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Effects of Chitosan and Silver Nanoparticles on Germination and Early Seedling Growth of Pea (*Pisum sativum* L.)

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تأثير الشيتوزان وجسيمات الفضة النانوية على الانبات ونمو البادرات المبكر في البازلاء

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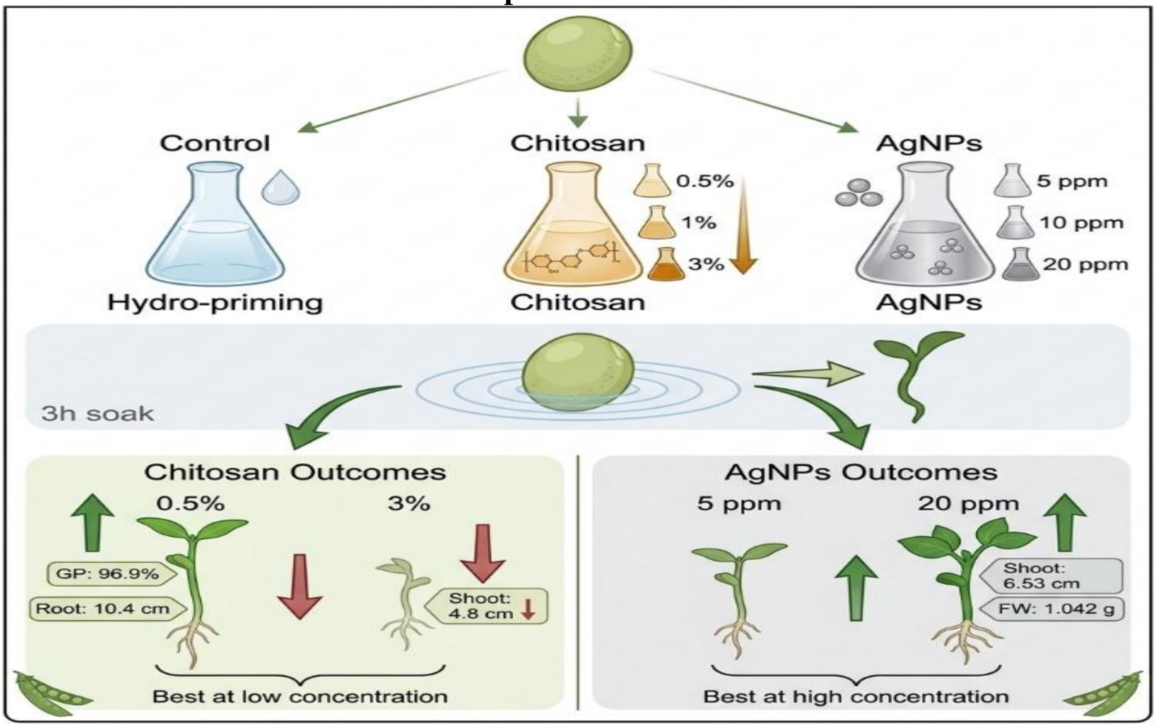
Abstract:
Seed priming is an important pre-sowing technique that enhances seed germination, seedling growth, and crop establishment. The current study was carried out during the 2025-2026 growing season in the Crops Laboratory, Department of Crops, Faculty of Agriculture, Sebha University, Libya to test the seed priming effects of distilled water (hydro-priming), various concentrations of chitosan (0.5%, 1%, and 3%) and silver nanoparticles (AgNPs) (5, 10, and 20 ppm) on the germination, Seeds were soaked in distilled water (control) or treatment solutions for 3 hours.
The findings revealed that the chitosan and the AgNPs influenced the germination and seedling growth parameters as compared to the control. The optimum chitosan concentration was 0.5 % with better germination percentage (96.9 %), germination index (10.00) and root length (10.4 cm) and when concentration was increased to 3 %, most of the growth characteristics were reduced especially shoot length (4.8 cm).
Conversely, AgNPs showed a concentration-dependent response, with 20 ppm showing the best performance in germination and some growth parameters. In addition, certain seedling growth phenotypes such as shoot length (6.53 cm) and fresh weight (1.042 g) but others were not consistently different from the control.
Overall, chitosan worked better at low concentrations, and AgNPs worked better at medium to high concentrations. These results indicate the need to maximize priming levels to promote seed germination and early seedling development of pea.

