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PSII Photosystem Protection by Soil Amendment with Biochar and Adequate Nitrogen Fertilizer Availability Attenuate Cadmium Induced-Photoinhibition and Phytotoxicity in *Solanum Lycopersicon*

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Abstract

The aim of this research was to assess the biochar ability to protect the photosynthesis process face to Cd contamination, especially, when nitrogen fertilizer was added at adequate amount. Culture of tomato seedlings was conducted on pots containing soil added with different portion of biochar (0, 3.5 and 7%) and nitrogen (3 or 6g NH4NO3). Irrigation was done by water (control condition) or Cd solution (150µM). Data showed that regardless presence of Cd, synchronously addition of high nitrogen fertilizer (6g) and biochar (7%) in the culture medium resulted in fresh matter production, photosynthetic pigment (Chlorophyll a) and mineral elements content enhancement. Similar observation was shown concerning the mineral nutrition (nitrogen, phosphorus and potassium contents). Significant increase in the maximum quantum efficiency of PSII (Fv/Fm) and photochemical quenching (qp) and decrease of the non-photochemical quenching (Npq) were observed in Cd-treated plants cultivated on soil containing the high amount of nitrogen and biochar level.

A positive correlation exists between growth enhancement and photosynthetic machinery protection protected against heavy metal contamination was detected when soil was amended by biochar and supplied with available nitrogen fertilizer quantity.

Keywords: Biochar; Nitrogen Fertilizer; Cadmium; Mineral Nutrition; Chlorophyll Fluorescence

Introduction

The discovery of substrate materials and their addition in heavy metal contaminated soil in order to optimize and support sensitive plant growth are one of the principal goals of researchers. One of these substrates is the biochar whose its addition to the soil contributes to different benefic effects [1,2]). The biochar properties are well studied and discussed suggesting its role in improvement of soil chemical properties. Some of these changes mentioned among literature are heavy metals adsorption, nutrient retention, nitrogen use efficiency, ion exchange capacity and others [3,4]. Biochar amendment to the soil were improved water holding capacity of the soils, in addition to developed crop nutrient availability, increased nitrogen use efficiency and hence yields [5]. Biochar have considerable influence on adsorption of heavy metals (Zn and Pb) in soil as mentioned in study of [6]. The concentration of available heavy metals such as Cd, Cu and Zn in soil decreased significantly when the soil amendment with biochar [7]. Nitrogen fertilizer in soluble is an important to its calm approval and assimilation during plant development stages. Therefore, mineral fertilizers are the major source of N affected to crops [8]. Soil amendments, nitrogen-biochar had some specific roles in improving soil acidity, increasing mineralization organic N, N uptake and decreased N transuding, so encouraging soybean growth [9].

This study aimed to investigate the mitigation effect of biochar and available nitrogen fertilization simultaneously application in alleviating cadmium-induced photo inhibition and growth drop alleviation in *Solanum lycopersicon*.

Material and Methods

Solanum lycopersicon culture was realized in plastic pots (11.5-cm and 10.5-cm of diameter and height) filled with a mix of soil (500g), biochar (0, 3.5 or 7 % w/w) and nitrogen fertilizer (3g or 6g NH_4NO_3).

Pepper crop green waste was used to product biochar. Pyrolysis temperature varied between 440 and 550 °C during the two runs. The content of total carbon, organic carbon and total nitrogen in the biochar used were respectively from 60, 57 and 0.7%. After pyrolysis, biochar was sifted through a 3 mm sieve before use. The pH of Biochar solution was from 10.2.

Culture was conducted in greenhouse under 25 °C temperature and 12-h photoperiod. Plants were irrigated with distillated water (control) or Cd ($CdSO_4$) solution (150µM). In order to reduce soil surface drying, a thin layer of quartz particles (2mm) were placed on the bottom of soil. Harvest was carried out five weeks later.

Atomic absorption spectrophotometer was used to determine Cd concentration, whereas nitrogen (N), phosphate (P) and potassium (K) contents were determined according to [10-12] respectively.

