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Research article

Nanostructured mesoporous silica materials induce hormesis on chili pepper (*Capsicum annuum* L.) under greenhouse conditions

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ABSTRACT

Current agricultural practices for vegetable production are unsustainable, and the use of certain nanomaterials has shown significant potential for either plant growth promotion or defense induction in crop species. The aim of the present work was to evaluate the possible effects of two SBA nano-structured silica materials differing in morphology; SBA-15, with porous structure in parallel and with a highly ordered hexagonal array and SBA-16, with spheric nano-cages located in cubic arrays, as plant growth promoters/eustressors on chili pepper (*Capsicum annuum* L.) during cultivation under greenhouse conditions. The study was carried out at three foliarly applied concentrations (20, 50 and 100 ppm) of either SBA materials to determine effects on seed germination, seedling growth, plant performance and cold tolerance under greenhouse. Phytotoxicity tests were carried out using higher concentrations (100, 1000 and 200 ppm) applied by dipping or spraying onto chili pepper plants. Deionized water controls were included. The results showed that the SBA materials did not affect seed germination; however, SBA-15 at 50 ppm and 100 ppm applied by imbibition significantly increased seedling height (up to 8-fold) and provided enhanced growth performance in comparison with controls under select treatment regimes. Weekly application of SBA-15 at 20 ppm significantly increased stem diameter and cold tolerance; however, SBA-16 showed significant decreases in plant height (20 ppm biweekly applied) and stem diameter (20, 50 and 100 ppm biweekly applied). The results demonstrate that both SBA materials provided hormetic effects in a dose dependent manner on chili pepper production and protection to cold stress. No phytotoxic response was evident. These findings suggested the nanostructured mesoporous silica have potential as a sustainable amendment strategy to increase crop production under stress-inducing cultivation conditions.

1. Introduction

It is widely accepted that in response to a growing population and a changing climate, achieving and maintaining global food security will be among the most significant challenges we face (White and Gardea-Torresdey., 2021). In addition, many current agricultural practices are unsustainable, especially in the face of climate change and the increased necessity to improve food production to support the growing global population (Lowry et al., 2019; Páramo et al., 2020). The application of nanotechnology in agriculture has shown promising results with regard to the development and use of products such as nano-enabled fertilizers, pesticides, and sensors that have significantly enhanced efficacy (Kah et al., 2019; Lowry et al., 2019; Páramo et al., 2020). There is great interest in novel agricultural practices that can sustainably produce vegetables, both by enhancing yield and by decreasing the negative environmental impact of food production (Vargas-Hernandez et al., 2017). Novel biostimulants and eustressors are approaches that are worthy of investigation. A biostimulant is a

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"formulated product of biological origin that improves plant productivity as a consequence of the novel or emergent properties of the complex of constituents, and not as a sole consequence of the presence of known essential plant nutrients, plant growth regulators or plant protective compounds" (Yakhin et al., 2017). Conversely, a eustressor is a "biological (also called elicitor), chemical or physical stress factor that when applied in low dose cause beneficial effects on plant immunity induction without significant negative affect on plant performance and yield" (Vázquez-Hernández et al. 2019a). Important, some of the beneficial effects that nanomaterials have displayed in agriculture are related to roles as plant growth promoters or eustressors, often very much dependent on the dose (Vázquez-Hernández et al., 2019a; Juárez-Maldonado et al., 2019).

There is a robust literature evaluating the interaction of nanomaterials with plants, and a number of studies have demonstrated many changes in exposed plants at the morphological, physiological, and molecular levels; with the extent of impact depending on properties as material chemical composition, size-morphology, surface coating, reactivity and dose (Siddiqui et al., 2015; Lowry et al., 2019). Nano-enabled agriculture has the promise of enhancing vegetable production while simultaneously reducing the negative environmental impacts of current agrochemicals. Innovations driven by nanotechnology can deliver a more sustainable, efficient and resilient agricultural system, while promoting food security. In the late 1990s, several mesoporous nano-structured materials based on silica were developed, including SBA-15 and SBA-16 (Santa Barbara Amorphous 15 and 16, respectively) (Lopes et al., 2013). Both SBA materials are of significant research and industrial interest due to features such as high surface area, well-defined porous structure, relatively inert nature, low biotic toxicity, high biocompatibility, thermal and hydro-thermal stability. These beneficial features have led to several applications in catalysis, adsorption, chemical detection, immobilization and drug delivery, among others (Lopes et al., 2013). In addition, the beneficial effects of silica nanoparticles such as SiO_2 (dose ranging from 2-14 g/L) on plants have been reported, including improving tomato germination (Siddiqui and Al-Whaibi, 2014); stimulating the growth and quality in *Changbai larx* (*Larix olgensis* A.Henry) seedlings measured by mean height, root collar diameter, main root length and the number of lateral roots (Bao-Shan et al., 2004); and promoting tolerance to salinity in tomato and squash (Haghighi et al., 2012; Siddiqui et al., 2015). However, SBA materials have only been explored in a limited number of crop species and only in early plant developmental stages (Sun et al. 2014, 2016). The effects of conventional silica on plants have been well documented, with this element being considered as beneficial to plant growth and to increasing resistance to biotic and abiotic stresses (Avila-Juarez et al., 2017).

Capsicum spp (Chili pepper) is an economically important crop, with a production in 2019 of ca. 35 000 000 tons, and China, Mexico and Turkey are the main producers (Rodríguez-Calzada et al., 2019). *Capsicum* spp have a flowering time between 55-60 days post-germination, with typical production of 35–50 fruits per plant (Mejia-Teniente et al., 2013). Some reports indicate that approximately 75% of the Mexican producers of *Capsicum* spp use several synthetic pesticides, primarily endosulfan (82 % of the cultivated surface), Mancozeb (65 %) and chloropyrifos (49%), to control pest and diseases during crop production (Mejia-Teniente et al., 2019). Importantly, the novel use of nano-enabled biostimulants and eustressors during cultivation could boost crop protection and enhance yield while simultaneously minimizing synthetic pesticide applications, thereby offering a more sustainable approach to crop production (Mejia-Teniente et al., 2019; Vázquez-Hernández et al. 2019a).

The goal of the current study was to evaluate the possible effects of SBA nano-structured materials as plant growth promoters/eustressors on chili pepper (*Capsicum annuum* L.) plants during cultivation under greenhouse conditions. The present study evaluated foliarly application of three concentrations (20, 50 and 100 ppm) of either SBA material to determine effects on seed germination, seedling growth, plant performance and cold tolerance under greenhouse conditions. In addition, toxicity evaluation was carried out using higher doses (100, 1000 and 200 ppm) by foliar dipping or spraying onto chili peppers. The development of Si-based novel and sustainable nanoscale technologies to increase crop production are critical to efforts to achieve global food security in sustainable way.

2. Materials and methods

2.1. Preparation of SBA-15 silica material

The SBA-15 mesoporous material was synthesized according to the sol-gel method of Flödstrom and Alfredsson (2003). Here, 4.8 g of the structural directing agent Pluronic P123 (Sigma-Aldrich, av. M_n~5,800) was dissolved by stirring in a solution consisting of deionized water and 4M hydrochloric acid at 35 °C. Then 11 mL of the silica precursor tetraethyl orthosilicate (Aldrich, 98%) was added and the mixture was stirred for 24 h. The resulting gel was transferred to a polypropylene bottle for incubation at 80 °C for an additional 24 h. The solid was recovered by filtration and washed with deionized water. The resulting solid was dried at 110 °C and then calcined for 4 h at 550 °C.

2.2. Preparation of SBA-16 silica material

The SBA-16 mesoporous silica material was synthesized via the sol-gel method as reported previously (Palos-Barba et al., 2020). A solution of deionized water and 2M HCl was prepared to dissolve the structure-directing agent Pluronic F127 triblock copolymer (Sigma-Aldrich). The mixture stirred for 1 h, and then 26 mL of silica precursor tetraethyl orthosilicate (Aldrich, 98%) was added. After 24 h of stirring at 30 °C, the suspension was transferred to a polypropylene bottle for incubation at 80 °C for 24 h. The solid was recovered by filtration and then washed with deionized water. Last, the solid was then dried at 110 °C and calcined for 6 h at 500 °C.

