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Article

Nano-sulfides of Fe and Mn Efficiently Augmented the Growth, Antioxidant Defense System, and Metal Assimilation in Rice Seedlings

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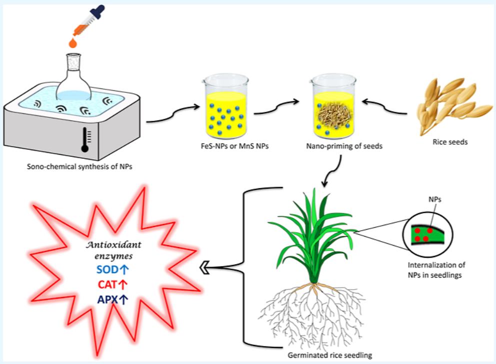
ABSTRACT: Physiological and biochemical mechanisms behind nanoparticle (NP)-induced seed germination by nanopriming with metal sulfide NPs are lacunae in the field of agriculture. Sonochemically synthesized aqua-dispersed ferrous sulfide NPs (FeS-NPs) and manganese sulfide NPs (MnS-NPs) were examined as nanopriming agents for physiological, pathological, and antioxidative defense parameters of rice in the present study. Under pot house conditions, in vivo nanopriming of rice seeds with FeS NPs and MnS-NPs at a concentration of 35 μg/mL for 8 h significantly improved the physiological parameters, viz., germination percentage, seed germination index, mean germination time, dry weight, and vigor index, and decreased the phytopathological parameters of nanoprimed rice seeds, viz., mortality, seed rot, and seedling blight. Stimulation of superoxide dismutase (SOD $\geq$ 28.16%), ascorbate peroxidase (APX $\geq$ 52.38%), and catalase (CAT $\geq$ 28.57%) enzymes in FeS-NP- and MnS-NP-nanoprimed seeds as compared to control (hydroprimed seeds) enhanced the fitness of rice seedlings. The augmented levels of Fe and Mn content in the shoots and roots of NP-treated seedlings as compared to hydroprimed seedlings confirmed the incorporation nanometals in rice seedlings as nanonutrients for effective plant growth. Inclusively, FeS-NPs and MnS-NPs were shown to be effective nanopriming agents for promoting the germination of naturally fungal infested rice seeds.



INTRODUCTION

Quick seed germination is necessary for augmented growth, successful plant establishment, and crop yield. The seeds are usually held for longer period, get aged, and degrade by various biotic and abiotic stresses that relay a cascade of events of their further oxidative damage, resulting in loss of their vitality and vigor.[1] Invigoration of seeds is an important trait that encompasses aging tolerance, viability, rapid germination, and seedling establishment.[2] These stresses can be circumvented by using seed enhancement techniques, viz., seed invigoration (priming), coating, hardening, soaking, and pelleting.

Seed priming is the practice of partly hydrating seeds in natural or synthetic composites in the definite setting until a spot at which germination-related metabolic activities are activated, but radicle has not yet emerged.[3] Priming agents have different characteristics and efficiencies, and therefore, optimization of the priming solution is essential for different plant species. Recently, the technique of seed priming with synthetic nanoparticles (NPs), typically called "nanopriming", has also been gaining importance in improving desired traits in crops.[4] Several NPs including silver NPs, gold NPs, copper NPs, titanium oxide NPs, Fe_2O_3 NPs, and carbon-based NMs have already been used as nanopriming agents.[5,6] NPs such as Ag, Zn, Fe, Mn, and Cu have also been used as pre-sowing agents that are testified to be supportive, where seeds are sown directly following the application lacking a preceding drying procedure.[4,7−12]

Metal NPs are often extremely reactive due to their high specific surface area, promoting catalytic activity and positive impacts on germination of seeds as compared to bulk equivalent molecules.[13] Seed priming with Fe-NPs was reported to be safer as compared to their bulk counterparts and had no toxic impact on seed germination, seedling development, and chlorophyll biosynthesis in watermelon seedlings.[14] Seeds primed with bulk ZnO did not endorse the beneficial effects in corn seed germination and seedling development.[15] Nanopriming of infested rice seeds with nano-ZnS (150 μg/mL) showed the invigorating effect on seeds by boosting the growth of seedlings as compared to bulk ZnS (150 μg/mL).[7]

Metal sulfides are of great interest to agriculturalists and environmentalists owing to their low nanotoxicological concerns.[7] Moreover, metal sulfides are water insoluble, and