Chlorophyll *a* content was determined as described by [13-15]. Chlorophyll fluorescence parameters were determined with a fluorometer (PAM-1200, Walz, Germany). The minimal (F0) and maximal Chl *a* fluorescence (Fm) emissions were assessed to calculate the maximum quantum yield of PSII (Fv/Fm = (Fm – F0)/Fm), [31]. The photochemical quenching (qp) and the Non-photochemical quenching of fluorescence (NPQ) which is proportional to the rate constant of thermal energy dissipation), were calculated by following [16,31] successively.

ANOVA analysis and Tukey's HSD tests were used to determine the significant differences between means (twelve replicates per treatment) treatments at a probability level of ≤0.05.

Results

Fresh weight, chlorophyll a, N, P and K and Cd tenors

Results showed that plants fresh weight (Figure 1a), chlorophyll a (Figure 1b), and mineral elements tenors (Table 1) were significantly reduced by Cd presence in soil. Whereas, addition of high biochar quantity (7%) simultaneously with high level of nitrogen fertilizer (6g) in Cd- treated culture medium resulted in growth increment and nitrogen (N), phosphorus (P), potassium (K) tenors increase.

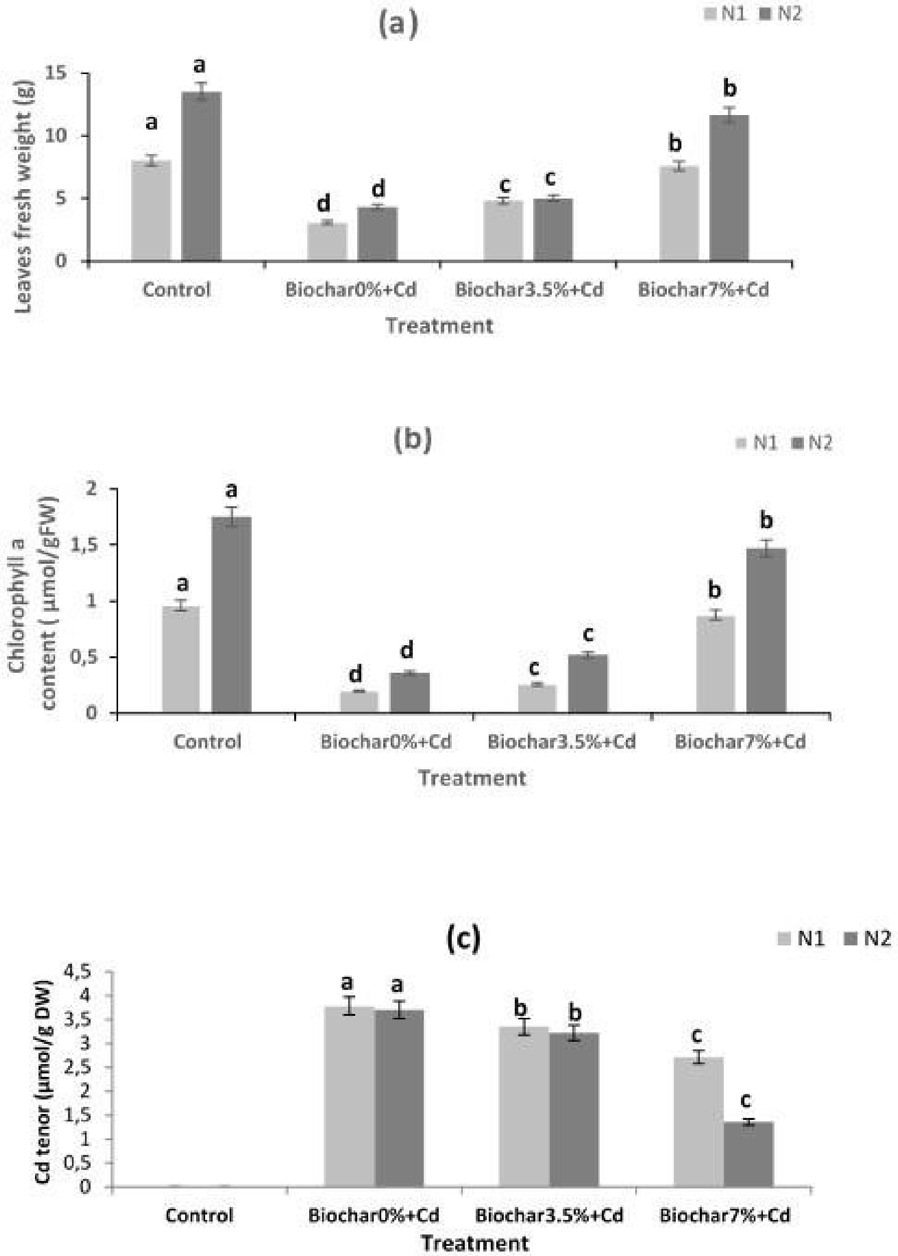

Figure 1: Leaves fresh weight (a), Chl a content (b) and Cd residue (c) in leaves of tomato plants cultivated under control condition (without Cd) and on Cd-treated soil in presence of different nitrogen fertilizer (N1: 3g - N2:6g) and biochar (0, 3.5 and 7 %) levels. The data are means (SE) from twelve determinations. Different letters above bars indicate significant differences between treatments (ANOVA)

