# BIOCHAR AND PGPR ALLEVIATED CADMIUM TOXICITY IN *ABELMOSCHUS ESCULENTUS* (L.) MOENCH THROUGH ORCHESTERATION OF IAA CONTENT AND SOME PHYSIOLOGICAL ATTRIBUTES

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**Abstract**

Current experiment was designed to investigate the potential of PGPR and biochar in mitigation of cadmium (Cd) stress in *Abelmoschus esculentus* (L.) Moench. Cadmium (Cd) reduced growth of *A. esculentus* seedlings. The consistency of Cd with the reduction of their toxicity in the *A. esculentus* (ladyfinger) plant by thiol-modified Biochar was scrutinized in experiment. An Examination was executed in a greenhouse to analyze the role of biochar in Cd absorption by the okra plant. In this experiment, formation of thiol-biochar, by mixing of simple biochar and 3-mercaptopropyltrithoxysilane (3-MPTS). This modification comes out as simple and environmentally friendly. Moreover, that *Bacillus subtilis* lessened the Cd effect on crop productivity; experiment signifies that PGPR inoculations could ameliorate *A. esculentus* grown in Cd toxic conditions.

**Keywords:** *Abelmoschus esculentus*, *Bacillus subtlis*, Cadmium, Heavy metal, Stress,

## Introduction

Nowadays, environmental pollution becomes one of the most technical problems throughout the world it harms the environment disastrously that is growing at an unavoidable. Due to rapid industrialization, a major concern has been created about the environmental toxicity of heavy metals because of explosions of heavy metals instantly into the environment. Proliferation of toxic elements as heavy metals in the animals and plants via food chains due to the drinking of water, dangerous for their health (Pehlivan *et al*., 2013). Cadmium (Cd) which is assumed as a biological extrinsic or unnecessary element, toxicity increase by increase concentrations (Li *et al*., 2016). Cd though an unnecessary element but its rapid bioavailability for animals and plant become a persistent carcinogen in the surroundings (Huang *et al*., 2019). The purpose of determining the biochar is the broad usage of biochar is due to abilities to reduce pollutants, remedial polluted soil, and lessen the greenhouse gas. This investigation showed better plant growth; enhance the photosynthetic aspects, chlorophyll number, transpiration rates, and stomata activities. The effect of biochar in the soil boosted the tolerance capacity in the okra plant also enhanced yield and nutrient infusions within the strawberry plant. Moreover, Biochar was also successes lowered the oxidative stress. It is also proved good for the maintenance of pH level of the soil. However, eventually, Biochar reduces the heavy metal stress as Cd in the ladyfinger field. When the Cd stress is introduced into the plant, there is the formation and compartmentalization of phytochelatins, a defense mechanism is activated. The proficiency gained by the experiment could be proved to help out for the hybrid planting of the heavy metal tolerant plants.

Plant growth-promoting rhizobacteria have great agricultural importance, especially *Bacillus* sp. The strains of *Bacillus subtlis* inoculate in the crops protected from the pathogens such as fungal infections such as *Phytophthora*, Powdery mildew (*Pedosphere aphanis*), common diseases diagnosed in strawberry plant and also effect on the growth of plants, increase chlorophyll contents, and nutrient content of element also increased resultant healthy and fresh plants grown in the crop. This research was accomplished to evaluates sewage sludge amendment (SSA) at approximately 19 .8 and 41% volumes for ladyfinger (*Abelmoschus esculentus)*, by the study of their yield figure, morphological and physical aspect as well as their chemical reaction.

Soil polluted with Cd evolved into prerogative global dilemmas. After the declaration of the US Environmental Protection Agency, Cd is recognized as an emphasized adulterant. The elimination of cadmium by anthropogenic workouts such as mining, smelting, and other manufacturer emissions into the fertile regions become one of the main reasons for the spreading of Cd contamination. Moreover, by the use of several industrial emanations such as spraying of pesticides, herbicides, and fertilizers and occasionally by an irrigation system which promotes the inoculation of this poisonous pollutant, Cd in the chain Food.it is easily put up with roots of plants then after realistic to several featured If plants even in minute quantity (Fan *et al*., 2020).

In plants, Cd has very destructive impacts as restricted growth, oxidative stress, associated with fluctuations in membranous permeability, and generates reactive oxygen species (ROS) at the subcellular level. The hydroxyl radical ($OH^-$), Superoxide anion (OH), and Hydrogen peroxide are produced due to the oxidative nature of Cd in results leakage of electrolyte in the membrane occurs (Anjum *et al*., 2015). The accidental death of cells occurred due to the unpredictable oxidative form of proteins and lipids within the membrane. Biological plants evolve a wide range of defensive systems, help plants as hostility against oxidative stress. Cd elicits the production of ROS.

