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PLANT TISSUE CULTURE

Effect of charcoal-impregnated calcium alginate spherules in mitigating polyphenolic interference in coconut (*Cocos nucifera* L.) suspension cultures

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Abstract

Phenolic oxidation and browning pose significant challenges in coconut cell suspension culture. Although activated charcoal is commonly used to mitigate phenolic interference, its powder form reduces medium clarity, thereby hindering precise quantification. The present study aimed to address this issue by developing an alternative method of incorporating activated charcoal that maintains its functional role while preserving culture transparency. For this, calcium alginate spherules of two sizes (4 and 6 mm) were prepared using varying concentrations of sodium alginate and calcium chloride, and their stability in suspension culture conditions was evaluated. Selected spherules were incorporated with activated charcoal and were further tested for polyphenol adsorption capacity in gallic acid solutions and coconut cell suspension cultures. While the spherules of treatment S_1T_{20} (6 mm diameter, 3.0% sodium alginate with 0.4 M calcium chloride), incorporated with 2.0 g L^{-1} of activated charcoal, adsorbed 68 to 80% of the polyphenols in coconut cell suspension, those devoid of activated charcoal exhibited no phenol adsorption. Proliferation of the cell and formation of cell clumps occurred in media with activated charcoal (either in the powder form or as incorporated in calcium alginate spherules), whereas the cells elongated and vacuolated in media without activated charcoal. This study introduced, for the first time, an effective alternative to the conventional approach of incorporating activated charcoal powder in suspension cultures using calcium alginate spherules. This approach effectively addressed the challenge of phenolic interference while simultaneously enhancing medium clarity facilitating accurate visualization and quantification of cultured cells. The proposed technology holds promise not only for coconut suspension cultures but also for application across a wide range of plant species where activated charcoal is essential during various stages of *in vitro* culture. The study provided important insights that can guide future research efforts to enhance the somatic embryogenesis protocol in coconut.

Keywords Callus · SEM · Turbidity · Nephelometry · Friable · *In vitro* culture

Introduction

Tissue culture of recalcitrant plants, particularly palms, poses significant challenges. One of the major impediments to successful coconut tissue culture is the interference caused by phenolic compounds, which are abundantly released from the explants as well as the growing cultures during the *in vitro* propagation (Ahmad *et al*. 2013; Aremu *et al*. 2013; Jones and Saxena 2013). These phenolics undergo rapid oxidation in the presence of air and polyphenol oxidases, leading to the formation of toxic quinones and browning of the culture medium. This oxidative stress not only inhibits cell division and morphogenesis but also results in cell death and tissue necrosis, thereby compromising the establishment and maintenance of viable cultures. The presence of polyphenols indicates a block in the biosynthesis pathway essential for embryogenesis in suspension culture. Effective management of phenolic exudation is therefore critical for improving the efficiency and reproducibility of coconut tissue culture protocols.

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A critical component in the media formulation for coconut tissue culture is activated charcoal (AC), which plays a vital role in influencing different growth stages, from callus induction to somatic embryogenesis and ultimately to plantlet in coconut as well as in other palms (Weatherhead *et al*. 1979; Pan and van Staden 1998; Fernando *et al*. 2007; Thomas 2008; Al-Khayri 2013; Nguyen *et al*. 2015; Adkins *et al*. 2016; Bandupriya *et al*. 2016; Weckx *et al*. 2019; Neema *et al*. 2024; Aparna *et al*. 2024). AC effectively absorbs the polyphenols secreted by the explants, thereby creating a polyphenol free growth medium that promotes healthier culture conditions (Dumas and Monteuuis 1995; Saenz *et al*. 2010; Sudheer *et al*. 2022; Permadi *et al*. 2024). Currently, the inclusion of AC is indispensable for the successful tissue culture of coconut. The unique properties of AC, with specific surface areas ranging from 600 to 2000 $m^2\ g^{-1}$ (Manocha 2003; Tsutomu *et al*. 2004; Mohammad-Khah and Ansari 2009), make it an essential additive in the media.

In cell suspension cultures, where the explants are grown in liquid medium, cultures undergo multiple developmental stages, including unsynchronized cell division and differentiation (Kato and Esaka 1999; Al-Khayri 2013; Banfalvi 2017; Zachleder *et al*. 2021). The presence of AC in such dynamic systems not only reduces oxidative stress but also supports cell proliferation. Accurate quantification and monitoring of these growth stages are essential, as a comprehensive understanding of cell behavior, division kinetics, and differentiation is fundamental for optimizing *in vitro* mass micropropagation strategies, particularly in recalcitrant species like coconut. To date, AC has been introduced into culture media as fine powder of particle size 38 to 100 µm. However, the introduction of AC into the liquid medium in the powder form presents challenges. Its presence obscures visibility and hinders the quantification of cell cultures (Fig. 1*A*, *B*). The AC can adhere to the cells, leading to inaccuracies in measuring the weight and volume of the cultures, thus complicating growth assessments. Hence, the possibility of an

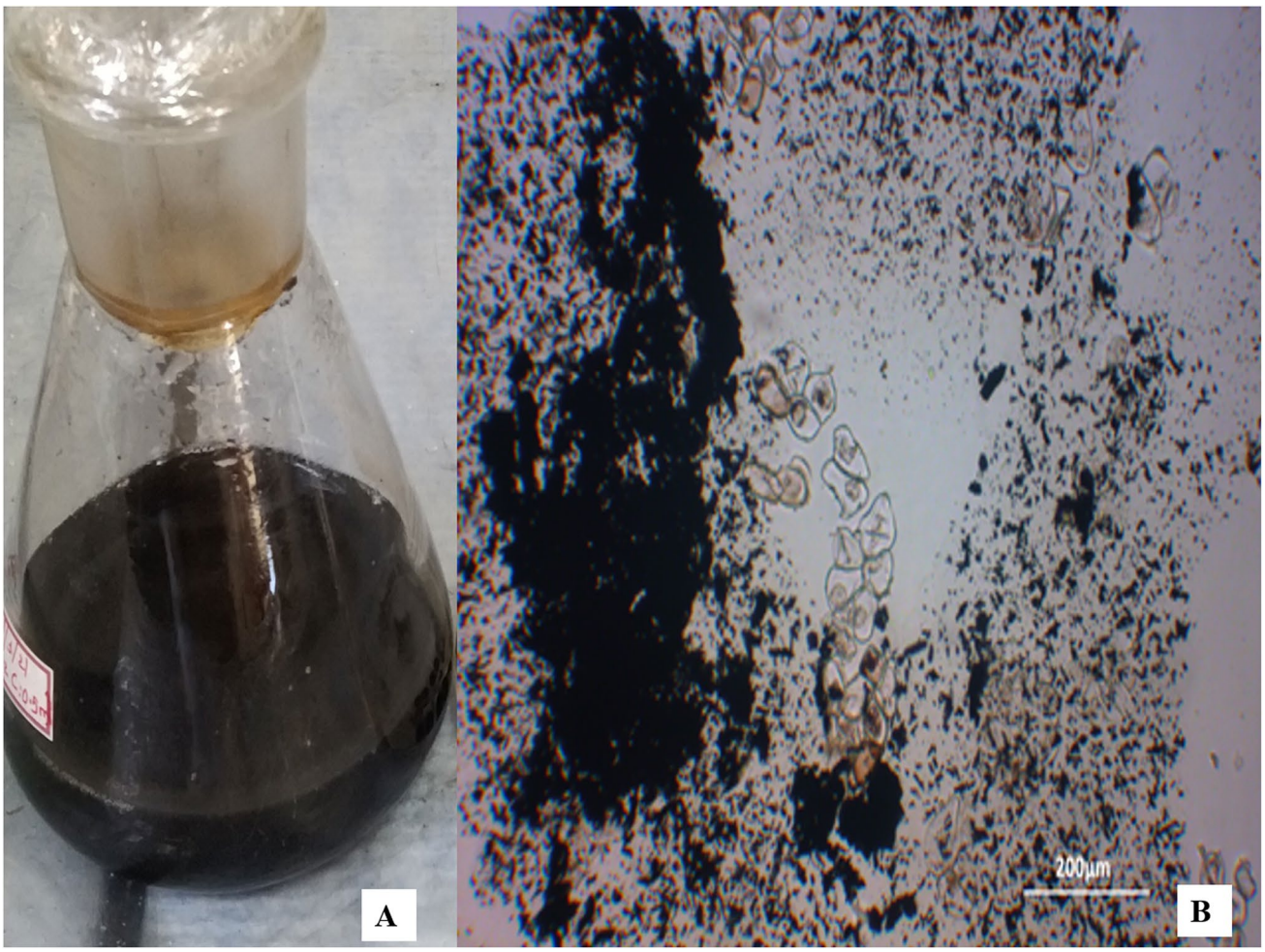

Figure 1. *Cocos nucifera* L. cell suspension culture with activated charcoal powder. Suspension culture in conical flask (*A*). Cells in suspension masked by activated charcoal (*B*).

alternative method to incorporate activated charcoal powder to suspension culture needs to be explored.

This paper discussed an innovative method to address this challenge, by incorporating activated charcoal powder in calcium alginate spherules (CAS). CAS are gel like spherical structures formed by the ionic cross-linking of sodium alginate (SA) with calcium ions. SA is a natural polysaccharide extracted from brown seaweeds, composed mainly of two monomeric units: mannuronic acid and guluronic acid. When a solution of SA is dropped into a calcium chloride ($CaCl_2$) solution, calcium ions displace the sodium ions and bind specifically to the guluronic acid blocks, forming a three-dimensional structure that results in gelation.