Graphical abstract



Keywords: Ag NPs, Germination parameters, Hydro-priming, Seedling growth, Seed priming.

الملخص

نقع البذور قبل الزراعة هي تقنية مهمة تهدف إلى تحسين إنبات البذور ونموها وتأسيس المحصول. أُجريت هذه التجربة خلال الموسم الزراعي 2025-2026 في معمل المحاصيل، قسم المحاصيل، كلية الزراعة، جامعة سبها، ليبيا، لاختبار تأثير النقع على البذور باستخدام الماء المقطر (نقع مائي)، وتراكيز مختلفة من الشيتوزان (0.5%، 1%، 3%) وجسيمات الفضة النانوية (5، 10، 20 جزء في المليون) على الإنبات. تم نقع البذور في الماء المقطر، ومحاليل الشيتوزان، وجسيمات الفضة النانوية لمدة 3 ساعات.

أظهرت النتائج أن الشيتوزان وجسيمات الفضة النانوية لها تأثير على خصائص الإنبات ونمو البادرات مقارنة بمعاملة الكونترول، حيث كان التركيز الأمثل للشيتوزان هو 0.5%، إذ سجل أعلى نسبة إنبات (96.9%)، ومؤشر إنبات (10.00)، وطول جذير (10.4 سم). ومع زيادة التركيز إلى 3%، انخفضت معظم صفات النمو، خاصة طول الرويشة (4.8 سم).

في المقابل، أظهرت جسيمات الفضة النانوية استجابة واضحة تعتمد على التركيز، حيث كان تركيز 20 جزء في المليون هو الأفضل في الإنبات وبعض صفات النمو. كما أظهرت بعض صفات نمو البادرات مثل طول الرويشة (6.53 سم) والوزن الطازج (1.042 جم) تحسنًا، بينما لم تختلف بعض الصفات الأخرى بشكل واضح عن الكونترول.

بشكل عام، كانت التراكيز المنخفضة من الشيتوزان أكثر فاعلية، بينما أظهرت جسيمات الفضة النانوية أداءً أفضل عند التراكيز المتوسطة إلى المرتفعة. وتشير هذه النتائج إلى ضرورة تحسين مستويات نقع البذور لتعزيز إنبات البذور والنمو المبكر لبادرات البازلاء.

الكلمات المفتاحية: جسيمات الفضة النانوية، قياسات الانبات، النقع المائي، نمو البادرات، نقع البذور.

Introduction

Pea (*Pisum sativum* L.) is a protein-rich legume crop with a stable balance of proteins, carbohydrates, vitamins, and minerals, which is why it can be considered an important element of the human diet and food security (Thirumalai et al., 2025; Janusauskaitė, 2025).

Seed priming (soaking) is a low cost, easy pre-sowing method that improves germination, seedling vitality and crop growth by increasing water absorption and the initiation of early metabolic events. It is also an effective way to reduce stress and achieve the consistency of seedling emergence in crop production, making it a useful tool in sustainable crop production (Farooq et al., 2022; Iqbal et al., 2021; FAO, 2020).

Nanotechnology is vital for agriculture, as it facilitates improved nutrient use efficiency and higher crop yields, and controlled release of agricultural chemicals (nano-fertilizers and nano-pesticides). It also supports sustainable agriculture by minimizing environmental impact, and by enhancing crop tolerance to various stresses. (Mridha et al., 2025; Usman et al., 2020).

AgNPs have become a topic of significant interest because of their biological activity and possible applications in agriculture. They have been known to increase seed germination, growth and tolerance of plant stress at the right levels. Moreover, AgNPs have good antimicrobial properties, which contribute to the protection of plants against the different pathogens. Thus, they can be regarded as a promising instrument of enhancing crop yields and promoting sustainable agriculture systems (Siddiqi and Husen, 2021; Rai et al., 2021).

Chitosan is a natural, eco-friendly biopolymer widely used as a plant bio stimulant it increases plant development, stimulates the uptake of nutrients and stimulates the plant defenses in relation to biotic and abiotic stresses. Moreover, chitosan enhances the photosynthesis performance and antioxidant properties, resulting in the increased plant performance and yield (Das et al., 2024; El-Araby et al., 2024; Román-Doval et al., 2023).