2.3. SBA materials characterization

The SBA-15 and SBA-16 mesoporous materials were evaluated by N_2 physisorption at 77 K with an Autosorb-IQ2 instrument (Anton Paar). For each sample, specific surface area was calculated by the Brunauer–Emmett–Teller (BET) method (ISO, 2010) and the adsorption-desorption data was analyzed using the Barrett-Joyner-Halenda (BJH) method (Barret et al., 1951) to acquire information about the pore size distribution and to confirm the mesoporous structure of the materials. The SBA-15 and SBA-16 surface topography was observed by Scanning Electron Microscopy (SEM) (TM3030Plus Hitachi). Material size and morphology was investigated by Scanning/Transmission Electron Microscopy (STEM) (Nanotech TEM JEOL JEM 2200FS + CS). Specifically, the materials were suspended in deionized water at 200 ppm and sonicated for 2 min. The ζ potential was measured by dynamic light scattering on a Malvern Zetasizer (Nanoseries ZS90) to analyze the electrostatic repulsion or attraction (charge) of the particles.

2.4. Chili pepper (Capsicum annuum L.) germination and seedling growth

SBA 15 and SBA-16 were suspended in deionized water (pH 6.4) and evaluated at low (25 mg/L), medium (50 mg/L) and high (100 mg/L) doses on chili pepper seeds (*C. annuum* L. cv. Dante-HMX 4664 F1, Harris Moran) based on previous reports using similar Si-based materials with maize (*Zea mays* L.) and tomato (*Solanum lycopersicum* L.) (Siddiqui et al., 2015). SBA suspensions were dispersed by sonication (Hielscher UP200Ht, Hielscher Ultrasonics, Teltow, Germany) and stored in the absence of light prior to use. The SBA materials were evaluated by imbibition and spraying assays. For imbibition, 50 seeds were immersed in 50 mL of either SBA 15 or 16 at 0, 25, 50 or 100 mg/L. The seeds were incubated for 1 h with slight shaking, and then the seeds were placed in a

filter paper (Whatman grade 1) within a petri dish. This experiment was carried out in triplicate (Total n = 150 seeds per treatment; 50 seeds per replicate). For the spraying assay, the seeds were placed within the petri dishes with filter paper (Whatman grade 1), and then were sprayed with 5 mL of the above-described SBA treatments. In both assays, the petri dish lids were secured to maintain humidity. Seed germination was evaluated daily as determined by emergence of the radicle. After 14 days, plant height was evaluated, and the seedlings were stored at – 80 °C for further biochemical and molecular analyses. Seedling length and root length were measured using a Vernier.

2.5. Capsicum annuum L. greenhouse assay

Both SBA materials were evaluated at 0, 25, 50 and 100 mg/L in a *C. annuum* L. cultivation study under greenhouse conditions. SBA materials were either weekly or bi-weekly sprayed onto the plants to drop point (i.e, saturated until run-off) according to Mejía-Teniente et al., (2019). The greenhouse conditions during cultivation were 17–28 °C and a relative humidity 60%. Seedlings with two true leaves were transplanted to pots containing tezontle (a substrate consisting in aluminum silicate formed by low weight porous lava fragments, commonly used as inert substrate in plant cultivation)-bokashi compost (organic fertilizer produced by fermentation of organic residues at high temperature during 21 days), and ant soil (consisting of residues from the ant species Atta Mexicana) (1:1:1). Steiner universal solution (100 %) was used to supplement plant nutrition and automated irrigation was used during cultivation. A factorial experimental design with 3 factors (type of SBA material, application time and concentration of SBA materials) was established, with deionized water as the control. Twelve replicate plants were grown for each treatment. This greenhouse study was evaluated during 15 weeks until fructification ends. During week 9 of cultivation, morphological data were measured in order to evaluate plant performance; moreover, during this time leaf samples were collected to carry out biochemical and molecular analyses.

2.6. Evaluation of SBA materials on cold stress response in Capsicum annuum L.

A separate experiment was conducted to evaluate the impact of SBA treatment on cultivation under cold stress. Here, the greenhouse design described above was used, with the exception that no supplemental heating was used during cultivation in order to evaluate possible cold tolerance induced by SBA treatment. The selected treatments to be evaluated in this part of the study were weekly SBA-15 foliar spray at 20 mg/L, and bi-weekly SBA-16 foliar spray at 20 mg/L foliarly. The experimental design consisted of a factorial experiment as described above. Plant necrotic zones on leaf and stem evaluated plant performance visually after 4 cold condition events (between -1 and -4 °C) that occurred during this experiment (January 2019). The experimental design consisted of 30 plants (n = 30) for each treatment.

2.7. Toxicity tests of SBA materials onto Capsicum annuum L.

A greenhouse experiment was conducted to evaluate potential phytotoxicity of SBA materials. Two factors were considered in this experiment: type of SBA material and concentration (100, 1000 and 2000 mg/L); deionized water was used as the control. This experiment was carried out at The Connecticut Agricultural Experiment Station (New Haven, CT, USA) under greenhouse conditions with automated lighting and at an average temperature 24 °C and 60% relative humidity. In this experiment, both foliar dipping and spraying were investigated as application methods for SBA materials onto plants. In the case of dipping, seedlings at the four true leaf-stage were inverted and immersed into respective SBA solutions for 10 s. The seedlings were allowed to dry (inverted) and were then transplanted in pots containing PRO-MIX BX® and amended with Miracle-Gro® as a fertilizer to support growth. For the spraying experiment, the seedlings were sprayed weekly to drop point with the SBA solutions. The plants were grown for 10 weeks, followed by evaluation of plant height, basal stem diameter, leaf number, relative foliar area (Easy Leaf Area), total chlorophyll (MultispeQ), fresh weight, dry weight, relative water content and flowering. In addition, 200 mg of samples from fruit and root tissues were collected for elemental analysis by inductively coupled plasma optical emission spectroscopy (ICP-OES) (ThermoScientific iCAP 6500) to determine Si content. For these analyses, plant samples were dried, homogenized and acid digested with nitric acid in a hot plate (45 min at 115 °C). Five replicate samples were then analyzed by ICP-OES for Si concentration. For quality control, a standard reference material (SRM 1573a, tomato leaves) was included in each batch of sample; yttrium as an internal standard, and a sample of a known concentration was read every 15 samples to verify the calibration of our instrument.

2.8. Determinations of total phenolics, flavonoids and antioxidant activity

Leaf samples (1 g) were collected at 9 weeks in the first cultivation greenhouse experiment to measure total phenolics, total flavonoids and antioxidant activities of the chili pepper plants in each treatment. Total phenolic content was evaluated with the Folin-Ciocalteu assay as described by Cardador-Martinez et al. (2002). The reaction is as follows: 40 μL of methanolic extracts were diluted using 460 μL of distilled water and 250 μL of the Folin-Ciocalteu reagent from SIGMA. After 5 min, the reaction was stopped with 1250 mL of 2% Na_2CO_3 and then incubated for 60 min at room temperature. The absorbance of each sample was measured at 765 nm using a UV–vis spectrophotometer. Gallic acid was used as phenolic standard and the total phenolic content was expressed as mg gallic acid equivalents/g sample. Total flavonoids were measured as mg of rutin equivalents per g of sample as reported by Oomah et al. (2005). Total flavonoids were determined using the 2-amino-ethyldiphenylborate assays by mixing 50 μL of a methanolic extract with 180 μL of distillate water and 20 μL of 1% of the 2-amino-ethyldiphenylborate solutions in a 96 wells microplate. The absorbance was determined at 404 nm on UV-vis spectrophotometer using rutin as flavonoid standard. Total flavonoid content was expressed as μg rutin equivalents/g sample. Antioxidant activity was determined by DPPH (Fukumoto and Mazza, 2000) and ABTS (Re et al., 1999) methodologies. DPPH method was as follows: 20 μl of the methanolic extract were mixed with DPPH (150 μM, 80% v/v aqueous methanol). The plate was covered and left in dark at room temperature. After 30, 60, 75, 90 and 120 min, absorbance was measured at 520 nm on a spectrophotometer. Antioxidant activity and total antioxidant content were expressed as μM Trolox equivalents/g sample. Antiradical activity (ARA) was determined by the Burda and Oleszek Eq. (1):

$$\% \text{ Inhibition} = [\text{Ac(o)} - \text{AA (t)}/\text{Ac(o)}] \times 100 \quad (1)$$

where Ac(o) is the absorbance of the control at t = 0 min and AA (t) is the absorbance of antioxidant at t = 1 h.

The ABTS (2,20-azino-bis-(3-ethyl benzothiazolin-6-ammonium sulphonate)) assay was performed by measuring triplicate samples at 734 nm (MULTISKAN GO, Thermo Fisher Scientific, Finland). The results were expressed as the percent inhibition of ABTS based on the following formula (2):

$$\%\text{ABTS\_inhibition.} [(\text{A.Control})-(\text{A.Sample})/\text{A.Control}]\times 100 \quad (2)$$

where A.Control represents the absorbance of the ABTS solution and A.

The results represent the absorbance of the sample with the ABTS solution.

