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Candle soot colloids enhance tomato (*Solanum lycopersicum*) seed germination and seedling quality

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Abstract

The effect of candle soot colloids, a novel nanomaterial from candle combustion that we developed previously, on tomato (*Solanum lycopersicum*) seed germination and seedling vigor was investigated. Candle soot colloids were applied at different concentrations and hydrophobicities to tomato seeds and germination parameters and seedling vigor characteristics, such as length, fresh weight, dry weight, chlorophyll, and carotenoids, were measured. It was found that candle soot colloids significantly improved germination speed, seedling length, and seedling fresh weight compared to the control. The adherence of soot particles to the trichomes on the seed surface, which may help seeds retain moisture and stay hydrated, was observed. The chemical composition of the soot samples was analyzed using gas chromatography-time of flight mass spectrometry and two different chemical constituents in hydrophilic and hydrophobic soot samples that may affect seed germination were identified. A new potential use of candle soot colloids as plant growth-promoting agents and insights into the effects of nanomaterials on plant physiology and biochemistry were revealed by this study.

Keywords Carbon · Colloidal · Nanomaterials · Chemical analysis · Nanoparticles · Seedling growth

1 Introduction

Agriculture is the process of harnessing solar energy through photosynthesis, which converts sunlight into chemical energy stored in the form of carbohydrates, primarily in plants [1]. Photosynthesis begins as soon as a plant has leaves with chlorophyll. The first leaves, often referred to as cotyledons, typically emerge after the germination phase [2]. During germination, a seed absorbs water and begins to swell, triggering various biochemical processes that lead to the emergence of seedling through the surrounding tissues. The initial growth during germination involves the

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development of the radicle (the embryonic root) and the hypocotyl (the stem region between the radicle and the cotyledons) [3]. Germination is therefore a crucial process for plant growth and development.

Germination is the initial and fundamental process of plant growth that affects yield and quality. Early germination has the advantage of providing better access to nutrients and space than late germination [4]. Seed germination performance is measured by several parameters, including final germination percentage, mean germination time, germination index, coefficient of velocity of germination, germination rate index, first day of germination, last day of germination, and time spread of germination. Seedling quality is assessed by parameters such as seedling vigor, which is based on seedling shoot length, root length, wet weight, and dry weight [5]. Various methods have been used to accelerate germination and improve seedling quality, including soaking seeds in pure water, immersing seeds in an osmotic solution with low water potential, adding plant growth regulators (hormones), adding bacterial inoculum, and using other chemical compounds such as antioxidants, chitosan, fungicide, and nanoparticles [6].

Chemical compounds in the form of particles can be obtained in a variety of types and sizes, including nano (below 100 nm), submicron, and other particle sizes [7]. Our group has previously reported that silica nanoparticles, an industrial nanomaterial, can improve tomato (*Solanum lycopersicum*) seed germination and seedling vigor [8]. On the other hand, carbonaceous particles are among the particulate matters that can be used for seed germination. These particles can be obtained from the combustion or thermal decomposition of carbon sources such as fossil fuels, biomass, and biofuels [9]. Previous studies have shown that carbonaceous particles can have both positive and negative effects on seed germination, depending on the type of plant and the particle concentration [10–12]. Despite numerous studies on the impact of carbonaceous particles on plants, there exists a notable void in our comprehension of how specific components of particulate matter, such as candle soot colloids, influence the early stages of plant development. Previous research have predominantly focused on the effects of carbonaceous particles such as biochar, graphene, and carbon nanotubes [12–14], neglecting the nuanced interactions with more unconventional sources such as candle soot. The specific properties and chemical composition of candle soot colloids remain largely unexplored, creating a gap in our understanding of their potential influence on seed germination and seedling vigor. In this study, we investigated the effect of candle soot colloids, a nanomaterial from candle combustion [15] on tomato (*S. lycopersicum*) seed germination and seedling vigor. We prepared aqueous colloids of candle soot at different concentrations and measured germination parameters and seedling vigor characteristics. We also analyzed the chemical composition of the soot samples using gas chromatography-time of flight mass spectrometry and compared the effects of hydrophilic and hydrophobic soot samples.

2 Materials and methods

2.1 Seeds viability test

The tomato seeds (*S. lycopersicum*) used in this study were obtained from Takii & Co., Ltd., Japan. Seeds were first rinsed and soaked with distilled water for 10 min [8]. This procedure was to select the viable seeds from those that were not. Viable seeds would sink to the bottom [16]. A total of 175 viable seeds were selected for the study.

2.2 Preparation of candle soot particles

Soot particles were collected as aerosols using a direct deposition method [15]. Candles used were obtained from Daiko Inc., Japan. A candle was placed under a support rack with a quartz glass plate attached. This glass served as the collection point for candle soot particles. Hydrophobic or hydrophilic soot (colloidal) samples were produced by changing the position of the glass plate. Hydrophilic particles were deposited from the tip of the flame, while hydrophobic particles were deposited from the interior of the flame [15]. After 1–2 min of settling time, the soot particles were collected and weighed. They were then mixed with distilled water to form colloidal suspensions at concentrations of 0 (control), 10^{-2}, 10^{-3}, and 10^{-4} wt%. To ensure homogeneity, the suspensions were ultrasonicated for 10 min at 35 kHz (UT-105S, Sharp Corp., Japan).

2.3 Particle characterization

The particle size and zeta potential of the candle soot suspension were analyzed using a dynamic light scattering instrument (Zetasizer Nano-ZS, Malvern Panalytical Ltd., UK) [8]. To measure particle size, 4 mL of the suspension was placed in a plastic cuvette and inserted into the instrument. To measure zeta potential, 1 mL of the suspension was placed in a folded capillary cell and inserted into the instrument. Measurements were performed three times for each treatment.