Stałanowska et al. (2025) demonstrated that bio-AgNPs have safe effects on oxidative status and metabolism in early stages of pea development without toxicity at the tested concentrations. However, a higher concentration had an antimicrobial effect at early stages but a negative impact on later development. Silver nanoparticles enhanced germination and seed quality in cowpea with 60 ppm identified as the most effective dose (Sattar and Din, 2024). Furthermore, silver nanoparticles (2.5 mM) positively influenced pea growth and productivity under both laboratory and field conditions as stated by (Rahman et al., 2023).

Szablińska et al. (2022) reported that AgNPs-enriched fertilizers have beneficial effects on plant growth and yield, suggesting their potential application in sustainable agriculture, while further studies are needed for their environmental safety. Germination, growth, and stress tolerance of bean seedlings were enhanced by low concentrations of AgNPs (Jaskulski et al., 2022). However, high concentrations showed initial antimicrobial effects negatively affected later growth due to disrupted symbiosis as mentioned by (Prażak et al., 2020).

Ali et al. (2025) reported that chitosan nanoparticles enhanced the growth and production of pea (Pisum sativum L.), either as single application or in conjunction with NPK fertilizers. They improved biomass, yield attributes and plant performance compared to the control plants. Likewise, chitosan nanoparticles improved seed germination and early growth of soybean seedlings, increasing seed quality and metabolic activity (Steven et al. 2024). Similar positive effects of chitosan nanoparticles on germination and seedling growth in soybean as noted by (Dawood et al. (2024)

Riseh et al. (2024) found that chitosan increased physiological indices and drought resistance in faba bean, including photosynthetic pigments, nutrient content, and antioxidants. In addition, chitosan is widely recognized as a bio stimulant to improve plant growth, photosynthetic capacity and stress tolerance by activating plant defense systems (Rojas-Pirela et al., 2024). Moreover, chitosan seed coating enhances germination, seedling growth and early development of seedlings in several crops mainly through the activation of metabolic and enzymatic activities during germination based on the findings of (Maluin and Hussein, 2020).

Therefore, this study aimed to evaluate and compare the effects the impacts of various levels of chitosan and silver nanoparticles (AgNPs) on the germination properties and initial seedling development of pea (*Pisum sativum* L.) in controlled laboratory environments.

Material and methods

Experimental site

The experiment was conducted during the 2025-2026 growing season in the Crop Laboratory, Department of Crops, Faculty of Agriculture, Sebha University, Libya, under controlled laboratory conditions.

Plant material

Pea (*Pisum sativum* L.) seeds were used in this study. Ten seeds were placed in each Petri dish for germination.

Treatments and design.

The study was done as a completely randomized design (CRD) having three replications. The experiment involved control (soaking in distilled water for 3 hours), seed priming using chitosan and silver nanoparticles (AgNPs) of varying concentrations.

Seed priming treatments
All treatments included soaking the seeds for 3 hours, and included:

Control (only in distilled water)

Three concentrations of chitosan (0.5%, 1% and 3%)

Three concentrations of silver nanoparticles (AgNPs) (5ppm, 10ppm and 20 ppm).

Seeds were soaked and then transferred directly to Petri dishes to germinate.

Measured parameters
Fresh Weight: Three germinated seedlings were also picked randomly at each replicate and weighted by use of sensitive electronic scale to get the average.

Dry Weight: The three germinated seedlings per replicate were randomly chosen (the same seedlings were used in fresh weight measurement), put in paper bags, and subjected to an oven at 70 degrees Celsius temperature over 72 hours. They were measured with a sensitive electronic scale (four decimal places) to get the average.

Shoot Length Measurement: Three germinated seedlings chosen randomly in each replicate were measured by means of a ruler at the point of separation of the stem. Ten days after making the planting, shoot length (cm) was measured.

Root Length Measurement: Three seedlings were uprooted randomly and with care, per replicate. The seedlings that were uprooted were washed with tap water. A ruler was used to measure root length; root length (cm) was measured ten days following planting.