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the dispersion of insoluble bioactive materials in water is also required to reduce the toxicity and percolation of materials into the groundwater.[8] Iron is an important trace element inflicting a vital role in bioinorganic chemistry as heme and Fe−S proteins playing crucial roles in nitrogen fixation, photosynthesis, and sulfur assimilation.[16] The iron requirement of rice (*Oryza sativa* L.) is greater than that of other plants, and iron deficiency is a common disorder of rice growing on well-drained (aerobic) soils, whether neutral, calcareous, or alkaline. Iron sulfide is a benign, natural, and nonhazardous material and less explored for remediation of agri-problems.[17] Efforts by our group have reported that the preparation, characterization, and application of FeS-NPs had a significant effect on the germination and vigor index of rice seeds.[9,10] The foliar applications of nano-FeS were also reported with better yielding effects.[18,19] Analogous results of FeS_2 by seed and root treatment have also shown promising results.[20−22]

Manganese(II) sulfide, a nontoxic, essential nutrient that is stable and sparingly water soluble, can be used as a safer material in bioapplications.[10] Mn-NPs have been reported as nanofertilizers that increased lettuce yield without causing toxicity at high concentrations as high as 50 ppm in a few reports of manganese nanoform.[23] Application of Mn NPs efficiently augmented nitrogen uptake, assimilation, and metabolism in mung bean plants and promoted crop management by bio-safe NPs.[24] The biochemical mechanisms behind NP-induced seed germination by nanopriming with metal sulfide NPs are needed to be explored in the agricultural field. The present study aims at synthesizing FeS-NPs and MnS-NPs using an environmentally friendly method and subsequently investigating its role and effectiveness as a seed priming agent. The growth promotion activity as a nanopriming agent (biophysical and biochemical parameters) was estimated and compared with that as a conventional priming agent.

■ RESULTS AND DISCUSSION

Morphological and Structural Details of FeS-NPs and MnS-NSs. The production of tiny, distorted round-shaped, slightly agglomerated NPs with an average size of 11.45 ± 1.07 nm was confirmed by a TEM nanograph of FeS-NPs (Figure S1).[9] Zeta potential analysis of the FeS-NPs exhibited a potential value of −6.5 mV (Figure S2a). The negative charge on the surface of NPs can be attributed to the presence of the PVP group as a capping agent over the NPs.[25] The NPs are said to be stable and highly charged if the potential exists in the range of +30 to −30 mV.[26] The broad surface plasma resonance band with absorption maxima at 285 nm in the UV−visible spectrum of FeS-NPs supported the existence of the nanoform of iron sulfide (Figure S2b).[9]

The morphological details of MnS-NPs by TEM revealed the formation of slightly aggregated and distorted spherical to semispherical shaped NPs with an average size of 56.26 ± 4.5 nm (Figure S3).[10] The negative zeta potential value of −5.2 mV represents the better stability index of the MnS-NP solution (Figure S4a). The optical absorption maxima analyzed by UV−visible spectroscopy was observed at 314 nm attributed to the formation of nano-MnS (Figure S4b).[10]

Physiochemical Characteristics of Loamy Sand Soil Used in a Pot House Study. The physicochemical characteristics of soil were determined by standard protocols and showed that the pH of loamy sand soil was 6.98 with an electrical conductivity (EC) value of 0.18 dS m^{-1}. The organic carbon (OC) content of the tested soil was 0.46% with available N, P, and K contents of 68.30, 12.58, and 116.60 kg ha^{-1}, respectively. The mechanical composition of sand, silt, and clay in loamy sand soil consists of 87.13, 2.31, and 10.56%, respectively. DTPA-extractable Fe and Mn were 16 and 8 mg kg^{-1} soil, respectively.

Impression of Nanopriming on Seed Quality Parameters of Rice Seeds. Eight hours of priming with FeS-NPs (35 μg/mL) and MnS-NSs (35 μg/mL) favorably affected the germination of rice seeds sown in pots. The dry weight and vigor index of seedlings were also affected positively. The germination percentage was increased with the DAS (days after sowing) and significantly affected by the type of the priming agent. After 16 days of sowing, the germination percentage was found to be highest in FeS-NP-primed seeds (91%) (Table 1). The germination percentage in priming treatments with MnS-NPs (86%) and captan (85%) was statistically similar and superior to hydropriming treatment (77%). The seed germination index showed a statistically different value for the FeS-NP (67.07)- and MnS-NP (61.80)-primed seeds and a significantly higher value than that of captan (60.31) and hydroprimed seeds (54.58). The mean germination time showed statistically similar results in all the priming treatments but significantly higher than control (hydropriming). The dry weight of FeS-NP (0.1421 g)-treated seedlings was significantly different and higher than rest of the treatments, but the dry weight of MnS-NP (0.1391 g)-primed seedlings was on par with that of captan (0.1336 g) and higher than hydropriming (0.1045 g) treatments. Notably, the overall seedling quality vigor index of FeS-NP-primed seedlings was recorded to be 12.93, which was much higher than that of MnS-NPs (11.96), captan (11.35), and hydropriming (8.04).

