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# Mixed Nanocomposite Fertilizers Influencing Endophytic Symbiosis and Nutritional and Antioxidant Properties of *Oryza sativa* as a Sustainable Alternative for Commercial Fertilizers

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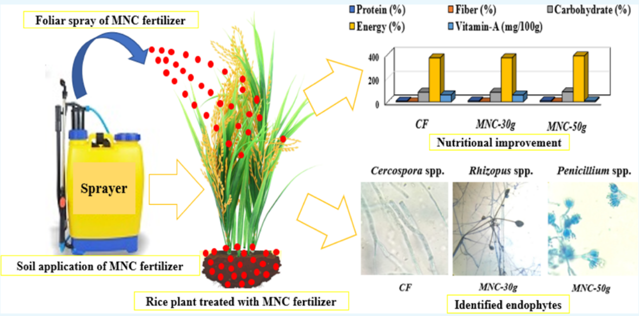

**ABSTRACT:** This study investigated the comparative effects of mixed nanocomposite (MNC) fertilizers as an alternative to commercial fertilizers (CFs) on endophytic symbiosis and nutritional properties of rice grains. We synthesized MNC fertilizers with different concentrations and characterized them by using scanning electron microscopy and Fourier transform infrared spectroscopy. The CF was applied as per the method followed by local farmers; however, for MNC fertilizers both foliar and soil applications were done. Comparative analysis of growth and development, rice−endophyte symbiosis, and nutritional properties of rice grains was conducted. The panicles per hill, length of panicles, grain per panicles, 1000-grain weight, and dry matter of rice plants treated with MNC fertilizers were found to be not statistically ($p > 0.05$) different compared to those of CF. However, growth parameters were significantly ($p < 0.05$) higher in MNC fertilizer-treated crops than in CF-treated crops. Several predominant endophytes such as *Penicillium* spp., *Aspergillus fumigatus*, *Rhizopus* spp., and *Fusarium* spp. that could have significant effects on the enhancement of growth and nutritional properties of rice grains were identified in rice plants treated with MNC fertilizers at different concentrations. Contrarily, stem-associated *Cercospora* spp. was found in the CF-treated field and fission yeast was observed in the blank-treated field. In addition, the contents of proteins, fibers, carbohydrates, energy-yielding components, vitamin A, and minerals were significantly increased in rice plants treated with MNC fertilizers. Thus, we would like to conclude that MNC fertilizers could be one of the most potential alternatives to CFs for achieving better rice−endophyte symbiosis as well as nutritional improvements in rice grains for sustainable production.

## 1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crops consumed by more than half of the world's population. At the same time, it is a staple food as well as a strategic asset for the national economy and food security in Bangladesh, where the annual per capita intake of rice is more than 170 kg.[1] To mitigate this huge rice consumption demand, farmers are using commercial fertilizers (CFs) indiscriminately because CFs play a pivotal role in improving the rice yield and quality. However, the increase of the crop yield is not linearly correlated to the increase of CF application rates.[2] This heavy reliance on CFs of modern agriculture has emerged as a significant threat to the agricultural production system, the environment, and the human health[3] It has been extensively documented that CFs have hazardous effects on humans, as well as on soil and water quality. Literature[4] show that excessive usage of N (nitrogen) fertilizers has resulted in a slew of environmental problems, including eutrophication of surface water, groundwater pollution, greenhouse gas emissions, and soil acidification. Besides, the increased fertilizer price increases the input cost by almost 20% in rice production,[5] which is the larger cost after labor. Therefore, alternative fertilizer management is crucial for minimizing environmental pollution in rice agroecosystems.

Nanotechnology is currently applied in modern agriculture extensively, which can improve the efficiency of agricultural inputs, and making nano fertilizers (NFs) an effective technology. Therefore, these NFs have been attracting great attention for the current agricultural practices. NFs are made by encapsulating plant nutrients in a thin layer of nanoparticles (NPs) and distributing them as nano-sized emulsions. There are various types of NPs (iron oxide NPs, zinc oxide NPs,

