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Chitosan-based NPK nanostructure for reducing synthetic NPK fertilizers and improving rice productivity and nutritional indices

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Researchers have repeatedly emphasized how urgently we have to decrease the massive nitrogen fertilizer consumption to support agricultural productivity and maintain a sustainable ecosystem. Using chitosan (CS) as a carrier for slow release is considered a potential tool for reducing synthetic fertilizer and improving crop productivity. Therefore, two field experiments were arranged in a randomized complete block design to investigate the effects of seven treatments including synthetic fertilizer and exogenous application of chitosan-based NPK nanostructure (Ch/NPs-NPK) on growth, productivity, and nutrient uptake traits of rice as a worldwide strategy crop during 2022 and 2023 growing seasons. The experimental treatments were: T1 = full recommended synthetic NPK (recommended urea, superphosphate, potassium sulfate; control treatment), T2 = 70% of T1 + Ch/NPs-NPK 100 ppm, T3 = 70% of T1 + Ch/NPs-NPK 200 ppm, T4 = 70% of T1 + Ch/NPs-NPK 300 ppm, T5 = 30% of T1 + Ch/NPs-NPK 100 ppm, T6 = 30% of T1 + Ch/NPs-NPK 200 ppm, and T7 = 30% of T1 + Ch/NPs-NPK 300 ppm. The results revealed that T4 (i.e., 70% of recommended NPK + Ch/NPs-NPK 300 ppm) and T1 (full recommended synthetic NPK) resulted in the highest and most significant growth and yield traits of rice as well as nutrient grain contents compared to other treatments. Therefore, combining 70% of recommended NPK with Ch/NPs-NPK 300 ppm as an exogenous application can be a smart choice for reducing synthetic NPK fertilizers by 30% in paddy fields without producing a significant decline in terms of growth, yield characteristics, or nutrient grain contents when applying the full recommended synthetic NPK.

KEYWORDS

chitosan-based NPK nanostructure, rice, productivity, sustainable agriculture, food security

1 Introduction

Synthetic fertilizers play a fundamental role in guaranteeing food for a growing population (Seleiman et al., 2021). However, many countries overapply fertilizers, resulting in huge ecological problems including contamination of underground water, biodiversity loss, and decreased soil fertility (Seleiman et al., 2020; Zou et al., 2021; Dimkpa et al., 2023). Egypt is an example of a highly fertilizer-consumed country, where, fertilizer consumption increased from 136.2 kg/ha in 1971 up to 473.4 kg/ha in 2020 with an annual increasing rate of 2.98%. Moreover, massive amounts of synthetic fertilizers are lost via leaching, degradation, runoff, and volatilization which has adverse environmental and economic impacts (Vejan et al., 2021; Almutari, 2023). Therefore, deep attention has to be paid to reduce the intensive utilization of agrochemical fertilizers to reinforce sustainable agriculture production (Seleiman et al., 2017).

The main cereals such as wheat, barley, rice, and maize are a daily basis for human food supply and the largest agrochemicals consumers in the agriculture sector (Ladha et al., 2016; Hafez and Seleiman, 2017; Elshayb et al., 2022b; Alhammad et al., 2023; Haydar et al., 2024). Of the major cereals, rice is regarded as an essential crop that provides food for almost half of the global population (Carrijo et al., 2017; Badawy et al., 2021). Rice nutrient requirements are driven by balanced applications among nitrogen, phosphorus, and potassium (NPK) at different rates (Vijayakumar et al., 2024). The synthetic fertilizers of NPK have been the cornerstone of rice production because of the critical role of these nutrients in many physiological pathways such as the photosynthesis process, mitotic activities, root proliferation, tissue growth, and development (Seleiman et al., 2022). Although the growers mostly relied on synthetic fertilizers to increase rice production, the excessive amounts and unbalanced NPK ratios led to several issues with the soil (e.g., soil acidification, decrease in both soil organic matter and salt accumulation, and reduced cation exchange capacity) plus the negative effects on environmental integrity (Upadhyay et al., 2023). Therefore, environmentally friendly solutions that aim to reduce NPK inputs, preserve crop production outcomes, and overcome negative climate perturbations should be considered when considering fertilizer inputs, particularly NPK, in paddy fields.

In such a scenario, nano-fertilizers are one of the most advanced interventions with appropriate formulations with a smart mechanism to achieve optimal plant uptake and avoid nutrient losses (Badawy et al., 2021; Elshayb et al., 2022a; Verma et al., 2022; Devi et al., 2023; Kumar et al., 2023; Tomar et al., 2024). The application of nano-fertilizers to soil or sprayed over the vegetative system greatly enhances plant nutrition (Batool et al., 2024; Mukhtiar et al., 2024; Singh et al., 2024). With lower molecular weights, better bioavailability, longer half-lives, and a higher surface area-to-volume ratio, nanoparticles are more effective (Sarkar et al., 2022; Ferradj et al., 2024). Particularly biological nano fertilizers hold a lot of potential as an instrument for sustainable agriculture. In contrast to mineral fertilizers, these fertilizers may have a high nutrient use efficiency and a slow-release nutrient profile, which means they give plants the nutrients they require over a longer time (Sangwan et al., 2023). Notably, there are various types of nano fertilizers, including those based on macronutrients (N, K, and P) (Chakraborty et al., 2023). As a result of its wide surface area and capability, the macronutrients (N, P, and K) in nanocomposite fertilizer encourage the absorption use of nutrients by crops resulting in increasing plant height, dry matter, leaf area,

chlorophyll synthesis, and photosynthesis rate (Sundararajan et al., 2024).

One of these smart nano-formulations, chitosan-based nanostructure (Ch/NPs) has gained more attention from scientists in the past few years owing to its biodegradable and biocompatible features (Abdel-Aziz et al., 2018; Ingle et al., 2022; Devi et al., 2023; Sangwan et al., 2023). It is worth mentioning that chitosan is a polycationic polymer that is isolated from chitin which is the second most abundant polysaccharide globally (a structural polymer in many different types of animals, mollusks, shrimp, fungi, and crabs) after it has been deacetylated (Ingle et al., 2022). One of the major advantages Ch/NPs typically degrade into non-toxic byproducts, reducing the risk of accumulation in the body or plant tissues and minimizing long-term side effects (Devi et al., 2023). In addition to their biocompatibility, Ch/NPs have been shown to stimulate a plant's immune system and aid in biotic and abiotic stress management (Sangwan et al., 2023). As a non-toxic biopolymer, Ch/NPs can be organized and integrated strategically with fertilizer sources (i.e., urea, potassium sulfate, and calcium phosphate) to produce control-release fertilizer of NPK. Loading urea fertilizer into Ch/NPs has proven its efficiency in reducing urea fertilizer quantity and enhancing rice grain productivity as documented in a previous study by Elshayb et al. (2022b). As a result, Corradini et al. (2010) loading NPK fertilizer into Ch/NPs can be a benign approach for making a complex NPK fertilizer with slow-release properties which is known as chitosan-based NPK nano-structure (Ch/NPs-NPK). Prior studies demonstrated the outstanding merits of Ch/NPs-NPK in improving wheat productivity under different soils compared with classical NPK fertilizer (Abdel-Aziz et al., 2018), enhancing both growth and potato tuber (Elshamy et al., 2019), and improving both nutrient uptake and water absorption of French bean (Hasaneen et al., 2016). A prior study by Deshpande et al. (2017) declared that the foliar application of zinc amalgamated with Ch/NPs on two durum wheat cultivars caused a significant increase in zinc grain content by 27 and 42% compared to zinc-deficient control plants. However, the foliar spray of Ch/NPs significantly increased the levels of Fe, Zn, Mn, P, Ca, and Mg in finger millet plants in comparison of the control treatment (Sathiyabama and Manikandan, 2021). However, the application of Ch/NPs-NPK in paddy schedule fertilization and its effect on nutritional grain content has remained unexplored until now.

