RESEARCH ARTICLE

Effects of seed pre-soaking with aqueous extract of coconut shell biochar on biochemical profile of chiltepín pepper (*Capsicum annuum* L. var. *glabriusculum*) under different light environments

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ABSTRACT

Chiltepín pepper (*Capsicum annuum* L. var. *glabriusculum* (Dunal) Heiser & Pickersgill) is a genetic resource with high economic potential; however, it shows high agronomic variability in response to the developmental environment. The aim of the present study was to investigate the effects of pre-soaking wild chiltepín seeds in a coconut shell biochar aqueous extract (BC) on the phytochemical profile (leaf and fruit) and yield of chiltepín peppers under different light environments. The BC treatments studied were 0.00%, 0.05%, 0.25%, and 0.75% (BC0, BC05, BC25, and BC75, respectively) and the light environments studied were low (LLE, 180 ± 26 µmol m^{-2} s^{-1}), medium (MLE, 437 ± 37 µmol m^{-2} s^{-1}), and high (HLE, 959 ± 49 µmol m^{-2} s^{-1}). In leaf tissue, BC25·LLE increased the total chlorophyll content by 7% and flavonoids by 13%. Vitamin C was higher in BC0·HLE (3.23 mg g^{-1} DW), while total phenols increased by 5.4% in BC25·HLE. RuBisCO presented the highest activity in BC05·MLE (126% higher than the control), whereas BC75·LLE increased phosphoenolpyruvate carboxylase (PEPC) activity by 92.9%. Fruit quality parameters such as phenols and flavonoids increased by 31.5% and 10.7% in BC25·HLE and BC05·LLE, respectively ($p < 0.05$). The BC05·MLE increased fruit production and yield by 118 % ($p < 0.05$) and 106%, respectively. Seeds pre-treated with 0.05% of BC and grown in an environment of 437 ± 37 µmol m^{-2} s^{-1} could be a reliable strategy to improve phytochemical profile and yield of chiltepin pepper.

Key words: Capsaicin, carotenoids, radiation, RuBisCO, total phenols.

INTRODUCTION

Chiltepín pepper (*Capsicum annuum* L. var. *glabriusculum* (Dunal) Heiser & Pickersgill) has high genetic diversity, is highly versatile, and is of great economic importance in Mexico. It is used as a source of nutrients, ornamental plants, and in the pharmaceutical industry (Jiménez-Viveros and Valiente-Banuet, 2023; Moreno-Contreras et al., 2024). However, inconsistencies in germination rate, morphological and genetic variability, scarce information on agricultural management, susceptibility to diseases, and little information on nutrition

and fertilization management and the impact of stresses (abiotic and biotic) (Valiente-Banuet and Gutiérrez-Ochoa, 2016; Mares-Quinones and Valiente-Banuet, 2019; Jiménez-Viveros and Valiente-Banuet, 2023) limit its productive potential.

In particular, high solar radiation intensity causes light stress in *Capsicum* plants (Fu et al., 2010) because modulating the photosynthetic properties of the canopy (Li et al., 2020) can negatively impact the growth, production, and fruit quality of chiltepín pepper (Jiménez-Leyva et al., 2022). Although wild, semi-wild, or semi-domesticated chiltepín pepper plants are highly adaptable to diverse climatic conditions (Moreno-Contreras et al., 2024), they are generally associated with low-intermediate solar radiation (100-737 μmol m^{-2} s^{-1}); therefore, high radiation (1906 $\pm$ 88 μmol m^2 s^{-1}) significantly alters the physiology and biochemistry of the plant (Jiménez-Leyva et al., 2022). This may be due to a reduction in the enzyme activity associated with photosynthesis (Li et al., 2020; Taylor et al., 2022; Amaral et al., 2024) and an increase in the production of free radicals in the chloroplast that causes oxidative stress (Halimeh, 2025).

Studies in greenhouses and/or with the use of shade nets have shown that chiltepín pepper plants respond favorably to low-intermediate light intensity conditions because they promote fruit production and quality (capsaicinoids, phenolic compounds, and flavonoids) (McCaughey-Espinoza et al., 2020; Díaz-Sánchez et al., 2021; Jiménez-Leyva et al., 2022; Jiménez-Viveros and Valiente-Banuet, 2023). However, it has been suggested that the phytochemical profile depends on the cultivar and water management of the plant (De la Cruz-Ricardez et al., 2024). However, a higher content of phenolic compounds, flavonoids, and carotenoids has been reported in chiltepín fruits grown in the open field (1635.55 μmol m^{-2} s^{-1}) compared to 35% and 70% shading (1098.3 and 586.2 μmol m^{-2} s^{-1}, respectively) (De la Cruz-Ricardez et al., 2023). This contrasting behavior was derived from the genetic diversity of chiltepín pepper. Understanding the alterations in growth, yield, and fruit quality under different light environments is important for establishing strategies to alleviate the high radiation stress in chiltepín plants.

In addition to manipulating light environments, it is necessary to integrate strategies to take advantage of the productive potential of chiltepín. In this context, biochar (BioC, solid product of pyrolysis of organic materials) as well as the aqueous extract of biochar (eBioC) can be alternatives that in addition to alleviating stress can act as biostimulants in plants (Ma et al., 2022). The use of eBioC from wheat straw and corn straw promotes vitamin C content in cabbage (Lou et al., 2016). Similarly, argan eBioC, in addition to improving germination rates, promotes chlorophyll pigments, carotenoids, and anthocyanins in sorghum (Benaceur et al., 2025).

This study establishes that the use of coconut shell biochar extract could be a strategy to promote the yield and quality of chiltepín crops in different light environments, allowing environmentally friendly management. The present study aimed to investigate the potential of pre-soaking seeds with biochar on the yield, enzymatic activity, and fruit quality of chiltepín pepper under different light environments.

MATERIALS AND METHODS

General protocol for pre-soaking and development plants

Chiltepin chili (*Capsicum annuum* L. var. *glabriusculum* (Dunal) Heiser & Pickersgill) seeds were obtained from various wild plants in the Sierra de San Bernardo (27°1'39" N, 108°56'24" W), Álamos, Sonora, Mexico, during 2022; this area has a temperature range of 23.7 to 45.4 °C and an average annual rainfall of 24 mm. Ripe red fruits (approximately 100 g) were collected and stored for 3 mo at 25 °C in a container, then washed with commercial sodium hypochlorite (20%, v/v) for 5 min, macerating manually to extract the seeds. They were rinsed three times with distilled water and finally dried at 25 °C for 30 d.

