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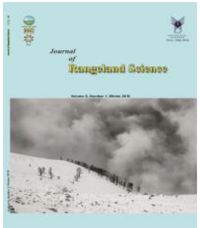

Research and Short Length Article:

Effect of Silver Nanoparticles on Seed Germination and Seedling Growth in *Thymus vulgaris* L. and *Thymus daenensis* Celak under Salinity Stress

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Abstract. Germination represents a fundamental stage of plant life highly responsive to change of environmental conditions. Low germination percent and seedling establishment are basic problems in saline regions. One of the sensitive stages of plants to salinity is the germination stage. This study was conducted using a factorial experiment with three factors such as species with two levels (*Thymus vulgaris* L. and *Thymus daenensis* Celak), nanosilver in 4 levels (0, 10, 20 and 30 ml) and salinity in 4 levels (0, 100, 200 and 300 mM NaCl) using a completely randomized design in four replications in University of Kashan, Iran in 2016. Results showed that the interaction among species, salinity and nanoparticles was significant only for germination rate ($P \le 0.01$). Silver nanoparticles increased germination percent, germination rate and root length up to 200 mM sanity, but they enhanced seed vigor and shoot length up to 100 mM salinity as compared to the control treatment. In 100 mM salt concentration, the 20 and 30 mL nano-silvers were effective, but for 200 mM salinity, the application of 10 mL nano-silver was effective.

Key words: Germination, Nanoparticles, *Thymus*, Salinity, Seed

Introduction

Now, a considerable amount of the world water resources is affected by salinity. Soil salinization is a progressive phenomenon and about 11% of the world's irrigated lands are affected by varying degrees of salinity (FAO, 2012). Iran with 8.6 million ha of saline lands (Momeni, 2010) is on top of the countries considered at risk of salinity. At present, the total areas of irrigated lands and agricultural lands with varying degrees of soil salinity level of soil, water or both are estimated as 3.7 million ha and 5.3 million ha, respectively (Banaii *et al*., 2004).

According to the researches, the seeds that have better germination cause the growth of plants with better vigor and stronger root system in the later stages (Opoku *et al*., 1996). Germination is strongly influenced by environmental factors, especially water soluble material (Soltani *et al*., 2006). Various studies on germination of crops reflect the fact that with increasing the levels of salinity, root length, shoot length and seedling dry weight were significantly reduced (Alebrahim *et al*., 2004). Reduced germination and seedling growth under osmotic stress and salinity may be due to the inhibition of absorption of water, Na^+ or Cl^- ion toxicity or nutrient imbalance (Lynch and Lauchli, 1988).

The atomic or molecular particles with a minimum size of between 1-100 nm have different physicochemical properties as compared to their bulk materials. Nanoparticles were effective in the increasing of germination and plant growth (Jagtap and Bapat, 2013).

Almutairi (2015) in studying the effects of silver nanoparticles on germination of tomato (*Solanum lycopersicum* L.) under salt stress conditions found that silver nanoparticles with concentration of 5.2 mL had increased the germination percent in salinity of 150 mM NaCl.

Razzaq *et al.* (2016) in the study of the effect of silver nanoparticles on seed germination of wheat showed that the silver nanoparticles had no effect on seed germination, but 25 and 50 ppm silver nanoparticles had significantly increased the root length more than the control treatment.

Most of the genus Thymus (family of Labiatae) are range plants that consist of about 215 species of herbaceous perennials and sub shrubs. They are originated from Mediterranean region (Jamzad, 2010). Several species of thyme have strong antibacterial, anti-fungal, anti-parasitic, anti-spasmodic and antioxidants effects. Medicinal properties of Thymus species plants have led to their excellent fame and popularity among the people around the world (Stahl-Biskup and Saez, 2002). 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).

This study aimed to investigate the effects of silver nanoparticles on increasing or improving the germination and seedling growth of two thyme species under salinity conditions.