In plant tissue culture and biotechnology, the calcium alginate beads are commonly used for encapsulation and synthetic seed production. In the pharmaceutical and biomedical sectors, calcium alginate beads serve as controlled drug delivery systems. In agriculture, they are applied for the encapsulation of biofertilizers and biopesticides. When activated charcoal powder is mixed with SA solution and the mixture is dropped into $CaCl_2$ solution, charcoal embedded calcium alginate beads are formed through a process of ionic gelation. This forms a three-dimensional gel network that traps the charcoal particles within the matrix, producing activated charcoal–impregnated calcium alginate spherules (ACICAS). This study focused on establishing stable calcium alginate spherules, incorporating AC to these beads and testing the adsorption of polyphenols by these beads and finally exploring the effect of these beads in coconut suspension culture.

Materials and methods

The study was conducted at ICAR-Central Plantation Crops Research Institute, Kasaragod, Kerala, India, from September 2021 to April 2023. All chemicals used in this study were of analytical grade. All the plant growth regulators and gallic acid was obtained from Sigma-Aldrich Co. (St. Louis, MO). Folin-Ciocalteu's phenol reagent (FCR) and SA were purchased from Merck KGaA (Darmstadt, Germany).

Effect of SA and $CaCl_2$ concentrations in spherule formation, autoclavability, and shaker tolerance in CAS - Preparation of CAS Seventy combinations of CAS of two different sizes (S_1, 6 mm; S_2, 4 mm diameter), 1.0, 2.0, 3.0, 4.0, and 5.0% SA, and 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 M $CaCl_2$, were prepared (Table 1). To prepare the spherules, SA was dissolved in water and dispensed into a beaker containing $CaCl_2$ solution using a pipette, resulting in the formation of spherules. The mixture was then left undisturbed for 1 h. The size of the formed spherules was precisely adjusted by varying the length of the pipette tips used during the dispensing process.

Stability evaluation of CAS in spherule formation, autoclavability and shaker tolerance Out of the seventy combinations tested, not all resulted in the formation of solid CAS. Some combinations produced spherules that were delicate and easily breakable while others failed to form spherules altogether (Table 2). Only those CAS that formed solid, well-defined spherical shapes were selected for autoclavability (Table 2) and shaker tolerance tests (Table 3). To assess the stability of properly formed spherules under sterilization conditions, these were subjected to autoclaving at 121 °C and 15 psi (pounds per square inch) for 20 min. The spherules that remained intact after autoclaving were considered stable and were subsequently subjected to mechanical agitation to determine the shaker tolerance. For this, intact autoclaved spherules were placed in an orbital shaker (Thermo Scientific Solaris Orbital Shaker, Waltham, MA) set at 100 revolutions per min (RPM) for a duration of 15 d. After the shaking period, the spherules were removed from the solution, and the turbidity of the surrounding medium was measured using a nephelometer (Thermo Scientific Eutech TN-100 Waterproof Turbidimeter). The turbidity

Table 1. Treatment details indicating combinations of sodium alginate (%) and $CaCl_2$ concentrations (M) used for preparation of S_1 (6 mm) and S_2 (4 mm) calcium alginate spherules

Treatment code	Spherule size	Sodium alginate (%)	$CaCl_2$ concentration (M)
S_1T_1 - S_1T_7	S_1 (6 mm)	1	0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5
S_1T_8 - S_1T_{14}	S_1 (6 mm)	2	0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5
S_1T_{15} - S_1T_{21}	S_1 (6 mm)	3	0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5
S_1T_{22} - S_1T_{28}	S_1 (6 mm)	4	0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5
S_1T_{29} - S_1T_{35}	S_1 (6 mm)	5	0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5
S_2T_1 - S_2T_7	S_2 (4 mm)	1	0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5
S_2T_8 - S_2T_{14}	S_2 (4 mm)	2	0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5
S_2T_{15} - S_2T_{21}	S_2 (4 mm)	3	0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5
S_2T_{22} - S_2T_{28}	S_2 (4 mm)	4	0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5
S_2T_{29} - S_2T_{35}	S_2 (4 mm)	5	0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5

was expressed as nephelometric turbidity unit (NTU). The higher the particles in the solution, the greater the turbidity and corresponding NTU value. The spherules less tolerant to mechanical agitation break down, thereby increasing the turbidity of the media in which it was placed. The spherules with low NTU were considered more stable.

Testing activated charcoal–impregnated calcium alginate spherules (ACICAS) for polyphenol adsorption - Preparation of ACICAS The spherules exhibiting the lowest NTU were selected for further analysis. Spherules of two diameters were prepared, including S_1, 6 mm and S_2, 4 mm diameter (Fig. 2). AC concentrations of 0, 1.0, 1.5, and 2.0 g L^{-1} were systematically incorporated into the selected spherules. This was accomplished by thoroughly mixing the designated amounts of AC into the SA solution prior to its dispensing into the $CaCl_2$ solution using a pipette, facilitating the formation of CAS with varying charcoal content for subsequent analysis.

Polyphenol adsorption by ACICAS in gallic acid (GA) In this study, GA was selected as the standard polyphenol compound to evaluate the adsorption capabilities of ACICAS. The polyphenol adsorption efficiency was assessed by measuring the decrease in GA concentration in solution over time. A standard 500.0 ppm GA solution was prepared, and 10 mL of the solution containing 5000.0 µg GA was transferred into a conical flask. Twenty charcoal-impregnated spherules were added to GA solution, which was then left to equilibrate at room temperature for 24 h. Detailed specifications of the spherules used in this experiment are provided in Table 4. After the equilibration period, the GA solution was filtered to separate the spherules from the liquid. The quantity of GA in the filtrate was measured using Folin-Ciocalteu reagent as described by Singleton *et al.* (1999). Ten milliliters of GA solution without spherules was used as control. The absorbance of each prepared solution was measured using a UV-visible spectrophotometer (model UV-160A, Shimadzu Corporation, Kyoto, Japan).

Quantity of phenol adsorbed by spherules = GA (µg) control − GA (µg) sample.

Polyphenol adsorption by ACICAS in coconut cell suspension culture Plumules extracted from embryos of West Coast Tall variety of coconut were inoculated in Y3 medium containing 74.65 µM 2,4-dichlorophenoxyacetic acid (2,4-D), 4.54 µM thidiazuron, and 0.1% AC, solidified with 6.5 g L^{-1} agarose. Cultures were maintained in the dark at 27 °C for callus initiation. Depending on the response, either friable or compact calluses were produced. One gram of friable callus formed (Fig. 3) was transferred to a suspension culture medium comprising Y3 medium supplemented with 4.09 µM biotin, 555.19 µM of myoinositol and 684.0 µM L-glutamine, 100.0 mg L^{-1} malt extract, 30.0 g L^{-1} sucrose, and 4.14 µM picloram. ACICAS prepared with concentrations of 0, 1.0, 1.5, and 2.0 g L^{-1} of AC were also incorporated into the media. After incorporating ACICAS, the polyphenol content was measured on the 15th day to assess the polyphenol adsorption capacity of ACICAS in coconut suspension culture. The cultures were placed in an orbital shaker at 100 RPM, 27 °C, in the dark, where friable callus tissues dissociated into small granular clumps. Ten milliliters of coconut suspension culture was centrifuged at 10,000 RPM for 5 min. The supernatant was collected, and the total phenol content in the supernatant was measured using the method discussed above. The quantity of polyphenols adsorbed by ACICAS was determined by subtracting

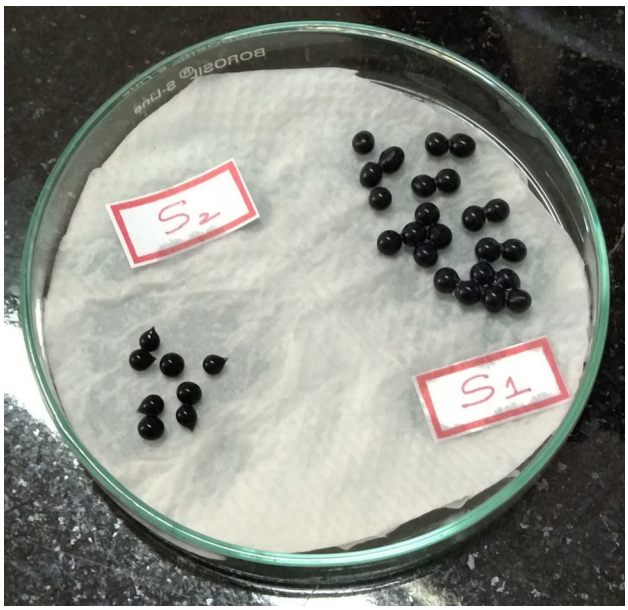

Figure 2. Charcoal-impregnated calcium alginate spherules: S_1, spherules with 6 mm diameter; S_2, spherules with 4 mm diameter.