Looking at the above objectives, this study focused on evaluating the application of chitosan-based NPK nano-structure at a different rate with a combination of T-NPK aiming to determine the optimal dose for enhancing growth properties, minimize T-NPK inputs, and maximize both grain productivity and nutrients (N, P, and K) uptake.

2 Materials and methods

2.1 Site, soil, and climatological properties

In the Agriculture Research Station, Kar El-Sheikh, Egypt's Experimental Farm of Sakha (31°05'17"N, 30°56'44"E), two field tests were carried out in 2022 and 2023 seasons.

The soil samples were taken from topsoil (depth of 0–20 cm), air-dried, and crushed (by passing through a 2-mm screen to remove big particles) before the setup of the experiments. The primary physiological properties were analyzed and recorded as described

before (Ryan et al., 2001). Across the 2022 and 2023 seasons, the analysis data declared that soil type was clayey texture, organic matter (OM%); 6.40 and 7.12), pH (1:2.5 water suspension; 8.16 and 8.35), Ec (dS m^{-1}); 3.08 and 2.90), available N (ppm; 585.4 and 592.3), available P (ppm; 5.68 and 6.00), available K (ppm; 1.44 and 1.65), available Zn^{+2} (ppm; 0.88 and 0.91), available Fe^{+2} (ppm; 4.97 and 5.00), and available Mn^{+2} (ppm; 3.14 and 3.82).

The weather databases were collected from a field observatory near the experimental site. The monthly meteorological particulars were codified annually from a field observatory and recorded in Figure 1.

2.2 Preparation of chitosan-based NPK nano-structure

Chitosan-based NPK nano-structure (Ch/NPs-NPK) was prepared according to Corradini et al. (2010) with some modifications. Briefly, chitosan (Ch) (molecular weight 100,000–300,000 Da, and viscosity: 50 to 200 mPa·s, ACROS, China) aqueous solution (0.2% w/v) was prepared by dissolving Ch in acetic acid solution (1% v/v) (99–100%, Riedel-de Haën) at ambient temperature. The pH of the solutions was adjusted to 5.5 with 0.5 M sodium hydroxide pellets ($\geq 95\%$, Sigma-Aldrich, US) solution. Subsequently, tripolyphosphate (TPP) (Technical grade, 85%, Sigma-Aldrich, US) as a cross-linking agent solution was dissolved in 10 mL deionized water to a final concentration of 0.6 mg/mL. After 30 min of vigorous magnetic stirring, the TPP solution was added dropwise (0.3 mL/min) to the chitosan solution in the Ch solution. Ch-NPK nano fertilizer was prepared by loading nitrogen (N), phosphorous (P), and potassium (K) into Ch/NPs by dissolving different amounts of NPK into 100 mL of nanoparticle solution under homogenizing at 18,000 rpm for 30 min in the presence of Tween 80 at 25°C. The resulting solution to incorporate NPK into the nanoparticles presents this final concentration: (i) 100, 200, and 400 ppm of N; (ii) 15, 30, and 60 ppm of P; (iii) 100, 200, and 400 ppm of K. The morphology of Ch/NPs-NPK was imaged by a High-Resolution Transmission Electron Microscope (HR-TEM) operating at an accelerating voltage of 200 kV (Tecnaï G2, FEI, Netherlands). Diluted Ch-NPK NF

solution was ultra-sonicated for 5 min to reduce the particle aggregation. Using a micropipette, three drops from the sonicated solution were deposited on a carbon-coated copper grid and left to dry at room temperature. HR-TEM images of the Ch/NPs-NPK that were deposited on the grid were captured for morphological evaluation. Dynamic light scattering (DLS) measurement of size and Zeta Potential was undertaken using a Nano-zeta sizer (Malvern, ZS Nano, UK). Synthesis and characterization of Ch/NPs-NPK were performed in Nanotechnology & Advanced Materials Central Laboratory (NAMCL), Agricultural Research Center (ARC), Giza, Egypt.

2.3 Plant material, and experimental details

During the dual seasons (2022 and 2023), the uniform size of rice grain (*Oryza sativa* L., Sakha super 300) was used in this study. After the nursery land was prepared, the rice grains at the rate of 120 kg/ha⁻¹ were soaked (for 24 h), incubated (for 48 h) to accelerate the germination process, and then the pre-germinated seed broadcasted in the nursery on 20 and 22 of May in both seasons, respectively. The experiments were organized in a randomized complete block design (RCBD) including 3 replicates with 7 treatments. In the permanent field, the 21-day-old seedlings were transplanted in the experimental unit with a size of 12 m² (4 m length \times 3 m width). The transplanted seedlings (2–3 seedlings/hill) were pulled and organized in the respective units at a 20 \times 20 cm distance between hills and rows. The studied treatments comprised factorial combinations among two fertilization levels (i.e., 70, and 30%) of traditional NPK fertilizer and three foliar doses of Ch/NPs-NPK (i.e., 100, 200, and 300 ppm) as well as the full recommended dose treatment (control treatment). The foliar application of Ch/NPs-NPK was applied twice (after transplanting at 20 and 40 D). The experimental treatment details are shown in Table 1.

According to the abovementioned amount of traditional soil application of NPK fertilizer, both potassium sulfate (48% K₂O) and mono-superphosphate (15% P₂O₅) were applied and integrated into the experimental soil before tillage. However, the amount of urea (CH₄N₂O)

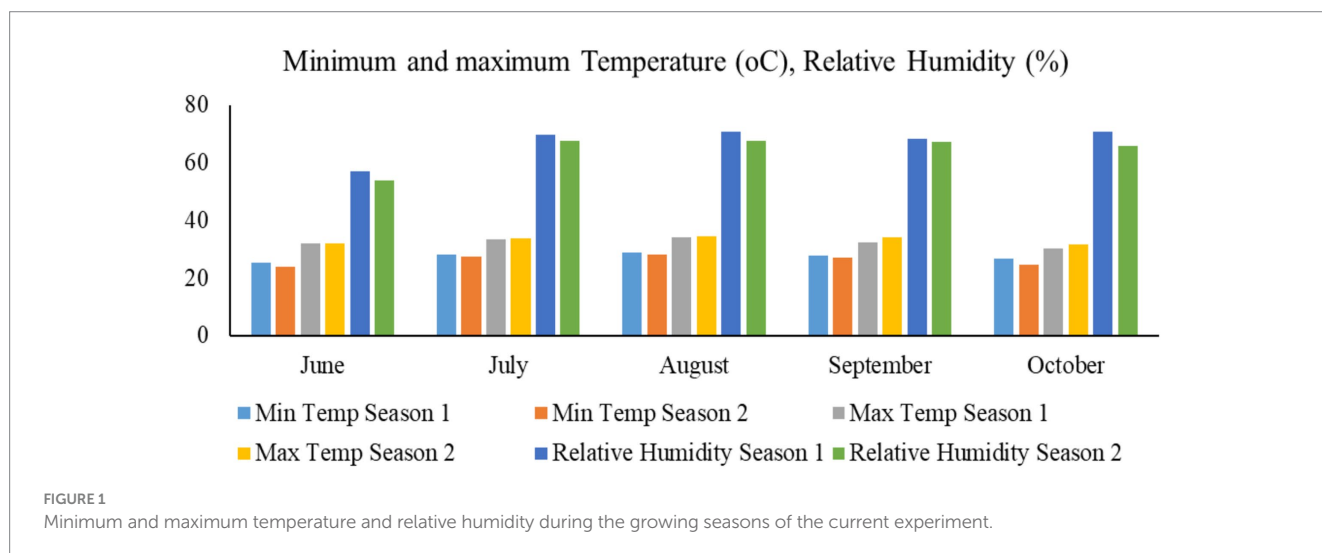


TABLE 1 The Schedule of the different fertigation treatments.

