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Research Article

Aqueous extract of coconut shell biochar as a pre-germination treatment increases seed germination and early seedling growth in chiltepín pepper (*Capsicum annuum* var. *glabriusculum*)

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Abstract

Since the fruit of the *Capsicum annuum* L. var. *glabriusculum* (Dunal) Heiser and Pickergil (chiltepín pepper) has a low germination rate, we sought to determine whether using an aqueous biochar extract could improve this. Germination tests were performed out in Petri dishes, using wild chiltepín pepper seeds collected in Sonora, México, which were exposed for 24 h to aqueous extracts of coconut shell biochar (CSBA) at different doses (0.05, 0.10, 0.25, 0.50, 0.75, and 1.00%, *w/v*) and a control comprising deionized water. In addition to quantifying the germination rate, we determined the physical quality, viability, imbibition, electrical conductivity, seed pH, and capsaicin content. The fast green test showed an ideal physical quality ($p = 0.5475$), an imbibition rate > 65% ($p > 0.05$), and high viability 98.4% ($p > 0.05$). The wild chiltepín pepper seeds exposed to the CSBA0.05 and CSBA0.25 treatments increased the percentage germination rate ($p < 0.001$) to 80.9% and 71.7%, respectively. A higher percentage of normal seedlings resulted from CSBA0.05, CSBA0.10 and CSBA1.00 ($p < 0.01$), and a greater shoot length was obtained with CSBA0.05 ($p < 0.01$). The exposure of wild chiltepín seeds to aqueous CSBA for 24 h at low doses (CSBA0.05 and CSBA0.25) increase the germination rate, while CSBA0.05 could enhance early seedling growth.

Keywords: capsaicinoids; dormancy; germinability; vigor index

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Introduction

The chili bell pepper crop is of great economic importance, particularly the chiltepín (or piquín) variety [*Capsicum annuum* L. var. *glabriusculum* (Dunal) Heiser and Pickergil], which is widely distributed from Peru to the Southwestern United States. Its fruit has great relevance in Mexico due to its high market demand (Cano-Vázquez *et al*., 2015) because of its rich, aromatic profile (Mares-Quiñones and Valiente-Banuet, 2019), high antioxidant capacity, and high phenol, flavonoid, capsaicinoid, capsinoid, carotenoid, tocopherol, tocotrienol, and ascorbic acid content (Hayano-Kanashiro *et al*., 2016; Quintero *et al*., 2018; Vazquez-Flores *et al*., 2020). This C3-type plant is characterized by its shrub-like wild growth, reaching heights of 55-150 cm; compact, berry-like, spherical or ovoid fruit (0.87-1.68 cm in length, 0.45-0.81 cm in diameter, 0.20-0.55 g in weight, and 0.45-0.82 mL by volume); 2.5-3.0-mm hard-coated seed, and maturity time of 6-10 months (Hayano-Kanashiro *et al*., 2016; Mares-Quiñones and Valiente-Banuet, 2019; Beltrán-Burboa *et al*., 2020; Cano-González *et al*., 2021). Due to the scarcity of information regarding an adequate production system, fruit collection usually involves cutting or removing whole branches, leading to a loss of genetic diversity (Mares-Quiñones and Valiente-Banuet, 2019).

Germination in chiltepín pepper is strongly influenced by the conditions of the environment in which it develops (Cano-Vázquez *et al*., 2015), its hard seed coat (Mares-Quiñones and Valiente-Banuet, 2019), and certain physiological factors, such as hormonal load (mainly gibberellins) and capsaicin content, which lead to seed dormancy and a low germination rate < 20% (Alcalá-Rico *et al*., 2019; Beltrán-Burboa *et al*., 2020). Studies by Sandoval-Rangel *et al.* (2018) and Mares-Quiñones and Valiente-Banuet (2019) have suggested that the seed embryo may require at least two months of rest after harvesting to reach maturity, whereas the seed can germinate 7-28 days after sowing. In this context, several strategies have been developed to promote chiltepín pepper seed germination, including the exogenous application of gibberellic acid (GA_3), indole-acetic acid, indole-3-butyric acid (IBA), naphthalene acetic acid (NAA), salicylic acid (SA), hydrochloric acid, nitric acid, sulfuric acid (H_2SO_4), hydrogen peroxide, kinetin, and potassium nitrate in various doses and at different priming times, achieving good results (Cano-Vázquez *et al*., 2015; Quintero *et al*., 2018; Sandoval-Rangel *et al*., 2018; Alcalá-Rico *et al*., 2019; Beltrán-Burboa *et al*., 2020; Cano-González *et al*., 2021; Junaidy and Shahruddin, 2022). Because there is no pre-germination protocol for this seed type (Cano-González *et al*., 2021), and given the requirement for adequate conditions, it has become necessary to expand the strategies used to improve chiltepín pepper' physiological germination potential, such as applying biochar.

Biochar is a by-product of the thermal transformation of organic matter under oxygen-limiting conditions (Joseph *et al*., 2021). During the pyrolysis process creates liquefied fumes, liquid compounds, and solid compounds that can be used in various agricultural contexts, such as promoting germination. Abdelhafez *et al.* (2021) reported improved germination rates of *Lactuca sativa* seeds by applying 0.1% and 0.25% of liquefied smoke obtained from the pyrolysis (at 600 °C) of nut shells and date-palm seeds. Biochar can also be mixed with the substrate to enhance seed germination, with French and Iyer-Pascuzzi (2018) demonstrating sprout induction in *Solanum* plants by mixing 4% biochar with peat. Given the diversity of functional groups (i.e., alcohols, aliphatics, hydroxyls, carboxyls, alkanes, and aromatic compounds) contained in biochars (Ma *et al*., 2022), their use can induce different responses in seed germination, including its inhibition (Joseph *et al*., 2021), the partial promotion of the gibberellin pathway (French and Iyer-Pascuzzi, 2018), or the triggering of chemical signals that increase the seed's light sensitivity, stimulating germination via their karrikinin content (Kochanek *et al*., 2016).

There is little to no information on the use of biochar in stimulating germination in chiltepín pepper and no knowledge about the seed's response to biochar exposure at different doses. Therefore, based on the hypothesis that biochar could improve chiltepín pepper seed germination, this study was aimed to verify the germination response of chiltepín pepper seeds exposed to aqueous biochar extract; determine whether pregerminative applications of aqueous biochar extract improved germination; and establish a dose adequate for improving the germination rate.

Materials and Methods

Experiment site

The experiment was performed in the Botanical Department of the Universidad Autónoma Agraria Antonio Narro in Saltillo, Coahuila, México.

Biological material: Fruit and seed selection

The biological material--mature red fruit--was collected from wild plants in the Sierra of San Bernardo, Alamos Sonora, México (2022). After three months of storage at room temperature in an airtight container (post-harvest treatment, which could improve embryo maturation of this species), the fruits were washed to eliminate agents for the possible presence of pathogens in commercial sodium hypochlorite (20%, *v/v*) for 5 min with continuous agitation, the pericarp was removed, and the seed was rinsed with distilled water. All floating seeds were discarded. The selected seeds were then air-dried at room temperature for 30 days, after which they were stored in a plastic container at room temperature (25 ± 5 °C) before analysis.

Coconut shell biochar pre-germination solutions

The biochar was prepared from coconut shells by controlled pyrolysis, performed with a maximum temperature of 500 °C, a slow heating rate, an iodine value of 70 mg g^{-1}, Brunauer-Emmett-Teller surface area of 70 m^2 g^{-1}, mean pore radius of 0.78 nm, and water solubility of 2% (Bio-C, Carbotecnia S.A. de CV, Jalisco México). The resulting biochar sample was analyzed in a certified laboratory (NAPT-PAP-accredited, certification number ER-0223/2020, MAE: ISO 17025, ISO-9001:2015). Its composition was determined to be 3.34% moisture, 3.96% ash, 55.70% organic carbon, 0.11% nitrate, 0.06% Olsen phosphorus, and 0.74% potassium, with a carbon: nitrogen ratio of 502, pH of 10.3, electrical conductivity (EC) of 4.3 mS cm^{-1}.

