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# Influence of Water Stress on Morphological and Physiological Changes in *Andrographis paniculata*

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**ABSTRACT**

*Present study investigates the morphological and physiological plant responses to water stress in Andrographis paniculata. The total experiment carried out under greenhouse conditions. Root and shoot lengths, Total plant fresh and dry weights, number of leaves, leaf area, chlorophyll content, DNA and RNA content were evaluated at different water stress levels such as control plants (daily watered), T2 plants(watered on every 2nd day), T4 plants(watered on every 4th day), T7plants(watered on every 7th day). In this experiment observed that in all characteristic features, that there was a decrease in them during severe stress conditions compared with those of control plants. water stress has not only affected growth parameters but also the levels of chlorophylls, DNA and RNA due to enhanced activities of various hydrolytic enzymes including proteases.*

***Key words**: Andrographis paniculata, Water stress, Chlorophyll, DNA and RNA.*

## INTRODUCTION

Medicinal plants constitute an important component of the plant resource and play a very important role in human life directly or indirectly. *Andrographis paniculata* is an annual herb extremely bitter in taste which belongs to the Acanthaceae family, it is commonly known as 'king of bitters'. The plant extracts exhibits anti-typhoid, anti fungal activity, antioxidants, anti inflammatory, anti snake venom and antipyretic properties. The plant contains a number of diterpenoids, major bitter constituent is andrographolide, which is diterpene lactone. Plant water stress usually caused by drought and can have major impacts on plant growth and development, it causes lower yields and can cause crop failure[4]. Water stress reduced the height of the plants and decreased shoot length and increased the root length. A sharp decline in water level has also reported in the photosynthesis, chlorophyll and nucleic acid synthesis due to short supply of water. Reduction in water supply has adverse effect on chlorophyll synthesis and chlorophyll a:b ratio. and plant size .Water stress is also responsible for rapid leaf shedding[17].

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## MATERIALS AND METHODS

*Andrographis paniculata* seeds were procured from Central Institute of Medicinal and Aromatic Plants(CIMAP), Hyderabad. The pot experimented was conducted under controlled water stress environment in glass house maintained in the botanical garden, Osmania university. Hyderabad. The experiment was laid out in completely randomized design with four treatment at different water stress levels i.e., control watered regularly(C), watered every 2nd day (T2), watered on every 4th day(T4), watered on every 7th day(T7) each with three replications was carried out. The plastic pots containing mixture of soil and sand (2:1). When the seedlings attained 15cm length(19day old plants), *Andrographis paniculata* were subjected to a progressive stress by with holding water. The growth parameters such as plant height, shoot length, root length, number of leaves, leaf area, fresh and dry weight were recorded every 30days, all the morphological and physiological parameters were measured. Leaves were oven dried (60$^{o}$C, 48h) and the mass of each leaf was weighed with an electric balance (to 0.001 g.). The chlorophylls were extracted with 80%acetone and quantified by the arnon[2]. DNA and RNA present in the ethanolic homogenate were separated by the procedure described by Ogur and Rosen[9]. The weight of the fresh leaves was measured (FW) and then the leaves were submerged in distil water for 24hrs at room temperature. After that blotted dry with filter paper and turged weight (TW) was determined. These leaves were over dried at 40$^{o}$C for 24 hrs to determine the dry weight (DW).RWC(relative water content) was calculated by using the following formula.

RWC (%) = (FW-DW)/(TW-DW)×100

## RESULTS

The effect of water stress on morphological and physiological responses in relation to growth of *Andrographis paniculata* at different stages (2nd, 4th,6th, and 8th month) is presented in tables.

**Results: Morphological parameters**

**Leaf number:** The change in leaf number affected by water stress is presented in **Table-1.** When compared with control Leaf number decreased when subjected to water stress. Reduction in number of leaves can be a phenomenon by the plants to reduce the transpiration surface. The number of leaves during water stress was maximum in T2 treated plants at 8thmonth stage with average of 62.33±2.12 and the minimum leaves were observed in T7 treatment with an average of 26.66±0.70 when compared to the control plants(69.33±2.12). In 4th month and 6th month old plants showed minimum leaf number in T2 treated plants with average of 21.66±0.70 and 52.33±0.70 respectively and least in T7 treated plants with an average of 15.33±1.4 and 38.66±1.41. In the 2nd month, water stress did not effect on leaf number much in all the treatments when compared to control due to the turgid pressure of the cells.

