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GROWTH AND BIOCHEMICAL RESPONSES OF LADY'S FINGER (*ABELMOSCHUS ESCULENTS*) GROWN IN FLUORIDE CONTAMINATED SOIL AUGMENTED WITH BIOCHAR

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ABSTRACT: The effect of fluoride on the growth and biochemical parameters was examined under the biochar mixed soil. A pot experiment was conducted, in which 0 mg/L, 15 mg/L, 30 mg/L, 45 mg/L and 60 mg/L of fluoride concentrations and two levels of wheat straw biochar (WSBC) at the rate of 3% and 5% by (w/w) were supplied to the plants at time of sowing seed. Growth and biochemical parameters including plant biomass, leaf surface area, plant height, photosynthesis, proline, ascorbic acid, phenolic content, peroxidase activity, etc. were studied. After 60 days harvest, it was observed that fluoride produced significant reduction in all parameters (plant height, plant biomass and leaf surface area) of plant. Maximum reduction in case of growth parameters was noticed. It was calculated that 55% leaf surface area under 3% of biochar treatment was recorded while at 5% of biochar the reduction rate reduced up to 21% as compared to that of control treatment. Similarly photosynthetic rate, transpiration rate and stomatal conductance showed the reduction of 53%, 67% and 54%, respectively, at 3% of biochar while at 5% these reduced to 41%, 52%, 44% as compared to that of control at $P \leq 0.05$. Fluoride stress also increased the lipid peroxidase, phenolic content and proline but its rate of increase was lesser at 5% of biochar as compared to that of control. Overall, it was also observed that adverse effect of fluoride was lesser at 5% of biochar as compared to that of 3% of biochar, which indicated that intensity of fluoride severity, can be minimized by increasing concentration of biochar for getting better yield of crops and vegetables.

Keywords: Biochar, Fluoride, Growth, Photosynthesis

INTRODUCTION

Fluoride contamination is one of the most emerging problem for the soil, water and environmental aspects as it does not occur naturally in its state but have always been found as reactive substance in the earth crest. It is always present in both organic and inorganic forms like cryolite, fluorspar and apatite which is released in to the atmosphere frequently from brick klins, fertilizers producing plants and several other industrial processes.[1-3] In gaseous form hydrogen fluoride (HF) is considered as one of the most toxic substance which produces adverse impacts on the health of plants as it can enter very easily through the stomatal pores of plants and then damages the internal metabolic process of plants while sodium fluoride as solid form is found most phytotoxic pollutants through soil entry.[4] The main source of fluoride in the soil is due to excessive use of phosphoric fertilizers which can produce about 1.5% of fluorine to the soil every year and it contaminates due to leaching fluoride in the soil, which now a days has become a global issue as it is regarded as environmental pollutants which has produced adverse impacts on plants and animals.[5-6] In Pakistan,

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fluoride has contaminated the air, soil and ground water which in turn produced negative effects on the living organism in the form of interaction between organisms and fluoride in the environment. This negative interrelation of fluoride with living organisms have not only produced the negative impacts on plants or animals but have also damaged the livestock and human being on large scale.[7] Fluoride contamination of soil is primarily because of consumption of phosphorous fertilizers on agricultural land which contain less than 1% to more than 1.5% fluorine but the availability of fluorine to crop is dependent on the soil pH and content of minerals in the soil. The normal concentration of fluoride in soil ranges from 150–400 mg/kg. However, WHO provided permissible limit of fluoride for human intake is 1.5 ppm. Fluoride can also cause the surface contamination of soil when it is released from the industrial sectors, while brick kilns are the main source of hydrogen fluoride pollution which has produced several adverse effects on the crops and vegetation.[8] This rapid contamination of fluoride is needing serious monitoring for the sake of reducing the losses of crops and foods stuff. In this regards, several tools have been applied so far for reducing its impacts but most of them have been found useless for controlling the problem of fluoride contamination. In this study biochar has been applied to reduce the effects of fluoride on the lady's finger as biochar has been considered as one of the most uplifting agent in case of contamination due to different pollutants in the soil. Since last two decades, biochar is being frequently used because of owing potential of carbon sequestration, bioremediation, soil fertility and overall environmental management for agriculture purpose.[9-10] different research studies revealed the importance of biochar against the organic and inorganic pollutants in agriculture sector because of having high adsorption capacity.[11-14] used compost along with biochar and found significant increase in the chlorophyll contents, photosynthetic rate and carboxylation as compared to that of non-amended biochar soil. As biochar has capacity to reduce the impacts of toxicity of soil so it can be used for reclamation of soil contaminated by fluoride. The aim of this study was also to reduce the adverse effects of fluoride by using biochar as up till now, no any role of biochar in reducing soil contamination by fluoride has been reported although several role of biochar in reducing toxicity of soil has been found extensively by many authors.

