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RESEARCH ARTICLE

Seed priming with chitosan and its nanoparticles improve physiological, biochemical and crop performances in lentil (*Lens culinaris* L.)

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Abstract

Chitosan, a derivative of chitin, is a natural elicitor known for its biocompatibility, biodegradability and non-toxic properties, offering significant yet underexplored potential in advancing sustainable agriculture. Its application enhances plant physiological responses and mitigates the detrimental effects of both abiotic and biotic stresses across various growth stages. This study evaluated the effects of seed priming with chitosan and chitosan nanoparticles (CNPs) on the germination and seedling development of lentil (*Lens culinaris* L.). Seeds were primed with varying concentrations of chitosan (0.5 %, 1.0 %, 1.5 % and 2.0 %) and CNPs (50, 100, 150, 200 and 300 ppm). The priming duration was optimized for each treatment based on water uptake kinetics and initial radical emergence. Seed priming with a low concentration of chitosan (0.5 %) significantly improved seedling growth parameters, hydrolytic enzyme activities (amylase, protease), dehydrogenase and phytase activity, as well as yield-associated traits. In contrast, CNPs priming at 200 ppm showed superior enhancement across most laboratory and field parameters. Primed seeds treated with CNPs showed marked improvements, including a 12.6 % increase in germination potential, a 65.9 % increase in root length, a 97.4 % increase in shoot length, a 73 % increase in total seedling length, a 19.7 % rise in seedling dry weight and significant increases in vigor indices (94.8 % and 34.8 %) compared to non-primed seeds. Additionally, hydrolytic enzyme activities, including amylase, protease, dehydrogenase and phytase, were substantially elevated in primed seeds relative to untreated controls. To corroborate these findings, field experiments were conducted to assess the crop performance upon priming. Results revealed that seed priming with chitosan (0.5 %) and CNPs (200 ppm) significantly ($P \leq 0.05$) enhanced seedling emergence, field establishment, plant height, pod and seed counts per plant, seed weight, overall yield and harvest index. These findings suggest that seed priming with 0.5 % chitosan or 200 ppm CNPs enhances seedling development, plant growth and yield by improving enzymatic activities and mobilization of food reserves.

Keywords: chitosan; chitosan nanoparticles; enzymes; germination; lentil; seed vigour; yield

Introduction

High-quality seeds, having germination percentage > 90 % and exhibit good plant vigor, are a fundamental input for crop establishment, directly influencing the yield and productivity of any crop. However, the unavailability of quality seeds particularly in pulses remains a significant constraint in narrowing the gap between potential and actual yields (1). Poor seed quality often results in reduced germination rates and suboptimal crop establishment, ultimately affecting both production and productivity (2).

Lentil (*Lens culinaris*), recognized as a promising legume crop due to its high content of plant-based protein and enriched micronutrient profile (Fe, Zn, Mg, K, Cu etc.), is cultivated across 3.85 million hectares globally, yielding 3.59 million tonnes. India ranks first in the area under cultivation (1.8 million hectares) and second in production (1.1 million tonnes) (3). However, despite leading in cultivation area, India's productivity (611 kg/ha) is significantly lower than that of Canada (1633 kg/ha) (3). Among the various factors contributing to this low productivity, seed quality is a critical determinant. The lack of high-quality seed prevents farmers from fully realizing the potential of improved

crop varieties. Over the years, numerous strategies (breeding for high yield cultivars, use of fertilizers and manures, seed treatments) have been explored to enhance the growth and development of crop species. Among these, seed priming has emerged as a widely adopted technique. This method involves controlled water uptake by seeds to initiate pre-germination metabolic processes, improving seed vigor and subsequent crop performance (4, 5).

Priming with chemicals and nanoparticles has been widely investigated to enhance the speed and uniformity of seed germination in various crops (6, 7). Chitosan, a chitin derivative, acts as a natural elicitor that induces plant resistance and has been extensively used for protection against bacterial, fungal and viral diseases (8-11). They offer an even greater advantage due to their high surface area, enhanced bioavailability and ability to penetrate plant tissues more effectively, leading to more robust and systemic defense activation (9, 12). Chitosan's role as a seed or plant treatment has also been assessed in multiple crops, including rice, wheat, maize, sunflower, tomato, grapes and beans, particularly in mitigating abiotic and biotic stress (12, 13). Studies have shown that chitosan suppress fungal pathogens such as *Fusarium oxysporum* in tomato (14) and *Alternaria solani* in potato (15). Several studies have reported their significant effects on seed germination, root and shoot growth, seedling biomass and vigor indices in agricultural crops such as pearl millet (16), maize (17), lentil (18, 19) and wheat (20). However, the potential application of chitosan and its nanoparticles as seed priming agent in Indian cultivars of lentil has not yet been documented.

The unique properties of chitosan and its nanoparticles highlight their potential for improving seed quality and stress resilience, particularly under changing climatic conditions. This study focuses on evaluating the efficacy of seed priming with chitosan and its nanoparticles in lentil, emphasizing seedling development, biochemical changes and field performance.

Materials and Methods

Priming agent

The study was conducted using medium-vigor seeds of lentil (*Lens culinaris* var. HUL 57), which were naturally aged. For seed priming, medium molecular weight chitosan powder with a deacetylation degree of 90 % and a viscosity range of 150-500 cP, along with CNPs with an average particle size of 80-100 nm was used as priming agents. Laboratory experiments were designed with ten different treatments and executed following a Completely Randomized Design (CRD) with three replications.

Priming solution

Chitosan (concentrations- 0.5 %, 1.0 %, 1.5 % and 2.0 %) and CNPs (concentrations- 50, 100, 150, 200 and 300 ppm) powder were dissolved in 1 % acetic acid solution under continuous stirring using magnetic stirrer for 1 hr at room temperature to ensure complete solubilization. Following dissolution, the pH of the solution was carefully adjusted to 6.5 by gradually adding 1 mL of 1 M sodium hydroxide (NaOH) solution while monitoring the pH to avoid overshooting. After achieving the desired pH, the solution was allowed to rest overnight at room temperature without further stirring. This resting period facilitated the stabilization of the solution and prevented the formation of clumps, ensuring uniform dispersion of the chitosan and CNPs.