**Leaf area**: *Andrographis paniculata* leaf area was affected by water stress is presented in **Table-1**. Leaf area of water stressed plants decreased when compared to control. The maximum leaf area was observed in T2 treatment with an average of 5.541±0.01 and minimum in T7 treatment with an average of 2.11±0.01 in the 8th month stage. In the 2nd, 4th, 6th and 8th month observed that T2 treatment shows highest leaf area with average of 0.118±0.003, 0.148±0.03 and 3.541±0.03 respectively when compared to the control plants.

**Plant height**: The difference in plant height at various growth stages in *Andrographis paniculata* as influenced by water stress is given in **Table-1**. The plant height decreased when compared to control plants. The maximum height was observed in T2 treatment with an average of 68.33±0.70 in 8th month, minimum plant height in T7 treatment with an average of 34.33±0.70. Least plant height was observed in the 2nd month in T2 treatment with an average of 15.66±1.52.

**Root length:** The change in the plant root length due to water stress in *Andrographis paniculata* is shown in **Table-2**. The root length was increased by water stress. Maximum root length was found in T7 treatment with an average of 29.66±2.82, 24.33±0.70,14.33±0.70 and 9.33±0.70 in 8th

month, 6th month, 4th month and 2nd month respectively. Minimum root length was found in T2 treated plants with an average of 3.64±1.41, 5.66±1.41, 1733±4.24 and 17.66±2.12 at different growth stages.

**Shoot length:** The change in the plant shoot length due to water stress in *Andrographis paniculata* is shown in **Table-2**. The shoot length was decreased by water stress in T2, T4 and T7 treatments when compared to the control. In T2 treatment, highest shoot length is observed at 8th month stage with an average of 51.33±2.12. In T7 treatment minimum shoot length with an average of 9.33±0.50, 14.33±0.70, 24.33±0.70 and 29.66±0.70 observed in different growth stages.

**Plant fresh and dry weight**: Effect of water stress on fresh weight and dry weight of *A.paniculata* are shown in **Table 3**. It is clear from the data that plant fresh weight and dry weight was decreased by water stress. In the T2 treatment, maximum plant fresh and dry weight was observed with an average of 8.386±0.14 and 1.428±0.01 respectively in the 8th month. The T7 treatment shows the minimum fresh and dry weight with average of 4.978±0.01 and 0.765±0.01 respectively in the 8th month stage.

**Root fresh and dry weight:** The root fresh weight, dry weight of *A.paniculata* subjected to water stress are shown in **Table-3**. It is clear from data that root fresh weight and dry weight decreased by water stress. The maximum root fresh weight and root dry weights were observed in T2 treated plants with average of 1.428±0.01 and 0.742±0.01 at 8th month stage. Minimum root fresh weight and root dry weights were observed in T7 treatment with an average of 0.765±0.01 and 0.320±0.01 respectively when compared to the control plants.

**Photosynthetic pigments:**

**Chlorophyll 'a':** Water stress resulted in decrease in chlorophyll 'a' content in all treatments. The difference in Chlorophyll 'a' effected by water stress are presented in Table-4. The total chlorophyll content among the treatments was maximum in T2 treatment with an average of 2.8±0.1mg/g.f.wt and minimum in T7 treatment with an average of 0.8±0.05 mg/g.f.wt when compared to control.

**Chlorophyll 'b':** The difference in Chlorophyll 'b' content at different growth stages of leaves was also affected by water stress are presented in Table-4. Chlorophyll 'b' content was decreased by water stress in all treatments expect control. Maximum content of Chlorophyll 'b' was observed in T2 treatment with an average of 1.2±0.1 mg/g.F.wt and minimum Chlorophyll 'b' content was observed in T7 treatment an average of 0.3±0.05 mg/g.F.wt when compared to the control plants.