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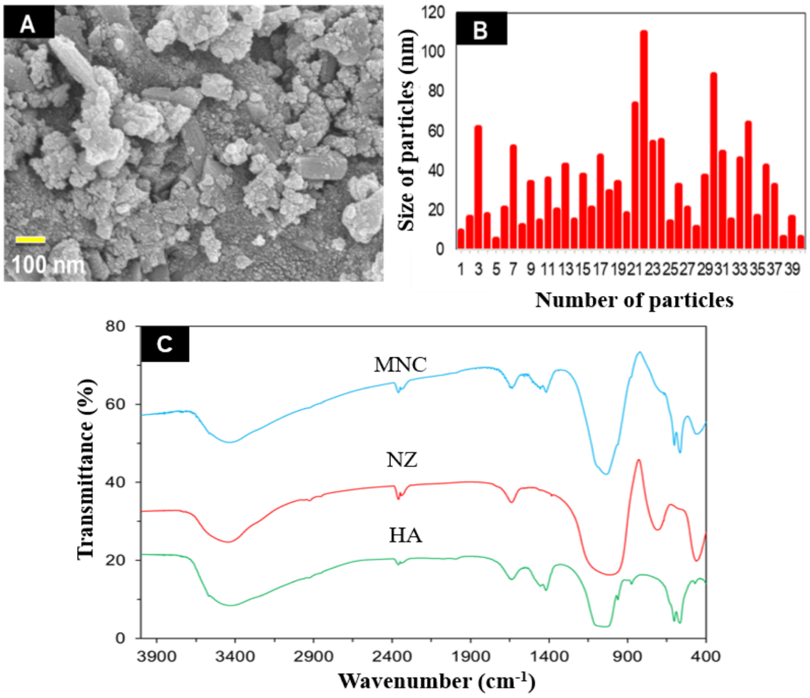

**Figure 1.** Structural characteristics of MNC fertilizers: (i) SEM image of the microstructure of the MNC fertilizer (A), (ii) particle size distribution histogram of the MNC fertilizer (B), and (iii) attenuated total reflectance (ATR)-FTIR spectra of HA, NZ, and MNC fertilizers (C). HA; hydroxyapatite, NZ; nanozeolite, and MNC; mixed nanocomposite.

silver oxide NPs, and titanium dioxide NPs) that are individually applied to enhance crop production. For example, Zn is necessary for stimulating the activity of enzymes, synthesis of tryptophan, cell division, maintenance of membrane structures, and photosynthesis, and it acts as a regulatory cofactor in protein synthesis. Iron (Fe) has also been discovered to play a vital role in plant development and metabolism, including chlorophyll synthesis, respiration, nitrogen fixation, reproduction, and shelf life extension, as it is the principal redox system of plants. Although Fe is an abundant element in soil, crops frequently suffer from Fe deficiency because it is not present in photoavailable forms. Thus, Fe deficiency in agricultural produce has also caused severe effects in human population. Similarly, copper (Cu) is another micronutrient required in minute amounts but essential for healthy plant growth and profitable crop production. Fe NPs also increased the stem length, yield, and Fe concentration in plants by 130 and 110%, respectively.[6] On the other hand, NPs range from 1 to 100 nm normally. However, the size of stomatal openings in rice leaves ranges from 17.1 to 25.6 $\mu$m, which is much larger than NPs. Therefore, plant leaves with nanopores and stomatal apertures enhance NF uptake and penetrate deep inside the leaves, resulting in improved nutrient use efficiency, leading to a higher productivity (6−17%) and nutritional quality of field crops.[7] Moreover, the hybrid NFs, nanozeolite (NZ)-based composite fertilizers, and mixed NFs (MNFs) were applied to okra (*Abelmoschus esculentus* L.), lettuce (*Lactuca sativa* L.), and tomato (*Solanum lycopersicum* L.) plants, respectively, and a potential impact on the growth, development, and nutritional values was found in our previous study.[8−10] Contrarily, microbes in soil, particularly mycorrhizal fungi, regulate the uptake of NFs by plants. They form a symbiotic association with the roots of plants and hence provide a better platform for the NF to get easily absorbed by the plants. However, understanding the holobiont ecology and association of beneficial microbes, viz., endophytes, with crop plants is crucial in modern eco-friendly agriculture as these could be affected by fertilizers. Non-pathogenic bacterial and fungal colonization, coevolution, and their intimate association with plant tissues are thus named endophytic relationship. These symbiotic relationships are yet to be explored but are already found to contribute to plants' nutrient acquisition, growth, and stress tolerance. Strategies for crop improvement by efficient uptake of soil nutrients and enhancing the yield should consider the use of endophytes as an emerging technology. In rice, endophytic fungi (e.g., *P. indica*) induce certain miRNA synthesis, which targets transcriptional activation of nutrient uptake, $Na^+$ transport, and auxin regulation, as well as upregulates genes responsible for stress tolerance.[11] In addition, many endophytes are claimed to control pests and diseases in plants. However, to the best of our knowledge, no study was found to investigate the effects of mixed nanocomposite (MNC) fertilizers on the physicochemical properties of rice and rice−endophyte symbiosis.

Therefore, the objectives of this present study were to investigate the effects of MNC fertilizers on the rice growth, development, and physicochemical properties of rice grains. Furthermore, the rice−endophyte association was extensively evaluated as mycorrhizal organisms having potential roles in NF uptake and translocation. We hypothesized that the application of MNCs as a potential alternative to CFs would increase the growth and grain quality of rice, enhancing the activity of the beneficial plant microbes (endophytes) as well as agroenvironments.

