) Interaction (T × Cr) (Means of 3 replicates)			Mean (T)	Shoot Cr concentration (µg g^{-1}) Interaction (T × Cr) (Means of 3 replicates)			Mean (T)
	0	35	70		0	35	70	
Control	1.36 ± 0.12 ^{e}	2.95 ± 0.20 ^{ab}	3.51 ± 0.14 ^{a}	2.61 ^{A}	6.22 ± 1.18 ^{ef}	22.79 ± 1.96 ^{b}	28.91 ± 1.80 ^{a}	19.30 ^{A}
Biochar	1.28 ± 0.04 ^{e}	2.29 ± 0.12 $^{b-d}$	2.71 ± 0.21 ^{bc}	2.09 ^{B}	5.06 ± 0.46 ^{ef}	17.93 ± 0.32 ^{bc}	21.09 ± 1.22 ^{b}	14.69 ^{B}
Foliar Fe	1.05 ± 0.07 ^{e}	1.67 ± 0.10 ^{de}	2.10 ± 0.13 ^{cd}	1.61 ^{C}	3.45 ± 0.58 ^{f}	14.32 ± 0.50 ^{cd}	10.86 ± 0.84 ^{de}	9.54 ^{C}
Mean (Cr)	1.23 ^{C}	2.30 ^{B}	2.77 ^{A}		4.91 ^{B}	18.34 ^{A}	20.29 ^{A}	
	Root Fe concentration (µg g^{-1})				Shoot Fe concentration (µg g^{-1})			
Control	157 ± 10.1 $^{a-c}$	122 ± 8.76 ^{c}	62 ± 7.00 ^{d}	114 ^{C}	230 ± 12.5 $^{a-c}$	180 ± 10.1 ^{cd}	137 ± 7.00 ^{d}	183 ^{C}
Biochar	164 ± 6.93 ^{ab}	147 ± 9.39 $^{a-c}$	125 ± 10.4 ^{bc}	145 ^{B}	258 ± 9.82 ^{ab}	212 ± 6.69 ^{bc}	186 ± 17.1 ^{cd}	219 ^{B}
Foliar Fe	188 ± 7.23 ^{a}	179 ± 8.50 ^{a}	163 ± 6.12 $^{a-c}$	177 ^{A}	274 ± 6.06 ^{a}	241 ± 10.3 ^{ab}	228 ± 11.1 $^{a-c}$	248 ^{A}
Mean (Cr)	170 ^{A}	149 ^{B}	117 ^{C}		254 ^{A}	211 ^{B}	184 ^{C}	

Means not sharing the same letter, within a column, differ significantly from each other at p ≤ 0.05 (n=3).

but only FFe performed significantly better as compared to control for shoot Cr concentration. However, at Cr0 both BC and FFe remained statistically alike with control for root Cr concentration. A significant maximum reduction of 59.1 and 166.2% in shoot Cr concentration was noted as compared to control where FFe was applied at Cr35 and Cr70, respectively.

Both main and interactive effects of T and Cr remained significant for Fe concentration in root but only main effects of T and Cr remained significant for shoot Fe concentration in *Spinacia oleracea* L. For root Fe concentration, BC and FFe remained statistically alike to each other but differed significantly as compared to control at Cr70 (Table 3). It was noted that at the Cr35, application of BC and FFe remained statistically similar to each other but only FFe remained significantly better as compared to control for root Fe concentration. However, at Cr0 both BC and FFe did not differ significantly as compared to control for root Fe concentration. Maximum increase of 46.7 and 163% in root Fe concentration was noted as compared to control where FFe was applied at Cr35 and Cr70 respectively. In case of shoot Fe concentration, FFe performed significantly the best as compared to control. Application of BC also differed significantly for shoot Fe concentration as compared to control (Table 3). Maximum increase of 35.5% in shoot Fe concentration was noted as compared to

control. Increasing level of Cr toxicity (Cr35) significantly decreased (16.9%) Fe shoot concentration as compared to control (Cr0). However, a significant maximum reduction (27.6%) in shoot Fe concentration was noted as compared to control where plants were cultivated under Cr70.

Both main and interactive effects of Cr and T differ significantly for electrolyte leakage in leaves of *Spinacia oleracea* L. At Cr35 and Cr70, application of FFe remained significantly better for less electrolyte leakage as compared to control (Figure 1). However, BC did not differ significantly as compared to control for electrolyte leakage at Cr35 and Cr70. It was observed that BC and FFe remained statistically alike to each other and with control at Cr0 for electrolyte leakage in leaves of *Spinacia oleracea* L. A significant maximum reduction of 34.9 and 53.7% in electrolyte leakage in leaves of *Spinacia oleracea* L. was noted as compared to control where FFe was applied at Cr35 and Cr70, respectively.

