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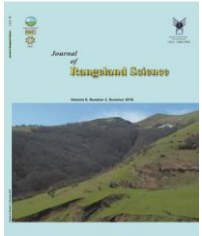

Research and Full Length Article:

Effects of Silica and Silver Nanoparticles on Seed Germination Traits of *Thymus kotschyanus* in Laboratory Conditions

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Abstract. The introduction of nanoparticles into seed germination and seedling growth of plants might have a significant impact and thus, it can be used for agricultural applications for better growth and yield. The purpose of this study was to compare the effects of silica and silver nanoparticles on seed germination and early growth traits as well as percent and rate of germination, root length, shoot length, seedling fresh and dry weight and seed vigor index of *Thymus kotschyanus*. Experiment was conducted using a completely randomized design with four replications in winter 2014. Seed sources were from Sabalan rangelands, Ardabil province, Iran. Treatments were control (distilled water) and silica and silver nanoparticles with the concentration of 20 and 60%. Thirty seeds were sown in each Petri dish. Seed germination began from the fourth day after sowing and they were counted every day until germination was stopped. Seed germination was controlled for 14 days. The statistical analyses were conducted using analysis of variance (ANOVA). Duncan test was performed to examine the differences between the treatments. Results showed that the germination of *T. kotschyanus* was strongly affected by nano-silver treatments in comparison with nano-silica and control treatments. Overall, higher values of seed germination traits were observed in nano-silver (20%). Moreover, increasing silica nanoparticle concentration had enhanced the seed germination. In contrast, the increase of silver nanoparticle concentration had decreased the germination traits.

Key words: Nanoparticles, Seedling growth, Seed germination, Vigor index, *Thymus kotschyanus*

Introduction

Nano-technology has emerged as a new discipline, and nanoparticles have become a center of attraction for researchers because of its unique physico-chemical properties as compared to their bulk particles (Monica and Cermonini, 2009). There is an increasing interest in the use of *ex vivo* synthesis of nanoparticles for diverse purposes such as medical treatments, use in various branches of industry production, and wide incorporation into diverse materials such as cosmetics or clothes (Lee *et al*., 2008; 2010b). Nanoparticles are microscopic particles with at least one dimension less than 1000 nm. For this, these particles are very attractive materials to handle in biological system. Nanoparticles are found to be very suitable in sensing and detection of biological structures and systems (Singh *et al*., 2008). Nanoparticles show a promise in different fields of agricultural biotechnology (Majumder *et al*., 2007). Agricultural application of nanoparticles is currently an interesting area of interest (Majumder *et al*., 2007; Lee *et al*., 2008; Siddiqui and Al-Whaibi, 2014). The introduction of nanoparticles into plants might have a significant impact and thus, it can be used for agricultural applications for better growth and yield (Josko and Oleszczuk, 2013). However, a thorough understanding of the role of Nano-sized engineered materials in plant physiology at the molecular level is still lacking (Khodakovskaya *et al*., 2011) whereas the action mode of nanoparticles on plant growth and development is still too scarce. Plants under certain conditions were reported to be capable of producing natural mineralized nano-materials necessary to their growth (Wang *et al*., 2001). As we know, seed germination provides a suitable foundation for plant growth, development and yield (Siddiqui and Al-Whaibi, 2014). Plants generally require silica to control biotic and abiotic stress (Ma, 2004). Silicon is one of the beneficial elements on plant growth under biotic and abiotic stresses. Some studies reported that silicon could ameliorate salt stress depression on plant species (Adatia and Besford, 1986; Wang *et al*., 2010; Wang *et al*., 2011; Zuccarini, 2008). Nano-silica is an important metal-oxide that covers all major fields of science and technology including industrial, electronics and biomedical applications (Paulkumar *et al*., 2011). Silica nanoparticle acts as a delivering agent that delivers DNA and chemicals into plants as well as animal cell and tissue (Torney *et al*., 2007). It has gained a greater attention because of its highly reactive surface-to-volume ratio property (Cheng *et al*., 2008). Short-term influence of silica, palladium, gold and copper nanoparticles on a soil microbial community and the germination of lettuce seeds are investigated at different concentrations of nanoparticles. Shoot and root ratio of lettuce seeds significantly reduced as compared to control when exposed to nanoparticle solution (Mazumdar, 2014). Nanoscale titanium dioxide was reported to promote photosynthesis, and growth of spinach (Hong *et al*., 2005; Yang *et al*., 2006). Similarly, mixture of nanoscale SiO_2 and TiO_2 had a significant effect on germination and growth of soya bean (Lu *et al*., 2002). Manzer *et al*. (2014) reported that nano-SiO_2 had a significant impact on the tomato seed germination potential. Silver nanoparticles are also interested because of the unique properties (e.g., size and shape depending optical, electrical, and magnetic properties) which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components (Senapati, 2005; Klaus-Joerger *et al*., 2001). Silver-based materials have been widely used over the last decades within medicine, electronic, photography, etc. (Ratte, 1999). Silver nanoparticles are currently receiving considerable attention because of their numerous applications in consumer products. By far, the greatest number of consumer product applications of nano-materials involves nano-silver effect of silver (El-Temsah and Joner, 2010). Silver nanoparticles may hold significant applications in agriculture and gardening by selectively inhibiting harmful fungi and bacteria on seeds and could provide an alternative source of fertilizer that may improve sustainable agriculture (Parveen and Rao, 2014). Nanoparticles on the reduction of biomass and transpiration rate were reported in *Cucurbita pepo*. The adverse effect on *C. pepo* was more prevalent in nanoparticles than bulk silver solutions (Musante and White, 2012). Zhu *et al*. (2008) reported that *Cucurbita maxima* growing in an aqueous medium containing magnetic nanoparticles can absorb, move and accumulate the particles in the plant tissues whereas *Phaseolus limensis* is not able to absorb and move particles. It indicates that different plants have different responses to the same nanoparticles.

