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Research article

Application of Chitin Nanofiber as A Nitrogen Source Improves Growth and Yield of BRRI Dhan28

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ABSTRACT

Rice is the principal crop, a key component of food security, and a cultural staple of Bangladesh. However, growing rice requires a lot of urea, which is easily lost through denitrification, volatilization, leaching, and runoff. A pot experiment was conducted to evaluate the effect of chitin nanofiber (CNF) as a nitrogen source on the growth and yield of BRRI dhan28 at the Nursery of Forestry and Wood Technology Discipline, Khulna University, Bangladesh, from February to May 2022. Four CNF concentrations—0.1%, 0.2%, 0.4%, and Control (without CNF)—were used in the completely randomized design (CRD) trial, which was duplicated seven times. Before and after transplanting, varying amounts of CNF were added to the soil. CNF application to the soil had a statistically significant effect on total biomass and leaf chlorophyll content index at 64 days after transplanting (DAT), tiller number hill-1 at 41 DAT. In comparison to the control treatment, which typically produced lower values, the experimental results showed that 0.4% CNF produced numerically higher plant height (87.88 cm), tiller number (26.00 hill-1), grain yield (10.08 g hill-1), total filled grain (580.50), straw weight (40 g hill-1), total biomass (50.08 g hill-1), 1000-grain weight (17.22 cm), and panicle length (21.05 cm). Heat stress was likely the cause of the low growth and yield characteristics, such as grain yield and harvest index. Thus, 0.4% CNF may be considered for rice cultivation based on the results. In Bangladesh and other countries, this knowledge may contribute to improved rice cultivation practices and a reduction in chemical fertilizer use; however, further research is warranted.

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Introduction

Rice (*Oryza sativa* L.) is the principal grain crop in Bangladesh, occupying approximately 75% of the country's cultivable land, contributing 5% to its total gross domestic product (GDP), and 46% to its agricultural GDP (BER, 2019, 2023). Rice is critical for national food security, supplying about 70% of caloric intake and 58% of protein consumption (BBS, 2019). Urea is the predominant nitrogen (N) fertilizer used by Bangladeshi farmers; however, nitrogen use efficiency (NUE) is low, with only 30–35% of applied nitrogen being utilized by rice plants (De Datta & Buresh, 1989). Losses occur through nitrification, denitrification, and ammonia volatilization, accounting for up to 26% of applied N (Phongpan et al., 1988). Nitrogen is often the most yield-limiting nutrient in tropical Asian soils, and reliance on costly nitrogenous fertilizers poses economic and environmental challenges (Islam et al., 2015). Consequently, improving NUE and developing sustainable nitrogen alternatives are major priorities for rice research.

Chitin nanofiber (CNF) has emerged as a promising candidate to address these challenges. CNF is produced by nanofibrillation of chitin, creating fibers with diameters below 100 nm and high aspect ratios (>100) (Xia et al., 2003; Li & Xia, 2004). CNF retains the native polymer structure and has a high nitrogen content (6–8%) and low C/N ratio, making it a potential direct source of nitrogen for crops (Egusa et al., 2019; Shams et al., 2025). Its high surface-to-volume ratio, unique nanofibrillar morphology, and remarkable mechanical strength (Ifuku et al., 2010, 2011, 2013) facilitate interactions with soil matrices that can enhance nutrient retention and gradual nitrogen release. CNF is also biodegradable, biocompatible, and antimicrobial (Al-Hetar et al., 2011; Azuma et al., 2014,

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2015; El Knidri et al., 2020; Sharp, 2013;), which may further support soil health and crop productivity.

Recent studies have demonstrated the potential of CNF as a sustainable nitrogen source in rice cultivation. Shams et al. (2025) showed that shrimp-derived CNF could partially replace urea in BRRI dhan67 cultivation, improving growth and yield parameters while reducing environmental nitrogen losses. Similarly, Afroj et al. (2025) reported that CNF supplementation enhanced physiological traits and yield components in BRRI dhan28, indicating its potential to increase NUE and support sustainable rice production. These findings highlight CNF not only as a bioactive growth enhancer but also as a functional nitrogen source that can contribute to both economic and environmental sustainability in rice-based agroecosystems. Despite these promising results, research on CNF in rice remains limited, particularly regarding its optimal application rates, timing, and interaction with different soil types and environmental conditions. Therefore, this study aimed to evaluate the effect of CNF as a nitrogen source on the growth and yield of BRRI dhan28, providing insight into its potential role in sustainable rice production in Bangladesh and similar agroecological regions.

