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Effect of Strengths and Exposure Durations of Magnetic Field on Seeds Germination of *Catharanthus roseus* cv. 'Acillata'

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Abstract

Magnetic field improved germination parameters. The germination of *Catharanthus roseus* seeds is lost under salinity conditions. The effect of different strengths of magnetic field and exposure durations on the germination of *C. roseus* seeds under salinity conditions (19 mS cm^{-1}) were investigated. The experiment was conducted with factorial arrangement based on a completely randomized design (CRD) with 3 magnetic field strengths (50, 100 and 150 mT) and 3 exposure durations (10, 20, and 30 minutes). It was found that exposure to magnetic field improved seed germination under salinity conditions, so that the highest germination percentages of (74.44 and 73.05%) were obtained from 20 and 30 minutes exposure to the 100 mT magnetic field, respectively, which resulted in 28% higher germination than control. Also, these treatments were found to be the best for germination rate and vigor indices I and II. The highest radicle length (2.37 cm) and plumule length (3.50 cm) were related to the 20 minutes exposure in 50 mT magnetic field and the 10 minutes exposure in the 100 mT magnetic field. The highest radicle dry weight of 0.24 g and plumule dry weight of 0.81 g was observed in treatment of 10 minute exposure to the100 mT magnetic field.

Keywords: Ornamental plant, Physical treatment, Salinity stress, Seed priming.

INTRODUCTION

Madagascar periwinkle (*Catharanthus roseus*) is an ornamental plant from the family of Apocynaceae grown as an annual ornamental plant in horticulture (Sain and Sharma, 2013; Aslam *et al.*, 2010). Although, the germination of Madagascar periwinkle is not fully understood; It is necessary to study its germination improvement under salinity because Madagascar periwinkle is classified as salinity-sensitive species whose germination percentage and rate decreases with salinity on the one hand (Rezaee *et al*., 2013; Saeb *et al.,* 2014); and the saline lands and waters are developing in Iran and Madagascar periwinkle is a drought-resistant ornamental plant on the other hand.

The adverse impact of salinity on germination is related to the presence of an excessive amount of cations and anions that results in toxicity by reducing water potential, hinders water uptake by seeds and consequently, cause the loss of germination rate and percentage (Ungar, 1996; Vicente *et al*., 2004; Khan and Gulzar, 2003). There are many studies already carried out on the inhibiting impact of salinity on germination of different plants (Burnett *et al*., 2005; Fallahi *et al.*, 2009; Voigt *et al.*, 2009; Ashraf and Foolad, 2005; Jamil *et al*., 2006).

Researchers have always been looking for methods to improve germination and plant growth and development under stressful conditions and have recommended methods like soil and water improvement, the use of saline-tolerant cultivars, and seed pretreatment with water to farmers in regions with saline water and soils. However, the use of non-chemical methods like magnetic field has been recently attracted attention for improving the plant growth under salinity. As is known, the use of physical methods like magnetic and electric fields has the favorable effects of chemical methods without damaging the environment. Various studies have reported the desirable effect of magnetic field on germination and plant growth and development (Kordas, 2002; Dorna *et al*., 2010; Martinez *et al.,* 2000; Carbonell *et al.,* 2004).

There are many reports about the improving effect of electromagnetic or magnetic field treatment on seed vigor and germination in different plant species (Martinez *et al.*, 2000; Carbonell *et al.,* 2004; Dorna *et al.,* 2010; Feisi *et al.*, 2012; Shams *et al*., 2013). Vashisth and Nagarajan (2008) reported 46-71% higher seed vigor in pea seeds exposed to magnetic fields than control. It is believed that magnetic or ultrasonic waves are high-energy mechanical waves that can increase the temperature of the tissues. In fact, seeds that pass through a magnetic field start swelling and then, the activity of their auxin increases. Also, their respiration is increased with higher energy and activities, resulting in their faster and more uniform germination. Then, the growing plants become resistant to stresses like salinity stress (Lipiec *et al.*, 2004; Aladjadjiyan, 2007; Marinkovic *et al.*, 2008).

The exposure of seeds to magnetic field results in the growth of salinity-resistant plants; the loss of seed germination in saline conditions is related to the presence of an excessive amount of cations and anions which reduce germination by creating a sort of water stress (Singh *et al*., 1988). On the other hand, the treatment of seeds with different strengths of magnetic field can improve water uptake by them. Therefore, it is trivial to see seed germination being improved with magnetic field under saline conditions (Fischer *et al.*, 2004; Renia *et al.,* 2001). Thus, the present study was carried out to examine the effect of different strengths and durations of magnetic field treatment on the germination of Madagascar periwinkle (Catharanthus roseus) in saline conditions.

