



Evaluation of the agronomic traits, chlorophyll content and bioactive components of *Andrographis paniculata* under various irrigation stresses

RK Upadhyay^{1*}, Pragya Pandey², RC Padalia³, Venkatesha KT⁴, Dipender Kumar⁵

¹ Principal Scientist, Department of Crop Production & Protection, CSIR-Central Institute of Medicinal and Aromatic Plants, Research Center, Pantnagar, PO Dairy Farm Nagla, Udham Singh Nagar, Uttarakhand, India

² Research Scholar, Department of Crop Production & Protection, CSIR-Central Institute of Medicinal and Aromatic Plants, Research Center, Pantnagar, PO Dairy Farm Nagla, Udham Singh Nagar, Uttarakhand, India

³ Sr. Principal Scientist, Department of Phytochemistry, CSIR-Central Institute of Medicinal and Aromatic Plants, Research Center, Pantnagar, PO Dairy Farm Nagla, Udham Singh Nagar, Uttarakhand, India

⁴ Senior Scientist, Department of Genetics & Plant Breeding, CSIR-Central Institute of Medicinal and Aromatic Plants, Research Center, Pantnagar, PO Dairy Farm Nagla, Udham Singh Nagar, Uttarakhand, India

⁵ Scientist Scientist, Department of Crop Production & Protection, CSIR - Central Institute of Medicinal and Aromatic Plants, Research Center, Pantnagar, PO Dairy Farm Nagla, Udham Singh Nagar, Uttarakhand, India

Abstract

The most crucial element in the area where abiotic stress is prevalent is irrigation. To assess the effects of various levels of stress on the development and bioactive components of *A. paniculata*, a pot experiment with five stress treatments and three replications was carried out. Results demonstrated the statistical significance of water stress on plant growth metrics. Finding showed that reducing irrigation supply to 50% (T3), deficit irrigation has significant effect on *A. paniculata*'s fresh weight, dry weight, leaf to stem ratio, leaf length and width all increased by 28.29%, 45.3%, 28.45 %, 27.59% and 19.54 %, respectively when compared to those of the control (T1) experiment. With just a slight drop in growth metrics (plant height, fresh and dry weight, etc.), chlorophyll content, and relative water content of leaves, rainfed irrigation had a substantial impact on plant survival. The plant in treatment T5 underwent extreme drought stress conditions, and the highest decrease was seen there. In contrast to the chlorophyll content and RWC, which have fallen as the shortage of water has become worse, root length and root to shoot ratio has grown massively as stress levels go up with the highest increase in rainfed irrigation (41.97 %). Maximum accumulation of active component andrographolide (2.46%) was found in treatment T₂ (Daily irrigation) with minor decrease in Neo-andrographolide (0.19%) and 14-deoxy-11,12-didehydroandrographolide (0.16%) as compared to control. According to the findings, *A. paniculata* may be grown in arid and semiarid regions as an alternative crop resistant to stress. In regions where water supply is very limited and irrigation management is subpar, deficit irrigation (T3) may be a viable water saving strategy. This approach can help cut the overall cost of irrigation.

Keywords: Water stress, irrigation system, relative water content, water retention, secondary metabolites

Introduction

All climatic disturbances that have a significant impact on plant growth and development are considered to be within the category of abiotic stress. Examples include high temperatures and water stress (either an excess or shortfall). Some areas rely only on rain-fed irrigation because they have an inadequate or no alternative irrigation resource, which has an indirect and direct impact on agricultural output. A yearly precipitation rate of 20 to 35 percent of potential evapotranspiration is seen in dry and semi-arid environments. In India, dry land agriculture accounts for 67 % of all net planted land (or around 95 million hectares), producing about 42 % of all crop yields. Abiotic stress is intermittent and a threat to the overall agricultural output due to prolonged dry spells and decreased rainfall in arid and semi-arid areas. Because of the sharp drops in rainfall, 90% of the groundwater used to irrigate agricultural land to overcome the productivity loss, which has made groundwater scarce in the affected areas. The early closing of stomata caused by a lack of water and a high evapotranspiration rate reduces their rate of transpiration, which in turn imbalances the entrance of carbon needed for photosynthesis and stunts development. The difficulties that

plants encounter in nature change their metabolic pathways, photosynthetic apparatus (PSII), and ability to absorb nutrients. All of these changes inhibit plant development and dramatically reduce crop output (Bechtold and field, 2018) ^[1]. A technique known as "deficit irrigation" uses less water for irrigation than what plants would need from a complete irrigation system without suffering any significant productivity losses. When plants receive enough water, they only obtain what they require to survive; as a result, their roots expand to take up more water. This irrigation system, according to Mirás-Avalos *et al.* (2016) ^[2], provides high chances for groundwater conservation without experiencing substantial agricultural losses. Agriculture on dry terrain also makes use of it. Regulation of irrational irrigation usage and management of agricultural loss in situations of water stress provide significant challenges. A lot of farmer in these areas also rely on rainfed irrigation, which is unsafe and fragile to both short-term and long-term droughts. The primary goal of choosing various irrigation stresses was to, first, preserve groundwater as much as possible by lowering or changing the irrigation dynamics below the demand of plants, and second, to choose a crop that could be grown on dry land and in rainwater in an arid and semi-arid

location with minimal to no yield loss. Hence, in this work, the impact of irrigation stress on the growth characteristics and bioactive components of *A. paniculata* is evaluated.

