

CHITOSAN CANOLA SEED COATING: ROOT SYSTEM AND DEFENCE RESPONSES

 Cyntia Lorena Szemruch^{1*},  Carola Gonçalves Vila Cova¹

¹*Institute of Research on Agricultural Production, Environment, and Health (IIPAAS), Faculty of Agricultural Sciences, National University of Lomas de Zamora. Route 4 and Juan XXIII, Llavallol, Lomas de Zamora, Argentina.*

**Corresponding author:*

E-mail: cszemruch@agrarias.unlz.edu.ar

(Received 25th December 2025; accepted 01th March 2026)

ABSTRACT. Chitosan is a natural polymer with biostimulant properties known to enhance plant defence responses. However, when applied as a seed coating technology, its effects on root growth are not fully understood. This study aimed to (i) develop a reproducible protocol for callose detection in canola roots as a functional marker of immune activation induced by chitosan under direct exposure; and (ii) evaluate the physiological, morphological, and growth effects of different chitosan seed coating formulations, with particular emphasis on distinguishing immune-mediated responses from the physicochemical properties of the coating matrix. In Stage 1, canola roots were immersed in 0.01% and 1% chitosan solutions to induce callose deposition as an immune response. In Stage 2, different seed coating formulations with chitosan (liquid and solid), sodium alginate, and talc were evaluated in terms of callose deposition, radicle and seedling emergence, seed water uptake, root length and electrolyte leakage. Callose deposition was observed under direct exposure, but not after seed coating treatment. Early physiological responses were instead primarily associated with the physicochemical properties of the coatings and their effects on water uptake. Liquid chitosan delayed radicle emergence by limiting hydration, whereas alginate and talc accelerated it. Seedling emergence, root length, and membrane integrity remained unaffected. The initial delay in radicle emergence caused by liquid chitosan did not compromise seedling growth. A reproducible protocol for callose detection as a marker of immune response was successfully developed. Under the tested conditions, these results highlight the critical role of the application method in determining chitosan bioactivity.

Keywords: *seed treatment, sodium alginate, biopolymers, Brassica napus L., elicitor*

INTRODUCTION

Chitosan is a natural polymer composed of glucosamine and N-acetylglucosamine monomers, derived from chitin found in the exoskeletons of arthropods and insects, as well as in fungal cell walls [1]. This biopolymer offers multiple benefits in agricultural production, functioning as a biopesticide, biostimulant, and biofertilizer, with positive effects on plant growth and resource-use efficiency [2]. Furthermore, it contributes to the mitigation of abiotic stress by activating key physiological processes, including enhanced photosynthetic rate, stomatal regulation, and the synthesis of antioxidant compounds, organic acids, sugars, and amino acids [3].

However, this promising evidence contrasts with reports of adverse effects on certain physiological and morphological traits, particularly those associated with the root system. In *Arabidopsis thaliana* L., high chitosan concentrations alter root morphology by inhibiting cell elongation and division. These effects have been linked to changes in gene

expression and to the synthesis, transport, and signaling of plant hormones [4; 5]. In *Capsicum annuum* L., chitosan concentrations of 10 or 20 mg L⁻¹ reduced root length by 34% and 18.5%, respectively [6]. Similarly, the use of chitosan as a seed coating agent in soybean and sunflower has been associated with reduced radicle emergence in both liquid and solid formulations [7; 8].

To elucidate the origin of these effects, the growth-defence trade-off should be considered. In this context, chitosan is perceived as a stressor, and the plant redirects its physiological resources, prioritising the immunological response over cell elongation and root system expansion [9]. This response is intensified when chitosan is dissolved in weak acids at high concentrations, activating multiple defence pathways that include the accumulation of phytoalexins and phenolic compounds, as well as the synthesis of lignin and suberin [9, 10]. Chitosan also forms a physical barrier against pathogen entry by altering plasma membrane stability and fluidity and induces Ca²⁺ displacement, which stimulates callose synthesis [11]. This polysaccharide, deposited in cell walls, plays a key role in plant immune responses. Studies on *Arabidopsis thaliana* L. root hairs have shown that callose deposition and growth responses are concentration dependent: low chitosan levels maintain an elicitor effect, whereas high concentrations inhibit growth and suppress callose synthesis [12]. Therefore, callose accumulation can be considered a functional marker of chitosan-induced immune activation.

Beyond chitosan concentration, its mode of application is particularly relevant. Substantial evidence demonstrates its effects when applied through seed priming, a technique involving controlled seed hydration followed by drying [13]. Across various crops, priming with chitosan has been shown to significantly enhance root system growth [14, 15, 16]. In contrast, results are less consistent when chitosan is applied as a seed coating. Unlike priming, this method does not involve seed imbibition; instead, seeds are enveloped in one or more thin layers of active and inert substances, which may or may not alter their size or weight [17]. Within the context of biopolymer use, coating allows incorporation of finely ground solids (e.g., talc, peat, lime, biochar) or liquids, which are fixed to the seed surface using an adhesive, often a natural gum (e.g., gum arabic, xanthan gum) or polysaccharide (e.g., sodium alginate or chitosan itself) [18]. This technique can modify both the chitosan release kinetics and its local concentration at the root surface. Furthermore, the biopolymers used as coating materials may affect water availability to the seed [19, 20, 21]. In particular, a chitosan coating could affect water uptake and germination physiology. Consequently, it is necessary to distinguish the biochemical immune activation, tested through callose deposition, from the physical and hydric properties of the coating matrix, which may alter seed water uptake dynamics. To explore this possibility, it is essential to consider the three phases of water uptake during germination: an initial rapid hydration phase (Phase I), followed by a stable absorption (Phase II), and a final phase of accelerated water uptake coinciding with radicle emergence (Phase III) [22].

Although chitosan is widely recognised as an elicitor capable of activating plant defence responses, including callose deposition, its biological activity strongly depends on exposure dynamics and delivery to plant tissues. Seed coating involves an indirect and delayed contact between the emerging radicle and the polymeric matrix, which may substantially modify both the intensity and timing of defence responses, as well as seed water uptake dynamics during germination. The objectives of this study were to (i) develop a reproducible protocol for callose detection in canola roots as a functional marker of immune activation induced by chitosan under direct exposure; and (ii) evaluate

the physiological, morphological, and growth effects of different chitosan seed coating formulations, with particular emphasis on distinguishing immune-mediated responses from the physicochemical properties of the coating matrix.

