

Comparative Effects of Chitosan and Nano Silver on Chickpea Germination and Seedling Growth

Najeeb Abdulsalm Alshami ¹, Abusif Sonousi ², Bilkess Meloud Salim ^{3*}

¹ Department of Horticulture, Faculty of Agriculture, Sebha University, Libya

^{2,3} Department of Crops, Faculty of Agriculture, Sebha University, Libya


*Email: Bil.salim@sebhau.edu.ly

التأثيرات المقارنة للكيتوسان وجسيمات الفضة النانوية على إنبات بذور الحمص ونمو البادرات

نجيب عبد السلام الشامي¹، أبوسيف سنوسي²، بلقيس ميلود سالم^{3*}

¹ البستنة، كلية الزراعة، جامعة سبها، ليبيا

^{2,3} المحاصيل، كلية الزراعة، جامعة سبها، ليبيا

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Abstract

Seed soaking is an important pre-sowing treatment that enhances germination and seedling establishment, improving germination under arid and stress conditions remains a major challenge in chickpea production, eco-friendly treatments such as chitosan and silver nanoparticles have gained increasing attention. The study was conducted during the 2025-2026 season in the Crops Laboratory, Crops Department, Sebha University, Agriculture Faculty, Libya.

Two experiments were carried out using different concentrations of chitosan (0.5%, 1% and 3%) and AgNPs (5, 10 and 20 ppm), relative to untreated seeds (control). Germination percentage (G %), germination power, germination index, shoot length, root length, fresh weight and dry weight were measured.

The findings indicated that the effect of chitosan (1%) was significantly improved all studied traits compared with other treatments recording the highest values of germination traits, including germination percentage (98), germination power (20000), germination index (10.4), shoot length (8.4 cm), root length (13.1 cm), fresh weight (3 g), and dry weight (0.8 g). In contrast, the highest concentration (3%) exerted a negative effect on most traits.

Regarding AgNPs, the 10 ppm treatment showed the best performance, with significant improvement of germination percentage (88), germination power (6000), germination index (6.2), shoot length (5.77 cm), root length (5.87 cm), fresh weight (1.04 g) and dry weight (0.259 g), whereas 20 ppm treatment showed inhibitory effects on most traits.

It is concluded that the application of 1% chitosan and 10 ppm AgNPs as seed treatments to improve chickpea germination and early seedling growth in the laboratory, and potentially in the field to improve crop establishment in arid conditions.

Keywords: Germ inability, Germination percentage, Germination power, Nano fertilization, Silver nanoparticles, Soaking seeds.

المُلخَص

نقع البذور يُعد معاملة مهمة قبل الزراعة تعمل على تعزيز الإنبات وتأسيس البادرات، إلا أن تحسين الإنبات تحت ظروف الجفاف والإجهاد ما يزال يمثل تحدياً رئيسياً في إنتاج الحمص، وقد حظيت المعاملات الصديقة للبيئة مثل الشيتوزان والجسيمات النانوية الفضية (AgNPs) باهتمام متزايد في الآونة الأخيرة. أُجريت هذه الدراسة خلال موسم 2025-2026 في مختبر المحاصيل بقسم المحاصيل، كلية الزراعة، جامعة سبها، ليبيا.