2.4 Germination with soot particles

Seven different treatments were used in this study, as shown in Table 1. The germination was carried out in Petri dishes lined with cotton puffs. Five tomato seeds were placed in each Petri dish and given 20 mL of suspension from each treatment. Each treatment consisted of five replications. The Petri dishes were then sealed with paraffin tape and placed in a closed box to ensure dark conditions [17]. Germination lasted for 8 days. The early tomato seedlings were obtained on the last day of germination and harvested to measure their vigor characteristics. Dry weight was obtained after the seedlings were dried in the oven for 48 h at 50 °C [18].

2.5 Germination parameters

The following parameters were used to assess germination performance: seed germination percentage (SGP), mean germination time (MGT), germination index (GI), and coefficient of velocity of germination (CVG). SGP indicates the proportion of seeds that germinated in each treatment on a given day. MGT measures the speed at which seeds germinate. GI combines the rate of germination and germination percentage [17, 19], while CVG reflects the speed at which seeds germinate [20]. The following formulas were used to calculate these parameters:

$$\mathrm{SGP} = \frac{\text{number of seeds germinated}}{\text{total number of seed}} \times 100\% \quad [17] \tag{1}$$

$$\mathrm{MGT} = \frac{\sum \mathrm{n} \times \mathrm{d}}{\sum \mathrm{n}} \quad [8] \tag{2}$$

$$\mathrm{GI} = \left(8 \times \mathrm{n}_1\right) + \left(7 \times \mathrm{n}_2\right)\left(6 \times \mathrm{n}_3\right) + \cdots + \left(1 \times \mathrm{n}_8\right) \quad [19] \tag{3}$$

$$\mathrm{CVG} = \frac{\left(\mathrm{n}_1 + \mathrm{n}_2 + \cdots + \mathrm{n}_8\right)}{\left(\mathrm{n}_1\mathrm{T}_1 + \mathrm{n}_2\mathrm{T}_2 + \cdots + \mathrm{n}_8\mathrm{T}_8\right)} \times 100 \quad [19] \tag{4}$$

where n is the number of seeds that germinated on day d and d is the number of days required for seeds to sprout. The number of seeds that germinated on the first, second, and every following day up until the seventh day is represented by the letters n_1, n_2, …, and n_8, and the first number in each term (8, 7, …, and 1) represents the weight that is given to the number of seeds that germinated on the first, second, and every following day. T represents the number of days since germination began. The vigor index (VI) was used to quantify seedling quality produced after germination [8, 17].

Table 1 Treatments and variables

Particles	Concentrations (wt%)	Treatment name
–	–	Control
Hydrophilic	10^{-2}	PH2
	10^{-3}	PH3
	10^{-4}	PH4
Hydrophobic	10^{-2}	PB2
	10^{-3}	PB3
	10^{-4}	PB4

Three parameters were considered for the measurement: seedling length (VI_{LENGTH}), fresh weight (VI_{FW}), and dry weight (VI_{DW}). The following formulas were used:

$$VI_{LENGTH} = SGP \times \text{mean of seedling length in cm} \quad [17] \tag{5}$$

$$VI_{FW} = SGP \times \text{mean of seedling fresh weight in g} \quad [17] \tag{6}$$

$$VI_{DW} = SGP \times \text{mean of seedling dry weight in g} \quad [17] \tag{7}$$

2.6 Analysis of the chemical composition

The chemical composition of the soot suspension was analyzed using a gas chromatograph (8890 GC System, Agilent Technologies, Inc., USA) and pyrolyzer (PY-3030D, Frontier Laboratories Ltd., Japan) combined with a spectrometer (JMS-T200GC, GC/HRTOFMS system, JEOL Ltd., Japan), which has a resolving power of 10,000 (FWHM) at *m/z* 613.9642 (perfluorotributylamine, Sigma-Aldrich Co. LLC, USA) [21]. The HRTOFMS system is a high-resolution time-of-flight mass spectrometer that provides accurate mass measurements of ions with high resolving power. The FWHM (full width at half maximum) is a measure of the resolution of the mass spectrometer, which indicates the ability to distinguish between ions with similar masses. Two different samples were used for analysis: hydrophilic and hydrophobic candle soot suspension with a concentration of 10^{-3} wt%. Data obtained from the chromatograms were analyzed using msFineAnalysis (JEOL Ltd., Japan) [21]. All conditions used in the analysis are listed in Table 2.

2.7 Analysis of the chlorophylls and carotenoids

A total of 0.1 g of seedlings leaves obtained from 8-days of germination was ground with mortar and pestle, and 5 mL of 96% ethyl alcohol was added [22]. The extract was left for 10 min then filtered with quantitative filter paper (Advantec® 4A, Advantec Toyo Kaisha, Ltd., Tokyo, Japan). The resulting filtrate was then poured into a 5 mL volumetric flask and filled to the volume with 96% ethanol. About 200 µL of the filtrate was taken and placed into a 96-well microplate (Nikkei Products Co. Ltd., Japan) and then the absorbance (470, 647, 663, and 750 nm) was measured using a multifunctional monochromator-based microplate reader (INFINITE M1000 Pro, Tecan Group Ltd., Switzerland). The following formulas were used to calculate the pigment content of leaves:

$$A_X = \frac{\left(OD_X - OD_{750}\right)}{\ell} \quad [22] \tag{8}$$

$$\text{Chl a} = \left(11.24 \times A_{662}\right) - \left(2.04 \times A_{645}\right) \times \frac{\nu}{\omega} \quad [22] \tag{9}$$