Materials and Methods

Pot preparation, climatic condition, planting material, and experimental design

The field experiment was carried out during the *Boro* season from February to May of 2022 at the Nursery of Forestry and Wood Technology Discipline, Khulna University, Khulna. Soil was collected from Nij Khamar, Khulna. A 809.29 kg of soil, combined with various fertilizers, including TSP (30.27g), MoP (24.23g), Gypsum (12.11g), Zn (2.72g), and cow dung (4.046kg), was weighed using a digital balance. Each bucket (black, 20 L) was filled with 13 kg of soil after thoroughly mixing. The Ganges Tidal Flood Plain (agroecological zone 13) covers the trial site. The climate of the experimental site was characterized by relatively high temperatures, heavy rainfall, and intermittent gusty winds during the Kharif season, and low temperatures and humidity during the Rabi season. The high-yielding boro rice variety utilized in this experiment is called BRRI dhan28. With 81 days of flowering, it is a short-duration cultivar. The life cycle lasted roughly 140 days, and the height was about 90 cm (Chhogyell et al., 2016). The experiment was laid out in a Completely Randomized Design (CRD) with seven replications and four treatments. About 30 days old seedlings were transplanted in the bucket on 11 February 2022.

Preparation of chitin nanofiber (CNF) and treatment applications

CNF was extracted from the shells tiger shrimp (*Peneus monodon*). 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 demineralized, deproteinized, and depigmented using HCI (37%), NaOH (99.9%), and Ethanol 50%, respectively (Ifuku et al., 2011). The suspension was blended with a super-speed blender (Vita-Mix Blender, Osaka Chem. Co. Ltd.) and a normal-speed blender (Panasonic MX Blender, Panasonic Holdings Corporation), and transferred to the super colloidal machine. The CNF was mechanically processed by passing it through a Super Masscolloider (MKCA6-51, Saitama-ken, Japan). The grinding stone clearance was set to -0.15 with a 1500 rpm rotating stone speed. After going through a super-speed blender for 10, 15, and 20 milling cycles, the CNF was extracted. The required concentrations (0.1%, 0.2%. and 0.4%) of CNF were prepared by adding more distilled water. The four treatments (control, 0.1%, 0.2%, and 0.4%) were given three times during the trial: ten days after transplanting (DAT), thirty days after transplanting (DAT), and sixty days after transplanting (DAT). The CNF solutions were applied to the soil each time.

Assessment of growth parameter

Data were recorded to determine growth parameters starting 41 days after transplanting (DAT) and continuing 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 tiller numbers were counted at 41 DAT, 52 DAT, 63 DAT, and 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 12 plants. From each plant, four leaves were taken and photographed. The area of the leaf was measured using ImageJ, and the mean leaf area was computed. Dry matter was estimated at 44 DAT, 55 DAT, and 66 DAT. The plants were uprooted and dried at 60 °C in an oven for 72 hours, and dry matter was weighed using an electronic balance.

Assessment of yield and yield attributes

When 90% of the grains had turned golden yellow, the crop was considered fully mature and ready for harvest. After being removed from each pot, the plants were tagged. Additionally, grain and straw yields were noted. The plants were properly sun-dried. The grains were separated from the straw and then weighed. Following harvesting, the mean number of efficient tillers per hill was determined. Each effective panicle's length was measured from the rachis' base node to the panicle's apex, and the mean was determined. The average was determined by counting the number of grains each hill. The means were computed by counting the number of unfilled spikelets per panicle. To determine the 1000-grain weight, 1,000 grains from each treatment were weighed on an electronic scale after sun drying. Additionally, each pot's grain weight was determined independently and expressed as g hill^{-1}. The straw weight of the harvested crop in each pot was determined. Each pot's sample was dried in an oven set at 60 °C for 72 hours. Each pot's results were separately recorded and represented as g hill-1. The harvest index (%) showed the ratio of grain yield to biological yield, which was calculated by combining grain yield and straw yield (Islam et al., 2013; Islam et al., 2025; Islam et al., 2025).

Statistical analysis

The data were analysed 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 Tukey's Honestly Significance Difference (HSD) Test @ 5%.

