Comparative analysis of synthetic and organic chitosan nanoparticles as seed primers on germinated soybeans

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ABSTRACT

The present study investigated the effect of synthetic and organic chitosan nanoparticles (CNPs) at different concentrations (20–100 ppm) on soybean germination performance, bioactive components, and antioxidant potential. Germination parameters including radical length, seedling weight, and germination percentage were significantly influenced by nanoparticle treatments. Compared with control (T), both synthetic and organic CNPs enhanced radical length and germination rate, with the highest improvements observed at 100 ppm (T5 and T50). Organic CNPs consistently showed slightly superior performance in stimulating seed vigor compared to synthetic counterparts. Analysis of bioactive components revealed that CNP application significantly elevated total phenolic content (TPC), flavonoids, and flavanols in germinated soybeans. At 60 ppm, organic CNPs (T30) recorded the highest levels of flavonoids (2.25 mg catechin/100 g) and flavanols (0.30 mg catechin/100 g), highlighting the role of chitosan in inducing secondary metabolite synthesis. These findings are consistent with previous reports that nanoparticles act as elicitors, activating phenolic and flavonoid biosynthesis pathways. Antioxidant activity, measured as radical scavenging capacity, increased in a concentration-dependent manner. The control (45.34%) was significantly lower than treated samples, with the maximum activity observed at 100 ppm organic CNPs (86.3%). The higher antioxidant potential corresponds with elevated phenolic and flavonoid content, suggesting that CNPs enhance reactive oxygen species (ROS) scavenging systems. In a nutshell, the study demonstrated that CNPs, particularly organic variants at 100 ppm, effectively improve germination, bioactive compounds, and antioxidant activity in soybeans. These results highlighted the potential of CNPs as sustainable, eco-friendly biostimulants to enhance crop quality and functional value.

Keywords: *Color profile and bioactive compounds, Germination, Nutritional composition, Organic and synthetic chitosan nanoparticle, Seed primers.*

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Highlights of this paper

- Organic Chitosan Nanoparticles significantly improved physical parameters of germination.
- Organic Chitosan Nanoparticles at 60 ppm showed the highest flavonoid and flavanol levels, indicating stimulation of secondary metabolite synthesis.
- Antioxidant activity peaked at 86.3% with 100 ppm organic organic Chitosan Nanoparticles linked to higher phenolics and ROS control.

1. INTRODUCTION

Chitosan nanoparticles (CNPs) are categorized as organic or synthetic, according to the way they are produced and used (Divya & Jisha, 2018). Organic nanoparticles are found in natural chitin deposits like the shells of shrimps, insect exoskeletons or fungi and are produced by processes that are non-polluting like ionic gelation or natural stabilizers (Rodríguez-Félix, Madera-Santana, & Freile-Pelegrín, 2017). They are inexpensive, biodegradable, and biocompatible, and thus suitable in agriculture, food, nutraceuticals, and cosmetics (Nawaz et al., 2025). Synthetic nanoparticles, by contrast, are chemically made via a variety of mechanisms, including crosslinking, polymer grafting, or nanofabrication to generate homogenous, soluble, and size-tunable structures to an extremely high level of homogeneity (Mavila, Eivgi, Berkovich, & Lemcoff, 2016). They are useful in very sophisticated biological applications such as drug delivery, cancer therapy and tissue engineering, but are more complex and expensive to produce (Prabaharan & Mano, 2004).

In recent decades, environmental stress and ecotoxicological factors have significantly affected the germination, development, and evaluation of cereal crops. Nearly every aspect of human existence has been revolutionized by nanotechnology, particularly in its vital contribution to agriculture (Thakur, Thakur, & Kumar, 2018). CNPs have been valued for their ability to improve plant defensive responses, seed growth, and sprout development. Topically applied CNPs enhanced the growth and yield of wheat, beans, and coffee (Channab et al., 2024).