Materials and Methods

This study was performed using a factorial experiment with three factors such as species with two levels (*Thymus vulgaris* and *Thymus daenensis*), nanosilver in 4 levels (0, 10, 20 and 30 ml) and salinity in 4 levels (0, 100, 200 and 300 mM NaCl) using a completely randomized design in four replications in University of Kashan, Iran in 2016. The seeds were disinfected by 1% sodium hypochlorite for three minutes and then washed with distilled water three times. 25 seeds of each thyme species were placed in each petri dish using the paper procedure. 10 ml of the solution with the determined stress levels was added to each petri dish. Then, the petri lids were sealed by parafilm. Then, they were maintained in a germinator with 26°C for 16 h photoperiod, 8 h darkness and the relative humidity of 70%. Germinated seeds were counted every day and at a definite time since the second day. The germination criterion of radicle emergence from seed was 2 mm. Counting continued until no more increase was observed in the number of the germinated seeds and this status remained constant for three successive days. In the last day of counting, five seedlings were randomly selected for measuring root and shoot length in mm. Then, the germination rate and seeds vigor were calculated based on the following equations (Panwar and Bhardwaj, 2005):

$$GR = \frac{\sum ni}{t} \quad (1)$$

Where:

GR = germination rate based on seed number per day

ni = the number of germinated seeds per day

t = day number

$$Vi = (RL + SL) \times GP \quad (2)$$

Where:

Vi: Seed Vigor

RL: root length (mm)

SL: shoot length (mm)

The collected data were analyzed using the SAS software version 9. First, data normalization was done using Kolmogorov–Smirnov test; then, to compare the means in the case of variance Homogeneity, Duncan's multiple range test was used.

Results

The results of the analysis of variance showed that the main effect of salinity was significant for all traits ($P<0.01$). The main effect of species was significant for germination percent, germination rate and seed vigor ($P<0.01$). The main effect of nanoparticles was significant for germination percent ($P<0.01$) and root length ($P<0.05$) (Table 1). The species × salinity interaction effects were significant for germination percent, germination rate and seed vigor ($P<0.01$); similarly, the salinity × silver interaction effects were significant for all of traits except germination rate ($P\leq0.01$). The interaction among species, salinity and nanoparticles was significant only for germination rate ($P\leq0.01$) (Table 1).

Table 1. Variance analysis of the effects of nanoparticles on the germination and growth indicators of *T. daenensis* and *T. vulgare* under salinity stress

Effects	DF	MS				
		Germination %	Germination rate	Shoot length	Root length	Seed vigor
Species(A)	1	14049.77**	608.96**	19.87	6.1 1	214.99**
Salinity (B)	3	36437.94**	1173.2**	918.46**	272.02**	1502.5**
Silver nanoparticle (C)	3	772.67**	13.77	12.79	16.37*	13.47
(A×B)	3	3336.03**	227.9**	17.01	2.45	47.65**
(A×C)	3	78.09	7.51**	18.5	2.07	10.33
(B×C)	9	394.7**	22.28	48.53**	12.72**	37.00**
(A×B×C)	9	144.44	18.02**	14.57	7.07	8.09
Error	95	143.45	4.86	14.72	4.76	75.00

* *, *= significant at 1% and 5% probability levels, respectively

Table 2. Means comparison of species × salinity interaction on seed germination and Seed vigor

Species	Salinity (mM)	Germination %	Seed vigor
T. vulgare	0	58b	12.47b
	100	26.5 c	3.25 d
	200	8.75 e	0.50 e
	300	4.50 e	0.20 e
T. daenensis	0	97.75 a	17.73 a
	100	66.25 b	7.50 c
	200	19.25 d	1.76 de
	300	1.86 e	1.05 e

Means of each column with the similar letters has no significant differences based on Duncan test (P<0.05)

Table 3. Means comparison of salinity × silver nanoparticle interaction on seed germination and Seed vigor

Salinity (mM)	silver nanoparticle (mm/lit)	Germination Percentage	Shoot length (mm)	Root length (mm)	Seed vigor
0	0	82.5a	15.55a	7.5a	18.96a
	10	78.00 a	17.77 a	7.97 a	15.31 a
	20	79.50 a	12.40 a	7.10 a	15.62 a
	30	71.5 a	10.47 a	5.16 ab	10.91ab
100	0	33.5 abc	3.55 cd	2.45 cde	2.50 cd
	10	58.5 ab	6.30 b	4.40 bc	5.86 bc
	20	45.5 ab	7.67 b	6.56 ab	6.92 bc
	30	45.0 ab	11.87 a	6.75 ab	6.23 bc
200	0	0.00 d	0.00 d	0.00 cde	0.00 c-f
	10	22.5 abc	3.84 cd	2.27 bcd	2.05 cd
	20	19.5 abc	3.21 cd	2.06 bcd	1.45 cde
	30	14.0 abc	2.11 cd	2.08 bc	1.02 cde
300	0	0.00 d	0.00 d	0.00 cde	0.00 c-f
	10	5.00 d	1.37 d	1.27 cde	0.23 ced
	20	2.50 d	0.57 d	0.50 cde	0.11 cde
	30	5.00 d	0.50 d	0.75 cde	0.17 cde

