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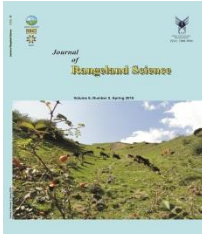

Research and Full Length Article:

Evaluation of SiO_2 Nanoparticle Effects on Seed Germination in *Astragalus squarrosus*

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Abstract. Improving seed germination rate accelerates the early seedling establishment which in turn, enhances the plant growth and forage production in rangelands and pastures. Rapid and simultaneous germination of seeds leads to successful plant establishment. The ingoing research aims to deal with the effects of SiO_2 nanoparticles at the concentrations of 0, 5, 20, 40, 60 and 80 mg/l on seed germination rate in *Astragalus squarrosus*. The experiment was conducted for 15 days under constant temperature of 20 °C for 16 and 8 hours at light and dark, respectively. The treatments were arranged as factorial ones based on a completely randomized design with four replicates in Department of Natural Resources at Ferdowsi University of Mashhad, Iran. The results of experiment showed that the germination percent of scarified seeds treated with SiO_2 nanoparticles with 40ppm concentration was improved as compared to control seeds and non-scarified ones treated with nanoparticles. At the same time, some other positive effects of other nanoparticle concentrations on germination rate and percent were obvious so that the effects of different concentrations of nanoparticles on seed germination traits of *Astragalus squarrosus* were found to be significant. The highest and lowest germination percent was recorded under the concentrations of 40 ppm and 80 ppm for those seeds treated with nanoparticles, respectively. As SiO_2 concentration increased, no enhancing positive effect on seed germination attributes of *Astragalus squarrosus* was found. In this experiment, it was found that seeds scarified and treated with 40 or 60 mg/l of SiO_2 nanoparticles showed the improved germination in *Astragalus squarrosus*; so, this treatment can be promising for the establishment and colonization of this species in natural landscapes.

Key words: Seed, Germination, Scarification, *Astragalus squarrosus*, Nanoparticles

Introduction

Nano is derived from the Greek word "dwarf" (short) that is an SI prefix meaning one billionth. Three atoms lined up are about one nanometer long (Thakkar *et al.*, 2009). Nanotechnology research is a High-Tech field which has increasingly boosted the development of electronics, biotechnology, medical, aerospace and defense industry.

To the best of our knowledge, there is paucity of literatures on the effects and mechanisms of nanoparticles on the plants growth (Zhang *et al.*, 2005). Some examples of unique properties of nanoparticles include high surface area, high surface energy and quantum confinement. Such foregoing unusual properties may even affect their environmental fate and behavior against bulk materials. Efficient seed germination and early seedling establishment are important for increasing forage production in rangeland. Rapid and homogeneous seedling emergence leads to successful establishment as it produces a deep root system before the upper layers of soil dry out, harden, or pose to adverse temperatures (Harris, 1996). However, seeds after dry storage often display slow and non-uniform germination due to the compromised vigor, especially when stored inappropriately. Moreover, the germinating seeds and young seedlings are susceptible to dehydration stress due, in part, to the progressive loss of desiccation tolerance upon seed hydration (Chen and Bradford, 2000).

Recently, some chemical substances were extremely used for the improvement of seed germination and breakdown of seed dormancy in plants. Applications of some nanomaterial can help faster plant germination/ production and effective plant protection with the reduced environmental impact as compared with traditional methods (Khot *et al.*, 2012). Silicon is a critical element for a number of metabolic and physiological plant activities. Application of silicon fertilizers in silicon deficient soil can encourage the plant growth, improve the plant resistance to disease, cold and heavy metals such as manganese, iron, aluminum and copper, and consequently enhance photosynthesis (Guo, 2000; Hu and Schmidhalter, 2005).

Plants serve as an integral part of all ecosystems, having a great contribution role in the fate and transport of nanoparticles in the environment through biological uptake and bioaccumulation. So nowadays, many efforts seek to find biocompatible production technologies based on physical treatments to increase vigor, yield and establishment of plant (Vashisth and Nagarajan, 2010).