The seeds were disinfected and soaked in an aqueous extract of concentrated coconut shell biochar (BC), prepared at 0.05%, 0.25%, and 0.75% (BC05, BC25, and BC75, respectively), in addition to a control (0.00%, BC0) consisting solely of distilled water. The treatments studied (BC0, BC05, BC25, and BC75) had pH values of 5.80, 6.68, 6.45, and 8.40, respectively. Meanwhile, electrical conductivity (EC) ranged from 0.02, 0.03, 0.07, and 0.16 dS m^{-1}, respectively. Finally, the redox potential (Eh) was 76, 100, 89, and 78 mV, respectively. After 24 h, the seeds were rinsed with distilled water and sown (1st February 2023) in polystyrene trays containing 30 mL fine mixture of perlite and peat moss (1:1, v/v); the seeds were watered with a 25% Steiner solution (Steiner, 1961). At 90 d after sowing plants with a uniform height of 10 cm were selected and

transplanted on black polystyrene containers with 5 L calcareous soil (pH 8.03, EC 1.13 dS m^{-1}, 0.99% organic matter, 17.8 mg kg^{-1} NO_3^--N, 28 mg kg^{-1} Olsen-P, 477 mg kg^{-1} K^+, 2,906 mg kg^{-1} Ca^{2+}, 268 mg kg^{-1} Mg^{2+}, 32.5 mg kg^{-1} SO_4^{2-}-S, 28 mg kg^{-1} Na^+, 0.53 mg kg^{-1} B, 0.30 mg kg^{-1} Cu, 2.04 mg kg^{-1} Fe, 3.05 mg kg^{-1} Mn, 1.67 mg kg^{-1} Zn). The containers were placed in a tunnel greenhouse (tunnel type, with 720-gauge white plastic with UV additives and 30% shade) at 60 cm between plants and 1 m between rows. After transplanting and until the end of the experiment, 100% Steiner nutrient solution was applied daily. Irrigation was applied according to the water requirements of the plant and readings were maintained at 20-25 kPa using a tensiometer (Irrometer Company, Riverside, California, USA). At the time of transplanting, the plants were dipped in Captan 50% WP (Rainbow Agro Science S.A. of C.V., Jalisco, Mexico). Likewise, during crop development, mancozeb + azoxystrobin + tebuconazole (Vencedor, UPL Agro S.A. of C.V., Mexico) was sprayed at a rate of 1.5 g L^{-1}, as well as 30 mL per plant of imidacloprid (1.5 mL L^{-1}) (Bayer de México, S.A. of C.V., Mexico).

Treatments and experimental design

A 4 × 3 factorial design (four biochar extracts and three light environments) was established under a split-plot arrangement distributed in randomized blocks: Light environment (low, medium, and high) in the plot and aqueous extract of coconut shell biochar (0.00%, 0.05%, 0.25%, and 0.75%) in the subplot for a total of 12 treatments. There were 16 replicates per treatment, with the experimental unit being one plant per container (192 total plants). The light environments inside the greenhouse were generated using high-density double-filament agricultural shade netting with pigmentation and UV additives (Hydro Environment, Tlalnepantla, Estado de México, Mexico) with an embroidery of 44 × 14 threads per 6.45 cm^2 for the low-light environment (LLE) and 25 × 15 threads per 6.45 cm^2 to generate a medium-light environment (MLE). For the high-light environment (HLE), no shadow mesh was used. The CO_2 mean in the greenhouse was 390 ± 50 mg L^{-1} (Desktop Indoor Air Quality CO_2 Monitors, CEM DT-802, Shenzhen, China). The photosynthetic photon flux density (PPFD) was monitored during the experimental period using a photosynthetically active radiation meter (PAR Light Scout Quantum Model 3415A, Spectrum, Technologies, Aurora, Illinois, USA) (Figure 1).
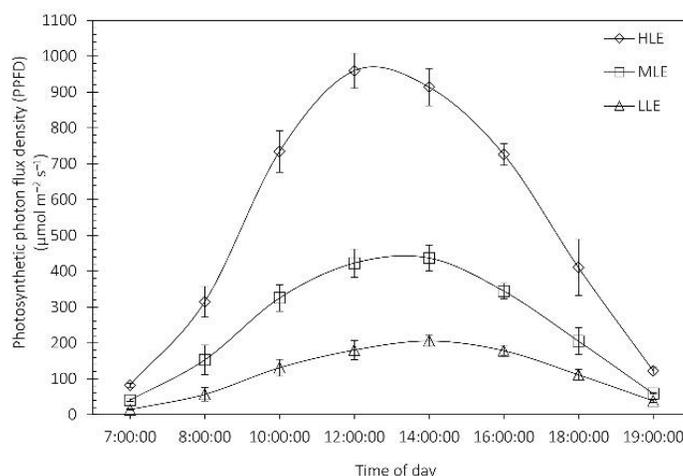

Figure 1. Dynamics of photosynthetic photon flux density (PPFD) in different light environments during the experiment. Vertical bars correspond to standard error. HLE: High-light environment; MLE: medium-light environment; LLE: low-light environment.

Leaf tissue and fruit sampling

During the BBCH-808 phase (Feldmann and Rutikanga, 2021) (173 d after transplanting), sampling of fully expanded young leaves and two fully red fruit sections was carried out. The general BBCH scale (Biologische Bundesanstalt, Bundessortenamt, and Chemical industry) allows codes to be established categorically that describe chili growth, which harmonizes agricultural practices. Leaf tissue and fruit sections (placed at -20 °C

and freeze-dried; model ECO-FD10PT, Biobase Meihua Trading, Shandong, China) were used to determine leaf and fruit quality traits, respectively. Fruit sections stored at 4 °C were used to determine fruit morphometry.

Leaf quality traits

Photosynthetic pigments (chlorophyll, Chl, *a* and *b*) were determined following the methodology cited by Nagata and Yamashita (1992); absorbances at 453, 505, 645 and 663 nm were taken in a spectrophotometer (ME-UV1800; MesuLab Instruments, Guangzhou City, China). Yellow (β-carotene, β-cryptoxanthin, and zeaxanthin) and red (capsanthin and capsorubin) carotenes were determined by the technique described by Hornero-Méndez and Minguez-Mosquera (2001), using hexane:acetone (3:2) as an extractant mixture and reading at 472 and 508 nm.

