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Determination of Effect of Spermine Treatment on Alleviation of the Drought Stress Caused by Polyethylene glycol in Soybean (*Glycine max*L.) Seeds

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ABSTRACT

The present study was formulated in order to determine the effect of spermine treatment on germination of soybean (*Glycine max* L.) seeds during drought-stress induced by polyethylene glycol (PEG).Soybean seeds were planted in petri dishes and irrigated with five solutions (namely control, drought, PEG, Spm, and PEG-Spm). Germination percentage was achieved by counting the number of germinated seeds per row. Germination criterion was appearance of radicle cap.Statistical analyses were performed by one-way ANOVA through Duncan Test at $p \leq 0.05$ in SPSS (Version 21) in three iterations. Graphs were drawn in Excel Software (Microsoft Office, 2010). The results obtained from the present study showed that addition of spermine 0.1 mM in normal condition led to significant reduction of germination compared with control treatment. Also, spermine treatment could not alleviate adverse effects of drought stress cause by PEG.

KEYWORDS: Spermine, PEG, Soybean, Germination, Drought, Gorgan.

1- INTRODUCTION

Drought stress influences on water holding capability in plant cells, tissues, and organs leading to specific and non-specific reactions as well as potential injury in tissues and induction of adaptive responses. Plants cope with drought stress by triggering their defensive mechanisms such as reduction of transpiration and photosynthesis in addition to accumulation of osmolites [1].

Osmoregulation is a mechanism in order to keep water potential in plant cells during water shortages. It includes accumulation of variety of osmoactive molecules and ions such as intracellular dissolved sugars, carbohydric alcohols, prolines, organic acids, calcium, potassium, and chloride ions. However, polyamines are also osmoactive substances with high importance in drought stress tolerance. Their role in osmoregulation, membrane stability, and free radicals scavenging has been pronounced. Biotic synthesis of three most well-known polyamines (i.e. putrescine, spermidine, and spermine) is performed either by direct decarboxylation of ornithine by ornithine decarboxylase or by intermediation of agmatine and N-carbomoylputrescine. Another important enzyme for polyamines synthesis is S-adenosylmethioninedecarboxylase which is necessary for production of aminopropyl groups found in spermine and spermidine [2].

Stress-tolerant plants usually have higher capacity to synthetize polyamines in response to stress compared to stress-sensitive ones. The stress-tolerant species have been found to increase their interna l polyamines in response to stressors. It has been proven that external spermine treatment results in higher drought and salinity tolerance in Arabidopsis. Furthermore, it has been suggested that transgenic plants with high contents of prolines and polyamines are more tolerant to stresses. Nevertheless, polyamine and proline contents can be interrelated because their biosynthetic and catabolic pathways have common intermediates [2].

The polyamine spermine plays role in cellular metabolism in eukaryotic cells. Spermine is derived from spermidine and is found in variety of organisms and tissues. Also, it is a necessary growth factor in some bacteria. Spermine is also seen in physiologic pH as polycation. It is also related to nucleic acids and is thought to stabilize helical structure, especially in viruses. Spermine phosphate crystals were first found in 1678 by Leeuwenhoek in human semen and that's why the polyamine has been called "spermine". Table 1 summarizes properties of spermineand Figure 1 depicts its chemical structure[3].

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Table 1: Properties of the polyamine spermine

Molecular formula	**$C_{10}H_{26}N_4$**
Molar mass	202.34 g.mol^{-1}
Appearance	Colorless crystals
Odor	Ichtyal, ammoniacal
Density	937 mg.ml-1
Melting point	28-30°C (82-86°F)
Boiling point	150°C (302°F) (at 700 Pa)

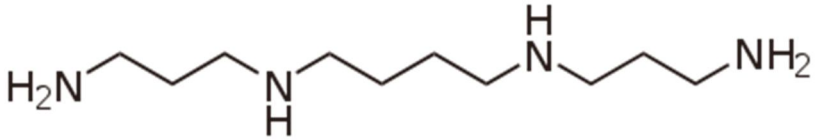


Figure 1: Chemical structure of spermine

Soybean is considered as one of the oldest agricultural plants. It contains abundant amounts of protein, carbohydrate, oil, phosphorus, calcium, iron, magnesium, zinc, fiber, and vitamins (thiamin, riboflavin, and niacin) [4]. Water is very important of growth and development of soybean. It is sensitive to drought in germination and great loss of germination occurs during drought stress. Also, lack of sufficient moisture in germination of soybean brings about slower growth [5]. With regard to what mentioned above, the present study was formulated in order to determine the effect of spermine treatment on germination of soybean (*Glycine max* L.) seeds during drought-stress induced by polyethylene glycol (PEG).

