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PRE-SOWING SEED TREATMENTS WITH SILICON NANO-IRON AND NANO-SILICON PARTICLES ON GERMINATION OF DRAGONHEAD

ABSTRACT

Nanotechnology is an emerging field of science widely exploited in agriculture in recent years. In this investigation, application of nanotechnology in agriculture via application of some nano-particles (nano-iron and nano-silicon) have been investigated in seed priming of dragonhead. Seeds were subjected to pre-hydration treatments by factor nano-silicon dioxide as; (S1) 0 mM or distilled water, (S2) 1 mM concentration and (S3) 2 mM concentration and and factor nano-iron oxide as; (F1) 0 Mm or distilled water, (F2) 1 mM concentration and (F3) 2 mM concentration. Germination percent, root fresh weight, shoot fresh weight, root length, shoot length, dry weight of the seed residue, root dry weight and shoot dry weight were measured. Analysis of variance showed significant variation for the main effect of nano-silicon dioxide as well as nano-iron dioxide in root length and dry weight of the seed residue. The interaction effect of nano-silicon × nano-iron priming treatments were significant in all of the measured traits except germination percentage and root fresh weight. The highest germination percentage was recorded in S2-F3, S3-F1 and S3-F3 while the root fresh weight was high in S2-F3 and treatments S1-F1 following to S2-F3 and S3-F2 produced the highest shoot fresh weight. Also, S2-F3 has the highest root length (16.1 cm) and the highest shoot length (18.4 cm). The best treatment combination suitable for obtaining of high values of germination characteristics of dragonhead was identified as S2-F3 (1 mM nano-silicon dioxide plus 2 mM nano-iron dioxide).

Key words: germination, nanoparticle, nanotechnology, *Dracocephalum moldavica* L.

INTRODUCTION

Dragonhead (*Dracocephalum moldavica* L.), hardy annual aromatic plant is belonging to family *Lamiaceae* and the composition of its essential oil is indicating a great variation due to plant origin. The dragonhead plants flower over a 30-day period and produce nectar, which supports production of honey at the rate of 200 kg

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per hectare (Domokos *et al.,* 1994). The maximum percentage of essential oil was 0.62% during the flowering stage and the oil contained 90% of oxygenated acyclic monoterpenes (Aziz and El-Sherbeny 2003). The fresh plant contains in average 0.4% volatile oils and this content may increase depending on the elevation of the growing condition. It is used in folk medicine as a painkiller and for the treatment of kidney complaints and its extracts are used against toothache and colds as a poultice against rheumatism (Chachoyan and Oganesyan, 1996). Dragonhead is distributed in high altitudes (2000–3000 m) in central and northern regions of Iran (Rechinger, 1986) and it has been used as analgesic agent and a part of the remedy against many cancers (Moghaddam *et al.,* 2011).

The response of crops to excess environmental stresses is complex phenomenon which involves changes in their different characteristics and seed germination is one of the most critical steps for any crop under such environmental stresses. In these conditions early sowing and suitable seedling establishment can hasten plant growth and may avoid the facing of critical stages of development with terminal environmental stresses (Janmohammadi *et al.,* 2013). Poor crop establishment was identified as a major constraint on crop production while seed priming would improve seed germination, plant growth, and crop yield (Ashraf and Foolad, 2005). Pre-sowing seed treatments are applied by various methods for enhancing pre- and post-germination activities of seeds in most crops. Priming is adjusting the hydration level within seeds to permit seedlings to emerge more rapidly and the main principle of this treatment is a controlled seed hydration to a point (Basra *et al.,* 2006; Hu *et al.,* 2005). Most priming efforts involve imbibing seed with constrained amounts of water to allow adequate hydration and progress of plant physiological processes.