An aqueous extract of coconut shell biochar (CSBA) was prepared from the characterized solid coconut shell biochar. 0.05, 0.10, 0.25, 0.50, 0.75 and 1.00 g of coconut shell biochar was weighed on an analytical balance and placed in a container with 100 mL of deionized water. The container was kept at room temperature (25 °C) and protected from light for 96 h with agitation. Then, the solution was filtered through a Whatman 42 filter and stored at 4 °C for later use. The CSBA solutions were prepared at doses reported in previous studies (Abdelhafez *et al*., 2021) and according to a rapid test developed on chili seeds. The CSBA were prepared at 0.05% (CSBA0.05), 0.10% (CSBA0.10), 0.25% (CSBA0.25), 0.50% (CSBA0.50), 0.75% (CSBA0.75), and 1.00% (CSBA1.00) *w/v*, corresponding to 0.91, 1.82, 4.54, 9.10, 13.61, and 18.15 Mg ha^{-1} at 15 cm depth and an apparent soil density of 1.21 g cm^{-3}. A deionized water control without CSBA (i.e., WCSBA) was also prepared. The treatment consisted only of soaking the seeds in the corresponding CSBA solution according to the test performed. The presence of probable functional groups in the CSBA were analyzed *via* Frontier attenuated total reflectance (ATR) Fourier-Transform Infrared Spectrometer (FTIR) (Frontier FT-IR/NIR, PIKE Technologies In., MA, USA) fitted with ATR MIRacle Diamond Frontier. Measurements and spectra were generated in the 4000 to 450 cm^{-1} wavenumber range with a resolution of 4 cm^{-1}. Each spectral image was collected with a method of 25 scans. The background was collected before each measurement (Figure 1).

Physical seed quality: Fast green test

One hundred chili seeds were collected and soaked for 24 h in the appropriate CSBA treatment, then removed and air-dried at room temperature. Once dry, the seeds were submerged in 10 mL of fast green (Jalmek®; San Nicolas de los Garza, Nuevo León, México) solution (0.1%, *w/v*) at room temperature for 5 min.

Next, they were rinsed with plenty of running water, followed by distilled water to remove the excess solution, and air-dried for 20 min (Olisa *et al*., 2021). Then, the dry seeds were inspected under a stereoscopic microscope (VE-S1, Velab Co., Parr, Texas, USA) to identify any pericarp damage. Images were captured using a Sony® DSC-H300 digital still camera (Sony Corp., Minato, Tokyo, Japan.), and processed using the Microsoft Office Picture software (Microsoft Corporation, Redmond, Washington, USA). The presence of parts with an intense dark green color indicated mechanical pericarp damage. These observations were expressed as the percentage of seeds with light damage (superficial damage to the testa away from the embryo), moderate damage (severe damage to the testa away from embryo), and severe damage (damage associated with the funiculus to embryo). Each treatment was replicated five times.

Imbibition tests

The seeds were dried at room temperature (25 ± 5 °C) for 24 h. The seeds weighed (0.2 g of dry weight) on an analytical balance (Ohaus PA224; Ohaus Corporation, Parsippany, New Jersey, USA), placed in beakers into which 2 mL the corresponding CSBA treatment was added, and then stored at room temperature (25 ± 5 °C). During the 4, 24, and 48 h imbibition periods, the solution was removed with a syringe, and seeds were immediately weighed before the solution was returned. Each treatment comprised five replicates of fifty seeds per replicate. The imbibition was expressed as the percentage difference seed's initial dry weight (DW) and the fresh weight (FW) of the seed after application of the treatment over time (Cano-González *et al*., 2021):

$$\% \text{ Imbibition} = [(FW - DW) / DW] \times 100 \tag{1}$$

EC, pH, and oxidation–reduction potential test

It was evaluated by placing the 0.2 g of seeds (fifty seeds) in a beaker containing 25 mL of the appropriate CSBA solution and incubated at 25 ± 5 °C. Readings were taken after 4, 24, and 48 h using an EC potentiometer (HI98130; Hanna Instruments, Woonsocket, RI, USA) in $\text{mS cm}^{-1}\ \text{g}^{-1}$. The pH was determination under the same conditions described previously. The solution's oxidation-reduction potential (Eh) was quantified using a potentiometer (ORP-200; HM Digital Inc., Los Angeles, California, USA). Five replications with fifty seeds per replicate were used for each treatment.

Capsaicin content

Two grams of seed (one gram equals approximately 250 seeds) were soaked for 24 h in the various CSBA treatments, then removed and air-dried at room temperature (25 ± 5 °C) for 24 h. Then, their capsaicin contents were determined based on the method used by Palma-Orozco *et al.* (2021). We used five replicates per treatment. The absorbance data were obtained at 286 nm using a spectrophotometer ME-UV1800; MesuLab Instruments Co., Ltd., Guangzhou City, China and interpolated on a standard capsaicin (Sigma-Aldrich, St. Louis, Missouri, USA) curve.

Seed viability: Tetrazolium test

Seed viability was assessed using 2,3,5-triphenyltetrazolium chloride (Sigma-Aldrich). The seeds were immersed for 24 h in the various CSBA treatments at room temperature. Next, they were dissected longitudinally through the embryo using a scalpel and placed in a 0.1% tetrazolium solution for 6 h at room temperature. Then, they were rinsed with deionized water and evaluated using an optical microscope (VE-S1). Five replications of fifty seeds, per replicate, were used for each treatment. Viable seeds were stained bright red, while unstained seeds indicated dead tissue. Images were processed as per the fast green test. The results are reported as degree of staining pattern-fully stained, fully unstained, and partially stained (International Seed Testing Association [ISTA], 2020).

Petri-dish germination test

The CSBA's seed germination-promoting ability was assessed using a 35-day laboratory germination test. First, the seeds were disinfected by soaking them in ethanol (70%, *v/v*) for 10 min. Next, they were rinsed in distilled water for 20 min and soaked in sodium hypochlorite (10%, *v/v*) for 5 min. Then, they were washed in distilled water for 20 min and finally soaked in a Captan 50-WP (N-trichlorormethylthio-4-cyclohexene-1,2-dicarboximide) solution for 5 min. Finally, they were washed with distilled water for 10 min and placed in Eppendorf tubes containing a solution with the appropriate CSBA concentration. They remained immersed for 24 h before being carefully removed and rinsed with deionized water. Next, the seeds were placed in Petri dishes (86 x 15 mm) lined with filter paper soaked in 5 mL of deionized water and stored in the total darkness for three days (25 ± 5 °C). Then, they were treated at room temperature (25 ± 5 °C), under 65 ± 10% relative humidity, to a daily regime of 9 h 30 min light to 14 h 30 min dark. The moisture content in the Petri dishes was monitored to ensure the filters did not dry out, and deionized water was sprayed on them as required. Each treatment comprised five replicates with 50 seeds per replicate. Seeds were counted daily and when they had a radicle length of ≥ 3 mm, seedlings were considered to have germinated (ISTA, 2020). To determine CSBA's effect on seed germination, the following parameters were determined: percentage of germinated seeds (%GS), percentage of non-sprouting seeds; (%NSS), the speed of seed germination (SSG); germination rate index (GRI) expressed as the number of seeds germinated in the time of germination; and seed vigor index (SVI) expressed as %germination * total length plant (Alcalá-Rico *et al.*, 2019). Similarly, the percentages of normal (%NS) and abnormal seedlings (%AS) were quantified. Germination test was conducted for 35-days, according to previous studies since chiltepín seed can take up to 28-days to germinate (Quintero *et al.*, 2018; Cano-González *et al.*, 2021). The percentage of abnormal seedlings included those where the roots were stunted, missing, broken, or split at the tip, as well as those with short, stout, slit, shrunken, twisted or rotten shoots, and deformed, damaged or missing leaves (ISTA, 2020). At the end of the test the primary root (PRL), shoot, (SL) and total plant lengths (TPL) were quantified for the normal seedlings and the %GS calculated as:

