Original Research

The Effect of Silicon Nanoparticles on the Seed Germination and Seedling Growth of Moso Bamboo (*Phyllostachys edulis*) under Cadmium Stress

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Abstract

In the current study, the impact of silicon nanoparticles on germination characteristics and key growth parameters was investigated in Moso bamboo seedlings under cadmium (Cd) stress. The seeds were germinated at a concentration of 100 μ M Cd alone and in combination with two levels of SiO₂ NPs (100 and 200 μ M), and the results were compared with those from a control treatment. The results indicated that SiO₂ NPs significantly improved germination characteristics (GP, GR, and GI) under Cd toxicity but also led to increased mean germination time (MGT). On the other hand, the results indicated that the seedling growth indexes, seedling biomass and vigor indexes of Moso bamboo improved under a combination of 100 μ M Cd and silicon nanoparticles. We concluded that SiO₂ NPs could ameliorate cadmium toxicity during seed germination and that the enhanced germination parameters improved seedling growth in Moso bamboo under Cd toxicity. We also found that SiO₂ NPs at 200 μ M rather than 100 μ M had a greater beneficial impact on the seed germination and the seedling growth under the Cd stress of 100 μ M. Thus, the use of SiO₂ NPs may help in controlling Cd toxicity at the stages of seed germination and seedling growth in Moso bamboo (*Phyllostachys edulis*).

Keywords: silicon nanoparticles, germination, Moso bamboo, cadmium

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Introduction

In recent decades, the contamination of soil and the environment caused by anthropogenic activities has led to rising levels of heavy metals in the soil and environment in the southern and eastern China, and heavy metal pollution is considered a major factor threatening human health [1]. Studies have reported that in China, more than 2×107 hm² (approximately 20%) of the total agricultural soils are polluted by heavy metal contamination caused by sewage [2]. Among heavy metals, cadmium (Cd) is one of the most widespread metals in this area and is one of the elements with the highest pollution levels in Chinese farmland soil [3]. Cadmium has polluted more than 1.133×10⁷ hm² of China's agricultural soil [2]. Extremely high levels of Cd can lead to a decrease in plant growth [4, 5] and development as well as a reduction in yield [6].

The seed germination stage is regarded as the most important part of the plant life cycle [7, 8]. The reduction of germination and seedling growth in many plant species under heavy metal and salt stress is observed [9]. Silicon, as the second most abundant element in surface soils, can help plant species under stress conditions [10]. Many researchers have reported that silicon can reduce Cd stress in plants by different mechanisms. As for wheat and rice plants, silicon reduces or entirely inhibits the translocation of Cd to shoot and reproductive organs [11, 12] and in cowpea, silicon is shown to effect the accumulation and deposition of Cd in the roots or (root zone) [13]. The reduced metal accumulation in plant species facilitated by silicon can positively contribute to plant growth and development [14, 15].

In recent years, researchers have focused on using nanoparticles for agricultural purposes [16]. Nanoparticles are made of three-dimensional spheres with a size between 1 and 100 nm [17, 18]. Nanoparticles have high surface area and high solubility, and they can change their physico-chemical properties. Therefore, they are well known as a unique material [19]. The adsorption mechanism by SiO, NPs when plants are subjected to the excess of heavy metals can lead to the reduction in lethal concentration of metals in plants [20]. There is some evidence that SiO₂ NPs impacts seed germination. Siddiqui and Al-whaibi reported that SiO₂ NPs improved seed germination in tomato [21]. Suriyaprabha obtained a similar result when studying the effect of SiO₂ NPs on Zea mays seeds [22]. Moreover, the growth indexes of Changbai larch seedlings [23], maize (Zea mays L.) [24], tomato seed germination [25, 26], and Arabidopsis thaliana [27] were reported to be positively influenced by SiO, NPs under various stress conditions. However, the impact of SiO, NPs on bamboo seed germination and growth under Cd stress is still not completely understood. To our knowledge, this study may be the first to investigate the role of SiO₂ NPs in the reduction of cadmium toxicity in Moso bamboo seed germination and seedling growth.

