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Carbonaceous Particles from Candle Soot Enhance Water Absorption and Modulate Starch-Sugar Metabolism in *Solanaceae* Seed Germination

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Abstract

Candle soot is a source of carbonaceous compounds that has been viewed as unwanted air pollution. Few have attempted to apply candle soot in agriculture, specifically for seed germination. This study was conducted to determine the effect of using candle soot on the germination of seeds from the *Solanaceae* family (*Capsicum annuum*, *Solanum lycopersicum*, and *Solanum melongena*). Three concentrations (10^{-2}, 10^{-3}, and 10^{-4} wt%) of candle soot were used. The results showed an improvement in measurable germination parameters and seedling quality parameters. The 10^{-3} wt% concentration was able to reduce the time needed for germination compared to the control by 11% in *C. annuum*, 12% in *S. lycopersicum*, and 10% in *S. melongena*. Further evaluation was conducted by analyzing the elements present in the seedlings. The results showed that the elemental information of seedlings treated with candle soot did not differ significantly from the control. This indicates that candle soot is biocompatible for agricultural applications. Further evaluation was also carried out to analyze biochemical components such as starch and soluble sugar, which play a crucial role in the seed germination process. The results showed a significant ($p < 0.05$) decrease in starch content compared to the control, while the soluble sugar content increased during treatment with candle soot. These carbonaceous particles could be a potential approach to enhancing germination and promoting sustainable agricultural practices, as revealed by this investigation.

Keywords: carbon; growth; hydrophilic; moisture; particles; seedlings

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INTRODUCTION

The increase in the global population demands adequate fulfilment of food needs (Raza et al., 2025). However, this contrasts with the decreasing availability of agricultural land due to land conversion for industrial activities, housing, and other purposes. From 2001 to 2016, 4.4 million hectares of farmland and ranchland in the United States were converted to urban, highly developed, or low-density residential area land use (Brain et al., 2023). Therefore, an approach is

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needed to increase agricultural yields and productivity without opening new agricultural land. This concept is known as agricultural intensification (Ndip and Sakurai, 2025). Intensification can be carried out at each life cycle stage of a plant, from germination, vegetative growth, and flowering to fruit production (Mas-Carrió et al., 2018).

Seed germination is a very crucial stage in the overall growth of plants (Farooq et al., 2021). Failure or delay in the germination process can reduce overall agricultural productivity (Nkosi et al., 2025). Several factors that can hinder seed germination include water availability, abiotic environmental factors (such as temperature, light intensity, and oxygen), seed viability, seed dormancy, pathogens, and pests (Tan et al., 2025). This could decrease overall plant productivity. Therefore, intensification is crucial at the seed germination stage to mitigate the negative effects of factors such as those mentioned earlier. One way to achieve this is by treating or priming the seeds before or during germination.

Seed treatment at the germination stage is a method aimed at improving germination performance and the quality of the resulting seedlings (Habibi et al., 2025). Seed priming is also a part of seed treatment, specifically a seed pre-sowing treatment that involves partially hydrating seeds but not allowing them to complete germination (Shrestha et al., 2021; Sembada and Lenggoro, 2024). Seed treatment and priming can be carried out biologically, physically, and chemically (Shrestha et al., 2021). Many types of chemical treatments can be used for seed germination, depending on the objectives to be achieved (Hafiz et al., 2025). The use of chemical compounds such as insecticides, fungicides, and herbicides is aimed at preventing pest and disease attacks that may occur during germination (Kamal et al., 2020). Meanwhile, the use of other chemical compounds, such as inorganic substances, nanoparticles, or plant growth regulators (hormones), aimed to accelerate germination and improve the quality of the resulting seedlings (Zaim et al., 2023).

Among these, nanopriming, using nanoparticles as priming agents, has emerged as a promising and innovative strategy to improve seed performance (Hameed et al., 2025). Nanopriming offers unique advantages due to the high surface area, reactivity, and tunable properties of nanoparticles (Amin and Aziz, 2025), which can enhance water uptake, enzyme activity, and stress tolerance during germination (Durgadevi et al., 2025). López-Vargas et al. (2020) demonstrated that the application of both carbon nanotubes and graphene for seed priming in tomatoes could improve root biomass by up to 127%. Recent studies continue to reinforce the effectiveness of various priming strategies. Tamimi (2024) reported that wheat seeds primed with 10% Dead Sea water (DS) achieved optimal germination and seedling growth under saline stress (up to 300 mM NaCl). The increased tolerance was attributed to improved water imbibition, antioxidant activity, and osmotic balance, including elevated proline and sugar levels, and reduced oxidative damage. In a nanotechnology approach, Handayani et al. (2025) found that silver nanoparticles (AgNPs) stabilized with tannic acid at ~27 nm and ~24 mg l^{-1} significantly enhanced the germination rate and growth of *Zea mays* compared to other sizes and concentrations. These findings highlight the role of nanoparticle size and formulation in optimizing seed performance.

Additionally, Yi et al. (2025) demonstrated that low concentrations (2 to 5%) of fermented liquid extract of *Padina australis* significantly improved germination and seedling vigor in curly lettuce, reinforcing the potential of eco-friendly, bio-based seed treatments. In addition, treatment and priming using such chemical compounds could provide resistance to seeds during germination under stress conditions such as drought and heavy metals (Prajapati et al., 2020). The researcher's previous study showed that tomato seeds primed with silica nanoparticles could alleviate the stress from copper and barium at concentrations of 1 to 10 ppm (Sembada and Lenggoro, 2024). González-García et al. (2022) showed that seed priming with carbon nanotubes could confer resistance to tomato seed germination under saline stress. Another study also demonstrated that the use of graphene oxide could alleviate salinity stress during the seed germination of *Cucumis melo* (Kaymak et al., 2022).

Exploring new sources for compounds that could be candidates for seed treatment and priming is an important area of study, especially in the context of promoting sustainable agriculture. The increasing demand for eco-friendly and cost-effective agricultural inputs has made it essential to identify alternative materials that support productivity while reducing environmental impacts (Yadav et al., 2025).

One such approach is the utilization of materials traditionally considered waste or pollutants. This aligns with the biorefinery and circular economy concepts, which aim to transform waste into valuable agricultural resources (Cherubini, 2010). In everyday life, we often encounter carbonaceous compounds, one of which is soot. Soot is a complex mixture of carbon-rich particles, primarily elemental carbon and amorphous carbon (Sembada et al., 2024b), along with organic compounds and trace metals, and it originates from the incomplete combustion of organic materials such as wood, coal, oil, or candles (Singh et al., 2024). Soot is a common air pollutant and is frequently viewed as an environmental nuisance (Mulay et al., 2019). To date, soot has been widely applied for pigments, inks, battery electrodes, and fuel cells (Gopal et al., 2021; Gregory et al., 2022). Few have used it in the field of agriculture, especially for seed germination.

The use of carbonaceous compounds in seed germination has been widely studied, though soot remains largely unexplored (Haghighi and da Silva, 2014). For instance, Zhang et al. (2020) used biochar for soil amendment to improve the seed germination of rice, tomato, and reed. Haghighi and da Silva (2014) demonstrated the use of carbon nanotubes with diameters of 8 to 15 nm applied for seed germination of tomato, onion, turnip, and radish. Similarly, graphene was also a carbonaceous compound that could improve the germination performance of *Solanum lycopersicum* seeds (Zhang et al., 2015). While these carbon-based materials have demonstrated efficacy, they often require complex synthesis processes, high production costs, and specialized handling protocols (Khan et al., 2025), which may limit their accessibility and practicality, especially in low-resource agricultural settings. In contrast, soot derived from candle burning is a freely available and extremely low-cost by-product of everyday combustion. The burning of candles is a routine activity, particularly in rural and underserved communities, where other lighting options may be limited. However, the resulting soot is usually discarded or regarded as waste (Mulay et al., 2019).

However, none have used soot from candle burning, or candle soot, as a compound for seed germination. This study explores the agricultural potential of candle soot as a novel, low-tech material for seed priming. Unlike carbon nanotubes or graphene, candle soot does not require any sophisticated synthesis or purification, making it a safer and more accessible alternative (Sembada et al., 2024b). The collected soot was suspended in water and applied to seeds during germination. Germination performance was analyzed using several key parameters. Furthermore, this study evaluated the elemental distribution in seeds using mapping to assess biocompatibility and analyzed the starch and soluble sugar content, which are closely linked to germination metabolism. This exploration supports sustainable agriculture by proposing a readily available and inexpensive resource that can enhance germination, reduce reliance on synthetic chemicals, and contribute to waste reduction. It also holds strong potential for application in developing countries, where access to commercial seed enhancers may be limited and agricultural sustainability is a key priority (Qaim, 2020). By transforming a common air pollutant into an agricultural input, this study contributes to both environmental remediation and agricultural innovation.