Germination percentage (G %) was characterized as the generation of normal seedling, was calculated for 5 and 10 days by using the following formula:

$$Germination\ (\%)\ (Germinated\ after\ 10\ days\ of\ planting)\ =\ (Number\ of\ germinated\ seedlings\ /\ Total\ number\ of\ seedlings)\ \times\ 100$$

Germination Power (for the first reading): Calculated using the following formula:

$$Germination\ Power\ (\%)\ =\ (Number\ of\ seeds\ germinated\ at\ first\ count\ /\ Total\ seeds)\ \times\ 100$$

Germination Power (second reading): was calculated as follows:

$$Germination\ Power\ (\%)\ =\ (Number\ of\ seeds\ germinated\ at\ first\ count\ /\ Total\ seeds)\ \times\ 100$$

Germination index (GI) was calculated to evaluate the speed and uniformity of seed germination during the experimental period. The number of germinated seeds was recorded daily, and the index was computed based on the cumulative germination over time using the following formula:

$$GI\ =\ \Sigma\ (Gt\ /\ Dt)$$

Where:

GI = Germination Index
Σ = Summation (sum of values over the counting period)
Gt = Number of seeds germinated on day t
Dt = Number of days from the beginning of the germination test

Statistical analysis
Analysis of variance (ANOVA) was used to analyze the data in a completely randomized design. The means of treatment were compared by LSD test at 0.05 probability level.

Results and discussion
Effect of Chitosan on pea germination and seedling growth.
All measured germination and growth parameters were significantly affected by chitosan treatments compared with hydro-priming (control).

Fresh weight ranged from 2.31 to 3.11 g, with the highest value recorded in the control (3.11 g) and the lowest in 1% chitosan Figure 1-A.

Dry weight ranged from 0.48 to 1.51 g, with the highest value in the control and the lowest in 0.5% chitosan. As observed in Figure 2-A.

Shoot length decreased with increasing chitosan concentration, ranging from 4.8 to 10.33 cm, with the highest value in the control Figure 3-A with the maximum length measured in the control. **Root length ranged** from 9.85 to 13.25 cm, with the highest value recorded in the control (Figure 4-A).

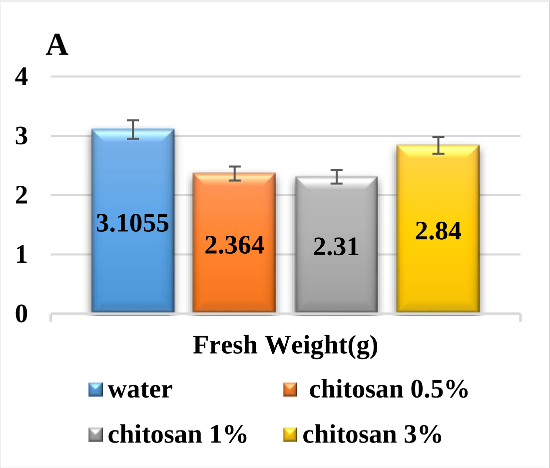
Germination percentage ranged from 84.3 to 96.9%, with the highest value at 0.5% chitosan and the lowest at 3% (Figure 5-A).and the lowest rate of 3 percent (Figure 5-A). The **Germination power** varied between 12,358.58 and 20,994.87 with the highest being in the control (Figure6-A). **Germination index** ranged from 8.66 to 10.00, with the highest value at 0.5% chitosan (Figure 7-A).

Effect of silver nanoparticles (AgNPs) on pea germination and seedling growth.
AgNPs treatments significantly affected all measured parameters compared with the control.

Fresh weight showed a concentration-dependent trend, with the highest value at 20 ppm (Figure 1-B. **Dry weight** ranged from 0.20 to 0.22 g, with the highest value at 10 ppm AgNPs. as shown in Figure (2 -B).

Shoot length ranged from 2.33 to 6.53 cm, with the highest value at 20 ppm AgNPs. Figure (3 -B). **Root length** ranged from 4.84 to 5.78 cm, with the highest value at 10 ppm AgNPs. Figure (4 -B).

The **Germination percentage** ranged from 78.0 to 85.6%, with the highest value at 20 ppm AgNPs (Figure 5-B). **Germination power** ranged from 5,378.88 to 8,179.55, with the highest value at 20 ppm AgNPs (Figure 6-B). **Germination index** ranged from 6.26 to 7.13, with the highest value at 20 ppm AgNPs (Figure 7-B).