Moso bamboo (Phyllostachys edulis) is one of the greatest hyperaccumulator species and makes up more than 70% of the bamboo species in forests in China [28,29,30,31]. This fast-growing plant covers more than three million hectares of forest in China [32-34], which are distributed in bamboo forest areas in 12-13 provinces in southern China [35,36]. Soil contamination by heavy metals has become a significant crisis in this region [3]. Moso bamboo, which has a large timber and shoot biomass, should be investigated as a potential heavy metal remediation species [32, 37]. Moso bamboo is also known for having a strong ability to accumulate silicon in the environment [38], which may help to reduce heavy metal contamination [39]. This study explored the possible ameliorative impacts of two levels of SiO, NPs on Cd toxicity in seed germination and seedling growth indexes of Moso bamboo (Phyllostachys edulis).

Materials and Methods

This study was conducted in the seed germination room of the Bamboo Research Institute, Nanjing Forestry University. For this purpose, the seeds of Moso bamboo (*Phyllostachys edulis*) were collected from the seed bank of the Bamboo Research Institute in Jiangsu Province. In the first step, the bamboo seeds were separated from the skin and sterilized with disinfection powder (Duo Jun Ling) twice for 30 min. The seeds were dispersed into the water for the separation of the empty seeds and were soaked in water for 12 hours. In this research, the treatments were in a completely randomized design with four replications for each treatment. The treatments included a control (Wild Type), 100 μ M Cd, 100 μ M Cd-100 μ M SiO₂ NPs, and 100 μ M Cd-200 μ M SiO₂ NPs (Fig. 1).

Approximately 100 seeds were used in each treatment. To separate the nanoparticles from each other and create a suspension, homogenization was conducted in solution with an ultrasonic device (100 W, 40 kHz). For the preparation of the treatments, the seeds

C Cd Cd/100 Si Cd/200 Si

Fig. 1. Moso bamboo as affected by 100 μ M Cd alone and in combination with 100 μ M or 200 μ M SiO, NPs.

were set on duplexed wet paper in a Petri dish that was stuffed with cotton. The experiment was conducted in a germination device with an optimal temperature of 25°C and 1000 lux (12 hours light - 12 hours darkness) light intensity, which was maintained at 60% humidity for 30 days.

To measure the germination characteristics during the seedling period, the number of germinated seeds was counted daily at a specified hour. Afterward, the germination characteristics were determined using the formulas below.

A. (Germination index) (Tekrony and Egli, 1990) [40].

$$GI = (n.t^{-1})$$

B. (Germination percentage) (Khodarahmpour, 2012) [41].

$$GP = (n/N) \times 100$$

C. (Germination rate) (Khodarahmpour, 2012) [41].

$$GR = f(n.t^{-1})$$

D. (Mean germination time) (Agrawal and Dadrani, 1995) [42].

 $MGT = \pounds(n.t). \ \pounds n^{-1}$ n = the number of seeds that germinated on day t. N = total number of seeds.

Seedling Growth Characteristics

The seedling growth was evaluated based on the shoot and root length. It was measured on the last day of the germination experiment and after separating irrigated seeds from hard and desiccated seeds. Ten germinated seeds were randomly selected from each treatment, and the growing parts of the embryo were separated from the storage parts (cotyledons). The root length, seedling height, and stem length were measured manually with a ruler [42].

Seedling Biomass Determination

The seedling roots and seedling shoots were carefully cleaned. In the first step, the water on all surfaces was removed by drying the samples in an oven at 110°C for 10 min. The samples were then dried for 20 min at 80°C to a constant dry weight.

Vigor Indexes

The vigor indexes of seed germination were obtained by the following formulas:

Vigor index I = standard germination percentage (SGP) × the mean seedling length (mm)

The measurement of cadmium and silicon contents in roots, and leaves of Moso bamboo.

The contents of Cd were measured in the roots, and leaves of Moso bamboo (Phyllostachys edulis) seedling at the end of the experiment. In this process, the samples were first cleaned and then dried and dissolved in 70% nitric acid for 15 min at the temperature of 70°C. Then, to collect the supernatants, the samples were centrifuged at 12000 g for 5 min. The cadmium and silicon contents in the roots and leaves were measured by atomic absorption spectrometry (AAS), which uses a spectrometer equipped with a furnace of graphite and a Zeeman-effect background correction system (AAnalyst 800, Perkin Elmer). To determine the amount of the elements, the parameters of the instruments were optimized. Then, the metal standards were calculated and recorded in the range of 2.5% nitric acid (Spectrascan). The calibration verification standard (Perkin Elmer) included all the elements in the inorganic target analyst list (TAL) and was run at optimal intervals in an unattended automatic analysis run.