2- MATERIALS AND METHODS

Polyamine spermine was purchased from Pajohan-Sanaat-Homehr. Characteristics of the materials are shown in Table 2.

Table 2: Characteristics of the purchased spermine

Assay ≥ 97%	$C_{10}H_{26}N$	Fw=202.34
bp 150°C / 5 mmHg	CAS 71-44-3	
mp 28-30	EC Number 200-754-2	
b 2-8°C	Sigma-Aldrich	

Figure 2: The purchased spermine

The required amount of PEG with molecular mass of 6000 was derived by the following relation in order to provide osmotic potential of 0.3 MPa:

$S = -(1.18\times10^{-2})\,C - (1.18\times10^{-4})\,C^2 + (2.67\times10^{-4})\,CT + (8.39\times10^{-7})\,C^2T$

where C, T, and S stand for concentration of PEG 6000 ($g.l^{-1}$), temperature (°C), and osmotic potential (MPa), respectively. PEG concentration was found to be 35.42 g.

Solutions were made on Aug 2013 in Research Laboratory of Islamic Azad University of Gorgan – Iran as follows:

Table 3: Spermine solution

Polyamine	Molar mass	Concentration of 0.1	Concentration of 0.5	Volume of 2 liter with concentration of 0.5
Spermine	88.15	0.009	0.0088×5=0.044	0.088

Spermine solution with concentration of 0.1 mM was made.

$$0.1 \quad mm = \frac{1}{10000}\text{M}$$

$$C_M = \frac{C}{M} \quad => \frac{1}{10000} = \frac{C}{202.34} \quad => C = \frac{202.34}{10000} = 0.02\ {}^{g}/_{lit}$$

Therefore, 0.04 $g.l^{-1}$spermine was dissolved in 2 lit of water in order to make 2 lit of spermine solution.

In order to provide PEG+Spm solution, 70.82 g PEG (2×35.42 for 2 lit) were weighed and the spermine required was achieved as follows:

$$Spm\,\frac{0.04}{2000} = \frac{x}{2058} \qquad => x = 0.04$$

Soybean (*Glycine max* L.) seeds were purchased from Araghi-Mahalleh Station – Gorgan – Iran and 600 seeds were placed in hypochloride sodium 10% for 10 min after rinsing with distilled water. Petri dishes were rinsed with boiling water and then disinfected with hypochloride sodium 10%. Petri dishes and seeds were rinsed immediately after disinfection. Cleansing fabrics were disinfected with distilled water and hypochloride sodium and finally they were washed with distilled water. The seeds were sorted in 5 petri dishes between two cleansing fabrics. In each petri dish, seeds were sorted in 5 rows each with 20 seeds. Then, the petri dishes were placed at 25°C at darkness and were irrigated with Spm, PEG, PEG+Spm, and control solutions every 8-hour intervals. Germination percentage was achieved by counting the number of germinated seeds per row. Germination criterion was appearance of radicle cap.

Statistical analyses were performed by one-way ANOVA through Duncan Test at $p\leq0.05$ in SPSS (Version 21) in three iterations. Graphs were drawn in Excel Software (Microsoft Office, 2010).

3- RESULTS

3-1- Germination percentage from the first to sixth 8-h intervals

Figure 3 depicts germination percentage achieved for the first to sixth 8-h intervals. As it can be seen, the highest and lowest germination percentage in soybean seeds in the first to sixth 8-h intervals were seen in control and Spm treatments, respectively. No significant difference was detected between the treatments in the first 8-h ($p>0.05$).However, there was a significant difference between control and other treatments in the 2nd 8-h ($p<0.05$).

In the 3rd 8-h, significant differences were seen between control and Spm in addition to PEG and Spm treatment ($p<0.05$). However, in the 4th 8-h, there was a significant difference between Spm and other treatments ($p<0.05$). Germination percentage in the 5th h-h followed a same trend as the 3rd 8-h. in the 6th 8-h, there was a significant difference between control and Spm treatments ($p<0.05$).