Ribulose 1,5-bisphosphate carboxylase activity (RuBisCO, EC 4.1.1.39) was assayed as described by Usuda (1985) and Khan et al. (2015), by monitoring nicotinamide adenine dinucleotide (NADH) oxidation at 340 nm for 1 min. The enzymatic activity of phosphoenolpyruvate carboxylase (PEPC, EC 4.1.31) was quantified following the methodology cited by Sun et al. (2012) and Studer et al. (2016), which contained 2 mM phosphoenolpyruvate and was determined from the NADH straight-line equation at 340 nm. Finally, β-carbonic anhydrase (β-CA) activity was estimated according to the Wilbur Anderson method (Makino et al., 1992; Mitra et al., 2019) by monitoring the change in pH from 8.3 to 6.3.

The vitamin C content (mg g^{-1} DW) was quantified using 2-6 dichlorophenolindophenol as an indicator at 515 nm (Hung and Yen, 2002). Briefly, 1 mL metaphosphoric acid (1%, w/v) was added to 10 mg freeze-dried tissue, filtered, and 75 μL collected, then 675 μL 2-6 dichlorophenolindophenol (50 μM) was added, reacting at room temperature for 15 s. Quantification was performed by spectrophotometry at 515 nm. The results were obtained from the equation of the straight line prepared with ascorbic acid (0-50 mg L^{-1}) and expressed in mg g^{-1} dry weight of ascorbic acid. Flavonoids were quantified following the methodology suggested by Arvouet-Grand et al. (1994), using methanol as the extractant solution and measuring the absorbance at 415 nm. Briefly, 20 mg tissue were weighed and 800 μL methanol (reagent grade) were added, then shaken for 30 s and filtered with Whatman No. 1 paper; 500 μL were taken and 1 mL 2% aluminum trichloride ($AlCl_3$) methanolic solution was added, allowing it to react for 20 min in the dark. The reading was taken in a spectrophotometer at 415 nm. The flavonoid content was expressed in milligrams of quercetin equivalent per 100 g dry weight (mg EQ 100 g^{-1} DW) by extrapolating the absorbances in the quercetin straight line equation (0-30 mg L^{-1}). Finally, total phenols were quantified using the Folin-Ciocalteu reagent, and the absorbance was determined at 750 nm (Singleton et al., 1999). Briefly, 20 mg tissue were taken and 400 μL water:acetone solution (1:1) were added, vortexed for 30 s, and sonicated for 5 min, then centrifuged (ST 16R, Thermo Scientific, Langenselbold, Germany) at 12 500 rpm for 10 min at 4 °C. Finally, the phenol content was tested by taking 12.5 μL supernatant, 50 μL Folin-Ciocalteu reagent, 125 μL Na_2CO_3 (20%), and 1.25 mL cold distilled water, vortexing (30 s), and placing in a water bath at 45 °C for 30 min. Finally, the absorbance was measured at 750 nm in a spectrophotometer. The results were expressed in milligrams of gallic acid equivalent per 100 g dry weight (mg EGA 100 g^{-1} DW), based on the gallic acid calibration curve (0-30 mg L^{-1}).

Fruit quality traits

The entire fruit (including pericarp, seeds, and placenta) was considered when determining fruit quality parameters. The contents of red and yellow carotenoids, vitamin C, phenols, and flavonoids were determined in freeze-dried fruits following the previously mentioned methodologies. The capsaicin content was determined following the method described by Palma-Orozco et al. (2021), with some modifications. This spectrophotometric method yields results that are significantly consistent with HPLC quantification. Briefly, 20 mg lyophilized tissue were added to an Eppendorf tube, and 1 mL methanol:ethanol:water solution (6:2:2, v/v) was added, shaken until homogenized, and sonicated (Sonicator Baku BK 2000, Guangzhou, Guangdong, China) for 20 min. The mixture was then centrifuged (10 min at 15 000 rpm at 4 °C; Microcentrifuge Frontier FC5515R, Ohaus Corporation, Parsippany, New Jersey, USA), the supernatant was recovered, and 12 mg activated charcoal was added and mixed for 10 min. The mixture was filtered (polytetrafluoroethylene, PTFE, 0.45 μm) and the absorbance was immediately quantified at 286 nm. Capsaicin content (μg·g^{-1} DW) was estimated from the capsaicin standard line equation (Sigma-Aldrich, St. Louis, Missouri, USA).

The fruits stored at 4 °C were homogenized to obtain a semi-liquid paste (1 g fruit:5 mL H_2O deionized) that was placed in a flask. The pH, EC (HI98130; Hanna Instruments, Woonsocket, Rhode Island, USA), and Eh (ORP-200; HM Digital, Los Angeles, California, USA) were quantified on this paste. Likewise, drops of the paste were taken and the total soluble solids (TSS, °Brix) were determined using a refractometer (HI96801; Hanna Instruments).

Morphometry and yield traits

To determine yield quality, fruit length from the tip to the base of the peduncle (equatorial diameter, ED) and fruit width (polar diameter, PD) were quantified using digital calipers (SureBilt Best Parts, Memphis, Tennessee, USA). The roundness index (RI) per fruit was calculated as the length-to-width ratio (IPGRI, AVRDC, CATIE, 1995). The fresh fruit weight (FWF) was determined with an analytical balance (Ohaus PA224; Ohaus Corporation, Parsippany, New Jersey, USA). The total fruit harvested per plant (TF) and yield per plant (Y) were also quantified.

Statistical analysis

To determine the differences between the doses of the biochar extract and the levels of light intensity, data were subjected to assumptions of normality, followed by two-way ANOVA followed by the least significant difference test ($p < 0.05$). All tests were performed using InfoStat v19 software (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina).

RESULTS AND DISCUSSION

The maximum radiation in different light environments occurred between 12:00 and 14:00 h, ranging between 180 ± 26, 437 ± 37, and 959 ± 49 µmol m^{-2} s^{-1} for low (LLE), medium (MLE), and high light environments (HLE), respectively (Figure 1). The open-field radiation was 1954 ± 101 µmol m^{-2} s^{-1}. The photosynthetically active radiation (PAR) of the treatments was within the ranges reported by Jiménez-Leyva et al. (2022) and Jiménez-Viveros and Valiente-Banuet (2023). It has been suggested that in chiltepín peppers, the light environment plays a fundamental role in the yield and biosynthesis of secondary metabolites in leaves and fruits (Jiménez-Viveros and Valiente-Banuet, 2023; Halimeh, 2025), as a mechanism for modulating light stress.