Results

Weather

During the experimental period, the majority of days experienced negligible rainfall, ranging from 0 to 53 mm, with an average of 1.83 mm. Minimum temperatures fluctuated between 12 and 29 °C (mean 22.92 °C), whereas maximum temperatures ranged from 23.5 to 39.3 °C (mean 33.41 °C). Relative humidity varied from 43–100% in the morning and 39–90% in the evening (Fig. 1), supporting the warm and humid weather of Bangladesh (Nahida et al., 2024). The combination of elevated temperatures during the flowering stage (30–36 °C), high humidity, and limited precipitation likely induced spikelet degeneration, reduced grain filling, and contributed to low yields. Temperature-sensitive physiological processes, including floral organ development and fertilization, were presumably impaired, resulting in a higher proportion of unfilled spikelets per hill and reduced harvest indices. Furthermore, the use of black pots positioned on concrete surfaces may have exacerbated heat stress, intensifying its adverse effects on reproductive development (Afroj et al., 2025).

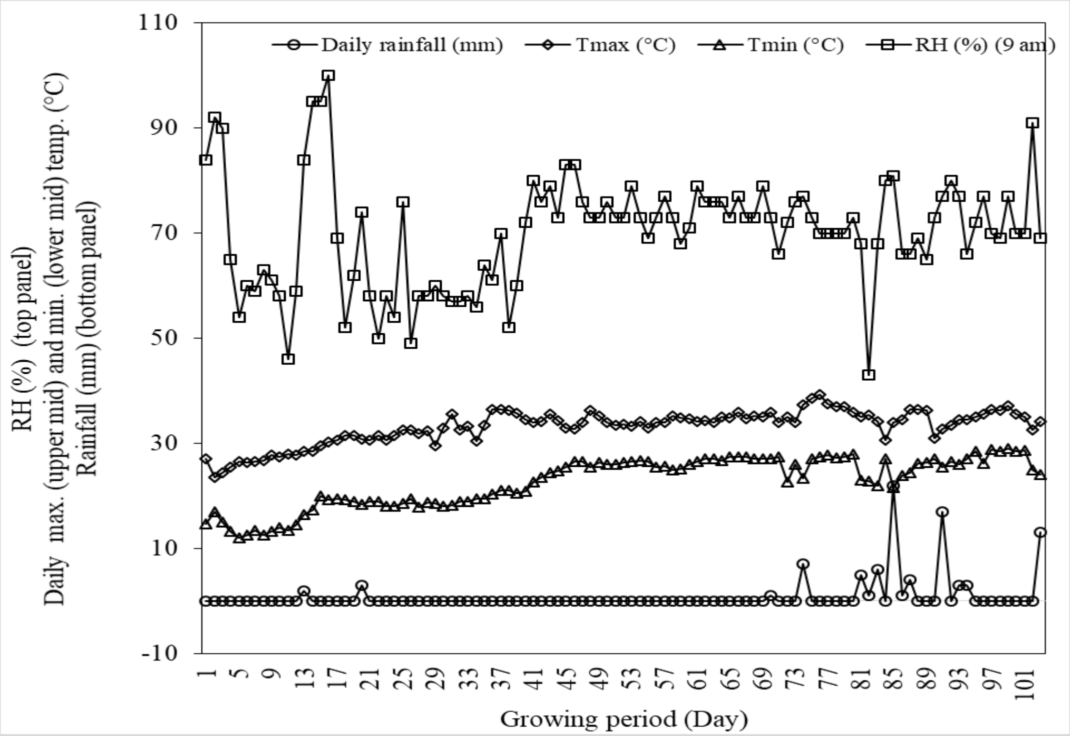

Figure 1: Relative humidity (RH, %), rainfall (mm), and temperatures [minimum (Tmax) and maximum (Tmin)] during the experiment. Data were collected from the nearby meteorological station of Khulna (within 100 m of the experiment field).

Growth attributes

Plant height (cm)

Plant height did not differ significantly due to the application of CNF (Table 1). However, numerically, the higher plant height (87.88 cm) was obtained from rice grown with 0.4% CNF for all growth stages, and the lower plant height (54.93 cm) was found from the control (without CNF) among different DATs (41, 63, 74), except 52 DAT (Table 1). At harvest, the highest plant height (76.13 cm) was obtained from 0.2% CNF, and the lowest plant height (72.00 cm) from the control (Table 1). Similarly, CNF increased, though non-significant, plant height of BRRI dhan28 and BRRI dhan67 (Billah et al., 2023; Biswas et al., 2023; Shams et al., 2025). Chitosan significantly increased heights of rice (Ahmed et al., 2013) and coffee (Dzung et al., 2011). However, chitosan did not affect the height of rice, as it primarily enhances physiological efficiency and yield-related traits rather than vegetative elongation, particularly under adequate soil fertility conditions. (Ahmed et al., 2020; Munshi, 2015).

