Nanopriming with polymeric nanoparticles improves biochemical attributes and germination in wheat seeds

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Abstract:

Nanomaterials have emerged as smart agents with growth-promoting and stress-emulating potential for sustainable agriculture. In recent years, biopolymers have shown great potential as plant growth stimulants. In this study, chitosan, alginate and BSA have been used to prepare CS-Alg-BSA NPs as a nanopriming agent for wheat seeds. The prepared samples remodeled biochemical attributes in primed seeds and improved the germination parameters. The priming treatments significantly increased the vital biomolecules, including proteins and sugars. It controlled ROS production, up-regulated antioxidants and significantly reduced MDA. These biochemical readjustments consequently improved the final germination percentage, germination energy and germination index. The priming memory imprints enabled the seeds to show sustainable germination and uniform seedling development.

Keywords: Sustainable growth; priming memory imprints; nanopriming, biopolymers; nanocoating

1. Introduction

Over the last few decades, abiotic stresses and ecotoxicological conditions have severely threatened crop productivity, posing challenges to sustainable agriculture [1, 2]. They hamper the productivity of highly demanded commercial crops creating food security issues, especially in developing countries. Among cereals, wheat is a highly demanded industrial crop and a major food around the globe. Over the years, there has been a

continuous decrease in its yield with compromised grain quality, due to the increasing severity of abiotic stresses. They delay seed germination, retards seedling growth and development, leading to low yield [3]. They induce ROS production, interrupting cellular signaling and antioxidant defense, consequently disrupt cellular integrity. Such physiological and biochemical complications delay seed germination, the first stage, which otherwise ensures better plant growth and final crop productivity. Germinating seeds adopt several mechanistic strategies at the sub-cellular level to counteract such negative impacts. Generally, the stress-mitigating approaches include activating antioxidants and readjustment of metabolic activities. They also increase the production of growth-promoting hormones, sugars, proteins and other biomolecules as stress-protective measures. Over the last decade, priming has become a valuable seed pre-treatment technique, enabling it to tolerate harsh environments. Priming helps the seed to readjust metabolic activities, signaling pathways and upregulate antioxidants [4]. All such physio-biochemical activations improve seed quality for fast germination. Furthermore, the priming-memory imprints facilitate the seed to acclimatize the environmental conditions during seedling establishment, plant growth and development stages. Over the years, various priming agents have shown appreciable growth-promoting and stress-insulating effects. In recent years, great efforts have been made to increase the applications of natural polymers as stimulants under the concept of sustainable agriculture. Recently, nanomaterials have emerged as ecofriendly and smart agents with growth-promoting and stress-emulating potential [5]. However, the applications of metallic-based nanomaterials as pesticides, fungicides and fertilizers have raised serious health concerns. Accordingly, efforts are underway to develop nonhazardous plant-growth stimulants from natural and sustainable sources.

Over the last decade, various polymeric materials have been evaluated as stimulants for seed germination and plant growth. Chitosan (CS) is a cationic polysaccharide, biocompatible and biodegradable biopolymer showing great potential for applications in sustainable agriculture. It can regulate carbon and nitrogen metabolism, antioxidants and cellular signaling for plant-growth stimulation [6]. The seed priming with CS mitigated the toxicity of salt stress and improved germination and growth performance [7-9]. Alginate (Alg), a natural polysaccharide, has shown potential for regulating seed germination and seedling development [10]. Alginate-based various nanocomposites have shown wide applications in abiotic stress management and sustainable agriculture [11]. Bovine serum albumin (BSA), the natural water-soluble protein, has demonstrated the potential to reduce the toxicity of AgNPs and ZnO NPs in germinating seeds and growing plants [12]. The CS, Alg and BSA have shown considerable applications in plant growth regulation; however, a study based on their combined potential is still lacking. Considering the efficiency of polymeric nanomaterials and the value of seed pre-conditioning, we envisioned preparing a biopolymer-based non-hazardous nanocoating for seeds. Herein, we report the synthesis of polymeric chitosan-alginate-BSA nanoparticles (CS-Alg-BSA NPs) and their subsequent use as a nanopriming agent for wheat seeds.

2. Materials and Methods

2.1 Preparation and characterization of polymeric nanopriming agents:

The ionic gelation method was used to prepare polymeric CS-Alg-BSA NPs as nanopriming agent. The ionic gelation method is well-established, fast and easily reproducible even at large scale. The electrostatic interactions between chitosan, alginate

and BSA lead to the production of nanoparticles. The electrostatic interactions between opposite charges fascilitates the polyelectrolyte complexation, which acts as a convenient alternative to chemical crosslinking agents. The interactions between the positively charged amino groups from chitosan and the negatively charged carboxylic acid groups from alginate and BSA led to NPs formation.

The CS (1.5%) was prepared in 0.5 % acetic acid solution and stirred at room temperature until the solution became transparent. Then sodium alginate (0.75 %) was prepared in distilled water and added dropwise to the CS solution with continuous stirring for 30 min. Afterward, the BSA solution was added slowly with constant stirring. The 0.25, 0.5 and 0.75% BSA solutions were used to prepare three different samples of CS-Alg-BSA NPs as A, B and C. The nanoparticles were precipitated after centrifugation at 4500 x g for 20 min, dried, ground and characterized.