Owing to the elevated to soluble property of water and portability, Cd is categorized at 7$^{th}$ position from all the lofty poisonous elements present in the periodic table and it is caused of their toxicity even in minutes' quantity. Nevertheless, the existence of cadmium in the field convinced its phytotoxicity for the production of the product. Some plants can tolerate the minute's quantity of Cd but still, get harmed by their toxicity which varies from species to species of plants. A fluctuated demeanor has been ascertained by various plants from a low to the intensive degree of perceptivity towards the Cd. Considerably, these metals are acquired in animals and humans through the food chain association, which may be proved lethal for the lives. These metals affect the yield of plants; some fruit-producing plants are badly affected by heavy metals contamination. The Strawberry plant is one of them; Cd also affects the crop of ladyfinger (Huang *et al*., 2019).

*A. esculentus* (Okra), which belongs to the Malvaceae also known as the Mallow family, this family produced nutritive fruits, it is an annular crop. Delicate and verdant fruit is capable to eat, commonly known as a vegetable. The succession of life does not only depend on assurance of food but also on the environmental dynamics. The dynamic environment is the embodiment for soil microbes, synergy co-evolution with the plant kingdom, and bio-mineralization of the soil (Gouda *et al*., 2018). As the population of World expanded over time, also demanding the increases of agricultural necessities, which directing the chemical contamination in the agricultural sector, become the due to the degradation of the purity of the environment degradation occurred in every passing year also effective in the quality of food and soil fertility. In this perspective, researchers and scientists diverted attention from chemical fertilizers and pesticides towards PGPR, which proved as a co-evolutionary factor among the plant and bacteria (Gouda *et al*., 2018).

Heavy Metal assemblage in the field of crop and various plant carcasses was also multitude in the regards of the exact figure of heavy metal, to learn their correlation between heavy metal and nutrition calculation in plants. There is also how the plant responds to the assemblage of Cd. The difficult agenda is sewage sludge is worldwide so there is no effective method launched yet, but the recycling the process introduced throughout the world. By this process scientist's efforts to recycle the useful components as organic compounds and other useful nutrients of the plant (Logan and Harrison, 1995; Singh, 2009). They also studied the benefit of micronutrients to other living organisms, which wasted by sewage. These micronutrients assist the other sources of nutrients and organics to contribute useful soil conditioning commodities. Sewages system receives industrial along with the storm water from the close industrial set set-Cd impurities in the soil become a widespread problem in the world, Pakistan also confronted this problem because their unplanned Secretary matters come from an industrial setup. The current study illustrated that the transitions of yield and production of Ladyfinger (*A. esculentus*), which consider a common crop in Pakistan and India (sharing almost the same climatic conditions) at several statuses of Cd in the soil.

Availability of the nutrients signifies the seed germination as well as survival of the plants. The limited bioavailability of phosphorous (P) and nitrogen (N) present in the rhizospheres, absorbed by the root of the plants (Sekar *et al*., 2018), these complex nutrients modify by *Bacillus* sp, into a bioavailable form for the plants. Commercially, the first bacteria used as a fertilizer in the crop, formulated from the *Bacillus* sp. named was Alinit and occurred more than 40 %, yielding of the crop increased. *B. subtilis* also prioritize nitrogen fixation (Islam *et al*., 2016).

Leading parameters are controlled the quantity of cadmium uptake by the ladyfinger plants such as the concerning proportion of Cd, pH, texture, and exchange of cation ability or (CEC) of the soil. Another crop species and their genotypes can be useful for the choice of ladyfinger plants for phytoremediation of polluted soil. The cadmium does not resist the chemical but highly bioavailable (Irshad *et al*., 2020) The Cd affected the growth of plants as stunted growth besides this lessened photosynthetic rate which eventually reduced the yield (Srivastava *et al*., 2019). Chlorosis, necrosis, and browning of roots (Irshad *et al*., 2010) detected in the plants. An elevated level of Cd resulted in oxidative damages in plant cells by heightening malondialdehyde (MDA) content, leakage of electrolyte is occured (EL), and lessened the actions of antioxidants enzymes (Khalid *et al*., 2018).

Relatively research efforts dedicated to the portability of techniques and sources to remove the heavy metals from the field. Presently, many techniques are operating such as chemical immobilization, chemical washing, physical methods, phytoremediation, and microbial ways (Peng *et al*., 2018) with variable expenses vary with the efficiency of the procedure. It is referred to as those chemicals that have high efficiency with low cost cataloged an improved option (Fan *et al*., 2020) though all these strategies sufficient for inconsistent removal of heavy metal but have various impacts on the production.

Immobilization of the chemicals declines the portability and bioavailability of heavy metal with the sequel of outcomes such as ion exchange and moisturizing. Also by the accumulation of organic and inorganic emendation in the field (Zhang *et al*., 2019). However, there is some narrative entity have formulated as immobilization deputies comprising Biochar, phosphates, potassium, Si-rich mineral (Wang *et al.*, 2019).