These results are in line with those of Ahuja et al. (2019)[9] and Ahuja et al. (2020),[10] which showed that the application of FeS-NPs and MnS-NPs had a significant effect on the germination and vigor index of rice seeds using the rolled paper towel method. The enhanced germination and dry weight in FeS-NP- and MnS-NP-primed seeds may be attributed to the absorption and utilization of metallic NPs by seeds which are covered in more detail later in this work. Invigoration of rice seeds on nanopriming with FeS-NPs and MnS-NPs demonstrated the nutritional modulation by enhanced uptake of nanoforms of iron and manganese. The enhanced germination of chick pea by priming the seeds with FeS_2 NPs (80 μg/mL) provided the better uptake of iron as an essential nutrient.[21] A significant increase in the growth of rice seedlings and seedling vigor was reported on nanopriming with 20 mg/L of zero-valent iron NPs (nZVI).[27] Similar results were found in peanut seeds, where seedling growth was increased significantly in plants treated with 2−5 mg/L of the nZVI solution.[28] Mn-NPs were reported as a better nutrient source in mung bean plants than commercial $MnSO_4$ for the sustainable agricultural productivity.[24] The reports on the concentration-dependent effect of green-synthesized MnO-NPs in watermelon seedlings recommended them as a safer seed priming agent to enhance agricultural crop production.[29]

In the case of rice seeds, various metal sulfide NPs have been explored as nanopriming agents invigorating the seed potential. In addition to MnS and FeS nanoforms, CuS NPs, ZnS NPs, and zinc-coated ZnS NPs have boosted the vitality of rice seeds during post-priming germination.[5,7,8] The results pertain to alleviating the biotic stress on the fungal infested seeds by acting as mycotoxic agents.

Effect of Seed Nanopriming on Disease Parameters of Rice. FeS-NPs and MnS-NPs successfully inhibited the mycelial growth of fungi in vitro;[9,10] thus, their ED_{90} concentration values were chosen, and their efficacy in pots under pot house conditions was assessed. In the present study, both FeS-NPs and MnS-NPs were effective in the reduction of the disease parameters of germinated rice seeds. The minimum occurrence of disease of seed rot (8.10 ± 0.31%) and seedling blight (3.20 ± 0.45%) was recorded at 35 μg/mL concentration of FeS-NPs (Figure 1). Similarly, low disease parameters were recorded when we used 35 μg/mL concentration of MnS-NPs, i.e., seed rot (11.15 ± 0.22%) and seedling blight (4.35 ± 0.35%). The seed rot and seedling blight in NP-primed seedlings were at par with the standard fungicide captan and significantly lowered than the hydroprimed treatment (control). Both FeS-NPs and MnS-NPs have strong ability to minimize the mortality rate in comparison to standard fungicide (captan) and hydropriming. The reduction in disease parameters was inferred due to the good antifungal activity of nanoforms of FeS and MnS as reported by Ahuja et al. (2019)[9] and Ahuja et al. (2020).[10] At the molecular biology scale, metal sulfide NPs interact better with sulfur-containing fungal proteins and other biomolecules by common ligand adsorptions through colloidal interfaces, endorsing the role of NPs on disrupting the external architecture of fungal hyphal and finally leading to fungal death to lower down the disease parameters.[7,30] The decrease in disease parameters in the present case can be hypothesized as the nanometals cause the disturbance in fungal hyphal wall composition by inhibition of vital enzymes involved in fungal cell wall synthesis. These NPs could be utilized as an effective and economical nano-alternative for mitigating myco-induced seed disease parameters.