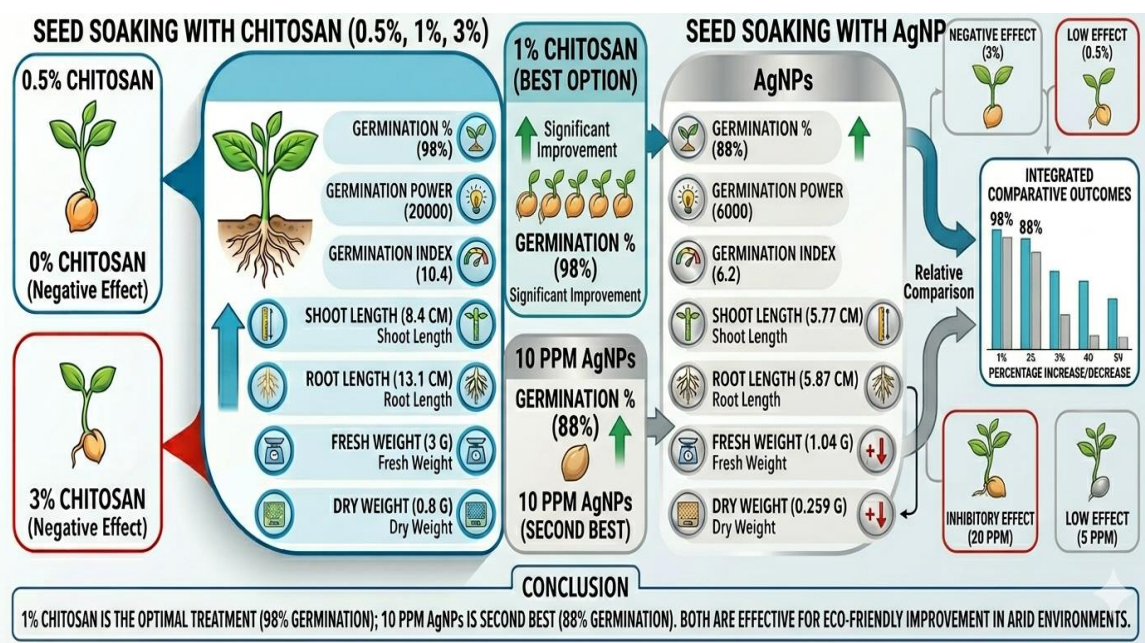
تم تنفيذ تجربتين باستخدام تراكيز مختلفة من الشيتوزان (0.5% و1% و3%) والجسيمات النانوية الفضية (5 و10 و20 جزء في المليون)، مقارنة بالبذور غير المعاملة (الشاهد). تم قياس نسبة الإنبات (G%)، قوة الإنبات، دليل الإنبات، طول الساق، طول الجذر، الوزن الطازج، والوزن الجاف.

أظهرت النتائج أن معاملة الشيتوزان بتركيز 1% أدت إلى تحسين معنوي في جميع الصفات المدروسة مقارنة ببقية المعاملات، وسجلت أعلى القيم في صفات الإنبات، بما في ذلك نسبة الإنبات (98%)، قوة الإنبات (20000)، دليل الإنبات (10.4)، طول الساق (8.4 سم)، طول الجذر (13.1 سم)، الوزن الطازج (3 غرام)، والوزن الجاف (0.8 غرام). في المقابل، كان للتركيز الأعلى (3%) تأثير سلبي على معظم الصفات.

وبالنسبة للجسيمات النانوية الفضية، أظهرت معاملة 10 جزء في المليون أفضل أداء، حيث أدت إلى تحسين معنوي في نسبة الإنبات (88%)، قوة الإنبات (6000)، دليل الإنبات (6.2)، طول الساق (5.77 سم)، طول الجذر (5.87 سم)، الوزن الطازج (1.04 غرام)، والوزن الجاف (0.259 غرام)، بينما أظهر تركيز 20 جزء في المليون تأثيرات مثبطة على معظم الصفات.

ويُستنتج أن استخدام 1% شيتوزان و10 جزء في المليون من الجسيمات النانوية الفضية كمعاملات للبذور يمكن أن يحسن إنبات الحمص ونمو البادرات المبكر في الظروف المعملية، مع إمكانية تطبيقها مستقبلاً في الحقل لتحسين تأسيس المحصول في الظروف الجافة.

الكلمات المفتاحية: الإنبات، التسميد النانوي، جسيمات الفضة النانوية، قوة الإنبات، نقع البذور، نسبة الإنبات



Graphical abstract depicting the effects of chitosan and silver nanoparticles (AgNPs) seed treatments on the germination and the early seedling development of chickpea (*Cicer arietinum* L.), showing the optimum concentrations and inhibitory concentrations.

Introduction

Chickpea (*Cicer arietinum* L.) is an important source of nutritious protein for animals, as they are high in protein and low in cellulose. Legumes play a significant role in atmospheric nitrogen fixation and are also an important source of essential amino acids...a significant source of amino acids that humans cannot produce, and 8 amino acids (isoleucine, lysine, leucine, methionine, phenylalanine, threonine, tryptophan and valine) must be obtained through the diet (Ahmad et al., 2022; Keskin et al., 2021).

Seed priming improves germination pre-sowing treatment that increases germination, seedling emergence and stress tolerance by bringing about physiological and biochemical changes. Seed priming improves germination, seedling establishment, and tolerance to various stresses (Ellouzi et al., 2024; Rawal, 2024).