2.9. Gene expression analysis

The expression of some plant health genes was determined by reverse transcription-real time PCR (RT-qPCR) (Rodriguez-Calzada et al., 2019).

Table 1. Structural and electrical characterization of SBA-15 and SBA-16 used in the study.

Type of nanostructured material	Surface* area (m^2/g)	Porous volumen (cm^3/g)	Porous Diameter (nm)	ζ potential (mV)
SBA-15	817.74_a	0.9142_a	5.65_a	-19.9_a
SBA-16	773.4_b	0.7342_b	3.41_b	-37.3_b

* Different letters in each column for each variable indicates significant difference according to Tukey's test (P = 0.05).

The genes evaluated corresponded to Mn-superoxide dismutase (*Mn-sod*, Genbank accession number AF036936.2), peroxidase (*pod*, Genbank accession number FJ596178.1), phenylalanine ammonia lyase (*pal,* Genbank accession number AF081215), and chalcone synthase (*chs*, Genbank accession number FJ705842.1). Leaf samples in triplicate were ground in liquid nitrogen, and RNA was extracted using a comercial kit (RNAeasy Plant Mini Kit, Qiagen). RNA of high purity (260/280 nm absorbance ratio above 2.0 and 260/230 nm absorbance ratio 1.8–2.0) was used to synthesize cDNA (Mastercycler gradient, Eppendorf) using the Maxima First Strand cDNA Synthesis #K1612 (ThermoFisher Scientific) for RT-qPCR according to the manufacturer (10 min at 25 °C followed by 15 min at 50 °C). Primer sequences used in the study were the following: *Mn-SOD* (forward 5′-CTC TGC CAT AGA CAC CAA CTT-3′; reverse 5′-CCA AGT TCG GTC CTT TAA TAA-3′), *POD*, (forward 5′-GCA GCA TTC CTC CTC CTA CT-3′; reverse 5′-ATT TCT TTG CCT TGT TGT TG-3′), *PAL* (forward 5′-ATT CGC GCT GCA ACT AAG AT-3′; reverse 5′-CAC CGT GTA AGG CCT TGT TT-3′), *CHS* (forward 5′-TCG ACC CTC AGT CAA ACG AC-3′; reverse 5′- TGG GCC ACG GAA AGT AAC TG -3′); and β-tubulin (β-TUB GenBank accession number EF495259.1, forward 5′- GAG GGT GAG TGA GCA GTT C-3; reverse 5′- CTT CAT CGT CAT CTG CTG TC). All primers were provided by T4 Oligo, (Irapuato, Gto, Mexico) and were used to amplify the genes using Applied Biosystems™ PowerUp™ SYBR™ Green Master Mix for qPCR analysis (Step One Plus Real-Time PCR System, ThermoFisher Scientific). Reaction conditions were: 2 s, 95 °C, and 40 cycles of 3 s, 95 °C and 30 s, 60 °C, quantitation was according to $\Delta\Delta^{Ct}$ method (Rodríguez-Calzada et al., 2019).

2.10. Statistical analysis

Statistical differences in the greenhouse study were determined by a two-way ANOVA; in the remainder of the experiments, a one-way ANOVA was carried out with a Tukey's test ($P < 0.05$) using GraphPad Prism 6.0 (GraphPad software, California, CA, USA). For the analysis, residuals were revised for normality using the Anderson-Darling test (P = 0.05). Data residuals were also revised for homoscedasticity and linearity using the Hawkins test and Q-Q plot graphical method (Hawkins, 1981; Holiday, 2017).

3. Results and discussion

3.1. Characterization of SBA-15 and SBA-16 materials

The characterization data of SBA-15 and SBA-16 are shown in Table 1. According to the Brunauer-Emmett-Teller (BET) method, SBA-15 displayed average surface area values of 817.74 m^2/g; pore sizes calculated with the Barrett-Joyner-Halenda method estimated pore volume at 0.9142 cm^3/g and a diameter of 5.65 nm. These values are significantly greater than those of SBA-16, which were 773.4 m^2/g (Fc =

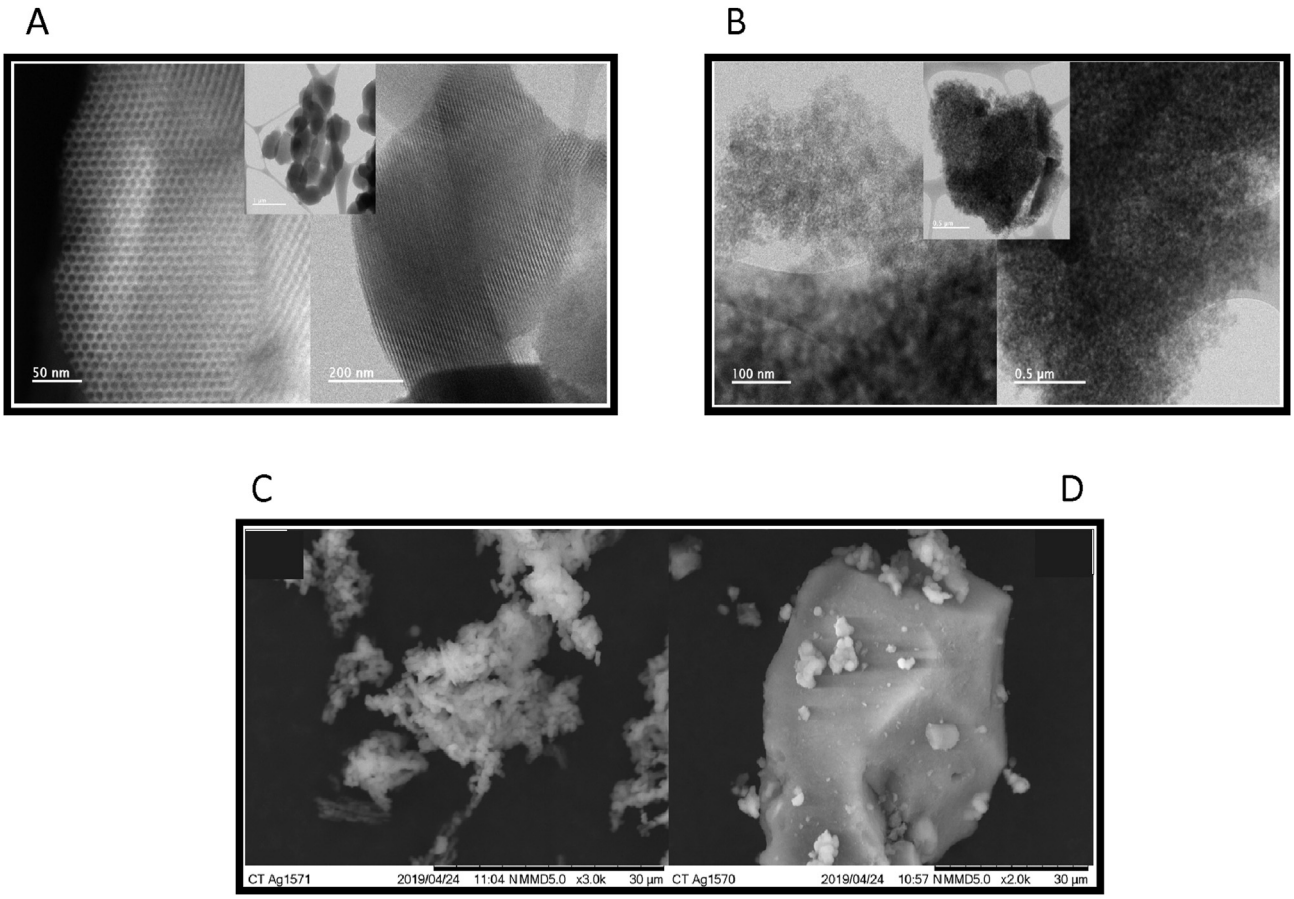

Figure 1. Electron microscopy images of the SBA-15 and SBA-16 used in the study. Panel A and B, SBA-15 and SBA-16 visualized by scanning transmission electron microscopy. Panel C and D, SBA-15 (left) and SBA-16 (right) by scanning electron microscopy.

