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Effects of corn cob biochar on physiological responses and yield of chili peppers

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Abstract

Biochar is a porous and carbon-rich material that is used in soil reclamation to improve the quality of acidic tropical agricultural soils. It has become an important tool for enhancing agricultural productivity. This study aims to investigate how corn cob biochar (CCB) affected the development, fruit yield, and photosynthetic efficiency of chili peppers (*Capsicum annuum* L.) grown for 91 days in acidic soils with a low pH of 4.3. Two treatments, CCB and non-CCB were established in a randomized complete block design. There were six replications in each treatment. The acidic soil was treated with 37.5 t/ha of CCB, and chili peppers were grown in the experimental plots containing the acid soil with CCB applications. The application of CCB considerably ($p < 0.05$) enhanced the performance index (Pi), single photon avalanche diode (SPAD) value, and chlorophyll fluorescence (Fv/Fm); however, it did not affect the total quantity of chlorophyll and carotenoids. Moreover, CCB also enhances plant growth and fruit production by increasing the sugar content of the leaves ($p < 0.05$) after 63–84 days of amendment. After treatment, the soil pH increased from 4.3 to 5.8. These findings confirm that CCB can be used for soil reclamation in acidic soil to efficiently increase soil pH and improve chili pepper productivity.
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Keywords: Amendment, Biochar, Chili pepper, Soil acidity, Soil reclamation

1. Introduction

Soil acidity (pH < 5.5) is one of the biggest issues in the natural environment for increasing crop yield and nutrient uptake [1]. In addition to developing naturally, acid soil can also emerge from long-term agricultural practices, ongoing chemical fertilizer applications, or other factors [1]. However, pyrite (FeS_2) buildup from the acidic parent rock in the soil layer is often the source of severely acidic soil (pH less than 3.0) [2]. The pyrite can react in a cascading effect of soluble Fe^{3+}, creating even more acidity. The reaction rate is controlled by the oxidation of Fe^{2+} to Fe^{3+} in the presence of O_2, which results in the soil pH decreasing. Moreover, the process can also occur biologically via sulfate-reducing bacteria, which can grow in the soil at pH 2–3 [2]. Because of tolerance traits like aluminum (Al) and acid tolerance, several plants may thrive in acidic soils. Unfortunately, very few commercially significant crops can thrive in severely acidic soil because the acidity of the soil alters plant processes, either directly or indirectly. For instance, Zhang et al. [3] found that pH 3.0 decreased the uptake and utilization efficiency of phosphorus (P) in *Juglans regia* seedlings. Low soil pH (3.0) can reduce the water content in eucalyptus roots, stems, and leaves, as well as inhibit CO_2 assimilation in eucalyptus, including decreasing the net leaf photosynthetic rate and transpiration rate in *J. regia* [4, 5]. Furthermore, Tóth et al. [6] have shown that acidic soil pH has significant effects on wheat lipid peroxidation and antioxidative capabilities, which eventually have a detrimental impact on the grain filling period yield.

Worldwide research has been done on how acidic soils affect plant development and agricultural productivity. Still, it is a significant issue, particularly in paddy soils where environmental reclamation requires revegetation.

In Thailand, highly weathered acidic soils occupy approximately 880 thousand ha. The most important extremely acidic soils in the country are those covering 80% of the delta flat in the Lower Central Plain, which accounts for 35.6% of the total land area of the Bangkok Plain, including small areas in the Southeast Coast and Peninsular regions [7]. Variations in pH levels caused by chemical changes in soils can impact plants' physiological, biochemical, and molecular mechanisms of adaptation [8]. A fall in soil pH during the dry season or an intense drought will make the soil conditions unsuitable for plants and soil microorganisms. Moreover, with soil drying, acidity will be a more important factor in providing agricultural capacity in the area of bulk density when pores in the soil, soil organic matter, soil organic carbon, as well as organic fertility or soil biomass, are considered. To overcome and/or reduce the effect of acidic soils on plant growth, biochar application is currently being considered as a means of enhancing soil productivity, which is an important requirement for increasing crop yields and improving the quality of agricultural soil. Biochar is a vegetable substance high in carbon that is used as a soil improvement; soil reclamation is the process of returning seriously damaged land to be useable once again [9]. It can be applied to the soil to enhance soil health and promote crop yield, including water retention [9]. The addition of biochar assisted the growth of plants and the stable carbon in biochar remains sequestered for considerably longer than in the original biomass [9]. However, further research into other factors that suggest the function of photosynthesis in times of plant stress is required to have a better knowledge of how plant development may be impacted. For instance, the changes in the primary photochemistry of photosystem II (PSII) in the plant are induced by environmental stress and caused by physiological stress, especially chlorophyll (Chl) fluorescence determination [10].