The detailed morphological structure of the prepared samples were studied using SEM analysis. The SEM image revealed the layered mesoporous structure of the polymeric nanocomposite. The low-intensity bands and a flat background in XRD of the prepared samples established the predominant amorphous nature of the nanocomposites. FTIR spectroscopic analyses were used to detect functional groups and their nature of interactions. The prepared samples exhibited FTIR spectra with characteristic peaks (Figure 1).

2.2 Priming treatments and germination study:

The spring wheat (*Triticum aestivum* L. cv. Akbar-2019) seeds were received from ARI, Faisalabad, Pakistan. The prepared CS-Alg-BSA NPs A, B and C samples were used as nanopriming agents (0.5 ppm (a), 1 ppm (b) and 1.5 ppm (c) solution) for 8 hrs. Later,

seeds were rewashed and dried to their original weight at 25±1 ºC. Some seeds were hydro-primed and used for comparison studies with nonprimed control.

Rules for seed testing set by the Association of Official Seed Analysts were followed for studying the germination of nanoprimed and control seeds [13]. In 12 cm diameter petri plates, three replicates of 24 seeds were allowed to germinate at 25±1 °C under normal conditions. Germinated seeds (radicle and coleoptile lengths 2-3 mm) were counted twice a day till maximum germination was observed.

2.3 Biochemical studies:

Seed samples were ground using specific extraction buffers and centrifuged for 25 minutes at 10,000×g. The supernatant was used for different biochemical studies employing the known spectrophotometric methods.

2.3.1 Enzymatic antioxidants:

The activity of superoxide dismutase (SOD) was assessed from its capacity to prevent the photochemical reduction of nitroblue tetrazolium (NBT) [14]. One unit of SOD activity corresponds to the amount of enzyme causing 50% inhibition of the photochemical reduction of NBT. The peroxidase (POD) and catalase (CAT) were also evaluated using the known methods [15]. An absorbance variation of 0.01 units/min corresponds to one unit of enzyme activity expressed on a seed weight basis. An increase in absorbance of the solution at 470 nm and a decline at 240 nm provided the values.

2.3.2 Proteins and sugars:

The bovine serum albumin was employed as standard for the estimation of total soluble proteins (TSP) following the known method [16]. A well-established method using dinitrosalicylic acid followed for the determination of reducing sugars [17]. The phenol sulfuric acid was used for the estimation of total sugars. The glucose was used for the

preparation of the standard curve and subsequent estimation of reducing and total sugars. Further, the difference between total sugars and reducing sugars provided the amount of non-reducing sugars.

2.3.3 Malondialdehyde contents:

The lipid peroxidation was assessed by measuring malondialdehyde (MDA) content using thiobarbituric acid (TBA). According to the procedure, 0.1% TCA and sample (0.2 g) were homogenized and centrifuged at 10000 x g. The mixture of 20% TCA, 0.05% TBA and supernatant was heated at 100 °C for 15 min. Then, the absorbance at 532 nm, and the extinction coefficient ($155 mM^{-1} cm^{-1}$) were used for the calculation of MDA [18].

2.4 Germination parameters:

The nanoprimed, hydroprimed and nonprimed control were allowed to germinate and used for the calculation of final and mean germination, germination energy, vigor and germination index.

2.4.1 Mean germination time:

The following equation was used to calculate mean germination time (MGT) [19].

$MGT = \sum Dn / \sum n$

Where n = the number of seeds germinated on day D

D = the number of days counted from the start of germination

2.4.2 Final Germination Percentage:

The final germination percentage (FGP) was calculated following known formula

FGP = No of seeds germinated on final day/Total no of seeds sown × 100

2.4.3 Germination index:

The germination index (GI) was calculated following formula provided by the AOSA [13].

GI = number of germinated seeds/Days of first count + ----------+ number of germinated seeds/days of final count

2.4.4 Germination Energy:

Germination energy was noted on post-planting day 4. It is the % of germinated seeds on post-planting day 4 relative to the total number of seeds [20].

2.5 Statistical analyses

The significance of data was measured using analyses of variance and the Tukey (HSD) Test at $p<0.01$ or $p<0.05$ using XLSTAT software.

3. Results

The untreated control, nanoprimed and hydroprimed seeds were subjected to germination in separate petri dishes. The abovementioned seeds were used for biochemical analysis and germination studies and compared with the control.

3.1 Effects of nanopriming on seed biochemistry:

3.1.1 Enzymatic antioxidants and lipid peroxidation markers:

Priming treatments significantly increased CAT (A (15-5%), B (23-63%), and C (19-51%)) in treated seeds compared to the control. All samples induced a concentration-dependent increase, and the maximum impact was observed with Ac. Nanopriming also caused a significant rise in POD; however, only samples B (50-88%) and C (80-91%) induced a concentration-dependent increase. The treatments with sample A triggered a substantial but somewhat similar impact. All priming treatments resulted in a significant increase in SOD (29-31%) compared to the control; however, there was a non-significant difference in the effect of all treatments. Thus, the priming treatments with the polymeric nanomaterial upregulated the enzymatic antioxidants. All nanopriming treatments

significantly reduced the MDA contents (A (9-22%), B (14-1%), and C (15-29%)) compared to the control. The maximum decrease in MDA was detected in seeds primed with Cc (Table 1).