Materials and Methods

1. Experimental site and preparation

A pot experiment was carried out in 2021 in the nursery of the Central Institute of Medicinal and Aromatic Plant, Pantnagar (latitude 29° N, longitude 79° 38'E), at an altitude of 243 m above sea level during the growing seasons of *A. paniculata*. *A. paniculata* (variety - cim Megha) nursery was set up in the greenhouse at the end of April. Plants that had been growing for two months were then transplanted into the pot.

2. Details of Imposition of water stress treatment

Three replications of the experiment were run in pots in an open field. After transplanting, water irrigation stress was applied at the vegetative stage and continued throughout the growing cycle. The unaffected plant designated as the control got uninterrupted watering up to harvest. We hypothesize the dry and moist situation to create the four different water irrigations stress plus control which includes:

- a. **Treatment 1(T₁):** 100% Proper irrigation (based on the full requirement of plants)
- b. **Treatment 2(T₂):** Irrigation regularly (water irrigated may be excess than the plants would require for optimal growth)
- c. **Treatment 3(T₃):** 50% reduction of full required irrigation (deficit irrigation)
- d. **Treatment 4(T₄):** Rainfed irrigation (completely depends on rainfall)
- e. **Treatment 5(T₅):** One-time irrigation provided at the time of transplantation (severe stress condition)

3. Determination of Relative water content and Water retention capacity

Utilizing the following formula, relative water content and water retention capacity were determined (Yamasaki and Dillenburg, 1999) [3]. After determining the fresh weight (FW) of the leaves, they were stored at room temperature for 24 hours in a petri dish with purified water. After 24 hours, leaves were dried using blotting paper, and turgid weight (TW) was noted. In order to calculate the dry weight, the leaves were dried for the following 24 hours at 40–50°C (DW).

Relative water content (RWC) (%) = $(FW - DW) \div (TW - DW) \times 100$

Water retention capacity (WRC) = TW / DW

4. Determination of chlorophyll content (mg/g fresh wt)

The method developed by Kamble *et al.* (2015) [4] was used to determine the amount of leaf chlorophyll. This approach involved carefully crushing 1gm of fresh leaf tissue in a mortar and pestle. The crushed leaves were combined with 20 ml of 80% acetone and 0.5 mg of MgCo₃ as needed. The mixture is centrifuged at 2500 rpm for 5 minutes after being held at 4 °C for 1-2 hours. The supernatant was decanted, and 80 percent acetone was added to bring the volume to 100 ml. Using 80% acetone as a blank, the absorbance of the extract was measured in a spectrophotometer at 645 and

663 nm. The following equation was used to determine the chlorophyll concentration (Ar non, 1949):

Chlorophyll "a" content (mg/g fresh wt.) = $12.7(A_{663}) - 2.69(A_{645}) \times V / 1000 \times W$

Chlorophyll "b" content (mg/g fresh wt.) = $22.9(A_{645}) - 4.68(A_{663}) \times V / 1000 \times W$

Total Chlorophyll content (mg/g fresh wt) = $20.2(A_{645}) + 8.02(A_{663}) \times V / 1000 \times W$

Where, A = absorbance at specific wavelengths, v = final volume of chlorophyll extract, W = fresh weight of sample.

5. Determination of active compounds

The entire plant, including the shoot, was picked, and after drying in the shade, the contents of the bioactive components were evaluated using an HPLC technique. A dried sample weighing 25 mg was combined with 20 ml of HPLC-grade methanol, and the mixture was then extracted for 60 minutes. A portion of the filtrate from the solution was evaporated to dry it off, and it was then dissolved in methanol. Filtration of the dissolved solution was done using a 0.45 μm membrane. Following the steps outlined in Chen *et al.* 2020 [6], the HPLC analytical process was completed.

6. Statistical analysis

The growth characteristics, including plant height, herb fresh and dry weight, leaf to stem ratio, and root length, were noted for agro morphological characterization. For the Randomized Block Design with three replications, all statistical analyses were completed. The means of the various test parameters under irrigation stress circumstances were compared at a p<0.01 level for analysis of variance and significant differences using a Statistical calculator.

Results

Data presented in Table and figure showed the significant (p<0.01) influence of various irrigation stresses on the agronomic characteristics, morphological data, Relative Water Content (RWC) Total Chlorophyll Content (mg/g) and bioactive compounds of *A. paniculata*. Following a 24-hour period of individual treatment, the soil moisture measurements in each pot were 16.5, 21.3, 12.8, 12.6 and 11.1 percent in the T₁, T₂, T₃, T₄, and T₅ treatments, respectively.