MATERIAL AND METHODS

Stage 1: development of a callose detection protocol in canola roots

Plant material

Seeds of a spring hybrid canola (*Brassica napus* L.) of Australian origin were used. Prior to experiments, seeds were stored at 10 °C for three months. To obtain the seedlings, three replicates of 50 seeds each were placed between two layers of saturated filter paper and covered with a third saturated paper layer. Seed packets were folded, placed into sealed cellulose bags to prevent moisture loss, and suspended vertically in a germination chamber maintained at 20 °C under a 12-h/12-h light/dark photoperiod.

Chitosan treatments

To evaluate the immunological response, three-day-old seedlings obtained from the previous trial were immersed in two chitosan solutions used as positive controls: 0.01% [12] and 1% (w/v). The latter corresponds to the concentration applied in seed coating treatments described in Stage 2 of this study. Both solutions were prepared by dissolving chitosan (medium molecular weight, Sigma-Aldrich®) in 1% (v/v) acetic acid. Control treatments included seedlings immersed in sterile water (negative control) and in 1% acetic acid (vehicle control). Groups of four seedlings were transferred using sterile forceps into test tubes containing 10 mL of the respective solutions and incubated for 24 h in a germination chamber set at 20 °C under a 12-h/12-h light/dark photoperiod.

Callose detection protocol

Callose detection was performed following the protocol of Zavaliev and Epel [23] with modifications. After the 24-h incubation, groups of four seedlings per treatment were fixed and dehydrated by immersion in 25 mL of 96% ethanol inside individual sealed containers on an orbital shaker (60 – 80 rpm) at 25 °C for 2 h. Rehydration was achieved by transferring seedlings, supported by the cotyledons, into new containers containing 25 mL of double-distilled water with 0.01% Tween-20, followed by incubation at room temperature on an orbital shaker (60 – 80 rpm) for 1 h. The staining solution consisted of 0.01% (w/v) Methyl Blue (Sigma-Aldrich M-6900) prepared in 0.15 M K₂HPO₄ buffer (pH 9.5), filtered through a 0.45 µm Millipore membrane, and stored in a sealed amber container at room temperature in darkness for at least 48 h to allow decolourisation to a bright yellow colour. After rehydration, the root apex (2 cm segment) was excised with a scalpel and immediately immersed in 15 mL of Methyl Blue solution in 25 mL glass tubes (one tube per treatment). Tubes were wrapped in aluminium foil and subjected to vacuum infiltration using a vacuum pump for 10 min, followed by gradual pressure release. Samples were then incubated for 2 h at room temperature on an orbital shaker set at 100 rpm. After staining, root apices were mounted directly on glass slides using an anti-fading medium. Callose deposits were observed under a Motic BA400 microscope equipped with a Moticom 1000 camera and an EF-UPR-III epifluorescence attachment using a DAPI filter. Images were captured with Motic Images Plus 3.0 software.

Stage 2: Evaluation of seed coating

Treatments

Canola (*B. napus* L.) seeds were coated either as single or successive layers (Table 1), using two biopolymers from Sigma-Aldrich®: chitosan, applied either in liquid (*ICH*) or solid (*sCH*) form combined with talc as a filler, and sodium alginate (*SA*). The latter, at 1.5% (w/v), was prepared by dissolving 1.5 g in 100 mL of distilled water and used as an adhesive layer. The liquid chitosan formulation (*ICH*) consisted of 1% (w/v) chitosan dissolved in 1% (v/v) acetic acid. The solid formulation (*sCH*) was prepared by mixing chitosan with talc [$\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$] at a 1:100 ratio (w/w), resulting in a final concentration of 1% (w/w) chitosan in talc [7]. To ensure uniform distribution and adhesion, 10 mL of each biopolymer solution or 30 g of the solid chitosan–talc mixture was progressively applied to 50 g of canola seeds under continuous rotation for 3 min. The control treatment (*W*) consisted of seeds coated with 10 mL of sterile distilled water under identical conditions. In those treatments where a second layer was applied, it was added 30 minutes after the first one had completely dried. After coating, all seed batches were air-dried for 24 h at room temperature (25 °C) and stored in paper bags at 10 °C until use.

Table 1. Composition and sequence of biopolymer coatings applied to canola seeds

Seed treatment *	First layer	Second layer
W	Water	–
SA	Sodium Alginate	–
ICH	Liquid Chitosan	–
ICH + SA	Liquid Chitosan	Sodium Alginate
SA + T	Sodium Alginate	Talc
SA + sCH (T)	Sodium Alginate	Solid Chitosan

* W: water, SA: sodium alginate, *ICH*: liquid chitosan, *ICH* + SA: liquid chitosan + sodium alginate, SA + T: sodium alginate + talc, SA + sCH(T): sodium alginate + solid chitosan and talc.

Callose detection in seedlings derived from coated seeds

The callose detection protocol was applied to roots of three-day-old seedlings derived from coated seeds. This sampling point was selected to ensure sufficient radicle development for reliable staining, to allow standardised comparisons among treatments, and to preserve tissue integrity and optical quality during ethanol dehydration and epifluorescence microscopy.

Radicle emergence rate

Radicle emergence was assessed according to ISTA [24] using four replicates of 100 seeds per treatment. The number of seeds showing radicle protrusion through the seed coat was recorded at regular intervals of 3 – 4 h, from 6 h to 36 h after sowing, based on preliminary trials conducted with the same genotype. The cumulative percentage of radicle emergence was plotted as a function of time. The time required for 50% of the seeds to exhibit radicle emergence (RE_{50}) was calculated following the method of Rana and García de Santana [25] and expressed in hours. Lower RE_{50} values indicate greater seed vigour [24].