Treatments	Soil application of traditional NPK	Foliar application of Ch/NPs-NPK	Designation
T1	The full recommended NPK	Without any foliar application	100% NPK (control treatment)
T2	70% of recommended NPK	100 ppm	70% NPK + 100 mg NPK/l
T3	70% of recommended NPK	200 ppm	70% NPK + 200 mg NPK/l
T4	70% of recommended NPK	300 ppm	70% NPK + 300 mg NPK/l
T5	30% of recommended NPK	100 ppm	30% NPK + 100 mg NPK/l
T6	30% of recommended NPK	200 ppm	30% NPK + 200 mg NPK/l
T7	30% of recommended NPK	300 ppm	30% NPK + 300 mg NPK/l

fertilizer was added as previously mentioned (twice at 2/3 as a basal application and 1/3 at panicle initiation). While observing the recommended doses of urea, mono-superphosphate, and potassium sulfate are 165 kg N/ha⁻¹, 36 kg P₂O₅/ha⁻¹, and 60 kg K₂O/ha⁻¹, respectively. Before seedling transplanting and after puddling, Zinc sulfate was broadcasted and applied at the rate of 24 kg/ha⁻¹. Six days after transplanting, 4.8 L/ha of the herbicide Saturn [S-(4-Chlorophenol methyl) diethyl carbamothioate] was sprayed to control weeds.

2.4 Data gathering

2.4.1 Physiological indicators

Photosynthetic pigments (i.e., chlorophyll a, chlorophyll b, and carotenoids) measured in fresh and fully expanded leaves (5 g) from the upper canopy and will be extracted in acetone (90% v/v) and then filtered to overact a final volume of 50 mL. Estimation of total chlorophyll (i.e., Ch. a and Ch. b) and carotenoid contents (C. content) was evaluated spectrophotometrically from the absorbance of the final extract as mg g⁻¹FW⁻¹ agreeing to the protocol given by Wellburn (1994) using Equations 1–3 as follows:

$$\text{Ch.a} = 12.25A_{663.2} - 2.79A_{646.8} \quad (1)$$

$$\text{Ch.b} = 21.5A_{466.8} - 5.1A_{663.2} \quad (2)$$

$$\text{C.content} = 1000A_{470} - 1.82\text{Ch.a} - 85.02\text{Ch.b} / 198 \quad (3)$$

Where, A_{663.2}, A_{646.8}, and A₄₇₀ represent the absorbance taken at 663.2 nm (Ch. a), 646.8 nm (Ch. b), and 470 nm (carotenoids) respectively.

At the heading stage, from a fixed point of each experimental unit, the rice leaf area index (LAI_s) was estimated from 10 tagged leaves which were randomly sampled and calculated according to the following Equation 4:

$$\text{LAI}_s = \frac{\text{Leaf area of tagged leaves (cm}^2\text{)}}{\text{Ground area occupied by these leaves (cm}^2\text{)}} \quad (4)$$

concerning the evaluation of dry matter production per hill (DMP/hill), five hills from each plot were pulled out, air-dried,

sealed into a kraft paper bag, and then exposed to oven-dried (70°C for 48 h) and stopped when a steady weight (g/hill) had been obtained according to the methodology proposed by Yoshida et al. (1971).

2.4.2 Yield causative components and yield outcomes

When the experimental field reached maturity, a certain area of the center of each experimental unit (10 m²) was harvested, well-dried, and mechanically threshed to evaluate the various studied characters. The estimation of plant height (cm²), number of panicles/m² (No. Panicles/m²), panicle characters (length, weight, filled grains percentage “Equation 5”), 10³ grain weight (g), biological yield (BY), final grain yield (GY), and harvest index (HIs) percentage “Equation 6” were calculated. During 2 years, both BY and GY weights were converted to t/ha in the results databases noting the adjustment of paddy grains to 14% moisture content as mentioned by Yoshida (1981).

$$\text{Filled grain (\%)} = \left[\frac{\text{Total filled grains number}}{\text{The total grains number}} \right] \times 100 \quad (5)$$

$$\text{HIs} = \frac{\text{final grain yield}}{\text{biological yield}} \quad (6)$$

2.4.3 Nutrient uptake indices

The uptake of N (N-uptake) indices was calculated by using the Micro-Kjeldahl methodology. Samples of both grain and straw (5 g of each) in each experimental unit were exposed to oven-dried (70°C) to obtain a constant weight. Thereafter, samples were ground and digested using H₂SO₄-H₂O₂ as the standard given earlier by Jackson (2005). However, the uptake of P (P-uptake) indices was estimated as the methodology indicated by Watanabe and Olsen (1965). About the uptake of K nutrient, (K-uptake) indices were computed by a flame photometer (RHYS international LTD, India) using atomic absorption as described by Peterburgski (1968). Whereby, nutrient uptake in both grains and straw parts was calculated as kg/ha using Equation 7:

$$\text{Nutrient uptake} \left(\frac{\text{kg}}{\text{ha}} \right) = \frac{\text{nutrient content (g/kg)} \times \text{grain or straw yield} \left(\frac{\text{kg}}{\text{ha}} \right)}{\text{grain or straw yield} \left(\frac{\text{kg}}{\text{ha}} \right)} \quad (7)$$

2.5 Statistical analysis

The obtained data was subjected to an analysis of variance (Gomez and Gomez, 1984). The means of different treatments were compared by Turkey's test to clarify the significant difference at $p \leq 0.05$ probability according to Hsu (1996). All statistical analyses were conducted utilizing the analysis of variance technique by the COSTATC computer software package.

3 Results

3.1 The characterization of chitosan-based NPK nano-structure

3.1.1 TEM analysis results

According to the findings displayed in Figure 2, Ch/NPs-NPK had a spherical surface, a uniform size distribution, was stable, and had a finely shaped exterior. The greatest surface area for loading NPK nutrients onto the surface of chitosan nanoparticles is found in their spherical shape, smooth surface, and an average size of about 25.7 nm.

3.1.2 Dynamic light scattering analysis

Hydrodynamic diameter was measured in the nanoscale range using the DLS technique. The zeta potential of Ch/NPs-NPK was +45.4 mV, and its size was 32.7 nm (Figure 3).

3.2 The effects of several rates of traditional NPK fertilizers and their combination with chitosan-based NPK nano-structure as a foliar application on physiological indicators

The physiological indicators of total photosynthetic pigments (Figure 4), LAIs, and DMP/hill were significantly (at $p \leq 0.05$) influenced

by several rates of traditional NPK fertilizers and their combination with Ch/NPs-NPK (Table 2). The higher values of total photosynthetic pigments (i.e., Ch. a, Ch. b, and C. content) showed in rice plots that fertilized with T1 (full recommended dosage of conventional NPK) which gave identical statistically with T4 (70% NPK + 300 mg NPK/l) in both seasons. Similar findings were noted for LAIs, where T4 and T3 (4.86 and 5.22) fertilization resulted in statistical conformity with T1 (5.48) in the 1st season. However, in the 2nd season, only T4 (5.37) recorded statistical similarity with T1. As for DMP/hill (g/hill), the highest weight of dry matter was rendered from the application of T1 with no significant difference between this treatment and T4 (81.9, 79.6, and 83.1, 80.3 g/hill respectively) in both cropping seasons. Whilst, the application of T5 gave the lowest values of total photosynthetic pigments (4.105 and 4.387 mg g⁻¹FW⁻¹), LAIs (4.12 and 4.32), and DMP/hill (63.8 and 65.2 g/hill) respectively in both sequential seasons.