MATERIALS AND METHODS

A pot experiment was conducted to examine the effect of fluoride present in the soil on the growth and biochemical attributes of lady's finger by using wheat straw synthesized biochar. In this regards, a completely randomized block design trail experiment was carried out in which sodium fluoride (NaF) was used as source of fluoride while wheat straw biochar (WSBC) synthesized at 600ºC by using vertical-silo kiln-type reactor. Stock solution of sodium fluoride was prepared for five treatments including 0 mg/L, 15 mg/L, 30 mg/L, 45 mg/L and 60 mg/L of fluoride concentrations and two levels of biochar at the rate of 3% and 5% by (w/w) were supplied respectively against each treatment. The treatments plan was designed as BC1F0, BC1F1, BC1F2, BC1F3, BC1F4 and BC2F0, BC2F1, BC2F2, BC2F3, BC2F4 in which biochar at the rate of 3% and 5% was also used respectively with each concentration of 0 mg/L to 60 mg/L of fluoride in which 0 mg/L was kept as controlled for its comparison with rest of fluoride and biochar treatments. Certified seeds of lady's finger were sown in clay pot containing eight kg of loamy soil mixed with two levels of biochar. Each pot was supplied with distilled water on each day to keep the water holding of soil up to 50% while fluoride treatments were done once in week. After sixty days of treatments, mature plants were harvested to examine the growth and biochemical activity of plants under controlled conditions. Growth parameters including plant height, plant biomass and leaf surface area was measured by using the method of Patil and Ayolagha.[15-16] Biochemical parameters including photosynthetic rate, transpiration rate (E) and stomatal conductance (CO_2) were observed by using potable infrared gas analyzer (IRGA) of Analytical Development Company, Hoddeson, England. Chlorophyll a and b were measured by following the method of Arnon, while protein was assessed by the Bradford method, and Ascorbic acid and carotenoid content were measured by method of Keller and Krick.[18] For the measurement of proline content, the method of Bates was followed while total phenol contents were calculated by the method of Bray.[17-22] Lipid peroxidase was observed by the following the method given by Heath and peroxidase activity was measured by the method of Fahey. [23-24] All the data was statistically analyzed with the help of software "Statistics" and MS Excel 2010 by using Two Way Analysis of Variance (ANOVA).

