Analysis of the Priming Effect of Iron Nanoparticle on the Germination Characteristics of *Melissa officinalis* under Salinity Stress

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Abstract: Regarding the importance of *Melissa officinalis*, especially in pharmaceutical industries, this experiment was conducted to analyze the effect of priming with iron nanoparticles on germination of *M. officinalis* under salinity stress using facotial experiment based on randomized complete block design, at the laboratory of Payam Nour University of Tehran, Iran in 2016. Priming improved the germination factors and the seedling growth of *M. officinalis* under salinity stress. By increasing the concentration of iron nanoparticles, more absorption occurred at the nanoscale and plant growth indices fell due to created toxicity and oxidative stress induced on the plant that led it to increase activities of its antioxidant enzymes in order to fight generated free radicals.

Keywords: *Melissa officinalis*, germination, nanoparticle, salinity.

1. INTRODUCTION

Applying nanotechnology in agriculture is an emerging technology even in worldwide scale. Nanotechnology suggests cheap solutions for better growth of plants. Among the most recent progresses made in technology, the nanotechnology has special role in agriculture and food production. (*Vashitsh, Nagarajan* 2010).

Salinity is one of the most common environmental stresses in nearly all over the world, reducing food production. (*Abdolzadeh, Kazuto* 1998).

Medicinal plants are attracting more attention every day. They are used both in the traditional medicine and also the modern medicine. *Melissa officinalis* L. is one of these important medicinal plants from mint family. It has special odor like the lemon and simple opposite leaves in dark green with short petiole. This plant is native to the Asia area and Mediterranean. This plant has many functions in human health. It cures neurological diseases, diseases of stomach, heart and intestine, and is used in food, cosmetic and pharmaceutical industries (*Mencherini et al*. 2007).

Essential oil of medicinal plants in pharmaceutical industries, this experiment was conducted to evaluate the effect of seed priming on the germination of *Melissa officinalis* L. under salinity stress.

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2. MATERIALS AND METHODS

This experiment was conducted in the laboratory of Payam Noor University of Tehran, in 2016. This research was conducted using factorial experiment based on randomized complete block design with 3 replications. Experimental factors were iron nanoparticles in five levels (0, 20, 40, 60 and 80 ppm) and salinity in four levels (0, 2, 5 and 10 ds/m NaCl). Seeds were disinfected by using 1% sodium hypochloride solution for 10 minutes and then were rinsed three times with distilled water. Seeds were naturally dried to get back to the original moisture level and then 40 seeds were put in each Petri disinfected. After that, 6 ml of the saline solution and 8 ml of colloidal nanoparticles of iron were added to the Petri dishes.

Petri dishes were packed with parafilm to prevent evaporation and were located in a germinator.

Temperature of the germinator was 20°C and the light period was 18 h light and 6 h dark with the humidity of 70%. Counting the number of germinated seeds was conducted every two days. Measuring the germinated seeds was stopped after 21 days when no more seed was germinated (radicle emergence was considered as germination). Then, measured germination attributes. The activity of catalase enzyme was measured in 240 nm wavelength based on the rate of H_2O_2 decomposition according to the method of (*Aebi*, 1974).

(*Khan and Ungar* 1998) method was used to measure germination percentage and germination rate (*Maguire* 1962, *Abdul-Baki and Anderson*, 1973)

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method was also used to measure seed vigor index and Allometric coefficient.

Data were analyzed using SAS 9.4 and means were compared according to LSD method at 0.05 level.

3. RESULTS AND DISCUSSION

The results of analysis of variance indicated the significant effect of iron nanoparticles (A), salinity (B) and also the interaction of these factors on most measured traits (Table **1**). Results showed the Increasing of salinity stress reduced germination characteristics (Table **2**).

3.1. Germination Percentage

In 20 ppm concentration of iron nanoparticles level, the highest germination percentage was achieved in zero salinity stress level (control) and the lowest was achieved in 10ds/m salinity level.

Table 1: Analysis of Variance of the Effect of Iron Nano Particles and Salinity Stress on the Measured Traits

SOV	df	Mean Squares (MS)							
		Germination percentage	Germination rate	Allometric coefficient	Seed vigor index	Plumule length	Radicle length	Fresh weight	Dry weight
Rep	2	2/41ns	2/31**	0/004*	9240/4ns	0/0043n	8/36ns	0/0003ns	0/00001ns
Iron nano particle (A)	4	620/51**	0/28ns	0/041**	344033/4**	0/113*	98/95**	0/012**	0/001**
Salinity (B)	3	3726/37**	30/17**	0/025**	1109920/4**	1/93**	213/32**	0/014**	0/0024**
A × B	12	1177/65**	212/29**	0/44**	1576850/1**	7/33**	463/04**	0/035**	0/001**
CV	-	11.79	6.53	6.75	13.65	9.20	14.85	19.37	20.04

ns, non significant; *, significant at P≤0.05; **, significant at P≤0.01.