Chitosan nanoparticles are now considered a potential alternative to conventional seed treatments because they enhance water absorption, enhance enzyme activity, and stimulate seed defenses (Sangwan, Sharma, Wati, & Mehta, 2023). Due to their nanoscale size, they are able to penetrate the seed coat effectively and interact with cellular structures, enzymes and signaling pathways to induce metabolism prior to germination. Such early germination enables the seeds to germinate at a faster rate, more uniformly and with higher germination percentages as compared to the untreated seeds (Durgadevi, Girigoswami, & Girigoswami, 2025).

Besides tolerance to stress, CNP seed priming has also been shown to promote nutrient uptake and metabolic efficiency. The mobilization of stored food reserves through the action of hydrolytic enzymes, including amylases and proteases, with the aid of chitosan nanoparticles leads to increased root and shoot development in the initial growth phase. In addition, their antibacterial properties can protect seeds against soil-borne diseases, reduce seed mortality, and promote healthier plant stands (Sen & Das, 2024).

Even though soybeans are vital for nutrition and the economy, it remains difficult to get them to germinate evenly and fast, posing a challenge for long-term production (Sedibe, Mofokeng, & Masvodza, 2023). Traditional seed germination processes often do not work well in terms of environmental safety, effectiveness, or long-term usefulness. A new and sustainable technique for enhancing plant performance and seed vigour is nanotechnology (Do Espirito, Caixeta, Fernandes, & Santaella, 2021). Due to their plant growth capabilities and eco-friendly nature, chitosan nanoparticles have become a popular nanomaterial of choice. It is, however, not clear that synthetic or organic chitosan nanoparticles are more effective as seed primers. Consequently, the aim of the present study is to determine the effectiveness of synthetic and organic chitosan nanoparticles in different concentrations on soybean germination and initial growth performance. Also, to determine the nutritional characteristics, colour profile and bioactive compounds.

2. MATERIAL AND METHODS

2.1. Procurement of Materials

Soybeans were bought from Multan local market, Pakistan. These were thoroughly cleaned to get rid of any contaminants that might prevent them from germinating normally, such as dust, soil particles, or cracked or shriveled seeds.

2.2. Synthetic Chitosan Nanoparticles

A commercial provider (Alibaba, China) provided synthetic chitosan nanoparticles. The nanoparticles were analytical grade and given in powdered form. They were received in airtight containers and maintained at room temperature under dry conditions until usage. A stock solution was made by dissolving 0.25 g of nanoparticles in 500 mL of distilled water, then swirling continuously with a magnetic stirrer to ensure uniform dispersion. Different concentrations of CNPs were produced from this stock solution (20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm) and utilized for seed priming treatments (Islam, Sattar, Sattar, Perveen, & Akhlaq, 2023).

2.3. Organic Chitosan Nanoparticles

Using distilled water, 2g of sodium alginate was dissolved in 100ml of distilled water to create a 2% sodium/calcium alginate solution. The beaker was then heated to 500ºC for 45 minutes on a hot plate. In order to obtain the required 0.04M solution, 4.4g of calcium chloride (hardening solution) was added to a beaker filled with 100 ml of distilled water. A beaker filled with regents was set on a hot plate at 50°C and 200 rpm to ensure adequate mixing. The straightforward emulsion extrusion method was used to complete the procedure. To thoroughly mix the $CaCl_2$ solution, the beaker will be placed on a hot plate and stirred with a magnetic stirrer. For 45 minutes, that treatment was administered at 50ºC. The 1 cc syringe with 36 G needle was filled with a mixture of sodium/calcium alginate and chitosan based on the treatments. Drop by drop, this solution was added to the $CaCl_2$ solution (which remained on the heated plate). The formation of the round-shaped nanoparticles began.

2.4. Seed Treatment

The soybean seeds were rinsed with distilled water after being surface sterilized with a 4% sodium hypochlorite solution for five minutes. The seedlings were subsequently immersed for two hours at ambient temperature in the designated chitosan nanoparticle solutions. Then they were rinsed with water and kept in water for 12hours. Seeds were employed for germination by keeping 100 grams on a wet jute bag for 48 hours. Sprouted beans were dried and kept in a polyethylene bag for further analysis.

2.5. Determination of Physical Parameter and Color Profile

Radical length, post-germination weight gain, and percentage germination were determined (Islam et al., 2023). The color of germinated soybean samples treated with organic and synthetic CNPs were examined using CR-10 Colorimeter (Minolta Co. Ltd., Japan) to determine any visual or biochemical changes that took place during germination.

