



## Effect of PEG induced drought stress on *Bacopa monnieri* (L.) Wettst. and *Adhatoda vasica* Nees

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### Abstract

The growth, development and biosynthesis of secondary metabolites in the plant is strongly affected by environmental stress. The pharmaceutical values of the medicinal plants depend on the presence of secondary metabolites that are affected by environmental stress. Drought stress is described as one of the most important abiotic factor that limits the growth and productivity of the plants. *Bacopa monnieri* has been used for centuries as a memory enhancer, anti-inflammatory, analgesic, antipyretic, sedative and antiepileptic agent. *Adhatoda vasica* is one of the important medicinal plants used with much success to treat asthma, chronic bronchitis, and other respiratory conditions. The aim of the present study was to study the effect of simulated water stress on medicinal plants *Bacopa monnieri* and *Adhatoda vasica*. Leaves of *Bacopa monnieri* and *Adhatoda vasica* were subjected to different levels of Polyethylene glycol (PEG) solution under room condition. In order to evaluate drought stress effect several parameters such as Proline, Relative water content (RWC) and Cell membrane stability were taken into consideration. The data obtained from the present study indicated that drought stress significantly affect proline, relative water content and cell membrane stability in both the plants.

**Keywords:** PEG, medicinal plants, proline, RWC, membrane stability

### Introduction

Abiotic factors such as light, temperature, water, salt etc. affect growth, development and geographical distribution of the plants and its productivity in agriculture. These environmental factors limit the growth and productivity of plants to varying degrees, depending upon severity of stress. To cope with these abiotic stress plants initiate a number of morphological, physiological and biochemical changes. The accumulation of certain organic metabolites of low molecular weight known collectively as compatible solutes helps the plant for survival and growth. These compounds are reported to play an important role in cellular osmotic adjustment in response to osmotic and salt stresses [1]. Drought is the major environmental factor that limits crop growth and yield globally. Therefore, improving crop performance under water stress conditions is an important area of research among plant scientists around the world. Although, the effect of drought stress has been studied extensively in different crop plants throughout the world, but there is few evidences available on the effects of different water stresses on growth, physiological and biochemical characteristics of medicinal plants [2, 3]. Biotic and abiotic factors are known to increase the production of secondary metabolites in medicinal plants [4].

*Bacopa monnieri* (L.) Wettst. is an important medicinal plant of the family Scrophulariaceae that has been consumed for thousands of year as a memory enhancing, anti-inflammatory, analgesic, antipyretic, sedative and anti-epileptic agent [5]. *Adhatoda vasica* Nees. belonging to family Acanthaceae, is an evergreen shrub found in many regions of the world. The plant is used in the indigenous system of medicine in India and is a well-known expectorant in Ayurvedic and Unani medicine. It has been used for the treatment of various diseases and disorders,

particularly for the respiratory tract ailments [6]. This study aims at understanding the response of these two plants to drought stress by examining the physiological and biochemical changes on plant leaves after subjecting to different level of PEG solution.

### Material and Methods

#### Stress treatment

Mature leaves of both *Bacopa monnieri* and *Adhatoda vasica* plants maintained in the garden were used for the stress treatments. For the screening leaves of similar stages were treated with various concentration of Polyethylene glycol (PEG) 6000 solution for different duration to simulate water stress condition. Total Proline concentration, Relative water content (RWC) and cell Membrane Stability Index were estimated after a period of stress treatment.

#### Proline

Proline content was measured as described by Bates *et al.* [7]. 0.5g of plant material (leaf) was homogenized in 5 ml of 3% sulphosalicylic acid and the residue was removed by centrifugation. 2 ml of the extract was thoroughly treated with 2 ml glacial acetic acid and 2 ml of Ninhydrin reagent (1.25 g Ninhydrin warmed in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid until dissolved) at 100°C in a boiling water bath for 30 min. The reaction was then terminated instantly by dipping in an ice bath. The reaction mixture was extracted with 4 ml toluene. The chromophore containing toluene was warmed to room temperature and its optical density was measured at 520 nm in a spectrophotometer against a toluene blank.

The amount of proline was determined from a standard curve in the range of 20 – 100 µg/ml.