Nowadays, impurities water and soil become a debatable issue throughout the world specifically with the heavy metal which directs to other environmental problems, significant falls in healthy productions of plants and crops and indirectly dangerous for human health. Susceptibility to toxic elements specifically heavy metal can consolidate the production of reactive oxygen species (ROS), which are constantly producing in any condition either stressed or unstressed situations of plants. With time vascular plants developed several defensive means to deal with the ROS circumstance (Hameed *et al*., 2011) as well as how to heal relative injuries.

Another change is functional enzymatic activities, containing APX, CAT, GR and SOD. Normally, these enzymes work in unstressed or normal conditions but if the stress hits the plants their reactive value boosted, these stressed including both biotic and maybe abiotic stress. This condition elicits several assortments of ROS like superoxide anion radical, hydrogen peroxide ($H_2O_2$), hydroxyl radicals (OH) and singlet oxygen (Elstner *et al*., 1982; Jung *et al*., 2004; Hameed *et al*., 2011). Further, along with the antioxidant enzymes, some of them non-enzymatic activities happened as generation of tocopherol, carotene, Glutathione One-S-Transferase (GSH) and ascorbate, basically these species performing scavenging of ROS in the stress of Cd (Rosen *et al*., 2010).

Biochar enriched with carbon, it is a mixture of organic compounds which is formed by the pyrolysis of organic substance when manure, sawdust, and food scraps are kept in low oxygen culture (Fan *et al*., 2020). Biochar has been noted to disable heavy metals founded in soil of the field of particular crops (Lu *et al*., 2017). It is noted that biochar as an apparent source for increasing soil fertility, immobilized soil potassium as well, so in yield which is contaminated with heavy metal we should also use potassium chloride, mix it up with biochar for the reduction of heavy metal stress. Potassium (K) plays a crucial role in the natural physiological process in the plants. K has benefits in plant growth and photosynthetic process which ultimately increases the crop production with better quality and importantly potassium is stress resistance for all the crops (Chen *et al.*, 2018). However, understanding of the consequences of biochar on soil potassium remains limited, so scientists have the main focus is the application of biochar.

Anyhow, pragmatic use of biochar has no consistently shown its attainment. Therefore, there prevails more research to assure the adequate outcomes of biochar. To heighten the efficiency of biochar several experiments are undergoing, comprising procedure with acid/base (Peng *et al*., 2017) loading with minerals (Liang *et al*., 2017), with Nanoparticles (Yu *et al*., 2018). Functional groups of organic compounds are also founded (Fan *et al*., 2010). Cd ions ultimately declines the bioavailability of this heavy metal from the soil it can be done by two other ways as plant physiological process and by soil chemical means (Huang *et al*., 2019). In the perspective of the biochar amendment, there are other suspicious biochar with a thiol group (Xia *et al*., 2019) supervised by modified thiol entities. These modifications are based on the 3-mercaptopropyltrimethoxysilane (3- MPTS), which raise their incredible to consume mercy and Cd heavy metals. Anyhow still have a low absorption rate as compared to carbon and graphene oxide (Huang *et al*., 2019).

**Materials and Methods**

**Preparation of biochar utilized by straw of Rice:** The biochar used in the experiment will be formed by raw straws obtained from the leftover of field Rice, currently harvested. After the collection, straws were cut down into pieces which will approximately 2 cm in length. These pieces underwent an Air-dried operation. Biochar will be prepared by slow-burned in the muffle furnace will pyrolysis under $N_2$, the temperature of the furnace was heated to 500°C with the rate 20°C per minute. The resultant trashed was collected, which was further crushed and ground out.

**Formation of thiol-modified biochar:** Furthermore, for the modification of biochar, we will take a glass container of about 20 mL. 5 g of prepared biochar will be added to that glass container. About 6 mL of Acetic anhydride proliferation with the 0.8 mL concentrated $H_2SO_4$ also added to the same container, cover with air free lid and leave it for 18 hours. When time passes solution would be settled, the required product was filtered through filtration and washed with distilled $H_2O$. Kept the substance in the preheated vacuum oven at the temperature of 32°C for 10 hours. Thereafter, the product (biochar) cool downed and graduate, pH will measure with a pH meter.

**Detection of the thiol group in the substance:** 1g of biochar mix with the 20 mL of ultra-pure water. For the detection of thiol contents present in the biochar, Ellan reagent will be used. 2 gm of biochar mix with the 0.2 mL of ethanol solution in a separate container. After 5 minutes, add the 0.2 mL phosphate buffer solution and leave the container for the next 20 minutes. Now add the 5.5' a-dithiobis-(2-nitro benzoic acid) solution in that adjacent container, that suspended biochar kept in the incubation for 6 minutes, the final product will be filtered with 0.22-micrometer membrane filter.