Seed water uptake

Three replicates of 100 treated seeds were placed between layers of water-saturated filter paper and enclosed in hermetically sealed polythene bags. Samples were incubated at 20 °C under a 12-h/12-h light/dark photoperiod. At predetermined intervals during the first 27 h after sowing, all three replicates were removed and weighed. The seeds were immediately weighed on an analytical balance to determine fresh mass and subsequently oven-dried at 60 °C to constant weight for dry weight determination. Seed water uptake was determined according to Moura et al. [26] and expressed as seed weight increase relative to the initial seed weight (g). The cumulative water uptake (g H₂O) was plotted against time to construct the water uptake curve. Interpolation between 12 and 24 h enabled estimation of the seed water content corresponding to 50% radicle emergence (RE₅₀), as determined in the previous step. A linear correlation analysis between RE₅₀ values and seed water content was performed, and the Pearson correlation coefficient (r) was calculated.

Seedling emergence and root length

Seedling emergence was assessed seven days after sowing by counting the number of normal seedlings, expressed as a percentage [24]. For each treatment, four replicates of 50 pre-chilled seeds were placed on moistened top paper and incubated at 20 °C under a 12-h/12-h light/dark photoperiod [24]. From each replicate, five seedlings were randomly selected, and their root length was measured in centimetres.

Indirect quantification of reactive oxygen species in the root

Increased reactive oxygen species (ROS) can compromise plasma membrane stability, resulting in leakage of cellular contents and loss of tissue integrity. Therefore, electrolyte leakage was used as an indirect indicator of ROS accumulation in root tissues. The method followed Poveda [27] with minor modifications regarding the sample size: 200 mg of fresh root tissue from seven-day-old seedlings grown on moistened paper immersed in 10 mL of double-distilled water. Electrolyte leakage was measured with a conductivity meter and expressed as the percentage of ions released to total ions.

Statistical analysis

All experiments were conducted under a completely randomised design. Seed water uptake was analysed considering two main factors: seed coating treatment and time. For each time point, independent and destructively sampled seed batches were used, and measurements at different times were therefore treated as independent observations. Percentage data were angularly transformed, and emergence data were log-transformed (Log₁₀) to meet assumptions of normality and homogeneity of variances. Analysis of variance was performed to detect significant differences among treatments, followed by Tukey's Honestly Significant Difference test at a 5% significance level for post-hoc comparisons. Data analysis was conducted using Navure Team statistical software (2023) [28].

RESULTS*Stage 1: Development of a callose detection protocol in canola roots*

Roots subjected to the control treatments, including sterile distilled water (negative control; Fig. 1a, b) and 1% acetic acid (vehicle control; Fig. 1c, d), showed no callose deposition, indicating the absence of immune activation. In contrast, roots treated with chitosan solutions at 0.01% and 1% (w/v), both dissolved in 1% (v/v) acetic acid and used as positive controls, exhibited an evident accumulation of callose in the cell walls. Under fluorescence microscopy, these callose deposits appeared well defined and sharply delimited (Fig. 1e, f).

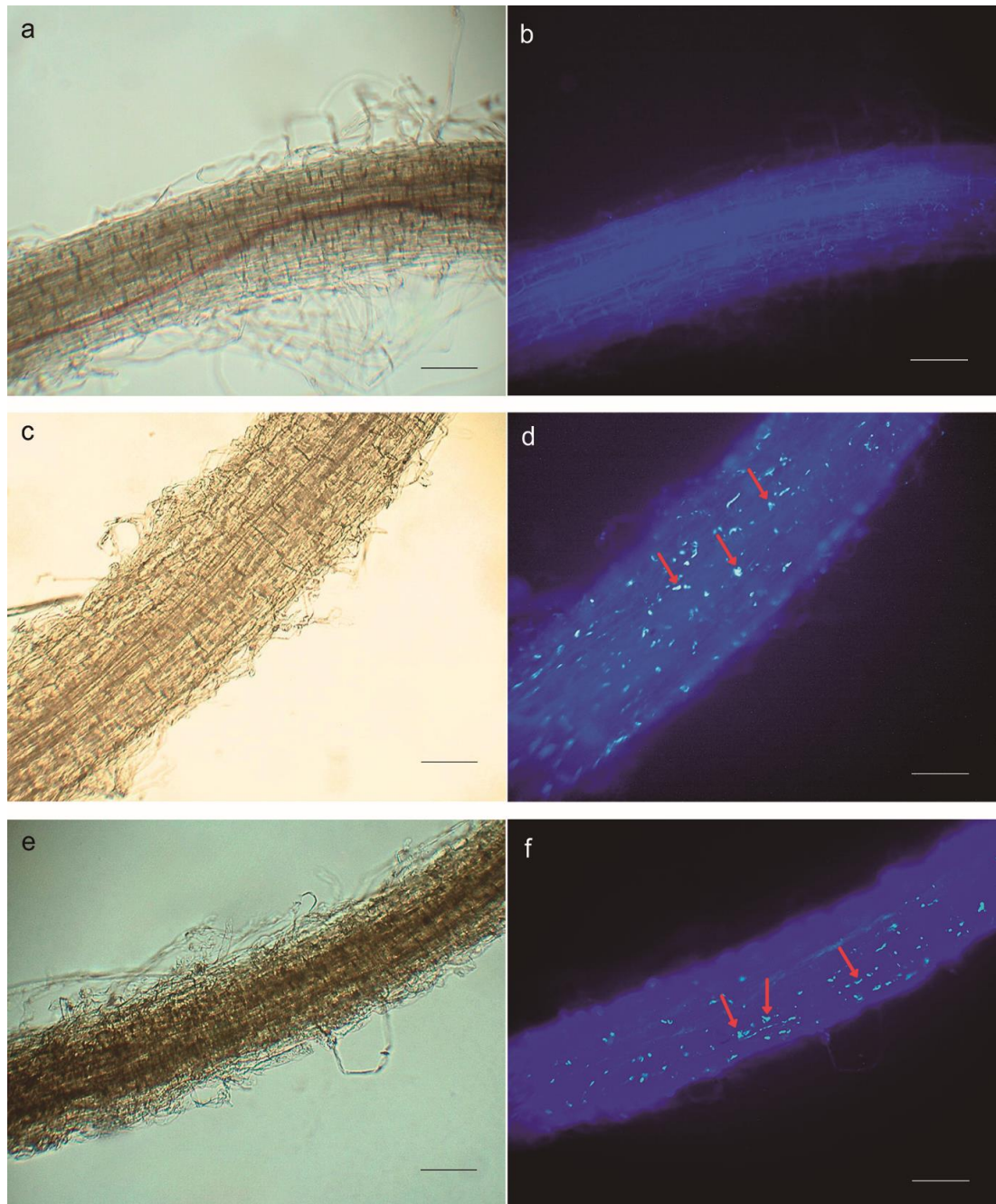


Fig. 1. Callose staining in canola roots after the following treatments: control (a, b), chitosan 0.01% (c, d), and 1% (e, f). Bright-field (a, c, e) and fluorescence (b, d, f). Red arrows indicate bright blue fluorescence corresponding to callose deposits. Scale bars: 150 μ m.