Means of each column with the similar letters has no significant differences based on Duncan test (P<0.05)

Table 4. Means comparison of salinity × silver nanoparticle interaction of species on seed germination rate

Salinity (mM)	silver nanoparticle (mm/lit)	*T. vulgare*	*T. daenensis*
0	0	9.41 a-e	22.37 a
	10	8.61 a-k	18.68 abc
	20	8.90 a-k	16.64 abc
	30	5.43 c-k	20.17 a
100	0	2.28 f-k	5.11 c-k
	10	7.51 a-k	8.88 a-k
	20	3.16 f-k	12.59 a-f
	30	5.26 f-k	14.47 a-e
200	0	0.00 k	0.00 k
	10	2.14 g-k	4.18 b-k
	20	1.68 g-k	2.86 f-k
	30	1.79 g-k	2.08 f-k
300	0	0.00 k	0.00 k
	10	0.87 h-k	043 jk
	20	0.81 h-k	0.00 k
	30	0.75 ijk	0.21 jk

Different letters in each column represent significant differences based on Duncan test P<0.05)

Discussion

Based on the results obtained (Table 2), increasing salinity significantly reduced germination percent in both *T. daenensis* and *T. vulgare* species. Bagheri *et al.* (2012) in *T. vulgare*, and Abdollahifar *et al.* (2013) in *T. daenensis* found similar results. The higher mean values were obtained in *T. daenensis* indicating its relative tolerance to salinity as compared to *T. vulgare.*

The highest seed vigor of *T. daenensis* (17.73) was obtained in the control treatment (Table 2). In both species, the vigor index were sharply decreased at salt concentration of 200 mM. However, its value for *T. vulgare* was lower at similar levels as compared to *T. daenensis* species. Germination decrease in salinity conditions may be resulted from low osmotic pressure and water intake prohibition, Cl^- or Na^+ ion toxicity, or nutrient imbalance (Lynch and Lauchli, 1988).

Results showed that silver nanoparticles enhanced germination percent at 100 and 200 mM salinity and increased seed vigor in 100 mM salinity as compared to the control treatment (Table 2). The lowest vigor indices were obtained in 200 and 300 mM salinity in different levels of Nano particles. This result was corresponding with that was reported by Aghajantabarali *et al.* (2014) on *Festuca ovina* and *Festuca arundinaceae* and Almutairi (2015) on *Solanum lycopersicum* L.

Effect of silver nanoparticles on 300 mM salinity treatments was significant for germination percent and vigor index; however, the values were much lower than other salinity levels. It might be due to the inability of 10, 20 and 30 mL silver nanoparticles to inhibit the adverse effect of salinity on seed germination in high salinity.

The differences between two species for shoot and root lengths were not significant (Table 1). However, results showed that the values of both traits were decreased remarkably with the increase of salinity levels (Table 3). Corresponding to our results, Abdollahifar *et al*. (2013), Alebrahim *et al*. (2004) and Bagheri *et al.* (2012) came to the same conclusion. Silver nanoparticles at the concentrations of 100 and 200 mM salt had increased shoot and root length more than that for the control treatment, specifically. In 100 mM salt, the highest values of both traits were obtained at Nano concentrations of 30 mL. The result is consistent with the findings of Aghajantabarali *et al*. (2014).

For shoot length, the effect of nanoparticles on salt concentration at 200 and 300 mM was not considerable. Despite a higher shoot growth in 200 mM salinity, there were no significant differences between two salinity treatments. So, silver nanoparticles in salinity levels greater than 100 mM had no ability to increase shoot length and did not overcome the negative effects of stress.