According to the problems with the germination of some plants and over grazing, the rate of forage production is greatly reduced. Thus, to take the advantages of such plants, it is necessary to identify and remove barriers of germination and establishment of suitable plants (Tavili *et al*., 2014). One of these plants is *T. kotschyanus* which has medicinal values and it is important for beekeeping and food production (Shahraki *et al*., 2015). *T. kotschyanus* which is from the genus *Thymus* is belonged to mint family (Lamiaceae). It is a wooden, short, perennial and grey colored plant with C_3 metabolism system with 20-40 cm tall depending on the climate of growth region and soil quality (Khorshidi *et al*., 2010). *T. kotschyanus* plays an important role in the economy of local people at most regions where its cultivation helps the regional economy (Darvishi *et al*., 2013). However, under the overgrazing and germination problem, there is evident of the reduction of that in many rangelands such as Sabalan rangelands in Ardabil province, Iran. *Thymus* spp. has a medicinal value which is widely used since many years ago (Darvishi *et al*., 2013; Tavili *et al*., 2014; Shahraki *et al*., 2015). They are fragrant, prominent and renowned species in terms of quality and quantity (Darvishi *et al*., 2013). Therefore, this study aimed to compare the effects of silica and silver nanoparticles on seed germination traits of *T. Kotschyanus,* and also to examine the effectiveness of using nano-particles in the accelerating plant growth and development.