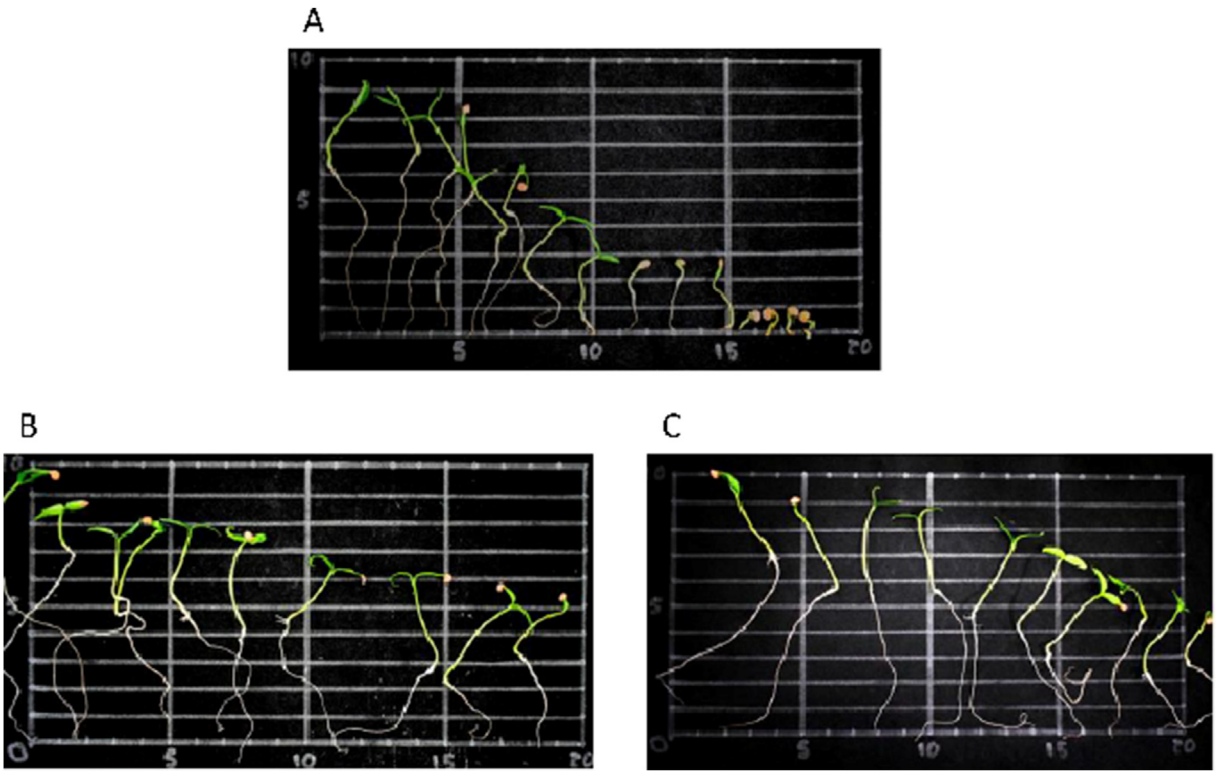

Figure 2. Phenotype of chili pepper seedings treated with SBA-15 and SBA-16 displaying significant biostimulant effects on growth at 14 days post-germination. Panel A, Control (non-treated with any SBA material); Panel B, seedlings treated with SBA-15 (100 ppm, the most significant treatment for SBA-15) by spraying; Panel C, seedlings treated with SBA-16 (50 ppm, the most significant treatment for SBA -16) by spraying.

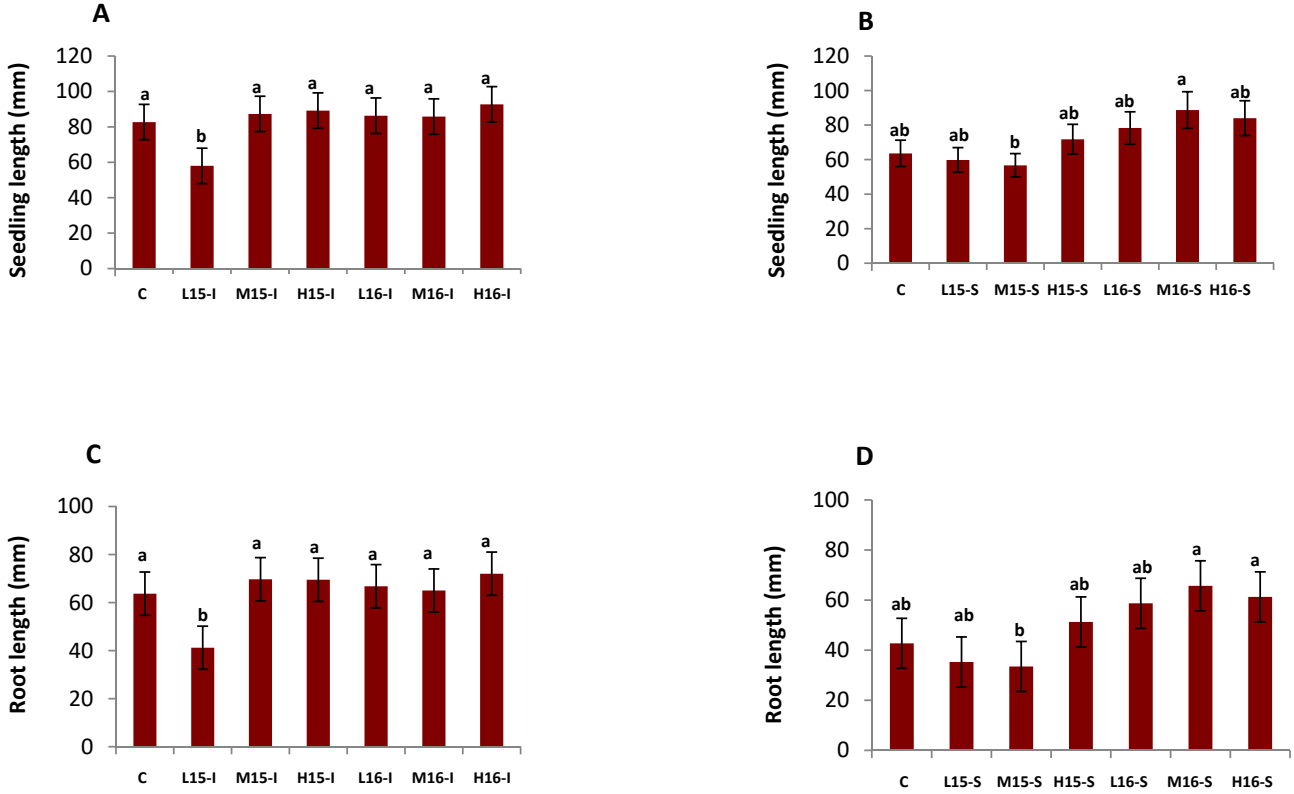

Figure 3. Effect of SBA-15 and SBA-16 on seedling length (Panels A and B) and root length (Panels C and D) either by imbibition (I) or spraying (S) application. Symbology: L (20 ppm), M (50 ppm) and H (100 ppm) of either SBA-15 (15) or SBA-16 (16). Different letters in each bar for each graph indicate significant difference according to Tukey's test (P = 0.05). Bars in the graphs indicate standard deviation.