$$\%GS = (\text{germinated seeds} / \text{total seeds}) \times 100 \quad (2)$$

Statistical analysis

A completely randomized design was used, with five replicates per treatment. The germination test, capsaicin content and tetrazolium test were performed out three times. The percentages corresponding to physical seed quality, seed viability, imbibition, germination, dormancy, and normal and abnormal seedlings were transformed using the arcsine square root. The Shapiro-Wilk and Levene tests were applied to the data of variables. Then, the data were subjected to a one-way analysis of variance, while the mean separation was evaluated by Fisher's least significant differences (LSD) test. The analyses were run at $p < 0.05$ using IBM Statistical Package for the Social Sciences 19.0 software (IBM Corp., Armonk, New York, USA).

Results

The FTIR spectra of CSBA used show the fingerprint region (400 to 1500 cm^{-1}) and functional groups region present in the sample (up to 4000 cm^{-1}). This study detected C=C, C≡C and O-H stretching (peaks at 1635.2, 2115.2 and 3305.5 cm^{-1}, respectively), which may indicate content of alkene, cyclic alkene, alkyne, alcohols, phenols, and alkyl compounds in the CSBA (Figure 1).

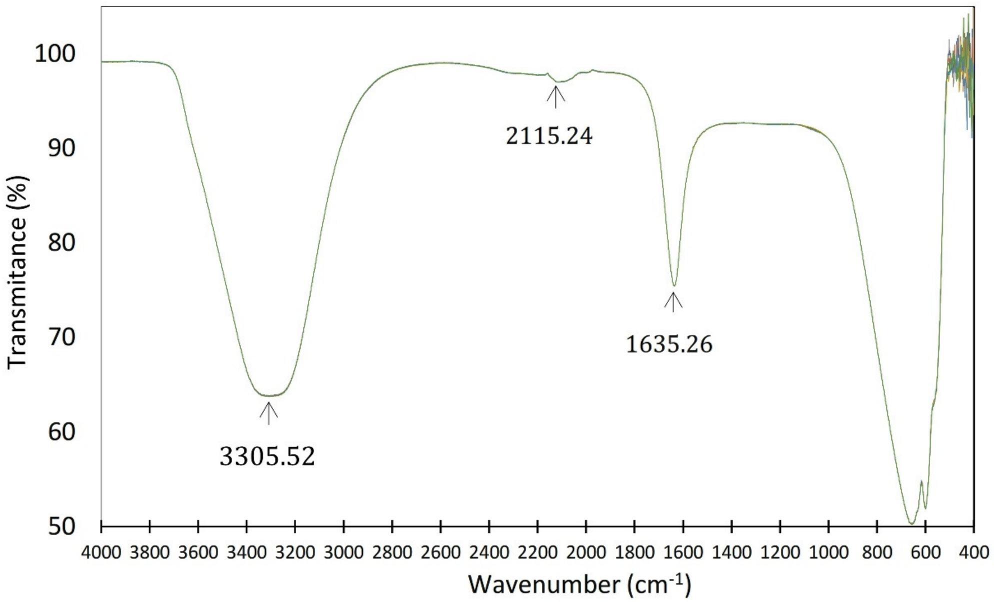

Figure 1. Attenuated total reflectance Fourier transform infrared spectrum of aqueous extract of coconut shell biochar

Physical seed quality: Fast green test

The seeds had adequate physical quality, with 87.4% of the mechanical pericarp damage being Category 1 (superficial damage to the testa away from the embryo, Figure 2A-B) and 12.6% Category 2 (severe damage to the testa away from the embryo, Figure 2C). No seeds had severe damage to the embryo (Figure 2). When comparing physical quality between treatments, there were no significant differences between Categories 1 and 2 ($p = 0.5475$).

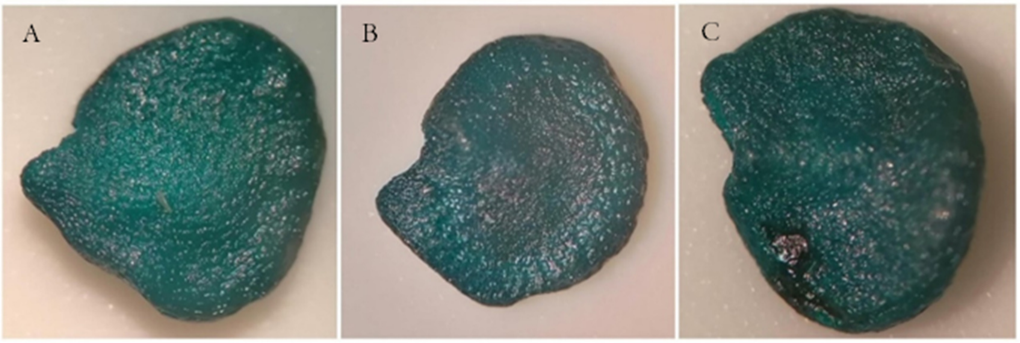

Figure 2. Physical quality of chiltepín pepper seed exposed to aqueous extract of coconut shell biochar
A= seed without any pericarp damage (category 1), B= seed light pericarp damage (classified as common, category 1), C= seed with moderate pericarp damage (category 2).

Imbibition tests

There seeds' imbibition increased with immersion duration. The average mean of all treatment imbibition was 65.8% at 4 h, increasing to 73.7% at 24 h and 81.3% at 48 h. The highest imbibition percentages were with CSBA0.25 (67.0%) at 4 h, CSBA1.00 (76.9%) at 24 h, and CSBA0.50 (86.8%) at 48 h. It should be noted that seed immersion in CSBA0.05 and CSBA0.10 led to a decrease in imbibed weight up to 3.6% (Figure 3).

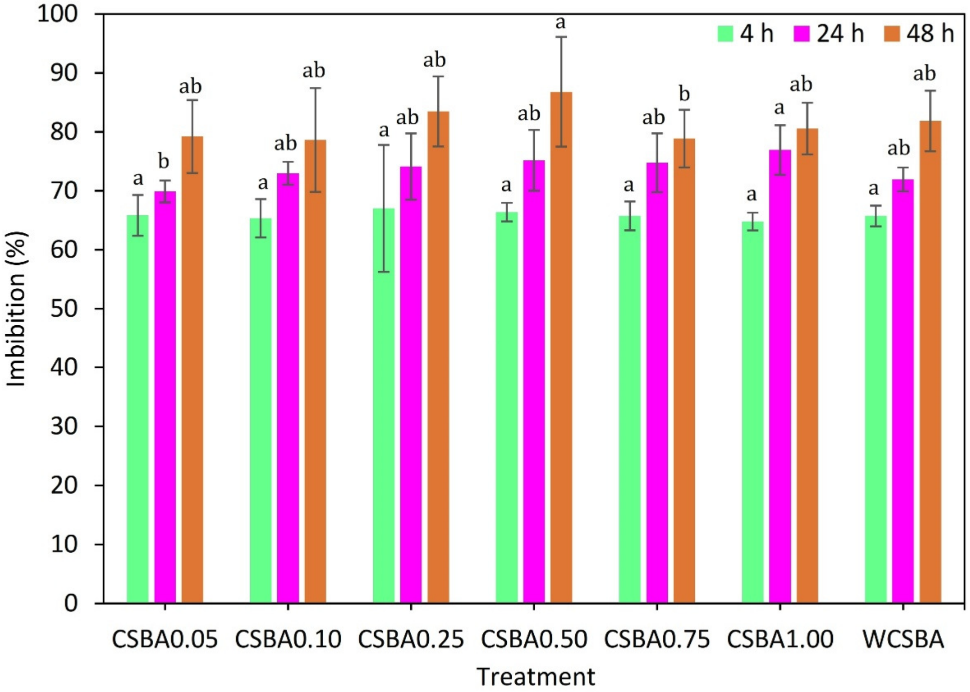

Figure 3. Imbibition of chiltepín pepper seed exposed to aqueous extract of coconut shell biochar
CSBA: aqueous extract of coconut shell biochar; 0.05, 0.10, 0.25, 0.50, 0.75, and 1.00 represent to % of extract. WCSBA: without aqueous extract of coconut shell biochar. Different letters above columns indicate a significant difference between treatments according to Fisher's LSD test ($p < 0.05$). n = 5. Vertical bars correspond to standard deviation.