Table 2 Analysis and data processing conditions for GC/MS

Analysis	Parameter	Condition
Pyrolysis	Temperature	600 °C
Gas chromatography	Column	30 m × 0.25 mm, 0.25 µm
	Oven temperature	100 °C (2 min)—10 °C/min—300 °C (2 min)
	Injection mode	Split (100:1)
Mass spectrometry	Ion source	EI Fragment Ion Analysis
	Ionization	EI+: 70 eV, 300 µA; FI+: – 10 kV, 40 mA/30 ms
	Mass range	*m/z* 30–800
Data processing	Library database	NIST17
	Tolerance	± 5 mDa
	Electron	Odd
	Element set	C: 0–50, H: 0–100, O: 0–10

$$\text{Chl b} = \left(20.13 \times A_{645}\right) - \left(4.19 \times A_{662}\right) \times \frac{\nu}{\omega} \quad [22] \tag{10}$$

$$\text{Crt} = \frac{\left(1000 \times A_{470}\right) - (1.9 \times \text{Chl a}) - (63.14 \times \text{Chl b})}{214} \times \frac{\nu}{\omega} \quad [22] \tag{11}$$

where A_x was the absorbance at x nm, OD_x was the measured optical density at wavelength x nm, ℓ was the path length of the microplate (= 0.876887 cm), Chl a was chlorophyll a (mg/g DW), Chl b was chlorophyll b (mg/g DW), Crt was total carotenoids (mg/g DW), ν was the volume of solvent (mL), and ω was the weight of the sample (mg).

2.8 Seed surface observation

The attachment of candle soot to tomato seed coat was analysed using a scanning electron microscope (SEM; JSM-6510, JEOL Ltd., Japan) at 24 h after treatment [8]. SEM analysis was only conducted for seeds treated with hydrophilic candle soot with concentration of 10^{-3} wt%. The seeds were coated with platinum (Pt) using an ion sputtering apparatus (Fine Coat Ion Sputter JFC-1100, JEOL Ltd., Japan) before SEM inspection. The SEM conditions were as follows: voltage = 2 kV, working distance (WD) = 11 mm, and strain sensors (SS) = 60.

2.9 Statistical analysis

Statistical analysis was performed to assess the effects of candle soot particle treatment on seed germination and seedling growth. The data for calculating seed germination parameters was 5 replications, while the data for calculating chlorophyll and carotenoids were triplicates. Data for pH of particle suspension, zeta potential, and average particle size were also in triplicates. All data obtained in this study will be evaluated using descriptive statistics, analysis of variance (ANOVA), and Duncan's multiple range test (DMRT) in IBM SPSS Statistics with *p-value* of 0.05 considered significant.

3 Results and discussion

3.1 Soot suspensions

The zeta potential and particle size distribution of candle soot particles obtained in this study, both hydrophilic and hydrophobic, were characterized. The zeta potential and the average particle size were shown in Table 3. The particle distribution was shown in Fig. 1. Hydrophilic soot particles tended to be larger than hydrophobic soot particles at the same concentration. Hydrophilic materials have an affinity for water. In the case of candle soot, which is primarily composed of carbonaceous primary particles, hydrophilic soot particles can interact more readily with water molecules, leading to their aggregation into larger clusters or agglomerates [23]. These larger agglomerates can result in an increase in the overall particle size of hydrophilic candle soot when suspended in water or other aqueous solutions. The particle size of candle soot suspensions increased directly with concentration. As the concentration of soot increases, particles are more likely to encounter each other and agglomerate. This can lead to agglomeration, where individual particles clump together to form larger aggregates [24]. There is a limit to how much agglomeration can occur, and beyond a certain point, further increases in concentration may not significantly increase particle size. Micrometer-sized aggregates were mostly observed

Table 3 pH, zeta potential, and average particle size from hydrophilic (PH2,3,4) and hydrophobic (PB2,3,4) candle soot suspensions

Treatment	pH	Zeta potential (mV)	Average particle size (nm)
PH2	6.77 ± 0.19^{a}	-34.2 ± 0.67^{c}	593.3 ± 13.7^{f}
PH3	6.33 ± 0.48^{a}	-33.23 ± 0.31^{bc}	380.53 ± 12.52^{d}
PH4	6.2 ± 0.22^{a}	-32.4 ± 0.62^{b}	196.24 ± 0.71^{b}
PB2	6.5 ± 0.5^{a}	-33.23 ± 0.98^{bc}	498.05 ± 6.48^{e}
PB3	6.07 ± 0.45^{a}	-31.83 ± 0.76^{b}	289.98 ± 4.41^{c}
PB4	5.9 ± 0.28^{a}	-29.33 ± 1.01^{a}	147.23 ± 3.63^{a}

Different letters (a, b, c, bc, d, e, f) indicate significance ($p \leq 0.05$) according to the Duncan's test

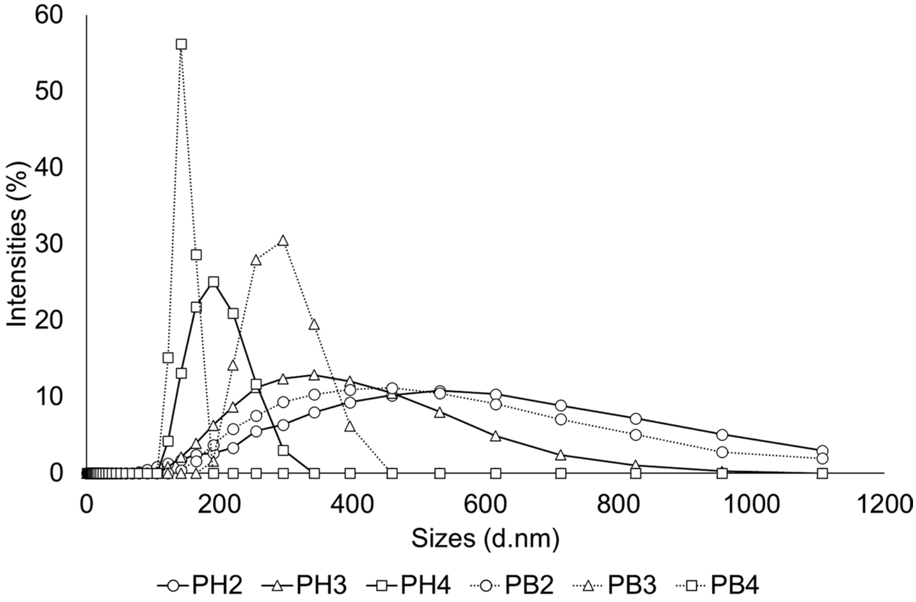

Fig. 1 Size distributions of soot particles measured in aqueous samples. PH2,3,4: hydrophilic; PB2,3,4: hydrophobic

