*J Bangladesh Agril Univ 23(3): 287–295, 2025* https://doi.org/10.3329/jbau.v23i3.84449

ISSN 1810-3030 (Print) 2408-8684 (Online)

Journal of Bangladesh Agricultural University

Journal home page: http://baures.bau.edu.bd/jbau

## Research Article

# Utilization of Chitin Nanofiber as a Supplement of Urea for BRRI dhan28 (*Oryza sativa* L. cv. BRRI dhan28) Cultivation

**Sumaiya Afroj [1], Md. Yasin Ali[1], Md. Iftekhar Shams [2], Tulshi Biswas [1] and Md. Yamin Kabir [1]✉**

[1]Agrotechnology Discipline, Khulna University, Khulna 9208, Bangladesh
[2] Forestry and Wood Technology Discipline, Khulna University, Khulna 9208, Bangladesh

**ARTICLE INFO**

**Article history**
Received: 04 May 2025
Accepted: 18 September 2025
Published: 30 September 2025

**Keywords**
Growth attributes,
Grain yield,
Snfilled spikelet,
Heat stress,
Harvest index

**Correspondence**
Md. Yamin Kabir
✉: yaminkabir@at.ku.ac.bd



## Abstract

Rice is the staple food for about half of the world's population and requires extensive urea fertilization. However, urea is prone to multifaceted losses, leading to environmental pollution. A pot experiment was conducted to investigate the suitability of using chitin nanofiber (CNF) on the growth and yield of BRRI dhan28. The experiment was laid out in a completely randomized design (CRD) with five treatments, e.g., 0.4% CNF, urea (recommended dose), CNF: urea (50:50), CNF: urea (75:25), and water (control) and replicated seven times. Among the treatments, CNF-urea (50/50) resulted in statistically similar plant height, tiller number, panicle length, and effective number of panicles with urea. CNF-urea (50/50) also had lower unfilled spikelets than the recommended dose of urea. Moreover, grain yield and harvest index were numerically higher for CNF-urea (50/50) than the recommended dose of urea. Therefore, CNF-urea (50/50) may produce a rice yield similar to urea, suggesting that half of the urea-nitrogen can be supplemented by CNF without significant yield loss of rice (BRRI dhan28). However, further experiments should be conducted in different seasons and in multilocation to get a final recommendation.



## Introduction

Bangladesh is an agrarian country where agriculture employs about 50% of the population, and about 70% of its land is under crop cultivation (FAOSTAT, 2022). Bangladeshi farmers produce rice following traditional practices using chemical fertilizers, including urea, MoP, TSP, and gypsum. Nitrogen fertilizers have been universally acknowledged for having a considerable impact on increasing rice yields (Karim et al., 2019), and the use of urea increased to be considered the chief N fertilizer worldwide (Bremner, 1995). To get an acceptable rice yield, practically all farmers use expensive N fertilizers like urea (Saleque et al., 2004). Moreover, initiatives to boost rice production are necessary for rice-growing countries like Bangladesh (Islam et al., 2025). Urea, a nitrogenous fertilizer, is extensively used and contributes to higher rice yields. However, the efficiency of urea ranges from 30% to 35% (IFIDC, 2007), and in wetland rice cultivation, N recovery is not more than 40% (Datta & Buresh, 1989). A substantial amount of N is lost through leaching, denitrification, runoff, and volatilization, causing environmental pollution. About 26% of the nitrogen in broadcast urea is lost as gaseous nitrogen through the nitrification-denitrification process and $NH_3$ volatilization (Phongpan et al., 1988). After application in the soil, urea is hydrolyzed in the oxidized layer, forming $NO_3^-$, which leaches from the soil and contaminates the groundwater. Besides, the price of urea is increasing day by day, and the Government of Bangladesh provides subsidies for urea. The amount of urea subsidies in the fiscal years 2020–21 and 2021–22 were Tk. 7,717 crore and Tk. 28,000 crore, respectively (The Business Standard, 2022). Therefore, the government increased the per kg urea from Tk. 16 to Tk. 22 on August 1, 2022.

**Cite This Article**
Afroj, S., Ali, M.Y., Shams, M.I., Biswas, T. and Kabir, M.Y. 2025. Utilization of chitin nanofiber as a supplement of urea for BRRI dhan28 (*Oryza sativa* L. cv. BRRI dhan28) cultivation. *Journal of Bangladesh Agricultural University,* 23(3): 287-295. https://doi.org/10.3329/jbau.v23i3.84449

Recent research has focused on finding organic substitutes that can replace urea without sacrificing rice yield. Chitin, a polymer of N-acetylglucosamine, is a N-containing biopolymer, which is nontoxic, biocompatible, and eco-friendly. However, the water insolubility of chitin limits its use in agriculture. Nanofibrilation of chitin, i.e., chitin nanofiber, can overcome this limitation. Chitin nanofiber (CNF) is defined as the aspect ratio greater than 100 and diameter in nanometer-scale (Xia et al., 2003; Li and Xia, 2004) and CNF has a unique morphology (Ifuku et al., 2010), a high surface-to-volume ratio (Tsutsumi et al., 2014), a high level of mechanical strength (Ifuku et al., 2011; Ifuku et al., 2013), and useful biological properties (Azuma et al., 2014; Azuma et al., 2015; Izumi et al., 2015; Izumi et al., 2016; Munira et al., 2025). Due to its fairly high N content (3.6%-6.8%) and low C/N ratio, CNF can be employed as a fertilizer or soil amendment to boost the growth and yield of crops (Egusa et al., 2019; Janssen et al., 2017). CNF can produce a similar rice yield to urea (Shams et al., 2025). To our knowledge, no experiments with the combination of urea and chitin nanofiber on rice (BRRI dhan28) have been reported. Under these circumstances, the present study is carried out on the BRRI dhan28 with CNF alone or in combination with urea to find out its effect on growth and yield attributes.