The germination percentage associated with treatments BC0, BC05, BC25, and BC75 ranged from 67.0%, 80.9%, 71.7%, and 68.5%, respectively. Meanwhile, analysis by Fourier transform infrared spectrometer (FTIR) with attenuated total reflectance (ATR) Frontier (Frontier FT-IR/NIR, PIKE Technologies, Madison, Wisconsin, USA) equipped with ATR MIRacle Diamond Frontier of the BC showed the presence of alkenes, cyclic alkenes, alkynes, alcohols, phenols, and alkyl compounds.

Leaf quality traits

The biochemical parameters of the leaf tissue of chiltepín plants showed significant responses (Table 1) because quality and quantity of solar radiation modulate the photosynthetic properties and biosynthesis of secondary metabolites in the plant (Li et al., 2020; Jiménez-Viveros and Valiente-Banuet, 2023; Halimeh, 2025). Chlorophyll content (*a*, *b*, and total) decreased in plants whose seeds were not subjected to coconut shell biochar aqueous extract (BC) pre-germinative treatment, in greater proportions under MLE and HLE. In contrast, BC25 treatment in LLE increased chlorophyll content, whereas the chlorophyll *a/b* ratio was higher in 0.25% BC (BC25) and 0.75% BC (BC75) under HLE. Shading environments (LLE) alter the light spectrum, which affects biosynthesis, chlorophyll content, and chlorophyll *a/b* ratio (De la Cruz-Ricardez et al., 2023). Therefore, alterations in photosynthetic pigments act as indicators of photosynthetic capacity (Chen et al., 2021) and reflect the physiological response of chiltepín plants to light. The promotion of chlorophyll in response to biochar extracts (from argan) has also been reported in sorghum plants under saline conditions (Benaceur et al., 2025).

In the case of β-carotene, a significant reduction (up to 58%) was documented under a high-light intensity environment without differences between BC treatments, and the highest values for this variable were observed under medium radiation intensity. Similarly, yellow and red carotenes showed a considerable reduction in the 0.00% BC (BC0)·HLE and BC0·MLE treatments. This response may be due to the fact that carotenes, as accessory pigments, respond to light conditions. Benaceur et al. (2025) reported the promotion of carotenes as an anti-stress response in sorghum plants under salinity when using argan BioC extracts.

Table 1. Leaf quality traits in chiltepín pepper plants grown in different light environments whose seeds were previously soaked with biochar (BioC). LE: Light environment; LLE: low-light environment; MLE: medium-light environment; HLE: high-light environment; BC: aqueous extract of coconut shell biochar; BC0: 0.00% BioC; BC05: 0.05% BioC; BC25: 0.25% BioC; BC75: 0.75% BioC; Ca: chlorophyll a (mg 100 g^{-1} DW); Cb: chlorophyll b (mg 100 g^{-1} DW); Ct: total chlorophyll (mg 100 g^{-1} DW); Ca/b: Ca/Cb ratio; βc: β-carotene (mg 100 g^{-1} DW); YC: yellow and red carotenes (mg 100 g^{-1} DW); VC: vitamin C (mg g^{-1} DW); TP: total phenols (mg GAE 100 g^{-1} DW); F: flavonoids (mg QE 100 g^{-1} DW); GAE: gallic acid equivalents; QE: quercetin equivalent. Different letters within the same column show significant differences at LSD test ($p < 0.05$).

Treatments	Ca	Cb	Ct	Ca/b	βC	YC	VC	TP	F
BC0·LLE	21.71[ab]	22.01[ab]	43.72[ab]	0.99[e-g]	133.50[a]	65.78[b]	1.54[g]	7.16[de]	49.96[b]
BC0·MLE	12.94[c]	13.93[e]	26.87[c]	0.91[g]	96.91[b]	20.22[e]	1.71[fg]	7.98[cd]	46.27[bc]
BC0·HLE	18.78[b]	19.65[a-c]	38.43[b]	0.96[fg]	67.81[c]	16.66[e]	3.23[a]	9.26[ab]	31.48[ef]
BC05·LLE	21.62[ab]	20.72[a-c]	42.34[ab]	1.04[d-f]	137.96[a]	59.25[bc]	2.05[d-f]	7.80[cd]	47.59[bc]
BC05·MLE	21.08[ab]	19.00[b-d]	40.08[ab]	1.11[cd]	140.96[a]	46.61[b-d]	2.55[bc]	8.67[bc]	43.75[c]
BC05·HLE	21.74[ab]	18.14[cd]	39.88[b]	1.20[bc]	66.99[c]	31.65[de]	2.63[b]	9.43[ab]	30.43[f]
BC25·LLE	24.10[a]	22.36[a]	46.46[a]	1.08[de]	131.65[a]	87.18[a]	2.63[b]	6.52[e]	56.48[a]
BC25·MLE	19.19[b]	19.47[a-c]	38.66[b]	0.99[e-g]	141.73[a]	29.56[de]	2.79[b]	7.20[de]	46.67[bc]
BC25·HLE	22.15[cb]	16.17[de]	38.32[b]	1.37[a]	58.96[c]	43.60[cd]	2.42[b-d]	9.76[a]	34.85[de]
BC75·LLE	21.91[ab]	21.71[ab]	43.62[ab]	1.01[d-g]	137.26[a]	62.15[bc]	2.21[c-e]	6.33[e]	31.44[ef]
BC75 MLE	19.60[b]	19.65[a-c]	39.26[b]	1.00[d-g]	139.69[a]	36.03[de]	1.96[ef]	8.20[c]	43.70[c]
BC75 HLE	23.71[a]	18.30[cd]	42.02[ab]	1.30[ab]	64.70[c]	42.84[cd]	2.02[d-f]	9.28[ab]	37.77[d]
BioC	0.0005	0.5528	0.0254	< 0.0001	0.0378	0.0116	0.0001	0.0300	< 0.0001
LE	0.0001	< 0.0001	0.0001	< 0.0001	< 0.0001	< 0.0001	0.0001	< 0.0001	< 0.0001
BioC×LE	0.0511	0.0065	0.0310	0.0002	0.0068	0.0261	< 0.0001	0.0718	< 0.0001