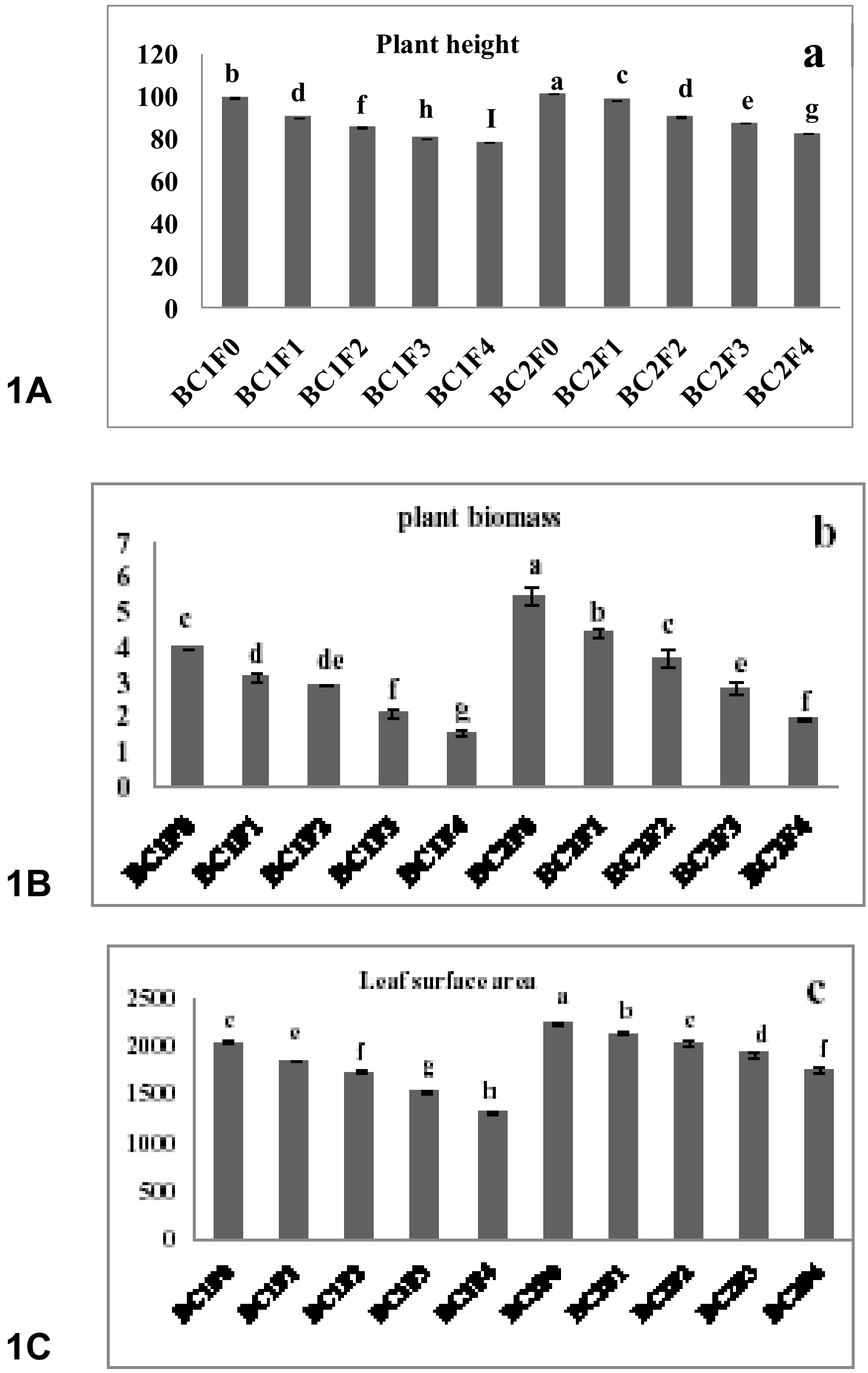
RESULTS AND DISCUSSION

The results have shown that there was significant effect of fluoride on growth and biochemical attributes of lady finger plants but at the same time biochar also expressed enhancing effect in all parameters of plants showing that biochar had ability to uplift the adverse effect of fluoride on plants. As shown in the Figure 1 (a, b, c) the fluoride produced adverse effect on the leaf surface area, total plant biomass and plant height as under treatment F1 these decreased by 10%, 21% and 9.3% respectively as compared to that of control at $P \leq 0.05$ in the presence of 3% of biochar. Similarly, highest reduction was also noted under treatment F4, which showed reduction by 55%, 61% and 0.21% in leaf surface area, plant biomass and plant height respectively. While in the presence of 5% of biochar the maximum reduction in the leaf surface area was recorded 21%, 64% in plant biomass and 1.8% in plant height as compared to that of control at $P \leq 0.05$.

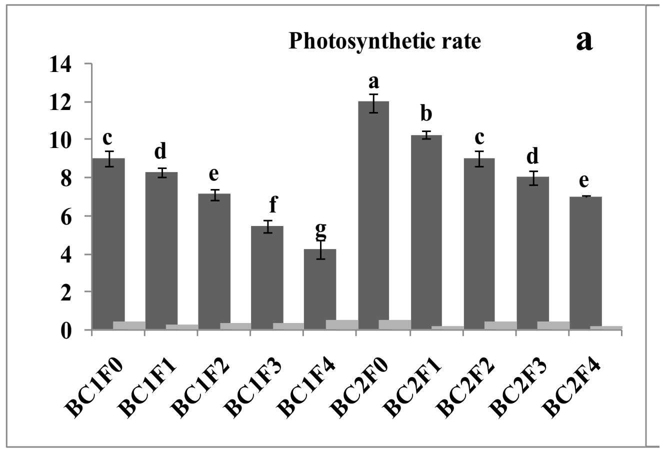
The trend of reduction in growth parameters was found lesser at 5% of biochar as compared to that of 3% in the soil. In case of photosynthetic parameters maximum reduction recorded in case of photosynthetic rate was 53% while stomatal conductance was reduced to 67%, transpiration rate 54% and chlorophyll a & b were found reduced to 46% and 68% respectively under fluoride stress in the presence of 3% of biochar as compared to that of control at $P \leq 0.05$. While slight relieving effect was observed in these parameters under the 5% level of biochar which showed maximum decrease of 41%, 52%, 44%, 38% and 53% respectively showing that 5% biochar had the capacity of neutralizing the impact of fluoride comparatively lesser than control as well as compared to that of 3% of biochar at $P \leq 0.05$ as shown in Figure 2 (a, b, c, d, e,). The response of biochemical parameters shown in Figure 3 (a, b, c, d, e) was also found similar under the effect of fluoride, maximum reduction recorded in carotenoid content was 65.45%, protein content 52.74%, ascorbic acid 68.53% while total phenol contents were increased 59.71% under the activity of 3%