MATERIALS AND METHOD

Collection and synthesis of carbonaceous particles from candle soot

Candle soot was collected by burning candles (Daiko Inc., Japan), and the soot was directly deposited onto the quartz glass from the tip of the burning flame (Faizal et al., 2018). The deposited soot was then collected and mixed with distilled water to create three different colloidal concentrations (10^{-2}, 10^{-3}, and 10^{-4} wt%, referred to as CS2, CS3, and CS4). These concentrations were selected based on the researcher's previous study (Sembada et al., 2024b), in which they demonstrated effective enhancement of seed germination without inducing phytotoxic effects. The suspensions were subsequently subjected to sonication (UT-105S, Sharp Corp., Japan) for 10 minutes at 35 kHz to ensure homogeneity (Qahtan et al., 2017). The characteristics of the derived carbonaceous suspensions from candle soot were evaluated based on zeta potential and particle size distributions. Zeta potential measurement was conducted by placing 1 ml of suspension on the folded capillary cell (DTS1070, Malvern Panalytical Ltd., UK) and inserting the cell into the dynamic light scattering instrument (Zetasizer Nano-ZS, Malvern Panalytical Ltd., UK). Particle size distribution measurement was conducted by placing 4 ml of suspension on the disposable polystyrene cuvette (DTS0012, Malvern Panalytical Ltd., UK) and inserting the cuvette into the same instrument (Sembada et al., 2024a). The measurements for all parameters were conducted three times.

Seed germination with carbonaceous particles

Seeds used for the experiment were obtained from Takii & Co., Ltd., Kyoto, Japan, with the specific varieties as follows: *C. annuum* var. Marokara, *S. lycopersicum* var. Momotaro, and *S. melongena* var. Shoya Daicho. Seeds were carefully selected for germination testing after undergoing a viability assessment. They were rinsed and soaked in distilled water for 5 minutes at 22 to 25 °C. Seeds that sank were considered viable and selected for the experiment (Siddiqui and Al-Whaibi, 2014). Seed priming was conducted by soaking viable seeds in candle soot suspensions (CS2, CS3, or CS4) for 24 hours at room temperature (22 to 25 °C). The control group was treated with distilled water only. After priming, seeds were transferred to 9 cm petri dishes lined with cotton and moistened with 20 ml of the corresponding suspension. Thirty seeds per treatment per species were used, with six replications. Germination was carried out in a controlled environment under complete darkness at 25±1 °C and relative humidity of 65 to 70% for 8 days, with germination progress monitored daily. On the final day, seedling length was measured, and seedling dry weight was determined by oven-drying samples at 50 °C for 24 hours (Sembada and Faizal, 2019).

Germination performances were assessed using several germination parameters. Seeds counted as germinated if the growing radicle showed a minimum length of 2 mm (Siddiqui and Al-Whaibi, 2014). Seed germination percentage (SGP, Equation 1) represents the proportion of seeds within a given sample that successfully commence sprouting and growth within a predetermined timeframe under specific conditions (Fernández-Pascual et al., 2021). Mean germination time (MGT, Equation 2) functions as a measurement utilized to assess the speed of seed germination within a specified experiment (Fernández-Pascual et al., 2021). The germination index (GI, Equation 3) serves as a quantitative measure utilized to assess both the speed and uniformity of seed germination within a designated experiment (Yang et al., 2021). The coefficient of velocity of germination (CVG, Equation 4) acts as a metric used to evaluate the velocity of seed germination (Ullah et al., 2022). The vigor index, which includes seedling length (VI_{LENGTH}, Equation 5) and dry weight (VI_{WEIGHT}, Equation 6), provides a comprehensive assessment of seedling growth and vitality (Siddiqui and Al-Whaibi, 2014). This combined measure offers a nuanced understanding of the overall health and robustness of seedlings as they emerge from seeds.

In the given context, x denotes the time taken for each germination event, measured in days, while f represents the number of seeds germinating at each time interval. The variables $n_1, n_2, \ldots, n_8$ indicate the frequency of germinated seeds on the first, second, and subsequent days until the final day. N represents the daily frequency of seeds germinating, and T signifies the duration from sowing to germination.

Analysis of elemental information in seedlings

Analyzing the elemental composition of the seedlings serves as a valuable tool in determining the potential impact of the application of carbonaceous particles on their physiological

$$SGP = \left(\frac{\text{Number of germinated seeds}}{\text{Total number of seeds}}\right) \times 100\% \tag{1}$$

$$MGT = \frac{\sum fx}{\sum f} \tag{2}$$

$$GI = (8 \times n_1)+(7 \times n_2)+\cdots+(1 \times n_8) \tag{3}$$

$$CVG = \left(\frac{N_1+N_2+\cdots+N_8}{100}\right) \times (N_1T_1+N_2T_2+\cdots+N_8T_8) \tag{4}$$

$$VI_{LENGTH} = SGP \times \text{seedlings length (cm)} \tag{5}$$

$$VI_{WEIGHT} = SGP \times \text{seedlings dry weight (g)} \tag{6}$$

status (Porfido et al., 2025). Seedlings from the final day of germination were dried in an oven at 50 °C for 24 hours. The dried seedlings were then weighed to 0.001 g and pressed into pellets using a mechanical press machine under 100 kg cm^{-2} pressure. These pellets were placed in the sample holder of an X-ray fluorescence (XRF) spectrometer (JSX-3100RII, JEOL Ltd., Tokyo, Japan) (Towett et al., 2016). The XRF conditions were set to a tube voltage of 30 kV, a collimator of 1, and a live-time value of 100 seconds. The intensity data obtained from the XRF analysis were then plotted on a heatmap.

Measurement of starch and soluble sugar during germination

The measurement of starch was conducted daily during the germination period. Seeds or seedlings were dried in an oven at 50 °C for 24 hours before undergoing the starch extraction process (Sembada and Faizal, 2022). The dried seeds or seedlings were weighed to 0.01 g, then ground into a homogenate with 2 ml of distilled water using a mortar and pestle. The mixture was then combined with 3 ml of 60% $HClO_4$ (Supelco 1.00519, Pennsylvania, USA) and centrifuged at 3,000 rpm for 5 minutes at room temperature (Huang et al., 2021). A milliliter of the resulting supernatant was mixed with 7 ml of distilled water and 2 ml of I_2KI (Sigma-Aldrich 1.09261, Missouri, USA). The absorbance of this mixture was read using a UV-Vis spectrophotometer (Shimadzu UV-VIS 1800, Shimadzu Corporation, Kyoto, Japan) at 660 nm (Huang et al., 2021). The absorbance values were then used to determine the starch concentration (mg g^{-1}) by fitting them to a standard curve depicting the relationship between starch concentration and absorbance.

Similarly, soluble sugar measurement was conducted daily, from the initial day to the last day. Seeds or seedlings were dried in an oven at 50 °C for 24 hours before undergoing the soluble sugar extraction process. After drying, seeds or seedlings were weighed to 0.01 g, ground into a homogenate with distilled water using a mortar and pestle, and centrifuged at 3,000 rpm for 5 minutes (Huang et al., 2021). A milliliter of the extracting solution was then mixed with 3.5 ml of distilled water, 5 ml of 98% H_2SO_4 (Sigma-Aldrich 339741, Missouri, USA), and 0.5 ml of anthrone (Sigma-Aldrich 801461, Missouri, USA). Subsequently, the mixture was heated in a water bath for 1 minute. The absorbance of this mixture was measured using a UV-Vis spectrophotometer at 630 nm (Huang et al., 2021). The absorbance values were used to determine the soluble sugar concentration (mg g^{-1}) by fitting them to a standard curve depicting the relationship between soluble sugar concentration and absorbance.

Observation of seed surface under treatment with carbonaceous particles

The attachment of candle soot particles to the seed surface was analyzed using a scanning electron microscope (SEM; model JSM-6510, JEOL Ltd., Japan) 24 hours after the treatment was applied (Sembada et al., 2024a). This detailed SEM analysis was conducted on seeds treated with a concentration of 10^{-3} wt% (CS3). Before SEM inspection, the seeds underwent a preparation process where they were carefully coated with a thin layer of platinum (Pt) using an ion sputtering apparatus (Fine Coat Ion Sputter JFC-1100, JEOL Ltd., Japan). The SEM was operated under specific conditions to optimize the imaging process: the accelerating voltage was set to 2 kV, the working distance (WD) was maintained at 11 mm, and the strain sensors (SS) were calibrated to a setting of 60.

Measurement of water absorption capacity

The water absorption capability was assessed following the method described by Chen et al. (2019). Seeds were placed on cotton moistened with 20 ml of candle soot suspensions at concentrations of 10^{-2}, 10^{-3}, and 10^{-4} wt%. The seeds were then weighed using an analytical balance after absorbing water for 3, 6, 12, 24, and 48 hours. For the control, cotton was moistened with 20 ml of distilled water only. The water absorption rate (mg hour^{-1}) was calculated by dividing the seeds' weight by the time.

Statistical analysis

The data collected was initially evaluated using descriptive statistics performed in Microsoft Excel. IBM SPSS Statistics software was utilized for a more comprehensive statistical analysis. A two-way analysis of variance (ANOVA) was conducted to assess the effects of treatments. Duncan's multiple range test (DMRT) was subsequently applied to identify and compare means with significant differences. All experiments were conducted with six biological replicates. Results are presented as the mean±standard deviation (SD), and differences were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Characteristics of carbonaceous particles

The experimental procedure involved the application of carbonaceous particles sourced from candle soot (CS2, CS3, and CS4) onto the seeds, administered as suspensions comprising these particles. Within the scope of this study, detailed characterization of these suspensions was conducted, such as pH, zeta potential, and average particle size, as outlined in Table 1. Furthermore, to provide a comprehensive understanding of the distribution of particle sizes across various concentrations of candle soot, the particle size distribution data for each concentration is visually depicted in Figure 1.

