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Effects of Nanosilica Powder from Rice Hull Ash on Seed Germination of Tomato (*Lycopersicon esculentum*)

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Abstract

Nanosilica powders were synthesized from rice hull ash (RHA) and their effects on seed germination of tomato plants were investigated. Synthesized nanosilica powder was subjected to various characterization studies for identification of the size, structure, morphology and elemental composition. Atomic force microscopy (AFM) and transmission electron microscopy (TEM) results showed that the nanoparticles were in agglomerated form with an average diameter of 46.5 nm and 40 nm, respectively. X-ray diffractometry (XRD) indicated that nanosilica powder is amorphous in nature. The nanosilica powder was also characterized as having a purity of 98.33% using EDXRF spectroscopy and having a surface area 172.19 $m^2\ g^{-1}$ using the Brunauer-Emmett-Teller (BET) method.

The study showed that nanosilica powder significantly improved germination parameters such as germination percentage, germination index, vigor index, mean germination time and average shoot length of tomato but not fresh weight and dry weight. The best results were found at $5gL^{-1}$ nanosilica powder. The increase over untreated control seeds was 22% for germination percentage, 47% for germination index, 92% for vigor index and 55% for average shoot length with the addition of 5 gL^{-1} nanosilica powder. Nanosilica powder-mediated improvement of seed germination in tomato suggests a potential application of nanosilica powder in seed germination of the plant. The study can serve as theoretical basis for further agricultural applications of nanosilica powder.

Keywords: seed germination, seedling growth, nanomaterial, nanopowder, nanosilica powder, nanotechnology, tomato (*Lycopersicon esculentum*)

Abbreviations: AFM– atomic force microscopy, BET – Brunauer-Emmett-Teller , RHA– rice hull ash, TEM – transmission electron microscopy, XRD – X-ray diffractometer, EDXRF– Energy Dispersive X-ray Fluorescence Spectroscopy

Introduction

Silicon (Si) has not yet been recognized as an essential element for plant growth (Ma & Yamaji, 2006). Although silicon is not considered an essential element, according to Datnoff, Deren, & Snyder (1997), addition of Si in soils with low Si concentration is essential to enhance plant development, growth and yield of many crop species. In several studies the beneficial effects of Si have been observed in agricultural crops by improving tolerance to drought (Ahmed, Hassen, & Khurshid, 2011; Gong, Zhu, Chen, Wang, & Zhang, 2005), resistance to pests and diseases (Côté-Beaulieu, Chain, Menzies, Kinrade, & Bélanger, 2009; Dannon & Wydra, 2004; Diogo & Wydra, 2007; Ghareeb, Bozśo, Ott, Repenning, Stahl, & Wydra, 2011; Kurabachew and Wydra, 2014), improving fruit yield (Rambo, Cardoso, Bevilaqua, Rizzethi, Ramos, Korndörfer, & Martins, 2011), and reducing the negative effects of some toxic elements in the soil (Rizwan, Meunier, Miche, & Keller, 2012; Marmiroli, Pigoni, Savo-Sardaro, & Marmiroli, 2014). A largely available source of Si is from harnessing rice hull ash (RHA) known to have high silica content (Amutha, Ravibaskar, & Sivakumar, 2010; Matori, Haslinawati, Wahab, Sidek, Ban, & Ghani, 2009). The use of RHA has both positive environmental and economic impact through the use of an abundant low-value agricultural by-product that can alleviate waste disposal problems.

Some authors reported the prophylactic effect of Si against several pathogens in tomato plants. Dannon & Wydra (2004) reported the influence of Si in reducing the disease incidence of bacterial wilt and its population density in stems of tomato while Ghareeb et al. (2011) concluded that Si in tomato plants alleviates biotic stress imposed by the pathogens. Improvements in salt tolerance by addition of Si have also been reported in tomato (Romero-Aranda, Jurado, & Cuartero, 2006).

In recent years, several studies have been conducted on the application of nanomaterials on plant germination and growth. Juhel, Batisse, Hugues, Daly, Van Pelt, O'halloran & Jansen (2011) reported that alumina nanoparticles increase the biomass accumulation of the water plant, such as an increase in root length and number of fronds per colony. Khodakovskaya, Dervishi, Mahmood, Xu, Li, Watanabe, & Biris (2009) studied the effects of carbon nanotubes on seed germination and plant growth of tomato seedlings. Carbon nanotubes were found to penetrate tomato seeds and affect their germination and growth rates. Germination was found to be significantly higher for seeds exposed to carbon nanotubes compared to control. Nair, Mohamed, Gao, Maekawa, Yoshida, Ajayan, & Kumar (2012) also studied the effects of carbon nanotubes on seed germination of the rice plant. Increase in water content was observed in seeds treated with the carbon nanomaterial during germination. Treated seedlings appeared to be healthier with well-developed root and shoot systems compared to untreated seedlings.