Chitosan is a natural, renewable biopolymer that is commonly used in agriculture as a seed treatment and bio stimulant; it increases germination, growth, efficiency of nutrient use and biomass production, activates plant defense and increases tolerance to environmental stresses, especially in semi-arid environments, thus offering a natural alternative to chemical fertilizers and pesticides. (Elmahdi et al., 2026; Hegde and Selvaraj, 2024; Bertrand and Bach. 2024).

Nano silver (AgNPs) are nanomaterials with distinct physicochemical characteristics that have recently received considerable attention in agriculture as they boost seed germination, promote plant growth, improve nutrient absorption and enhance tolerance to biotic and abiotic stresses, and possess antimicrobial properties against plant pathogens, which makes them a potential alternative for sustainable agriculture (Alabdallah and Latif, 2025; Kale et al., 2021).

Alfosea-Simón et al., 2025 reported that the increased germination and seedling growth under nano silver treatments could be explained by the stimulatory effect of nano silver (AgNPs) on plant physiological and metabolic mechanisms. Nano silver has been reported to enhance nutrient and water uptake and stimulate enzyme activity, resulting in improved germination percentage, germination index and seedling vigor. Moreover, it enhances stress tolerance by

stimulating antioxidant enzymes, reducing oxidative stress, and maintaining cellular integrity, leading to better shoot and root length and fresh and dry matter (Alabdallah and Latif, 2025). Moreover, the antibacterial activity of nano silver reduces pathogenic effects during the initial plant growth, thereby indirectly improving germination and seedling growth (Martínez-Cisterna et al., 2024).

Rocha et al. (2024) reported that the green-synthesized AgNPs are highly effective against microorganisms and had high catalytic properties, which highlight the potential for AgNPs to have a multifunctional effect beyond plant growth. AgNPs influence plant physiological traits under stress conditions, with positive or negative effects depending on their environmental conditions (salinity). (Bsoul et al., 2023). Silver nanoparticles are important in improving plant response to abiotic stresses by activating antioxidant and metabolic responses as stated by (Alabdallah and Hasan, 2021).

Hidangmayum and Dwivedi, 2023 indicated that chitosan seed priming enhances, seedling establishment and stress tolerance under abiotic stresses, Chitosan has amino groups that can interact with other molecules and improve its mobility in plant tissues and activation of biological processes (Riseh et al., 2023). Chitosan-based materials have been investigated for use in slow-release fertilizers (SRF) using various cross-linking strategies, to enhance nutrient delivery efficiency (Chiaregato et al., 2022).

Ling et al., 2022 found chitosan increases antioxidant activity and decreases damage by activating important defense pathways, Chitosan-based materials have been used in slow-release fertilizers (SRF) with different cross linking methods, enhancing nutrient delivery efficiency (Chiaregato et al., 2022). Chitosan has been reported to function as a bio stimulant even in the absence of stress conditions to improve root system, chlorophyll content and total biomass (lettuce and soybean).

Therefore, the objective of this study was to evaluate the effect of different concentrations of chitosan and nano silver (AgNPs) on seed germination and early seedling development of chickpea (*Cicer arietinum* L.) under laboratory conditions, to improve crop stand and sustainable food production under arid climatic conditions in southern Libya for food security.

Material and Methods

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 on the chickpea seeds (*Cicer arietinum* L.). Ten seeds were placed in each Petri dish for germination. With completely randomized design (CRD) with three replications. The experiment involved control, chitosan and silver nanoparticles (AgNPs) of varying concentrations. The seeds were priming for 3 hours).

Seed priming treatments:

Control (only in distilled water)

Chitosan 0.5%

Chitosan 1%

Chitosan 3%

Nano silver 5ppm

Nano silver 10ppm

Nano silver 20 ppm.

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

Measured parameters:

Fresh Weight: Three randomly selected germinated seedlings from each replicate were weighed using a sensitive electronic scale to determine the average weight.

Dry Weight: Three germinated seedlings per replicate were randomly selected (the same seedlings utilized for fresh weight measurement), placed in paper bags, and treated to an oven at 70 degrees Celsius for 72 hours. The measurements were obtained using a precise electronic scale (four decimal places) to calculate the average.

Shoot Length: Three randomly selected germinated seedlings from each replicate were measured using a ruler at the stem separation point. Ten days post-planting, shoot length (cm) was recorded.