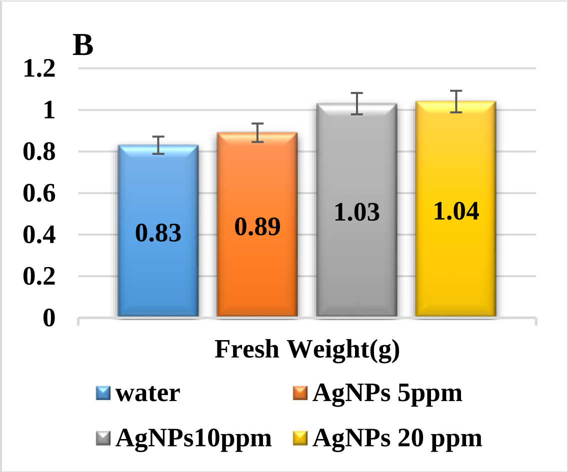


Figure 1. Fresh Weight (g) of pea with chitosan Concentrations (A) and silver nanoparticles Concentrations (AgNPs) (B).

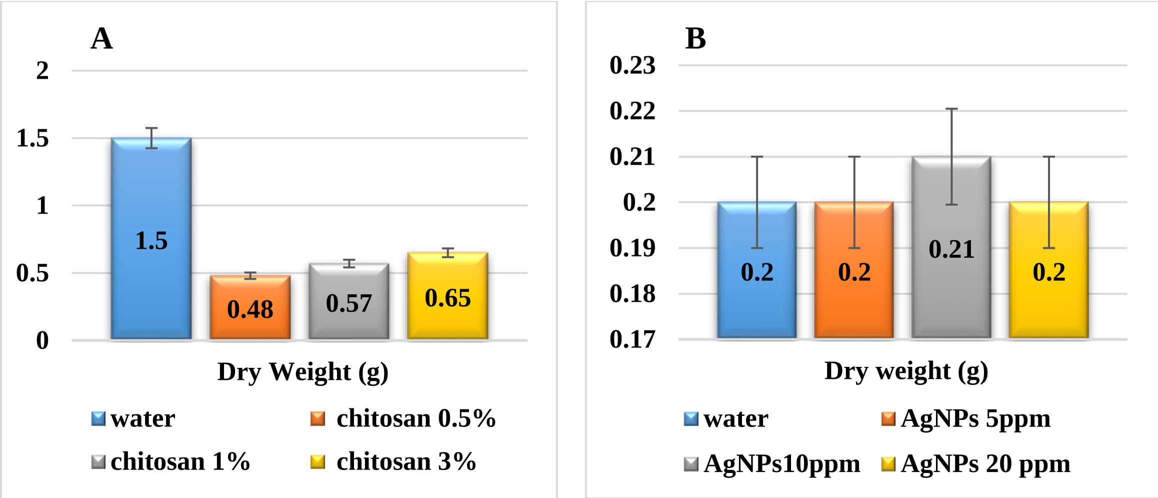


Figure 2. Dry Weight (g) of pea with chitosan Concentrations (A) and silver nanoparticles Concentrations (AgNPs) (B).