Water uptake curve study and optimization of priming duration

This study investigated the effects of four concentrations of chitosan (0.5 %, 1.0 %, 1.5 % and 2.0 %) and five concentrations of CNPs (50, 100, 150, 200 and 300 ppm) on seed imbibition in lentil, with water-treated seeds serving as the control. The optimal priming duration was determined based on the time required for the first radical protrusion (Fig. 1b). To standardize the priming duration, a preliminary experiment was conducted using three replicates of 50 seeds, all of uniform weight. These seeds were placed between two layers of filter paper moistened with 10 mL of the respective priming solutions. The experimental setups were maintained in a seed germination chamber under controlled conditions: a constant temperature of 20 ± 2 °C and a relative humidity of 80 - 85 %. To monitor water uptake, the seeds were periodically surface-dried using tissue paper to

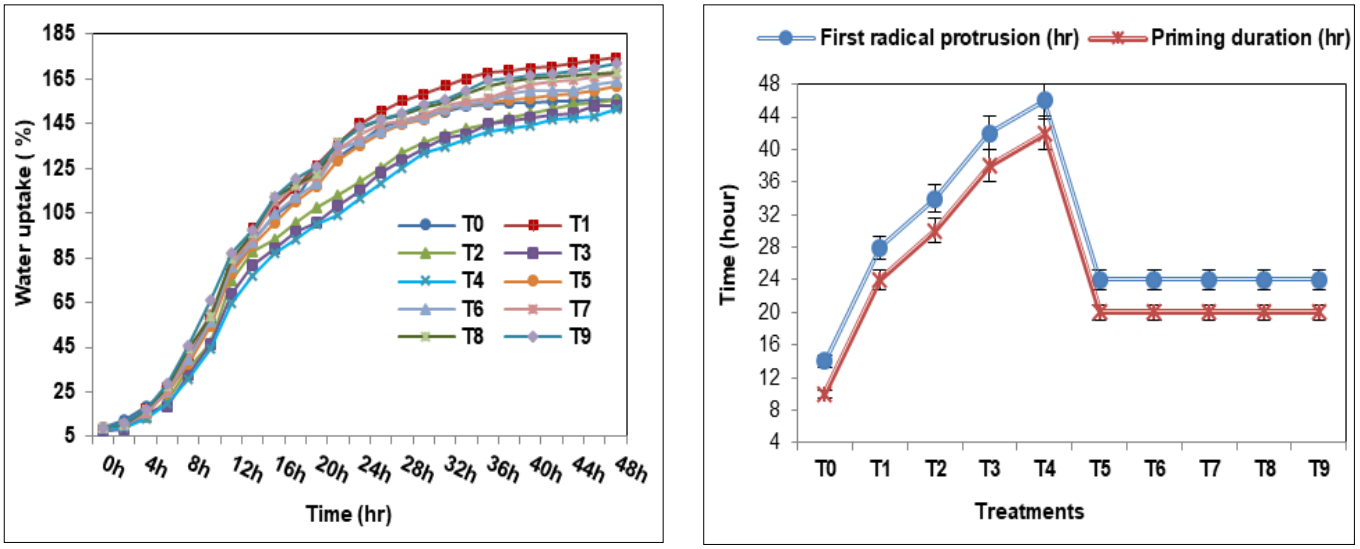

Fig. 1. Optimization of priming duration for different treatments. (a) Water uptake pattern over 48 hrs of time (b) time of radical protrusion for different treatments. T0=Control (water), T1=0.5 % Chitosan, T2=1.0 % Chitosan, T3=1.5 % Chitosan, T4=2.0 % Chitosan, T5=50 ppm CNPs, T6=100 ppm CNPs, T7=150 ppm CNPs, T8=200 ppm CNPs,T9=300 ppm CNPs.

prevent damage and then weighed to record their dry weight. This systematic evaluation of water absorption patterns provided critical data for optimizing the seed priming process and ensuring its effectiveness in enhancing seed performance.

The water uptake was calculated based on increase in seed moisture at each 2 hrs interval and it was calculated by the following formula:

$$\text{The moisture increase (\%)} = \frac{\text{FW-DW x 100}}{\text{DW}}$$

Where, FW=Fresh weight (g) of seed at 2 hrs interval

DW= Dry weight (g) of seed taken at the end of imbibition period (after 48 hrs)

The priming duration for each concentration was finalized based on the first radical protrusion. The duration of first radical protrusion for each concentration of chitosan and CNPs was recorded and a duration of 4 hrs before the radical protrusion was selected for each concentration of chitosan and CNPs.

Physiological study on seedling characteristics

To evaluate the effects of chitosan and CNP priming on seed germination, seedling growth and vigor in lentil, a standard germination test was conducted. The test involved 100 seeds per replicate, with three replicates for each treatment. The procedure adhered to the International Rules for Seed Testing (21). Final germination percentages were calculated based on the number of normal seedlings observed at the end of the experiment. Seedling length (including root and shoot) and seedling dry weight were measured for 10 randomly selected seedlings from each treatment group.

The seedling vigour index I and II were calculated (22).

Seedling vigour index (SVI I) = Germination (%) x Seedling length (cm)

Seedling vigour index (SVI II) = Germination (%) x Seedling dry weight (g)

The most effective priming concentrations 0.5 % chitosan and 200 ppm CNP were further assessed for their performance in pot trials. Sterilized plastic pots (10 × 10 cm) filled with silica sand served as the germination medium. The moisture content of the sand was maintained at approximately 80 % of field capacity by watering with distilled water as required.

For each treatment, 25 seeds (primed and non-primed) were sown at a depth of 2 cm in moist silica sand, with three replicates per treatment. The pots were placed in a seed germinator set to a temperature of 25 ± 2 ℃ and a relative humidity of 80 - 85 %, ensuring optimal conditions for germination and seedling development.