As shown in Figure 2c, the less Cd tenor was detected in plants derived from medium containing 6g of nitrogen fertilizer and 7% dose of biochar.

Fluorescence measurements

As shown in Figure 2a, the maximum quantum efficiency of PSII photochemistry (Fv/Fm) is highly affected by the furniture of different nitrogen fertilizer also than biochar when soil in contaminated with Cd. The results indicated that parameter (Fv/Fm) is dramatically reduced by Cd whatever the quantity of nitrogen fertilizer used. Whereas, when soil amendment with high level of biochar in the Cd contaminated soil, the negative effects of the metal are reduced especially if important nitrogen fertilizer was co-supplied.

Regarding the photoquenching (pq) coefficient which indicate the level of energy light conversion to chemical one is very sensitive to presence of Cd. Generally, presence of the metal inhibited the photoquenching in tomato leaves fed with the different nitrogen fertilizer amounts. But, in Figure 2b results indicated that addition of an important level of biochar (7%) simultaneously with 7g of nitrogen fertilizer resulted in alleviation of the negative effects of Cd.

Under light these results, investigated that the dissipation of light energy process (Npq coefficient) was increased in Cd-treated tomato leaves (Figure 2c). The dramatic effect of biochar application parallel with nitrogen fertilizer one on the photosynthetic parameter in tomato leaves cultivated on Cd- treated soil is underlined in results. Results depicted that the dissipation of energy as fluorescence (Npq) was reduced in Cd treated leaves tomato cultivated in presence of both important amount of nitrogen fertilizer and biochar.

