EFFECT OF WHEAT STRAW BIOCHAR ON THE GROWTH OF FENUGREEK (TRIGONELLA FOENUM-GRAECUM L.) IN CADMIUM SPIKED SOIL

Tanveer M.a, Shaikh I.A.a, Irfan M.a, Noor H.a and Farooq U.b

a. College of Earth and Environmental Sciences, University of the Punjab, Lahore-Pakistan.
   b. Department of Chemistry, University of Education, Lahore-Pakistan.

Corresponding Author’s Email: muhadinfan6167@gmail.com

Received: 04th Sep. 2019 Revised: 14th Sep. 2019 Accepted: 30th Sep. 2019

Abstract: Cadmium (Cd), from different sources such as wastewater, fertilizers and pesticides, alters the soil microbial community, physical and biochemical properties. It can also accumulate in plant tissues, reduce the plant growth and yield, and cause chlorosis in leaves. In the current study, the effect of the wheat straw biochar on the growth and yield of fenugreek was observed in Cadmium contaminated soil using Cadmium chloride (60 mg/kg). Two treatment levels with varying concentrations of biochar (2.5% and 5% w/w) were prepared along with the control group (no biochar) and replicated three times. Each experiment was conducted in a plastic pot with 30 fenugreek seeds sown in 5-kilogram soil (dry weight). NPK fertilizer (60-80-40 kg/ha) was applied after 30 days of sowing and the number of plants was reduced to 10 plants in each pot. The plants were harvested after 90 days of planting and different growth parameters were measured. It was observed that the addition of biochar significantly increased the germination rate and growth of the plants. Moreover, the accumulation of Cd in different parts of the plants was reduced with increase in biochar concentration.

Keywords: Biochar; Cadmium; Fenugreek; Growth; Wheat straw biochar; Yield.

Postal Address: College of Earth and Environmental Sciences, University of the Punjab, Lahore-Pakistan, 54500. Tel.: +92-300-7806683

INTRODUCTION

The anthropogenic and natural sources contribute toxic elements in the agricultural soils which affect the plants growth and yield. The most toxic elements are heavy metals e.g. Cadmium (Cd), Chromium (Cr), Lead (Pb) due to their non-biodegradable, persistent and toxic nature (Rehman et al., 2018). Pesticides, fertilizers, sewage sludge, solid waste disposal and waste water are the potential sources of these heavy metals into the agricultural soil. Waste water irrigation for a long period of time saturates the soil with heavy metals (Khan et al., 2014). At the same time, when Cadmium contributes to osteoporosis, Itai-Itai disease and cardiovascular diseases in human, it is also considered toxic and hazardous for the soil environment and plants. Its high concentration in the soil (>1000 mg/kg) disturbs the soil respiration, nitrogen fixation and organic matter decomposition (Johansen et al., 2018). Previous studies showed that Cadmium reduced the growth and yield of wheat (Triticum aestivum), rice (Oryza sativa), maize (Zea mays), radish (Raphanus raphanistrum), cucumber (Cucumis sativus), pepper (Capsicum Sp.), potato (Solanum tuberosum), cabbage (Brassica oleracea), spinach (Spinacia oleracea), lettuce (Lactuca sativa) and in many other vegetables and caused chlorosis in leaves (Abdelgani et al., 1999; Prades, 2000; Moradi, 2013; Ghosh and Biswas, 2017; Sanderson et al., 2019). Cadmium affects the plant morphology and physiochemical processes including the stomata conductance (Sanderson et al., 2019).

