Impact of Water Stress on Morphological Traits of Henna (*Lawsonia inermis* L.) in Arid Conditions in Shahdad

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ABSTRACT

Objective: This study aimed to investigate the effects of water stress on the morphology of *Lawsonia inermis* L. (henna) plants, specifically in the arid region of Shahdad, where severe drought conditions are prevalent.
Methods: A four-month experiment was conducted in a personal greenhouse in Shahdad, where henna plants were subjected to three different irrigation regimes: full field capacity (control, T0), 50% of the control (moderate stress, T1), and 25% of the control (severe water scarcity, T2). Morphological parameters, including leaf area (LA), leaf number, and stem length, were measured to assess the impact of varying water availability.
Results: The results indicated a decrease in leaf area, leaf number, and stem length in henna plants under drought stress conditions. Moderate drought stress (T1) did not significantly impair plant morphology and exhibited better growth compared to the control (T0). However, severe water scarcity (T2) led to a significant reduction in total leaf number and LA, with a 65.79% decrease in LA after 60 days of measurement and a 44% reduction in stem length. The responses to water stress were found to be both time and intensity-dependent.
Conclusions: This research provides valuable insights into the morphological responses of henna plants to varying levels of water stress, highlighting the resilience of henna under moderate drought conditions and the detrimental effects of severe water scarcity. The findings contribute to understanding how henna cultivation can be managed in arid regions facing water limitations.

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1- Introduction

Henna (scientific name: *Lawsonia inermis*) is one of the valuable plants of the genus Lawsonia, which holds significant importance in the dyeing industry. This plant is a shrub native to North Africa, South and West Asia, and Northern Australia.

Historically, henna has been used as a cosmetic substance in ancient Egypt, parts of North Africa, West Africa, the Horn of Africa, the Arabian Peninsula, the Near East, the Indian subcontinent, and South Asia. It was also popular among women in the Iberian Peninsula and 19th-century Europe. The dye obtained from henna is used for colouring the skin, hair, and nails, as well as fabrics including silk, wool, and leather. It possesses medicinal properties for treating kidney stones, jaundice, wound healing, and preventing skin inflammation. Traditionally, henna has been used to treat jaundice, splenomegaly, nephritis, leprosy, and stubborn skin diseases. The species is named after the Scottish physician Isaac Lawson, a close friend of Linnaeus.

Iranians have long recognised the magical properties of henna in medicine and the treatment of skin diseases. Consequently, henna has held a very special place in the development of agriculture, trade, export, and job creation in Iran. Many mills were constructed for grinding henna leaves, which operated using water; however, as henna fields and gardens were gradually replaced by other crops, all mills, dyeing industries, and businesses associated with the henna industry also gradually disappeared. A compound in henna leaves not only possesses dyeing properties but also exhibits antibacterial and antifungal effects.

Henna is cultivated in various regions of Iran, particularly in the south and southeast, including Sistan and Baluchestan and Kerman, where it is of high quality. Among these, the henna from Shahdad in Kerman is particularly distinguished and is renowned as "fiery henna." Phenological studies of this plant have shown that, on average, seeds germinate and emerge from the soil 12 to 14 days after planting. Flowering in the plant typically begins around 90 days after sowing and continues consistently. The rate of flowering slows down with the onset of the warmer months (Tir and Mordad) but accelerates again towards the end of the growth period as the weather cools. In various regions, the complete ripening of fruit occurs from late Mehr to late Aban.

The number of harvests is influenced by the planting date, the frequency of irrigation, and the perennial nature of the plant, varying between two to three harvests. The first harvest of henna is the most valuable and of the highest quality compared to subsequent harvests. The timing of henna harvesting varies depending on the climate type and number of harvests, occurring from Tir to Aban. The total growth period of the plant varies on average between 190 to 220 days in the studied regions.

The aim of this study is to describe the effects of three different irrigation regimes on the morphological traits of the henna plant, including stem length and leaf number, which are some of the repeated traits used to assess the physiological responses of plants under water stress.

