

Potential of Iron Nanoparticles to Increase Germination and Growth of Wheat Seedling

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Abstract

Rapidly expanding use of nanoparticles in every discipline of life necessitates finding their potential to increase food production to match with burgeoning population. This study was conducted first time in Bangladesh to determine the potentiality of iron nanoparticles for enhancing germination and growth of BARI Gom 25. Two experiments were conducted for this purpose. One was synthesis of iron nanoparticles. Another was seed priming effect on germination and growth of wheat seedlings. An aqueous method has been considered to prepare iron nanoparticles in water by reducing their ionic salts such as FeCl_3 in presence of surfactant (PVP) under oilbath heating for several minutes. The synthesized nanoparticles were purified by precipitation method. A sand culture seedlings growth experiment was conducted to quantify nanomaterials delivered to wheat seedlings. The treatments of the experiment were determined, including nanoparticles of iron at two levels like before germination and seven days of germination; doses of priming at five concentration levels of nanoparticles were 0, 1.0, 1.5, 2.0 and 2.5 ppm. Seed germination and seedlings growth was regularly affected with 1.0 to 2.0 ppm but decreased significantly at 2.5 ppm of iron nanoparticles. This study concluded that optimum level of iron nanoparticles concentration helps to increase germination and growth response of wheat seedlings. Further experimentation is required to explore the internal mechanism of iron nanoparticle absorption and mode of application for yield maximization of wheat.

Keywords: Nanomaterial, Aqueous method, Seed priming, Oilbath heating.

Introduction

Nanoparticles are fundamental to modern science and technology. Changes in agricultural technology have been a major factor shaping modern agriculture. Among the latest line of technological innovations, nanotechnology occupies a prominent position in transforming agriculture and food production. The development of nanodevices and nanomaterials could open up novel applications in agriculture [1]. Nanotechnology may offer

an important role in improving the existing crop management techniques in Bangladesh. Mastery over the shape of a nanocrystal enables control of its properties and enhancement of its usefulness for a given application. Application of nanotechnology in agriculture, even at its global level is at its nascent stage. Nanoscience is leading to the development of a range of inexpensive nanotech applications for enhanced plant growth. Nanoparticulate material delivery to plant technology also holds the promise of controlled release of agrochemicals and site targeted delivery of various macromolecules needed for improved plant disease resistance, efficient nutrient utilization and enhanced plant growth. The uptake efficiency and effects of various nanoparticles on the growth and metabolic functions may vary differently among plants. Nanoparticle mediated plant transformation has the potential for genetic modification of plants for further improvement [2]. The present study was therefore, undertaken to quantify the role of nanoparticle on seed priming for germination and seedlings growth performance of sustainable wheat production in Bangladesh.

In fact, nanotechnology is a rapidly developing discipline substantially influencing every field of science and biology. Nanotechnology certainly holds the potential to rejuvenate agriculture [3] and is expected to become a dynamic economic force in the near future. Convincingly nanotech based reorientation of agriculture can boost production of quality food in a resource and environment friendly manner. Exploring comprehensive application profile of nano-particles may revolutionize research in crop science and transform agriculture into industry. Copper nanoparticles show positive effects on germination [4] but are phytotoxic at seedling growth [5]. However, fewer studies have been reported on exploring the potential of copper nanoparticles in crop growth. Visualizing enormous beneficial aspects of metal nanoparticles present study was conducted to find out the possible role of copper nanoparticles can play in enhancing growth and increasing yield of wheat crop.

Sustainable wheat production, in view of lessening the burden on rice, has been a major concern for the agricultural sector of Bangladesh. Various conventional studies have been undertaken

already for this purpose. The aim of this study is to quantify the role of iron nanoparticle on seed priming for germination and seedling growth response of wheat. Thus, the delivery of nanoparticle to plants and their ultimate effects which could provide some insights for the safe use of this novel technology for the improvement of sustainable wheat production in Bangladesh.

Materials and methods

Experimental materials and methodologies which were used for experiments within this paper are outlined below.

Nanoparticle synthesis technique

The aquostic method was a typical technique to prepare metallic nanoparticles in water by reducing their ionic salts. In general, a mixture of reagent and polymer surfactant in water was heated in an oilbath heater for several minutes, as a result of heating nanoparticles are prepared. The overall technique was shown as below.

Materials

FeCl₃.H₂O (99.8%), and PVP powder (average molecular weight MW: 40000 in monomer units) used for this study were purchased from Sigma - Aldrich Industries Ltd. All these reagents were used without further purification.