2.6. Determination of Nutritional Composition

Nutritional composition i.e., crude ash (protocol no. 923.03), moisture (protocol no. 925.10), fat (protocol no. 920.85), protein (protocol no. 920.87), and fiber (protocol no. 32–10) of the raw samples were determined by the

working standard protocols as mentioned in Official Methods of Analysis in Association of Official Analytical Chemists (AOAC) (Pojić, Kravić, & Stojanović, 2015).

2.7. Determination of Bioactive Compounds

2.7.1. Extract Preparation for Determination of Bioactive Compounds

Accurately measured 10 g of flour was added in 100 ml 100% (v/v) ethanol and homogenized by using an orbital shaker for 8 h. To separate the extract, the mixture was filtered by using filter paper (Whatman No.1). Rotary evaporator (Rotary Vacuum Evaporator, EYELA N.N. Series) was used to remove the residual solvent of ethanolic extract at 40 °C under reduced pressure. The obtained extract was then used to analyze the DPPH activity, Total phenolic content (TPC), Total Flavonoid Content (TFC) and Total Flavanols (TF) (Islam et al., 2023).

2.7.2. Determination of Free Radical Scavenging Activity by DPPH

Accurately 2.9 ml sample extract was dissolved with 0.1 ml of 0.0 mM DPPH and placed in the dark at room temperature for 30 min at 23 °C. The sample was filtered after 30 min to measure the absorbance at 517 nm by using a spectrophotometer (UV–Vis 3000, ORI, Germany). A control solution was prepared by mixing 0.1 ml of methanol in 2.9 ml DPPH solution (Aryal et al., 2019).

DPPH activity was measured by using the following equation.

$$\text{DPPH } \% = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} * 100 \quad (1)$$

2.7.3. Determination of Total Phenolic Content

Accurately measured sample extract of 1 ml was dissolved in 1 ml of Folin-Ciocalteus reagent and 1 ml of sodium carbonate solution (7.5%). The solution was allowed to stand for 30 min and then absorbance was measured at 765 nm by using a UV–VIS spectrophotometer (UV–Vis 3000, ORI, Germany). The total phenolic content was expressed as mg GAE/g (Aryal et al., 2019).

2.7.4. Determination of Total Flavonoid Content

The accurately measured 1 ml sample extract was dissolved with 0.2 ml of AlCl3 (10% solution prepared in Methanol), 0.2 ml of potassium acetate (1 M), and 5.6 ml of distilled water. The solution was kept for 30 min at room temperature. Then absorbance was measured at 415 nm by using a UV–Vis spectrophotometer (UV–Vis 3000, ORI, Germany). A standard solution was prepared by dissolving 0–500 µg/mL of quercetin in ethanol (Aryal et al., 2019).

2.7.5. Determination of Flavanols

Extract of samples (200 µL) was mixed with 2000 µL of AlCl3 (2%, w/v) solution, and 3000 µL (50 g/L) of sodium acetic acid solution. This solution was shaken well and allowed to stand at 20 °C for 2.5. After stay time absorbance was measured against a blank solution at 440 nm, the total flavonol contents were mentioned as mg of quercetin equivalents (QE) per gram of dry weight (mg QE/g extract) by using the standard calibration curve prepared with quercetin (Aryal et al., 2019).

3. RESULTS AND DISCUSSION

3.1. Physical Parameters and Color Profile

The results as shown in Table 1 demonstrated that chitosan nanoparticle (CNP) priming significantly enhanced soybean seed germination, radical growth, and early seedling performance compared with the untreated control (T). Radical length increased steadily with nanoparticle treatment, with the highest value recorded in T50 i.e organic CNPs (1.80 cm), suggesting that higher concentrations of CNPs stimulate cell elongation and root extension. Similar findings were reported by Malerba and Cerana (2018) who showed that chitosan nanoparticles enhanced seedling vigor by modulating water uptake and stimulating phytohormone activity.