Vitamin C in foliar tissue showed a particular behavior; the highest content ($p < 0.05$) was documented in plants grown at high-intensity (BC0·HLE), whereas the rest of the treatments presented a reduction of up to 52%. This response contrasts with data obtained from foliar applications of aqueous BioC extracts of wheat straw and corn straw in cabbage, where vitamin C content was promoted (Lou et al., 2016); this discrepancy may be due to the fact that in our study the response is dependent on the light environment. Similarly, total phenols showed a significant increase in high-intensity light environments regardless of the pre-germinative treatment with BC, this may be due to the fact that phenolic compounds have the ability to limit chlorophyll excitation in conditions of high solar radiation (Hassanpour and Hassanpour, 2021), which would imply a protective function in the presence of high solar radiation. In the case of flavonoids, significant accumulation was found in LLE and MLE, being promoted by the BC25·LLE treatment, which represented 11.5% more than the treatment without pre-soaking in BC under the same light environment (Table 1).

Regarding the enzymes associated with the photosynthetic process, the analyses showed that RuBisCO activity (Figure 2a) was higher in MLE; likewise, the pre-soaking of seeds in 0.05% BC (BC05) promoted this enzyme. Maximum RuBisCO activity (≈ 100 µmol CO_2 fixed mg^{-1} min^{-1}) was documented in plants treated with BC05·MLE, followed by BC05·HLE. In LLE, nonsignificant alterations were observed. RuBisCO activity was extremely low (≈ 20 µmol CO_2 fixed mg^{-1} min^{-1}) in non-BC treated plants grown under HLE. RuBisCO is a key enzyme in photosynthesis because it controls the rate of C fixation (Li et al., 2020), net photosynthesis, and crop productivity (Taylor et al., 2022), which is reflected in the decrease in fruit yield per plant (Table 2). Maximum values found in plants of the BC05·MLE treatment can be explained because RuBisCO is strongly regulated by adjustments in the chloroplast stromal environment as a function of light and temperature variations (Amaral et al., 2024; Halimeh, 2025) so that in such an environment (437 ± 37 µmol m^{-2} s^{-1}) RuBisCo activase promoted RuBisCo carbamylation due to adequate tilacoidal electron transfer that allowed ATP synthesis (Amaral et al., 2024). In contrast, the minimal RuBisCO activity in LLE (180 ± 26 µmol m^{-2} s^{-1}) may be due to the rapid decarbamylation of RuBisCO due to low ATP synthesis in the thylakoid (Taylor et al., 2022).
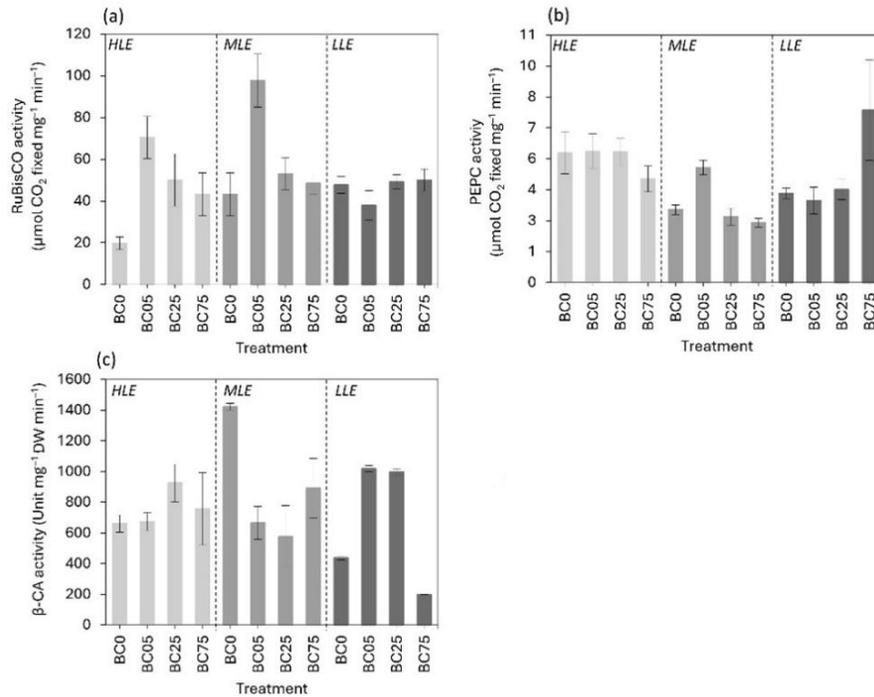
Figure 2. RuBisCO (a), phosphoenolpyruvate carboxylase (PEPC) (b) and β-carbonic anhydrase (β-CA) (c) activity in chiltepín pepper plants grown in different light environments whose seeds were previously soaked with biochar. Vertical bars correspond to standard error. n = 5. LLE: Low-light environment; MLE: medium-light environment; HLE: high-light environment; BC: aqueous extract of coconut shell biochar (BioC); BC0: 0.00% BioC; BC05: 0.05% BioC; BC25: 0.25% BioC; BC75: 0.75% BioC.

Table 2. Morphometry and yield traits of chiltepín pepper fruits developed in different light environments whose seeds were previously soaked with biochar (BioC). LE: Light environment; LLE: low-light environment; MLE: medium-light environment; HLE: high-light environment; BC: aqueous extract of coconut shell biochar; BC0: 0.00% BioC; BC05: 0.05% BioC; BC25: 0.25% BioC; BC75: 0.75% BioC; ED: equatorial diameter; PD: polar diameter; RI: roundness index; FWF: fresh weight per fruit; TF: total fruits; Y: yield per plant. Different letters within the same column show significant differences at LSD tests ($p < 0.05$).