1. Impact of irrigation stress on agronomic characteristics of *A. paniculata*

1.1. Plant height (cm)

When the collected data were statistically analyzed, they revealed a significant impact (p<0.01) on plant height at each irrigation stress treatment. The highest plant height under the T₃ treatment (deficit irrigation), where water supply to the crop was decreased to 50% of the needed irrigation, was measured at 61.0 ± 0.57 cm. When comparing the findings of plant height with control (50.28 ± 0.36 cm), T₃ had a 21.32% rise in plant height, whereas T₂ had a 16.69% increase. Stressed *A. paniculata* in treatment T₄ (rainfed irrigation) caused a 9.17 percent reduction in plant height compared to control (T₁). The crop underwent extreme stress and received little to no water in treatment T₅, which resulted in a 72.16 percent reduction in plant height (Table 1).

Table 1: Characteristics trait of *Andrographis paniculata* observed under different stress 423 conditions

Treatment	Plant height (cm)	Fresh weight (gm/plant)	Dry weight (gm/plant)	Leaf- stem ratio	Leaf length (cm)	Leaf width (cm)
1(Control)	50.28 ± 0.36	129.16± 0.52	27.66± 1.2	1.23± 0.04	7.03± 0.09	3.07± 0.03
2(Daily Irrigation)	58.67± 0.88	152.27± 2.09	35.67± 0.33	1.46± 0.05	9.37± 0.09	4.27± 0.03
3(Deficit Irrigation)	61.00± 0.57	165.7± 1.64	40.19± 1.60	1.58± 0.04	8.97± 0.09	3.67± 0.17
4(Rainfed Irrigation)	45.67± 0.67	115.0± 1.73	26.66± 1.202	1.20± 0.02	6.42± 0.04	2.10± 0.06
5(No Irrigation)	14.00± 0.57	5.00± 0.577	0.16± 0.04	0.38± 0.06	3.47± 0.09	1.27± 0.03
C.D. (p < 0.01)	2.15**	4.66**	2.88**	0.14**	0.27**	0.23**
SEm (±)	0.71	1.54	0.95	0.04	0.09	0.08
C.V. (%)	2.67	2.35	6.30	6.60	2.22	4.63

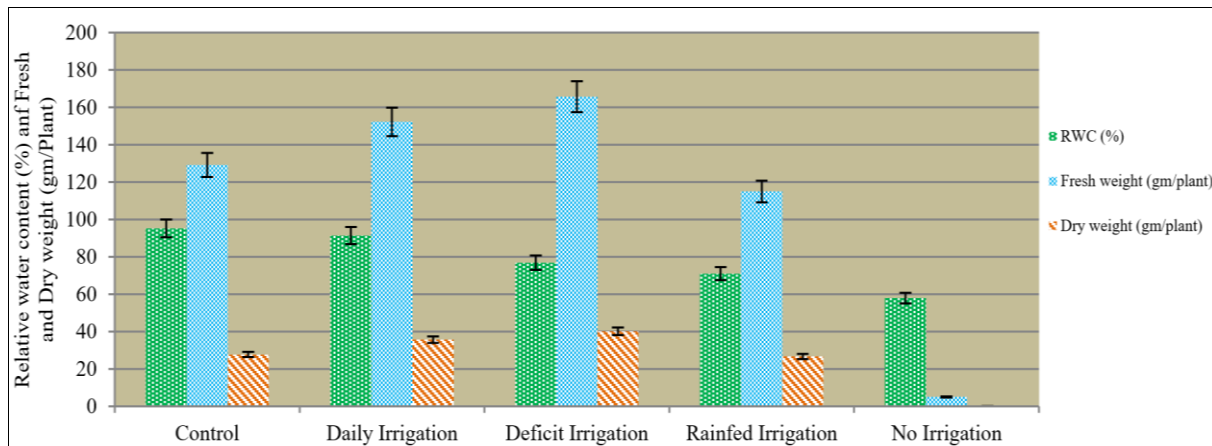


Fig 1: Effects of different irrigation stress on relative water content (%), fresh and dry weight of *A. paniculata*

1.2. Fresh and dry weight of plant

The fluctuation in irrigation level had a significant impact on the plant's fresh and dry weight, according to Table 1 and Figure 1 analysis of variance at 1%. The outcome demonstrates *A. paniculata* tolerance for limited water availability. With a maximum increase in fresh weight of 28.29 percent and dry weight of 45.3 percent as compared to control, as shown in Table 1, the effect of limiting the irrigation supply to 50% in T3 treatment had a substantial influence on the fresh and dry weight of herb. Daily irrigation (T2) created temporary waterlogging stress, which resulted in a considerable increase in the fresh and dry weight of *A. paniculata* by 17.89% and 28.95%, respectively, in comparison to control (T1). The plant that depends on rainfed irrigation (T4) reduces the fresh and dry weight by 10.96% and 3.62%, respectively, when the stress data of herb yield are compared. An increase in stress level, T5 had led to a significant yield loss by lowering the fresh weight by 96.13 percent and the dry weight by 99.42 percent per plant.