**Total chlorophylls:** Total chlorophyll content was decreased by water stress in all the treatments is presented in Table-4. Maximum total chlorophyll content was observed in T2 treatment with an average of 4.2±0.1 mg/g.F.wt and minimum in T7 treatment with an average of 2.5±0.1 mg/g.F.wt when compared to the control plants.

**Nucleic acids**

The effects of water stress on nucleic acid levels in *Andrographis paniulata* presented in Table-5. Water stress suppressed the nucleic acid in all the treatments.

**DNA:** The difference in Deoxyribonucleic acid (DNA) content observed due to water stress. The maximum DNA content observed in T2 treatment with an average of 6.79±0.12 mg/g.F.wt and minimum DNA content observed in T7 treatment with an average of 4.31±0.06 mg/g F.wt in the 8th month stage when compared to the control plants(7.54±0.07 mg/g.F.wt).

**RNA:** The difference in Ribonucleic acid (RNA) content observed due to water stress. The maximum RNA content observed in T2 treatment with an average of 11.15±0.45 mg/g.F.wt and minimum RNA content observed in T7 treatment with an average of 6.26±0.26 mg/g.F.wt in the 8th month stage.

**Relative water content(RWC):**

Stress to tolerance in terms of the leaf water status and relative water content (RWC) was measured in all plants subjected to different water stress levels and results were depicted in the table 9. The leaf relative water content

was found to be decreased in all treatment over the control plants during water stress. However the per cent decrease in RWC was comparatively high in T2 treatment with average of 85.49±1.21% and less in T7 treatment with average of 54.47±0.19 at 8th month old plants. It is also clear from the results that T2 treated plants maintained relatively higher relative water content than T4 and T7 treatment.

**Table 1: Effect of water stress on leaf number, leaf area and plant height of *Andrographis paniculata* at different growth stages**

| Treatments | Leaf number (mean±SD) | Leaf area ($cm^2$) (mean± SD) | Plant height(cm) (mean± SD) |
|---|---|---|---|
| **2nd months old plants** | | | |
| Control | 15.23±0.12 | 0.117±0.0005 | 15.29±0.34 |
| T2 plants | 14.18±0.14 | 0.114 ±0.001 | 13.42±0.45 |
| T4 plants | 12.83±0.65 | 0.123±0.0011 | 11.12±0.19 |
| T7plants | 11.20±0.18 | 0.106±0.0005 | 10.48±0.35 |
| **4th month old plants** | | | |
| Control | 21.08±0.67 | 0.14±0.002 | 28.66±1.52 |
| T2plants | 18.90±0.75 | 0.12±0.0005 | 27.66±1.50 |
| T4plants | 14.80±0.62 | 0.13±0.049 | 22.33±1.52 |
| T7 plants | 12.11±0.19 | 0.11±0.03 | 20.33±1.52 |
| **6th month old plants** | | | |
| Control | 53.33±0.70 | 3.52±0.028 | 54.66±1.41 |
| T2plants | 52.33±0.70 | 3.20±0.115 | 53.66±1.40 |
| T4 plants | 44.66±1.41 | 2.2±0.017 | 43.66±0.70 |
| T7 plants | 38.66±1.41 | 1.10±0.014 | 35.66±0.70 |
| **8th month old plants** | | | |
| Control | 69.33±2.12 | 5.57±0.063 | 69.33±0.70 |
| T2 plants | 62.33±2.01 | 5.10±0.028 | 68.33±0.70 |
| T4 plants | 39.66±1.41 | 4.17±0.69 | 46.33±0.70 |
| T7 plants | 26.66±070 | 2.14±0.05 | 34.33±0.70 |