Stage 2: Evaluation of seed coating treatments

Radicle emergence

Liquid chitosan (*ICH*) delayed radicle emergence by more than two hours compared with the control, whereas sodium alginate (*SA*) significantly accelerated it (Table 2). The combined treatment (*ICH* + *SA*) also promoted earlier radicle emergence. Solid chitosan mixed with talc (*sCH*) showed an intermediate effect between the control and sodium alginate, further supporting the opposing effects of these compounds. This pattern was also observed in the cumulative radicle emergence over time, where liquid chitosan maintained a sustained delay, particularly between 16 and 32 hours after sowing (Fig. 2a).

Table 2. Time of radicle emergence, seedling emergence, root length and root ion leakage in canola after the seed coating treatments.

Treatments*	Radicle emergence [h]	Seedling emergence [%]	Root length [cm]	Root ion leakage [%]
W	20.4 ± 0.27 b	95 ± 1.1 a	5.34 ± 0.85 a	32.7 ± 5.35 ab
SA	19.5 ± 0.44 c	97 ± 2.5 a	4.69 ± 0.26 a	38.3 ± 4.99 a
<i>ICH</i>	22.8 ± 0.35 a	98 ± 2.5 a	4.68 ± 0.45 a	32.0 ± 0.98 ab
<i>ICH</i> + <i>SA</i>	19.7 ± 0.13 c	95 ± 2.6 a	5.36 ± 0.55 a	24.3 ± 0.24 b
<i>SA</i> + <i>T</i>	19.3 ± 0.19 c	97 ± 2.8 a	5.54 ± 0.49 a	34.3 ± 3.46 ab
<i>SA</i> + <i>sCH</i> (<i>T</i>)	19.9 ± 0.17 bc	94 ± 2.8 a	5.11 ± 0.36 a	29.3 ± 4.65 ab

*W: water, SA: sodium alginate, *ICH*: liquid chitosan, *ICH* + *SA*: liquid chitosan + sodium alginate, *SA* + *T*: sodium alginate + talc, *SA* + *sCH*(*T*): sodium alginate + solid chitosan and talc. Different letters indicate significant differences ($p < 0.05$). Values are average ± SD.

Callose detection

No callose deposition was observed in roots from any of the seed coating treatments at three days after sowing (Fig. 3), indicating that, under the evaluated conditions and time points, the coating formulations did not trigger a sustained callose-mediated immune pathway.

Seed water uptake

Seed water uptake showed high variability among treatments following application of the different coatings. During Phase I, the positive effect of talc on imbibition dynamics was evident in treatments *SA* + *T* and *SA* + *sCH*(*T*), which exhibited higher rates of water absorption (Fig. 2b). In Phase II, seeds coated with liquid chitosan (*ICH*) displayed the lowest water content, whereas treatments containing sodium alginate (*SA* and *ICH* + *SA*) showed higher hydration levels (Fig. 2b). Compared with the sodium alginate plus talc formulation (*SA* + *T*), the inclusion of solid chitosan [*SA* + *sCH*(*T*)] resulted in high initial water uptake that gradually decreased toward the end of the phase (Fig. 2b).