at the concentration of 10^{-2} wt%. At the lower particle concentration of 10^{-4} wt%, the particle size distributions were narrow. These results agree with the research conducted by Faizal et al. on candle soot particles dissolved in ethanol [15]. The zeta potential values of hydrophilic and hydrophobic candle soot suspensions were similar. The pH of the suspension can affect the zeta potential. If the pH results in similar surface charges on both types of soot particles, their zeta potentials may be similar [25]. However, zeta potential of PB4 showed significant differences ($p < 0.05$) compared to other candle soot suspensions. Hydrophobic particles tend to have lower zeta potentials compared to hydrophilic particles at the same concentration. This is because hydrophobic particles often attract fewer water molecules to their surfaces, leading to a less developed electric double layer [26]. The electric double layer is crucial in determining the zeta potential, and its development is influenced by the interaction between the particle surface and the surrounding solvent [27].

3.2 Chemical compositions of candle soot particles

Candle soot consists of various chemical components that result from the incomplete combustion. The type of candle wax, additives, and burning conditions can affect the composition of candle soot. In this study, we demonstrated that different combustion conditions could produce candle soot with different characteristics: hydrophilicity and hydrophobicity. We examined the chemical constituents of hydrophilic and hydrophobic candle soot as shown in Fig. 2 and Table 4. Carbon was the dominant component of candle soot, accounting for a large fraction of its mass. Candle soot also contained various organic compounds, mostly hydrocarbons. These compounds included alkanes, alkenes, and aromatic hydrocarbons [28]. We identified at least 4 compounds that were present in both hydrophilic and hydrophobic candle soot: pentadecane, cetene, n-hexadecanoic acid (palmitic acid), and octadecanoic acid (stearic acid). Pentadecane and cetene were straight-chain hydrocarbons [29], while palmitic and stearic acids were saturated fatty acids [30].

Some compounds were specific to either hydrophilic or hydrophobic candle soot and were not found in both. The compound with the highest intensity in hydrophobic candle soot was tritriacontane ($C_{33}H_{68}$), while in hydrophilic candle soot it was nonadecanenitrile ($C_{19}H_{37}N$). These differences in chemical composition might affect the responses of seeds during germination. Identifying the compounds in hydrophilic and hydrophobic soot could be useful for assessing the impact of these particles in other research areas (e.g. seed germination or other agricultural or biological studies) to explore the potential of candle soot. The concentration of hydrocarbons in the germinating and growing medium is an important factor that affects seed germination [31, 32]. High concentrations of certain hydrocarbons, especially petroleum-based products such as 3-chlorobenzoate and 4-chlorobenzoate, can be toxic to seeds and inhibit germination [31, 33]. Lower concentrations may have positive, mild, or negligible effects [31, 34]. For example, *Lycopersicum esculentum* var. Moench seeds showed a germination percentage of 86% when exposed to 50 mg/L of 3-chlorobenzoate ($C_7H_4ClO_2$) or 4-chlorobenzoate ($C_7H_4ClO_2$). However, when the concentration was increased to 400 mg/L, seed germination was completely inhibited [31]. The presence of n-hexadecanoic acid could inhibit the germination of *Tillandsia*