## Materials and methods

### *Pot preparation, planting material, experimental design, and treatments*

The pot experiment was conducted at the Nursery of Forestry and Wood Technology Discipline of Khulna University, Khulna, Bangladesh, from February to May 2022. Soil was collected from Nij Khamar, Khulna. Exactly 460 kg of soil was weighed with an electrical balance, spread over a concrete floor, and mixed thoroughly with TSP (17.21 g), MoP (13.77 g), gypsum (6.88 g), zinc-sulphate (1.54 g) and cow dung (4 kg). A 20-kg-sized black plastic pot was filled with 13 kg soil, and 35 pots were prepared. 30-day-old seedlings of BRRI dhan28 were transplanted in the pot on 11 February 2022. BRRI dhan28 is a high-yielding *Boro variety* cultivated widely throughout Bangladesh. The rice is about 90 cm tall and medium-sized with strong culm. It requires about 140 days to harvest the rice, and it yields, on average, 6 tons per hectare. The single-factor experiment was arranged following Completely Randomized Design (CRD) with seven replications and five treatments such as T0 = control (water), T1 = urea (0.65g) (recommended dose), T2 = 0.4% CNF (450 ml), T3 = 75% CNF (375 ml CNF) + 25% urea (0.1613g urea), and T4 = 50% CNF (225 ml CNF) + 50% urea (0.325g urea). Three identical doses of treatments (T0 – T4) were administered on 11 February ($1^{st}$ dose, just before transplanting), 32 days after transplanting ($2^{nd}$ dose), and 62 days after transplanting ($3^{rd}$ dose). Thus, a total of 1.95 g urea was applied per pot (13 kg soil) according to BRRI recommendation (300 kg urea $ha^{-1}$; i.e., 300,000 g urea for 20,00000 kg soil) (BRRI factsheet). All the treatments were applied by mixing with the soil. A layer of standing water was maintained until the grain-filling stage of rice.

### *Preparation of chitin nanofiber (CNF)*

Shells of tiger shrimp (*Peneus monodon*) were collected from Khulna, Bangladesh. Shrimp shells were meticulously cleaned in water to wash the clinging dust, dirt, and other debris. Before letting them dry in the air, the shrimp shells were repeatedly cleaned with tap water. Using a typical grinder, the dry shells were reduced in size to particles between 2 and 4 mm. About 300 g dried, crushed shrimp shells were used for the next step. The shells were demineralized, deproteinized, and depigmented using HCI (37%), NaOH (99.9%), and Ethanol 50%, respectively, following standard procedure (Ifuku et al., 2010, 2011; Shams et al., 2012). The obtained suspension was then placed in a super-speed blender (Vita-Mix Blender, Osaka Chem. Co. Ltd.) and mixed for 10 minutes at 37,000 rpm to produce chitin nanofiber. Next, the suspension was placed in a normal-speed blender (Panasonic MX Blender, Panasonic Holdings Corporation) and mixed for 10 minutes at 11,000 rpm. The suspension is then ready to be added to the super colloidal machine once the necessary amount of water has been added.

The chitin was mechanically processed by passing it through a Super Masscolloider (MKCA6-51) from Masuko Sangyo, Honcho, Kawaguchi-shi, and Saitamaken, Japan, which has many milling functions. The grinding stone clearance was set to -0.15 with a 1500 rpm rotating stone speed. After going through a super-speed blender 10, 15, and 20 milling cycles, the sample chitin was extracted. The solution was stored at 4 °C, and the concentration was adjusted to 0.4% by adding distilled water at the time of use. CNF contains 3.6%-6.8% N (Janssen et al., 2017).

### *Determination of growth attributes*

To determine different growth parameters, data were recorded starting at 41 days after transplanting (DAT) and continued till maturity. Plant height was estimated from the base to the tip of the tallest leaf or the panicle of each plant in each pot five times at 41 DAT, 52 DAT, 63 DAT, and 74 DAT and harvest. The number of tillers per hill was recorded and means were computed. The tiller number was counted at 41 DAT, 52 DAT, 63 DAT, 74 DAT, and harvest. The chlorophyll content of leaves was recorded through the SPAD 502 Plus Chlorophyll meter at 42 DAT, 53 DAT, 64 DAT, and 75 DAT for all the plants.

Leaf blades were collected from 15 plants. Four leaves were taken from each plant and photographed. The area of a leaf was measured using ImageJ and the mean leaf area was computed. Dry matter was calculated at 44 DAT, 55 DAT, and 66 DAT and the average value was presented. The plants were uprooted, and dried at 60 °C in an oven for 72 hours, and dry matter was weighed using an electronic balance.

*Determination of yield and yield attributes*

The crop was harvested at its full maturity when 90% of the grain turns into golden yellow. The plants from each pot were uprooted, bundled separately, and tagged properly. Grain yield and straw yield were also recorded. The plants were sun-dried well. After separating the grains from the straw, they were weighed separately. After harvesting, the number of effective tillers was recorded per hill, and the mean was calculated. Panicle length was measured for each effective panicle from the base node of the rachis to the apex of each panicle, and the mean was calculated. The number of grains per hill was counted and the average was calculated. The total number of unfilled spikelets per panicle was also counted and the means were calculated. After sun drying, an electric balance weighs 1,000 grains from each treatment to have a 1000-grain weight. Similarly, grain weight was recorded separately pot-wise and expressed as g hill$^{-1}$. The straw-weight of the harvested crop of each pot was determined. Each pot's sample was dried in an oven at 60 °C for 72 hours and was recorded separately pot-wise and expressed as g hill$^{-1}$. To have biological yield, grain yield and straw yield were added together and the ratio of grain yield and biological yield was expressed as harvest index (%).

*Statistical analysis*

The data were analyzed using IBM SPSS Statistics for Windows (Version 27.0.1.0) [IBM Corp. (2020), Armonk, NY, USA] following analysis of variance (ANOVA) for CBD design. Treatment means were compared using the same software Tukey's Honestly Significance Difference (HSD) Test with a 5%.