MATERIALS AND METHODS

The study was carried out as a factorial experiment on the basis of a Randomized Complete Design with two factors, three replications and 30 experimental units. The factors included magnetic field strength at three levels of 50 (M1), 100 (M2), and 150 (M3) mT and magnetic field exposure duration at three levels of 10 (T1), 20 (T2), and 30 (T3) minutes as well as a control (no exposure). The seeds of Madagascar periwinkle (*Catharanthus roseus* cv. 'Acllata') were procured from Parmis Institution of Mahallat, Iran. The generator used for generating magnetic field had a pair of strong magnets whose distance to each other can be adjusted. The strength of a magnetic field at different distance of two poles of the magnets was measured with a Tesla meter (model Lybold Heraeus 51652, Germany). The seeds packed in plastic bag containing 100 seeds were placed between two magnets for different durations. Then, they were disinfected with sodium hypochlorite 5% solution for one minute. After that, they were washed with distilled water, were bundled in the groups of 30 seeds, and were planted in sterile Petri-dishes with 7 cm diameter containing filter paper and sea water (salinity = 19 mS cm^{-1}). Then, the Petri-dishes were placed in controlled chamber at 21-24°C and 60-70% humidity until the end of the experiment (20 days) (Figs. 1 and 2).

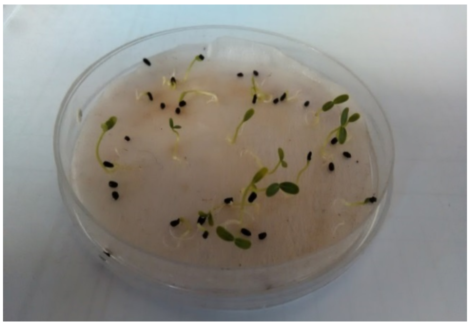

Fig. 1. Germinated seeds of *Catharanthus roseus* in Petri dish.

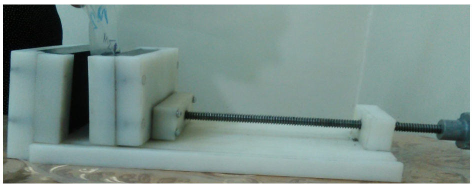

Fig. 2. Magnetizing machine and the application of the treatments.

Measurement of traits

Germination percentage

The germinated seeds were daily counted until the final day (20^{th} day). That the seeds of the root length were more than 2 mm were counted as germinated seeds. Then, germination percentage was determined by the following equation (Florez *et al.,* 2007):

$$GP = 100 \times n/N$$

Where; GP shows the germination percentage, n shows the number of seeds germinated at the final day, and N shows the total number of planted seeds.

Germination rate

The germination rate was estimated by Maguire's (1982) method in which the following equation is used:

$$GR = \left(\frac{a}{1}\right) + \left(\frac{b-a}{2}\right) + \left(\frac{c-b}{3}\right) + \quad + \left(\frac{n-n-1}{N}\right)$$

Where; GR shows the germination rate in terms of seeds germinated per day, and *a, b, c,* and *n* show seeds germinated 1, 2, 3, and N days after seeds imbibitions, respectively.

Vigor index I and II

Vashisth and Nagarajan's (2010) method was used to measure vigor index:

Vigor index I = germination percentage × seedling length (cm)

Vigor index II = germination percentage × seedling dry weight (g)

Seedling length, radicle length, plumule length

Seedling, radicle and plumule lengths were measured with a ruler at the end of the experiment.

Radicle and plumule fresh and dry weights

Fresh weight was measured with a 0.001 precision digital scale. Then, the seedlings, radicles and plumules were dried at 70°C for five hours, and their dry weight was measured with a 0.001-precision digital scale.

Data analysis

Finally, the data were analyzed with MSTAT-C statistical software package, means were compared with LSD test, and graphs were drawn with MS-Excel software package.

RESULTS

The analysis of variance showed that the interaction effect of different strengths of magnetic

Table 1. Analysis of variance (ANOVA) of the effect of different treatments on studied characteristics.