Preparation of nanoparticles

Iron nanoparticles are prepared under oilbath heating. A mixture of FeCl₃ solution and PVP as a polymer surfactant in 25 mL of water was heated at 70 °C for 45 min under oil bath heater. The final concentrations of FeCl₃ and PVP were 50 milimolar and 250 milimolar, respectively.

Characterization of nanoparticles

The synthesized nanoparticles was purified by precipitation method. Firstly, we would like to study crystal structures and growth mechanisms of nanoparticles prepared by aquostic method on the observation of AFM. Morphologies of Fe nanoparticles were characterized using atomic force microscope AFM (Park Systems, XE-70, South Korea). Product solutions were centrifuged at 6,000 rpm three times for 30 min to ensure complete collection of the products each time. The precipitates are collected and then re-dispersed in ethanol. Samples for AFM

measurements were prepared by dropping a droplet of the colloidal solutions on the glass slides.

Plant material

The BARI Gom 25 (wheat) was used as a testing plant. The pedigree of BARI Gom 25 is shown in Table 1.

The characteristics of the BARI Gom 25 are presented in Table 2. Their performance was evaluated under two growing environments.

Rational for selecting the plant material

The grain size of BARI Gom 25 was highest among the recently BARI released wheat varieties. During the delivery of nanomaterials within seeds it will helpful to absorb more iron nanoparticles due large surface area. That's why we have used the BARI Gom 25 in this experiment to absorb maximum amount of nanomaterials by the wheat seed. It helped to use wheat seedlings to utilize nutrients from seed reserve.

Seed priming for germination

Synthesized nanoparticles are used for seed treatment as a priming material for the BARI Gom 25 seed germination. These nanoparticles are used as a soaking agent for pre-germination. Pre-germinated seeds were sown in petri dishes in sand medium culture. Different concentration of Fe nanomaterials are used as per treatment.

Experimental design

The experiment was carried out by randomized complete block design (RCBD) with five replications. The experiment was consisted with different concentration of iron nanoparticles. These were 0, 1.0, 1.5, 2.0 and 2.5 ppm of nanomaterials. Five seeds of wheat that are treated with nanomaterials were considered for each treatment. The experiment was divided into two folds. Firstly, synthesis of iron nanoparticle under oil bath heating. Secondly, seedling growth experiment. This seedlings growth experiment was RCBD. The seedling growth experiment was divided into categories such as variation of iron nanoparticles concentration and variation of days for seed germination, five treatments like 2 days, 4 days, 6 days, 8 days and 10 days. Variation of concentration was 0, 1.0, 1.5, 2.0 and 2.5 ppm.

Experimental procedure

Table 1: Pedigree of recently released wheat varieties in Bangladesh

Variety	Accession No.	Pedigree/ Cross	Year of release
BARI Gom 25	BAW 1059	ZSH 12/HLB 19//2*NL297	2010

Source: Raj et al. [6]

Table 2: Characteristics of wheat varieties used this experiment

Variety	Stress tolerance capacity	Duration (Days)	Yield (kg ha ⁻¹)	Suitable area for cultivation	Sowing time	Harvest time	Major diseases and pests
BARI Gom 25	Tolerant to terminal heat stress in late seeding	104-110	3500 to 5000	Possible to grow throughout the country except in areas with salinity level >6 dS/m	Nov. 15-30	Mar.-Apr.	Tolerant to <i>bipolaris</i> leaf blight and resistant to leaf rust diseases (stem rust) race, Ug 99

