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Studies on Seed Germination and Seedling Growth of *Silybum marianum* (L.) Gaertn under Water-Deficit Stress Conditions

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ABSTRACT

Among the environmental stresses, water-deficit stress is one of the most adverse factors to plant growth and development. The present study was undertaken to study the seed germination and seedling growth of Silybum marianum (L.) Gaertn. to water-deficit stress under laboratory conditions.Water stress was generated using different concentrations of PEG-6000 (Polyethylene glycol 6000) and the seed germination, seedling growth, proline, lipid and chlorophyll (a, b and total) content were evaluated. The increase in osmotic potential showed significant decrease in germination. Seedling growth parameters decreased with an increase in PEG concentration, except the seedling fresh weight which increased in all the treatments. Proline and MDA content increased with increase in PEG concentrations but chlorophyll content decreased with an increase in water stress.

Key words: *Water-deficit, Polyethylene glycol 6000, Osmotic potential, Germination, Proline, MDA, Chlorophyll content.*

INTRODUCTION

Silybum marianum (L) Gaertn is an annual or biannual medicinal plant belonging to the Asteraceae family. It is also known as Milk Thistle due to the presence of milky white sap that oozes out from the leaves when they are crushed. It is a native of Southern Europe and is now found throughout the world. In India, Western Himalayas and Kashmir are its natural habitat, whereas in all over the country, it is highly cultivated in Rajasthan. Its medicinal effects are documented among the alternative medicines referred to as liver and bile-related diseases remedy[14]. Milk thistle oil has been suggested as being suitable edible oil and a vitamin E rich source[7]. It contains a phenolic compound known as Silibinin which is a major constituent of Silymarin, a extract of milk thistle seeds. Silibinin has hepatoprotective properties that protect liver cells against toxins. Silymarin and its components have also been shown effective against UV damage and skin cancer. Its fruits are edible.

Plants, because of their sessile nature, are the foremost organisms which always face several environmental stresses such as extreme temperatures, drought, water logging, salinity and heavy metals, which severely affect their productivity.

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Out of all the stresses, water deficit stress is the most influential stress. Extensive studies have been conducted for understanding the plant tolerance in response to water-deficit. It is a major abiotic factor affecting the global crop yield[15]. In arid and semiarid regions, plants are often exposed to water deficit stress, also known as drought stress. It has been known to induce a sequence of morphological, biochemical and molecular alterations that negatively affect plant growth and productivity[6]. Responses of plants to water deficit result in alternation of morphology, chloropohyll content, free proline and lipid content. Plants have evolved several mechanisms that allow perceiving the stresses and rapidly regulating their physiology and metabolism to cope them. Osmotic solution such as PEG (Polyethylene glycol) has been used to impose water stress in different experimental approaches[12].

The major objective of the present investigation was to study the water deficit stress impact on germination and growth of seedling, free proline, MDA level and chlorophyll contents in *Silybum marianum* (L.) Gaertn.

MATERIAL AND METHODS

Seed Source:

The seeds of *S. marianum* (L) Gaertn were obtained from Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.). The experiment was conducted in laboratory conditions of Shoolini University of Biotechnology and Management Sciences, Solan.

Germination test:

The seeds were selected on the basis of uniformity and were surface sterilized with 0.1% $HgCl_2$ for 5 minutes. Thereafter, the seeds were washed under distilled water and then soaked in control (distilled water), 1%, 2.5 %, 5%, 7.5%, 10%, 15% and 20 % PEG solution for 24 hours. The seeds were transferred to petriplates lined with three layers of filter papers moistened either only by distilled water (control) or by different concentrations of PEG. Fifteen seeds, in triplicate, were used for each treatment. The seeds were then allowed to germinate in an incubator at 25±2ºC under continuous illumination provided by fluorescent white light. After 24 days of germination, seedling growth was measured in terms of root length shoot length and seedling fresh weight. Seedlings (15 days old) were shifted to hydroponic culture containing Hoagland nutrient solution[10] and allowed to grow in BOD incubator at 25±2ºC. Nutrient medium was replaced by fresh one at regular intervals. After 14 days of shifting to BOD, the plants were treated with 1%, 2.5 %, 5%, 7.5%, 10%,15% and 20 % PEG through appropriate addition to the nutrient medium. After 21 days of treatment, determination of proline was done by following the method of Bates *et al*.[2]. The estimation of lipid peroxidation and chlorophyll contents were done by following the method of Dhindsa *et al*.[5] and Hiscox and Israelstam[9] respectively.