The results indicated that the measured pH and zeta potential values across these three concentrations did not exhibit significant statistical differences, suggesting a consistency in these properties irrespective of concentration. It showed that the particles were well-dispersed in the suspension. A stable dispersion ensured that the electrostatic repulsion between particles was sufficient to keep them uniformly distributed (Azizov et al., 2025). Hu et al. (2005) indicated the Tyndall effect observed in an aqueous dispersion of single-walled carbon nanotubes, and this effect was aligned with the consistent zeta potential values measured across different pH. The pH of the suspension remained constant across different concentrations, indicating that the particle surface did not interact strongly with the surrounding medium to change its acidity or basicity (El Badawy et al., 2010). This suggested minimal release of acidic or basic groups from the particles. The study conducted by Kumar and Bohidar (2012) showed that the zeta potential value of candle soot dispersed in water is -22 mV, indicating good stability. Similarly, in the present study, the candle soot particles obtained were dispersed in a water sample. The fact that candle soot showed good stability when dispersed in water highlighted water's effectiveness as a dispersant. Water facilitated the separation and distribution of soot particles, likely due to its polar nature and ability to interact with the charged

Table 1. Physicochemical characteristics of carbonaceous particles derived from candle soot at three concentrations

Treatment	pH	Zeta potential (mV)	Average particle size (nm)
CS2	6.63±0.09[a]	-33.67±0.84[a]	414.1±14.89[c]
CS3	6.56±0.09[a]	-33.17±0.41[a]	313.9±5.33[b]
CS4	6.43±0.10[a]	-32.67±0.39[a]	209.2±1.98[a]

Note: Data are presented as mean±standard deviation (n = 3). Different letters (a, b, c) within the same column indicate statistically significant differences among treatments at $p \leq 0.05$ based on DMRT (following two-way ANOVA)

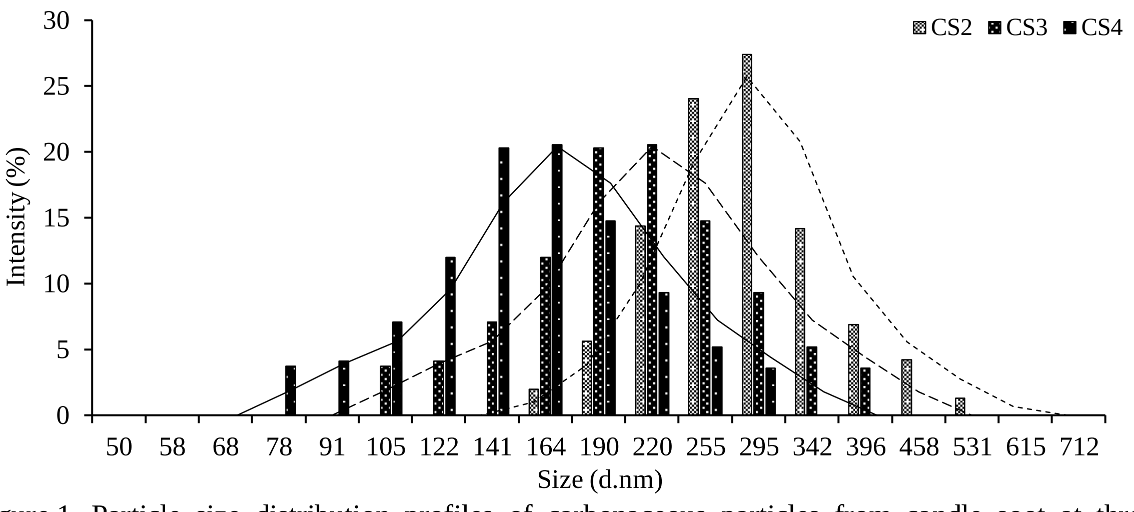

Figure 1. Particle size distribution profiles of carbonaceous particles from candle soot at three concentrations

Note: Measurements were obtained using dynamic light scattering (DLS) and represent the average of three replicates (n = 3). The distributions show differences in average particle sizes related to soot concentration surfaces of the particles (Qahtan et al., 2017; Mulay et al., 2019).

However, the situation was notably different when it came to the average particle size. The average particle size values showed significant ($p \leq 0.05$) statistical differences between the treatments. Specifically, it was observed that as the concentration increases (notably in CS2), the measured average particle size also increases, indicating that higher concentrations lead to larger particle sizes. As concentration increased, the likelihood of particle-particle interactions and collisions also increased (Nayak et al., 2025). This could lead to the formation of larger aggregates or agglomerates, increasing the measured average particle size. Wang et al. (2017) indicated an increase in the hydrodynamic diameter of multi-walled carbon nanotubes with concentration. The hydrodynamic diameter tended to increase with increasing concentration (Wang et al., 2017). Attractive forces arise between particles due to temporary dipoles induced by fluctuations in the electron distribution (Martínez-Ballesta et al., 2020). As concentration increases, more particles come into closer proximity, leading to stronger van der Waals interactions and an increased tendency for aggregation (Wang et al., 2017; Martínez-Ballesta et al., 2020). At higher concentrations, collisions between particles occur more frequently, potentially leading to particle coalescence.

Germination performance of seeds under the influence of carbonaceous particles

The first parameter used to measure seed germination performance during the application of carbonaceous particles is SGP, as shown in Table 2. The results indicated that on the 8th day,

Table 2. SGP of three *Solanaceae* species under different concentrations of candle soot-derived carbonaceous particles, compared to the untreated control

Species	Treatment	SGP (%) for each day								
		0	1	2	3	4	5	6	7	8
C. annuum	Control	0.0	0.0	0.0	50.0	86.7	100.0	100.0	100.0	100.0
	CS2	0.0	0.0	10.0	66.7	83.3	100.0	100.0	100.0	100.0
	CS3	0.0	0.0	6.7	70.0	100.0	100.0	100.0	100.0	100.0
	CS4	0.0	0.0	10.0	60.0	76.7	100.0	100.0	100.0	100.0
S. lycopersicum	Control	0.0	0.0	10.0	63.3	90.0	100.0	100.0	100.0	100.0
	CS2	0.0	0.0	20.0	70.0	96.7	100.0	100.0	100.0	100.0
	CS3	0.0	0.0	36.7	66.7	100.0	100.0	100.0	100.0	100.0
	CS4	0.0	0.0	13.3	66.7	93.3	100.0	100.0	100.0	100.0
S. melongena	Control	0.0	0.0	0.0	20.0	83.3	100.0	100.0	100.0	100.0
	CS2	0.0	0.0	3.3	33.3	90.0	100.0	100.0	100.0	100.0
	CS3	0.0	0.0	13.3	43.3	86.7	100.0	100.0	100.0	100.0
	CS4	0.0	0.0	0.0	40.0	80.0	93.3	100.0	100.0	100.0

Note: Data is presented as the average of six replicates (n = 6)

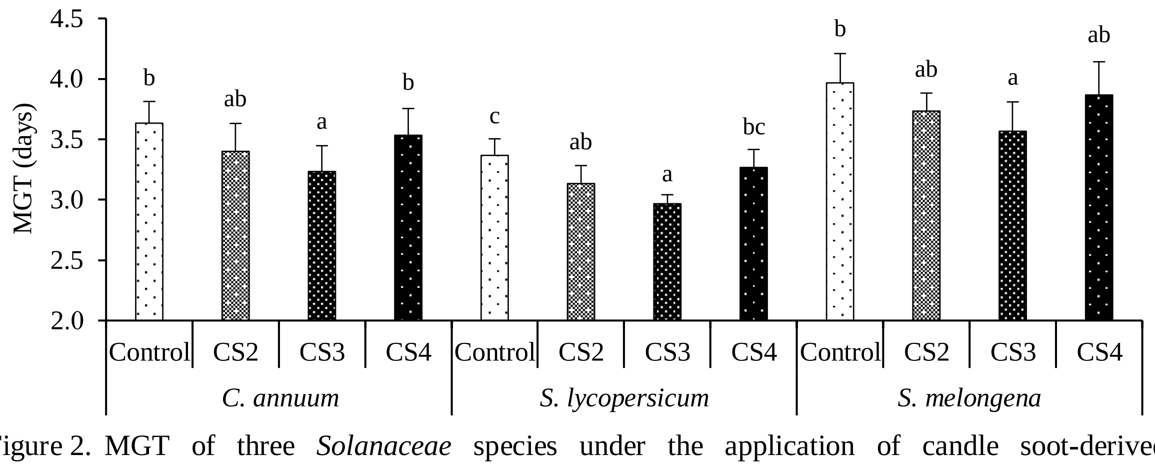

Figure 2. MGT of three *Solanaceae* species under the application of candle soot-derived carbonaceous particles at three concentrations, compared to the untreated control

Note: Data are presented as mean±standard error (n = 6). Different lowercase letters (a, ab, b, bc, c) indicate significant differences ($p \leq 0.05$) within each species based on DMRT following two-way ANOVA