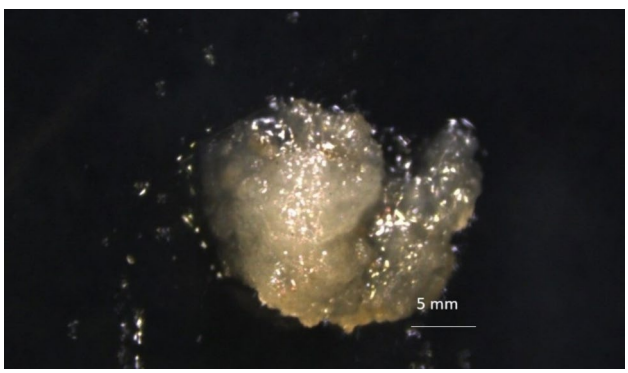

Figure 3. Friable callus obtained from *Cocos nucifera* L. plumular explant.

the polyphenol content remaining in this sample from the total phenol content in the original suspension culture. The experiment was replicated thrice.

Comparing Polyphenol adsorption of ACICAS with that of activated charcoal powder (ACP) The effect of ACICAS in cell suspension, with respect to ACP, was tested with cell viability as the criterion. One gram of friable callus was inoculated into cell suspension culture medium and incubated on a rotary shaker at 100 RPM for 15 d to facilitate callus dissociation. From the resulting suspension, 10-mL aliquots were transferred into nine separate conical flasks, each containing 15 mL of fresh suspension culture medium. Three flasks were supplemented with 2.0 g L^{-1} ACICAS. Another three flasks received ACP at 2.0 g L^{-1}, while the remaining three flasks served as controls without any charcoal supplementation. A viability test was carried out after 60 d of inoculation using trypan blue staining. Ten microliters of 0.4% trypan blue stain was added to 10.0 µL of cell suspension. The stain and cell suspension were mixed using a pipette. The stained suspension was placed on the glass slide and viewed under a microscope.

Scanning electron micrograph (SEM) studies To obtain SEM, charcoal spherules were affixed to aluminum stubs using double-sided transparent tape and coated with a 0.02-mm layer of gold using an EMITECH SC7620 sputter coater (Quorum Technologies Ltd., Lewes, UK). The samples were then examined at an accelerating voltage of 15 kV with an FEI-Quanta 250 SEM (FEI, Tokyo, Japan) (Tisserat and DeMason 1982). Micrographs of 70×, 500×, and 1000× magnifications were obtained. Two types of samples were used for the study: sample 1 included activated charcoal–impregnated calcium alginate spherules, and sample 2 included activated charcoal–impregnated calcium alginate spherules immersed in gallic acid for an hour.

Statistical analysis The experiment for determining stability of CAS was conducted in completely randomized design (CRD) with 51 treatment combinations (wherever spherules were properly formed) out of 70 combinations of different levels of SA and $CaCl_2$, each with three replications. The experiment to determine the polyphenol adsorption capacity of ACICAS was also conducted in CRD with 12 treatment combinations (Table 4) comprising four concentrations of charcoal with three different types of spherules, each replicated three times. Studies on cell viability were set up in CRD with three treatments and three replications. Data were subjected to one-way analysis of variance (ANOVA) using SAS software (Ver.9.3.), and the treatment means were compared using Tukey's HSD test at 5% level of significance ($p < 0.05$). Values are presented as mean ± standard deviation (SD), and error bars in figures represent SD based on three replicates.

Results and discussion

Effect of SA and $CaCl_2$ concentrations in spherule formation, autoclavability, and turbidity in CAS Ionic interactions occur when SA comes into contact with $CaCl_2$, leading to the formation of calcium alginate spherules. This gelation process results in the immediate formation of stable spherules, which were subjected to autoclaving to ensure that the spherules maintain their structural integrity and functionality, confirming their suitability for use in subsequent experiments. Subjecting the spherules to orbital shaking simulated the conditions typically encountered in suspension cultures and assessed the ability of the spherules to maintain integrity and functionality under continuous agitation. The nephelometric observations provided an indication of the particle suspension and helped determine the effectiveness of the spherules in maintaining a clear growth medium, which was crucial for assessing cell culture growth and development.

The formation and stability of spherules depended upon the concentration of SA and $CaCl_2$ used (Table 2). In the case of S_1 spherules with a larger diameter, at very lower concentration of sodium alginate (1.0%), spherules were not formed in any concentration of $CaCl_2$. Also, at 0.025 M $CaCl_2$, no spherules were formed at any given concentration of sodium alginate. But, in the case of S_2 spherules, with a smaller diameter, spherules were formed even at 1.0% sodium alginate when the $CaCl_2$ concentration was equal to or greater than 0.3 M. But no spherules were formed when $CaCl_2$ concentration was 0.025 M. Thus, it can be seen that

Table 2. Effect of sodium alginate and $CaCl_2$ concentrations on spherule formation and stability

Spherule type	SA (%)	$CaCl_2$ (M)	Spherule formation	Stability during autoclaving
S1 (larger)	1.0%	0.025 to 0.5	Not formed	—
S1 (larger)	2 to 3%	0.025 to 0.05	Formed	Not stable
S1 (larger)	2 to 5%	0.05 to 0.5	Formed	High stability at > 0.3 M $CaCl_2$
S2 (smaller)	1.0%	≥ 0.3	Formed	Moderate
S2 (smaller)	1.0%	0.025	Not formed	—
S2 (smaller)	2 to 5%	0.05 to 0.5	Formed	High stability at > 0.3 M $CaCl_2$

treatments with 2.0% sodium alginate and above with $CaCl_2$ concentrations from 0.2 to 0.5 M generally yielded successful spherule formation. Treatments with sodium alginate concentrations of 2.0 to 5.0% and high $CaCl_2$ concentrations (above 0.3 M) demonstrated high autoclavability, indicating that these concentrations enhanced stability. The results on the formation of CAS are summarized in Table 2. Out of the 70 treatments, only 51 resulted in the formation of spherules and were stable at autoclaved condition. The effect of SA and $CaCl_2$ concentrations and spherule size on turbidity is shown as Fig. 4. Treatments with sodium alginate concentrations of 2.0 to 5.0% and $CaCl_2$ concentrations of 0.3 to 0.5 M produced stable beads that could withstand autoclavability and shaker tolerance. The treatments were ranked based on their stability as indicated by their NTU values (Table 3). The NTU value represents the turbidity of the solution. Lower NTU values indicate lower turbidity, suggesting less particle dissociation from the beads under stress, and therefore, these beads are more stable. More stable spherules were assigned higher ranks. Of these 51 combinations of spherules prepared, three types (namely, S_1T_{20}, 3% SA, 0.4 M $CaCl_2$; S_2T_{13}, 2% SA, 0.4 M $CaCl_2$; and S_2T_{28}, 4% SA, 0.5 M $CaCl_2$) exhibited the lowest NTU (Table 3) and hence were selected for further analysis.

Successful spherule formation depended on adequate sodium alginate and $CaCl_2$ concentrations. Studies indicated that sodium alginate concentrations above 2% are optimal for creating stable, uniform spherules due to sufficient cross-linking. Bennacef *et al*. (2023) discussed how increased $CaCl_2$ concentration improves spherule stability but may also lead to brittleness beyond certain levels due to excessive cross-linking. CAS were formed by cross-linking sodium alginate with calcium ions, resulting in a stable hydrogel that has broad applications in drug delivery and plant cell encapsulation due to its biocompatibility and structural integrity under diverse conditions. Recent studies have shown that spherule stability can be enhanced by factors like polymer concentration, reaction time, and environmental conditions,