Treatments	ED	PD	RI	FWF	TF	Y
	mm	mm		g		g plant^{-1}
BC0·LLE	7.62[e]	6.59[b]	0.87[c]	244.72[c-e]	188.83[b-d]	53.07[bc]
BC0·MLE	7.80[c-e]	7.02[b]	0.90[a-c]	226.56[de]	132.67[c-e]	40.42[c-e]
BC0·HLE	7.93[b-e]	7.39[ab]	0.93[a-c]	190.56[e]	37.75[f]	17.63[e]
BC05·LLE	8.54[ab]	7.82[ab]	0.91[a-c]	387.93[a]	144.67[c-e]	55.75[bc]
BC05·MLE	8.56[ab]	8.55[a]	1.00[a]	391.23[a]	289.50[a]	83.21[a]
BC05·HLE	8.48[ab]	7.47[ab]	0.88[bc]	285.22[b-d]	113.42[d-f]	34.78[c-e]
BC25·LLE	8.87[a]	8.49[a]	0.96[a-c]	344.17[ab]	232.92[ab]	74.33[ab]
BC25·MLE	7.70[de]	7.66[ab]	1.00[ab]	283.90[b-d]	196.33[bc]	55.62[bc]
BC25·HLE	8.34[a-c]	7.83[ab]	0.90[a-c]	344.54[ab]	80.75[ef]	34.99[c-e]
BC75·LLE	8.31[a-d]	7.46[ab]	0.90[a-c]	317.18[a-c]	231.00[ab]	72.09[ab]
BC75·MLE	8.40[a-c]	7.19[b]	0.86[c]	317.34[a-c]	174.33[b-d]	44.94[cd]
BC75·HLE	8.32[a-d]	7.83[ab]	0.93[a-c]	285.96[b-d]	73.42[ef]	27.48[de]
BioC	0.0007	0.0286	0.2939	< 0.0001	0.0485	0.0162
LE	0.3620	0.9921	0.5323	0.0953	< 0.0001	< 0.0001
BioC×LE	0.0544	0.3023	0.1953	0.2507	0.0091	0.0635

This agrees with Fu et al. (2010), who reported that *Capsicum* presents better photosynthetic efficiency in environments with intermediate incident radiation (450-500 μmol m^{-2} s^{-1}), and with Jiménez-Leyva et al. (2022) who reported that chiltepín plants seem to be physiologically acclimatized to low and intermediate irradiance (90-737 μmol m^{-2} s^{-1}), where greater biomass gain is promoted with an investment of 14% to develop fruit; that is, there is a promotion of vegetative growth that promotes fruit load and fruit production (Valiente-Banuet and Gutiérrez-Ochoa, 2016), which agrees with the trend found in this study, where there was higher fruit production in MLE (Table 2). However, the studies cited did not directly quantify light intensity but only suggest which light environment may promote the growth and development of chiltepín plant. Furthermore, since this species requires host plants throughout its life cycle, it is possible that some of the phenological results observed are, at least partially, due to the fact that no host plants were used in this study.

Phosphoenolpyruvate carboxylase (PEPC) activity (Figure 2b) was high in plants grown in HLE and LLE environments. The BC75 treatment at LLE promoted the highest activity (≈ 7 μmol CO_2 fixed mg^{-1} min^{-1}). Alterations in PEPC activity appear to reflect the activation of complementary pathways in C fixation, modulation of cellular respiration, and/or the induction of anaplerotic C fluxes in the Calvin-Benson-Bassham cycle. These mechanisms appear to maintain adequate levels of photosynthetic activity and redox balance in both low and high light intensity conditions (Wieloch et al., 2022). In the case of β-carbonic anhydrase (β-CA), the highest activity was documented in MLE and HLE but not in plants whose seeds were not treated with BC (Figure 2c). Similarly, plants untreated with BC and grown under MLE presented higher activity (≈ 1400 unit mg^{-1} DW min^{-1}), followed by BC75 treatment in the same environment. The β-CA activity is associated with the conversion of CO_2 to carbonic acid (H_2CO_3), and then to bicarbonate anions (HCO_3^-) (Amaral et al., 2024), which could reflect an adaptation of the sub-stomatal C sequestration mechanisms to promote photosynthesis in chiltepín plants.

Fruit quality traits

The fruit quality parameters showed significant responses to different seed pre-soaking treatments and light conditions (Table 3). The light environment altered the conditions of pH (LLE), Eh (MLE), total soluble solids (TSS) (LLE), and yellow carotene (HLE) in fruit. Likewise, fruits from seeds pretreated with BC25 altered ($p <$ 0.05) all parameters, except TSS.

Table 3. Quality traits of chiltepín pepper fruits developed in different light environments whose seeds were previously soaked with biochar (BioC). LE: Light environment; LLE: low-light environment; MLE: medium-light environment; HLE: high-light environment; BC: aqueous extract of coconut shell biochar; BC0: 0.00% BioC; BC05: 0.05% BioC; BC25: 0.25% BioC; BC75: 0.75% BioC; EC: electrical conductivity; Eh: redox potential; TSS: total soluble solids; RC: red carotenoids; YC: yellow carotenoids. Different letters within the same column show significant differences at LSD tests ($p <$ 0.05).

Treatments	pH	EC	Eh	TSS	RC	YC
		dS m^{-1}	mV	°Brix	mg g^{-1} DW	mg g^{-1} DW
BC0·LLE	4.81[bc]	1.10[d]	151.4[cd]	0.88[a]	94.23[de]	6.64[ef]
BC0·MLE	4.78[cd]	0.86[f]	151.4[cd]	0.56[d]	100.80[b-d]	11.53[b-d]
BC0·HLE	5.02[a]	1.34[a]	147.2[f]	0.86[a]	90.21[e]	14.53[ab]
BC05·LLE	4.77[c-e]	1.24[ab]	153.6[b]	0.82[ab]	94.69[c-e]	6.93[d-f]
BC05·MLE	4.73[de]	1.10[d]	153.0[bc]	0.76[a-c]	95.63[c-e]	8.87[de]
BC05·HLE	4.98[a]	0.98[e]	145.8[f]	0.68[cd]	104.77[a-c]	14.30[ab]
BC25·LLE	4.72[de]	0.96[ef]	133.4[g]	0.84[ab]	114.15[a]	4.09[f]
BC25·MLE	4.85[b]	1.11[cd]	151.4[cd]	0.82[ab]	103.98[a-d]	9.29[c-e]
BC25·HLE	4.81[bc]	1.06[de]	147.6[ef]	0.72[bc]	108.84[ab]	16.95[a]
BC75·LLE	4.71[e]	1.00[de]	133.6[g]	0.78[a-c]	107.88[ab]	15.28[ab]
BC75·MLE	4.72[de]	1.22[bc]	156.2[a]	0.82[ab]	100.75[b-d]	5.95[ef]
BC75·HLE	4.76[c-e]	0.98[e]	149.4[de]	0.78[a-c]	94.70[c-e]	13.66[a-c]
BioC	< 0.0001	0.1770	< 0.0001	0.5766	0.0002	0.6176
LE	< 0.0001	0.7614	< 0.0001	0.0110	0.4431	< 0.0001
BioC×LE	< 0.0001	< 0.0001	< 0.0001	0.0001	0.0132	0.0003