Chili pepper is an economically significant vegetable in Thailand, accounting for approximately 45 million USD of the country's export earnings each year [11]. However, nothing is known about how biochar affects crop development and chili pepper production in reclaimed acid sulfate soil in terms of chlorophyll fluorescence under field circumstances. Although chili peppers can grow well in several seasons and agricultural soil with a pH value range from 5.6 to 6.8, soil acidification has direct negative effects on growth and photosynthetic performance. It is essential to raise the pH of acidic soil before planting to maximize the fruit output of chili peppers. Thus, increasing agricultural output will likewise depend on improving the acidic soil's quality [12]. Thus, this study aims to investigate the effects of high-acidity soil amendments on the photosynthetic efficiency, shoot development, and crop production of chili peppers by utilizing corn cob biochar (CCB).

2. Materials and methods

2.1 Experimental design

The present study was conducted at the experimental site in the Botanical Learning Center, Srinakharinwirot University, Amphur Ongkharak, Nakhon Nayok province, Thailand (Figures 1A–B). The CCB was made and obtained from the Phetchabun province, Thailand. In the experiment, it was homogenized to less than 2 mm and mixed with extremely acidic soil. The soil background and biochar were analyzed for characterization according to the standard methods of the Department of Soil Science, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand. Table 1 lists the physicochemical characteristics. Biochar (37.5 t/ha) was applied as an amendment to very acidic soils, whereas a control group did not receive any amendment. After soil preparation for each treatment, the soil was incubated to equilibrate for 2 weeks before planting. The chili pepper cultivar supper-hot (*C. annuum*) was used in the present study. Seeds were sown in the soil mixture (50% (w/w) coconut peat, 50% (w/w) loam soil). The seedlings were transferred to the experimental plot (2×2 m) located at 14°7'17" N and 101°0'14" E (6 replications per treatment and 25 seedlings per replication) when each seedling exhibited 8–10 leaves. Basal NPK fertilizers (16:16:16) were applied as NH_4NO_3 and KH_2PO_4 and grown in 70% shading conditions for 49 days after transplanting. Plants were irrigated twice a day and soil moisture levels were kept between 70 and 80 percent of the field capacity during the growth season. The average air temperature is between 28 and 33°C, and the mean maximum rainfall occurs between 77 and 97 days after planting (DAP), measuring 57.23±14.83 mm.