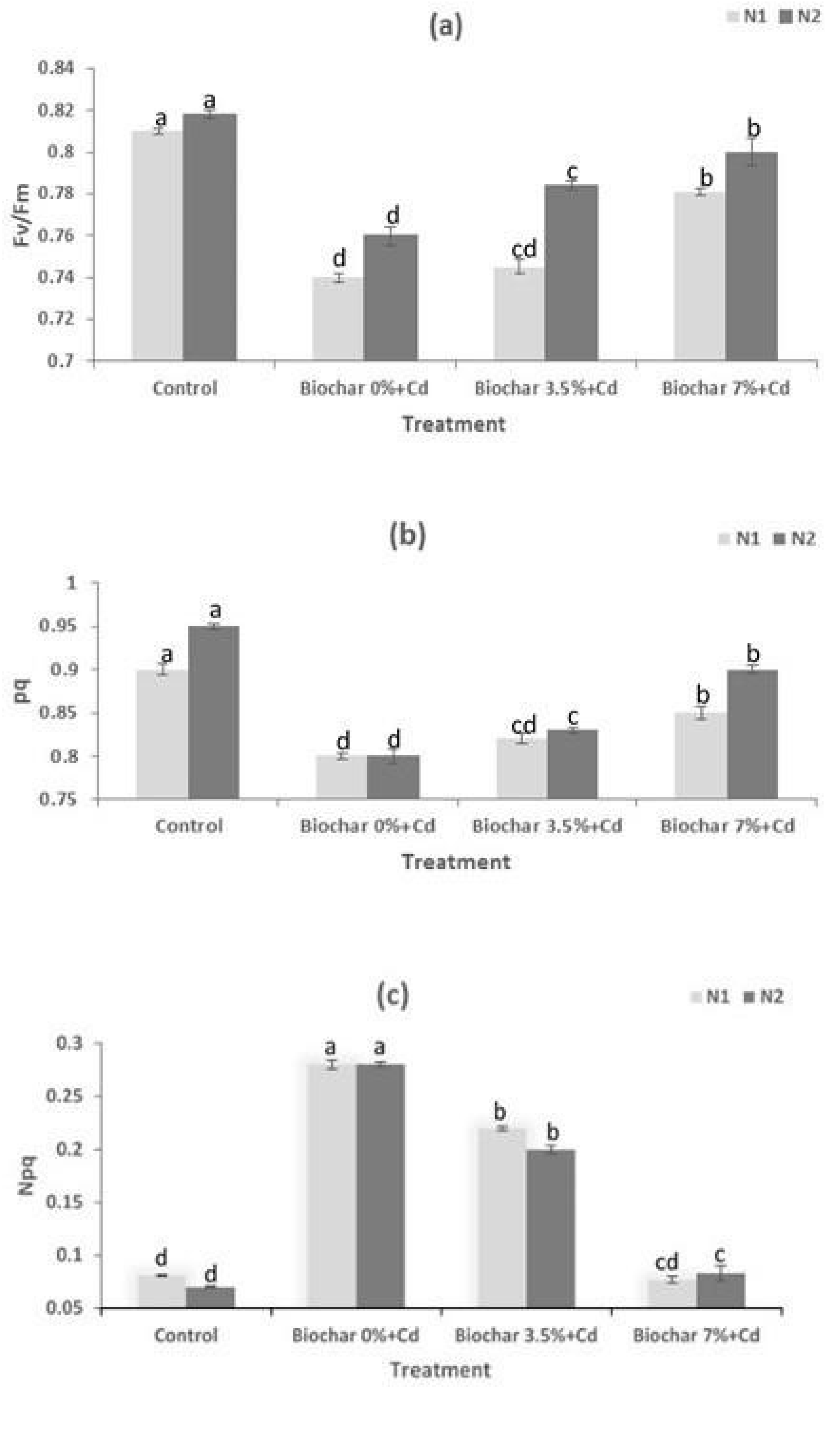

Figure 2: Maximum quantum efficiency of PSII photochemistry (Fv/Fm) (a), Photoquenching (pq) (b), Non photoquenching (Npq) (c) of tomato leaves cultivated on Cd-treated soil or no in presence of different nitrogen fertilizer (N1: 3g - N2:6g) and biochar (0, 3.5 and 7 %) levels. The data are means (SE) from twelve determinations. Different letters above bars indicate significant differences between treatments (ANOVA)

Discussion

Tomatos are ranged among heavy metals susceptible glycophyte plants. Accordingly to this reality, we found that several studies suggested that tomato seedlings growth and productivity were negatively affected by presence of heavy metals [17,18]. Data showed that regardless contamination of soil with Cd, presence of biochar resulted in enhancement of growth plants. To more precise, the addition of 7% of biochar simultaneously with high nitrogen fertilizer amount (6g) was reduced by the most important growth level of tomato on Cd- contaminated soil. In fact, the inhibition of tomato growth by Cd reflected its directly and/or indirectly negative effects on mineral nutrition, nitrogen and carbon metabolisms, photosynthetic pigment synthesis, and photosystem II (PSII) efficiency [19,20]. In addition, it is known that biochar can reduce the bioavailability and leachability of heavy metals in soil, through adorption, immobilization soil pH increase and other factors [21]. Results shown in this experiment are in agreement with the previous suggestions. Biochar amendments of soil not only have a benefic effect on nutrient elements availability, but also reported positive interactions between biochar and application of nitrogen fertilizer on plant growth [22-24]. Results presented in Table 1, were in agreement with these suggestions. Leaves N, P and K tenors reduced by Cd (contaminated soil in absence of biochar), were dramatically increased by presence of biochar. Their more important tenors were detected in samples derived from high- level nitrogen fertilization (6g) and high applied quantity of biochar (7%). It seemed that this previous described condition was the more effective in enhancing N, P and K absorption and translocation to shoot part. This is in accord with several other authors [21,25] they have well discussed the impact of biochar on soil N, P and K dynamics. Such effects have as consequence, the enhancement of tomato plant growth cultivated on Cd-contaminated soil and added with biochar parallel with an optimal nitrogen fertilization.

