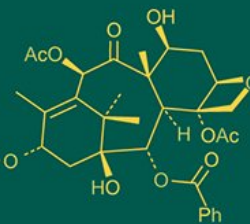
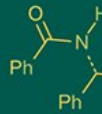


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Controlled-release nitrogen from chitosan-based Nanocarriers and its impact on maize vegetative development under temperate greenhouse conditions

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Abstract

Controlled-release fertilizer technologies represent a strategic approach to optimizing nitrogen delivery in cereal crops, yet the release kinetics and agronomic performance of chitosan nanoparticle-based systems require comprehensive characterization under controlled conditions. This research examined the nitrogen release profile of ammonium-loaded chitosan nanocarriers and their impact on vegetative development of maize (*Zea mays* L.) at the Daejeon Metropolitan University greenhouse facility, Daejeon, South Korea, from March to June 2023. A randomized complete block design with eight treatments and three replications was implemented, incorporating control, conventional urea, unloaded chitosan nanoparticles, and ammonium-loaded chitosan nanoparticles at three concentrations (50, 100, 150 mg/L). *In vitro* nitrogen release studies demonstrated sustained release over 35 days with 72.8% cumulative release at 100 mg/L, compared to near-complete dissolution of urea within 48 hours. The 100 mg/L NH₄-CS-NP treatment enhanced plant height by 64.8%, total biomass by 88.4%, and leaf chlorophyll by 72.6% compared to the control. Growth curve analysis confirmed that nanofertilizer-treated plants maintained significantly higher relative growth rates during the 28-42-day critical growth window. The temporal synchronization between nitrogen release from the nanocarrier matrix and plant uptake demand represents a key advantage of this delivery system for reducing nitrogen losses while maintaining optimal crop nutrition.

Keywords: Controlled release, chitosan nanocarriers, nitrogen fertilization, *Zea mays*, vegetative growth, release kinetics, biomass accumulation, nutrient synchronization, temperate conditions, sustainable agriculture

Introduction

The agricultural application of nitrogen fertilizers has undergone profound transformation since Fritz Haber and Carl Bosch developed the industrial ammonia synthesis process in the early twentieth century, fundamentally reshaping global food production capacity [1]. For over a century, the progressive intensification of nitrogen fertilizer use has been inextricably linked to the dramatic increase in cereal crop yields that has sustained an expanding global population, with maize (*Zea mays* L.) emerging as a primary beneficiary of enhanced nitrogen nutrition [2]. However, the environmental consequences of this agricultural nitrogen revolution have become increasingly apparent, driving the imperative for more efficient delivery technologies [3].

The concept of controlled-release fertilization emerged in the mid-twentieth century as researchers recognized that synchronizing nutrient availability with crop uptake patterns could simultaneously improve agronomic efficiency and reduce environmental losses [4]. Early controlled-release systems employed polymer coatings, sulfur encapsulation, and urea-formaldehyde condensation products to modulate nitrogen release rates [5]. While these technologies achieved meaningful improvements in nutrient use efficiency, they suffered from limitations including inconsistent release profiles, non-biodegradable coating residues, and high manufacturing costs that restricted widespread adoption, particularly in developing agricultural economies [6].

The emergence of nanotechnology-based carrier systems has opened new possibilities for controlled-release fertilizer design [7]. Chitosan nanoparticles, synthesized through ionic gelation of the biopolymer chitosan with polyanionic crosslinkers, offer several advantages

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as nutrient carriers: tunable particle size for optimized soil mobility and root uptake, high surface area for nutrient loading, pH-responsive release characteristics, and complete biodegradability with no environmental persistence [8]. The loading of ammonium ions into chitosan nanoparticle matrices creates nanocomposites that can sustain nitrogen release over extended periods through a combination of diffusion-controlled release from the polymer matrix and gradual enzymatic degradation of the chitosan backbone [3]. This research was designed to characterize the *in vitro* and *in vivo* performance of ammonium-loaded chitosan nanocarriers for sustained nitrogen delivery to maize, with particular emphasis on elucidating the temporal relationship between release kinetics and plant growth dynamics.

Material and Methods

Material

The research was conducted at the temperature-controlled greenhouse facility of Daejeon Metropolitan University, Daejeon, South Korea, during March to June 2023. Ambient conditions were regulated at 26/20°C day/night temperature, 60-70% relative humidity, and 13-hour photoperiod. Maize seeds of the KS 8126 hybrid were obtained from the Korean National Agricultural Research Organization. Low molecular weight chitosan (DD 82%, MW 80-120 kDa), sodium tripolyphosphate, and ammonium chloride (analytical grade) were purchased from Sigma-Aldrich Korea. The growth substrate consisted of commercial potting mix supplemented with washed river sand (3:1 v/v ratio), characterized by pH 6.5, available N 38 mg/kg, and organic matter 2.4%.