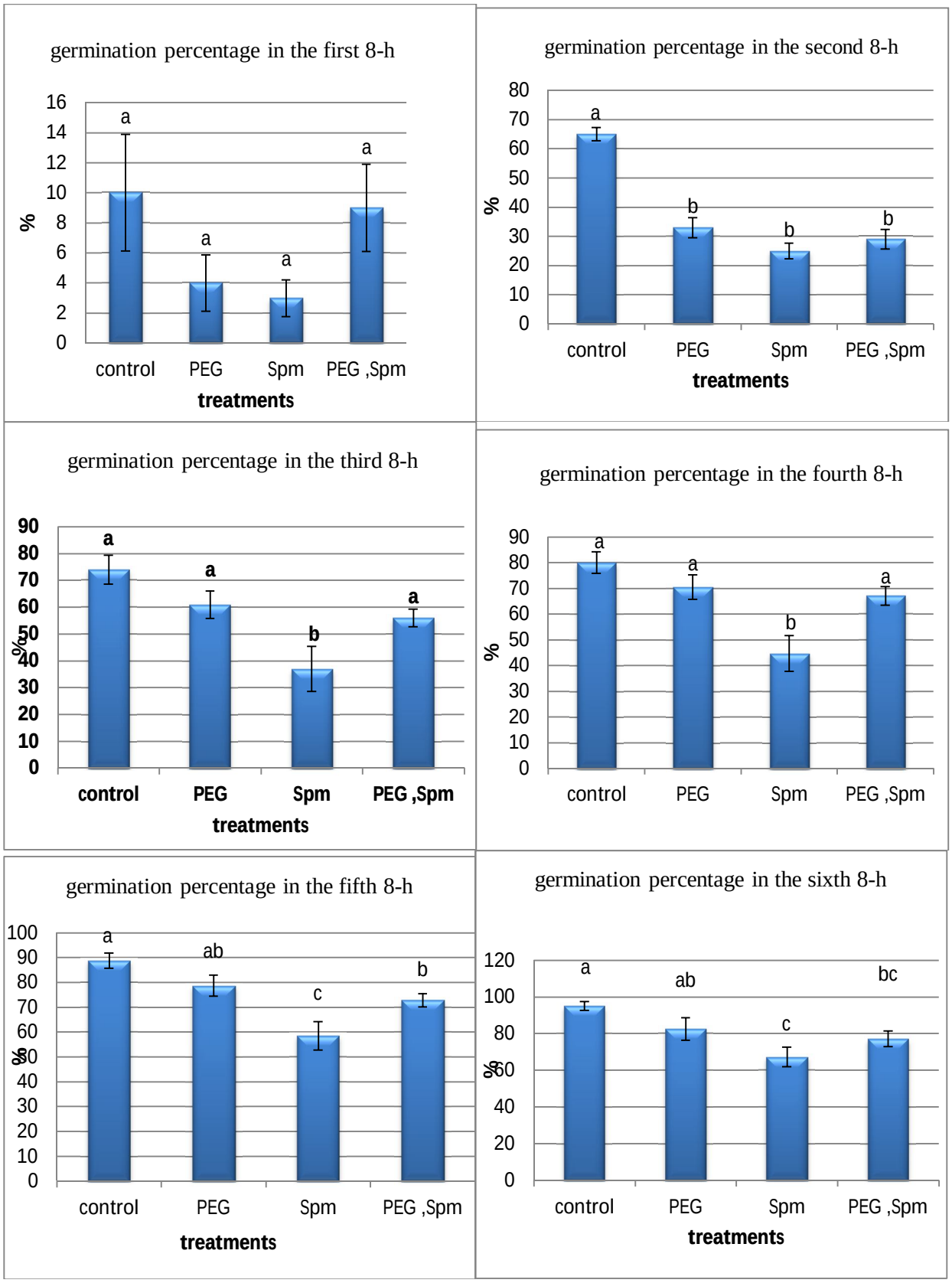


Figure 3: Germination percentage in the first to sixth 8-h intervals between the treatments

Figure 4 shows germination percentage in all the treatments from the 1st to 6th 8-h. as it is seen, in all 6 8-h intervals, the highest and lowest germination rates are for control and Spm treatment, respectively.