## 2. RESULTS AND DISCUSSION

**2.1. Characterization of MNC Fertilizers.** Scanning electron microscopy (SEM) analysis was used to investigate the microstructure of the MNC fertilizer, which is presented in Figure 1A. The average size of the MNC fertilizer ranges from

20 to 100 nm, which agrees well with the particle size distribution histogram (Figure 1B). In addition, Figure 1C shows the spectra of nanohydroxyapatite (HA), NZ, and MNC fertilizers. However, the Fourier transform infrared (FTIR) spectra of nano HA show characteristic absorption bands at wavelengths in the range 560−600 and 1000−1100 $\text{cm}^{-1}$ for bending and stretching vibrations of $PO_4^{3-}$ respectively.[12] The absorption peaks at 882 and 1432 $\text{cm}^{-1}$ were observed for $CO_3^{2-}$.[12,13] The FTIR spectra of NZ show characteristic absorption peaks at wavelengths in the range 900−1200 $\text{cm}^{-1}$ for bending and stretching vibrations of Al−O and Si−O, respectively, which confirmed the presence of zeolite.[14] Characteristic absorption bands of nano HA and NZ were found in the FTIR spectra of MNC fertilizers (HA/NZ/NPs), which reveal that HA successfully binds with NZ. Some additional absorption bands found in the MNC fertilizer spectra represent the presence of metal NPs.

**2.2. Effects of MNC Fertilizers on Chlorophyll Contents.** The effects of MNC fertilizers on the growth and development as well as chlorophyll contents of matured rice plants were studied and compared to those of CF. The results showed that the number of plants, the maximum height of plants, the maximum length of shoot, and the number of leaves per hill were significantly ($p < 0.05$) increased in the MNC-50g-treated rice field at 90 DAS (Table S1). In addition, the chlorophyll pigments in rice leaves were also significantly ($p < 0.05$) increased while the MNC fertilizer was applied (Figure 2). The Chl-*a* content was significantly higher than that of Chl-*b* in the MNC fertilizer-treated fields as compared to CF treatment, indicating that the rice plants are illuminated by the MNC fertilizer. Similarly, the total chlorophyll ($a + b$) and carotenoid contents were significantly ($p < 0.05$) increased with MNC fertilizer treatments as compared to CF treatment. This might be attributed to the balanced and controlled distribution of the MNC fertilizer, consisting of Fe, nano HA, and NZ that could have played a pivotal role in chlorophyll biosynthesis in rice plants. Similar findings were observed in grapefruit plants treated with a mixture of Cu and chelated Fe,[15] *Stipa tenacissima* treated with foliar application of Fe and Zn,[16] and tomato fruits treated with a MNF prepared using Fe, Cu, and Zn.[10]

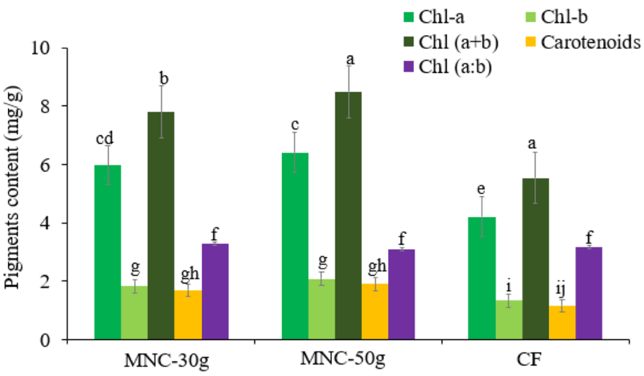

**Figure 2.** Photosynthetic pigments in rice leaves treated with MNC-30g, MNC-50g, and CF (CF; commercial fertilizer, MNC-30g; applied MNF was 30 g per time, MNC-50g; applied MNF was 50 g per time, Chl-*a*; chlorophyll *a*, Chl-*b*; chlorophyll *b*, Chl ($a + b$); total chlorophyll *a* and chlorophyll *b*, and Chl ($a/b$); the ratio of chlorophyll *a* and chlorophyll *b*). All different letters (a−j) on the error bars in the figure are significantly different ($p < 0.05$).

