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# Effect of Polyethylene Glycol induced Water- deficit stress on the Seed Germination and Seedling Growth of *Cynara scolymus* L.

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**ABSTRACT**

*Water stress is generally the most important factor which influence the development and productivity of the plants. The present study aims to know the effects of water stress on the seed germination and seedling growth of Cynara scolymus L. Water stress was generated using different concentrations of PEG-6000 (Polyethylene glycol 6000). Proline, MDA and chlorophyll content were also assessed. The seedling growth and seed germination of C. scolymus L. were restricted further decreasing the shoot length, root length and seedling fresh weight at higher stress conditions. It caused increase in the level of proline and MDA of both shoots and roots. Chlorophyll content decreased with an increase in water stress.*

***Key words:*** *Stress, Polyethylene glycol, Proline, MDA, Chlorophyll content.*

## INTRODUCTION

The growth, development and spatial distribution of plants are severely restricted by a variety of environmental stresses. Among the major environmental stresses, water deficit stress is the most critical one[4]. It is characterized by the reduction of water potential, water content, closure of stomata and decrease in cell growth further inhibiting the growth of plant[16]. When a plant is exposed to high water stress, its major processes such as seed germination, vegetative growth, flowering, fruiting, lipid peroxidation, photosynthesis and protein synthesis are affected. The response of plants to water stress depends upon the intensity and duration of stress as well as on plant species and its stage of growth. For the purpose of crop production, yield improvement and yield stability under water stress conditions, developing of drought tolerant varieties is the best option[18]. Osmotic solution such as PEG (Polyethylene glycol) has been used to impose water-deficit stress[11].

*Cynara scolymus* L., a perennial herb of Asteraceae family is known for its nutritional and curative properties. In India, it is cultivated as vegetable crop on hills and on plains. It contains some bioactive components that have an antioxidant and antibiotic properties. Many parts of *C. scolymus* L. have been widely used as an astringent, blood cleanser, cardio tonic, detoxifier, digestive stimulant, diuretic as well as medicine for liver complaints[12].

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It also contains caffeoylquinic acid derivatives (cynarin and chlorogenic acids) and flavonoids such as *luteolin* and apigenin[22]. The purpose of the present study was to evaluate the effects of water- deficit stress on the seed germination and seedling growth, free proline, MDA level and chlorophyll contents of *C. scolymus* L.

## MATERIAL AND METHODS

The seeds of *C. scolymus* L. were obtained from Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan. The experiment was conducted in laboratory conditions of Shoolini University of Biotechnology and Management Sciences, Solan. The seeds were selected on the basis of uniformity and were surface sterilized. After surface sterilization with 0.1% $HgCl_2$ for 5 minutes, 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. Thereafter, 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. Emergence of 2-5 mm radical was taken as seed germination. 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 and allowed to grow in BOD incubator at 25±2ºC. After 14 days of shifting to BOD, the plants were treated with 1%, 2.5 %, 5%, 7.5%, 10%, 15% and 20 % PEG. After 21 days of treatment, determination of proline, lipid peroxidation and chlorophyll contents were done.

**Determination of free Proline content:** Free Proline was estimated spectrophotometrically following the method of Bates *et al*.[2]. The leaves/roots weighing 250 mg were homogenized with 3 % sulphosalicyclic acid (SSA). The homogenate was centrifused at 10,000 rpm for 10 minutes and supernatant was collected. Supernatant (2ml) was then reacted with 2 ml of freshly prepared ninhydrin (1.25 g of ninhydrin dissolved in a mixture of 30 ml glacial acetic acid and 20 ml of 6 M orthophosphoric acid with warming and stirring) and 2 ml of glacial acetic acid in a test tube and then was kept in a boiling water bath at $100^0$C for 1 hour. The reaction was terminated in an ice bath and then shifted to room temperature. Thereafter, the reaction mixture was extracted with 4 ml of toluene, mixed vigorously with test tube stirrer for 15-20 seconds. The chromophore containing toluene was aspirated from aqueous phase and absorbance was read at 520 nm using toluene as a blank. The proline concentration was determined from the calibration curve.