3.1.2 Potent biomolecules:

Compared to the control, a concentration-dependent and significant increase in TSP (A (4-17%), B (3-9%), and C (5-16%)) was observed in nanoprimed seeds. However, the maximum increasing impact was recorded in seeds treated with Ac and Cc. All nanopriming treatments significantly increased the reducing sugars (A (31-33%), B (20-27%), and C (18-30%)). The maximum increment was observed with treatments of sample A. Nanopriming treatments also induced a significant increase in non-reducing sugars (A (3-7%), B (5-6%), and C (4-9%)). The priming treatment Cc resulted in the maximum increase compared to the control. All priming treatments significantly increased the total sugars (A (5-9%), B (5-8%), and C (4-10%)) in treated seeds compared to the control. However, the priming with Cc resulted in a maximum increase in total sugars compared to the control (Figure 2).

3.1.3 Hydrolytic enzymes:

Both the hydrolytic enzymes viz. α-amylase (A (39-51%), B (45-57%), and C (42-56%)) and protease (A (9-26%), B (6-29%), and C (19-31%)) increased significantly in nanoprimed seeds compared to the control. Priming with Cc caused a maximum increase in α-amylase and protease compared to the control (Figure 3).

3.2 Effects of nanopriming on germination parameters:

The nanopriming resulted in significant improvements ($p<0.05$) in final germination compared to the control. The treatment with A (8-10%), B (7-9%) and C (7-10%) increased the final germination of primed seeds. A significant reduction in mean germination time was observed in seeds primed with A and B (3-6%) and C (4-5%). There was a significant increase in the germination index with A (15-19%), B (9-15%) and C (7-9%). The nanopriming reduced the germination energy significantly with A (6-12%), B (9-12%) and C (16%) (Table 2).

4. Discussion

Germinating seeds activate their antioxidant machinery to counter the stress-induced overproduction of ROS. Accordingly, the upregulation of enzymatic antioxidants like CAT, SOD and POD is considered an important ROS-mitigating approach [21]. The CS activates the antioxidant machinery, regulates metabolism and other biochemical pathways for smooth germination and sustained plant growth [6]. Thus, it prevents lipid peroxidation and keeps cellular structures intact for smooth physiological and biochemical functioning. The CS could also control oxidative stress by regulating the non-enzymatic antioxidants through several cascade reactions. It could maintain the biochemical pathways for the synthesis of secondary metabolites, including polyphenols, as a stress acclimation approach. It conferred insulation against abiotic stress by upregulating the activity of CAT, POD and SOD in tomatoes, eggplants and milk thistle [22, 23]. It also stimulated growth parameters in soybean plants and provided antioxidant protection to sorghum plants under salt stress [24, 25]. The alginate gel reduced the phytotoxicity of silver NPs and upregulated the antioxidant enzymes including CAT and SOD in germinating cucumber seeds [26]. The BSA-capped AuNCs increased the

antioxidant capacity and promoted growth parameters in *A. thaliana* seedlings [27]. Accordingly, in our case, seed preconditioning with the CS-Alg-BSA significantly upregulated the expression of enzymatic antioxidants. Therefore, the activated antioxidant machinery provided insulation against ROS, which was duly represented by low MDA contents compared to the control.

Proteins are the major source of amino acids, available as potent reservoirs of carbon and nitrogen for germinating seeds and developing seedlings. Various enzymes, being proteins in nature, control metabolism, coordinate signaling pathways and manifest a series of physiological functions. The CS induces the synthesis of important molecules like proteins under stress conditions. The CS increased protein contents by controlling vital enzymes of glycolysis and regulated the protein expressions as a stress countermeasure in tomato seedlings [28]. Seed treatments with Ca-alginate submicroparticles (100 µg/ml) enhanced the TSP (4.5%) compared to the control [29]. In another study, the alginate increased heat shock proteins in corn plants grown in Ce-mixed soil [30]. The BSA acts as a pool of amino acids; thus, it could regulate various functions in germinating seeds [31]. In our case, nanopriming induced a substantial increase in TSP, which could have regulated the metabolic activities and enzymatic antioxidants.

Sugars are potent for metabolic activities and energy generation, mediating smooth seed germination and seedling establishment. They could protect biological membranes by acting as ROS scavengers and osmoprotectants under stress conditions. The seed pretreatments with CSNPs (50 mg/ml) increased TSS in Lupine termis L. plants [32]. The film coating of *Z.* mays seeds with Alg (1% sol) induced a substantial increase in soluble

sugars under osmotic stress conditions [33]. In our case, a significant increase in sugars highlighted the positive role of nanopriming in modulating the biochemical attributes in treated seeds.