S.o.V	df	Germination percentage	Germination rate	Vigor index I	Vigor index II	Seedling length	Radicle length	Plumule length	Plumule fresh weight	Plumule dry weight	Radicle fresh weight	Radicle dry weight
Time	1	135.27**	0.77ns	12278.5*	155.6**	0.374**	0.048ns	0.067*	0.073*	0.007*	0.027**	0.004**
Strength	1	275**	8.34**	19010**	549.9**	0.302**	0.076*	0.275*	0.055*	0.039**	0.031**	0.002**
Time× Strength	6	96.8**	11.56**	14840.5**	416.9**	0.322**	0.100**	0.31*	0.047*	0.027**	0.026**	0.002**
Error		44.63	0.967	150	39.53	0.08	0.03	0.03	0.008	0.004	0.008	0.0002
CV (%)		7.42	9.96	8.4	10.21	3.95	6.33	4.31	10.00	5.43	3.20	6.12

* and **: Significant at $P<0.05$ and $P<0.01$, respectively.

Table 2. Mean comparisons of the effect of different treatments on studied characteristics.

Treatments	Germination percentage	Germination rate (day)	Vigor index I	Vigor index II	Seedling length (cm)	Radicle length (cm)	Plumule length (cm)	Plumule fresh weight (g)	Plumule dry weight (g)	Radicle fresh weight (g)	Radicle dry weight (g)
Control	46.38 c	4.99 ef	205.5 e	24.11 e	4.58 b	1.74 c	2.85 d	0.72 f	0.54 c	0.39 b	0.15 de
M1T1	48.60 c	4.23 f	175.0 e	16.59 e	4.45 b	2.02 bc	2.43 e	0.70 f	0.36 d	0.24 c	0.13 e
M1T2	60.55 b	6.01 de	309.0 d	39.42 d	5.30 a	2.37 a	2.96 cd	0.98 a	0.64 bc	0.45 ab	0.19 bc
M1T3	68.88 ab	7.28 bcd	372.7 a-d	54.09 ab	5.21 a	2.06 b	3.21 abc	0.96 a	0.79 ab	0.50 ab	0.18 bc
M2T1	61.38 b	6.87 cd	330.6 bcd	43.19 cd	5.28 a	1.91 bc	3.50 a	0.91 ab	0.81 a	0.57 a	0.24 a
M2T2	74.44 a	9.80 a	390.3 ab	56.02 a	5.13 a	1.99 bc	3.13 bcd	0.76 bc	0.55 c	0.38 bc	0.16 cd
M2T3	73.05 a	8.67 ab	422.8 a	57.07 a	5.44 a	2.00 bc	3.44 ab	0.89 ab	0.73 ab	0.56 a	0.18 bc
M3T1	69.72 ab	6.65 cd	375.1 abc	50.21 abc	5.17 a	1.92 bc	3.20 abc	0.85 b	0.73 ab	0.53 ab	0.20 b
M3T2	66.11 ab	8.21 abc	325.5 cd	45.59 bcd	5.04 a	1.92 bc	3.11 cd	0.79 bc	069 abc	0.52 ab	0.17 cd
M3T3	66.94 ab	7.07 bcd	362.5 a-d	49.02 a-d	5.24 a	2.04 b	3.13 bcd	0.98 a	0.69 abc	0.50 ab	0.18 bc

* Abbreviation include the M1: 50 mT, M2: 100 mT, M3: 150 mT,T1: 10 min, T2: 20 min, T3: 30 min. In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test.

field and exposure durations was significant at 1 and 5% probability level on the germination parameters of Madagascar periwinkle (Table 1).

Germination percentage

According to the results of means comparison, the highest germination percentage was obtained from the treatments of M2T2 (74.44%) and M2T3 (73.05%) without statistically significant differences with those obtained from the treatments of M1T3 (68.88%), M3T1 (69.72%), M3T2 (66.11%), and M3T3 (66.94%). Control seeds (46.38%) showed no statistically significant difference with the treatment of M1T1 (48.60%). They produced the lowest germination percentage (Table 2).

Germination rate

The treatment of Madagascar periwinkle seeds with different strengths and exposure durations of magnetic field increased germination rate significantly as compared to control, except for the treatment of M1T1. Table 2 shows that, the treatment of M1T1 (4.23%) and control (4.99%) had the lowest germination rate among all studied treatments. The highest germination rate (9.80%) was obtained from the treatment of M2T2, but it showed no statistically significant differences with the treatments of M2T3 (8.67%) and M3T2 (8.21%). They, altogether, were come to be known as the best treatment for the germination rate of Madagascar periwinkle seeds (Table 2).