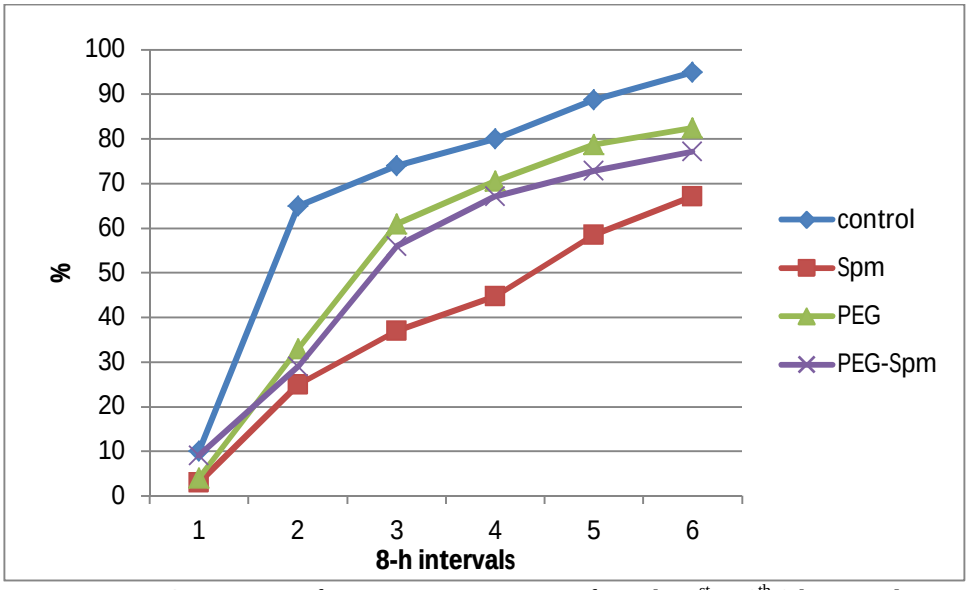
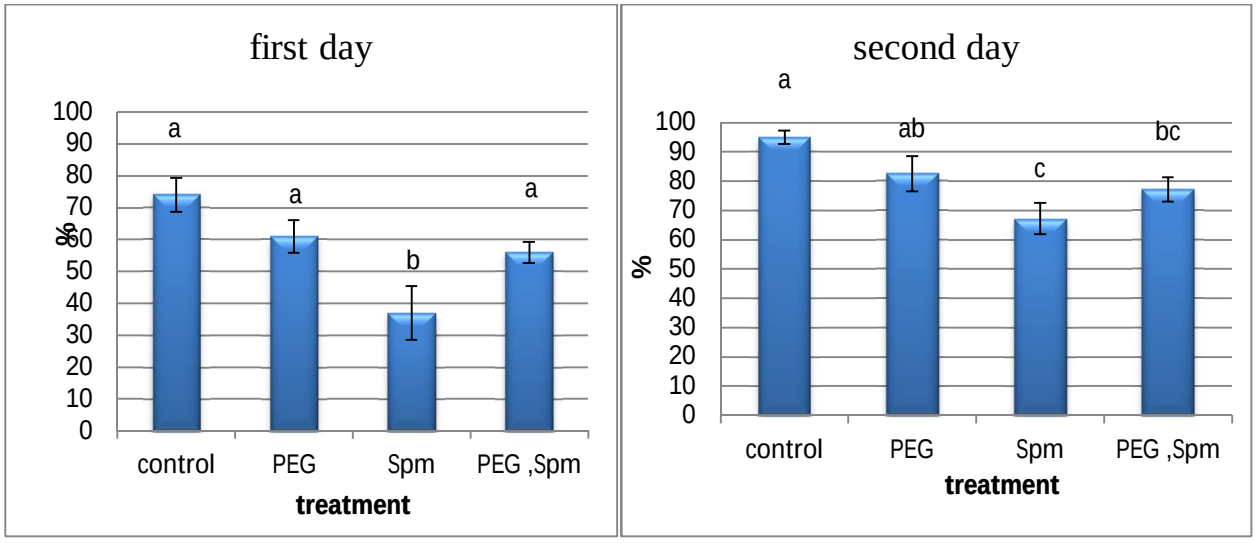


Figure 4: Comparison of germination percentage from the 1st to 6th 8-h intervals

3-2- Germination percentage from the first to seventh days

Again, the highest and lowest germination percentage in soybean seeds in the first to seventh days were seen in control and Spm treatments, respectively. In the first day, significant differences were detected between Spm and other treatments ($p<0.05$).in the 2nd day, a significant difference was detected between control and Spm treatments ($p<0.05$). However, no significant differences were seen between control and PEG, PEG and PEG-Spm, and PEG-Spm and Spm ($p>0.05$).