Nanotechnology is becoming an important subject in agriculture, as additives, growth stimulators and nano-fertilizers and it appears that their utilization could increase efficiency and lead to more environmentally sound applications. Nanoparticles can easily enter into plant system by overcoming the cell wall barrier in comparison with bulk materials and they have achieved greater consideration because of their highly reactive surface-to-volume ratio property (Lyons *et al.,* 2011). Behavior of nanoparticles inside plant system is unpredictable and among various nanomaterials there were lots of interests in effect of nano-silicon dioxide on plant growth. It is probably due to its beneficial effects on plant growth and production such as activation of some defense mechanisms (Liang *et al.,* 2007). It has an important function in plant protection due to its role in physiological barrier (Ma and Yamaji, 2008). Nano-iron oxide particles facilitate the photosynthate and iron transferring to the leaves of peanut (Liu *et al.,* 2005) and the use of nano-iron oxide increase total iron concentration and total absorption of iron in soybean (Sheykhbaglou *et al.,* 2011). Seed prehydration by nano-silicon dioxide and nano-iron oxide can be considered as a seed vigour enhancement treatment.

The use of nano-particles has given a lot of interesting by different investigators especially by those studying seed properties and some useful nano-particles were reported to have a useful effect in plants such as by nano-silicon dioxide and nano-iron oxide. Although the earlier investigations revealed that some advantages of seed priming for vigour improvement, there is dearth of information about the germination performance of primed seeds of dragonhead with nano-materials. Therefore, it was essential to evaluate the application of nano-silicon dioxide and nano-iron oxide particles in order to improve dragonhead seed germination capability. The purpose of present study was to investigate the effect of seed priming in nano-silicon dioxide and nano-iron oxide particle treatments on seed germination properties of dragonhead to find out the most promising concentration.

MATERIALS AND METHODS

Uniform seeds of dragonhead were immersed in a 5% sodium hypochlorite solution for 5 min to ensure surface sterility and washed in distilled water and their primary germination rate was greater than 90%. Seeds were subjected to pre-hydration treatments by soaking in three levels of factor nano-silicon dioxide as; (S1) 0 mM nano-silicon dioxide or distilled water, (S2) 1 mM nano-silicon dioxide concentration and (S3) 2 mM nano-silicon dioxide concentration and three levels of factor nano-iron oxide as; (F1) 0 Mm nano-iron oxide or distilled water, (F2) 1 mM nano-iron oxide concentration and (F3) 2 mM nano-iron oxide concentration. Of 50 g seeds in each treatment were placed between two wetted germination papers and temperature was maintained at 20 °C and were retrieved at 4 hours in the solutions. At the end of treatment superficial water present on seed surface was removed and seeds were dried back to their original moisture content under shade. One piece of filter paper was put into each 100 mm × 15 mm Petri dish, and 10 mL of each test solution was added to each experimental sample. Forty-five seeds were selected and placed in each Petri dishes and then were covered and sealed with tape, placed in an incubator (with 16/8 h photoperiod, 20±2 ºC temperature and relative humidity of 75%). Nano-silicon dioxide and nano-iron oxide particles were prepared from Pishgaman of *Nano-Materials Company, Iran*. The result of large area TEM image of the used nanoparticles (Fig. 1) is displayed.