## Results and discussion

*Growth Parameters*

*Plant height (cm)*

Plant height varied significantly due to the application of CNF and urea at 52 DAT and harvest (Table 1). The tallest plant (83.13 cm) was recorded from urea and the shortest (72.00 cm) from the control treatment. At 52 DAT, the highest plant height was obtained from T1 (urea) which was statistically similar to T3 (75/25-CNF/urea) and T4 (50/50-CNF/urea). At 74 DAT, the highest plant height (93.25 cm) was observed from urea.

**Table 1. Effects of CNF and urea on plant height of BRRI dhan28 at different growth stages**

| | Plant height (cm) | | | | |
|---|---|---|---|---|---|
| Treatment | 41 DAT | 52 DAT | 63 DAT | 74 DAT | At Harvest |
| Control ($T_0$) | 54.93 | 70.67b | 80.38 | 85.25 | 72.00b |
| Urea ($T_1$) | 59.79 | 79.79a | 89.52 | 93.25 | 86.13a |
| CNF ($T_2$) | 55.21 | 71.92b | 83.00 | 86.63 | 76.62ab |
| 75/25-CNF/urea ($T_3$) | 58.64 | 75.83ab | 82.10 | 84.50 | 79.50ab |
| 50/50-CNF/urea ($T_4$) | 61.79 | 79.00a | 89.80 | 92.25 | 79.88ab |
| *p* | 0.101 | 0.031 | 0.096 | 0.273 | 0.011 |
| CV (%) | 4.56 | 4.84 | 4.63 | 4.12 | 5.85 |

In a column, CNF = Chitin nanofiber; DAT = Days after transplanting; same letter indicates statistically similar and different letter indicates statistically dissimilar results among the treatments. $p<0.05$, CV = Coefficient of variation.

*Tiller number per hill*

Although the number of tiller hill$^{-1}$ differed significantly at all DATs (41, 52, 63, 74, and at harvest), a highly significant difference was observed for 52, 63, and 74 DATs and at harvest. The highest tiller number per hill was found urea at harvest (35.75), which was not different from the treatment urea-CNF combination (Table 2). The lowest number was found in the control (18.00).

*Leaf chlorophyll content index*

Although there was no significant difference in chlorophyll content at 42 DAT, 53 DAT, 64 DAT, and 75 DAT, the values were numerically different (Table 3). Numerically, the highest chlorophyll content (48.11) was obtained from urea and CNF (50:50), and the lowest chlorophyll content was obtained from the control (27.68) at 75 DAT.

**Table 2. Effects of CNF and urea on tiller number per hill of BRRI dhan28 at different growth stages**

| | Tiller number hill$^{-1}$ | | | | |
|---|---|---|---|---|---|
| Treatment | 41 DAT | 52 DAT | 63 DAT | 74 DAT | At Harvest |
| Control ($T_0$) | 11.29b | 18.50b | 18.20c | 20.55b | 18.00b |
| Urea ($T_1$) | 17.14a | 28.33a | 29.80a | 33.00a | 35.75a |
| CNF ($T_2$) | 11.71b | 21.50ab | 22.00bc | 21.50b | 25.50b |
| 75/25-CNF/urea ($T_3$) | 11.14b | 21.17ab | 21.80bc | 23.00b | 26.00ab |
| 50/50-CNF/urea ($T_4$) | 14.78ab | 24.00ab | 23.60b | 23.00b | 26.25ab |
| *p* | 0.020 | 0.002 | 0.001 | 0.002 | 0.018 |
| CV (%) | 17.95 | 14.58 | 16.45 | 18.55 | 21.44 |

In a column, CNF = Chitin nanofiber; DAT = Days after transplanting; same letter indicates statistically similar and different letter indicates statistically dissimilar results among the treatments. $p<0.05$, CV = Coefficient of variation.

**Table 3. Effects of CNF and urea on SPAD Readings (CCI) of BRRI dhan28 at different growth stages**

| | SPAD Readings (CCI) | | | |
|---|---|---|---|---|
| Treatment | 42 DAT | 53 DAT | 64 DAT | 75 DAT |
| Control ($T_0$) | 46.54 | 40.87 | 37.90 | 27.68 |
| Urea ($T_1$) | 45.40 | 43.70 | 45.02 | 41.60 |
| CNF ($T_2$) | 43.96 | 43.23 | 38.96 | 39.62 |
| 75/25-CNF/urea ($T_3$) | 44.70 | 42.00 | 41.20 | 39.08 |
| 50/50-CNF/urea ($T_4$) | 48.11 | 40.87 | 44.72 | 36.95 |
| *p* | 0.722 | 0.472 | 0.118 | 0.062 |
| CV (%) | 3.20 | 2.78 | 6.99 | 13.20 |

CNF = Chitin nanofiber; DAT = Days after transplanting; $p<0.05$, CV = Coefficient of variation.

*Leaf area (cm$^2$)*

The individual leaf area did not differ significantly at 42, 53 and 64 DATs (Table 4). At earlier stage (42 DAT), leaf area was numerically higher in urea ($T_1$), CNF ($T_2$) and control ($T_0$) but it was higher in CNF-urea combination ($T_3$ and $T_4$) in later stages (53 DAT and 64 DAT).

**Table 4. Effects of CNF and urea on leaf area of BRRI dhan28 at different growth stages**

| | Leaf area (cm$^2$) | | |
|---|---|---|---|
| Treatment | 42 DAT | 53 DAT | 64 DAT |
| Control ($T_0$) | 22.31 | 10.61 | 16.24 |
| Urea ($T_1$) | 25.80 | 6.24 | 15.89 |
| CNF ($T_2$) | 21.84 | 8.30 | 11.86 |
| 75/25-CNF/urea ($T_3$) | 18.27 | 17.07 | 19.57 |
| 50/50-CNF/urea ($T_4$) | 12.80 | 10.85 | 18.50 |
| *p* | 0.150 | 0.096 | 0.769 |
| CV (%) | 21.79 | 34.28 | 16.20 |

CNF = Chitin nanofiber; DAT = Days after transplanting; $p<0.05$, CV = Coefficient of variation.