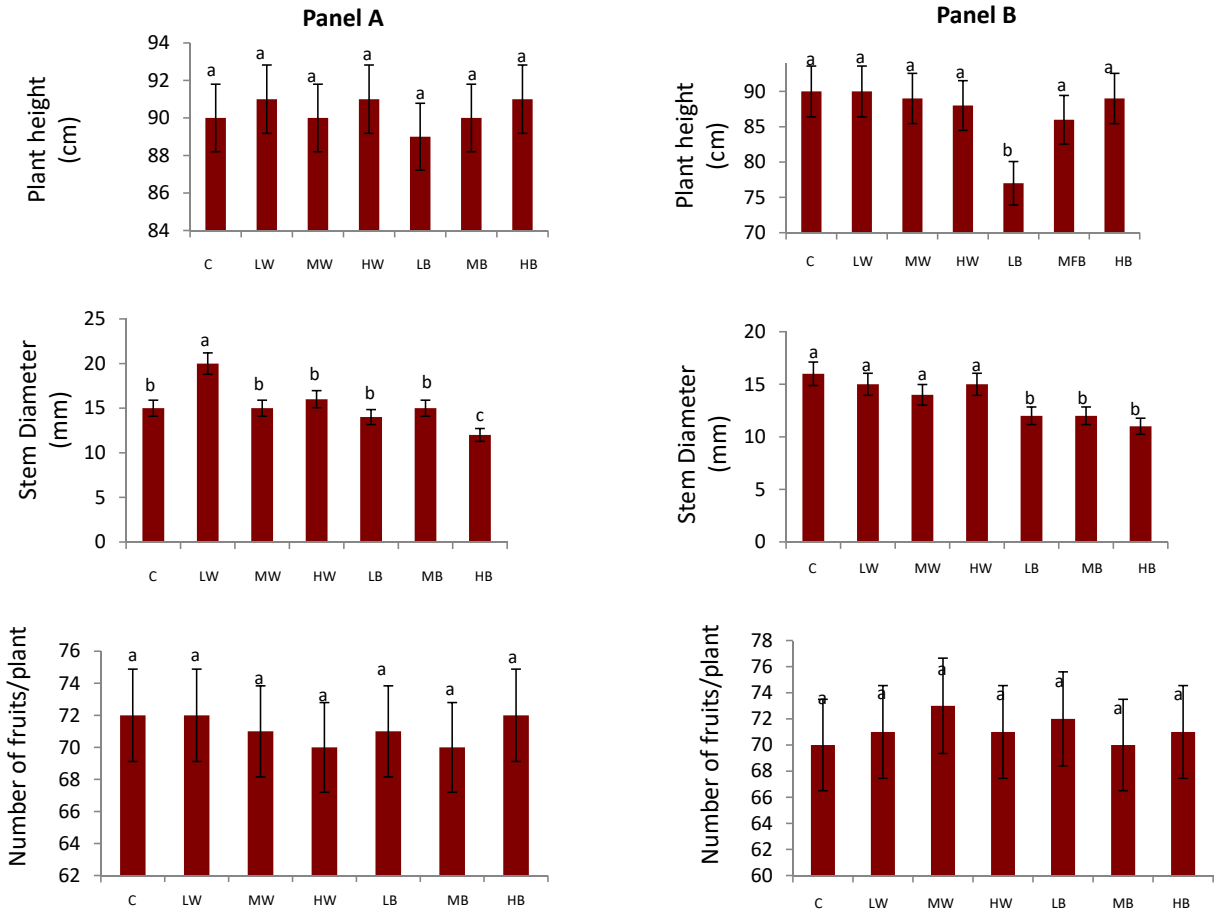

Figure 4. Effect of SBA-15 (Panel A) and SBA-16 (Panel B) on some plant performance variables in chili pepper grown under greenhouse conditions. Results shown correspond to 9 weeks post-transplant. Symbology: C, control plants non-treated with any SBA material; L (25 ppm), M (50 ppm) or H (100 ppm) indicates low, medium or high dose of SBA material, respectively. W or B indicates weekly or biweekly applications, respectively. Different letters in each bar indicate significant difference according to Tukey's test (P = 0.05). Bars in the graphs indicate standard deviation.

1946569.88; df = 1; p-value<0.00001), 0.7342 cm^3/g (Fc = 13503000.17; df = 1; P-value<0.00001) and 3.41 nm (Fc = 208693.5; df = 1; p-value<0.00001), respectively (Table 1). During the textural characterization of SBA-15 and SBA-16 materials, type IV isotherms were observed (Sing, 2009), demonstrating the existence of mesopores. SBA-15 showed a H1 hysteresis loop (not shown), indicating cylindrical pores, whereas the SBA-16 material exhibited a H2 hysteresis loop (not shown) corresponding to cage-like interconnected mesopores (Palos-Barba et al., 2020). These textural properties for SBA-15 and SBA-16 align well with the SBA materials reported in the literature (Katiyar et al., 2006; Liu et al., 2013). The zeta potential of SBA-15 and SBA-16 were significantly different, with average values of -19.9 and -37.3, respectively (Table 1). The SBA-16 materials displayed a higher stability in the dispersion when forming aqueous suspensions in comparison with SBA-15. The results of the zeta potential obtained in the present study for both SBA materials were similar to those reported by Andrade et al. (2013).

Images obtained by S/TEM and SEM of both SBA materials are shown in Figure 1. SBA-15 observed by S/TEM displayed amorphous particles with mesoporous structure in highly ordered hexagonal conformation (Figure 1). The size of SBA-15 particles ranged from 400 to 800 nm, which were smaller than those reported by Katiyar et al. (2006) for SBA-15 materials. S/TEM results for SBA-16 also displayed amorphous particles but with less order than SBA-15 (Figure 1). The size of SBA-16 particles obtained was smaller than 100 nm, which was similar to reports in literature for this type of material (Andrade et al., 2013). The SEM results of SBA materials showed that SBA-15 displayed heterogeneous morphology in comparison with SBA-16 (Figure 1). Importantly, the SBA-15 and SBA-16 materials produced in the current study displayed generally similar features (Figure 1 and Table 1) as reported for these materials in the literature (Zhao et al., 1998; Katiyar et al., 2006; Liu et al., 2013).

3.2. *Capsicum annuum* L. germination and seedling growth

Treatment with SBA-15 and SBA-16 at 0–100 mg/L did not significantly impact seed germination; the percent germination was 90–93% across all treatments and the control. Similar results using SBA materials have been reported in lupin (*Lupinus albus* L.), maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) using higher doses than the ones evaluated in the present study, ranging from 0.2 to 20 mg/mL (Hussain et al., 2013; Sun et al., 2014).

With regard to subsequent seedling phenotype, SBA-15 100 mg/L and SBA-16 50 mg/L clearly improved growth (Figure 2). The remainder of the treatments had no impact relative to the untreated control plants (data not shown). For seedling length, SBA-15 20 mg/L by imbibition showed significant decreases of 1.3-fold in comparison with control (Fc = 2227.27; df = 6; p-value<0.00001) (Figure 3). Root length was significantly decreased by the same treatments, with the remainder of the treatments displaying no difference in comparison with control (Fc = 807.03; df = 6; p-value = 0.0000) (Figure 3). Sun et al. (2014) reported no negative effects from exposure to mesoporous silica nanoparticles in *T. aestivum* L., *L. albus* L., *Z. mays* L. and *Arabidopsis thaliana* L. in doses ranging from 0.2 to 20 mg/L. In the present, the mesoporous silica

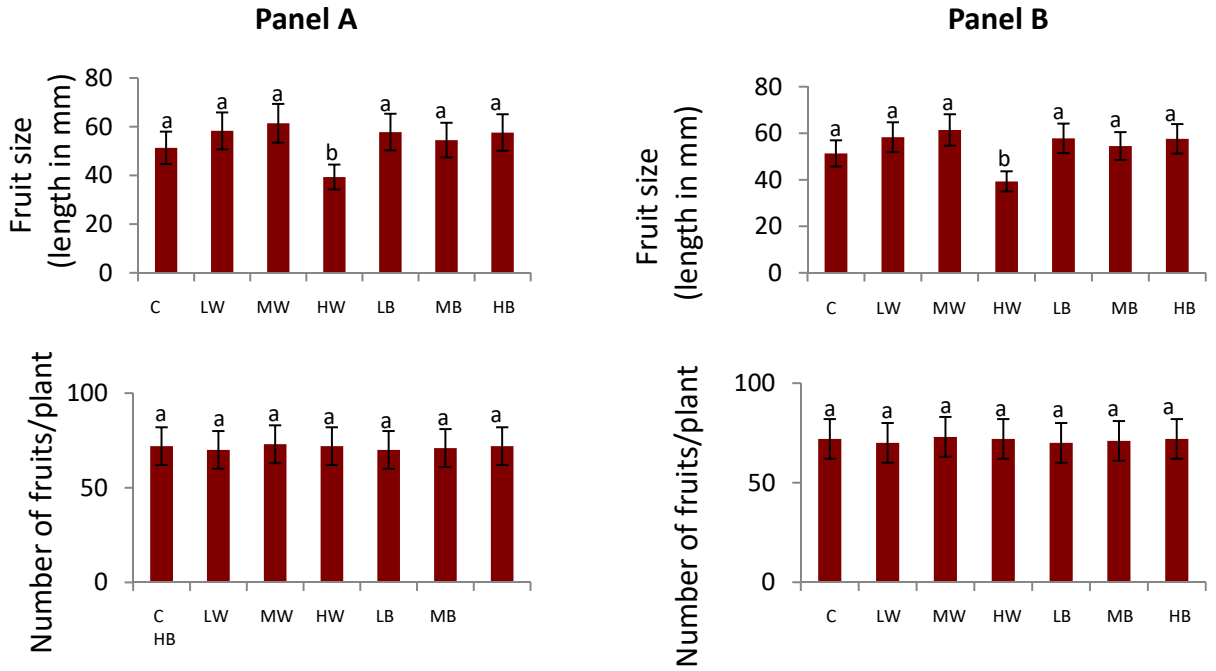

Figure 5. Effect of SBA-15 (Panel A) and SBA-16 (Panel B) on some plant performance variables in chili pepper grown under greenhouse conditions. Results shown correspond to 15 weeks post-transplant. Symbology: C, control plants non-treated with any SBA material; L, M or H indicate low (25 ppm), medium (50 ppm) or high (100 ppm) dose of SBA material, respectively. W or B indicate weekly or biweekly applications, respectively. Different letters in each bar indicate significant difference according to Tukey's test (P = 0.05). Bars in the graphs indicate standard deviation.

materials tested onto chili pepper are lower doses than the ones in the report of Sun et al. (2014), suggesting that both dose and plant species are important to overall response.