**Inoculation of *B. subtilis* S4:** The selective bacteria *B. subtilis* inoculated from the conservatory sector of the University of Punjab, Lahore. The bacteria injected from Luria-Bertani media culture (in Petri dish) was situated in the stirrer for 15 hours, was then kept in a centrifugal machine after the cultured was decomposed by distilled water, while a suspension in the solution. Later on, *B. subtilis* S4 was inoculated in each allocated pot.

**Soil incubation experiment**: Sample of soil was collected from the Cd contaminated soil from the area of interest. The collected sample passed through the air-dried procedure and departed into a 2 mm sieve. The substance is kept in the incubator for 20 minutes. The collected sample of soil (32 g) poured into a centrifuge tube (50 mL). Thiol-biochar was added in the test tubes and sterilized gently.

**Sterilization of seeds:** Before going to any experiment, the *A. esculentus* seeds were dipped in NaOCl solution, after 3 and half minute, transfer in the Petri dish with 80 % ethanol for approximately 1 minute. The concerned seeds rinsed with distilled water and dried less than 65 °C of dry air.

**Study Area:** The project was performed at the field is having a greenhouse of the University of Narowal (UON), a suburban area of Panjab, Pakistan. The trials performed between April and May 2021. These months of the year maintain the temperature between 37.7 and 40.2 % ºC, approximately 183 mm with rainfall, the humidity level changes between that period of the experiment if figure out it was approximately varied maximally from 57.2 % - 79 % and 24.4 % - 55.8 % minimally.

**Planation of seedlings:** The trial was executed in plastic pots of 35 cm in diameter and 35 cm in depth. There are total 5 treatments of different combinations of biochar, PGPR and stress of heavy metal (Cd) as;

*Treatment 1*: unimproved soil (taken from field of native area) control group

*Treatment 2*: Soil and PGPR

*Treatment 3*: 20 % Cd in soil

*Treatment 4*: 10 % Biochar + 30 % Cd + PGPR in soil

*Treatment 5*: 20 % Biochar +20 % Cd in soil

The soil taken from the field that was dugout approximately 35 cm, that soil passed in air- drying procedure after analyzed and then pots filled with the soil.

**Pots treatments Experiment:** The experiment was performed in seven to eleven weeks in early summer, which is the favorable growing season for ladyfinger plants. 5 treatments were investigated with 1 control in a 30 mL to 50 mL enclosed pot filled with the organic peat in minimum quantity. as; T1 was filled with the contaminated 30 mL soil with 5 % Cd stress and 5 g biochar, T2 with 10 % cadmium stress and 10 g of biochar, T3 treated with 20 % Cd stress and 20 g of thiol-biochar, T4 treated with 40 % stress of Cd and 40g biochar, and T5 treated 60 % of Cd and 50 g of thiol-biochar was added in the pots and water with the same quantity of $H_2O$ and was leave for a minimum of one day. The very next step was sowing of sterilized seeds. The bacterial suspension of *B. subtilis* was inoculated in all allocated experimental pots. All these pots were kept in the greenhouse under 25°C

**Growth Parameters:** In this experiment, we used the Plant growth regulator technique (PGR), is the most valuable technique for the investigation of parameters like, total percentage for the survival of germinations, height of the seedling, total length of the shoot, and root, the total surface area of the young leaves as comparing the control group. The germination rate was also determined after 3 to 4 days. The survival rate was analyzed within 7 to 8 weeks. The plants taken out of the soil and was examine the other parameters such as root and shoot length, fruit size, and quality of fruit.

**Analysis of Cd tolerance:** The tolerance of Cd was estimated by inoculation of PGPR. The Cd was poured into different test tubes as 0, 10, 20, 30, 40, and 50 mL/L. In each test tube, bacterial suspension was added and stirred those test tubes and placed under 25 °C.

**Determination of photosynthetic pigment content, Gaseous exchange phenomena, and rate of photosynthetic:** In the Lichtenthaler method was applied, detection of photosynthetic pigment content was determined in the growing plant; the plant content (leaves and stem) was obtained and kept in the Petri dish. About 80 % of acetone was also added to the adjacent Petri dish and leave for 7 days in-room temperature, was no light in the room. After 7 days, a medium was obtained, and resultant material was observed in a spectrophotometer.

The determination of the whole photosynthetic rate and all attributed to gaseous exchange in the plant was also by applying the (Pan *et al*., 2006) technique. An infrared gas analyzer was used for analyzing gaseous exchange in transpiration and stomata phenomenon, examined in the early morning for three days.