1.3. Leaf to stem (L: S) ratio of *A. paniculata*

As can be shown in Table 1, the response to water deprivation had a substantial impact on plant growth (p<0.01). With a 28.45 percent increase, deficit irrigation (T3) had the highest leaf-to-stem ratio. T2 (daily irrigation) had the next-highest leaf-to-stem ratio, increasing by 18.70 percent, as shown in Table 1. When compared to control, rainfed irrigation has a modest effect, with a 2.44 percent drop in the leaf-to-stem ratio (T1). In treatment T5, when the plant was severely stressed by dryness, the leaf-to-stem ratio deteriorated to a high of 69.11 percent.

2. Influence of irrigation stress on morphology of *A. paniculata*

2.1. Leaf length and width (cm)

Tremendous impact of irrigation stress on *A. paniculata* leaf growth. With maximum increases of 33.28 percent and

39.08 percent in leaf length (9.37± 0.09 cm) and width (4.27± 0.03 cm), respectively was obtained in the T2 treatment as compared to control (T1). Furthermore, deficit irrigation (T3) showed a comparable increase of 27.59 percent and 19.54 percent in leaf length (8.97± 0.09 cm) and leaf width (3.67± 0.17 cm), respectively (Table 1). By modifying the leaf trait (leaf length and width), which controls the transpiration rate, *A. paniculata* has been able to overcome circumstances of water stress (deficit and surplus). By narrowing the leaf width by 31.59% and length by 8.67 % compared to the control, rainfed irrigation affects the transpiration rate (T4). Having a severe water shortage, T5 has a detrimental impact (p<0.01) on leaf characteristics, resulting in a drop-in leaf length and width of 50.64 and 58.63 %, respectively.

2.2. Root length

Table 2 displayed the root length statistical information for which significant (p0.01) outcomes were noted for all stress treatments. The irrigation method with the largest root length (25.20±0.46 cm), which grew by 41.97 % in comparison to control was rainfed irrigation (T4). Root length (22.22±0.35 cm) reported in deficit irrigation (T3) treatment was considerably higher by 25.18 % than control (T1) having root length of 17.75±0.30 cm When compared to the control, root length (14.74 ±0.33 cm) was affected by excessive water stress caused by daily irrigation treatment (T2). Results unmistakably show that plants grow deeper roots to obtain enough moisture and nutrients to endure irrigation stress. The severe water shortage in treatment T5 leads to a drought stress state, which negatively affects the root of *A. paniculata* by inhibiting root development. As a result, treatment T5 showed a 63.61% drop with a minimum root length of 6.463 ±0.14 cm.

Table 2: Influence of irrigation stress on root length, fresh and dry weight of *A. paniculate*

Treatment	Root length (cm)	Shoot length (cm)	Root-shoot ratio	Roots fresh weight (g/plant)	Roots Dry weight (g/plant)
1(Control)	17.75± 0.30	50.28 ± 0.36	0.353± 0.01	1.89± 0.01	0.39± 0.01
2(Daily Irrigation)	14.74± 0.33	58.67± 0.88	0.251± 0.00	1.91± 0.01	0.40± 0.01
3(Deficit Irrigation)	22.22± 0.35	61.00± 0.57	0.364± 0.00	2.10± 0.02	0.41± 0.01
4(Rainfed Irrigation)	25.20± 0.46	45.67± 0.67	0.552± 0.01	1.92± 0.01	0.32± 0.01
5(No Irrigation)	6.463± 0.14	14.00± 0.57	0.463± 0.02	0.32± 0.01	0.05± 0.01
C.D. (p<0.001)	0.97**	2.15**	0.02**	0.033**	0.02**
SEm (±)	0.32	0.71	0.01	0.01	0.01
C. V. (%)	3.21	2.67	3.51	1.06	3.94