Silicon oxide and titanium dioxide (SiO_2) are nanoparticles most commonly used in industry. One-year-old pine seedlings of *Larix olgensis* were treated with SiO_2 nanoparticles for 6 hours at concentrations of 62, 125, 250, 500, 1000, 2000 µl.l. Results showed that seedlings growth and quality were greatly improved. The best result was attributed to the treatment 500 µl.l where average length, root diameter length, main root length and number of lateral roots were increased to 42.5%, 30.7%, 14% 31.6% respectively as compared to control. Similarly, under the same treatment, the highest chlorophyll content was recorded (Lin *et al.*, 2004). Mixture of SiO_2 and TiO_2 (Titanium Dioxide) nanoparticles at low concentrations enhanced nitrate reductase activity in soybean rhizosphere and thus the germination and growth of soybean among others (Lu *et al.*, 2002). In a study, nanoparticles of palladium, copper, silicon and gold were added to soil in planting and 15 days before the seeds of lettuce. The results showed that when the soil was treated for 15 days with nanoparticles and then, the seeds were planted, higher root /shoot length ratio was observed under nanoparticles as compared to control (Shah and Belozerova, 2009).

Astragalus squarrosus belonging to Papilionaceae family is a perennial undershrub, root woody characterized with ecological adaptability and tolerance to the arid environments. Thanks to rooting depth (2 m) and horizontal root extension in the arid and semi-arid area, it plays an important role in soil erosion control and conservation as well as stabilization of dunes. In addition, its aerial branches act as windbreak. It provides palatable forage, especially in spring and early fall for livestock as sheep. Because of silvery leaves, young stems covered with white spreading hairs which remain for 9 months of the year as well as very beautiful and fragrant pink flowers can be used for landscape aesthetics along the roads and highways. *Astragalus squarrosus* is colonized through seed propagation (Mahdavi and Jouri, 2009).

To the best of our knowledge, there have been studies on nano-particles mechanisms regarding the germination and development of rangeland species (Zhang *et al*., 2005).

Nowadays, there is an increasing interest in the use of *ex vivo* synthesis of nanoparticles (NPs) for diverse purposes such as medical treatments used in various branches of industry production, and wide incorporation into diverse materials such as cosmetics or clothes (Rogers, 2005; Lee *et al.*, 2008; Lee *et al*., 2010a). They have a high surface to volume ratio that increases their reactivity and possible biochemical activity (Dubchak *et al*., 2010).

However, the interaction mechanisms at the molecular level between nanoparticles and biological systems are largely unknown (Barrena *et al*., 2009).

Also, a thorough understanding of the role of nano-sized engineered materials on plant physiology at the molecular level is still lacking (Khodakovskaya *et al*., 2011). Plants under certain conditions were reported to be capable of producing natural mineralized nano-materials (NMs) necessary to their growth (Wang *et al*., 2001).

Nano-TiO_2 treatment in proper concentration accelerates the germination of the aged seeds of spinach (Zhang *et al*., 2005) and wheat (Feizi *et al.*, 2012) in comparison to bulk TiO_2. Similarly, carbon nanotubes improve the seed germination and root growth by penetrating into the thick seed coat of tomato and support water uptake inside seeds (Khodakovskaya *et al*., 2009).

The effect of NPs on plants varies from plant to plant and species to species. With respect to the acclaimed reports on the use of nanotechnology as an emerging discipline in almost all fields of technology, it is important to understand the course of germination in relation to nanoparticles.

The recent advances in nanotechnology and its use in the field of agriculture are astonishingly increasing; therefore, it is tempting to understand the role of nano-silicon dioxide (SiO_2) in the germination of seeds. Considering available literature, the present experiment was designed to investigate the effects of SiO_2 on the characteristics of germination of *Astragalus squarrosus* seed.

Materials and Methods

Laboratory tests

Astragalus squarrosus seeds collected from rangeland of Neyshabor rangeland were obtained from the Department of Natural Resources, Khorasan Razavi province. Germination tests were carried out in two stages.