Fenugreek (Trigonella foenum-graecum L.) is a winter seasoned, herbaceous, leguminous and self-pollinated crop commonly known as
kasuri methi in Kasur, Pakistan. Being a diploid plant, it has 16 chromosomes and an average height of 25-60 cm. It is native to central Asia and mainly grown in India, Pakistan, China, Turkey, Egypt, Ethiopia, Greece, Morocco and Ukraine. Its seeds and leaves are rich in vitamins and minerals and its cultivation improves the soil nitrogen due to nitrogen fixation from the atmosphere up to 48% (Ratnakar and Rai, 2013; Seghatoleslami et al., 2013; Khan et al., 2014). Leaves of fenugreek are used as vegetable, seeds as feed for animals and as food for humans. The plant is also used for the treatment of diabetes, chronic dysentery and diarrhea while the seeds are considered to be anti-inflammatory, anti-cancerous and anti-microbial (Kausar et al., 2017). In ancient times, fenugreek was used for the preservation of dead bodies and removal of toxic substances from the blood. It also helps in losing weight, improving the memory, strengthening the hairs and solving the skin problems in human. Constipation, emphysema, hay fever and influenza can also be cured using its leaves and seeds (Pradesh, 2000; Soughir et al., 2013; Alaraidh et al., 2018). A number of heavy metals were found to be adversely affecting the growth and yield of fenugreek. For example, Copper (Cu) reduced the radical length, germination rate, fresh root mass, root length, lateral roots numbers, \( \text{H}_2\text{O}_2 \) production, chlorophyll and carotenoid contents (Elleuch et al., 2013). Similarly, Cd, Cr and Pb reduced the germination rate, root and shoot length, fresh and dry weight of fenugreek grown in Saudi Arabia. Heavy metals also altered the gene expression in this plant (Alaraidh et al., 2018). Cd in sandy loam reduced fresh and dry weight of fenugreek and bioaccumulation in the roots, shoots and leaves was significantly increased in the contaminated soil (Bashri and Prasad, 2016). During an experiment in Ethiopia, it was observed that the bioaccumulation of toxic, trace and major elements takes place in the plant. The most accumulated heavy metals were Cd (285 mg/kg), Pb (615 mg/kg), Cr (3-552 mg/kg), Ni (31-108 mg/kg), Fe (6041-18584 mg/kg), Zn (15-33 mg/kg) and Co (4-15 mg/kg) (Hagos and Chanravanshi, 2016).

Biochar is a stable black solid produced by pyrolysis of biomass that can lift soil fertility, advance the soil pH, improve the moisture holding capacity, preserve nutrients for a long period and promote the advantageous fungi and microbes in soil. Large surface area and negatively charged surface of biochar help in the sorption of contaminants and pollutants (Wu et al., 2012; Hu et al., 2014). The elemental composition of biochar contains four major plant growth promoting elements i.e. Nitrogen, Phosphorus, Potassium and Carbon. Due to high carbon content, large surface area and porosity, biochar can hold large amount of pesticides. Biochar controls acidity, upturns the soil cation exchange capacity, develops the soil fertility and immobilizes heavy metals. By reducing bio-availability of heavy metals, biochar increases the plant growth and yield. The main objective of the study was to measure the influence of wheat straw biochar on the growth and yield of fenugreek in cadmium contaminated soil (Ali et al., 2015; Svoboda et al., 2017; Chen et al., 2018).

**EXPERIMENTAL**

**Site Description**

This study was conducted under the environmental conditions of College of Earth and Environmental Sciences, University of the Punjab, Lahore-Pakistan at an average altitude of 213 m from the sea level (Figure 1). The mean temperature of the site ranges from 25 to 33°C with an average relative humidity of 55 to 67% and rain fall of about 628.8 mm/month (Shah and Ahmed, 2017).
Muhammad et al., 2019; Effect of wheat straw biochar on the growth of fenugreek (Trigonella Foenum-Graecum L.) in Cadmium spiked soil

Figure 1. Study Area

Soil Collection and Preparation
The soil was collected from the agricultural land located around College of Earth and Environmental Sciences from a depth of 0-30 cm. It was assured that there was no use of any fertilizer or pesticide on the sampling site within the past 5 years. After sampling, stones were removed by hand, the soil was air dried and sieved through 2 mm sieve at room temperature (25°C). The soil was contaminated by using Cadmium chloride salt (CdCl₂·H₂O) at the basic rate of 60 mg/kg dry soil. After the addition of biochar in the defined amount, the contaminated soil was added into the plastic pots (20 cm wide at top and 25 cm in length), covered with plastic sheets and was kept undisturbed in the field conditions for three months (September to November, 2018).

Soil Analysis
Soil saturate paste was prepared by dissolving 200 g of dry soil in distilled water. One drop of hexametaphosphate solution as dispersing agent was added and it was kept overnight. Soil saturated paste percentage was measured by following formula (US Salinity Lab, 1954):

\[
\text{Saturated Paste Percentage} = \frac{\text{Loss in Mass of Saturated Paste}}{\text{Oven Dried Soil Mass}} \times 100
\]

pH of the saturated paste was measured by pH meter (Hannah, HI 8424) and EC of soil extract was measured by EC meter (Hannah, HI 9811-5). Hydrometer method (Bouyoucos, 1962) was used to estimate the soil textural class of 50 g soil following the international textural class. Walkey-Black method was used for the estimation of organic carbons contents of the soil (Walkley and Black, 1934). Different properties of the sampled soil are given in Table 1.