Scanning Electron Microscope (SEM) Analysis of Leaf and Root Surfaces

Observations of the germination organs (leaves and roots) were performed using a scanning electron microscope (SEM) (JSM-6380, JEOL, Tokyo, Japan) operating at 15-25 kV. Briefly, the selected leaves and roots were fixed in 70% FAA consisting of a formaldehyde solution and glacial acetic acid and kept at 4°C overnight in a vacuum. The next day, the samples were cut horizontally or vertically from the surface to meet the requirements of imaging, and the final wash was administered with 80% acetone. Then, the samples were transferred to the Test Center Lab at Nanjing Forestry University, where the samples were dried on a platform before microscopic observation.

Statistical Analysis

This study was carried out using a completely randomized design (CRD) with four replicates. Analysis of variance (ANOVA) was performed using the statistical software package R. The mean differences were determined using Tukey's test at the p<0.05 probability level.

Results

Seed Germination Characteristics

In the studied species, the differences in the germination index (GI) among the various concentrations of Cd and SiO, NPs and the control treatment were significant at the 1% level (p<0.01). According to the results, the 100 μ M Cd+200 μ M SiO₂ NPs treatment had the highest germination index (GI), with a 15% increase over that of the control treatment. The 100 µM Cd treatment had the lowest germination index, with a 28% reduction compared with that of the control treatment. The results obtained by our data analysis indicated that there were significant differences in the germination rate (GR) among the different concentrations of Cd and SiO, NPs and the control treatment (P<0.01). The germination rate (GR) in the 100 µM Cd+200 µM SiO, NPs, 100 µM Cd+100 µM SiO₂ NPs, and 100 µM Cd+0 µM SiO₂ NPs treatments showed 1.40-, 1.28- and 1.22-fold increases, respectively, in comparison with that of the control treatment. The germination percentage (GP) showed similar results as the germination rate. There were significant differences in germination percentage between the various concentrations of Cd and SiO, NPs and the control treatment. The results indicated that the germination percentage (GP) increased with the addition of SiO, NPs. The germination percentage (GP) in the 100 μ M Cd+100 µM SiO, NPs and 100 µM Cd+200 µM SiO, NPs treatments were 12% and 24% higher than that in the control treatment, respectively. The data analysis also revealed a significant reduction in the germination percentage (GP) in the 100 µM Cd treatment without SiO, NPs; in that treatment, the germination percentage (GP) decreased by 25% in comparison with that of the control treatment. The mean germination time (MGT) was another critical seed germination index. According to the data analysis, there were significant differences between the different concentrations of SiO₂ NPs and the control treatment (p<0.01), showing that the mean germination time increased with the addition of silicon NPs and Cd. The mean germination times were 3.9, 4.6, 4.6 and 6.5 days in the control, 100 μ M Cd+0 μ M SiO, NPs, 100 µM Cd+100 µM SiO, NPs, and 100 µM Cd+200 µM SiO, NPs treatments, respectively.

Seedling Growth Characteristics

Shoot Length and Root Length

The results obtained from the different combinations of Cd and SiO₂ NPs for the root and shoot length indicated that there were significant differences between the various concentrations of Cd and SiO₂ NPs and the control treatment (p<0.01). In treatments with a high concentration of silicon in combination with Cd (100 μ M Cd+200 μ M SiO₂ NPs), the shoot and root lengths were 24% and 55% higher, respectively, than those of the control. However, the results showed that exposure to 100 μ M Cd reduced the shoot and root length by 21% and 10% compared with the control treatment, which revealed the role of SiO₂ NPs in the reduction of Cd toxicity in the seedling root and shoot growth stages.

Seedling Biomass Characteristics

Shoot and Root Dry Weight

In this study, the seedling biomass was calculated according to the measurements of root and shoot dry weight. The data analysis of the effects of the different treatments on the root and shoot dry weight indicated that there were significant differences between the different concentrations of Cd and SiO₂ NPs and the control treatment (p<0.01). A high concentration of SiO₂ NPs resulted in the highest root and shoot dry weight, with 1.28- and 1.51-fold increases, respectively, in comparison with those of the control treatment. This result demonstrates that SiO₂ NPs could help to increase biomass in seedlings growing under cadmium toxicity. On the other hand, the results revealed that 100 μM Cd reduced root and shoot dry weight by 1.32- and 1.45-fold, respectively, compared with those in the control treatment. Moreover, this result indicated the negative impact of 100 µM cadmium on seedling biomass.