2-Materials and Methods

2.1. Plant Materials and Growth Conditions

Henna seeds (Lawsonia inermis L.) were grown in 4-liter plastic pots filled with a mixture of sand and soil (1:2). Two seeds were planted in each pot, and the soil was irrigated with distilled water until the henna germinated. Maximum germination rate was observed after 15 days. At this stage, the seedlings in each pot were reduced to one, and they were watered three times a week. Subsequently, the plants were grown under the following conditions in a greenhouse: a temperature of ± 25 degrees Celsius, 50% relative humidity during the day / 75% relative humidity at night, and a light cycle of 16 hours of light / 8 hours of darkness. The experiment was conducted in a growth chamber with three treatments and three replicates. At two months of age, nine plants (three plants per treatment) were irrigated to field capacity (control, T0), 50% of control (moderate stress, T1), and 25% of control (severe water deficit, T2). The experiments were conducted from August and continued until November.

2.2. Measurements

Leaf area (LA) was measured at 15, 30, 45, and 60 days after the start of the bioassay on three young, fully expanded leaves from each pot. LA (cm^2 $plant^{-1}$) was determined by scanning the leaves using an ADC area meter (Analytic Development Co. Ltd; AM 100). The measured parameters included the leaf emergence rate (leaf day^{-1} $plant^{-1}$), which was assessed biweekly over two months by counting the number of green leaves. Stem growth characteristics were evaluated by measuring the length from the soil surface to the apical meristem.

2.3. Statistical Analysis

Data collection was performed in three replicates for a single henna cultivar for each measurement. A multifactorial analysis (3 irrigation levels versus 4 assessment dates) was conducted to statistically analyze the behavior of the similar henna cultivar. Analysis of variance (ANOVA) at α=0.05 indicated significant differences between and within the factorial combinations. The new Duncan's multiple range test (MRT) was employed to compare means and distinguish between the responses of henna to each irrigation regime for each assessment date.

3-Results and Discussion

3.1. Changes in henna leaf area growth under water stress

Leaves, as the first organs to exhibit visible signs of drought, may provide inexpensive and easy traits for selection under water scarcity conditions. The LA (Leaf Area) for each treatment used in Figure 1 represents the average of three replicates under a treatment. Our results indicated that the LA values in the control plants were higher than those in the water-stressed plants. Generally, LA was higher under optimal conditions (T0) compared to water deficit conditions T1 and T2 (Figure 1). In fact, we observed that under good irrigation conditions (T0), the LA of henna varied from 2.45 (cm^2 $plant^{-1}$) at 15 days after the start of irrigation to 8.88 (cm^2 $plant^{-1}$) at 60 days.
Leaves, as the first organs to exhibit visible signs of drought, may provide traits that are inexpensive and easy to record for selection under water scarcity conditions. LA (Leaf Area) for each treatment used in Figure 1 represents the average of three replications under a treatment. Our results indicated that LA values in control plants were higher than those in plants subjected to water stress. Generally, LA was higher under optimal conditions (T0) compared to water-deficient conditions T1 and T2 (Figure 1). We observed that under good irrigation conditions (T0), the LA of henna varied from 2.45 (cm^2 $plant^{-1}$) at 15 days after the onset of irrigation to 8.88 (cm^2 $plant^{-1}$) at 60 days.
Under mild stress T1 (50% FC), LA decreased from 8.88 to 5.51 (cm^2 $plant^{-1}$), indicating a reduction of approximately 37.98% compared to the control. Therefore, under severe stress (T2), the average LA value was 3.04 (cm^2 $plant^{-1}$). The percentage reduction after the application of this severe water stress compared to T0 was 65.79%. This implies that henna exhibited less tolerance to water deficiency (25% FC). Furthermore, exposure to drought stress led to significant changes in LA, which were confirmed using ANOVA, indicating that the effect of irrigation T2 was significant at $P<0.0001$. Several studies have shown that water deficit results in a substantial reduction in LA development. The limitation of leaf growth is an adaptive mechanism to reduce transpiration.

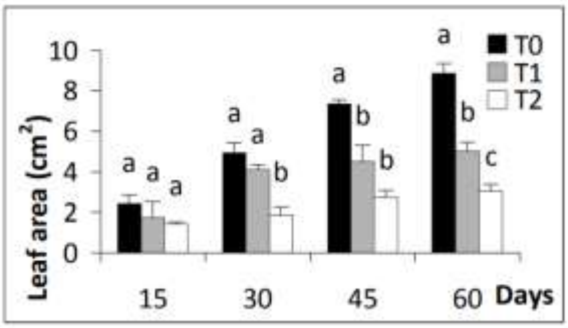

Figure 1. Leaf area (LA) change in henna plants under T0 (control), T1: moderate deficit irrigation (50% FC) and T2: severe deficit irrigation (25% FC) at 15, 30, 45 and 60 days, after the initiation of irrigation regimes. Data represent mean ± S.E. (n = 3). Means with different letters are significantly different at $P < 0.05$.