Wheat Straw Biochar (WSB)
The wheat straw biochar was purchased from the local market in Lahore, Pakistan which was produced at 500°C to 900°C temperature by double container kiln method. The pH, EC and total carbon contents were almost same as described in (Zama et al., 2018).

Experimental Design and Plant Growth
Three groups (one control and two treatments) with three replications each were prepared for the study. Biochar was added into the experimental groups at a rate of 2.5% and 5% w/w, respectively while the control consisted of contaminated soil with no biochar. Seeds of fenugreek were purchased from the local market in Lahore, Pakistan, packed by an Indian


132
Muhammad et al., 2019; Effect of wheat straw biochar on the growth of fenugreek (Trigonella Foenum-Graecum L.) in Cadmium spiked soil company moon star under the variety name of sortex clean. Initially, 30 seeds were sown in each pot but only 10 plants were kept after 30 days of sowing. Nitrogen, Phosphorus and Potassium (N, P, K) were applied in the form of fertilizer at a basic rate of 60, 80 and 40 kg/ha, respectively and the crop was harvested after 90 days.

Table 1. Basic Physical and Chemical Properties of Soil

<table>
<thead>
<tr>
<th>Soil properties</th>
<th>Units</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texture class</td>
<td>-</td>
<td>Sandy loam</td>
</tr>
<tr>
<td>Sand</td>
<td>%</td>
<td>66.70</td>
</tr>
<tr>
<td>Silt</td>
<td>%</td>
<td>15.40</td>
</tr>
<tr>
<td>Clay</td>
<td>%</td>
<td>17.90</td>
</tr>
<tr>
<td>SP</td>
<td>%</td>
<td>30.85</td>
</tr>
<tr>
<td>pHs</td>
<td>-</td>
<td>7.78</td>
</tr>
<tr>
<td>EC</td>
<td>dSm⁻¹</td>
<td>1.96</td>
</tr>
<tr>
<td>OM</td>
<td>%</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Measurement of Growth and Yield Parameters

Seeds germination potential was measured by the following formula developed by Association of Official Seeds Analyst (AOSA, 1990):

\[
\text{Final Germination (\%)} = \frac{\text{Total Seeds Germinated}}{\text{Total Seeds Planted}} \times 100
\]

Number of leaflets, branches and lateral roots for each plant were counted manually. Shoot and root lengths were measured using a centimeter scale while grid counting method was exercised for the calculation of leaf area (Chaudhary et al., 2012). Vernier caliper (precision: 0.05mm) was used to measure the stem and root diameter (Khan et al., 2014). An analytical balance (OEM, 3003D) was used to measure the fresh and dry weight of roots and shoots. Harvest index (%) was estimated by method used in (Chen et al., 2018) by formula as described below:

\[
\text{Harvest Index (\%)} = \frac{\text{Fresh Leaves Weight per Pot}}{\text{Total Biomass Weight per Pot}} \times 100
\]

Water contents of leaves was calculated by using the following formula (Barrs and Wheatherley, 1962).

\[
\text{Leaves Water Content (\%)} = \frac{\text{Fresh Weight – Dry weight}}{\text{Turgid Weight – Dry Weight}} \times 100
\]

Determinaton of Cadmium

The Cadmium concentration in shoots, roots and leaves of fenugreek was measured on Atomic Absorption Spectrophotometer using WinLab-32 software. Plant sample (0.5 g) was dissolved in 5 ml of di-acid digestion mixture (HNO₃ and HClO₄ by ratio 2:1). After digestion, sample was increased to 25 ml by adding distilled water. The solution was then used to measure Cadmium concentration (AOAC, 1990; Rehman et al., 2018).