**2.3. Effects of MNC Fertilizers on Grain Quality.** The grain quality was estimated in terms of panicles per hill, length of panicles, grains per panicle, 1000-grain weight, and total production (Table 1). The field treated with a minute amount of MNC fertilizer showed very similar results to the CF-treated field in terms of panicles formation per hill, length of panicles, 1000-grain weight, and total production. It might be due to the proper uptake and balanced distribution of essentially required micronutrients supplied as MNC fertilizers: zeolite and HA increased the rice grain quality. These micronutrients greatly contributed to the plant's growth and development (Tables S1, S2), which could have enhanced the grain quality through rice−endophyte symbiosis (Figures 4, S1). A similar result was found with the application of ZnO NPs in wheat,[17] nanoCF in date palm,[18] and $TiO_2$ in rice.[19] In addition, grains per panicle were significantly higher in the CF-treated rice; however, the effects of MNC fertilizers on grain quality were non-significantly ($p > 0.05$) different except for grains/panicles (Table 1), indicating the MNC fertilizers as a suitable alternative to CF. Besides, the total rice production was lower in MNC fertilizer treated plants as compared to CF-treated ones; however, it was higher than the average rice production in Bangladesh.[20]

**Table 1. Comparative Effects of MNC Fertilizers with Different Concentrations and CF on the Grain Quality of Rice**[a]

| parameters | CF | MNC-30g | MNC-50g |
|---|---|---|---|
| panicles/hill | 13.60 ± 1.08a | 13.0 ± 1.15a | 13.40 ± 1.23a |
| length of panicles/hill (cm) | 25.26 ± 1.50a | 24.96 ± 1.70a | 25.24 ± 1.33a |
| grain/panicles | 215.2 ± 5.20a | 203.8 ± 5.56c | 208.2 ± 4.35b |
| 1000-grain weight (g) | 21.62 ± 0.87a | 21.18 ± 0.95a | 21.46 ± 0.96a |
| total production (t/ha) | 5.267 ± 1.05a | 3.278 ± 1.10c | 4.354 ± 1.35b |

[a] Values are expressed as means ($n = 5$) ± standard deviation. All similar subscript letters in the same column are non-significantly different ($p > 0.05$). CF; commercial fertilizer, MNC-30g; applied MNC fertilizer was 30 g per application time, and MNC-50g; applied MNC fertilizer was 50 g per application time.

**2.4. Effects of MNC Fertilizers on Nutritional Properties of Rice.** *2.4.1. Effects on the Proximate Composition of Rice.* The proximate nutritional composition of rice grain is shown in Table 2. The results showed that the protein, fiber, carbohydrate, and energy contents of rice treated with the MNC-50g fertilizer were significantly ($p < 0.05$) increased as compared to those of both MNC-30g fertilizer- and CF-treated rice plots. It might be due to the fortified Zn and Fe micronutrients supplied by the MNC fertilizer including NZ and HA that might have regulated the biochemical mechanisms including protein synthesis, enzyme activation, and nucleic acid metabolism.[10] An increased total protein content was also observed in coriander treated with $TiO_2$-NPs by Pošćić et al.[21] However, the ash and fat contents were non-significantly different for all treatments. It is worth mentioning that with the increase of the concentration of the MNC fertilizer, all the parameters were increased except moisture. A similar agreement was found for the application of Zn as a nutrient in wheat plants.[22]

*2.4.2. Effects on Mineral and Vitamin A Contents.* The comparative effects of MNC fertilizers (MNC-30g and MNC-

Table 2. Comparative Effects of MNC Fertilizers with Different Concentrations and CF on the Proximate Nutritional Compositions of Rice Grains[a]

| | proximate composition of rice (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| treatments | moisture | ash | protein | fiber | fat | carbohydrate | energy (kcal//100 g) |
| CF | 8.0 ± 0.8a | 1.2 ± 0.2a | 6.7 ± 0.83b | 0.73 ± 0.02b | 1.7 ± 0.23a | 82.4 ± 1.23b | 371.6 ± 1.79c |
| MNC-30g | 7.8 ± 0.7b | 1.1 ± 0.1a | 7.9 ± 0.65b | 0.25 ± 0.01c | 1.6 ± 0.13a | 81.6 ± 1.06c | 372.2 ± 1.96b |
| MNC-50g | 3.6 ± 0.4c | 1.0 ± 0.1a | 8.7 ± 0.68a | 0.96 ± 0.04a | 1.5 ± 0.10a | 85.0 ± 1.02a | 388.1 ± 1.84a |

[a] Values are expressed as means ($n = 5$) ± standard deviation. All similar subscript letters in the same column are non-significantly different ($p > 0.05$). CF; commercial fertilizer, MNC-30g; applied MNC fertilizer was 30 g per application time, MNC-50g; applied MNC fertilizer was 50 g per application time.