Root Length: Three seedlings were meticulously uprooted at random for each replicate. The uprooted seedlings were rinsed with tap water. A ruler was employed to measure root length; root length (cm) was recorded 10 days post-planting.

Germination percentage (G %) was defined as the emergence of normal seedlings and was calculated for 5 and 10 days using the following formula:

$$\begin{aligned} \text{Germination (\%)} & \text{ (Germinated after 10 days of planting)} \\ & = (\text{Number of germinated seedlings} / \text{Total number of seedlings}) \\ & \times 100 \end{aligned}$$

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

$$\begin{aligned} \text{Germination Power (\%)} \\ & = (\text{Number of seeds germinated at first count} / \text{Total seeds}) \times 100 \end{aligned}$$

Germination Power (second reading): was calculated as this formula:

$$\begin{aligned} \text{Germination Power (\%)} \\ & = (\text{Number of seeds germinated at first count} / \text{Total seeds}) \times 100 \end{aligned}$$

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

The effect of chitosan:

The concentrations of chitosan had a substantial impact on all the characteristics under study, including germination parameters, and seedling growth characteristics.

Best performance was at 1% chitosan, which recorded the highest values of germination (98%), germination power (20000), germination index (10.4), shoot length (8.4 cm), root length (13.1 cm), fresh weight (3.0 g), and dry weight (0.8 g).

The 0.5% treatment ranked second, which had relatively high values on most traits with slight variations between 0.5% and 1%.

The intermediate values in the control treatment were smaller than 0.5% and 1% treatment.

In contrast, 3% chitosan recorded the lowest values of all traits, including dry weight (0.3 g), fresh weight (1.0 g), shoot length (4.4 cm), and root length (8.0 cm), as well as decreased germination performance.

The effect of nano silver

Nano-silver (AgNPs) had a significant impact on all the measured characteristics.

The greatest values were recorded at 10 ppm, which showed the highest values in percentage of germination (88%), germination power (6000), germination index (6.2), shoot length (5.77 cm), root length (5.87 cm), fresh weight (1.04 g), and dry weight (0.259 g).

The 5-ppm treatment was second with moderate to high values in most of the traits.

The control exhibited moderate values in all the parameters.

Conversely, 20 ppm had the lowest values of all the traits, including dry weight (0.19 g), fresh weight (0.87 g), shoot length (3.72 cm), and root length (4.88 cm), and reduced germination performance.