of biochar while in case of 5% of biochar less reduction was noted in all biochemical parameters e.g. carotenoid content 59.6%, protein content 46.4% and ascorbic acid 67% while total phenol content increased slightly 45.45% as compared to that of control at $P \leq 0.05$. Under the effect of fluoride, increase in lipid peroxidase was recorded 201% in 3% of biochar concentration while only 108% increase was observed in lipid peroxidase in the presence of 5% of biochar, similar results were also obtained in case of peroxidase activity in which increase under 3% of biochar was 81.9% as compared to that of control treatment at $P \leq 0.05$ while surprisingly under 5% of biochar peroxidase activity decreased to 6.9% as compared to that of control as well as 3% of biochar at $P \leq 0.05$ as shown in Figure 4(a,b). Overall, it was observed that in case of all parameters of plants the activity of 3% of biochar was found lesser than 5% of biochar with significant differences in all treatments as compared to that of control at $P \leq 0.05$ (Tables.1A and 1B).

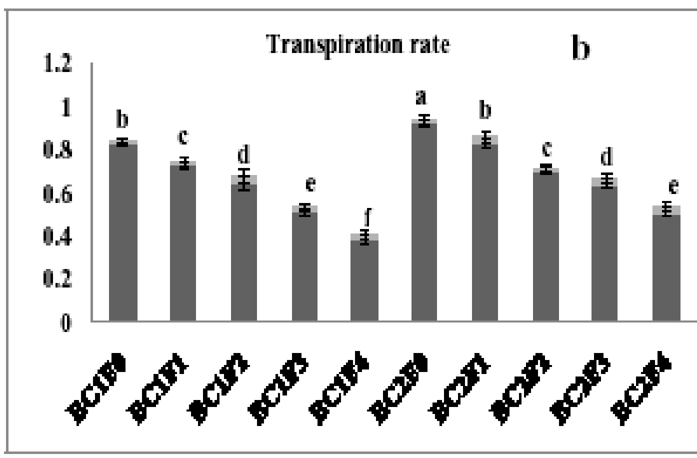
The study showed that fluoride produced prominent effect on the growth, photosynthetic as well as biochemical attributes of lady's finger in the presence of both levels of biochar which is in line of findings of Pelc et al. showing reduction in the growth pattern of wheat crop and found prominent decrease in root, shoot as well as biomass of the plants.[25] But the results of research conducted by Sanjay et al. showed no morphological changes in cotton under maximum of 1000 ppm of fluoride concentration.[26] At the same time at this concentration photosynthetic activity was found reduced, which might be due to nature and duration of exposure. Several authors also noticed similar pattern of changes in the form of reduction in all growth, photosynthetic and biochemical attributes of the plants which correlates with the present findings.[27] One of the most concerned phenomena with fluoride exposure to plants is based on its frequent availability in the soil and water with its strong residing ability in the roots, shoots and leaves of plants. The authors reported the effect of fluoride contaminated soil on the yield and growth of some plants by obtaining results with reduction in growth and biochemical attributes of plants.[28-30] The role of biochar has also been found very remarkable in this study because of its potential to detoxify the soil contaminants along with its positive effect on the physiological, biochemical and photosynthetic parameters of plants under the stress of selenium.[31] In this study, it has been observed that due to fluoride there was decrease in all parameters of plants along with increase in the peroxidase activity, lipid peroxides and phenol content but at 5% treatment of biochar, the degree of decrease or increase in the plant parameters were suppressed which seemed to be very similar with the study of in which it was observed that there was decrease in all above discussed parameters of cabbage and lettuce under the influence of Cd but rice husk was found helpful in reducing the adverse impacts of copper.[32] Many authors have investigated the positive role of biochar for decreasing the toxicity of various heavy metals by increasing yield and growth of some vegetables. [10,32-34]



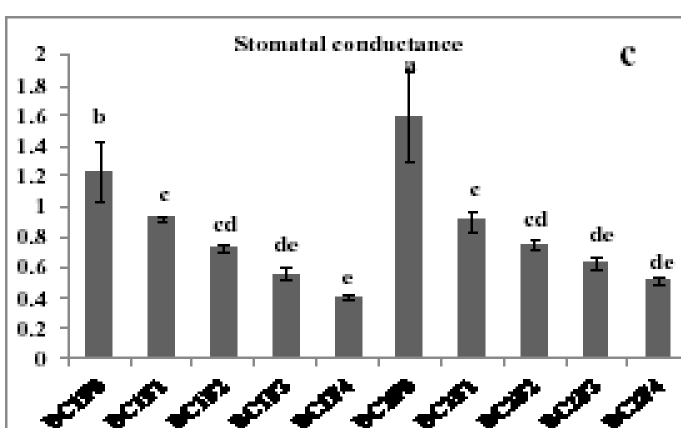
Figures 1A–1C. Effect of fluoride and biochar on:
1A: plant height (cm)
1B: plant biomass (g/plant)
1C: leaf surface area (cm^2)