Biochemical analysis

α-amylase assay

α-amylase was extracted from primed seeds using a modified Bernfeld method (23), by homogenizing seeds in 10 mM calcium chloride at 4 ℃. To initiate the enzymatic reaction, 1 mL of 1 % starch solution and 1 mL of diluted enzyme were mixed and incubated at room temperature for 15 min. The reaction was stopped by adding 2 mL of 1 % 3, 5-dinitrosalicylic acid (DNS) and incubating at 100 ℃ for 5 min. After cooling, 1 mL of 40 % potassium tartrate (Rochelle salt solution) was added and mixed. The solution was then diluted with 5 mL of water and the absorbance was measured at 560 nm to assess enzyme activity.

Protease assay

Protease was extracted from primed seed tissues using a modified Carrie Cupp-Enyard method (24). Seeds were homogenized in 100 mM potassium phosphate buffer at 4 ℃. The enzymatic reaction was initiated by adding 1 mL of crude enzyme to 1 mL of 1 % casein (w/v) and incubating at 37 ℃ for 30 min. The reaction was stopped by adding 2 mL of chilled 5 % trichloroacetic acid (TCA) and freezing for 10 min to precipitate the protein. Afterward, 1 mL of Folin-Ciocalteu reagent (1:1 v/v) was added and the mixture was incubated in the dark at room temperature for 30 min. Absorbance was measured at 660 nm after the blue color developed, with the standard curve based on tyrosine digestion (µg).

Dehydrogenase assay

Dehydrogenase assay was carried out using the method of Kittock and Law (25). Soaked seeds were mashed and mixed with 5 mL of 0.5 % TTC solution (pH 7.0), incubated at room temperature for 4 hrs and then centrifuged at 10000 rpm for 3 min. The supernatant was discarded and the residue was mixed with 10 mL acetone for formazan extraction, followed by 16 hrs of incubation at room temperature. After centrifugation, the absorbance of the supernatant was measured at 520 nm using a spectrophotometer. A standard curve was generated based on the concentration of TPF (µmol/mL).

Phytase assay

Germinated seeds, both primed and non-primed, were homogenized in 0.1 M sodium acetate buffer and centrifuged at 12000 x g for 5 min. The assay mixture consisted of 350 µL of 0.1 M sodium acetate buffer (pH 5.0) and 100 µL of 2 mM sodium phytate. The reaction was initiated by adding 100 µL of crude enzyme and incubating at 40 ℃ for 30 min with gentle shaking once. Phosphate release was measured using the ammonium molybdate method (26). The absorbance at 355 nm was recorded after adding acetone/ H_2SO_4/ammonium molybdate solution and citric acid. Enzyme activity (units) was calculated using a calibration curve, with results expressed as µmol of phosphate liberated per minute. Blanks were prepared by adding ammonium molybdate solution before the enzyme.

Field experiments

The best performing concentrations of chitosan and chitosan nanoparticles were selected for further evaluation under field conditions. The seeds primed with 0.5 % chitosan and 200 ppm CNPs for 24 hrs and 20 hrs respectively. Once primed, the seeds were dried under ambient conditions and used for sowing in the field following randomized block design with five replicates.

Statistical analysis

Data were statistically analysed using the statistical software IBM SPSS Statistics, Version 22.0 (Armonk, NY: IBM Corp.). Significant differences were tested using a general linear model and means were compared using Tukey's test at 5 % probability level ($p \leq 0.05$). The results are expressed as means ± standard error of the mean. Microsoft Excel was used for graphing and data visualization.

Results

Effect of chitosan and CNPs concentrations on water uptake curve and radicle protrusion

The lentil seeds were allowed to imbibe priming solution of different concentrations of chitosan and CNPs along with control (water) for 48 hrs and the imbibition patterns (water uptake curves) at varying concentrations were recorded. The graph represents the data of three replicates and vertical bars above and below each mean denote the standard error of mean (Fig. 1a and 1b). The results showed that seed moisture varied from 7.6 - 9.2 % at the start and increased to 152 - 175 % after 48 hrs of imbibition in different treatments. Water uptake curve for all the priming treatments was similar. After 2 hrs of imbibitions, the control (water-treated) seeds showed the highest moisture increase (12.65 %) where water was used. The initial hours after imbibition, which lasted up to 12-14 hrs, were characterized by rapid moisture increase. Water uptake was slowed with increasing concentration (0.5 % to 2.0 %) of chitosan and it was found that seed moisture increased to 104 % in control (water), 108 % in 0.5 % chitosan, 93 % in 1.0 % chitosan, 89 % in 1.5 % chitosan and 87 % in 2.0 % chitosan at 16 hrs of imbibition. In contrast, the water uptake did not vary significantly among the concentrations of CNP used and the seed moisture increased to 100 % in 50 ppm, 104 % in 100 ppm, 110 % in 150 ppm and 112 % in 200 and 300 ppm over control (104 %) at 16 hrs of imbibition.

The water uptake pattern in lentil followed the sigmoidal curve, where the water uptake was rapid during initial hours of imbibition (phase I), afterward the uptake slowed down upon saturation, which is aligned with the previous report on soybean (27).

Enhancement of seed germination and seedling characteristics by chitosan and CNPs priming

To examine the effect of chitosan and CNPs priming on seed germination and other seedling characteristics of lentil, the standard germination test was conducted and the results are presented in Table 1. Significant change in the seed germination percentage was observed among different priming concentrations. The seed germination was significantly (p=0.05) increased only at 0.5 % of chitosan priming and germination was negatively affected when concentration was increased thereafter. In CNPs priming, increasing trend of germination percentage was observed with increasing concentrations of CNPs. Highest germination percentage (93.75 %) was observed in T8 (200 ppm CNP) followed by 92.25 % in T1 (0.5 % chitosan) over control (83.25 %).