Source: BARI 2012

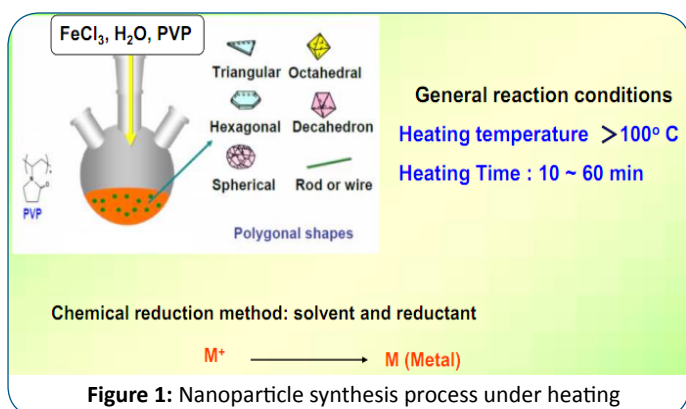
Five petri dishes were taken for this experiment. Each Petri dish was filled with adequate amount of sand. Petri dishes were labeled as 0.0 ppm, 1.0 ppm, 1.5 ppm, 2.0 ppm, 2.5 ppm respectively. Five seeds of BARI Gom 25 were sown in each petri dish. 0.5 mL of synthesized nanoparticles solution was taken in a beaker containing 100 mL of distilled water. Then 10 mL of distilled water was applied to the seeds in the petri dish labeled as 0 ppm concentration of Fe nanoparticles. At the same time 0.0 ppm, 1.0 ppm, 1.5 ppm, 2.0 ppm, 2.5 ppm of the prepared Fe nanoparticles solution was applied to the seeds of corresponding petri dishes drop by drop. All the petri dishes were kept at room temperature in the laboratory.

Seed germination

The sown seeds were observed for germination at 2 days interval. A 50 mL of distilled water was added daily to each petri dish drop by drop on 3rd, 5th and 7th day. A second treatment was undertaken and the measurements of the germinated seeds were taken on 2nd, 4th, 6th, 8th and 10th day.

Seedlings emergence

The BARI Gom 25 were started emergence after 2 days of sowing (DAS). The seedling was emergence within petri dishes (Figure 1). We did not transfer the seedling in pot because we have to add different doses of Fe nanoparticles solution in every alternative days of seedlings emergence. Sand was used a medium of seedling



emergence to measure whole seedlings biomass as well as root.

Seedlings growth conditions in control environments

Seedlings were grown in a growth cabinet with day/night temperature of 20±2°C, with 10 hours of dark and 14 hours of light conditions and average light intensity of 210 μM photons/m²/s. All petri dishes were re-randomized within the growth chamber on alternate days during the growing period for the seedlings, to minimize positional effects.

Seedlings harvesting and live root collection procedure

Whole seedlings and roots with surrounding sands were removed from petri dishes by gentle agitating of the seedlings to provide minimum disturbance to the roots and shoots. Whole seedlings were then lifted gently from the sand and shaken lightly to remove sand from the roots. Whole seedlings including roots (after removal of sand) were then placed in a leveled snap polyethylene bag as well as kept 20-30 minutes for air dry. The seedlings were harvested on the 7th days after sowing and measurements of the roots and shoots were taken by a ruler.

Root and shoot weight

Roots and shoots were dried at 70°C for at least 72 h and weighed.

Statistical analysis

Results were analyzed by a one-way analysis of variance (ANOVA) using Genstat 12th edition for Windows (Lawes Agricultural Trust, UK).

Results

The focus of this paper has been to determine the effect of delivery of nanomaterials to wheat seedlings. The main objective of this study was to quantify Fe nanoparticles effect on seed priming effect of BARI Gom 25 germination.

Atomic force microscope (AFM) observation

Freshly prepared nanoparticles were centrifuged with ethanol, followed by casting onto glass slide for film and subsequent dried in open air at room temperature. Figure 3 depicts AFM images of

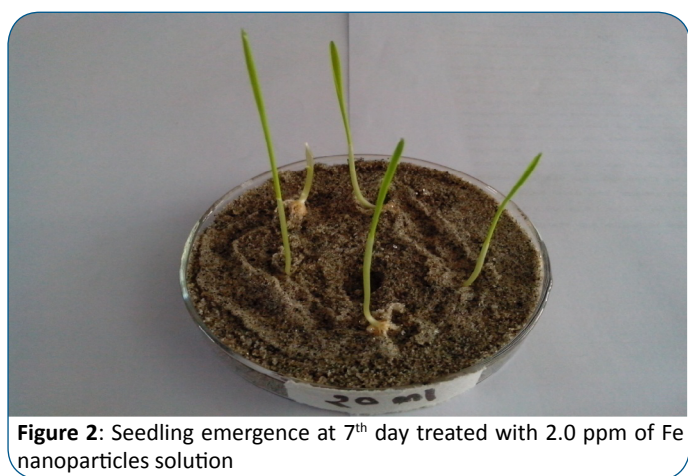


Figure 2: Seedling emergence at 7th day treated with 2.0 ppm of Fe nanoparticles solution