Various letters (a, b, c, d, etc.) denoted a statistically significant variation in the treatment means at $p < 0.05$. As for treatments abbreviation (see Table 1).

3.3 The effects of several rates of traditional NPK fertilizers and their combination with chitosan-based NPK nano-structure as a foliar application on yield causative components

The appraisal data in Table 3 imparted that a meaningful impact has been made by several treatments concerning plant height, No. panicles/m², and panicle length in both seasons. The tallest plants (115.1 and 116.9 cm) were recorded under the T1 treatment without significant differences between this treatment (T1) and both T3 and T4 (115.1, 114.3, and 114.5 cm) treatments in the 2022 season. Whilst, in the 2023 season, T1 treatment gave identical statistically with T4 only. As for the result of counting panicles number /m², T1 gave the best value (574.4 and 583.2 panicles/m²) which was statistically identical with T4 (556.4 panicles/m²) treatment in the 1st season and

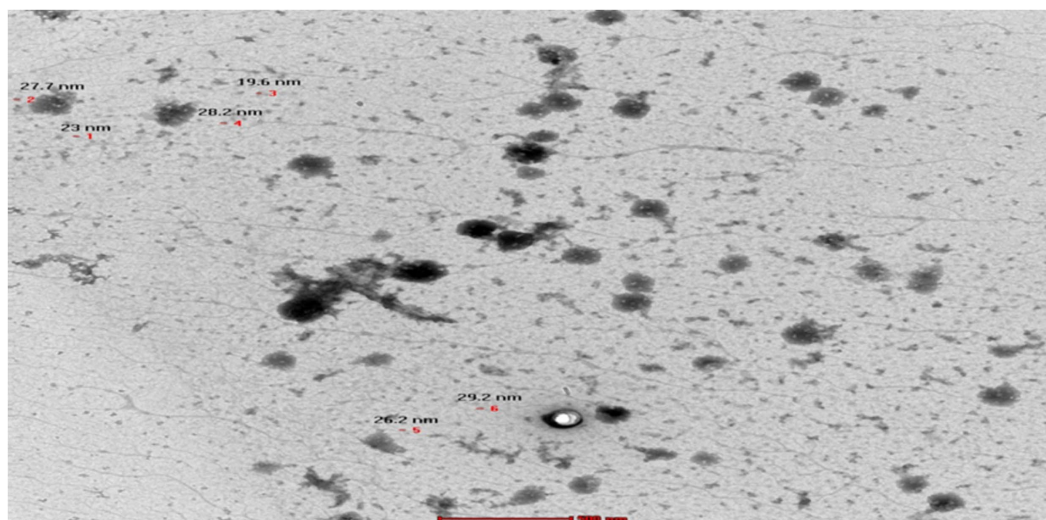


FIGURE 2
TEM image of Ch/NPs-NPK.

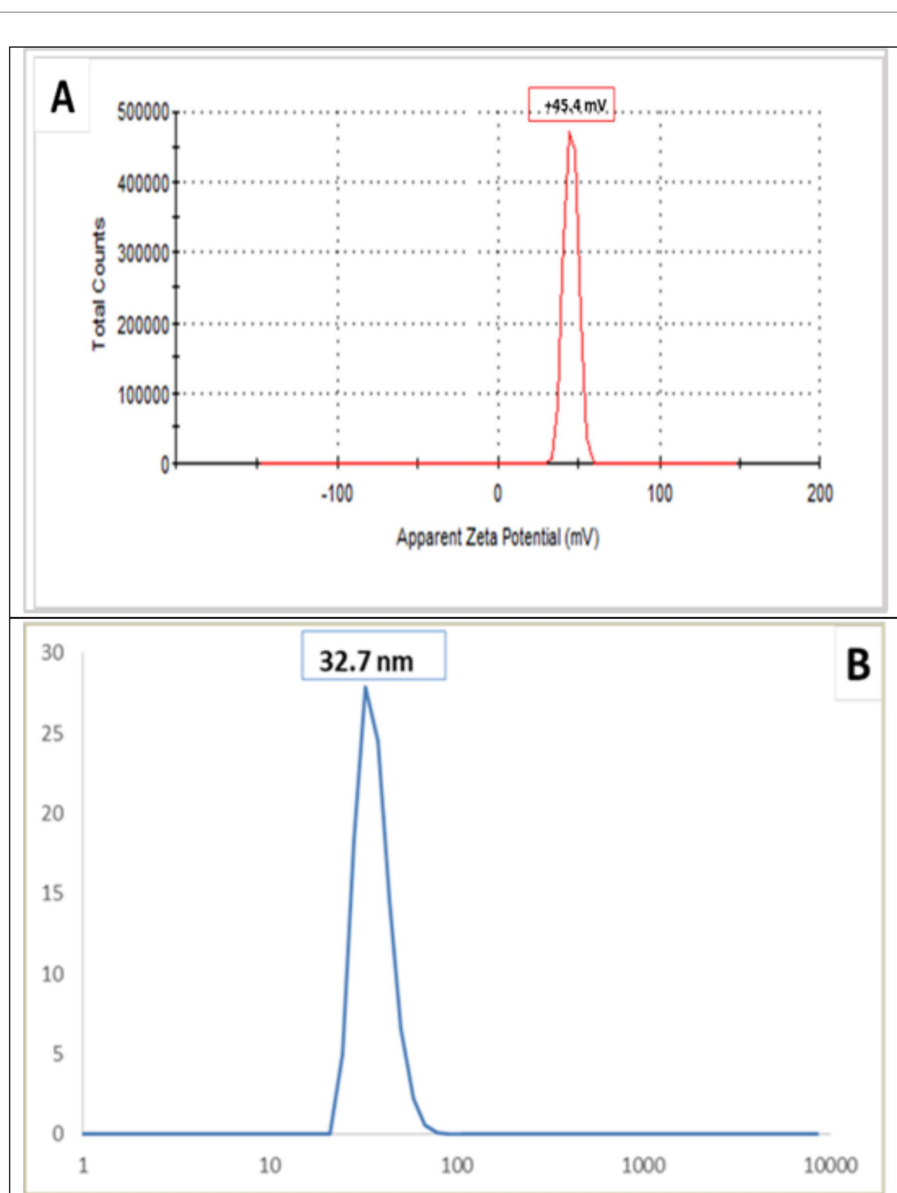


FIGURE 3
DLS analysis of Ch/NPs-NPK. Zeta potential (A) and Particle size (B).

with both T3 and T4 (551.3 and 568.2 panicles/m² respectively) treatments in the 2nd season. Likewise, T4 rendered a statistical match with T1 (22.8, and 24.1 cm, respectively in both seasons) concerning the length of the panicle. Again, the lowest values of these characters, i.e., plant height (103.3 and 104.3 cm), No. panicles/m² (454.4 and 455.7), and panicle length (18.3, and 18.0 cm) respectively during both planting, seasons were witnessed under T5 treatment.