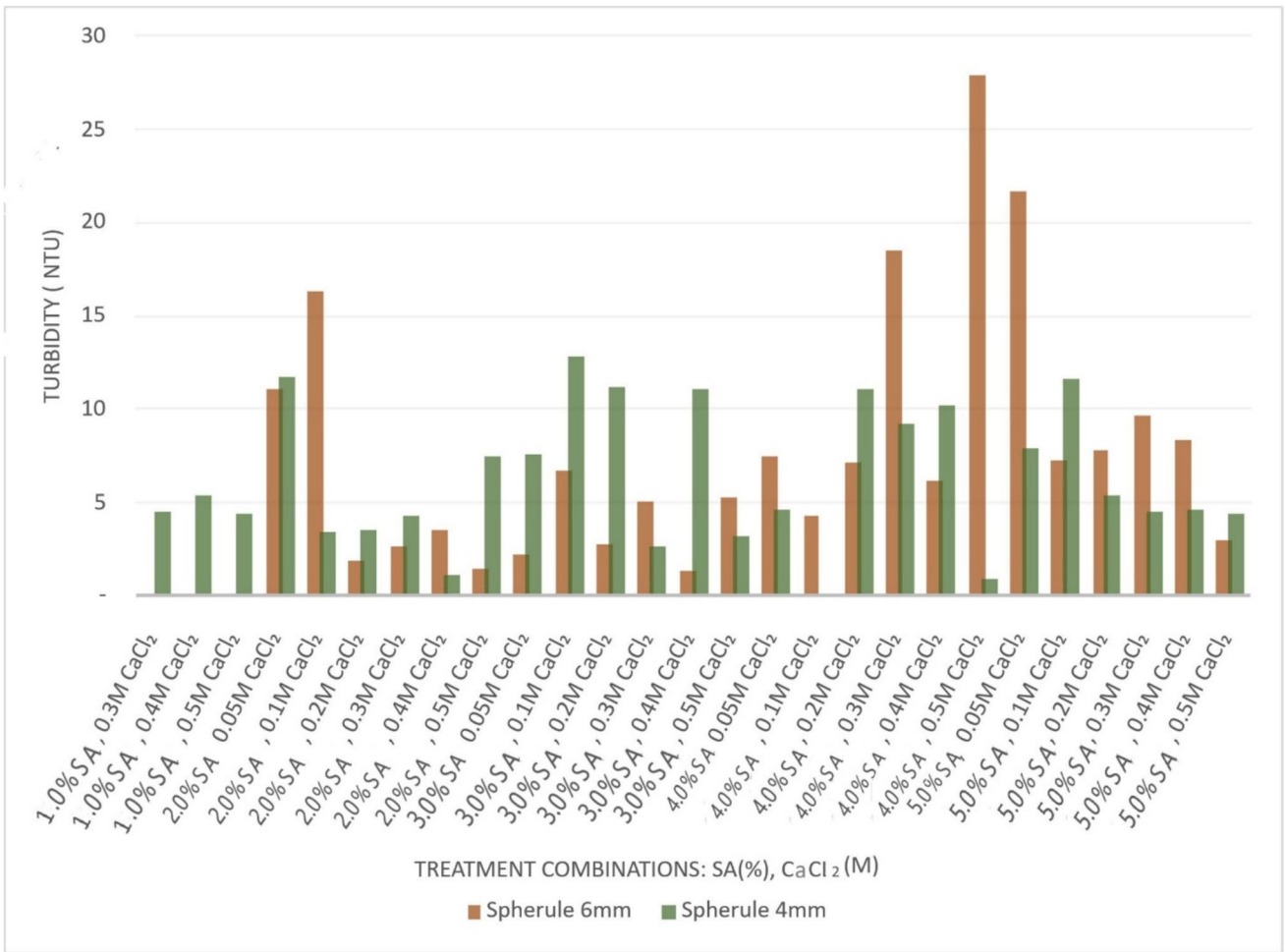

Figure 4. Effect of sodium alginate, $CaCl_2$ concentrations, as well as bead size on spherule stability. Each group on the *x-axis* represents combination of sodium alginate and $CaCl_2$ concentrations, and the *bars* compare the turbidity values for S_1 (6 mm spherules) and S_2 (4 mm spherules).

Table 3. Comparative turbidity of different treatments

Treatment code	Nephelometric turbidity unit	Rank of treatment
S_1T_{10}	16.37^{D}	4
S_1T_{11}	$1.90^{YZ(2)A}$	47
S_1T_{12}	2.61^{WXY}	45
S_1T_{13}	3.49^{TUVW}	39
S_1T_{14}	$1.43^{Z(2)AB}$	48
S_1T_{16}	2.22^{XYZ}	46
S_1T_{17}	6.67^{MN}	23
S_1T_{18}	2.81^{VWXY}	43
S_1T_{19}	5.11^{PQRS}	29
S_1T_{20}	$1.40^{Z(2)AB}$	49
S_1T_{21}	5.30^{OPQR}	28
S_1T_{23}	7.52^{KLM}	19
S_1T_{24}	4.34^{STU}	36
S_1T_{25}	7.18^{LM}	22
S_1T_{26}	18.50^{C}	3
S_1T_{27}	6.16^{NO}	24
S_1T_{28}	27.87^{A}	1
S_1T_{30}	21.67^{B}	2
S_1T_{31}	7.30^{LM}	21
S_1T_{32}	7.75^{KL}	17
S_1T_{33}	9.63^{HI}	13
S_1T_{34}	8.30^{JK}	15
S_1T_{35}	3.00^{VWX}	42
S_1T_9	11.07^{FG}	11
S_2T_{10}	3.43^{UVW}	40
S_2T_{11}	3.57^{TUV}	38
S_2T_{12}	4.33^{STU}	37
S_2T_{13}	$1.13^{(2)AB}$	50
S_2T_{14}	7.43^{KLM}	20
S_2T_{16}	7.60^{KL}	18
S_2T_{17}	12.8^{5E}	5
S_2T_{18}	11.23^{F}	8
S_2T_{19}	2.67^{VWXY}	44
S_2T_{20}	11.09^{FG}	10
S_2T_{21}	3.23^{VW}	41
S_2T_{23}	4.63^{PQRS}	30
S_2T_{25}	11.12^{FG}	9
S_2T_{26}	9.20^{IJ}	14
S_2T_{27}	10.22^{GH}	12
S_2T_{28}	$0.89^{(2)B}$	51
S_2T_{30}	7.93^{KL}	16
S_2T_{31}	11.63^{F}	7
S_2T_{32}	5.39^{OPQ}	26
S_2T_{33}	4.50^{QRS}	32
S_2T_{34}	4.60^{PQRS}	31
S_2T_{35}	4.40^{RST}	34
S_2T_5	4.50^{QRS}	33
S_2T_6	5.44^{OP}	25
S_2T_7	4.37^{ST}	35
S_2T_9	11.74^{F}	6
General mean	7.14	
p-value		
CV(%)	3.83	
SE(d)	0.223	
Tukey HSD at 5%	0.9234	

The same *superscript* refers to non-significant difference between treatments; higher rank indicates lower turbidity

allowing improved encapsulation efficiency and extended-release times, which might be beneficial for maintaining the structural stability of spherules in phenolic environments (Semanate 2024). Higher $CaCl_2$ concentrations enhanced spherule resistance to autoclave stress. Murata *et al*. (2004) found that spherules prepared with 0.1 M $CaCl_2$ withstood temperature and pressure better due to stronger ionic bonding, ensuring structural stability during sterilization. Turbidity values indicated spherule stability over time. Research by Lee and Mooney (2012) suggested that combining higher alginate and moderate $CaCl_2$ concentrations reduces spherule leakage, resulting in lower turbidity. Optimal concentrations ranged from 2 to 3% sodium alginate with $CaCl_2$ at 0.2 to 0.5 M for stability and low turbidity over time. Calcium alginate spherules with 2 to 4% alginate and moderate to high $CaCl_2$ concentrations maintained low turbidity and structural integrity, making them ideal for long-term culture stability (Draget *et al*. 1997). This matched findings in the present study, where 2% alginate concentrations and above showed low turbidity results in treatments, such as S_1T_{20}, S_2T_{13}, and S_2T_{28}.

Testing ACICAS for polyphenol adsorption - Polyphenol adsorption by ACICAS in gallic acid The adsorption of polyphenols by spherules of two different diameters (S_1, 6 mm and S_2, 4 mm) at various AC concentration is shown in Table 4. Spherules of treatments T_1, T_2, and T_3 did not adsorb any polyphenol from the medium, likely indicating that the phenol binding capacities of the spherules were due to the AC embedded in them. As AC concentration increased, polyphenol adsorption increased significantly, indicating a positive relationship between AC concentration and adsorption (Table 4). This effect aligned with recent literature indicating that AC's porous structure provides ample sites for adsorption, particularly for phenolic compounds, due to the high surface area and favorable interaction with hydrophobic molecules (Jiao *et al*. 2024). Studies by Stavropoulos *et al*. (2008) demonstrated similar trends, where increased AC dosage in adsorbent matrices corresponded with greater removal of phenolic pollutants from aqueous

Table 4. Phenol adsorption by activated charcoal–impregnated spherules

Treatment code	Spherules	AC concentration ($g\ L^{-1}$)	Phenol adsorption (µg)
T_1	S_1T_{20}	0	0.000 (0.707[i])
T_2	S_2T_{13}	0	0.000 (0.707[i])
T_3	S_2T_{28}	0	0.000 (0.707[i])
T_4	S_1T_{20}	1	127.820 (11.326[f])
T_5	S_2T_{13}	1	116.953 (10.837[g])
T_6	S_2T_{28}	1	107.693 (10.402[h])
T_7	S_1T_{20}	1.5	206.187 (14.376[c])
T_8	S_2T_{13}	1.5	159.993 (12.667[d])
T_9	S_2T_{28}	1.5	144.780 (12.051[e])
T_{10}	S_1T_{20}	2	278.340 (16.698)
T_{11}	S_2T_{13}	2	253.423 (15.934[a])
T_{12}	S_2T_{28}	2	235.700 (15.368[b])
C.D.	7.349		
SE (m)	2.503		
SE (d)	3.540		
C.V.	3.190		

solutions due to enhanced adsorption surface area and pore volume.

S_1 spherules with larger diameter consistently showed higher polyphenol adsorption than S_2 spherules (smaller diameter) at all AC levels, suggesting that polyphenol adsorption increases with the surface area of the spherules. This phenomenon was supported by the study of Roostaei and Tezel (2004), which highlighted the importance of particle size in adsorption efficiency, noting that larger particles often presented more effective adsorption surfaces due to their greater contact area and increased access to internal pores.

At 1.5% AC (T_7 to T_9), polyphenol adsorption significantly increased, with S_1 (6 mm) spherules (T_7) adsorbing more polyphenols than S_2 spherules (T_8 and T_9). At the maximum 2% AC concentration (T_{10} to T_{12}), both spherules reached peak adsorption, with the highest adsorption in T_{11} (S_2T_{13}) among S_2 samples; but T_{10} (S_1T_{20}) had the highest overall value. This supported findings by Bazrafshan *et al.* (2016), where higher adsorbent dosages in structured media led to saturation thresholds characterized by peak pollutant removal efficiencies.

The study showed that larger S_1 spherules incorporated with 2.0 $g\ l^{-1}$ of activated charcoal (S_1T_{20}) exhibited higher polyphenol adsorption (278.340 µg) compared to those with lower charcoal concentrations. This suggested that larger spherules with higher AC concentrations possess greater adsorption capacity. The enhanced performance may be attributed to the increased surface area associated with larger bead size, which allowed for more AC particles to come into closer contact with polyphenols, thereby facilitating more efficient adsorption.