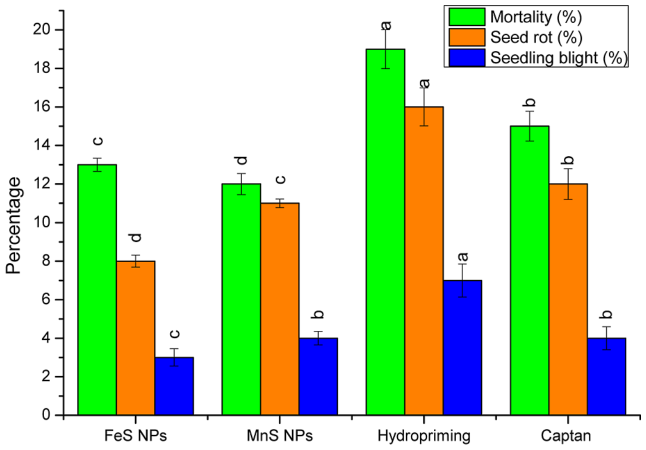

Figure 1. Comparative evaluation of FeS NP and MnS NP nanopriming on the disease parameters of rice with respect to captan (standard fungicide) and hydroprimed seeds (control). Each value represents the mean ± SD (standard deviation) (n = 3). Different lowercase letters indicate the significant difference among different treatments, followed by Tukey's test at $P < 0.05$.

Assessment of Antioxidant Enzyme Activity in Nanoprimed Rice Seeds. The antioxidant enzymatic [stimulation of superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT)] activities in primed seeds fluctuated

Table 1. Effect of Nanopriming of FeS-NPs and MnS-NPs on Germination Parameters of Rice[a]

treatment	percent germination (days after sowing)								germination index[b]	mean germination time (days)[c]	dry weight (g)	vigor index[d]
	2	4	6	8	10	12	14	16				
FeS-NPs (35 μg/mL)	18 ± 1^{d}	38 ± 4^{d}	70 ± 2^{c}	74 ± 2^{c}	82 ± 1^{d}	88 ± 1^{c}	90 ± 1^{c}	91 ± 3^{c}	67.06 ± 1.02^{c}	5.36 ± 0.25^{b}	0.1421 ± 0.001^{d}	12.93 ± 0.59^{c}
MnS-NPs (35 μg/mL)	15 ± 4^{b}	36 ± 2^{c}	64 ± 2^{b}	70 ± 1^{b}	77 ± 2^{c}	80 ± 2^{b}	86 ± 1^{b}	86 ± 3^{b}	61.80 ± 1.41^{b}	5.20 ± 0.12^{b}	0.1391 ± 0.020^{c}	11.96 ± 0.12^{b}
hydropriming	14 ± 3^{a}	26 ± 3 ^{a}	60 ± 2^{a}	65 ± 3^{a}	68 ± 1^{a}	71 ± 2^{a}	76 ± 2^{a}	77 ± 3^{a}	54.58 ± 0.92^{a}	4.88 ± 0.35^{a}	0.1045 ± 0.009^{a}	8.04 ± 1.25^{a}
captan	16 ± 2^{b}	30 ± 3 ^{b}	65 ± 3^{b}	68 ± 2^{a}	75 ± 1^{b}	80 ± 1^{b}	84 ± 1^{b}	85 ± 2^{b}	60.31 ± 1.95^{b}	5.24 ± 0.12^{b}	0.1336 ± 0.010^{b}	11.35 ± 0.75^{b}

[a] Values are means ± SD (standard deviation), and different lowercase letters (a, b, c, and d) indicate the significant difference among different treatments ($P < 0.05$). [b] Germination index (TGI) = $\Sigma G/T$, where G is the percentage of seeds germinated per day, and T is the germination time (days). [c] Mean germination time (MGT) = $\sum(n \times d)/N$, where n = number of newly germinated seeds, d = number of days from the beginning of the test, and N = total number of seeds sown. [d] Vigor index (VI) = germination percentage × dry weight (g).