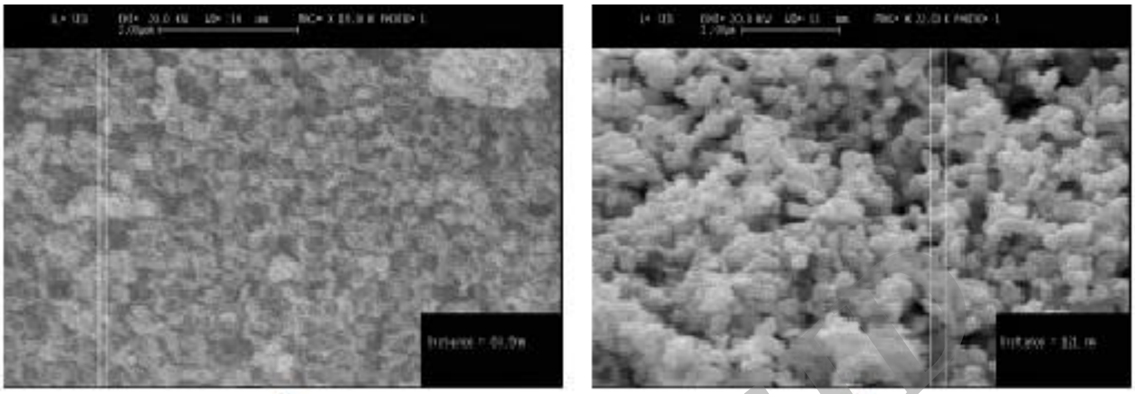
Materials and Methods

Nano-particle suspension

As the aim was to evaluate the effects of silica and silver nanoparticles on *T. kotschyanus* seed germination and seed vigor index, the experiment was conducted in a completely randomized design with four replications in winter 2014 at the Rangeland laboratory of Agricultural Technology and Natural Resources College in the University of Mohaghegh Ardebili, Ardabil, Iran. Treatments were control (distilled water) and Silica and silver with the concentrations of 20 and 60% for each nanoparticle (Azimi *et al*., 2014). Thirty seeds were placed in each Petri dish. The seeds were collected from Sabalan rangelands, Ardabil, Iran. Seed viability, purity and seed moisture content of *T. kotschyanus* were 50, 96 and 10%, respectively. In addition, 1000 seed weight (gr) of it was 0.18 g.

Initially, the seeds were disinfected with a solution of sodium hypochlorite with 10% for 30 seconds, and then, were rinsed with distilled water. Seeds for the treatments of nanoparticles were placed in different nano-solutions for 2 h, and after washing them with distilled water, they were sown on a filter paper in Petri dishes and seeds were irrigated with 10 ml of distilled water (Bekhrad *et al*., 2015). The experiment was performed in a germinator with an average temperature of 25/15±1ºC for 8/16 h in darkness and light, respectively.

The characteristics of silica and silver nanoparticles were subjected to the identification and morphology that are given in Fig. 1, respectively. The morphological study of these nanoparticles was done by scanning electron microscope (SEM).



a b

Fig. 1. Scanning electron microscopy (SEM) image of Silica nanoparticles (a) and Silver nanoparticles (b)

Seed germination traits

Seed germination began from the fourth day after cultivation and the germinated seeds were counted and recorded on the same day. Exiting of 2 mm root length was the germination criterion (Seyedi *et al*., 2002). Seed counting continued for 14 days (Haghighi *et al*., 2012). On the tenth day, the shoot and root of seedlings were measured using a ruler. Seedling fresh and dry weights were recorded using a digital scale.

Germination percent (GP) was calculated using the equation 1 (Parveen and Rao, 2014) and the Germination Rate (GR) was calculated using the equation 2 (Panwar and Bhardwaj, 2005). Vigor index (Vi) was calculated using the equation 3 (Vashisth and Nagarajan, 2010). Mean germination time (MGT) which is an indicator of the speed and acceleration of germination was calculated based on equation 4 (Ellis and Roberts, 1981).

$$GP = \frac{ni}{N} \times 100 \qquad (1)$$

$$GR = \sum \frac{ni}{t} \qquad (2)$$

$$Vi = GP \times (rl + sl) \qquad (3)$$

$$MGT = \frac{\sum ni \times di}{N} \qquad (4)$$

Where:

n_i= the number of seeds germinating on the d_i^{th} day of germination testing,

t= the number of days since sowing,

rl and sl= root and shoot length, respectively

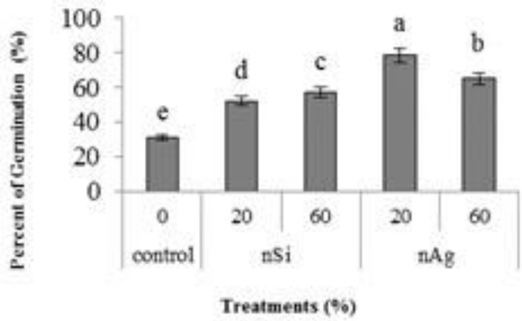
N= total number of seeds germinating during the experiment