A different and significant response on panicle weight, filled grains percentage, and 10³-grain weight characters were observed under various treatments (Table 4). Compared to a traditional application of NPK (control treatment), both T3 and T4 (3.73 and 4.11 g respectively) gave statistical conformity with the T1 (4.12 g) concerning panicle weight in the 2022 season. However, T4 treatment only provided (4.30 g) a statistical match with T1 (4.13 g) in the 2023 season. Concerning the filled grains percentage character, there wasn't a significant difference between T1 and T4 (89.6 and 92.2% respectively) in the 2022 season but in the 2023 season, T3 and T4 (88.6 and 92.6% respectively) treatments

gave identical statistics to the application of T1. As regards 10³-grain weight, the highest weight was observed in both treatments of T1 and T4 (26.93, 28.90, and 27.49, 28.16 g) respectively in the 2022 and 2023 seasons and without significant difference between them. Nevertheless, the lowest values of these characters, i.e., panicle weight (3.03 and 3.11 g), filled grain percentage (77.8 and 79.9%), and 10³-grain weight (23.20 and 23.49 g) were noted under the treatment of T5.

3.4 The effects of several rates of traditional NPK fertilizers and their combination with chitosan-based NPK nano-structure as a foliar application on yield outcomes

The obtained data about yield outcomes (BY and GY) and HIs are recorded in Table 5. During both cultivated seasons, a significant influence ($p \leq 0.05$) appeared among all treatments related to BY which recorded the highest value (24.16 and 24.93 t/ha⁻¹) respectively

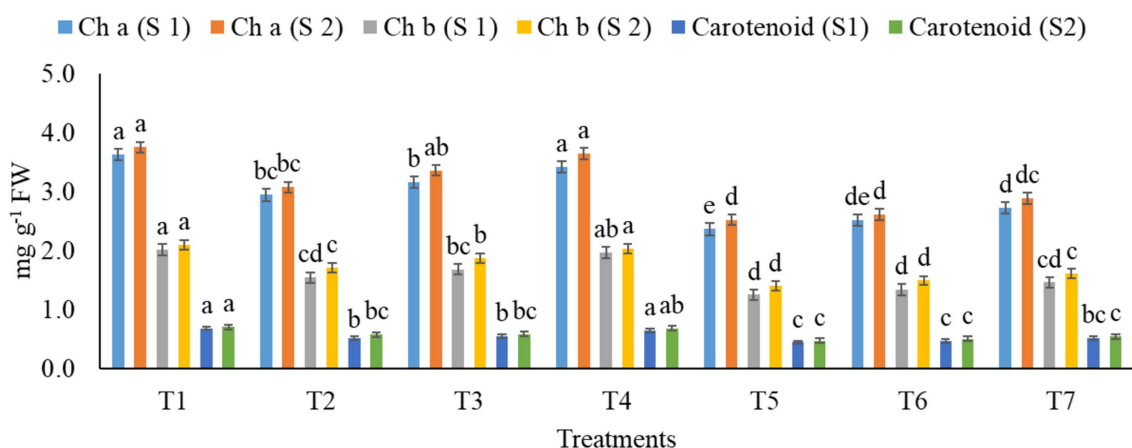


FIGURE 4 The effects of several rates of traditional NPK fertilizers and their combination with chitosan-based NPK nano-structure as a foliar application on total chlorophyll (i.e., Ch. a and Ch. b) and carotenoid contents (C. content). Various letters (a, b, c, d, etc.) denoted a statistically significant variation in the treatment means at $p < 0.05$. As for 366 Treatments treatments abbreviation (see Table 1).

TABLE 2 Effects of synthetic NPK fertilizers and their combination with chitosan-based NPK nano-structure as a foliar application on total pigment contents, leaf area index, and dry matter production.

Traits treatments	Total pigments contents (mg g ⁻¹ FW ⁻¹)		Leaf area index (LAIs)		Dry matter production (g/hill)	
	2022	2023	2022	2023	2022	2023
T1	6.326 a	6.558 a	5.48 a	5.82 a	81.9 a	83.1 a
T2	5.009 bc	5.366 c	4.61 bc	4.59 c	72.5 bc	73.1 bc
T3	5.391 bc	5.805 b	4.86 ab	4.98 bc	76.3 ab	75.2 bc
T4	6.028 ab	6.354 a	5.22 a	5.37 ab	79.6 ab	80.3 ab
T5	4.105 d	4.387 d	4.12 d	4.32 d	63.8 d	65.2 d
T6	4.323 d	4.598 cd	4.23 cd	4.60 c	66.1 cd	66.7 d
T4	4.691 cd	5.030 c	4.59 bc	4.76 bc	68.3 cd	70.1 cd
F. test	**	**	**	**	**	**

** implies $p \leq 0.05$; Data followed by the same letter were not significantly different for probability. Each column's various letters (a, b, c, d, etc.) denoted a statistically significant variation in the treatment means at $p < 0.05$. As for treatments abbreviation (see Table 1).

from the T1 treatment and on par with the T4 treatment. Also, the same trend was revealed concerning GY which represented the highest value underlying T1 treatment (10.93 and 11.04t/ha, respectively, over both seasons). Notably, the T1 and T4 treatments did not significantly differ in either of the two seasons. For HIs during both seasons, the implications exhibited that T1 and T4 contributed the highest HIs value (0.448, 0.451, and 0.447, 0.449 respectively) without significant difference between them. The lowest value of BY (20.83, and 21.27 t/ha), GY (8.35, and 8.56 t/ha), and HIs (0.403 and 0.410) is seen under the application of T5 in the 1st and 2nd seasons.

3.5 The effects of several rates of traditional NPK fertilizers and their combination with chitosan-based NPK nano-structure as a foliar application on nutrient uptake indices

From the results set (Figure 5), a substantial change in nutrient uptake (i.e., N, P, and K nutrients) of rice grain was indicated by the

effect of various applications during the 1st and 2nd seasons. Concerning N uptake indices by grains, the control treatment (T1) gave statistics similar to the T4 application in both seasons. Also, the same trend was observed concerning P uptake indices whereby the highest value was recorded by T1 without a significant difference between T1 and T4 in both sequential seasons. Whilst, a variable increase was noted concerning K uptake indices underlying rice plants treated with T3 and T4 applications and with no substantial difference between T1, T3, and T4 in both cropping seasons. Substantial difference between T1, T3, and T4 in both cropping seasons. The lowest values of N, P, and K elements uptake were presented from the plots that fertilized with T5 application in both of 2022 and 2023 seasons.

Concerning nutrient uptake (i.e., N, P, and K nutrients) of rice straw parts, the resultant data set in Figure 6 illustrated a variable significant influence in these indices via the effect of various applications. The control treatment gave the highest rank concerning the indices of N uptake by rice straw without a significant difference between T1 and T4 treatments in both seasons. Once more, T1 reaffirmed superiority in the indices of P uptake by rice straw with

TABLE 3 Effects of synthetic NPK fertilizers and their combination with chitosan-based NPK nano-structure as a foliar application on some yield components traits.

Traits treatments	Plant height (cm)		No. panicles/m ²		Panicle length (cm)	
	2022	2023	2022	2023	2022	2023
T1	115.1 a	117.0 a	574.4 a	583.2 a	23.3 a	23.4 a
T2	111.2 b	112.6 b	616.3 c	529.5 b	20.3 b	20.9 b
T3	114.3 a	111.8 b	541.4 bc	551.3 ab	20.9 b	21.3 b
T4	114.5 a	116.5 a	556.4 ab	568.2 a	22.8 a	24.1 a
T5	103.3 d	104.3 c	454.4 d	455.7 d	18.3 c	18.0 d
T6	105.8 c	107.1 c	467.6 d	474.5 cd	19.7 bc	20.1 c
T4	109.5 b	110.9 b	482.2 cd	488.6 c	1020.1 bc	20.3 c
F. test	**	**	**	**	**	**

** implies $p \leq 0.05$; Data followed by the same letter were not significantly different for probability. Each column's various letters (a, b, c, d, etc.) denoted a statistically significant variation in the treatment means at $p < 0.05$. As for treatments abbreviation (see Table 1).