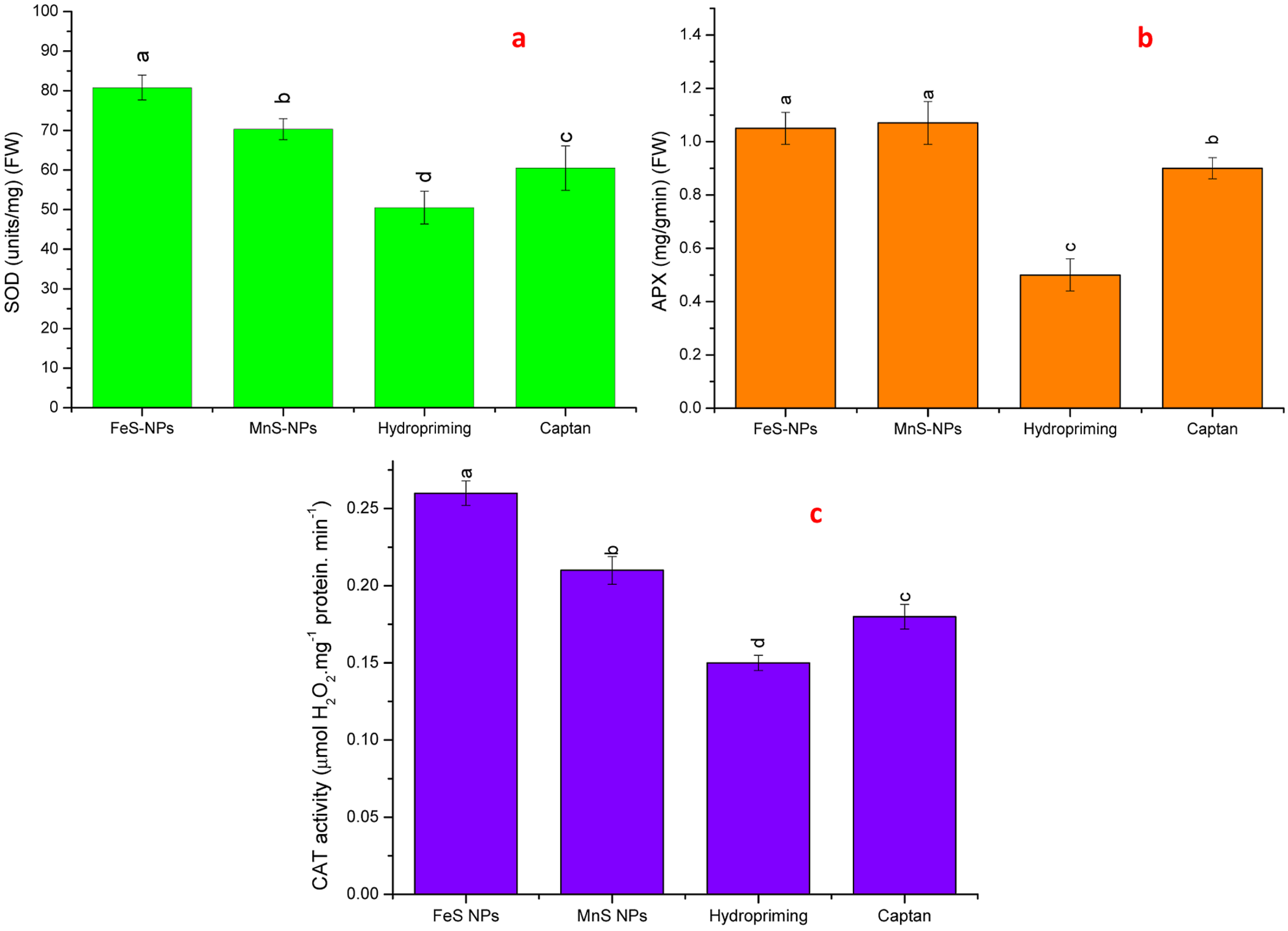

Figure 2. Antioxidant activity of (a) SOD, (b) APX, and (c) CAT enzymes in rice seeds primed with FeS NPs, MnS NPs, hydropriming, and captan. Each value represents the mean ± SD (standard deviation) (n = 3). Different lowercase letters indicate the significant difference among different treatments, followed by Tukey's test at P < 0.05.