2A



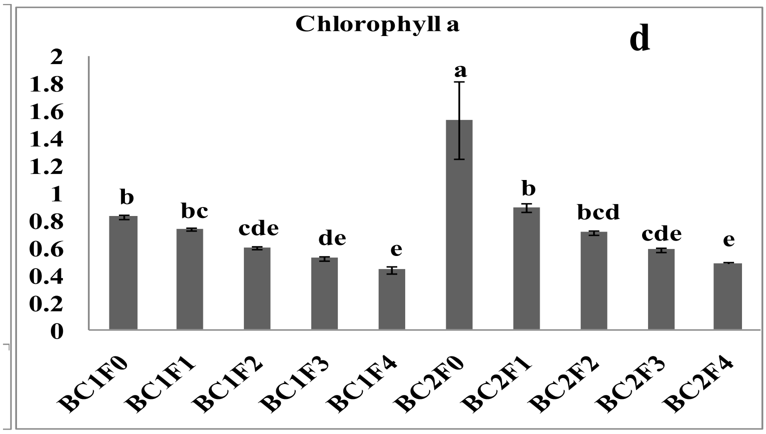
2B



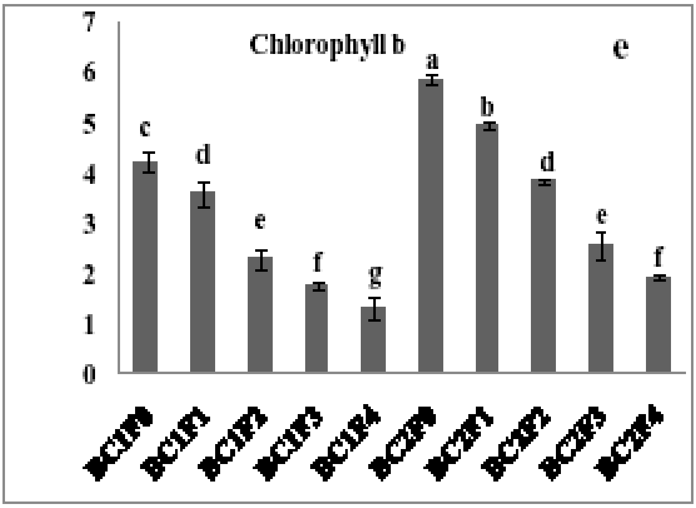
2C

Figures 2A–2C. Effect of fluoride and biochar on:
2A: Photosynthetic rate (μmole (CO_2) m^2s^{-1}
2B: Transpiration rate (mmole m^2s^{-1})
2C: Stomatal conductance (m (H_2O) m^2s^{-1}