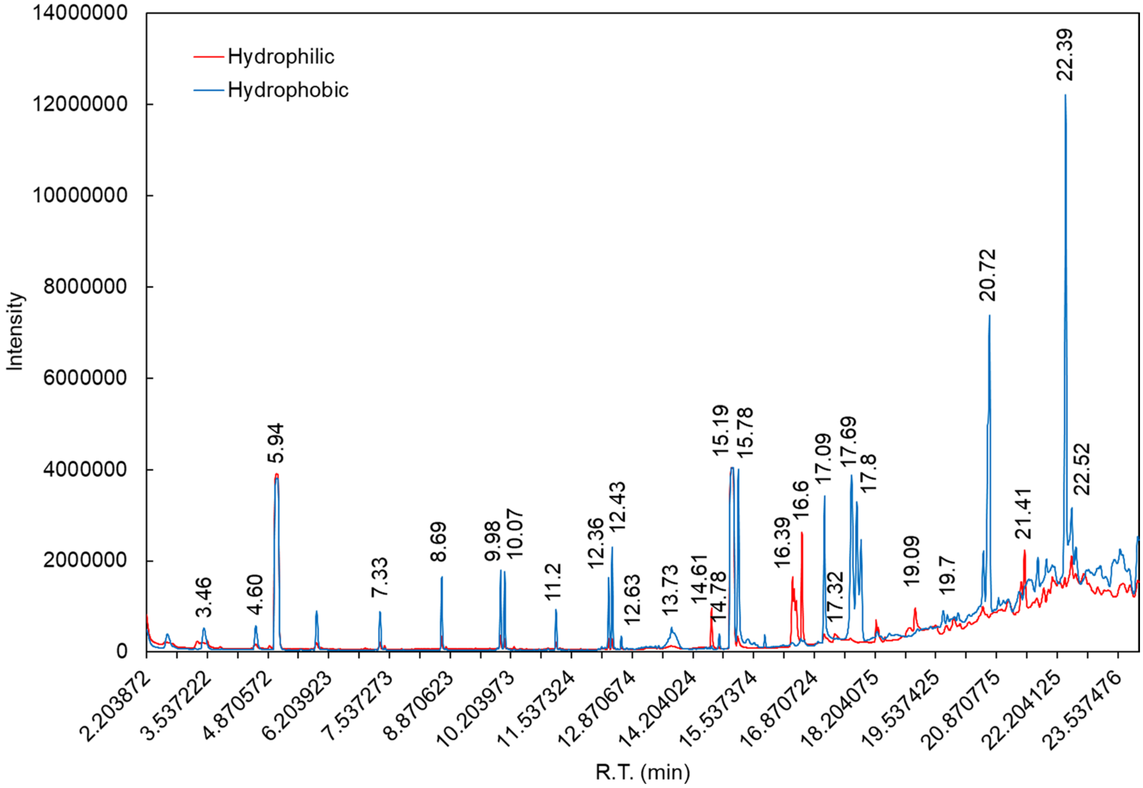

Fig. 2 GC/MS analysis of the hydrophilic and hydrophobic candle soot suspensions with concentration of 10^{-3} wt%

recurvata seeds. *n*-Hexadecanoic acid ($C_{16}H_{32}O_2$) was one of the candle soot components found in both the hydrophilic and hydrophobic candle soot [33]. A study by Varjani et al. showed that high hydrocarbon concentrations in the soil inhibited the germination of *Vigna radiata* seeds [32]. Another study showed that hydrocarbons altered the properties of soil microbes, which could interfere with seed germination [35]. A study conducted by Dominguez-Rosado et al. demonstrated positive feedback of hydrocarbons on germination [34]. Three seed species (*Phaseolus vulgaris*, *Glycine max*, and *Zea mays*) germinated better than other treatments at used oil concentrations up to 10% (w/w). The oil was dominated by medium and long chain aliphatic, benzene and naphthalene-based hydrocarbons, especially 1-ethyl-2methyl benzene, 1-methyl-2-(2 propenyl)-benzene, decane, hexadecane, and eicosane [34]. The hydrocarbons presented are similar to those obtained from candle soot as shown in Table 4. A possible explanation for this unexpected behavior, hydrocarbons improved seed germination, could be attributed to additional sources of C acting as plant nutrients, thereby stimulating growth [34, 36]. This aligns with the fact that carbon is a crucial element for plant development and is a major component of organic molecules, including carbohydrates, proteins, and lipids [37]. Plants typically obtain carbon through the process of photosynthesis, where they convert carbon dioxide into organic compounds using light energy [38]. However, in certain situations, external sources of carbon, such as hydrocarbons, could potentially contribute to the plant's nutrient supply [36].

3.3 Seed germination and seedling growth

Several parameters were used to analyse germination performance under candle soot suspensions application. The first parameter was seed germination percentage shown in Fig. 3. All seeds were observed to be germinated in all treatments. This indicates that the use of candle soot particles at concentrations of 10^{-2} to 10^{-4} wt% did not inhibit the germination. However, the germination time differed between treatments. Treatment with candle soot concentration of 10^{-3} wt%, both hydrophilic and hydrophobic, gave germination percentage of 100% on the 4th day, while other treatments did not show 100% germination on that day. A study conducted by Baz et al. showed that the used of the commercial carbonaceous nanoparticles could improve the germination and growth of lettuce seeds [39]. We observed the attachment of candle soot particles to the hairy surface of seeds as shown in Fig. 4. These tiny hair-like structures on the surface of tomato

Table 4 GC/MS identification of the chemical compounds in hydrophilic and hydrophobic candle soot

R.T. (min)	Compound	Formula	Intensity	
			Hydrophilic	Hydrophobic
3.46	1-Decene	$C_{10}H_{20}$	–	2416954
4.6	1-Undecene	$C_{11}H_{22}$	–	2054900
5.94	1-Dodecene	$C_{12}H_{24}$	–	2460205
7.33	1-Tridecene	$C_{13}H_{26}$	–	1847977
8.69	1-Tetradecene	$C_{14}H_{28}$	–	3038858
9.98	1-Pentadecene	$C_{15}H_{30}$	–	2976363
10.07	Pentadecane	$C_{15}H_{32}$	395836	2847853
11.2	Cetene	$C_{16}H_{32}$	260462	1519167
12.36	E-14-Hexadecenal	$C_{16}H_{30}O$	–	2705571
12.43	Heptadecane	$C_{17}H_{36}$	–	3660403
12.63	Oxirane, dodecyl-	$C_{14}H_{28}O$	–	546155
13.73	Pentadecanal-	$C_{15}H_{30}O$	–	1382427
14.61	Hexadecanenitrile	$C_{16}H_{31}N$	1810603	–
14.78	13-Methyltetradecanal	$C_{15}H_{30}O$	–	648325
15.19	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	590149	8278732
15.78	Isopentadecanal	$C_{15}H_{30}O$	–	495912
16.39	9-Octadecenenitrile, (Z)-	$C_{18}H_{33}N$	1082971	–
16.6	Nonadecanenitrile	$C_{19}H_{37}N$	4537804	–
17.09	Octadecanoic acid	$C_{18}H_{36}O_2$	696246	6667749
17.32	Nonanamide	$C_9H_{19}NO$	1026554	–
17.69	Hentriacontane	$C_{31}H_{64}$	–	10913449
17.8	*n*-Hentriacontane	$C_{31}H_{64}$	–	5718289
19.09	Octadecanamide	$C_{18}H_{37}NO$	1642221	–
19.7	Tritriacontane, 2-methyl-	$C_{34}H_{70}$	–	914185
20.72	Dotriacontane	$C_{32}H_{66}$	–	14731030
21.41	Nonadecane	$C_{19}H_{40}$	874574	–
22.39	Tritriacontane	$C_{33}H_{68}$	–	22985060
22.52	Eicosane	$C_{20}H_{42}$	–	3016538