**Lipid Peroxidation:** Lipid peroxidation was estimated from accumulated malondialdehyde (MDA) following the method given by Dhindsa *et al*.[5] The leaves/root weighing 200 mg were homogenized in 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for 10 minutes and supernatant was collected. Supernatant (2ml) was reacted with 4 ml of 0.5 % thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) and kept at $95^0$ C in a water bath for 30 minutes. The reaction was terminated by cooling the reaction mixture in ice for 5 minutes. Absorbance was read at 532 nm. Measurements was corrected for unspecific turbidity by subtracting the absorbance at 600nm. MDA content was determined using the extinction coefficient of 155 $mM^{-1}$ $cm^{-17}$.

**Chlorophyll estimation:** Cholorophyll extraction was done by using dimethyl sulphoxide (DMSO) chlorophyll extraction technique of Hiscox and Israelstam[8]. Test tubes containing 7 ml DMSO were preheated to 65°C in a water bath for 15 minutes. Leaf tissue weighing 100 mg was homogenized with DMSO. Final volume was made approximately 10 ml with DMSO. The spectrophotometer was calibrated to zero

absorbance using a blank of pure DMSO. Absorbance of both blank and sample were measured at 645 and 663 nm.

Amount of total chlorophyll, chlorophyll a and chlorophyll b was estimated by using following equations given by Arnon's[1] recommended by Hiscox and Israelstam[8]:

Chl 'a' (mg/g) $= 0.0127\ A_{663} - 0.00269\ A_{645}$

Chl 'b' (mg/g) $= 0.0229\ A_{645} - 0.00468\ A_{663}$

Total Chl (mg/g) $= 0.0202\ A_{645} + 0.00802\ A_{663}$

**Statistical analysis**

All experiments were conducted with six replicates and results were expressed as mean ± standard error (SE). 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

In the present study, the effects of water deficit stress were monitored on seed germination and seedling growth of *C.scolymus* L. The seeds were non-dormant and started germinating within 3 days of incubation. The seeds responded differently to PEG concentrations (Fig.1). At lower (1% and 2.5%) concentration of PEG, the seed germination increased slightly as compared to control but PEG at higher concentration i.e. 5%, 7.5%, 10%, 15% and 20% decreased germination gradually. This variability in response to seed germination by PEG or water stress can be attributed to genetic or species variability. Similar results were found in barley and *Brassica*[9] and *Aeluropus lagopoides*[6]. On the other hand, in *Echinochloa crusgalli*, it was reported that various concentrations of PEG accelerated seed germination[23].