3.3. Greenhouse study

The results of either weekly or bi-weekly application of SBA-15 or SBA-16 on chili pepper plant height, stem diameter, and fruit production are shown in Figures 4 and 5. The results demonstrate variability in plant response as a function of treatment regime and dose. For example, amendment with SBA-15 did not affect plant height in comparison to controls after 9 weeks of cultivation; conversely, SBA-16 caused a significant decrease in plant height upon 25 mg/L bi-weekly application (Fc = 244.26; df = 6; p-value<0.00001) (Figure 4). Conversely, weekly SBA-15 application at 20 mg/L increased stem diameter (Fc = 221.59; df = 6; p-value = 0.0000), whereas bi-weekly treatment with the same material at 100 mg/L significantly decreased this parameter (Fc = 221.59; df = 6; p-value = 0.0000) (Figure 4). In addition, bi-weekly application of SBA-16 at 20 mg/L decreased both plant height (Fc = 92.77; df = 6; p-value = 0.0000) and stem diameter (Fc = 67.48; df = 6; p-value = 0.0000), and SBA-16 at 50 and 100 mg/L under bi-weekly application decreased stem diameter (Fc = 92.77; df = 6; p-value = 0.0000) (Figure 4). Interestingly, at 9 weeks of cultivation none of the treatments affected fruit number (Fc = 1.62977; df = 6; p-value = 0.20586) (Figure 4), however, 100 mg/L weekly application of either SBA material significantly decreased fruit size at 15 weeks of cultivation (Figure 5). To our knowledge, this is the first report on the effect of SBA materials on fruit parameters in a horticultural crop. Collectively, these findings on plant performance suggest that the SBA materials induce a hormetic response, with either benefit (eustressic) or toxicity (distressic) as a function of dose (Vargas-Hernández et al., 2017; Vázquez-Hernández et al. 2019a). A two-way ANOVA analysis of these variables did not identify interactions among the factors that resulted in a significant biostimulant effect in chili pepper under the greenhouse conditions (P = 0.05). Moreover, SBA-15 and SBA-16 also showed no difference on the plant performance variables evaluated (not shown). In general, as mentioned above the biostimulant effect depended on the dose more than on the type of SB and the time of application; the stem diameter was the only variable that displayed significant differences dependent on SB type, dose and the time of application (with the treatment SBA-15, 20 mg/L weekly applied as the optimum strategy, Figure 4). Future research with SBs materials must consider comparing possible differences with no-nanostructured Si, especially taking into account that many materials (including Si) behave differently at the nanoscale with biological implications (White and Gardea-Torresdey, 2021; Tripathi et al., 2016).

3.4. Biochemical and molecular endpoints

Both SBA materials significantly increased select biochemical endpoints at 9 weeks of cultivation, often in a dose-dependent fashion (Figure 6). Specifically, bi-weekly application of SBA-15 and SBA-16 at 20 mg/L increased total phenolics by as much as 4-fold and at 100 mg/L (Fc = 1413.36; df = 6; p-value = 0.0000), increased flavonoids content by as much as 8.7-fold (Fc = 5958.05; df = 6; p-value = 0.0000), in comparison with controls (Figure 6). In addition, weekly application of SBA-15 at 100 mg increased phenylpropanoid content by 3-fold (Fc = 1413.36; df = 6; p-value = 0.0000) and bi-weekly application at 20 mg/L increased content by 4-fold (Fc = 1413.36; df = 6; p-value = 0.0000) (Figure 6). SBA-15 and SBA-16 exhibited similar impacts on antioxidant activity, with significant increases for 100 mg/L weekly and bi-weekly applications at 1.4 and 3-fold for the DPPH test in comparison with the control (Fc = 3040.48; df = 6; p-value = 0.0000). Interestingly, the 50 mg/L bi-weekly application of both SBA materials significantly decreased this value (Fc = 3040.48; df = 6; p-value = 0.0000) (Figure 6). Antioxidant activity measured by ABTS inhibition was also similarly affected by SBA-15 and SBA-16 (Figure 6). Weekly application of 50 and 100 mg/L, as well as 50 mg/L bi-weekly-spraying, significantly increased ABTS inhibition by 3- and 8-fold in comparison with the control (Fc = 1578.18; df = 6; p-value = 0.0000), respectively (Figure 6). In addition, bi-weekly application of 25 and 100 mg/L caused a significant decrease (5-fold) in antioxidant activity compared to control (Fc = 1578.18; df = 6; p-value = 0.0002) (Figure 6). Given these findings, it is clear that both SBA-15 and

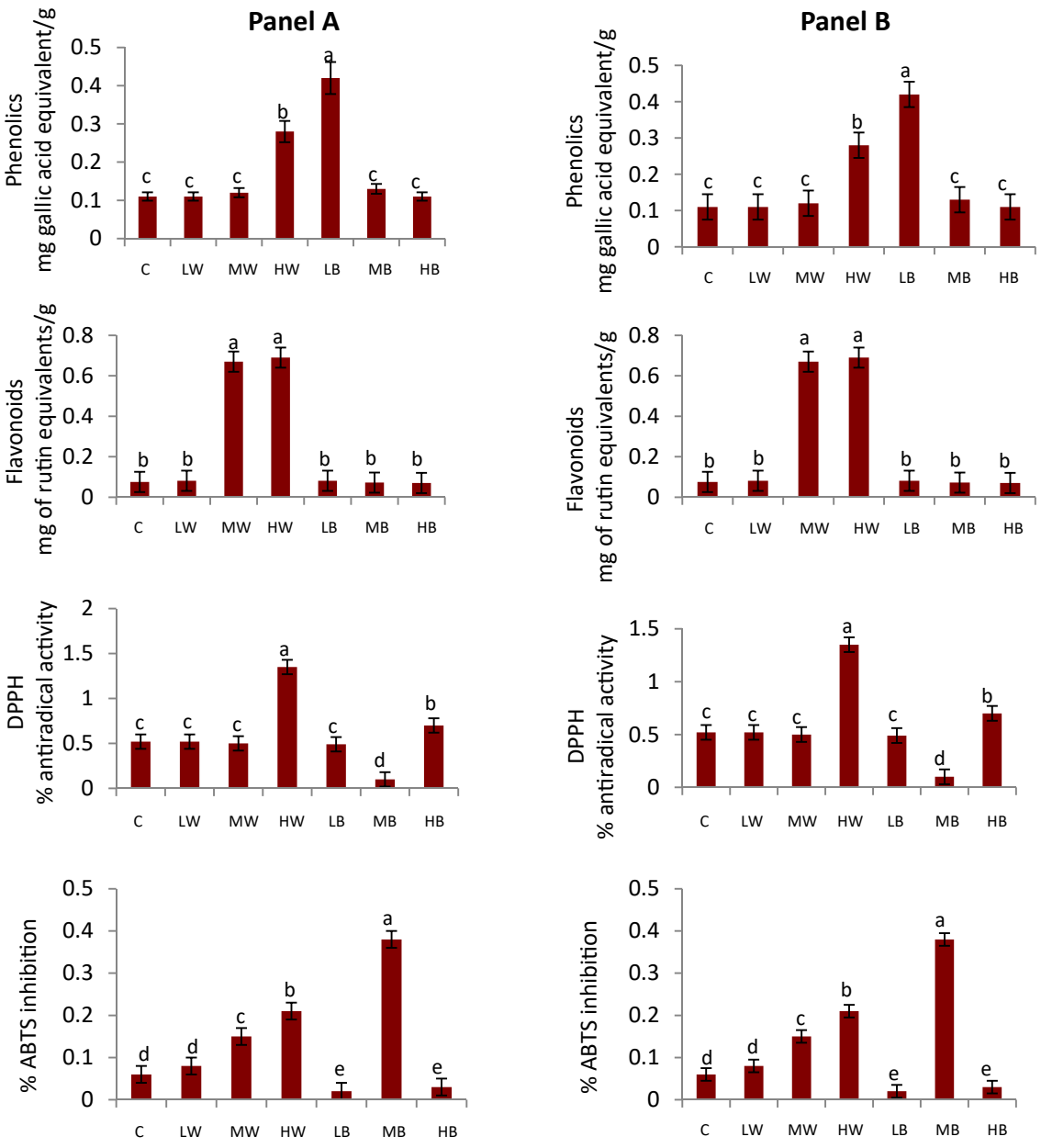

Figure 6. Effect of SBA-15 (Panel A) and SBA-16 (Panel B) on phenolics, flavonoids and antioxidant activity (DPPH and ABTS) in leaves of chili pepper grown under greenhouse conditions. Results shown correspond to 9 weeks post-transplant. Symbology: C, control plants non-treated with any SBA material; L, M or H indicate low (25 ppm), medium (50 ppm) or high (100 ppm) dose of SBA material, respectively. W or B indicate weekly or biweekly applications, respectively. Different letters in each bar indicate significant difference according to Tukey's test (P = 0.05). Bars in the graphs indicate standard deviation.