Vigor Indexes

Vigor Index (I) and Vigor Index (II)

The investigation of vigor indexes during seed germination indicated significant differences between the various concentrations of Cd and SiO₂ NPs and the control treatment (p<0.01). According to the results, SiO₂ NPs significantly increased seed vigor in bamboo seeds under cadmium stress. The data indicated that the concentrations of Cd and SiO₂ NPs enhanced vigor index (I) and vigor index (II) by 62% and 137%, respectively, compared with those of the control treatment. On the other hand, cadmium had a damaging impact on seed germination. The data showed a reduction of 41% and 44% in vigor index (I) and vigor index (II), respectively, compared with those in the control treatment.

The Measurement of Heavy Metal and Silicon Contents in the Bamboo Seedling Roots, and Leaves

It is obvious that silicon has the ability to regulate metal accumulation in leaves through mechanisms such as decreasing metal distribution (immobility) within leaves cells and the limitation of metal translocation from root to shoot, which can reduce metal toxicity in plants. Cd accumulation in the roots and leaves of the bamboo seedling displayed significant differences between the accumulated amounts of cadmium and the various levels of cadmium (p<0.01). Based on the results, the combination of 100 μ M cadmium, 100 μ M cadmium- (100 μ M SiO₂ NPs) and, 100 μ M cadmium- (200 μ M SiO₂ NPs) resulted in accumulation of 3.27 μ g, 1.56 μ g, and 0.76 μ g of Cd in the roots and 1.87 μ g, 0.91 μ g and 0.46 μ g of Cd in the leaves, respectively.

In this study, the reduction of cadmium concentration in the leaves, and roots was related to the enhanced content of SiO_2 NPs. Thus this can prove the hypothesis that SiO_2 NPs may act as an adsorbent of ions of cadmium, leading to the removal of excess levels of cadmium from the bamboo seedling organs (Table 1).

Microscopy Observation

Scanning Electron Microscopy Imaging of Cd and SiO₂ NP Absorption by Leaf and Root Surfaces in Moso Bamboo (Phyllostachys edulis)

Generally, silicon is absorbed as silicic acid from the soil mixture [43]. Silicon can participate in plant organs in different forms, including primarily as amorphous silica (Opal A; SiO₂•nH₂O) in the cell wall and lumen and as phytoliths in the intercellular space [44,45], where water evaporates from plants [46,47]. Defense mechanisms involving silicon appear throughout plants. It has been shown that the silicon in leaves is used in some leaf structures, such as epidermal hair and trichomes. It has also been reported that silicon can accumulate as amorphous silica and phytoliths in spines [48]. Therefore, in this study, we aimed to demonstrate SiO₂ NPs precipitation and accumulation on the leaf and root surfaces of Moso bamboo (Phyllostachys edulis) after seedling growth using SEM. In Figure 6, electron microscopy images show the formation of the Cd and SiO₂ NPs in glands on the leaf surface and the SiO₂ NP accumulation (white spot) in the roots of Moso bamboo (Phyllostachys edulis) seedling.

Discussion

It is clear that seed germination plays an important role in plant growth and development, as it is the first stage of plant structure formation [49]. On the other hand, it was reported that NPs might help seed germination, seedling vigor and plant photosynthesis [50-52]; it has also been reported that NPs may improve plant vigor under stress conditions [53, 54]. Among them, SiO₂ NPs can ameliorate heavy metal stress in plants [55-57] e.g. in *Pisum sativum* exposed to Cr at seedling stage [58]. The main mechanism involved in the amelioration of Cd toxicity in plants by SiO₂ NPs is related to the stimulation of antioxidant activity so silicon can scavenge ROS compounds by stimulation of antioxidant capacity [59]. Also, SiO, NPs with the formation of Si-heavy metals complexes reduces cadmium mobility via some chemical mechanisms [60]. In this study, the results obtained by our data analysis revealed that SiO₂ NPs can reduce Cd toxicity in the Moso bamboo (Phyllostachys edulis) at germination stage by increasing the germination index (GI), germination percentage (GP) and germination rate (GR). However, the results showed that SiO₂ NPs increased the mean germination time (MGT) in comparison with that in the control. Thus we hypothesized that the adsorption of cadmium ions onto SiO, NPs could decrease cadmium accumulation in the bamboo seedling leaves. On the other hand, SiO, NPs led to the restriction of cadmium translocation from the root to the shoot and leaves, which can be cited as a reason for the enhanced germination and seedling growth indexes in our study.