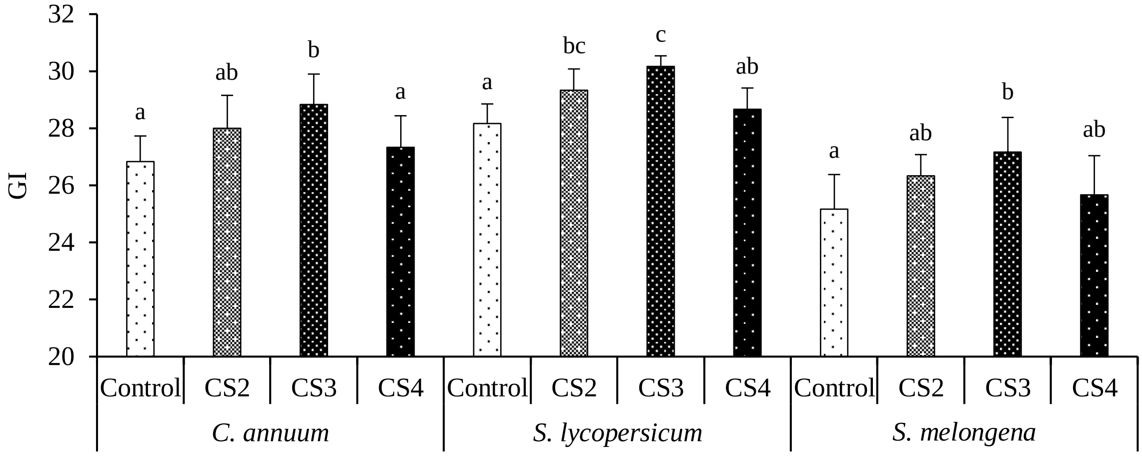

Figure 3. GI of three *Solanaceae* species under the application of candle soot-derived carbonaceous particles at three concentrations, compared to the untreated control

Note: Data are presented as mean±standard error (n = 6). Different lowercase letters (a, ab, b, bc, c) indicate significant differences ($p \leq 0.05$) within each species based on DMRT following two-way ANOVA

all seeds from all species (*C. annuum*, *S. lycopersicum*, and *S. melongena*) achieved 100% germination. However, there was one instance where the CS3 treatment in *C. annuum* and *S. lycopersicum* showed 100% germination earlier, on the 4th day. This remarkable finding suggested that the use of carbonaceous particles derived from holds significant promise as a potential priming agent to enhance germination performance.

The second parameter employed is MGT to further examine the potential of these carbonaceous particles, as shown in Figure 2. Analysis of this parameter revealed that the CS3 treatment had a significant ($p \leq 0.05$) impact, accelerating the germination time for all the species tested. This observation underscored the effectiveness of the carbonaceous particles in improving the velocity of seed germination across different species. The CS3 treatment was able to reduce the time required for germination compared to the control by 11% in *C. annuum* (from 3.63±0.18 to 3.23±0.21 days), by 11.88% (from 3.37±0.14 to 2.97±0.07 days) in *S. lycopersicum*, and by 10.08% (from 3.97±0.24 to 3.57±0.24 days) in *S. melongena*.

The findings revealed through the initial and secondary parameters suggested tentatively that the CS3 treatment emerged as the most favorable option. To solidify the claim that the CS3 treatment indeed stands as the optimal choice, it becomes imperative to delve deeper into the analysis of additional parameters. These include, but are not limited to, the 3rd and 4th parameters: GI and CVG. These parameters are showcased in Figures 3 and 4. Across various plant species, including *C. annuum*, *S. lycopersicum*, and *S. melongena*, the CS3 treatment exhibited a remarkable improvement in GI and CVG. The CS3 treatment improved GI compared to the control by 7.45% in *C. annuum* (from 26.83±0.9 to 28.83±1.07), by 7.1% in *S. lycopersicum* (from 28.17±0.69 to 30.17±0.37), and by 7.95% in *S. melongena* (from 25.17±1.21 to 27.17±1.21). Additionally, the CS3 treatment enhanced CVG compared to the control by 12.59% in *C. annuum* (from 27.59±1.30 to 31.06±20), by 13.37% in *S. lycopersicum* (from 29.75±1.21 to 33.73±0.89), and by 11.33% in *S. melongena* (from 25.3±1.53 to 28.17±1.95).

The culmination of the outcomes derived from the assessment of the four parameters, namely SGP, MGT, GI, and CVG, collectively affirmed the overarching conclusion that the CS3 treatment emerged as the optimal choice. Furthermore, in gauging the quality of the burgeoning seedlings, meticulous attention is directed towards the vigor index, a comprehensive metric derived from various parameters such as seedling length (VI_{LENGTH}, as in Figure 5) and seedling dry weight (VI_{WEIGHT}, as in Figure 6).

Consistent findings underscored the beneficial impact of the CS3 treatment on the parameters of VI_{LENGTH} and VI_{WEIGHT}, revealing a significant deviation from the control group. Across diverse plant species, including *C. annuum*, *S. lycopersicum*, and *S. melongena*, the CS3 treatment demonstrated notable enhancements in VI_{LENGTH}, with respective increments of 28.76% (from 1,352.67±195.07 to 1,741.67±121.75), 33.77% (from 1,406±104.4 to 1,880.83±216.4), and 37.92% (from 1,244.67±73.87 to

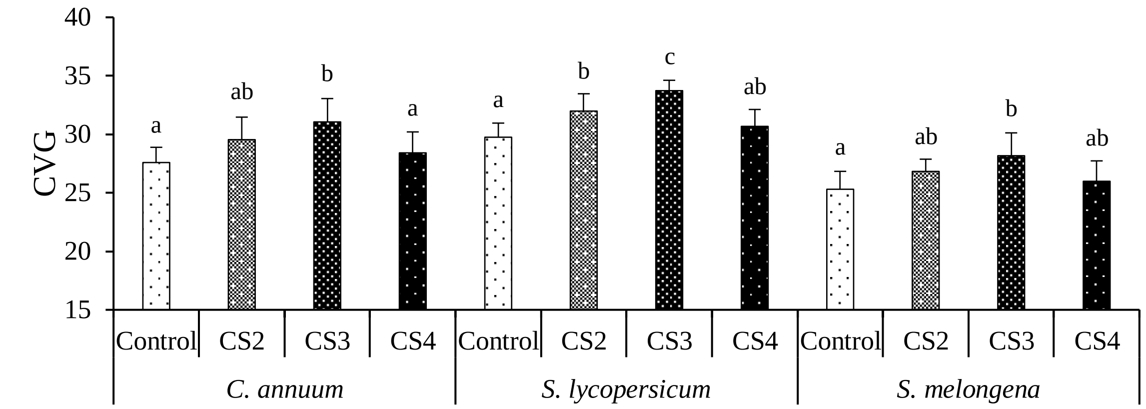

Figure 4. CVG of three *Solanaceae* species under the application of candle soot-derived carbonaceous particles at three concentrations, compared to the untreated control

Note: Data are presented as mean±standard error (n = 6). Different lowercase letters (a, ab, b, c) indicate significant differences ($p \leq 0.05$) within each species based on DMRT following two-way ANOVA

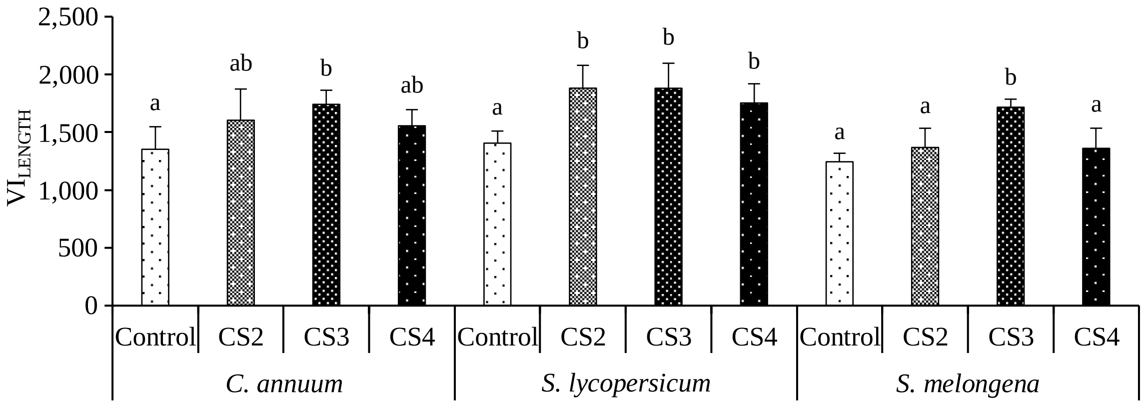

Figure 5. VI_{LENGTH} of three *Solanaceae* species under the application of candle soot-derived carbonaceous particles at three concentrations, compared to the untreated control

Note: Data are presented as mean±standard error (n = 6). Different lowercase letters (a, ab, b) indicate significant differences ($p \leq 0.05$) within each species based on DMRT following two-way ANOVA