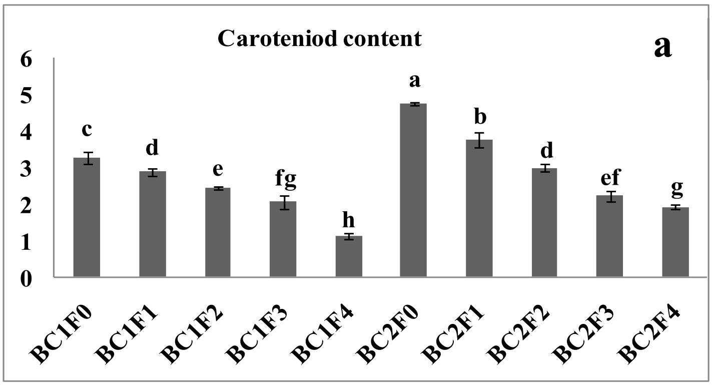
2D



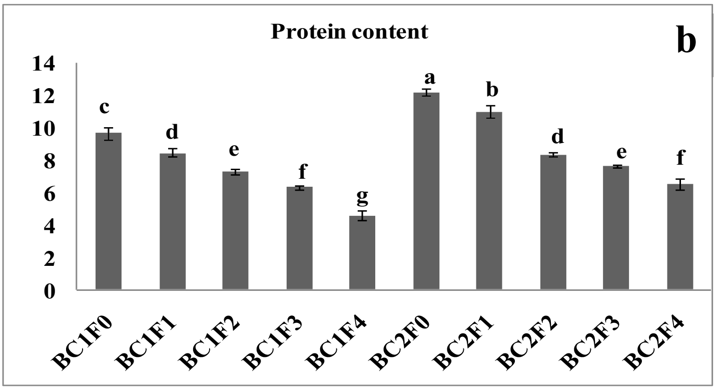
2E



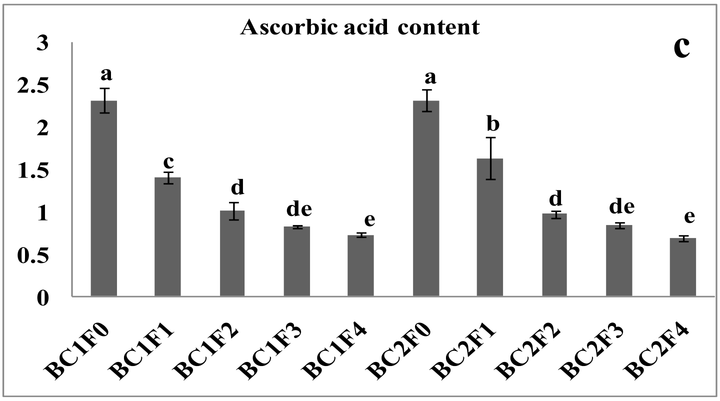
Figures 2D and 2E. Effect of fluoride and biochar on:
2D: Chlorophyll a (mg/g dw)
2E: Chlorophyll b (mg/g dw
(dw = dry weight)
Groups from left:
BC1F0
BC1F1
BC!F2
BC1F3
BC1F4
BC2F0
BS2F1
BC2F2
BC2F3
BC2F4



3A



3B



3C

Figures 3A–3C. Effect of fluoride and biochar on:
3A: Carotenoid content (mg g^{-1} dw)
3B: Protein content (mg g^{-1} dw)
3C: Ascorbic acid (mg g^{-1} fw) \
(dw = dry weight; fw = fresh weight)

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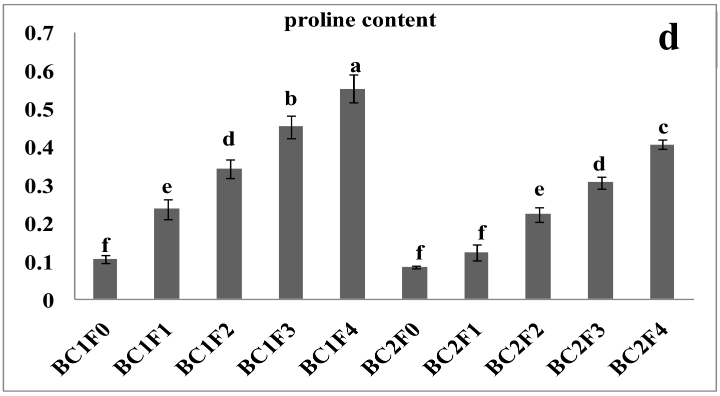
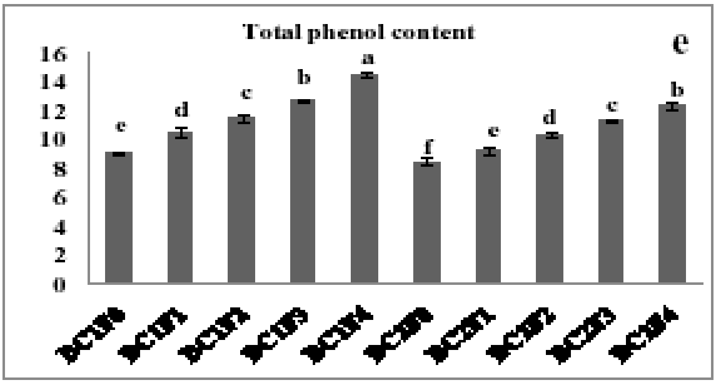
3D



3E



Figures 3A–3C. Effect of fluoride and biochar on:
3D: Chlorophyll a (mg/g dw)
3E: Chlorophyll b (mg/g dw)