Root, shoot and seedling length was also significantly regulated upon different priming treatments. The root, shoot and seedling length were recorded higher in T8 (200 ppm CNPs concentration) followed by T7 (150 ppm CNPs). Similarly, seedling dry weight, vigour indices (Vigor Index I and II) were also recorded higher in T8 as compared to control and other treatments (Fig. 2).

Chitosan and CNPs priming effect on enzymes modulation

The activities of key biochemical enzymes were assessed to find out the effect of selected chitosan (0.5 %) and CNPs (200 ppm) priming on hydrolytic enzymes viz amylase and protease, dehydrogenase and phytase during seed germination and growth. Non primed seeds and seedlings of the same age were taken as control. The result graph represents the data of four replicates and vertical bars denote the standard deviation. Bars designated with different letters indicates significant difference among treatments and bars designated with the same letter are not significantly different based on Tukey's test at p=0.05 (Fig. 3). The results exhibit that in chitosan priming, the activity of α-amylase, protease, dehydrogenase and phytase were increased. The seeds primed with 0.5 % chitosan exhibited the highest amylase activity, registering at 0.042 mg of maltose per minute. This was closely followed by seeds treated with CNPs at 200 ppm, which demonstrated an amylase activity of 0.041 mg maltose per minute (Fig. 3a). Both treatments resulted in a noteworthy enhancement of amylase activity compared to the control group. In contrast, the Bavistin-primed seeds (conventional fungicide) showed the lowest amylase activity and there was no significant difference when compared to the control. Statistical analysis affirmed the presence of significant differences among the various treatments and the control group, highlighting the effectiveness of chitosan and CNPs in boosting enzyme activity.

In case of protease activity, results ranged from 8.90 to 7.68 µmoles of tyrosin/min (Fig. 3b). Notably, 0.5 % chitosan again led to the highest protease activity with a measurement of 8.90 µmoles of tyrosin/min, followed by CNPs at 200 ppm with 8.24 µmoles of tyrosin/min. Both treatments resulted in a statistically significant increase in activity over the control, while Bavistin primed seeds showed the least activity, yet this was also not significantly different from the control.

Table 1. Effect of chitosan and CNPs priming on seed germination and seedling characteristics in lentil. Where, FGP: Final germination percentage

Concentration of Ch/CNPs	Final germination	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling dry Weight (g)	Vigour Index I	Vigour Index II
T_0	83.25±3.30^{def}	6.37±0.34^{d}	6.85±0.39^{ef}	13.20±0.39^{f}	0.066±0.001^{c}	1108.75±59.58^{e}	5.52±0.27^{de}
T_1	92.25±2.63^{ab}	8.83±0.43^{bc}	9.48±0.26^{d}	18.26±0.66^{d}	0.075±0.001^{ab}	1680.00±77.03^{c}	6.88±0.15^{ab}
T_2	85.00±2.94^{cde}	8.03±0.24^{c}	7.40±0.36^{e}	15.43±0.54^{e}	0.072±0.001^{b}	1310.50±32.92^{d}	6.15±0.30^{cd}
T_3	80.00±2.58^{ef}	6.43±0.22^{d}	6.93±0.31^{ef}	13.37±0.50^{f}	0.065±0.002^{c}	1068.75±29.92^{e}	5.22±0.09^{ef}
T_4	78.00±2.94^{f}	6.20±0.39^{d}	6.24±0.31^{f}	12.44±0.65^{f}	0.062±0.002^{c}	971.50±84.85^{e}	4.82±0.30^{f}
T_5	86.00±2.58bcde	8.83±0.39^{bc}	10.92±0.45^{c}	19.75±0.75^{cd}	0.072±0.003^{b}	1698.12±71.58^{c}	6.21±0.17^{bc}
T_6	89.00±2.94abcd	9.08±0.32^{b}	11.26±0.67^{bc}	20.34±0.89^{bc}	0.075±0.003^{ab}	1811.50±24.66^{bc}	6.69±0.16^{abc}
T_7	90.00±2.58abc	9.66±0.45^{b}	11.49±0.43^{bc}	21.15±0.52^{bc}	0.076±0.0.004^{ab}	1903.37±66.68^{b}	6.8±0.50^{abc}
T_8	93.75±1.71a	10.55±0.23^{a}	13.39±0.27^{a}	23.68±0.23^{a}	0.079±0.002^{a}	2202.88±87.77^{a}	7.28±0.32^{a}
T_9	89.50±2.38^{abcd}	9.34±0.39^{b}	12.06±0.43^{b}	21.62±0.82^{b}	0.076±0.002^{ab}	1950.48±84.25^{b}	6.95±0.33^{a}

RL: Root length, StL: Shoot length, SL: Seedling length, SDW: Seedling dry weight, VI I: Vigour index I, VI II: Vigour index II. T_0=Control, un-primed, T_1=Chitosan 0.5 %, T_2=Chitosan 1.0 %, T_3=Chitosan 1.5 %, T_4=Chitosan 2.0 %, T_5=CNPs 50 ppm, T_6=CNPs 100 ppm, T_7=CNPs 150 ppm, T_8=CNPs 200 ppm, T_9=CNPs 300 ppm