Table 1. Influence of variable concentrations of synthetic and organic chitosan nanoparticles on the percentage of germination, gained weight and length of radicle on Soy beans.

Sample	Radical length (cm)	Weight (gm)	Germination percentage (%)
T	1.36±0.05^{a}	320±1.00^{e}	85.66±0.57^{a}
T1	1.63±0.01^{b}	319±0.57^{c}	88.00±1.00^{b}
T10	1.66±0.05^{c}	320±0.05^{d}	89.36±0.05^{a}
T2	1.66±0.00^{c}	334±1.00^{d}	88.33±3.05^{b}
T20	1.68±0.05^{c}	331±0.05^{c}	89.36±0.05^{b}
T3	1.44±0.00^{a}	314±0.57^{b}	89.33±2.08^{b}
T30	1.52±0.05^{b}	317±0.05^{b}	88.36±0.05^{a}
T4	1.56±0.02^{b}	311±2.88^{b}	91.00±1.00^{c}
T40	1.61±0.05^{c}	309±0.05^{a}	91.36±0.05^{c}
T5	1.70±0.01^{d}	316±1.00^{b}	91.33±0.57^{c}
T50	1.80±0.05^{d}	318±0.05^{b}	94.36±0.05^{d}

Note: Each data is represented as the mean SD of three replicates. Significant differences are seen at $P < 0.05$ between values in a column with distinct superscript letters. Abbreviations: T: Germination, T1: 20ppm Synthetic Chitosan Germination, T10: 20 ppm Organic Chitosan Germination, T2: 40ppm Synthetic Chitosan Germination, T20: 40 ppm Organic Chitosan Germination, T3: 60ppm Synthetic Chitosan Germination, T30: 60 ppm Organic Chitosan Germination, T4: 80ppm Synthetic Chitosan Germination, T40: 80 ppm Organic Chitosan Germination, T5: 100ppm Synthetic Chitosan Germination, T50: 100 ppm Organic Chitosan Germination.

Seedling weight showed more variable responses. While T2 and T20 exhibited higher seedling weights (334 g and 331 g, respectively), some treatments (e.g., T40 and T4) showed lower weights compared with control. This indicates that while CNPs improve germination and root elongation, biomass accumulation may depend on concentration and interaction with seed metabolism. A comparable trend was observed by Aslam et al. (2024) in maize, where low-to-moderate doses of nanoparticles improved growth, but excessive concentrations led to reduced biomass due to possible oxidative stress.

Germination percentage was strongly improved across treatments, reaching its maximum at T50 (94.36%), compared with 85.66% in the control. The results aligned with Kashyap, Xiang, and Heiden (2015) who reported that chitosan-based nanoparticles enhanced seed germination by improving seed coat permeability and stimulating enzymatic activity required for early growth. Moreover, organic nanoparticle treatments (e.g., T4, T40, T5, T50) consistently supported higher germination percentages, highlighting their potential for eco-friendly seed priming strategies. Thus, CNPs priming improves soybean germination efficiency, radical elongation, and antioxidant defense, though the optimum concentration appears critical to balancing seedling vigor and biomass.

The effect of synthetic and organic chitosan nanoparticles (CNPs) on the soybean color profile revealed significant variations in lightness (L), redness (a), and yellowness (b) values depending on concentration as shown in Table 2. The control (T) had moderate lightness (L = 80.02) and relatively balanced redness and yellowness. At lower concentrations (20–40 ppm), both synthetic (T1, T2) and organic (T10, T20) treatments increased lightness, reaching the highest at T20 (L = 89.51), indicating brighter and more visually appealing seeds (Malerba & Cerana,

2016). Redness (a value) decreased sharply with 20 ppm treatments (T1 and T10, ≈1.3), suggesting a suppression of red pigmentation. As concentrations increased to 80 ppm (T4, T40), redness values slightly rose, though still lower than control, which reflects a concentration-dependent influence. This agrees with Kashyap et al. (2015) who suggested that nanoparticle coatings may modify pigment oxidation and phenolic metabolism, affecting seed coat coloration. Yellowness (b value) increased significantly at 20–40 ppm treatments, peaking at T2 (27.04) and T5 (29.87). Interestingly, at the highest concentration (100 ppm), both synthetic (T5) and organic (T50) treatments reduced lightness (L = 71–72) but increased redness and yellowness, causing darker, more pigmented seeds (Kashyap et al., 2015)).