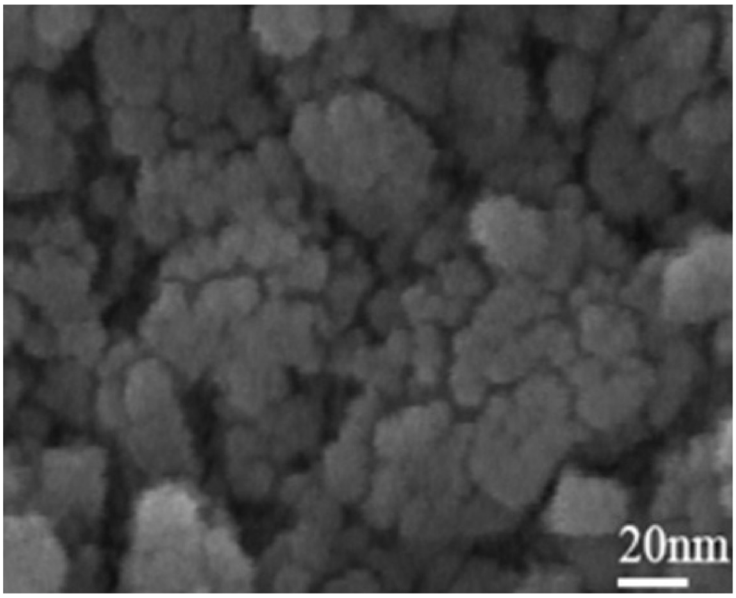

Fig. 1. Scanning Electron Microscope (SEM) image of synthesized nanoparticles of ferric oxide

Daily observation for germinating seed continued for ten days and germination percent (GP) was calculated which the seeds were considered to be germinating at the moment of radical emergence (1-2 mm in length). All evaluations were executed as described in Seedling Evaluation Handbook (AOSA, 1991). Also, RFW, root fresh weight (g); SFW, shoot fresh weight (g); RL, root length (cm); SL, shoot length (cm); DWS, dry weight of the seed residue; RDW, root dry weight (g); And SDW, shoot dry weight (g) were recorded. Each treatment combination (nano-silicon dioxide × nano-iron oxide) was conducted with five replications. The fresh weight of seedling roots and shoots was determined by weighing the roots and the shoots separately on electric balance. After the fresh weight was taken, the seedlings were kept in a hot air oven at 60°C for 48 h and then the weight of dry matter was recorded. The data was analyzed employing one way ANOVA according in factorial experiment layout in a complete randomized design with SAS 9.1 (SAS, 2004) software and the means compared by least significant differences (LSD) method ($P < 0.05$). The datasets were tested for normality by the Anderson and Darling normality test (Minitab, 2005).

RESULTS AND DISCUSSION

Analysis of variance showed significant variation for the main effect of nano-silicon dioxide seed priming treatments in root fresh weight (RFW), root length (RL), dry weight of the seed residue (DWS) and root dry weight (RDW) at 5% probability level (Table 1). Also, we found significant differences for the main effect of nano-silicon dioxide seed priming treatments in germination percentage (GP) and shoot length (SL) at 1% probability level while there were not any significant variation in shoot fresh weight (SFW) and shoot dry weight (SDW). The main effect of nano-iron dioxide priming treatment was significant in shoot fresh weight (SFW), root length (RL), dry weight of the seed residue (DWS) and shoot dry weight (SDW) but it was not significant for germination percentage (GP), root fresh weight (RFW), shoot length (SL) and root dry weight (DW). The interaction effect of nano-silicon × nano-iron priming treatments were significant in all of the measured traits except GP and RFW (Table 1). The present findings seem to be consistent with other researches, which found that the significant beneficial influences of the nano-silicon particles on seed germination properties of lentil and sunflower (Sabaghnia and Janmohammadi, 2014; Janmohammadi and Sabaghnia, 2015).

Table 1

Analysis of variance for the measured traits of dragonhead (*Dracocephalum moldavica*)

SOV	DF	GP	RFW	SFW	RL	SL	DWS	RDW	SDW
Nano-Si (S)	2	54.2^{*}	61.77^{**}	0.29^{ns}	34.1^{**}	49.0^{*}	15.824^{**}	0.350^{**}	0.153^{ns}
nano-Fe (F)	2	39.8^{ns}	7.81^{ns}	11.32^{*}	29.3^{*}	26.0^{ns}	20.857^{**}	0.099^{ns}	1.322^{**}
S× F	4	7.4^{ns}	12.25^{ns}	12.60^{**}	36.5^{**}	43.0^{*}	16.755^{**}	0.178	0.607^{**}
Error	36	16.6	6.42	3.06	5.9	14.7	0.598	0.065	0.119
CV		10.0	25.7	17.4	18.6	25.7	5.0	27.8	34.6