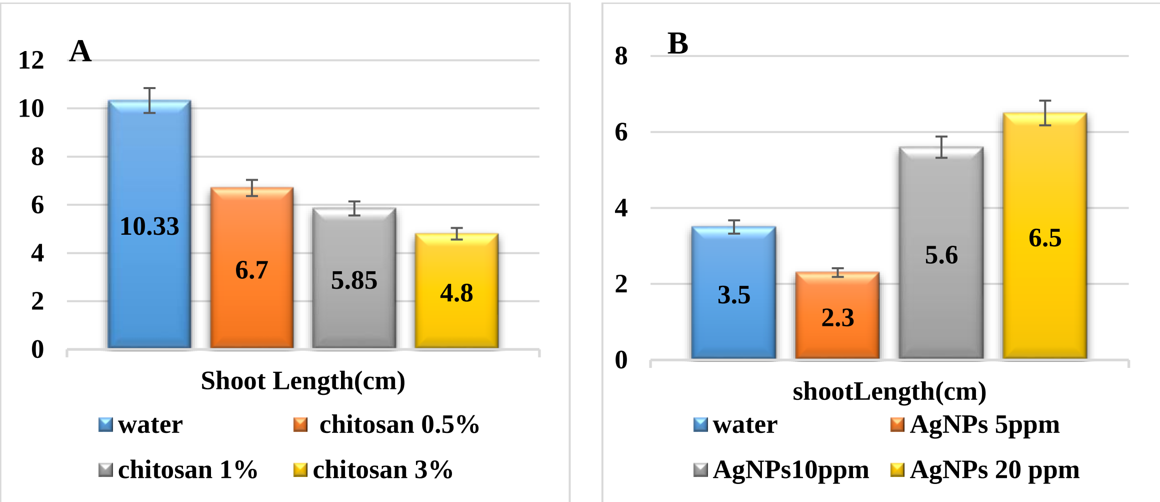


Figure 3. Shoot Length (cm) of pea with chitosan Concentrations (A) and silver nanoparticles Concentrations (AgNPs) (B).

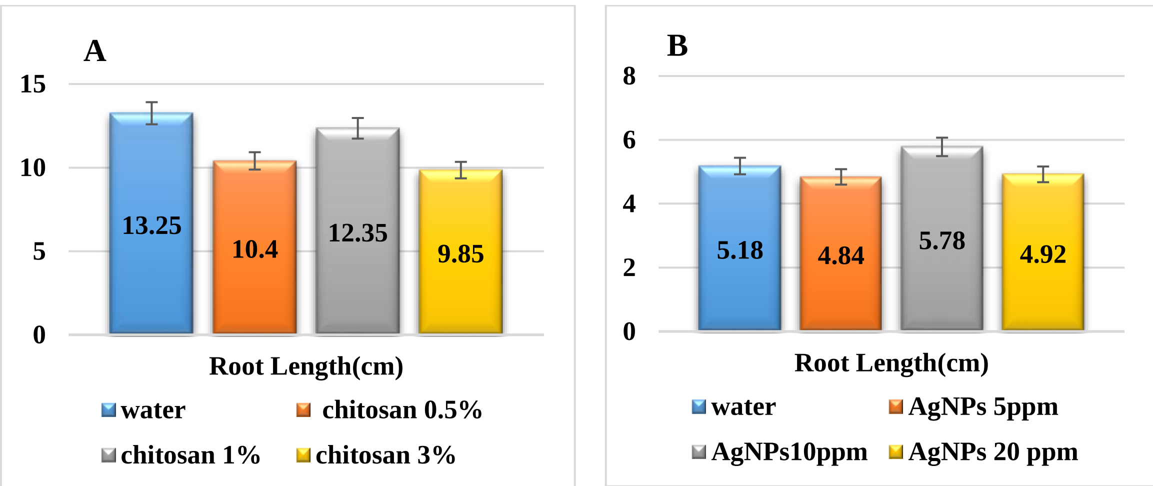


Figure 4. Root Length (cm) of pea with chitosan Concentrations (A) and silver nanoparticles Concentrations (AgNPs) (B).

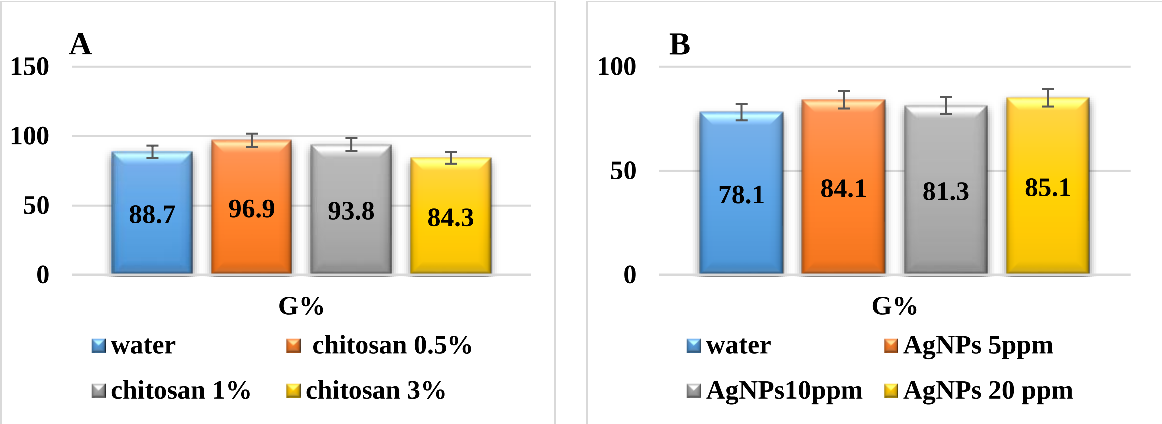


Figure 5. Germination Percent of pea with chitosan Concentrations (A) and silver nanoparticles Concentrations (AgNPs) (B).