The CS-Alg-BSA NPs-mediated nanopriming is envisioned to have integrated the antioxidative machinery to maintain ROS at threshold gradient. It has protected the membranes from cellular damage and reduced MDA. The oxidative stress could damage functional biomolecules like nucleic acids, proteins and lipids, leading to atypical cell structure and functional abnormalities [34, 35]. The controlled oxidative stress avoids negative impacts on metabolism, signaling pathways and subcellular structures, warranting membrane integrity [36]. The upregulated hydrolytic enzymes, increased TSP and sugar contents positively influence the germination.

The nanopriming caused a significant increase in the final germination percentage and germination index. Furthermore, its growth-stimulating potential significantly reduced the mean germination time and energy. The seed preconditioning with nanocomposite stimulated the priming memory, ensuring sustainable germination and seedling establishment.

5. Conclusions

Over the last decade, climate change and abiotic stresses have severely threatened crop productivity, posing challenges to sustainable agriculture. Germinating seeds adopt several mechanistic strategies at the sub-cellular level to counteract such negative impacts. Recently, priming has become a valuable seed pre-treatment technique, empowering seeds to tolerate harmful environments. In this study, the priming treatments with polymeric NPs controlled the ROS production, activated antioxidants and hydrolytic

enzymes in wheat seeds. The nanopriming caused a substantial increase in proteins and sugars in primed seeds. All such biochemical readjustments reduced MDA and improved membrane stability, ensuring sustainable germination.

A future mechanistic study is recommended to elucidate the interaction of polymeric NPs with enzymatic antioxidants and other biomolecules, resulting in improved germination performance.

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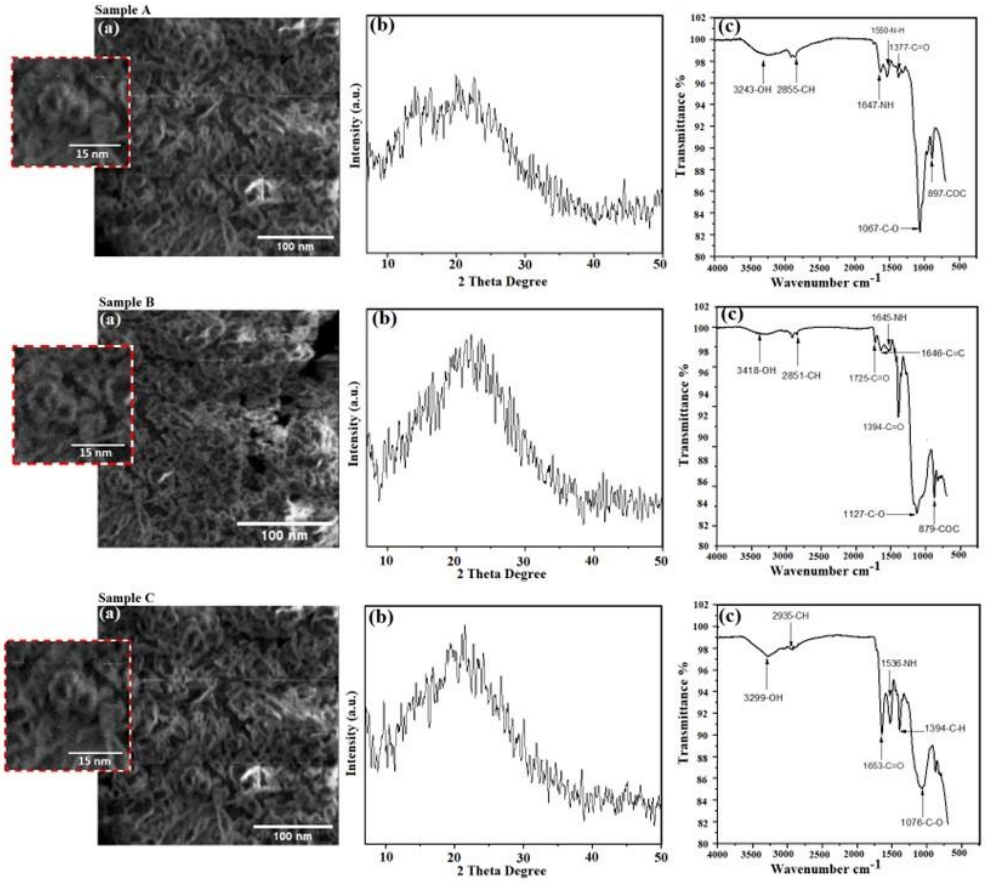
Conflicts of Interest: "The authors declare no conflict of interest."

References:

1. Verma, K.K., et al., *Climate change adaptation: Challenges for agricultural sustainability.* Plant, Cell & Environment, 2025. **48**(4): p. 2522-2533.
2. Zou, F., et al., *Do technological innovation and environmental regulation reduce carbon dioxide emissions? evidence from China.* Global NEST Journal, 2024. **26**(7): p. 1-15.
3. Mukhopadhyay, R., et al., *Soil salinity under climate change: Challenges for sustainable agriculture and food security.* Journal of Environmental Management, 2021. **280**: p. 111736.
4. Wojtyla, Ł., et al., *Molecular processes induced in primed seeds—increasing the potential to stabilize crop yields under drought conditions.* Journal of Plant Physiology, 2016. **203**: p. 116-126.
5. An, C., et al., *Nanomaterials and nanotechnology for the delivery of agrochemicals: strategies towards sustainable agriculture.* Journal of Nanobiotechnology, 2022. **20**(1): p. 11.
6. Stasińska-Jakubas, M. and B. Hawrylak-Nowak, *Protective, Biostimulating, and Eliciting Effects of Chitosan and Its Derivatives on Crop Plants.* Molecules, 2022. **27**(9): p. 2801.