**Analyses of indole acid (IAA) production:** *B. subtilis*, stain S4 was prepared in artificial culture by 00 mg NL of 1- tryptophan for one day. That matters having homogeneous nature for the bacterial culture got through by the process of centrifugation. Later on, 1 mL supernatural was hybrid and reaction happened between orthophosphoric acid 10 m M and Salkowski's. After sometime at 530 nm, the pink color was observed and that mixture pertains to the IAA solutions (Bodel *et al*., 1999).

**For analysis of Cd:** Took 1 g of soil from the pot and passed the air-dried procedure and after that plant also dried in the oven and their different parts of body digested in Petri dish which contained 15 mL of tri-acid solution (Allen *et al*., 1980; Sharma *et al*., 2010). The final matters filtered and exactitude and accuracy were determined through the spectrometer AA- 7000. The total quantity of Cd in specimens was carried out by the multiplication of the sample with the overall dry weight of the plant.

## Results

The study revealed that the *A. esculentus* plant survived in the stress condition, applied different treatments 5, 10, 20, 40 and 50 g of thiol-modified biochar against the 20 mg kg¹ Cd 15,40,70, and 80 per cent respectively.

**Concentration of Cadmium in the *A. esculentus*:** Biochar and Cd showed a substantial variation in the level of Cd in *A. esculentus* 0.69 to 1.98 mg g¹ in the stem, 2.5 to 4.8 mg kg¹ in the lower portion as in roots, and 1.9 to 2.3 mg k¹ in the leaf area of the ladyfinger. Human activities increased the level of Cd and other toxic metals as depicted in fig. 01.

**4.2 Measurements of growth parameters**

The influence of the biochar and *Bacillus subtilis* S4 on *A. esculentus* plant's growth of different parts as root, shoot and leaf area with different types of weight under the Cd stressed conditions. As ±SD nearly equal to 5. According to the fact and figure, It is concluded that biochar and *B. subtilis* S4 with the combination of Thiol-modified biochar effectively showed different growth rate as shown in table 01.

**4.3 Effect on photosynthetic rate (l M $CO_2$ $m^{-2}$ $s^{-1}$):** Biochar and PGPB (Bacillus *subtilis)* affected the photosynthetic rate of plants. The values noted before and after the dry procedure. Concentration of Cd was taken different in every treatment with respective to soil as shown in table 02.

**4.4 Rate of Transpiration:** Consequence of biochar and *B. subtilis* on the rate of gaseous exchange in the *A. esculentus* when it was treated with different treatments. Graphic demonstration in fig. 2 showed ± SD taken a (n=5), different concentration of Cd added in the pots, minimum 10 % - 80 % along with the mixture of biochar and *B. subtilis*.

**4.5 Analysis of indole acid (IAA)**

Figure 3 showed that production of IAA within a plant effected by the accumulation of Cd in stress condition but the biochar and *Bacillus subtilis* S4 suppressed the effect and produce well concentration of IAA.

**4.6 Evaluation of Antioxidant Enzymes:**

Cd increases the production of ROS species whereas BC and PGPB (*Bacillus esculentus)* in the seedlings of *A. esculentus* as shown in figure 4.