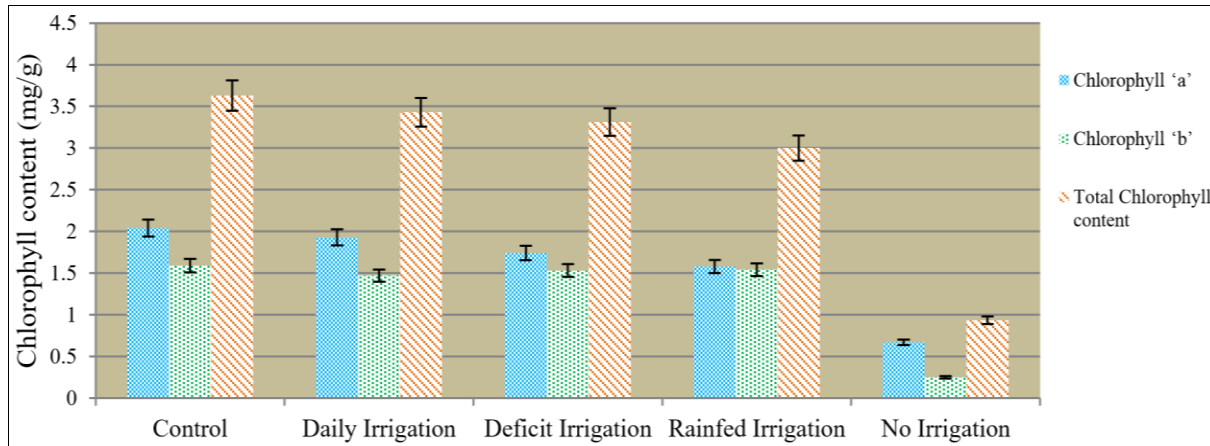


Fig 2: Impact of different irrigation stress on Total Chlorophyll content in *A. paniculata* leaves

2.3. Roots fresh and dry weight

Table 2 displayed the measured data for various irrigation stresses that had a significant (p<0.01) effect on root development. With maximum increases of 11.11 percent and 5.13 percent, respectively, compared to control, deficit irrigation (T3) had a substantial impact on the fresh and dry weight of *A. paniculata* roots. Daily water supply (T2) resulted in a 1.05 % rise in fresh root weight and a 2.56 % growth in dry root weight. *A. paniculata* demonstrated a tolerant response to rainfed irrigation in root development with an increase in root fresh weight by 1.59%, while reducing root dry weight by 17.95% in comparison to the control (T1). *A. paniculata* roots' fresh and dry weights decreased by 81.48 % and 87.18%, respectively, as a result of the plant's acute stress brought on by the severe water scarcity.

2.4. Root to shoot ratio

The root-to-shoot ratio underwent analysis, which is depicted in Table 2, to determine the effects of various irrigation stresses on the plant. As soil water shortage stress increased (decrease in soil moisture content), the table revealed a considerable rise in the root-to-shoot ratio. Plants under treatment T₄ that rely on rainfall irrigation have produced a dry stress environment that affects plant growth and modifies the root-to-shoot ratio (0.552 ± 0.01), which increases by a maximum of 56.37% in comparison to the control and has a regulating effect on plant growth. Poorly developed *A. paniculata* in treatment T₅ attempted to adapt to a severe drought stress environment by changing the root and shoot system, which increased its root-to-shoot ratio (0.463 ± 0.02) by 31.61% in comparison to the control. When compared to the control T₁ (0.353±0.01), *A. paniculata*'s root-to-shoot ratio (0.251±0.00) is reduced by 28.89% due to excessive water stress in treatment T₂

(wetting condition). The root-to-shoot ratio in Treatment T₃'s deficit irrigation was 0.364 ± 0.00 with a slight rise of 3.12%, which aided in *A. paniculata*'s growth.

3. Influence of water stress on Physiological process

3.1. Relative water content (RWC)

In contrast to control T₁, which had 95.24 % RWC and a water retention capacity of 2.91, the results shown in Figure 1 reveal a decline in RWC ranging from 91.30 % to 57.89 %. The daily irrigation treatment (T₂) resulted in a reduction in water content (91.30 percent) of 4.14 percent with water retention of 3.30. During deficit irrigation T₃, exhibited a considerable fall in relative water content to 19.24 percent with a water retention capacity of 3.89, when the water supply was cut in half. To survive under rainfed irrigation (T₄), the recorded leaf size was reduced, which finally resulted in a reduction in water content of 25.49 %. In treatment T₅, which was under severe drought stress, there was a maximum loss of 39.22% in relative water content (57.89%) with a water retention capacity (7.33).
3.3.2, Chlorophyll 'a' and Chlorophyll 'b' content (mg/g)
 Under various irrigation stresses, Figure 2 clearly shows that chlorophyll a content was severely impacted whereas chlorophyll b content had a modest impact. Chlorophyll a and b levels peaked at 2.04 and 1.59 mg/g, respectively, in the control (T₁), and they gradually decline as the level of water stress rises. With a 5.39 % drop in chlorophyll a content (1.93 mg/g) and a 7.55 % fall in chlorophyll b content (1.47 mg/g) when compared to control, daily irrigation (T₂) has a substantial impact. The chlorophyll a (1.74 mg/g) and chlorophyll b (1.53 mg/g) concentrations decreased by 14.71 and 3.77 %, respectively, in deficit irrigation (T₃ treatment), as compared to control. Similar to the above, under rainfed irrigation, *A. paniculata* leaves' chlorophyll a and chlorophyll b contents have reduced by

22.55 % and 3.14 %, respectively. In treatment T5, where plants encountered severe water deficit/drought stress conditions, there was a significant fall in chlorophyll content by 67.16 % and 84.27 %, respectively.