The BC-treated fruits from different light environments consistently showed lower pH (acidic), specifically in BC75·LLE, BC75·MLE, and BC25·LLE. It appears that chiltepín fruits harvested from plants under greenhouse conditions tend to have pH < 6.0 (McCaughey-Espinoza et al., 2020) values that agree with those found here (Table 3); similarly, they are below those reported by Díaz-Sanchez et al. (2021), who cite fruit pH values between 5.1 and 5.6.

The same trend was observed for EC, with the lowest values found for BC0·MLE, BC05·HLE, BC25·LLE, and BC75·HLE. The Eh was altered ($p < 0.05$) in response to the light environment, BioC pretreatment, and interaction; in that sense, LLE conditions promoted reduced values (≈ 143 mV), while soaking with BC25 induced ≈ 144 mV. Consistently, reductions of 11.8% and 11.7% were documented in the Eh of the BC25·LLE and BC75·LLE interactions, respectively, compared with the respective control treatment (Table 3). The TSS showed a considerable increase ($p < 0.05$) under the BC25·LLE and BC75·LLE treatments. Several reports have mentioned that chiltepín fruits from plants grown in greenhouses show lower acidity (% citric acid) (McCaughey-Espinoza et al., 2020), ranging from 2.0 °Brix (Díaz-Sánchez et al., 2021), which is higher than that reported here. The TSS values increase as the fruit ripens; therefore, a higher TSS content would mean better fruit quality, as these sugars would act as substrates during the activation of cellular respiration (Díaz-Sánchez et al., 2021).

Red carotenes showed a high content under the BC25·LLE treatment, whereas yellow carotenes responded significantly to high-light environments, increasing up to 73.3% (particularly in BC25·HLE) with respect to MLE and LLE (Table 3). Light conditions affect carotenoid biosynthesis (Halimeh, 2025) by altering the conversion of α-carotene or β-carotene through several reactions to phytoene and lycopene from geranylgeranyl pyrophosphate (GGPP) (De la Cruz-Ricardez et al., 2024). Carotenoid content in chiltepín fruits was higher under high radiation (1635.55 µmol m^{-2} s^{-1}) with respect to lower radiation (1098.3 and 586.2 µmol m^{-2} s^{-1}) (De la Cruz-Ricardez et al., 2023). Interestingly, it has been documented that irradiance > 1000 µmol m^{-2} s^{-1} could reduce carotenoid content in fruit (Jiménez-Viveros and Valiente-Banuet, 2023).

Figure 3a shows that the capsaicin content in chiltepín fruits decreased significantly in LLE, particularly with BC75. In HLE, BC05 increased capsaicin content by 66% compared to untreated seeds. In the case of MLE environment, more stable values are documented. High capsaicin content indicates the antioxidant activity of the fruit (Halimeh, 2025). The data found here show a trend similar to that reported by De la Cruz-Ricardez et al. (2024), who reported a higher concentration of capsaicin in plants under open sky conditions with respect to environments with 70% shading. However, they contrast with Diaz-Sanchez et al. (2021), who reported a substantial increase in the pungency of chiltepín fruits in greenhouses (29 485 Scoville heat units, SHU; 57% capsaicin and 43% dihydrocapsaicin) with respect to fruits collected in the field (6114 SHU; 40% capsaicin and 60% dihydrocapsaicin). The same authors pointed out a higher probability of *Capsicum* fruits synthesizing more capsaicinoids under favorable conditions. Similarly, red fruits grown in a black-shaded environment (650-850 µmol m^{-2} s^{-1}) promoted high levels of capsaicinoids (Jiménez-Viveros and Valiente-Banuet, 2023). These discrepancies may be due to the fact that capsaicin synthesis is affected by light environments and by the genetic variation of the chiltepín plant (Díaz-Sánchez et al., 2021; Jiménez-Viveros and Valiente-Banuet, 2023; De la Cruz-Ricardez et al., 2024; Halimeh, 2025); however, other biotic stressors (pests, diseases) and abiotic stressors (water deficit, drought, salinity, alkalinity, microplastics, among others) can also alter its synthesis.

For vitamin C content, there were nonsignificant modifications in light environments, but it can be seen that BC75 promoted this biocompound in greater proportions in LLE (Figure 3b). Total phenols showed a positive response to light environments and pre-soaking with BC, with BC25·HLE and BC75·MLE being the treatments with the highest accumulation of these compounds (Figure 3c). This response is contrast to that reported by Jiménez-Viveros and Valiente-Banuet (2023), who found a higher content of total phenolic compounds in the fruits of plants under shading (650-850 µmol m^{-2} s^{-1}). The accumulation of total phenolics in fruits may be a protective response to high solar radiation and reflect high antioxidant capacity, as these biomolecules have the ability to reduce oxidative stress by scavenging reactive oxygen species (ROS) (Hassanpour and Hassanpour, 2021). The profile of phenolic compounds varies among genotypes and fruit maturity stages; however, they are significantly affected by the level of light, mainly red and blue radiation (De la Cruz-Ricardez et al., 2024; Halimeh, 2025).