EC, pH, and Eh seed test

The aqueous extracts of biochar in deionized water resulted in EC values of 0.02, 0.03, 0.07, 0.12, 0.16, and 0.21 mS cm^{-1} for the CSBA0.05, CSBA0.10, CSBA0.25, CSBA0.50, CSBA0.75, and CSBA1.00 treatments, respectively, while WCSBA resulted in an EC value of 0.08 mS cm^{-1}. Similarly, the extracts' initial pH values were 6.68, 6.51, 6.45, 7.71, 8.40, and 8.74, respectively, with the Eh values of 100, 90, 89, 85, 78 and 78 mV, respectively. In the WCSBA, these values were pH = 5.80 and Eh = 76 mV.

There were highly significant differences (Table 1) between treatments in the seeds' EC, pH, and Eh values. The EC and pH values increased considerably in seeds immersed in the different CSBA treatments (by 0.19 mS cm^{-1} g^{-1} and 1.62 units, respectively), while the Eh value deceased (to 43.4 mV), compared with the WCSBA. While the pH and EC values remained stable for the different immersion durations, the Eh values increased faster in CSBA-treated than in control seeds up to 24 h. For the different seed imbibition durations, the CSBA1.00 treatment considerably increase in EC (at 4, 24, and 48 h) and pH (42.0%, 24.6%, and 20.2% at 4, 24, and 48 h, respectively) compared to the control treatment. The biochar treatments caused a significant reduction in Eh compared to the WCSBA treatment, with the seeds immersed in CSBA0.05 having the lowest Eh values, corresponding to reductions of 78.4%, 17.0%, and 58.1% at 4, 24, and 48 h, respectively.

Table 1. pH, electrical conductivity, and potential oxidation-reduction of seeds exposed to aqueous extract of coconut shell biochar

Treatment	EC (mS cm^{-1} g^{-1})			pH			Eh (mV)		
	4 h	24 h	48 h	4 h	24 h	48 h	4 h	24 h	48 h
CSBA0.05	$0.042 \pm 0.004^{e\dagger}$	0.062 ± 0.029^{e}	0.064 ± 0.023^{e}	6.44 ± 0.21^{d}	6.66 ± 0.44^{a}	6.12 ± 0.34^{c}	15.0 ± 22.60^{f}	67.4 ± 7.04^{d}	44.6 ± 3.21^{f}
CSBA0.10	0.040 ± 0.000^{e}	0.042 ± 0.004^{f}	0.050 ± 0.000^{f}	6.16 ± 0.04^{e}	6.06 ± 0.08^{c}	5.90 ± 0.07^{d}	42.8 ± 12.79^{c}	72.4 ± 0.84^{c}	62.8 ± 0.89^{e}
CSBA0.25	0.086 ± 0.005^{d}	0.086 ± 0.005^{d}	0.088 ± 0.004^{d}	6.30 ± 0.03^{d}	6.19 ± 0.05^{bc}	5.87 ± 0.11^{d}	50.8 ± 10.82^{b}	74.8 ± 1.92^{bc}	69.8 ± 1.48^{d}
CSBA0.50	0.118 ± 0.008^{c}	0.130 ± 0.000^{c}	0.126 ± 0.005^{c}	6.73 ± 0.07^{c}	6.40 ± 014^{b}	6.16 ± 0.16^{bc}	48.6 ± 12.66^{b}	76.2 ± 2.07^{b}	71.0 ± 3.77^{d}
CSBA0.75	0.164 ± 0.005^{b}	0.168 ± 0.004^{b}	0.170 ± 0.000^{b}	7.51 ± 0.14^{b}	6.69 ± 0.05^{a}	6.35 ± 0.07^{ab}	34.4 ± 20.32^{d}	73.0 ± 3.58^{c}	78.2 ± 0.71^{b}
CSBA1.00	0.208 ± 0.004^{a}	0.206 ± 0.005^{a}	0.210 ± 0.000^{a}	8.15 ± 0.07^{a}	6.84 ± 0.03^{a}	6.49 ± 0.09^{a}	25.4 ± 22.72^{e}	69.6 ± 2.79^{d}	73.8 ± 0.55^{c}
WCSBA	0.020 ± 0.000^{f}	0.020 ± 0.000^{g}	0.020 ± 0.000^{g}	5.74 ± 0.09^{f}	5.49 ± 0.09^{d}	5.40 ± 0.13^{e}	69.6 ± 16.09^{a}	81.2 ± 4.16^{a}	106.4 ± 0.84^{a}
p-value	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*

† = distinct letters in the row indicate significant differences according to Fisher's LSD test ($p < 0.05$). CSBA: aqueous extract of coconut shell biochar; 0.05, 0.10, 0.25, 0.50, 0.75, and 1.00 represent to % of extract. WCSBA: without aqueous extract of coconut shell biochar. EC: electrical conductivity. Eh: oxidation-reduction potential. n = 5. ± standard deviation.

Capsaicin content

An average of 91.9 ± 4.9 μg capsaicin g^{-1} dry seed of chiltepín pepper was observed. There was no difference between treatments. The CSBA1.00 and CSBA0.75 treatments had the highest numerical means compared to the WCSBA treatment, at 93.7 and 92.9 μg capsaicin g^{-1} dry seed, respectively (Figure 4).