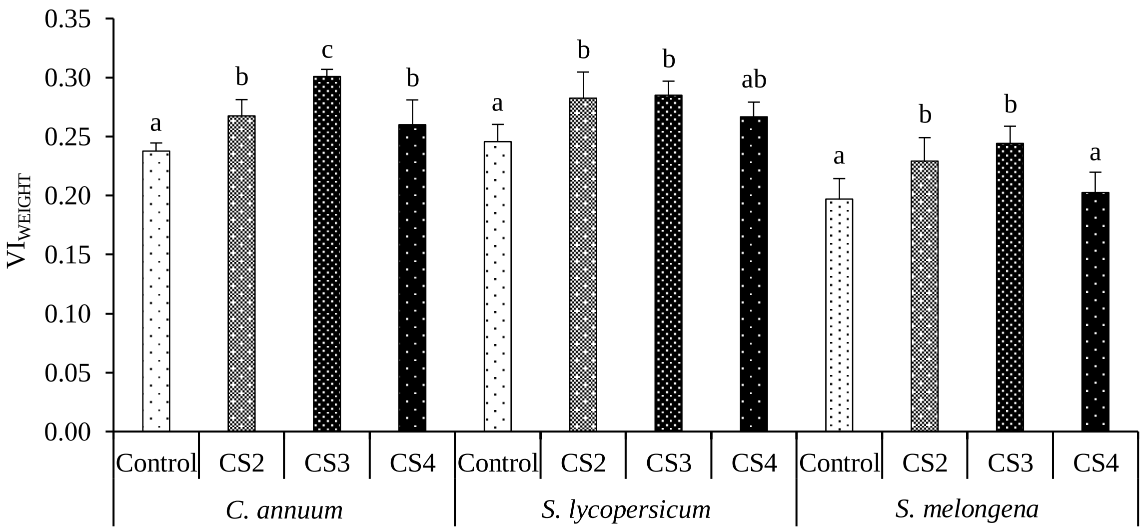

Figure 6. VI_{WEIGHT} of three *Solanaceae* species under the application of candle soot-derived carbonaceous particles at three concentrations, compared to the untreated control

Note: Data are presented as mean±standard error (n = 6). Different lowercase letters (a, ab, b, c) indicate significant differences ($p \leq 0.05$) within each species based on DMRT following two-way ANOVA

1,716.67±69.74) compared to their respective controls. Additionally, under the CS3 treatment, the VI_{WEIGHT} values witness considerable upticks, with respective increases of 26.58% (from 0.24±0.01 to 0.3±0.01), 16.01% (from 0.25±0.01 to 0.29±0.01), and 23.94% (from 0.2±0.02 to 0.24±0.01) observed in *C. annuum*, *S. lycopersicum*, and *S. melongena* when compared with the controls.

Observation of carbonaceous particles on the seed surface and the water absorption capacity

The comprehensive evaluation of the four germination parameters, SGP, MGT, GI, and CVG, collectively underscored the conclusion that the CS3 treatment stands out as the optimal choice. The parameters used to measure seedling quality (VI_{LENGTH} and VI_{WEIGHT}) also consistently indicated that the CS3 treatment was the most effective. To delve deeper into this discussion, researchers correlated the presence of carbonaceous particles during germination. As depicted in Figures 7, 8, and 9, it was evident that carbonaceous particles were present on the seed surfaces throughout the germination process.

When carbonaceous particles attach to the seed surface, they could form a thin layer that acts as a barrier. Zhang et al. (2015) indicated that tomato seeds treated with graphene showed deposition of graphene on their surfaces. The densely deposited graphene formed a graphene sheet that could penetrate the seed husks. Lahiani et al. (2013) investigated the effects of multi-walled carbon nanotube deposition on seed surfaces, finding that it could affect germination performance and growth of seedlings. The deposition of carbonaceous compounds on the seed surface, which was also observed in this study as well as in past studies, could create a thin layer on the exterior of the seeds. This layer could help to reduce water evaporation from the seed surface by providing a physical barrier between the seed and the surrounding environment, as reported by Zhang et al. (2015). By reducing water evaporation, the thin layer of carbonaceous particles helped the seeds to retain moisture for a longer period (Zhang et al., 2015). This was crucial for germination because seeds require a certain level of moisture to imbibe water and initiate the germination process (Bareke, 2018). In this study, the water absorption capacity during treatment with carbonaceous particles derived from candle soot was investigated, as shown in Figure 10.

Treatment with carbonaceous particles from candle soot significantly enhances water absorption in all species. Over time, water

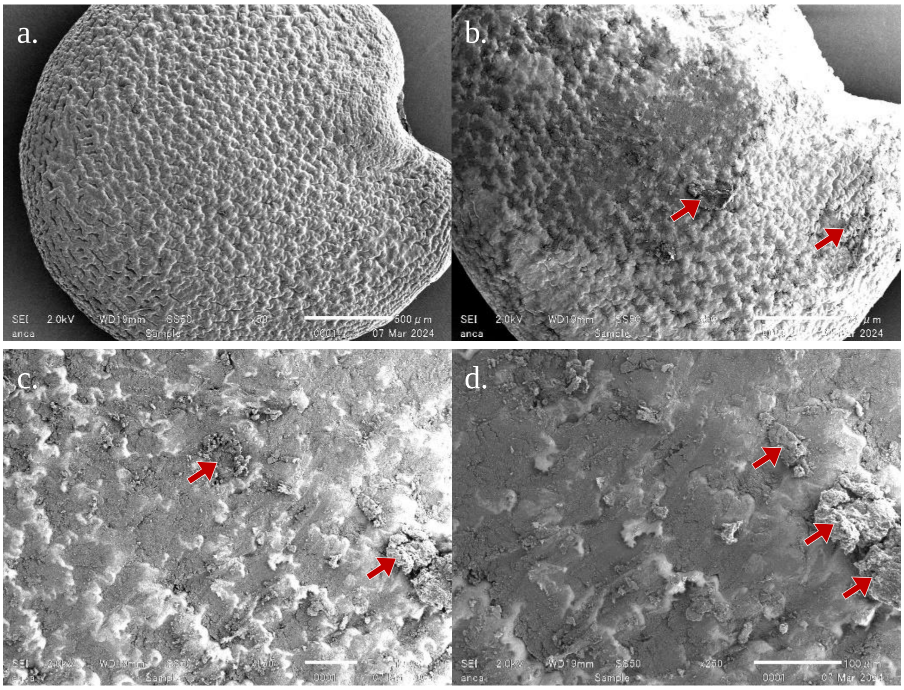

Figure 7. SEM images of *C. annuum* seed surfaces under different treatments with candle soot-derived carbonaceous particles. (a) Untreated control seed; (b–d) Seeds treated with 10^{-3} wt% candle soot suspension (CS3)

Note: Red arrows indicate aggregated carbonaceous particles deposited on the seed coat. Magnifications: (A, B) = 50×; (C) = 150×; (D) = 250×

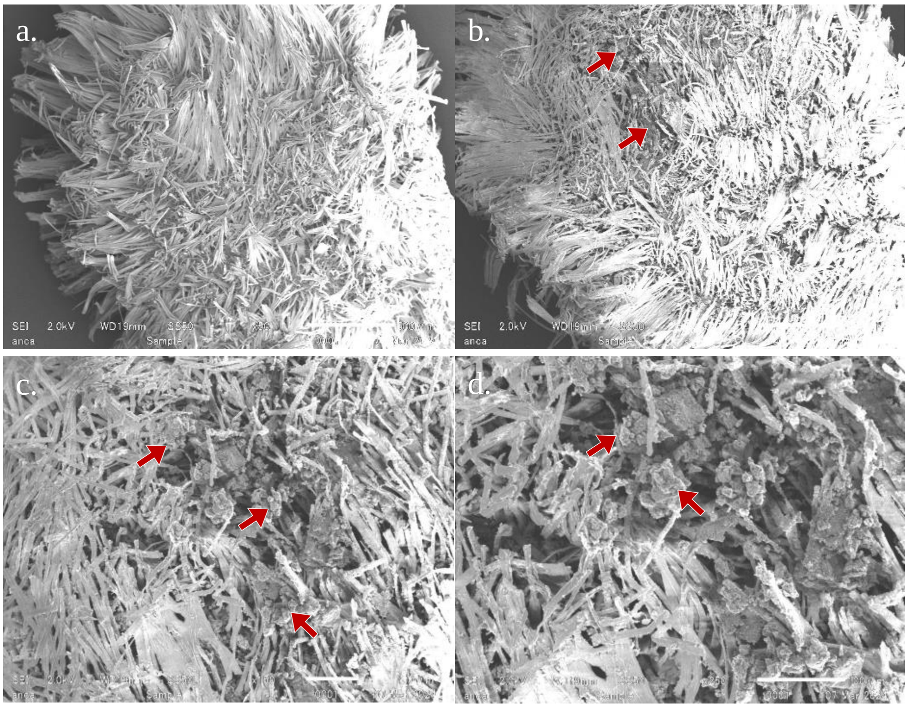

Figure 8. SEM images of *S. lycopersicum* seed surfaces under different treatments with candle soot-derived carbonaceous particles. (a) Untreated control seed; (b–d) Seeds treated with 10^{-3} wt% candle soot suspension (CS3)

Note: Red arrows indicate aggregated carbonaceous particles deposited on the seed coat. Magnifications: (A, B) = 50×; (C) = 150×; (D) = 250×