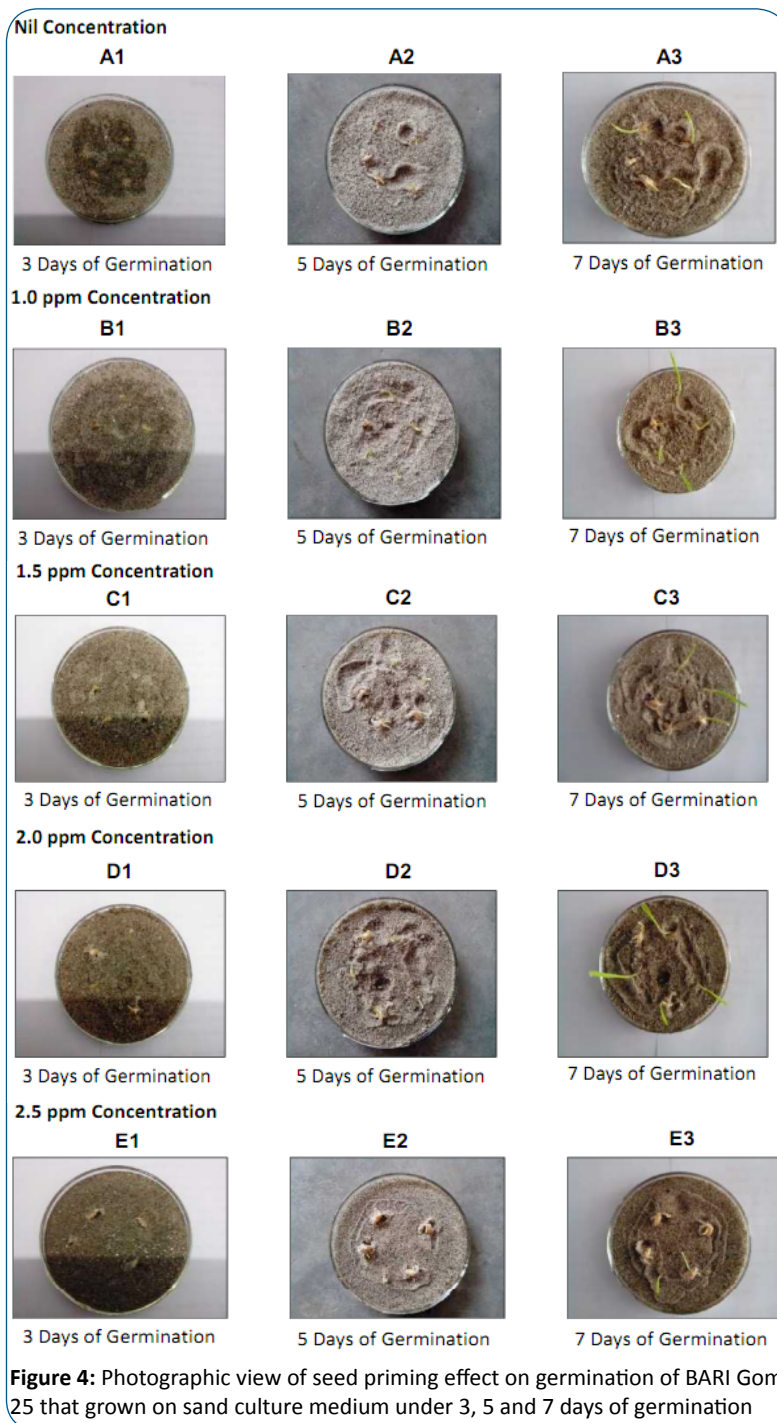
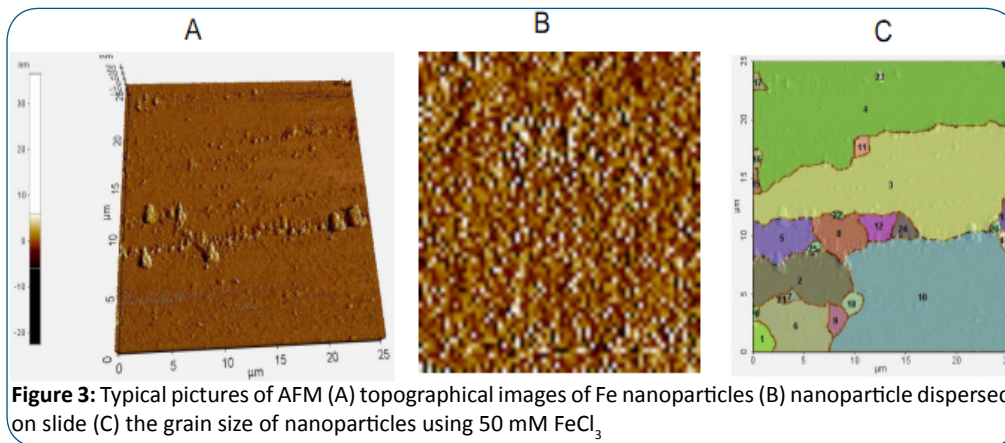
Fe nanoparticles with metal ion concentration 50 mM.