3-2- Change in the number of henna leaves under water stress

These traits showed significant differences depending on the irrigation factor and drought period (Figure 2). Water deficiency (T1 and T2) reduced the number of leaves per plant by up to 50% compared to the control group (T0). Under normal conditions, the average number of leaves for henna (0.9 ± 40) was recorded more than 60 days after the start of the biosurvey. In response to the initial water stress (T1), the total number of leaves varied from 40 to 29 leaves per plant over a period of 60 days. The analysis of variance (ANOVA) allowed us to indicate that the effect of T1 is highly significant ($P<0.0001$). Furthermore, the Duncan test revealed that after the onset of stress, between the second and sixth weeks (from 15 to 45 days), henna responded similarly under moderate and severe water deficit conditions (Figure 2).
After 45 days, the response of the henna plant to water deficiency was highly dependent on the intensity of water stress. Plants that experienced drought during the vegetative growth stage exhibited a reduction in the total number of leaves

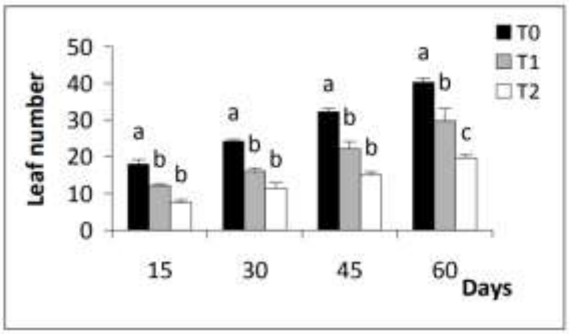

Figure 2. Change in total leaf number in henna plants under T0 (control), T1: moderate deficit irrigation (50% FC) and T2: severe deficit irrigation (25% FC) at 15, 30, 45 and 60 days after the start of irrigation regimes. Data represent mean ± S.E. (n = 3). Means with different letters are significantly different at $P < 0.05$.

3-3-Changes in stem elongation under water stress

The initial length of the main stems was 8.5 centimeters. Under controlled conditions for more than 15 days, the stems continued to elongate during the 8-week experiment, showing an extension of 15 centimeters (Figure 3). Under moderate stress (T1), stem growth was maintained, and the length of the stems significantly increased by 12 centimeters after two months of water stress. The plants measured at 60 days exhibited a significant reduction in stem length under severe drought conditions (T2). This reduction was approximately 44 percent compared to T0. Water deficiency had a substantial impact on the growth of the plant's stem length. In fact, our results indicated that the reduction in growth of the henna plant was noticeable following the application of the irrigation regimes T1 and T2 (Figure 3). Therefore, after 8 weeks of severe stress T2 (25% FC), the highest percentage reduction in stem length compared to the control was 41.38 percent.

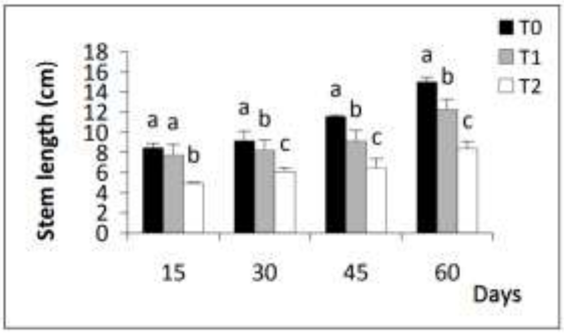

Figure 3. Change in stem length in henna plants under T0 (control), T1: moderate deficit irrigation (50% FC) and T2: severe deficit irrigation (25% FC) at 15, 30, 45 and 60 days after the start of irrigation regimes. Data represent mean ± S.E. (n = 3). Means with different letters are significantly different at $P < 0.05$.

4.Conclusion

Henna, as a relatively drought-tolerant plant, responds accordingly. The mechanisms of drought response exhibited by the henna plant varied to some extent depending on the irrigation dosage. Under moderate stress (T1), henna plants were able to tolerate water stress by adapting their morphological mechanisms to cope with drought damage. However, under severe water deficit stress (T2), the plants appear to be more sensitive to drought. In summary, henna plants may be utilized to overcome drought rather than salinity issues in their natural habitats; however, further research is essential to test the physiological mechanisms for coping with drought in semi-arid regions and to enhance henna production in Shahdad.

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