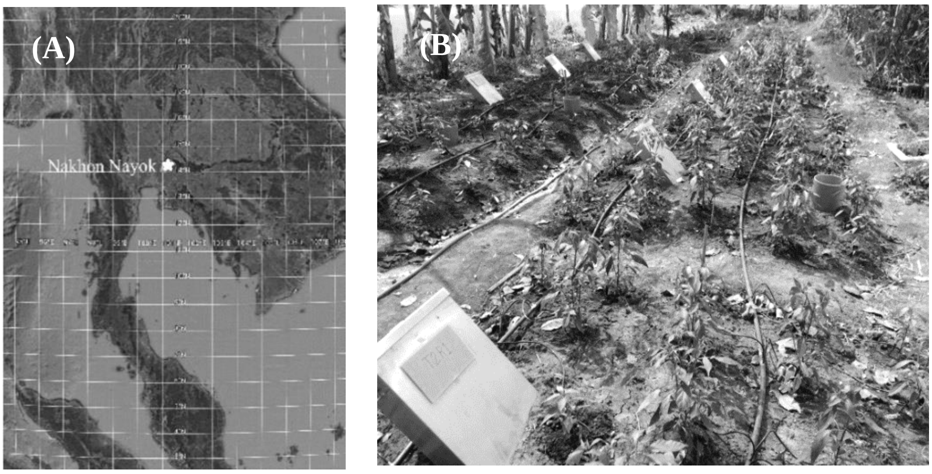

Figure 1 Location of the experimental site in the Botanical Learning Center, Srinakharinwirot University, Amphur Ongkharak, Nakhon Nayok province, Thailand (A). Field layout of a hot chili plantation applied with CCB (B).

Table 1 The physicochemical properties of extremely acidic soils and biochar made from corn cobs.

Properties	Soil	Biochar	Methods [13–15]
pH (1:5 H_2O)	4.10	10.43	pH meter (1:1 soil to water ratio)
CEC ($cmol_c$/kg)	34.20	43.33	Sodium acetate method
O.M. (%)	3.53	10.94	Walkley-Black method
Total N (%)	0.17	0.61	Kjeldahl method
Total P (%)	0.03	nd	Perchloric acid digestion
Total K (%)	0.73	nd	Hydrometer method
Total S (%)	0.16	0.70	Turbidimetric method
Total SO_4^{2-} (%)	0.48	2.10	Turbidity method
Total Ca (g/kg)	1.61	1.00	Ammonium acetate method
Total Mg (g/kg)	2.61	1.30	Ammonium acetate method
Total Mn (g/kg)	0.08	0.03	Nitric acid extraction
Total Fe (g/kg)	28.54	0.37	Nitric acid extraction
Total Al (g/kg)	89.10	0.19	McLean method
Total Zn (g/kg)	nd	0.04	Acetic acid extraction method

nd refer to not determination

2.2 Leaf greenness index, single photon avalanche diode (SPAD) and chlorophyll content determination

The leaf greenness SPAD value was measured using a SPAD-502 Plus chlorophyll meter (Konica–Minolta, Inc., Japan). The SPAD value of pepper plants was calculated by counting their third and fourth completely grown leaves from the top. Between 8:00 and 12:00 a.m., the SPAD value was recorded for each leaf four times. Each SPAD value expresses a mean of at least 8 readings per plant, with 80 readings per treatment every 7 DAP for the same plant till maturity. The 0.5 g of leaf tissue was cut into fine strips and placed in a test tube containing 5 mL of dimethyl sulfoxide (DMSO) and incubated at 25°C for 24 h in the dark. After incubation, a 3 mL aliquot was determined by spectrophotometry at 470, 649, and 664 nm. The Chl a, Chl b, and Carotenoid concentrations were determined according to the methods of Lichtenthaler and Buschmann [16].

2.3 Chlorophyll fluorescence determination

It was found that PSII's maximal quantum efficiency (Fv/Fm) and overall performance index (PI total) indicate quantum efficiency from photons absorbed by PSII to the reduction of photosystem (PS) and acceptors. The dissipation energy per active reaction center (DIo/RC) and absorption flux per RC (ABS/RC) were measured to evaluate specific energy fluxes per reaction center (RC) in PSII using a Pocket PEA chlorophyll fluorimeter (Hansatech, UK). Leaves were pinched with leaf clips for 30 min of dark adaptation. A rapid pulse of high-intensity light of 3,500 $\mu mol/m^{-2}/s$ (600 W/m^{-2}) was administered to the leaf, inducing fluorescence.

2.4 Total soluble sugar determination

Leaf samples were ground in liquid nitrogen before extracting total soluble sugar with 5 mL of 80% (v/v) ethanol for 30 min at 25°C. Following centrifugation, 0.5 mL of the supernatant and 4.5 mL of anthrone reagent were used to calculate the amount of total soluble sugar. For fifteen minutes, incubate the mixed solution at 95°C. After that, halt the reaction for ten minutes in the ice bath. According to Strasser et al., the combination solution's absorbance was measured at 620 nm [17]. By utilizing a standard glucose standard to compare the data to the standard curve, the values are given in mg/g leaf FW.