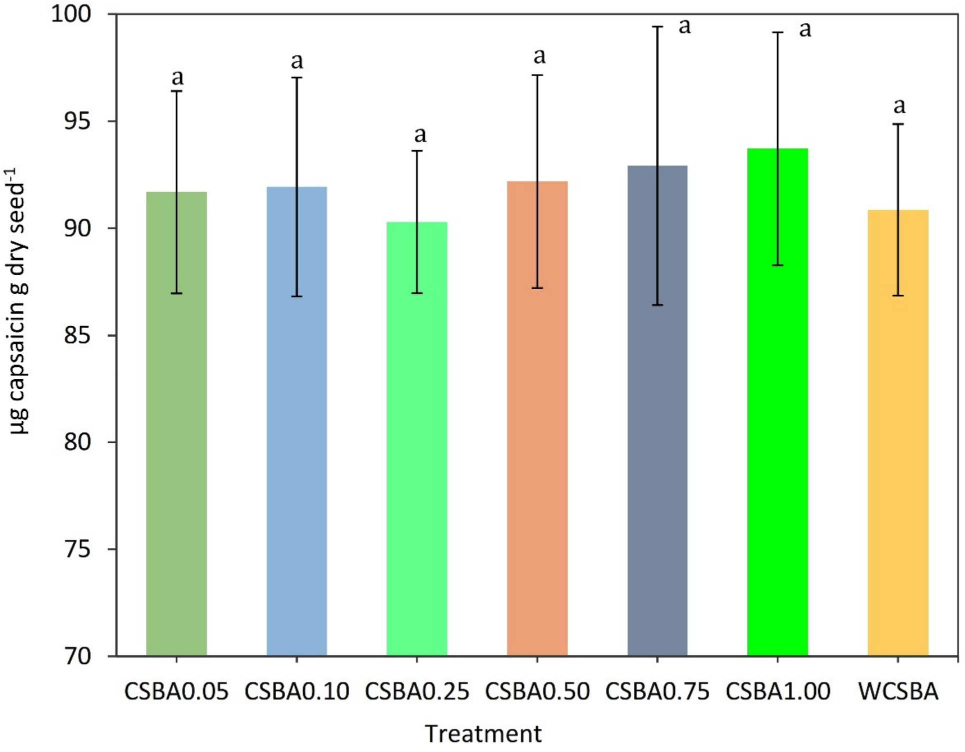

Figure 4. Capsaicin content in chiltepín pepper seed exposed to aqueous extract of coconut shell biochar CSBA: aqueous extract of coconut shell biochar; 0.05, 0.10, 0.25, 0.50, 0.75, and 1.00 represent to % of extract. WCSBA: without aqueous extract of coconut shell biochar. Different letters above columns indicate a significant difference between treatments according to Fisher's LSD test ($p < 0.05$). n = 5. Vertical bars correspond to standard deviation.

Seed viability: Tetrazolium test

The tetrazolium test indicated no significant differences between treatments in viable ($p = 0.9981$) and non-sprouting seed ($p = 0.9990$) seeds, with a mean of 98.4% seed viability (Figure 5).

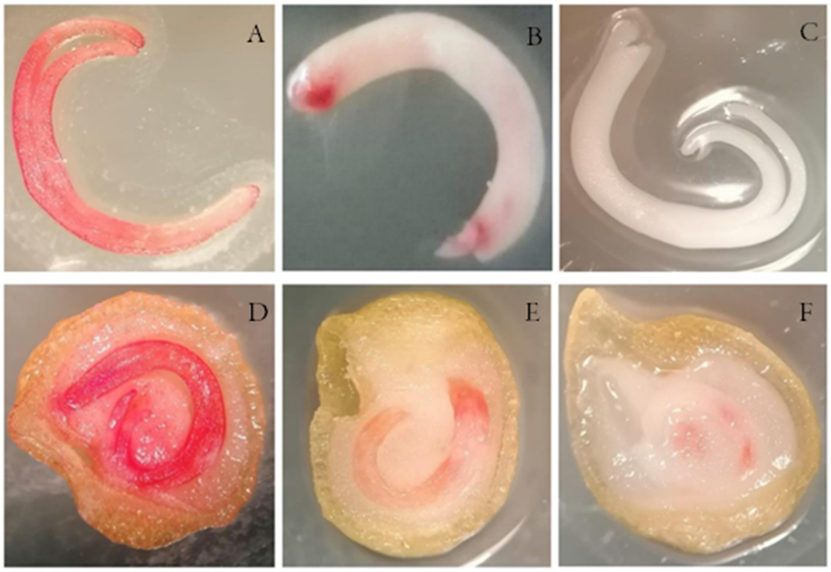

Figure 5. Viability of chiltepín pepper seed exposed to aqueous extract of coconut shell biochar
A= viable embryo, B-C= non-viable embryo. D= viable seed, E-F= non-sprouting seed.

Seed germination and early seedling growth

Differences were found in the germination percentages of chiltepín pepper seeds between the biochar doses explored. Relative to WCSBA, seed germination increased by 20.7% (to 80.9%) after treatment with CSBA0.05, followed by 7.0% (to 71.7%) with CSBA0.25. Eventually, these same treatments showed 42.4% and 14.3% reductions in non-sprouting seeds. The seeds treated with CSBA0.50 had a reduced germination rate of 2.5%, with a 5.1% increase in non-sprouting seeds, statically similar to WCSBA treatment (Figure 6). The percentages of normal and abnormal seedlings different significantly between treatments, with a higher percentage of normal seedlings with CSBA0.05 and CSBA0.10, which caused increases of 12.3% and 11.0% compared to WCSBA, respectively. In contrast CSBA0.75% caused a 4.1% reduction in normal seedlings. The CSBA0.75 treatment caused a 17.7% increase in abnormal seedlings (Table 2).

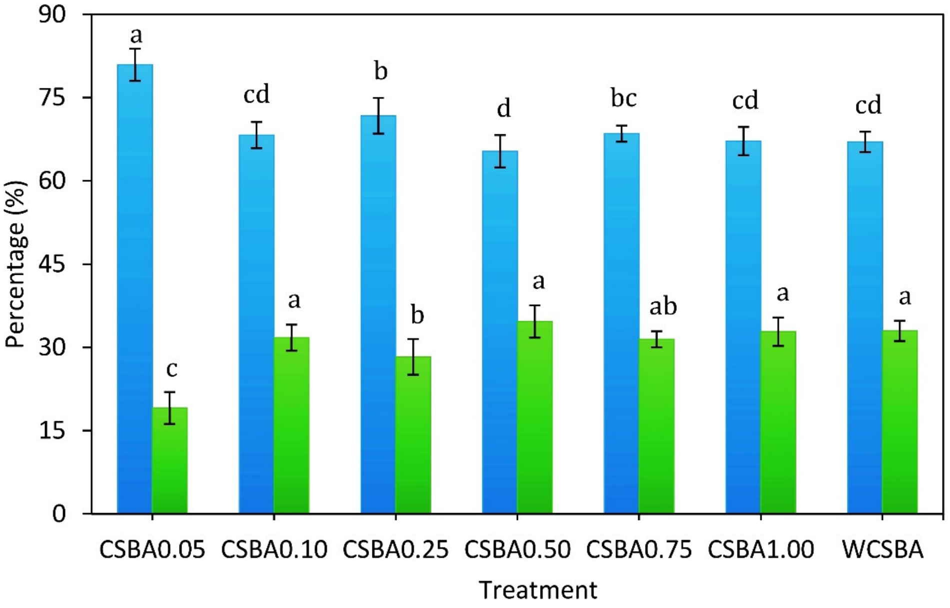

Figure 6. Germination test on chiltepín pepper seed exposed to aqueous extract of coconut shell biochar. Blue bar= percentage of germination. Green bar= percentage of non-sprouting seeds. CSBA: aqueous extract of coconut shell biochar; 0.05, 0.10, 0.25, 0.50, 0.75, and 1.00 represent to % of extract. WCSBA: without aqueous extract of coconut shell biochar. Different letters above columns indicate a significant difference between treatments according to Fisher's LSD test ($p < 0.05$). n = 5. Vertical bars correspond to standard deviation.