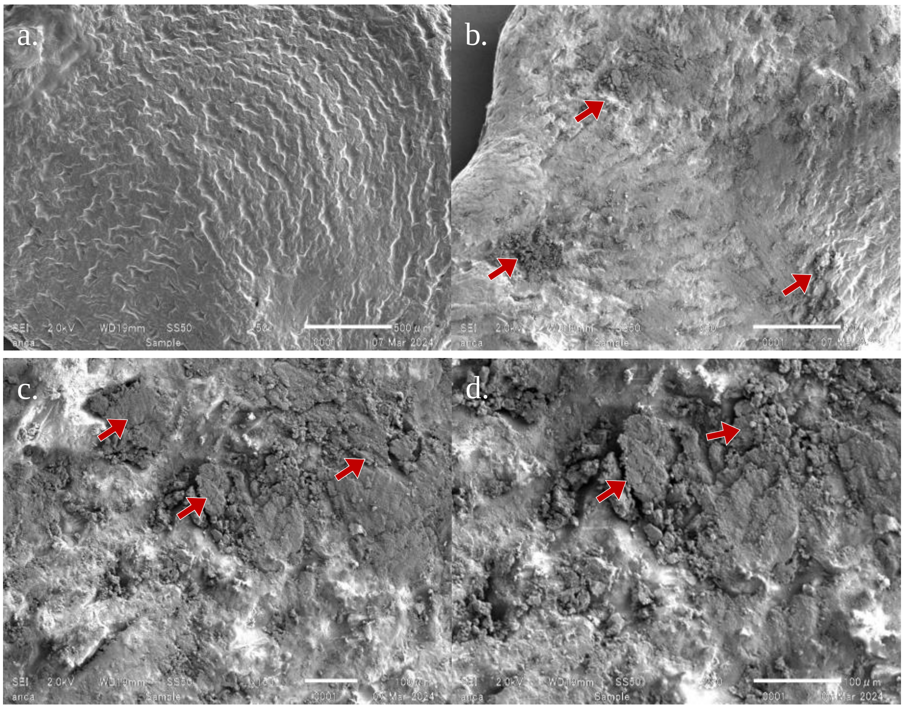

Figure 9. SEM images of *S. melongena* seed surfaces under different treatments with candle soot-derived carbonaceous particles. (A) Untreated control seed; (B–D) Seeds treated with 10^{-3} wt% candle soot suspension (CS3)

Note: Red arrows indicate aggregated carbonaceous particles deposited on the seed coat. Magnifications: (A, B) = 50×; (C) = 150×; (D) = 250×

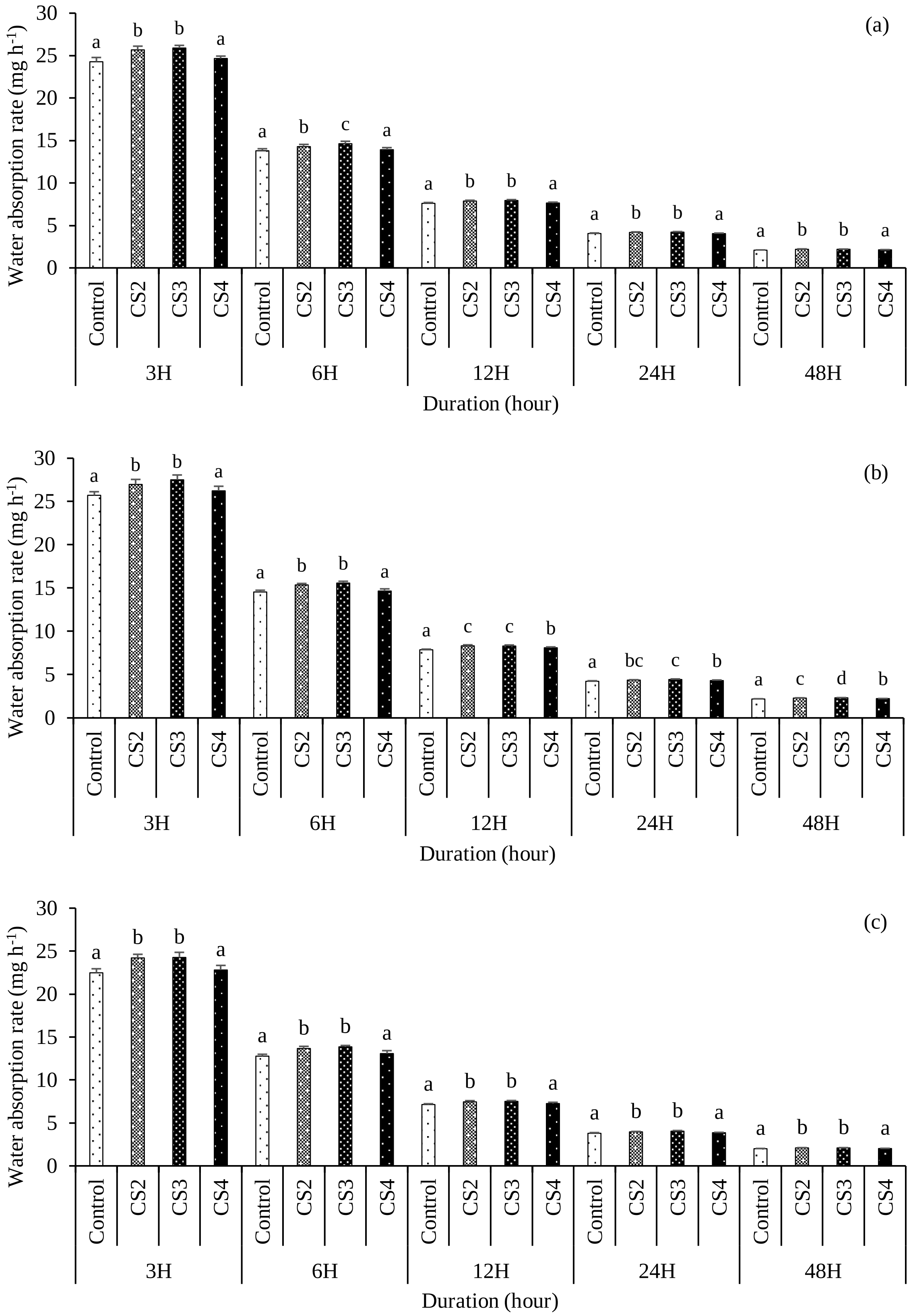

Figure 10. Water absorption capacity of seeds treated with carbonaceous particles derived from candle soot. (a) *C. annuum*, (b) *S. lycopersicum*, and (c) *S. melongena*

Note: Treatments include CS2 (10^{-2} wt%), CS3 (10^{-3} wt%), and CS4 (10^{-4} wt%) candle soot suspensions. Bars represent mean±standard deviation (n = 6). Different letters (a, b, c) within each species and duration indicate significant differences between treatments ($p \leq 0.05$) according to DMRT absorption decreased as the seeds became saturated and began to germinate. This evidence aligns with the findings of Chen et al. (2019), who also observed higher water absorption in seeds treated with graphene compared to the control. A study by Mushtaq (2011) also showed that the presence of carbonaceous particles facilitated water uptake inside the embryo during cucumber seed germination, which impacts the overall germination performance (Mushtaq, 2011). He et al. (2018) indicated that the use of graphene oxide could accelerate spinach seed germination because it promoted water absorption by the seeds. Another study also used graphene oxide for treating rice seed germination. The results showed that water absorption significantly increased during the testith 5 to 10 g ml^{-1} graphene oxide treatments compared to the control (Chen et al., 2019). Consistently, graphene oxide alone at 10 g ml^{-1} also accelerated rice seed germination and root growth due to improved water uptake (Li et al., 2020).

Another study using carbon nanotubes also exhibited the same mechanism of retaining seed moisture, increasing water content, and reducing water evaporation (Khodakovskaya et al., 2009). Tomato seeds exposed to carbon nanotubes could accumulate about 57.6% of moisture, whereas unexposed seeds retained only 38.9% (Khodakovskaya et al., 2009). The application of multi-walled carbon nanotubes could significantly enhance root dehydrogenase activity, which, in turn, improved the water uptake ability of *Triticum aestivum* seedlings (Wang et al., 2012). Based on the information presented in past studies, it can be concluded that the deposition of carbonaceous compounds or particles on the surface of seeds can help retain moisture and water, thereby improving germination performance. Researchers hypothesize that candle soot, also a carbonaceous compound, may have a similar mechanism to other carbonaceous compounds in enhancing germination performance.

The carbonaceous particles from candle soot used in the present study also exhibited hydrophilic properties, as they were collected from the soot originating from the tip of a burning flame (Faizal et al., 2018). Hydrophilic particles have a strong affinity for water, allowing them to absorb moisture from the surrounding environment efficiently (Ahmad et al., 2018). This property ensured that water was readily available for seed hydration, a critical step in the germination process. Hydrophilic particles could retain moisture effectively, creating a favorable microenvironment around the seeds (Zvinavashe et al., 2019). Another study utilizing hydrophilic particles, specifically silica nanoparticles, for germination also demonstrated improved germination performance (Sembada and Lenggoro, 2023). Hydrophilic particles can create a hydrophilic environment around the seeds, thereby enabling the seeds to absorb more water, which is beneficial for germination (Siddiqui and Al-Whaibi, 2014).

Elemental analysis of seedlings: An insight into plant physiological status under the influence of carbonaceous particles

Equally important to note was that the presence of carbonaceous particles from candle soot on the seed surface during germination was within a range that did not adversely affect the overall physiological status of the plants. This observation was supported by the elemental information provided in Figure 11, which suggested that the interaction between the carbonaceous particles and the seeds during germination did not lead to any significant disruptions in the plants' physiological processes. Although XRF is less common in plant science compared to inductively coupled plasma (ICP) or atomic absorption spectrometry (AAS), it was selected in this study due to its non-destructive nature, rapid processing, and ability to detect a wide range of elements without requiring chemical digestion. The "intensity" values shown in the heatmap correspond to the fluorescence signal strength for each element, which is proportional to its relative abundance in the sample. Each treatment is represented by six color gradients in the heatmap, reflecting the six biological replicates analyzed per treatment group. This visual representation allows for easy comparison of elemental distribution patterns across treatments and species. The absolute elemental concentration values derived from XRF analysis are provided as a supplementary file to support transparency and facilitate interpretation.