2.5 Shoot growth and fruit yield

Following 105 days of seeding, the plant was harvested, and the shoot dry weight was documented. For the fruit yield analysis, ripening fruit with a 95% red color was picked once a week. The fruit was harvested, and its fresh weight was measured. The ripening fruit was kept at 35°C for 5 days in a hot air oven to assess its dry weight.

2.6 Statistical analysis

Statistical analysis was performed using SPSS software version 23.0 (SPSS, Inc., Chicago, IL, USA). Differences among physiological parameters and treatments were analyzed using the t-test significance level of 0.05. Each value was shown as the mean ±standard error (SE).

3. Results

3.1 Photosynthetic pigment and chlorophyll fluorescence

As seen in Figures 2A and 2B, the results demonstrated that the CCB treatment did not affect the Chl a content in chili leaves, however from day 70 to day 91, it was considerably lower than the control ($p < 0.05$). Regarding Chl b, on day 70, the concentration in chili leaves was substantially greater than the control ($p < 0.05$). However, from day 77 to day 84, there was no significant change ($p > 0.05$) when compared to the control. At the end of the experiment, the Chl b content was much higher than the control.

However, the leaves of plants grown in the control treatment (solid line) showed no significant difference ($p > 0.05$) in SPAD values compared with the CCB treatment (dashed line) between 49 and 91 DAP but showed a significant difference at 70 DAP ($p < 0.05$) (Figure 2C). The Carotenoid content of both treatments consistently increased but showed no significant ($p > 0.05$) difference between treatments during 49–70 DAP. Although the carotenoid content in chili leaves treated with CCB was significantly lower than the control on day 77, there was no significant difference ($p > 0.05$) between treatments during 84–91 DAP (Figure 2D).