In view of the increasing applications and advantages of using Si in agricultural crops, this study was conducted to prepare and characterize nanosilica powder from RHA and consider the possibility of using this powder as Si source for seed germination of tomato plants. It is important to observe its effects at the initial stages of plant development. The benefits of Si in the growth and development of tomato should manifest early in the form of improved germination characteristics.

Materials and Methods

Preparation and Characterization of Nanosilica Powder

The preparation of nanosilica powder was fully described in our previous work (Lu, Peralta, Elepaño, Yaptenco, Suministrado, & Peralta, 2013). Nanosilica powder was extracted from RHA (with some modifications) and used in the study. RHA was obtained from uncontrolled burning of rice hull. RHA was digested at 95°C for 2 h in 200 mL of 0.001 N HCl to remove soot and dissolve metal oxide contaminants. The residue was ignited at 650°C for 6 h in a furnace to completely burn off any remaining sooty contaminants.

Ignited RHA samples were dissolved in 3 N NaOH solution and boiled for 3 h. The pH was adjusted to pH 2 using concentrated H_2SO_4 in such a way as to obtain the maximum yield of precipitated silica. Then, it was thoroughly washed with boiling distilled water. The mixture was filtered and the residue was dried at 120°C for 12 h to come up with a white silica powder. Silica powder was dissolved in 50 mL 2.5 N NaOH solution with continuous stirring for 10 h. Concentrated H_2SO_4 was added to adjust the pH to a range of 7.5–8.5 and the mixture was sonicated (Heat Systems-Ultrasonics, USA) for 1 h. The precipitated silica was washed with distilled water until the filtrate became alkali-free. The solution was filtered and nanosilica powder was collected after drying the residue at 50°C for 48 h.

The nanosilica powder thus obtained was subjected to various characterization studies for identification of their structure and morphology. The morphology and particle size of nanosilica powder was studied using atomic force microscopy (AFM) (XE-70, Park systems, South Korea) and transmission electron microscopy (TEM) (JEOL USA, Inc., JEM-1010, USA) operated at an accelerating voltage of 100 kV. The samples were dispersed in ethanol for 30 min before imaging. The X-ray diffractometer (XRD) (MAXima XRD-7000, Shimadzu, Japan) was employed to examine the crystalline structure of nanosilica powder using Cu K_{α} (λ = 1.5406 Å) as a radiation source, acceleration voltage of 40 kV and current of 30 mA. The diffraction angle (2θ) was scanned from 3°–90° at a rate of 1°min^{-1}.

The elemental composition of nanosilica powder in the form of oxides and the purity of synthesized nanosilica powder were determined using Energy Dispersive X-ray Fluorescence Spectroscopy (EDXRF) (EDX-720, Shimadzu, Japan). The surface area was calculated from the linear portion of the Brunauer-Emmett-Teller (BET) plot at a relative pressure (P/P_o) range 0.05–0.30, where P is the system pressure and P_o is the initial pressure. Samples were degassed at 300°C for 3 h and a six-point BET analysis was conducted using the surface area and pore size analyzer (Nova 2200e, Quantachrome, USA) to obtain the surface area of nanosilica powder.

Plant Materials and Treatments

Seeds of tomato cv. Magilas were procured from Pilipinas Kaneko Seeds Corporation, Batangas, Philippines. A laboratory experiment was performed with ten concentrations of Si. A total of 150 seeds in three replicates (50 seeds $replicate^{-1}$) were utilized for each treatment. The procedure was repeated for a second trial.

Nanosilica powder was dispersed at different concentrations in de-ionized water using a sonicator (Heat Systems-Ultrasonics, USA) at amplitude of 30 kHz for 1 hr. Silicon concentrations used in the study were; 1 g L^{-1}, 2 g L^{-1}, 3 g L^{-1}, 4 g L^{-1}, 5 g L^{-1}, 6 g L^{-1}, 7 g L^{-1}, 8 g L^{-1}, 9 g L^{-1} and 10 g L^{-1}. All treatment concentrations are in g nanosilica powder L^{-1} of de-ionized water.