This observation was particularly noteworthy concerning critical elements such as phosphorus and potassium. The lack of adverse effects on plant physiology indicated that the carbonaceous particles from candle soot were biocompatible and safe for use in seed germination processes (Singh et al., 2024). However, adverse effects from using candle soot may be observed at higher dosages or concentrations. Additionally, such effects might occur in seed species other

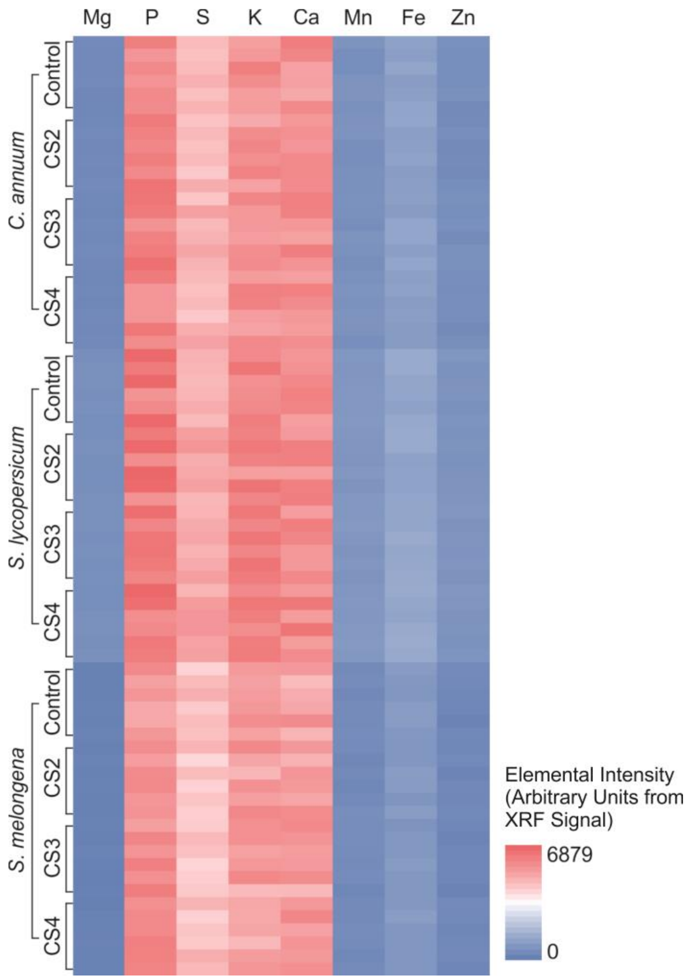

Figure 11. Heatmap of elemental distribution in seedlings treated with carbonaceous particles derived from candle soot

Note: Elemental mapping was performed using XRF spectrometry. The heatmap displays signal intensity (arbitrary units) from 0 to 6,879, representing elemental presence. Each treatment includes six biological replicates, visualized individually across the gradient

than the three used in this study. Therefore, future research should focus on the phytotoxicity of candle soot specifically for seed germination across various seed species and concentrations.

Analysis of starch and soluble sugar contents: Insights into metabolism under the influence of carbonaceous particles

The temporal dynamics of starch and soluble sugar profiles relating to germination time are illustrated in Figures 12 and 13, respectively. The general trend observed from the starch content data illustrated a continuous and consistent decrease as the germination time progressed, evident in both the control group and the treatments with CS2, CS3, and CS4 across all species examined. Notably, the CS3 treatment exhibited the most pronounced reduction in starch content when compared to the other treatments. The initial starch content measured in *C. annuum*, *S. lycopersicum*, and *S. melongena* was 155.31±5.54, 165.06±6.59, and 144.74±4.18 mg g^{-1}, respectively. Following the germination process, which spans a duration of 8 days, the starch content in the CS3 treatment underwent significant changes, ultimately resulting in values of 61.55±2.59, 72.55±4.96, and 52.21±5.48 mg g^{-1} for the respective species. In contrast, under the control treatment conditions, the starch content experienced a different trajectory, culminating in final values of 76.74±6.36, 87.67±5.43, and 67.83±2.91 mg g^{-1} for *C. annuum*, *S. lycopersicum*, and *S. melongena*, respectively. These changes underscored the impact of the CS3 treatment compared to the control on starch metabolism during the germination period.

In contrast to the trend observed in starch content, the general trend for soluble sugar exhibited an increase as germination progressed, evident in both the control and treatments with

CS2, CS3, and CS4 across all species. Initially, the soluble sugar content measured in *C. annuum*, *S. lycopersicum*, and *S. melongena* was 30.65±3.81, 40.68±3.69, and 20.74±3.33 mg g^{-1}, respectively. For the 8-day germination, the soluble sugar content in the CS3 treatment displayed significant increases, ultimately reaching values of 58.04±3.7, 69.22±4.77, and 49.46±5.02 mg g^{-1} for the respective species. By contrast, under the control treatment conditions, the soluble sugar content culminated in final values of 51.4±4.15, 60.96±4.99, and 40.08±4.61 mg g^{-1} for *C. annuum*, *S. lycopersicum*, and *S. melongena*, respectively. These findings highlight the pronounced effect of the CS3 treatment on enhancing soluble sugar production during the germination period compared to the control.

The previous explanations highlight the notable role played by carbonaceous particles during the germination phase, particularly relating to water dynamics. The influx of water into the seed triggers a cascade of metabolic processes crucial for germination to proceed (Farooq et al., 2022). The presence of a naturally formed layer of carbonaceous particles on the seed surface serves to mitigate water evaporation, thereby ensuring a sustained water supply and uninterrupted metabolic activity (Lahiani et al., 2013; Zhang et al., 2015). Consequently, this mechanism contributes to enhanced germination performance when compared to conditions without such particles. Moreover, observations indicate an elevated consumption of starch (Figure 12) and an increased production of soluble sugars (Figure 13) relative to the control group. Hatami et al. (2017) also demonstrated increased activity of α-amylase, an enzyme that converts starch into soluble sugars, during treatment with single-walled carbon nanotubes in *Hyoscyamus niger* seed germination. Starch and soluble sugars play critical roles as major metabolites during the initial establishment phase of seedlings (Steinbrecher and Leubner-Metzger, 2017). These compounds provide essential energy and nutrients necessary for seedling growth and development. Consequently, enhancing the regulation of this metabolic process could lead to notable improvements in the overall productivity of the seedlings, ultimately contributing to their robustness and vigor (Martínez-Ballesta et al., 2020).

In addition to enhanced water dynamics, the data indicate increased starch consumption (Figure 12) and elevated production of soluble sugars (Figure 13) in seeds treated with candle soot. This suggests that carbonaceous particles may influence enzyme regulation associated with carbohydrate metabolism. Specifically, the enzyme α-amylase, which hydrolyzes starch into soluble sugars, is known to be upregulated during the germination process and plays a central role in energy mobilization (Bozdar et al., 2025). For example, Wang et al. (2024) demonstrated that treatment with multi-walled carbon nanotubes significantly increased amylase activity in maize grains, thereby accelerating starch hydrolysis and enhancing seed germination characteristics. Although α-amylase activity was not directly measured in the present study, the metabolic patterns observed imply a similar enhancement.

Moreover, emerging evidence suggests that carbon-based nanomaterials may generate low, non-toxic levels of reactive oxygen species (ROS), which function as signaling molecules to activate germination-related gene expression and enzymatic pathways. For instance, Mazhar et al. (2025) showed that graphene oxide nanoparticle (GONP) seed priming in soybean modulated hormonal signaling and improved the antioxidant defense system, reducing oxidative stress markers like hydrogen peroxide while enhancing the activities of antioxidant enzymes, such as peroxidase, superoxide dismutase, and catalase. These biochemical shifts were associated with improved plant growth and stress tolerance under arsenic exposure. Based on this, researchers hypothesize that candle soot, being a carbonaceous material, may exert similar regulatory effects on ROS signaling and enzyme activation during seed germination. In addition to improving water retention, it may influence internal signaling pathways that promote radicle protrusion and enhance metabolic activity required for early seedling development.

Beyond its efficacy in enhancing seed germination, the use of candle soot introduces broader implications for sustainable agriculture and environmental stewardship. Candle soot is a widely available, low-cost by-product of daily activities, particularly in regions where candles remain a common light source. Its repurpose aligns with the principles of circular economy and waste valorization by transforming an air pollutant into a functional agricultural input. This approach minimizes waste while reducing the reliance on synthetic chemical priming agents, many of which are costly, potentially harmful to the environment, or inaccessible in rural settings.