In this study we used AFM to investigate the morphological view of Fe nanoparticles under heating time. The AC mode was used to acquire both the topographical and phase AFM images during the scan (Figure 3). The phase image provides the degree of phase shift of the cantilever oscillation relative to the signal sent to the base of the piezo driving the cantilever. By recording the phase shift of the cantilever oscillation, the phase imaging goes beyond simple topographical mapping to detect variations in chemical composition, adhesion and other surface properties. The topographical and the corresponding phase images in Figures 3(A-C) show the typical near spherical shape.

Figures 3(A-C) showed that AFM data at 50 mM FeCl₃ salt solution after heating for 45 min. Figure 3A and 3B showed the topographical images of Fe nanoparticles and nearly spherical particles were dispersed on slide respectively. Grain boundaries in Figure 3C of particles depict that the average size of Fe nanoparticles was 10.25 ± 7.7 nm and major products were nearly spherical shape. The collected nanoparticles were used as promoter of wheat germination.

Effect of iron nanomaterial concentration variations on seed priming

The results indicate that the different doses of iron enriched nanomaterials had a significant effect on seed priming of BARI Gom 25. The effect of nanomaterial concentration variation



was prominent on the seed priming (Figure 4). Also the results showed that the priming treatment before germination of the wheat seeds enhances the shoot and root proliferation in all doses. The nanomaterial effect on seed priming was highest at 2.0 ppm concentration among 0.0 ppm, 1.0 ppm, 1.5 ppm and 2.5 ppm concentration of Fe nanoparticles. Seed priming effect also indicated that 7 days of germination was highest on 2.0 ppm Fe nanoparticle concentration. Seed priming effect was increased with the increase of seedlings growth stage in Figure 2. However, our predictions suggested that excess amount of Fe nanoparticles has negative impact on seed priming. The evidences depicted that 2.5 ppm concentration of Fe nanoparticles has an adverse effect on seed priming, even sometimes lower than 0.0 ppm concentration of Fe nanoparticles. Table 3 showing analysis

Table 3: Significance levels from the analysis of variance for the main effects of growth parameters among various nanomaterial concentrations

Source of variation	Root length	Shoot length
Iron nanoparticle concentration	(0.009)**	(0.037)*

Where * and ** represents probability ≤ 0.05 and ≤ 0.01 . Values were means of five replicates.

of variance for the main effects of growth parameters among various nano-material concentrations.

In general, shoot length of BARI Gom 25 increase with the increase

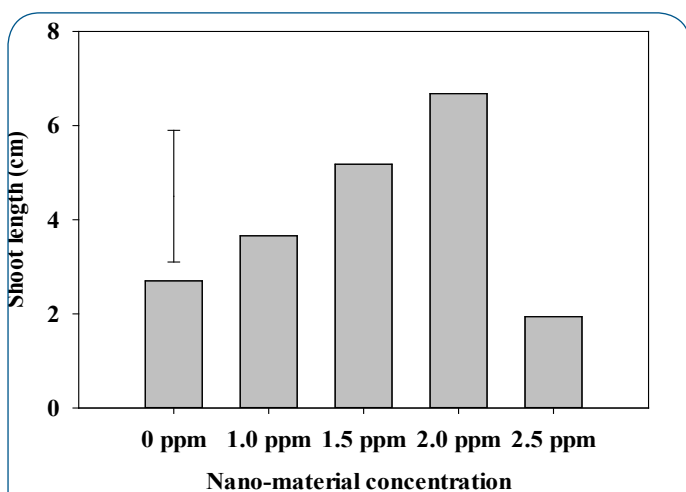


Figure 5: Effect of Fe nanoparticles delivery on shoot length of BARI Gom 25 under various concentrations of Fe nanoparticles. Vertical bar represents LSD ($P \geq 0.05$) for various Fe nanoparticles concentrations

of delivery of Fe nanoparticles upto 2.0 ppm concentration (Figure 5). The shoot length for 0, 1.0, 1.5, 2.0 and 2.5 ppm Fe nanoparticles were 2.7, 3.7, 5.2, 6.7 and 1.9 cm respectively.