In the first step to check the quality and germination percent of *Astragalus squarrosus*, 25 intact seeds in four replications were placed in Petri-dish; then, 15-day germination test was performed with the distilled water. After 15 days, it was found that the germination percent in the intact seeds was very low (25%).

For this reason, the treatment of seed scarification and different concentrations of nanoparticles were used to improve germination attributes. In order to evaluate various concentrations of SiO_2 nanoparticles on seed germination, 12 treatments involving different concentrations of 0, 5, 20, 40, 60 and 80 mg/l SiO_2 and two scarification treatments (no scarification, scarification before adding SiO_2) in a completely randomized design with four replications were considered.

SiO_2 nanoparticles were provided from a company branch of Spain TECNAN characterized with purity 99% and the average particle size of 15-10 nm and specific surface area was 600m^2/g. Before testing, nanoparticle size was specified using a scanning tunneling microscope STM in the central laboratory of Ferdowsi university of Mashhad (Fig. 1). Its purity and composition were determined by X-ray diffraction (XRD) in Damghan University of Basic Sciences, Iran.

To obtain foregoing concentration, some SiO_2 nanoparticles were first weighed and solved in distilled water. In order to bring up uniform suspension, an ultrasonic bath for 20 minutes was used.

The suspension was prepared and poured into 2 ml per Petri dish at 25 seeds. 2 ml distilled water was added into control treatment. If needed later, distilled water was added to the dishes. Scarification was used to increase the seed germination percent (ISTA, 2009). The experiment was conducted in Department of Natural Resources and Environment Laboratory of Ferdowsi university of Mashhad in a germinator at 20°C for 16/8 h (light/dark).

To prevent moisture and extract evaporation, Petri dishes were sealed with plastics.

Germinated seeds were counted and recorded in a daily manner. Counting was continued till 15 days after seed germination.

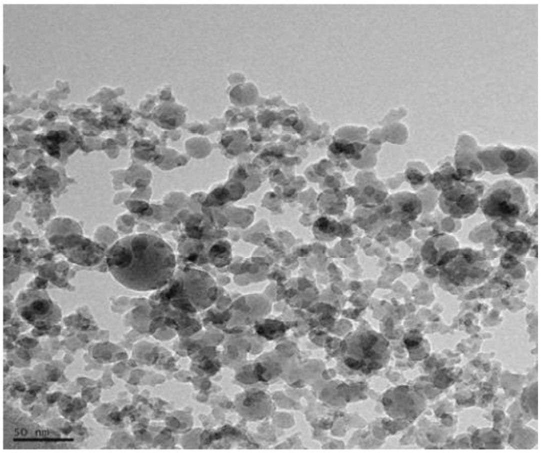

Fig. 1. Images of Nano-sized SiO_2 by Scanning Tunneling Microscope (STM)

Data analysis

The collected data were entered into Excel spreadsheet and then, analysis of variance was done using Minitab16 and means comparisons were made using Duncan method at 5% probability level.

Mean Germination Time (MGT) was calculated based on Matthews and Khajeh-Hosseini (2007) as follows (Equation 1):

$$\mathrm{MGT} = \frac{\sum F.X}{\sum F} \qquad \text{(Equation 1)}$$

Where

F = number of germinated seeds

X= hours from the beginning of germination test,

Mean Daily Germination (MDG), Pick Value (PV) and Germination Value (GV) were also calculated by the following Equations 2, 3 and 4 (Hartmann *et al.*, 1990):

$$\mathrm{MDG} = \text{Germination\% / total experiment days}$$

(Equation 2)

Pick Value =

$$\mathrm{PV} = \text{Maximum germinated seed number at one day / day number}$$

(Equation 3)

Germination Value= $\mathrm{GV} = \mathrm{PV} \times \mathrm{MDG}$

(Equation 4)

Results and Discussion

According to analysis of variance, concentration of SiO_2 nanoparticles and scarification significantly influenced seed germination attributes of *Astragalus squarrosus* (Table 1). Given this, various concentrations of SiO_2 nanoparticles significantly improved final germination percent and germination time ($p<0.01$). For the effect of scarification treatments on final germination percent and germination rate, a significant effect was observed at 1% probability level ($p<0.01$), but it had no effect on pick value (Table 1).