In the 3rd day, a significant difference was seen between control and Spm treatments ($p<0.05$). Moreover, in the 4th and 5th days, significant differences were detected between control and Spm treatments ($p<0.05$). In the 6th day, the only change compared to last day was in PEG which reached 96.25%. no significant differences were seed between control and PEG treatments and also between Spm and PEG-Spm treatments ($p>0.05$). But, significant differences were seen between control and PEG treatments and Spm and PEG-Spm treatments ($p<0.05$).a same trend as the 6th day was seen in the 7th day.



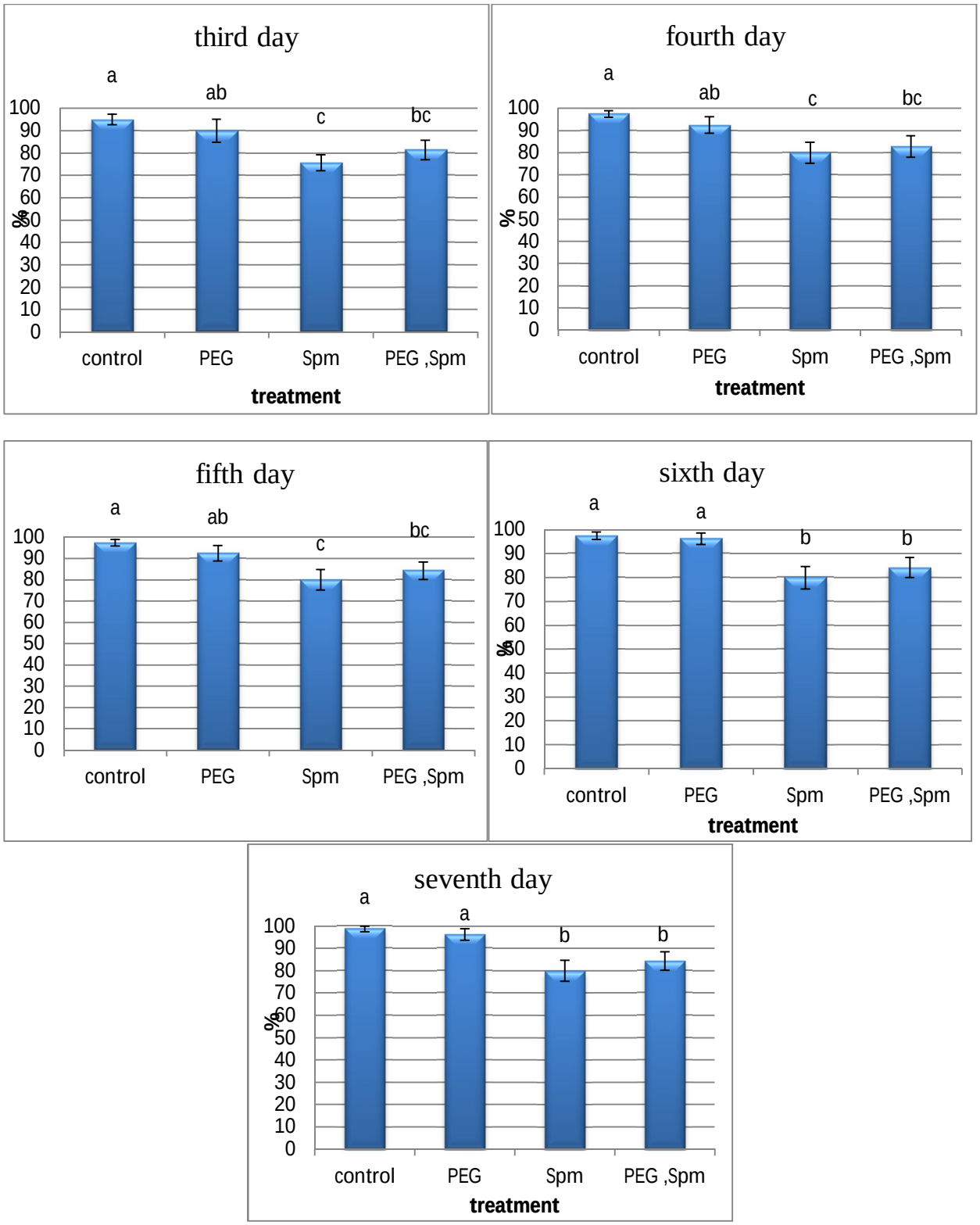


Figure 5: Germination percentage from the 1st to 7th days

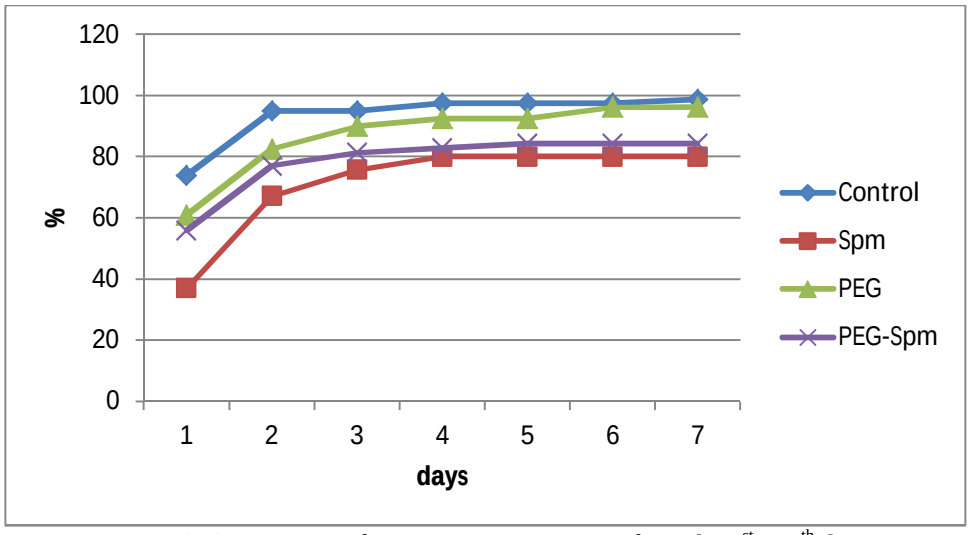


Figure 6: Comparison of germination percentage from the 1st to 7th days

As it can be seen in Fig. 6, germination percentage in soybean seeds in all the treatments increased dramatically from the 1st to 2nd days; however, it leveled off from the 2nd to 7th days. In all seven days, the highest and lowest germination percentages were seen in control and Spmtreatments, respectively.

4- DISCUSSION AND CONCLUSION

Plant abilities in tolerance to different stress conditions are different. The tolerance can be classified into stress avoidance and stress tolerance. By providing some physical and/or metabolic barriers, a plant can avoid stress conditions. In tolerance condition, plants tolerate the damages and losses caused by stresses and try to minimize such effects. In this condition, the plants are subjected to stresses but the damages are expected to be alleviated [6]. The results obtained from the present study showed that the highest and lowest germination percentages were in control and spermine treatments. Drought stress was induced in petri dishes by use of polyethylene glycol. PEG is very popular because of its ability to make more realistic drought conditions [7]. PEG results in reduction of hydrolysis of seeds' stored materials and consequently, lower germination percentages by making drought