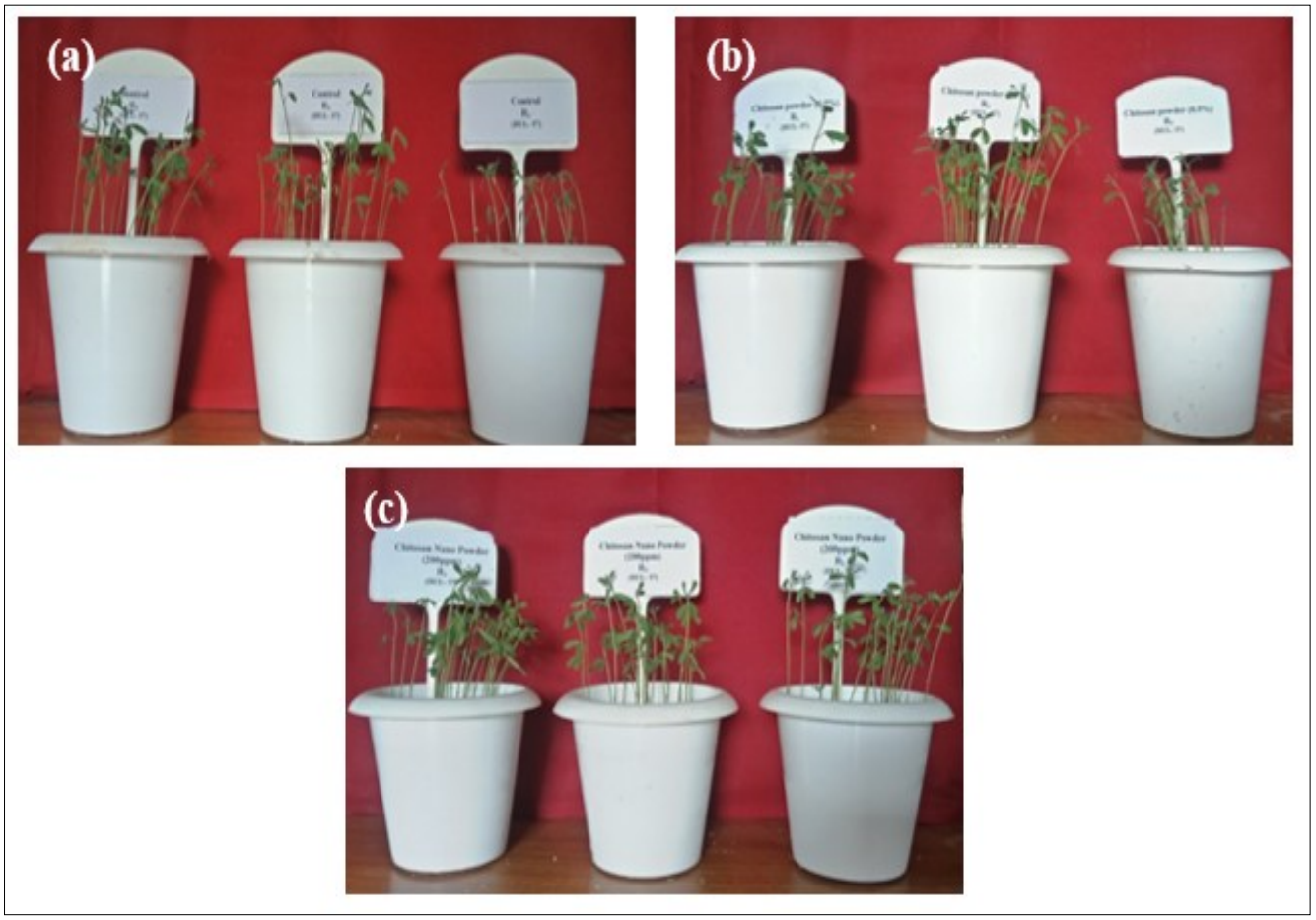

Fig. 2. Effect of chitosan and its nanoparticles priming on seedling growth of lentil (a) Un-primed (control), (b) 0.5 % chitosan primed for 24 hrs and (c) 200 ppm CNPs primed for 20 hrs.