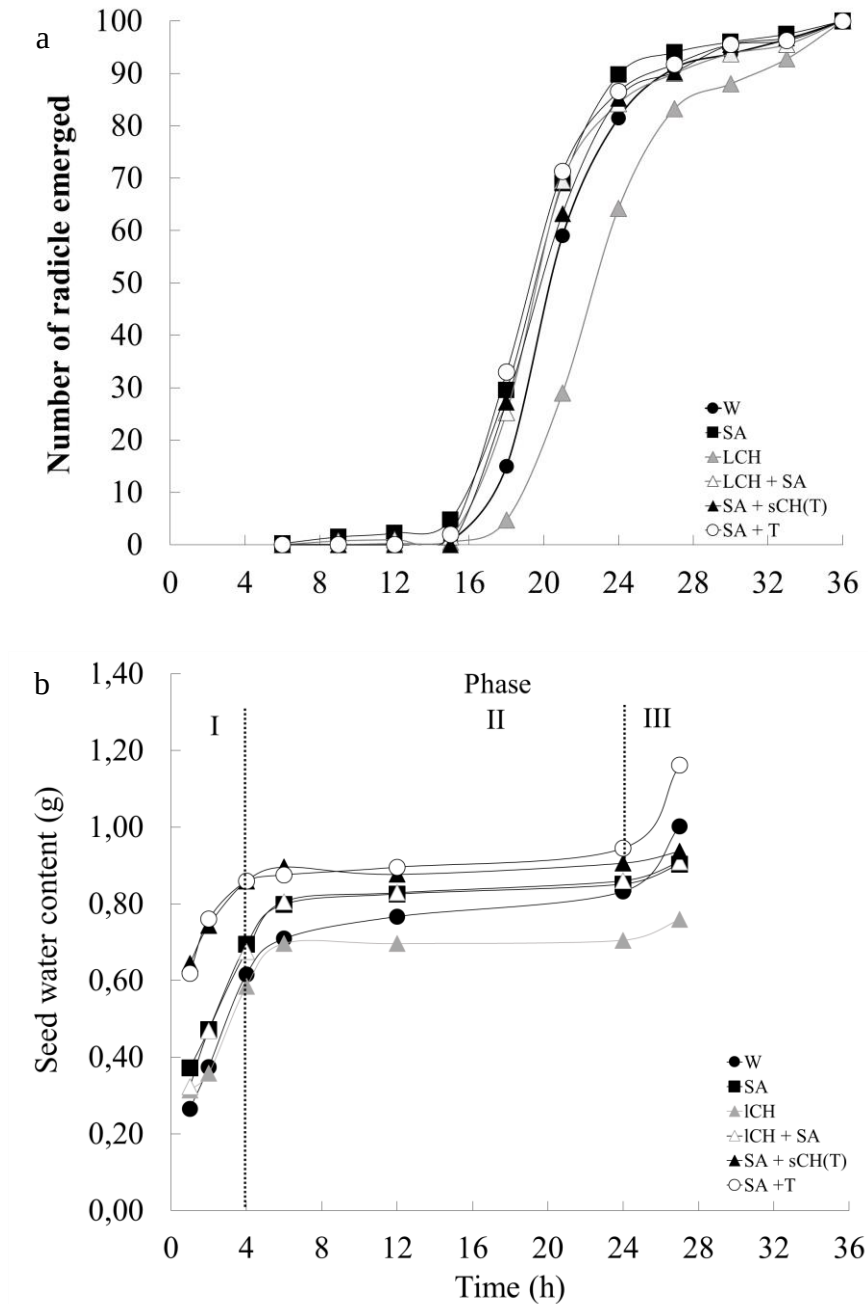


Fig. 2. Dynamics of radicle emergence (a) and seed water uptake over time (b) in canola after the seed coating treatments: W = water, SA = sodium alginate, LCH = liquid chitosan, LCH + SA = liquid chitosan + sodium alginate, SA + T = sodium alginate + talc, SA + sCH(T) = sodium alginate + solid chitosan and talc. Roman numerals indicate the phases of water absorption.

Linear regression analysis between seed water content and the time required for 50% radicle emergence (RE_{50}) revealed a significant negative correlation, with a Pearson coefficient of $r = 0.77$.

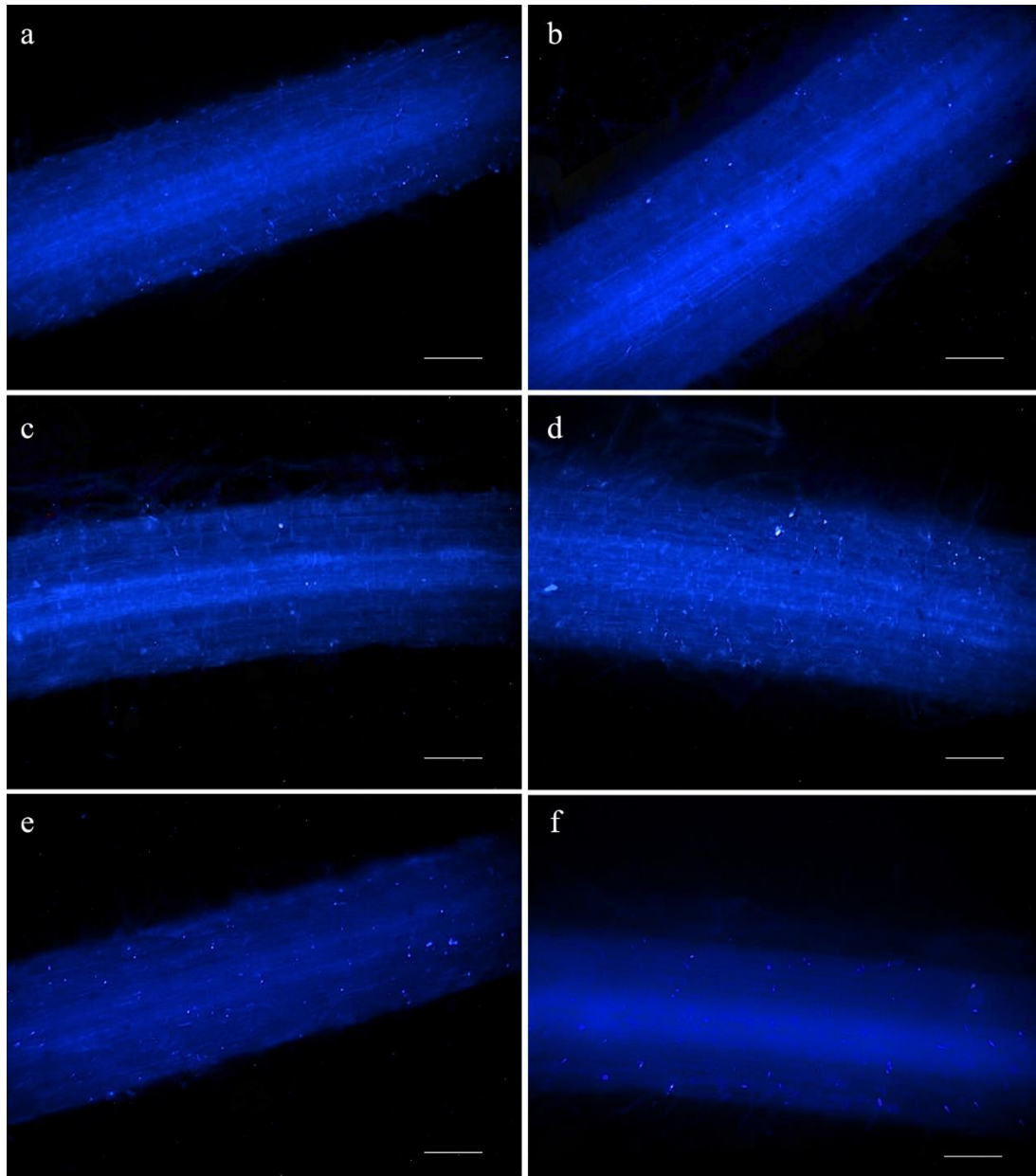


Fig. 3. Callose staining in canola radicles after the following seed coating treatments: W = water (a), SA = Sodium Alginate (b), ICH = Liquid Chitosan (c), ICH + SA = Liquid Chitosan + Sodium Alginate (d), SA + T = Sodium Alginate + Talc (e), SA + sCH = Sodium Alginate + Solid Chitosan (f). Scale bars: 150 μ m.