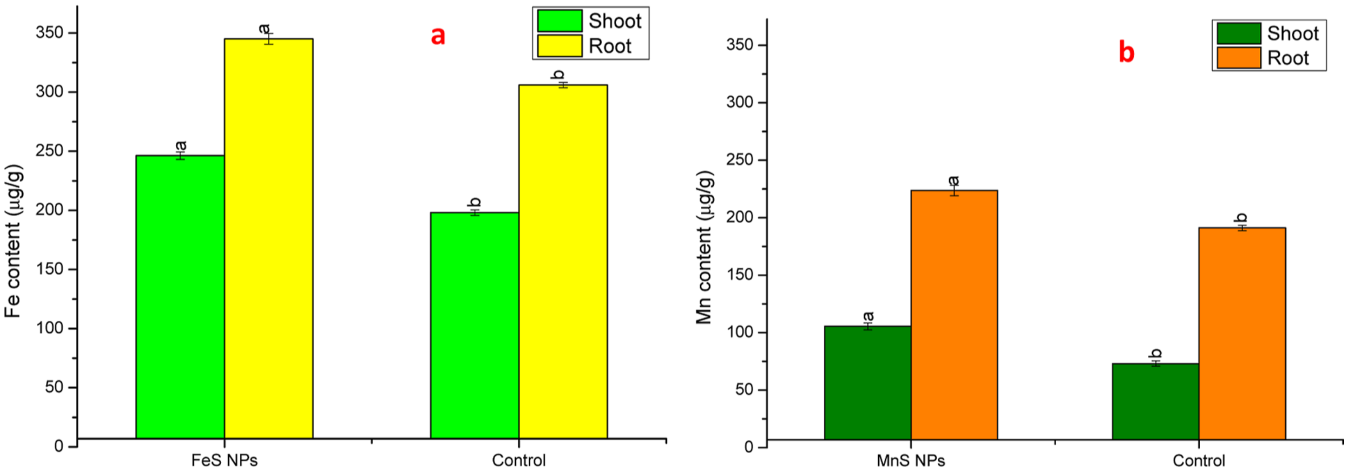

Figure 3. (a) Internalization of Fe in FeS NP-treated seedlings. (b) Modulation of Mn in MnS NP-nanoprimed seedlings. Each value represents the mean ± SD (standard deviation) (n = 3). Different lowercase letters indicate the significant difference among different treatments, followed by Tukey's test at P < 0.05.

substantially in retort to the different seed priming treatments. The priming of seeds with FeS-NPs and MnS-NPs for 8 h significantly changed the antioxidant enzymatic activity levels. Stimulation in the enzymatic activity of SOD, APX, and CAT to be 37.50, 52.38, and 42.30%, respectively, was observed in FeS-NP-primed seeds as compared to control (Figure 2). Similarly, upregulation of SOD (28.16%), APX (53.27%), and CAT (28.57%) activity was observed in MnS-NP-primed seeds as compared to control. Nanopriming induces the formation of nanopores in the shoot and helps in the uptake of water absorption and activates the antioxidant mechanisms in seeds.[31] Nanoprimed seeds showed the more vigorous antioxidant defense system due to the oxidative burst stimulation during germination and early seedling establishment.[32] The increased antioxidant properties can enhance the fitness of seedlings and plants during post-priming germination.[33] Similar results have also been reported by the application of chitosan NPs in wheat for the upregulation of antioxidant enzymes.[34] Mahakham et al. (2017) reported the stimulation of SOD and CAT enzymatic activity in seedlings by priming the seeds with green synthesized silver NPs.[35] Sharma et al. (2020) demonstrated that rice seedling treated with different concentrations of molybdenum NPs shows altered activities of antioxidant enzymes (SOD, CAT, and APX).[36] Mechanistically, the seeds primed with NPs undergo various synergistic events as a result of enhanced metabolism, modulating biochemical signaling pathways, trigger hormone secretion and upregulation of aquaporin gene expression, and reduce reactive oxygen species leading to increased seed germination with improved disease resistance.[37] Overall, FeS-NPs and MnS-NPs triggered the upregulation of potential antioxidant metabolism with an increase in enzymatic activity to manage the stress involved in the rice defense mechanism.

Internalization of Fe and Mn in Rice Seedlings. FeS-NP and MnS-NP nanopriming to rice cultivars significantly increased the Fe and Mn content in their respective treatments, considerably higher from hydropriming. In FeS-NP-treated seedlings, the highest Fe content in the shoot (246.36 μg/g) and root (345.17 μg/g) was observed in comparison to hydroprimed seedlings (Figure 3a). A higher Mn content of 105.44 μg/g in the shoot and 223.71 μg/g in the root was observed in MnS-NP-primed seedlings with respect to hydroprimed treatment (Figure 3b). The augmented Fe and Mn levels in nanoprimed seedlings confirmed the accumulation of NPs in seedlings by enhancing their nutritive values for the better fitness of rice seedling. Results are in consonance with our previous studies, where the enhancement in the uptake of nutritional metal in rice seedling was reported by priming the seeds with metal sulfide NPs.[8] Guha et al. (2018) reported the augmented growth of seedlings by priming the seeds with low concentration of NPs (<80 ppm).[27] Seeds priming with Fe (micronutrient) can stimulate the activation of enzymes to enhance the nutritive value for improved productivity of crops.[38] The essential micronutrient Mn, required in trace amounts by plants, is involved in photosynthesis, respiration, and nitrogen metabolism, affecting the germination profile of seedling. The higher uptake of intact Mn NPs (20 nm) was reported in the root and shoot of germinated mung bean seedling relative to control.[24] The present work substantiated that the increase in seed germination and seedling vigor of rice seeds is attributable to the enhanced nutritional modulation by absorption and translocation of iron and manganese in rice seedlings.