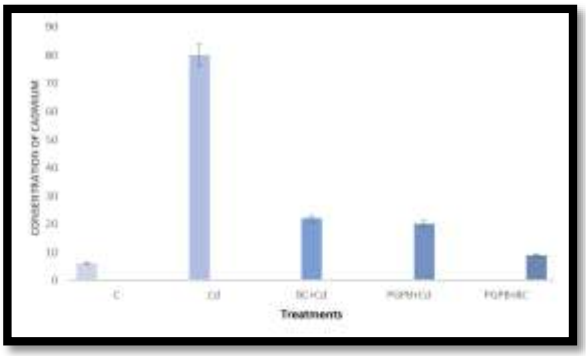


Fig.1

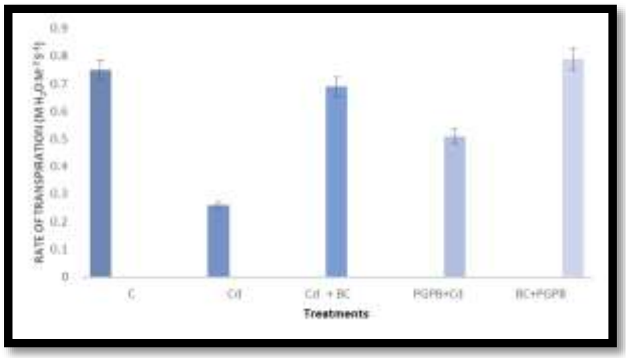


Fig. 2

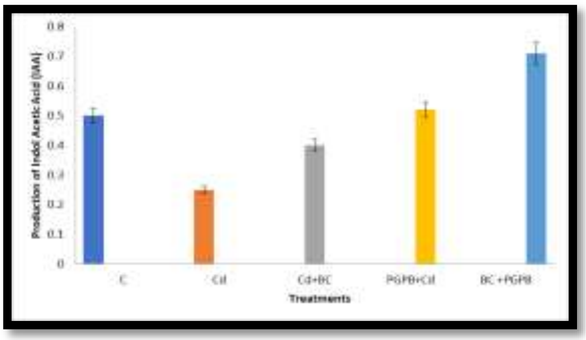


Fig. 3

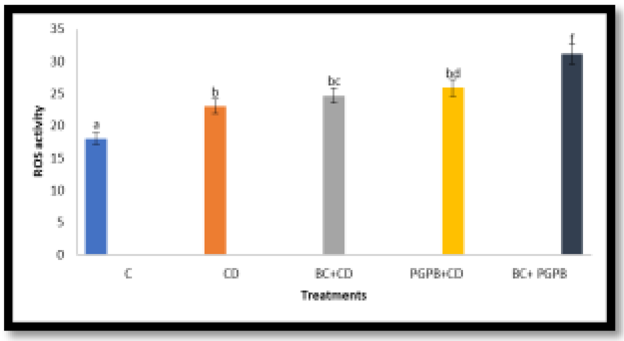


Fig. 4

Fig. 1: Illustartion of Cd concentration in *A. esculentus* in different treatments.

Fig. 2. Effect of biochar and *B. subtilis* on rate of transpiration of *A. esculentus* in different treatments.

Fig. 3. Effect of production of IAA by of *A. esculentus* under different treatments.

Fig. 4. Production of ROS in the seedlings of *A. esculentus* under different treatments.

**Table 01: Influence of biochar and *Bacillus subtilis* S4 on growth of different parts of *A. esculentus.***

| Treatments | Parts of *A. esculentus* | | | | | |
|---|---|---|---|---|---|---|
| | *Root length (cm)* | *Shoot shoot (cm)* | *FW root (g)* | *FW shoot (g)* | *DW root (g)* | *DW shoot (g)* |
| *C* | 59 ±3.02[c] | 201 ±7.92[d] | 60.2 ±3.21[d] | 199.2 ±8.11[d] | 7.9 ±2.12[b] | 28.2 ±1.21[c] |
| *Cd* | 39 ±1.7[d] | 194 ±4.3[f] | 41.18 ±2.3[d] | 172 ±6.23[e] | 5.91 ±1.21[d] | 21.1 ±1.32[d] |
| *Cd +BC* | 71 ±3.00[bc] | 297 ±7.91[c] | 71.15 ±2.91[bc] | 212. ±5.73[bc] | 7.91 ±2.01[bc] | 28.12 ±1.12[c] |
| *PGPB +Cd* | 62 ±1.26[cd] | 221 ±5.21[e] | 63.12 ±1.82[c] | 197.12 ±4.8[d] | 6.72 ±1.23[c] | 24.1 ±1.2[cd] |
| *BC +PGPB* | 78 ±2.3[a] | 289 ±08.42[b] | 73.12 ±2.21[b] | 267.2 ±5.23[b] | 8.91 ±2.24[d] | 32.54[c] |

**Table 02: Effect of Biochar and PGPB (*Bacillus subtilis*) on photosynthetic rate of *A. esculentus.***

| Treatments | Chl A | Chl B | Total chlorophyll pigments |
|---|---|---|---|
| *C* | 1.43±0.11[a] | 0.65±0.11[ab] | 2.08±0.22[b] |
| *Cd* | 0.92±0.04[b] | 0.58±0.68[cd] | 1.5±0.72[c] |
| *Cd +BC* | 1.81±0.13[b] | 1.42±0.39[b] | 3.23±0.52[b] |
| *PGPB +Cd* | 1.36± 0.15[c] | 1.03±0.12[b] | 2.39±0.27[bc] |
| *BC +PGPB* | 2.22±0.32[a] | 1.54±0.12[a] | 3.76±0.44[c] |

**Discussion**

Heavy metal stress decreases yield of crops grown in higher contaminated conditions. The outcome shows the agreement with the previous studies (de Silva *et al*., 2012; Moosavi *et al*., 2014; Ma *et al*., 2106; Abbas *et al*., 2018) showed that not only plant growth but also biomass affected by the Cd stress. As the previous experiment of red maple indicated the other parts of plants also affected as the diameter of the stem and root length along with their dry-biomass when compared to the control group (de Silva *et al*., 2012) leaf area shorten in the stressed conditions (Abbas *et al*., 2018). CD might prevail to the rate of the change of the plants (Moosavi *et al*., 2015). Under this experiment, it could be concluded that this research also showed that the role of biochar on Cd proliferation in the crop of ladyfinger under both heavy metal (Cd) as well as drought stress. The result revealed that the soil filled with biochar could enhance the growth of the plant when the Cd stress hits the crop. According to the study of Cornelissen *et al*. (2013), adding the biochar in the stressed soil could boost their qualities.