In treatments where spherules lacked AC, no phenol adsorption was observed. This can be attributed to the inert nature of the calcium alginate matrix, which does not possess significant affinity or active binding sites for phenolic compounds. AC, by contrast, provided a large surface area, high porosity, and abundant functional groups (for example hydroxyl and carboxyl) capable of interacting with and adsorbing phenolic molecules through mechanisms, such as hydrogen bonding, van der Waals forces, and π-π interactions. Thus, in the absence of AC, the spherules were unable to retain or adsorb polyphenols from the medium, highlighting the critical role of AC in phenol removal.

Among the smaller sized spherules (S_2, 4 mm diameter), both S_2T_{13} and S_2T_{28} exhibited a positive correlation between AC concentration and polyphenol adsorption, indicating that adsorption was influenced by the amount of charcoal embedded in the matrix. However, S_2T_{13} consistently showed higher adsorption capacity than S_2T_{28} at comparable charcoal concentrations. This difference may be attributed to the composition of the spherules, particularly the lower sodium alginate (2%) and $CaCl_2$ concentration (0.4 M) in S_2T_{13} compared to the higher polymeric density in S_2T_{28} (4% and 0.5 M, respectively). The denser gel matrix in S_2T_{28} likely resulted in reduced porosity and diffusivity, which can hinder the movement of polyphenols into the interior of the spherule and limit the accessibility of charcoal adsorption sites. In contrast, the less compact structure of S_2T_{13} may have allowed better diffusion of polyphenols and greater exposure of charcoal surfaces, leading to enhanced adsorption efficiency despite the same spherule size.

Polyphenol adsorption by ACICAS in coconut cell suspension culture Spherules from the S_1T_{20} treatment were found to adsorb the maximum quantity of polyphenol in the previous experiment. Hence, these spherules were incorporated into the coconut suspension culture. The polyphenols present in coconut suspension ranged from 6.2 to 8.1 µg g^{-1} dry weight while the polyphenol adsorbed by the charcoal beads ranged from 2.5 to 5.5 µg g^{-1} dry weight. This range suggested that the spherules were effective in removing up to 68 to 80% of phenolic compounds, a result consistent

with previous research indicating that AC can effectively reduce phenolic concentrations in plant suspension cultures (Olah 2017). Figure 5 illustrates the microscopic observations during the transition from coconut friable callus to suspension culture. The friable callus (Fig. 5*A*) exhibited a loose and granular texture, suitable for suspension initiation. Upon inoculation into liquid medium supplemented with activated charcoal–impregnated calcium alginate spherules (ACICAS), proliferation of dispersed cells was observed at 10× magnification (Fig. 5*B*), which became more distinct at 25× magnification (Fig. 5*C*). As the culture progressed, formation of true cell suspensions was evident (Fig. 5*D*), followed by the development of aggregated cell clumps (Fig. 5*E*). The morphology of the suspension culture at 40× magnification showed actively dividing, viable cells (Fig. 5*F*). AC's efficacy as an adsorbent was attributed to its large surface area and high porosity, which enhanced its capacity to bind phenolic molecules (Dąbrowski *et al*. 2005; Thomas 2008). Several studies have reported the use of AC in suspension cultures. Abohatem *et al*. (2025) observed an eight-fold increase in biomass production in date palm suspension culture when AC was combined with glutamine. Likewise, Teng (1997) reported that the incorporation of AC in leaf cell suspension culture of *Platycerium bifurcatum* markedly increased the number of regenerated sporophytes, even in growth regulator–free medium. Moreover, immobilization of AC within calcium alginate beads (spherules) further improved the process by preventing dispersal of charcoal particles, thereby ensuring a stable and controlled adsorption environment (Kajani *et al*. 2010).

Comparing Polyphenol adsorption of ACICAS with that of ACP The impact of ACICAS on cell viability in suspension cultures was evaluated in comparison to ACP. The viability of cells was evaluated 60 d after inoculation of the friable callus into suspension medium supplemented with activated charcoal in two different forms. In one treatment, 2.0 g L^{-1} activated charcoal was incorporated in the form of ACICAS, while in another, the same concentration of activated charcoal was added as powder. A third treatment, where the medium lacked activated charcoal, served as the control. The viability of the cultures was determined using trypan blue stain. Trypan blue does not stain live cells but stains dead cells blue (Strober 2015). Results showed that cell viability remained high in both the charcoal spherule (Fig. 6*A*) and charcoal powder treatments (Fig. 6*B*), but low in treatment devoid of charcoal (Fig. 6*C*).

Statistical analysis using ANOVA followed by Tukey's HSD test ($p < 0.05$) indicated no significant difference in cell viability between the two treatments, suggesting that the physical form of activated charcoal did not significantly affect cell survival (Fig. 7). However, both treatments with activated charcoal showed improved viability compared to

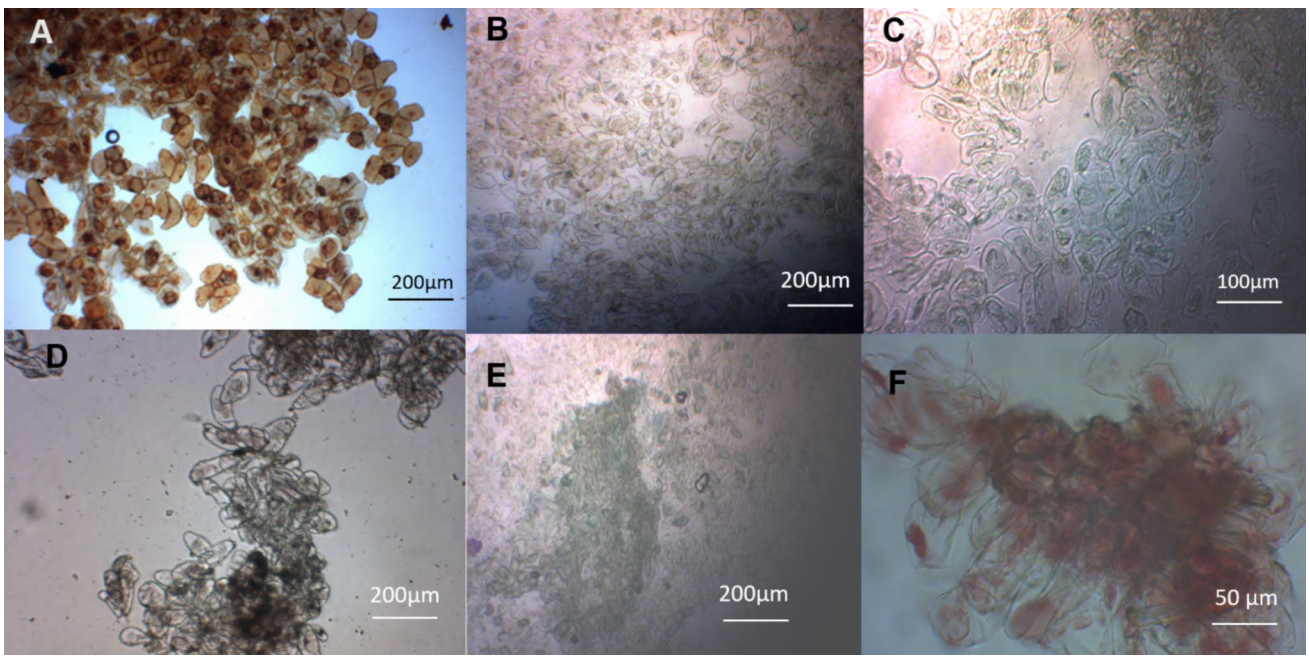

Figure 5. Microscopic view of *Cocos nucifera* L. friable callus and subsequent formation of cell suspension. Friable callus (10×; *scale bar* = 200 µm) (*A*); proliferation of cells in suspension medium supplemented with activated charcoal–incorporated calcium alginate spherules (10×; *scale bar* = 200 µm) (*B*); proliferating cells (25×; *scale bar* = 100 µm) (*C*); formation of suspensions (10×; *scale bar* = 200 µm) (*D*); formation of suspension clumps (10×; *scale bar* = 200 µm) (*E*); *Cocos nucifera* L. suspension (40×; *scale bar* = 50 µm) (*F*).

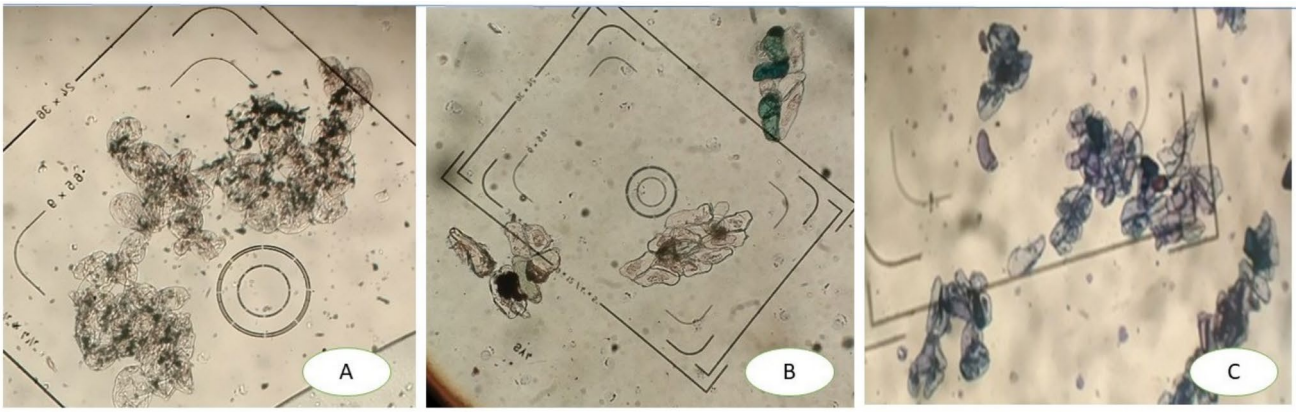

Figure 6. Trypan blue viability test for *Cocos nucifera* L. suspension culture in medium supplemented with activated charcoal powder (*A*), activated charcoal–incorporated calcium alginate spherules (*B*), charcoal-free media (*C*).