R.T. is retention time in minutes

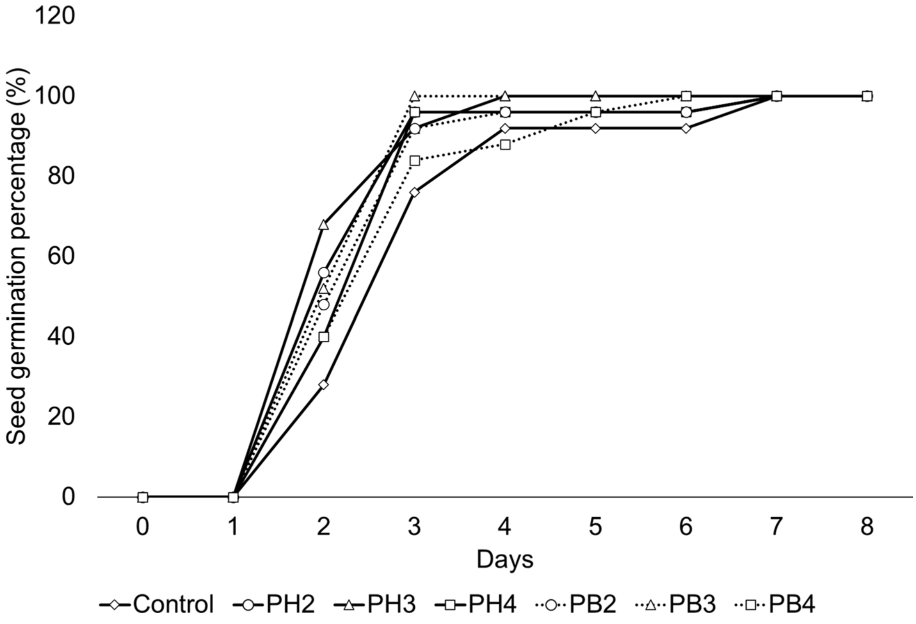

Fig. 3 Seed germination percentage of tomato seeds under application of hydrophilic (PH2,3,4) and hydrophobic (PB2,3,4) candle soot suspensions