Table 2. Initial growth of seedlings of chiltepín pepper exposed to aqueous extract of coconut shell biochar

Treatment	%NS	%AS	SL	PRL	TPL	SSG	SVI	GRI
CSBA0.05	$90.13\pm2.43^{ab\dagger}$	9.87 ± 2.43^{cd}	$19.42\pm0.53^{a\dagger}$	7.98 ± 0.97^{bc}	27.39 ± 1.43^{a}	1.13 ± 0.05^{a}	21.12 ± 1.26^{a}	4.97 ± 0.35^{a}
CSBA0.10	91.16 ± 2.46^{a}	8.84 ± 2.46^{d}	17.39 ± 0.74^{bc}	6.85 ± 0.84^{d}	24.24 ± 1.20^{c}	0.98 ± 0.03^{bc}	15.70 ± 0.58^{cd}	3.93 ± 0.20^{e}
CSBA0.25	84.31 ± 6.28^{bcd}	15.69 ± 6.28^{abc}	17.40 ± 0.61^{bc}	7.42 ± 0.36^{cd}	24.83 ± 0.76^{bc}	1.02 ± 0.05^{b}	17.37 ± 0.87^{b}	4.04 ± 0.21^{de}
CSBA0.50	82.34 ± 7.48^{cd}	17.66 ± 7.48^{ab}	17.34 ± 1.33^{bc}	7.09 ± 0.43^{cd}	24.83 ± 1.73^{c}	0.93 ± 0.04^{c}	15.22 ± 1.09^{d}	4.26 ± 0.19^{cd}
CSBA0.75	77.88 ± 5.83^{d}	22.12 ± 5.83^{a}	17.65 ± 1.27^{b}	8.95 ± 1.24^{ab}	26.60 ± 2.44^{ab}	0.98 ± 0.03^{bc}	16.93 ± 1.38^{bc}	4.77 ± 0.22^{ab}
CSBA1.00	88.81 ± 4.64^{abc}	11.19 ± 4.64^{bcd}	17.69 ± 0.55^{b}	9.09 ± 0.91^{a}	26.78 ± 1.17^{ab}	0.96 ± 0.03^{c}	17.16 ± 0.30^{b}	4.03 ± 0.25^{de}
WCSBA	81.21 ± 3.57^{d}	18.79 ± 3.57^{a}	16.41 ± 1.07^{c}	8.63 ± 0.78^{ab}	25.01 ± 1.34^{bc}	0.96 ± 0.04^{c}	15.73 ± 0.78^{cd}	4.46 ± 0.30^{bc}
p-value	*0.0018*	*0.0017*	*0.0019*	*0.0004*	*0.0101*	*<0.0001*	*<0.0001*	*<0.0001*

† = distinct letters in the row indicate significant differences according to Fisher's LSD test ($p < 0.05$). CSBA: aqueous extract of coconut shell biochar; 0.05, 0.10, 0.25, 0.50, 0.75, and 1.00 represent to % of extract. WCSBA: without aqueous extract of coconut shell biochar. %NS = normal seedlings (%), %AS = anormal seedlings (%). SL = shoot length (mm), PRL = primary root length (mm), TPL = total plant length (mm). SSG = speed seed germination (number of seeds germinated in the time of germination), SVI = seed vigor index (%germination * total length plant), GRI = germination rate index (%germination in 35-days). n = 5. ± standard deviation.

Initial seedling development showed with the length of the seedlings' shoot increasing by 18.3% with CSBA0.05 treatment. Principal radicle length was statistically similar in CSBA1.00, CSBA0.75 and WCSBA treatments, showing a decrease by 20.6% with CSBA0.10 treatment. Seedling length was 9.5% greater with CSBA0.05 treatment than with the WCSBA treatment. With CSBA0.05 treatment increasing SSG, SVI, and GRI by 17.7%, 34.3%, and 11.4%, respectively. CSBA0.25 treatment increased SSG and SVI values by 6.3% and 10.4%, respectively, compared to WCSBA. However, CSBA0.50 treatment performed statistically similar to WCSBA in SSG, SVI and GRI parameters. Finally, GRI was significantly reduced with treatments CSBA0.10 (11.9%), CSBA1.00 (9.6%), and CSBA0.25 (9.4%) compared to WCSBA (Table 2).

Discussion

Seeds of the genus *Capsicum* usually have low or poor germination rates (Bissoli *et al.*, 2022) due to their high dormancy rates (Beltrán-Burboa *et al.*, 2020). The seeds' good physical quality illustrates the minimal damage that genetic material from wild plants can present, allowing for a quick and easy determination of their germination potential. Generally, seed damage can occur during fruit harvesting or the seed aging process during storage (Satya Srii *et al.*, 2022). Here, we demonstrated that the collected chiltepín pepper seeds had minimal to no physical damage to the pericarp. Therefore, the compounds necessary for the metabolic process once imbibition began were still present.

Imbibition tests

The imbibition rates determined here were similar to those of Cano-Vázquez *et al.* (2015) and Cano-González *et al.* (2021). Imbibition percentages > 50% after the first 4 h of seed immersion in the CSBA solution (highest with CSBA0.25, CSBA0.50, and CSBA1.00; Figure 3) reflect the absence of morphological germination problems due to the presence of a too-thick testa, since high testa lignification can prevent or reduce imbibition and gaseous exchange between the embryo and the environment (Bissoli *et al.*, 2022). During this process, which follows the Lucas-Washburn law, there is a gradual swelling from water penetration into the seed tissues caused by gradients and capillary pressure (Louf *et al.*, 2018). Therefore, the chiltepín seed coat was not a barrier to water imbibition since the was no physical dormancy and a strong association with the morpho-physiological conditions, which allowed the seeds to maximize the water conditions to which they were exposed (Cano-González *et al.*, 2021).

EC, pH, and Eh seed test

The seeds' EC, pH, and Eh values may have largely resulted from the characteristics of the biochar used as the raw material in the aqueous solutions, with high pH (10.3) and EC (4.3 mS cm^{-1}) recorded. However, the CSBA did not cause an ionic concentration high enough to inhibit germination (Bai *et al.*, 2022), except for CSBA0.50. Because EC increases in the treatment solutions were low compared to the pure extract, we can assume the seeds had high vigor because a low EC indicates no loss of electrolytes from the cell membrane into the solution (Prado *et al.*, 2019). This finding is consistent with the good physical quality and high viability mentioned above. The reduction in Eh values with the biochar treatments (greatest with CSBA0.05) might have created an environment conducive to germination since it can be associated with shifts in the redox state of seed proteins. Oxidized proteins are reduced during the early imbibition stages, prompting signalling to remobilize reserves and allow for proper radicle and shoot tissue development (Nietzel *et al.*, 2020).

Seed germination and early seedling growth

The chiltepín chili plants in Sonora are photosynthetically adapted to low photon fluxes (~500 µmol m^{-2} s^{-1}), show ecophysiological fluctuations that can alter their phenological stages (Jiménez-Leyva *et al.*, 2022), and can modify their yields, fruit quality, and even their content of aromatic compounds, such as capsaicin. Díaz-Sánchez *et al.* (2021) reported appreciable variability in the levels of this latter component in several creole chiltepín pepper varieties, particularly wild species from Sonora, which have a fruit content of 8,220 µg g^{-1} capsaicin. The capsaicin content of chiltepín pepper seeds can be classed as low because this compound is synthesized and accumulated in the epidermal cells of the placental tissue stored on the placental surface

(Hayano-Kanashiro *et al*., 2016). Barchenger and Bosland (2016) reported that capsaicin could reduce or minimize seed germination in some chili cultivars. Quintero *et al.* (2018) reported that the capsaicinoids content in seeds could be decreased by pre-germination treatment (seed priming), reducing their dormancy, and increasing their germination rate. This response was not found in this study, suggesting that the various biochar doses used did not alter the content of this compound.

In addition to their genetic quality, the seeds of the diverse chiltepín ecotypes can have high germination potential rates (Bissoli *et al*., 2022), consistent with the high proportion of viable seeds found in the fruits of wild pepper plants (Figure 5D). CSBA's germination stimulation (imbibition, metabolic activity reinitiation, and root protrusion) can be explained by the production of volatile organic compounds as alkene, cyclic alkene, alkyne, alcohols, phenols, and alkyl groups (Socrates, 2004) during its pyrolysis that might help seeds to germinate rapidly (Głodowska *et al*., 2016).