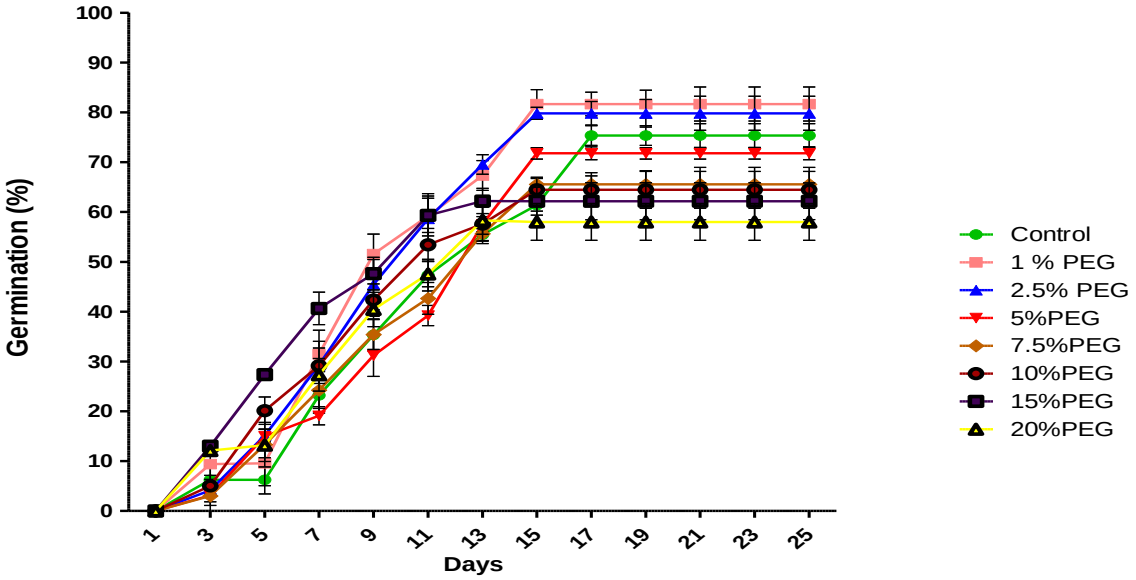


**Fig. 1: Time-course of germination of *Cynara scolymus* L. seeds as affected by 1%, 2.5%, 5%, 7.5%, 10%, 15% and 20% PEG treatment. Values are mean ± S.E**

The seedling growth was measured in terms of root length shoot length and seedling fresh weight. The different concentrations of PEG affected the seedling growth (Fig. 2). The root length of seedling increased at lower concentration of PEG (1% and 2.5%) but as the concentration of PEG increased (5%, 7.5%, 10%, 15% and 20%), the root length decreased gradually. This suggests that *C. scolymus* L. can tolerate water stress up to a certain degree only as root length is an important trait against water stress. Similarly, Smita and Nayyar[19] observed reduction in root length of *Cicer arietinum* under water stress. However, an increased root growth due to water deficit stress was reported in *Catharanthus roseus*[10]. Further, the shoot length decreased in all the treatments. There was a significant reduction in shoot height under water deficit stress in tomato[21]. The

seedling fresh weight also increased in 1% and 2.5% PEG treatments but decreased in higher concentrations of PEG (5%, 7.5%, 10%, 15% and 20%). Similar results were found in *Ziziphus rotundifolia* by Tsialtas *et al*.[20].