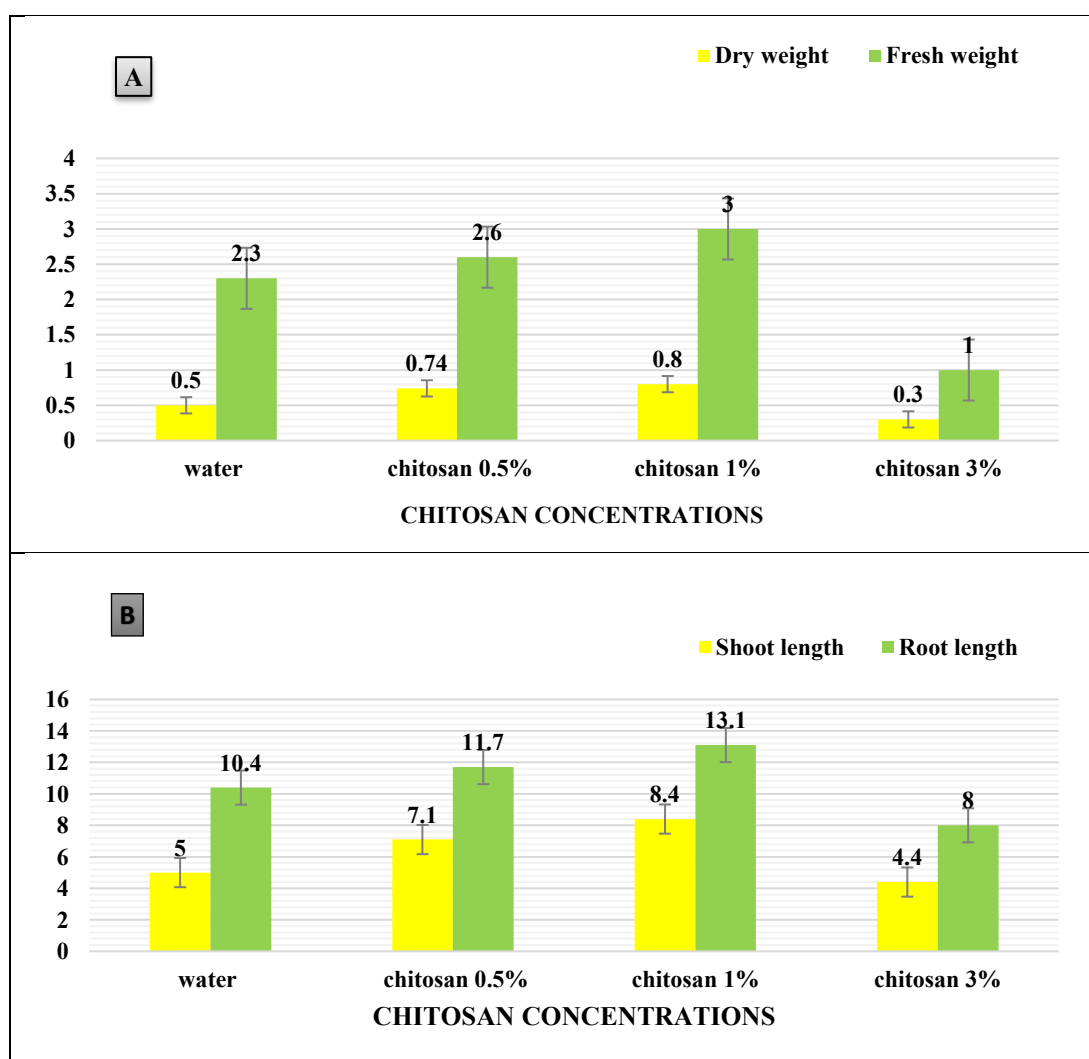
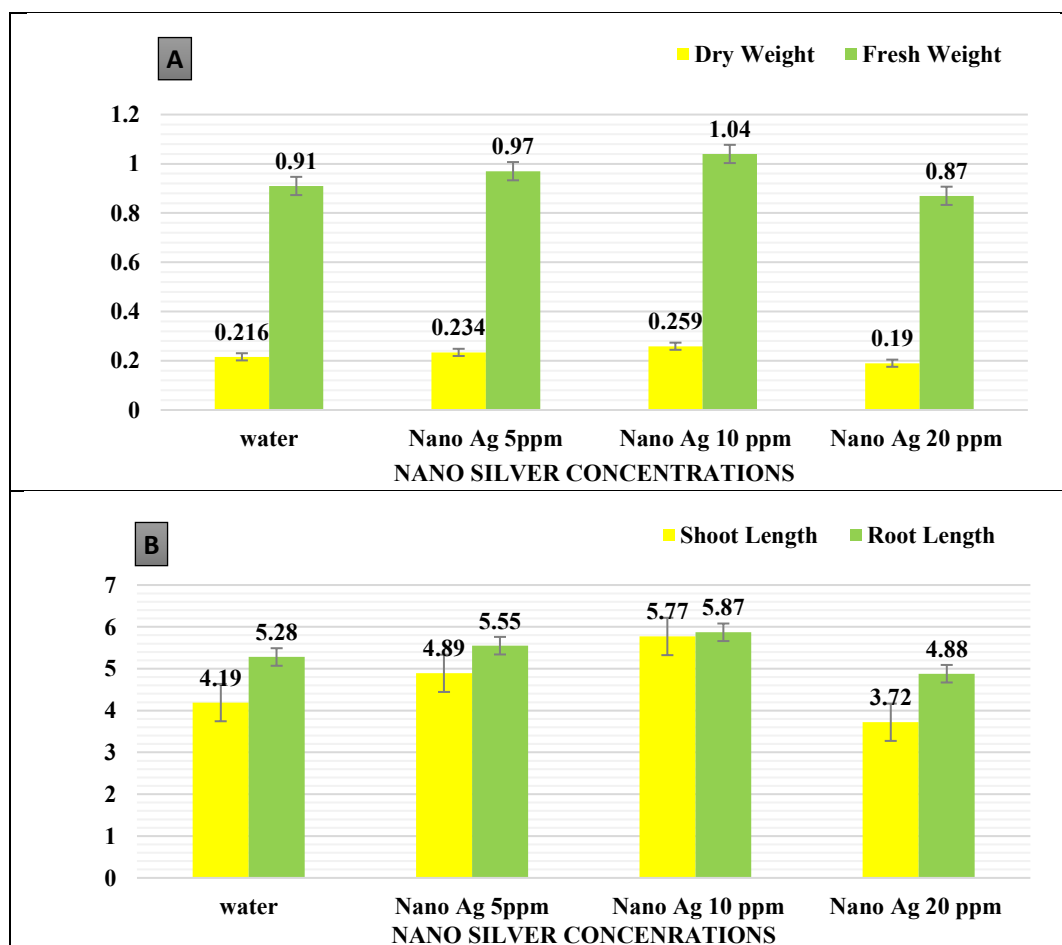