Germination tests were carried out using 9-cm-diameter plastic Petri dishes (50 seeds Petri $dish^{-1}$) fitted with one-layer of Whatman No. 2 filter paper that was moistened with 5-mL of the test solution. Seeds treated with de-ionized water only served as a control. After treatment, the Petri dishes were placed in a dark room. Germination study was conducted at the Institute of Chemistry, University of the Philippines Los Baños on June–August 2014.

Measurement of Germination Parameters

Seeds were considered germinated when the radicle at least 2-mm long has protruded through the seed coat (He, Ren, Chen, & Chen, 2014; Sharma, Rathore, Srinivasan, & Tyagi, 2014; Zheng, Hong, Lu, & Liu, 2005). Germinated seeds were counted every day for 10 days. Germination percentage (GP), mean germination time (MGT), germination index (GI) and vigor index (VI) were calculated on the last day using the following equations:

GP = n/N x 100%, where n is the total number of seeds germinated and N represents the total number of tested seeds (Shi, Zhang, Yao, Wu, Sun, & Gong, 2014; Yu, Duan, Xu, Zhang, & Jin, 2014);

MGT = Σ (A x B) / C, where A is the number of seeds germinated on day B, B is the number of days counted from the beginning of germination test and C is the total number of seeds germinated at the end of the experiment (Amooaghaie, Tabatabaei, & Ahadi, 2015; Ellis & Roberts, 1981);

GI = Σ (Gt /Dt), where Gt is the number of seed germinated at t day and Dt represents the corresponding day of germination (Shi et al., 2014; Muharrem, Gamze, Mehmet, Mehmet, Sevil, Khalid, & Cemalet-Tin, 2008);

VI = mean shoot length (cm) x GP % (Abdul-Baki & Anderson 1973; Amooaghaie et al., 2015; Dhindwal, Lather, & Singh, 1991; Siddiqui & Al-Whaibi, 2014).

At the end of the germination experiment; the average shoot length, fresh and dry weights were measured. Shoot length was manually measured for all seedlings using a ruler. Seedling dry weights were determined from all the seedlings that were produced in each Petri dish. Seedlings were dried in the oven at 70°C to a constant weight.

Statistical Analysis

Data were statistically analyzed by one-way analysis of variance (ANOVA) using Minitab software, version 16 (Minitab Inc., USA). Differences among treatment means were tested for significance using the Tukey's test (honestly significant differences, HSD) at the 0.05 level (P<0.05).

Results and Discussion

Characteristic Analysis of Nanosilica Powder

The AFM and TEM analyses confirmed earlier studies of Lu et al. (2013) that the silica powder obtained using this preparation method had an average size in the nanometer range. The average diameter of nanosilica powder as determined by AFM and TEM was approximately 46.5 nm and 40 nm, respectively. The AFM and TEM micrographs of the synthesized nanosilica powder (Figure 1) showed that the particles were in agglomerated form with spherical morphology. These results are in agreement with those previously reported by Liu, Guo, Zhu, An, Gao, Wang, Ma, & Wang (2011) who reported that nanosilica from RHA has average dimensions of 40–50 nm.

Nanosilica powder was examined by X-ray Diffractometry to determine the extent of crystallinity. Crystallization is a disadvantage in preparing silicon-based materials, because silica is rendered inactive in its crystalline form (Payá, Monzó, Borrachero, Mellado, & Ordoñez, 2001). According to Ghorbani, Younesi, Mehraban, Celik, Ghoreyshi, & Anbia (2013), sharp diffraction peaks observed in XRD pattern indicate the presence of crystalline forms of prepared silica while a typical amorphous structure exhibits a hill-like peak in the range of (2θ) = 15°–30°, indicating the absence of any ordered crystalline structure and a highly disordered structure of silica. The XRD pattern of the prepared nanosilica powder shows a broad peak centered at 2θ angle =22°, which is indicative of its amorphous nature (Figure 2). This XRD pattern is in good agreement with the values for silica derived from RHA as reported in the literature (Amutha et al., 2010; Gu, Zhou, Yu, Luo, Wang, & Shi, 2015; Kamath & Proctor, 1998; Thuadaij & Nuntiya, 2008; Tzong-Horng, 2004).