Tiller number hill^{-1}

The tiller numbers hill^{-1} were influenced by the CNF levels of only 41 DAT (Table 2). The highest number of tillers hill^{-1} (17.00) was obtained from 0.1% CNF, which was statistically similar to 0.4% CNF. The lowest number of tiller hill^{-1} (18.50) was obtained from the control (without CNF). However, tiller number hill-1 did not vary at 52 DAT, 63 DAT, 74 DAT, and the harvesting stage. Numerically, the highest tiller number was obtained from 0.4% CNF at all DATs, except at 63 DAT (Table 2). The highest tiller number (26.00) was obtained from 0.4% CNF, and the lowest tillers (20.55) were in control treatment at harvest. The doses and sources of nitrogenous fertilizers had a substantial impact on the number of tillers hill^{-1} (Alim, 2012; Khatun, 2015). Similarly, the tiller number plant^{-1} was significantly influenced by the application of chitosan (Boonlertnirun et al., 2008;

Theerakarunwong and Phothi, 2016), which is attributed to its biostimulatory effects on rice physiology. Chitosan enhanced root growth, nutrient uptake, improved photosynthetic activity and chlorophyll content, together increasing assimilate availability for tiller formation. Thus, chitosan promotes tillering indirectly through enhanced physiological efficiency rather than direct growth regulation (Boonlertnirun et al., 2008; Theerakarunwong and Phothi, 2016).

Leaf chlorophyll content index

The leaf chlorophyll content index differed only at 64 DAT. The highest leaf chlorophyll content index (43.48) was recorded from 0.4% CNF, and the lowest chlorophyll (36.88) was obtained from 0.1% CNF (Table 3). Similarly, chitin nanofiber treatments produced rice plants with SPAD readings and chlorophyll indices statistically similar to urea-fertilized controls, indicating no significant enhancement of leaf greenness (Afroj et al., 2025) However, leaf chlorophyll did not differ at other DATs. 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.

Table 1: Effect of CNF on plant height of BRRI dhan28

	Plant height (cm)				
Treatment	**41 DAT**	**52 DAT**	**63 DAT**	**74 DAT**	**At Harvest**
Control	54.93	70.67	80.30	67.50	72.00
0.1% CNF	57.91	69.75	80.90	81.88	72.13
0.2% CNF	59.57	71.67	82.30	82.88	76.13
0.4% CNF	56.63	72.83	86.70	87.88	75.38
***P* value**	0.333	0.557	0.209	0.474	0.236
CV (%)	3.43	1.86	3.60	10.94	2.91

CNF stands for chitin nanofiber, and CV for coefficient of variation.

Table 2: Effect of CNF on tiller number hill^{-1} of BRRI dhan28

	Tiller number hill^{-1}				
Treatment	**41DAT**	**52DAT**	**63DAT**	**74DAT**	**At Harvest**
Control	11.29b	18.50	18.20	20.55	20.55
0.1% CNF	17.00a	20.33	20.40	18.25	21.25
0.2% CNF	14.14ab	20.00	18.80	18.50	21.75
0.4% CNF	15.14a	20.67	20.60	19.75	26.00
P value	0.014	0.586	0.379	0.792	0.318
CV (%)	16.56	4.81	6.07	5.61	10.98

Different letter in a column indicates statistical differences. CNF stands for chitin nanofiber, and CV for coefficient of variation.

Table 3: Effect of CNF doses on SPAD reading (CCI) of BRRI dhan28 at different growth stages

	SPAD reading (CCI)			
Treatment	**42 DAT**	**53 DAT**	**64 DAT**	**75 DAT**
Control	46.54	40.87	37.90b	27.68
0.1% CNF	42.16	39.22	36.88b	33.10
0.2% CNF	47.62	40.02	41.64ab	30.95
0.4% CNF	44.20	41.77	43.48a	39.20
P value	0.275	0.127	0.033	0.130
CV (%)	5.41	2.71	7.77	14.83

Different letter in a column indicates statistical differences. CNF stands for chitin nanofiber, and CV for coefficient of variation.