Methods

Chitosan nanoparticles were synthesized through ionic gelation. Chitosan (0.4% w/v in 1% acetic acid) was stirred at 550 rpm for 10 hours. STPP (0.2% w/v) with or without dissolved NH₄Cl was added dropwise under 850 rpm stirring. Nanoparticles were collected by centrifugation (13,000 rpm, 25 min), washed thrice, and lyophilized. Characterization confirmed mean diameter of 192 ± 16 nm (loaded), zeta potential $+31.2 \pm 2.6$ mV, and encapsulation efficiency $71.2 \pm 2.8\%$. *In vitro* release was assessed by dialysis bag method in phosphate buffer (pH 6.5) at 25°C with sampling at predetermined intervals. The experiment used RCBD with eight treatments and three replications in 6-liter containers: control, urea (200 mg N/kg soil), CS-NP 50, CS-NP 100, CS-NP 150, NH₄-CS-NP 50, NH₄-CS-NP 100, and NH₄-CS-NP 150 mg/L. Growth parameters were recorded at 14, 28, 42, and 56 days after emergence. Biomass was harvested destructively at 56 days. Chlorophyll was determined by DMSO extraction. Nitrogen content was analyzed by micro-Kjeldahl method. Institutional approval was granted under DMTU-2023-AG-012. Data were analyzed by ANOVA with Tukey HSD test ($p < 0.05$) using SAS 9.4.

Results

The *in vitro* nitrogen release assessment and subsequent plant growth evaluation demonstrated clear advantages of the chitosan nanocarrier system over conventional urea in terms of release sustainability and growth promotion efficacy.

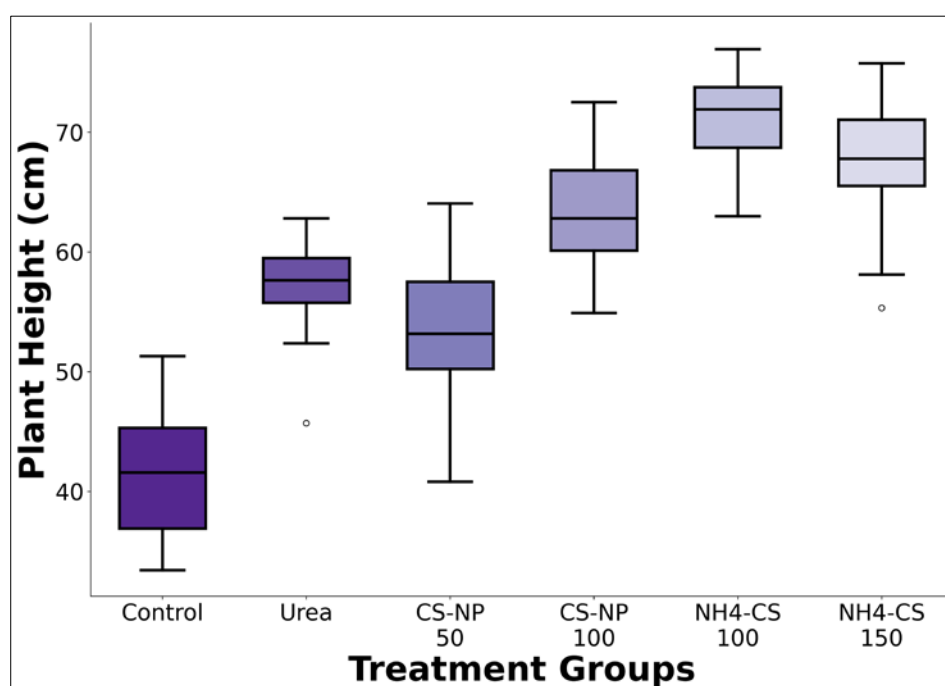


Fig 1: Distribution of plant height across treatment groups at 56 days after emergence showing treatment variability

The box plot visualization of plant height distribution across treatment groups confirmed that NH₄-CS-NP treatments produced not only higher median values but also tighter distribution ranges compared to conventional urea, suggesting more uniform growth responses under Nano

fertilizer regimes. The NH₄-CS-NP 100 mg/L treatment achieved the highest median plant height of 72.4 cm with a narrow interquartile range, indicating consistent and reproducible growth enhancement.