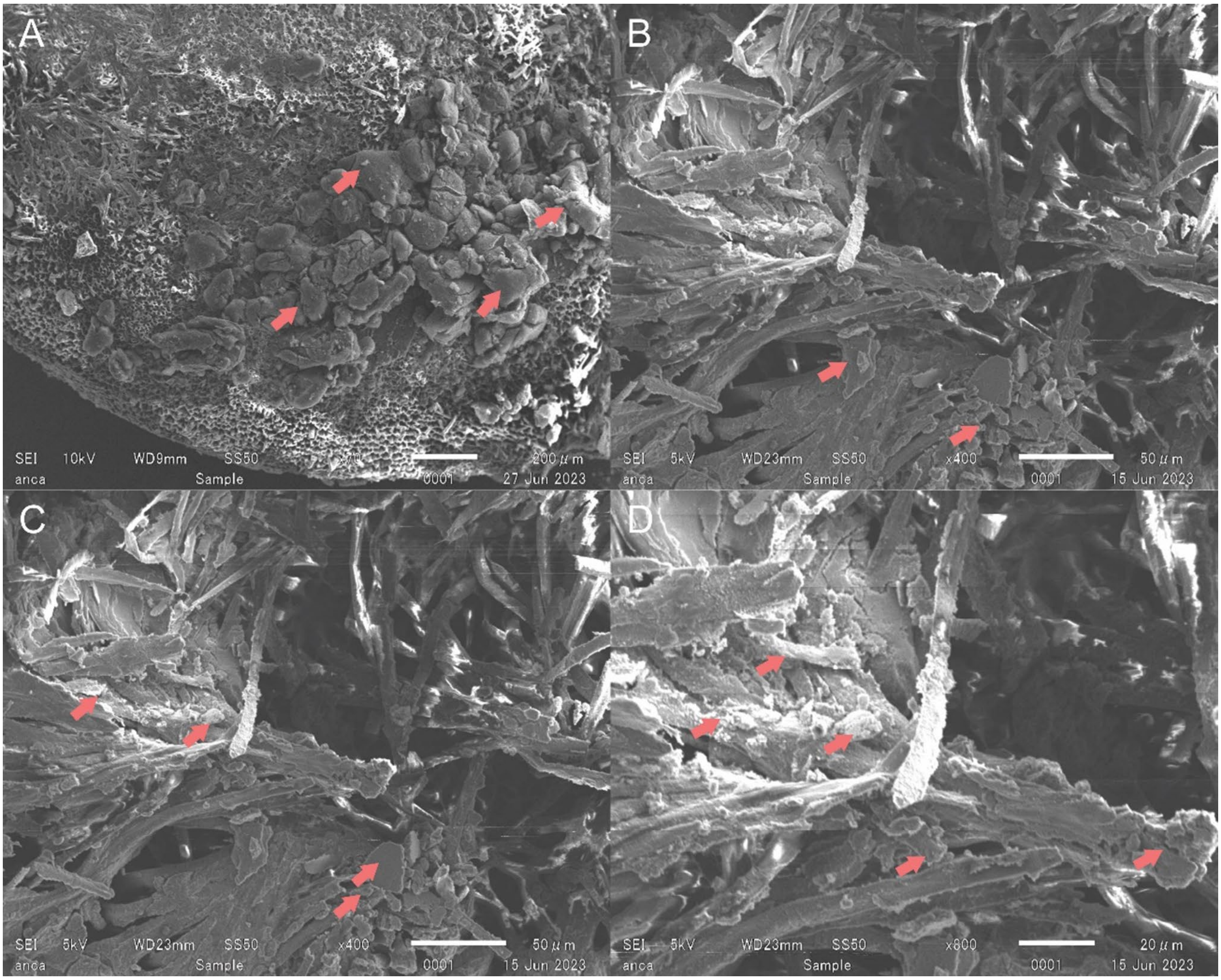

Fig. 4 SEM images of the surface of tomato seeds treated with hydrophilic (**A** & **B**) and hydrophobic (**C** & **D**) soot suspensions (10^{-3} wt%). The red arrows indicate the aggregated soot particles. The magnifications are 70x (**A**), 400x (**B** & **C**), and 800x (**D**)

seeds are known as trichomes [40]. Trichomes are specialized epidermal cells that can take various forms and serve different functions across plant species. The interaction of trichomes and soot particles may help the seeds retain moisture by forming a thin layer that reduces water evaporation from the seed surface. Another study showed that carbonaceous particles could help seeds retain water during germination. Tomato seeds treated with 40 µg/mL of carbon nanotubes during germination. The treated seeds contained 19% more water than the control seeds [41]. This can be advantageous during germination by maintaining adequate hydration for the seeds.

The other parameters, mean germination time, germination index, and coefficient of velocity of germination, are shown in Table 5. All of the parameters showed similar results, and the concentration of 10^{-3} wt% was considered the optimal concentration for both hydrophilic and hydrophobic conditions. Significant differences ($p \le 0.05$) between treatments were seen in particle concentration but not in particle hydrophilicity or hydrophobicity. This indicates that particle concentration may be the most prominent factor affecting particle behavior in a system. If one treatment has a significantly higher particle concentration than another, it can lead to more noticeable differences in various aspects, such as the physical properties of the suspension or the interaction of particles with the surrounding seed microenvironment. Differences in concentration may also lead to variations in the size distribution, which can influence the observed differences. Even if treatments have similar particle surface properties (hydrophilicity or hydrophobicity), variations in the particle size distribution can affect particle behavior in a suspension [42]. Also, hydrophilic particles tend to give better results than hydrophobic particles at the same concentration. Hydrophilic particles have an affinity for water and can absorb and retain moisture more effectively [43]. This property is advantageous for seed germination because it

Table 5 Mean germination time (MGT), germination index (GI), and coefficient of velocity of germination (CVG) evaluated under the application of hydrophilic (PH2,3,4) and hydrophobic (PB2,3,4) soot suspensions

Treatment	MGT (days)	GI	CVG
Control	3.2 ± 0.44^{b}	29 ± 2.19^{b}	31.79 ± 3.94^{b}
PH2	2.6 ± 0.55^{a}	32 ± 2.76^{a}	40.03 ± 7.53^{a}
PH3	2.4 ± 0.13^{a}	33 ± 0.63^{a}	41.78 ± 2.22^{a}
PH4	2.76 ± 0.34^{ab}	31.2 ± 1.72^{ab}	36.74 ± 4.12^{ab}
PB2	2.72 ± 0.41^{ab}	31.4 ± 2.06^{ab}	37.59 ± 5.52^{ab}
PB3	2.48 ± 0.2^{a}	32.6 ± 1.02^{a}	40.59 ± 3.3^{a}
PB4	2.92 ± 0.37^{ab}	30.4 ± 1.85^{ab}	34.82 ± 4.57^{ab}

Different letters (a, b, ab) indicate significance ($p \leq 0.05$) according to the Duncan's test

ensures a consistent water supply to the seeds, allowing for better water imbibition. We also observed positive feedback from the use of hydrophilic silica nanoparticles on seed germination in a previous study [8], which is in accordance with several other studies [17].

The shortest mean germination time was obtained in the treatment of 10^{-3} wt% hydrophilic candle soot, which was 33.33% lower than the control. This shows that hydrophilic candle soot can hasten seed germination. Candle soot particles, being hydrophilic, can absorb and retain water. When applied to cotton puffs or seeds, they can help maintain a consistently moist environment, which is essential for seed germination. Adequate moisture softens the seed coat and allows for water uptake and the initiation of the germination process [44]. Improved germination performance was observed in both hydrophilic and hydrophobic candle soot at the same concentration. Hydrophilic and hydrophobic candle soot at a concentration of 10^{-3} wt% increased the germination index by 12.12% and 11.04%, respectively. The attachment of soot particles, both hydrophilic and hydrophobic, to the seed surface may have directly benefited seed germination by providing a protective or moisture-retaining layer. A concentration of 10^{-3} wt% was also increased the coefficient of velocity of germination by 23.93% for hydrophilic and 21.7% for hydrophobic compared to the control.

The effect of candle soot particles on seedling vigor after 8 days of germination is shown in Table 6. Hydrophilic candle soot at a concentration of 10^{-3} wt% gave the highest values for all vigor index parameters. Hydrophilic soot at that concentration increased VI_{LENGTH} by 19.14%, VI_{FW} by 20.98%, and VI_{DW} by 21.11%. Hydrophilic particles gave higher values for all vigor index parameters than hydrophobic particles. However, hydrophobic candle soot particles still gave significantly higher values ($p \leq 0.05$) than the control. Our present study is the first to show that particles from burning candles can provide benefits for seed germination. However, several studies have shown that other carbonaceous materials, such as biochar, can also improve seed germination. Ali et al. showed that biochar in maize increased shoot dry biomass, root dry biomass, total chlorophyll content, germination percentage, seedling vigor, and relative water content [45]. Biochar also contributed to the regulation of some enzymes, such as superoxide dismutase and catalase [46, 47]. Further study is needed to determine the mechanism of action of hydrophilic and hydrophobic candle soot particles in regulating germination performance and seedling quality.