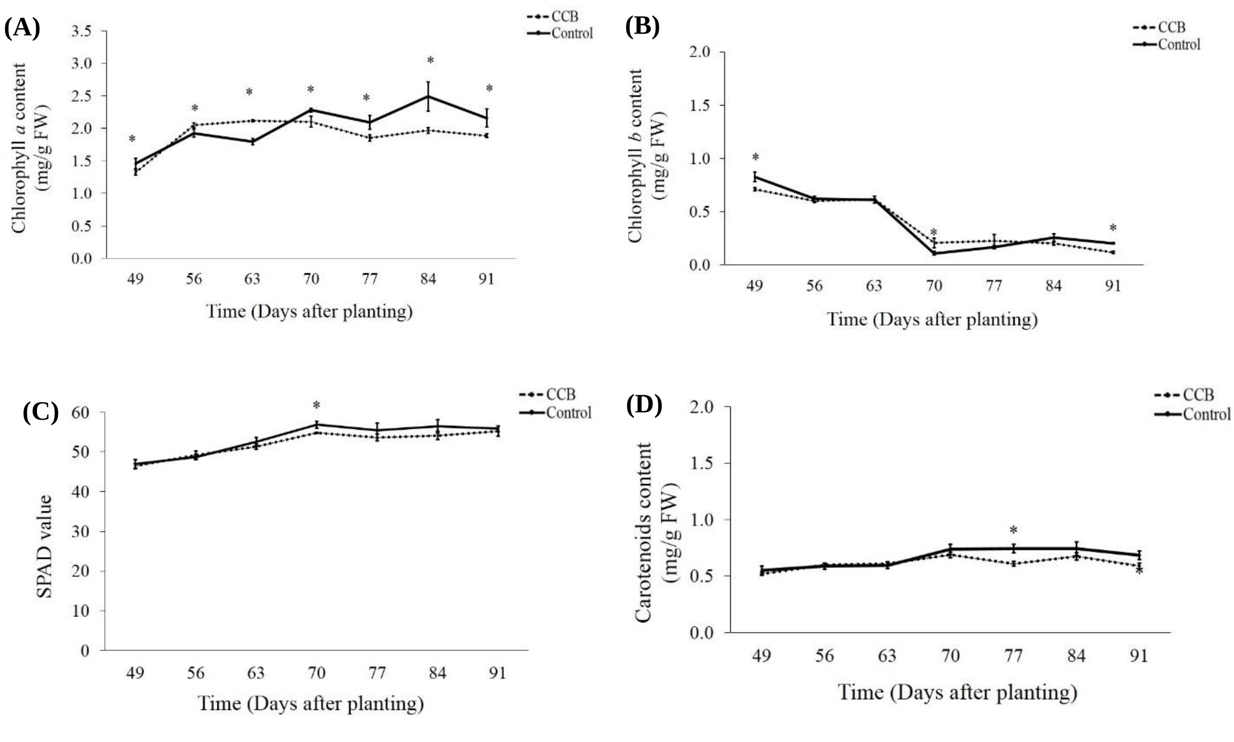

Figure 2 The effect of the acidic soil amendment containing CCB on Chl a content (A), Chl b content (B), SPAD values (C) and carotenoids content (D) in chili plant leaves. Values are the mean ±SE of each plant (n = 6). The CCB treatment was shown as a dash line and the control was shown as a solid line. The star indicates significant differences compared to control values, which were calculated using the independent sample t-test ($p < 0.05$).

The analysis of chlorophyll fluorescence in plant leaves revealed that the CCB treatment showed significantly higher ($p < 0.05$) Fv/Fm and performance index (PI) values than the control from 63 to 84 DAP (Figures 3A–B). While the results in chili plants treated with CCB were observed during the same period of the experiment, they showed a significantly lower ($p < 0.05$) rate of energy dissipated by PSII per reaction center (DIo/RC) and ABS/RC than the control (Figures 3C–D).