Treatment						
	N (meq/g DW)		P (meq/g DW)		K (meq /g DW)	
Nitrogen Fertilization level	N1	N2	N1	N2	N1	N2
Biochar 0%	8.8±0.78[a]	19.3±1.01[a]	2.08±0.19[a]	5.35±0.046[a]	1.01±0.01[a]	2.77±0.173[a]
Biochar 0%+Cd	0.25±0.023[d]	1.82±0.93[d]	0.17±0.001[d]	0.91±0.083[d]	0.14±0.002[d]	0.029±0.002[d]
Biochar 3.5%+Cd	0.37±0.065[cd]	5.66±0.044[c]	0.21±0.053[cd]	2.02±0.117[c]	0.18±0.01[cd]	0.96±0.072[c]
Biochar	5.28±0.438[b]	12.04±1.15[b]	1.33±0.101[b]	4.17±0.024[b]	0.92±0.082[b]	1.35±0.124[b]

Table 1: Leaves FW (mg); N, P and K (meq /gDW) tenors in tomato leaves cultivated on Cd-treated soil or no in presence of different nitrogen fertilizer (N1: 3g - N2:6g) and biochar (0%, 3.5% and 7 %) levels. The data are means (SE) from twelve determinations. Different letters above bars indicate significant differences between treatments (ANOVA)

The thought about studying the chlorophyll *a* fluorescence parameters was ideal. Data demonstrated that the efficiency of the PSII photochemistry was negatively affected by Cd stress similarly as results shown by [18]. However, presence of Biochar in Cd-contaminated soil was traduced by enhancement of the efficiency, especially when high level of nitrogen fertilizer was applied. Results showed that Fv/Fm ratio on the leaves was negatively affected by Cd. Moreover that increased by adding biochar and nitrogen fertilizer doses that enhanced the previous parameter in Cd-treated leaves. A similar alleviation of Cd-induced effects on PSII photochemistry efficiency in tomato plants was previously detected when ammonia was used as nitrogen fertilizer in presence of Cd contamination of soil [17]. These observed variations of PSII photochemistry efficiency, could explain the change in light energy conversion process (photochemical quenching pq,) detected in tomato plants cultivated in our experimental conditions. The results suggested that the furniture of an important nitrogen fertilizer simultaneously with high biochar amount advanced the photochemical quenching. Noteworthy chlorophyll fluorescence dynamics are directly related to redox reactions in PSII and the all photosynthetic electron transport chain. Therefore, we can understand the advance of photochemical quenching shown, through the increment of the primary electron acceptor of PSII oxidation. On the other side, the photochemical conversion and the capacity of the electron transport for the reduction of NADP were not affected by Cd stress, because the metal small amount absorbed and transmitted to leaves. [26-28] were in agreement with the fact that most heavy metals disturbed photosynthetic electron transport processes which led to suppression of the efficiency of energy transformation in photosystem II. The results explained, provided additional evidence, and examined the non-photochemical quenching coefficient (NPQ) values. So, results noted that the co-presence of high biochar level and nitrogen fertilizer dose in soil was accompanied by rise of the previous coefficient (NPQ). These results indicated that in this precisely described culture conditions, the dissipation of absorbed photon energy was limited and reduced and could reflect the photosynthesis apparatus protection against Cd toxicity. [29-31] explained how plants adapt their photosynthesis apparatus face to the different environmental changes and endogenous cues to maintain their photosynthetic process.

Conclusion

All results suggested a positive relationship between high biochar level, optimal nitrogen fertilization, and growth enhancement of tomato seedlings despite presence of Cd in soil. The improvement of nitrogen fertilizer availability simultaneously to high biochar amount promotes mineral nutrition and chlorophyll a content resulting in alleviation of negative Cd-induced effects on photosynthesis process.

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