Statistical analysis

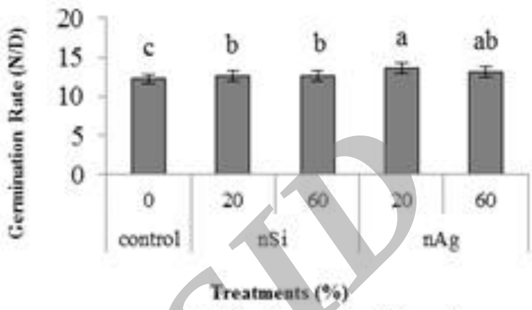
Germination percent, germination rate, mean germination time, root length, shoot length, fresh and dry weights and vigor index were measured in this study. The statistical analysis was conducted using ANOVA and Duncan method was used to examine the differences between treatments. Before carrying out the analysis, the normality and homogeneity of data were evaluated by Kolmogorov-Smirnov's and Leven's tests, respectively. Data were analyzed using SPSS version 22.

Results and Discussion

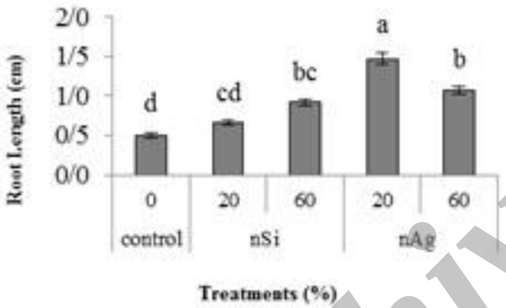
The results of ANOVA showed a significant effect of Silica and Silver Nanoparticles on all of seed germination traits (P≤0.01) (Table 1). Thus, the application of silica nanoparticles had enhanced the seed germination potential. The means comparisons among treatments are presented in Fig. 2. For all the seedling parameters, nano-silica with 60% concentration showed higher mean values than that for 20% concentration and control. Vice versa, in the silver nanoparticles (nAg), treatment with the concentration of 20% showed higher mean values than that for nano-silver 60% concentration and control. In addition, the seedling properties values in silver nanoparticles treatments were always higher than silica nanoparticles (both in 20 and 60% concentrations) (Fig. 2).