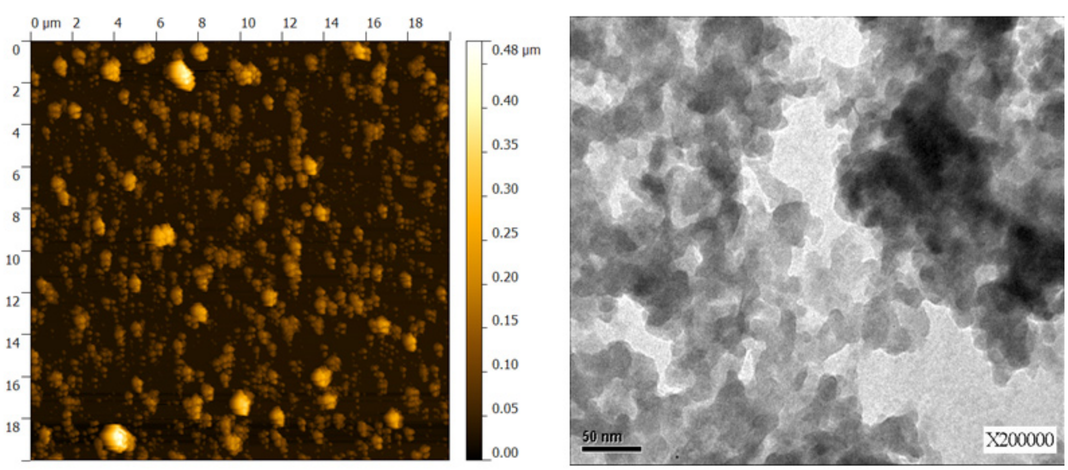

Figure 1. Images of rice hull ash nanosilica obtained using the XE-70 atomic force microscope from Park Systems (left) and the JEM-1010 transmission electron microscopy from Jeol, USA (right).

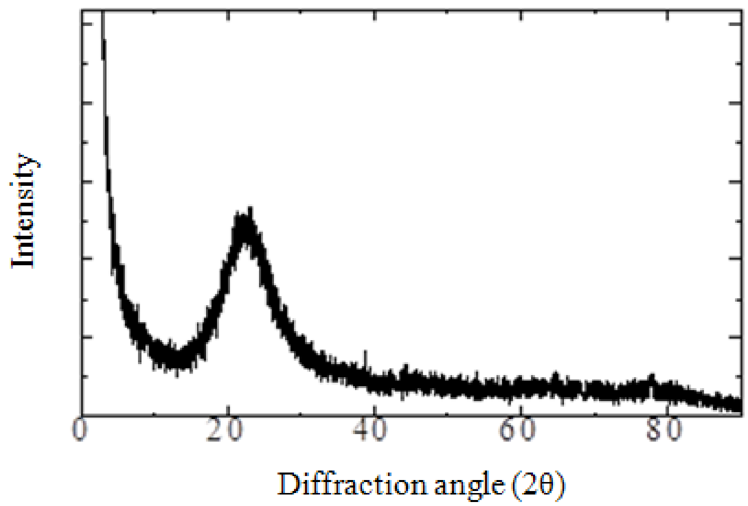

Figure 2. X-ray diffraction (XRD) pattern of nanosilica powder

The surface area is a crucial parameter for nanomaterials since materials with high surface area possess a high surface energy (Lazaro, Van De Griend, Brouwers, & Geus, 2013). Therefore, it is beneficial to tailor the surface area of nanosilica to fit the requirements of different applications (Gu et al., 2015). Figure 3 shows the BET surface area plot of nanosilica powder. The BET plot of nanosilica powder was found to be linear up to a relative pressure of 0.3 from the minimum relative pressure observed (P/Po~0.05). The surface area of nanosilica powder was found to be 172.19 $m^2\ g^{-1}$. The high value for the surface area of nanosilica powder corresponds to the values found in the literature; nanosilica treated with 2 N NaOH and 3 N NaOH with surface area 184 and 187 $m^2\ g^{-1}$, respectively (Thuadaij & Nuntiya, 2008).