Similarly, results showed significant interaction effects of scarification treatments x Nano concentrations of SiO_2 for germination%, mean daily germination and pick value at 1% probability level ($p<0.01$) and germination time and germination value at 5% probability level ($p<0.05$) (Table 1).

Table 1. Variance analysis of different concentrations of SiO_2 nanoparticles and scarification on germination attributes of *Astragalus squarrosus*

SOV	Df	MS				
		Germination %	Mean Germination Time	Mean Daily Germination	Pick Value	Germination Value
Treatments (T)	1	816.75**	9.75**	4.17**	0.01^{ns}	0.84**
Nanoparticles (N)	5	1092.43**	3.37**	5.57**	0.13**	2.5**
N x T	5	61.55**	0.45*	0.31**	0.02**	0.08*
Error	36	29.17	0.29	0.15	0.01	0.05

^{ns},*,** represent non-significant, significant at probability level of 1% and 5%, respectively

Nanoparticles and scarification interaction effects

Results of analysis of variance showed a significant interaction of scarification and Nano concentrations for all the traits (Table 1). The means comparison of interaction effects is presented in Table 2.

Results showed intact seed germination percent and controls were 25%, whereas those scarified showed 33%; however, they did not differ significantly. The scarified and nano SiO_2-dipped ones were accounted for the highest germination percent given about 57% (Table 2).

Table 2. The interaction of different concentrations of SiO_2 nanoparticles + scarification on germination attributes of *Astragalus squarrosus*

Pre Chilling	SiO_2 (mg L^{-1})	Germination (%)	MGT (day)	MDG	PV	GV
Intact seed	0	25.00 ^{de}	8.33 ^{a}	1.79 ^{de}	0.18 ^{ef}	0.32 ^{de}
	5	28.40 ^{cd}	7.47 ^{b}	2.03 ^{cd}	0.25 ^{def}	0.51^{de}
	20	28.00 ^{cd}	6.98 ^{bc}	2.00 ^{cd}	0.27 ^{cde}	0.53 ^{e}
	40	41.75 ^{b}	6.20 ^{def}	2.98 ^{b}	0.57 ^{a}	1.68 ^{a}
	60	41.25 ^{b}	6.90 ^{bcd}	2.95 ^{b}	0.29 ^{cde}	0.88 ^{c}
	80	13.00 ^{f}	6.50 ^{cde}	0.93 ^{f}	0.15 ^{f}	0.14 ^{e}
Scarified seed	0	35.50 ^{bc}	7.46 ^{b}	2.54 ^{bc}	0.15 ^{f}	0.37 ^{de}
	5	40.00 ^{b}	5.90 ^{ef}	2.86 ^{b}	0.37 ^{bc}	1.08 ^{bc}
	20	36.75 ^{bc}	6.10 ^{ef}	2.63 ^{bc}	0.34 ^{bcd}	0.91 ^{c}
	40	57.00 ^{a}	6.00 ^{ef}	4.07 ^{a}	0.44 ^{b}	1.80 ^{a}
	60	41.50 ^{b}	5.70 ^{ef}	2.96 ^{b}	0.44 ^{b}	1.32 ^{b}
	80	17.00 ^{ef}	5.60 ^{ef}	1.2 ^{ef}	0.18 ^{ef}	0.23^{de}

Those values found with common letter per each column are not differed significantly ($p<0.05$)