TABLE 4 Effects of synthetic NPK fertilizers and their combination with chitosan-based NPK nano-structure as a foliar application on some yield components traits.

Traits treatments	Panicle weight (g)		Filled grains (%)		1,000 grain weight (g)	
	2022	2023	2022	2023	2022	2023
T1	4.12 a	4.13 a	89.6 ab	91.1 a	26.93 ab	28.90 a
T2	3.59 bc	3.81 bc	82.7 c	86.8 c	25.33 bc	26.15 b
T3	3.73 ab	3.90 bc	86.2 b	88.6 ab	25.95 b	26.75 b
T4	4.11 a	4.30 a	92.2 a	92.6 a	27.49 a	28.16 a
T5	3.03 c	3.11 d	77.8 d	79.9 d	23.20 d	23.49 d
T6	3.42 bc	3.53 c	80.1 cd	83.7 cd	24.06 d	23.98 cd
T4	3.43 bc	3.70 bc	80.6 cd	84.8 c	24.06 cd	25.10 bc
F. test	**	**	**	**	**	**

** implies $p \leq 0.05$; Data followed by the same letter were not significantly different for probability. Each column's various letters (a, b, c, d, etc.) denoted a statistically significant variation in the treatment means at $p < 0.05$. As for treatments abbreviation (see Table 1).

statistical conformity with the application of T4 in the 1st and 2nd seasons. As for the indices of P uptake by rice straw, the treatment of T1, and T4 occupied the highest rank without significant difference among them in the 1st season. Whilst there was a statistical match between T1, T3, and T4 in the 2nd season. Across both seasons, the treatment of T5 submitted the lowest rank concerning all studied nutrient uptake in rice straw.

4 Discussion

Undoubtedly, crop agrarian productivity necessitates the usage of NPK-containing fertilizers because of their vital role in macronutrient (i.e., N, P, and K) homeostasis that determines biomass synthesis and dry matter production (Moe et al., 2019; Krasilnikov et al., 2022; Sarkar et al., 2022). More critically, the tiny particle size and wide surface area of nano fertilizers lead to an increase in particle number per unit of weight as well as high reactivity, which improves the fertilizer's contact with the plant and boosts both fertilizer use efficacy and nutrient uptake (Abdel-Aziz et al., 2018; Abd-Elrahman et al., 2023; Devi et al., 2023; Elshayb et al., 2022a). Hence, modified NPK fertilizer with nanostructure and slow-release properties has a special potential to enhance the usage and uptake of nutrients.

Photosynthetic pigments have a cardinal role in light energy's absorption, transmission, and transformation processes during photosynthesis (Moe et al., 2019). Numerous investigations have demonstrated that the regulation of photosynthetic pigment synthesis in crop leaves is significantly influenced by nitrogen nutrition (Vijayalakshmi et al., 2015; Peng et al., 2021). Also, an appositive correlation is well-documented between N-level supply and both Ch. content and C. content (Kumari et al., 2024; Srikanth et al., 2023). Therefore, the results in Figure 4 and Table 2 may be due to the different variations of N quantities supply which reflect on total photosynthetic pigments (i.e., chlorophyll a, chlorophyll b, and carotenoids) that were affected positively with several studied traits. The coprecipitation of chemical fertilizer of NPK and exogenous application of Ch/NPs-NPK 300 ppm underlying T4 may give plants adequate P nutrient addition that has a monumental role in several metabolic processes (i.e., photosynthesis, energy storage, and cell division) as a result of an integral constituent of phospholipids, nucleic acid, and co-enzymes that ultimately activate amino acid formation (Bechtaoui et al., 2021; Gao et al., 2023; Vance et al., 2003). On the other side, sufficient K supply plays an influential function in photosynthetic CO₂ fixation and preservation of chloroplast status about enzyme activities, PH, and turgor (Dreyer et al., 2017). In this regard, Sheoran et al. (2021) found that wheat plants sprayed with

TABLE 5 Effects of synthetic NPK fertilizers and their combination with chitosan-based NPK nano-structure as a foliar application on yield outcomes.

Traits treatments	Biological yield (t/ha)		Grain yield (t/ha)		Harvest index (%)	
	2022	2023	2022	2023	2022	2023
T1	24.16 a	24.93 a	10.93 a	11.04 a	0.448 a	0.451 a
T2	22.11 bc	22.20 bc	9.37 c	9.54 bc	0.424 c	0.430 bc
T3	22.72 bc	22.79 bc	9.66 bc	9.93 bc	0.436 bc	0.438 b
T4	23.40 ab	23.92 ab	10.45 ab	10.69 ab	0.447 a	0.449 a
T5	20.83 c	21.27 c	8.35 d	8.55 d	0.403 d	0.410 c
T6	21.36 c	21.86 c	8.58 cd	8.83 cd	0.415 cd	0.414 c
T4	21.94 bc	22.25 bc	8.91 cd	9.27 cd	0.419 cd	0.422 bc
F. test	**	**	**	**	**	**

** implies $p \leq 0.05$; Data followed by the same letter were not significantly different for probability. Each column's various letters (a, b, c, d, etc.) denoted a statistically significant variation in the treatment means at $p < 0.05$. As for treatments abbreviation (see Table 1).

nano-K utilized the exchangeable K in the sandy loam soil to the highest extent possible, resulting in the least amount of K leaching. This may be explained by the increased absorption of K and its related functions in cation-anion balance, energy transfer, stomatal movement, osmoregulation, photosynthesis, enzyme activation, protein synthesis, and phloem transport (Elshamy et al., 2019; Abd-Elrahman et al., 2023). Therefore, applying Ch/NPs-NPK 300 ppm can probably compensate for the missing quantity of chemical NPK fertilizer under the condition of T4 (when T-NPK decreases by up to 30%). Hence, compared to T1 (control), total pigment contents did not give a statistical difference with the T4 treatment (Table 2). Indeed, the higher efficacy of the photosynthesis process reflected positively on cell properties (e.g., elongation, division, and expansion) (Moe et al., 2019). It is obvious that the sole application of Ch/NPs (at the rate of 90 ppm) has been proven beneficial to wheat plants undergoing water stress conditions via enhancing Ch. Content (Behboudi et al., 2019). Another article given by Devi et al. (2023), indicated that Ch/NPs played a versatile growth promoter role by underpinning leaf area and total Ch. Content of rice plants. On the above basis, the higher contents of photosynthesis pigment led to higher values of LAIs which was witnessed clearly under the treatments of T1, and T4 (in both seasons). Consequently, the increase of LAIs values causes a benign utilization and capture of solar energy that leads to more photo-assimilated formation and ultimately generates higher DMP (Moe et al., 2019; Elshayb et al., 2022a; Elshayb et al., 2022b; He et al., 2024). Hereby, the perusal results (Table 2) deduced that the application of Ch/NPs-NPK 300 ppm concerning the treatment of T4 may be synchronized and underpinned with the plant's nutrient needs despite the decrease of traditional NPK fertilizers till 30%.

After Ch/NPs-NPK is applied exogenously, it penetrates the epidermal of plant leaves, allowing for easy absorption through the plant's organs. Due to the noncirculatory system of plant vascular systems, exogenous nanoscale material moves directly into the xylem (the shoot's internal structure) and phloem (the root's internal structure) (Gangwar et al., 2023). Probably, the foliar application of Ch/NPs-NPK (at the proper dose) supported and enhanced the translocation process from paddy shoots to their root. Moreover, a facility of molecule absorption (due to its tiny size) may be reflected favorably on enhancing the physiological processes that work on encouraging meristematic activity, cell division, and both stem and

internode elongation Subsequently improving plant height. A previous study by Hasaneen et al. (2016), found that the Ch/NPs-NPK treatment had a significant favorable impact on the growth characteristics of the plants, and they attributed this to an enhanced nutritionally balanced state resulting from an increase in NPK flux within plant tissue.