The EDXRF results in Table 1 show that RHA nanosilica has a purity of 98.33% with negligible sulfur, copper and zinc contamination. Sulfur and copper have been reported to exist as impurities in synthesized nanosilica (Lu et al., 2013). The presence of sulfur as an impurity in the sample is probably due to the use of H_2SO_4 (Kamath & Proctor, 1998) for the extraction of silica/nanosilica while copper and zinc may be present in RHA (An, Guo, Zhu, & Wang, 2010;

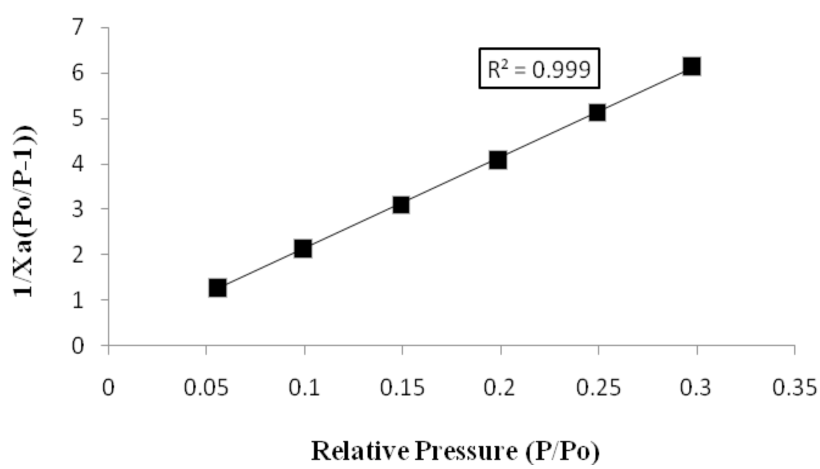

Figure 3. Brunaeur-Emmett-Teller (BET) surface area plot of nanosilica powder.

Carmona, Oliveira, Silva, Mattoso, & Marconcini, 2013; Liu et al., 2011; Matori et al., 2009) and may have not been completely removed in the purification process.

Table 1. Chemical composition of nanosilica powder using Energy Dispersive X-ray Fluorescence Spectroscopy (EDXRF).

Element	Silicon (Si)	Sulfur (S)	Zinc (Zn)	Copper (Cu)
Wt. (%)	98.33	1.50	0.09	0.08

Effects of Nanosilica powder on Tomato Seed Germination

Seed germination is a mechanism in which morphological and physiological alterations result in activation of the embryo. When the radicle has grown out of the covering seed layers, the process of seed germination is completed (Hermann, Meinhard, Dobrev, Linkies, Pesek, Heß, Macháčková, Fischer, & Leubner-Metzger, 2007). Seed germination provides a suitable foundation for plant growth, development and yield (Siddiqui & Al-Whaibi, 2014).

The germination percentage of tomato seeds treated with different amounts of nanosilica powder is presented in Figure 4. Significant differences in seed germination rate were observed among the means of samples for the different treatments at $P < 0.05$. Figure 4 shows the groupings assigned using the Tukey's method which explains that treatments that do not share a letter label are significantly different.

An increase in germination percentage was observed in nanosilica-powder-treated samples compared to control. Seeds treated with de-ionized water only yielded an average seed germination rate of 57.00%. The addition of 2 g L^{-1} already showed a significant difference from the control. The treatment with the highest number of seeds germinated was observed with the addition of 5g L^{-1} at an average seed germination rate of 70.33%. No significant effect was further observed in germination percentage of tomato by the addition of more nanosilica from 4g L^{-1} to 10g L^{-1}.

The results of the study are in agreement with other studies done using nanoparticles which enhanced percentage of seed germination: carbon nanotubes observed in tomato seedlings (Khodakovskaya et al., 2009); FITC-labeled silica nanoparticles observed in rice (Nair, Poulose, Nagaoka, Yoshida, Maekawa, & Kumar, 2011); silver nanoparticles observed in Pearl millet seeds (Parveen & Rao, 2013); nanosilica (hydrophilic fumed silica purchased from the Evonik Industries, Germany) observed in tomato (Siddiqui & Al-Whaibi, 2014); nanosilica (extracted from RHA by acid precipitation and alkali extraction methods) observed in maize seed germination (Suriyaprabha, Karunakaran, Yuvakkumar, Rajendran, & Kannan, 2012);