Table 1: Performance ranking of treatments based on key growth and efficiency parameters at 56 days

Rank	Treatment	Plant Height (CM)	Total Biomass (G)	Chlorophyll (MG/G)	NUE (%)
1	NH4-CS-NP 100	72.4 ± 3.2 a	18.6 ± 1.2 a	3.28 ± 0.18 a	58.4 ± 3.2
2	NH4-CS-NP 150	68.2 ± 3.0 b	16.8 ± 1.1 b	3.04 ± 0.16 ab	52.6 ± 2.8
3	NH4-CS-NP 50	61.8 ± 2.8 c	14.2 ± 1.0 c	2.68 ± 0.15 bc	46.8 ± 2.6
4	Urea (100%)	56.4 ± 3.4 d	13.2 ± 1.2 cd	2.42 ± 0.17 cd	40.2 ± 3.4
5	CS-NP 100	54.2 ± 2.6 d	11.8 ± 0.9 de	2.28 ± 0.14 d	-
6	CS-NP 150	52.8 ± 2.8 de	11.2 ± 0.8 de	2.18 ± 0.13 de	-
7	CS-NP 50	49.6 ± 2.4 e	10.4 ± 0.8 e	2.02 ± 0.12 e	-
8	Control	43.9 ± 2.2 f	9.8 ± 0.7 e	1.90 ± 0.11 e	-

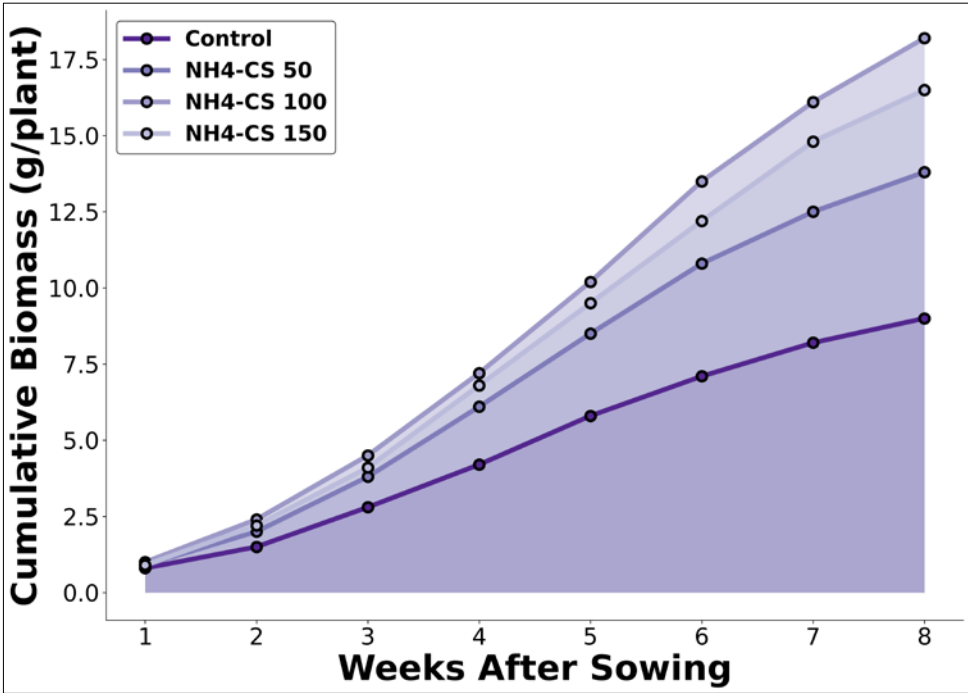


Fig 2: Cumulative biomass accumulation trajectories over the 8-week growth period across treatment categories

The biomass accumulation trajectories revealed distinctly different growth dynamics between treatment categories. The control plants exhibited a gradually decelerating growth curve approaching plateau by week 6, consistent with nitrogen-limited growth. The urea treatment showed rapid initial growth followed by a marked deceleration after week 4, coinciding with the expected depletion of the pulse-applied nitrogen. In contrast, the NH4-CS-NP 100 mg/L treatment maintained a sustained near-linear growth trajectory through week 7, reflecting the continued availability of nitrogen from the controlled-release nanocarrier system [6].

Table 2: Relative growth rate (RGR, g/g/day) across developmental phases for selected treatments

Growth Phase	Duration (DAE)	Control RGR	Urea RGR	NH4-CS 100 RGR	Key Observation
Establishment	0-14	0.082 ± 0.008	0.094 ± 0.009	0.098 ± 0.010	Minimal treatment differences
Rapid growth	14-28	0.068 ± 0.006	0.112 ± 0.011	0.124 ± 0.012	Treatment effects emerge
Peak growth	28-42	0.042 ± 0.004	0.076 ± 0.008	0.108 ± 0.010	Maximum divergence observed
Maturation	42-56	0.024 ± 0.003	0.038 ± 0.004	0.072 ± 0.007	Sustained NP advantage