*Dry matter (g hill$^{-1}$)*

The dry matter was taken from the whole plant including its root, leaf sheath, leave, stem and panicle which was measured at three dates (44 DAT, 55 DAT and 66 DAT) and it was non-significant (data not shown). However, data was presented from DAT 66 (Fig. 1). Numerically the highest dry matter was obtained from $T_3$ (75/25-CNF/urea) (25.12 g) and the lowest dry matter was collected from control (18.65 g).

*Yield and yield contributing attributes*

*Panicle length (cm)*

Panicle length varied significantly among the treatments. The highest panicle length (21.53 cm) was metered from the urea-CNF (50/50) treatment, which was statistically similar to urea and control, and the lowest panicle length (18.93 cm) was obtained from the CNF treatment (Table 5).

*Effective panicle number hill$^{-1}$*

The number of effective panicles per hill differed significantly among the treatments. The highest number of panicles (12) was recorded from urea, which was statistically similar to CNF and urea (50/50) (Table 5).

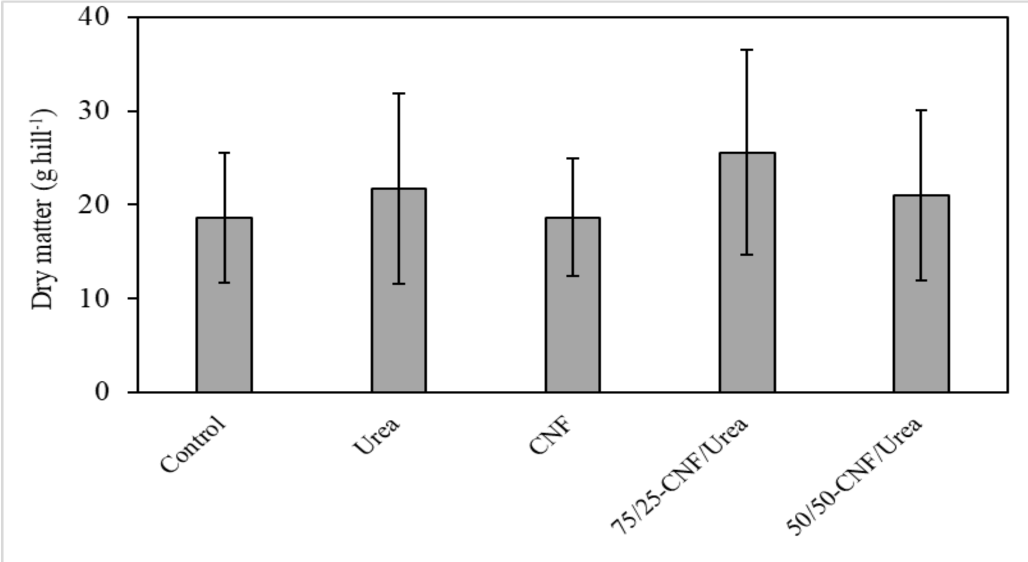


Fig. 1. Effects of Chitin nanofiber CNF and urea on the dry matter of BRRI dhan28. The data represent mean of at least three measurements. The error bar represents mean ± SE (standard error). The coefficient of variation and p value were 11.08% and 0.98, respectively.

### *Numbers of filled grain and unfilled spikelet hill$^{-1}$*

Though the number of filled grains did not vary among the treatments, the number of unfilled spikelets per panicle varied statistically. The highest number of unfilled spikelets per panicle (1694.25) was obtained from urea, which was statistically similar to CNF and urea (75/25), and the lowest number of unfilled spikelets (826.25) was obtained from the control treatment (Table 5).

### *1000 Grains weight (g hill$^{-1}$)*

The 1000-grain weight did not differ among the treatments (Table 5).

### *Straw weight (g hill$^{-1}$)*

Straw weight varied significantly among the treatments. The highest straw weight (59.50 g hill$^{-1}$) was obtained from urea and the lowest was measured from control (28.50 g hill$^{-1}$) (Table 5).

### *Grain yield (g hill$^{-1}$)*

Though grain yield (g hill-1) did not vary among the treatments, numerically higher grain yield (11.54 g hill-1) was found in CNF-urea (50/50) and lower grain yield (5.05 g hill-1) in CNF treatment (Table 5).

### *Total biomass (g hill$^{-1}$)*

Total biomass (g hill$^{-1}$) varied significantly among the treatments. The highest biomass (70.69 g hill$^{-1}$) was recorded from urea, and the lowest (36.68 g hill$^{-1}$) from control (Table 5), indicating that an increased rate of N application increased rice biomass production.

### *Harvest index (%)*

Though the harvest index (%) did not vary among the treatments, a numerically higher harvest index (HI) (20.04%) was calculated from the control treatment, and the lower harvest index (10.52 %) from CNF (Table 5).

**Table 5. Effects of CNF and urea on yield and yield contributing attributes of BRRI dhan28 at harvest**

| | At harvest | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatment | Panicle length (cm) | Effective panicle number hill$^{-1}$ | Filled grain hill$^{-1}$ | Unfilled grain hill$^{-1}$ | 1000 grains weight (g) | Straw weight (g) hill$^{-1}$ | Grain weight (g) hill$^{-1}$ | Total biomass (g) hill$^{-1}$ | Harvest Index (%) |
| Control ($T_0$) | 20.25abc | 5.75b | 420 | 826.25c | 16.98 | 28.50c | 7.18 | 36.68d | 20.04 |
| Urea ($T_1$) | 20.98a | 12.00a | 642.50 | 1694.25a | 17.18 | 59.50a | 11.19 | 70.69a | 15.01 |
| CNF ($T_2$) | 18.93c | 6.25b | 305 | 1198.50bc | 16.55 | 43.25b | 5.05 | 48.30c | 10.52 |
| 75/25-CNF/urea ($T_3$) | 19.23bc | 5.25b | 300.50 | 1495.50ab | 23.19 | 47.75b | 5.91 | 53.66bc | 11.04 |
| 50/50-CNF/urea ($T_4$) | 21.53a | 10.75ab | 674 | 1255.50b | 17.08 | 48.75b | 11.54 | 60.29b | 19.51 |
| *p* | 0.040 | 0.037 | 0.259 | .002 | 0.481 | 0.000 | 0.286 | 0.000 | 0.563 |
| CV (%) | 4.94 | 35.02 | 33.40 | 22.66 | 13.77 | 22.07 | 32.93 | 21.15 | 26.47 |

In a column, CNF = Chitin nanofiber; DAT = Days after transplanting; same letter indicates statistically similar and different letter indicate statistically dissimilar results among the treatments. $p<0.05$, CV = Coefficient of variation.