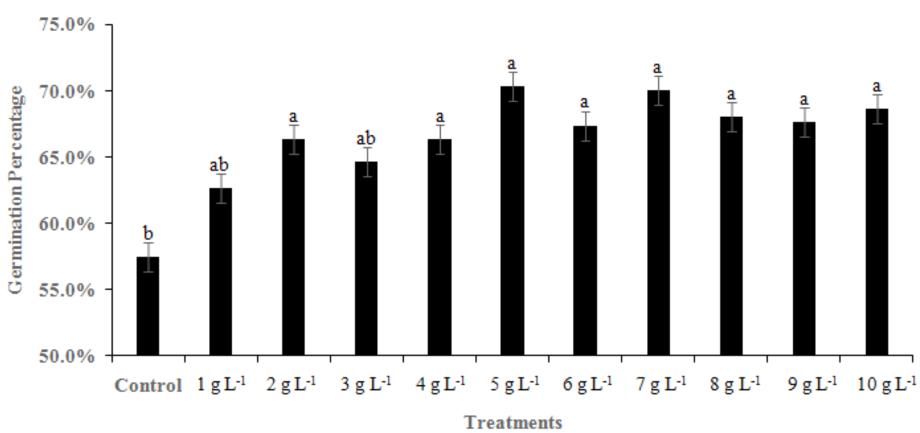

Figure 4. The effect of different amounts of nanosilica powder on germination percentage of tomato.

nanotitania effect on germination and growth of spinach seeds (Zheng et al., 2005); and graphene effects on seed germination and seedling growth of tomato (Zhang, Gao, Chen, & Li, 2015).

Our results showed that the germination percentage of tomato seeds was increased by 22% relative to the control when 5 g L^{-1} was applied. The increase in germination percentage agrees with the results of Siddiqui and Al-Whaibi (2014) who reported that 5 g L^{-1} nanosilica increased tomato seed germination by approximately 10% relative to the control. Suriyaprabha et al. (2012) concluded that an increase in germination percentage may be due to the absorption and utilization of nanoparticles by seeds. Zheng et al. (2005) also mentioned that due to small particle size, nanotitania penetrates into the seeds of spinach during the treatment period and enhances the germination characteristics of the seed. Therefore, nanosilica powders would have directly transported into tomato seeds because of its smaller size and therefore, might have improved the germination percentage of treated samples.

The result of Tukey's method for the determination of germination index is shown in Figure 5. The study showed that germination index of tomato gradually increased with the addition of RHA nanosilica powder. Compared to the control, the addition of 1 g L^{-1}–4 g L^{-1} did not lead to a significant increase in the germination index of tomato seeds. A significant difference on the germination index was noted starting with the addition of 5g L^{-1} relative to the control.

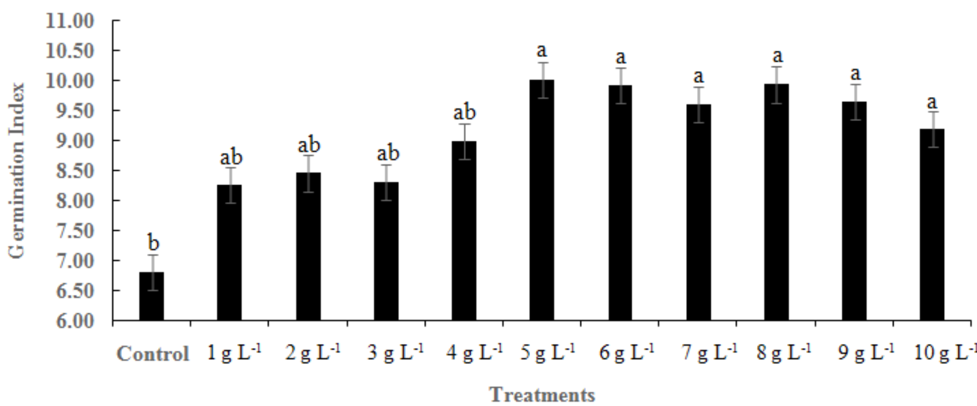

Figure 5. The effect of different amounts of nanosilica powder on germination index of tomato.