Table 2: The Effect of Interaction of Seed Priming x Salinity Stress on the Measured Traits

Iron nano particles levels (ppm)	Salinity levels (ds/m)	Germination percentage	Germanton rate	Seed vigor index	Allometric coefficient	Plumule length (mm)	Radicle length (mm)	Fresh weight (g)	Dry weight (g)
0	0	56.66a	15.41a	1922.66a	0.83a	3.005a	25.23a	0.28a	0.07a
	2	54.16a	15.28a	801.33a	0.66a	2.46b	8.70b	0.10b	0.047a
	5	17.0b	15.19a	800.04a	0.60b	2.12b	6.41b	0.041c	0.016b
	10	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0d	0.0c
20	0	71/66a	15.22a	1907.50a	0.79a	3.41a	17.36a	0.22a	0.043a
	2	40.0a	14.93b	1129.50a	0.75a	2.81a	14.62b	0.16b	0.017b
	5	20.0b	14.77b	369.92b	0.65a	2.09b	8.01b	0.10b	0.011b
	10	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0d	0.0c
40	0	31.66b	15.55a	730/89b	0.70a	3.49a	25.67a	0.11b	0.015b
	2	24.16b	15.34a	569.55b	0.60b	2.67b	19.51a	0.086c	0.015b
	5	19.16b	14.84b	363.61b	0.58b	2.24b	16.72b	0.069c	0.013b
	10	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0d	0.0c
60	0	39.16a	15.98a	1318.85a	0.84a	3.32a	32.92a	0.17b	0.020b
	2	20.83b	15.18a	1121.66a	0.70a	3.17a	25.08a	0.14b	0.017b
	5	10.0b	15.09a	123.84b	0.61b	1.88b	6.26b	0.092c	0.016b
	10	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0d	0.0c
80	0	56.66a	16.55a	1444.83a	0.64a	3.14a	27.66a	0.33a	0.054a
	2	35/83a	15/01a	337.76b	0.59b	2.82a	17.86a	0.15b	0.018b
	5	12.50b	14.98b	255.53b	0.40b	2.60b	5.89b	0.13b	0.014b
	10	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0d	0.0c

Means in a column followed by the same letter are not significantly different at P≤0.01 according to the LSD test.

Table 3: Analysis of Variance of the Effect of Seed Priming with Iron Nano Particles and Salinity Stress on Catalase and Hydrogen Peroxide Activity

SOV	df	Mean Squares (MS)	
		Catalase	Hydrogen peroxide
Rep	2	0/0002^{ns}	2/24**
Iron nano particle (A)	4	0/003**	11/75**
Salinity (B)	3	0/0146**	94/41**
A × B	12	0/00006^{ns}	0/068^{ns}
CV		7.66	3.47

ns, non significant; *, significant at P≤0.05; **, significant at P≤0.01.

Table 4: The Effect of Iron Nano Particles on Catalase and Hydrogen Peroxide

Iron nano particles (ppm)	Catalase (mg Pro/min)	Hydrogen peroxide (mg Pro/min)
0	0.11d	10.25d
20	0.12c	10.55d
40	0.12c	11.05c
60	0.13b	11.81b
80	0.15a	12.69a

Means in a column followed by the same letter are not significantly different at P≤0.05 according to the LSD test.

3.2. Germination Rate

In 80 ppm concentration of ironnano particles level, the highest germination rate was achieved in zero salinity stress level (control) and the lowest germination rate was achieved in 10ds/m salinity level.

3.3. Allometric Coefficient

In 60 ppm concentration of iron nanoparticles level, the highest allometric coefficient was achieved in zero salinity stress level (control) and the lowest allometric coefficient was achieved in 10 ds/m salinity level.

3.4. Seed Vigor Index

In 20 ppm concentration of iron nano particles level, the highest seed vigor index was achieved in zero salinity stress level (control) and the lowest seed vigor index was achieved in 10 ds/m salinity level.

3.5. Plumule Length

In 40 ppm concentration of iron nano particles level, the highest plumule length was achieved in zero salinity stress level (control) and the lowest plumule length was achieved in 10 ds/m salinity level.

3.6. Radicle Length

In 60 ppm concentration of iron nano particles level, the highest radicle length was achieved in zero salinity stress level (control) and the lowest radicle length was achieved in 10 ds/m salinity level.

3.7. Fresh and Dry Weight

In 80 ppm concentration of iron nano particles level, the highest fresh weight and in 0 ppm of iron nano particles level, the highest dry weight were achieved in zero salinity level (control) and the lowest both of them were achieved 10 ds/m salinity level.

3.8. Catalase and Hydrogen Peroxide Activity

The effect of seed priming and salinity was significant on this trait (P≤0.01) but their interaction had no effect (Table **3**).

In 80 ppm concentration of iron nanoparticles levels, also salinity level 10 ds/m enhancement of the activity of catalase and hydrogen peroxide. The lowest both of them were in the control (Tables **4-5**).

Results of this experiment indicated that when the concentration of iron nanoparticles and the level of salinity stress increased, the germination of *Melisa*

Table 5: The Effect of Salinity Stress Levels on Catalase and Hydrogen Peroxide

Salinity (ds/m)	Catalase (mg Pro/min)	Hydrogen peroxide (mg Pro/min)
0	0.102c	8.93d
2	0.107c	9.52c
5	0.140b	12.22b
10	0.169a	14.41a

Means in a column followed by the same letter are not significantly different at P≤0.05 according to the LSD test.

seed decreased and plant produced more antioxidant to fight the toxicity of iron nano particles.

Absorption of nano particles by plants is a new subject for researches. There are several experiments on onion, broccoli, turnip and seed indicating that priming increases seed germination percentage and rate, which are in agreement with the findings of our experiment (*Basra, et al*. 1994; *Jett, et al*. 1996; *Zheng, et al*. 1994;). Salinity stress reduces the germination features such as plumule length, radicle length, fresh and dry weight *(Pandya, et al*. 2004).

Absorption, translocation and accumulation of nano particles depend on plant species and the type of nano particles (*Rico, et al*. 2011). Most of these experiments have been conducted in germination stage and there is low information on this subject so there is a great need to study the effect of ecological toxicity of nano particles and to evaluate their environmental risk for all life forms, to understand their bioavailability and bioaccumulation in the food chains (*Lin, Xing* 2008).

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