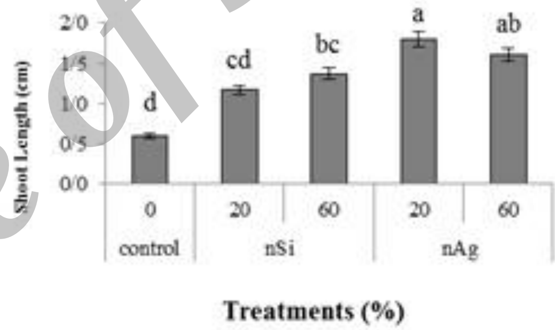
a) percent of seed germination



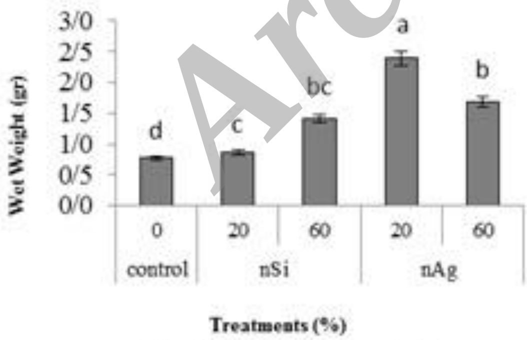
b) Seed germination rate



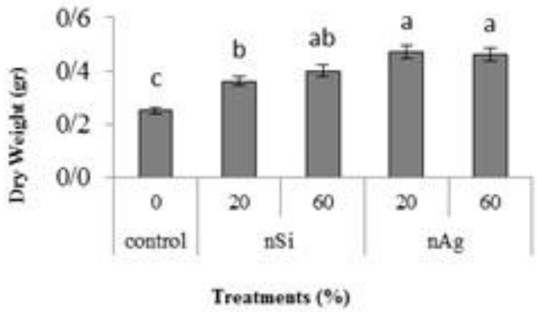
c) root length



d) shoot length



e) Seedling fresh weight



f) Seedling dry weight

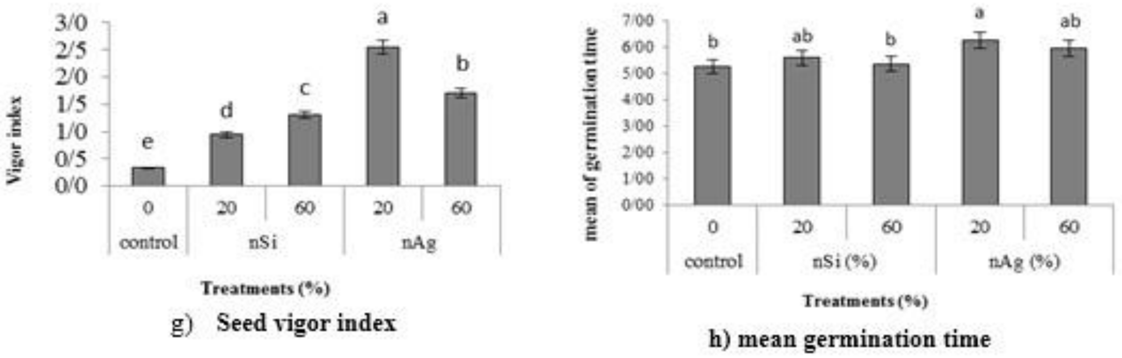


Fig. 2. Effects of nanoparticles (nSi= nano-Silica; nAg= nano-Silver) on seed germination traits of *T. kotschyanus*. Vertical error bars represent the ±SE. Different letters in each bar differ significantly according to DMRT test ($p<0.05$)

Table 1. ANOVA results for root and shoot length, speed and rate of germination, fresh and dry weight and vigor index of *T. kotschyanus*

Source of variation	df	MS							
		Percent of Germination	Speed of Germination (n/d)	Mean Germination Time	Root Length (cm)	Shoot Length (cm)	Fresh Weight (g)	Dry Weight (g)	Seed Vigor Index
Treatments	4	1237.4**	1.21**	0.006*	0.56**	0.85**	1.72**	0.03**	2.75**
Error	15	3.76	0.47	0.000	0.03	0.02	0.03	0.00	0.02