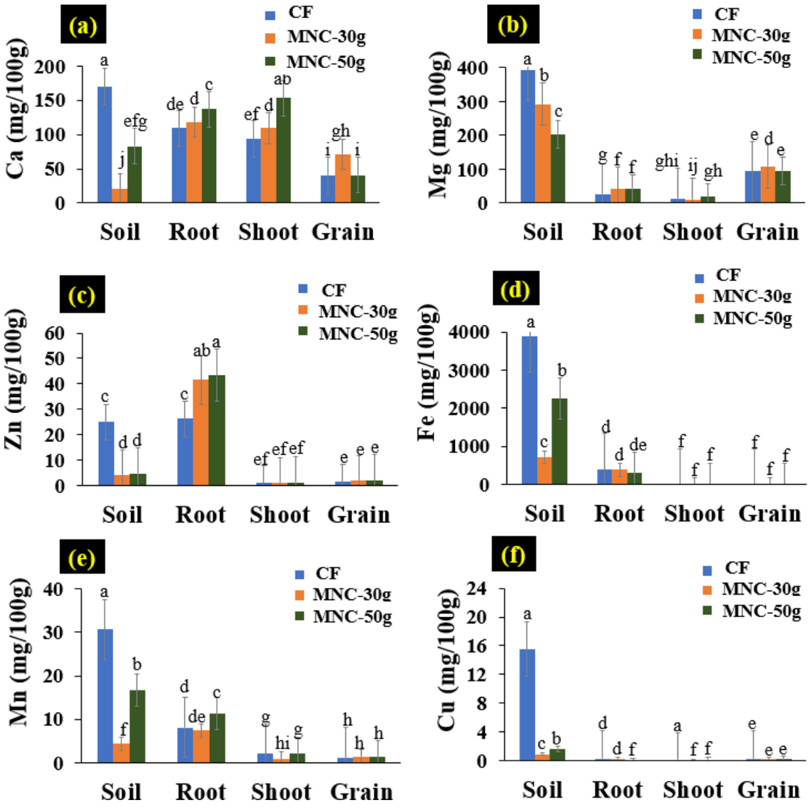

Figure 3. Essential micronutrients (mg/100 g) detected: Ca (a), Mg (b), Zn (c), Fe (d), Mn (e), and Cu (f), from different parts of the rice plant (root, shoot, and grain) and soil treated with CF, MNC-30g (applied MNC fertilizer was 30 g per application time), and MNC-50g (applied MNC fertilizer was 50 g per application time). All different letters (a−j) on the error bars in the figure are significantly different ($p < 0.05$).

Table 3. Comparative Effects of MNC Fertilizers with Different Concentrations and CF on Antioxidant Properties of Rice Grains[a]

| | | antioxidant compositions | | |
|---|---|---|---|---|
| treatments | vitamin A (μg/100 g) | TPC (mg GAE/100 g) | TFs (mg/100 g) | tannins (mg/100 g |
| CF | 59.5 ± 3.27a | 8.03 ± 0.91a | 1.91 ± 0.39a | 34.7 ± 1.02bc |
| MNC-30g | 58.1 ± 3.68ab | 7.85 ± 0.96a | 1.22 ± 0.28ab | 35.2 ± 1.10ab |
| MNC-50g | 8.7 ± 1.25c | 7.39 ± 0.87a | 1.81 ± 0.0.56a | 36.6 ± 1.23a |

[a] Values are expressed as the means ($n = 5$) ± standard deviation. All similar subscript letters in the same column are non-significantly different ($p > 0.05$). CF; commercial fertilizer, MNC-30g; applied MNC fertilizer was 30 g per application time, MNC-50g; applied MNC fertilizer was 50 g per application time.

50g) and CF on different mineral contents in different parts of the rice plant are shown in Figure 3. There are six essential micronutrients such as Ca, Mg, Mn, Zn, Fe, and Cu presented here. Figure 3 illustrates that the mineral contents in the CF-treated soil were significantly ($p < 0.05$) higher than those in the MNC fertilizer- treated soil. It explains the availability of micronutrients in the soil. However, the MNC-50g fertilizer-treated soil showed a higher micronutrient content than MNC-30g fertilizer-treated soil. The micronutrient uptaken by root was significantly higher in the MNC-50g fertilizer-treated rice plot than in the CF-treated field except for Fe and Cu. This result indicated that the MNC has been uptaken by the plants' roots easily due to their nanosize and higher surface ratio, and most of them were plant-available form into the soil. Similarly,