At the end of experiment, statistical analysis was carried out by using GraphPad Prism® (Version 5.02). The one-way or two-way ANOVA analysis was performed and means comparison analysis was achieved using Tukey's Multiple Comparison Test ($P < 0.05$).

RESULTS AND DISCUSSION

The seeds of *S. marianum* (L) Gaertn were non-dormant and responded differently to PEG concentrations. PEG at higher concentration i.e. 5%, 7.5%, 10%, 15% and 20% decreased germination gradually. At lower concentration the seed germination increased compared to control (Fig. 1).

The seedling growth of *S. marianum* (L) Gaertn was measured after 24 days of incubation on substratum containing different concentration of PEG. The growth was measured in terms of root length shoot length and seedling fresh weight. Root length increased at lower concentration of PEG (1%, 2.5%, 5%, 7.5%) but with the increase in concentration of PEG, root length decreased. Shoot length increased at 1%, 2.5%, 5% PEG concentration but as the concentrations of PEG increased (7.5 %, 10 %, 15 % and 20 %), shoot length decreased gradually. Seedling fresh weight increased in all the treatments (Fig.2).

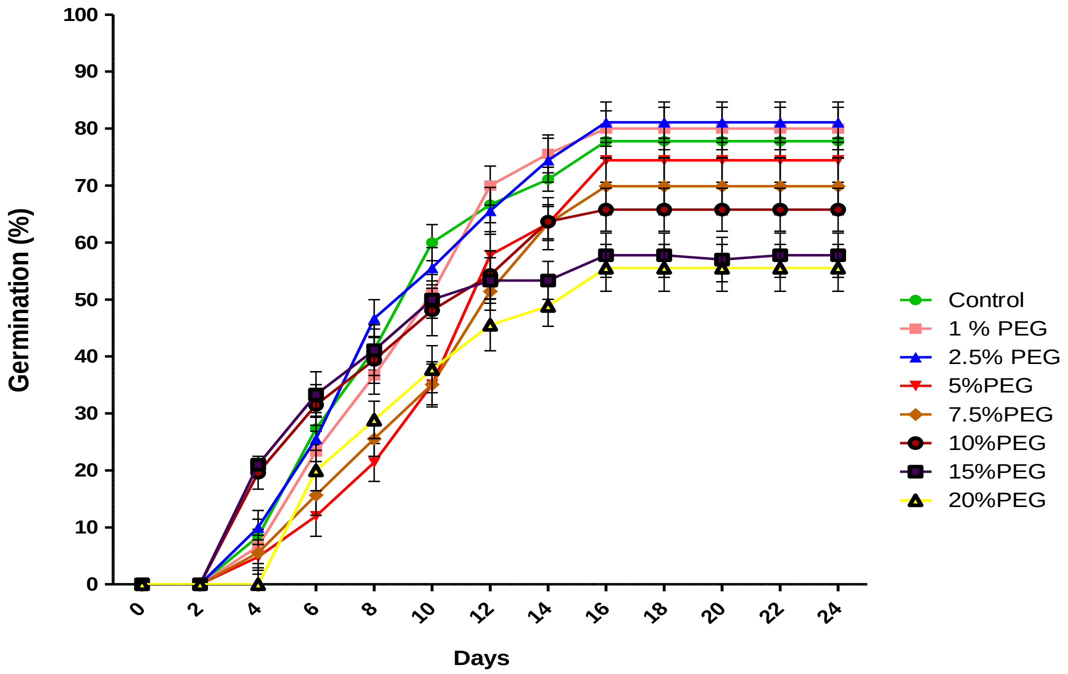
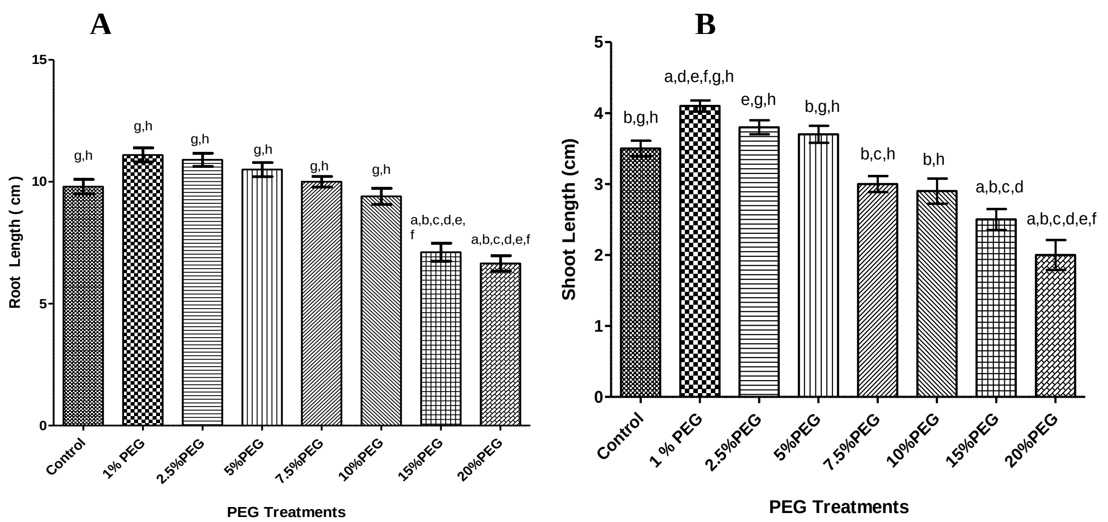