Table 2. Effect of different concentration of synthetic and organic chitosan nanoparticles on soybeans color profile.

Samples	L	a	b
T	80.02±2.08^{b}	3.83±0.18^{c}	22.85±1.83^{a}
T1	82.02±2.08^{b}	1.33±0.33^{a}	26.46±1.16^{b}
T10	84.02±2.08^{b}	1.35±0.33^{a}	25.46±1.16^{b}
T2	88.51±0.99^{c}	2.81±0.19^{b}	27.04±0.54^{b}
T20	89.51±0.99^{c}	2.85±0.19^{b}	25.04±0.54^{b}
T3	84.57±4.78^{bc}	1.71±0.37^{a}	24.66±0.41^{ab}
T30	86.57±4.78^{bc}	1.60±0.37^{a}	23.66±0.41^{ab}
T4	84.06±1.71^{bc}	2.51±0.39^{b}	24.99±0.88^{ab}
T40	86.06±1.71^{bc}	2.43±0.39^{b}	23.99±0.88^{ab}
T5	71.14±1.55^{a}	5.93±0.04^{d}	29.87±0.66^{c}
T50	72.14±1.55^{a}	4.93±0.04^{d}	27.87±0.66^{c}

Note: Each data is represented as the mean SD of three replicates. Significant differences are seen at $P < 0.05$ between values in a column with distinct superscript letters. Abbreviations: T : Germination, T1: 20ppm Synthetic Chitosan Germination, T10: 20 ppm Organic Chitosan Germination, T2: 40ppm Synthetic Chitosan Germination, T20: 40 ppm Organic Chitosan Germination, T3: 60ppm Synthetic Chitosan Germination, T30: 60 ppm Organic Chitosan Germination, T4: 80ppm Synthetic Chitosan Germination, T40: 80 ppm Organic Chitosan Germination, T5: 100ppm Synthetic Chitosan Germination, T50: 100 ppm Organic Chitosan Germination.

3.2. Proximate Composition

The proximate composition of soybean seeds was significantly influenced by priming with synthetic and organic chitosan nanoparticles (CNPs) as shown in Table 3. Moisture content was substantially reduced in treated samples compared with the control (9.10%), reaching the least values at 60–100 ppm treatments (4.0%). Reduced seed moisture enhanced storage stability by limiting microbial activity and lipid oxidation consistent with the findings of Do Espirito et al. (2021) who reported that nanoparticle seed coatings can improve water regulation.

Table 3. Influence of various concentration of synthetic and organic chitosan nanoparticles on soybeans proximate composition.

Sample	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Crude Fibre (%)	Carbohydrate (%)
T	9.10 ± 0.15^{d}	2.90 ± 0.05^{b}	2.90 ± 0.10^{d}	25.00 ± 0.25^{a}	6.00 ± 0.10^{a}	54.10 ± 0.20^{c}
T1	5.90 ± 0.10^{b}	3.10 ± 0.05^{c}	1.80 ± 0.05^{b}	25.60 ± 0.15^{b}	10.50 ± 0.10^{c}	53.10± 0.20^{c}
T10	5.70 ± 0.10^{b}	3.00 ± 0.05^{c}	1.60 ± 0.05^{b}	26.00 ± 0.15^{b}	11.50 ± 0.10^{c}	52.20 ± 0.15^{b}
T2	5.78 ± 0.12^{b}	2.80 ± 0.05^{a}	1.90 ± 0.05^{b}	28.30 ± 0.20^{b}	11.80 ± 0.10^{c}	49.42± 0.15^{a}
T20	5.60 ± 0.12^{b}	2.60 ± 0.05^{a}	1.50 ± 0.05^{b}	26.30 ± 0.20^{b}	11.70 ± 0.10^{c}	52.30 ± 0.10^{b}
T3	4.00 ± 0.10^{a}	2.80 ± 0.04^{b}	1.60 ± 0.04^{b}	27.20 ± 0.20^{c}	11.90 ± 0.08^{c}	52.50 ± 0.12^{b}
T30	4.00 ± 0.10^{a}	2.80 ± 0.04^{b}	1.60 ± 0.04^{b}	27.20 ± 0.20^{c}	11.90 ± 0.08^{c}	52.50 ± 0.12^{b}
T4	4.70 ± 0.15^{ab}	2.90 ± 0.05^{b}	1.20 ± 0.03^{a}	27.00 ± 0.15^{c}	12.70 ± 0.12^{d}	51.50 ± 0.15^{a}
T40	4.70 ± 0.15^{ab}	2.90 ± 0.05^{b}	1.20 ± 0.03^{a}	27.00 ± 0.15^{c}	12.70 ± 0.12^{d}	51.50 ± 0.15^{a}
T5	4.00 ± 0.12^{ab}	3.00 ± 0.05^{c}	1.00 ± 0.05^{a}	29.90 ± 0.20^{d}	12.90 ± 0.15^{d}	49.20 ± 0.15^{a}
T50	4.80 ± 0.12^{ab}	3.10 ± 0.05^{c}	1.10 ± 0.05^{a}	28.90 ± 0.20^{d}	12.80 ± 0.15^{d}	49.30 ± 0.12^{a}