*Climatic condition during the growing period*

During the growing period, most of the days remained rainless. The rainfall ranged from 0 mm to 53 mm, an average of 1.83 mm. The minimum temperatures ranged from 12 °C to 29 °C with an average of 22.92 °C and the maximum temperatures ranged from 23.5 °C to 39.3 °C with an average of 33.41 °C (Fig. 2). The relative humidity at 9:00 am ranged from 43% to 100% which decreased to 39% to 90% at 6:0 pm (Fig. 2). The high average minimum (22.92 °C) and maximum (33.41 °C) temperatures along with high relative humidity (100% in the morning, 90% in the evening) indicates warm and humid nature of Bangladeshi weather. The scanty rainfall during January and February shows the dry nature of Bangladeshi winter. However, more rainfall in the last week of May indicates a wet summer – the characteristic summer in Bangladesh.

## Discussion

*Growth Parameters*

Nitrogenous realizers increased plant height, tiller number, leaf chlorophyll, leaf area, and dry matter of rice. CNF increased the plant height of BRRI dhan28 and BRRI dhan67 (Biswas et al., 2023; Billah et al., 2023) and the application of N fertilizers at different doses greatly boosted plant height (Chaturvedi, 2005; Rony et al., 2019; Alim, 2012). However, the plant height did not differ at 41, 52, and 74 DATs. Moreover, plant height remained unaffected by the application of chitosan with various molecular weights (Boonlertnirun et al., 2006) and foliar spray of oligomeric chitosan did not influence soybean plant height (Khan et al., 2002). Nitrogen also increased tillers per $m^2$ when rice was harvested 95 days after transplanting (Chaturvedi, 2005). Both the doses and sources of nitrogenous fertilizers had a substantial impact on the number of tillers $hill^{-1}$ (Alim, 2012; Khatun, 2015). However, the tiller number was maximum if rice seeds were soaked in a chitosan solution before planting, and for soil application, they were not substantially different from the control (Boonlertnirun et al., 2008).

The leaf greenness of rice did not always differ, as reported in the present study. Similar findings were reported by Boonlertnirun et al. (2008), who stated that when rice plants' leaf greenness was assessed using a chlorophyll meter, treatments using chitosan in various methods did not significantly alter it. However, tomato leaves treated with chitin revealed high chlorophyll concentrations (SPAD readings), which anticipated high leaf nitrogen contents (Egusa et al., 2020).

Nitrogen affected leaf area of rice. The results of the present study are in accordance with the findings of Theerakarunwong et al. (2016) who reported that when compared to the control, the chitosan-treated rice's leaf area was higher (14.96-24.12 $cm^2$ $blade^{-1}$) than average leaf area (11.85-20.57 $cm^2$ $blade^{-1}$). However, leaf area index varied depending on the techniques of urea fertilizer applications (Azam et al., 2012). Though, dry matter content did not differ among the treatments, N-fertilizer administration boosted dry matter accumulation significantly in rice at all growth stages (Chaturvedi, 2005).

*Yield and yield contributing attributes*

Nitrogen affected panicle length, effective panicle number, numbers of filled grain and unfilled spikelets, 1000-grain weight, straw weight, grain yield, and harvest index of rice. Similar to the present study, the longest panicle (22.84 cm) was produced by BRRI dhan28, and the shortest panicle (20.47 cm) by the BRRI dhan36 (Alim, 2012). However, panicle length was only affected a little by the treatments (Ahmed et al., 2020). Similarly, panicle numbers varied based on nitrogen treatment (Boonlertnirun et al., 2008). Soaking rice seeds in chitosan solution before planting and subsequent soil application produced a maximum number of panicles (Boonlertnirun et al., 2006), and rice panicle counts increased after being watered with chitosan at a rate of 0.4 g/50 cm3 (chitosan: water) (Lu et al., 2002). However, chitosan treatment did not affect the number of filled grains (Boonlertnirun et al., 2006).

Similar to our findings, 1000-grain weight was not influenced by the nitrogen sources, doses or interactions of sources and doses (Alim, 2012) and it was not affected by chitosan application techniques (Boonlertnirun et al., 2006). Straw weight also varied based on nitrogen sources and amount of nitrogen applied (Alim, 2012). However, straw weight was shown to be statistically negligible for different treatments (Ahmed et al., 2013, 2020).

Rice grain yield was affected by the nitrogenous fertilizers and nitrogen-containing biomolecules. Chitosan application methods did not influence the grain yield of rice (Boonlertniru et al., 2008). However, CNF increased the grain yield of BRRI dhan28 and BRRI dhan67 (Biswas et al., 2023; Billah et al., 2023); BRRI dhan28 resulted in higher grain yield compared to BRRI dhan36, and the higher yield was contributed by effective tiller number in a hill, length of panicle, filled grains in a panicle, and 1000-grain weight (Alim, 2012). Higher doses of N also increased rice biomass production (Hossain et al., 2008). Similar to our findings, the rice variety Kachra had a harvest index of 25.48% (Islam et al., 2013). However, HI varied due to the source and level of N applied (Alim, 2012), and chitosan concentration also affected HI (Ahmed et al., 2013).

*The mechanism of influencing growth and yield of rice by CNF*

Though CNF contains low nitrogen (3.6–6.8%) compared to urea (46.6%), CNF influences the growth and yield of rice similar to urea (Shams et al., 2025) due to the higher availability of nitrate-N and ammonium-N. Moreover, CNF may enhance microbial activities in the root zone, favoring better nutrient availability (Shams et al., 2025). On the other hand, urea is prone to losses. However, further investigations are required to elucidate the mechanisms encouraging the growth and yield of rice.