Finally, flavonoids responded positively to LLE and MLE and pre-soaking in BC25 and BC75 (Figure 3d). Like phenols, flavonoids are significantly affected by light environments and play an important role in fruits by acting

as antioxidants (De la Cruz-Ricardez et al., 2024). Both phenolic compounds and flavonoids are synthesized from the shikimate pathway with phenylalanine or tyrosine as intermediates and present a protective function in the photosynthetic process (De la Cruz-Ricardez et al., 2024), which explains their high content in the fruit. According to De la Cruz-Ricardez et al. (2023) the content of phenolic compounds and flavonoids in chiltepín fruits increased when grown under open sky conditions (1635.55 µmol m^{-2} s^{-1}) with respect to 35% and 70% shading meshes (1098.3 and 586.2 µmol m^{-2} s^{-1}, respectively).
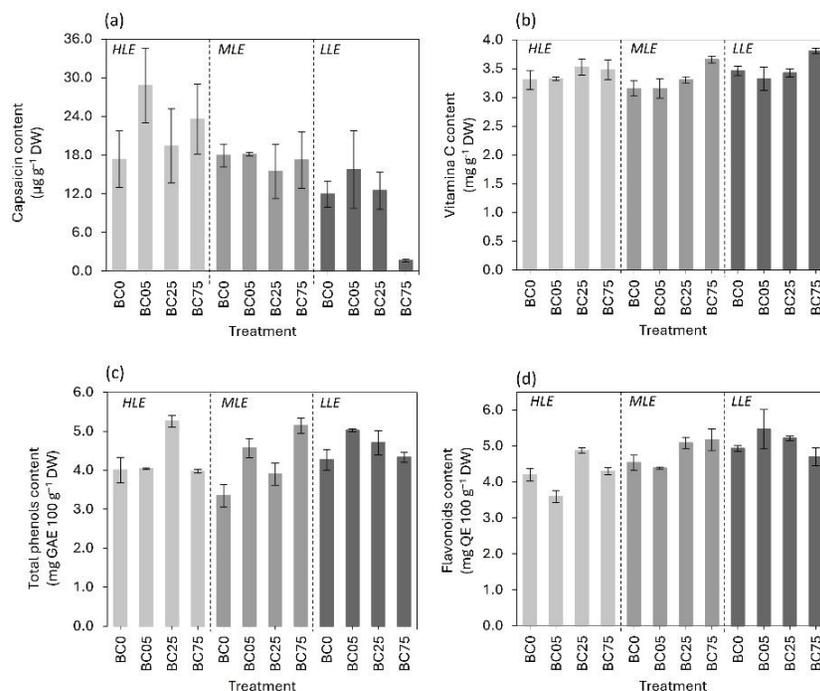

Figure 3. Capsaicin (a), vitamin C (b), total phenols (c) and flavonoids (d) content in fruits of chiltepín pepper plants grown in different light environments whose seeds were previously soaked with biochar. Vertical bars correspond to standard error. n = 5. LLE: Low-light environment; MLE: medium-light environment; HLE: high-light environment; BC: aqueous extract of coconut shell biochar (BioC); BC0: 0.00% BioC; BC05: 0.05% BioC; BC25: 0.25% BioC; BC75: 0.75% BioC; GAE: gallic acid equivalents; QE: quercetin equivalent.

Morphometry and yield traits

The morphometric parameters of polar diameter, equatorial diameter, and roundness index did not show significant responses to light environments and interactions, only to the pre-germinative treatment with BioC. This may be due to the fact that wild, semi-wild and semi-domesticated phenotypes have a high adaptability to diverse climatic conditions (Moreno-Contreras et al., 2024). Values close to 1 in the roundness index indicate a chiltepín fruit with a rounded-to-oval shape. According to Díaz-Sanchez et al. (2021), under greenhouse conditions, chiltepín plants produce larger fruits (47% longer, 43% wider, and 100% heavier) than those harvested in the field, probably owing to nutritional management. The same authors reported fruits with an average length of 6 and 37 mm, with an average weight of 240 mg; these data agree with those found in this study.

The fresh weight per fruit increases in BC05 pretreated plants grown in a MLE. A similar response was documented in chiltepín fruits grown in a light environment between 650-850 µmol m^{-2} s^{-1} (Jiménez-Viveros and Valiente-Banuet, 2023). The values revealed that the control plants (BC0) in HLE reduced 37% the fresh weight per fruit and by 80% the number of fruits harvested per plant, while BC05·MLE, BC25·LLE, and BC75·LLE promoted this variable (p = 0.0091). In the case of yield, a similar trend was found, since high-light intensity

reduced this variable ($p < 0.05$), while the highest yield was found in BC05·MLE (83.21 g plant^{-1}), followed by BC25·LLE (74.33 g plant^{-1}) and BC75·LLE (72.09 g plant^{-1}), representing increases of 105.8%, 40.0%, and 35.8%, respectively, compared to the control treatment (Table 2).

Under protected agriculture, chiltepín production can be increased (McCaughey-Espinoza et al., 2020); however, light conditions must be properly managed to avoid negatively impacting its productive potential. As an agricultural strategy, the aqueous extract of BioC promotes the evaluated parameters because of its biostimulant effect on the seed, which is due to the diversity of molecules present in the extract (water-soluble organic compounds, humic substances, low-molecular-weight acids, neutral and aromatic compounds, triethyl phosphate, 2,4-bisphenol, alkanes, and mineral nutrients) (Lou et al., 2016; Ma et al., 2022). Our results reflect how different light conditions in combination with a pre-germinative treatment with an aqueous extract of coconut shell biochar can promote the production, quality, and quantity of phytochemical compounds in native chiltepín fruit (Díaz-Sánchez et al., 2021; Jiménez-Viveros and Valiente-Banuet, 2023). Future studies should verify whether the benefits of pregerminative treatment and light environments prevail during long cycles (> 1 yr) and establish the intricate mechanisms of ecophysiological and biochemical regulation of plants under these management conditions.

CONCLUSIONS

The use of an aqueous extract of coconut shell biochar as a pre-germinative treatment promotes the production and biochemical profile of chiltepin peppers grown under different light conditions. The maximum yield and most favorable biochemical profile were documented in plants whose seeds were previously soaked in a 0.05% aqueous extract of coconut shell biochar and developed in an environment with medium light (437 ± 37 µmol m^{-2} s^{-1}).

Author contributions
Conceptualization: F.P-L. Methodology: MC.L-P., F.P-L. Validation: A.J-M., E.F-H. Formal analysis: MC.L-P., F.P-L., R-S. Investigation: R-S., MC.L-P., E.F-H. Resources: A.J-M., F.P-L. Data curation: MC.L-P., F.P-L. Writing-original draft: MC.L-P., F.P-L. Writing-review & editing: MC.L-P., R-S., A.J-M., F.P-L. Visualization: A.J-M., E.F-H. Supervision: F.P-L. Project administration: F.P-L. Funding acquisition: F.P-L. All co-authors reviewed the final version and approved the manuscript before submission.

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