Effect of iron nanoparticles concentration variations on root length of wheat seedlings

On average, the root length increase with the increase of nanoparticles concentration (Figure 6). There was an exceptional case for the 2.5 ppm of Fe nanoparticles concentration. At this highest concentration (2.5 ppm), seed priming effect of nanomaterial did not occur. The root length for 0, 1.0, 1.5, 2.0 and 2.5 ppm of Fe nanoparticles were 3.7, 5.1, 7.02, 7.96 and 2.3 cm respectively.

Effect of day variation at constant iron nanoparticles

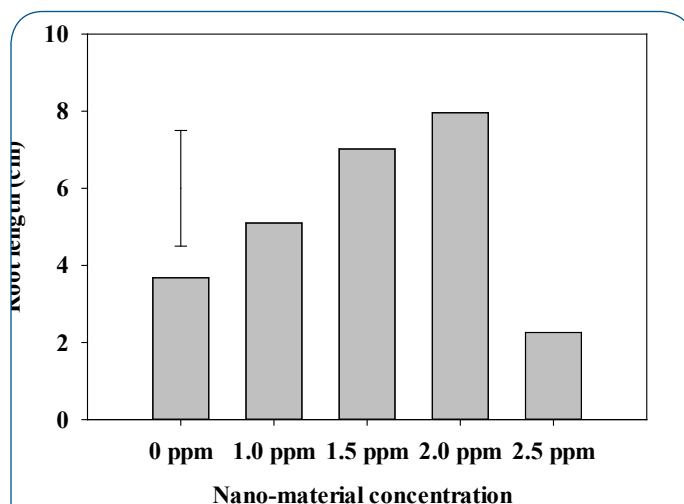


Figure 6: Effect of Fe nanomaterial delivery on root length of BARI Gom 25 under various concentrations of nanomaterials. Vertical bar represents LSD ($P \geq 0.05$) for various Fe nanoparticles concentrations

The variations of days on germination were prominent at constant iron nanoparticles concentration such as 2.0 ppm in Figure 7. The day variation effect was started on 2 days of germination (Figure 7A). The coleoptiles tends to appear at 4 days of germination (Figure 7B). The variation effect was prominently observed at 6 days of germination (Figure 7C).

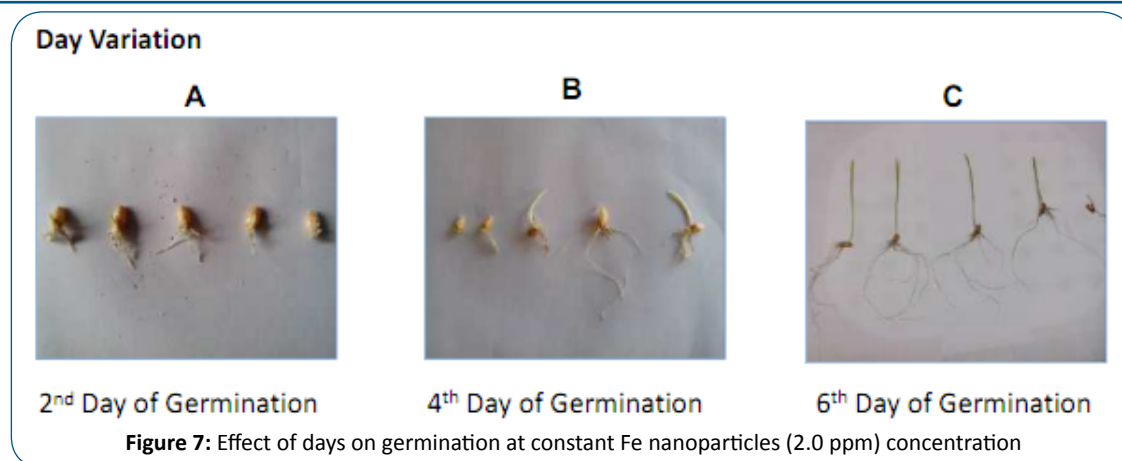
Discussion

The key findings have been presented in results section. A number of additional issues relating to these experiments will be discussed in this section.