Indeed, reproductive tiller per unit area is an important morpho-physiological characteristic of paddies and is considered an indicator of high N content (Banayo et al., 2021; Elshayb et al., 2022a; Tyagi et al., 2024). Moreover, the higher reproductive tillers under T4 treatment may be due to P sufficiency status in early stages that perhaps led to encouraging the formation of roots, tillers, and flowering because plants remobilize it within their tissues throughout the advanced growth stages (Miranda-Villagómez et al., 2019). These striking data concerning T1 and T4 treatments (Tables 3, 4) signified that sufficient and adequate absorption of N, P, and K nutrients before the panicle initiation stage perhaps occurred and caused a positive effect on panicle numbers per meter, panicle primordia formation, number of spikelet's per panicle, and panicle branching (Yoshida, 1981). Thus, across both seasons, the increase in N, P, and K nutrients was more predominant with the application of T4. The increase in panicle length is related to the increase concurrently with N addition (Liu et al., 2020; He et al., 2024). This implies that utilization applications of T1 (applying the full recommended dose of NPK), and the T4 (applying 70% of NPK + 300 ppm Ch/NPs-NPK) treatments possibly gave the plants with optimum nitrogen dose. On the other side, chitosan promotes phytohormone formation, especially cytokinin which enhances cell proliferation in the filling stage resulting in the developing sink size (Behboudi et al., 2019; Sangwan et al., 2023). In this sense, the production and translocation of the pre-sorted assimilated output from enriched plant tissues can easily transfer from source to sink, indicating a notable improvement in yield-causative components including panicle qualities (i.e., length and weight), filled grains percentage, and 1,000-grain weights.

Overall, benign DMP is the infrastructure for producing a distinguished yield component and grain outcomes (Abdel-Aziz et al., 2018; Elshayb et al., 2022a; Seleiman et al., 2022; He et al., 2024). The photosynthetic synthesis of leaves (especially flag leaves) is responsible for 90% of rice grain output (Hai-Yan et al., 2009) Also, higher N intake during the vegetative period until panicle initiation is associated

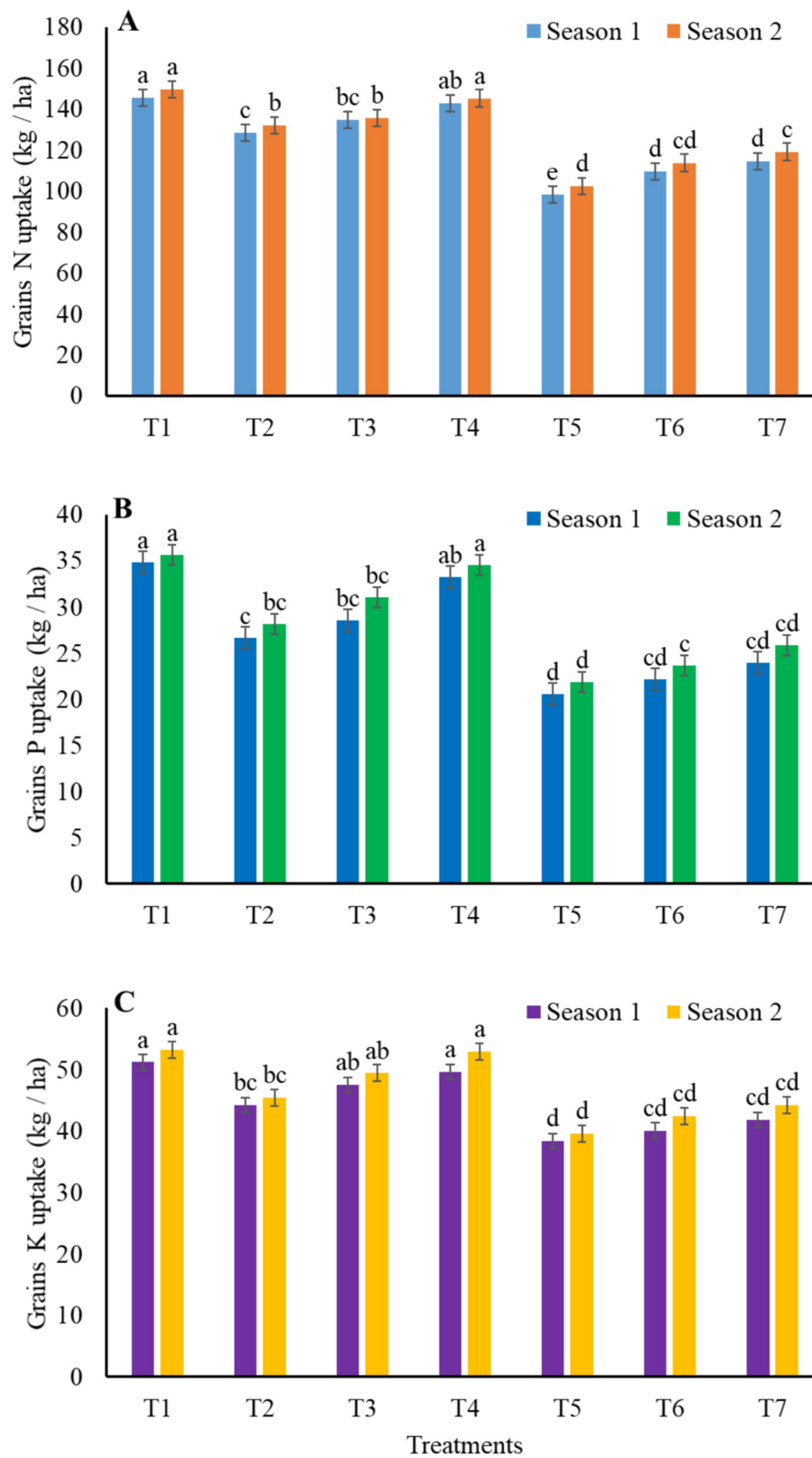


FIGURE 5
 Effects of traditional NPK fertilizers and their combination with chitosan-based NPK nano-structure as a foliar application on (A) grain N uptake, grain P uptake (B), and grain K uptake (C) of rice. Various letters (a, b, c, d, etc.) indicate a statistically significant variation among treatment means at $p < 0.05$. As for treatments abbreviation (see Table 1).