Fig. 1: Time-course of germination of *Silybum marianum* seeds as affected by 1%, 2.5%, 5%, 7.5%, 10%, 15% and 20% PEG treatment. Values are mean ± S.E



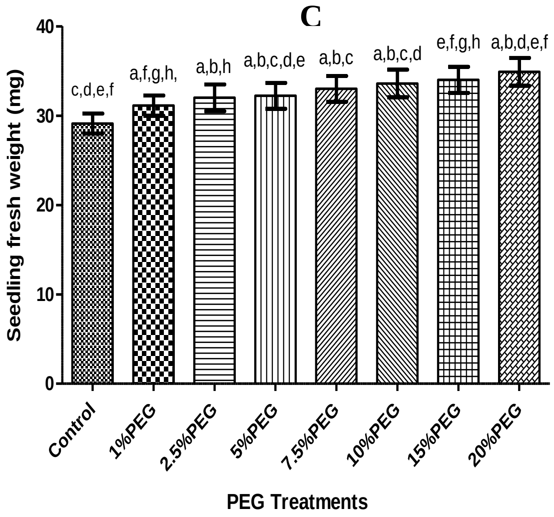


Fig. 2: Effect of PEG treatments on root length (A), shoot length (B) and seedling fresh weight (C) of *Silybum marianum*. Values are mean ± SE

Free Proline Content:

Free proline content increased with an increase in PEG concentrations (Fig. 3).

MDA Content:

Lipid peroxidation, a measure of free radical generation, was determined in terms of malondialdehyde (MDA) content. Leaf and root MDA content increased with increase in PEG concentration (Fig. 4).

Chlorophyll Content:

PEG induced drought stress imposed to plants, significantly decreased chlorophyll 'a', chlorophyll 'b' and total chlorophyll content (Fig. 5). Chlorophyll a and total chlorophyll content decreased with increase in concentration except at 1% PEG. Chlorophyll b content decreased with increase in concentration except at 1%, 2.5% and 5% PEG.

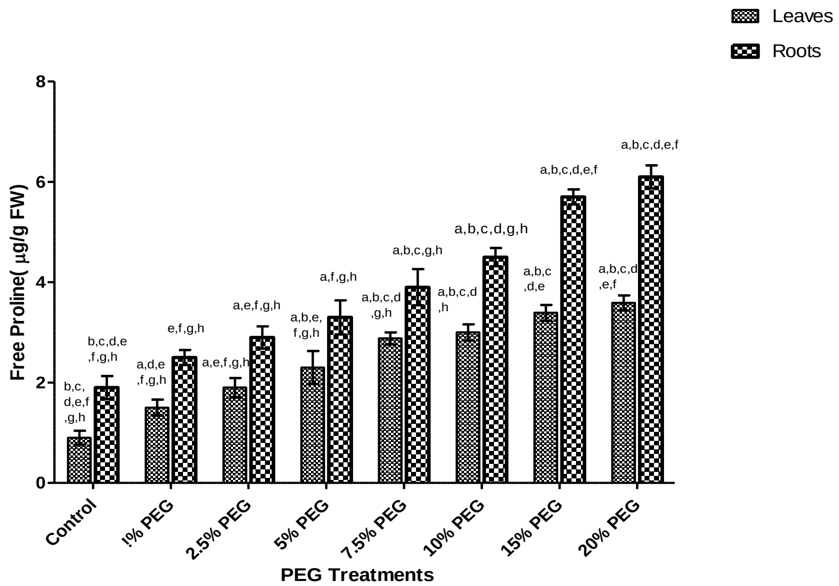


Fig. 3: Effect of PEG treatments on free proline content in leaves and roots of *Silybum marianum*. Seedlings were grown hydroponically for 15 days and subsequently exposed to stress for 21 days. Values are mean ± SE