Note: Each data is represented as the mean SD of three replicates. Significant differences are seen at $P < 0.05$ between values in a column with distinct superscript letters. Abbreviations: T : Germination, T1: 20ppm Synthetic Chitosan Germination, T10: 20 ppm Organic Chitosan Germination, T2: 40ppm Synthetic Chitosan Germination, T20: 40 ppm Organic Chitosan Germination, T3: 60ppm Synthetic Chitosan Germination, T30: 60 ppm Organic Chitosan Germination, T4: 80ppm Synthetic Chitosan Germination, T40: 80 ppm Organic Chitosan Germination, T5: 100ppm Synthetic Chitosan Germination, T50: 100 ppm Organic Chitosan Germination.

Ash content increased slightly in CNP-treated seeds, with the highest values at 20–100 ppm synthetic and organic treatments (3.10%) indicating improved mineral retention. This observation aligned with Suwanchaikasem, Idnurm, Selby-Pham, Walker, and Boughton (2024) who suggested that chitosan enhances mineral uptake and metabolic activity.

Fat content showed a marked decline with increasing nanoparticle concentrations, decreasing from 2.90% in the control to nearly 1.0% at 100 ppm treatments (T5, T50). This reduction may be linked to enhanced lipid metabolism during germination, which is accelerated by nanoparticle-induced enzymatic activity (Ingle et al., 2022).

Protein content increased significantly across treatments, with the highest value in T5 (29.90%). Synthetic nanoparticles at higher concentrations had stronger effects on protein enrichment than organic nanoparticles, likely due to differences in how they interact with seed metabolism (Ingle et al., 2022).

Crude fiber content also increased progressively reaching its maximum in T5 and T50 (12.9%), suggesting enhanced cell wall development and metabolic restructuring. Carbohydrate levels, however, decreased compared

with the control due to their utilization as energy sources for rapid germination and protein biosynthesis (Ingle et al., 2022).

3.3. Total Phenolic Content, Total Flavanoid Content and Total Flavanols

The application of synthetic and organic chitosan nanoparticles (CNPs) had a notable impact on the bioactive profile of soybean seeds, particularly on total phenolic content (TPC), flavonoids, and flavanols as shown in Table 4. Total phenolic content (TPC) values ranged between 0.07–0.16 mg GAE/g. The highest TPC was observed at 60 ppm organic CNPs (T30, 0.16 mg GAE/g), followed by 60 ppm synthetic CNPs (T3, 0.14 mg GAE/g). This demonstrated that moderate concentrations, especially organic forms, are more effective in enhancing phenolic accumulation. Similar results were reported by Kashyap et al. (2015) who emphasized the role of chitosan nanoparticles in stimulating phenylpropanoid metabolism, leading to higher phenolic biosynthesis. Flavonoid content increased substantially under CNP priming, with the highest values again at 60 ppm treatments (T3: 2.24 mg; T30: 2.25 mg catechin/100 g) far exceeding the control (1.14 mg). This sharp increase highlights the concentration-dependent effect of CNPs, which likely activate secondary metabolite pathways as stress signaling molecules (Ingle et al., 2022). Flavanol showed a smaller but consistent increase with CNP treatments, ranging from 0.24 to 0.30 mg catechin/100 g. The highest values were observed in T30 (0.30 mg) and T3 (0.29 mg) suggesting that synthetic and organic nanoparticles are equally effective in enhancing flavanol synthesis at optimal concentrations. Nanoparticles may enhance enzyme activities involved in flavonoid biosynthesis, leading to such improvements (Do Espirito et al., 2021).