For root length, higher mean values in 100 mM salt were obtained in 20 and 30 mL Nano than those for 10 mL Nano. In 200 and 300 mM salt, the silver nanoparticles had no significant effects on root length. These results were in contrast with the findings of Aghajantabarali *et al.* (2014) that found the reduction of high salinity effect by increasing the concentration of silver nanoparticles in root length of *Festuca arundinaceae* and *Festuca ovina*.

For germination rate, the results showed that salinity had a negative effect on the germination rate of both species under study. Similar to our result, Abdollahifar *et al*. (2013) and Bagheri *et al.* (2012) on *T. daenensis* and Fateh and Alimohammadi (2010) on *T. vulgare* came to the same conclusion. For 100 mM NaCl treatments, the lower germination rate of both species was obtained in the control treatment (without nanoparticles). There were also no differences between 10, 20 and 30 mL Nano at 100 mM salt for germination rate (Table 4). However, in all of 0, 100 and 200 mM salt, the mean values of *T. daenensis* were always higher than *T. vulgare*. The result indicated that in 100 mM salt, the treatment of 30 mL Nano had the best performance in both species. In contrast, for 200 mM salt, lower Nano (10 mL) had higher effects (Table 4). Therefore, we can see that nano treatment in mild and gentle tensions could remove harm effect of tensions, but in severe tensions, it could damage the plant and had no ability to recover the plant damage.

Also, *T. vulgaris* had the highest rate of germination (9.41) in the control treatment (distilled water); however, this trait was reduced by increasing concentrations of nanoparticles and this was consistent with the findings of Ramezani *et al*. (2014) on *Medicago sativa*. Nanoparticles had little impact on this character at higher salinity levels and have no significant differences with control. So nanosilver particles failed to prevent from the negative impact of severe stress levels of sodium chloride to speed germination.

The overall conclusion in the genus Thymus in terms of salinity was that the silver nanoparticles increase germination percent, germination rate and root length up to 200 mM salinity, but they enhance seed vigor and shoot length up to 100 mM salinity as compared to the control treatment. In 100 mM salt concentration, the 20 and 30 mL nano-silver were effective, but for 200 mM salinity, the application of 10 mL nano-silver was effective.

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تاثیر نانو ذرات نقره بر جوانه‌زنی و رشد گیاهچه دو گونه آویشن باغی و آویشن دنایی در شرایط تنش شوری

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چکیده. جوانه‌زنی یک مرحله اساسی زندگی گیاه است که به‌شدت تحت تأثیر عوامل محیطی قرار می‌گیرد. کاهش درصد جوانه‌زنی و استقرار گیاهچه از مشکلات اصلی خاک‌های شور است. یکی از مراحل حساس گیاهان به شوری، مرحله جوانه‌زنی است. این مطالعه در سال ۱۳۹۵ به‌عنوان یک طرح فاکتوریل با سه فاکتور گونه در دو سطح شامل گونه آویشن باغی *Thymus vulgaris* L. و آویشن دنایی *Thymus daenensis* Celak و محلول نانو نقره در چهار غلظت (شاهد، ۱۰، ۲۰ و ۳۰ میلی‌گرم در لیتر) و شوری در چهار سطح (شاهد، ۱۰۰، ۲۰۰ و ۳۰۰ میلی‌مولار نمک طعام) در قالب طرح کاملا تصادفی در چهار تکرار در دانشگاه کاشان انجام شد. نتایج نشان داد برهمکنش گونه، تنش شوری و نانو ذرات فقط بر سرعت جوانه‌زنی اثر معنی‌دار در سطح احتمال یک درصد داشت ($P<۰/۰۱$). نانو ذرات نقره، میانگین صفات درصد جوانه‌زنی، سرعت جوانه‌زنی و طول ریشه‌چه را تا شوری ۲۰۰ میلی‌مولار و همچنین شاخص بنیه و طول ساقه‌چه را تا شوری ۱۰۰ میلی‌مولار نسبت به شاهد افزایش داد. برای شوری ۱۰۰ میلی‌مولار کاربرد نانو ذرات با غلظت‌های ۲۰ و ۳۰ میلی‌گرم بر لیتر و برای شوری ۲۰۰ میلی‌مولار کاربرد نانو ذرات با غلظت ۱۰ میلی‌گرم بر لیتر توصیه شد.

کلمات کلیدی: جوانه‌زنی، نانو ذرات، آویشن، شوری، بذر