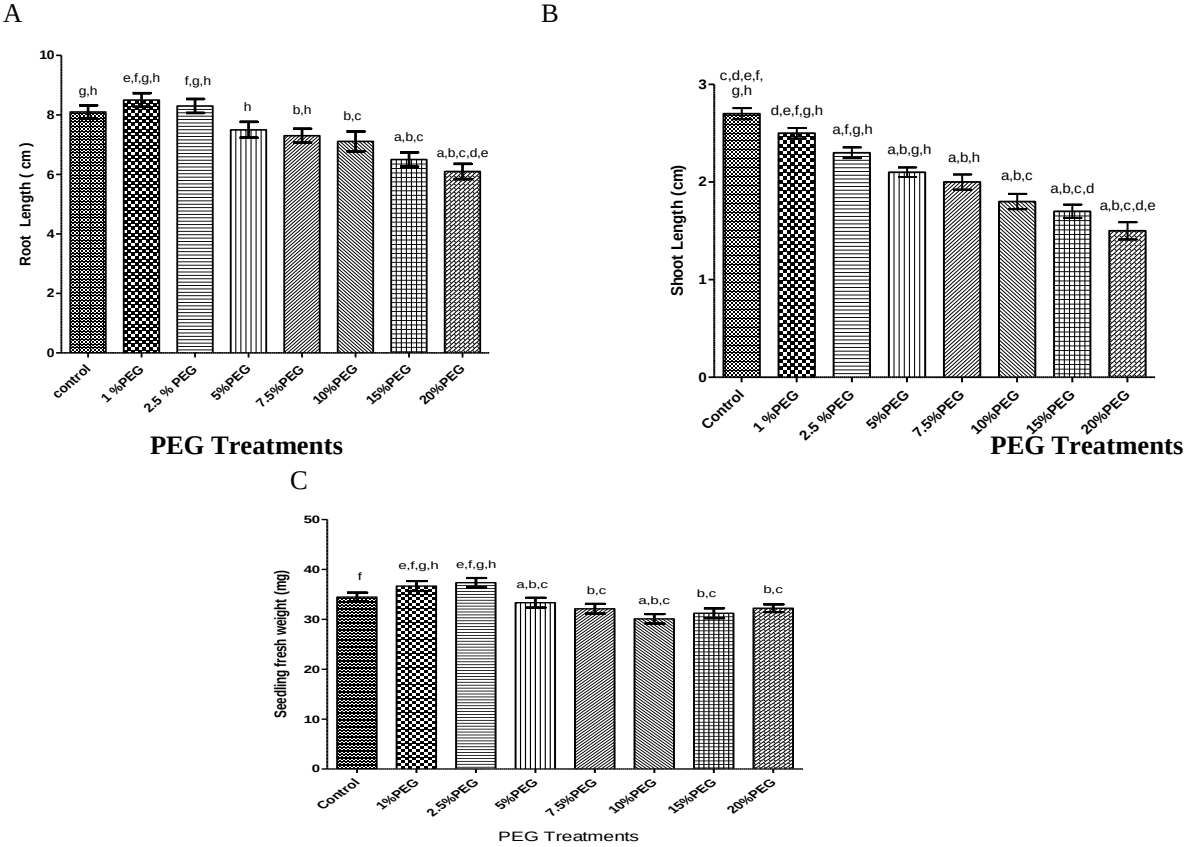


**Fig 2: Effect of PEG treatments on root length (A), shoot length (B) and seedling fresh weight (C) of *Cynara scolymus* L. Values are mean ± SE; n=15.Tukey's analysis of variance showed significance difference ( $^{a}p<0.05$ Vs control, $^{b}p<0.05$ Vs 1%PEG, $^{c}p<0.05$ Vs 2.5% PEG, $^{d}p<0.05$ Vs 5% PEG, $^{e}p<0.05$ Vs 7.5% PEG, $^{f}p<0.05$ Vs 10% PEG, $^{g}p<0.05$ Vs 15% PEG, $^{h}p<0.05$ Vs 20% PEG)**

In another experiment, physiological / metabolic responses of *C. scolymus* L. under water deficit stress were assessed in hydroponic system. The estimation of Proline content was done in leaves and roots of *C. scolymus* L. after treating the plants with PEG treatments in hydroponic culture for 21 days. In *C. scolymus* L. proline content in leaves and roots increased with an increase in PEG concentrations (Fig. 3). The most prominent function of proline might be to act as an osmoregulator and thus keeping the cells turgid. Free proline accumulation in water stressed plants has also been suggested to serve as an index of drought hardness; higher proline accumulation being a characteristic of resistant cultivars. Similar results were found by Zaifnejad *et al*.[24].

Lipid peroxidation, a measure of free radical generation, was determined in terms of malondialdehyde (MDA) content. The estimation of MDA content was done in leaves and roots of *C. scolymus* L. It increased with an increase in PEG concentrations (Fig. 4). Generally, water stress caused an extensive lipid per oxidation which has been often used as an indicator of stress induced oxidative damage of membrane. The increase in MDA contents might be due to the increased antioxidative enzymes which have scavenged various reactive oxygen species (ROS) produced due to water stress. Earlier, Shin *et al*.[17], found increased MDA concentration in *Arabidopsis* plant under water stress. Lipid peroxidation also increased in PEG stressed seedling of *Capparis ovata*[14].