3.2. Total Chlorophyll content (mg/g)

As can be seen from the data in Figure 2, there was a correlation between the total chlorophyll content and the relative water content since a considerable decline was observed in the same sequence (T2 > T3 > T4 > T5). Treatment T2 demonstrated a marginal decline of 5.51 % in total chlorophyll content as compared to the control, which had 3.63 mg/g of it. Following the directive, under deficit irrigation (T3) treatment, a loss of 8.82 percent in the total chlorophyll content (3.31 mg/g) was noted. The amount of total chlorophyll content (3.00 mg/g) in rainfed irrigation (T4) has decreased dramatically by 17.36 percent. Figure 2 illustrates the significant decrease in total chlorophyll content (0.935 mg/g) under extreme stress conditions (T5) with a 74.24 percent drop rate.

4. Influence of water stress on the biochemical profile of *A. paniculata*

4.1. Bioactive compound content (%)

The bioactive substance found in *A. paniculata* was significantly affected, according to the statistical analysis given in Figure 3. Deficit irrigation (T3) showed in minimal reductions of 16.96 % in andrographolide (AG) level (1.86) in *A. paniculata* as compared to control. By lowering the level of Neo-andrographolide and 14-deoxy-11, 12-didehydroandrographolide by 17.37 % and 11.11 %, respectively in daily irrigation (T2), the amount of andrographolide (AG) in leaves (2.46%) was found to be considerably greater than control, with rises of 9.02 %. This can be brought on by plants under stress accumulating secondary metabolites. Figure 3 illustrates a significant reduction in other bioactive compounds under rainfall irrigation (T₄), including a 17.37 and 44.44 percent reduction in Neo-andrographolide and 14-deoxy-11, 12-didehydroandrographolide, respectively. Control and other treatment have recorded lower andrograpanin levels of 0.1 percent.

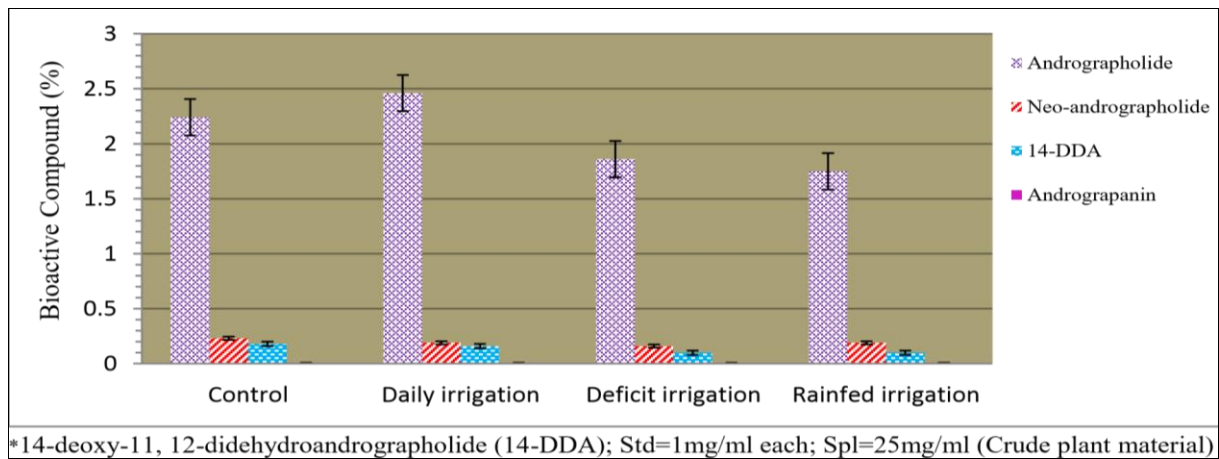


Fig 3: Bioactive content of *A. paniculata* under control and irrigation stressed condition

Discussion

Plants have been exposed to substantial abiotic stress, which affects their growth, yield, and biologically active compounds. The amount of rain that falls each year decreases as a result of climate change, along with the precipitation rate, which creates a water shortage or puts inhabitants under stress from a dry spell. Because of the sharp drops in rainfall, 90% of the groundwater used to irrigate agricultural land, which has made groundwater scarce in the affected areas. Groundwater depletion has sparked concern about the need to save water resources and the necessity of concentrating on dryland farming or modifying the irrigation system to the restricted amount of water delivered to plants in arid and semi-arid regions. From species to species, there are differences in how plants react to abiotic stress. *A. paniculata* and other investigated plants have shown both positive and adverse effects on growth when subjected to abiotic stress, according to the literature. In the current study, each applied treatment has targeted a specific problem brought on by abiotic stress and has a substantial impact on growth parameters (plant weight, leaf stem ratio, leaf length, root length, etc.), total chlorophyll content (mg/g), and bioactive substances (Figure 1,6 and 7). By affecting the chlorophyll content, leaf water content, leaf size, and transpiration rate, water stress has a long-term