**Relative water content**

Leaf relative water content (RWC) was determined from the fully expanded young leaves according to Galmes *et al.* [8]. Fully expanded young leaves from each treatment were weighed to obtain fresh leaf weight. Immediately leaves were hydrated by floating on de-ionized water in a closed petri dish. After hydration turgid weight were taken and oven dried at 70°C for 48hr. Finally, dry weight of leaves was measured. Leaf relative water content was calculated according to the equation:

$$RWC (\%) = (Fresh\ weight - Dry\ weight / Turgid\ weight - Dry\ weight) \times 100$$

**MSI (Membrane stability index)**

Leaf membrane stability index (MSI) was determined according to the method of Premchandra *et al.* as modified by Sairam [9, 10]. Leaf discs (100 mg) were thoroughly washed in running tap water followed by washing with double distilled water thereafter the discs were heated in 10 ml of double distilled water at 40° C for 30 min. Then electrical conductivity (C1) was recorded by EC (Electrical Conductivity) meter. Subsequently the same samples were placed in a boiling water bath (100° C) for 10 min and their electrical conductivity was also recorded (C2). Membrane stability index was calculated as follows:

$$Membrane\ stability\ index\ (MSI) = [1 - (C_1/C_2)] \times 100.$$

**Result**

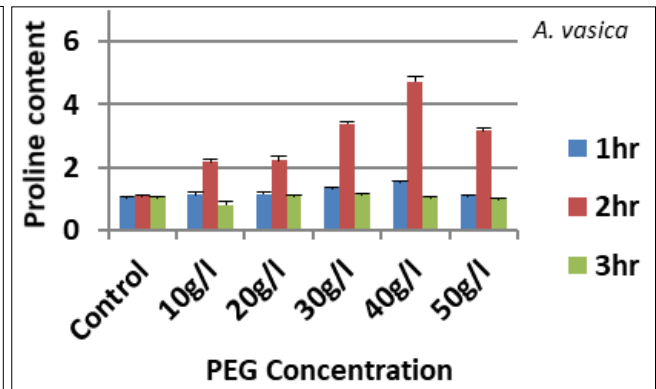
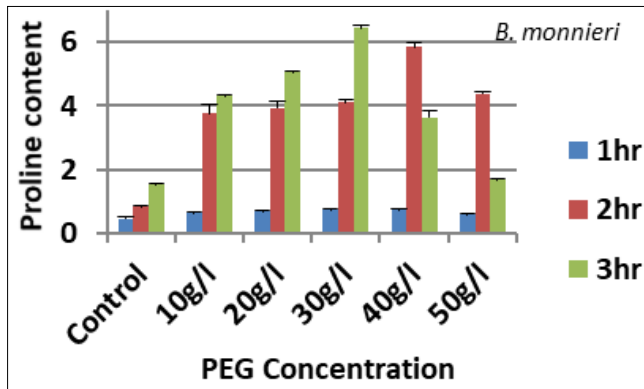
**Proline content**

In the present study increase in free proline content has been observed in stress induced leaves with increasing PEG concentration. The results showed that free proline

increased exponentially with increasing stress level. In *Bacopa monnieri* maximum proline content was observed after 3hrs stress treatment. However, in *Adhatoda vasica* significant increase in proline content was observed at 2hr stress treatment. In case of *Bacopa* 2hr stress treatment at 40g/L of PEG concentration, the free proline had been prominently increased to 5.84 µM/g tissue than the control 0.84 µM/g. In 3hr of stress treatment at 30g/L of PEG concentration, proline content increased to 6.44 µM/g than the control 1.53 µM/g. At 40g/L and beyond the stress level becomes harmful for plant. In *Adhatoda vasica* significant increase in proline content was observed at 2hr stress treatment. In 30g/L and 40g/L of PEG concentration, proline content increased to 3.38 µM/g and 4.73 µM/g than the control 1.09 µM/g.