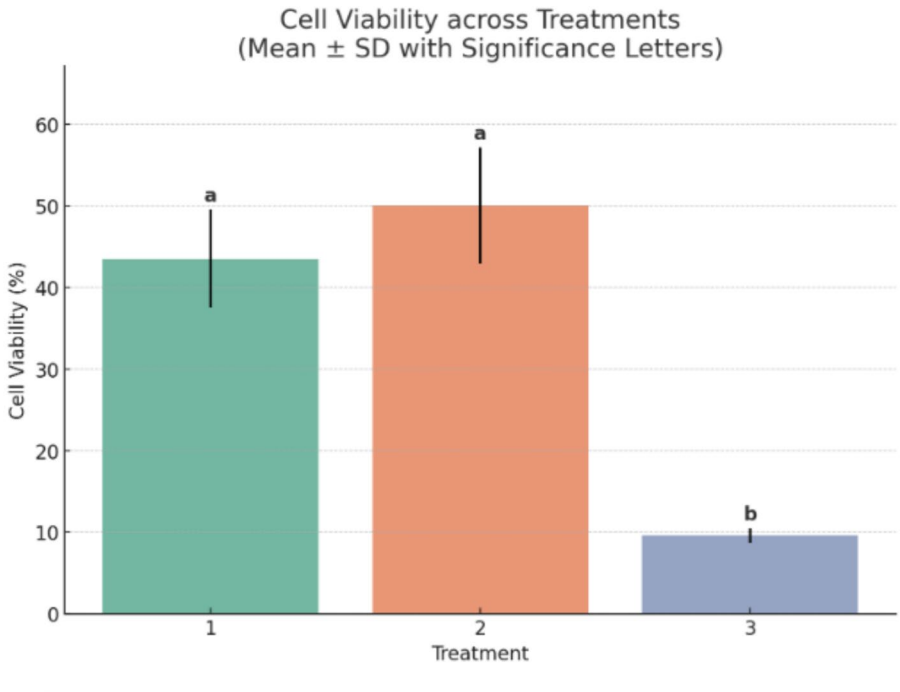

Figure 7. Viability of the cells after inoculating in medium with activated charcoal–incorporated calcium alginate spherules (ACICAS), activated charcoal powder (ACP), and charcoal-free media. Treatments 1 and 2, S_1T_{20} treatment with 2.0 g L^{-1} activated charcoal (AC) incorporated as ACICAS and as ACP; T3, S_1T_{20} treatment without AC. There is no significant difference between treatments 1 and 2.

the control, devoid of activated charcoal. Similar results were obtained by Abohatem *et al*. (2025) in date palm and Teng (1997) in *Platycerium bifurcatum.*

Proliferation of cells in suspension culture was observed predominantly in media containing AC. This stimulatory effect can be attributed to the adsorptive properties of AC, which helps remove inhibitory compounds, such as oxidized polyphenols, toxic metabolites, and excess plant growth regulators, from the culture medium (Thomas 2008). Phenolic exudation, commonly released from wounded plant tissues, can rapidly oxidize and generate reactive quinones, which are known to be cytotoxic and inhibitory to cell growth (Xu and Wang 2025). AC selectively adsorbed these phenolic compounds, thereby reducing oxidative stress and creating a more favorable environment for cell division and metabolic activity. The current study demonstrated that both ACICAS and powdered forms of activated charcoal, when applied at 2.0 g L^{-1}, were equally effective in maintaining high cell viability in coconut cell suspension cultures. The absence of a significant difference between the two treatments indicated that the physical form of activated charcoal does not markedly influence its efficacy in promoting cell survival, at least within the concentration and duration tested. Activated charcoal has long been recognized for its adsorptive capacity,

particularly in neutralizing phenolic compounds, toxic exudates, and residual plant growth regulators in plant tissue culture media (Thomas 2008). Phenolic oxidation, common in plant cell cultures, can severely reduce cell viability. By adsorbing such inhibitory substances, activated charcoal created a more favorable environment for cell proliferation (Fridborg *et al*. 1978).

While powdered activated charcoal has a high surface area, making it efficient in adsorption, it can also lead to media darkening, complicating observations and downstream processing. The comparable viability between spherule-based and powdered charcoal treatments suggested that ACICAS was a viable alternative to powdered charcoal, particularly when clarity or control over medium composition is needed (Fig. 8*A*, *B*, and *C*). The control treatment, lacking any form of charcoal, showed relatively lower viability, reinforcing the protective role of activated charcoal in plant suspension cultures. This aligned with earlier reports where the omission of activated charcoal led to browning, reduced cell division, and lower biomass production (Thomas 2008).

In summary, both forms of activated charcoal significantly enhanced cell viability compared to the control. Although no significant differences were observed between the bead and powder forms under these specific conditions, the operational benefits of charcoal beads made them promising for routine application in suspension culture systems.

SEM studies Micrographs of charcoal-impregnated calcium alginate spherules were examined before (Fig. 9*A*, *C*, *E*, *G*, and *I*) and after immersion in gallic acid (Fig. 9*B*, *D*, *F*, *H*, and *J*) at magnifications of 70× to 5000×. At 70×, untreated spherules displayed a rugged, porous surface with exposed charcoal particles, while treated spherules appeared comparatively smoother (Fig. 9*A*, *B*). This roughness was attributed

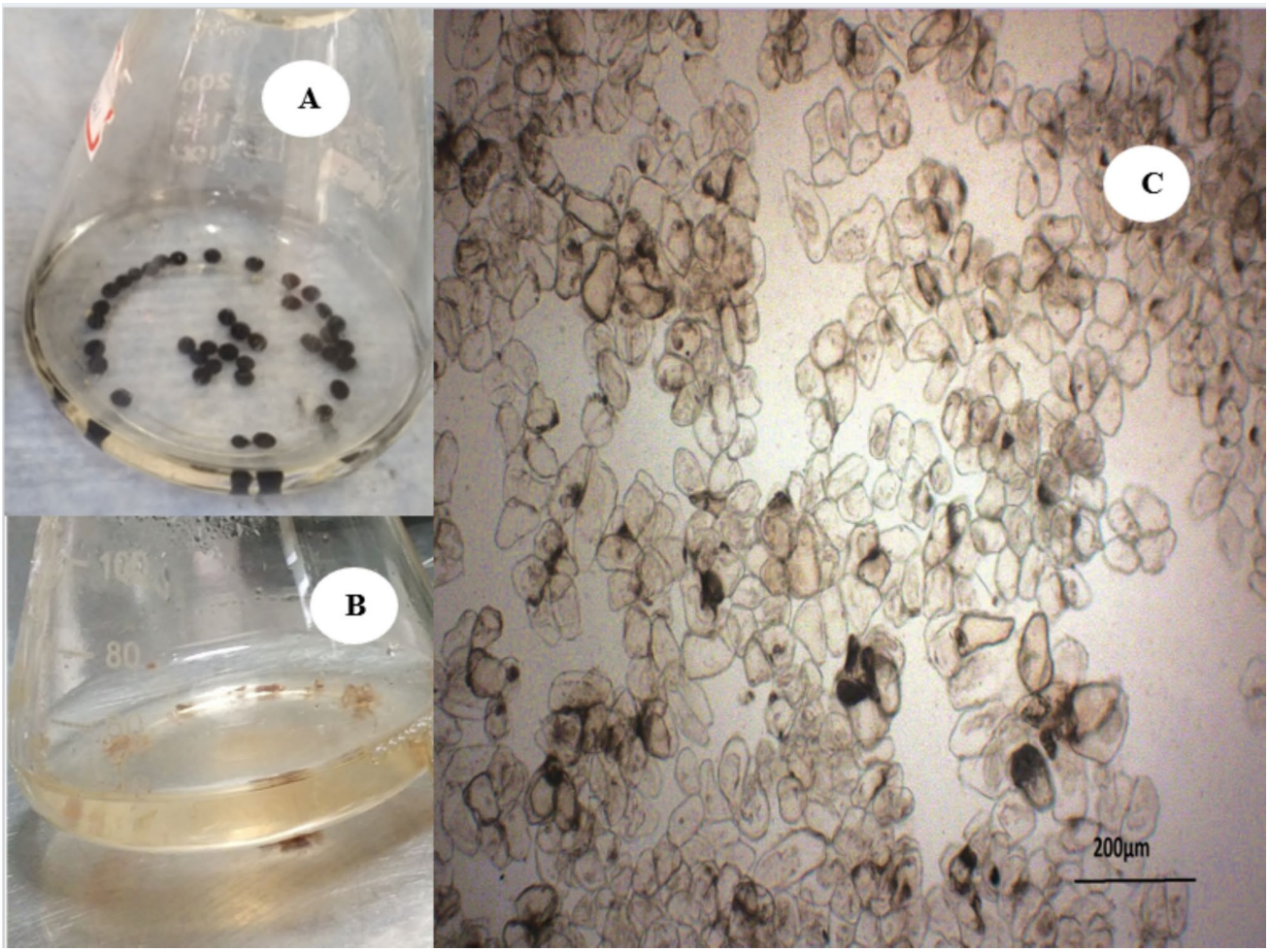

Figure 8. Suspension culture in *Cocos nucifera* L. with reduced phenolic interference achieved by the addition of activated charcoal–incorporated calcium alginate spherules (ACICAS). Suspension culture in conical flask with ACICAS (*A*), clear suspension after removing spherules (*B*), enhanced visibility in coconut suspension cells due to addition of ACICAS (*C*; 10× magnification, *scale* 200 µm).