SBA-16 at the evaluated doses elicited plant defense pathways, aligning with several published reports demonstrating controlled stress induction as an elicitation strategy for plant performance improvement (Vázquez-Hernández et al., 2019b; Parola-Contreras et al., 2020). Select SBA treatments evaluated in the present study clearly caused controlled elicitation in chili pepper based on demonstrated increases in immunity indicators such as phenylpropanoids and antioxidant activity. It is likely that these treatments induced a systemic tolerance to either biotic or abiotic environmental stresses in comparison to control. These findings align with controlled elicitation strategies such as those reported for *C. annuum* L. and *Nicotiana tabacum* L. using hydrogen peroxide as elicitor and UV-B as eustressor, that have shown to be effective inducing tolerance to geminivirus disease and drought stress in these species (Cardenas-Manríquez et al., 2016; Mejia-Teniente et al., 2019; Saenz de la O et al., 2021).

With regard to gene expression, all SBA-15 and SBA-16 treatments significantly enhanced manganese superoxide dismutase (*Mn-sod*) and peroxidase (*pod*) expression, with increases of 1.7 and 1.9-fold in comparison with controls (Fc = 2074.16; df = 6; p-value = 0.0000) (Figure 7). In addition, phenylalanine ammonio lyase (*pal*) expression was significantly increased by 100 mg/L weekly application (1.86-fold) and 25 mg/L biweekly application (LB, 2-fold) for both materials compared with the control (Fc = 2496.2; df = 6; p-value<0.0000) (Figure 6). *Chalcone synthase* (*chs*) gene expression was increased by 50 (2-fold) and 100 mg/L (2.3-fold) weekly treatment for both SBA materials in comparison with controls (Fc = 2165.15; df = 6; p-value<0.0001) (Figure 7). This expression data also demonstrates an induction of *Mn-sod* and *pod* genes, as well as *pal* and *chs*, under certain treatments, supporting our hypothesized mechanism of action (Figure 7). Taken together, our findings indicate that certain hormetic doses of both SBA

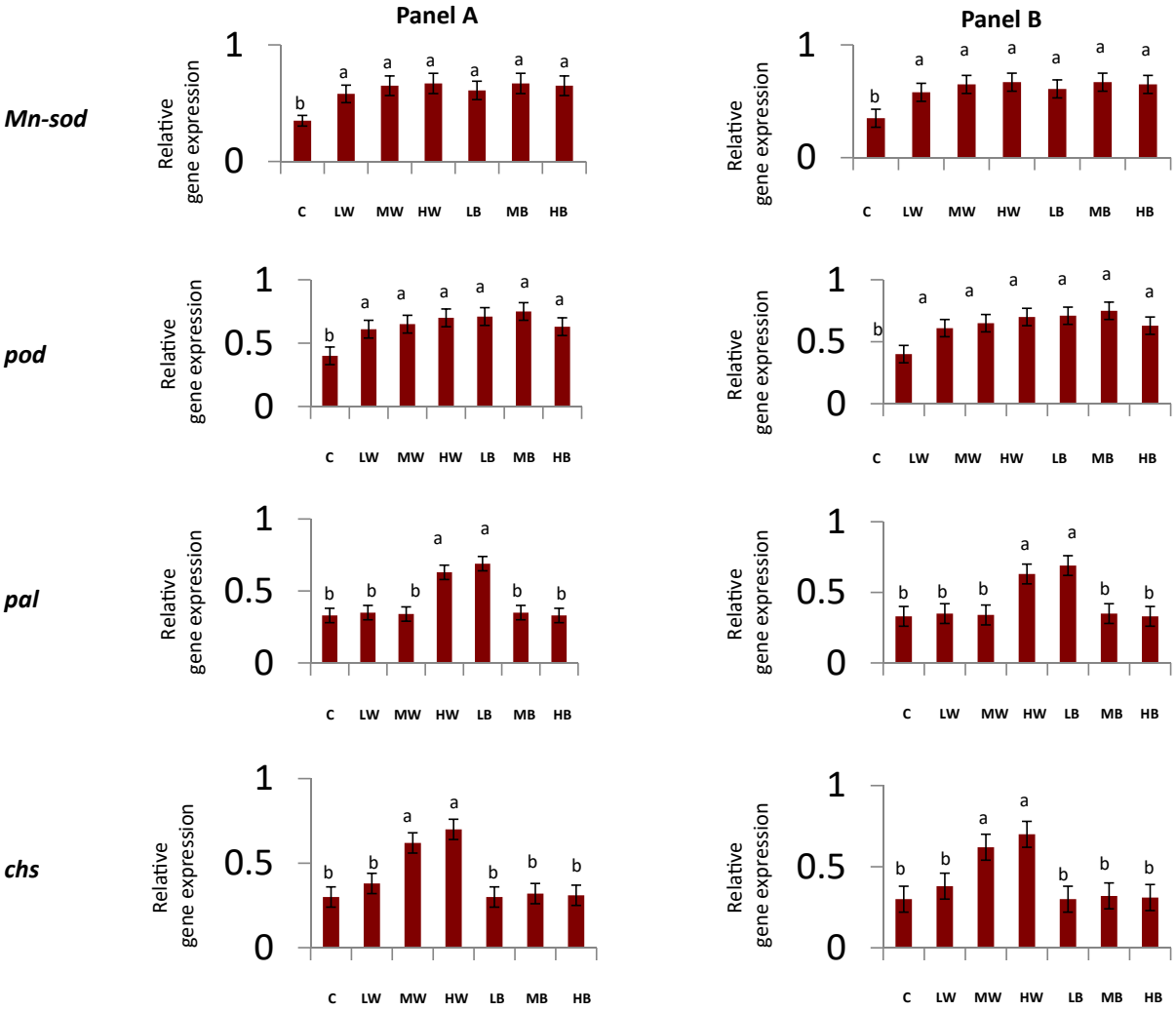

Figure 7. Effect of SBA-15 (Panel A) and SBA-16 (Panel B) materials on gene expression associated with plant defense in leaves of chili pepper grown under greenhouse conditions. Results shown correspond to 9 weeks post-transplant. Symbology: C, control plants non-treated with any SBA material; L, M or H indicate low (25 ppm), medium (50 ppm) or high (100 ppm) dose of SBA material, respectively. W or B indicate weekly or biweekly applications, respectively. Different letters in each bar indicate significant difference according to Tukey's test (P = 0.05). Bars in the graphs indicate standard deviation.

materials function as eustressors, inducing elicitation of molecular and biochemical endpoints related to plant defense, and highlighting a novel way to increase stress tolerance in plants (Feregrino-Perez et al., 2018; Vazquez-Hernandez et al., 2019).

3.5. Cold stress response of Capsicum annuum L. with SBA-15 and SBA-16

With regard to cold tolerance in *C. annuum* L., the selected treatments were chosen based on either no effect (SBA-15 20 mg/L weekly-sprayed) or actual growth inhibition (SBA-16 20 mg/L bi-weekly-sprayed) in comparison with control plants during the greenhouse study. These treatments were evaluated in the cold tolerance study, which included four cold conditions events between -1 and -4 °C during the experiment. Both control plants and those treated weekly with SBA-15 at 20 mg/L showed 100% mortality, visualized as extensive necrotic organ damage (stem and leaves) (Figure 8). Conversely, bi-weekly treatment with SBA-16 resulted in 95% of plant survival, demonstrating significantly greater cold tolerance (Figure 8). This enhancement in cold tolerance is likely related to increases in health indicators noted above, including phenylpropanoids, antioxidant capacity and gene expression-associated to plant defense as a function eustressor-induced plant defense (Vázquez-Hernández et al. 2019a). There are several reports of nanoparticles (NPs) providing abiotic stress tolerance, such as reported for silicon-NPs with maize (*Zea mays* L.) for arsenate toxicity (Tripathi et al., 2016), silica-NPs in salt stress with strawberry (*Fragaria x ananasa* Duch.) (Avestan et al., 2019) and Ag-NPs in heat stress for *T. aestivum* L. (Iqbal et al., 2019).