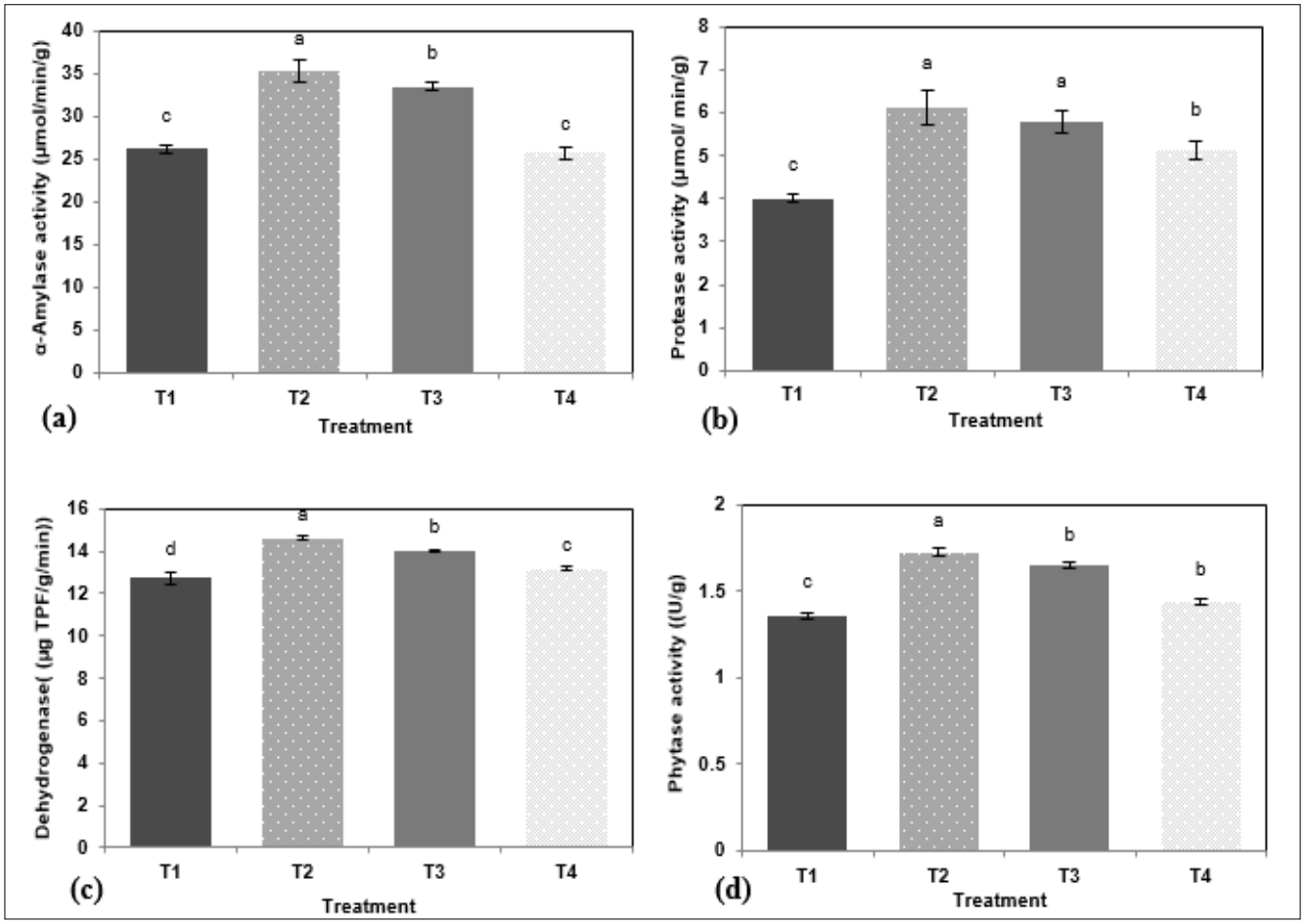

Fig. 3. Effect of chitosan and CNPs priming on (a) α-amylase, (b) protease, (c) dehydrogenase and (d) phytase activity. T1=Control (un-primed), T2=0.5 % chitosan primed, T3=200 ppm CNPs primed, T4=Conventional seed tretament (Carbendazim@2g/kg seed).

The evaluation of seed priming effects on enzyme activity revealed that 0.5 % chitosan primed seeds exhibited the highest dehydrogenase activity at 0.540, followed closely by CNPs at 200 ppm, which showed an activity level of 0.503. Both priming treatments resulted in a significant increase in enzyme activity compared to the control. In contrast, Bavistin primed seeds demonstrated the lowest dehydrogenase activity; however, this difference was not statistically significant when compared to the control (Fig. 3c).

For phytase activity, levels ranged from 0.30 to 0.36 µmoles phosphate/min. CNPs at 200 ppm achieved the highest phytase activity at 0.36 µmoles phosphate/min, with 0.5 % chitosan following closely at 0.35 µmoles phosphate/min. Both treatments led to a significant enhancement in activity compared to the control, while bavistin primed seeds recorded the lowest phytase activity without any significant difference from the control group (Fig. 3d). Overall, the findings highlight the potential of 0.5 % chitosan and CNPs as effective seed priming agents to enhance enzyme activity, which may contribute positively to seed performance.

Chitosan and CNPs priming effect on yield and its attributing traits

Fig. 4 illustrates the effects of different treatments with chitosan and CNPs on seedling emergence, plant growth and yield-related parameters in lentils. The results demonstrate the superiority of chitosan (T_2) and chitosan nanoparticle (T_3) priming over both the control/untreated (T_1) and conventional seed treatment (T_4) in improving yield-related traits in lentils. The number of seeds per plant was significantly influenced by the treatments. T_2 (0.5 % chitosan) and T_3 (200 ppm CNPs) yielded the highest number of seeds per plant, with significantly more seeds per plant than the control (T_1) and T_4 (Carbendazim).

T_2 (0.5 % chitosan) also exhibited the highest seed weight, which was significantly higher than the control (T_1). T_3 and T_4 also showed higher seed weight compared to the control (T_1). Seed yield per plant was maximized in T_2 (0.5 % chitosan) and T_3 (200 ppm CNPs), with both treatments significantly outperforming the control (T_1). T_4 showed moderate improvements over the control but was less effective than T_2 and T_3. The harvest index, which measures the efficiency of the plant in converting total biomass into economic yield, was highest in T_2 and T_3. Both treatments significantly outperformed the control, indicating that chitosan and CNPs improve resource use efficiency (Fig. 5).

Discussion

Seed priming is a widely adopted technique to enhance the seed quality and improve performance across diverse environmental conditions. We conducted the present experiment to optimize the optimum dose and observe the effect of chitosan and CNPs priming for lentil seed enhancement. In the present study, we observed that seeds priming with chitosan and its nanoparticles resulted in increased germination percentage, seedling vigour index, length and dry weight of hypocotyl and radical compared to control. The germination percentage was significantly up to 10 % in 0.5 % chitosan primed seed and 9 % in CNP (200 ppm) over control.

The significant increase in the germination and physiological characteristics upon chitosan priming was also reported in maize (28) and vegetable crops (29, 30). In support of the present findings, the enhanced effect of chitosan priming on germination, root length, shoot length, vigour index, fresh weight and dry weight were found in wheat (31); groundnut (32); in ajwain (33); in mung bean (34,35); under adverse condition. Chitosan priming enhanced the disease resistance, germination and vigor in pearl millet (16) and improved germination rates, seedling length, seedling weight increased the length and weight of hypocotyls and radicles in rapeseed under salinity stress (36). The role of chitosan and its nanoparticles in mitigating abiotic stresses, particularly drought and salinity by alleviating physiological and biochemical attributes in the crops has been studied by several research (37-39). Low concentrations of nanomaterials have been shown to significantly boost plant tolerance to abiotic stresses by activating plant cell signaling pathways by inducing the controlled generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), enhancing the plant's defense systems by increasing the efficiency of antioxidants. This dual action helps mitigate oxidative damage and maintain cellular stability under stressful environmental conditions (37, 39).