7. Soni, A.T., J.E. Rookes, and S.S. Arya, *Chitosan nanoparticles as seed priming agents to alleviate salinity stress in rice (Oryza sativa L.) seedlings.* Polysaccharides, 2023. **4**(2): p. 129-141.
8. Riseh, R.S., et al., *Chitosan coating of seeds improves the germination and growth performance of plants: A Rreview.* International Journal of Biological Macromolecules, 2024: p. 134750.
9. Nandini, T., et al., *A review-chitosan nanoparticles towards enhancing nutrient use efficiency in crops.* International Journal of Biological Macromolecules, 2025: p. 141433.
10. Aly, A.A., N.E. Eliwa, and G. Safwat, *Role of gamma-irradiated sodium alginate on growth, physiological and active components of iceberg lettuce (Lactuca sativa) plant.* BMC Plant Biology, 2024. **24**(1): p. 185.
11. Ray, A., et al., *Nanocomposite-based agricultural delivery systems: a sustainable approach to enhanced crop productivity and soil health.* Journal of Nanoparticle Research, 2025. **27**(4): p. 110.
12. Janani, B., et al., *Impact of bovine serum albumin–A protein corona on toxicity of ZnO NPs in environmental model systems of plant, bacteria, algae and crustaceans.* Chemosphere, 2021. **270**: p. 128629.
13. Anonymous., *Association of Official Seed Analysts (AOSA). Rules for testing seeds.* Seed Science & Technology, 1990. **12**: p. 1-112.
14. Giannopolitis, C.N. and S.K. Ries, *Superoxide dismutases: I. Occurrence in higher plants.* Plant Physiology, 1977. **59**(2): p. 309-314.
15. Chance, B. and A. Maehly, *Assay of catalases and peroxidases*, in *Methods in Enzymology*. 1955, Academic Press. p. 764-775.
16. Bradford, M.M., *A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding.* Analytical Biochemistry, 1976. **72**(1-2): p. 248-254.
17. Miller, G.L., *Use of dinitrosalicylic acid reagent for determination of reducing sugar.* Analytical Chemistry, 1959. **31**(3): p. 426-428.
18. Heath, R.L. and L. Packer, *Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation.* Archives of Biochemistry and Biophysics, 1968. **125**(1): p. 189-198.
19. Ellis, R. and E. Roberts, *The quantification of ageing and survival in orthodox seeds.* Seed Science and Technology (Netherlands), 1981.
20. Ruan, S., Q. Xue, and K. Tylkowska, *The influence of priming on germination of rice (Oryza sativa L.) seeds and seedling emergence and performance in flooded soil.* Seed Science and Technology, 2002. **30**(1): p. 61-67.
21. Hasanuzzaman, M., et al., *Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator.* Antioxidants, 2020. **9**(8): p. 681.
22. Safikhan, S., et al., *Role of chitosan on the growth, physiological parameters and enzymatic activity of milk thistle (Silybum marianum (L.) Gaertn.) in a pot experiment.* Journal of Applied Research on Medicinal and Aromatic Plants, 2018. **10**: p. 49-58.
23. Ortega-Ortiz, H., et al., *Enzymatic activity in tomato fruits as a response to chemical elicitors.* Journal of the Mexican Chemical Society, 2007. **51**(3): p. 141-144.
24. Mulaudzi, T., et al., *Antioxidant Capacity of Chitosan on Sorghum Plants under Salinity Stress.* Agriculture, 2022. **12**(10): p. 1544.
25. Nedved, E., et al., *Growth parameters and antioxidant activity in cucumber seedlings with the application of chitosan and hydroxycinnamic acids conjugates under salt stress.* Applied Biochemistry and Microbiology, 2022. **58**(1): p. 69-76.