negative impact on a plant's productivity. Plant species can only withstand a specific amount of stress for a brief period of time without having an impact on their physiological growth factors. According to Singh *et al.* (2019) [7], some crop species that were vulnerable to water stress experienced a modest drop in yield and growth. Our findings did not agree with the research conducted by Wakchaure *et al.* (2018) [8]. They subjected the plant to several abiotic stresses and noticed a decline in yield and other growth variables. Our findings were consistent with Parkash and Singh's (2020b) [9] research, which demonstrated that a scarcity of water slows the rate of leaf expansion and, thus, reduces water loss as well as the total chlorophyll content and net photosynthetic activity of the plant. The plant increases the accumulation of ABA phytohormones, which activates SnRK2s kinase, stimulates the transport of sucrose from the shoot to the root, and improves root development. In turn, it improves root function under stress conditions and raises the root-to-shoot ratio in plants (Chen *et al.* 2022) [10]. Researchers studying water stress situations in plants, including Jarvis *et al.* (2011) [11], reported a process that stimulates the formation of secondary roots which, in turn, enhances the accumulation of mineral acquisition and absorbs the water the plants require. These results were consistent with our

study, which found that crops with inadequate water shrunk leaf size and grow longer roots than control, except for the extreme drought conditions (Table 2). According to a recent study, regular irrigation (T2) results in increased water stress, which causes *A. paniculata* roots to grow shorter by 16.96% in comparison to the control plant yet heavier by 1.05% (Table 2). Similar to earlier findings, maximum leaf length and width were seen when plants received regular irrigation (T2), which may have increased transpiration rate in comparison to controls, while minimum leaf size was noticed in conditions of extreme drought stress (T5), which may have decreased transpiration rate as a result of partial stomatal closure. However, with increased leaf length during deficit watering, leaf width has nearly matched that of the control (Table 1). Each irrigation stress strategy showed a link between total chlorophyll concentration and relative water content (RWC) (Figure 1 and 6). The findings were consistent with those made by Heba and Samia, (2014)^[12], which show a considerable decline in total chlorophyll content under various irrigation stresses. Another study on *Helianthus annuus* (Garofalo and Rinaldi, 2015)^[13], *A. paniculata* (Talei *et al.*, 2015)^[14], and Ratoon Banana (Surendar *et al.*, 2013)^[15] discovered that under stressful conditions, total chlorophyll content, photosynthetic activity decreases with an adjustment in plant survival mechanisms, which effects yield improvement with a modest rise in crop output (Begna, 2020)^[16].

Plants produce more secondary metabolites while under stress, which helps them build a defense mechanism. This study shows that bioactive compounds such as andrographolide, neo-andrographolide, and others found in *A. paniculata* has effected by irrigation stress condition when compared to the control (Figure 3). *A. paniculata* (Chen *et al.* 2020)^[6], and *Phellodendron amurense* (Li *et al.* 2007)^[17] have all previously been shown to produce more alkaloids and other secondary compounds when exposed to water stress. When the growth of *Labisia pumila* Benth (Jaafar *et al.* 2012)^[18] and *Salvia miltiorrhiza* (Liu *et al.* 2011)^[19] was hampered by stress, as a result, malondialdehyde (MDA) and secondary metabolites were advantageously accumulated, serving as a protective mechanism against stress and promoting the growth and development of the plants. With the exception of high-stress circumstances, which result in a considerable loss in bioactive compounds, daily and rainfed irrigation has affected secondary metabolites and produced a drop in the content of andrographolides and other compounds when compared to the control. This is even though the fact that water deficit stress has a favorable influence on secondary metabolite accumulation. Therefore, it is clear that *A. paniculata* may be planted in locations with limited water supply and irrigation sources as well as in places where water logging stress was a short-term issue owing to poor drainage system.

Conclusion

In this study, each treatment showed measurable morphological and physiological impacts on plant attributes. Plants are challenged by water stress to evolve physiologic and biochemical defenses and adaptive mechanisms. The findings show that the extreme water stress condition (T5) has had an impact on the growth characteristics of *A. paniculata*, including plant height and yield, development of the leaves and roots, chlorophyll content, and leaf water

content. By modifying the morphological features of the plant, such as reducing leaf growth and changing root function, deficit irrigation (T3) has greatly increased the output of *A. paniculata*. In addition to boosting the accumulation of bioactive compounds in irrigation stress comparison to conventional watering (control). *A. paniculata* has further demonstrated a tolerance response by evolving a few adaptation techniques under rainfed irrigation with just a little production loss, and it is suitable for use as a selected crop for dry land farming. This crop can also be helpful in locations that are temporarily under water logging stress, and it can employ defensive mechanisms to enhance its growth by changing some morphological traits, such as increasing leaf size and modifying root development, without suffering significant losses. A study demonstrates that deficit irrigation and rainfed irrigation systems may offer a promising growth and production in agriculture without suffering any significant losses. In the arid and semi-arid regions, the cultivation of stress-tolerant crops with controlled irrigation systems may be helpful for water-conserving methods and dry land farming while causing no or minimal yield loss.