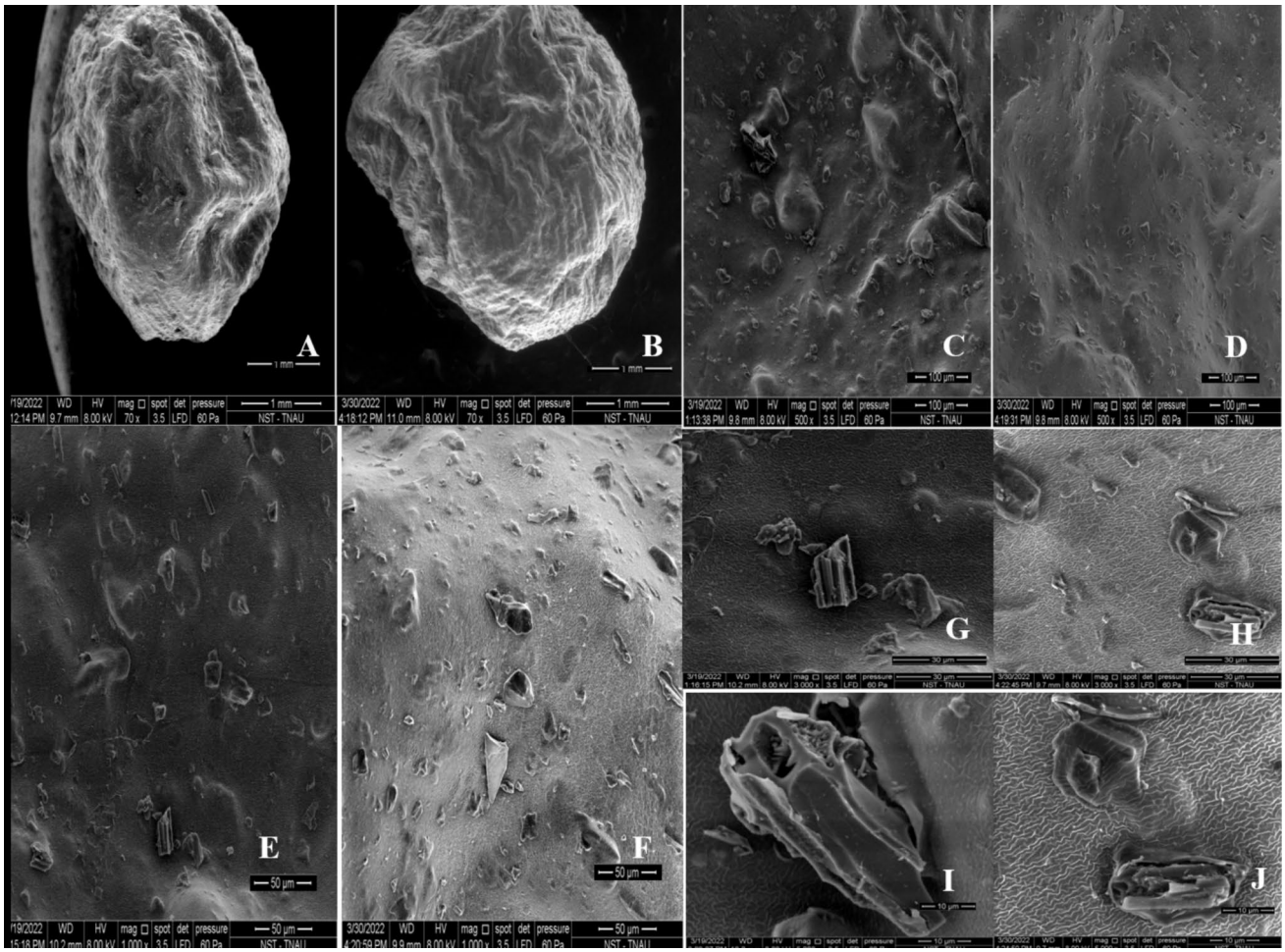

Figure 9. SEM images of activated charcoal–incorporated calcium alginate spherules (ACICAS) with and without gallic acid (GA) treatment. Untreated ACICAS: (*A*), (*C*), (*E*), (*G*), (*I*); ACICAS treated with gallic acid: (*B*), (*D*), (*F*), (*H*), (*J*). Images were captured at magnifications of 70× (*A*, *B*; *scale bar* = 1000 µm), 500× (*C*, *D*; *scale bar* = 100 µm), 1000× (*E*, *F*; *scale bar* = 50 µm), 3000× (*G*, *H*; *scale bar* = 30 µm), and 5000× (*I*, *J*; *scale bar* = 10 µm).

to the porous nature of activated charcoal (AC), which increased surface area and facilitated adsorption of organic compounds, including polyphenols (Leng *et al*. 2021).

At 500×, untreated spherules revealed a flaky, layered microstructure, whereas treated spherules showed a more compact and regular surface (Fig. 9*C*, *D*). The irregular structure of AC, known to promote adsorption, was consistent with observations by Ryu *et al*. (2002), who reported enhanced adsorption capacity due to AC's microstructural complexity.

At 1000× and 3000× magnification, the untreated spherules exhibited well-distributed, porous charcoal embedded within the alginate matrix (Fig. 9*E*, *G*). Such a structure provided multiple adsorption sites and a synergistic effect between calcium alginate and AC, improving polyphenol capture (Nadavala *et al*. 2009). After gallic acid immersion, these features became less distinct, with a smoother surface observed (Fig. 9*F*, *H*), suggesting pore filling or surface coating by adsorbed phenols. This morphological change supported findings by Mashoene *et al*. (2023), who reported reduced roughness due to polyphenol induced pore blockage.

At 5000×, untreated spherules (Fig. 9*I*) showed rough charcoal surfaces within the alginate, while treated spherules (Fig. 9*J*) exhibited smoother textures and reduced pore visibility, indicating successful adsorption of gallic acid. Similar trends have been described by González-García *et al*. (2013), who noted surface texture changes following phenolic adsorption on AC. The smoother appearance confirmed the interaction between phenolic molecules and the adsorbent surface, as polyphenols fill micropores and active sites, forming a uniform layer (Mojoudi *et al*. 2019). Overall, these microstructural changes validated the adsorption potential of AC-alginate spherules, demonstrating the role of surface texture and porosity in enhancing polyphenol sequestration in suspension cultures.

While the current study demonstrated the efficacy of AC in mitigating phenolic interference in suspension cultures, it is important to recognize that AC also exhibits a non-specific adsorptive nature, removing not only polyphenolic compounds but also plant growth regulators, vitamins, and essential nutrients from the culture medium. This dual role presented both challenges and opportunities. Future research would be focused on modulating the release and retention kinetics of bioactive compounds in AC-embedded systems, possibly through co-encapsulation strategies or controlled release formulations. Additionally, optimizing the composition and porosity of the alginate matrix may help balance the beneficial removal of phenolics with the retention of critical growth-promoting substances, thereby fine tuning the microenvironment for enhanced cell proliferation and metabolite production across various plant species. Furthermore, molecular level investigations into the pathways of phenolic attenuation and cellular stress responses could provide mechanistic insights, supporting the rational design of AC-based delivery systems.

Conclusion

The results indicated that larger spherules with higher charcoal content adsorbed more polyphenols when compared with smaller spherules with less charcoal while the spherules without charcoal did not adsorb polyphenols. The best treatment S_1T_{20} possessed a turbidity value of 1.40 NTU and adsorbed about 80% of the polyphenols present in coconut cell suspension culture. SEM analysis underscored the potential of charcoal-impregnated calcium alginate spherules as effective adsorbents for polyphenols. The structural integrity and surface modifications resulting from polyphenol immersion highlighted the feasibility of utilizing these spherules in suspension culture, reducing phenolic interference while enhancing visibility and quantification. This study emphasized an alternate method of incorporating AC as AC impregnated calcium alginate spherules in cell suspension cultures, thereby reducing phenolic interference as well as enhancing the visibility and quantifiability of the cultures. Further investigations into the kinetics and mechanisms of adsorption will provide a deeper understanding of the interactions at play and may open avenues for optimization in practical applications.

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Data Availability All data generated or analysed during this study are included in this published article.