*Climate and rice growth and yield*

Rice is a temperature-sensitive crop, particularly during the blooming stage. Exposure of rice to temperatures higher than 35 0C for more than an hour during the growth phase causes injury. For BRRI dhan28, the crucial nighttime and daytime temperatures are 12 0C and 280C, respectively, at the beginning of panicle initiation (The Daily Star, 2014). High temperatures hinder the development of floral organs at the panicle initiation stage and exacerbate spikelet degeneration (Wang et al., 2019). High temperature also declines the spikelet fertility, which reduces rice yield (Shi et al., 2017). In the present study, it is noticed that the majority of the spikelets did not form at the flowering stage and were also worn out and had a delicate white structure with black spots, which may happen due to temperature stress as the highest temperature ranged from 30 0C to 36 0C during flowering stage (Fig. 2). This resulted in too many unfilled spikelets and the number of unfilled spikelets was at least double compared to the number of filled grains (Table 5). Even the number of unfilled spikelets was almost five times compared to the number of filled grains, which resulted in a very low harvest index. Moreover, using black pots in the cultivation and placement of those pots on the concrete may augment the high-temperature stress that may worsen the situation.

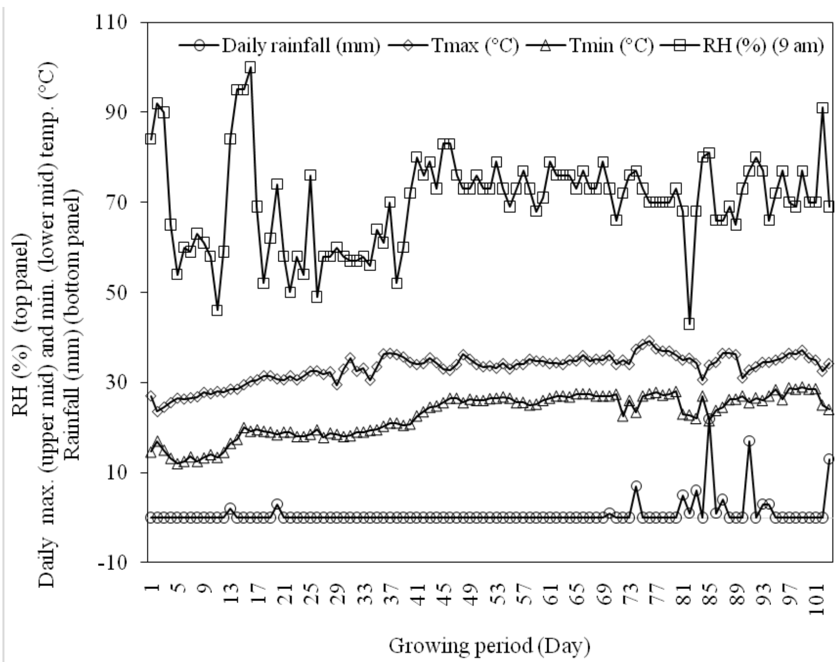


Fig. 2. Relative humidity (%), daily maximal and minimal air temperatures (°C), and daily rainfall (mm) during the study period. Tmin and Tmax are daily minimal and maximal air temperatures, respectively. Data were collected from Khulna Meteorological Station, <100 m from the study field.

## Conclusion

Among the treatments, CNF-urea (50/50) resulted in statistically similar plant height, tiller number, panicle length, and the effective number of panicles to urea. CNF-urea (50/50) also had a lower number of unfilled spikelets compared to the recommended dose of urea. Moreover, grain yield and harvest index were numerically higher for CNF-urea (50/50) than urea alone. Therefore, CNF-urea (50/50) may produce a similar rice yield to urea, suggesting that half of the urea-nitrogen can be supplemented by CNF without significant yield loss of rice (BRRI dhan28). However, further experiments should be conducted in multilocation to get a final recommendation.

## Competing Interest

The authors report that there are no competing interests to declare.

## Reference


Ahmed, F., Issak, M., & Sultana, A. (2020). Effect of chitosan-raw-materials on grain yield and agronomic traits of transplanted aman rice (BRRI dhan49). *Eco-friendly Agril. J.* 13(10), 38-46.

Ahmed, R. A. S. E. L., Hossain, M. E., Ullah, M. A., Gazi, A. R., & Rahman, M. Z. (2013). Effect of chitosan on growth and yield of rice cv. BRRI dhan29. *J. Agrofor. Environ*, *7*(2), 33-35.

Alim, M. A. (2012). Effect of organic and inorganic sources and doses of nitrogen fertilizer on the yield of Boro rice. *Journal of Environmental Science and Natural Resources*, 5(1), 273-282.https://doi.org/10.3329/jesnr.v5i1.11592

Azam, M., Ali, M., Karim, M., Rahman, A., Jalal, J., & Mamun, A. (2012). Growth and yield of boro rice as affected by different urea fertilizer application method. *Interaction*, *1*(3), 2.DOI: 10.5829/idosi.ijsa.2012.04.03.312

Azuma, K., Ifuku, S., Osaki, T., Okamoto, Y., & Minami, S. (2014). Preparation and biomedical applications of chitin and chitosan nanofibers. *Journal of biomedical nanotechnology,* 10(10), 2891-2920. DOI: https://doi.org/10.1166/jbn.2014.1882

Bangladesh Rice Research Institute. Factsheet: *Dhan utpadon proshikkan module*. Gazipur. Bangladesh.

Billah, M., J. S. Bristi, M. Y. Ali, M. I. Shams, and M.Y. Kabir. (2023). Use of Chitin Nanofiber (CNF) on Growth and Yield of BRRI dhan67. Abstract presented at the International Conference on Climate Smart Agriculture and the 4th Agricultural Revolution; Organized by Agrotechnology Discipline, Khulna University, Khulna 9208, Bangladesh, 19 – 20 October, 2023.