condition [8].It is noteworthy that in all the cases, except for the 2nd 8-h, no significant differences were seen between control and PEG treatments ($p>0.05$). Drought stress is globally known as one of the most important abiotic factors to limit germination rate [9]. Water availability and water absorption by seeds are necessary for germination processes. Reduction of water potential in seeds is one of the consequences of drought stress. High negative potential of water, especially in the first stages of germination, leads to reduction of water absorption by seeds and hinders continuation of germination processes. Increased drought stress reduces water availability and exerts unfavorable effects on germination rate and percentage [9].Furthermore, a same trend was seen in the germination percentage from the 1st to 7th days as the 1st to 6th 8-h intervals where the highest and lowest germination percentages were detected in control and spermine treatments, respectively. However, no significant difference was detected between these two treatments ($p>0.05$). It has been reported that exertion of drought stress in germination stage resulted in significant reduction of germination rate [10] which is not in agreement with the results obtained from the present study. Reduction of germination percentage under other stress conditions has also been reported. For instance, Zapata et al (2004) reported that salinity stress reduces germination percentage in the species studied in their investigation [11].

Finally, addition of spermine 0.1 mM in normal condition led to significant reduction of germination compared with control treatment. This is not consistent with the results obtained by Liu et al (2012); they claimed that polyamines stimulate germination [12]. Also, it has been stated that putrescine, spermidine, and spermine prevent seeds from germination.

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The authors declare that they have no conflicts of interest in the research.

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