26. Rutkowski, M., et al., *Impact of silver nanoparticles in alginate gels on seed germination, growth and stress biochemical parameters of cucumber seedlings.* Plant Stress, 2024: p. 100491.
27. Yan, Y., et al., *Enhancement of Photosynthesis and Alleviation of Abiotic Stress in Plants through Bovine Serum Albumin-Capped Gold Nanoclusters.* ACS Applied Nano Materials, 2023. **6**(22): p. 21101-21112.
28. Attia, M.S., et al., *Impact of Foliar Application of Chitosan Dissolved in Different Organic Acids on Isozymes, Protein Patterns and Physio-Biochemical Characteristics of Tomato Grown under Salinity Stress.* Plants, 2021. **10**(2): p. 388.
29. He, J., et al., *Effects of Calcium Alginate Submicroparticles on Seed Germination and Seedling Growth of Wheat (Triticum aestivum L.).* Polymers, 2018. **10**(10): p. 1154.
30. Zhao, L., et al., *Alginate modifies the physiological impact of CeO2 nanoparticles in corn seedlings cultivated in soil.* Journal of Environmental Sciences, 2014. **26**(2): p. 382-389.
31. Rogalinski, T., S. Herrmann, and G. Brunner, *Production of amino acids from bovine serum albumin by continuous sub-critical water hydrolysis.* The Journal of Supercritical Fluids, 2005. **36**(1): p. 49-58.
32. Bakhoum, G., M. Sadak, and M. Tawfic, *Chitosan and chitosan nanoparticle effect on growth, productivity and some biochemical aspects of Lupinustermis L plant under drought conditions.* Egyptian Journal of Chemistry, 2022. **65**(5): p. 537-549.
33. Behboud, R., A. Moradi, and H. Farajee, *Film coating with sodium alginate improves seed germination of sweet corn (Zea mays var. Saccharata) under osmotic stress conditions.* Plant Process and Function, 2021. **9**(40): p. 33-42.
34. Sachdev, S., et al., *Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms.* Antioxidants, 2021. **10**(2): p. 277.
35. Kerchev, P.I. and F. Van Breusegem, *Improving oxidative stress resilience in plants.* The Plant Journal, 2022. **109**(2): p. 359-372.
36. Considine, M.J. and C.H. Foyer, *Oxygen and reactive oxygen species-dependent regulation of plant growth and development.* Plant Physiology, 2021. **186**(1): p. 79-92.



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2 **Figure 1: Characterization of prepared polymeric CS-Alg-BSA NPs**

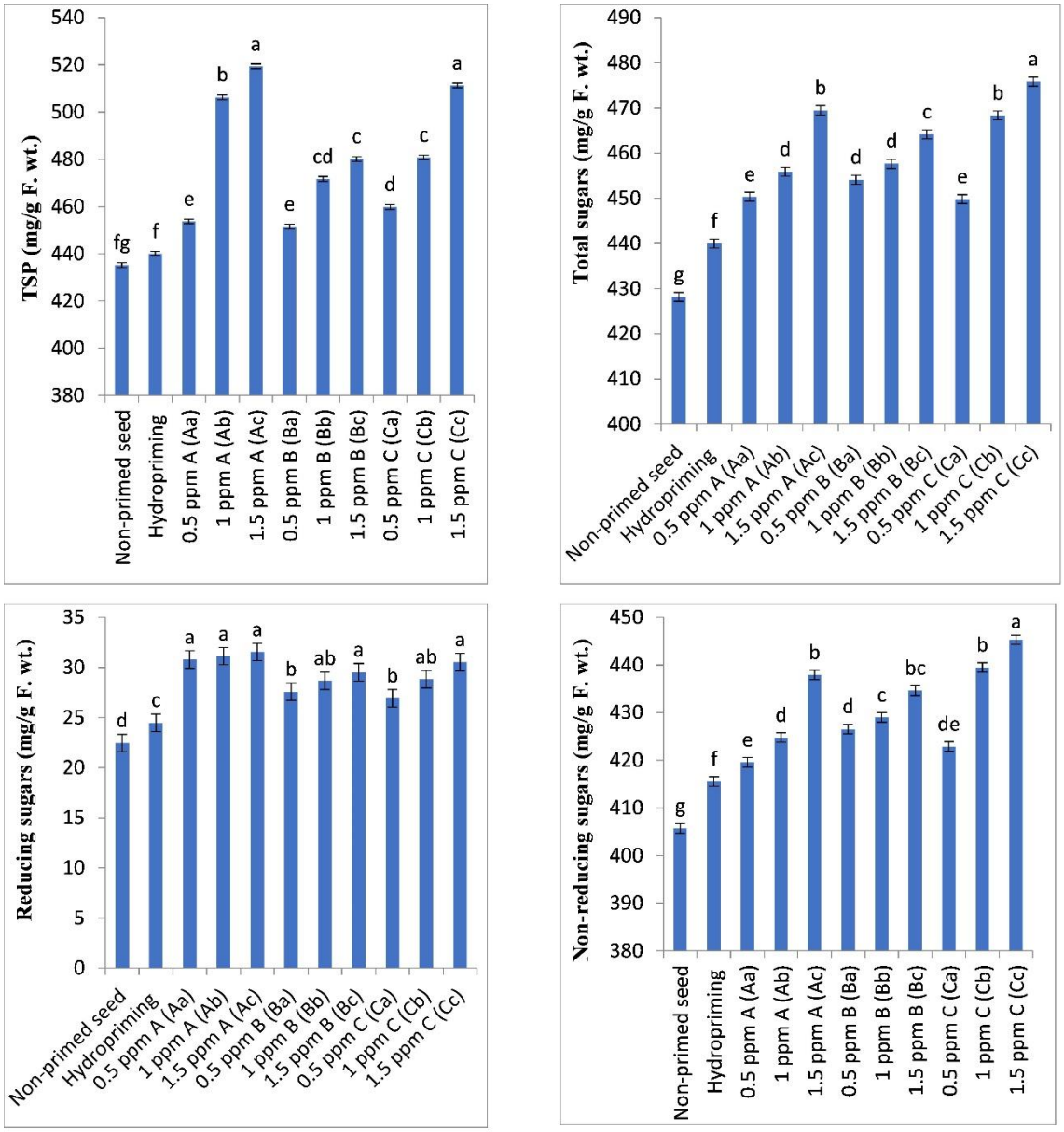

Figure 2: Effect of CS-Alg-BSA NPs priming on biomolecules in wheat seeds.