Figure 1. Effect of different concentrations of chitosan on (A) fresh and dry weight, and (B) shoot and root length of pea seedlings under laboratory conditions.

Table 1. Effect of various concentrations of chitosan on the traits of pea seed germination (germination index, germination power and germination percentage (G %)) in laboratory conditions.

Treatments	G index	G Power	G%
water	8 c	14000 c	87 c
chitosan 0.5%	9.7 b	16000 b	92 b
chitosan 1%	10.4 a	20000 a	98 a
chitosan 3%	8.1 d	11000 d	81 d
LSD at 0.05	0.40010	1900	0.41111

**Figure 2.** Effect of different concentrations of Nano silver on (A) fresh and dry weight, and (B) shoot and root length of pea seedlings under laboratory conditions.**Table 2.** Effect of various concentrations of Nano silver on the traits of pea seed germination (germination index, germination power and germination percentage (G %)) in laboratory conditions.

Treatments	G index	G Power	G%
water	5.5 bc	4600 bc	88 bc
Nano Ag 5ppm	5.9 ab	5300 ab	84 ab
Nano Ag 10 ppm	6.2 a	6000 a	88 a
Nano Ag 20 ppm	5.2 c	4200 c	77c
LSD at 0.05	0.40444	800	0.422233

Discussion:**Comparative discussion: Chitosan vs Nano silver (AgNPs):**

Chitosan enhances **fresh weight** primarily through osmoregulation, membrane integrity, and increased water holding capacity. AgNPs also enhance fresh weight by improving water uptake and decreasing membrane damage (oxidative stress). (Gebril et al., 2026; Stałanowska et al., 2025; Saini et al., 2024; Lyalina et al.(2023) .

Both chitosan and AgNPs improve plant water status, although through different mechanisms. In addition, water balance necessary for seed germination and plant growth, but in different ways. Chitosan primarily works through osmoprotection, by enhancing the accumulation of osmolytes, cell wall and membrane stabilization, and water loss prevention, thus promoting cell turgor and physiological activity during stress. On the other hand, AgNPs affect water status by enhancing membrane permeability and water absorption during imbibition, leading to faster water uptake and better physiological performance

Chitosan improves **dry weight** by increasing metabolic activity, nutrient absorption and decreasing oxidative stress, whereas AgNPs increase dry weight primarily by increasing photosynthesis, chlorophyll and maintaining high carbon assimilation. (Elmahdi et al 2026; Gebril et al., 2026;Sadeghi, 2024; Divya et al., 2021; Tripathi et al., 2020).

Thus, chitosan enhances biomass accumulation under stress conditions by enhancing antioxidant capacity, preventing oxidative damage and preserving cellular integrity under stress, which helps to sustain minimum metabolic activity. On the other hand, AgNPs enhance the photosynthesis-based biomass accumulation through enhanced chlorophyll, photosynthetic rate, and carbon assimilation efficiency and increased structural biomass formation under normal growth conditions.

Chitosan significantly enhances **root length** through increased cell division, root branching and osmotic regulation through proline. AgNPs also promote root length but mainly via increased nutrient absorption and regulation of root water permeability. (Gebril et al., 2026; Saini et al., 2024; Lyalina et al. (2023); Godínez-Garrido et al. (2022); Malerba and Cerana, 2020; Tripathi et al., 2020).

Chitosan is more important in root architecture modification, stimulating root length, root branching and root hair by enhancing cell division in root meristems, hormone regulation (particularly auxins), and nutrient absorption, resulting in enhanced root development. However, AgNPs primarily improve root physiological efficiency by improving membrane transport, water and nutrient uptake, and regulating metabolic processes and oxidative stress, thus improving root function with only limited changes in root architecture.