Reference

1. Bechtold U, Field B. Molecular mechanisms controlling plant growth during abiotic stress. *Journal of Experimental Botany*,2018;69(11):2753–2758. doi:10.1093/jxb/ery157.
2. Mirás-Avalos JM, Pérez-Sarmiento F, Alcobendas R, Alarcón JJ, Mounzer O, Nicolás E. Using midday stem water potential for scheduling deficit irrigation in mid-late maturing peach trees under Mediterranean conditions. *Irrig. Sci*,2016;34(2):161–173. <https://doi.org/10.1007/s00271-016-0493-9>
3. Yamasaki S, Dillenburg LC. Measurements of leaf relative water content in *Araucaria angustifolia*. *Revista Brasileira de Fisiologia Vegetal*,1999;11:69–75.
4. Kamble PN, Giri SP, Mane RS, Tiwana A. Estimation of Chlorophyll content in young and adult leaves of some selected plants. *Universal journal of environmental research and technology*,2015;5(6):306–310.
5. Arnon DI. Copper enzymes in isolated chloroplasts phenoloxidase in *Beta vulgaris*. *Plant physiology*, 1949;24:1. doi: 10.1104/pp.24.1.1
6. Chen X, Xie Y, Wei K, Lan Z, Li C, Li Y, Guo X. Drought stress enhanced andrographolides contents in *Andrographis paniculata*. *Acta Ecologica Sinica*,2020;40(2):113-121. <https://doi.org/10.1016/j.chnaes.2020.02.003>
7. Singh M, Saini RK, Singh S, Sharma SP. Potential of integrating biochar and deficit irrigation strategies for sustaining vegetable production in water-limited regions: A review *Hort Science*,2019;54(11):1872-1878. <https://doi.org/10.21273/HORTSCI14271-19>
8. Wakchaure G, Minhas P, Meena KK, Singh NP, Hegade PM, Sorty AM. Growth, bulb yield, water productivity and quality of onion (*Allium cepa* L.) as affected by deficit irrigation regimes and exogenous application of plant bio-regulators. *Agric. Water Manag*,2018;199:1-10. <https://doi.org/10.1016/j.agwat.2017.11.026>

9. Parkash V, Singh S. A review on potential plant-based water stress indicators for vegetable crops Sustainability,2020b:12:3945.
10. Chen Q, Hu T, Li X, Song CP, Zhu JK, Chen L, Zhao Y. Phosphorylation of SWEET sucrose transporters regulates plant root: shoot ratio under drought. Nature Plants,2022:8:68-77. <https://doi.org/10.1038/s41477-021-01040-7>
11. Jarvis NJ. Simple physics-based models of compensatory plant water uptake: concepts and eco-hydrological consequences. Hydrol Earth Syst Sci,2011:15:3431–3446. doi.10.5194/hess-15-3431-2011.
12. Heba IM, Samia AA. Influence of garlic extract on enzymatic and non-enzymatic antioxidants in soybean plants (*Glycine Max*) grown under drought stress. Life Science Journal,2014:11(3):47-58.
13. Garofalo P, Rinaldi M. Leaf gas exchange and radiation use efficiency of sunflower (*Helianthus annuus L.*) in response to different deficit irrigation strategies: from solar radiation to plant growth analysis. Eur J Agron,2015:64:88–97. doi: 10.1016/j.eja.2014.12.010.
14. Talei D, Valdiani A, Maziah M, Sagineedu SR, Abiri R. Salt stress induced protein pattern associated with photosynthetic parameters and andrographolide content in *Andrographis paniculata* Nees. Biosci. Biotechnol. Biochem,2015:79(1):51–58, <https://doi.org/10.1080/09168451.2014.963499>.
15. Surendar KK, Devi DD, Ravi I, jeyakumar P, Velayudham K. Water Stress Affects Plant Relative Water Content, Soluble Protein, Total Chlorophyll Content and Yield of Ratoon Banana. International Journal of Horticulture,2013:17:96103. doi: 10.5376/ijh.2013.03.0017.
16. Begna T. Effects of Drought Stress on Crop Production and Productivity. International Journal of Research Studies in Agricultural Sciences (IJRSAS),2020:6(9):3443. <https://doi.org/10.20431/2454-6224.0609005>.
17. Li X, Wang Y, Yan X. Effects of water stress on berberine, jatrorrhizine and palmatine contents in Amur corktree seedlings, Acta Ecol. Sin,2007:27:58–63, [https://doi.org/10.1016/S1872-2032\(07\)60011-0](https://doi.org/10.1016/S1872-2032(07)60011-0).
18. Jaafar HZE, Ibrahim MH, Mohamad NF. Impact of soil field water capacity on secondary metabolites, phenylalanine ammonia-lyase (PAL), maliondialdehyde (MDA) and photosynthetic responses of Malaysian kacip fatimah (*Labisia pumila Benth.*), Molecules,2012:17:7305–7322. <https://doi.org/10.3390/molecules17067305>.
19. Liu H, Wang X, Wang D, Zou Z, Liang Z. Effect of drought stress on growth and accumulation of active constituents in *Salvia miltiorrhiza* Bunge, Ind. Crop. Prod,2011:33:84–88, <https://doi.org/10.1016/j.indcrop.2010.09.006>.