Vigor index I

Between all studied treatments, the highest vigor index I was related to the treatment of M2T3 (422.8), but with no statistically significant differences with those obtained from the treatments of M2T2 (390.3), M3T1 (375.1), M3T3 (362.5) and M1T3 (372.7). Control (205.5) and M1T1 (175.0), without showing significant difference to each other, produced the lowest seed vigor I (Table 2).

Vigor index II

Means comparison revealed that the highest vigor index II was devoted to the treatments of M2T3 (57.70), M2T2 (56.02), M1T3 (54.09), M3T1 (50.21) and M3T3 (49.02). They had no statistically significant differences with each other. The lowest seed vigor indices II of 16.59 and 24.11 was related to the treatments of M1T1 and control, respectively, though without significant differences with each other (Table 2).

Seedling length

According to the results, the treatments of control and M1T1 produced the shortest seedlings with the lengths of 4.58 and 4.45 cm, respectively. The highest seedling length of 5.44 was related to the treatment of M2T3. This treatment did not exhibit significant differences in seedling length with the treatments of M3T3, M3T2, M3T1, M2T2, M2T1, M1T3, and M1T2 (Table 2).

Plumule length

Means comparison of the effect of magnetic field strength and exposure duration on plumule length revealed that the treatment of M2T1 resulted in the highest plumule length of 3.50 cm, but without significant differences with the treatments of M2T3 (3.44 cm), M3T1 (3.20 cm), and M1T3 (3.21 cm). The shortest plumules were recorded for the treatment of M1T1 (2.43 cm) and then, control (2.85 cm) (Table 2).

Radicle length

As means comparison showed, radicle length in plants exposed to magnetic field was higher than those in control, so that control produced the lowest radicle length (1.74 cm). The highest radicle length of 2.37 cm was devoted to the treatment of M1T2 (Table 2).

Plumule fresh weight

Among studied treatments of magnetic field strength and exposure duration, the highest plumule fresh weight was related to the treatments of M1T2 and M3T3 (0.98 g), but with no significant differences with those obtained from the treatments of M1T3, M2T1, and M2T3. The treatment of M1T1 and control produced the lowest plumule fresh weight of 0.70 and 0.72 g, respectively (Table 2).

Radicle fresh weight

As can be seen in Table 2, the treatment of M1T1 produced the lowest root fresh weight of 0.24 g. Also, the treatment of M2T1 recorded the highest root fresh weight of 0.57 g without significant differences with those obtained from the treatments of M1T2, M3T3, M3T2, M3T1, M2T3, and M1T3 (Table 2).

Plumule dry weight

According to the results of means comparison for the effect of different treatments on plumule dry weight, the lowest plumule dry weight of 0.36 g was related to the treatment of M1T1. The treatments of M2T1 (0.81 g), M1T3 (0.79 g), M2T3 (0.73 g), M3T1 (0.73 g), M3T2 (0.69 g), and M3T3 (0.69 g) had no statistically significant differences and produced the highest plumule dry weight (Table 2).

Radicle dry weight

The application of different strengths and duration of magnetic field resulted in significant differences in radicle dry weight except in the treatment of M1T1 that showed no significant difference with control. As is evident in Table 2, the lowest radicle dry weight of 0.13 g was related to the treatment of M1T1 and then to control with radicle dry weight of 0.15 g. The highest radicle dry weight of 0.24 g was obtained from the treatment of M2T1 (Table 2).

DISCUSSION

Germination is a critical phase in the growth period of the plants that is usually affected by environmental stresses, particularly drought and salinity. Salinity reduces the germination percentage of Madagascar periwinkle seeds (Jaleel *et al.*, 2008). But, the present study shows that the treatment of seeds with different strengths and durations of magnetic fields of increased germination percentage in this species. The positive impact of magnetic field on seed germination under salinity conditions can be related to the impact of magnetic field on increasing the energy and activity of seeds, respiration, and auxin activity (Lipiec *et al.*, 2004; Aladjadjiyan, 2007; Marinkovic *et al.,* 2008); It is believed that the exposure of seeds to magnetic field produces plants resistant to stresses included salinity (Aladjadjiyan, 2007; Marinkovic *et al.,* 2008).

In a study on germination percentage of tobacco seeds as affected by magnetic field with the strength of 0.15 T for 10, 20 and 30 minutes, it was found that germination percentage was linearly increased as affected by magnetic field (Aladjadjiyan and Ylieva, 2003). Feizi *et al.* (2012) studied the influence of different intensities and exposure durations of magnetic field on germination and growth of wheat seedling and reported that 20-minute exposure of seeds to the 10-T magnetic field had an inductive effect and that stronger treatments had an inhibitory effect on germination traits.