**Table .2: Effect of water stress on Root length and Shoot length of *A.paniculata* at different growth stages**

| Treatments | Root length (cm) (mean± SD) | Shoot length(cm) (mean± SD) |
|---|---|---|
| **2nd month old plants** | | |
| Control plants | 3.37±0.44 | 12.29±0.57 |
| T2 plants | 3.72±0.14 | 12.06±0.04 |
| T4 plants | 4.52±0.23 | 10.25±0.13 |
| T7 plants | 5.29±0.05 | 9.43±0.086 |
| **4th month old plants** | | |
| Control plants | 5.63±0.40 | 20.29±0.069 |
| T2 plants | 5.83±0.98 | 19.83±0.092 |
| T4 plants | 6.30±0.04 | 16.62±0.069 |
| T7 plants | 7.62±0.05 | 14.29±0.064 |
| **6th month old plants** | | |
| Control plants | 17.00±0.005 | 46.33±2.12 |
| T2 plants | 17.94±0.075 | 45.33±2.12 |
| T4 plants | 19.57±0.19 | 34.33±2.12 |
| T7 plants | 21.69±0.015 | 24.33±0.70 |
| **8th month old plants** | | |
| Control plants | 17.65±0.005 | 51.31±0.028 |
| T2 plants | 19.58±0.05 | 43.59±0.023 |
| T4 plants | 23.26±0.22 | 35.63±0.092 |
| T7 plants | 29.62±0.55 | 29.62±0.098 |

**Table 3: Effect of water stress on shoot and root fresh weight and dry weight of *A. paniculata* at different growth stages**

| Treatment | fresh weight of the shoot(gm) (mean±SD) | Dry weight the of shootgm) (mean±SD) | fresh weight of the root(gm) (mean±SD) | Dry weight of the Root(gm) (mean±SD) |
|---|---|---|---|---|
| **2 months old plants** | | | | |
| Control | 1.76±0.04 | 0.41±0.02 | 0.19±0.03 | 0.07±0.01 |
| T2 plants | 1.65±0.03 | 0.37±0.05 | 0.18±0.04 | 0.06±0.01 |
| T4 plants | 1.13±0.02 | 0.18±0.04 | 0.15±0.01 | 0.02±0.001 |
| T7plants | 0.70±0.03 | 0.15±0.01 | 0.14±0.01 | 0.01±0.05 |
| **4th month old plants** | | | | |
| Control | 2.02±0.08 | 0.98±0.02 | 1.19±0.02 | 0.23±0.03 |
| T2plants | 1.79±0.05 | 0.89±0.02 | 1.09±0.02 | 0.19±0.03 |
| T4plants | 1.16±0.01 | 0.72±0.03 | 0.79±0.04 | 0.38±0.05 |
| T7 plants | 1.01±0.01 | 0.56±0.04 | 0.55±0.03 | 0.32±0.05 |
| **6th month old plants** | | | | |
| Control | 4.08±0.38 | 1.92±0.06 | 1.18±0.06 | 0.31±0.05 |
| T2plants | 3.38±0.32 | 1.83±0.01 | 1.14±0.05 | 0.30±0.05 |
| T4 plants | 1.98±0.16 | 1.48±0.05 | 0.58±0.04 | 0.23±0.03 |
| T7 plants | 1.11±0.12 | 1.21±0.08 | 0.55±0.04 | 0.19±0.002 |
| **8th month old plants** | | | | |
| Control | 9.08±0.06 | 1.48±0.05 | 1.38±0.05 | 0.94±0.02 |
| T2 plants | 8.38±0.14 | 1.42±0.05 | 1.32±0.05 | 0.74±0.03 |
| T4 plants | 6.98±0.27 | 0.85±0.02 | 0.85±0.02 | 0.34±0.05 |
| T7 plants | 4.97±0.07 | 0.76±0.03 | 0.76±0.03 | 0.31±0.01 |

**Table 4: Effect of water stress on chlorophyll 'a', chlorophyll 'b' and total chlorophyll content of *Andrographis paniculata* in different treatments.**

| Treatments | Chlorophyll a Mg/g .F.wt mean±SD | Chlorophyll b Mg/g .F.wt mean±SD | Total chlorophylls Mg/g .F.wt mean±SD |
|---|---|---|---|
| Control plants | 2.36±0.37 | 1.38±0.36 | 4.4±0.43 |
| T2 plants | 2.03±0.36 | 1.1±0.1 | 4.2±0.41 |
| T4 plants | 1.37±0.34 | 0.6±0.25 | 3.4±0.38 |
| T7 plants | 0.56±0.032 | 0.3±0.05 | 2.47±0.34 |