Shoot length were enhanced by chitosan through improved antioxidant activity and cell integrity. AgNPs boost shoot growth by enhancing chlorophyll content and photosynthetic productivity. (Gebril et al., 2026; Saini et al., 2024; Hidangmayum and Dwivedi (2023); Tripathi et al., 2020; Malerba and Cerana, 2020).

Chitosan protects growth processes under stress by enhancing antioxidant enzyme activity through increased activity of antioxidant enzymes, reduced stress-induced oxidative damage and reestablishment of cell metabolism, and hence normal physiological processes, leading to better germination and seedling establishment under stressful conditions. However, AgNPs promote photosynthetic-mediated shoot growth through improved chlorophyll content,

photosynthetic efficiency and nutrient uptake, which lead to increased energy generation, carbon fixation and enhanced plant growth.

Chitosan improves **germination index** and uniformity by facilitating the activation of stored resources through enzyme degradation and by eliminating the oxidative lag during embryo activation. AgNPs increase the germination percentage but primarily by enhancing hydration, membrane properties and cell water balance, promoting radicle penetration. (Gebril et al., 2026; Saini et al., 2024; Lyalina et al. (2023); Godínez-Garrido et al. (2022); Rai et al., 2021). Overall, chitosan improves germination through by activating biochemical and metabolic processes by enhancing hydrolytic enzymes, reserve mobilization and antioxidant defense mechanisms, while AgNPs mainly improve the physical and physiological conditions of germination by increasing membrane permeability, water holding capacity and cellular hydration, which in turn contribute to increasing germination efficiency.

Both chitosan and AgNPs improve the **germination power**, leading to better early growth. Chitosan increases vigor by activating antioxidant defense systems and regulating stress-response genes, whereas AgNPs increase vigor by enhancing photosynthesis and nutrient uptake efficiency. (Gebril et al., 2026; Saini et al., 2024; Godínez-Garrido et al. (2022); Rai et al., 2021; Tripathi et al., 2020).

Chitosan promotes stress tolerance by stimulating antioxidant defense mechanisms, limiting oxidative stress, and triggering stress-related pathways that promote cellular homeostasis and metabolic stability during stress. In contrast, AgNPs enhance growth efficiency and biomass accumulation under normal conditions by improving water uptake, membrane transport, and metabolic activity. by enhancing water absorption, membrane transport, and metabolic reactions to accelerate cell division and cell elongation, which result in an increased plant growth and Root length .

Chitosan and nano silver (AgNPs) improved **germination percentage** under normal and abiotic stress conditions but by different mechanisms. Chitosan enhances germination mainly by regulating hydrolytic enzyme activity (particularly α -amylase), reserve mobilization, and oxidative stress during imbibition. (Particularly α -amylase) and reserve mobilization as well as relieving oxidative stress during the imbibition period. In contrast, AgNPs enhance germination primarily through the regulation of water uptake and early metabolic activation at optimal concentrations and early metabolic activation at optimal concentrations (Gebril et al., 2026; Stałanowska et al., 2025; Saini et al., 2024; Riseh et al., 2023; Hidangmayum and Dwivedi, 2023).

In general, Chitosan is primarily involved in biochemical activation of germination by promoting hydrolytic enzymes (especially α -amylase) activity and reserve release, antioxidant defense and hormone balance, thus contributing to seed vigor and seedling establishment. On the other hand, whereas AgNPs predominantly influence physiological and cellular processes by increasing membrane permeability and water absorption, facilitating seed hydration and metabolic activation, and controlling reactive oxygen species (ROS) at optimal levels, which leads to more rapid and uniform germination.

Conclusion

Chitosan and silver nanoparticles (AgNPs) enhance germination and seedling performance of chickpea in laboratory conditions. The highest values for most traits were obtained at 1%

chitosan and 10 ppm AgNPs. These treatments can be considered potential seed priming agents for enhancing crop establishment under semi-arid conditions of southern Libya, thereby contributing sustainable food production and security.

It is recommended that these treatments be further tested in field conditions their usefulness under different environmental conditions. Others aspects that should be addressed in future studies include optimizing application methods, evaluation of the long-term effects on plant growth and yield and understanding physiological and molecular mechanisms of the observed responses.

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Compliance with ethical standards*Disclosure of conflict of interest*

The authors declare that they have no conflict of interest.

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