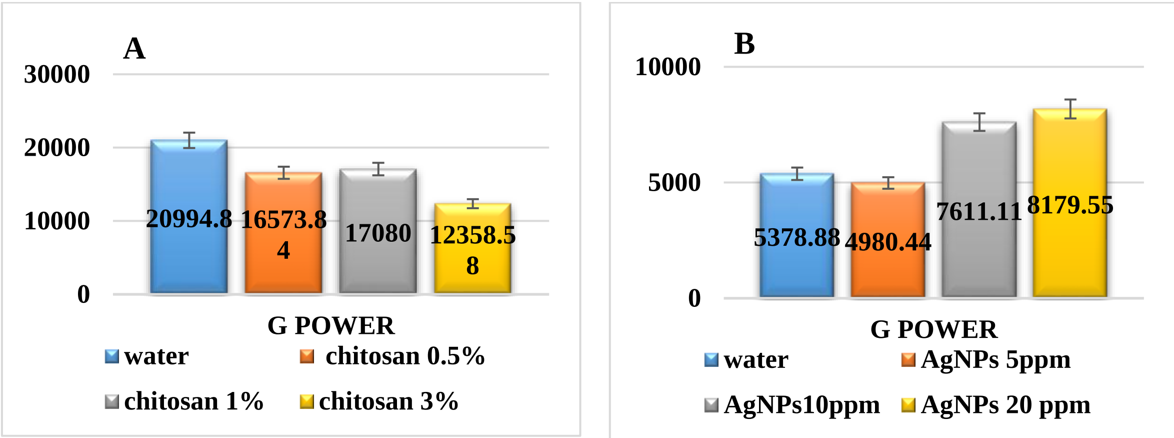


Figure 6. Germination Power of pea with chitosan Concentrations (A) and silver nanoparticles Concentrations (AgNPs) (B).

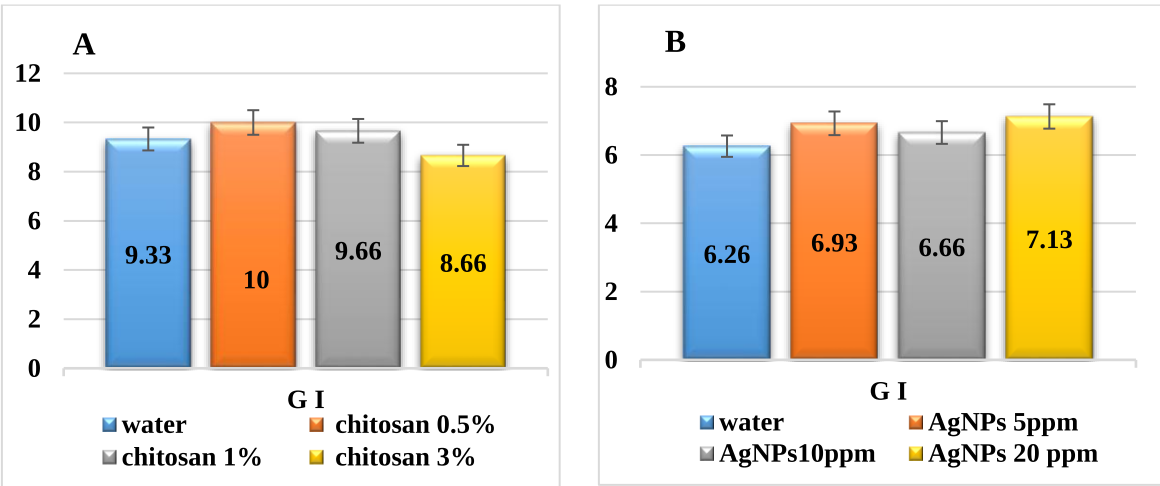


Figure 7. Germination Index of pea with chitosan Concentrations (A) and silver nanoparticles Concentrations (AgNPs) (B).