Both main and interactive effects of Cr and T differ significantly for chlorophyll a content in leaves of *Spinacia oleracea* L. At Cr35 and Cr70, application of FFe differed significantly better for chlorophyll a content as compared to control (Figure 2). However, BC did not differ significantly for chlorophyll a content at Cr35 but differed significantly at Cr70 as compared to control. Application of BC performed significantly better (42.9%) but FFe did not differ significantly at Cr 35 as compared to Cr70 for chlorophyll a content. It was observed that BC remained statistically alike but FFe remained significant with control at Cr0 for chlorophyll a content in leaves of *Spinacia oleracea* L. Maximum increase of 39 and 108% in chlorophyll a content in leaves of *Spinacia oleracea* L. was noted as compared to control where FFe was applied at Cr35 and Cr70, respectively.

Both main and interactive effects of Cr and T differ significantly for chlorophyll b content in leaves of *Spinacia oleracea* L. At Cr35 and Cr70, application of FFe performed significantly the best for chlorophyll b content as compared to control (Figure 3). However, BC did not differ significantly for chlorophyll b content at Cr35 and Cr70 as compared to control. It was observed that BC and FFe remained statistically alike with control at Cr0 for chlorophyll b content in leaves of *Spinacia oleracea* L. Maximum increase of 47 and 118% in chlorophyll b content in leaves of *Spinacia oleracea* L. was noted as compared to control where FFe was applied at Cr35 and Cr70, respectively.

Both main and interactive effects of Cr and T differ significantly for total chlorophyll content in leaves of *Spinacia oleracea* L. At Cr35 and Cr70, application of FFe differed significantly as compared to control (Figure 4). However, BC did not differ significantly for total chlorophyll content at Cr35 but performed significantly better at Cr70 as compared to control. It was observed that BC remained statistically alike but FFe remained significantly better as compared to control at Cr0 for total chlorophyll content in leaves of *Spinacia oleracea* L. Maximum increase of 42 and 107% in total chlorophyll content in leaves of *Spinacia oleracea* L. was noted as compared to control where FFe was applied at Cr35 and Cr70, respectively.

Both main and interactive effects of Cr and T differ significantly for carotenoids in leaves of *Spinacia oleracea* L. At Cr70, application of FFe and BC differed significantly as compared to control. However, FFe and BC did not differ significantly for carotenoids at Cr35 as compared to control (Figure 5). It was observed that BC remained statistically alike, but FFe was significantly better as compared to control at Cr0 for carotenoids in leaves of *Spinacia oleracea* L. Maximum increase of 19 and 154% in carotenoids in leaves of *Spinacia oleracea* L. was noted as compared to

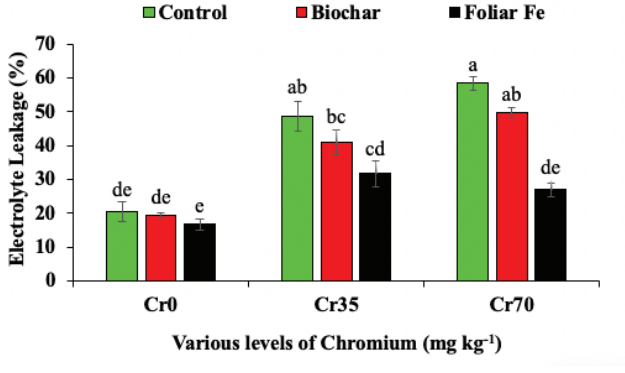

Figure 1: Effect of biochar and foliar application of Fe (1000 mM) on electrolyte leakage (%) in *Spinacia oleracea* L. leaves under various levels of Cr induced toxicity

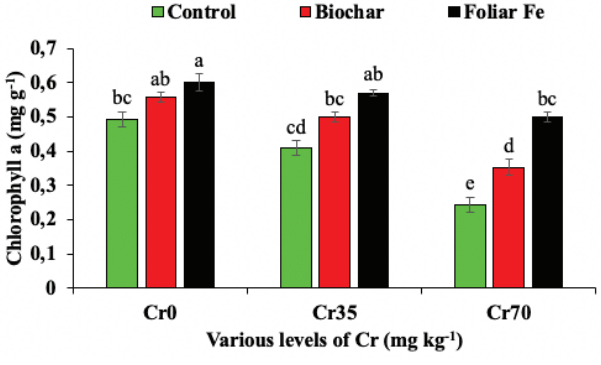

Figure 2: Effect of biochar and foliar application of Fe (1000 mM) on chlorophyll a (mg g^{-1}) in *Spinacia oleracea* L. leaves under various levels of Cr induced toxicity

control where FFe was applied at Cr35 and Cr70, respectively.