Those scarified seeds treated with SiO_2 (nano+scarfication) showed the best results and germination rate and percent were recorded about 57% and 5.7 day (Table 2). Foregoing treatment enhanced the germination rate from 8.33 days under control treatment to 5.7 days (Table 2). The treatment characterized with scarification first followed by SiO_2 was found to be most effective in breaking the seed dormancy and improving the germination attributes as compared to the others. Although scarification is necessary to break the seed dormancy in *Astragalus squarrosus*, those seeds first scarified and then treated with SiO_2 proved further increase in germination percent as compared with non-scarified seeds. Khodakovskaya *et al.* (2009) showed that carbon nanotubes in the concentration of 10 to 40 m.l increased germination and growth of tomato probably due to the ability of carbon nanotubes to penetrate into the seed coat and stimulate water absorption. Azimi *et al.* (2013) showed that the application of bulk TiO_2 particles in 80 ppm concentration greatly decreased the majority of studied traits. Therefore, a phytotoxicity effect was observed on wheatgrass seedling by the application of bulk TiO_2 particles in 80 ppm concentration. Exposure of wheatgrass seeds to 5 ppm nano-sized TiO_2 and bulk and nano-sized TiO_2 at 60 ppm obtained the lowest mean germination time but higher concentrations did not improve the mean germination time (Azimi, *et al.*, 2013). Germination percent was increased by increasing SiO_2 concentration to 40 mg and then, decreased from 60 mg (Table 2). The highest germination rate was recorded at concentrations of 40 and 60 mg.l, respectively (Table 2). Magnetic nanoparticles at low concentrations had the inducing effects and in high concentrations, showed inhibitory effects on the growth of some plants (Racuciu and Creanga, 2007). Azimi *et al.* (2014) showed that the application of SiO_2 nanoparticles significantly increased seed germination of tall wheatgrass from 58 percent in control group to 86.3 and 85.7 percent in 40 and 60 mg L^{-1}, respectively. Applying SiO_2 nanoparticles increased dry weight of shoot, root and seedling of tall wheatgrass. Increasing concentration of nanoparticle from 0 up to 40 mg L^{-1} increased seedling weight around 49 percent as compared to the control; nevertheless, it was decreased under 60 and 80 mg L^{-1} treatments. In conclusion, seed pre-chilling in combination with SiO_2 nanoparticles largely broke the seed dormancy for *Agropyron elongatum*. Among nanoparticle concentrations, the 80 mg concentration had the highest inhibitory effect on seed germination (Azimi *et al.*, 2014). Very low concentrations of silver nanoparticles (less than 1 ppm) may be toxic to seedlings of *Arabidopsis thaliana*. Nanoparticles sized with 80-20 nm substantially stunted growth and their toxicity varied on concentration and particle size (Lee *et al.*, 2008). Zhang *et al.* (2005) showed that older spinach seed with low germination rate under TiO_2 treatment showed the 23% increase but under Nano-TiO_2 its germination rate, germination index, seedling dry weight and seed vigor index were increased significantly. Superoxide and hydroxyl ions may increase the seed permeability and facilitate entry of water and oxygen into the cell, triggering germination metabolism (Zhang *et al.*, 2005). In addition, penetration of TiO_2 nanoparticles into cells triggers redox reactions via superoxide ions radical during germination in the dark, causing the excretion of free radicals in germinating seeds. Oxygen generated in this process can be used to respiration that in turn will further accelerate germination. In case of spinach, the most suitable TiO_2 concentration treated was 2.5 ppm under which both fresh and dry weights per plant were increased about 63 and 76 percent, respectively (Zhang *et al.*, 2005).

All treatments significantly influenced all the seed germination traits. The highest germination was attributed to those scarified seeds impregnated with SiO_2 nanoparticles at concentrations of 40 (57%) and 60 mg (41.5%) and the lowest values were related to those scarified seeds dipped with SiO_2 nanoparticles at concentrations of 40 (6 days) and 60 (5.7 days) (Table 2). SiO2 changed seed germination percent in *Arabidopsis thaliana*. Rootlet length was affected by all the concentrations of nano-Al_2O_3 and concentration of 400 ml nano- SiO_2 in a positive and significant way and the concentration of nano-Fe_3O_4 and also ZnO imposed the inhibitory effects on rootlet length. Under all ZnO concentrations, small number of leaves was found (Lee *et al.*, 2010b). Khodakvfskaya *et al.* (2009) proved that 40- 10 mg/l carbon nanotubes improved tomato germination and growth probably due to the ability of carbon nanotubes to penetrate into the seed coat and stimulate water uptake.