 SiO_2 NPs can improve plant photosynthesis and plant biomass under stress conditions [61]. Accumulation of excess heavy metals in plants can lead to significant changes in their physiological and biochemical processes, resulting in alterations of vital structures such as plant cell membranes [62, 63] and hampering the translocation and accumulation of nutritional elements within plants [64, 65,]. The alleviative impact of silicon on heavy metal toxicity in plants might be attributed to the reduction of heavy metal accumulation in plant organs, as observed in the previous studies [66,67]. This reduction of metal accumulation can

Table 1. Differential accumulation of cadmium and silicon in the leaves and roots of bamboo seedling.

Leaves			
Cadmium treatment concentration	SiO ₂ nanoparticles concentration	Cadmium accumulation	Silicon accumulation
μΜ	μΜ	μg	μg
100	0	1.88± 0.12 ^A	0
100	100	$0.91{\pm}~0.02^{\scriptscriptstyle \rm B}$	1.35±0.39 ^B
100	200	0.46±0.01 ^c	2.74±0.80 ^A
Root			
Cadmium treatment concentration	SiO ₂ nanoparticles concentration	Cadmium accumulation	Silicon accumulation
μΜ	μΜ	μg	μg
100	0	3.32±0.21 ^A	0
100	100	1.57±0.10 ^B	1.63±0.04 ^B
100	200	0.77±0.06 ^c	3.32±0.07 ^A

be ascribed to silicon-related mechanisms that block metal transfer and reduce metal uptake in plants [68]. Consequently, silicon reduces metal uptake by limiting or immobilizing metal ions in the growth media and/or influencing influx transporters, which can decrease the transport of metals from the root surface to the shoot xylem [68]. This phenomenon was observed in our study showing that Cd concentrations were significantly lower in the aerial organs of the bamboo seedling due to the presence of silicon nanoparticles in the growth medium. Nevertheless, the efficiency of this mechanism might depend on various factors, such as plant species, growth



Fig. 2. Effects of the combination of Cd and SiO₂ NPs on the germination index (GI), germination rate (GR), germination percentage (GP) and mean germination time (MGT) of moso bamboo (*Phyllostachys edulis*). The treatments included a control treatment and 100 μ M Cd alone or in combination with two levels of SiO₂ NPs (100 μ M and 200 μ M). The letters indicate statistically significant differences among the different treatments according to Tukey's test (P<0.05).



Fig. 3. Effects of the combination of Cd and SiO₂ NPs on the shoot length and root length of moso bamboo (*Phyllostachys edulis*). The treatments included a control treatment and 100 μ M Cd alone or in combination with two levels of SiO₂ NPs (100 μ M and 200 μ M). The letters indicate statistically significant differences across different treatments according to Tukey's test (P<0.05).

conditions, plant genotype, and type and concentration of element, among others [68].

The concentration of silicon increases in the root and shoot with the addition of SiO₂ NPs in the growth medium and in soil [70]. Silicon accumulation in leaf apoplasts plays an essential role as a barrier for plant protection under stress conditions [71]. Additionally, silicon in the cell membrane can regulate the cell membrane under stress conditions [72]. Moreover, enhanced binding of Cd to the cell wall leads to the translocation of Cd to vacuoles and Cd compartmentalization [73], which is one of the vital mechanisms involved in heavy metal amelioration by silicon. Silicon involved in protein synthesis increased the concentration of protein in fenugreek [74], which can improve plant photosynthesis and plant growth under stress conditions. The results obtained by our study showed that silicon increased the shoot and root length, and reduced the uptake of Cd by the roots and leaves, which, in turn, can boost the bamboo seedling growth and development under Cd stress. Silicon improves plant growth and biomass by improving cell



Fig. 4. Effects of the combination of Cd and SiO₂ NPs on the root and shoot dry weight of moso bamboo (*Phyllostachys edulis*). The treatments included a control treatment and 100 μ M Cd alone or in combination with two levels of SiO₂ NPs (100 μ M and 200 μ M). The letters indicate statistically significant differences among the different treatments according to Tukey's test (P<0.05).