Table 4. Effect of different concentration of synthetic and organic chitosan nanoparticles on soybeans bioactive components.

Sample	TPC (mg Gallic Acid/g)	Flavonoids (mg Catechin/100g)	Flavanols (mg Catechin/100g)
T	0.13±0.00^{c}	1.14±0.01^{b}	0.28±0.00^{b}
T1	0.12±0.00^{b}	0.84±0.01^{a}	0.24±0.00^{a}
T10	0.14±0.00^{b}	0.86±0.01^{a}	0.27±0.00^{a}
T2	0.07±0.00^{b}	1.71±0.00^{d}	0.25±0.00^{b}
T20	0.08±0.00^{b}	1.73±0.00^{d}	0.28±0.00^{b}
T3	0.14±0.00^{a}	2.24±0.01^{d}	0.29±0.00^{c}
T30	0.16±0.00^{a}	2.25±0.01^{d}	0.30±0.00^{c}
T4	0.14±0.00^{d}	1.67±0.0^{e}	0.26±0.00^{a}
T40	0.15±0.00^{d}	1.77±0.0^{e}	0.27±0.00^{a}
T5	0.12±0.00^{a}	1.39±0.00^{c}	0.27±0.01^{b}
T50	0.13±0.00^{a}	1.36±0.00^{c}	0.28±0.01^{b}

Note: Each data is represented as the mean SD of three replicates. Significant differences are seen at $P < 0.05$ between values in a column with distinct superscript letters. Abbreviations: T: Germination, T1: 20ppm Synthetic Chitosan Germination, T10: 20 ppm Organic Chitosan Germination, T2: 40ppm Synthetic Chitosan Germination, T20: 40 ppm Organic Chitosan Germination, T3: 60ppm Synthetic Chitosan Germination, T30: 60 ppm Organic Chitosan Germination, T4: 80ppm Synthetic Chitosan Germination, T40: 80 ppm Organic Chitosan Germination, T5: 100ppm Synthetic Chitosan Germination, T50: 100 ppm Organic Chitosan Germination.

3.4. Antioxidant Activity

The data clearly demonstrated in Figure 1 that both synthetic and organic CNPs enhanced antioxidant activity in a concentration-dependent manner. Organic CNPs consistently showed slightly higher antioxidant activity than synthetic counterparts at equivalent concentrations. This could be explained by the natural polymeric structure of organic chitosan which might provide better biocompatibility, solubility, and interaction with plant biochemical pathways compared to synthetic forms (Golkar, Taghizadeh, & Yousefian, 2019). Chitosan nanoparticles are known to act as elicitors, stimulating the production of phenolic compounds, flavonoids, and antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (Stasińska-Jakubas & Hawrylak-Nowak, 2022). The

increase in antioxidant activity observed here aligned with previous reports where nanoparticle-based treatments enhanced ROS-scavenging capacity and improved seedling vigor. The highest antioxidant activity at 100 ppm organic CNPs (T50 = 86.3%) suggested that this concentration is most effective for maximizing antioxidant potential. This indicated a promising application of organic nanomaterials in sustainable agriculture, as they not only outperform synthetic variants but also provide an eco-friendly approach to enhancing crop resilience and nutritional quality.