Current study showed that, the impact of different treatments of magnetic field strengths and exposure durations was significant on germination rate, and the seeds exposed to 100-T treatment for 20 and 30 minutes had the highest germination rate. It is suggested that the positive effect of magnetic field on seeds germination rate is associated with its effect on changing water relations in seeds (Garcia and Arza, 2001). Researchers believe that magnetic field reduces water surface tension and viscosity as well as latent heat of vaporization that finally, entails rapid vaporization of water. The variations of this index resulted in more rapid and effective penetration of water into seeds and so, higher germination rate (Pang and Deng, 2008). Renia *et al*. (2001) suggested that magnetic field causes essential changes in ion concentration of cell membrane, controlling osmotic pressure and finally, the rate of water inflow to seeds. The final result is a higher germination rate.

According to the International Seed Testing Association's definition (ISTA, 2009), seed vigor is the set of seed qualities that determine seed or seed lot's activity and performance during germination and seedling emergence. In fact, seed quality is composed of numerous factors, but germination potential, seed vigor, durability and seed health are the main components of seed quality, playing an important role in its quality (van Gastel *et al.,* 1996). According to our results, the vigor indices I and II as factors representing plant growth potential and determining plant's annual yield were influenced by magnetic field. Podlesny *et al.* (2005) reported that exposure to magnetic field improved the vigor of pea seeds. Vashisth and Nagarajan (2010) found that the exposure of sunflower seeds to magnetic field improved seed vigor by 18-74%.

The present study revealed the significant influence of different strengths of magnetic field and exposure times on the length of seedlings, radicles, and plumules. According to Marghaeizadeh *et al.* (2015), magnetic field improves the first growth phase of most plants. In their study on the impact of ultrasonic waves and magnetic field on germination and growth indices of *Carum copticum* L., they reported that radicle and plumule lengths were increased under the treatment of magnetic field and ultrasonic waves. They related this observation to the increased membrane permeability that enhanced the inflow of nutrients and also to the reduced distribution of nutrients in the lack of magnetic field treatment. Similar results have been reported by Aladjadjiyan (2002), Soltani and Kashi (2004) and Podlesny *et al.* (2003). Martinez *et al*. (2009) stated that lentil and pea seeds exposed to 125 T magnetic field for 10 minutes grew significantly longer plumules and seedlings as compared to control.

Improvement of seedling fresh and dry weight with magnetic field in the present study can be associated with the impact of different strengths of magnetic field and exposure durations on the production of resistant seedlings. Researchers believe that magnetic field not only accelerates the inflow of water into seeds, but also affects the rate of enzymatic reactions (Tieu *et al*., 2001). Higher water uptake in the first step accelerates the swelling of seeds under magnetic field which results in their higher fresh weight. In addition, higher fresh weight of seedlings may be related to more rapid metabolism and higher water content of the plants (Fischer *et al.*, 2004). These researchers related the effect of static magnetic field on sunflowers and wheat to higher radicle fresh and dry weight and total plant fresh and dry weight. Aladjadjiyan (2002) revealed that the application of the 150 mT magnetic field increased germination and stem fresh weight of corn significantly. The increasing effect of magnetic field has been also reported on fresh and dry weight of tomatoes (de Souza *et al.,* 2006) and seedling dry weight of pea (Vashisth and Nagarajan, 2010). In a study on marigold, Salehi Arjmand and Sharafi (2015) showed that higher strength of the magnetic field and exposure duration reduced seedling dry weight.

CONCLUSION

The exposure to magnetic field increased germination percentage and rate of Madagascar periwinkle seeds in a medium with 19 mS cm^{-1} salinity. Also, seed vigor indices I and II and seedling, plumule and radicle lengths and fresh and dry weights were improved by magnetic field. In fact, it can be said that the exposure of seeds to different intensities of magnetic field induced germination and increased plumule and radicle lengths resulting in the growth of salinity-resistant plants. In the present study, 20 minute exposure to the 100 mT magnetic field and 30-minute exposure to the same magnetic field intensity produced the highest germination percentages of 74.44 and 73.05%, respectively, as compared to control with the germination percentage of 46.38%. Also, these treatments had the highest germination rate and seed vigor indices I and II. Since vigor indices I and II represent plant growth and establishment potential, it can be said that 20 or 30 minute exposure to the 100 mT magnetic field were the best treatments for the germination of Madagascar periwinkle (*Catharanthus roseus* cv. 'Acillata') seeds in salinity conditions.

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