**Table 5: Effect of water stress on Nucleic acids in *Andrographis paniulata* at 8th month growth stage**

| Treatments | DNA content (mg/gm F.wt) | RNA content (mg/gm F.wt) |
|---|---|---|
| control plants | 3.54±0.03 | 12.80±0.50 |
| T2 plants | 2.94±0.02 | 11.15±0.45 |
| T4 plants | 1.64±0.01 | 9.13±0.38 |
| T7 plants | 1.20±0.03 | 6.26±0.26 |

## DISCUSSION

Determination of growth at different stages has shown that water stress has caused significant reduction in shoot length, number of leaves, plant height and fresh, dry weight production. With increase in water stress, shoot growth notably diminished, fresh weight and dry weight in *Andrographis paiculata* plants. Inhibition of the root growth and the biomass reduction are usually observed in the plants under water stress[18]. Drought stress effects on elongation and expansion of plant growth[1]. In soya bean, the stem length was decreased under water deficit conditions[14] . The reduction in plant height was associated with a decline in the cell enlargement and

more leaf senescence in *A.esculentus* under water stress. The decreased growth of the plant height was reported by Cowett and Sprague , Seetharama *et al*[12] and stout in Sorghum. Present study also observed that reduction in leaf number, plant height, fresh and dry weights of leaf, stem all treatments compare to the control plants. The maximum growth parameters were observed in T2 treated plants(68.33±0.70) of *Andrographis paniculata.*Water stress mostly reduced leaf growth and in turns the leaf areas in *Populous* . It is evident from data that water stress has resulted in reduction of leaf area in all the four treatments. Fischer and Hagan stated that leaf area is a sensitive parameter to water stress. The leaf area in T7 plants shows the so much reduction in leaf area is compared to the other treatment plants. Leaf area in the plants receiving water stress may be explained by the reports of IIuna , Slatyer were they all stated that cell enlargement is more sensitive to water stress. The development of root system increase the water uptake and maintains osmotic pressure through higher proline levels in *phoenix dactylifera* [5] increased root growth due to water stress was reported in sunflower[15] and *Catharanthus roeus*[7]. In the present study, root length increased in T2(17.66±1.41cm), T4(23.33±0.70cm), T7 (29.66±2.82cm)plants when compare to the control( 17.66±2.12cm). The root dry weight was decreased under water stress in maize, wheat. The decline in RWC was reported by several investigators under water stress conditions Ramanjulu and sudhakar observed a decrease in RWC with response to gradually induced water stress in two mulberry cultivars. In the present study, it was observed that *Andrographis paniculata* plants subjected to water stress displayed reduced RWC of the leaves. In the present study, analysis for RWC revealed that *Andrographis paniculata* plants showed decrease in the RWC in treatments. It is observed that T7 treatment showed very less RWC compare to the T2 treatment of *Andrograrphi paniculata.* Both chlorophyll a and b are prone to water stress[6]. The results are not in agreement with findings of Megdiche that drought stress increased chlorophyll contents in *Withania somnifera*. Decrease of photosynthesis due to water stress has been attributed to both stomatal and non-stomatal limitations[13]. A reduction in DNA, RNA content was decreased in all treatments compared to the control plants. In the present study nucleic acids were decreased by water stress in T4, T7 plants compared to the control plants at all the growth stages (Tables 5). The percentage decrease in DNA and RNA was more in T2 plants with average of( 2.94±0.02, 11.15±0.45mg/gm f.wt). Similar results have been reported in Sugarcane[10], wheat[3] *et al*.,

## CONCLUSION

In the present study observes that water stress has affected the plant growth, metabolism.With the effect of water stress the growth parameters such as leaf area, plant height, number leaves, shoot length, fresh weight, and dry weight decreased with increased in water stress. Root length increased when compared to shoot length with increase in water stress. *Andrographis paniculata* is moderately tolerant to water stress conditions. At various levels of water stress had a highly significant effect upon the survival %age, plant height, shoot fresh and dry weight, root fresh and dry weight and number of leaves also varied significantly. The findings suggest that the response of *Andrographis paniculata* to water stress depends on the concentration of the water stress applied. Focusing at the survival percentage, growth and biomass production of *A.paniculata*, it is suggested that the *Andrographis paniculata* could be tried on moderately water stress habitat.

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