Leaf area (cm^2)

Leaf area hill^{-1} was statistically insignificant at all growth stages (Table 4). At 42 DAT, the highest leaf area hill^{-1} (20.31cm^2) was obtained from the control treatment, and the lowest (14.95 cm2) was found from 0.2% CNF (Table 4). Similarly, leaf area was statistically insignificant for maize or soybean due to the application of chitosan (Khan et al., 2002). However, chitosan increased the leaf area of rice (Theerakarunwong and Phothi, 2016) and waxy corn (Suvannasara et al., 2011).

Dry matter production (g hill^{-1})

The dry matter was taken from the whole plant, including its root, leaf sheath, leaves, stem, and panicle, which was measured at 44 DAT, 55 DAT, and 66 DAT. The dry matter did not differ at any growth stages (Table 5). However, numerically, the higher dry matter (20.30 g) was obtained from 0.1% CNF. Similarly, dry matter production of rice did not differ due to chitosan (Boonlertnirun et al., 2012, 2015). However, chitosan increased the accumulation of dry matter in rice (Ahmed et al., 2020; Ohnishi, 2008; Theerakarunwong and Phothi, 2016).

Table 4: Effect of CNF doses on leaf area hill^{-1} of BRRI dhan28 at different growth stages

	Leaf area (cm^2)		
Treatment	**42 DAT**	**53 DAT**	**64 DAT**
Control	20.31	10.61	16.24
0.1% CNF	19.95	10.13	11.88
0.2% CNF	14.95	10.29	8.65
0.4% CNF	17.97	13.64	16.94
P value	0.526	0.531	0.100
CV (%)	13.42	14.49	29.64

CNF stands for chitin nanofiber, and CV for coefficient of variation.

Table 5: Effect of CNF doses on dry matter production hill^{-1} of BRRI dhan28

	Dry Matter production (g hill^{-1})
Treatment	**Dry matter**
Control	18.65
0.1% CNF	20.30
0.2% CNF	19.20
0.4% CNF	18.98
P value	0.998
CV (%)	3.71

CNF stands for chitin nanofiber, and CV for coefficient of variation.

Yield and yield contributing characters

Panicle Length and Effective Panicle per Hill

Panicle length and the number of effective panicles per hill did not vary significantly among treatments; however, 0.4% CNF resulted in a numerically higher panicle length (21.05 cm, Table 6). This aligns with previous studies where panicle traits were largely unaffected by chitosan under normal or stress conditions (Ahmed et al., 2020; Boonlertnirun et al., 2007; Munshi, 2015), although some studies reported significant improvements in panicle length due to chitosan (Ahmed et al., 2020; Boonlertnirun et al., 2012; Sultana et al., 2015). In the present study, high temperatures during panicle initiation and flowering likely limited panicle elongation, as rice is highly sensitive to temperatures above 35 °C, which can damage floral organs and hinder panicle development (Shi et al., 2017; Wang et al., 2019).

Total Filled and Unfilled Grains per Hill

Total filled and unfilled grains per hill did not differ significantly among treatments, although 0.4% CNF produced numerically higher values (580.55 filled and 985.50 unfilled grains, Table 6). Similar non-significant effects were observed with chitosan (Boonlertnirun et al., 2007; Munshi, 2015). Conversely, Ahmed et al. (2020) reported that chitosan enhanced filled grains per panicle through improved physiological efficiency, nutrient uptake, and assimilate partitioning. In this study, high temperatures (30–36 °C) during flowering likely caused spikelet degeneration and increased unfilled spikelets, limiting grain filling despite CNF application. Additional stress may have resulted from the use of black pots on concrete.

Thousand-Grain Weight

Thousand-grain weight did not differ significantly among treatments, although 0.4% CNF showed a numerically higher value (17.22 g). Similar trends were reported with chitosan, showing no significant changes in rice (Ahmed et al., 2020; Boonlertnirun et al., 2006, 2007), whereas Munshi (2015) observed significant increases under modified chitosan application. High temperatures during reproductive stages may have limited grain filling, preventing statistically significant differences among treatments.