RESULTS AND DISCUSSION

Effect on Plant Growth Parameters

Results of different plant growth parameters are given in the table 2. Application of wheat straw biochar (5% w/w) significantly increased the germination rate, number of leaves, branches, lateral roots, stem diameter, root diameter, leaf area, root length, shoot length, and dry weights of roots, shoots and leaves. The results of current study can be justified by many previously published studies. Rehman et al. (2018) found that Cadmium significantly reduced the germination percentage in wheat whereas organic amendments such as rice husk biochar significantly increased the germination percentage. Combined with farmyard manure, Rice husk biochar favored the root and shoot length, dry weight, productive tillers and grain yield in Cadmium spiked soil and similar results were also observed by our study. Biochar significantly reduced the uptake of cadmium in leaves, shoots and roots of wheat grown in Faisalabad-Pakistan (Rehman et al., 2018). Tan et al. (2018) concluded that rice husk biochar significantly increased the germination percentage, plant height, root length, shoot length and grains per spike in wheat grown in
Wheat straw biochar improved the soil quality and carbon content for long time (Toole et al., 2013).

In another experiment, wood biochar reduced the cadmium level in tissues of tomato plant and improved the soil nutrients, and microbial activities in psammanquent and plinthudult soils of China (Muhammad et al., 2017). Hu et al. (2014) reported that wheat straw biochar increased the soil carbon sequestration, NO$_3$-N concentrations, soil gross nitrification rate, soil C/N ratio and reduced the emissions of CO$_2$ and N$_2$O. Biochar changed the soil carbon and hydrogen cycling processes in the black chernozem soil of Canada. Ali et al. (2015) presented that biochar enhanced wheat growth, preserved long term fertility of soil, increased spikes, grains / spike, 1000 grain weight, grain yield, soil potassium, soil phosphorus, soil carbon, spike, grain phosphorus uptake and phosphorus use efficiency. Kavitha et al. (2018) concluded from previous studies in a review articles that biochar improved crop yield as a fertilizer and conditioner. In plants, Biochar improved the germination, yield, root development, biomass yield, sucrose contents, biomass, growth and nodulation development, nutrient cycling, efficiency of nitrogen, water use sufficiency and reduced the uptake of heavy metals in plants because of adsorption of metals to negative charges on the surface of biochar particles. The surface area of biochar helps in immobilization of heavy metals such as Cd, Pb, Cu and Zn and in this way decreased the uptake of heavy metals in the plant parts.

Thu et al. (2017) described that Bamboo biochar increased the fresh weight, dry weight, total sugar contents, diameter and length in sweet potato, carrot and radish grown in Japan. Turan et al., (2018) reported that the different levels of biochar increased the plant height, shoot dry weight, root dry weight, fruit dry weight, superoxide dismutase, carbohydrates contents, total phenolic contents, catalase, peroxidase, ascorbic acid, protein contents, fat contents, fiber contents and decreased the uptake of heavy metals such as Nickle (Ni) in fruits, Cadmium (Cd) in shoots and fruits, Cobalt (Co) in shoots, Chromium (Cr) in shoots and fruits, and Iron (Fe) in shoots in Brinjal plant (Solanum melongena) grown in Faisalabad, Pakistan. In China, bamboo biochar (normal and Silicon modified) increased dry biomass and reduced uptake of arsenic in roots and shoots of Spinach grown in arsenic contaminated soil (Zama et al., 2018).

### Table 2: Effect of WSB on Different Growth Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatments</th>
<th>Percentage increase (T$_3$ over T$_1$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (T$_1$)</td>
<td>Cd+ 2.5% WSB (T$_2$)</td>
</tr>
<tr>
<td>Germination Rate (%)</td>
<td>53.33</td>
<td>76.67</td>
</tr>
<tr>
<td>Leaf Area (cm$^2$)</td>
<td>49</td>
<td>112</td>
</tr>
<tr>
<td>Shoot Length (cm)</td>
<td>25.83</td>
<td>32.57</td>
</tr>
<tr>
<td>Root Length (cm)</td>
<td>5.68</td>
<td>8.37</td>
</tr>
<tr>
<td>Shoot Dry Weight (g)</td>
<td>3.67</td>
<td>9.8</td>
</tr>
<tr>
<td>Root Dry Weight (g)</td>
<td>0.27</td>
<td>0.8</td>
</tr>
<tr>
<td>Leaves Dry Weight (g)</td>
<td>1.6</td>
<td>4.25</td>
</tr>
<tr>
<td>Leaves Water Content (%)</td>
<td>81.79</td>
<td>81.96</td>
</tr>
<tr>
<td>No. of branches</td>
<td>4.5</td>
<td>6.27</td>
</tr>
<tr>
<td>No. of leaves</td>
<td>25.7</td>
<td>41.7</td>
</tr>
<tr>
<td>No. of lateral roots</td>
<td>8.47</td>
<td>15.07</td>
</tr>
<tr>
<td>Stem Diameter (mm)</td>
<td>1.65</td>
<td>2.4</td>
</tr>
<tr>
<td>Root Diameter (mm)</td>
<td>1.32</td>
<td>2.41</td>
</tr>
<tr>
<td>Harvest Index (%)</td>
<td>39.13</td>
<td>41.29</td>
</tr>
</tbody>
</table>