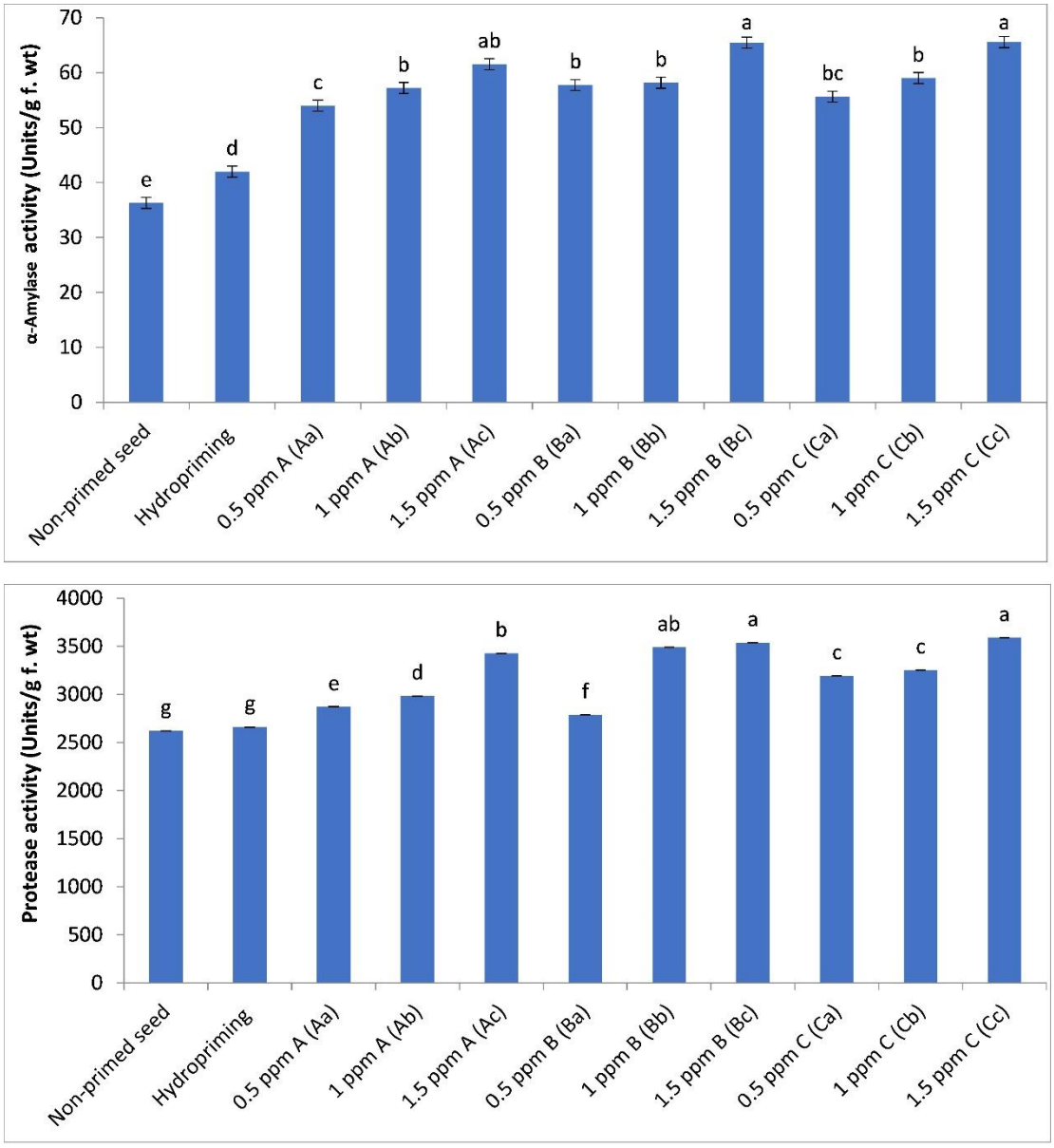

Figure 3: Effect of CS-Alg-BSA NPs priming on Hydrolytic enzymes in wheat seeds.

Table 1. Effect of CS-Alg-BSA NPs priming on enzymatic antioxidants and lipid peroxidation markers in wheat seeds.