Discussion

The present study demonstrated that the influence of chitosan and silver nanoparticles (AgNPs) on germination and initial seedling development of pea depended greatly on concentration and varied across the various parameters measured.
The result of the fresh weight was that the control treatment was the highest followed by chitosan and AgNPs, which had relatively lower values but slight improvements were observed at particular concentrations. This indicates that hydro priming was more effective in supporting seed hydration under the experimental conditions. In addition, biomass accumulation under the present conditions. However, the relative increase at some AgNPs concentrations can be explained by the increased membrane permeability, better uptake of water, and the increase in the metabolic activity during germination. Previous studies have reported that AgNPs can enhance cellular hydration, metabolic activity and antioxidant defense mechanisms, which aid in biomass accumulation at optimum concentrations (Stałanowska et al., 2025; Saini et al., 2024; Rai et al., 2021).

Similarly, the control treatment recorded the highest dry weight compared with chitosan and AgNPs treatments. This suggests that these treatments activated the physiological processes while also inducing mild stress responses or divert metabolic energy into defense processes instead of biomass accretion. AgNPs have been reported to affect the activity of enzymes, nutrient uptake, and ROS homeostasis, which can promote growth at optimal concentrations but may reduce biomass accumulation under suboptimal conditions (Sadeghi, 2024; Tripathi et al., 2020; Rai et al., 2021). Chitosan was also shown to have a concentration-dependent effect, with lower concentrations being relatively more favorable, and higher concentrations inhibiting growth, probably because it activates plant defense mechanisms (Divya et al., 2021).

The results of shoot and root length indicated that the control treatment usually gave the highest results, with an increment in concentration of chitosan causing a decrease in growth. However, AgNPs demonstrated moderate improvements at certain concentrations, especially in the growth of shoots at 20 ppm and roots at 10 ppm. The results indicate that AgNPs can stimulate cell division, elongation, and nutrient uptake at optimal concentrations, whereas high concentrations of chitosan may cause growth retardation by changing metabolism towards stress responses. Previous studies also noted similar reports; with AgNPs, positively influencing growth parameters at the correct concentrations, whereas higher concentrations had a negative impact on the growth of plants (Tripathi et al., 2020; Saini et al., 2024; Malerba and Cerana. 2020).

The highest percentage of germination was recorded in the chitosan treatment at 0.5 and the highest concentrations reduced germination. AgNPs also performed better than some treatments in enhancing germination with the highest performance at 20 ppm, but lower than the control in some situations. These findings suggest that the two treatments are capable of stimulating the germination process within optimal concentrations since they stimulate metabolic processes and enzyme systems like α-amylase that facilitates the mobilization of reserves. Nevertheless, at excess levels, it can cause disregard of physiological balance and the decline of germination. These results are in line with the previous reports that AgNPs and chitosan have a dual role in stimulating growth in lower concentrations and inhibiting in higher concentrations (Stałanowska et al., 2025; Saini et al., 2024; Riseh et al., 2024).

A similar trend was observed with germination power and germination index in which moderate changes were realized at optimal concentrations of both treatments; especially with AgNPs and the control treatment was performing well. This indicates that nanopriming may be effective with respect to increasing metabolic activation and speeding up the germination process, but only when specific concentration optimization is applied. AgNPs have also been found to increase enzyme activity, energy metabolism, and stress tolerance, resulting in seed vigor and quicker germination (Saini et al., 2024; Rai et al., 2021; Tripathi et al., 2020). Chitosan also helped to increase the performance of germination by increasing water uptake and the activation of early metabolic activities (Divya et al., 2021; Maluin and Hussein, 2020).

Conclusion

Chitosan and silver nanoparticles (AgNPs) produced a significant influence on seed germination and early seed development of pea (*Pisum sativum* L.) in a concentration-dependent manner. The highest germination percentage and germination index were observed with chitosan at 0.5% and AgNPs at 20 ppm, while 10 ppm was most effective for root growth. Enhanced most of the seedling growth parameters. Hydro priming tended to give higher values on certain biomass related characteristics than most chemical treatments.

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