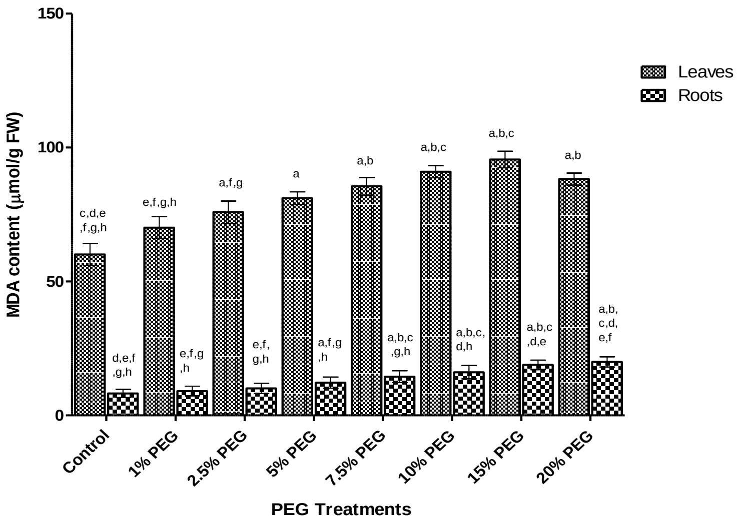


Fig. 4: Effect of PEG treatments on MDA content in leaves and roots of *Silybum marianum*. Seedlings were grown hydroponically for 15 days and subsequently exposed to stress for 21 days. Values are mean ± SE