3.4 Seedling pigments

The progeny of plants depends on maternal photosynthesis, via substances of reserves accumulated in the seeds, which it is necessary to supply energy for seed germination and early seedling establishment [48]. In this study, the chlorophylls

Table 6 Seedlings vigor based on length (VI_{LENGTH}), fresh weight (VI_{FW}), and dry weight (VI_{DW}) evaluated under the application of hydrophilic (PH2,3,4) and hydrophobic (PB2,3,4) soot suspensions

Treatment	VI_{LENGTH}	VI_{FW}	VI_{DW}
Control	1193.2 ± 71.71^{d}	2.42 ± 0.17^{c}	0.22 ± 0.02^{c}
PH2	1424 ± 58.58^{abc}	2.94 ± 0.14^{ab}	0.26 ± 0.01^{ab}
PH3	1475.6 ± 83.21^{a}	3.06 ± 0.16^{a}	0.27 ± 0.01^{a}
PH4	1454.4 ± 94.09^{ab}	3.05 ± 0.14^{a}	0.27 ± 0.02^{a}
PB2	1340 ± 63.33^{c}	2.76 ± 0.29^{b}	0.25 ± 0.03^{b}
PB3	1401.2 ± 46.02^{abc}	2.94 ± 0.15^{ab}	0.26 ± 0.01^{ab}
PB4	1360.4 ± 57.12^{bc}	2.82 ± 0.05^{ab}	0.25 ± 0.01^{ab}

Different letters (a, b, ab, c, abc, bc, d) indicate significance ($p \leq 0.05$) according to the Duncan's test

Table 7 Chlorophylls and carotenoids under application of hydrophilic and hydrophobic candle soot suspensions

Treatment	Chlorophyll a (mg/g DW)	Chlorophyll b (mg/g DW)	Total carotenoids (mg/g DW)
Control	0.15 ± 0.01^{e}	0.07 ± 0.02^{b}	0.1 ± 0.01^{b}
PH2	0.21 ± 0.01^{ab}	0.1 ± 0.01^{ab}	0.14 ± 0.03^{ab}
PH3	0.23 ± 0.01^{a}	0.11 ± 0.02^{a}	0.16 ± 0.02^{a}
PH4	0.18 ± 0.01^{cd}	0.08 ± 0.01^{ab}	0.12 ± 0.01^{ab}
PB2	0.19 ± 0.01^{bcd}	0.08 ± 0.01^{ab}	0.13 ± 0.01^{ab}
PB3	0.2 ± 0.02^{bc}	0.1 ± 0.03^{ab}	0.13 ± 0.02^{ab}
PB4	0.16 ± 0.02^{de}	0.07 ± 0.01^{ab}	0.11 ± 0.01^{ab}

Different letters indicate significance ($p \leq 0.05$) according to the Duncan's test

and carotenoids were measured in seedling, after the completion of germination in the strict sense. No measurement of chlorophylls and carotenoids were performed during germination. The quantification of chlorophylls and carotenoids in seedlings reflects their growth [49]. Furthermore, the chlorophylls and carotenoids are essential biochemical indicators of plant photosynthesis and photoprotection [50]. We found significant differences ($p \leq 0.05$) in chlorophyll a between treatment and control, but not in chlorophyll b or total carotenoids. Hydrophilic candle soot at a concentration of 10^{-3} wt% increased chlorophyll a by 35.43% compared to the control (Table 7). During the germination stage, the seed does not perform photosynthesize [51]. The seedling stage begins when the seed has germinated and the root and cotyledon have developed sufficiently to enable photosynthesis [52]. Chlorophyll a is the main pigment involved in light absorption and photosynthesis: it participates in the conversion of light energy into chemical energy, which is essential for providing energy to the developing seedling [53]. Chlorophyll a also plays a key role in electron transfer during the light-dependent reactions of photosynthesis, which generate energy-rich molecules (such as ATP) that drive the carbon-fixing reactions of the Calvin cycle [54]. Chlorophyll a is an integral part of both Photosystem I and Photosystem II, the two major photosynthetic complexes that absorb light and transport electrons in photosynthesis [55]. These complexes are responsible for converting light energy into chemical energy, enabling carbon fixation and energy production.

4 Conclusions

The potential of using candle soot colloids, a nanomaterial from candle combustion, to improve tomato (*S. lycopersicum*) seed germination and seedling vigor was demonstrated. Hydrophilic and hydrophobic candle soot samples improved tomato seed germination and seedling vigor at an optimal concentration of 10^{-3} wt% (aqueous sample). This concentration reduced germination time, enhanced germination parameters, and increased seedling quality indicators such as length, fresh weight, dry weight, chlorophyll, and carotenoids. The adherence of soot particles to the trichomes on the seed surface may help seeds retain moisture and stay hydrated. The chemical composition of hydrophilic and hydrophobic soot samples was analyzed for future studies to better understand the impact of these nanomaterials on seed germination. Hydrophilic soot samples were found to be more effective than hydrophobic soot samples at the same concentrations. Compared to our previous study using silica particles [8], candle soot particles can be used as a novel and low-cost method to improve tomato seed germination and seedling vigor. Further studies are needed to elucidate the mechanisms of action of candle soot colloids on seed germination and to test their effects on other plant species and under different environmental conditions.

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Data availability Further data and details are provided in Additional Material file.

Declarations

Competing interests The authors declare that they have no competing interests, financial or non-financial, that are directly or indirectly related to the work submitted for publication.

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