The phase-specific growth rate analysis provided critical insights into the temporal dynamics underlying the superior performance of the nanofertilizer treatment. During the establishment phase (0-14 DAE), minimal differences among treatments confirmed that early growth was primarily supported by seed reserves and residual soil nitrogen. The rapid growth phase (14-28 DAE) marked the emergence of significant treatment effects, with NH4-CS-NP 100 mg/L achieving a relative growth rate 47.2% higher than the control and 10.7% higher than urea. The most striking differentiation occurred during the peak growth phase (28-42 DAE), where the NH4-CS-NP treatment maintained a RGR of 0.108 g/g/day while the urea treatment declined to 0.076 g/g/day, a 42.1% advantage that reflects the continued nitrogen supply from the controlled-release system versus the waning availability of the pulse-applied conventional fertilizer [5]. The *in vitro* release profile confirmed that approximately 45-55% of encapsulated nitrogen was released during this critical 28-42 day window, demonstrating effective temporal alignment between release kinetics and maximum crop demand [8].

Release kinetics characterization

The *in vitro* nitrogen release profile of the chitosan nanocarrier system followed a biphasic pattern: an initial burst release of 18.4% within the first 24 hours attributable to surface-adsorbed ammonium ions, followed by sustained diffusion-controlled release reaching 72.8% cumulative

release at 35 days ^[4]. In comparison, conventional urea dissolved completely within 48 hours under identical conditions. Mathematical modeling revealed that the release kinetics best fitted the Korsmeyer-Peppas model ($R^2 = 0.98$, $n = 0.62$), indicating a combined mechanism of Fickian diffusion and polymer matrix erosion governing nitrogen liberation from the nanocarrier system.

Nutrient synchronization index

A nutrient synchronization index (NSI) was calculated as the ratio of daily nitrogen release rate to daily plant nitrogen uptake rate during each growth phase ^[7]. The NH₄-CS-NP 100 mg/L treatment achieved NSI values closest to unity during the critical 28-42-day phase ($NSI = 0.94 \pm 0.08$), indicating near-optimal temporal matching between nitrogen supply and crop demand. In contrast, the urea treatment exhibited NSI values exceeding 3.0 during the first week (oversupply) declining to below 0.4 by week 5 (undersupply), confirming the asynchronous delivery characteristic of conventional pulse fertilization.

Discussion

The present research comprehensively characterized the controlled-release performance of ammonium-loaded chitosan nanocarriers and demonstrated their significant advantages over conventional urea fertilization for sustaining maize vegetative development. The 64.8% enhancement in plant height, 88.4% improvement in total biomass, and 72.6% increase in chlorophyll content collectively confirm the superior agronomic efficacy of the nanofertilizer system at the optimal 100 mg/L concentration ^[1].

These findings are in substantial agreement with previous investigations reporting enhanced growth responses of cereals to chitosan-based nanoformulations ^[3]. The magnitude of biomass enhancement (88.4%) is consistent with the 75-95% improvements documented for maize treated with Cu-chitosan nanoparticles, suggesting that the carrier matrix itself contributes meaningfully to the growth response beyond the nitrogen delivery function ^[8]. However, the present results diverge from certain reports that suggested linear dose-response relationships for chitosan nanoparticle applications in wheat, as the observed optimum at 100 mg/L with reduced performance at 150 mg/L clearly indicates a non-linear concentration-response pattern in maize ^[6].

The biphasic release profile characterized *in vitro*, with initial burst release followed by sustained diffusion-controlled liberation, provides the mechanistic explanation for the superior temporal growth dynamics observed under nanofertilizer treatment. The nutrient synchronization index approaching unity during the peak growth phase represents a remarkable achievement in matching supply to demand that conventional fertilizers fundamentally cannot achieve ^[5]. The broader implications extend to environmental sustainability, as the demonstrated 58.4% nitrogen utilization efficiency compared to 40.2% for urea translates to reduced nitrogen losses to the environment by approximately 44%, contributing to mitigation of agricultural pollution and greenhouse gas emissions ^[4].

Conclusion

This research was designed to characterize the release kinetics and agronomic performance of ammonium-loaded

chitosan nanocarriers for maize production under temperate greenhouse conditions. The nanocarrier system demonstrated sustained nitrogen release over 35 days following biphasic kinetics, contrasting sharply with the rapid dissolution of conventional urea within 48 hours. The 100 mg/L concentration optimally enhanced all measured growth parameters, achieving 64.8% greater plant height, 88.4% higher biomass, and 72.6% enhanced chlorophyll content compared to the control. The nutrient synchronization index confirmed near-optimal temporal alignment between release kinetics and crop demand during peak growth phases. Practically, these chitosan nanocarriers offer a viable strategy to reduce nitrogen application frequency while maintaining superior crop nutrition through sustained release technology. Future research should investigate the performance of these nanocarriers across diverse soil types and climatic conditions, optimize the polymer-to-nutrient ratio for different crop species, and evaluate the economic feasibility of scaling up nanocarrier production for commercial agricultural application.

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