Role of iron nanoparticles on seed germination

Different nanoparticles have been applied to several plant species with variable and contrasting effects. Metal nanoparticles are considered to modify physiological and biochemical processes in plants thereby affecting their germination and growth favorably or otherwise. Several studies conducted to investigate the role of nanoparticles on germination of seeds have reported variable and concentration dependent effects of nanoparticles (Figure 4). Our study revealed that iron based nanoparticles did not affect seed germination and wheat seedling growth at higher concentration (Figures 4, 5 and 6). Application of 2.0 ppm of Fe nanoparticle in MS medium seems better for good seedling growth. However, higher concentration proved phytotoxic for wheat plants. This might be due to more bioavailability, absorption and accumulation of nanoparticles leading to toxic effects. Concentration of iron nanoparticle higher than 2.0 ppm in MS medium produced declining trend in growth parameters.

Application of 1.0 ppm of iron nanoparticle applied to petri dishes in sand did not produce significant effects as compared to control indicating low bioavailability and absorption of sand applied nanoparticles. Nonetheless, application of 2.0 ppm of iron nanoparticle to sand in petri dishes proved best for growth of wheat seedlings. Therefore, 2.0 ppm of Fe nanoparticle applied to sand depicted that best response of germination and growth of wheat seedlings. Declining trend in germination and growth of wheat seedlings at higher concentrations than 2.5 ppm might be occur due to more absorption of nanoparticles leading to phytotoxic effects. Several studies have reported that



the phytotoxic effects of excess Fe nanoparticles on germination and growth that predictions are match with our results. Many authors speculated in reputed journals that the phytotoxic effects were concentration dependent [7, 8].

Effect of iron nanomaterials

Findings from this experiment showed that there were significant differences in shoot and root proliferation affected by Fe nanoparticles concentration at various levels of nanoparticles (Figures 4, 5, and 6). In the analysis of variance, it was observed that both stages of seed priming with different doses of iron nanoparticles increased the shoot and root of the wheat seedlings (Figures 5 and 6). Other study found similar observations. Many authors suggested that the nanoparticles cause increasing the percentage of germination, length of shoot and establishment of the wheat seedling [9]: nanoparticles include those compounds having at least one dimension must be less than 100 nanometers in size [10]. Plant cell wall acts as a barrier for easy entry of any external agent including nanoparticles into plant cells. The sieving properties are determined by pore diameter of cell wall ranging from 5 to 20 nm [11]. Hence, only nanoparticles or nanoparticle aggregates with diameter less than the pore diameter of the cell wall could easily enter through and reach the plasma membrane [12,13]. Advances in supra molecular chemistry, nanotechnology, metal complexes and motivation for goal oriented application have made it possible to achieve results in the fields of agriculture which was unimaginable even a few decades.

Conclusion

Iron nanoparticles certainly have a potentiality to enhance germination and growth of wheat seedlings. Nanotechnology may play an important role in improving the existing wheat production techniques in Bangladesh. The main objective of this study was to quantify the iron nanoparticles effect on seed priming of BARI Gom 25 germination. The BARI Gom 25 seeds are large and well absorbing that encourage me to use BARI Gom 25 as a testing plant. The results depicted that the different doses of iron nomaterials had a significant effect on seed priming of BARI Gom 25. The effect of iron nanoparticle concentration variation was prominent on the seed priming. Also the results suggested that the priming treatment before germination of the wheat seeds enhances the shoot and root proliferation in all doses. The effect of nanomaterial on seed priming was highest at 2.0 ppm concentration among 0.0, 1.0, 1.5 and 25 ppm concentrations of Fe nanoparticles. Seed priming effect also

indicated that 3 days of germination was highest on 2.0 ppm of iron nanoparticles concentration. Seed priming effect was increased with the increase of seedlings growth stage. However, our findings reported that excess amount of nanomaterial has a negative impact on seed priming. These provided evidence proof that 2.5 ppm concentration of Fe nanoparticles did not show good response of seed priming effect lower than 0.0 ppm concentration of iron nanoparticles. This could be due to the reason that excess iron nanoparticle delivery to the wheat seedlings causes toxic effect. So, there must always be an optimum concentration of nanomaterial where plants show the best response. This study revealed that iron nanoparticle has the potential to enhance germination and growth of wheat seedlings but their effect is concentration dependent. Further experimentation is needed to precisely explore impact of iron nanoparticle on germination of wheat seed for conclusive results.

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