Considering the significance of the interaction effect in most traits, the mean comparison was performed only for treatment combinations (three levels of nano-silicon dioxide × three levels of nano-iron dioxide). The highest values of germination percentage were recorded in seeds primed in S2-F3 (1 mM nano-silicon dioxide plus 2 mM nano-iron dioxide), S3-F1 (2 mM nano-silicon dioxide plus 0 mM nano-iron dioxide) and S3-F3 (2 mM nano-silicon dioxide plus 2 mM nano-iron dioxide) (Table 2). The lowest values of germination percentage were recorded in seeds primed in S1-F2 (0 mM nano-silicon dioxide plus 1 mM nano-iron dioxide). However, priming at high concentration of nano-silicon as well as nano-iron particles could improve seed germination percentage. The beneficial effects of nano-silicon dioxide on the seed germination of tomato is reported by Siddiqui and Al-Whaibi (2014.) An increase of seed germination and plant growth factors of spinach was observed at nano-TiO_2 treatment (Zheng *et al.,* 2005). According to El-Temsah and Joner (2012), positive impact of iron nanoparticles on seed germination characteristics in aqueous suspension is demonstrated.

Table 2

Mean comparison of the measured morphological traits of dragonhead (*Dracocephalum moldavica*) for three levels of nano-silicon (Si) and three levels of nan-iron (Fe)

Si	Fe	GP		RFW		SFW		RL	
0 mM Si	0 mM Fe	90.7	AB	9.8	BC	12.7	A	14.7	AB
	1 mM Fe	81.3	B	6.4	D	8.0	D	6.9	C
	2 mM Fe	88.0	AB	7.7	CD	9.7	BCD	12.5	B
1 mM Si	0 mM Fe	89.3	AB	12.0	AB	9.7	BCD	13.6	AB
	1 mM Fe	86.7	AB	10.4	BC	8.8	CD	12.8	B
	2 mM Fe	96.0	A	13.7	A	11.3	AB	16.1	A
2 mM Si	0 mM Fe	97.3	A	9.5	BCD	10.1	BCD	12.4	B
	1 mM Fe	92.0	AB	10.4	BC	10.5	ABC	14.8	AB
	2 mM Fe	96.0	A	8.9	BCD	9.8	BCD	13.8	AB

Si	Fe	SL		DWS		RDW		SDW	
0 mM Si	0 mM Fe	17.1	AB	13.7	E	0.89	B	0.95	BCD
	1 mM Fe	9.7	D	19.5	A	0.53	C	0.51	D
	2 mM Fe	11.7	CD	16.3	B	0.90	B	1.84	A
1 mM Si	0 mM Fe	15.6	ABC	15.7	BC	1.27	A	0.95	BCD
	1 mM Fe	13.4	BCD	15.6	BC	0.93	B	0.86	BCD
	2 mM Fe	18.4	A	13.3	E	1.04	AB	1.19	B
2 mM Si	0 mM Fe	14.7	ABC	15.0	C	0.83	BC	0.73	CD
	1 mM Fe	17.1	AB	14.9	CD	1.04	AB	0.98	BC
	2 mM Fe	16.5	ABC	13.9	DE	0.79	BC	0.98	BC