4A

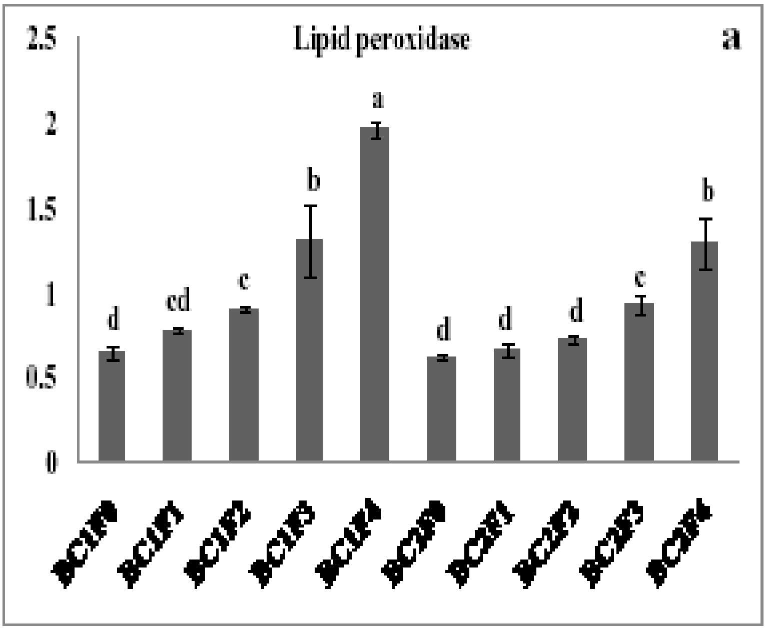


Figure 4A. Effect of fluoride and biochar on: 4A: Lipid peroxidase (nM MDA mL^{-1}).

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4B

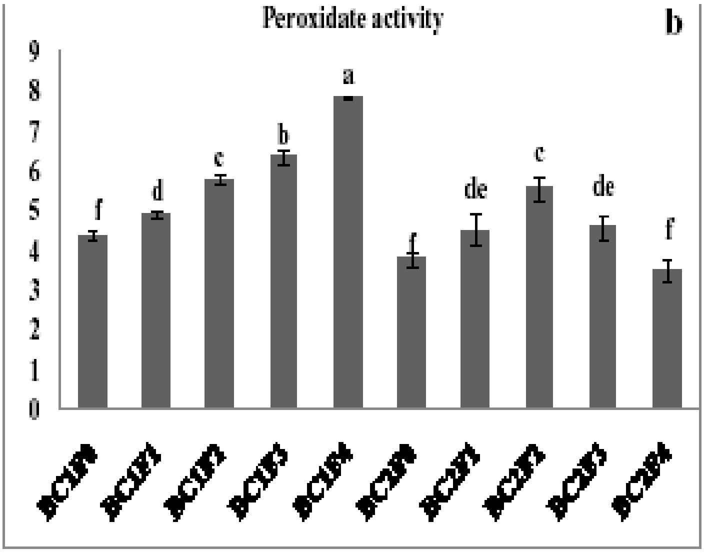

Figure 4B. Effect of fluoride and biochar on: 4B: Peroxidase activity (IM $min^{-1}g^{-1}$ fw)

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Table 1A. Comparative effect of biochar at 3% (w/w) on the growth, photosynthetic and biochemical attributes of lady fingers grown in contaminated soil (Mean±S.E) at P≤0.05

Parameters	Biochar at 3% (w/w)				
	F0	F1	F2	F3	F4
Leaf surface area (cm^2/ plant)	2050 ±2.6c	1855 ±5.6e	1729.66 ±4.6f	1541.33 ±7.4g	1320 ±2.6h
Total plant biomass (g $plant^{-1}$)	3.95 ±0.01c	3.12 ±0.03d	2.90 ±0.01de	2.13 ±0.04f	1.51 ±0.02g
Plant height (cm $plant^{-1}$)	99.5 ±0.06b	90.2 ±0.02d	85.3 ±0.04f	80.2 ±0.03h	78.3 ±0.02i
Stomatal conductance (mH_2Om^{-2} s^{-1})	1.23 ±0.15b	0.92 ±0.02e	0.72 ±0.02cd	0.56 ±0.03de	0.40 ±0.01e
Photosynthesis rate µmole(CO_2) $m^{2\ s-1}$	9 ±0.13c	8.26 ±0.08d	7.10 ±0.09e	5.43 ±0.09f	4.22 ±0.15g
Transpiration rate (mmole m^{-2} s^{-1})	0.83 ±0.01b	0.73 ±0.01c	0.64 ±0.02d	0.51 ±0.01e	0.38 ±0.02f
Chlorophyll a (mg g^{-1} dw)	0.83 ±0.01a	0.74 ±0.004bc	0.60 ±0.003cde	0.52 ±0.01de	0.44 ±0.01e
Chlorophyll b (mg g^{-1} dw)	4.21 ±0.07c	3.57 ±0.08d	2.26 ±0.06e	1.76 ±0.02f	1.31 ±0.07g
Carotenoid content (mg g^{-1} dw)	3.28 ±0.05c	2.88 ±0.03d	2.44 ±0.02e	2.05 ±0.06fg	1.13 ±0.03h
Lipid peroxidation (nM MDA mL^{-1})	0.65 ±0.01d	0.77 ±0.01cd	0.90 ±0.00e	1.30 ±0.07b	1.96 ±0.01a
Protein content (mg g^{-1} dw)	9.67 ±0.12c	8.46 ±0.08d	7.27 ±0.05e	6.35 ±0.04f	4.57 ±0.10g
Proline content (mg g^{-1} fw)	0.11 ±0.003f	0.24 ±0.008e	0.34 ±0.008d	0.45 ±0.009b	0.55 ±0.012a
Total phenol content (mg g^{-1} fw)	9.06 ±0.03e	10.53 ±0.09d	11.50 ±0.08c	12.68 ±0.04b	14.47 ±0.06a
Ascorbic acid content (mg g^{-1} fw)	2.32 ±0.05a	1.40 ±0.02c	1.02 ±0.03d	0.83 ±0.01de	0.73 ±0.01e
Peroxidase activity (IM $min^{-1}g^{-1}$ fw)	4.34 ±0.04f	4.91 ±0.03d	5.77 ±0.04c	6.33 ±0.05b	7.87 ±0.01a