Under natural conditions, chiltepín pepper seed has low germination rates (< 20%) (Beltrán-Burboa *et al*., 2020). Therefore, the 20.7 and 7.0% increases in germination rates of for seeds immersed in CSBA0.05 and CSBA0.25, respectively, (compared to WCSBA) might be associate with the organic groups probable dissolved in these aqueous extracts. These groups might have caused seed testa scarification, prompting a specific and functional reorganization of the seed membrane's lipid compositions (Yu *et al*., 2015), improving the embryo's protrusion and reserves consumption. Similarly, karrikins (mainly karrikinolide, KAR1), produced during pyrolysis, might promote germination by enhancing water uptake, break the hormonal balance by reducing the abscisic acid (ABA) concentration, prompting GA_3 enzyme synthesis, maintaining reactive oxygen species homeostasis, stimulating enzyme activity, or induce gene regulation (Joseph *et al*., 2021; Sami *et al*., 2022; Gokdas *et al*., 2022). This study's germination percentages are similar to those reported by Cano-Vázquez *et al.* (2015), Quintero *et al.* (2018), Beltrán-Burboa *et al.* (2020), and Cano-González *et al.* (2021), who all applied growth regulators (GA_3, NAA, IBA, SA) or acids (H_2SO_4) to chiltepín seeds collected in different municipalities of the Mexican Republic (i.e., San Luis Potosí, Sonora, Tuxpan, and Tamaulipas). Ultimately, the responses found differed due to genetic fluctuations in the varieties used in these studies.

According to Bai *et al.* (2022), the use of a biochar pyrolyzed at 500 °C (the same temperature as the biochar used in this study) can result in low heavy metals and polycyclic aromatic hydrocarbon contents, reducing the presence of $^{\bullet}OH$ and $^{\bullet}O_2^-$, and thus minimizing the oxidative damage to the seed and seedling. In this context, decrease seed germination with CSBA0.50 (compared to CSBA0.05) and increase abnormal seedling occurrence with CSBA0.75 (compared to CSBA0.05) can be partially explained by the probable high level of these groups in the aqueous extract and possible changes in the osmotic potential due to the salts in the biochar (Joseph *et al*., 2021). However, under the highest dose of biochar (CSBA1.00) an increase in %NS and SVI, as well as a reduction in %AS and GRI, statistically different from the WSCBA. The statistically similar behaviors of CSBA0.50, CSBA0.75, CSBA1.00, and WCSBA contrast with the findings of Bai *et al.* (2022), who noted that high doses of aqueous biochar extract could lead to improvements in seed germination. This difference likely reflects the type of seed and the raw biochar material used, and dose applied. However, it is important to note that aqueous biochar extract can reduce the adverse effects on seed germination compared to solid biochar (Bai *et al*., 2022). Ma *et al.* (2022) reported that biochar solution extracts containing various organic fractions could positively or negatively affect seed growth at low concentrations.

The differences in radicle and shoot length observed in seeds submerged in CSBA may result from a supply of dissolved ions to the seed (Głodowska *et al*., 2016) or the presence of functional groups in the biochar extract (Ma *et al*., 2022) that can enhance the development of these tissues. It should be noted that while using biochar extracts at high concentrations can reduce bud and root lengths (Ma *et al*., 2022), this study's findings tend not to support this (Table 2), likely due to the type of biochar material used and the doses explored. The significant improvements in SSG, SVI, and GRI values with CSBA0.05 and SSG and SVI, with CSBA0.25 suggests that the seedlings that developed under these treatments have a high likelihood of developing and surviving--parameters of vital importance for piquín chili growing in the wild. A similar response was reported by (Głodowska *et al.*, 2016) who, after coating corn seeds with biochar, finding faster germination and a better germination speed index. The same authors proposed that the increase in this variable could be due to stimulation by growth-promoting substances and the eventual translocation of secondary metabolites.

Conclusions

In this study, aqueous extracts of coconut shell biochar were tested as pre-treatments on wild chiltepín pepper seeds to improve their germination and early seedling growth. The results showed that the chiltepín pepper seeds had ideal physical quality, enabling imbibition rates > 65%, in addition to their high viability (98.4%) and average capsaicin content (91.9 μg g^{-1} dry seed). The different aqueous biochar extract concentrations modified the seed's electrical conductivity, pH, and oxidation-reduction potential values. Seed germination improved with biochar exposure, with the highest percentages observed with 0.05% aqueous extracts of coconut shell biochar (80.9%) and 0.25% of aqueous extracts of coconut shell biochar (71.7%). The highest percentages of normal seedlings were found with exposure to 0.05%, 0.10% and 1.00% of aqueous extracts of coconut shell biochar. Greater shoot length occurred with 0.05% of aqueous extracts of coconut shell biochar. Our results provide useful information to improve the germination of wild chiltepín seed, the exposure of chiltepín pepper seeds to low aqueous extracts of coconut shell biochar doses (0.05% and 0.25%) for 24 h may increase the germination rate and the 0.05% doses could improve early seedling growth.

Authors' Contributions

Conceptualization: F P-L, MC L-P; Methodology: F P-L, MC L-P; Software: MC L-P, A J-M, Validation: F P-L, MC L-P; Formal analysis: F P-L, MC L-P, A J-M; Investigation: F P-L, MC L-P, S G-M, A B-M, A J-M; Resources: F P-L, MC L-P, A J-M; Data curation: MC L-P, S G-M, A B-M, A J-M; Writing-original draft preparation: F.P.-L., M.C.L.-P., S.G.-M., A.B.-M., A.J.-M., Writing-review & editing: F P-L, MC L-P, S G-M, A B-M, A J-M; Visualization: MC L-P, S G-M, A B-M, A J-M, Supervision: F P-L, MC L-P, Project administration: F P-L.; Funding acquisition: F P-L, MC L-P. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abdelhafez AA, Zhang X, Zhou L, Cai M, Cui N, Chen G, … Hamed MH (2021). Eco-friendly production of biochar via conventional pyrolysis: application of biochar and liquefied smoke for plant productivity and seed germination. Environmental Technology & Innovation 22:101540. *https://doi.org/10.1016/j.eti.2021.101540*

Alcalá-Rico JSGJ, López-Benítez A, Vázquez-Badillo ME, Sánchez-Aspeytia D, Rodríguez-Herrera SA, Pérez-Rodríguez MA, Ramírez-Godina F (2019). Seed physiological potential of *Capsicum annuum* var. *glabriusculum* genotypes and their answers to pre-germination treatments. Agronomy 9(6):325. *https://doi.org/10.3390/agronomy9060325*

Bai X, Zhang S, Shao J, Chen A, Jiang J, Chen A, Luo S (2022). Exploring the negative effects of biochars on the germination, growth, and antioxidant system of rice and corn. Journal of Environmental Chemical Engineering 10(3):107398. *https://doi.org/10.1016/j.jece.2022.107398*

Barchenger DW, Bosland PW (2016). Exogenous applications of capsaicin inhibits seed germination of *Capsicum annuum*. Scientia Horticulturae 203:29-31. *https://doi.org/10.1016/j.scienta.2016.03.009*

Beltrán-Burboa JN, López-Peralta MCG, Hernández-Meneses E, Cruz-Huerta N (2020). *In vitro* seed germination of chiltepin pepper (*Capsicum annuum* L. var. *glabriusculum*) and plant regeneration via organogenesis. Agrociencia 54(2):195-208. *https://www.agrociencia-colpos.org/index.php/agrociencia/article/view/1901/1898*

Bissoli G, Bono M, Martínez-Almonacid I, Moreno-Peris E, Renard J, Espinosa A, … Rodríguez-Burruezo A (2022). Seed coat lignification level is crucial in *Capsicum spp* seed longevity. Physiologia Plantarum 174:e13600. *https://doi.org/10.1111/PPL.13600*

Cano-González MÁ, Ayil-Gutiérrez BA, Delgado-Martínez R, Osorio-Hernández E, Rangel-Lucio JA, Poot-Poot WA (2021). Physiological potential of piquin pepper seeds in response to pregermination treatments. Ciência e Agrotecnologia 45:e019521. *https://doi.org/0.1590/1413-7054202145019521*