The root fresh weight (RFW) of dragonhead seedlings (Table 2) increased with increase in priming with 1 mM nano-silicon dioxide, but decreased with increase in priming with 1 mM nano-iron dioxide. In general, the highest values of root fresh weight was recorded in seeds primed in S2-F3 (1 mM nano-silicon dioxide plus 2 mM nano-iron dioxide) with 13.7 g weight while the lowest values of root fresh weight was recorded in seeds primed in S1-F2 (0 mM nano-silicon dioxide plus 1 mM nano-iron dioxide) with 6.4 g weight (Table 2). Treatment combination S1-F1 (control, without any nano-particles) following to S2-F3 (1 mM nano-silicon dioxide plus 2 mM nano-iron dioxide) and S3-F2 (2 mM nano-silicon dioxide plus 1 mM nano-iron dioxide) produced the highest shoot fresh weight (SFW) as 12.7, 11.3 and 10.5, respectively. These results confirm the earlier study on improved root and shoot fresh weight by utilization of nano-silicon in germination of tomato seeds (Siddiqui and Al-Whaibi, 2014) and seed properties of sunflower (Janmohammadi and Sabaghnia, 2015).

The results of Table 2 showed that pre-hydration treatment of seeds before germination significantly influenced root length (RL) and demonstrated that S2-F3 (1 mM nano-silicon dioxide plus 2 mM nano-iron dioxide) has the highest root length (16.1 cm) while S1-F2 (0 mM nano-silicon dioxide plus 1 mM nano -iron dioxide) has the lowest root length (6.9 cm).

Also, the highest shoot length (18.4 cm) was seen in S2-F3 (1 mM nano-silicon dioxide plus 2 mM nano-iron dioxide) while the lowest shoot length (9.7 cm) was observed in S2-F3 (1 mM nano-silicon dioxide plus 2 mM nano-iron dioxide). Therefore, for obtaining the high values of root and shoot length of dragonhead, 1 mM nano-silicon plus 2 mM nano-iron treatment combination could be useful. These results confirm the pervious investigations' results on improved root and shoot length by utilization of nano-iron (El-Temsah and Joner, 2012) and nano-silicon (Siddiqui and Al-Whaibi, 2014; Janmohammadi and Sabaghnia, 2015).

Dry weight of the seed residue (DWS) is an index of the weight of utilized reserves of cotyledons and shows how much of this reserves is utilized by seedling. According to Table 2, control (without any nano-particles) and S2-F3 (1 mM nano-silicon dioxide plus 2 mM nano-iron dioxide) with 13.7 and 13.3 g had the lowest reserves and have used this reserve efficiently but S1-F2 (0 mM nano-silicon dioxide plus 1 mM nano-iron dioxide) treatment could not use its reserves of cotyledons and had the highest amounts (19.5 g). The increase of the weight of utilized reserves caused to increase of the other seedling characteristics like fresh and dry weight of shoot and root as well as length of shoot and root. Similarly, our findings are in agreement with the report of Soltani *et al.,* (2006) and Sabaghnia and Janmohammadi (2014).

Root dry weight (RDW) had the highest values (1.27 g) for S1-F2 (0 mM nano-silicon dioxide plus 1 mM nano-iron dioxide) treatment and had the lowest values (0.53 g) for S2-F1 (1 mM nano-silicon dioxide plus 0 mM nano-iron dioxide) treatment (Table 2). The highest values of shoot dry weight (SDW) were recorded in seeds primed in S1-F3 (0 mM nano-silicon dioxide plus 2 mM nano-iron dioxide), while the lowest values of shoot dry weight (SDW) were recorded in seeds primed in S1-F2 (0 mM nano-silicon dioxide plus 1 mM nano -iron dioxide) (Table 2). According to Asadi *et al.,* (2016), the shoot and root dry weight were increased under salinity stress by applying nano-zinc oxide particles. Applying SiO_2 nanoparticles increased dry weight of shoot and root in seedling of tall wheatgrass (*Agropyron elongatum* L.) (Azimi *et al.,* 2014).