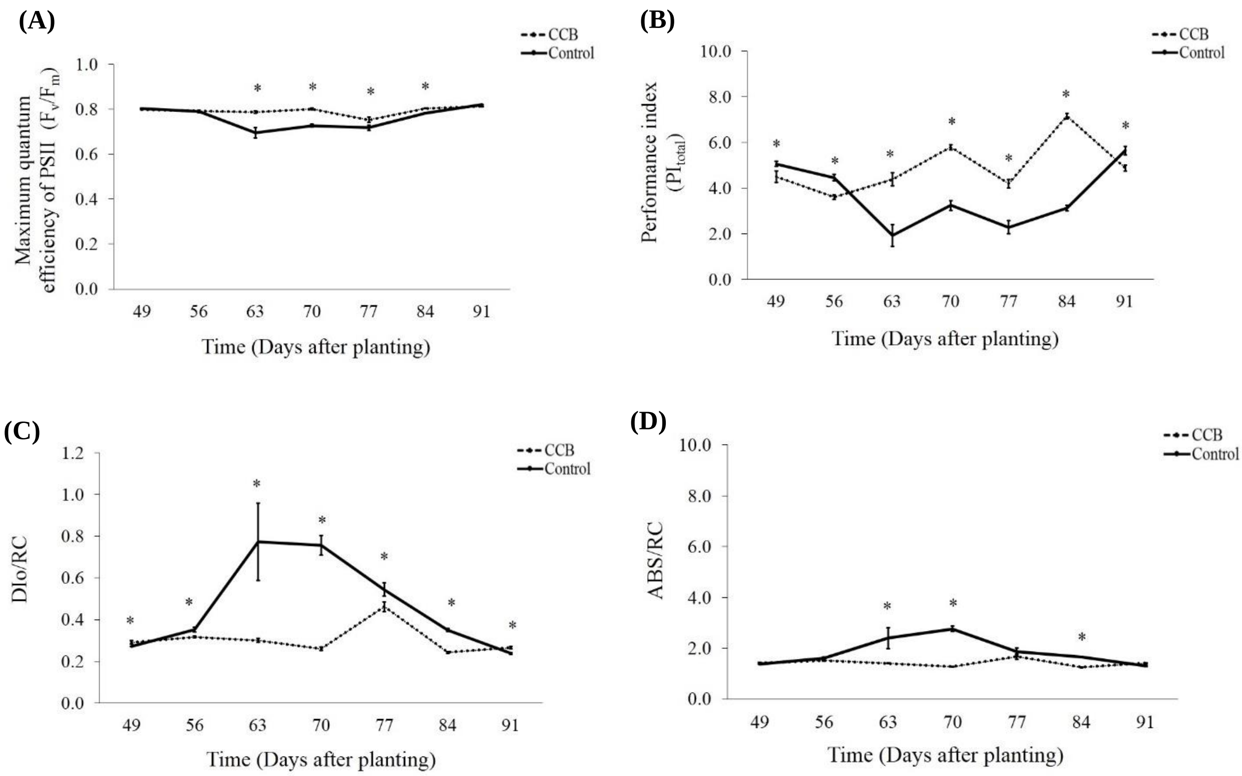

Figure 3 Maximum quantum efficiency of PSII (Fv/Fm, A), performance index (PI, B), rate of energy dissipated by PSII per reaction center (DI_0/RC, C) and absorption flux per RC (ABS/RC, D). Values are the mean ±SE of each plant (n = 6). The CCB treatment was shown as a dash line and the control was shown as a solid line. The star indicates significant differences compared to control values, which were calculated using the independent sample t-test ($p < 0.05$).

3.2 Total soluble sugar content

Figure 4 illustrates how CCB did not affect the total soluble sugar concentration of leaf tissues. After 63 days of the experiment, the total soluble sugar content in the leaves increased quickly, despite being considerably lower in both the control and CCB treatments during the first non-shading stage (49–63 DAP) with significant differences ($p < 0.05$). Furthermore, from 70 to 91 DAP, the total soluble sugar level in the CCB treatment rose considerably in comparison to the control.

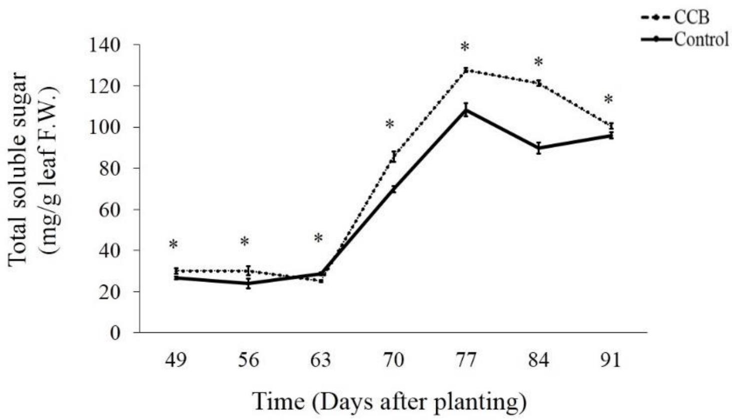

Figure 4 The effect of a highly acidic soil amendment containing CCB on total soluble sugar in plant leaves. The data is the mean of six replicates. The CCB treatment was shown as a dash line and the control was shown as a solid line. The star indicates significant differences compared to control values, which were calculated using the independent sample t-test ($p < 0.05$).

3.3 Shoot growth and fruit yield

The experiment conducted under natural growth circumstances revealed that the shoot length (82.5±5.5 g/plant) and shoot dry weight (66.9±12.0 g/plant) of the CCB treatment were considerably greater than those of the control group (Table 2). Additionally, as shown in Table 3, the fruit yield in the CCB treatment was considerably greater in both fresh weights (141.9±10.9 g/plant) and dry weights (33.9±2.9 g/plant).

Table 2 The effect of a highly acidic soil amendment containing CCB on chili pepper shoot growth 91 days after planting.