The content of chlorophyll a and b in leaves decreased with an increase in PEG concentration (Fig. 5). The chlorophyll a: b ratio altered marginally with the PEG (Fig. 6). The total chlorophyll content of leaves also decreased with an increase in PEG concentrations (Fig. 7). The chlorophyll content was suppressed by the stress conditions. Similar results were observed from the fact that total chlorophyll content decreased when exposed to water deficit stress in soybean[15] and cotton[13]. However Beeflink *et al*.[3] reported increase in chlorophyll in onion under drought stress.

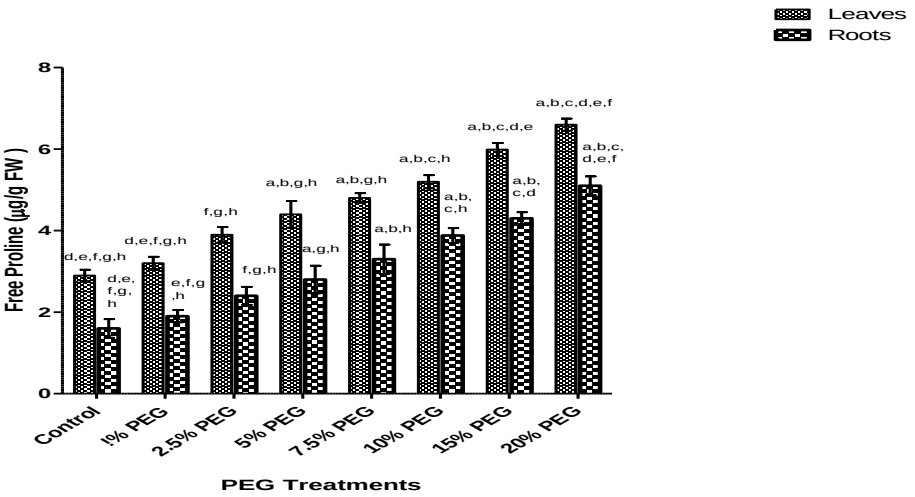


**Fig. 3: Effect of PEG treatments on free proline content in leaves and roots of *Cynara scolymus*. Seedlings were grown hydroponically for 15 days and subsequently exposed to stress for 21 days. Values are mean ± SE; n=15. Tukey's analysis of variance showed significance difference ($^{a}p<0.05$ Vs control, $^{b}p<0.05$ Vs 1%PEG, $^{c}p<0.05$ Vs 2.5% PEG, $^{d}p<0.05$ Vs 5% PEG, $^{e}p<0.05$ Vs 7.5% PEG, $^{f}p<0.05$ Vs 10% PEG, $^{g}p<0.05$ Vs 1% PEG, $^{h}p<0.05$ Vs 20% PEG)**