Both main and interactive effects of Cr and T differ significantly for anthocyanin in leaves of *Spinacia oleracea* L. At Cr35 and Cr70, application of FFe and BC remained statistically alike to each other, but differed significantly for anthocyanin as compared to control (Figure 6). However, FFe and BC did not differ significantly for anthocyanin in leaves of *Spinacia oleracea* L. at Cr0 as compared to control. A significant maximum reduction of 0.33 and 47.6% in anthocyanin in leaves of *Spinacia oleracea* L. was noted as compared to control where BC and FFe were applied at Cr35 and Cr70, respectively.

Both main and interactive effects of Cr and T differ significantly for lycopene in leaves of *Spinacia oleracea* L. At Cr70, application of FFe and BC differed significantly as compared to control (Figure 7). However, FFe remained significantly better for lycopene at Cr35 as compared to control. It was observed that BC and FFe remained statistically alike with control at Cr0 for lycopene in leaves of *Spinacia oleracea* L. However, the lycopene content at Cr35 was significantly lower only for FFe as compared to control. Maximum significant reduction of 39 and 54% in lycopene in leaves of *Spinacia oleracea* L. was noted as compared to control where FFe was applied at Cr35 and Cr70 respectively.

4 Discussion

Results of current experiment showed that without BC and FFe, toxicity induced by Cr35 and Cr70 significantly decrease shoot length, root length, plant fresh weight, plant dry weight in *Spinacia oleracea* L. Barceló et al. (1986) suggested that higher concentration of Cr resulted in restriction of cell division in root and shoot due to which shoot and length are decreased. The findings of Panda and Patra (2000) also supported our resulted as they observed a significant reduction in the root length of wheat seedlings when they were cultivated in Cr toxic

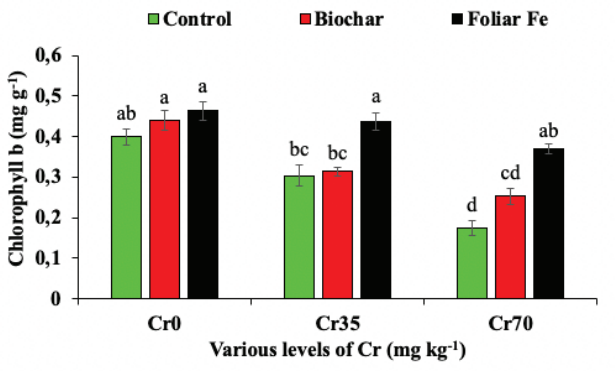

Figure 3: Effect of biochar and foliar application of Fe (1000 mM) on chlorophyll b (mg g^{-1}) in *Spinacia oleracea* L. leaves under various levels of Cr induced toxicity

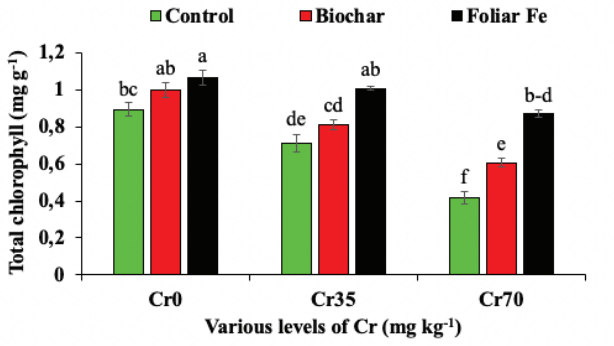

Figure 4: Effect of biochar and foliar application of Fe (1000 mM) on total chlorophyll (mg g^{-1}) in *Spinacia oleracea* L. leaves under various levels of Cr induced toxicity

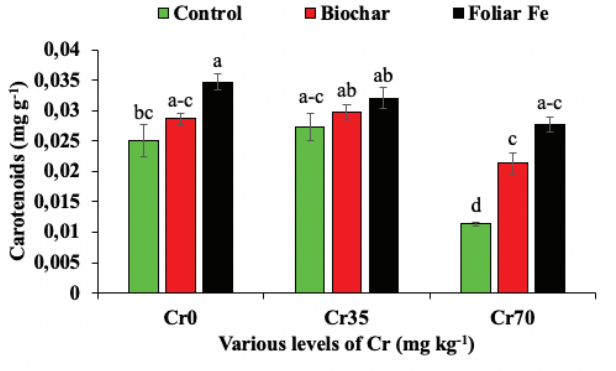

Figure 5: Effect of biochar and foliar application of Fe (1000 mM) on total carotenoids (mg g^{-1}) in *Spinacia oleracea* L. leaves under various levels of Cr induced toxicity