Fig. 5. Effects of the combination of Cd and SiO₂ NPs on vigor index (I) and vigor index (II) in moso bamboo (*Phyllostachys pubescens*). The treatments included a control treatment and 100 μ M Cd alone or in combination with two levels of SiO₂ NPs (100 μ M and 200 μ M). The letters indicate statistically significant differences among the different treatments according to Tukey's test (P<0.05).



Fig. 6. Leaf and root surfaces from bamboo specimens exposed to the Cd and SiO₂ NP combined treatment visualized under an environmental scanning electron microscope: (a) bamboo leaf surface with an intact epidermal layer and Cd and SiO₂ NPs in glands; (b) Cd and SiO₂ NPs in glands on the bamboo leaf surface at high magnification; (c) Cd and SiO₂ NPs in glands on the bamboo leaf surface at the highest magnification; and (d) the epidermal layer of the root surface covered by Cd and SiO₂ NPs in Moso bamboo (*Phyllostachys edulis*) at high magnification.

wall extensibility [69] as well as increasing antioxidant activity in bamboo plants under Cd stress, which can lead to increased plant photosynthesis and eventually to plant growth and biomass increase. It is reported that SiO₂ NPs can raise biomass and yield quality in Cd-stressed rice through reducing Cd accumulation in roots and increasing antioxidant capacity [75]. Moreover, foliar spray or soil application of SiO, NPs are shown to enhance dry biomass of roots, shoots, grains, and spikes in wheat [76]. In this study, the results obtained by our data indicated that SiO₂ NPs application resulted in the increased seedling biomass in Moso bamboo (Phyllostachys edulis) species under Cd stress. This increase can be attributed to the role of silicon in improving plant nutritional status [75-77]. Alzahrani has reported that silicon reduces cadmium toxicity by increasing the content of K+ in wheat [78], which also showed increased uptake of S, K, Mg, and P with silicon treatment under stress conditions [12]. Therefore, we conclude that silicon improves seedling biomass in Moso bamboo (Phyllostachys edulis) under Cd stress by increasing plant nutrient uptake.

The results obtained from our data indicated that silicon could increase seedling vigor in Moso bamboo (*Phyllostachys edulis*) under Cd stress. Ali et al. (2019) reported that increased plant vigor and plant photosynthesis were related to the reduction of Cd levels in plant tissue by SiO, NPs, which can improve plant growth under Cd stress. This may have occurred in our study, so we suggest that silicon NPs improves plant vigor in Moso bamboo (*Phyllostachys edulis*) by reducing its Cd levels [76].

Conclusion

In our study, the application of SiO₂ NPs significantly ameliorated Cd toxicity by increasing the seed germination characteristics, i.e., seed germination index (GI), germination percentage (GP), germination rate (GR) and mean germination time (MGT). This enhancement in germination characteristics from the application of SiO₂ NPs improved the seedling growth parameters, i.e., shoot and root length and dry weight, as well as seed vigor indexes under Cd stress. We concluded that the accumulation of silicon in the shoots and roots protects bamboo species against Cd toxicity; so we suggest that the formation of Simetal ion complexations may be responsible for the reduction of cadmium accumulation in the roots and leaves of the bamboo seedling. However, the adsorption of ions of cadmium onto SiO, NPs could decrease metal accumulation in the roots and leaves. On the other hand, the reduction of silicon translocation from the root to shoot, as well as the stimulation of antioxidant activities by silicon can impact the reduction of cadmium stress in our bamboo seedling study. Additionally, we found that the combination of 100 μ M Cd with 200 µM SiO₂ NPs had a greater effect on the reduction of Cd toxicity in our Moso bamboo than 100 µM Cd with 100 µM SiO, NPs (Phyllostachys edulis).

Abbreviations

Germination index (GI), Germination percentage(GP), Germination rate (GR), Mean germination time (MGT).

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Author Contributions

A.E., Y.D and Y.X: statistical analysis A.E and Y.D and Z.A: writing-original draft preparation, A.E., Y.D., and Y.X: Investigation, A.E., Y.D: Supervision, Y.D: Project administration, Y.D.: Funding acquisition, Y.D.,A.E., F.M; revising and English editing of the reviewed manuscript; English editing, by American Journal Express.

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