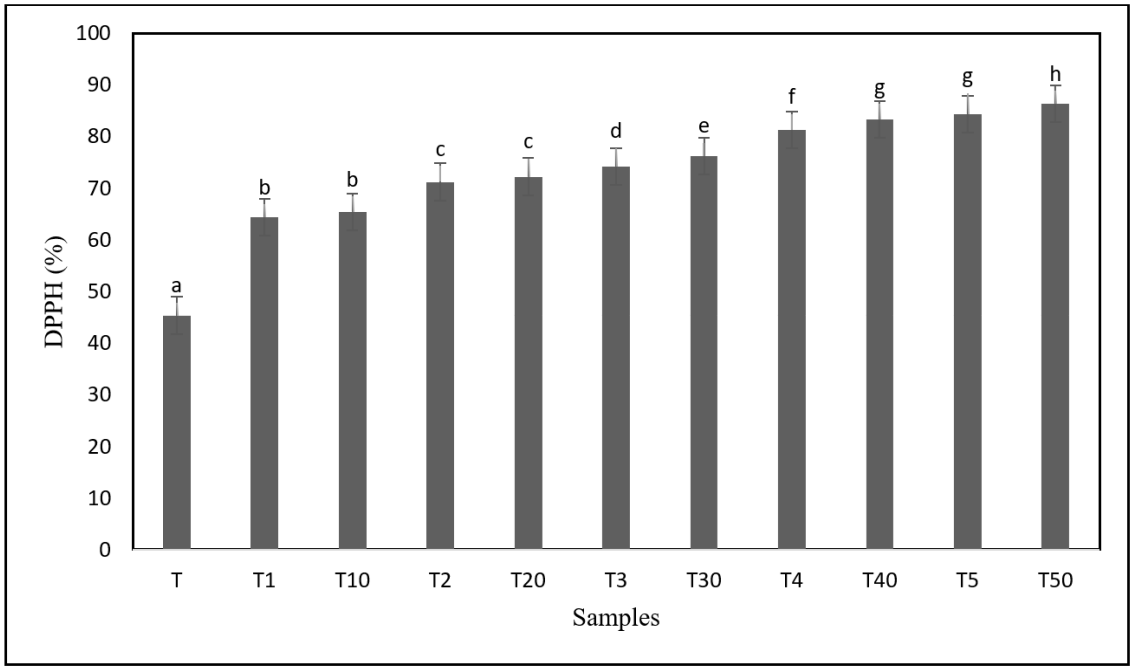

Figure 1. Effect of different concentration of synthetic and organic chitosan nanoparticles on antioxidant activity.

Note: Each data is represented as the mean SD of three replicates. Significant differences are seen at $P < 0.05$ between values in a column with distinct superscript letters. Abbreviations: T : Germination, T1: 20ppm Synthetic Chitosan Germination, T10: 20 ppm Organic Chitosan Germination, T2: 40ppm Synthetic Chitosan Germination, T20: 40 ppm Organic Chitosan Germination, T3: 60ppm Synthetic Chitosan Germination, T30: 60 ppm Organic Chitosan Germination, T4: 80ppm Synthetic Chitosan Germination, T40: 80 ppm Organic Chitosan Germination, T5: 100ppm Synthetic Chitosan Germination, T50: 100 ppm Organic Chitosan Germination.

4. CONCLUSION

This study demonstrated the potential of synthetic and organic chitosan nanoparticles (CNPs) as effective seed priming agents in soybeans, influencing germination, proximate composition, and antioxidant properties. The findings revealed that both nanoparticle types significantly altered seed quality, although their effects varied in magnitude and specificity depending on concentration and origin. Organic CNPs were more effective in promoting early germination and radical elongation, while synthetic CNPs favored greater seedling biomass accumulation. This suggests that organic nanoparticles are particularly suited for initiating germination processes, whereas synthetic nanoparticles play a stronger role in supporting subsequent growth. Antioxidant profiling further showed a complementary relationship between the two nanoparticle types. Synthetic CNPs significantly increased total phenolic and flavonoid levels, pointing to their ability to stimulate secondary metabolite production, while organic CNPs enhanced DPPH radical scavenging capacity, offering superior free radical quenching efficiency. This dual benefit highlights how synthetic CNPs primarily boost nutritional fortification, whereas organic CNPs enhance functional and sustainable antioxidant activity.

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