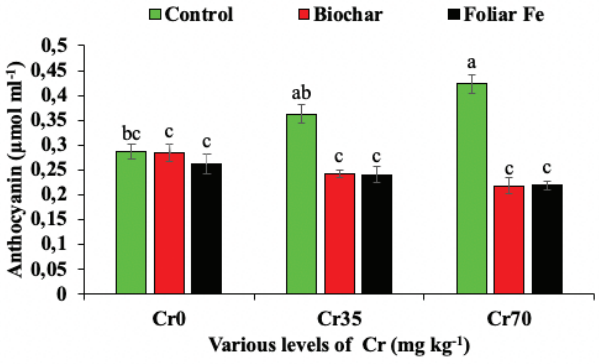

Figure 6: Effect of biochar and foliar application of Fe (1000 mM) on anthocyanin (µmol ml^{-1}) in *Spinacia oleracea* L. leaves under various levels of Cr induced toxicity

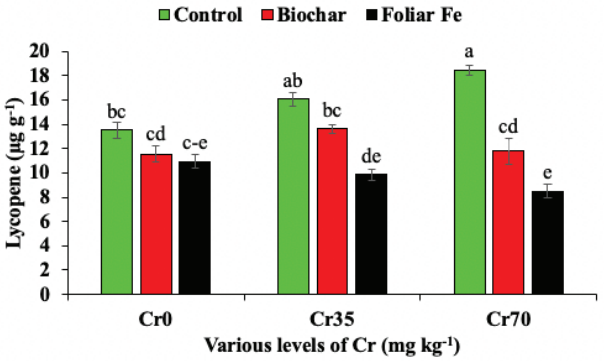

Figure 7: Effect of biochar and foliar application of Fe (1000 mM) on total lycopene (µg g^{-1}) in *Spinacia oleracea* L. leaves under various levels of Cr induced toxicity

conditions. However, Chen et al. (2001) noted that 20 mg Cr(VI) kg^{-1} of soil as $K_2Cr_2O_7$ build a significant toxicity that decreased root dry weight and root length in wheat. In current study, application of BC and FFe significantly enhanced shoot length, root length, plant fresh weight, plant dry weight in *Spinacia oleracea* L. This improvement might be due to less intake of Cr and fortification of Fe in shoot and root of *Spinacia oleracea* L. Choppala et al. (2012) argued that presence of functional groups and dissolved organic carbon in BC provide electrons for the reduction of toxic Cr(VI) into non-toxic Cr(III) due to which toxic effects of Cr are decreased. Similarly, Choudhary et al. (2017), reported that it is high sorption of Cr by phenolic functional groups in eucalyptus bark biochar that played an efficacious role in the reduction of Cr(IV) into nontoxic form Cr(III). According to Gao et al. (2016), BC also enhanced the Fe retention and modified physio-chemical properties of soil that improve the intake of Fe. Bishnoi et al. (1993) suggested that the low production of dry matter in plants under Cr toxicity is due to the destruction of chlorophyll. Davies et al. (2002) noted that restriction of electron transport chain resulted in inactivation of enzymes due to which chloroplasts become disorganized and CO_2 fixation is reduced (Bishnoi et al. 1993; Shanker 2003). In current study similar kind of results were noted where Cr35 and Cr70 significantly decreased chlorophyll a, chlorophyll b, total chlorophyll and carotenoids contents in *Spinacia oleracea* L. However, application of BC and FFe significantly improved chlorophyll contents while decreased anthocyanin and lycopene in *Spinacia oleracea* L. due to better intake of Fe in shoot and root that alleviate Cr toxicity. According to Shanker (2003), it is restriction of plasma membrane H^+ ATPase under Cr induced toxicity that inhibit the intake of nutrients (i.e. N, P and Fe) in plant tissue (Adriano 1986b). Miller et al. (1982) argued that deficiency of Fe resulted in reduction of chlorophyll content because it plays an imperative role in the formation of glycine and succinyl Co A to δ-damino laveulinic acid, (precursor of porphyrins). Gao et al. (2016) also reported an increase in Fe content of dry beans when they applied wood waste BC in the soil as an amendment.

5 Conclusion

From results it is concluded that foliar application of Fe at the rate of 1000 mM or application of 1% BC are effective approaches to mitigate Cr induced toxicity by decreasing its intake, improve chlorophyll contents and growth attributes in *Spinacia oleracea* L. Comparative to BC, foliar application of Fe is more efficacious to alleviate Cr toxicity due to antagonistic relation of Cr and Fe. However, more investigation is yet suggested to introduce optimum level of Fe foliar application to improve the growth of *Spinacia oleracea* L. in Cr toxic field conditions.

Conflict of interest: Authors declare no conflict of interest.

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