DISCUSSION

Exposure of roots to both chitosan concentrations enabled clear and well defined visualization of callose deposits, comparable to those reported by Yi et al. [29]. The presence of these deposits indicates that chitosan acts as an elicitor capable of inducing defence mechanisms in canola root tissues, confirming both the immunological response and the effectiveness of the developed protocol. Although the method was originally designed for leaves [23], the alcohol bleaching and subsequent washing durations proved

suitable for roots of the size obtained three days after sowing, ensuring tissue integrity. Unlike Drs et al. [12], who used lower dye concentrations, the 0.01% concentration employed here was compatible with the selected root size, allowing clear visualisation of callose deposits. Smaller roots or prolonged alcohol incubation could compromise tissue structural integrity, while larger roots might hinder microscopic observation due to overlapping tissue layers, limiting deposit resolution.

The contrasting patterns of callose deposition observed between the two experimental stages highlight that immune activation by chitosan in this species is strongly dependent on the mode of application. In Stage 1, direct immersion of already developed roots in chitosan solutions ensured immediate exposure to defined concentrations, resulting in a clear accumulation of callose, in agreement with reports in other species [12,29,30]. In contrast, when chitosan was applied as a seed coating, roots only came into contact with the polymer after radicle emergence, probably at lower or spatially heterogeneous concentrations due to the release kinetics from the coating matrix. The absence of detectable callose deposition in the coating treatments does not imply a lack of elicitor capacity of chitosan; rather, it indicates that callose-mediated immune activation was not induced under the selected conditions and evaluation time. Because chitosan defence mechanisms may occur rapidly, earlier responses should be assessed, for example, during imbibition in the seed tissues or immediately after radicle emergence. With radicles measuring approximately 2 mm, the protocol should be adapted to avoid tissue damage and ensure accurate microscopic visualization. Although callose is a widely used marker of certain defensive pathways, others may operate independently of its deposition. Therefore, it is also important to examine the activation of hormones, immune-related genes, specific immune receptors, and molecular patterns [31,32]. Moreover, these responses could be compared against different pathogens to characterise multiple defence pathways.

The significant increase in water uptake rates observed in treatments containing talc can be attributed to the predominantly physical nature of the initial imbibition phase, driven by the gradient between internal matric forces and external water availability [22]. Within the coating matrix, talc enhances water absorption by forming, at the microscopic level, a porous structure with large interparticle spaces [33]. Comparative analysis of the biopolymers regarding water uptake revealed an antagonistic response: sodium alginate accelerated absorption, whereas liquid chitosan delayed it. In combined formulations, the effect of alginate predominated over chitosan. According to Behboud et al. [20], coating seeds with chitosan improves germination and root growth, suggesting that both compounds can enhance water uptake by maintaining a humid microenvironment around the seed. These effects occur via different mechanisms: chitosan limits water loss, whereas alginate facilitates water absorption through swelling upon hydration. Therefore, it is important not only to consider absorption capacity but also the water transfer properties of the polymer matrix [34]. Furthermore, coating films made from these biopolymers for medical or food applications have demonstrated differential water absorption [35,36]. Consistently, our results showed significant differences in water uptake among treatments, with alginate promoting higher seed hydration. Sodium alginate forms highly permeable hydrogels capable of retaining and releasing large water volumes [37,38], optimizing water transfer to the seed and promoting germination [39, 40]. In contrast, due to its cationic nature, chitosan forms insoluble complexes and dense, low permeability films [41,42], limiting water diffusion toward the seed and reducing the imbibition rate [39]. These physicochemical properties of the coatings may affect canola

seed germination physiology, since slower water uptake in seeds coated with liquid chitosan may delay the reactivation of essential metabolic processes for germination, affecting the synchrony and efficiency of initial root system development.

The observed delay in radicle emergence under liquid chitosan treatments suggests the presence of early physiological effects associated with alterations in water availability or polymer seed interactions, rather than callose-mediated immune activation. Consequently, the results indicate that chitosan coatings did not induce detectable callose-dependent responses, while not excluding the involvement of alternative stress or signalling pathways.

The absence of cellular damage and alterations in membrane integrity, together with stable seedling morphology, suggests that early stage effects are transient and manifest exclusively at the radicle level. This response suggests that roots compensate for initial growth restrictions during subsequent seedling development. Although improvements in germination as reported for priming techniques were not observed [14,16], this compensatory growth indicates that early effects did not necessarily translate into negative impacts on crop establishment. Moreover, the inclusion of chitosan in seed coatings remains relevant due to its biocidal activity against insects and fungi, contributing to the sustainability of production systems.

CONCLUSION

A reproducible protocol was successfully developed for callose detection in canola roots under conditions of direct exposure to chitosan, confirming its utility as a marker of immune activation in this species. At the evaluated developmental stage, when chitosan was applied as a seed coating, in both liquid and solid formulations, no callose-mediated immune responses were detected. Instead, early effects on radicle emergence were primarily associated with differences in water absorption determined by the physicochemical properties of the coating materials.

The initial delay in radicle emergence observed in the liquid chitosan treatments did not translate into a reduction in seedling emergence, root growth, or membrane integrity, indicating that these early effects were transient and compensated for during subsequent development. Altogether, these results highlight the critical role of the application method in determining chitosan bioactivity. To contribute to the safe design of chitosan seed coating technology, it would be advisable to analyse more genotypes and substrates.

REFERENCES

- [1] Poznanski, P., Hameed, A., Orczyk, W. (2023): Chitosan and chitosan nanoparticles: parameters enhancing antifungal activity. *Molecules*, 28: 2996. <https://doi.org/10.3390/molecules28072996>
- [2] Boamah, P. O., Onumah, J., Aduguba, W. O., Santo, K. G. (2023): Application of depolymerized chitosan in crop production: A review. *International Journal of Biological Macromolecules*, 242: 124706. <https://doi.org/10.1016/j.ijbiomac.2023.124706>
- [3] Hidangmayum, A., Dwivedi, P., Katiyar, D., Hemantaranjan, A. (2019): Application of chitosan on plant responses with special reference to abiotic stress. *Physiology and Molecular Biology of Plants*, 25: 313–326. <https://doi.org/10.1007/s12298-018-0633-1>
- [4] Lopez-Moya, F., Escudero, N., Zavala-Gonzalez, E. A., Esteve-Bruna, D., Blázquez, M. A., Alabadí, D., Lopez-Llorca, L. V. (2017): Induction of auxin biosynthesis and WOX5