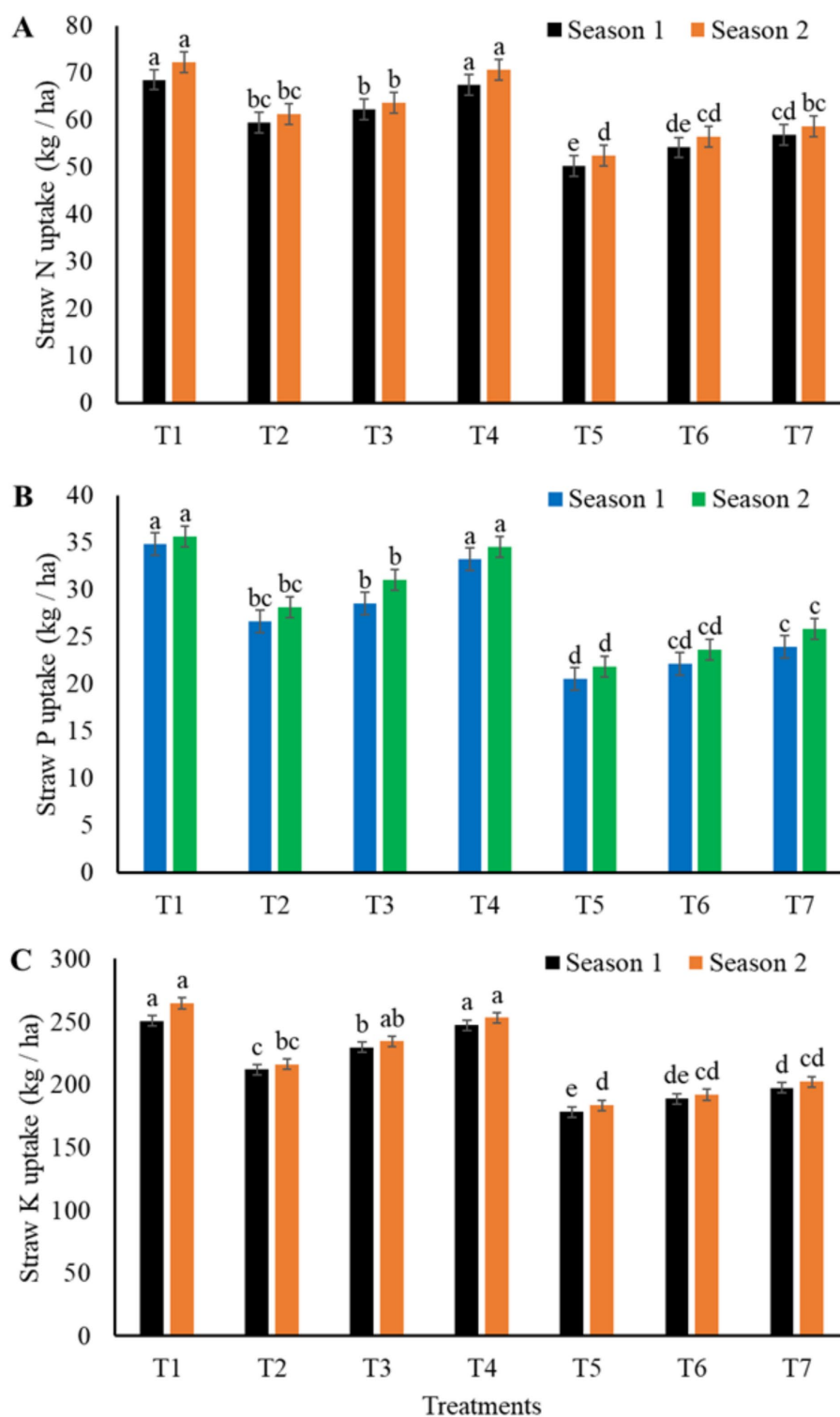


FIGURE 6

Effects of traditional NPK fertilizers and their combination with chitosan-based NPK nano-structure as a foliar application on (A) N uptake (B) P uptake, and (C) K uptake of rice straw. Various letters (a, b, c, d, etc.) indicate a statistically significant variation in the treatment means at $p < 0.05$. As for treatments abbreviation (see Table 1).

with benign carbohydrate buildup which could minimize tiller abortion, enhance reproductive tillers, and eventually maximize GY (Seleiman et al., 2022; Tyagi et al., 2024). Therefore, probably, T1 and T4 provided a benign content of N in rice tissues (Table 5). Previous

research by Elshayb et al. (2022a), demonstrated that rice crops GY and BY were optimized and 40% less urea was required in paddy fields when urea chitosan nanoparticles were sprayed instead of the highly recommended traditional approach. On the other side, the presence

of potassium may boost the GY magnitude underlying T1 and T4 treatment (Table 5) because of its vital role in converting photosynthetic products into starch and protein which are transported directly to grains (Al-Shahmani and Al-Juthery, 2021). An earlier investigation by Sirisena et al. (2012), declared that using potassium fertilizer in nanosizing form greatly improved GY outcomes. Also, Poudel et al. (2023), noted that the foliar application of P in the nanoscale form during tillering and panicle initiation stages enhanced some bioprocess that led to increasing rice grain and straw yields.

As for, nutrients (i.e., N, P, and K) uptake indices by grains, the obvious result (Figure 5) was the increase of nutrient uptake with increasing both Ch/NPs-NPK and T-NPK levels which perhaps produced sufficient nutrition for rice production. This study hypothesis was that Ch/NPs were grabbed by NPK nutrients which added a slow-release feature to the composite of Ch/NPs-NPK resulting in higher efficacy concerning nutrient uptake. As per Abdel-Aziz et al. (2018), using Ch/NPs-NPK in wheat fertilization has been able to lower N loss and increase N uptake's effectiveness. Another investigation by Elshayb et al. (2022a), observed that utilizing urea-chitosan nanocomposite by 500 ppm led to optimizing N-uptake in both grain and straw rice yields. Another investigation by Abdel-Hakim et al. (2023) revealed that the leaf tissues of lettuce plants treated with NPK as a nano formulation had the largest accumulation of nutrients (N, P, K, Ca, Mg, Fe, Mn, and Zn). Recent studies pointed to the effective role of Ch/NPs in improving nutrient cycling and providing essential nutrients over an extended period, reducing the frequency of applications (Sarkar et al., 2022; Singh et al., 2024). Therefore, it is possible that the foliar application of Ch/NPs-NPK 300 ppm underlying T4 treatment was a good route to obtain the mechanism of uptake and transport of NPK nutrients into intact plant organs.

An analysis of the current findings showed that the nutrients (i.e., N, P, and K) uptake indices by straw (Figure 6) increased progressively by increasing both Ch/NPs-NPK and T-NPK levels. Meanwhile, T4 treatment gave a substantial increase in nutrient uptake by straw which may be due to the mass flow phenomenon via a pressure differential between the leaves and stems, which is what allows nanoparticles to travel from stomata and make their way to the xylem vessels and then move the phloem tissue where they were carried nutrients to reach all plant tissues (Hasaneen et al., 2016). A prior study by Corradini et al. (2010) pointed to the ability of endosomes (parts of the endocytic membrane transport pathway) to transport the nanoparticles to various sites within the cells. Notably the most important aspect of utilizing Ch/NPs associated with synthetic fertilizers contributes to reducing environmental pollution because it's biodegradable (Sangwan et al., 2023). Consequently, even though T-NPK dropped by up to 30%, the application of T4 improved the status of nutrient (i.e., N, P, and K) absorption indices in grain and straw yields and can be considered a smart and eco-friendly application in paddy fields.

5 Conclusion

The findings of the current study reported that utilizing Ch/NPs-NPK with 300 ppm was considered a significant application to compensate synthetic NPK fertilizers by 30% without a significant reduction in growth, yield, and nutrient content of rice. Therefore, using Ch/NPs-NPK can be recommended as an exogenous application in the paddy fields to reduce the excessive use of synthetic NPK fertilizers and

enhance physiological traits that lead to sustaining a satisfactory grain outcome. Accordingly, this approach not only can mitigate the environmental impact associated with excessive fertilizer use but also can enhance the physiological traits of the rice plants, contributing to sustainable agricultural practices. The biodegradable and slow-release feature of Ch/NPs makes it an alternative to smart-safe use in the agriculture sector. Ultimately, utilizing such modern and modified technologies to produce compensatory alternatives for balanced plant nutrition provides environmental and economic usefulness in the ecosystem. Moreover, there's a dire demand to examine and explore the potential of Ch/NPs-NPK on different rice varieties, and other plant species for a deep and thorough evaluation of these materials.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

OE: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Software, Writing – original draft, Writing – review & editing. HG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing. MW: Investigation, Methodology, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. KF: Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. DW: Methodology, Software, Writing – original draft, Writing – review & editing. MS: Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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