**Table 1:** Proline content (expressed in µM/g tissue) in *Bacopa monnieri* and *Adhatoda vasica* leaves exposed to different concentration of PEG solution for different time duration (Mean±SE)

Plant	PEG concentration	1hr	2hr	3hr
<i>Bacopa monnieri</i>	Control	0.45±0.04	0.84±0.02	1.53±0.02
	10g/l	0.63±0.02	3.76±0.27	4.29±0.05
	20g/l	0.67±0.04	3.94±0.23	5.07±0.04
	30g/l	0.72±0.01	4.11±0.07	6.44±0.07
	40g/l	0.75±0.03	5.84±0.15	3.63±0.23
	50g/l	0.59±0.03	4.38±0.04	1.67±0.01
<i>Adhatoda vasica</i>	Control	1.02±0.04	1.09±0.03	1.03±0.04
	10g/l	1.13±0.07	2.17±0.11	0.8±0.11
	20g/l	1.15±0.06	2.22±0.16	1.09±0.02
	30g/l	1.31±0.05	3.38±0.08	1.11±0.05
	40g/l	1.51±0.04	4.73±0.17	1.02±0.05
	50g/l	1.07±0.03	3.17±0.05	0.96±0.04



**Fig 1:** Proline content in *Bacopa monnieri* and *Adhatoda vasica*

**Relative water content**

To understand the dehydration tolerance which shows the metabolic activities in the tissues of plant, RWC is considered to measure water status in plant. In the present study RWC decreased as a result of water stress in both the selected plant. In *B. monnieri* at 50g/L PEG concentration, RWC decreased to 90.94, 83.99 and 80.98 % as compared to control 96.83, 96.19 and 96.1% in 1hr, 2hr and 3hr stress respectively. Similarly, in *A. vasica* it decreased at 50g/L to 89.31, 82.53 and 80.1% as compared to control 95.85, 93.53 and 93.48% in 1h, 2h and 3h exposure to stress conditions (Table 2). In *B. monnieri*, the lowest RWC in relation to control (96.1%) was observed in 50g/l stress treatment (80.98%) for 3hr duration. Similarly lowest RWC in relation to control (93.48%) was also observed in 50g/l stress treatment (80.1%) for 3hr duration in *A. vasica*.

**Table 2:** Relative water content (%) in *Bacopa monnieri* and *Adhatoda vasica* leaves exposed to different concentration of PEG solution for different time duration (Mean±SE)

Plant	PEG concentration	1hr	2hr	3hr
<i>Bacopa monnieri</i>	Control	96.83±0.39	96.19±0.28	96.1±0.29
	10g/l	96.18±0.24	94.77±0.31	94.76±0.16
	20g/l	93.27±0.03	93.05±0.65	93.03±0.16
	30g/l	93.23±0.02	92.47±0.11	92.46±0.09
	40g/l	92.67±0.1	92.4±0.1	91.98±0.06
	50g/l	90.94±0.1	83.99±0.07	80.98±0.25
<i>Adhatoda vasica</i>	Control	95.85±0.16	93.53±1.15	93.48±0.62
	10g/l	94.83±0.18	90.62±0.84	90.26±0.53
	20g/l	94.57±0.1	88.15±0.44	83.04±0.62
	30g/l	92.88±1.55	87.47±0.53	81.95±0.98
	40g/l	92.71±0.53	82.83±0.98	80.95±0.36
	50g/l	89.31±0.48	82.53±0.87	80.1±0.13