Straw Weight and Total Biomass

Straw weight did not vary significantly; however, total biomass differed among treatments, with 0.4% CNF producing the highest total biomass (50.08 g) and the control the lowest (35.68 g, Table 6). This suggests that CNF can enhance vegetative growth even under stress. While straw yield is generally insensitive to chitosan (Ahmed et al., 2013, 2020), biomass production can respond to nitrogen availability (Alim, 2012; Hossain et al., 2008).

Grain Yield

Grain yield did not differ significantly among treatments, although 0.4% CNF produced a numerically higher yield (10.08 g hill^{-1}) and 0.2% CNF the lowest (5.94 g hill^{-1}, Table 6). Effects of chitosan on rice yield have been inconsistent, with some studies reporting no effect (Boonlertnirun et al., 2008) and others showing increases depending on application methods and environmental conditions (Ahmed et al., 2013, 2020; Boonreung & Boonlertnirun, 2013). In this study, low yields were likely caused by high-temperature stress during flowering, resulting in spikelet sterility and a high proportion of unfilled grains.

Harvest Index

Harvest index (HI) did not differ significantly, with 0.1% CNF producing the numerically highest HI (21.19%) and 0.2% CNF the lowest (14.72%, Table 6). Chitosan has been reported to significantly influence HI in rice and mung bean (Ahmed et al., 2020; Mondal et al., 2013). In this experiment, adverse weather conditions, particularly high temperatures and limited rainfall, likely constrained grain filling and reduced HI across treatments.

Weather Impact on Observed Traits

During the experimental period, most days were rainless, with rainfall ranging from 0–53 mm (average 1.83 mm). Minimum temperatures ranged from 12–29 °C (average 22.92 °C), and maximum temperatures from 23.5–39.3 °C (average 33.41 °C). Relative humidity ranged from 43–100% in the morning and 39–90% in the evening. The combination of high temperatures during flowering (30–36 °C), warm and humid conditions, and limited rainfall likely caused spikelet degeneration, poor grain filling, and low yields. Temperature-sensitive processes such as floral organ development and fertilization were impaired, resulting in a high number of unfilled grains per hill and low harvest indices. The use of black pots on concrete may have further amplified heat stress.

Although CNF treatments, particularly 0.4%, tended to improve numerical values of panicle length, filled grains, grain yield, and biomass, extreme weather conditions during the flowering and grain-filling stages limited statistical significance. Nevertheless, CNF shows potential to mitigate stress-induced yield losses and enhance rice growth under suboptimal conditions, warranting further investigation (Afroj et al., 2025; Shams et al., 2025).

Table 6: Effect of different CNF doses on yield and yield attributes of BRRI dhan28

Treatment	**Panicle Length (cm)**	**Effective No. of Panicle hill^{-1}**	**Total Filled Grain hill^{-1}**	**Total unfilled spikelet hill^{-1}**	**1000 grain weight (g)**	**Straw weight (g)**	**Grain yield (g)**	**Total biomass (g)**	**Harvest Index (%)**
Control	20.25	5.75	420.00	826.25	16.98	28.50	7.18	35.68b	20.04
0.1%CNF	19.73	12.25	485.00	763.75	17.20	30.75	8.60	39.35b	21.19
0.2% CNF	20.13	6.75	344.75	829.75	17.17	33.50	5.94	39.44b	14.72
0.4% CNF	21.05	9.50	580.50	985.50	17.22	40.00	10.08	50.08a	20.06
P value	0.365	0.171	0.696	0.663	0.974	0.064	0.708	0.003	0.876
CV (%)	2.72	34.16	21.85	11.10	0.6	15.08	22.49	15.11	15.29

Different letter in a column indicates statistical differences. CNF stands for chitin nanofiber, and CV for coefficient of variation.

Conclusion

The application of 0.4% chitin nanofiber (CNF) improved key physiological and yield-related traits of rice, including leaf chlorophyll content, leaf area, panicle length, filled grains, grain yield, and straw yield, even under adverse conditions such as heat stress during the grain-filling stage. These findings highlight the potential of CNF as a biostimulant to enhance rice productivity and support more sustainable cultivation practices by reducing dependence on chemical fertilizers. Further research is needed to optimize CNF application rates and timing, as well as to evaluate its effectiveness across different rice varieties and environmental conditions. Overall, this study provides a promising approach for improving rice production in Bangladesh and similar agroecological regions.

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Conflict of Interest

There is no conflict of interest.

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