*, **=Corresponding MS significant at 5% and 1% respectively

Seed germination, germination rate and germination time

Results showed that nanoparticle treatments significantly increased the germination percent and rate in comparison with the control. The maximum values of germination percent (78.5%) and rate (13.65 n/d) were recorded in the nano-silver 20% concentration; the maximum average of mean germination time of seeds belonged to silver nanoparticles with 20% concentration (6 days) and minimum values were recorded in control (Figs. 2a, 2b and 2h). Raskar and Laware (2014) investigated the effects of different concentrations of Zinc Oxide nanoparticle treatments on onion seed germination and early seedling growth. Seed germination and related indices were increased in lower concentrations; however, they were decreased at higher concentrations. Karimi *et al*. (2012) indicated that silver nanoparticles did not reduce wheat seed germination. Silver nanoparticles can protect seeds against fungi as well as the conventional fungicide, Carboxitiram and seed protection afforded by silver nanoparticles. Similarly, nano-SiO_2 significantly enhanced the seed germination in tomato (Siddiqui and Al-Whaibi, 2014). Germination improvement as a result of silica nanoparticles application demonstrated the effects of this nano-material. Similar resultes were reported by other studies such as those done by Zuccarini (2008), Lee *et al*. (2010a), Wang *et al*. (2010) and Wang *et al*. (2011) who had confirmed the positive effects of silicon at salinity stress. Yin *et al*. (2012) examined the effects of silver nanoparticles on the germination and growth of eleven wetland common plant species. They compared the effects of two silver nanoparticles as 20 nm polyvinylpyrrolidine coated silver nanoparticles (PVP-AgNPs) and 6-nm gum arabic coated silver nanoparticles (GA-AgNPs). In the direct exposure experiments, PVP-AgNP had no effect on germination while 40 mg Ag L21 GA-AgNP exposure significantly reduced the germination rate of three species and enhanced the germination rate of *Eupatorium fistulosum* species. Parveen and Rao (2014) reported that biologically synthesized silver nanoparticles enhanced the percentage of seed germination in *Pennisetum glaucum* but higher concentration of silver nanoparticles decreased the root, shoot and total seedling length in this species. They observed that higher concentration of nanoparticles had adverse effects on plant species. Inhibition of seed germination and root elongation had been found to be highly dependent on both plant type and nano-particle properties. Karimi *et al*. (2012) suggested that silver nano-solution coated seeds recorded better germination than the seeds treated with fungicide and concluded to use silver nano-coating instead of using fungicides. Silver nanoparticles even at the highest concentration did not disturb the germination traits and seedlings growth of *Ricinus communis* (Jyothsna and Pathipati, 2013). Results of Karimi´s *et al*. (2012) showed that treating seeds with silver nanoparticles did not reduce germination; it is possible to use this treatment in the agricultural practices. In other words, silver nanoparticles did not affect the seed living process adversely. Results of Haghighi *et al*. (2012) that studied the effect of nano-Si on tomato seed germination demonstrated that mean germination time had low reduction of 1 and 2 ppm nano-Si application.

Seedling root and shoot length

According to Duncan tests, the highest root and shoot lengths were observed in nano-silver with 20% concentration (1.47 and 1.8 cm, respectively). Moreover, the lowest root and shoot length was observed in control treatment. The silica nanoparticles had increased root and shoot length with increasing the concentration of the nanoparticles, and silver nanoparticles decreased root and shoot length in 60% concentration. Shoots and roots growth responded differently to nanoparticles with relative inhibition of root growth generally being lower for shoot growth (Figs. 2c and 2d). Vashisth and Nagarajan (2010) studied germination and early growth characteristics in sunflower and showed that the effects of treatments of ZnO nanoparticles at different concentrations. They had related the ZnO nanoparticles as a good effect on root and shoot growth. Bao-shan *et al*. (2004) examined nano-structured silicon dioxide on growth of Changbai larch (*Larix olgensis*) seedlings. They observed that seedling growth and quality of *Larix* treated with silicon dioxide was promoted. 500 μL/L showed the highest seedling length, root collar diameter, root length and the number of lateral roots of seedlings. Tan *et al*. (2009) concluded that silver nanoparticles treatment mostly affected the shoot and root growth in comparison with seed germination.

Seedling Fresh and Dry weight

Results showed that the maximum means of seedlings fresh and dry weights were obtained for silver nanoparticles with 20% concentration (2.39 and 0.47 g, respectively) while they were at the minimum in the control treatment. In silver nanoparticles, by increasing nano concentration, fresh and dry weights were reduced; however, the increase of silica nanoparticles concentration was decreased for fresh and dry weights (Figs 2e and 2f). The results of Zhu *et al*. (2008) on the *Phaseolus vulgaris* showed that shoot length, fresh and dry weights of shoot had influenced by different concentrations of ZnO nanoparticles and dry shoot weight was significantly different among the treatments. However, the lowest fresh and dry weight was observed in control. However, the maximum shoot length was recorded in 20mg as compared to the other concentrations and control. Moreover, Haghighi *et al*. (2012) expressed that great effects of silica nanoparticles had improved salinity stress on tomato seed germination. 25 ppm silica nanoparticles showed great enhancement on germination characteristics such as germination rate, root length and dry weight.