In our present study, seed priming with 0.5 % chitosan and CNPs, 200 ppm significantly enhanced amylase, protease and dehydrogenase activities were while phytase activity was found lower. These enzymes are crucial for seed germination, facilitating the breakdown of stored reserves like starch, proteins and lipids to supply energy and nutrients for embryonic development (40, 41). The increased amylase activity in chitosan-primed seeds, consistent with early works highlights enhanced starch hydrolysis and sugar mobilization, which support energy-intensive early growth (41, 42, 28). Elevated protease activity was also observed, promoting nitrogen metabolism and soluble protein levels, in line with previous works (43, 44), who linked protease activity to improved root and shoot development through efficient nitrogen recycling. Increased dehydrogenase activity in chitosan-primed seeds indicates enhanced respiratory efficiency (45, 46), with improved ATP production essential for embryonic growth. Interestingly, phytase activity peaked in CNP-treated seeds, aligning with early results, who noted increased phytase activity during germination, reducing phytate levels and improving phosphorus availability. The superior performance of nanoparticles in nutrient mobilization as suggested (48), likely explains the enhanced phytase activity observed in this study. These findings collectively underscore the potential of chitosan and its nanoparticles to modulate key enzymatic processes, promoting germination and seedling vigor. Significant differences were observed in the number of pods, seed yield and harvest index in chitosan primed seed over control. The results are in accordance with the studies on maize and chickpea, where seeds primed with botanical leaf extracts led to higher seed yield and yield-related parameters. The findings of this study indicate significant improvements in the number of pods, seed yield and harvest index when seeds were primed with chitosan and CNPs compared to untreated controls. These results align with previous research demonstrating the beneficial effects of seed priming on yield-related traits across various crops. For instance, in maize and chickpea, seed priming with botanical leaf extracts resulted in enhanced seed yield and yield-contributing parameters (6, 49). Similarly, chitosan-based priming has been shown to improve reproductive traits in diverse agricultural crops by enhancing nutrient mobilization and hormonal regulation, leading to better pod and seed formation (6, 12, 13, 50-54). The application of

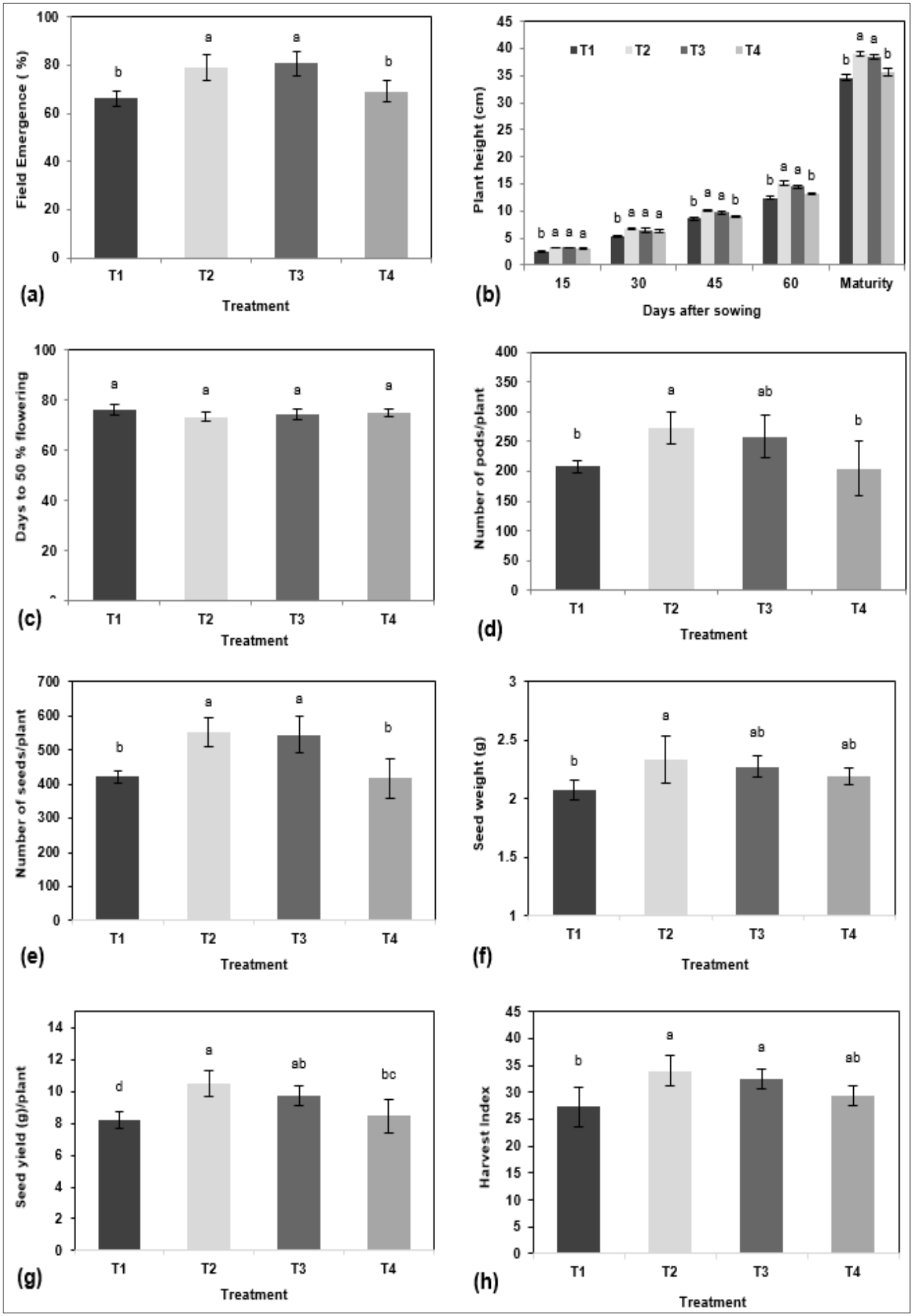

Fig. 4. Effect of chitosan and CNPs priming on crop performance based on (a), plant height at 15, 30, 45, 60 DAS and at maturity. T1=Control (un-primed), T2=0.5 % Chitosan primed, T3=200 ppm CNPs primed, T4=Conventional seed tretament (Carbendazim@2g/kg seed).