Harvest Index and leaves’ Water Content
Wheat straw biochar non-significantly encouraged the harvest index and leaves water contents. The maximum harvest and water
Muhammad et al., 2019; Effect of wheat straw biochar on the growth of fenugreek (Trigonella Foenum-Graecum L.) in Cadmium spiked soil

contents were observed in biochar modified pots and minimum in cadmium contaminated pots. The increasing order of both harvest index and water contents was T1 > T2 > T3. Similar results were also observed by Rehman et al. (2018) in wheat in soil of Faisalabad, Pakistan.

Effect of WSB on Bioaccumulation of Cadmium in Roots, Shoots and Leaves

Cadmium concentrations in roots, shoots and leaves are shown in figure 2. Higher cadmium concentrations were observed in roots as compared to shoots and leaves. The decreasing order of cadmium concentrations was roots > shoots > leaves. Wheat straw biochar modified soils significantly reduced the cadmium in roots, shoots and leaves at 5% level of significance over control (T1). The decreasing order of cadmium concentrations in treatments was T1 > T2 > T3. Like our results, similar results were found by Liu et al. (2018) in which wheat straw biochar reduced the uptake of cadmium in ears, leaves, stems and roots of maize and promoted the maize growth and yield. They found that biochar boasted maize height and fresh weights of shoots, roots and leaves by minimizing the uptake and toxicity of cadmium. Wheat straw biochar added alkalization in amended soil and stimulated cadmium immobilization. Sanderson et al. (2019) estimated the concentrations of cadmium in potatoes grown in Jamaica, west indies. It was estimated that the minimum concentrations were 0.01 mg/kg and maximum were 0.22 mg/kg in potato-tubers. Bashir et al. (2018) observed the effect of rice straw biochar on the uptake of cadmium in water spinach (Ipomoea iquatica) grown in china. It was found that rice straw biochar significantly reduced the Cd solubility and uptake in shoots and roots and boasted plant growth, biomass, chlorophyll a, chlorophyll b and carotenoid contents. Khan et al. (2018) observed that rice straw, maize comb, bagasse and wood biochar reduced the uptake of cadmium in tomato and cucumber and increased the chlorophyll contents and biomass. Benabid and Ghorab, (2013) investigated that cadmium was accumulated more in roots than in stem and leaves of bean plants grown in Algeria. With respect to toxicity, cadmium was more toxic for leaves as compared to roots and stem. Cadmium caused chlorosis in the leaves of bean which suggested that cadmium metabolism takes place in leaves. Leaves are more sensitive to cadmium toxicity than other parts of bean plant.

![Figure 2. Cd Accumulation in Roots, Shoots and Leaves of Fenugreek](image_url)

CONCLUSION

Wheat straw biochar (at 2.5% and 5%) significantly increased the plant growth and yield parameters such as dry weights of leaves, shoots and roots, length of roots and shoots, number of leaves, lateral roots and branches, stem and root diameter, leaves area and reduced the concentrations of cadmium in leaves, shoots and roots over control (60 mg/kg of cadmium) with application of NPK (60-80-40) fertilizer at the 5% level of significance.
REFERENCES


Muhammad, N., Aziz, R., Brookes, P.C. and Xu, J.
Muhammad et al., 2019; Effect of wheat straw biochar on the growth of fenugreek (Trigonella Foenum-Graecum L.) in Cadmium spiked soil


US Salinity Lab (US Salinity Laboratory Staff) (1954). Diagnosis and improvement of saline and alkali soils. US Department of Agriculture Handbook 60, Washington, DC.


Source of Financial Support: Nil
Conflict of Interest: None, Declared.