Cano-Vázquez A, López-Peralta MC, Zavaleta-Mancera HA, Cruz-Huerta N, Ramírez-Ramírez I, Gardea-Béjar A, González-Hernández VA (2015). Variation in degrees of seed dormancy among collections of chile piquin (*Capsicum annuum* var. *glabriusculum*). Botanical Science 93:175-184. *https://doi.org/10.17129/botsci.138*

Díaz-Sánchez DD, López PA, López-Sánchez H, Silva-Rojas HV, Gardea-Béjar AA, Cruz-Huerta N, … González-Hernández VA (2021). Pungency and fruit quality in Mexican landraces of piquín pepper (*Capsicum annuum* var. *glabriusculum*) as affected by plant growth environment and postharvest handling. Chilean Journal Agricultural Research 81(4):546-556. *https://doi.org/10.4067/S0718-58392021000400546*

French E, Iyer-Pascuzzi AS (2018). A role for the gibberellin pathway in biochar-mediated growth promotion. Scientific Reports 8:5389. *https://doi.org/10.1038/s41598-018-23677-9*

Głodowska M, Husk B, Schwinghamer T, Smith D (2016). Biochar is a growth-promoting alternative to peat moss for the inoculation of corn with a pseudomonad. Agronomy for Sustainable Development 36:21. *https://doi.org/10.1007/s13593-016-0356-z*

Gokdas Z, Yildirim E, Gupta S, Demir I (2022). Karrikinolide stimulated seed germination of artificially aged marrow, cabbage and pepper seeds through repair of cell structure and enzyme activity. South African Journal Botany 151:208-213. *https://doi.org/10.1016/j.sajb.2022.09.049*

Hayano-Kanashiro C, Gámez-Meza N, Medina-Juárez LÁ (2016). Wild pepper *Capsicum annuum* L. var. *glabriusculum*: taxonomy, plant morphology, distribution, genetic diversity, genome sequencing, and phytochemical compounds. Crop Science 56:1-11. *https://doi.org/10.2135/cropsci2014.11.0789*

International Seed Testing Association ISTA (2020). International Rules for Seed Testing. International Seed Testing Association. Switzerland, NW Bassersdorf.

Jiménez-Leyva A, Gutiérrez A, Ojeda-Contreras ÁJ, Vargas G, Esqueda M, Orozco-Avitia JA (2022). Seasonal phenology, shade reliance, and ecophysiology of wild *Capsicum annuum* var. *glabriusculum* in Sonoran Desert. Journal of Arid Environments 201:104736. *https://doi.org/10.1016/j.jaridenv.2022.104736*

Joseph S, Cowie AL, Van Zwieten L, Bolan N, Budai A, Buss W, … Lehmann J (2021). How biochar works, and when it doesn't: A review of mechanisms controlling soil and plant responses to biochar. Global Change Biology Bioenergy: Bioproducts for a Sustainable Bioeconomy 13(11):1731-1764. *https://doi.org/10.1111/gcbb.12885*

Junaidy RB, Shahruddin S (2022). Germinability and seedling growth performance of chilli (*Capsicum annuum*) seeds in response to different gibberellic acid concentrations pre-treatment. AgroTech Food Science, Technology and Environment 1:10-16. *https://doi.org/10.53797/agrotech.v1i1.2.2022*

Kochanek J, Long RL, Lisle AT, Flematti GR (2016). Karrikins identified in biochars indicate post-fire chemical cues can influence community diversity and plant development. PLoS One 11(8):e0161234. *https://doi.org/10.1371/journal.pone.0161234*

Louf JF, Zheng Y, Kumar TA, Bohr T, Gundlach C, Harholt J, … Jensen KH (2018). Imbibition in plant seeds. Physical Review E 98(4):042403. *https://doi.org/10.1103/PhysRevE.98.042403*

Ma J, Quan G, Yan J, Ippolito JA, Cui L, Wang H, Sun Y (2022). Biochar extract compounds alter germination and growth of crop seed. BioResources 17(3):4151-4166. *https://doi.org/10.15376/biores.17.3.4151-4166*

Mares-Quiñones MD, Valiente-Banuet JI (2019). Horticultural aspects for the cultivated production of piquin peppers (*Capsicum annuum* L. var. *glabriusculum*)-A review. HortScience 54(1):70-75. *https://doi.org/10.21273/HORTSCI13451-18*

Nietzel T, Mostertz J, Ruberti C, Née G, Fuchs P, Wagner S, … Schwarzländer M (2020). Redox-mediated kick-start of mitochondrial energy metabolism drives resource-efficient seed germination. Proceedings of the National Academy of Sciences 117(1):741-751. *https://doi.org/10.1073/pnas.1910501117*

Olisa BS, Awosanmi FE, Akinropo MS, Ojo PO, Ishiak K, Danlami A, … Okeke CU (2021). Differential response of commercial hybrid and open pollinated maize seeds to mechanical damage during seed processing. Notulae Scientia Biologicae 13(4):10738-10738. *https://doi.org/10.15835/nsb13410738*

Palma-Orozco G, Orozco-Álvarez C, Chávez-Villeda AA, Mixtega-Martínez A, Castro-Muñoz R (2021). Capsaicin content in red habanero chilli (*Capsicum chinense* Jacq.) and its preservation after drying process. Future Foods 4:100070. *https://doi.org/10.1016/j.fufo.2021.100070*

Prado JPD, Krzyzanowski FC, Martins CC, Vieira RD (2019). Physiological potential of soybean seeds and its relationship to electrical conductivity. Journal of Seed Sciece 41(4):407-415. *https://doi.org/10.1590/2317-1545v41n4214988*

Quintero CMF, Castillo OG, Sánchez PD, Marín-Sánchez J, Guzmán AI, Sánchez A, Guzmán JM (2018). Relieving dormancy and improving germination of Piquín chili pepper (*Capsicum annuum* var. *glabriusculum*) by priming techniques. Cogent Food & Agriculture 4:1550275. *https://doi.org/10.1080/23311932.2018.1550275*

Sami A, Zhu ZH, Zhu TX, Zhang DM, Xiao LH, Yu Y, Zhou KJ (2022). Influence of KAR1 on the plant growth and development of dormant seeds by balancing different factors. International Journal Environmental Science and Technology 19(4):3401-3410. *https://doi.org/10.1007/s13762-021-03282-6*

Sandoval-Rangel A, Tapia-González A, Cabrera-De la Fuente M, González-Fuentes JA, Benavides-Mendoza A (2018). Age, benefit and gibrellic acid affect the germination and production of piquín pepper plant. Revista Mexicana de Ciencias Agrícolas 9(special issue 20):4199-4209. *https://doi.org/10.29312/remexca.v0i20.990*

Satya Srii V, Nagarajappa N, Vasudevan SN (2022). Is seed coat structure at fault for altered permeability and imbibition injury in artificially aged soybean seeds? Crop Science 62(4):1573-1583. *https://doi.org/10.1002/CSC2.20750*

Socrates G (2004). Infrared and raman characteristic group frequencies: tables and charts. John Wiley & Sons Ltd. (3rd ed), Chichester, UK. Volume 35.

Vazquez-Flores AA, Góngora-Pérez O, Olivas-Orduña I, Muñoz-Bernal ÓA, Osuna-Avila P, Rodrigo-García J, … Alavarez-Parrilla E (2020). Pytochemical profile and antioxidant activity of chiltepin chili (*Capsicum annuum* var. *glabriusculum*), Sonora, Mexico. Journal of Food Bioactives 11:57-65. *https://doi.org/10.31665/jfb.2020.11237*

Yu X, Li A, Li W (2015). How membranes organize during seed germination: three patterns of dynamic lipid remodelling define chilling resistance and affect plastid biogenesis. Plant, Cell & Environment 38(7):1391-1403. *https://doi.org/10.1111/pce.12494*

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