We did not find any interaction effects between nano-SiO_2 and nano-iron on germination percentage and root fresh weight of dragonhead. Application of silica nanoparticles largely increased seedling, root and shoot elongation as compared to the control. Use of 1 mM nano-silicon dioxide plus 2 mM nano-iron dioxide concentration enhanced most of the seed germination characteristics of dragonhead around 22% more than other treatment combinations. In other word, S2-F3 treatment enhanced germination percentage around 6.5%, root fresh weight around 45.9%, shoot fresh weight around 14.0%, root length around 26.9%, shoot length around 27.1%, dry weight of the seed residue around -14.6%, dry weight of the seed residue around 15.9% and shoot dry weight around 22.1% more than average of the other treatment combinations. Lin *et al.,* (2004) demonstrated that treatment of Changbai larch seedlings by silicon dioxide nanoparticles significantly increased mean height and root traits. Seed priming with SiO_2 nanoparticles largely broke the seed dormancy for tall wheatgrass, hence it seems that nanoparticles could be an alternative potential for breaking of seed dormancy (Azimi *et al.,* 2014).Clement *et al.,* (2012) reported that the soaking of flax seeds in the suspensions of nanoparticles increased seed germination and root growth and these positive effects could be due to antimicrobial properties of TiO_2 that increase plant resistance to stress. Feizi *et al.,* (2013) demonstrated that use of TiO_2 nanoparticles enhanced fennel seed germination, while it decreased from exposure to bulk TiO_2 compared to the control.

We believe that high concentration of SiO_2 nanoparticles (2 mM) could be toxic for dragonhead seed, therefore high concentration adversely affected on seed performance. In previous work, it was demonstrated that using nano-sized TiO_2 in low concentration could encourage seed germination of lentil in comparison to untreated control, but in high concentrations it had an inhibitory or no effect on seed (Sabaghnia and Janmohammadi, 2014). Our results suggested that response of dragonhead germination to pre-hydration at various concentrations of nano-silicon and nano-iron solution were very different which indicates the influences of the nanoparticles on early seedling growth of plants are almost unpredictable (Khodakovskaya *et al.,* 2011). Exogenous application of bulk silicon at high concentration considerably improved germination characteristics of borage seeds (Torabi *et al.,* 2012), our findings emphasized that the best result could be obtained at low concentration of nano-silicon. Previous reports have suggested that nano-silicon and nano-iron application may alleviate the adverse effects of salinity stress on seed germination (Haghighi *et al.,* 2012; Shi *et al.,* 2014; Sabaghnia and Janmohammadi, 2015) and increase water-use efficiency in plants (Ma, 2004).

One of the most important applications of nanotechnology in agriculture is using nano-particles for priming crops under environmental and biological stresses. There is awareness created on the risks of consuming few operations rather than the benefits of the technology and in spite of all these drawbacks there is continuous research carried out in nanotechnology, there will be a day which will come in near future for an accepted nanotechnology. Exploiting the unique properties of nanoparticle nutrient formulations could be an effective strategy for using in agriculture. Another study examining a variety of crops noted that nano-ZnO increased seed germination while a bulk form of ZnO used for comparison had a negative impact on germination (Singh *et al.,* 2013). In the present study, we found that pre-sowing treatment with nano-silicon plus nano-iron particles could invigorate the germination of dragonhead. However, to the present, very low is understood about physiological roles of nano-silicon and nano-iron in improvement of seed germination and further research should be done to investigate the interaction between nano-silicon and nano-iron with seed at cellular and molecular levels.

CONCLUSIONS

Applications of nano-materials can encourage earlier seed germination and improve crop production of dragonhead. To our knowledge, this effort is the first information related to the effects of SiO_2 and iron nanoparticles on dragonhead. A significant increase in seed germination percentage, root fresh weight, shoot fresh weight, root length, shoot length, dry weight of the seed residue, root dry weight and shoot dry weight were found by priming with SiO_2 and iron nanoparticles suggest that nanoparticles may be contributed in the metabolic or physiological activity in dragonhead seed. Consequently, it has proposed that applying nanoparticles with treatment combination S2-F3 (1 mM nano-silicon dioxide plus 2 mM nano-iron dioxide) could use as a new alternative potential for seed germination properties in dragonhead.

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