Conclusion

In light of above discussion, the effects of different concentrations of SiO_2 nanoparticles significantly improved the germination percent and rate in *Astragalus squarrosus* at 1% probability level ($p<0.01$). At 40 and 60 mg/l, SiO_2 nanoparticles imposed stimulatory and an inhibitory effect on seed germination of *Astragalus squarrosus* at higher concentrations. High SiO_2 nanoparticle concentrations negatively affected MGT in the germination stage; however, the most suitable concentration for growth and germination was found to be 40 and 60 mg/l nanoparticle treatments. The treatment characterized with scarification first followed by SiO_2 was found to be the most effective one in breaking the seed dormancy and improving the germination attributes among the others. The application of SiO_2 nanoparticles in concentrations of 40 and 60 mg/l and scarification (scarification + nano) was accounted for the highest germination percent. As a whole, it was found that low SiO_2 nanoparticle concentrations and scarification improve germination percent and growth of *Astragalus squarrosus* so that this species can be established in field.

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بررسی تاثیر غلظت‌های مختلف نانو ذرات اکسید سیلیسیم (SiO_2) بر خصوصیات جوانه‌زنی بذر گیاه مرتعی(*Astragalus squarrosus*)

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چکیده. بهبود در سرعت و میزان جوانه‌زنی بذرها تاثیر بسیار مهمی بر استقرار نهال‌های اولیه و افزایش تولید مراتع دارد. جوانه‌زنی سریع و یکنواخت بذرها منجر به استقرار موفقیت آمیز گیاهان می‌شود. هدف از این مطالعه بررسی اثرات نانو ذرات SiO_2 در غلظت‌های ۰، ۵، ۲۰، ۴۰، ۶۰ و ۸۰ میلی گرم بر لیتر بر میزان و سرعت جوانه‌زنی بذرهای ***Astragalus squarrosus*** بود. این طرح به مدت ۱۵ روز در دمای ثابت ۲۰ درجه سانتی‌گراد در شرایط ۱۶ ساعت نور و ۸ ساعت تاریکی در ژرمیناتور دانشکده منابع طبیعی دانشگاه فردوسی مشهد در قالب طرح کاملا" تصادفی با چهار تکرار انجام شد. نتایج آزمایش نشان داد که درصد جوانه‌زنی بذرهای خراشدهی و سپس تیمار شده با نانوذرات SiO_2 به غلظت ۴۰ppm نسبت به بذرهای سالم شاهد و بذرهای سالم تیمار شده با نانوذرات افزایش داشت. همچنین تاثیرات مثبتی نیز در سایر غلظت‌های دیگر نانوذرات بر سرعت و درصد جوانه‌زنی مشاهده شد به طوری که اثر غلظت‌های مختلف نانوذرات بر خصوصیات جوانه‌زنی بذرهای گیاه مرتعی نتر معنی‌دار بود. بیشترین درصد جوانه‌زنی در غلظت ۴۰ppm و کمترین سرعت جوانه‌زنی در بذرهای تیمار شده با نانوذرات مشاهده شد. در غلظت‌های بالای نانوذرات SiO_2 تاثیرات مثبتی بر خصوصیات جوانه‌زنی بذرنتر مشاهده نشد. در این آزمایش مشخص شد که بذرهای خراشدهی و تیمار شده با نانوذرات SiO_2 می‌تواند موجب بهبود خصوصیات جوانه‌زنی بذر این گیاه مرتعی شده و در نتیجه به استقرار این گیاه در عرصه‌های طبیعی کمک بسزایی می‌کند.

کلمات کلیدی: بذر، جوانه‌زنی، خراش‌دهی، گیاه نتر، نانو ذرات