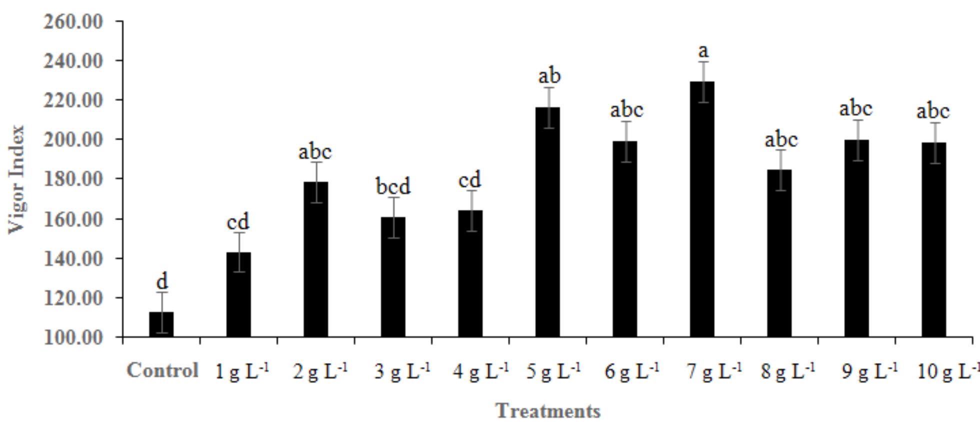

Figure 6. The effect of different amounts of nanosilica powder on vigor index of tomato.

Thereafter, no significant difference was observed with the addition of more nanosilica. Germination index was increased by 47% with the addition of 5 g L^{-1} compared to control.

According to Deng, Yuan, Feng, Ding, Song, & Wang (2014), vigor index represents the germination capacity and growing tendency of seedling. The decrease of vigor of seeds slows the plant's growth rate, reducing crop yields (Zheng et al., 2005). The results showed that the vigor index of tomato was significantly increased with the addition of nanosilica powder (Figure 6). The result of the study on vigor index is comparable with the germination percentage where the addition of 2 g L^{-1} also showed significant difference from the control; however, the level of significant change was not sustained upon further addition of 3 g L^{-1} to 4 g L^{-1} of nanosilica. Vigor index was significantly increased by 92% with the addition of 5 g L^{-1} compared to control. The addition of 7g L^{-1} yielded the largest vigor index, but no further significant increase was observed relative to the addition of 5g L^{-1} to 10g L^{-1} nanosilica. The positive effects of nanosilica on germination index and vigor index of tomato seeds observed in the study agree with the findings of Siddiqui & Al-Whaibi (2014).

The mean germination time of tomato seeds treated with different amounts of nanosilica powder is shown in Figure 7. Significant differences in mean germination time were observed among the means of samples for the different treatments. As shown in Figure 7, the addition of nanosilica powder was found to accelerate the process of tomato seed germination and significantly shortened the mean germination time. The control showed an average germination time of 5.5 days while the treatment with the lowest mean germination time was observed with the addition of 6 g L^{-1} at an average germination time of 4.4 days. No significant effect was observed in mean germination time of tomato by the addition of more nanosilica from 4 g L^{-1} to10 g L^{-1}.

Khodakovskaya et al. (2009) demonstrated that carbon nanotubes had a positive impact on the germination of tomato seeds. Carbon nanotubes were able to penetrate seed husks, causing the husks to break and thereby initiate water uptake, resulting in rapid seed germination. Zhang et al. (2015) showed that graphene sheets also penetrated the seed husks of tomato seeds which allowed water uptake inside the seeds. Therefore the results suggested that synthesized nanosilica powder would have penetrated the seed husks of tomato seeds and therefore shortened the mean germination time. Similar results have been reported for the effects on germination of nanosilica in tomato (Siddiqui & Al-Whaibi, 2014) and maize seed (Suriyaprabha et al., 2012) germination.

The results of the study on the effects of nanosilica powder on the average shoot length of tomato are shown in Figure 8. Shoot length was observed to be highest in the 7g L^{-1} treatment and the values were significantly higher than the control in the 2 g L^{-1} and 5 g L^{-1}–10 g L^{-1} treatments.