Biswas, T., M. Y. Ali, M. I. Shams, and M.Y. Kabir. (2023). Chitin Nanofiber for Growth and Yield of BRRI dhan28. Abstract presented at the International Conference on Climate Smart Agriculture and the 4th Agricultural Revolution; Organized by Agrotechnology Discipline, Khulna University, Khulna 9208, Bangladesh, 19 – 20 October, 2023.

Boonlertnirun, S., A. Sarobol, S. Mechoui and A. Sooksathan. (2006). Effect of Molecular Weight of Chitosan on Rice Yield Potential cv. Suphanburi 1. Kasetsart J. *(Nat. Sci),* 40(4).

Boonlertnirun, S., Boonraung, C., & Suvanasara, R. (2008). Application of chitosan in rice production. *Journal of metals, materials and minerals*, *18*(2).

Bremner, J. M. (1995). Recent research on problems in the use of urea as a nitrogen fertilizer. *Nitrogen economy in tropical soils*, 321-329.

Chaturvedi, I. (2005). Effect of nitrogen fertilizers on growth, yield and quality of hybrid rice (Oryza sativa). *Journal of Central European Agriculture*, 6(4), 611-618. https://hrcak.srce.hr/17330

De Datta, S. K., & Buresh, R. J. (1989). Integrated nitrogen management in irrigated rice. *In*: Stewart, B.A. (eds) Advances in Soil Science. *Advances in Soil Science,* vol 10. Springer, New York, NY. https://doi.org/10.1007/978-1-4613-8847-0_4

Egusa, M., Matsukawa, S., Miura, C., Nakatani, S., Yamada, J., Endo, T., ...& Kaminaka, H. (2020). Improving nitrogen uptake efficiency by chitin nanofiber promotes growth in tomato. *International journal of biological macromolecules*, 151, 1322-1331.DOI: 10.1016/j.ijbiomac.2019.10.178

Egusa, M., Parada, R., Aklog, Y. F., Ifuku, S., & Kaminaka, H. (2019). Nanofibrillation enhances the protective effect of crab shells against Fusarium wilt disease in tomato. *International journal of biological macromolecules*, 128, 22-27.

FAOSTAT. (2022). https://www.fao.org/asiapacific/perspectives /agricultural-statistics/global-strategy/results-in-the- region/ bangladesh /en/, Accessed on 12December, 2022.

Hossain M B, Islam M O, Hasanuzzaman M. (2008). Influence of different nitrogen levels on the performance of four aromatic rice varieties. *Intl.J. of Agril. & Bio.* 10: 693-696.

Ifuku, S., Nogi, M., Abe, K., Yoshioka, M., Morimoto, M., Saimoto, H., & Yano, H. (2011). Simple preparation method of Chitin Nanofibers with a uniform width of 10–20 nm from prawn shell under neutral conditions. *Carbohydrate Polymers,* 84(2), 762-764. https://doi.org/10.1016/j.carbpol.2010.04.039

Ifuku, S., Nogi, M., Yoshioka, M., Morimoto, M., Yano, H., & Saimoto, H. (2010). Fibrillation of dried chitin into 10–20 nm nanofibers by a simple grinding method under acidic conditions. *Carbohydrate Polymers,* 81(1), 134-139. https://doi.org/10.1016/j.carbpol.2010.02.006

Islam, N., Kabir, M. Y., Adhikary, S. K., & Jahan, M. S. (2013). Yield performance of six local aromatic rice cultivars. *IOSR Journal of Agriculture and Veterinary Science*, 6(3), 58-62.

Izumi, R., Azuma, K., Izawa, H., Morimoto, M., Nagashima, M., Osaki, T., ... &; Ifuku, S. (2016). Chitin nanofibrils suppress skin inflammation in atopic dermatitis-like skin lesions in NC/Nga mice. *Carbohydrate polymers,* 146, 320-327. https://doi.org/10.1016/j.carbpol.2016.03.068

Izumi, R., Komada, S., Ochi, K., Karasawa, L., Osaki, T., Murahata, Y., ... &; Ifuku, S. (2015). Favorable effects of superficially deacetylated chitin nanofibrils on the wound healing process. *Carbohydrate polymers,* 123, 461-467. https://doi.org/10.1016/j.carbpol.2015.02.005

Janssen, R.H.; Vincken, J.P.; Van Den Broek, L.A.M.; Fogliano, V.; & Lakemond, C.M.M. 2017. Nitrogen-to-protein conversion factors for three edible insects: Tenebrio molitor, Alphitobius diaperinus, and Hermetia illucens. J. Agric. Food Chem., 65: 2275–2278. doi: 10.1021/acs.jafc.7b00471.

Karim, M., Kashem, M. A., Huda, A., Aziz, M. A., & Goswami, B. K. (2019). Effect of Different Doses of Urea on the Yield of Boro Rice Varieties in Haor Areas of Bangladesh. *Asian Plant Research Journal*, 3(2), 1-9, 2581-9992. https://doi.org/10.9734/aprj/2019/v3i230061

Khan, W.M., B. Prithiviraj and D.L. Smith. (2002). Effect of foliar application of chitin and chitosan oligosaccharide on photosynthesis of maize and soybean. *Photosynthetica,* 40, 624-624.

Lu, J., Zhang, C., Hou, G., Zhang, J., Wan, C., Shen, G., ...& Hou, T. (2002). The biological effects of chitosan on rice growth. *Acta Agriculture Shanghai*, 18(4), 31-4.

Munira, S., Khan, S.A.K.U., Shams, M.I., & Kabir, M.Y. 2025. Potentials of Chitin Nanofiber for Postharvest Quality of Banana. *Journal of Food Processing and Preservation*, volume 2025, ID 2776934. https://doi.org/10.1155/jfpp/2776934

Phongpan, S., Vacharotayan, S., & Kumazawa, K. (1988). Fate and efficiency of urea fertilizer in wetland rice soil. *Soil Science and plant nutrition*, 34(1), 117-126. https://doi.org/10.1080/00380768.1988.10415585

Rony, I. A., Das, A., Quddus, K. G., & Sarker, B. C. (2019). Effect of nitrogen on growth and yield of aman rice (Brri dhan 51) in battaghata upazila of khulna dtstrict. *Bangladesh J. Agric. and Environ*, 15(1), 19-26.