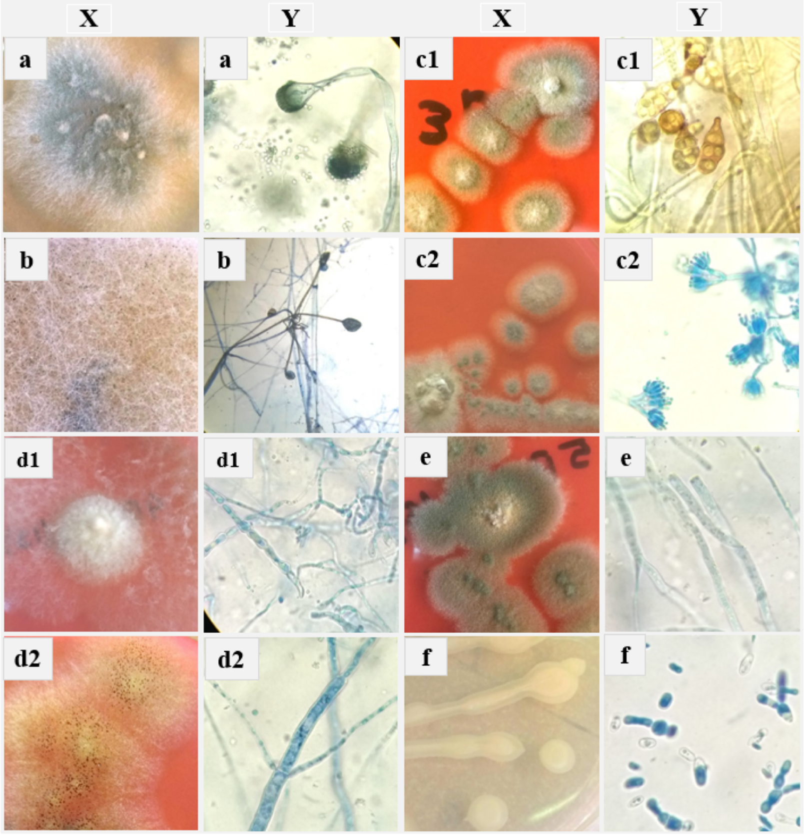

**Figure 4.** Identified fungal genera with colony (*X*) and morphological features (*Y*) under MNC fertilizer treatments with different concentrations: treatment 1 (15 g NC fertilizer): *Aspergillus* spp. (a), treatment 2 (30 g NC fertilizer): *Rhizopus* spp. (b), treatment 3 (50 g NC fertilizer): *Alternaria* spp. (c1), *Penicillium* spp. (c2), treatment 4 (30 g NC fertilizer applied into the soil): *Fusarium* spp. (d1), *Rhizopus* spp. (d2), treatment 5 (urea: 700 g and Zn: 500 g as traditional hand-spray): *Cercospora* spp. (e), and treatment 6 (no fertilizer, blank plot): fission yeast (f).

rice shoots treated with MNC-50g have uptaken comparatively higher Ca, Mg, Mn, and Zn contents than the CF-treated rice. However, all rice grains treated with MNC fertilizers contained very minute mineral contents, which are not lower than those of the CF-treated rice grains. This proves that the drastic use of CF does not significantly affect the mineral contents in rice grains. However, a minute amount of MNC fertilizers give almost equal contents of minerals for rice cultivation. Similarly, the vitamin A content was significantly ($p < 0.05$) increased with the application of a lower concentration of MNC fertilizers (MNC-30g fertilizer-treated field), which was non-significantly different as compared to CF-treated rice grains (Table 3). The improved mineral content was also observed by Poščić et al.,[21] who found that Zn and Mn concentrations were improved in barley kernels treated with nano-$TiO_2$.

*2.4.3. Effects on Antioxidant Properties of Rice.* The total phenolic content (TPC), total flavonoids (TF), and tannins (T) of rice grains treated with MNC fertilizers were determined and are shown in Table 3. All antioxidant compounds found in the MNC fertilizer-treated rice were statistically non-significant ($p > 0.05$) as compared to CF-treated rice. These findings imply that a minute amount of MNC fertilizers can maintain the antioxidant properties of rice grains as a sustainable alternative to CF. It might be due to the supplementation of combined essential micronutrients as the MNC fertilizer, which could have a potential impact on the regulation of bioactive compounds in rice grains.[10] It is worth mentioning that the concentrations of MNC fertilizers used in this study did not have a significant effect on antioxidant properties. Also, this finding is in agreement with Zhu et al.,[23] who observed that the foliar application of Se increased the bioactive compounds such as flavonoids, glutathione, vitamin C, and vitamin E in pink tomato fruits.