Table 1B. Comparative effect of biochar at 5% (w/w) on the growth, photosynthetic and biochemical attributes of lady fingers grown in contaminated soil (Mean±S.E) at P≤0.05

Parameters	Biochar at 5% (w/w)				
	F0	F1	F2	F3	F4
Leaf surface area (cm^2/ plant)	2240 ±3.4a	2138.33 ±6.1b	2045.33 ±8.0c	1923.66 ±6.3d	1763.33 ±7.9f
Total plant biomass (g $plant^{-1}$)	5.47 ±0.09a	4.41 ±0.04b	3.68 ±0.09c	2.81 ±0.05e	1.96 ±0.01f
Plant height (cm $plant^{-1}$)	101.4 ±0.0a	98.3 ±0.05c	90.4 ±0.09d	87.4 ±0.07e	82.5 ±0.09g
Stomatal conductance ($mH_2Om^{-2} s^{-1}$)	1.60 ±0.21a	0.90 ±0.04c	0.75 ±0.02cd	0.63 ±0.03de	0.52 ±0.02de
Photosynthesis rate µmole(CO_2) $m^{2\ s-1}$	11.94 ±0.15a	10.24 ±0.06b	9.01 ±0.12c	8.00 ±0.12d	6.95 ±0.04c
Transpiration rate (mmole $m^{-2} s^{-1}$)	0.92 ±0.01a	0.83 ±0.02b	0.7 ±0.01c	0.64 ±0.02d	0.51 ±0.02e
Chlorophyll a (mg g^{-1} dw)	1.53 ±0.09a	0.89 ±0.01b	0.71 ±0.01bcd	0.59 ±0.00cde	0.48 ±0.004e
Chlorophyll b (mg g^{-1} dw)	5.86 ±0.03a	4.91 ±0.02b	3.83 ±0.02d	2.54 ±0.08e	1.90 ±0.01f
Carotenoid content (mg g^{-1} dw)	4.75 ±0.02a	3.78 ±0.07b	3.00 ±0.03d	2.22 ±0.05ef	1.92 ±0.02g
Lipid peroxidation (nM MDA mL^{-1})	0.62 ±0.004d	0.66 ±0.01d	0.72 ±0.01d	0.93 ±0.02c	1.29 ±0.05b
Protein content (mg g^{-1} dw)	12.20 ±0.07a	11.00 ±0.13b	8.33 ±0.04d	7.62 ±0.03e	6.54 ±0.11f
Proline content (mg g^{-1} fw)	0.09 ±0.001f	0.12 ±0.006f	0.22 ±0.006e	0.31 ±0.005d	0.41 ±0.004c
Total phenol content (mg g^{-1} fw)	8.47 ±0.06f	9.21 ±0.07e	10.36 ±0.05d	11.26 ±0.04c	12.32 ±0.10b
Ascorbic acid content (mg g^{-1} fw)	2.32 ±0.04a	1.63 ±0.08b	0.97 ±0.01d	0.84 ±0.01de	0.69 ±0.01e
Peroxidase activity (IM $min^{-1}g^{-1}$ fw)	3.73 ±0.05f	4.50 ±0.13de	5.53 ±0.09c	4.57 ±0.10de	3.47 ±0.09f

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CONCLUSIONS

Being agricultural based country Pakistan is facing biggest challenge for crop production due to effects of pollutants on crop's yield that decrease due to the toxic effect of pollutant like fluoride which cause reduction in growth and production of crop particularly in vicinity of kiln area. It has been concluded from the present research study that wheat straw biochar played important role in reducing toxic effect of fluoride. Though, fluoride produced negative effects on the growth and physiology of lady's finger but its effects was significantly reduced as the concentration of biochar was increased in soil. So present study not only revealed the effect of fluoride toxicity on lady finger yield but also shown that intensity of fluoride toxicity was found lesser at higher level of biochar in soil.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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