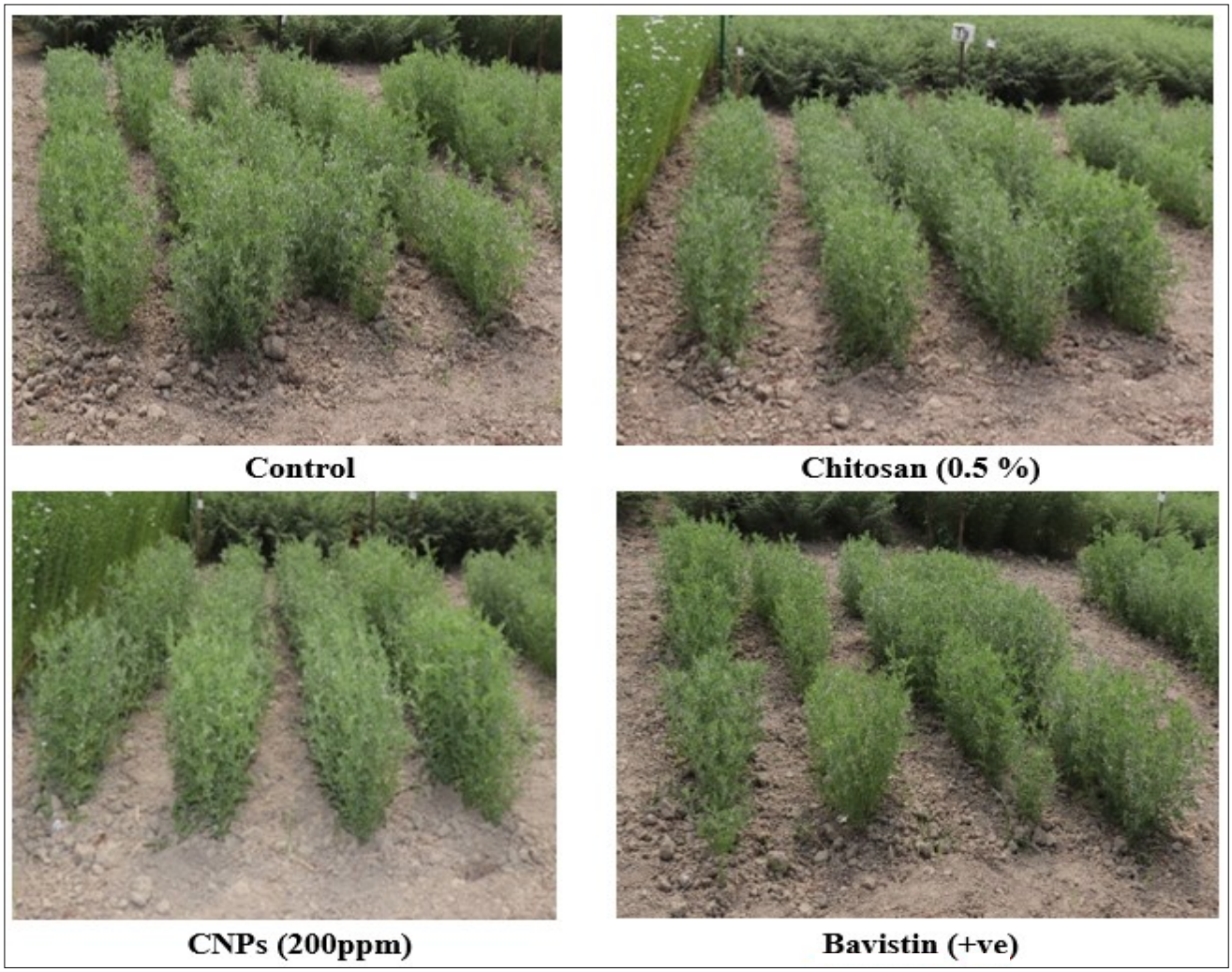

Fig. 5. Field view of assessment of effect of chitosan and its nanoparticles on crop performance in lentil.

chitosan has demonstrated a notable enhancement in yield and associated phenotypic traits, including flowering, pod maturation, seed set, grain filling and overall agricultural productivity under both optimal and stress-induced conditions (53-57). This improvement is largely attributed to the biostimulatory properties of chitosan, which trigger a range of physiological and biochemical pathways that favorably influence plant growth and development. Chitosan exhibits remarkable versatility in enhancing crop yield across diverse agricultural settings and stress conditions, positioning it as a valuable sustainable input. Its capacity to modulate physiological, biochemical and molecular mechanisms emphasizes its significance in bolstering crop resilience and productivity. This adaptability will be particularly critical for bolstering crop resilience and productivity in the context of climate change and the escalating global food demand.

Conclusion

The present study showed that seed priming using chitosan and chitosan nanoparticles enhances seed quality by improving germination percentage, root and shoot lengths. It not only improves seed growth but also provide durable resistance against several diseases. The improvement by chitosan treatment was associated with the enhancement of amylase and protease which is thought to involve in providing energy for seed growth. The enhancement in hydrolytic enzymes like amylase and protease, dehydrogenase and phytase are thought to play a role in seed germination process by loosening the endosperm around the radicle or by protecting seeds from pathogens. Results in present study clearly demonstrate that chitosan and CNPs act as seed quality and growth enhancers, modulate the activities of antioxidant and defense enzymes which could be a promising approach to improve plant growth and disease resistance in lentil. Chitosan and CNPs can be effectively used in agriculture to improve the yield and production as they are safe due to their biocompatible and biodegradable nature. The ability of these treatments to promote starch hydrolysis, nitrogen metabolism, lipid mobilization and phosphorus release underscores their potential as sustainable seed priming agents for improving germination, stress tolerance and crop productivity in pulse and cereal crops. Future research focused on the mechanistic pathways behind enzyme activation by CNPs could further expand their utility in sustainable agriculture.

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Authors' contributions

KR, RRK and TK participated in the conceptualization, supervision, validation, manuscript writing and editing. SK, PK and VK performed the research work. AK, VK, SKB and BMB assisted in data analysis and validation. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration : The authors declare that generative AI or AI-assisted technologies were not used in the writing of this manuscript. All content was developed through original analysis, interpretation and drafting by the authors.

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