Vigor index

Seed vigor index in control of 20 and 60% nanoparticle concentrations was significantly different and the highest value was found for 20% silver nanoparticles treatment (2.55) (Fig. 2g). Aghajantabar Alee *et al*. (2014) reported that silver nanoparticles had enhanced root and shoot in both species of *Festuca ovina* and *Festuca arundinaceae*; however despite of the positive effects on both species, the effect on *Festuca arundinaceae* was lower. In addition, the concentration of 20 ppm silver nanoparticles to improve in terms of speed germination, seedling vigor index and shoot length was compared to control salinity. Moreover, Siddiqui and Al-Whaibi (2014) studied the role of SiO_2 nanoparticles in germination of tomato (*Lycopersicum esculentum*) and reported that among the treatments, application of 8 ppm of SiO_2 nanoparticles proved to be the best by giving the highest values of seedling vigor index and seed germination. Ushahra *et al*. (2014) by evaluating the effects of biogenic nanoparticles on shoot and root length, fresh and dry matter, and vigor index reported that nanoparticles caused to improve the seed parameters.

Conclusion

In general, results of the current study reveal that the application of silica and silver nanoparticles has significantly enhanced the seed germination potential. Application of silica and silver nanoparticles improved all of early growth characteristics of *T. kotschyanus* seedlings. The results showed that the germination of *T. kotschyanus* was more affected by nano-silver treatments in comparison with silica nanoparticles and control treatments. In overall, higher values of seed germination traits were observed in silver nanoparticles with 20% concentration. By increasing the silica nanoparticle concentration, the seed germination had also increased; however, by increasing the silver nanoparticle concentration of 60%, the seed germination was decreased.

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مقایسه اثر نانو ذرات سیلیس و نقره بر پارامترهای جوانه‌زنی بذر آویشن کوهی (*Thymus kotschyanus*) در شرایط آزمایشگاهی

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چکیده. معرفی نانو ذرات ممکن است تأثیر قابل توجهی روی جوانه‌زنی و رشد گیاهان داشته و در نتیجه، می‌توان از آن برای برنامه‌های کاربردی کشاورزی برای رشد و عملکرد بهتر استفاده نمود. هدف از انجام این مطالعه مقایسه اثرات ذرات نانو سیلیس و نانو نقره بر خصوصیات جوانه‌زنی و صفات اولیه رشد بذر گیاه آویشن کوهی (*Thymus kotschyanus*) از جمله؛ درصد جوانه‌زنی، سرعت جوانه‌زنی، طول ریشه‌چه، طول ساقه-چه، وزن تر گیاهچه، وزن خشک گیاهچه و شاخص بنیه بذر می‌باشد. آزمایش در قالب طرح کاملاً تصادفی با ۵ تیمار و ۴ تکرار در زمستان ۹۳ انجام شد. بذر این گونه از مراتع سبلان جمع‌آوری شد. تیمارها شامل؛ شاهد (آب مقطر)، نانو نقره و نانو سیلیس هر کدام با غلظت‌های ۲۰ و ۶۰ درصد بودند. تعداد ۳۰ بذر در هر پتریدیش کشت شد. جوانه‌زنی بذرها از روز چهارم پس از کشت شروع شده و خصوصیات مورد مطالعه از همان روز ثبت شدند و جوانه‌زنی بذور به مدت ۱۴ روز کنترل شد. برای بررسی اختلاف معنی‌داری بین تیمارها، از تجزیه واریانس یک‌طرفه و برای مقایسه میانگین تیمارها از آزمون دانکن استفاده شده است. نتایج نشان داد که جوانه‌زنی بذور آویشن به‌شدت تحت تاثیر تیمارهای نانو نقره قرار گرفته است. همچنین بیشترین مقدار خصوصیات جوانه‌زنی بذر، در تیمار نانو نقره با غلظت ۲۰٪ مشاهده شد. افزایش غلظت نانو سیلیس باعث افزایش جوانه‌زنی بذور شده در حالیکه، افزایش غلظت نانو نقره موجب کاهش جوانه‌زنی گردیده است.

کلمات کلیدی: نانو ذرات، صفات اولیه رشد، خصوصیات جوانه‌زنی، شاخص بنیه، آویشن کوهی