- repression mediate changes in root development in *Arabidopsis* exposed to chitosan. *Scientific Reports*, 7: 16813. <https://doi.org/10.1038/s41598-017-16874-5>
- [5] Lopez-Moya, F., Suarez-Fernandez, M., Lopez-Llorca, L. V. (2019): Molecular mechanisms of chitosan interactions with fungi and plants. *International Journal of Molecular Sciences*, 20: 332. <https://doi.org/10.3390/ijms20020332>
 - [6] Asgari-Targhi, G., Iranbakhsh, A., Ardebili, Z. O. (2018): Potential benefits and phytotoxicity of bulk and nano-chitosan on the growth, morphogenesis, physiology, and micropropagation of *Capsicum annuum*. *Plant Physiology and Biochemistry*, 127: 393–402. <https://doi.org/10.1016/j.plaphy.2018.04.013>
 - [7] Szemruch, C., Astiz Gassó, M. M., García, F., Sanchez, S., Martinez, P. M., Cerdá, M. (2022): Biopolymer and *Trichoderma harzianum* compatibility for sunflower seed coating. *Journal of Experimental Research*, 10: 203–212.
 - [8] Szemruch, C. L., Gonçalves Vila Cova, C., García, F. A., Astiz Gassó, M. M., Sanchez, S. E., Colinas, A. (2024): Optimizing biopolymer seed coatings: Impact of sodium alginate and chitosan on the viability of *Trichoderma harzianum*, *Bradyrhizobium japonicum* and soybean seed quality. *SSRG International Journal of Agriculture and Environmental Science*, 11: 1–8. <https://doi.org/10.14445/23942568/IJAES-V11I6P101>
 - [9] Suwanchaikasem, P., Idnurm, A., Selby-Pham, J. (2024): The impacts of chitosan on plant root systems and its potential to be used for controlling fungal diseases in agriculture. *Journal of Plant Growth Regulation*, 43: 3424–3445. <https://doi.org/10.1007/s00344-024-11356-1>
 - [10] Rojas-Pirela, M., Carillo, P., Lárez-Velásquez, C., Romanazzi, G. (2024): Effects of chitosan on plant growth under stress conditions: similarities with plant growth promoting bacteria. *Frontiers in Plant Science*, 15: 1423949. <https://doi.org/10.3389/fpls.2024.1423949>
 - [11] Calabuig-Serna, A., Sancho-Oviedo, D., Rivas-Sendra, A., Mata-Nicolás, E., Arjona-Mudarra, P., Mir, R., Seguí-Simarro, J. M. (2025): Modulation of calcium, callose synthesis, membrane permeability and pectin methyl-esterase activity affect cell wall composition and embryo yield during *Brassica napus* microspore embryogenesis. *Annals of Botany*, 136: 99–110. <https://doi.org/10.1093/aob/mcaf054>
 - [12] Drs, M., Krupa, P., Škrabáľková, E., Haluška, S., Müller, K., Potocká, A., Brejsková, L., Serrano, N., Voxeur, A., Vernhettes, S., Ortmannová, J., Caldarescu, G., Fendrych, M., Potocký, M., Žárský, V., Pečenková, T. (2024): Chitosan stimulates root hair callose deposition, endomembrane dynamics, and inhibits root hair growth. *Plant Cell and Environment*, 48: 451–469. <https://doi.org/10.1111/pce.15111>
 - [13] Pedrini, S., Balestrazzi, A., Madsen, M. D., Bhalsing, K., Renzaglia, M. (2020). Seed enhancement: getting seeds restoration-ready. *Restoration Ecology*, 28 (Suppl. 2): S568. <https://doi.org/10.1111/rec.13184>
 - [14] Lyalina, T., Shagdarova, B., Zhuikova, Y., Il'ina, A., Lunkov, A., Varlamov, V. (2023): Effect of seed priming with chitosan hydrolysate on lettuce (*Lactuca sativa*) growth parameters. *Molecules*, 28: 1915. <https://doi.org/10.3390/molecules28041915>
 - [15] Sen, S. K., Das, D. (2024): A sustainable approach in agricultural chemistry towards alleviation of plant stress through chitosan and nano-chitosan priming. *Discover Chemistry*, 1: 44. <https://doi.org/10.1007/s44371-024-00046-2>
 - [16] Steven, S., Islam, M. S., Ghimire, A., Methela, N. J., Kwon, E. H., Yun, B. W., Lee, I. J., Kim, S. H., Kim, Y. (2024): Chitosan-GSNO nanoparticles and silicon priming enhance the germination and seedling growth of soybean (*Glycine max* L.). *Plants*, 13: 1290. <https://doi.org/10.3390/plants13101290>
 - [17] Weissmann, E. A., Raja, K., Gupta, A., Patel, M., Buehle, A. (2023): Seed quality enhancement. In: Weissmann, E. A. (ed.) *Seed Science and Technology*, 1st ed., Springer, Singapore, pp. 391–414.
 - [18] Skrzypczak, D., Jarzembowski, Ł., Izydorczyk, G., Hoppe, V., Mielko, K. A., Pudełko-Malik, N., Młynarz, P., Chojnacka, K., Witek-Krowiak, A. (2021): Hydrogel alginate