3.6. Phytotoxicity and Si accumulation

Treatment with SBA-15 and SBA-16 induced no toxicity, even at doses as high as 1000 and 2000 mg/L, regardless of application method. The level of silica (Si) in the fruit was evaluated in order to determine possible accumulation in plant organs after SBA exposure. The fruit Si content from plants treated (dipping) with SBA-16 at 2000 mg/L was significantly increased ($Fc = 555.1304$; $df = 4$; p-value<0.00001); levels were 41 μg/g as compared with 30 μg/g in the controls (Figure 9). Interestingly, the dipping treatment with SBA-15 at 2000 ppm resulted in a significant decrease in Si fruit content (23 μg/g) (Figure 8). No significant changes in Si content were detected in the leaves and roots across all treatments (data not shown). Similar toxicity results were reported with

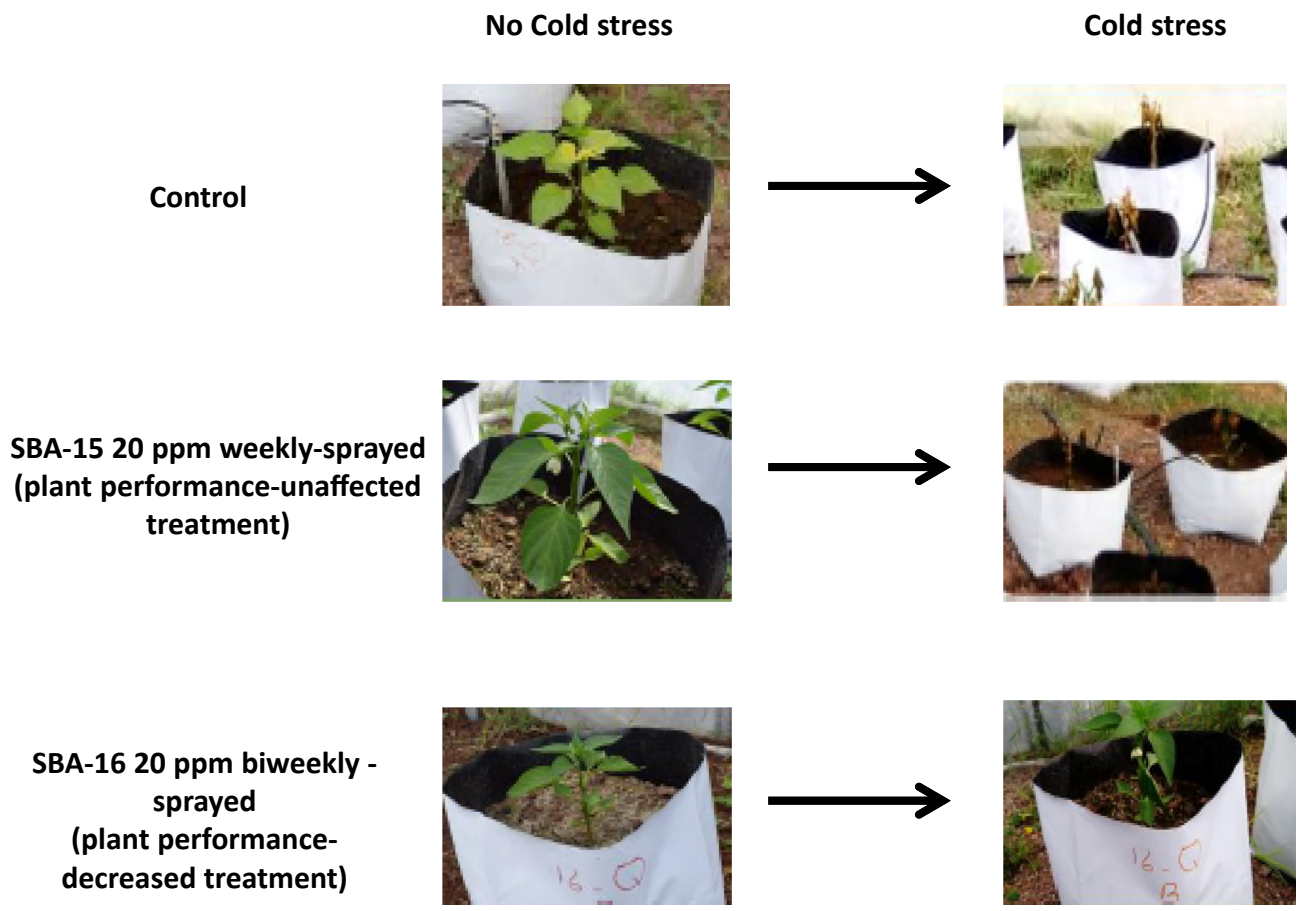

Figure 8. Typical phenotypic response of chili pepper grown under greenhouse in cold stress conditions and how treatment with SBA-15 and SBA-16 affect plant performance. The SBA-15 and SBA-16 materials were used at 20 mg/L either weekly or biweekly-spraying, respectively. Control plants were either weekly or biweekly-spraying with deionized water (only weekly-sprayed is shown, biweekly–spraying displayed the same results.

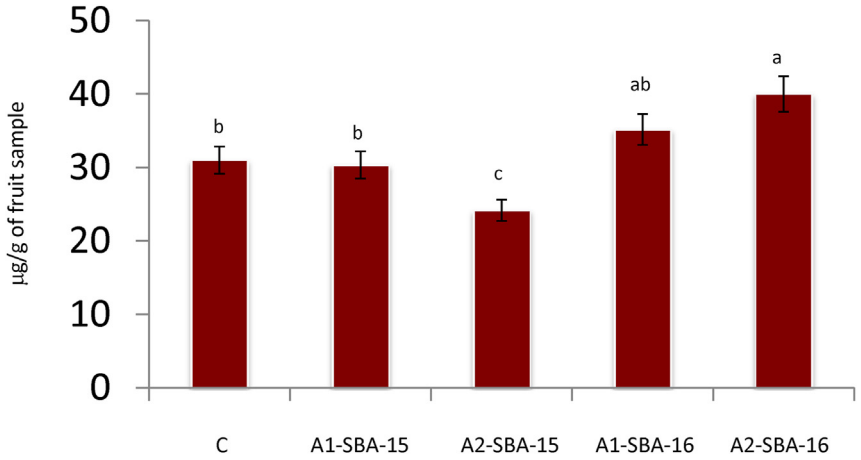

Figure 9. Determination of Si levels in fruits of chili pepper plants treated with 1000 ppm (A1) or 2000 ppm (A2) of SBA-15 or SBA-16 materials in toxicity tests. Control plants were treated only with deionized water. Different letters in each bar indicate significant difference according to Tukey's test (P = 0.05). Bars in the graphs indicate standard deviation.

SBA materials in *T. aestivum* L. and *L. albus* L. at doses up to 20,000 mg/L (Sun et al., 2016). These results suggest that SBA materials exert minimal toxicity even at excessive doses in several plant species, and suggest that higher doses could be used to further enhance chili pepper health and cold tolerance.

As noted above, the accumulation of Si in plant organs was largely unaffected by treatment, and there was no visual damage on the fruit tissue (Figure 9). In contrast, watermelon (*Citrullus lanatus* Thunb.) seeds treated with mesoporous silica nanoparticles (MSN) at 500 mg/L had significantly increased Si content (Buchman et al., 2019). Moreover, Kang et al. reported that in *C. lanatus* Thunb. treated with silica nanoparticles with different dissolution rates, significant increases in leaf Si content of both healthy and *Fusarium*-infected plants during 4 weeks of growth (Kang et al., 2021). These results suggest that the silica

nanoparticle application will lead to the accumulation Si in different plant organs, depending on the dose, as well as plant development stage and species.

4. Conclusion

The findings demonstrate that nano-structured mesoporous SBA-15 and SBA-16 induced plant growth promotion and eustressic effects on chili pepper (*C. annuum* L. cv. Dante-HMX 4664 F1) upon cultivation under greenhouse conditions. Specifically, seedling performance was improved with both SBA materials through the induction of plant defense indicators and improvement in the physiological functions during cultivation that lead to increased health and cold tolerance. Importantly, the SBA beneficial are dose dependent. Although the data is promising the complexity of applications at field scale could present unknown challenges and need to be investigated. In spite of there are still important unknowns, the need for sustainable strategies such as this one evaluated in the present study is critical given the pressure on food security from population growth and climate change.

Declarations

Author contribution statement

Ernesto Magaña-López; Viviana Palos-Barba; Nubia Zuverza-Mena; Ma.

Cristina Vázquez-Hernández: Performed the experiments.

Rufino Nava-Mendoza: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Ana A. Feregrino-Pérez: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Irineo Torres-Pacheco: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Jason C. White; Ramón G. Guevara-González: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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