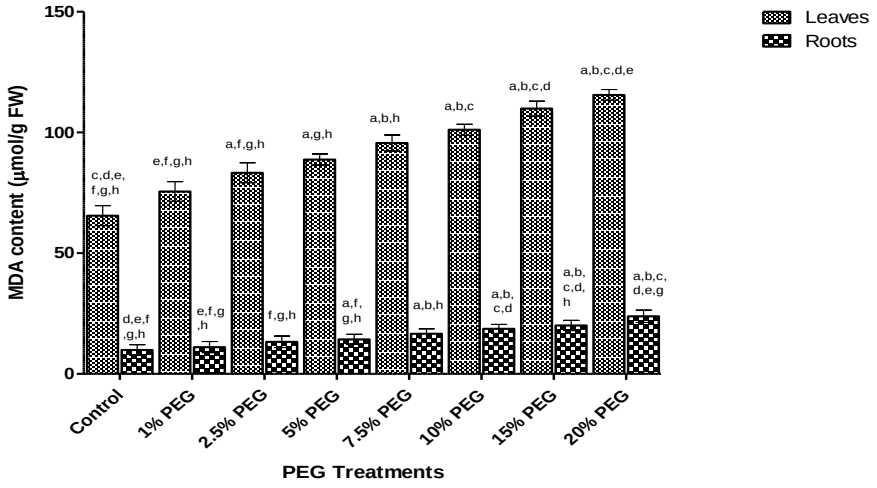


**Fig. 4: Effect of PEG treatments on MDA content in leaves and roots of *Cynara scolymus*. Seedlings were grown hydroponically for 15 days and subsequently exposed to stress for 21 days. Values are mean ± SE; n=15. Tukey's analysis of variance showed significance difference ($^{a}p<0.05$ Vs control, $^{b}p<0.05$ Vs 1%PEG, $^{c}p<0.05$ Vs 2.5% PEG, $^{d}p<0.05$ Vs 5% PEG, $^{e}p<0.05$ Vs 7.5% PEG, $^{f}p<0.05$ Vs 10% PEG, $^{g}p<0.05$ Vs 15% PEG, $^{h}p<0.05$ Vs 20% PEG)**

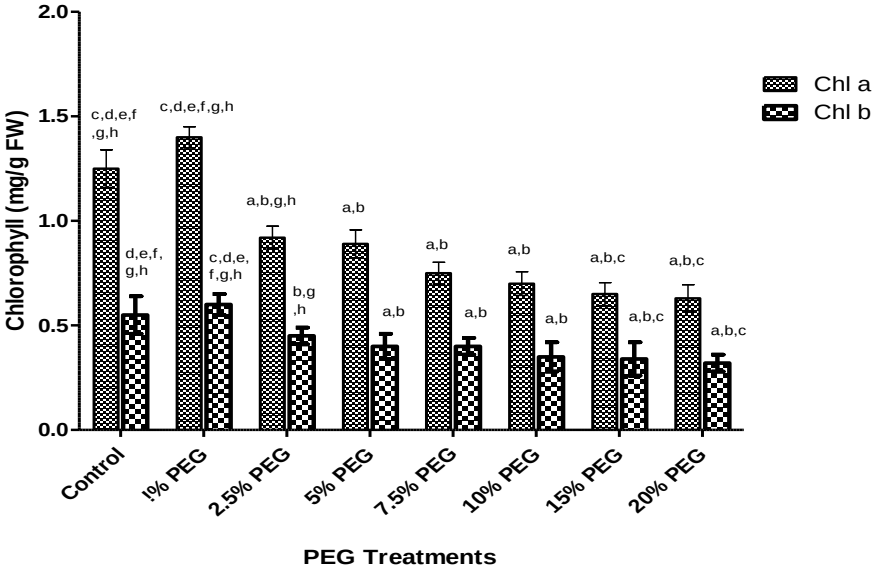


**Fig. 5: Effect of PEG treatments on chl a & chl b in *Cynara scolymus*. Seedlings were grown hydroponically for 15 days and subsequently exposed to stress for 21 days. Values are mean ± SE; n=15. Tukey's analysis of variance showed significance difference ($^{a}p<0.05$ Vs control, $^{b}p<0.05$ Vs 1%PEG, $^{c}p<0.05$ Vs 2.5% PEG, $^{d}p<0.05$ Vs 5% PEG, $^{e}p<0.05$ Vs 7.5% PEG, $^{f}p<0.05$ Vs 10% PEG, $^{g}p<0.05$ Vs 15% PEG, $^{h}p<0.05$ Vs 20% PEG)**