Treatments	Shoot length (cm±SE)	Shoot dry weight (g/plant±SE)
Extremely acid soil (control)	66.0±2.5	31.4±4.4
37.5 t/ha CCB	82.5±5.5	66.9±12.0
F-test	*	*

Table 3 The effect of a highly acidic soil amendment containing CCB on chili pepper fruit yield 91 days after planting.

Treatments	Fruit fresh weight (g/plant±SE)	Fruit dry weight (g/plant±SE)
Extremely acid soil (control)	93.6±8.6	24.9±2.4
37.5 t/ha CCB	141.9±10.9	33.9±2.9
F-test	*	*

4. Discussion

4.1 Photosynthetic pigment and chlorophyll fluorescence

An increase in photosynthetic pigment content in plants is the basic process for their survival and response to abiotic stresses. In the present study, Chl a and carotenoids in the leaves of the CCB treatment increased from 70 to 91 DAP. With the ability to shield plants from oxidative damage, carotenoids may prove to be vital antioxidants. Carotenoids are required for optimal development during the detoxification process of plants from the impacts of reactive oxygen species (ROS) and stress adaption [18]. When plants are grown in highly acidic soil, the increased effectiveness of plant systems in decreasing ROS levels in leaf cells is a result of carotenoids. The Chl a fluorescence-derived parameter was used to evaluate the PSII activity of the plant. The Fv/Fm ratio indicates the maximal efficiency of PSII photochemistry in chili plants. Although a reduction of Fv/Fm below 0.8 after exposure to extreme conditions in plants is commonly considered [19], the results of this study prove that plants treated with CCB had significantly higher Fv/Fm (≥ 0.80) during the growth period (63–84 days). This could be the case because, when Fv/Fm increases throughout leaf growth until it reaches the fully expanded stage, CCB contributes to signaling conditions that improve plant metabolic processes by maintaining leaf stomatal conductance. The higher values of PI between 63 and 84 DAP (4.38, 5.79, 4.19, and 7.15) also indicated higher light use efficiency and photosynthesis performance in CCB-treated plants.

Additionally, at 63–84 DAP, CCB-treated plants exhibited considerably lower DIo/RC and ABS/RC than control plants. Since photons in the control group could not be trapped by the inactive light reaction centers, an increase in the rate of energy dissipation of untrapped excitations might lead to an increase in the absorption flux per RC and the dissipation energy flux per RC. These results indicated that, in acidic soil, reaction centers were rendered inactive because they released most of the absorbed energy as heat instead of putting it to use in the photochemical quenching of photosynthesis. Full utilization of the light energy received in leaves for photosynthesis and an increase in yield was made possible by biochar, which likewise improved the activity patterns of protective enzymes and delayed the control of photosynthetic physiological activities and cytoplasmic membrane peroxidation. Similar to a previous experiment in rice (*Oryza sativa* L.) and peanut leaves (*Arachis hypogaea* L.), PSII reaction centers and photosynthetic electron transfer rates were changed by biochar application [20, 21]. Changes in the energy distribution of the photosynthetic apparatus are necessary, whereas plants exposed to acid soil have lower vulnerability [22].

Various abiotic stress conditions, especially low pH, have been discovered to have a deleterious effect on how well different plant species operate photosynthetically. It is plausible that the reduced ABS/RC ratios, which were the result of a smaller antenna, had an impact on the extent of damage to the photosystem's response center [22]. Similar results utilizing JIP-test settings have also been reported for stressed plants. High levels of Al buildup in the cells of the root apical meristem and other plant parts that develop in very acidic soil can be harmed by Al toxicity in the soil solution [23].

Water deprivation in plants is frequently shown to cause stress under physiologically extreme conditions. High DIo/RC values have often been linked to the occurrence of photoinhibition in plants, with Fv/Fm decreases occurring when the structure and function of PSII are disturbed [24]. Likewise, water stress reverses defense mechanisms, leading to damage to membranes [8]. Water stress in plants can be observed by changing the maximum quantum yield (Fv/Fm) of PSII photochemistry. The changing DIo/RC levels appeared to support the photosynthetic system's conversion of received energy into heat. Accordingly, while taking into account the decrease in electron transport and a high deviation level of absorbed light energy, our results showed stronger photoinhibition in the control [25].