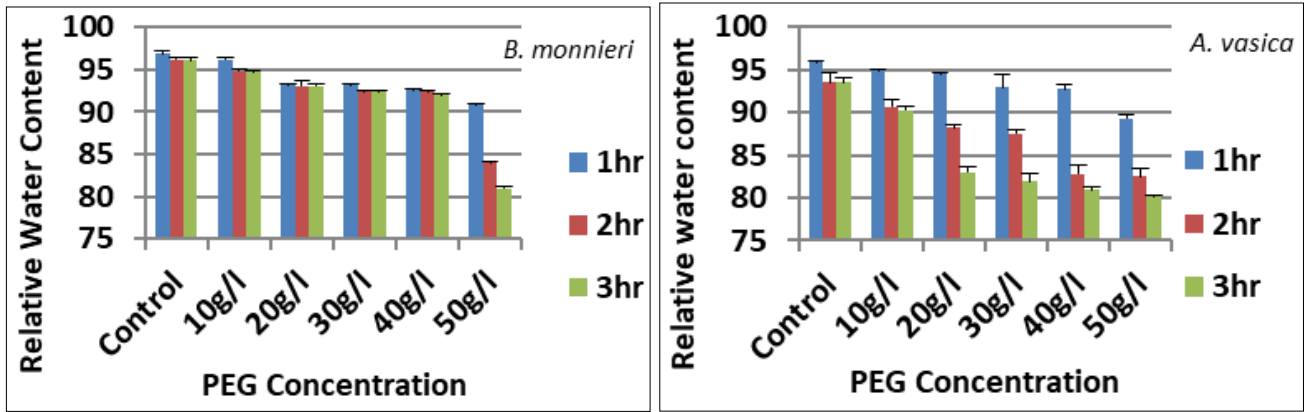


Fig 2: Relative water content *Bacopa monnieri* and *Adhatoda vasica*

**Membrane stability index (MSI)**

In the present study we observed that the MSI decreases with the increase level and duration of stress. Significantly higher MSI value was recorded in control plant. In *B. monnieri* at 50g/L PEG concentration, MSI decreased to 70.69, 69.19 and 65.92% as compared to control 74.28,

73.86 and 73.31% in 1hr, 2hr and 3hr stress respectively. Similarly, in *A. vasica* MSI decreased at 50g/L upto 85.38, 82.22 and 79.61% as compared to control 94, 93.56 and 93.5% in 1h, 2h and 3h exposure to stress conditions (Table 3).

Table 3: Membrane Stability Index of *Bacopa monnieri* and *Adhatoda vasica* leaves exposed to different concentration of PEG solution for different time duration (Mean±SE)

Plant	PEG concentration	1hr	2hr	3hr
<i>Bacopa monnieri</i>	Control	74.28±0.39	73.86±0.41	73.31±0.52
	10g/l	74.21±0.29	73.74±0.37	73.25±0.71
	20g/l	73.57±0.38	73.31±0.41	72.75±0.19
	30g/l	73.52±0.25	72.54±0.13	72.53±0.36
	40g/l	71.62±0.39	71.49±0.31	71.26±0.37
	50g/l	70.69±0.46	69.19±0.24	65.92±0.42
<i>Adhatoda vasica</i>	Control	94±0.17	93.56±0.24	93.5±0.27
	10g/l	93.74±0.23	93.18±0.55	87.59±0.33
	20g/l	92.69±0.22	91.01±0.38	87±0.13
	30g/l	91.8±0.29	85.89±0.46	84.84±0.45
	40g/l	89.66±0.39	84.64±0.23	81.84±0.46
	50g/l	85.38±0.45	82.22±0.36	79.61±0.52

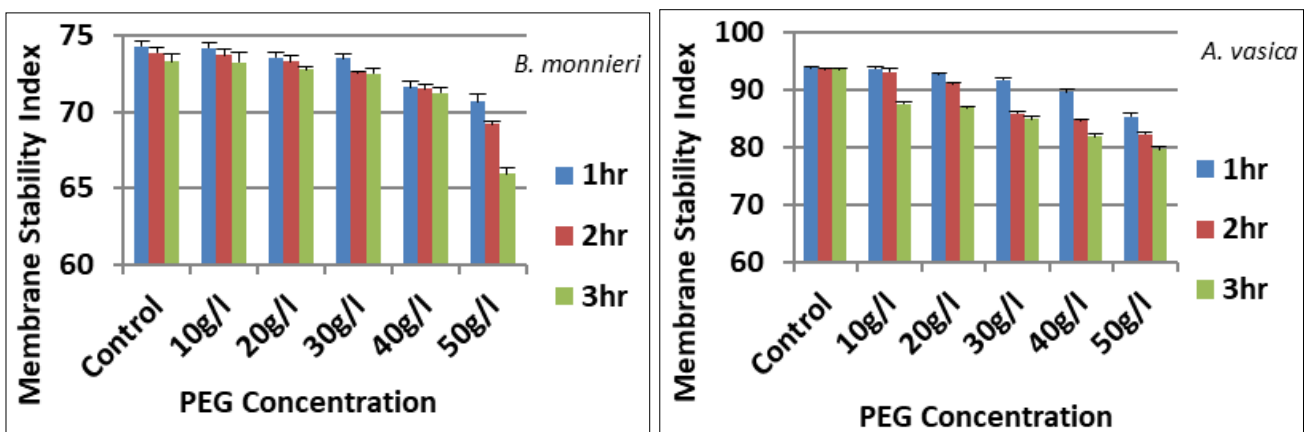


Fig 3: Membrane Stability Index (MSI) of *Bacopa monnieri* and *Adhatoda vasica*

**Discussion**

Proline, an amino acid, plays an important role in plants. It accumulates in many plant species in response to environmental stress and helps plants to recover from stress more rapidly. The results of present study are in agreement with the earlier reports on the free proline accumulation under stress in seedlings, plants as well in callus cultures [11, 12, 13]. When proline is applied exogenously to plants exposed to stress, results in increased growth and other