References

Abohatem MA, Baaziz M, Jaiti F (2025) Morphological stages of date palm cell suspension culture and production of biomass, phenolics, and proteins under elicitors of activated charcoal and glutamine. Preprint, Res Square, 17 June 2025

Adkins S, Nguyen QT, Foale M (2016) Improving the availability of valuable coconut germplasm using tissue culture techniques. Cord 32:27–36

Ahmad I, Hussain T, Ashraf I, Nafees M, Maryam RM, Iqbal M (2013) Lethal effects of secondary metabolites on plant tissue culture. Am Eurasian J Agric Environ Sci 13:539–547

Al-Khayri JM (2013) Factors affecting somatic embryogenesis in date palm (*Phoenix dactylifera* L.). In: Aslam J, Srivastava PS, Sharma MP (eds) Somatic embryogenesis and genetic transformation in plants. Narosa Publishing House, New Delhi, pp 5–38

Aparna V, Neema M, Shil S, Prakash K, Kavya K, Anand S, Sudha R (2024) Standardization of in *vitro* multiplication technique for *Areca concinna* Thwaites, an endangered palm species for its conservation and utilization. *In Vitro* Cell Dev Biol - Plant 60:215–221

Aremu AO, Gruz J, Šubrtová M, Szüčová L, Doležal K, Bairu MW, Finnie JF, Van Staden J (2013) Antioxidant and phenolic acid profiles of tissue cultured and acclimatized *Merwilla plumbea* plantlets in relation to the applied cytokinins. J Plant Physiol 170:1303–1308

Bandupriya HDD, Fernando SC, Vidhanaarachchi VRM (2016) Micropropagation and androgenesis in coconut: an assessment of Sri Lankan implication. COCOS 22:31–47

Banfalvi G (2017) Overview of cell synchronization. In: Banfalvi G (ed) Cell cycle synchronization. Methods in molecular biology, vol 1524. Humana Press, New York, NY, pp 3–27

Bazrafshan E, Amirian P, Mahvi AH, Ansari-Moghaddam A (2016) Application of adsorption process for phenolic compounds removal from aqueous environments: a systematic review. Glob NEST J 18:146–163

Bennacef C, Desobry S, Jasniewski J, Leclerc S, Probst L, Desobry-Banon S (2023) Influence of alginate properties and calcium chloride concentration on alginate bead reticulation and size: a phenomenological approach. Polymers 15:4163

Dąbrowski A, Podkościelny P, Hubicki Z, Barczak M (2005) Adsorption of phenolic compounds by activated carbon—a critical review. Chemosphere 58:1049–1070

Draget KI, Skjak-Braek G, Smidsrod O (1997) Alginate-based new materials. Intl J Biol Macromol 21:47–55

Dumas E, Monteuuis O (1995) In *vitro* rooting of micropropagated shoots from juvenile and mature *Pinus pinaster* explants: influence of AC. Plant Cell Tiss Org Cult 40:231–235

Fernando SC, Vidhanaarachchi VRM, Weerakoon LK, Santha ES (2007) What makes clonal propagation of coconut difficult? Proc Asia Pac Conf Plant Tiss Agribiotechnol 18:163–165

Fridborg G, Pedersen M, Landström LE, Eriksson T (1978) The effect of activated charcoal on tissue cultures: adsorption of metabolites inhibiting morphogenesis. Physiol Plant 43:104–106

González-García P, Centeno TA, Urones-Garrote E, Ávila-Brande D, Otero-Díaz LC (2013) Microstructure and surface properties of lignocellulosic-based activated carbons. Appl Surf Sci 265:731–737

Jiao H, Guo X, Shu F, Zhang Q, Wu W, Jin Y, Jiang B (2024) Structure-property-function relationships of wood-based activated carbon in energy and environment materials. Sep Purif Technol 353:128607

Jones AMP, Saxena PK (2013) Inhibition of phenylpropanoid biosynthesis in *Artemisia annua* L.: a novel approach to reduce oxidative browning in plant tissue culture. PLoS One 8:e76802

Kajani AA, Mofid MR, Abolfazli K, Tafreshi SAH (2010) Encapsulated activated charcoal as a potent agent for improving taxane

synthesis and recovery from cultures. Biotechnol Appl Biochem 56:71–76
Kato N, Esaka M (1999) Changes in ascorbate oxidase gene expression and ascorbate levels in cell division and cell elongation in tobacco cells. Physiol Plant 105:321–329
Lee KY, Mooney DJ (2012) Alginate: properties and biomedical applications. Prog Polym Sci 37:106–126
Leng L, Xiong Q, Yang L, Li H, Zhou Y, Zhang W, Jiang S, Li H, Huang H (2021) An overview on engineering the surface area and porosity of biochar. Sci Total Environ 763:144204
Manocha SM (2003) Porous carbons. Sadhana 28:335–348
Mashoene TN, Leudjo Taka A, Akpotu SO, Lawal IA, Klink MJ (2023) Surface modification of tea-waste-based biochar adsorbent: synthesis, characterization, and batch adsorption for the removal of zidovudine ARV drug and phenol. Appl Sci 13:7493
Mohammad-Khah A, Ansari R (2009) Activated charcoal: preparation, characterization and applications: a review article. Intl J Chem Tech Res 1:859–864
Mojoudi N, Mirghaffari N, Soleimani M, Shariatmadari H, Belver C, Bedia J (2019) Phenol adsorption on high microporous activated carbons prepared from oily sludge: equilibrium, kinetic and thermodynamic studies. Sci Rep 9:19352
Murata Y, Jinno D, Kofuji K, Kawashima S (2004) Properties of calcium-induced gel beads prepared with alginate and hydrolysates. Chem Pharm Bull 52:605–607
Nadavala SK, Swayampakula K, Boddu VM, Abburi K (2009) Biosorption of phenol and o-chlorophenol from aqueous solutions onto chitosan–calcium alginate blended beads. J Hazard Mater 162:482–489
Neema M, Aparna V, Aparnna W, Zaeema F, Sudha R, Nagaraja NR (2024) Morphohistological changes during arecanut (*Areca catechu* L.) somatic embryogenesis. Propag Ornam Plants 24:31–36
Nguyen QT, Bandupriya HD, López-Villalobos A, Sisunandar S, Foale M, Adkins SW (2015) Tissue culture and associated biotechnological interventions for the improvement of coconut (*Cocos nucifera* L.): a review. Planta 242:1059–1076
Olah R (2017) The use of activated charcoal in grapevine tissue culture. Vitis 56:161–171
Pan MJ, van Staden J (1998) The use of charcoal in in *vitro* culture—a review. Plant Growth Regul 26:155–163
Permadi N, Akbari SI, Prismantoro D, Indriyani NN, Nurzaman M, Alhasnawi AN, Doni F, Julaeha E (2024) Traditional and next-generation methods for browning control in plant tissue culture: current insights and future directions. Curr Plant Biol. https://doi.org/10.1016/j.cpb.2024.100339
Roostaei N, Tezel FH (2004) Removal of phenol from aqueous solutions by adsorption. J Environ Manage 70:157–164
Ryu Z, Rong H, Zheng J, Wang M, Zhang B (2002) Microstructure and chemical analysis of PAN-based activated carbon fibers prepared by different activation methods. Carbon 40:1144–1147
Sáenz L, Herrera-Herrera G, Uicab-Ballote F, Chan JL, Oropeza C (2010) Influence of form of activated charcoal on embryogenic callus formation in coconut (*Cocos nucifera*). Plant Cell Tiss Org Cult 100:301–308
Semanate EC (2024) Evaluating the swelling/degradation behavior of calcium alginate beads in synthetic wastewater under varied environmental conditions (Doctoral dissertation, Rice University)
Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 299:265–275
Stavropoulos GG, Samaras P, Sakellaropoulos GP (2008) Effect of activated carbons modification on porosity, surface structure and phenol adsorption. J Hazard Mater 151:414–421
Strober W (2015) Trypan blue exclusion test of cell viability. Curr Protoc Immunol 111:A3.B.1-A3.B.3
Sudheer WN, Praveen N, Al-Khayri JM, Jain SM (2022) Role of plant tissue culture medium components. In: Al-Khayri JM, Jain SM, Johnson DV (eds) Advances in plant tissue culture. Academic Press, pp 51–83
Teng WL (1997) Activated charcoal affects morphogenesis and enhances sporophyte regeneration during leaf cell suspension culture of *Platycerium bifurcatum*. Plant Cell Rep 17:77–83
Thomas TD (2008) The role of activated charcoal in plant tissue culture. Biotechnol Adv 26:618–631
Tisserat B, DeMason DA (1982) A scanning electron microscope study of pollen of *Phoenix* (Arecaceae). J Am Soc Hortic Sci 107:883–887
Tsutomu I, Takashi A, Kuniaki K, Kikuo O (2004) Comparison of removal efficiencies for ammonia and amine gases between woody charcoal and activated carbon. J Health Sci 50:148–153
Weatherhead MA, Burdon J, Henshaw GG (1979) Effects of activated charcoal as an additive in plant tissue culture media: part II. Z Pflanzenphysiol 94:399–405
Weckx S, Inzé D, Maene L (2019) Tissue culture of oil palm: finding the balance between mass propagation and somaclonal variation. Front Plant Sci 10:722
Xu L, Wang X (2025) A comprehensive review of phenolic compounds in horticultural plants. Intl J Mol Sci 26:5767
Zachleder V, Ivanov IN, Kselíková V, Bialevich V, Vítová M, Ota S, Takeshita T, Kawano S, Bišová K (2021) Characterization of growth and cell cycle events affected by light intensity in the green alga *Parachlorella kessleri*: a new model for cell cycle research. Biomolecules 11:891