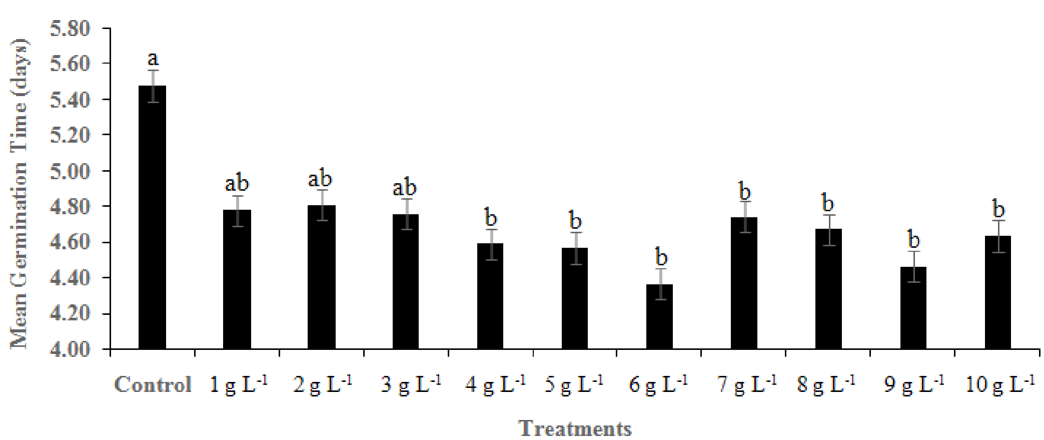

Figure 7. The effect of different amounts of nanosilica powder on mean germination time of tomato.

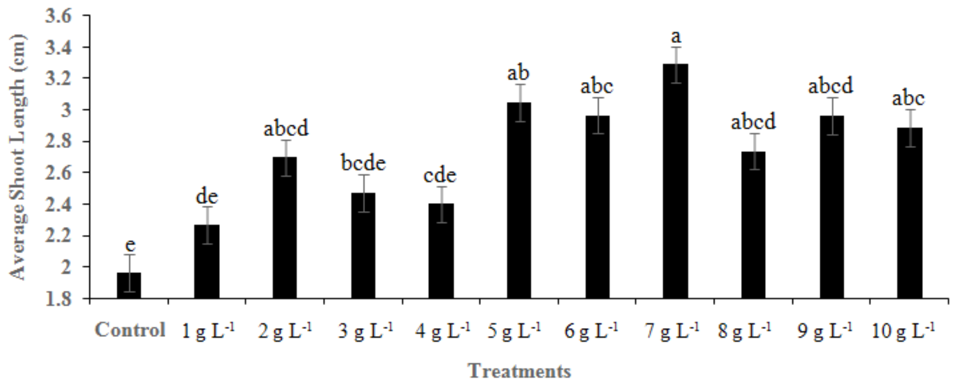

Figure 8. The effect of different amounts of nanosilica powder on average shoot length of tomato.

The effect of nanosilica powder on fresh and dry weights of tomato is shown in Table 2. The positive effects of nanosilica on fresh weight and dry weight of tomato seedlings were observed in tomato cv. Super Strain B by Siddiqui & Al-Whaibi (2014) and in maize seeds by Suriyaprabha et al. (2012). However, in this study, the addition of nanosilica powder did not significantly affect the fresh and dry weights of tomato. In agreement with this work, previous studies also reported neither positive nor negative effects of nanomaterials on fresh weight or dry weight of seedlings (Haghigni, Afifipour, & Mozafarian, 2012; Zhang et al., 2015).

Table 2. Effects of nanosilica powder applied on fresh and dry weights of tomato

Treatments	Fresh Weight (mg)*	Dry Weight (mg)*
Control	300	90
1g L^{-1}	330	88
2g L^{-1}	410	82
3g L^{-1}	460	83
4g L^{-1}	390	87
5g L^{-1}	440	87
6g L^{-1}	500	88
7g L^{-1}	460	93
8g L^{-1}	340	93
9g L^{-1}	440	95
10g L^{-1}	400	93

*Values are not significant at $P< 0.05$ level by Tukey's test

Germination percentage, germination index and vigor index are some of the most common indices of germination which are used to estimate the germinating performance and seedling growth. After assessment of germination percentage and other indices in the study, it was found that tomato seed germination was enhanced by the addition of nanosilica powder and its effects optimized in lower concentrations of nanosilica.

The best treatment should consistently prove significant differences from the control in terms of the desirable characteristics of germinated seeds, while utilizing the least amount of nanosilica. The addition of 5g L^{-1} nanosilica showed the most promising results that yielded significant differences from the control in terms of seed germination rate, germination index, vigor index, average shoot length and mean germination time. The addition of more nanosilica showed no significant effect in further enhancing these characteristics. Although the addition of 5g L^{-1} nanosilica showed no significant effect in increasing the fresh and dry weights of germinated seeds, the same was observed for all the treatments used in the experiment.

With the present study, the beneficial responses of tomato to nanosilica powder from RHA were noted to be significant for germination parameters. Therefore, the nanosilica powder-mediated improvement of seed germination of tomato suggests a potential application of the nanomaterial in the said plant. The study can serve as theoretical basis for further agricultural applications of nanosilica powder.

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