To assure an environmentally safe application of stabilized metal sulfide NPs, it is important to understand their potential environmental risks to the ecosystem and human health. The transport, transformation, and accumulation of metal sulfide NPs through food chains have limited impacts on acute toxicity to organisms.[39] Our previous studies reported that the nonhepatotoxic and biodegradable nature of aqua-dispersed metal sulfide nanoformulation makes them a safer alternative than organic pesticides which have toxic residues with long-term impacts.[7] Due to the soil-degradable and chemically unstable (at low pH) nature of metal sulfide NPs, they can dissociate into nutritious compounds which can be assimilated by the plants for the better growth and development.[39] Inclusively, these outcomes suggest that the applied lower concentrations of FeS-NPs and MnS-NPs can be used as eco-safer, bio-assimilative, and agro-compatible nanoseed priming agents over commercially used seed treatment chemicals.

■ CONCLUSIONS

The present effort demonstrated that FeS NPs and MnS NPs might function as an efficient seed priming agent to enhance the germination potential and reduce the disease parameters of rice seedlings. Crop development and production may be benefit from the application of these NPs with twin affirmative effects on seed health and quality. Stimulation of antioxidant enzymes and internalization of NPs into the seed supported the effective use of FeS and MnS as nanonutrients for the efficient defense and plant growth. Hence, the application of FeS NPs and MnS NPs as seed nanopriming agents can be utilized as a nanofertilizer with fungicidal effects on rice seeds and can be commercialized after extensive field trials for sustainable agriculture.

■ MATERIALS AND METHODS

Chemicals Used. Ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), manganese sulfate monohydrate ($MnSO_4 \cdot H_2O$), sodium sulfide ($Na_2S \cdot 9H_2O$), Triton-X, and polyvinyl pyrrolidone (PVP) were purchased from HiMedia Laboratories Pvt. Ltd, Mumbai, India, for the synthesis of NPs. All the chemicals were of analytical grade and used without further purification.

Synthesis of FeS-NPs and MnS-NPs. Iron sulfide NPs (FeS-NPs) were prepared using the standard methodology given by Ahuja et al. (2019).[9] In brief, an aqueous solution of sodium sulfide was added dropwise to ferrous sulfate solution, containing a drop of Triton-X (surfactant), under sonicating conditions, and PVP (0.3 g) was added to aid in the stabilization of the synthesized NPs. The solution was sonicated for 30 min to get PVP-coated FeS-NPs.

MnS-NPs were synthesized using the sonochemical methodology given by Ahuja et al. (2020).[10] An aqueous solution of manganese sulfate monohydrate (0.0015 M) was added dropwise to sodium sulfide solution (0.0015 M), containing a drop of Triton-X, under sonication. PVP (0.3 g) was added to the sonicating solution to get PVP-stabilized MnS-NPs.

Characterization of FeS-NPs and MnS-NPs. The morphological details and particle size of the prepared NPs were measured using transmission electron microscopy (TEM) in the Electron Microscopy and Nanoscience Laboratory, Punjab Agricultural University, Ludhiana. The zeta potential of FeS NPs and MnS-NPs was measured by using a Malvern Zeta Potential Analyzer at the Central Instrumentation Facility, Lovely Professional University, Phagwara. The UV−visible spectrum of NPs was recorded on a UV-1800 Shimadzu double-beam spectrophotometer in Punjab Agricultural University, Ludhiana. The uptake of Fe and Mn in rice seedlings was determined by using an inductively coupled plasma−atomic emission spectrophotometer (ICP−AES) (Agilent Technologies, Japan) at the Department of Soil Science, Punjab Agricultural University, India.

POT HOUSE STUDIES

Physiochemical Properties of Experimental Soil. Pots were filled with approximately 2000 g of loamy sand soil for the sowing, germination, and growth of rice seeds under pot house conditions. The international pipette method was used to determine the soil texture.[40] The pH was determined using a 1:2 soil−water suspension using a glass electrode pH meter,[41] and the EC of soil was determined from soil−water suspension equilibrated for 24 h using a conductivity bridge.[42] Soil OC was determined through wet combustion by the Walkley and Black (1934) rapid titration method.[43] Briefly, 2 g of the soil sample was taken in a conical flask; 10 mL of 1 N $K_2Cr_2O_7$ was added with 20 mL of concentrated H_2SO_4 to the flask. Excess of $K_2Cr_2O_7$ was determined by titration with 0.5 N ferrous ammonium sulfate in the presence of a diphenylamine indicator and NaF, which gives a clear solution. The DTPA-extractable Fe and Mn in soil was analyzed on ICP−AES.[44]

Collection of Seeds for Priming. Infested seeds of Pusa basmati rice were procured from the Seed Technology Laboratory, Punjab Agricultural University, India. Surface-sterilized seeds were dried in the shade at room temperature (28−30 °C) with constant aeration.