Treatments	Seed Biochemistry			
	Enzymatic Antioxidants and lipid peroxidation markers			
	Catalase (CAT) (units/g f.wt)	Peroxidase (POD) (units/g f.wt)	Superoxide dismutase (SOD) (units/g f.wt)	Lipid peroxidation MDA (µM/g f. wt)
Control	42 ± 1.101 f	202.4 ± 0.6 h	117.721 ± 0.279 c	922.177 ± 0.823 a
Hydro-primed	43.5 ± 0.950 f (3.508%)	203.9 ± 0.099 h (0.738%)	140.675 ± 0.325 b (17.77%)	899.339 ± 0.661 b (-2.51%)
0.5ppm A (Aa)	49 ± 0.055 e (15.385%)	281.7 ± 0.3 g (32.76%)	162.41 ± 0.590 a (31.906%)	835.145 ± 0.855 c (-9.90%)
1ppm A (Ab)	62 ± 1.005 d (38.46%)	283.9 ± 0.41 g (33.52%)	160.936 ± 0.064 a (31.017%)	805.258 ± 0.742 d (-13.54%)
1.5ppm A (Ac)	105 ± 1 a (85.71%)	285.4 ± 0.36 g (34.04%)	161.556 ± 1.044 a (31.39%)	734.048 ± 1.095 e (-22.72%)
0.5ppm B (Ba)	53 ± 1 e (23.16%)	339 ± 0.255 f (50.46%)	157.072 ± 0.928 a (28.640%)	794.565 ± 0.435 d (-14.87%)
1ppm B (Bb)	62.± 0.255 d (38.46%)	402.4 ± 0.155 e (66.14%)	162.163 ± 0.837 a (31.76%)	787.355 ± 0.645 d (-15.77%)
1.5ppm B (Bc)	81 ± 0.600 b (63.41%)	524.5 ± 0.753 c (88.62%)	163.105 ± 0.895 a (32.32%)	766.597 ± 0.597 de (-18.42%)
0.5ppm C (Ca)	51 ±0.702 e (19.35%)	476.6 ± 0.3 d (80.76%)	159.067 ± 0.933 a (29.875%)	793.258 ± 1.258 d (-15.03%)
1ppm C (Cb)	62 ± 0.1 d (38.46%)	540.4 ± 0.76 b (91%)	161.37 ± 0.630 a (31.279%)	761.758 ± 0.324 de (-19.05%)
1.5ppm C (Cc)	71 ± 1 c (51.33%)	544.7 ± 0.4 a (91.63%)	161.658 ± 1.342 a (31.45%)	687.194 ± 0.806 f (-29.20%)

Note: Values of three replicates with S.D and relative percentage presented. Within a column, means sharing the same letters are non-significantly different (P>0.05) according to the Tukey's Test (HSD).

Table 2. Effect of CS-Alg-BSA NPs priming on germination parameters in wheat seeds

Treatments	Germination Parameters			
	Final Germination Percentage (%)	**Mean Germination Time (hours)**	**Germination index**	**Germination Energy**
Control	90 ± 0.082 d	56.425 ± 0.040 a	13.919± 0.015 f	11.333 ± 0.333 a
Hydro-primed	95 ± 0.732 c (5.41%)	56.379±0.054 a (- 0.082%)	14.245 ± 0.059 f (2.32%)	10.997 ± 0.548 a (-3.00%)
0.5ppm A (Aa)	98 ± 0.786 a (8.51%)	54.341± 0.072 c (- 3.76%)	16.305 ± 0.035 b (15.79%)	10.333 ± 0.125 b (-9.23%)
1ppm A (Ab)	100 ± 0.018 a (10.53%)	53.139± 0.041 d (- 5.99%)	17.338 ± 0.061 a (21.87%)	10.000± 0.575 b (-12.50%)
1.5ppm A (Ac)	100 ± 0.333 a (10.53%)	52.754±0.006 e (- 6.72%)	16.989 ± 0.102 a (19.86%)	10.667 ± 0.333 ab (-6.05%)
0.5ppm B (Ba)	97 ± 0.028 ab (7.49%)	55.989 ± 0.001b (- 0.78%)	15.334 ± 0.001 c (9.67%)	10.333 ± 0.345 b (-9.23%)
1ppm B (Bb)	98 ± 0.082 a (8.51%)	54.448± 0.009 c (- 3.57%)	15.667 ± 0.166 c (11.82%)	10.000 ± 0.027 b (-12.50%)
1.5ppm B (Bc)	99 ± 0.728 a (9.52%)	53.124 ± 0.030 d (- 6.03%)	16.337 ± 0.0247 b (15.98%)	10.000 ± 0.078 b (-12.50%)
0.5ppm C (Ca)	97 ± 0.631 a (7.49%)	55.940 ± 0.025 b (- 0.84%)	14.980 ± 0.0125 d (7.34%)	10.000 ± 0.0245 b (-12.50%)
1ppm C (Cb)	100 ± 0.082 a (10.53%)	53.944 ± 0.005d (- 4.50%)	15.330 ± 0.004 c (9.65%)	10.000 ± 0.018 b (-12.50%)
1.5ppm C (Cc)	100 ± 0.075 a (10.53%)	53.459± 0.045 d (-5.40%)	15.338 ± 0.009 c (9.70%)	10.000 ± 0.0545 b (-12.50%)

Note: Values of three replicates with S.D and relative percentage presented. Within a column, means sharing the same letters are non-significantly different (P>0.05) according to the Tukey's Test (HSD).