physiological characteristics of plants [14]. Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress [15]. The reason behind the increased proline content under stress conditions might be due to changes in proline metabolism under stress condition and increased expression of proline synthetic enzymes. In the present study increase in free proline content has been observed in

stress induced leaves with increasing PEG concentration. Proline concentration increase with time even in control in *Bacopa* which means it perceives stress quickly with increased level & duration of stress. In *Bacopa monnieri* maximum proline content was observed after 3hrs stress treatment. However, in *Adhatoda vasica* significant increase in proline content was observed at 2hr stress treatment. An increase in proline accumulation in *C. roseus* under drought stress induced by PEG 6000 of different concentrations was also observed [3]. The accumulation of proline also increased significantly in cotton with increasing water stress [16]. *Mentha piperita* and *Catharanthus roseus* increased accumulation of osmolytes (such as proline, sugars, glycine betaine) and secondary metabolites in response to stress, which contribute to the maintenance of growth under stress conditions by improving the plant water potential and increasing ROS scavenging, thereby preventing the damaging effects of drought and heat stress [17]. Result of the present study is in agreement with earlier reports on proline accumulation under stress conditions in plants.

Relative water content (RWC) is proposed as a most appropriate measure of plant water status under drought stress conditions. It expresses the relative amount of water present in the plant tissue and may indicate the level of stress in plants. In the present study there were significant differences between all treatments of drought stress for this parameter also. Highest RWC was recorded in control leaf, which decreased gradually with increasing stress level. However, the percent decrease was comparatively more in *Adhatoda vasica*. A decrease in the relative water content (RWC) in response to drought stress has been noted in wide variety of plants. Drought stress induced by 2% and 4% PEG (6000) significantly reduced RWC from 80% under control condition to 62% and 28%, respectively, in *Thymus citriodorus* [2]. RWC in the *C. roseus* leaves decreased under the treatment of PEG 6000 at different concentrations, especially, when treated with 35% PEG 6000, which caused a sharp decrease [3]. Significant decrease in relative water content was also observed in some other plant species [16, 18]. One of the major impacts of environmental stress is cellular membrane modification in plants, which results in its perturbed function or total dysfunction. The cellular membrane dysfunction due to stress causes increased permeability and leakage of ions. The estimation of membrane dysfunction under stress by measuring cellular electrolyte leakage from affected leaf tissue into an aqueous medium is used to measure Cell Membrane Stability and as a screen for stress resistance. The cell membrane stability test can also be used under *in vitro* conditions to differentiate the drought tolerant and susceptible cultivars (19). Water stress had significant effect on membrane stability index. The degree of cell membrane injury induced by water stress can be estimated through measurements of electrolyte leakage from the cells. Membrane stability index under a given level of stress has also been used as a measure of stress tolerance by many researcher [9, 11, 19, 20, 21]. A significant decrease in MSI in salinity stress was reported in mulberry (11). A significant decrease in cell membrane stability index was also observed in response to drought and salinity combined stress during anthesis in Tibetan wild and cultivated barleys (20). In an experiment performed on groundnut cultivars a negative relationship between PEG concentration in the medium and membrane stability measured as electrolyte leakage was observed (19).

## Conclusion

The reasons behind the medicinal use of both the selected plant are the presence of different phytochemicals. The type and concentration of secondary molecule produced by a plant are determined by the species, genotype, physiology, developmental stage and environmental factors during growth. In the present study significant change in the physiological and biochemical parameter were observed in stressed leaves as compared to control in both medicinal plant. Therefore, Relative water content, proline content and membrane stability index can be used for screening drought tolerance genotypes that maintain cell turgor under water stress conditions. Biotic and abiotic factors are known to increase the production of secondary metabolites in medicinal plants. Therefore, further study may be carried out for the screening of stress tolerance in potted plant and the production of active compounds in plants subjected to different stressed conditions. If the production increases on stressed condition, the data obtained can be used for enhanced secondary metabolite production.

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