Seed Priming and Sowing. A pot experiment was conducted to check the effect of FeS-NPs and MnS-NSs on the growth potential and disease resistance of rice. Based on previous in vitro mycelial growth inhibition trials, the ED_{90} values (i.e., the effective dose at which 90% inhibition of fungus has occurred) of 35 μg/mL of FeS-NPs and 35 μg/mL of MnS-NSs were selected for priming of rice seeds by following the standard methodology of Ahuja et al. (2019).[9,10] After 8 h of seed priming, FeS-NP- and MnS-NS-treated seeds were sown in loamy sand soil. Captan-primed seeds were used as a standard, and hydroprimed seeds sown in soil were served as a control. In each pot, 100 seeds were sown and placed in a pot house for the observation of physiological and pathological parameters after a regular interval of time until maximum germination.[45]

Physiological Parameters. The physiological parameters, viz., germination percentage, mean daily germination and mean germination time, seed germination index, dry weight, and vigor index, were studied after the emergence of seeds in loamy sand soil under pot house conditions. After 6 days of sowing, the number of germinated seeds and seed germination percentage were recorded after an interval of 48 h until 16 days. Seeds were considered as germinated when their radicle showed at least 2 mm length. The mean germination time, mean daily germination, and germination index were calculated.[46] The fresh weight of shoots was calculated by carefully removing (without any root loss) 10 plants per treatment, using a sensitive weighing balance. For the calculation of dry weight, the samples were placed in an oven at 70 °C for 24 h, the dry weight of the seedlings was recorded after 72 h, and the vigor index was calculated.[7]

Measurement of Disease Parameters. To investigate the efficacy of FeS-NP and MnS-NP nanopriming for 8 h on rice seeds under pot house conditions, the phytopathological parameters in terms of mortality, seed rot, and seedling blight were recorded. Visual assessment of seed rot and seedling blight were observed in the plants of various treatments by following a well-defined protocol and compared with the standard fungicide (captan) and hydropriming (control).[47,48] The seedling mortality percentage of germinated plants was measured and calculated after 16th day of sowing in all the treatments.

Antioxidant Enzymatic Activity. The activity of SOD, APX, and CAT enzymes in FeS-NP- and MnS-NP-primed seeds was evaluated by following the methodology of Afzal et al. (2020).[26] Seed embryos (1 g) were homogenized in a pre-chilled mortar pestle containing 10 mL of 50 mM buffer (pH = 7.8), 1% PVP (polyvinylpyrrolidone), 0.5% Triton X-100, and 1 mM ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged at 6000 g for 30 min at 4 °C, and the supernatant was used for the detection of antioxidant enzymes. The antioxidant enzymatic activities of SOD, APX, and CAT in NP-treated seeds were evaluated by the standard methodology of Giannopolitis and Ries (1977),[49] Chen and Asada (1989),[50] and Aebi (1984),[51] respectively. One unit (U) activity of SOD is the particular quantity of enzyme needed for 50% inhibition of NBT, APX (U) is the amount of enzyme needed for oxidizing 1 nmol ascorbate min^{-1}, and CAT (U) is equal to 1 nmol H_2O_2 dissociated min^{-1}.

Determination of Internalization of Fe and Mn in Rice Seedlings. To visualize the capability of NPs to influence the content of Fe and Mn in primed seedlings as compared to control (hydropriming), the oven-dried tissues (separate roots and shoots) of NP-treated seedlings and hydroprimed seedlings were weighed, crushed to powder, and digested in aqua regia (i.e.. HNO_3/HCL; 3:1). The digested samples were diluted with 1% nitric acid. The diluted solutions were analyzed by using inductively coupled plasma atomic emission spectroscopy (ICAP−OES) for Fe and Mn contents in treated seedlings.[8]

Statistical Analysis. The entire data was expressed as mean ± standard deviation of three independent replicates. A one-way analysis of variance (ANOVA) followed by Tukey's test was performed to test the significance of differences between means obtained among the treatments at the 5% level of significance using the SPSS statistical software package release 16.0 (SPSS Inc., Chicago, IL, United States).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c03012.

TEM nanographs, particle size distribution, zeta potential, and UV−visible spectrum of FeS-NPs and MnS-NPs (PDF)

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Notes

The authors declare no competing financial interest.
Human and animal rights—No humans and animals were used in this research.

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