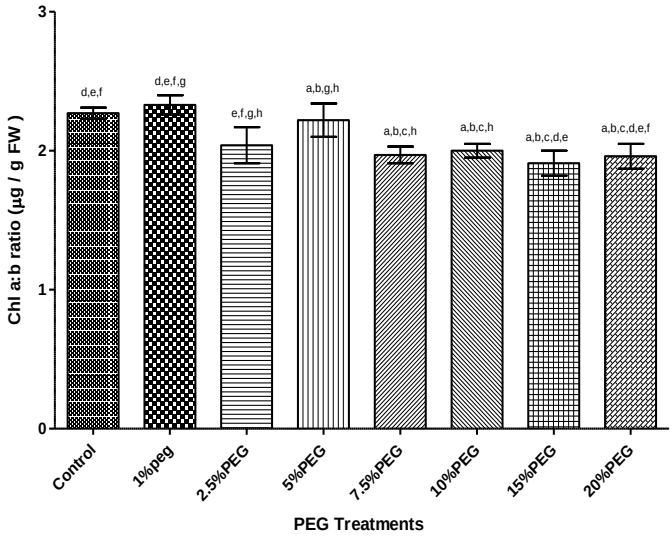


**Fig. 6: Effect of PEG treatments on chlorophyll a: b ratio in *Cynara scolymus*. Seedlings were grown hydroponically for 15 days and subsequently exposed to stress for 21 days. Values are mean ± SE; n=15. Tukey's analysis of variance showed significance difference ($^{a}p<0.05$ Vs control, $^{b}p<0.05$ Vs 1%PEG, $^{c}p<0.05$ Vs 2.5% PEG, $^{d}p<0.05$ Vs 5% PEG, $^{e}p<0.05$ Vs 7.5% PEG, $^{f}p<0.05$ Vs 10% PEG, $^{g}p<0.05$ Vs 15% PEG, $^{h}p<0.05$ Vs 20% PEG)**

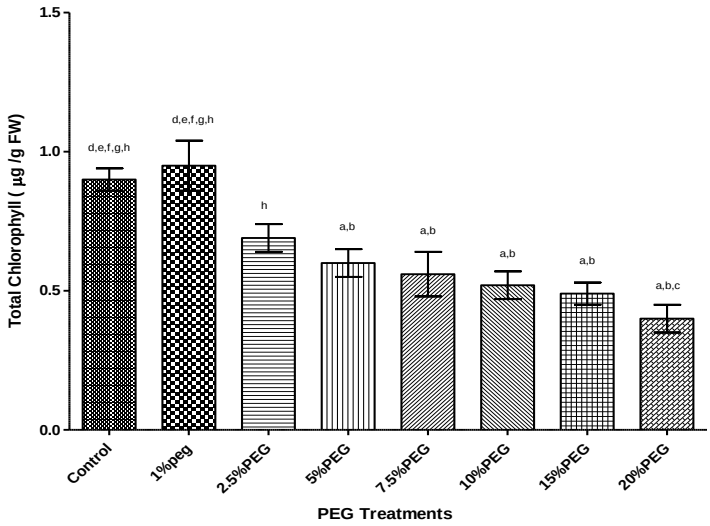


**Fig. 7: Effect of PEG treatments on total chlorophyll content in *Cynara scolymus*. Seedlings were grown hydroponically for 15 days and subsequently exposed to stress for 21 days. Values are mean ± SE; n=15. Tukey's analysis of variance showed significance difference ($^{a}p<0.05$ Vs control, $^{b}p<0.05$ Vs 1%PEG, $^{c}p<0.05$ Vs 2.5% PEG, $^{d}p<0.05$ Vs 5% PEG, $^{e}p<0.05$ Vs 7.5% PEG, $^{f}p<0.05$ Vs 10% PEG, $^{g}p<0.05$ Vs 15% PEG, $^{h}p<0.05$ Vs 20% PEG)**

## CONCLUSION

The findings from the present study reveals the nature and magnitude of the responses of *C. scolymus* L. to water deficit stress. This study shows that water stress restricts the seed germination and seedling growth of *C. scolymus* L., further decreasing the shoot length, root length and seedling fresh weight at higher stress conditions. Although, the loss in chlorophyll content leads to disruption of photosynthetic machinery, the proline and MDA accumulation might help plants in stressful conditions to some extent.

## Acknowledgements

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