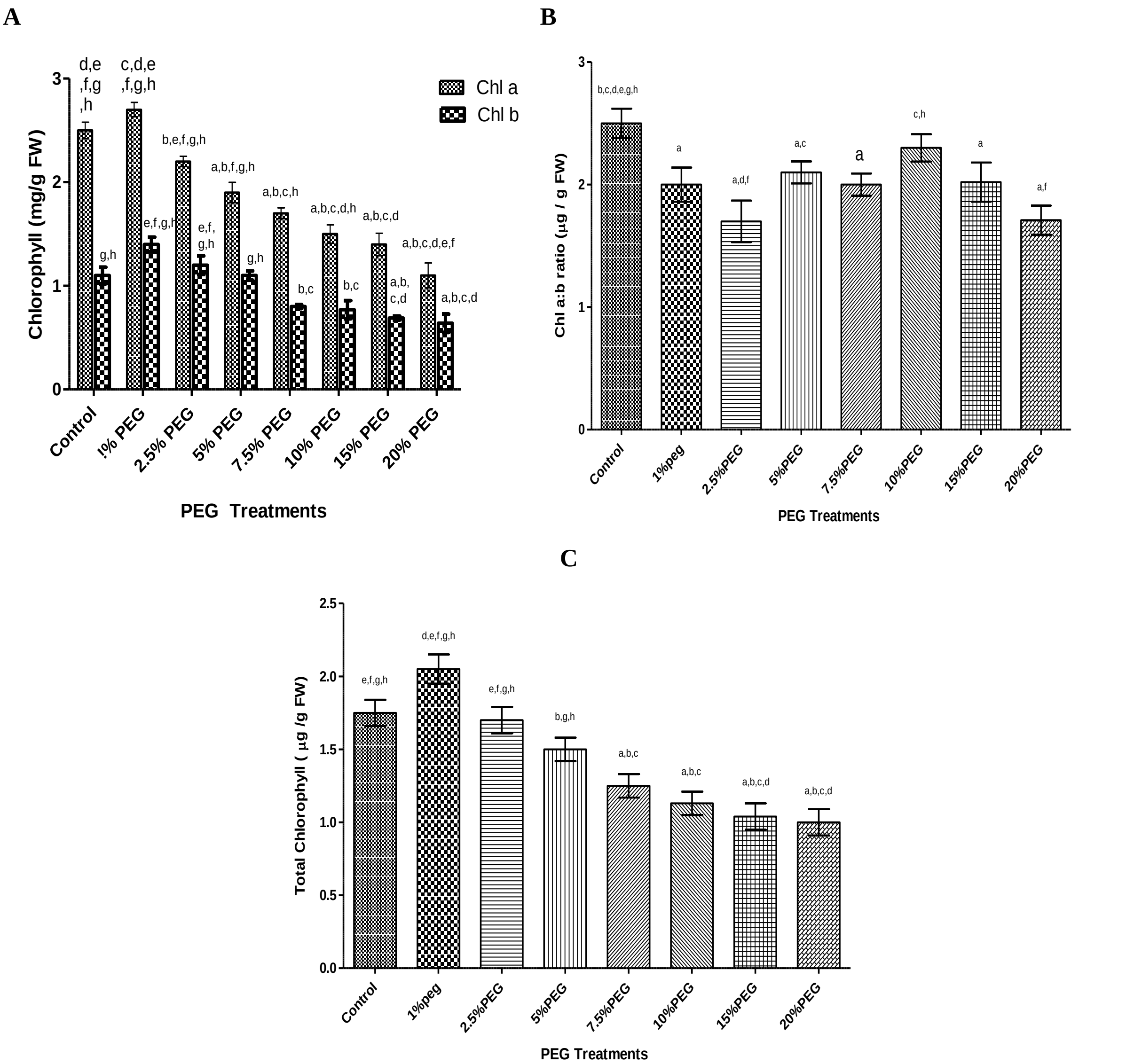


Fig. 5: Effect of PEG treatments on Chlorophyll a and b (A), Chlorophyll a:b (B) and total chlorophyll content (C) in *Silybum marianum*. Seedlings were grown hydroponically for 15 days and subsequently exposed to stress for 21 days. Values are mean ± SE; n=15

DISCUSSION

Environmental stresses trigger a wide variety of plant responses, ranging from altered gene expression and cellular metabolism to changes in growth rate and plant productivity. Plant reaction/ response are to circumvent the potentially harmful effects caused by a wide variety of both abiotic and biotic stresses. The present study was undertaken to know the effects of water stress on *S. marianum* (L.) Gaertn In the present study, seed germination increased at lower concentration of PEG (1 % and 2.5 %). Wenfan *et al.*[21] attributed increase in germination due to PEG.

In the present study, there is an increase in the root length at lower concentration of PEG (1%, 2.5%, 5%, 7.5%) which leads to the inference that *S. marianum* (L.) Gaertn might tolerate drought upto a certain degree. An increased root growth due to water stress was reported in sunflower[19] and *Catharanthus roseus*[11]. The present study revealed a gradual decrease in shoot length at higher concentration of PEG except at 1%, 2.5% and 5% PEG. PEG (6000) caused a significant reduction in the shoot growth of *Anthxanthum odoratum*[1]. Similarly, Okçu *et al*.[17] observed that water stress depressed the shoot growth. Further, at various concentrations of PEG, a gradual increase in seedling fresh weight was observed. According to Bloor[3], the increase in seedling fresh weight could be due to high-light treatment.

In the present investigation, it was observed that proline content in leaf and root increased with increase in PEG concentration. Increased level of proline may be an adaptation to overcome the stress conditions. Tatar and Gevrek[20] showed that proline content increased under drought stress. The increased level of MDA is an indicator of membrane damage that is closely associated with the uncontrolled accumulation of ROS caused by water stress[4]. In the present study, the MDA content in root and leaves of *S. marianum* (L.) Gaertn increased under drought stress. The earlier studies reported that MDA content increased under PEG stress[18]. The photosynthetic ability of the plant is also affected by the stress conditions as in the present study, the chlorophyll content decreased at higher PEG concentrations. It is because of production of reactive oxygen species (ROS), such as O_2 and H_2O_2, which lead to lipid peroxidation and consequently, chlorophyll destruction[13]. This result was in agreement to the finding of Manivannan *et al.*[16] according to which drought stress reduced chlorophyll *a* content, the chlorophyll *b* content and the total chlorophyll content in all sunflower varieties.

CONCLUSION

Our present results indicate that PEG 6000 induced water stress caused significant morphological, physiological and biochemical changes in *S. marianum* (L.). The seed germination along with root length decreased at higher PEG concentration. On the other hand, seedling fresh weight increased. Also, enhanced proline accumulation during stress indicates that proline play a cardinal role as an osmo-regulatory solute in plants. MDA content also increase due to reactive oxygen species. In addition to other factors, changes in photosynthetic pigments are of paramount importance to drought tolerance. Finally, the present findings revealed that, *S. marianum* (L.) may cope water-deficit stress conditions to some extent.

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