Due to the non-degradable character, heavy metals become the vital source of soil pollution, as heavy metals having a higher mobility rate and readily for uptake by the plant (Abbas *et al*., 2018). Many strategies prevailed to ameliorate metal toxicity in the soil which indirectly accumulated in the plants and human beings (Rehman *et al*., 2018). Thiol- Modified Biochar can reduce the devastating effect of metal by lowering or sometimes completely zeroed in the bioavailability of the Cd and made the soil stable for the uptake of only necessary nutrients from the soil (Adrees *et al*., 2020). The results of the research showed that the biochar enhanced the morpho-physiological as well as the chemical metabolic associates of the plant *A. esculentus.* The vigorous molecules of the Cd were substituted into the immobile components due to the biochar. The studies showed that the growth of the plant also increased by adding the biochar.

Cd stress also affected the chlorophyll contents as it reduces it and ultimate the rate of photosynthesis decreased which is not beneficial for the proper growth of the plant Zu *et al*. (2016), the experiments proved that the biochar increases the Chl content which also increases the photosynthetic rate of the ladyfinger. Hussain *et al*. (2019) described through their experiment that the biochar also increased the final production of crop by alleviating the effect of Cd present in the soil. Furthermore, the previous study demonstrated that the biochar also improved the photosynthesis of the tobacco plants and the final yield of the crop bestowed more profit (Alkhatib *et al*., 2019). Moreover, the research revealed that the other metabolic activities showed a positive response by the application of biochar as shown in the results in which, as under the stresses of Cd the production of the MDA, $H_2O_2$, and ROS species also increased, Biochar activates the other species that balanced these including the SOD, CAT and POD. Biochar made the Cd less mobile and not uptake by the roots and transportation of the Cd is less in all the parts of the plant of *A. esculentus* (Abbas *et al*., 2018). Likewise, it was also presented by Qiao *et al*. (2018) as uptake in the rice field dealt with the biochar.

Another important element that applied was PGPB, as the previous the study already depicted the importance of PGPB as a growth regulator (Ma *et al*., 2016) also revealed that the PGPB is a bacterium that can eliminate or completely reduces poisonous impurities from the soil specifically the lethal effect of heavy metals (Ma *et al*., 2016). Biochar and PGPR intercourse proved the one of good method d for the reduction of harmful effects of heavy metals, as Ar, Cd, Al (Anjum *et al*., 2015).

This research indicated that the lab strain of *B. subtilis* S4 had the ameliorative ability of Cd that existed in the polluted soil. Moreover, the previous study shows that the PGPB increased the production of auxin in the plants likewise, in the *Catharanthus roses* when the plant faced by abiotic stress such as heavy metal stress and sometimes drought stress (Yasin et al. 2018) which already proved by the current results. As IAA, directly regulates the growth factor within the plant, it increases the roots and shoots promptly. The Auxin synthesis by the PGPB, *B. subtilis* S4 become the source of the exogenous IAA to boosted plants growth and development under stressed situation (Saleem *et al*., 2018) noted the growth rate of a sunflower when it was faced the stress condition. The experiment demonstrated the plant *A. esculentus* seedling when it was treated with the stressed as well as the normal condition along with the growth regulator, PGPB (a strain of *B. subtilis* S4) prepared in the lab medium.

There were many characters were studied under that experiments regarding the leaf. As gaseous exchange through leaf also inferred in the presence of Cd, the plant of Ladyfinger faced heavy metal stress. The transportation process disturbed, shows the less rate as compared to the normal conditions, when the Cd pertained to the soil of the ladyfinger, can't in the pots experiment (Ma *et al*. 2006) proved that the metal stress (Cd) decreased the number of chlorophyll pigments in the leaf and stem areas in *B. oxyrrhina,* that is directly affected the photosynthesis process. Likewise, Xia *et al.* (2015) presented their study that the Cd also decreases the gases exchange in the peanut plant. Metal stress (Cd) also affected the overall stomata density, photosynthetic pigment decreased due to the Cd and the hydraulic activities (Silva *et al*., 2012; Abbas *et al*., 2018). Along with the biochar and the PGPR proved the enhancer elements in the soil and contribute the better production of the adjacent plant.

Our inquiry showed the decrease in the number of chlorophyll pigments which directly affect the lessen photosynthesis process was independently affected the transpiration process. Formerly, a survey also confirmed that the application of biochar could increase both these attributes in the plant under heavy metal stress as well as drought stress (Yousaf *et al.*, 2016) and the condition of the extra water (Ali *et al*., 2017).