**2.5. Rice−Endophyte Symbiosis.** The effects of treatment with MNC fertilizers with different concentrations and CF on rice−endophyte symbiosis were extensively studied, and the results are presented in Figure 4. The experimental results showed that *Penicillium* spp., *Aspergillus fumigatus*, and *Fusarium* spp. as predominant endophytes of rice plants (mainly seed- and stem-associated) are available in the MNC fertilizer-treated fields (Figure S1). Numerous fungal metabolites produced by *Aspergillus* spp., endophytic fungi have already been reported.[24] Some of them are potent antifungal agents and inhibit the growth of phytopathogenic fungi. *Fusarium* spp. produces bioactive molecules, some of which are biocontrol agents. The genus *Penicillium* has more than 200 species and is well known as a source of antibiotics.[25] In addition to traditional antibiotic potential, endophytic *Penicillium* species produce hundreds of compounds exhibiting antimicrobial, antiviral, antioxidant, antiparasitic, insecticidal, and biocontrol activities. We found *Rhizopus* spp. to be both stem- and seed-associated in rice plants (Figure S1), which received 30 g of MNC fertilizer (Figure 4b,d). This particular observation made us believe that the type and amount of MNC fertilizer select the endophytic population. *Rhizopus oryzae* isolates produce plant growth-promoting phenolics, flavonoids, salicylic acid, and indole 3-acetic acid and show a significant growth enhancement in rice seedlings.[26] These secondary metabolites also induce the transcription of several antistress genes. Rice plants receiving treatment 3 (50 g MNC fertilizer) also showed *Alternaria* spp. as endophytes (Figure 4c1). However, in our rice plants, no symptoms of pathology were found. Dalinova et al.[27] reported hundreds of metabolites of *Alternaria* spp. So far, most of them were reported to have potential antibiotic, antifungal, and antiprotozoal activities that influence the healthy growth of rice plants and rice grains (Table 1). In the rice plants receiving traditional CF (treatment 5, Figure S1), stem-associated *Cercospora* spp. were identified (Figure 4e). Although *Cercospora* spp. are relatively well-studied phytopathogenic fungi causing cercosporin spots on leaves, any sort of disease spots was not observed in the field. There are countless species of these genera that are not yet described. It might have any endophytic benefits to plants, especially when the association was found to be non-pathogenic or asymptomatic. From the rice plants in the field, where no fertilizers were used, only a fission yeast associated with seed was isolated (Figure 4f).

## 3. CONCLUSIONS

As a potential alternative to CFs for rice cultivation, MNC fertilizers with two different concentrations were studied in terms of rice−endophyte symbiosis, biochemical properties, and growth and development of rice plants. The obtained findings indicated that the application of MNC fertilizers is an efficient and effective fertilization management for significantly ($p < 0.05$) increasing the beneficial endophytes (*Penicillium* spp., *A. fumigatus*, *Rhizopus* spp., and *Fusarium* sp.), while the CF-treated rice field showed the stem-associated *Cercospora* spp., which are phytopathogenic fungi causing cercosporin leaf spots in rice leaves; however, no disease spots were observed in the blank-treated field. In addition, the overall growth parameters were significantly ($p < 0.05$) increased in the MNC-50g-treated field, suggesting the potential role of endophytic symbiosis in rice plants. Similarly, the nutritional properties of rice grains were also significantly increased in MNC fertilizer-treated rice grains; however, their antioxidant compositions were non-significantly different as compared to CF. In conclusion, MNC fertilizers could be one of the most effective and potential alternatives to CF, for sustainable rice production.

## 4. MATERIALS AND METHODS

### 4.1. Selected Land, Chemicals, and Rice Variety.

The experiment was conducted from January to April 2021 in the field just beside the north side of Jashore University of Science and Technology, Bangladesh. The field is at 23° 10′ North latitude and 89° 13′ East longitude at a height of 9 m above the sea level, according to the global positioning system (GPS). It belongs to the Gopalganj−Khulna Beels in the agro-ecological zone (AEZ) no. 14. The research field is situated in a subtropical climatic zone having an average annual temperature range from 15.4 to 34.6 °C, an average annual rainfall of 1651 mm, and an average annual relative humidity of 57%. The soil of the experimental field is medium-high to high land with silt or silt-clay loam (Table 4). All other chemicals and reagents required for the preparation of the MNC and nutritional analysis of rice grains were purchased from Sigma-Aldrich, China, and were of analytical grade. IRRI rather than-63 rice cultivar was used in this experiment as a rice variety.

**Table 4. Soil Chemical Properties of the Selected Land for Rice Cultivation**

| soil properties | amount |
|---|---|
| electric conductivity (dS/m) | 7.38 |
| pH | 0.79 |
| total N (%) | 0.15 |
| P (μg/g) | 154.5 |
| K ($\mathrm{Cmol_ck/g}$) | 0.37 |
| S (μg/g) | 14.87 |
| Na ($\mathrm{Cmol_ck/g}$) | 0.114 |
| organic matter (%) | 1.66 |

### 4.2. Preparation and Characterization of MNC.

To prepare Zn NPs, 10 g of NaOH (1.0 M) was dissolved in 250 mL of ultrapure water and stirred at 90 °C. Then, 17.0358 g of $ZnCl_2$ (0.5 M) solution was prepared and kept in a burette. The solution was then dropwise added into the NaOH solution for 26 min and continuously stirred for 2 h at 90 °C. The obtained solution was kept overnight to be settled down from the precipitate. The collected suspension was washed with absolute ethanol and ultrapure water several times to remove the unreacted molecules. Similarly, Fe and Cu NPs were prepared by the chemical reduction method using $FeCl_2$ and $CuCl_2$, respectively.[8] Similarly, NZ and HA were also prepared according to our previous study.[8,9] The above-prepared and synthesized fertilizers were homogenously mixed together. Then, SEM analysis was performed to gain insights into the MNC fertilizer. In addition, to confirm the interaction of different NFs (HA, NZ, and NPs), ATR-FTIR spectrum analysis was carried out by using a Nicolet iS20 ATR-FTIR spectrometer.