- seed coating as an innovative method for delivering nutrients at the early stages of plant growth. *Polymers*, 13: 4233. <https://doi.org/10.3390/polym13234233>
- [19] Peltonen-Sainio, P., Känkänen, H., Pahkala, K. (2006): Polymer coated turnip rape seed did not facilitate early broadcast sowing under Finnish growing conditions. *Agricultural and Food Science*, 15: 152–165. <https://doi.org/10.2137/145960606778644511>
- [20] Behboud, R., Moradi, A., Piri, R., Dedicova, B., Fazeli-Nasab, B., Ghorbanpour, M. (2024): Sweet corn (*Zea mays* L.) seed performance enhanced under drought stress by chitosan and minerals coating. *BMC Plant Biology*, 24: 991. <https://doi.org/10.1186/s12870-024-05704-2>
- [21] Kintl, A., Huňady, I., Vymyslický, T., Ondrisková, V., Hammerschmiedt, T., Brtnický, M., Elbl, J. (2021): Effect of seed coating and PEG-induced drought on the germination capacity of five clover crops. *Plants*, 10: 724. <https://doi.org/10.3390/plants10040724>
- [22] Vertucci, C. W. (2015): The kinetics of seed imbibition: controlling factors and relevance to seedling vigor. In: Stanwood, P. C., McDonald, M. B. (eds.) *Seed Moisture*, 1st ed., Crop Science Society of America, Madison, Wisconsin, USA, pp. 93–115.
- [23] Zavaliev, R., Epel, B. L. (2015): Imaging callose at plasmodesmata using aniline blue: quantitative confocal microscopy. In: Heinlein, M. (ed.) *Plasmodesmata. Methods in Molecular Biology*, 1st ed., Humana Press, New York, USA, pp. 173–180.
- [24] ISTA (2023): *International Rules for Seed Testing*. International Seed Testing Association, Bassersdorf, Switzerland.
- [25] Ranal, M. A., Santana, D. (2006): How and why to measure the germination process? *Brazilian Journal of Botany*, 29: 1–11. <https://doi.org/10.1590/S0100-84042006000100002>
- [26] Moura, S. S. S., Silva, R. dos S., Alves, E. U., Gonçalves, E. P., Araújo, L. D. A. de, Alves, M. M., Araújo, P. C. (2019): Morphology of seeds, seedlings, and young plants of *Dimorphandra gardneriana* Tul. *Semina: Ciências Agrárias*, 40: 1063–1078. <https://doi.org/10.5433/1679-0359.2019v40n3p1063>
- [27] Poveda, J. (2020): *Trichoderma parareesei* favors the tolerance of rapeseed (*Brassica napus* L.) to salinity and drought due to a chorismate mutase. *Agronomy*, 10: 118. <https://doi.org/10.3390/agronomy10010118>
- [28] Navure Team. (2023): Navure (3.0.0): A data-science-statistic oriented application for making evidence-based decisions. Software. Available from: <http://www.navure.com> (last consult: 2025/07/01).
- [29] Yi, G., Wu, W., Wei, T. (2021): Delivery of rice gall dwarf virus into plant phloem by its leafhopper vectors activates callose deposition to enhance viral transmission. *Frontiers in Microbiology*, 12: 662577. <https://doi.org/10.3389/fmicb.2021.662577>
- [30] Suwanchaikasem, P., Idnurm, A., Selby-Pham, J. (2024): The impacts of chitosan on plant root systems and its potential to be used for controlling fungal diseases in agriculture. *Journal of Plant Growth Regulation*, 43: 3424–3445. <https://doi.org/10.1007/s00344-024-11356-1>
- [31] Wang, Y., Li, X., Fan, B., Zhu, C., Chen, Z. (2021): Regulation and function of defense-related callose deposition in plants. *International Journal of Molecular Sciences*, 22: 2393. <https://doi.org/10.3390/ijms22052393>
- [32] Luna, E., Pastor, V., Robert, J., Flors, V., Mauch-Mani, B., Ton, J. (2011): Callose deposition: a multifaceted plant defense response. *Molecular Plant-Microbe Interactions*, 24: 183–193. <https://doi.org/10.1094/MPMI-07-10-0149>
- [33] Sun, J., Liu, F., Xu, Y., Hu, W. (2025): Coatings applied to the optimization of *Portulaca oleracea* L. seed pellet formulation based on mixture design. *Coatings*, 15: 341. <https://doi.org/10.3390/coatings15030341>

- [34] Martínez-Cano, B., Mendoza-Meneses, C. J., García-Trejo, J. F., Macías-Bobadilla, G., Aguirre-Becerra, H., Soto-Zarazúa, G. M., Feregrino-Pérez, A. A. (2022): Review and perspectives of the use of alginate as a polymer matrix for microorganisms applied in agro-industry. *Molecules*, 27: 4248. <https://doi.org/10.3390/molecules27134248>
- [35] Cruz Sánchez, E., García, M. T., Pereira, J., Oliveira, F., Craveiro, R., Paiva, A., Gracia, I., García-Vargas, J. M., Duarte, A. R. C. (2023): Alginate–chitosan membranes for the encapsulation of lavender essential oil and development of biomedical applications related to wound healing. *Molecules*, 28: 3689. <https://doi.org/10.3390/molecules28093689>
- [36] Wibowo, A. H., Fehragucci, H., Purnawan, C. (2023): Effect of plasticizer addition on the characteristics of chitosan alginate edible film. *Alchemy Journal of Chemical Research*, 19: 123–129. <https://doi.org/10.20961/alchemy.19.2.71348>
- [37] Eslami, Z., Elkoun, S., Robert, M., Adjallé, K. (2023): A review of the effect of plasticizers on the physical and mechanical properties of alginate-based films. *Molecules*, 28: 6637. <https://doi.org/10.3390/molecules28186637>
- [38] Mehta, C., Pawar, S., Suvarna, V. (2024): Recent advancements in alginate-based films for active food packaging applications. *Sustainable Food Technology*, 2: 1246–1265. <https://doi.org/10.1039/d3fb00216k>
- [39] Peña-Datoli, M., Hidalgo-Moreno, C. M. I., González-Hernández, V. A., Alcántar-González, E. G., Etchevers-Barra, J. D. (2016): Recubrimiento de semillas de maíz (*Zea mays* L.) con quitosano y alginato de sodio y su efecto en el desarrollo radical. *Agrociencia*, 50: 1091–1106. Available from: <http://www.redalyc.org/articulo.oa?id=30249305011>
- [40] Durgadevi, P., Girigoswami, K., Girigoswami, A. (2025): Biodegradable nanomaterials in boosting seed vigor and germination: seed coating towards sustainability. *Discover Applied Sciences*, 7: 695. <https://doi.org/10.1007/s42452-025-06737-4>
- [41] Xing, Y., Xu, Q., Li, X., Chen, C., Ma, L., Li, S., Che, Z., Lin, H. (2016): Chitosan-based coating with antimicrobial agents: preparation, property, mechanism, and application effectiveness on fruits and vegetables. *International Journal of Polymer Science*, 2016: 4851730. <https://doi.org/10.1155/2016/4851730>
- [42] Yadav, A., Yadav, K., Ahmad, R., Abd-Elsalam, K. A. (2023): Emerging frontiers in nanotechnology for precision agriculture: advancements, hurdles and prospects. *Agrochemicals*, 2: 220–256. <https://doi.org/10.3390/agrochemicals2020016>