Furthermore, the research examined the amount of reactive oxygen species (ROS) and the pace at which lipid peroxidation reactions occur in other leaf structures, which might account for the control group's reduced capacity for photosynthesis. Therefore, reductions in Fv/Fm imply reduced efficiency of the photochemical conversion process in plants, which may either damage or inhibit PSII activity [25]. PI_{total} values represent the energy flow efficiency of the photosynthetic transport chain. PI_{total} highly contributes to the overall growth and survival of plants under stress conditions and has been a valuable JIP test-based parameter because of its sensitivity [25]. Herbicide exposure had comparable effects in rice plants, as stated by Sousa et al. [26]. These findings demonstrated biochar's capacity to enhance photosynthesis's chlorophyll fluorescence characteristics. However, more research is required to further understand how biochar enhances the characteristics of chlorophyll fluorescence in chili plants.

4.2 Total soluble sugar content

Soluble sugar is not only the main form of photosynthesis in plants but also the main substrate for carbohydrate metabolism and temporary storage in plant cells. Our research demonstrated that soluble sugars are important in the stress response of chili peppers, with a substantial build-up of soluble sugars in plants under soil acidity stress at 63 DAP. In the case of acid soil treated with CCB, biochar might promote feedback regulation of sugar signals to enhance photosynthesis. In addition, sucrose phosphate synthase (SPS) and sucrose synthase (SS) are involved in stimulating the high production of sucrose and also increasing total soluble sugar content [27]. Likewise, by raising the transcript levels of genes encoding starch synthases, biochar amendments improved the synthesis of carbohydrates, which may raise crop output [28]. The importance of sugars was not only in the synthesis of biochemical compounds and the production of energy but also in membrane stabilization and coordination of gene expression, including signal molecule regulation. The addition of biochar improves plant health in the current study, which is intriguing since it may be crucial for maintaining plasma membrane integrity and reducing oxidative stress in stressed plants.

4.3 Shoot growth and fruit yield

Plant growth and productivity depended on soil chemical properties, including Fe solubility and plant uptake. Furthermore, soil pH is frequently a highly variable property due to the various processes of soil-plant-microorganism interactions. According to our findings, CCB raised the pH of acid soil from 4.3 to 5.8 and improved photosynthetic efficiency. Higher chlorophyll content leaves can absorb more light, which is necessary for photosynthesis. Because the high rate of photosynthesis is proportionate to the chlorophyll concentration, the more the soluble sugar content of the plants increases, the more the improvement in plant growth and production.

Not only can biochar improve soil health by functioning as a soil dietary supplement, but it also includes nutrients for plants. By lowering soil exchangeable acidity, which raises soil exchangeable base cations and base saturation, biochar can lessen soil acidity [4]. The soil pH measurement after extremely acidic soil was amended, with 37.5 t/ha of CCB, which was lower in the experiment than in the control treatment.

According to the findings, the soil pH of CCB treatments increased from 4.3 to 5.8. Increasing the soil pH had a significant impact on the root growth rate and nutrient uptake (P, K, Ca, and Mg) of plants. The low pH of extremely acidic soil very often affects the uptake of nutrients and water by plants. These findings were supported by the results of reduced water content in low pH-treated eucalyptus trees [5]. Likewise, Malkanthi et al. [29] and Dhaliwal et al. [30] stated that at a soil pH of 3.8 wheat, barley, and chili absorbed less K, Ca, and Mg, whereas cowpea plants absorbed more N, Ca, and Mg.

5. Conclusion

The control of soil acidity improved as a result of using biochar. Our findings indicate that the qualities of acid soil may be effectively enhanced by biochar. By improving soil quality, the application of CCB boosted the potential for plant growth and production, photosynthetic efficiency, PI, and SPAD values. In addition to being economically useful for crop growth in natural agricultural systems or for the rehabilitation of acid soil settings, our findings pave the way for further research aiming at comprehending and controlling acidic soil.

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