Saleque MA, Abedin MJ, Bhuiyan NI, Zaman SK and Panaullah GM. (2004). Long-term effects of inorganic and organic fertilizer sources on yield and nutrient accumulation of lowland rice. *Field Crops Research,* 86, 53-65.

Shi C L, Luo Z Q, Jiang M, Shi Y L, Li Y X, Xuan S L, Liu Y, Yang S B, Yu G K. (2017). An quantitative analysis of high temperature effects during meiosis stage on rice grain number per panicle. *Chin J Rice Sci,* 31(6), 658–664.

The Business Standard, (2022). https://www.tbsnews.net bangladesh/reducing-use-urea-fertiliser-20-will-not-harm-production -agri-minister-471134, Accessed on 12 December, 2022.

The Daily Star, (2014). Growing rice under stress environment. https://www.thedailystar.net/growing-rice-under-stress-environment-15447, Accessed on 27 December, 2022.

The Financial Express, (2018). BRRI develops two high yielding rice varieties.https://thefinancialexpress.com.bd/national/brri-develops-two-high-yielding-rice-varieties-1539174235, Accessed on 12December, 2022.

Theerakarunwong, C. D., & Phothi, R. (2016). Physiological and photosynthesis enhancement of Thai rice (Oryza sativa L.) cultivars by chitosan. NU. *International Journal of Science*, 13(1), 37-49.

Tsutsumi, Y., Koga, H., Qi, Z. D., Saito, T., & Isogai, A. (2014). Nanofibrillar chitin aerogels as renewable base catalysts. *Biomacromolecules,* 15(11), 4314-4319. https://doi.org/10.1021/bm501320b

Wang, Y., Wang, L., Zhou, J., Hu, S., Chen, H., Xiang, J. ... & Zhang, Y. (2019). Research progress on heat stress of rice at flowering stage. *Rice Science*, 26(1), 1-10. doi.org/10.1016/j.rsci.2018.06.009

Xia, Y., Yang, P., Sun, Y., Wu, Y., Mayers, B., Gates, B. ... &; Yan, H. (2003). One-dimensional nanostructures: synthesis, characterization, and applications. *Advanced materials,* 15(5), 353-389. https://doi.org/10.1002/adma.200390087

**Appendix I. Analysis of variance for the effect of CNF and urea on plant height of BRRI dhan28 at different growth stages**

| Source of variance | Degrees of freedom | Mean sum of square | | | | |
|---|---|---|---|---|---|---|
| | | Plant height (cm) | | | | |
| | | 41 DAT | 52 DAT | 63 DAT | 74 DAT | At harvest |
| Treatment | 4 | 44.63 | 126.05 | 111.22 | 10130 | 142.26 |
| Error | 30 | 25.83 | 23.790 | 26.83 | 29.43 | 21.37 |
| P value | | 0.101 | 0.031 | 0.094 | 0.273 | 0.011 |
| F value | | 1.73 | 5.30 | 4.15 | 3.44 | 6.66 |

**Appendix II. Analysis of variance for the effect of CNF and urea on tiller number hill$^{-1}$ and dry matter of BRRI dhan28 at different growth stage.**

| Source of variance | Degrees of freedom | Mean sum of square | | | | | |
|---|---|---|---|---|---|---|---|
| | | Tiller number hill$^{-1}$ | | | | | Dry matter (g hill$^{-1}$) |
| | | 41 DAT | 52 DAT | 63 DAT | 74 DAT | At harvest | |
| Treatment | 4 | 60.53 | 88.12 | 96.94 | 126.99 | 180.18 | 19.72 |
| Error | 30 | 13.48 | 12.04 | 10.64 | 14.11 | 33.35 | 243.77 |
| P value | | 0.020 | 0.002 | 0.001 | 0.002 | 0.018 | 0.988 |
| F value | | 4.49 | 7.32 | 9.11 | 9.00 | | 0.08 |

**Appendix III. Analysis of variance for the effect of CNF and urea SPAD readings (CCI) and leaf area of BRRI dhan28 at different growth stages**

| Source of variance | Degrees of freedom | Mean sum of square | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | SPAD readings (CCI) | | | | Leaf area (cm$^2$) | | |
| | | 42 DAT | 53 DAT | 64 DAT | 75 DAT | 42 DAT | 53 DAT | 64 DAT |
| Treatment | 4 | 34.44 | 16.44 | 70.65 | 118.92 | 93.63 | 60.51 | 35.18 |
| Error | 15 | 37.32 | 11.40 | 21.26 | 41.63 | 48.85 | 26.38 | 66.91 |
| P value | | 0.722 | 0.472 | 0.118 | 0.062 | 0.150 | 0.096 | 0.769 |
| F value | | 0.92 | 1.44 | 3.32 | 2.86 | 1.92 | 2.29 | 0.53 |

**Appendix IV. Analysis of variance for the effect of CNF and urea on yield and yield contributing attributes of BRRI dhan28 at different growth stages**

| Source of variance | Degrees of freedom | Mean sum of square | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Panicle length (cm) | Effective panicle number hill$^{-1}$ | Filled grain hill$^{-1}$ | unfilled grain hill$^{-1}$ | Grain weight (g) | Straw weight (g) | 1000 grain weight (g) | Total biomass (g) | Harvest index (%) |
| Treatment | 4 | 3.44 | 45.05 | 96910.7 | 664438 | 24.16 | 688.17 | 29.55 | 846.94 | 69.78 |
| Error | 15 | 1.67 | 17.40 | 97254.1 | 88019 | 30.60 | 43.62 | 34.64 | 24.88 | 135.72 |
| P value | | 0.040 | 0.037 | 0.259 | 0.002 | 0.286 | 0.000 | 0.481 | 0.000 | 0.563 |
| F value | | 2.06 | 2.70 | 1.00 | 7.55 | 0.79 | 15.78 | 0.85 | 34.04 | 0.51 |