The cellular function of the plant affected by the oxidative stress because of the lipid accumulation in the cell which due to the lipid peroxidation, these changes in the cell fluid could damage the nucleic acids (Schutzendubel and People, 2002). The more concentration of the ROS due to the activation of different antioxidant in which SOD, POD and CAT etc. It shows that the SOD occurred by the conversion of the hydroxyl to basic form as dihydro-oxide species in the cell membrane. The free oxygen may cause a catalytic converter of the $H_2O_2$. That's why the detoxification of the basic species as ROS happened in the membrane of the plant cell but for the normal growth and the development of the plants compelled the harmonious level of the ROS released in the cell membrane but after detoxification the degradation of the membrane and the leakage of the membrane (Duman and Koca, 2014; Priscila *et al*., 2015).

Concentration also increased of the MDA we're observed in the leaf areas under Cd stress, but previous study showed that these level may less harmful in the grafted plant as compared to the non-grafted plant under stress conditions, the stress of heavy metal and drought stress (Priscila *et al*., 2015). But in the grafted plant the MDA content was observed in the scions and not in the lower part of the granted plant, it showed that the accumulation of the Cd in the soil harmed the plant also by the production of MDA and ROS in the cell if the plant which also played the redox signed in metabolic of the leaf (Deck *et al*., 2014; Wang *et al*., 2014).

There was also observed that the Cd also enhanced the proliferation of the other metabolites in the tissue of the plant likely proline, which affects their protective character towards the plant tissues (Gratao *et al*., 2012; Mishra *et al*., 2014; Priscilla *et al*., 2015). The defensive mechanism of the plant directly associated with the mesh of both enzymatic and enzymatic processes which also become the signal for the production of ROS because capable of suppression of the stress.

In current study, it was observed that the Cd had a disastrous effect not only on the plant's growth but also another metabolism also affected in *A. esculentus* but also get to know about the $CdCl_2$ positive effect on the growth of the plant. The growing parts of the plants as root, stem showed a decrease in growth rate in the treatments of the Cd but 0.05 mM of $CdCl_2$ showed a little increase in the growth rate. A prior study had evidence that just not only Cd but all heavy metal, like Cu, Zinc, Mercury, and Aluminium had negative effects on the crop (Barnabas *et al*., 2000; Horvath *et al*., 1996; Hameed *et al*., 2011). The recent data described that it is also in the line with the impression that the external appearance of the plants clearly showed the effect of the Cd as stunted growth, leaf chlorosis and change in the other internal metabolic activities of different enzymes that could be affected by various metabolic processes (Arduini *et al.*, 1996; Abbas *et al*., 2018)

Every species of plants having tolerance ability to the stress, one of them is heavy metal stress that capacity of tolerance fluctuated from species to species, these plants able to assimilated and acquire heavy metals. Likewise, that identified different variations in the growth rate with time as well as their physical and chemical characteristic of soil changes in which accumulation of Cd occurred by the different anthropology activities of human beings. Specifically, it noted that the crops cultivated in the areas having any kind of industrial setups nearly had decreased final production of the crops because that soil contaminated with different types of heavy metal released by the industries and other local setups. As plants can take the nutrients from the soil so when they absorbed nutrient the heavy metals, Cd which is already present in the soil or that soil irrigated by the contaminated water, that crop plant has prevailed in the chemical properties (Pandey *et al*., 2009). The exploration has been shown that the Thiol-modified biochar has a positive effect on the Cd, as the ability of the plant enhanced against the Cd presenting the soil. Our findings confirmed that they pertain to the Biochar (0.5 %) and Thiol-modified BC (1.5 %) approximate to the limited to Cd bioavailability that's why plant becomes unable to uptake through the roots. Very clearly, the accompaniment of the soil, the BC contributed to the betterment and fertility of the soil by lowering the bioavailability of the Cd in the soil as a result the growth of the ladyfinger increase.

**Conclusion**

This experimentation work revealed that a high amount of Cd moved from the contamination soil to all the plant body through the root system. Cd proved as lethal for the healthy plant as it directly affected on the basic metabolism of the plant. It lessens the number of photosynthetic pigments that's caused the decreased the photosynthesis process within the plant of *A. esculents*. It also stunted the growth of ladyfinger plant. Moreover, gaseous exchange also disturbed as Cd caused the closure of stomata, electrolyte leakage also noted, production of antioxidant enzymes against the ROS initiated by the high concentration of Cd including SOD, CAT, APX. Further, the strain of *B. subtilis* S4 applied with thiol-biochar, that combination worked positively for the *A. esculentus* in the stressed of toxic heavy metal Cd, both balanced the metabolic rate within the *A. esculentus*, increases the photosynthetic pigments which directly increases the photosynthesis process and transpiration rate, growth of the plant was observed. As biochar lessen the bioavailability of the toxic Cd which was present in the contamination field of soil.

**Conflict of Interest:**

All the authors declare no conflict of interest.

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