### 4.3. Agronomic Practices and Management.

The cultivated land was plowed well to get fine tilths. Then, the 30 day old, healthy, and vigorous rice seedlings were transplanted into every bed with an equal area of 100 × 33 $ft^2$. The plant density was 4 plants/$ft^2$. Standard management practices such as intercultural operations such as weeding, watering, and insecticide applications were done as per traditional methods. The whole area was divided into three for treatment: (1) CF-treated field (2.2 kg CF/application interval), (2) MNC-30g-treated field (NZ: 10 g, HA: 10 g, Fe: 2 g, Cu: 2 g, Zn: 2 g, and salts: 4 g), and (3) MNC-50g-treated field (NZ: 10 g, HA: 20 g, Fe: 5 g, Cu: 5 g, Zn: 5 g, and salts: 5 g).

*4.3.1. Application of MNC and CF Fertilizers.* The prepared 30 and 50 g of MNC fertilizers were each diluted with 8 L water in two different buckets. Then, half of each solution was applied to leaves with a foliar fertilizer sprayer pot and the rest half was applied to the soil every time of fertilizer application. However, the foliar spray of the MNC was done for the first two times during the month of sowing. The third foliar application of MNC was done at the time of panicle initiation of rice plants, and the fourth and final applications were performed when the panicles of rice came out. However, the CF was applied to the CF-treated field as per the traditional method with a rate of 2.2 kg/application at the same date of foliar application of the MNC.

### 4.5. Rice−Endophyte Symbiosis Analysis.

Healthy indigenous rice plant varieties popularly grown in Bangladesh were sampled on March 3, 2021 from the pilot study field at Jashore, Bangladesh (GPS position). The whole rice plant was sampled during the late-ripening phase of growth before harvesting. The freshly collected samples were brought to the laboratory in a sterile package system and washed with autoclaved distilled water with 0.1% Tween 20 (Sigma-Aldrich). Large portions of different plant parts (leaves, stem, root, and seeds) are cut and surface-sterilized by dipping in 5% hypochlorite solutions (Merck, Germany) and then rinsed thoroughly with plenty of autoclaved distilled water. The parts were then dipped in 70% ethanol and allowed to dry in a laminar flow system. The large portions were cut into smaller pieces, the outer edge portions were removed maintaining aseptic conditions under laminar flow, and inoculated on Sabouraud dextrose agar medium (Oxoid, UK). The plates were incubated at 30 ± 2 °C for 2 weeks with daily monitoring. The endophytic fungal growth on media was subcultured further for isolation of pure colony. Growth characteristics such as mycelium type, color, and sporulation were observed and recorded. Microscopic identification of the hypha type and arrangement, fruiting body, and conidiophores was performed using a light microscope (magnification 1000×), followed by staining with lactophenol cotton blue.

### 4.6. Nutritional Analysis of Paddy.

*4.6.1. Major Nutrients and Energy-Yielding Components.* The major proximate components moisture, protein, fat, ash, fiber, and carbohydrate were determined by following the official methods of analysis followed by Ojo et al.[28] The contents of energy-yielding components such as protein, fat, and carbohydrate were multiplied with respective water conversion factors to derive the total energy potential in terms of kcal/100 g of the rice samples.

*4.6.2. Vitamin and Mineral Determination.* The vitamin A content of rice samples was determined following the procedures described by Awolu.[29] However, the minerals contained in the soil, root, shoot, and rice grains were determined using an inductively coupled plasma atomic emission spectrometer (model, Trilogy-7).[30] The results were expressed as mg/100 g of samples.

*4.6.3. Determination of Antioxidants.* The TPC and the total tannin content (TTC) were measured using the Folin–Ciocalteu assay previously reported by Bao et al.,[31] with a slight modification. The TPC and TTC results were, respectively, expressed as milligrams of gallic acid equivalent (GAE) and milligrams of tannic acid equivalent (TAE) per 100 g of paddy sample. In addition, the TF content (TFC) was also measured following the method previously reported by Alenazi et al.,[32] with a slight modification. The TFC of the extracts (mg QE/100 g) was estimated by comparing their concentration against the standard curve. All samples were determined as triplicate.

**4.7. Statistical Analysis.** The obtained data were the mean values of three replications that were statistically analyzed and scientifically presented. The significance of the differences was estimated and compared using the Duncan test at a 5% level of probability ($p < 0.05$). Finally, all statistical analyses were carried out using the "SPSS version 20" computer software package (2016).

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c06395.

Detailed information on the growth and development of rice plants as well as endophytes identified from the rice field treated with MNC fertilizers (PDF)

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### Notes

The authors declare no competing financial interest.

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