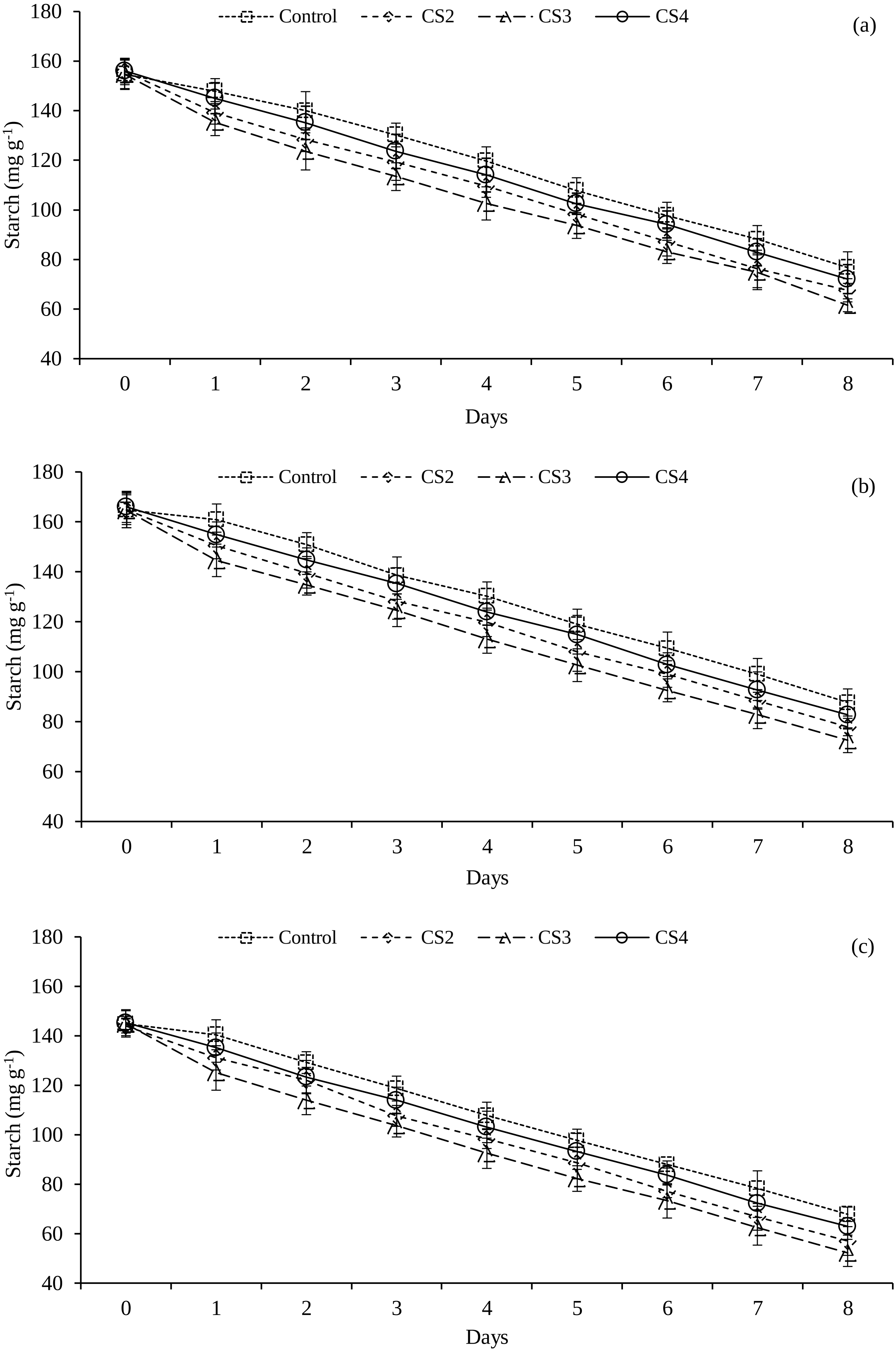

Figure 12. Starch content profile (mg g^{-1}) in germinating seeds of *Solanaceae* species under the application of carbonaceous particles from candle soot. Subfigures: (a) *C. annuum*, (b) *S. lycopersicum*, and (c) *S. melongena*

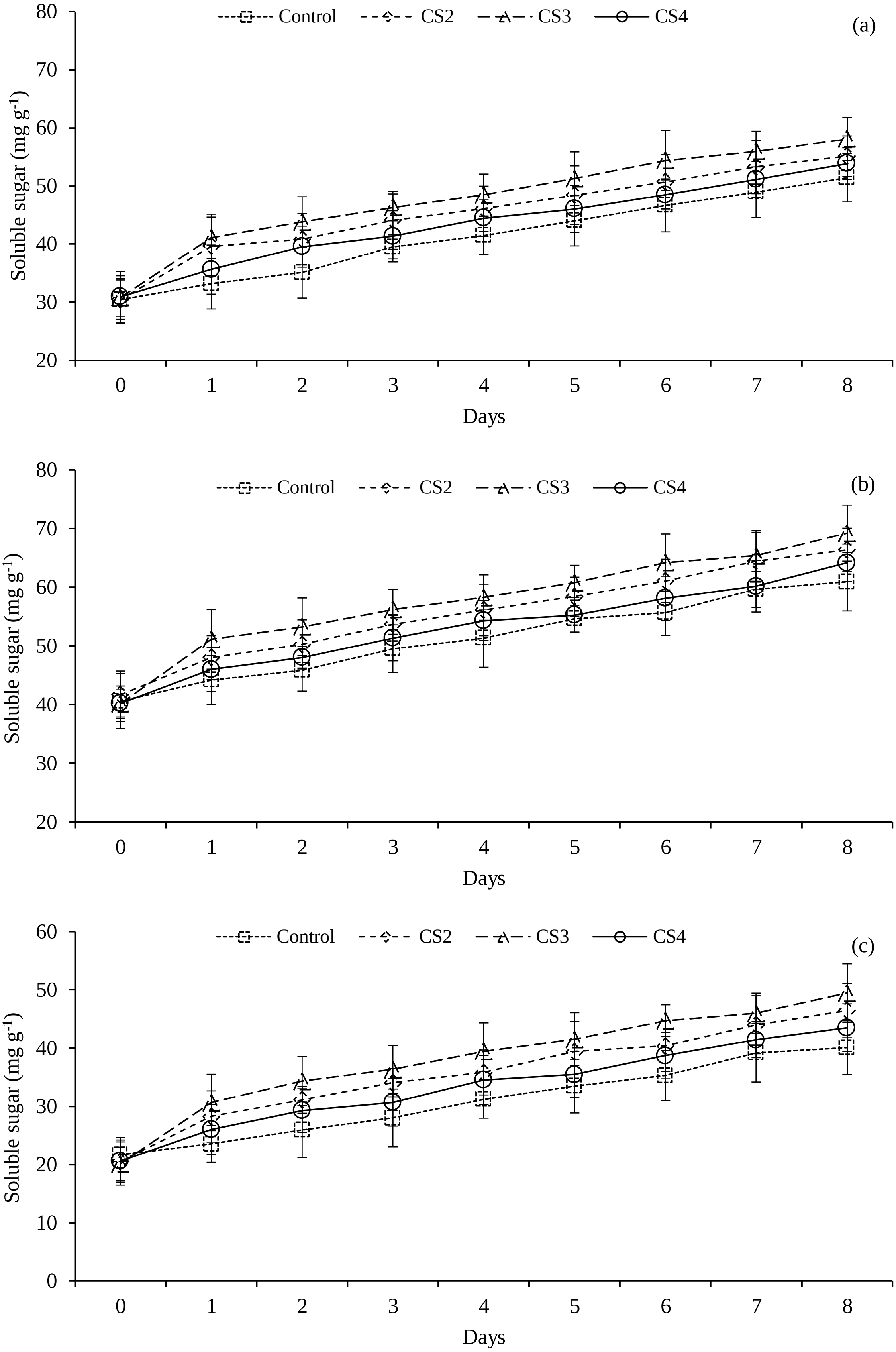

Figure 13. Soluble sugar content profile (mg g^{-1}) in germinating seeds of *Solanaceae* species under the application of carbonaceous particles from candle soot. Subfigures: (a) *C. annuum*, (b) *S. lycopersicum*, and (c) *S. melongena*

In addition, unlike engineered nanomaterials such as carbon nanotubes or graphene oxide, candle soot requires no complex synthesis, making it more accessible and safer for large-scale use. By supporting seedling vigor through natural metabolic enhancement without compromising plant health, this method offers an eco-friendly, scalable alternative that can be adopted particularly in resource-limited and developing regions. As such, candle soot-based seed treatment represents a promising step toward integrating low-tech, environmentally safe innovations into sustainable agricultural practices.

Moving forward, further investigations could delve into the precise mechanisms underlying the interaction between carbonaceous particles and seeds during germination. First, future studies should focus on the water used and water dynamics during germination with candle soot. The relationship between the formation of a thin layer of candle soot on the seed surface and moisture retention inside the seeds needs to be clarified. Second, regarding the hydrophilicity of candle soot, future studies need to clarify its use for alleviating drought stress during germination. Third, regarding the phytotoxicity of candle soot, its use across a broader range of concentrations and various seed species will provide a comprehensive understanding of its biocompatibility. Furthermore, considering the environmental implications, future studies could focus on the scalability and eco-friendliness of producing carbonaceous particles from renewable sources or waste materials. Developing sustainable methods for obtaining these particles would align with efforts to promote environmentally conscious agricultural practices.

CONCLUSIONS

This study demonstrates that carbonaceous particles derived from candle soot can enhance seed germination performance in *C. annuum*, *S. lycopersicum*, and *S. melongena*. Physicochemical characterization confirmed that the suspension was stable, and scanning electron microscopy showed the deposition of particles on seed surfaces. The 10^{-3} wt% treatment (CS3) significantly improved germination metrics. Enhanced starch degradation and soluble sugar production suggest improved energy mobilization during early seedling development. These findings introduce a low-cost, biocompatible material for seed treatment and propose a novel mechanism for improving seed vigor using recycled carbonaceous matter. Future studies should examine the molecular pathways involved in starch-sugar metabolism under candle soot exposure and validate its applicability under field conditions for broader crop types and climate scenarios.

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